Improving Smallholder Livelihoods through Improved Casuarina Productivity

Proceedings of the 4th International Casuarina Workshop
Haikou, China
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Preface

The substantial socio-economic importance of casuarinas (several members of the family Casuarinaceae) has ensured ongoing global interest in research and development of this group of nitrogen fixing trees. Plantings are invariably associated with agricultural systems and there are about 2 million ha planted in the tropics and sub-tropics, offering stability to fragile sandy coastal ecosystems and providing services of protection, soil organic matter, poles, fuelwood and commercial wood fiber. Following earlier successful international meetings in Canberra (1981), Cairo (1990) and Da Nang (1996), the 4th International Casuarina Workshop was held in Haikou, Hainan, People's Republic of China, 21-25 March 2010. The objectives of the meeting were to update and collate current information and advances in the research and development of this interesting group of species, with particular reference to their impact on smallholder livelihoods, reflected in the title for the Workshop of: *Improving smallholder livelihoods through improved casuarina productivity.*

A total of 74 participants from 14 countries attended the Workshop and presented 37 papers and 8 short communications covering agroforestry, tree improvement, silviculture, nitrogen fixation, environment and utilization. Several papers not presented at the Workshop have been included in these proceedings. A one-day field trip was arranged for participants to view tree improvement activities, utilization of casuarinas for coastal stabilization, charcoal production and plywood factory. This Workshop demonstrated the continuing interest in research and development of casuarinas.

Chonglu Zhong
Khongsak Pinyopusarerk
Antoine Kalinganire
Claudine Franche

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Many organizations provided financial support for participants to attend this workshop. These organizations are CAF (Chinese Academy of Forestry), CSIRO (Commonwealth Scientific and Industrial Research Organisation), ICRAF (World Agroforestry Centre), APAFRI (Asia Pacific Association of Forestry Research Institutions), IRD (Institut de Recherche pour le Développement), and Consulat Général de France à Canton. In addition, forestry agencies in southern coastal provinces of Fujian, Guangdong, Hainan and Zhejiang supported their staff to attend and present research papers.

The organizers of the workshop thank the Hainan Forestry Bureau for facilitating the local organization. Many staff and post-graduate students from the Research Institute of Tropical Forestry are thanked for looking after the needs of overseas participants during the workshop.

The authors assume full responsibility for the contents of their papers. The views expressed in each paper are those of the authors. Editing of the original papers has been made only to improve and maintain the uniformity of the proceedings.

Chonglu Zhong
Khongsak Pinyopusarerk
Antoine Kalinganire
Claudine Franche

Summary of Discussions and Recommendations

After the completion of the formal presentation programmes, the meeting divided into three broad groups to discuss major issues arising from the presentations. The groups were:

1. Agroforestry, Tree Improvement and Silviculture
2. Nitrogen Fixation, Symbiosis, Biotechnology and Genetics
3. Environmental Amelioration and Pests and Diseases

The following recommendations were brought forwarded by each group at the plenary session:

Group 1. Agroforestry, Tree Improvement, Silviculture

1. Tree improvement

- Explore variation in lesser known species like *Casuarina oligodon* and *C. obesa* and other alternative species for effective use in special situations like salt affected lands, heavy metal accumulation, cold and drought, cyclones, mine rehabilitation, saline and water-logged areas and changing climate.
- Maintain a broad genetic base of most suitable provenances in the form of Provenance Resource Stands (gene banks or ex-situ conservation stands) with fresh seed collection to 'fall back' in case of disease/insect epidemics.
- Facilitate exchange of progeny/new clones and other forms of germplasm from breeding programmes in different countries to widen genetic base of both breeding and biomass producing populations. Make available improved germplasm to smallholding farmers through strategies like community seed production systems.
- Tailor-made clones for diverse end uses and sites. Advanced breeding programmes should focus on carbon sequestration, wood properties and resistance to disease/insect attack. Number of clones for planting should be increased to secure clonal plantations from disease/insect attack.
- Widen hybridization with productive provenances and introduce new traits through inter-specific hybridization. Enhance understanding of flower and pollen biology especially species other than *C. equisetifolia* to higher hybridization success. Efficient micropropagation techniques to mass multiply hybrids and marker systems to verify parent and hybrid identity.

2. Silviculture and agroforestry systems

- Develop effective methods to cultivate casuarinas in combination with different and agricultural crops. The socio-economic and environmental advantages and sustainability need to be assessed.
- Precision establishment techniques for growing casuarinas in diverse environments like shelterbelts, mined areas and farmlands. Spacing and fertilizer application and utilization of coppicing ability in farmland and in different agroforestry conditions need to be optimized. Develop silvicutural techniques versus harvest/optimal rotation age.

- Widen knowledge on different end uses of casuarinas like in green energy, charcoal, craft, high value products, and industrial uses like paper, plywood, etc.
- A monograph compiling available latest information on management and utilization of casuarinas.

Group 2. Nitrogen Fixation, Symbiosis, Biotechnology and Genetics

1. Biotechnology of symbiosis

– *Frankia*

- Collect *Frankia* strains and constitute an international collection - share the isolation protocols and optimize the protocols for storage of the actinorhizal strains.
- Share the inoculation procedures for forestry, clonal and seedlings.
- Sequence more *Frankia* strains that have the ability to nodulate in stressed areas and marginal lands such as salt and heavy metals.
- Characterize the signal molecules that are involved in the molecular dialogue with casuarinas; molecules should improve the nodulation and the root growth.

– *Mycorrhizae: ecto and endomycorrhiza*

- Share knowledge on strain isolation for mycorrhiza.
- Share the *Frankia* /mycorrhizal inoculation protocols (use *Frankia* then mycorrhiza, or vice versa or together).
- Collect mycorrhiza and use molecular markers for taxonomic identification; constitute a well characterized strain collection.
- Study the role of mycorrhiza for protection of the root system against biotic and abiotic stress such as salt stress and nematode infection.
- Use of molecular markers, such as phosphate transporter, to study the efficiency of the symbiotic association.
- Use of the symbiotic MYC factors to improve the association.

– *Rhizospheric helper microorganisms*

- Develop metagenomics to understand the diversity of the microorganisms in the rhizosphere of casuarina plantations; characterize the effect of degraded land on rhizospheric biodiversity of microorganisms.
- Physiological studies on the contribution of helper bacteria (*Pseudomonas* and *Bacillus*) to improve the symbiotic processes.
- Improve the characterization of other helper bacteria and study their impact on plant growth in the field (i. e. *Azospirillum*).

2. Plant biotechnology

– *Tissue culture*

- Test and improve the micropropagation protocols for adult elite trees.
- Develop micropropagation from callus for biotechnological purposes (e. g. gene transfer for valuable traits); *ex vitro* studies of tissue culture plants.
- Transfer techniques to other potential but lesser known *Casuarina* species (e. g. *C. obesa*).
- Obtain a better root system after auxin treatment for a better soil anchorage (improve resistance to typhoons).

– Molecular biology

- Approach Joint Genome Institute (JGI) for the sequence of a *Casuarina* genome; need support from the casuarina community.
- Use microarray and deep sequencing techniques to understand the symbiotic process, stress resistance, adaptation to heavy metals.
- Develop molecular markers for breeders and for the study of plant biodiversity.

– Genetic transformation

- Develop genetic transformation procedures for valuable tree species.
- Targets: tree architecture, disease resistance, salt tolerance, drought, cold tolerance, metal uptake for soil rehabilitation, lignin for pulp, charcoal and for improving the wood quality.

Groups 3. Environmental Amelioration and Pests and Diseases

1. Safe transfer of germplasm to avoid dissemination of pests and diseases between countries

- Strengthen quarantine procedures for germplasm exchanges.
- Information (including on web site) about pests and diseases containing a variety of information e. g. pest and disease agent, prevention and control.

2. Management

- Use of biological/cultural strategies for preventing/controlling pests and diseases.
- Use of mixed plantations and appropriate management strategies for mixed plantation.
- Monitoring/investigation of pests and diseases in natural stands.
- Adaptation of new pests and diseases to improved hybrids/clones.
- Effects of pests and diseases on productivity - how to improve nutrient cycling.
- Monitoring diseases in nursery that are transmitted to plantations.
- Indicator host of pests and diseases.
- Monitoring of tree decline occurrence - study biotic/abiotic contributors/stressors.
- Increased cold tolerance, salinity tolerance and other stress adaptation.

3. Impacts of climate change

- Permanent monitoring plots to document the impacts of climate change, e. g. rising sea levels.
- Impacts to casuarinas on distribution, pests and disease occurrence.
- Role of casuarinas in addressing impacts of climate change - establish trials to identify character traits adaptable to climate change impacts.

Status of Casuarina Research and Development

Casuarina Research and Development in China

Chonglu Zhong[1*], Yong Zhang[1], Yu Chen[1], Qingbin Jiang[1], Zhen Chen[1], Chong Wu[1], Khongsak Pinyopusarerk[2], Claudine Franche[3] and Didier Bogusz[3]

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Abstract Casuarina trees are planted along the coastal area of southern China as windbreaks, in agroforestry systems and for wood and fuelwood production. At present, casuarina plantations cover about 300,000 ha. *Casuarina equisetifolia*, *C. cunninghamiana*, *C. glauca* and *C. junghuhniana* are the most commonly planted species. Series of field trials have been carried out using different *Casuarina* species, provenances and clones to screen for adaptability to biotic and abiotic stress in different areas. A simple technique for the mass propagation of casuarina seedlings is rooting of cuttings in water culture. Experiments conducted in the nursery, glasshouse and field showed that ectomycorrhizal (ECTM) or arbuscular mycorrhizal (AM) fungi or *Frankia*-tree genotype symbiotic associations played an important role in improved management of casuarina growth.

1 Introduction

The family Casuarinaceae includes tree and shrub species that have the capacity to enter different endosymbiotic interactions with soil microbes including the nitrogen-fixing actinomycete *Frankia*, ectomycorrhizal and endomycorrhizal fungi (Zhong *et al*., 1995). Many casuarinas are economically and ecologically important as they provide a wide range of goods and services. Their wood is a main source of fuelwood and charcoal, and is also used for general construction and other wood-based industries, e.g. woodchips for paper pulp and veneer for plyboard. Along the southern coast of China, casuarinas are commonly planted as windbreaks to stabilize the moving sand, in agroforestry, and for general rehabilitation programmes, e.g. pioneer trees for degraded sites and soil improvement. Very few species can replace casuarinas at the foreshores.

Casuarinas were introduced to the tropical and sub-tropical zones of China in the early 1960s, and currently cover about 300,000 ha mostly in the coastal areas. Among the large number of species introduced, *Casuarina equisetifolia* L. Johnson, *C. cunninghamiana* Miq., *C. glauca* Sieber ex Sprengel and *C. junghuhniana* Miq., are the most successful, and are the focus of ongoing research and development. This paper summarizes research and development on casuarinas conducted in China over the past 25 years.

2 Improvement Programmes for Key *Casuarina* Species

2.1 Provenance/family/clone selection

Since 1984, with the support from projects funded by ACIAR, CSIRO-coordinated international trials, FAO and the Chinese government, 23 *Casuarina* and *Allocasuarina* species comprising 204 bulked seedlots, 230 individual families and more than 100 clones have been field tested in China. The results showed that *C. cunninghamiana*, *C. equisetifolia* and *C. junghuhniana* were most suitable for the tropical region. *C. equisetifolia*, *C. glauca* and some provenances of *C. cunninghamiana* were suitable for the subtropical region along the coast in the southern part of Zhejiang province and the north-eastern part of Fujian province. The summarized results are as follows:

C. equisetifolia: By ACIAR projects and CSIRO-IPTC projects, more than 10 international provenance trials have been established in Guangdong, Hainan, Fujian and Guangxi in southern China since 1985. Based on more than ten quantitative and qualitative traits (Pinyopusarerk *et al.*, 1995), better-than-average provenances were 14233, 18008, 18013, 18015, 18086, 18118, 18119, 18127, 18128, 18134, 18143, 18153, 18154, 18244, 18288, 18348 and 18355, with possible suitable areas predicted by means of Booth's model (Zhong, 1990; Zhong and Bai, 1996; Zhong, 2000; Zhong *et al.*, 2001).

C. cunninghamiana: Around 35 seedlots have been introduced into China since 1980s. Based on results from ACIAR projects 8457 and 8848, and FAO project GCP/CRP/005/FRA, better-than-average seedlots were 13513, 13514, 13515, 13516, 13518, 13519, 13520 and 15574. In southern China, the provenances from northern Queensland performed better than those from southern Queensland and New South Wales (Zhong and Bai, 1996).

C. glauca: Around 28 seedlots have been introduced to China since 1984. Based on the results from ACIAR project 8457 and 8848, FAO project GCP/CRP/005/FRA and Chinese projects, the better-than-average seedlots were 13141, 14146, 15217, 15218 and 15579. In southern China, northern provenances from its native distribution performed better than those from southern provenances (Zhong and Bai, 1996).

C. junghuhniana: Since 1985, over 36 provenances have been introduced and tested in southern China. In 1986, two provenances were tested in Qionghai of Hainan and Zhangzhou of Fujian. In 1991, seven provenances were planted in Yangxi of Guangdong. In 1996, two international provenance trials involving 28 seedlots were established in Zhangzhou, Fujian and Dianbai, Guangdong. The results showed that better-than-average provenances were 17877, 18844, 18847, 18852, 18853, 18949, 18950, 19238, 19239, 19240, 19489, 19490 and 19491 (Zhong and Bai, 1996; Zhong, 2000; Zhong *et al.*, 2003).

In 2001, a progeny test of *C. equisetifolia* with 230 individual families was set up. In 2008, more than 400 genetic resources of the four key *Casuarina* species were used to establish seed orchards in Hainan Island. Since 2002, tests including more than 50 clones have been carried out at six sites with a total area of over 10 ha in Hainan and Guangdong provinces.

2.2 Hybridization

Based on introduction, conservation, provenance trials and progeny and clone tests, Chinese researchers have been working on the selection of new *Casuarina* varieties to enhance productivity and adaptability. Chinese researchers created new varieties by producing *Casuarina* hybrids during the 1950s. The following hybrids were reportedly produced: *C. equisetifolia* × *C. glauca*, *C. glauca* × *C. equisetifolia*, *C. cunninghamiana* × *C. equisetifolia* and *C. cunninghamiana* × *C. glauca* (Xu and Lao, 1984). Unfortunately, these hybrid resources have been lost. At present, *Casuarina* hybrid clones in China are natural hybrids selected from clonal plantations of mainly *C. cunninghamiana*, *C. equisetifolia* and *C. glauca*. Current hybridization work has been conducted in India (Nicodemus *et al*., 2010). El-lakany (1983) reported a hybrid of *C. cunninghamiana* × *C. glauca* in Egypt. Clonal plantations of a male hybrid of *C. junghuhniana* × *C. equisetifolia* have been established in Thailand and India (Kondas, 1983).

From 2007 to 2010, 136 crosses were performed by controlled pollination, and seeds were harvested from 64 successful crosses. Progeny tests of these seeds were conducted in 2008 in Hainan and Fujian provinces.

2.3 Molecular biotechnology

The genetic structure and diversity of *C. equisetifolia* provenances were revealed by RAPD and AFLP markers (Guo *et al*., 2003; Huang *et al*., 2009). The information has provided baseline data for selection and breeding of this species in China. Meanwhile, *C. equisetifolia* and *C. cunninghamiana* have been used for genetic transformation in China (Zhong, unpublished data).

3 Propagation Techniques

3.1 Propagation by cuttings

Branch cuttings are used to propagate planting material. The vegetative material is the young needle-like branchlet, preferably less than three months old taken from stock plants in hedge orchards. Water culture is now a common method for propagation of casuarina cuttings in China (Liang and Chen, 1982). The procedure consists of soaking the bottom part of 8-10 cm long branchlets in 50-100 mg kg^{-1} of naphthalene-acetic acid (NAA) or indole-butryic acid (IBA) solution for 24 hours. The bottom 3-4 cm of the plant material is then soaked in tap water and placed near sunlight. The tap water is changed every day. At 25-32°C water temperature, cuttings will root after 7-10 days, and the rooting percentage reaches over 80% after 15 days. If the water temperature is lower than 25°C, it may take 15-30 days to root. The rooted branchlets are then transplanted in growing containers filled with standard potting mix. This technique has been extended to county foresters and farmers.

Rooting in moist, fine sand, and applying the same hormone treatment as described above for water culture, is an alternative method. Generally, a sand bed 15-18 cm in depth, 80-100 cm in width and 5-10 m in length, is built with bricks. A plastic sheet is placed inside the bed to keep the sand clear of soil, and water pipes are laid at one end to supply water. The sand bed is always kept moist and is covered with a clear plastic sheet to increase the temperature in winter. This method is also suitable for mass production of planting material.

Both water culture and sand culture are used successfully for *C. equisetifolia*, *C. cunninghamiana*, *C. glauca* and *C. junghuhniana*. Other species such as *C. cristata*, *Allocasuarina littoralis* and *A. torulosa* are more difficult to root.

3.2 *In vitro* tissue culture

In vitro tissue culture propagation of casuarina is also an important technique to produce seedlings, particularly species that are difficult to root or when propagating old trees. Since 1980s, tissue culture methods for propagating casuarinas have been reported (Abo El-Nil, 1987; Cao *et al*., 1990;

Duhoux *et al*., 1990). Suitable tissue materials include slender branches, young buds, immature male inflorescence and female flower buds. The success of the approach depends on appropriate disinfection of the plant material, the composition of the nutrient medium, rooting hormone (NAA, 6-BA or IBA), temperature, light, and the biological characteristics of the species. Generally, slender tissue-cultured material is easier to root than material from cuttings. In 2000, Chinese researchers started to work on *in vitro* casuarina trees. Up to now, *in vitro* plants of *C. cunninghamiana*, *C. equisetifolia* and *C. glauca* have been obtained, but there are considerable differences in organogenesis among species (Liu *et al*., 2003).

4 Selection for *Frankia*/Mycorrhizal Fungus-Association of Tree Genotypes and Application of Inoculum

Over 20 strains of *Frankia*, eight genera of ectomycorrhizal fungi and 15 AM fungi have been collected from casuarina plantations in China (Kang and Zhong, 1999; Zhong *et al*., 2010). Mycorrhizal fungi and *Frankia* can improve the growth and biomass production of casuarina seedlings or saplings. Chinese researchers have been working on the selection of casuarina symbiotic genotypes, and inoculation with ectomycorrhizal fungus significantly improved the diameter and height of seedlings of *C. equisetifolia* and *C. junghuhniana* (Zhong, 1993; Zhong, 2000). There was also variation among seedlots in response to the inoculation (Zhong *et al*., 2003). A *Frankia* inoculation experiment on *C. cunninghamiana* was carried out in the hot, dry river valley in Yuanmou, Yunnan province, which is located far from the coastal area and there is no natural *Frankia* presence in the soil. Survival of inoculated seedlings was 10-20% higher than that of uninoculated seedlings. Tree height after two years differed significantly among *Frankia* treatments, and not all *Frankia* strains improved tree growth (Yang *et al*., 2007). It is important to screen suitable *Frankia* strains for different species and different sites. Since 1989, many field inoculation trials have been conducted in Hainan, Guangdong and Yunnan provinces. More than 18 years of experience in applying casuarina inocula in China have shown that application of a symbiotic microorganism (*Frankia* and mycorrhizal fungi) can effectively improve survival and biomass productivity of casuarina plants (Zhong *et al*., 2010), and is recommended when casuarina trees are planted at sites where casuarinas have not previously been planted. Pure culture strains or isolate mycorrhizal fungus inoculants in liquid form can be used to inoculate seedlings in the nursery.

5 Casuarina Trees for Degraded Lands

Based on previous experiments, we studied pest and disease resistance at seedlot and clone levels; wind resistance at seedlot and clone levels; salt tolerance at species and clone levels; and cold tolerance at species and clone level. Tissue-cultured seedlings of three *C. equisetifolia* clones were subjected to salt-tolerant tests, and the best clone was able to grow with 0.3% NaCl in the growing medium (Liu *et al*., 2003). Tolerance to salt, drought and disease of cuttings material using some physiological traits and growth indexes have been reported (Yang *et al*., 2003; Zhang *et al*., 2008). Since 2001, we have introduced *C. equisetifolia*, *C. glauca*, *C. cunninghamiana*, *C. junghuhniana*, *C. obesa*, *C. cristata* and *A. littoralis* to the hot dry river valley in Yuanmou, Yunnan province, which is characterized by degraded soil, low annual rainfall (600 mm) and high evaporation (3,800 mm). The first five species have potential but further observations are needed (Yang *et al*., 2007).

6 Wilt Disease

Dieback of casuarina trees caused by bacterial wilt disease *Ralstonia solanacearum* (formerly

Pseudomonas solanacearum) is a very serious problem in many plantations in southern China. Selection of wilt-resistant genotypes started in the 1980s. Some clones have shown resistance but their growth is slow. In addition, new strains of *R. solanacearum* tend to develop in new environments. A precautionary measure is to broaden the genetic diversity by planting many clones in bacterial-wilt prone areas. A minimum of 10 clones may be required in large-scale planting by forest farms. However, in China, plantation stakeholders often only consider the short-term financial gain and plant only very few highly productive clones.

7 Conclusions

Four *Casuarina* species, *C. equisetifolia*, *C. cunninghamiana*, *C. glauca* and *C. junghuhniana* are the most promising for plantation establishment in southern China. These species have shown good adaptability to degraded sites, e. g. salt alkaline land in coastal areas and stone mountains and hot dry river valleys in inland areas. Casuarina trees display marked variation in quantity and quality traits. The extent of these variations needs to be studied for the selection of superior materials. Among propagation methods, root cuttings of young branchlets yields very good results and is the most commonly used method for mass propagation. Inoculation experiments in China have also shown that symbiotic microorganisms can improve survival and growth, especially on degraded lands. It is thus important to screen the best *Frankia* and mycorrhizal strains for different species and different sites.

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Research and Development of Casuarinas in India

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Abstract India has around half a million hectares of casuarina plantations grown in a range of environmental conditions and with diverse silvicultural practices. Introduced in the late nineteenth century mainly as a fuelwood species, it has acquired multiple end uses like pulpwood for paper making, poles for construction, shelterbelts, windbreak and amelioration of salt-affected and mined areas. Its nitrogen-fixing ability and sparse branching pattern causing low shade make it a highly preferred species to grow along with agriculture crops in various combinations. Casuarina is the major component of shelterbelts in the 6, 100 km long coastline of mainland India protecting human habitations, agricultural fields and vital installations like spaceports and atomic energy stations. Casuarina research and development in India dates back to its early introduction time. Initially the research focus was on perfecting the planting techniques and as the area under cultivation increased emphasis was given to increasing plantation productivity. The most significant of them is the assemblage of a broad genetic base of *Casuarina equisetifolia* and *C. junghuhniana* in the form of provenances and open-pollinated families from the entire natural and planted distribution of these species. One generation of breeding through the new germplasm has yielded 13-28% increase in wood production from plantations compared to the unimproved seed sources. Intra and interspecific hybrids developed with outstanding provenances and clones are under various stages of field testing. Biotechnological interventions are underway to produce transgenic casuarinas tolerant to salinity, disease and drought. Various DNA markers have been developed to evaluate breeding populations, clonal accessions and hybrids. Species and clone specific strains of different biofertilizers like *Frankia*, ecto and endomycorrizhae have been developed and successfully employed to improve the planting stock quality. Various agroforestry models have been developed to combine food and wood production in farmlands involving casuarinas with agricultural and horticultural crops. The major pest and disease problems of casuarina in India, the 'wilt' disease and attack by the bark feeding insect have been studied in detail and seed sources and clones tolerant to them have been identified. The priorities for the future include breeding for adaptability to grow in low rainfall and saline-affected sites and for wood traits to meet the different end uses and to carry forward the biotechnology research aiming at producing transgenic casuarina.

1 Introduction

Casuarina (*Casuarina equisetifolia* subsp. *equisetifolia*) has been under cultivation for over a century in India especially in the coastal areas. Introduced into India in the late nineteenth century mainly to meet increasing fuelwood demand, it has become a multipurpose tree of significant environmental and socio-economic importance. The nitrogen-fixing ability and adaptability to grow in a wide range of soil and climatic conditions including moisture and nutrient limited sites makes Casuarina a preferred choice for commercial and environmental planting programmes. The short-rotation period of 3-4 years (the shortest for tree crops in India) suits well the average Indian farmer with small landholding. It is also amenable to grow along with agricultural and horticultural crops in different agroforestry conditions. Casuarina is the principal species planted in shelterbelts especially in the cyclone-prone east coast of India.

According to the National Forest Policy 1988, the wood-based industries have to source their raw material requirements from outside the natural forests. As a consequence the industries turned to farmers for their wood requirements resulting in increase of farmland area planted with short-rotation tree crops. With this encouraging trend extensive research and development work has been undertaken in Casuarina in India during the last two decades. The Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore has consistently worked on Casuarina improvement since its inception in 1988. In addition, forest departments and agricultural universities in the States of Andhra Pradesh, Orissa, Puducherry and Tamil Nadu have also carried out basic and applied research work. An All India Coordinated Project on Casuarina is being implemented with ICFRE/IFGTB as the lead organization and State Forest Departments, Universities and wood-based industries as partners. This paper presents an overview of recent research and development work on Casuarina in India and sets out priorities for the immediate future.

2 Extent of Cultivation and Utilization

Casuarina equisetifolia constitutes more than 90% of Casuarina plantations in India. Although it is cultivated throughout Peninsular India, plantations are concentrated in the coastal areas of Andhra Pradesh, Puducherry, Orissa and Tamil Nadu which account for about 80% of total plantations. Since the Casuarina plantations are scattered and predominantly raised by numerous smallholding farmers no precise information on the extent of its cultivation is available. Based on the annual utilization of Casuarina wood by the paper industry, it is estimated that at least 500,000 ha are planted with casuarinas in the above mentioned States. Growing Casuarina is also steadily increasing in inland areas where it is not traditionally grown. Shortage of farm labour, insufficient water availability for agriculture, non-remunerative prices for farm produce and increase in frequency of absentee farming are the major reasons for shifting to casuarinas cultivation. A male clone of a hybrid between *C. junghuhniana* and *C. equisetifolia* introduced from Thailand is widely grown for its adaptability to different environments, fast growth and desirable stem form. Wood based industries exclusively use this clone in their farm forestry and contract farming programmes in a large scale.

The principal uses of casuarinas wood in India are fuelwood, poles and pulpwood. All parts of Casuarina trees including the fallen needles and cones are used for many purposes. While the main stem is used as poles and pulpwood, the branches, stumps and roots are used as direct fuelwood and for charcoal making. Poles find use in rural construction, as props for agricultural crops like banana and for scaffolding in the urban areas. It is extensively planted in shelterbelts, windbreaks, sand dune stabilization, amelioration of mine overburdens and afforesting saline and sodic soils.

3 Silviculture and Agroforestry

Casuarina is raised in high density plantations (about 10,000 stems ha^{-1}) and it responds well for irrigation and nutrient application. The commonly followed rotation period is 4 years with irrigation and 6 years under rainfed conditions. But the duration varies greatly in different areas and between farmers. In a few places of coastal Tamil Nadu irrigated Casuarina is harvested as early as 2.5 years of age whereas Forest Department plantations without irrigation are retained up to 8 years. Wood production varies greatly across location, cultivation techniques adopted and age of the trees. Plantations with irrigation and fertilizer application yield 100-150 tonnes ha^{-1} of air dried wood (up to 7 cm dbh). Under rainfed conditions an average yield of 75-100 tonnes ha^{-1} in 6 years depending upon soil quality and amount of rainfall during the cultivation period. An additional 12-18 tonnes of miscellaneous wood is produced per hectare in the form of branches, tops and roots. At the time of harvest the average height is 12 m and dbh is 8 cm. The best trees may measure 20 m height and 16 cm dbh.

Casuarinas have association of N_2 fixing actionmycete, *Frankia* which forms root nodulation. Specific types of *Frankia* strains have been identified and used for rapid growth enhancement of Casuarina seedlings. *Frankia* inoculated seedlings showed increased number of nodules and nodule biomass. *Frankia* inoculated Casuarina was also found suitable for mine spoil rehabilitation. Microbial inoculation has been found very effective in increasing the productivity, even to the extent of 350%. Casuarina also hosts VAM like *Glomus fasciculatum* and ectomycorhizae like *Pisolithus tinctorius*, all showing a high degree of coordination and synergistic effect.

4 Genetic Improvement

Selection of outstanding phenotypes from the plantations and establishment of clonal seed orchards (CSO) were major improvement activities prior to 1988. To facilitate cloning of selected trees a simple and cost-effective vegetative propagation technique of rooting sprig cuttings was developed. Efficient orchard designs were developed to suit the dioecious and wind-pollinated nature of Casuarina. The earliest clonal seed orchard was established in Neyveli, Tamil Nadu with 29 female and 20 male clones (Kumaravelu and Paramathma, 2001).

4.1 Breeding programme

A well-defined long term Casuarina improvement programme was initiated in 1995 with international provenance testing. A written breeding programme (Pinyopusarerk, 1996) was launched in 1998. New Germplasm in the form of provenances and families were assembled from 40 locations in 15 countries spread in three continents (Africa, Asia and Australia) and tested in different locations. Provenances from Thailand, Malaysia and Solomon Islands showed up to 40% faster growth than local Casuarina seedlots in the coastal regions. In areas away from coast (inland region) provenances from Kenya and Thailand showed fast growth. Similarly provenances with higher wood density, straight stems and freedom from diseases and insects were also identified (Nicodemus, 2007). A new species of Casuarina (*Casuarina junghuhniana*) was introduced from Indonesia, Timor and Kenyan sources and tested in coastal and inland sites. Provenances from Timor grew 38% faster than the local seedlot with almost equal wood density (Nicodemus *et al.*, 2005; Pinyopusarerk *et al.*, 2005). This species was also found to be more drought and disease tolerant compared to Indian seedlots. It also showed ability to coppice and produce more than one crop from a single planting.

The first generation provenance-progeny tests were thinned out and converted into seedling seed orchards to produce improved seeds. Outstanding individuals in these test plots were identified for growth, stem form and wood traits. These 'plus trees' were vegetatively multiplied and used for establishing clonal seed orchards. On-farm gain tests showed that seeds from seed orchards provided up to 50% gain in *C. equisetifolia* and 112% in *C. junghuhniana* compared to the unimproved local seed source. Four outstanding clones of *C. equisetifolia* have been released for commercial cultivation after a decade of multilocation testing and selection. Second generation breeding orchards have been developed with selections from the first generation breeding populations. Intra and interspecific hybrid families between the two species were produced through control pollination and are being field tested for selecting outstanding hybrid individuals. A new concept of Community Seed Orchards has been developed for large scale production of genetically improved seeds with the involvement of farmers, nursery operators and forest department with the technical support of CSIRO, Australia and funding support under the PSLP programme of AusAID.

4.2 Biotechnological interventions

In Casuarina species, genetic diversity at different levels of populations was assessed using ISSR markers. The genetic diversity estimates within and between five species of *Casuarina* (*C. equisetifolia*, *C. junghuhniana* and *C. glauca*) and *Allocasuarina* (*A. littoralis* and *A. heugeliana*) revealed the genetic distance between *C. equisetifolia* and *C. junghuhniana*. Similarly, 18 morphometric parameters were analyzed to estimate the diversity existing between the species and compared across the ISSR profile generated for genetic diversity. Both procedures revealed high genetic distance between *C. equisetifolia* and *C. junghuhniana*. Species-diagnostic SCAR (Sequence Characterized Amplified Regions) markers were developed for *C. equisetifolia* and *C. junghuhniana* and were validated on 10 randomly selected hybrids in a multiplexed reaction. The presence of amplicons specific to both species validated their hybridity (Dasgupta *et al.*, 2009).

Studies have been initiated through collaborative efforts with the Rhizogenesis group, Institut de Recherche pour le Développement (IRD), France, for functional analysis of genes involved in salt tolerance in casuarinas for use in marker assisted and transgenic programmes. Fifty-two clones of *C. equisetifolia* were assessed for salt stress response using gradually increasing concentrations of sodium chloride ranging from 50 mM to 550 mM in Hoaglands solution, and highly tolerant and susceptible clones were identified and characterized for their sodium content. These clones will be used for differential transcriptome and functional analysis of gene homologues for development of association based molecular marker approaches. Work is also in progress to isolate pathogen—defense related genes from *C. equisetifolia*.

5 Seed and Nursery Technology

In India, casuarinas are propagated mainly through seeds. Casuarina seeds are small consisting 500,000-600,000 seeds kg^{-1} in *C. equisetifolia* and 1.4-1.6 million seeds kg^{-1} in *C. junghuhniana*. Although the *C. equisetifolia* seeds do not have any dormancy, germination is only 40-50% while *C. junghuhniana* recorded germination up to 80%. For testing *C. equisetifolia* seeds, a temperature of 30℃ and relative humidity of 90 ± 3% with 12 hours photoperiod are recommended. *C. equisetifolia* seeds could be conveniently stored at ambient conditions for 1-2 years and storage at 10℃ prolonged its viability. Pre-storage treatment with botanicals also increased the shelf life of *C. equisetifolia* seeds (Anandalakshmi *et al.*, 2001; Sivakumar *et al.*, 2007).

Seeds are sown in raised sand beds (called 'mother beds') of the size 10 m × 1 m. Generally no pretreatment is necessary for casuarina seeds. In each bed about 200 g of seeds (100 g for *C. junghuhniana*) are evenly spread by mixing with fine sand. They are overlaid with a thin layer of sand. The sand bed is covered with rice straw to prevent washing off of seedlings while watering. Water is provided through a rose can or a sprayer. A suitable insecticide is applied along the periphery of the bed to prevent ants removing the seeds. Seeds start germinating from the 5^{th} day and the straw is removed on the 7^{th} day. They are grown in the mother beds for next 3-4 weeks. The beds have to be kept moist by watering through rose can but water stagnation should be avoided to prevent fungal diseases. After 4 weeks when the seedlings attain 8-10 cm height they are transferred either to a secondary bed or polythene bags. Secondary beds are also of the same size as the mother beds but in addition to sand, farm manure and soil (2 : 1 : 1) are also added to increase nutrient availability and water holding capacity.

Seedlings pricked from the primary beds are transplanted in the secondary bed at approximately 4 cm apart. Seedlings are grown in the secondary beds for 3 months to obtain a height of 30-45 cm and a collar diameter of 3-5 mm. Growing seedling in polybags and root trainers is better than bare root seedlings especially for planting in rainfed areas. Seedlings raised in containers establish well in plantations and record vigorous growth in the first year. Seedling may attain plantable size within 2 months but may be maintained for another 4-6 months if planting is delayed.

6 Insect Pests and Diseases

Although Casuarina is reported to be attacked by about 40 species of insect pests, only nine of them frequently occur in nurseries and plantations capable of causing severe damage. The bag worm *Eumeta crameri* defoliates Casuarina by feeding on the needles in nurseries and plantations. The cottony cushion scale, *Icerya purchasi*, *Ferrisia virgata* and aphids are sap feeders causing damage in nurseries and saplings. Grubs of *Celosterna scabrator* and *Sinoxylon* sp. bore into the stem. *Sahyadrassus malabaricus* also cause damage to stems in young plantations by boring the stem. The bark feeder, *Indarbela quadrinotata*, is considered as a serious pest which often causes economic damage in plantations.

Screening of extensive germplasm of *C. equisetifolia* involving 55 seed sources (provenances and land races) assembled in international provenance trials was conducted to select resistant seed sources against the bark-eating caterpillar infestation. It was observed that, the seed sources like Kilifi and Robinson Island from Kenya, and Queensland, Australia were completely free from the bark-eating caterpillar infestation. The seed sources such as Wangetti Beach and Northern Territory from Australia, Mamorah Alexandria and Montazah from Egypt, Vanua Levu from Fiji, and Kenya were found to be moderately susceptible to the bark-eating caterpillar. The Indian and Malaysian seed sources were found to be highly susceptible. The bark of resistant seed sources contained large number of tanniferous cells, compared to that of susceptible ones. There was a significant negative correlation between susceptibility and total tannin content (Sasidharan *et al.*, 2005).

In Casuarina, collar rot disease caused by *Lasiodiplodia theobromae*, leaf blight and seedling wilt or blister bark are the common pathological problems in nurseries. In plantations, blister bark disease caused by *Trichosporium vesiculosum*, root rot disease by *Ganoderma lucidum* and bacterial wilt by *Ralstonia solanacearum* cause severe mortality of saplings and trees. Provenances from Australia (Northern Territory and Queensland) and Kenya consistently remained free from the blister bark and root rot diseases.

7 Priorities for Future

The major Casuarina research and development priorities for future in India include advancing the breeding programme to produce end use-specific and site-specific planting material, scaling up production of high quality seeds and other propagules to increase the area planted with high yielding material. Precision silviculture methods like trickle irrigation, liquid fertigation and weed management have to be standardized for the newly developed improved planting material. Basic research on developing transgenics, screening *Frankia* isolates, understanding the resistance to blister bark disease and bark feeding insects has to continue to provide necessary support to the genetic improvement programmes. Conservation and enrichment of existing Casuarina germplasm in India also needs high priority. It is also essential to understand and document the socio-economic impacts of research and development efforts especially on the small land holding farmers.

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Research and Development of *Casuarina equisetifolia* in Vietnam

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Abstract Vietnam has over 500,000 ha of coastal sandy soil of which some 400,000 ha along the central coast have been subject to desertification with an estimated 20 ha of agricultural soil being encroached annually by moving sands. To prevent coastal sandy soil areas from becoming desertified, Vietnam has enacted measures to improve the ecological conditions and environments of these sites. Over many years, experiments were carried out to select suitable plant species and *Casuarina equisetifolia* has shown good adaptability to drought and grown well on these coastal sites. Over the past century about 100,000 ha of this species have been planted mainly on coastal sandy soils with high annual rainfall. This article summarizes the findings of research and development of *C. equisetifolia* in Vietnam including: selection of provenances with good growth in various ecological areas; assessment of growth performance on poor sites; planting techniques in moving sand dunes; use of microorganisms to enhance growth and adaptability on poor sites; and management measures for pests and diseases.

1 Introduction

Vietnam has over 500,000 ha of coastal sandy soil (Phan Lieu, 1987) which is critical ecological area where the risk of moving sand is overwhelming. Some 400,000 ha of coastal sandy soil spreading along Central Vietnam have been subject to desertification, with about 20 ha of agricultural soil being encroached annually by moving sand (Nguyen Van Dang, 2002). To prevent coastal sandy soil areas from becoming desertified, Vietnam has enacted measures to improve the ecological conditions and environments of these sites. Species trials were conducted and various plant species, including *Casuarina equisetifolia*, which are drought resistant and grow well on poor sites, selected. This casuarina was introduced to Vietnam in 1886 and over the past century appropriately 100,000 ha have been planted mainly on sandy soils with high annual average rainfall. On high and sloping moving dunes afforestation has rarely been successful. At the bottom dunes survival rate is very low due to moving sand burying the planted seedlings. Under dry weather conditions the lack of water causing wilt and death of shoots. Following the rainy season these casuarinas trees produce new shoots. If the trees survive they will produce a large number of shoots and most of these droop down onto the sandy ground. If only protection against moving sand is taken into consideration, casuarinas plantations partly meet the planting objective.

This paper summarizes the findings of research and development of *C. equisetifolia* in Vietnam including: selection of provenances with good growth in various ecological areas; assessment of growth performance on poor sites; planting techniques in moving sand dunes; use of microorganisms to enhance growth and adaptability on poor sites; and management measures for pests and diseases.

2 Research Activities and Results

Research on casuarina in Vietnam can be categorized into the following.

2.1 Provenance trials

Provenance trials of *C. equisetifolia* involving 50 seedlots provided by CSIRO were carried out in the early 1990s at four sites representing four ecological areas and different soil types. These sites were Bau Da, Tuy Phong (Binh Thuan province); Binh Minh, Thang Binh (Quang Nam province); Nghi Xuan, Nghi Loc (Nghe An province) and Quang Hung, Quang Xuong (Thanh Hoa province). Experimental designs were randomized complete block with 4 replicates, 25 trees per plot and 2 m × 2 m spacing.

Only a few provenances showed good performances across the four test sites. At the low annual rainfall (700 mm) in Binh Thuan, high mortality rates (81-100%) were recorded for provenances 18244 (Bako National Park, Malaysia), 18086 (Hai Thinh, Nam Dinh, Vietnam), 18127 (Thach Lien, Ha Tinh, Vietnam), 18153 (Ela Beach, Papua New Guinea) and 18040 (Navutoca, Tonga). Height growth of provenances 18008, 16166, (Australia), 18120, 18013, 18014, 18015, 18118, 18119 (India), 18185, 18086, 18217, 18152 (Vietnam) and 18154 (Philippines) was significantly greater than the overall average, with provenance 18008 (Darwin, Australia) showing the greatest average height. Diameter growth for the above 13 provenances was also greater than the overall average. Among these, 18013 (India) and 18015 (Vietnam) provenances had the largest diameter.

In Quang Nam province, four provenances were ranked the highest for diameter and height growth. These were 18297 (Ranong, Thailand), 18287 (Hambantota, Sri Lanka), 18321 (Efate, Vanuatu) and 18121 (Mariana Islands, Guam).

In Nghe An province, the best diameter and height growth was recorded for provenance 18117 (Mindore, Philippines).

In Thanh Hoa province, seven provenances: 18296, 18299 (Thailand), 18152 (Vietnam), 18015, 18119 (India), 18355 (Benin) and 18288 (Sri Lanka) displayed the best diameter and height growth.

There was also a provenance trial of *C. junghuhniana* planted in Ba Vi, north of Hanoi in 1997. The trees of all provenances grow very poorly and the trial was later discarded.

2.2 Growth assessment of *C. equisetifolia* in coastal moving sand areas

The assessment was carried out in sample plots. Diameter and height were measured and data analyzed by single-factor analysis of variance.

2.2.1 Seedling plantation

In Ninh Thuan province trees were planted at a density of 5,000-10,000 trees ha^{-1}, however, survival was very low due to the moving sand. When there was strong sand movement young plants were buried in the sand. Growth of *C. equisetifolia* planted on moving sandy dunes in Ninh Thuan province is shown in Table 1. Plantations which were planted on moving sandy dunes and aged between 2 and 21 years formed into clumps. On average there were 6-10 stems per clump which were 9.8-11.6 m in height, 6.7-7.2 cm in diameter and 8.1-9.2 m in crown width (up to 10 m). Even at low densities between 300 and 326 clumps ha^{-1}, plantations still reached ground coverage of up to 40-50% and returns to the soil humus layer of 2 cm to 5 cm.

Table 1 Growth of *C. equisetifolia* plantations on moving sandy dunes

Age (year)	Number of tree clumps ha^{-1}	DBH (cm)	Total height (m)	Crown width of clumps (m)
21	300	7.2	11.6	9.2
18	326	6.7	9.8	8.1
15	1,109	buried	1.3	2.5
10	2,600	5.9	2.6	2.1
3	3,100	1.2	0.7	0.6
2	3,520	2.2	1.9	1.5

In Quang Binh province, density of casuarina planted on moving sandy dunes at age 15 years was 1,109 clumps ha^{-1}, but many trees were buried by sand and failed to grow above the sand. Almost all shoots sprouted horizontally at heights under 1.3 m, with crown diameter per clump of 2.5 m and ground coverage of 28-40%. In ten-year-old plantations, trees were not buried by sand and the density was 2,600 trees ha^{-1}, with diameter reaching 5.9 cm and height 2.6 m. In many moving sand dunes, *C. equisetifolia* plantations have not established well because of slow growth, low tree density and poor crown closure, but these plantations still bring some environmental values and supply firewood to local people.

In Binh Thuan province, *C. equisetifolia* plantations were planted on moving sand dunes in 1974, with a density at planting of 10,000 trees ha^{-1}. At 26 years of age, density of the plantation was 1,100-2,000 trees ha^{-1}, with an average height of 15-18 m, diameter of 25.2 cm, and crown diameter of 4.6 m. The humus layer was 5-7 cm thick, improving fertility of the soil and creating favorable conditions for plantation growth on sandy areas, and contributing to improving the climate for crop production.

Overall, planting of *C. equisetifolia* on moving sand dunes in Vietnam has not been successful for many reasons:

- Bare-root seedlings were used for planting, especially in Quang Binh province. The root systems might have been damaged before they were planted out.
- Planting holes were not filled with organic matters (e.g. grass, leaves, manure or compost). Thus, during the dry season and cold winds, young plants could not absorb enough water causing shoots to dry off and die.
- Late planting in November and December was subject to strong north-easterly season winds, causing seedling roots to become exposed up to 50-60 cm in depth. As a result, many trees died, were blown over or sometimes the whole plots were buried.

2.2.2 Clonal plantations

In Quang Binh province, an Agricultural Resource Conservation and Development (ARCD) project established 13 ha of *C. equisetifolia* plantations in 1999 using clones 601 and 701, with a density of

5,000 stems ha^{-1} and 3,300 stems ha^{-1} respectively, in non-annual moving dunes (Dao Cong Khanh and Dang Van Thuyet, 1997). The nursery seedlings (cuttings) were 1 m tall and planted 40 cm deep into the sand. In the first year these seedlings grew slowly, reaching an average height of 1 m above ground.

In Binh Thuan province, growth and survival of clones 701 and 601 were better than those of normal seedlings. Some characteristics of these clones included a pyramidal crown, high rising shoots and low branching to the sandy ground. Growth of these clones of was twice faster than that of normal seedlings. Clone 701 had better growth and greener foliage and branches than clone 601.

2.3 Assessment of the effect of *Frankia* inoculum and phosphate-dissolving microorganism inoculums on growth of *C. equisetifolia* seedlings in nurseries and plantations

2.3.1 Nursery experiment

Frankia inoculum coded FprHN1, phosphate-dissolved inoculum No. 1 and other phosphate-dissolved inoculum No. 2 were used (No. 1 was produced from *Aspergillus niger* and No. 2 from *Bacillus* sp. DL1). Tests were carried out at the Forest Science Institute of Vietnam, Hanoi, and included 6 treatments, 5 replicates, 50 seedlings per plot and 1 g of the inoculum per seedling. Experimental treatments were as follows:

Treatment 1: No. 1 inoculum
Treatment 2: No. 2 inoculum
Treatment 3: FprHN1 + No. 1 inoculum
Treatment 4: FprHN1 + No. 2 inoculum
Treatment 5: FprHN1 inoculum
Treatment 6: Control

Results of seedling growth two months after application are shown in Table 2. There were clear differences in seedling growth between treatments two months after inoculation. Seedlings treated with No. 1 inoculum showed no difference in growth compared with the control. Separate inoculums of phosphate-dissolved microorganisms and *Frankia* had a clear influence on the growth of seedlings. However, the influence of separate inoculums on the seedling height was much less than that of mixed inoculums such as the *Frankia* inoculum + No. 1 inoculum and *Frankia* inoculum + No. 2 inoculum. Among these mixtures, the *Frankia* inoculum + No. 1 inoculum had the greatest influence on seedling height. Compared with the control treatment, the increase in seedling height was 146% and 152% for the *Frankia* inoculum + No. 1 inoculum treatment, 174% and 193% for the *Frankia* inoculum + No. 2 inoculum treatment, and 137% and 150% for the *Frankia* inoculum treatment, respectively.

Table 2 Growth of *C. equisetifolia* seedlings two months after application of *Frankia* and microorganism inoculums

No.	*Frankia* treatment	Mean height (cm)
1	Treatment 1	24.84a
2	Treatment 2	27.80ab
3	Treatment 3	42.70d
4	Treatment 4	35.90cd
5	Treatment 5	33.70bc
6	Treatment 6	24.60a

Mean values followed by the same letter do not significantly differ at $P = 0.05$ using Duncan's new multiple range test.

2.3.2 Field experiment

The effects of *Frankia* inoculum FprHN1 and phosphate-dissolved inoculum No. 2 on *C. equisetifolia* grown in coastal areas were studied. The experiment was located in Dien Trung commune, Dien Chau district, Nghe An province. There were 4 treatments, 4 replicates and 2 m × 2 m spacing. The treatments were designed as follows:

Treatment 1: Seedlings planted without *Frankia* inoculum and uninfected by *Frankia* in the nursery (control).

Treatment 2: Seedlings inoculated with *Frankia* inoculum in the nursery.

Treatment 3: *Frankia* inoculum applied to seedlings at planting at 100 g per tree.

Treatment 4: *Frankia* inoculum FprHN1 + phosphate-dissolved inoculum No. 2 applied at planting at 100 g per tree.

Growth data of young plantations at 7 and 29 months of age was collected and assessed by ground diameter (Do), diameter at breast height (DBH) and height (H). Results of analysis of variance among experimental treatments are shown in Table 3. There was a significant difference in seedling growth between treatments. Height growth of all treatments which obtained fertilized microorganism inoculum was significantly greater than that of the control treatment. In treatment 2, with fertilization with *Frankia* in both the nursery and the field, there was not a clear difference in diameter and height growth. At seven months after planting, height growth of seedlings reached its highest level in the treatment fertilized with *Frankia* inoculum FprHN1 and phosphate-dissolved inoculum No. 2. At 29 months after planting, there were clear differences in diameter and height growth of all experimental treatments compared to the control.

Table 3 Growth of *C. equisetifolia* plantations treated with *Frankia*

Experimental treatment	7 months after planting		29 months after planting	
	Height (m)	Ground diameter (mm)	Height (m)	DBH(cm)
Treatment 1	0.95a	11.02a	6.83a	5.38a
Treatment 2	1.00ab	12.07b	7.37b	5.97b
Treatment 3	1.09ab	13.54b	7.20b	5.73b
Treatment 4	1.15b	14.25b	7.25b	5.98b

Mean values followed by the same letter in the same column do not significantly differ at $P = 0.05$ using Duncan's new multiple range test.

2.4 Pest and disease situation of *C. equisetifolia*

Pests and diseases of *C. equisetifolia* were surveyed and sampled in plantation areas across Vietnam. Damage was classified into 3 levels:

Severe (+ + +): widespread infestation of plantations by pests and diseases causing significant impact on growth and development of trees.

Moderate (+ +): infestation by pest and diseases of more than 2 plantations, causing some impact on growth and development trees.

Minor (+): low incidence of pests and diseases, having little impact on growth and development of trees.

The survey results are shown in Table 4. Pests and diseases were relatively abundant in *C. equisetifolia*. The stem borer *Zeuzera coffeae* was by far as the most serious damaging pest species on coastal moving sand sites. This stem borer caused die-back of shoots or broken top of shoots, preventing the trees from establishing apical dominance, with shoots hanging down onto the sandy ground. This was a serious problem, and effective prevention measures should be taken against this damaging insect for the success of plantation establishment in moving sand areas.

Table 4 Composition of *Casuarina equisetifolia* pest and disease species and damage levels

Pest and disease species	Common name	Order	Damage part	Damage level
Hypomyces squamosus	Cau cau	Coleoptera	leaf	+
Eumata wallacci	Sau ken	Lepidoptera	leaf	+
Pagodia hekmeyeri	Sau chua	Lepidoptera	leaf	+
Dappula sp.	Sau ken	Lepidoptera	leaf	+
Zeuzera coffeae	Sau duc than	Lepidoptera	leaf	+ + +
Anoplocnemis sp.	Bo xit	Hemiptera	shoot	+ +
Erthrina fullo	Bo xit	Hemiptera	shoot	+ +
Icerya purchasi	Rep sap	Homoptera	leaf	+ +
Ralstonia solanacearum	Heo xanh	Pseudomonales	root	+ +
Trichosporium vesiculosum	Loet than	Trichosphaeriales	stem	+ +

3 Conclusion

Studies in Vietnam suggest that more successful planting of casuarina can be achieved by using appropriate provenances on specific sites and application of micro-organisms to enhance productivity.

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Agroforestry/Farm Forestry/Livelihoods

Enhancing Food Security for Smallholder Farmers through Increased Soil Fertility: Case Studies for the Sahel

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Abstract The decline of soil fertility in smallholder farming systems is the greatest biophysical constraint to increasing agricultural productivity especially in Sub-Sahara Africa. This fertility depletion is the fundamental biophysical root cause of declining per capita food production in Africa, and its replenishment should be considered as an investment in natural resource capital. The need to improve soil fertility management is a very important issue in the development policy agenda, because of the strong linkage between soil fertility and food insecurity. Land degradation is a major threat to agricultural productivity in most tropical countries as it represents a loss of natural capital, the value to society of land, water, plant and animal resources. Land degradation results from natural processes, land uses or other human activities resulting in soil erosion and the destruction of the vegetation cover. In order to address these challenges, low cost agroforestry options to replenish soil fertility are being investigated in many tropical countries. Sustainable land management technologies, including agroforestry practices, to increase the productivity of subsistence and smallholder farmers are discussed. Agroforestry practices are among the proposed options and now widely recognized as a viable concept of creating a sustainable agriculture in the tropics. Such practices have the potential to significantly enhance agricultural productivity, while at the same time contributing to natural resources conservation under changing weather patterns. They also increase substantially and sustainably crop production making a difference to food security.

1 Introduction

Trees are a natural component of the Sahelian landscapes as parklands. Although the rise in human populations and shifting cultivation has caused pronounced reductions in tree cover, trees remain an important element of the wets African Sahel. Parkland trees and shrubs provide many functions for the rural poor (Kalinganire *et al.*, 2008). They are sources of foods, including fruits, fats, oils, leafy vegetables, nuts and condiments that complement staple food crops in the local diet. Some of these foods are particularly important during the months when grains are in short supply, and during years of intense drought. In addition, parkland trees and shrubs provide firewood and construction materials, and a range of services such as shade for humans and animals, wind protection and aesthetic and spiritual value. Moreover, less visibly but not less importantly, both exotic and native trees and shrubs play a crucial role in maintaining and regenerating soil fertility through the action of their roots and litter (Bayala *et al*., 2008). Such species that sustain soil fertility, and providing many other functions, are deliberately maintained on farms and in the landscapes by rural communities. They also fix atmospheric nitrogen. Species such as *Acacia* and *Faidherbia* species are also drought tolerant and thrive in environments receiving annual rainfall as low as 300 mm, they are well adapted to poor soils, fix nitrogen, and have relatively early rapid growth rates.

Lack of access to food and its availability is a main concern in most Sahelian countries and a fundamental challenge for human welfare and economic growth. Furthermore, low agricultural production results in low incomes, poor nutrition, vulnerability to risks and lack of empowerment. Doubling food production by mid-century, particularly in Africa, will require non-conventional approaches, particularly since so many of the continent's soils are depleted, and farmers are faced with a changing climate.

Poverty in Sub-Sahara Africa is largely a rural phenomenon as 85% of the poor reside in rural areas. Agriculture remains the principal economic sector upon which the rural population depends for their livelihoods and one of the most alarming aspects of rural poverty is the lack of food security. Poor food production is mainly due to drought and the loss of the fragile soil and deterioration of its quality which represent the greatest threat to food security in the Sahel. This loss of vegetation cover exacerbates soil erosion and degradation of its quality through loss of organic matter while soil carbon constitutes one of the factors essential for assessing the sustainability of cropping systems.

Soil degradation is a biophysical process, but is driven by social, economic and political forces. Minimizing degradation and enhancing restoration depends on addressing both the biophysical and the human dimensions that drive land misuse. There is an urgent need to break the cycle between poverty and land degradation inAfrica by employing strategies that empower farmers economically and promote sustainable agricultural intensification using efficient, effective and affordable agricultural technologies.

Mineral fertilizers are the most efficient way to reverse soil nutrient depletion, and thus improve livelihoods. However, fertilizers are used in Sub-Sahara Africa at a very low rate and that is one of the main causes for environmental degradation. Low fertilizer inputs and poor farming practices by the smallholders are due to their limited financial capacity, continuously mine the soil of its limited nutrient base. A first step in designing sustainable and productive farming system, therefore, is to restore perennial vegetative cover. The strategy of constant vegetation cover is achievable through agroforestry, which provides the basic function of forestry in the form of perennial canopy cover, while allowing field crop production.

This paper gives examples about the roles and effects of trees, including both native and exotic species, on soil fertility with special reference to the Sahel. Maintaining soil fertility is the basis of all forms of sustainable land use that remains productive in the long term. Such land use improves crop productivity contributing to food security of the Sahel.

2 Trees and Soil Fertility

2.1 Soil fertility management

In Sub-Sahara Africa, especially for smallholder farming, soil fertility decrease is due mostly to nutrient mining through crop harvest with little or no replenishment. The failure to address conservation and improvement of soil largely explains why majority of the agricultural improvement technologies have failed in the Sahel.

In the Sahel, organic matter is mainly composed of manure, compost and crops residues. However, crop residues are collected by farmers, leaving the soil bare exposed to wind and water erosion, and used as animal feed, construction materials or fuel. All these uses constitute a loss for nutrient cycling in the production areas. Trees have a different impact on soil properties than annual crops, because of their longer residence time, larger biomass accumulation, and longer-lasting, more extensive root systems. By accumulating biomass and carbon via the photosynthesis process, trees contribute to reduce the carbon in the atmosphere. This proven contribution of trees to soil carbon build up shows their importance in carbon sequestration in semi-arid zones where soil carbon is also a major factor controlling soil fertility both for nutrients release and soil organic matter formation. In addition, when they are N-fixing trees, trees increase soil N content while producing good quality fodder for livestock thus reducing the pressure on crop residues and soil trampling that can be left in the fields to protect against erosion and favour a recycling of the nutrients they contain.

Agroforestry practices have been shown to influence chemical, physical and biological components of soil fertility (Bayala *et al.*, 2011). Trees can improve nutrient balance of a site both by reducing unproductive nutrient losses from erosion and leaching and by increasing nutrient inputs through nitrogen fixation; they can improve soil structure, water holding capacity and crop rooting volume; and they can increase the biological activity in the soil by providing biomass and a suitable microclimate. If for example a site is deficient in nitrogen and phosphorous, leguminous trees may be able to increase the availability of nitrogen through biological nitrogen fixation, however phosphorus may be added from external sources through mineral fertilizers such as the natural phosphate of Tilemsi, Mali.

2.2 Fallows with legume-based trees and shrubs practices

Successful agroforestry systems increase nutrient inputs, enhance internal flows, decrease nutrient losses and provide environmental benefits when the competition for growth resources between trees and crop component is well managed (Bayala *et al.*, 2008). It is clear that agroforestry practices can increase the total quantity of nutrients in the soil-plant system by increasing the nutrient transfers into the system and by reducing nutrient losses from the system. Moreover, the incorporation of legumes into cropping systems provides additional benefits besides nitrogen input, particularly in terms of pest and disease control, e. g. role of legumes in striga management.

Increased nutrients inputs may originate either from the atmosphere through biological nitrogen fixation by legume and/or nitrogen-fixing trees, or from soil compartments which are outside the reach of crop plants. If deep-rooting trees are associated or grown in rotation with shallow-rooting crops at a site where nutrients are available in deeper soil horizons, nutrients which were previously below the crop rooting zone may be made available to the crops via tree litter or prunings. Moreover, trees could also increase the total amount of nutrient recycling in a system by accessing nutrient pools that are not accessible to crops, for example through more efficient mycorrhizal associations or solubilization of recalcitrant phosphorus forms in the atmosphere.

There are a certain number of tested fast-growing species that can produce a high biomass that can be used as mulching in a cut and carry system. In the Sahel, such species include: *Cassia sieberiana*, *Combretum lecardii*, *Gliricidia sepium*, *Guiera senegalensis*, *Khaya senegalensis*, *Piliostigma* *reticultatum*, *Piliostigma thonningii*, *Prosopis africana*, *Pterocarpus erinaceus*, etc. (Bayala *et al.*, 2011). Many of these species are able to produce more than 3 tonnes ha^{-1} and 7 tonnes ha^{-1} of leaf and wood biomass respectively in 5 years (IER, 2002). Leaf biomass nitrogen content ranged between 1.9% and 2.8%.

The transferred biomass will typically be leaves and twigs that are spread over the soil surface to influence the physical, chemical and biological properties of the soil and its micro-climate with the aim of improving the productivity of a site (Sileshi *et al.*, 2008; Bayala *et al.*, 2011). Such practice, by increasing soil organic carbon, will also improve the efficiency of mineral fertilizers applied. In addition, the above mentioned species will increase the vegetation cover, provide environmental services (soil protection, increased soil biological activities, etc.) while providing products like fodder, wood, medicine, etc. Soil that receives adequate inputs of organic matter will provide a higher level of buffering functions that support water and nutrient availability in variable-weather environments. Critical uncertainties exist in quantification of the soil fertility replenishment functions across climatic zones and stages of intensification of land use. Various efforts at 'ecological restoration' and enhancement of landscape multi-functionality and complexity require critical evaluation and emerging ideas on 'good practice', as they deal with multiple system scales and the interactions between them. Socio-economic conditions make it even more difficult to make blanket recommendations justifying the need to come up with specific solutions for specific socio-ecological niches.

2.3 Australian tree species for soil fertility

Australian acacias and some casuarinas are adapted to a wide range of environments: tropical and temperate, humid and arid, acidic and alkaline and saline and nutrient deficient. Their adaptation and early rapid growth have made them popular for planting on degraded lands in most parts of the Sahel (Bayala *et al*., 2011). A number of Australian acacias were first introduced and tried in the Sahel in the 1980s, with the aim of identifying adapted tree species for fuelwood and windbreaks (Cossalter, 1987). Multipurpose Australian acacias have the potential to form an integral component of agricultural systems in the Sahel. In Maradi in southern Niger, where the annual rainfall is less than 500 mm, well-adapted Australian acacia species such as *Acacia colei* var. *ileocarpa*, *A. torulosa*, *A. tumida* and *A. elachantha* have been identified after more than a decade of research work (Bayala *et al*., 2011). These acacias grow rapidly, with annual mean tree height of more than 2.8 m after 15 months. They are well adapted to infertile soils, produce large quantities of fuelwood and construction timber. They can also be used as windbreaks and to improve soil fertility. In Senegal, under comparable ecological conditions, *A. holosericea*, *A. linarioides* and *A. tumida* showed the best performance (Diallo, 2008; ISRA, personal communication). In Mali where the annual rainfall is estimated at 900 mm, results from trials show potential of *A. colei* var. *ileocarpa* and *A. mountforidaea*.

Casuarina equisetifolia proved its adaptation mostly in Senegal where it was first introduced one hundred years ago to provide various functions, including poles for construction, firewood and timber (Ousman Diagne, Institut Sénégalais de Recherches Agricoles (ISRA), unpublished information). However, due to its adaptability and its ability to grow better than the other tree species introduced at the same period, *C. equisetifolia* was selected to border the coastal part of the North of Senegal in monospecific plantations. The aim was to reinforce road stabilization between the coast and the countryside, stop sand dune movements and protect market gardens. The following years the impact of *C. equisetifolia* plots was established: access to coastal villages became easy;

wind erosion was slowed down; agriculture was promising along the coast; sand dunes were stabilized; and building infrastructures were possible. Subsequently, rural populations introduced the species along the coast and on farms. As the species is well adapted, and a very good nitrogen fixing species, it should be encouraged for on-farm plantings for the replenishment of soil fertility.

2.4 Fertilizer trees and shrubs including *Faidherbia albida*

The agroforestry parklands in the Sahel is a result of a deliberate effort by farmers to allow the seeds of useful tree species to regenerate naturally in their fields, a practice known as assisted natural regeneration or farmer-managed natural regeneration (FMNR). One of the important naturally regenerated tree species in the Sahel is *Faidherbia albida*. The species helps mitigate the consequences of the reduction of the doses of mineral fertilizers due to the removal of input subsidies (Bayala *et al.*, 2011).

Farmers in Niger exploit this leguminous tree species given its role in soil fertility improvement and provision of fodder and other products. Its characteristics of '*reverse phenology*' whereby the trees shed their leaves during the rainy season mean that there is little competition with associated food crops. The leaf litter during the rainy season contributes to the enrichment of the soil, increasing biological activity and as a result higher crop yields (millet, sorghum, maize or groundnuts) are obtained under such trees (Sileshi *et al.*, 2008). Moreover, it is deep rooted and hence access nutrient and water at lower levels while leaving the upper levels for the short rooted food crops. Given the relaxed forest laws in Niger, it is estimated that there are close to 4.8 million ha (up to 160 trees per hectare) of *Faidherbia*-dominated agro-ecosystems generated through FMNR in the Maradi and Zinder regions alone. In Malawi farmers growing crops under the canopy of *Faidherbia albida* have seen corn fields yield increase up to 280% (Sileshi *et al.*, 2008).

Soil fertility decline is a major limiting factor for agricultural production and economic growth in the Sahel. Inadequate land management and unfavorable price ratios between food crops and fertilizers are important elements in the process of land degradation occurring in the Sahel, resulting in soil nutrient depletion and declining crop productivity. The combined effects of soil-fertility depletion, active soil erosion and frequent periods of drought in the region have accelerated the degradation of the natural resource base by a poverty-stricken population forced to overexploit natural resources in order to subsist (Bocary Kaya, MDG West and Central Africa Project, unpublished data). Previous research in the Sahel (Harouna Yossi, IER, unpublished data) has established that short duration improved fallows are promising soil-fertility replenishment technology. Thus, the following tree/shrub species have proved promising for such practices in the Sahel: *Senna siamea*, *Senna spectabilis*, *Albizzia lebbeck*, *Gliricidia sepium* and *Flemingia macrophylla*, *Crotalaria* species, *Tephrosia* species, *Sesbania sesban* and *Cajanus cajan* (Kaya, 2002). Improved fallows using leguminous tree and shrub species have shown maize yields increase on average by about 70% in Cameroon (Degrande *et al.*, 2007), but in some areas 3- or 4-fold gains are possible (Sileshi *et al.*, 2008; Assah *et al.*, 2011; Bayala *et al.*, 2011).

3 Scaling up Efforts for Agroforestry Practices in the Sahel

The tree/shrub based practices are important in restoring soil fertility in the tropics and particularly in the Sahel if they are massively propagated and adopted. Rural communities and other investors should, therefore, be actively planting such trees and shrubs on private farms and community land with the aim of enhancing local livelihoods and environmental services in the Sahel. It should be stressed, however, that there are several obstacles to the adoption of such technologies in the Sahel. These obstacles generally include economic, cultural and political factors.

Three main problems associated with the scaling-up of tree/shrubs related technologies include: (a) the availability of high quality germplasm, (b) lack of appropriate environmental and natural resource management policy, and (c) lack of marketing system of agroforestry tree products. There is a general shortage of good quality germplasm for most developed environmental technologies. The collected germplasm is often of low genetic and physiological quality. The lack of seed, seedlings and other forms of improved propagules is hindering the scaling-up of the tree/shrub-based technologies. In addition, there is a need for a better germplasm diffusion system in order to obtain more results and impact. For example, farmers and NGOs should be independent in germplasm production by establishing their own seed production areas and hedge plants on their farms. Training sessions on mass production of seedlings, vegetative propagation and horticultural techniques, FMNR technique to enhance reforestation in the Sahel should be encouraged. This will help maximize the production of high quality germplasm by the farmers for on-farm planting and agroforests.

The evaluation of the legislation on land-use policy, land tenure, and forest management in order to understand their implications in the adoption of agroforestry technologies has been carried out in some countries in the Sahel (Kalinganire *et al*., 2009). The study showed that the various forest and land-related texts do not sufficiently take into account agroforestry systems now in practice. The situation does not favour the scaling-up and management of agroforestry technologies on-farm and in the parklands. It was made clear that inappropriate government laws have negative effect on afforestation initiatives by the communities. Thus, if the farmer does not have the right to harvest the trees he has protected for years before maturity, there will be little incentive for him to adopt any technology. Policy practices which would help to better manage Sahelian agroforestry parklands are recommended.

Furthermore, better marketing of agroforestry products provides a way for poor farming households to generate income. Such markets would help farmers make more profits, motivate them to invest in planting more trees and thus contribute to less environmental degradation. The key challenge, therefore, is to improve the structure, conduct and performance of agroforestry tree product markets, including carbon markets, and make them accessible to the rural communities. It is beyond any doubt that developed tree/shrub-based technologies, including *Casuarina* species, contributing to soil fertility enhance agricultural production and improve food security in the Sahel. However considerable work needs to be done to understand how farmers would adopt such technologies in order to enhancing the performance of rural communities farming systems by reducing poverty and creating impacts on livelihoods.

4 Conclusions

The paper reviews the role of trees in replenishing soil fertility in the tropics and particularly in the Sahel, thus contributing to food security in general. Examples of agroforestry practices and case studies contributing to sustainable management of the tropical drylands are providing multiple livelihoods and landscape benefits including food, carbon sequestration, and increased vegetation cover are given. The whole environment may benefit from effective soil fertility management. It is clear that efforts are needed for scaling up such sustainable tree/shrub-based practices for soil fertility restoration in the tropics and mostly in West African Sahel.

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Improved Casuarina for Farm Forestry in South India and Its Socio-economic Implications for Small-holding Farmers

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Abstract Casuarina (*C. equisetifolia* ssp. *equisetifolia*) has been in cultivation for over a century in India essentially for producing fuelwood and poles. It is grown in about half a million hectares mainly by farmers with a small land holding of 0.5 to 5 ha signifying its socio-economic importance in rural regions. In recent years due to acute shortage of raw material, casuarina wood is widely used for papermaking. Four major paper mills in southern India alone consume more than a million tonnes of casuarina pulpwood every year. The increased industrial demand and a stable price regime resulted in spread of casuarina cultivation from coastal to inland region and created need for new and improved planting material. A systematic casuarina breeding programme implemented during last 15 years through collaboration between IFGTB and CSIRO with funding support from AusAID, ACIAR and FAO has resulted in significant gains for casuarina growers. Extensive testing of large germplasm in the form of provenance-progeny tests revealed that the natural provenances from SE Asia possessing faster growth (up to 40%) than the local seedlots. The Australia Pacific provenances and African land races had good stem form and were more tolerant to insects and diseases. The newly introduced species, *C. junghuhniana* was found to be faster growing and more drought and disease tolerant than *C. equisetifolia*. Seeds from seedling seed orchards developed by thinning provenance-progeny tests are supplied to wood-based industries, forest departments and farmers. Plantations raised from orchard seeds recorded more wood production compared to that of local origin significantly improving the income to the tree farmers. Awareness on the benefit of using improved seed was created through on-farm demonstration plots which resulted in heavy demand for genetically improved seed which was beyond the production capacity of existing orchards. The current focus is to decentralize seed production and to involve farming communities and local government units to establish and manage community seed orchards to meet the seed demand.

1 Introduction

Casuarina is an important multipurpose tree crop in the eastern Peninsular India where it is under cultivation for over a century. It is predominantly cultivated by farmers of small land holdings mainly due to the ease of cultivation and the stable market for its wood. Casuarina wood has diverse uses like poles for rural construction and scaffolding, pulpwood for papermaking, and fuelwood. Four major paper mills in South India alone consume more than a million tonnes of casuarina wood annually. It is also a preferred species for environmental plantings like shelterbelts and afforesting mined areas due to its adaptability to grow close to the high tide level in the coast and nitrogen fixing ability. The wide interest in casuarina cultivation in India during the last two decades has resulted in many research and development initiatives to increase plantation productivity. In particular new germplasm from a wide genetic base was introduced and a systematic breeding programme was launched and the output from such programmes has started reaching the end users, the farmers. This paper reviews the gains realized from recent casuarina improvement programme and its socio-economic impact on smallholding farmers in South India.

2 Current Estate

In terms of area of cultivation casuarina is the fourth major species next to eucalypts, teak and phyllodinous acacias (*Acacia auriculiformis* and *A. mangium*). While a significant portion of plantations of other species are owned by government (Forest Department/Forest Corporations) or wood-based industries, casuarina is almost exclusively raised by farmers except the shelterbelt plantations in the shoreline. Since the area cultivated by a single farmer ranges from 0.5 ha to 5 ha, it is difficult to precisely estimate the total area under casuarinas cultivation. It could be in the range of 500,000 ha with a major portion falling in the States of Tamil Nadu, Andhra Pradesh and Orissa in the east coast. Growing casuarina is also steadily increasing in inland areas where it is not traditionally grown. Shortage of farm labour, insufficient water availability for agriculture, non-remunerative prices for farm produce and a growing trend of absentee farming are the major reasons for farmers shifting to casuarina cultivation.

3 Cultivation Practices

Casuarina is generally raised in block plantations of high density (about 10,000 stems ha^{-1}). The rotation age was 30 years at the time of introduction and it has gradually reduced to 2.5 to 4 years under irrigation and 5 to 6 years when grown rainfed. In order to cost-effectively meet the requirement of large number of seedlings for the high density plantations, bare-root seedlings are used. More than 90% of the estimated 200 million seedlings used every year is produced by landless farm labourers for whom casuarina nursery is the main source of livelihood support.

Field planting is usually synchronized with one of the two monsoon rains (south west and north east) to enhance the survival of bare-root seedlings planted in pits 30 cm × 30 cm × 30 cm. Usually an intercrop is grown during the first 3 months after planting especially where irrigation is available. This practice helps manage the plantation weed-free and also to recover the cost of tree planting. Fertilizer application is generally restricted to irrigated plantations and mostly between first and second years. Pruning of side branches is carried out between first and second years and second and third years. The expenditure for pruning is met by the sale of pruned material which is used for fuel.

4 Management and Yield

The commonly followed rotation period is 4 years with irrigation and 6 years under rainfed conditions. But the duration varies greatly in different areas and between farmers. Soil quality, water availability, amount of rainfall, farmers' economic needs, and the prevailing market demand and prices influence the age at which the crop is harvested. In a few places of coastal Tamil Nadu irrigated casuarina is harvested as early as 2.5 years of age whereas Forest Department plantations without irrigation are retained up to 8 years. Once harvested, the stumps are dug out and the land is prepared for the next planting.

Wood production varies greatly across locations, cultivation techniques adopted and age at which harvested. Plantations with irrigation and fertilizer application yield 100-150 metric tonnes (MT) ha^{-1} of air dried wood (up to 20 cm girth) in 4 years. Under rainfed conditions an average yield of 75-100 MT ha^{-1} is obtained in 6 years depending upon soil quality and amount of rainfall during the cultivation period. An additional 12-17 MT of miscellaneous wood is produced per hectare in the form of branches, tops and roots. At the time of harvest the average height of the tree is 12 m and the dbh is 8 cm. The best trees may measure 20 m height and 15 cm dbh. Poles (up to 7 cm in diameter) are the most valuable end product fetching between USD 75-100 per MT depending on the quality of poles, place of availability and prevailing demand. Pulpwood prices remain more stable than that of poles from USD 40-50 MT. Fuelwood is priced between USD 40 and 60 based on local demand and dryness of wood.

5 Genetic Improvement

As with the many exotics introduced with a limited genetic base, the Indian land race of *C. equisetifolia* became highly variable in terms of adaptability and growth (ICFRE, 1994). Systematic introduction of new germplasm as natural provenances and land races was carried out only since 1990 under various internationally coordinated programmes supported by CSIRO, FAO (FORTIP), AusAID and ACIAR. A brief summary of these introductions and the details of seed sources and their performance are provided in Table 1 and Table 2.

Table 1 Details of introductions of *Casuarina* germplasm in India since 1990

Location	Lat. (°′N)	Long. (°′E)	Alt. (m asl)	Rainfall (mm)	No. of seed sources	No. of families	Year of planting
			Casuarina equisetifolia				
Sadivayal	11 00	76 58	500	1,200	7	Bulk	1991
Neyveli	11 30	79 30	300	1,000	7	Bulk	1991
Neyveli	11 30	79 30	300	1,000	7	Bulk	1992
Puducherry	11 59	79 50	30	1,200	35	Bulk	1995
Rajahmundry *	17 00	81 48	500	1,100	27	100	1997
Kakinada *	16 56	82 13	10	1,500	27	100	1997
Balukhanda *	19 53	85 53	10	1,420	32	180	1997
Sadivayal *	11 00	76 58	500	1,200	33	108	1998
			Casuarina junghuhniana				
Puducherry	11 59	79 50	30	1,200	21	Bulk	1996
Panampalli	10 52	76 46	500	1,500	21	Bulk	1996

* Main populations of breeding programme.

Table 2 Performance of *Casuarina* provenances/land races in comparison with Indian land race form growth, form, health and flowering characteristics

CSIRO No.	Provenance	Country	Growth				
			Coast	Inland	Form	Health	Flowering
Casuarina equisetifolia							
15958	Wangetti Beach, QLD	Australia	–	–	–	+	–
18378	Prince of Wales Is., QLD	Australia	–	–	–	+	–
18008	Darwin, NT	Australia	0	–	0	+	–
16166	Danger Point, NT	Australia	0	0	0	+	–
18402	Kolombangara	Solomon Is.	+	0	–	+	–
18153	Ela Beach	PNG	0	+	+	+	–
18374	Kedah	Malaysia	+	+	+	–	–
18244	Sarawak	Malaysia	+	+	+	–	0
18348	Kuantan Penang	Malaysia	+	+	+	–	–
18355	Cotonou	Benin	+	0	+	0	–
18299	Had Samira, Songkhla	Thailand	+	+	–	0	–
18298	Had Chao Mai, Trang	Thailand	+	+	–	0	–
18296	Ban Bang Sak, Phang Nga	Thailand	+	+	–	0	–
18297	Ban Kam Phuam, Ranong	Thailand	+	0	–	0	–
18141	Robinson Island	Kenya	0	+	+	+	–
18135	Malindi	Kenya	0	+	+	+	–
18142	Kilifi	Kenya	0	0	+	+	–
18134	Kenyatta Beach	Kenya	0	0	+	+	–
18144	Baobob	Kenya	0	+	+	+	–
18152	Ninh Thuan	Vietnam	+	0	0	0	+
Casuarina junghuhniana							
19489	Kapan, Kupang	East Timor	+	+	0	+	0
19490	Camplong	East Timor	+	+	0	+	0
17878	Noelmina River	East Timor	+	+	+	+	0
17877	Sw Soe, Timor	East Timor	+	+	0	+	0
19491	Buat, Soe	East Timor	+	0	0	+	0
17844	Old Uhak, NE Wetar	East Timor	+	+	–	+	0

0, + and - indicate equal, greater and less than the Indian land race respectively.

5.1 Selection of provenances and land races

The extensive field testing of a wide range of provenance and land race seedlots provided consistent results on their suitability for deployment in plantation and breeding programmes. The broad trends of these tests summarized in Table 2 are: (1) Although growth of Indian land race was above average, it was outperformed by provenance/land race seedlots from South East Asia (Thailand and Malaysia), East Africa (Kenya) and the Pacific Islands (Papua New Guinea (PNG) and Solomon Islands) in terms of growth (up to 40%). (2) With regard to stem form, land races including those from India in general possessed better stem straightness than natural provenances. (3) There was a clear coast to inland trend in the growth performance of seedlots. The Thailand and Malaysian provenances performed well both in coastal and inland sites, the Kenyan land race and PNG provenances were more suited to inland regions and the Solomon Islands performed better only in coastal site. (4) The provenances from Australia-Pacific region and land race from Kenya possessed harder wood and were more tolerant to stem borer attack and wilt disease than other seedlots. (5) No single seedlot was found to have all the desirable characteristics but many families and individuals possessed such combination of traits. These findings are consistent with the outcome of the internationally coordinated provenance trials of *C. equisetifolia* conducted in 20 countries with 60 seed sources (Pinyopusarerk *et al*., 2004).

Provenance trials of *C. junghuhniana* involving seedlots from its natural (Indonesia) and planted (Kenya) distribution showed that those from the low elevation (5 to 800 m asl) in Timor and Wetar islands were well suited to the plains in South India (Table 2). The altitudinal adaptation was consistent in field trials conducted outside India with the similar set of seedlots (Mwihomeke *et al*., 2002; Pinyopusarerk *et al*., 2005). *C. junghuhniana* scored over *C. equisetifolia* in many ways: (1) faster growth (30% and above over *C. equisetifolia*), (2) more drought tolerant; well suited for rainfed cultivation in inland regions, (3) freedom from wilt disease, and (4) coppicing ability with a potential to have more than one crop from a single planting (Pinyopusarerk and House, 1993; Nicodemus *et al*., 2003; 2005).

5.2 Ongoing breeding programme

With the understanding of geographic variation in *C. equisetifolia* through the early field tests, a detailed breeding programme was prepared for implementation by IFGTB with technical collaboration of CSIRO with the objective of maximizing the yield and quality of poles and pulpwood (Pinyopusarerk, 1996). The two-tier breeding populations include a 'Main Population' established as large provenance-family trials (100 + open-pollinated families) and a 'Core Population' consisting of clones from the best 25 families selected from the Main Population. Four Main Populations were established at Kakinada, Rajahmundry, Nellore (Andhra Pradesh) and Sadivayal (Tamil Nadu) in 1998 (Table 1). They underwent two thinnings after ranking the trees based on growth and form traits at four and six years of age. They now function as Seedling Seed Orchards to supply seeds for planting programmes.

The Core Population comprised 50 outstanding clones (25 male and 25 female) selected from the Main Population based on superiority in growth and stem form. They were drawn from the best provenances and assembled in a clone bank through rooting of cladode cuttings. Two clonal tests were established with these clones at Sadivayal (Tamil Nadu) and Hosekote (Karnataka). Clones selected from outstanding provenances like those from SE Asia outperformed selections from unimproved plantations. Four clones which showed consistently outstanding growth were short listed for commercial release in 2010. These clonal tests were subjected to two thinnings to remove inferior clones and converted into Clonal Seed Orchards.

5.3 Output from breeding programme

Although the breeding programme prescribed collection and supply of seeds from SSOs from the fifth year (i.e. after the first thinning in the fourth year), commercial supply was deferred till the eighth year to have adequate flowering and panmixis in the orchards. Flowering in the natural provenances especially those from the South East Asia was quite low up to the age six whereas heavy flowering occurred in the Indian land race seedlots (Nicodemus *et al*., 2001; Pinyopusarerk *et al*., 2004; Nicodemus, 2007). Since these provenances are among the best in both coastal and inland sites, low reproductive output from them will affect the genetic gain that can be realized from the SSO progeny. Genetic gain tests involving seeds collected from SSOs at age four revealed they were not significantly different from natural provenance (Thailand) and CSO seed of Indian land race (Varghese *et al*., 2010).

At around the age six, the overall proportion of flowering trees increased to over 80% in particular that of the outstanding provenances like Thailand, Malaysia and PNG. Between the years 2006 and 2009, more than 100 kg of seeds were collected from the orchards of the two species and supplied to different user groups. Approximately one third of the seed supplied was that of *C. equisetifolia* and the rest *C. junghuhniana*. Since both seed rate and germination of *C. junghuhniana* are far higher than that of *C. equisetifolia*, the seed supplied could cover planting in six times more area. Generally seeds were supplied to a particular planting site from an orchard located in a site similar in soil type, rainfall and distance from the sea coast. Growth of orchard progeny was better than that of the local unimproved seedlot in all planting sites. Data obtained from sample plots laid out in plantations raised with orchard and local seeds are shown in Table 3. *C. equisetifolia* orchard progeny recorded 50% more volume growth over the unimproved *C. equisetifolia* at 3 years old. Similarly the *C. junghuhniana* orchard seedlot produced more than twice the volume growth than the local *C. equisetifolia* seedlot at 5 years old. A minimum of 20% better growth was obtained in low input plantations raised under rainfed conditions.

Table 3 Comparison of growth by seed orchard progeny with unimproved seedlot in Veedur, Tamil Nadu, India

Species, seedlot and age	Height (m)	DBH (cm)	Conical volume (m^3)	Volume gain over local seedlot (%)
Casuarina equisetifolia: 3 years				
Seed orchard progeny	14.36	7.06	0.0225	50
Unimproved local seedlot	12.82	6.36	0.0150	
Mean	13.59	6.71	0.0188	
SED	0.714	0.225	0.0025	
P	ns	0.05	0.05	
Casuarina junghuhniana: 5 years				
Seed orchard progeny	19.07	12.4	0.085	112.5
Unimproved local seedlot	18.06	9.43	0.040	
Mean	18.84	10.91	0.0625	
SED	0.98	0.5806	0.01	
P	ns	0.014	0.046	

6 Socio-economic Impact of New Planting Material

The linking of breeding programme to plantation development programme has tremendously benefited both. The potential economic gain from the use of genetically improved seeds from seed orchards has been realized by the farmers as the plantations raised with improved seed come to harvest. A mere 10% increase in wood production results in an additional income of USD 417 ha^{-1} at the time of harvest. Assuming this minimum gain from all the 4,312 ha of plantations raised during the last four years with seed orchard seeds, an additional income of around USD 1.8 million will be realized.

Since it is difficult for the smallholding farmers to raise their own nursery for planting in a small area, a partnership with paper industry was helpful in disseminating the new planting material to individual farmers. All major paper industries in South India are now using orchard seeds to raise a part of their total seedling production and distribute them to smallholding farmers. Farmers' plantations raised with improved seeds and demonstration plots established by IFGTB and the industries have created the awareness on use of high quality planting material.

7 Challenges in Meeting Seed Demand

The current demand for casuarina seeds in South India is about 2 tonnes a year and the seed produced from existing orchards is less than one percent of it. It is not possible to produce such large amounts of seed by any single organization. Although clonal propagation is easy for casuarina, rooted cuttings will still be costlier than seedlings for the resource-poor farmers. Some of the industries have started establishing their own orchards with families/clones developed through the breeding programme. A new project funded by the AusAID has been initiated jointly with CSIRO and the forest departments of Tamil Nadu and Puducherry. This project aims at capacity building of farmers and forest managers in developing and managing 'community seed orchards' at village level or for a cluster of villages. Model seed orchards are being developed with the involvement of farmers in areas where casuarina is the principal tree crop. These orchards will be managed as a community resource to benefit all its members. Another approach is to train the traditional nursery growers in modern nursery techniques and make orchard seeds and other inputs accessible to them. This will help make available high quality seedlings at affordable cost to a large number of smallholding farmers who are dependent on these nurseries and also improve the livelihood support for the nursery growers.

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Modern Silviculture Practices versus Livelihood Needs: The Case of Casuarina Growing along the Kenyan Coastal Region

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Abstract The main *Casuarina* species being grown in Kenya are *C. equisetifolia* and *C. cunninghamiana*. Other minor species are *C. junghuhniana*, *C. glauca* and *C. torulosa*. The trees have been grown mainly for poles and as ornamentals, with firewood, charcoal and windbreak being minor uses. *C. equisetifolia* is the most widely grown in the country and has been grown commercially both in plantations by individuals, companies and multi-national organizations and small-scale farmers who comprise the bulk of the producers. The poles are used in the local construction industry especially the tourist hotels, villas and homes. On-station and on-farm spacing trials were established to determine the spacing providing high yields for improved farmers' livelihood. The trials had six spacings with five farmer-researcher designed and the sixth one farmer-designed spacing. Farmers' comparative analyses were meant to facilitate potential adoption of the best spacing in relation to high yields. This was enhanced further through annual review field days rotationally held in participating farmers' farms. Though the farmers participated both directly as implementers and indirectly as participants during the field days, the majority of them continued to plant the trees at their preferred (farmer designed) spacing. "Their" spacing provided higher returns and the income was spread over a longer period as the farmers removed (cut or thinned) the marketable poles leaving the small trees to grow. From the trial it was evident that livelihood improvement was the main criteria determining the spacing to be adopted by the farmers.

1 Introduction

Casuarina is a genus of 17 species in the family Casuarinaceae, native to Australasia, South East Asia and many of the Pacific Island countries. Casuarina is commonly known as the she-oak, ironwood or beefwood. It is commonly grown in the tropical and subtropical areas throughout the world. *Casuarina equisetifolia* is a common tropical tree known as Whistling-pine and is often planted as a windbreak along the sea coast. The *C. equisetifolia* wood is used for shingles, fencing, and is excellent firewood.

In Kenya *C. equisetifolia* is widely grown along the coast both in plantations and on-farms. From existing government records, *C. equisetifolia* was first raised in Jilore tree nursery in the late 1960s and the first planting done in Gede Malindi district in 1971 as part of potential plantation species trials (Choge and Mbuvi, 2002). Casuarina growing on private land is estimated at 2,000 ha and is much more than that on the gazetted forests. On average, one out of every four farmers in the region cultivates *C. equisetifolia* as a source of income. Cultivation of *C. equisetifolia* within the region for livelihood improvement is carried out by small landholding farmers who account for 82% of the productionm and large scale producers. However, shortage of land is a major limitation to cultivation of *C. equisetifolia* by most farmers. Among the small scale farmers, 92% of all cultivators were those with less than one hectare of land area (Choge and Mbuvi, 2002).

The growing of *C. equisetifolia* in Kenya is strongly linked to the tourism industry where the hotels remain the main buyers. A decline in the tourism industry in the mid 1990s adversely affected the cultivation of *C. equisetifolia*. The quantity of wood available within the region in 2002 was sufficient to satisfy a moderate demand for pole export (Choge and Mbuvi, 2002). The major use of this species is construction, fuelwood, windbreak, ornamental, rehabilitation of degraded or mined sites and nitrogen fixation.

The coastal region of Kenya is a timber and pole deficit region due to the ban on exploitation of natural forest, controlled exploitation of mangroves and low development of on-farm and plantation forestry. Farm forestry offered a solution to this problem and *C. equisetifolia* was the species preferred by farmers. Unfortunately farmers were growing *C. equisetifolia* at close spacing despite recommendations by researchers and Kenya Forest Service extension staff of wider spacing of 2.5 m × 2.5 m and 3 m × 3 m under a perceived faster growth. To address the above problem, in 1998 KEFRI established participatory experimental spacing trial at the coast through GOK/GTZ funding. It was researcher-farmer designed experiment and the spacing used was: 0.5 m × 1.0 m, 1.0 m × 1.0 m, 1.0 m × 2.0 m, 1.5 m × 1.5 m, 2.0 m × 2.0 m and 2.5 m × 2.5 m with each spacing plot having 49 trees. Due to lack of enough space on the individual farmer's land for replication of the treatment on the same piece of land, different farmers acted as the replicates. This paper presents the income a farmer would add to livelihood on growing *C. equisetifolia* at the above six espacements at the fourth year of growth.

2 Materials and Methods

The participatory experimental woodlot plots were located along the *C. equisetifolia* growing zone of Kilifi County. The total number of plots established was 20. Six plots failed at the initial establishment after browsing by Dik Diks and elephants. Another 3 plots failed because of various management problems and 3 more plots had been harvested by the fourth year when the experiment was closed. Therefore, the results presented are for 8 plots except the one showing the harvesting trends where all farmers who had harvested are shown. The trees were inter-cropped with maize until the trees canopy closed.

The criteria for selecting participating farmers included: individual land ownership, having grown and/or sold casuarina poles, sociable person, no known anti-social attitudes, farm location to enhance chances of exposure of the plots to many farmers, and large people concentrated places like schools which were selected as they would reach as many farmers as possible through parents and the school children - "the future farmers" (Wairungu *et al*., 2002).

The experiments were assessed annually on the following parameters: survival, height and root collar diameter initially at six months and thereafter at the end of every year. The mean root collar diameter for each espacement at age 4 years was used to obtain the current market price of poles at farm gate. The income a farmer would add to livelihood on growing *C. equisetifolia* was calculated by multiplying the market price of the mean root color diameter by the number of surviving trees per hectare. The approximate actual number of trees the farmer would have sold by the fourth year was calculated based on the trees the farmers had sold each year from each spacing.

3 Results and Discussion

The mean survival, mean height and mean root collar diameter at 4 years old are presented in Table 1. There was a clear distinction in mean root collar diameter between the narrow and wider spacings.

Table 1 Average survival percentage, height and mean root collar diameter of 4 years old *Casuarina equisetifolia* spacing trial at Kenya coast

Farmer/Spacing (m)	0.5 × 1.0	1 × 1	1 × 2	1.5 × 1.5	2 × 2	2.5 × 2.5
Average survival (%)	80.0	82.3	82.2	76.9	82.0	79.5
Average height (m)	4.0	4.4	5.4	5.6	6.7	7.3
Mean root color diameter (cm)	4.0	4.4	5.4	5.6	6.7	7.3

Though wider spacing resulted in faster growth and greater mean height, it produced conical shaped poles which are in less demand in the market. The buyers preferred cylindrical shaped poles produced by close spacing.

Root collar diameter is the main yardstick used to determine the selling price followed by pole height and shape. The communities use inches instead of centimetres and smooth cylindrical shaped poles are preferred for roof construction by tourist hotels.

The market price for *C. equisetifolia* poles at the farm gate is shown in Table 2. In 2002 when the experiment was concluded the price per inch was 100 Kenya Shillings (KES). The market price in 2010 is KES 200 per inch. The current market demand is poles between 4 inches (10 cm) and 6 Inches (15 cm).

Table 2 Gross and actual cash income from one ha plantation of 4-year-old *Casuarina equisetifolia* at Kenya coast

Spacing (m)	Total No. of trees	Survival (%)	Actual No. of trees	DGL (inch)	2010 pole price (KES)	Gross income (KES)	% of poles sold at 4 yrs	No. of marketable poles	Actual income (KES)
0.5 × 1.0	20,000	80	16,000	1.6	318.4	5,093,877.55	0	0	0
1 × 1	10,000	82.3	8,230	1.8	359.2	2,956,081.63	0	0	0
1 × 2	5,000	82.2	4,110	2.2	440.8	1,811,755.10	0.45	1,850	815,289.80
1.5 × 1.5	4,445	76.9	3,418	2.3	457.1	1,562,608.00	0.75	2,564	1,171,956.00
2 × 2	2,050	82	1,681	2.7	546.9	919,404.08	1	1,681	919,404.08
2.5 × 2.5	1,600	79.5	1,272	3.0	595.9	758,008.16	1	1,272	758,008.16

1 USD equals to 77 Kenya shillings.

If trees were to be harvested at age four, Table 2 gives the gross and actual incomes using the means of root collar diameter and the current farm gate prices. This is the perceived incomes that drive farmers to plant trees at very close spacing not realizing that at age four hardly any tree planted at close spacing would have attained the marketable size.

Casuarina poles are marketable when root collar diameter is more than 2 inches. The highest demand for casuarina in 2002 was 4 inches, but in 2011, 6 inches is the one that is in high demand. Table 2 further provides cumulatively the poles farmers had sold after attaining the marketable diameter size of over 2 inches in each spacing by age four.

Trees attain marketable size at different ages for the different spacings. In some spacings, few trees annually attain the right size. This allows the farmers spread income over a longer period. Trees under close spacings had not attained marketable size by four years of age.

If the area was put under maize, the major crop grown in the region, the returns would be very low.

4 Conclusions

Farmers have now experienced through practice and have decided the best spacing to be 1.5 m × 1.5 m and 2.0 m × 2.0 m. These two espacements ensure faster growth, early maturing and produce poles of high quality which are in high demand by the customers ensuring farmers get cash income for better livelihood.

5 Recommendations

The farmer-researcher post experiment consultative workshop developed the following recommendations:

- There is need for on-going officer-farmer engagement and to formulate strategies for better extension programmes for information sharing.
- There is need for policy to address the needs of the poor community members to increase social awareness on *C. equisetifolia* cultivation for livelihoods.
- Forest produce movement needs to be regulated through forest produce movement permits issued by Kenya Forest Service as a way of ensuring that casuarina trees belonging to farmers who live far away from their farms are not stolen.
- The farmers have a limit how far they can share information. This calls for partnerships between farmers, government officers and civil society.
- Farmers need to form associations to avoid exploitation by agents, middle men and brokers.
- Adoption of "the quarter acre model" for sustained income generation and better environment. This is being promoted in recognition that farmers will plant trees which provide income and improve their livelihoods while at the same time feeding their families. It also recognizes that most of the farmers do not have enough resources to manage large areas under trees. Moreover the poor are more concerned with their survival than ecological sustainability. Casuarina growing in the coastal region in Kenya offers this solution through "the quarter acre model" where the small scale farmers with one acre can grow casuarina and crops on the same piece of land.

This starts with clearing the one acre in year 1 and planting the first quarter acre with casuarina and compatible food crops. Each year the farmer plants a quarter acre up to year 4. The intercropping is done up to the second year for each quarter, therefore in year 3 the first quarter is not suitable for inter-cropping, and by the fourth year, the farmer starts to harvest what is growing in the first quarter and then replants casuarina after harvest. This process is repeated once again with the farmer assured of cash from pole sale and food every year. This is as shown in Table 3.

Table 3 The quarter acre model

Area	Year 1	Year 2	Year 3	Year 4
1st quarter	Casuarina and food crops	Casuarina and food crops	Casuarina	Casuarina (harvesting)
2nd quarter	Food crops	Casuarina and food crops	Casuarina and food crops	Casuarina
3rd quarter	Food crops	Food crops	Casuarina and food crops	Casuarina and food crops
4th quarter	Food crops	Food crops	Food crops	Casuarina and food crops

The farmer would make an average of KES 6,408.8 each year (based on 2010 farm gate price) from the quarter area under maize farming while from casuarina based on the three spacings that would be undergoing harvesting the farmer would make KES 99,869 from 1 m × 2 m, KES 143,784 from 1.5 m × 1.5 m and KES 137,340 from 2 m × 2 m and KES 92,853 from 2.5 m × 2.5 m. From the foregoing, the quarter acre model needs to be adopted and popularized but more research is required to have it internalized.

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Casuarina in Farm Forestry for Sustainable Livelihoods: The Andhra Pradesh Paper Mills Experience

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Abstract Casuarinas are among the most adaptable perennials in the tropics and subtropics. This tree species also offers reliable solutions in farm forestry ensuring the sustainability of different terrestrial eco-systems. Land use options that increase resilience and reduce vulnerability of contemporary societies are fundamental to livelihood improvement and adaptation to environmental change. Farm forestry contributes to diversified end uses for society by self sufficiency of raw material to user industries, biodiversity conservation, additional benefits like income generation to society, augmentation of the carbon storage in eco-systems, enhancing the fertility of the soils and providing social and economic well being to the people. For casuarina cultivation on coastal marginal lands which are low in soil nutrient status and mostly rainfed areas and even if available irrigation is scarce beyond first year, economic considerations need to be taken into account as farmers can hardly meet the high initial expenditure for better inputs to obtain higher returns. On the other hand in inland areas with irrigation, the demand for sound management practices arises in order to obtain maximum productivity. In both scenarios Research and Development is a pre requisite in order to get more productivity from different agro climatic zones. Introduction of superior planting material by seeds or clones is vital in developing manageable and profitable plantations. Transfer of technology with *Casuarina equisetifolia* by low cost planting methods and upgrading farming practices has catered the needs of farming community, making them affordable to adopt clonal planting material with casuarina hybrid by doubling of yield. This has ensured higher returns from the land holdings, additional income with intercropping and meeting needs like fuelwood. Farm forestry has been a main stay in sustaining the livelihoods of agrarian communities.

1 Introduction

In developing countries, especially in the tropics enhanced productivity on sustainable basis without disturbing natural resources needs to identify alternative land use systems. In India, besides land under agricultural use, 105 million ha is lying unutilized (Singh and Upadhaya, 1999). Introduction of tree species and inputs like research and extension can however aid in proper utilization and afforestation of these wastelands. Farming systems in waste and degraded lands are circumscribed by several factors like climate, soil, topography and permeability besides other physical and chemical properties. Utilization of these wastelands by means of farm forestry is essential in order to address the needs of the industry and for the social well-being of the local communities.

Casuarina species, noted for their fast growth and rapid colonizing ability on disturbed soils, are also well known for their quality of wood and utility properties. It is, perhaps, these features together with their propensity that make them ideal plantation trees. *C. equisetifolia* is the most widely planted casuarina and other species like *C. junghuhniana* are also wide spread (Bourke, 1989; Askin *et al*., 1990). The introduction of casuarina hybrid between *C. junghuhniana* and *C. equisetifolia*, being propagated by vegetative means, is a productive planting stock (Thirawat, 1953; Boontawee and Wasuwanich, 1980). There is also potential to improve productivity through domestication. Domestication in casuarina can also be considered by strategy designed to genetically improve either the productivity or performance of trees for social or industrial use. El-lakany (1996) also drew attention to the wide provenances of *C. equisetifolia* and the possibility of exploiting this variation through vegetative propagation. The first recorded introduction of casuarina to the Indian subcontinent, which was way back in 1868 (Pinyopusarerk *et al*., 1996), has become pioneer in the area of forestry programmes. Different species of the genus *Casuarina* can easily establish themselves overcoming environmental stress, even in poorer edaphic conditions. Thus they are an asset for sustainable forestry programmes. *C. equisetifolia* is the most widely studied species and finds extensive application in India.

The ever increasing demand of this versatile tree occupying key position in afforestation needs improvement of yield and quality. Vegetative propagation techniques and multiplication of *C. equisetifolia* by rooting of cladode segments under controlled condition are handy for clonal multiplication (Gurumurthi and Bhandari, 1988). For all these reasons casuarina has become popular among farmers for its multiple uses (Subba Rao and Rodriguez-Barrueco, 1995).

2 The Andhra Pradesh Paper Mills Ltd (APPM)

The Andhra Pradesh Paper Mills Ltd., located in East Godavari district, of Andhra Pradesh, India is an ISO 9001, ISO 14001 and OHSAS 18001 company. It is one of the largest integrated pulp and paper manufacturers in India with a rated capacity to produce 107,000 tonnes of finished paper production per annum and 182,000 tonnes bleached pulp production per annum. The annual requirement of fibrous raw material is around 700,000 tonnes. APPM initiatives to achieve the raw material sufficiency and sustained availability is ensured by its farm forestry programme emphasizing the need for conservation of natural resources, clean environment by massive plantations on marginal and degraded farmlands along with sustainable livelihood to the agrarian community.

3 Methodology

APPM Farm Forestry was formulated in 1989. Under this scheme, after careful study of the socio-economic conditions of the farmers in the area, multi-pronged strategy was adopted. APPM farm forestry approach for augmenting sustainable livelihood focuses particularly on small and marginal farmers, ensuring self sufficiency of raw material to mills and adequate income to economically vibrant society. The sustainable livelihood approach is used to identify the main constraints and opportunities faced by the agrarian communities. The key parameters considered for methodology are as follows.

3.1 Project area

The farm forestry project area comprises marginal and degraded lands with low productivity in coastal districts of Andhra Pradesh which are mostly rainfed and agro-ecologically suitable for casuarina cultivation. Andhra Pradesh lies between 12°41′N and 22° N latitude, and 77° E and 84°40′E longitude.

3.2 Research and development

The APPM Farm Forestry Programme has been sustaining agrarian community by providing quality-planting material of casuarina and extending continuous technical know-how at all the stages. Constant efforts in Research and Development through introduction of low-cost planting technology by means of "treated bare rooted seedlings" was introduced by which seedlings were transferred from primary bed to secondary bed for root shock and vigorous root growth. The introduction of casuarina hybrid by means of macro propagation was intended to test the adaptability of clones to diverse agro-climatic regions and edaphic conditions.

Clonal seed orchard (CSO) is the right option for quality seed production with known origin. Employing seeds from proven seed sources and identified seed stands have benefited in development of superior planting stock in the farm forestry programme for the benefit of farmers. The mill has taken up a collaborative project on casuarina improvement by means of CSO with the Institute of Forest Genetics and Tree Breeding, under Indian Council of Forestry Research and Education.

3.3 Employment generation

The scheme aims to create huge employment for the rural communities from pre planting to post harvest stage, apart from addressing the local needs of the people by making available to them wood for additional benefits like scaffolding and fuel purpose, along with assured market for pulpwood.

4 Results

Farm forestry activities in the project area since 1989 to 2009 have recorded development and distribution of 603 million quality casuarina seedlings to 32,775 beneficiaries (families) covering an area of 80,720 ha (Fig. 1). During 2008-2009, around 97% of the mills' raw material requirement was met from its farm forestry sources (Fig. 2).

4.1 Adaptability

Low cost planting technique by way of treated bare root seedlings proved to be advantageous over that of bag and conventional seedlings.

Treated bare root seedlings are cost effective and have better survival, uniform growth with less variation in growth and easy transportation with no damages.

Treated bare root seedlings are cost effective and eco-friendly. Transportation is easy and less expensive. It avoids root coiling, ensures better growth without distortion.

4.2 Reduction in initial investment

Treated bare root seedlings require low initial expenditure, in comparison to bag or conventional casuarina seedlings. This has helped reduce initial plantation establishment cost by up to 63% (Table 1).

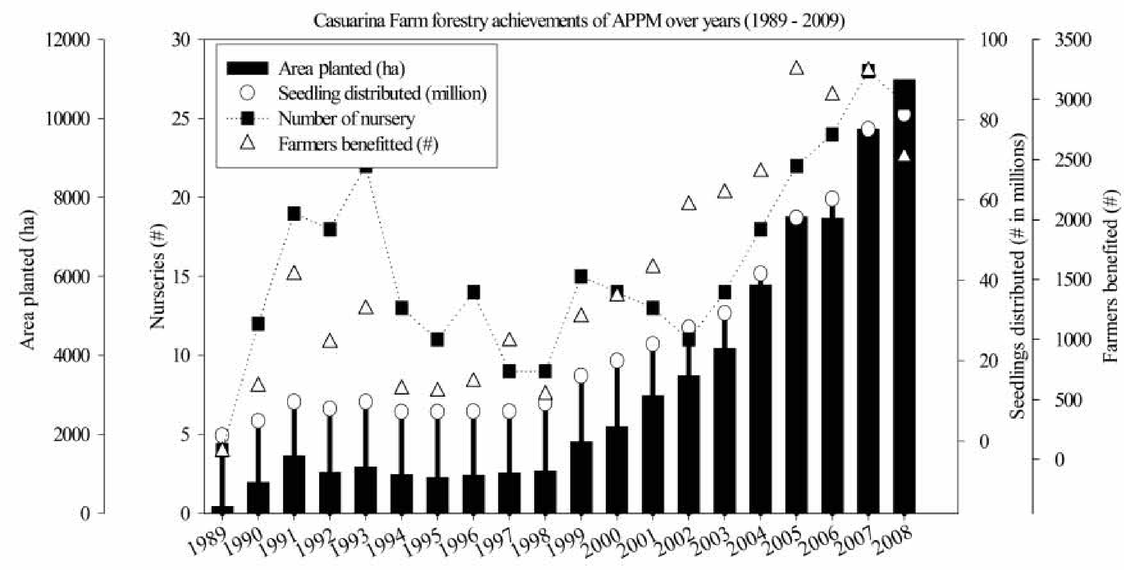

Fig. 1　Farm forestry plantation activities

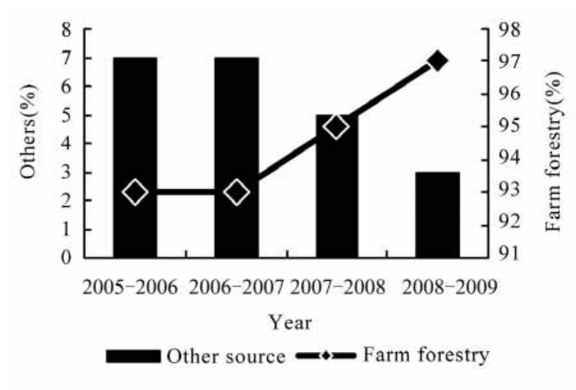

Fig. 2　Pulpwood procurement pattern

Table 1　Reduction per hectare in establishment cost expenditure

Activity	Bag plants/conventional ($US ha^{-1})	Treated bare root seedlings ($US ha^{-1})
Seedling cost	163	4
Transportation cost	22	2
Transportation damage	5	0
Planting	163	122
Total	353	128

Reduction in initial establishment cost USD: 225 or 63%.

4.3　Productivity improvement by research and development

The yield of conventional bare rooted seedlings at 4 years rotation is 50 tonnes ha^{-1}. Poly bag seedlings recorded a yield of 75 tonnes ha^{-1} at the same rotation age. Subsequently, the concept of treated bare root seedlings showed a better yield of 80 tonnes ha^{-1} at 4 years rotation with great reduction in initial investment. Finally the yield was doubled with casuarina hybrid clones reaching up to 165 tonnes ha^{-1} at 4 years rotation (Fig. 3).

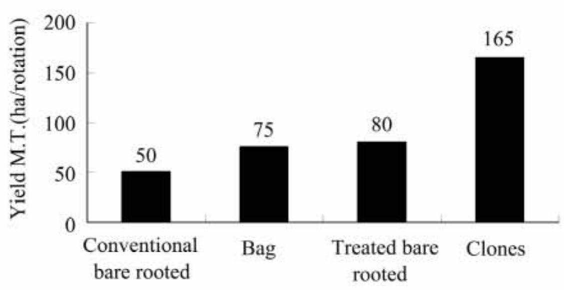

Fig. 3 Casuarina productivity improvement

4.4 Employment generation

The scheme has attracted, channelized and mobilized small, marginal and backward farming community groups making productive use of wastelands and ensuring quality raw material sustainability for the industry. It has been instrumental in creating employment by people participation in nursery seedling development and plantation activities besides logging and depot operations (Table 2).

Table 2 Farm Forestry Programme-employment generation (1989-2009)

No.	District	Area (ha)	No. of seedlings (million)	No. of beneficiaries	Employment generation (million man day)
1	Srikakulam	5, 043	34.7	1, 651	2.52
2	Vijayanagaram	921	6.4	63	0.46
3	Visakhapatnam	43, 676	323.6	17, 016	21.84
4	East Godavari	18, 034	139.0	10, 047	9.02
5	West Godavari	3, 800	33.4	2, 367	1.9
6	Krishna	6, 034	46.8	1, 154	3.02
7	Prakasam	2, 905	17.8	475	1.45
8	Nellore	307	2.2	2	0.15
	Total	80, 720	603.9	32, 775	40.36

4.5 Tangible and intangible benefits of plantations

Casuarina species are good at nitrogen fixing. The long term farm forestry activities have helped in soil nitrogen fixation and carbon sequestration (NRC 1982, Subbarao and Rodriguez-Barrueco, 1995). The farm forestry activities with casuarina have been instrumental in sustaining social, economical and environmental needs of the society. The fuelwood needs are being catered by lopping and topping of this species which is known for its high calorific value and the root portion serving the purpose of incineration in industrial usage. Therefore continual inflow of benefits from seedlings to post harvest stage is assured (Table 3).

Table 3 Casuarina Farm Forestry project asset creation (1989-2009)

Particulars	Quantum
No. of Casuarina seedlings distributed (million)	603
Area covered under plantation (hectare)	80, 720
No. of beneficiaries in the project area	32, 775
Total estimated wood yield out of above plantations (million metric tonne)	6.08

Adhoc estimates of tangible and intangible benefits out of casuarina plantations of APPM

Particulars	Quantum (million)	Value (million USD)
Fuel wood available to beneficiaries (metric tonne)	1.21	13
Employment generation (man day)	40.36	44
Asset creation out of yield (metric tonne)	6.08	132
Carbon sequestration (metric tonne) on yield Carbon credit value	3.04	13
Soil Nitrogen Fixation (metric tonne)	0.02	6
Addition of Organic Manure (metric tonne)	0.68	1
Rain water fixation in soil (m^3)	6, 222	*
Total estimated value (million)		209

5 Discussion

The stupendous efforts of APPM have helped in sourcing almost its entire raw material requirement, generated through its farm/social forestry initiatives. It is evident that casuarina development under farm forestry has contributed to achieve sustained supply of raw material to mills as well as improving socio-economic status of local inhabitants. More over these casuarina plantations along the coastal belt has proved as a savior of the lives and property of the local population during natural calamities.

It has paved the way for the paper industry to become self-sustained in the generation of the much needed pulpwood resource while ensuring the growth of the farmers by raising the socio-economic status of the local populace and more importantly, improving the environment and maintaining the ecological balance.

6 Conclusions

The Farm Forestry programme with casuarina have been a dynamic force in sustaining raw material requirement and promoting a sustainable livelihood approach. During inception of the programme, selection of *C. equisetifolia* suitable for the project area and introduction of cost effective treated bare root seedlings for farmers holding marginal and degraded lands was vital for success. Constant innovative techniques and tree improvement programme with introduction of casuarina hybrid have helped farmers to achieve enhanced productivity. It has addressed the essential areas like social, economic segments of the community as well as environment amelioration of the area and has also ensured quality raw material supply on a sustained basis to mills.

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Tree Improvement

Variation in Growth and Morphological Characteristics of *Casuarina junghuhniana* Provenances in Thailand

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Abstract Variation in growth, stem form, branching characteristics and flowering of *Casuarina junghuhniana* was assessed at 4 years of age in a provenance/progeny trial planted in Kanchanaburi province, Thailand. The trial consisted of 146 families from 20 natural provenances and land races from Indonesia (Bali, Java, Timor and Wetar), Australia and Kenya, and a local commercial hybrid clone of *C. junghuhniana* × *C. equisetifolia*. In addition to significant differences among families within provenances, there were clear differences between regions and between provenances within regions for most of the characteristics assessed. Low-altitude sources from Timor and Wetar grew faster than high-altitude sources from Bali, Java and Kenya. The local hybrid clone was among the tallest entries and had the best stem form while the material from Australia also grew fast but had the most crooked stem. Seed sources from lower altitudes had a higher number of flowering trees than those from higher altitudes. Multivariate analysis revealed a pattern of geographic variation among natural provenances from Indonesia with Bali and Java clustering in one group and separating from Timor and Wetar. Land races from Australia and Kenya aligned closely with provenances from Bali and Java suggesting the original introductions might have come from these two Indonesian islands.

1 Introduction

Casuarina junghuhniana Miq. (syn. *C. montana*) of the Casuarinaceae family is a deciduous tree, 25-35 m tall and 50-80 cm in diameter. The natural distribution is restricted to the eastern part of the Indonesian Archipelago where it occurs on Java, Bali, Lombok, Sumbawa, Flores, Alor, Sumba, Wetar and Timor Islands (Pinyopusarerk and House, 1993). It grows in various habitats such as hard stony ground, steep slopes of water-ways, valley sides and mountains, and in crater valleys. It is a principal species on the slopes of volcanoes at altitudes of 1, 500-3, 100 m but also at lower altitudes in Wetar and Timor, where it occurs from near sea level to 1, 500 m altitude. It is long-lived and well adapted to a monsoonal climate with a distinct dry season. Rainfall in the natural distribution ranges from 700 mm to 1, 500 mm, and up to 2, 500 mm in highland places in Java (Mile,1996). Temperature varies widely with altitudes with mean annual minimum of 13℃ and mean annual maximum of 31℃. The species tolerates a variety of soil conditions, from compact clays to coastal volcanic soils and alkaline shales on Timor.

Casuarina junghuhniana is a pioneer species of deforested lands, moderately to very drought tolerant and especially good as a pioneer of soils prone to land-slides (Djogo, 1992). It is planted to improve soil fertility and rehabilitate degraded soils and as a windbreak. Branches and foliage are burnt and the ash is spread on village gardens in Timor. The wood is heavy, air dry density 900-1,000 kg m^{-3}, and makes good firewood and charcoal. The energy value of the charcoal is 34,500 kJ kg^{-1}, which is among the highest for firewood species (Pinyopusarerk, 1997). *C. junghuhniana* has been introduced successfully to some countries in Eastern Africa including Kenya, Uganda and Tanzania and, is increasingly being planted as an agroforestry tree (Okorio *et al.*, 1994; Mwihomeke *et al.*, 2002). It has shown potential in early species introduction trials in southern China (Zhong and Bai, 1996), and was found to be more productive than the commonly planted *C. equisetifolia* L. in coastal area of southern India (Varghese *et al.*, 2010). A male hybrid with *C. equisetifolia* was introduced to Thailand in about 1900 and has become a commercial clonal plantation species for production of piling poles (Chittachumnonk, 1983).

There has been increasing interest in planting *C. junghuhniana* and hence the search for suitable seed sources. However, information on genetic variation within the species is limited to a small progeny trial in southern China established with seed from a small number of trees from Timor and from planted stands in Kenya and Tanzania. Not until around the mid 1990s when the CSIRO Australian Tree Seed Centre organized individual-tree seed collection from major occurrences in Indonesia and planted stands in Australia and Kenya was it possible to investigate the genetic variation within the species in progeny trials. This paper presents results from a provenance/progeny trial planted in Thailand at age four years.

2 Materials and Methods

2.1 Planting material

Planting stock was raised as tube stock from seed obtained from 146 individual trees from 15 natural provenances from Indonesia and five planted stands from Australia and Kenya (Table 1). In addition, cuttings of the local hybrid clone of *C. junghuhniana* × *C. equisetifolia* were included as a control. These seedlot entries can be divided into seven groups or regions according to the country of origin, and in the case of seedlots from Indonesia according to the distribution in different islands.

Table 1 Origins of provenances of *Casuarina junghuhniana* planted in a progeny trial in Thailand

Group	Prov. No.	CSIRO seedlot	Provenance	Country	Latitude	Longitude	Altitude (m)	Rainfall (mm)	No. of families
1	1	17559	Coffs Harbour, NSW	Australia	30°18′S	153°08′E	100	1,800	2
2	2	17844	Old Uhak, NE Wetar	Indonesia	7°36′S	126°30′E	5	1,000	10
3	3	17877	25 km SW Soe, Timor	Indonesia	9°54′S	124°14′E	550	1,500	3
	4	17878	Noelmina River, Timor	Indonesia	9°59′S	124°06′E	170	1,400	2
	5	19489	Kapan, Kupang, Timor	Indonesia	10°13′S	123°38′E	600	1,300	4
	6	19490	Camplong, Timor	Indonesia	10°05′S	123°57′E	600	1,300	4
	7	19491	Buat, Soe, Timor	Indonesia	9°51′S	124°16′E	800	1,500	4
4	8	18848	Mt Abang, Bali	Indonesia	8°55′S	115°25′E	1,500	1,200	10

(continued)

Group	Prov. No.	CSIRO seedlot	Provenance	Country	Latitude	Longitude	Altitude (m)	Rainfall (mm)	No. of families
	9	18849	Kintamari, Bali	Indonesia	8°13′S	115°20′E	1,500	1,300	9
5	10	18948	Mt Kawi, Java	Indonesia	7°55′S	112°25′E	2,000	1,900	10
	11	18949	Mt Argopuro, Java	Indonesia	8°00′S	113°3′E	1,500	2,500	5
	12	18950	Mt Bromo, Java	Indonesia	7°55′S	112°55′E	1,600	2,300	7
	13	18951	West of Mt Arjuno, Java	Indonesia	7°45′S	112°33′E	1,350	2,100	11
	14	18952	Mt Willis, Java	Indonesia	7°50′S	111°47′E	1,500	2,500	8
	15	18953	East of Mt Arjuno, Java	Indonesia	7°45′S	112°35′E	1,350	2,200	9
	16	18954	Mt Bromo, Java	Indonesia	7°55′S	112°55′E	2,500	2,500	10
6	17	19237	Meru Forest, Meru	Kenya	0°07′N	37°37′E	1,750	1,400	10
	18	19238	KEFRI, Muguga, Kiambu	Kenya	1°13′S	36°39′E	2,080	1,300	8
	19	19239	KARI Muguga, Kiambu	Kenya	1°16′S	36°36′E	2,060	1,300	10
	20	19240	Muka Mukuu, Machakos	Kenya	1°05′S	37°12′E	1,460	1,000	10
7	21	Thai	Local commercial hybrid clone	Thailand					

2.2 Planting site

The planting site belongs to the Thai Royal Forest Department's Lumpao-Lumsai Forest Tree Experimental Station in Kanchanaburi province (latitude 13° 58′N, longitude 99° 18′E, altitude 45 m). Mean annual temperature is 29.9℃ and mean annual rainfall is 900 mm with 4-6 months of dry season. The soil is sandy and silty loam, with a pH of 6.7.

2.3 Experimental design

The experiment was laid out in a latinized row-column design (Williams *et al.*, 2002). The design generation package CycDesigN (Whitaker *et al.*, 2002) was used to produce the design and randomization for eight replicates each containing 10 rows of 15 columns. The local hybrid clone was repeated four times to make up the total 150 treatments. Each family plot consisted of a row of four trees spaced at 1.5 m between trees. The space between rows was 3 m. Two guard rows of the same species were planted surrounding the entire experiment. The experimental area was ploughed prior to the planting in August 2004.

2.4 Assessment

At 4 years of age, all trees were measured for height (*Ht*) and diameter at 1.3 m above ground (*DBH*). In addition, each tree was scored subjectively for eight morphological characteristics: axis persistence; stem straightness; density, thickness and angle of permanent branches; length and thickness of branchlets; and flowering status. Details of the assessments are given in Table 2.

Table 2 Characteristics assessed on each tree in the *Casuarina junghuhniana* provenance/progeny trial at Kanchanaburi, Thailand

Characteristic	Unit	Explanation
Quantitative traits		
Height (*Ht*)	m	Height of the tallest stem
Diameter (*DBH*)	cm	Measured at 1.3 m above ground

(continued)

Characteristic	Unit	Explanation
Qualitative traits		
Axis persistence (*Axpst*)	1-6	Ability of tree to retain its primary stem axis 1 = Multiple stems from ground level 2 = Forking in 1^{st} (lowest) quarter of stem 3 = Forking in 2^{nd} quarter 4 = Forking in 3^{rd} quarter 5 = Forking in 4^{th} quarter 6 = No forking
Stem straightness (*Strst*)	1-4	1 = Very crooked, > 2 serious bends 2 = Slightly crooked, >2 small bends or <2 serious bends 3 = Almost straight, 1-2 small bends 4 = Completely straight
Branching habit		
Permanent branches		First order branches originating from the main stem
Density (*Denpb*)	1-4	1 = Very high, regularly branched, internode mainly 15 cm 2 = High, irregularly branched, internode around 15 cm 3 = Low, irregularly branched, internode around 30 cm 4 = Very low, sparsely branched, internode >30 cm
Thickness (*Thkpb*)	1-4	1 = Very heavy, >3 branches diameter >1/3 of adjacent stem 2 = Heavy, 1-3 branches diameter > 1/3 of adjacent stem 3 = Light, branch diameter ≤1/3 of adjacent stem 4 = Very light, branch diameter ≤1/4 of adjacent stem
Angle (*Angpb*)	1-2	1 = Upright, <60° 2 = Horizontal, >60°
Branchlets		Deciduous branchlets (needles)
Length (*Lenbl*)	1-2	1 = Short, <15 cm 2 = Long, >15 cm
Thickness (*Thkbl*)	1-2	1 = Fine 2 = Coarse
Flowering (*Flowr*)	1-2	1 = No flowering 2 = Yes (regardless of sex)
Sex (*Sex*)	percent	Sex of each flowering tree was recorded as: male, female or monoecious, and percentage of each sex calculated for data analysis

2.5 Data analysis

Each characteristic was analysed to determine differences (1) between groups (regions) of provenances; (2) between provenances within regions; and (3) between families within provenances. Measurements on the trees within each plot were summarised to plot mean, variance and count using data pre-processing package DataPlus 3.0 (Williams *et al*., 2000). Normality checks were conducted on the plot mean, especially the categorical measurements.

Analysis of variance (ANOVA) was carried out on the plot mean using the statistical package GenStat Discovery Edition 3 (Buysse *et al*., 2004). The mean squares for groups, provenances within groups and families within provenances were tested against the residual mean square from the initial analysis. Estimated provenance means for growth and morphological characteristics were subjected to a canonical variate analysis. This multivariate analysis was performed to investigate the combinations of these characteristics which would help elucidate the differences between provenances from different groups as well as between groups. Height was not included in the analysis because of its strong correlation with diameter while monoecious tree sex was not included due to the lack of variation between regions or between provenances. Similarly for the local commercial hybrid clone, its far better-than-average stem straightness over all other seed sources would dominate the analysis.

3 Results

Except for branchlet thickness at the group level, significant differences ($P < 0.05$-0.001) were found for all of the characteristics measured between groups, between provenances within groups and between families within provenances (Table 3). Provenance means, group means, overall trial mean and standard errors of differences for all growth characteristics are given in Table 4. Despite highly significant ($P < 0.001$) variation among families within provenances, the results show clear evidence of significant variation among groups and among provenances within groups.

Table 3 Summarised results of analysis of variance for characteristics showing mean square values and significant difference levels

Growth trait	Group	Provenance	Family	Residual
1. Height	280.718***	11.020***	7.012***	3.152
2. Diameter	197.000***	8.351***	5.968***	2.933
3. Axis persistence	9.604***	3.544***	3.105***	1.006
4. Stem straightness	3.502***	1.175***	0.607***	0.217
5. Branch density	3.137***	0.994***	0.510***	0.164
6. Branch thickness	1.912***	0.733***	0.513***	0.259
7. Branch angle	1.077***	0.340***	0.256***	0.088
8. Branchlet length	0.629***	0.413***	0.121***	0.071
9. Branchlet thickness	0.016ns	0.021*	0.019***	0.011
10. Flowering	6.378***	0.942***	0.442***	0.109
11. Sex - male	6010.1***	919.5ns	–	546.8
12. Sex - female	5930.2***	895.3ns	–	541.6
13. Sex - monoecious	1.488ns	3.296ns	–	3.300

*, *** indicate significant differences at $P < 0.05$ and 0.001 respectively;
ns indicates no significant differences.

3.1 Characteristics discriminating groups and provenances

3.1.1 Growth

Compared with other seed sources, the provenances from Bali, Java and Kenya were significantly slower growing with mean height of 5.90-8.01 m and mean diameter of 5.54-7.10 cm. The Timor provenance group was most consistent with mean height of 9.59-10.63 m and mean diameter of 8.23-8.92 cm (Table 4). The two Bali provenances differed considerably from each other with Mt

Abang (mean height 8.01 m and mean DBH 7.10 cm) grew significantly faster than Kintamani (mean height 6.63 m and mean DBH 6.05 cm).

Seedlots from Wetar, Australia and local hybrid clone all recorded mean height over 9-10 m. However, the diameter of the local hybrid clone (7.78 cm) was smaller than material from Wetar (9.58 cm) and Australia (8.67 cm), but it was still significantly greater than seedlots from Bali, Java and Kenya.

3.1.2 Stem form

The majority of seedlots scored highly in axis persistence, reflecting the propensity of the species to be single-stemmed or to fork at the higher part of the stem. Nevertheless seedlots from Australia, Bali and Java scored significantly less than other sources (Table 4). The top score in axis persistence (5.82) belonged to the local commercial hybrid clone, which was also the best in terms of stem straightness (3.47). The Australian material was the worst in stem straightness with a mean score of 2.15. All other seed sources had reasonable to good stem straightness, mean values in the high 2's or close to 3. Of the two Bali seedlots, Kintamani had significantly straighter stem than Mt Abang.

3.1.3 Branching habit (permanent branches)

Branch density can generally be divided into two groups. The first group was characterised predominantly by densely but irregularly branched trees. The seed sources from Bali, Java and the local hybrid clone were included in this group (mean score ≤ 2.0). The second group having lower branch density, included mainly seed sources from Timor, mean score around 2.5. Land races from Kenya were very variable consisting of both high and low branch density, mean score ranging from 1.89 to 2.46.

Branch thickness varied between provenances although the majority of provenances tended to have thick branches, i.e. branch diameter greater than 1/3 of the adjacent main stem. The local hybrid clone clearly had the smallest branches, diameter $\leq 1/3$ of the adjacent main stem.

For branch angle, although the majority of provenances had horizontal branching (i.e. the angle $>60°$), those from Bali and Java had more upright branch angle than others. Of the two Bali seedlots, branches of Mt Abang (No.8) were more horizontal than Kintamani (No.9).

3.1.4 Deciduous branchlets

All but one seedlot from Meru Forest, Kenya had long branchlets. At the region or group level, Wetar seedlots had shortest branchlet, mean score 1.63 compared to 1.81 or greater by other groups.

3.1.5 Flowering

There were clear differences between groups and between provenances within groups in the number of flowering trees, irrespective of male, female or monoecious trees. None of the trees of the local male hybrid clone had flowered at 4 years of age while provenances from Timor, Wetar and Australia had very high proportions of flowering trees.

3.1.6 Sex ratio

The results confirm that *C. junghuhniana* is a monoecious tree species as observed in two seedlots Timor (No.3) and Kenya (No.18) (Table 4).

There were clear differences in the sex ratio between regions of provenances. The groups from Australia, Bali, Java and Kenya consisted of more male than female trees, with Wetar and Timor having more female trees.

Table 4 Mean values for 13 growth and morphological characteristics at 4 years of age of a *Casuarina junghuhniana* provenance/progeny trial in Thailand

No.	CSIRO seedlot	Seed source	Origin of mother trees	Ht	D	Axpst	Strst	Denpb	Thkpb	Angpb	Lenbl	Thkbl	Flowr	Sex		
														Male	Female	Mono-ecious
1	17559	Australia	Coffs Harbour	9.29	8.67	4.92	2.15	2.21	1.29	2.00	1.90	1.00	1.85	61.10	38.90	0.00
2	17844	Wetar, Indonesia	Old Uhak	10.44	9.58	5.43	2.55	2.27	1.49	1.93	1.63	1.00	1.77	36.70	63.30	0.00
3	17877	Timor, Indonesia	25 km SW Soe	9.59	8.76	5.21	2.32	2.60	1.34	1.88	1.97	1.00	1.90	47.60	50.00	2.38
4	17878		Noelmina River	10.63	8.92	5.64	2.87	2.41	1.51	1.98	1.94	1.10	1.94	54.00	46.00	0.00
5	19489		Kapan, Kupang	10.08	8.52	5.56	2.79	2.54	1.71	1.91	1.92	1.00	1.86	36.00	64.00	0.00
6	19490		Camplong	9.82	8.23	5.67	2.94	2.48	1.75	1.91	1.88	1.05	1.93	45.80	54.20	0.00
7	19491		Soe, Buat	9.75	8.38	5.81	2.97	2.54	1.74	1.99	1.92	1.06	1.89	40.70	59.30	0.00
		Mean		9.92	8.51	5.59	2.79	2.52	1.64	1.93	1.92	1.04	1.90	44.80	54.70	0.48
8	18848	Bali, Indonesia	Mt Abang	8.01	7.10	4.88	2.67	2.06	1.52	1.89	1.84	1.00	1.51	66.40	33.60	0.00
9	18849		Kintamani	6.63	6.05	5.04	2.98	2.01	1.51	1.68	1.87	1.00	1.19	100.00	0.00	0.00
		Mean		7.36	6.61	4.96	2.82	2.04	1.51	1.79	1.85	1.00	1.36	83.20	16.80	0.00
10	18948	Java, Indonesia	Mt Kawi	6.66	5.87	5.14	2.96	2.12	1.62	1.65	1.92	1.00	1.35	76.00	24.00	0.00
11	18949		Mt Argopuro	6.35	5.70	5.56	2.90	2.25	1.51	1.72	1.78	1.00	1.43	91.70	8.30	0.00
12	18950		Mt Bromo	7.04	6.38	5.07	2.58	2.14	1.67	1.89	1.78	1.00	1.37	84.70	15.30	0.00
13	18951		Mt Arjuno West	6.99	6.41	5.39	2.99	2.04	1.58	1.78	1.84	1.02	1.35	69.20	30.80	0.00
14	18952		Mt Willis	6.36	5.54	4.95	3.02	1.88	1.59	1.73	1.87	1.02	1.26	87.50	12.50	0.00
15	18953		Mt Arjuno East	7.04	6.37	4.92	2.78	2.10	1.52	1.72	1.87	1.03	1.38	66.50	33.50	0.00
16	18954		Mt Bromo	6.51	5.65	4.42	2.79	2.05	1.49	1.61	1.89	1.01	1.34	89.60	10.40	0.00
		Mean		6.73	6.01	5.04	2.87	2.07	1.57	1.72	1.86	1.01	1.35	79.40	20.70	0.00
17	19237	Kenya	Meru	5.90	5.54	5.25	2.91	2.46	1.44	1.90	1.57	1.00	1.21	75.00	25.00	0.00
18	19238		KEFRI Headquarters	7.14	6.18	5.64	2.86	2.15	1.65	1.93	1.94	1.00	1.73	55.60	42.30	2.08
19	19239		KARI-Muguga	6.50	5.87	5.49	2.75	1.89	1.88	1.78	1.89	1.05	1.49	69.70	30.30	0.00
20	19240		Muka Mukuu	7.18	6.56	5.44	2.72	2.07	1.52	1.89	1.87	1.00	1.68	96.70	3.30	0.00
		Mean		6.66	6.03	5.44	2.81	2.14	1.62	1.87	1.81	1.01	1.52	74.20	25.30	0.57
21	Thai	Thailand	Local hybird clone	10.03	7.78	5.82	3.47	2.11	2.19	1.86	1.91	1.00	1.00			
	Trial mean			7.52	6.69	5.24	2.82	2.15	1.59	1.81	1.84	1.01	1.48	66.10	33.60	0.24
	s. e. d. region			0.52	0.50	0.29	0.14	0.12	0.15	0.09	0.08	0.03	0.10	13.50	13.44	0.80
	s. e. d. provenance			0.56	0.54	0.32	0.15	0.13	0.16	0.09	0.08	0.03	0.10	16.54	16.46	1.17
	s. e. d. family			1.03	0.99	0.58	0.27	0.23	0.29	0.17	0.15	0.06	0.19			

Table 5 Canonical variate loadings weighted according to the standard deviations of the characteristics in the *C. junghuhniana* provenance/progeny trial at Kanchanaburi, Thailand

Variate		Loadings	
		CV1	CV2
1	Diameter	8.69	2.57
2	Axis persistence	-5.07	-0.11
3	Stem straightness	12.35	4.08
4	Permanent branch density	7.52	-17.31
5	Permanent branch thickness	19.42	-0.62
6	Permanent branch angle	-22.28	-13.90
7	Branchlet length	-24.43	-35.53
8	Branchlet thickness	13.91	-33.90
9	Flowering	1.75	4.68
10	Sex-male	-3.24	-1.41
11	Sex-female	-3.35	-1.40

3.2 Multivariate analysis

The results of multivariate analysis are shown in Fig. 1 where the first canonical variate is plotted against the second. The first accounted for 78.5% of the variance and the second 16.4%. The figure clearly shows seedlots from Australia, Bali, Java and Kenya cluster in one group while Wetar and Timor separate on their own.

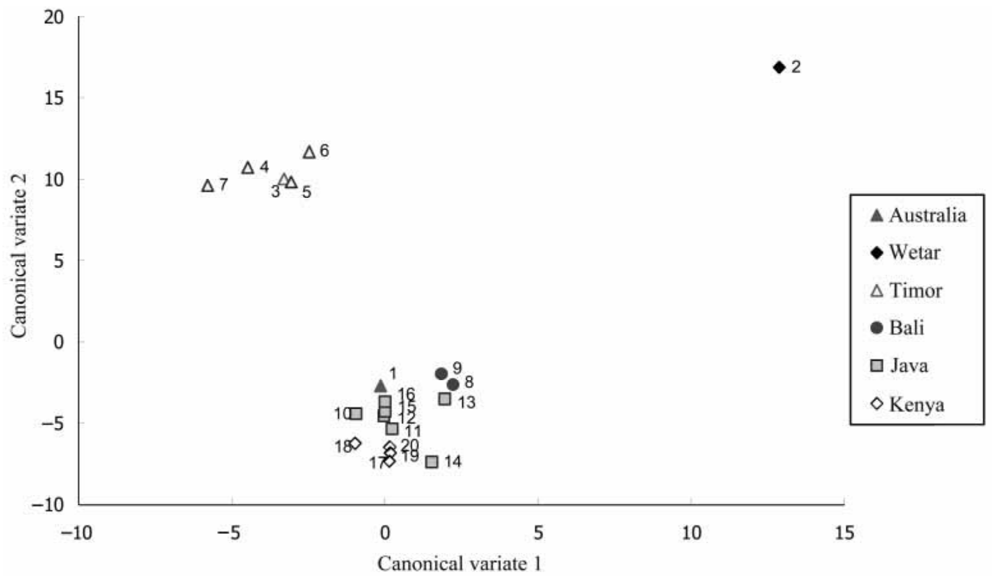

Fig. 1 Plots of first two canonical variates from the canonical variate analysis of 11 growth and morphological characteristics of *C. junghuhniana*

Loadings for the first two canonical components, weighted according to the standard deviation of the characteristics, are given in Table 5. A large absolute value for a variate (characteristic) loading indicates the importance of the variate in the formation of the canonical variates. It can be seen that the first canonical variate was based largely on thickness and angle of permanent branches and length of branchlets, while the second was related to permanent branch density and length and thickness of branchlets.

4 Discussion

The results indicate marked variation among populations of *C. junghuhniana* in many growth and morphological characteristics. Despite significant differences between provenances within groups, the results showed some evidence of variation among groups.

There was an indication, as shown in the canonical variate, of geographic variation among natural provenances from Indonesia. Data supported a separation of populations into three groups: (1) high-altitude provenances from Bali and Java, and (2) Wetar and (3) Timor, which were low-altitude provenances. Land races from Australia and Kenya were grouped closely with provenances from Bali and Java suggesting that their original introductions might have come from these Indonesian islands. Growth, stem form, branching habits and flowering were all contributing characteristics.

There was a tendency for provenances from high altitudes to grow slower than those from low altitudes. Provenances from Bali, Java and Kenya, which came from high altitudes 1,350-2,500 m grew slowly compared to Wetar, Timor and Australia, which came from lower altitudes 5-800 m. The altitude of the trial site in Thailand is 45 m. The results thus indicated better adaptation to low-elevation of low altitude provenances from Wetar and Timor. Similar results were obtained from a *C. junghuhniana* provenance trial on a low altitude site (8 m) at Dian Bai, Guangdong, China where Timor provenances grew better than Java and Bali provenances (Zhong and Bai, 2003). Nicodemus *et al*. (2005) also reported better performance of low-altitude-provenances from Wetar and Timor in India where the trial sites were located between 10 and 500 m asl. However, the results are contrary to those obtained from a provenance trial established in Tanzania with more or less the same seedlots. In Tanzania, high altitude provenances from Bali and Java outperformed low altitude provenances from Wetar and Timor (Mwihomeke *et al*., 2002). The trial site in Tanzania was located at 1,500 m altitude. These results thus suggest a possible environment-by-provenance interaction for *C. junghuhniana*, with low-elevation provenances better adapted to the low-elevation tropics and high-elevation provenances better adapted to tropical highlands.

The land races from Kenya had poor growth with the seedlot from Meru Forest (No. 17) being the slowest growing in the trial, while other seedlots (Nos. 18-20) were comparable to the slow-growing provenances from Java. *C. junghuhniana* was believed to have been introduced to Kenya from Indonesia. The poor performance of the Kenyan land races could have been due to genetic deterioration over successive generations through build up of inbreeding. Rapid genetic deterioration has been documented in several unmanaged land races of other species including tropical acacias (Luangviriyasaeng and Pinyopusarerk, 2001).

This provenance/progeny trial demonstrated considerable variation not only in growth but also stem form, branching habit and flowering among provenances and land races of *C. junghuhniana*. The local commercial hybrid clone showed good growth and stem form as expected. Other seed sources showing good growth and stem form were from Wetar, Timor and Australia. With further selection and improvement in stem form and branching characteristics, these seed sources will offer a wider range of utilization potential especially as sawn timber in addition to the conventional use as poles.

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Production of Inter-provenance and Inter-specific Hybrids of *Casuarina equisetifolia* and *C. junghuhniana* and Their Early Evaluation for Growth and Form Traits

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Abstract Members of the genus *Casuarina* sensu stricto share a common chromosome number (2n = 18) providing opportunity for hybridization among species. Outstanding male and female clones drawn from different provenances of *C. equisetifolia* and *C. junghuhniana* were used in a hybridization programme. The mean fruitset was 0.2% and 0.1% respectively when *C. equisetifolia* and *C. junghuhniana* were used as the female parent. Twelve fullsib families were produced through control pollination and were studied for variation in growth and stem straightness in field tests at three locations up to 2 years of age. Twenty open-pollinated families harvested from an 8 year old hybridization orchard, bulked seedlot from a seedling seed orchard of each species and a putative hybrid clone were included for comparison. In general control-pollinated hybrid families performed better than the open-pollinated seedlots. The mean volume growth of the best 5% of hybrid trees was 88% to 108% greater than the best seed orchard seedlot in a given site. There was a coastal-inland trend in the performance of the hybrid combinations: families with *C. equisetifolia* as the female parent performed the best in coastal site whereas those with *C. junghuhniana* as female parent were superior in inland locations. The strategies for testing and deploying hybrid clones for increasing plantation productivity in south India are discussed.

1 Introduction

All species of the genus *Casuarina* have a similar chromosome number (2n = 18) and polyploidy has not been recorded (Barlow, 1983). Reports on artificial hybrids are scanty but natural hybrids from both natural and introduced locations are known. Hybrids of *C. cunninghamiana* × *C. glauca* and *C. cunninghamiana* × *C. cristata* were found in natural stands (Wilson and Johnson, 1989). In cultivation, hybrids of *C. cunninghamiana* × *C. glauca* were reported in Egypt (Badran *et al.*, 1976) and between *C. equisetifolia* and *C. glauca* in Taiwan (Wang *et al.*, 1984).

Most species of *Casuarina* are not sympatric in their native habitat in Australia and their occurrence together in a new environment may have removed the isolation barriers leading to hybridization. This evidence suggests that there may be many more opportunities to create new hybrid combinations, adapted to new climatic and soil environments, even for *Casuarina* species whose natural distributions do not overlap (Boland *et al.*, 1996).

The present study reports results from a control pollination experiment to produce intra and interspecific hybrids of *C. equisetifolia* and *C. junghuhniana* and the early performance of various hybrid combinations in comparison with pure species seedlots.

2 Materials and Methods

2.1 Selection and clonal propagation of parent trees

Twenty outstanding individuals were selected from the seedling seed orchards (SSO) of the two species and used as parents for intra and interspecific crossing. Fullsib seeds were obtained for crosses involving only 11 of these 20 clones details of which are provided in Table 1.

Table 1 Details of parent trees of *Casuarina equisetifolia* and *C. junghuhniana* selected at 4 years for hybridization programme

S. No.	Clone No.	CSIRO number	Provenance	Country	Location of SSO	Sex *	Height (m)	DBH (cm)	Stem St'ness
Casuarina equisetifolia									1 to 6
1	11	18296	Ban Bangsak	Thailand	Karunya	F	9.5	9.6	6
2	26	18298	Had Chaomai	Thailand	Karunya	F	11.0	9.1	5
3	31	18297	Ranong	Thailand	Puducherry	M	9.9	8.2	5
4	33	…	Salem	India	Karunya	F	9.8	8.8	6
5	35	…	Neyveli	India	Karunya	M	9.3	8.6	6
6	46	18153	Ela Beach	PNG	Puducherry	M	10.3	9.2	6
				Mean of selected trees			9.9	8.9	5.7
				Mean of all trees in the trial			8.5	6.6	4.8
Casuarina junghuhniana									
7	2	17878	Noelmina R	E. Timor	Puducherry	M	13	10.8	6
8	5	17878	Noelmina R	E. Timor	Karunya	M	11.5	10.2	6
9	11	17878	Noelmina R	E. Timor	Puducherry	M	12	10.2	6
10	12	17844	Wetar	Indonesia	Karunya	F	14	11.1	5
11	14	17844	Wetar	Indonesia	Karunya	M	13	9.2	5
				Mean of selected trees			12.6	10.2	5.5
				Mean of all trees in the trial			7.1	5.1	4

* M: Male; F: Female.

All the selected trees were clonally propagated through rooting of softwood cuttings ("sprigs") using low-cost polytunnels. Cuttings were mostly collected from matured branches in which flowering and/or fruiting was observed. However, the amount of flowering and fruiting among trees especially those belonging to different provenances varied considerably. While the *C. equisetifolia* clones of Indian land race flowered in the nursery itself, low flowering and fruiting was observed in clones of native provenances like Thailand and PNG.

Five ramets of each clone was planted in earthern pots and grown for 6 months to produce sufficient branches to bear flowers and fruits. All the control pollination experiments were conducted in this potted hybridization orchard assembled in the Silviculture Research Nursery of IFGTB (Fig. 1).

Fig. 1 A potted hybridization orchard of *Casuarina equisetifolia* and *C. junghuhniana* for control pollination experiments

2.2 Control pollination experiments

Nearly one hundred combinations (between different provenances of the same species and between the two species) were attempted in three consecutive years between 2004 and 2006 but sufficient seedset was obtained for only 12 hybrid combinations.

Two flowering seasons were observed in both species, the first one during June - July and the second during November - December. Since the male and female clones of different provenances and species showed differences in flowering phenology, storage of pollen was necessary. Pollen from those male clones that completed their flowering before the initiation of flowering in the desired female clones was stored for use in the next season (6 months later). Branches bearing male inflorescences were covered with pollination bags made from butter papers one day before anthesis. Inflorescences were collected in clean Petri plates just before anther dehiscence and dried with silica gel. Pollen was separated from the anthers and stored in 5℃ until use.

Branches bearing female inflorescences were covered with pollination bags one day before anthesis. Pollen was applied to stigma through a paint brush between 8.00 and 9.00 hours in the morning when the stigma unwound. The branches were again covered with pollination bags to prevent pollen contamination from other plants. The bags were retained for the next 5 days or until the stigma dried up. Casuarina fruits ('cones') matured in about 2 months after pollination. Fruits of each cross were harvested separately and seeds were extracted by drying in partial shade.

2.3 Nursery development

Seeds of 12 control-pollinated families harvested from 2004 to 2006 were stored and sown together in nursery in March 2007. These 12 families included two intraspecific crosses each of *C. equisetifolia* (EE) and *C. junghuhniana* (JJ), five crosses involving *C. equisetifolia* as female parent (EJ) and the other three with *C. junghuhniana* as mother (JE). Ten open-pollinated families each from *C. equisetifolia* (EHO) and *C. junghuhniana* (JHO) female trees from a Hybridization Orchard were included to identify putative hybrid progeny. The 8-year-old hybridization orchard was planted with alternate rows of trees of the two species to promote natural hybridization between them. A bulked seedlot from SSO of two species (ESSO and JSSO) and the putative hybrid clone were also included as control. Four-month-old, polybag-grown seedlings inoculated with *Frankia* were used for field planting.

2.4 Field testing of hybrid progeny

Three hybrid progeny tests were established in contrast sites to identify the outstanding families and individuals in terms of growth and form. The test locations include a coastal farmland, Veedur, Tamil Nadu (latitude 11°55′N; longitude 79°52′E; altitude 10 masl; rainfall 1,250 mm), an inland high rainfall site, Panampalli, Kerala (latitude 10°52′N; longitude 76°46′E; altitude 500 masl; rainfall 1,500 mm) and sea shore, Sriharikota, Andhra Pradesh (latitude 13°45′N; longitude 80°10′E; altitude 0 masl; rainfall 1,200 mm) to select hybrids suitable for different environments and end uses.

The three field trials were laid out in incomplete block designs with 4 rows and 8 columns (32 accessions) in Sriharikota and Veedur and five rows and seven columns (35 accessions) in Panampalli. Each trial had five replications with four (Veedur) or five (Sriharikota and Panampalli) trees per accession per replication planted at a spacing of 2 m × 1 m. A guard row was planted along the perimeter of the trial plots.

All trees in the field trials were assessed for height growth at the age of 6 months and height, dbh and stem straightness at 12 and 24 months. Stem straightness was scored on a 4 point scale: 1- crooked, more than 3 bends; 2- slightly crooked, 2-3 bends; 3- almost straight with one bend; 4- straight). Analysis of variance was done using the softwares DataPlus (version 1.1) and Genstat (version 3.12) to test the significance of differences among different crosses and the control. An across-site analysis was performed with 27 families common to all three test sites to determine the G × E interaction for the different characters assessed. Using block adjusted values of individual trees, the best 5% of trees (Panampalli: 43; Veedur: 30; Sriharikota: 40) were selected for clonal propagation and multilocation testing as hybrid clones.

3 Results

3.1 Control pollination

The overall mean fruitset for all combinations tried was less than 1%. In general, better fruitset was obtained when *C. equisetifolia* was involved as female parent (0.2%) than when *C. junghuhniana* was as female parent (0.1%). Fruitset gradually increased from the first year of experiments (2004) to the third year (2006) due to standardization of protocol for pollen storage and control pollination techniques.

3.2 Field evaluation of hybrid families

The various cross combinations showed significant differences ($P < 0.001$) in terms of survival, height, diameter, volume and stem straightness at 6, 12 and 24 months in all test locations. Since the trend in the performance of families and crosses was almost similar at the three ages, only the results obtained at 24 months are discussed in detail. The mean survival was 96% in Panampalli and Veedur and 86% in Sriharikota. In all sites the JE clone had the lowest survival (68-85%).

The control-pollinated hybrid combinations (both intra and interspecific) generally performed better than the open-pollinated seedlots but the best combination was not the same in different locations (Table 2). The difference in the growth of intra and interspecific crosses was not large in Panampalli and Veedur whereas in Sriharikota intraspecific crosses of EE recorded better growth than interspecific crosses. The JE crosses recorded the best volume growth in Panampalli followed by JJ crosses. In Veedur, the EE crosses were the best closely followed by the interspecific crosses JE and EJ. While the volume growth of the intraspecific JJ crosses were comparable to trial mean in this site, the open-pollinated seedlots of *C. junghuhniana* performed below average. The best growth in Sriharikota was recorded by the EE combinations followed by the ESSO seedlot and EH families. The JJ and JE crosses as well as the JH open-pollinated seedlots showed poor growth. Growth of JH clone was consistently less than the trial mean in all three sites.

Generally the intraspecific EE crosses possessed straighter stems compared to other control and open-pollinated seedlots, closely followed by EJ and JE crosses. These crosses scored close to 3 or more in a 4-point scale which means they possess 0 to 1 bend. JJ crosses and JH open pollinated seedlots generally scored less than 3 indicating presence of 1 to 2 bends in a tree. Stem straightness of the JH clone was among the best in all sites. The across-site analysis involving 27 families common to all three test locations showed significant site, family and site × family variations for growth and stem straightness (Table 3). The variation due to the site effect was the greatest, ranging from 88% to 96% of total variation observed. The significant site × family interaction is also supported by the fact that no cross combination was found to be consistently performing well in all three sites (Table 2).

Table 2 Survival, growth and stem straightness at 2 years age for intra and interspecific crosses of *Casuarina equisetifolia* and *C. junghuhniana*

Cross	Panampalli					Veedur					Sriharikota				
	Surv (%)	Height (m)	DBH (cm)	Volume (dm^3)	SST (1-4)	Surv (%)	Height (m)	DBH (cm)	Volume (dm^3)	SST (1-4)	Surv (%)	Height (m)	DBH (cm)	Volume (dm^3)	SST (1-4)
EE	96	5.84	4.42	3.09	2.99	98	9.65	6.49	11.14	3.65	94	7.81	6.38	10.08	2.91
EJ	99	6.07	4.56	3.46	2.89	100	9.04	6.14	9.33	3.45	98	6.71	4.95	5.74	2.89
JJ	96	6.14	4.69	3.75	2.23	90	8.62	6.07	8.77	2.94	84	4.31	2.36	1.28	2.32
JE	96	6.12	4.73	4.06	2.68	96	9.42	6.46	10.90	3.30	80	4.61	2.58	1.26	2.60
ESSO	95	6.02	4.13	3.18	2.84	95	9.39	5.76	8.33	3.93	92	7.41	6.05	7.88	3.00
JSSO	98	5.97	4.76	2.45	3.81	95	9.16	6.10	9.39	3.58	72	4.59	2.50	1.55	2.46
EHO	95	5.81	4.21	2.92	3.05	94	8.82	5.65	7.84	3.52	96	7.11	5.46	6.43	2.92
JHO	96	5.84	4.44	3.28	2.36	98	8.20	5.50	7.28	2.97	71	3.85	2.21	1.08	2.25
JH clone	80	4.91	3.37	1.64	3.83	85	7.94	5.04	6.27	3.60	68	3.15	2.01	1.33	2.57
Mean	96	5.9	4.4	3.2	2.8	96	8.7	5.8	8.4	3.3	86	5.6	3.9	4.1	2.6
SED	6.67	0.24	0.24	0.45	0.24	6.27	0.38	0.31	1.02	0.20	10.07	0.57	0.39	0.96	0.19
CD	13.19	0.48	0.47	0.89	0.47	12.40	0.75	0.61	0.86	0.39	19.92	1.13	0.77	1.89	0.37
P	0.04	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3 Means squares and their significance for differences due to site, family and site × year for survival, growth and stem straightness of Casuarina hybrid families at 2 years age

Source of variation	df	Survival		Height		DBH		Volume		Straightness	
		MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*
Site	2	1152.46	0.017	78.83	0.000	22.86	0.000	184.50	0.000	3.60	0.000
Family	26	89.31	0.002	1.757	0.288	1.38	0.005	6.84	0.051	0.37	0.001
Family × site	52	92.73	0.000	1.04	0.000	1.25	0.000	5.18	0.000	0.06	0.015
Pooled residual		33.51		0.16		0.09		0.64		0.04	

Table 4 summarizes the extent of gain in volume and stem straightness likely to be obtained by selecting the best 5% individuals, families and cross combinations over seed orchard seeds. The volume gain over the best suited seed orchard progeny by using the selected 5% of trees ranged from 88% to 108%. Gain in volume was accompanied by loss of stem straightness in Panampalli and Veedur where hybrids involving *C. junghuhniana* were among the best (4.5-22%). But in Sriharikota the best 5% of trees almost exclusively belong to the EE combination and recorded a gain of 33.6% to 50% in stem straightness.

Table 4 Percentage gain in volume from selecting the best 5% of trees in each site relative to SSO seed, best cross and family at 2 years of age

Item	Panampalli		Veedur		Sriharikota	
	Volume	St'ness	Volume	St'ness	Volume	St'ness
Gain relative to CE SSO Seedlot(%)	152.8	−22.2	133.9	−14.9	107.8	33.6
Gain relative to CJ SSO Seedlot(%)	88.5	1.0	107.5	−6.6	956.6	50.0
Gain relative to best family(%)	50.2	−10.7	57.4	−4.5	8.9	34.5
Gain relative to best cross(%)	76.8	−7.7	74.9	−8.4	62.5	35.7
Gain relative to site mean(%)	123.0	−11.3	133.1	1.6	300.4	44.1

4 Discussion

The results indicate that hybrids between different provenances and species of *C. equisetifolia* and *C. junghuhniana* have the potential to increase plantation productivity in South India. Control pollination helps in combining the outstanding provenances/individuals which is not possible under open-pollinated conditions. Since casuarina trees are grown for multiple enduses like fuelwood, poles and pulpwood, which require different set of characters, specific crosses/individuals can be designed to meet these diverse needs.

The marked superiority of the selected individuals can be exploited through clonal forestry following selection of outstanding clones from clone tests established with rooted cuttings. The simple hydroponic method of rooting casuarina young shoot cuttings developed in China (Zhong Chonglu, pers. comm.) offer the scope for cost-effective mass vegetative propagation of hybrid clones. These clones will widen the choice of clones in India which is at present limited to a single putative hybrid clone introduced from Thailand. With further selection and testing clones with adaptability to specific sites and end uses can be developed.

The significant site × family interaction demands that families and clones will have to be selected specific to a given planting site. Hybrids generally need a distinct 'hybrid habitat' for optimal expression of their growth potential (Anderson, 1948). There seems to be a coast to inland trend for growth and stem straightness. Hybrids with *C. equisetifolia* as mother are well suited for coastal areas whereas those with *C. junghuhniana* as female parent are the best for inland areas. *C. junghuhniana* is not suitable for shelterbelt conditions both as pure species and as a female parent for hybrids.

It is possible to select parents based on the performance of their progeny for developing bi-clonal orchards to produce seeds of fullsib families under open-pollinated conditions. The prerequisite for such a strategy is overlapping phenology of the two parent clones. This approach will be particularly useful to produce large number of seedlings required for planting in shelterbelts. Since the difference in gain from the best 5% of trees and the best family is not high under shelterbelt conditions in Sriharikota, mass production of seeds of selected families will be a cost-effective method of realizing genetic gain.

The stem straightness of *C. junghuhniana* is generally poorer than that of *C. equisetifolia*. As a result, hybrid selections involving *C. junghuhniana* showed inferior stem form. Since there is no adverse correlation between growth and stem straightness in Casuarina, it is still possible to simultaneously select parents and progeny for both traits (Pinyopusarerk *et al*., 2005; Nicodemus, 2007). Although the present study reports results from an early age of 2 years, it is half-rotation age for Casuarina in India and a strong correlation has been reported for growth at second and later years (Nicodemus, 2007).

5 Conclusions

Intra and interspecific hybrids of *C. equisetifolia* and *C. junghuhniana* showed faster growth than seed orchard seeds of pure species. Stem straightness of intraspecific hybrids of *C. equisetifolia* was superior to crosses involving *C. junghuhniana*. A strong site × family interaction exists for growth and stem straightness indicating selection of specific hybrid families and individuals for different planting sites. Hybrid vigour can be exploited through clonal propagation of outstanding individuals and by establishing biclonal orchards with parents of best families.

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A Key for Nursery Identification of Interspecific Hybrids of *Casuarina equisetifolia* and *C. junghuhniana*

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Abstract Detecting hybrids at seedling stage especially among progeny raised from open-pollinated seeds has considerable utility in further testing and deployment of hybrids to increase plantation productivity. The sexual system of *Casuarina equisetifolia* and *C. junghuhniana* is predominantly dioecious in India. This enables production of interspecific hybrids through open pollination by assembling male and female clones of different species with overlapping flowering phenology in a hybridization orchard. The present study describes a simple key developed based on morphological characteristics viz. colour and thickness of deciduous branchlet, colour of leaf tip and number of leaves per node for the identification of hybrids in the nursery. Since these characteristics are highly species-specific, the hybrids were found to be intermediate for any or all of them. Ten putative hybrids randomly chosen among those identified through the key were validated by species-specific sequence characterized amplification region (SCAR) markers.

1 Introduction

Identification of hybrids prior to field planting is highly profitable since hybrids can be allowed for planting (Gan and Sim, 1992). If they can be identified through simple morphological markers, seedlings can easily be segregated in nursery itself and hybrid individuals selected for planting. It is also essential to evaluate the discriminating ability of morphological traits through a more full proof marker systems like DNA markers. If molecular markers confirm high level of precision in segregation based on morphological characters, a simple key for identifying hybrids could be developed.

Rufelds (1987) indicated that it was possible to differentiate seedlings of *Acacia mangium*, *A. auriculiformis* and their hybrids on the basis of leaf development, morphology and taxonomic characteristics. The Leaf Taxonomic Characteristics (LTC) include pinnule margin pubescence, colour of the margin and underside of the pinnule, as well as the phyllode morphology. The Lead Development Pattern (LDP) includes the developmental sequences of the different pinnae leaves and phyllode. Although the extent of hybrids identified by the Rufeld's method was consistent across different locations and assessors, it overestimated the frequency of hybrids (Gan and Sim, 1992).

The predominantly dioecious sexual system of *Casuarina* species provides scope for producing specific hybrid combinations under open-pollinated conditions in bi-clonal orchards. A simple key involving vegetative characters preferably leaf morphological characters will help identifying hybrids in the nursery. In an earlier study, seven morphological characters viz., branchlet length, internode length, sheath teeth number, cone fresh weight, cone length, cone diameter and seed weight grouped trees of *C. glauca*, *C. equisetifolia* and the suspected hybrids between them separately. The branchlet anatomy of *C. glauca* and *C. equisetifolia* were different and that of the putative hybrids was intermediate in nature. An index based on the assessment of the above mentioned traits was found to be useful in identifying the hybrids (Wang *et al.*, 1984).

The objective of the present study was to identify interspecific hybrids of *C. equisetifolia* (CE) and *C. junghuhniana* (CJ) in the nursery through a simple key involving morphological characters of the deciduous branchlet ('needle').

2 Materials and Methods

The present study was conducted in the Silviculture Research Nursery of the Institute of Forest Genetics and Tree Breeding, Coimbatore, India during 2008.

2.1 Study material

Seedlings raised with control pollinated fullsib seeds and open-pollinated seeds from a Hybridization Orchard were studied for 'needle' morphology. Twenty four seedlings each of 9 control pollinated families and twelve open-pollinated families (6 each with CE and CJ as female parent) were assessed. The polybag grown seedlings were 3 months old at the time of assessment. Eight parent clones that produced the control pollinated families were also studied for comparison with their progeny.

2.2 Morphological characters

Seven morphological traits were studied to discriminate the two species and their hybrids. They are colour of the stem, number of pronounced bends, colour and thickness of the branchlet, colour of the leaf tip, and number of leaves per node and branch angle. Out of these four characteristics were distinct for the two species and the hybrids showed either intermediate characters or resembled one of the parent species. All the seedlings were assessed for colour and thickness of needle, leaf tip colour and using score classes as explained in Table 1. The number of leaves per node was counted for each seedling using a hand lens. The sum of values obtained for the four traits were used to assign them to the two species or hybrids (Table 2). *C. equisetifolia* and *C. junghuhniana* were designated as class 11 and 33 respectively and plants considered as hybrids were scored as classes 12, 13, 31 and 32.

Table 1 Details of morphological characters of branchlet studied to identify *C. equisetifolia*, *C. junghuhniana* and their hybrids

S. No.	Character	Classes	Score assigned
1	Colour of needle	Dark green	1
		Intermediate	2
		Yellowish green	3
2	Thickness of needle	Thick (>1 mm)	1
		Intermediate (0.5 to 1 mm)	2
		Thin (<0.5 mm)	3

(continued)

S. No.	Character	Classes	Score assigned
3	Colour of leaf tip	Green	1
		Very light pink	2
		Light pink	3
		Deep pink	4
4	Number of leaves per node	6 to 12	6 to 12

Table 2 Details of classes to which individuals of *C. equisetifolia*, *C. junghuhniana* and their hybrids were assigned based on needle morphology

Class	Female parent	Morphology shown
11	*C. equisetifolia*	*C. equisetifolia*
12	*C. equisetifolia*	Intermediate
13	*C. equisetifolia*	*C. junghuhniana*
31	*C. junghuhniana*	*C. equisetifolia*
32	*C. junghuhniana*	Intermediate
33	*C. junghuhniana*	*C. junghuhniana*

Table 3 Proportion of seedlings that showed branchlet morphology similar to *C. equisetifolia* (CE), *C. junghuhniana* (CJ) and intermediate (hybrid) between them

S. No.	Family	CE	CJ	Hybrid
Control pollinated families				
1	CE33 × CE35	92	4	4
2	CE26 × CE35	83	4	13
3	CJ12 × CJ2	0	79	21
4	CJ12 × CJ14	0	88	13
5	CE33 × CJ2	0	4	96
6	CE11 × CJ14	0	0	100
7	CJ12 × CE5	0	8	92
8	CJ12 × CE35	0	4	96
9	CJ12 × CE46	13	13	75
Open pollinated families				
10	CE13	96	0	0
11	CE15	88	0	13
12	CE16	88	0	13
13	CE19	79	0	17
14	CE21	54	0	46
15	CE24	96	0	4
16	CJ25	8	46	46
17	CJ26	4	38	58
18	CJ29	0	46	54
19	CJ30	0	75	25
20	CJ31	0	63	38
24	CJ33	0	13	88

2.3 Data analysis

The sample means were calculated for morphological characters and multivariate analysis based on stepwise canonical discriminant analysis was computed using SPSS. In this analysis, characters were entered one by one and the process stopped when none of the remaining characters significantly improved the discriminant capacity ($P \leqslant 0.05$). Characters with the highest coefficient of the canonical structure on the first canonical function were selected which explained the highest proportion of variance between groups.

2.4 Validation of hybrids through SCAR markers

Ten randomly selected hybrid individuals were screened with SCAR markers developed for *C. equisetifolia* and *C. junghuhniana* (Dasgupta *et al*., 2009) for validation of the identification through morphological characters (Table 4). Species specific SCAR markers for both *C. equisetifolia* and *C. junghuhniana* were developed from ISSR markers. Twenty five individuals from five species (*C. equisetifolia*, *C. junghuhniana*, *C. glauca*, *Allocasuarina littoralis* and *A. heugeliana*) were randomly collected for DNA isolation and amplified with seven ISSR primers. Species specific bands were cloned and sequenced. Primer pairs were designed from the edited sequence data using Primer 3 software and subsequently synthesized.

Table 4 Scores for branchlet morphology of selected accessions of *C. equisetifolia* (CE), *C. junghuhniana* (CJ) and their hybrids

Accession number	Species/Cross combination	Needle colour	Needle thickness	Leaf tip colour	No. of leaves per node	Total score	Designation
CE 11	CE Clone	1	1	1	7	10	PS
CE 33	CE Clone	1	1	1	8	11	PS
CE 35	CE Clone	1	1	1	8	11	PS
CE 46	CE Clone	1	1	1	7	10	PS
3/24	CE 33 × CJ 14	2	2	3	10	17	Hybrid1
10/8	CE 11 × CJ 14	2	2	2	10	16	Hybrid2
11/22	CJ 12 × CE 35	2	3	2	10	17	Hybrid3
12/4	CJ 12 × CE 46	1	2	2	8	13	Hybrid4
16/17	CE-OP	1	2	3	9	15	Hybrid5
19/3	CE-OP	2	2	2	10	16	Hybrid6
21/15	CE-OP	1	1	2	10	14	Hybrid7
25/10	CJ-OP	2	2	3	10	17	Hybrid8
26/2	CJ-OP	2	2	2	8	14	Hybrid9
29/15	CJ-OP	1	1	3	12	17	Hybrid10
CJ2	CJ Clone	3	1	4	12	20	PS
CJ 5	CJ Clone	3	3	4	11	21	PS
CJ12	CJ Clone	3	3	4	10	20	PS
CJ 14	CJ Clone	3	3	4	11	21	PS

PS = pure species.

The SCAR primer pair specific to *C. equisetifolia* (IFGTBCE-01) were amplified in 30 individuals representing 10 provenances while SCAR primer pair specific to *C. junghuhniana* (IFGTBCJ-02) was amplified in 20 individuals. Both the SCAR primer pairs were further amplified individually in ten randomly selected individuals of other four species to ascertain the specificity of the primers. An internal control reaction was conducted using multiplexed PCR with CESSR 22 and IFGTBCE-01. Ten randomly selected hybrids were amplified in a single reaction by multiplexing the two primer pairs IFGTBCE-01 and IFGTBCJ-02 and resolved by 15% agarose gel electrophoresis.

3 Results and Discussion

3.1 Seedling morphology

The seven morphological characteristics viz. stem colour, number of bends per seedling, needle colour, leaf tip colour, branch angle, needle thickness and number of leaves per node discriminated the two species and their hybrids fairly accurately. Scores obtained by different cross combinations and families are provided in Table 3. The proportion of seedlings of different families that were characterized as parent species and hybrids is given in Table 3. In case of intraspecific crosses, the key detected 88-92% as that of pure species. The detection of a small number of seedlings as hybrid may be due to contamination during control pollination experiments or inability of the characters used to distinguish them. In case of control pollinated interspecific crosses the proportion of hybrids detected ranged from 75% to 100%. Among the open pollinated families 0-46% of hybrids were found with *C. equisetifolia* as female parent and 25-88% *C. junghuhniana* mother trees. Those hybrid individuals which possessed morphological characters similar to any of the parent species cannot be determined as hybrids if at least one of the parents is not known. Among the seven characters studied the colour of the leaf tip and the number of leaves per node resolved the status of individuals better than the other characters. Kamalakannan *et al.* (2006) reported that morphological traits grouped three species of *Casuarina* and two species of *Allocasuarina*.

The Canonical Discriminant Analysis grouped the 6 classes to which the seedlings were placed based the total score obtained for the morphological characters assessed (Fig. 1). Classes 12 and 32 which had individuals possessing morphological traits intermediate between the two species were placed exactly in between the two pure species (classes 11 and 33). The other two classes 13 and 31 aligned close to the species whose morphology they resembled. The fairly distinct grouping of the individuals into pure species and hybrids indicate the effectiveness of the four morphological characters in identifying hybrid individuals. Since these characters can be assessed at an early stage in the plant's life with the use of simple field lens, they are useful in identifying hybrids.

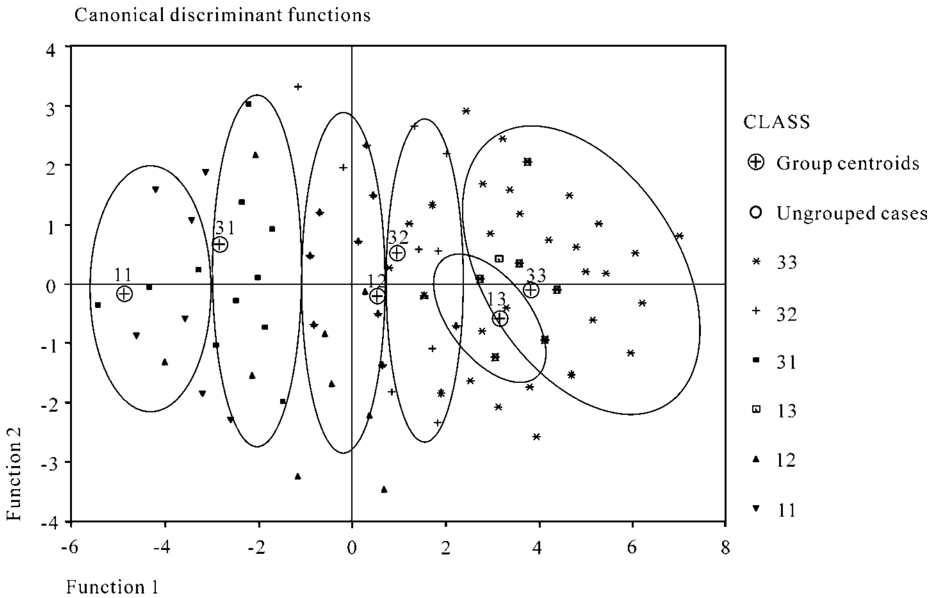

Fig. 1 Cluster diagram for grouping *C. equisetifolia*, *C. junghuhniana* and their hybrids based on four needle morphological characters

3.2 Hybrid validation with SCAR markers

Seven ISSR primers produced distinct profiles in all 120 individuals and generated a total number of 241 scorable products in the size range of 220 to 1, 710 bp. Two amplicons at 410 bp and 550 bp amplified by the primer UBC842 in *C. junghuhniana* and *C. equisetifolia* respectively were found to be specific and repeatable. Primer pairs were synthesized from the sequence data. IFGTBCE-01 specific to *C. equisetifolia* amplified in all 30 individuals at 500 bp while its absence was documented in other 4 species where the internal CESSR 22 amplified at 210 bp. IFGTBCJ-02 amplified in all 20 individuals at 250 bp and no amplification was observed in other species. The primer pairs IFGTBCE-01 and IFGTBCJ-02 were multiplexed and amplified in both CJ × CE and CE × CJ hybrids and amplicons specific to both species at 500 bp and 250 bp amplified in all individuals confirming hybridity.

SCAR markers confirmed the identification of hybrids based on the morphological characters (Fig. 2). Since *Casuarina* species are predominantly dioecious, desired hybrid combinations can be produced through open-pollinated hybrid orchards housing male and female clones with overlapping phenology. The resulting progeny could be screened early in the nursery to select the hybrid individuals. DNA markers are widely used to determine hybrids before deploying them for plantation development. Putative and control pollinated interspecific hybrids of *Acacia mangium* and *A. auriculiformis* were identified through RAPD markers and individuals selected for vegetative propagation and use in establishing plantations (Wickneswari and Lee, 1993). The identified SCAR markers in the two important species of *Casuarina*, *C. equisetifolia* and *C. junghuhniana* can be used for the accurate and rapid identification of both species for certification and determination of putative hybrids during selection and hybridization programmes of casuarinas.

Lanes 1 to 10 refer to the hybrid accessions listed in Table 4.

Fig. 2 Multiplexed PCR profile of SCAR primer pairs in *Casuarina* hybrids

4 Conclusions

Interspecific hybrids of *C. equisetifolia* and *C. junghuhniana* can be detected in nursery based on branchlet colour, thickness, leaf tip colour and number of leaves per node. SCAR markers validated the hybrids identified through the morphological key.

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Fertility and Progeny Performance in Seedling Seed Orchards of *Casuarina equisetifolia* and *C. junghuhniana*

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Abstract Two seed orchards each of *Casuarina equisetifolia* and *C. junghuhniana* established by selectively thinning provenance trials located in coastal and inland regions in southern India were evaluated at four years of age for fertility, sex ratio and performance of progeny. The *C. equisetifolia* orchards had similar proportion (84-87%) of fertile trees in both locations whereas *C. junghuhniana* differed in fertility between the coastal (99%) and inland (55%) sites. Sibling coefficient, which gives an estimate of fertility variation, was low ($\Psi < 3$) at the coastal site in both species and at least two times higher in the inland sites, indicating predicted deviation from random mating. Land races of *C. equisetifolia* had high fecundity in the inland site as compared to the coastal site. The Kenyan land race of *C. junghuhniana* had mostly male trees, but differed in their reproductive output and contribution to gene pool, between coastal (29%) and inland (88%) sites. Natural seedlots from Timor, Indonesia produced almost all the fruits in *C. junghuhniana* orchards, but the Kenyan land race produced 71% of the male flowers in the inland site. Bulked seed from each of the orchards were tested in genetic gain trials in a coastal and inland site along with a Thailand and local seedlot of *C. equisetifolia* as control. At three years *C. junghuhniana* seedlots were superior to *C. equisetifolia* in growth at the coastal site whereas they were on par with the control in the inland location. *C. junghuhniana* and Thailand seedlot of *C. equisetifolia* had poor stem form compared to the other seedlots. As seen in the parent orchards, fecundity of progeny was higher in the inland test location. The newly introduced *C. junghuhniana* and Thailand seedlot of *C. equisetifolia* had low fecundity, which raises difficulties in capturing the potential gain associated with their introduction.

1 Introduction

Casuarina is raised as a short rotation crop, both as monoculture and mixed with other agricultural crops in various agroforestry models in the coastal areas of India. The eastern coast of peninsular India extending from the southern tip of Tamil Nadu to Orissa accounts for the major planted area.

It is also being planted on light deep soils in inland regions with irrigation. The Indian land race of *Casuarina equisetifolia* is quite popular among farmers, who follow a traditional cultivation practice. New seedlots from several provenances of *C. equisetifolia* and *C. junghuhniana* were tested in coastal and inland regions of southern India with a view to identify high yielding provenances and produce good quality seed of wide genetic base. After early evaluation, the provenance trials were thinned and converted to seedling seed orchards by removing the inferior trees (Nicodemus *et al.*, 2001). New introductions of natural provenances to a new region has to be monitored for its fecundity and fertility variation along with the growth to actually produce improved seed for large scale planting. Casuarina orchards have not been adequately studied in terms of fertility variation and contribution of parental genotypes to the seed crop. This study was initiated to evaluate the performance of Casuarina seed collected from provenance and progeny trials established in India. The provenance trials were progressively thinned to promote seed production in the retained trees. *C. junghuhniana* was newly introduced and showed great promise due to its greater drought tolerance than the currently planted species. Domestication of a new species has to be done scientifically with proper care since the first generation is the entry point to a new site where high genetic drift and coancestry (Kang and Lindgren, 1998; Lindgren and Mullin, 1998) can result in poor capture of the potential gain in the next generation. Thus parent trees have to be monitored for its contribution to the gene pool and suitable measures taken to ensure that adequate diversity is maintained in the seed crop. This paper reports the fecundity and fertility variation among parent trees in two seedling seed orchards each of *C. equisetifolia* and *C. junghuhniana* and the performance of the progeny in relation to natural provenance and land race controls.

2 Materials and Methods

2.1 Seedling seed orchards

Two provenance trials each of *C. equisetifolia* and *C. junghuhniana* located in a coastal (Pondicherry) and inland site (Panampally and Karunya) in southern India were converted to seedling seed orchards by thinning the inferior trees after evaluation at four years of age. Bulked seed of 10-20 trees was used to establish the provenance trials except the *C. equisetifolia* trial at the inland site which was established initially as a provenance-progeny trial of 100 families from the same provenances as that of the coastal site (Nicodemus *et al.*, 2001). Table 1 provides details of the location and climate of the orchard sites. Details of number of trees of each provenance and the contribution of each tree to the gene pool are provided in Table 3 and Table 4.

Table 1 Location and climatic details of Casuarina orchard sites

Trait	*C. equisetifolia* Karunya (CE Kar - Inland)	*C. equisetifolia* Pondicherry (CE Pon - Coast)	*C. junghuhniana* Panampally (CJ Pan- Inland)	*C. junghuhniana* Pondicherry (CJ Pon - Coast)
Latitude (N)	11°00′	11°55′	10°52′	11°55′
Longitude (E)	76°58′	79°52′	76°46′	79°52′
Rainfall (mm)	1,000	900	1,200	900
Altitude (m asl)	300	10	400	10
Mean min-max temperature range (℃)	20-36	21-38	22-39	21-38

The orchard trees were evaluated for individual tree fertility at four years of age when the trees were suitable for seed production. Trees were assessed for sex, number of fruits and the male and female fecundity of each tree (Varghese *et al.*, 2002). The number of primary, secondary and tertiary branches was counted in each tree and the flowers per tertiary branch recorded for all the trees. Number of fruits per secondary branch was recorded and estimates of fruits and flowers produced per tree were obtained by extrapolating the counts made on flowers and fruits (Kang and Lindgren, 1998; Bila *et al.*, 1999). The fecundity of each tree was used to compute the following two parameters for the seedling seed orchards.

2.2 Relative status number

Relative status number (N_r) was used to compare the effective number of trees (N_s) contributing to random mating, with the actual number of trees retained (N) in the orchard.

$$N_r = \frac{N_S}{N}$$

$$N_S = \frac{1}{\sum_{i=1}^{N} p_i^2} \qquad [1]$$

where

(p_i is the contribution from individual genotype i to the gamete pool and N the census number of trees in the orchard.)

2.3 Sibling coefficient

Sibling coefficient (ψ) was calculated from the number of trees in the orchard (N) and individual fertility (p_i) of each tree. It was used to describe fertility variation among the trees.

$$\psi = N \sum_{i=1}^{N} p_i^2 \qquad [2]$$

2.4 Genetic gain trials

Seed was collected from 25 average or above-average trees in each orchard at four years of age. Equal weights of seed from each mother tree were combined to give four orchard bulk seedlots that were tested in genetic gain trials in two locations in southern India (Table 3). A single bulked seedlot collected from 10 trees in a clonal seed orchard of *C. equisetifolia* at Neyveli in Tamil Nadu and a bulked seedlot of natural Thailand provenance of *C. equisetifolia* (that was used for establishing the parent orchards) were used as control. The trials tested the seedlots using randomized complete block designs with 49-tree (7 × 7) plots in five replications. A spacing of 3 m between planting rows and 2 m between trees within rows was used. One month after planting NPK 17 : 17 : 17 fertilizer was applied at 25 g per plant. Complete manual weeding was done twice each year. Tree height (ht), diameter at breast height (dbh), axis persistence (scale of 1-6) and stem straightness (scale of 1-4) were recorded, and the number of fruits (cones) was counted on each tree, three years after planting.

2.5 Statistical analysis

At each site, plot mean values for height, dbh, plot conical volumes, survival and number of fruits per tree were subjected to analysis of variance using the following linear model:

$$Y = \mu + \text{REPL} + \text{TREAT} + \text{RESIDUAL} \qquad [3]$$

where Y is the vector of plot observations, μ is the site mean, REPL is the vector of fixed replicate effects, TREAT is the vector of fixed genetic treatment effects, and RESIDUAL is the vector of

Table 2 Fertility, fecundity (per fertile tree) and proportion of different sexes in Casuarina orchards

Orchard	Non-fertile trees(%)	Male trees		Female trees		Monoecious		
		%	Flowers $tree^{-1}$	%	Fruits $tree^{-1}$	%	Male flowers $tree^{-1}$	Fruits $tree^{-1}$
C. equisetifolia Karunya (inland)	13.8	24.7	3,090,000	46.8	3,262	14.7	533,437	2,186
C. equisetifolia Pondicherry (coast)	12.6	26.3	967,000	48.6	1,953	12.5	877,628	2,113
C. junghuhniana Panampally (inland)	54.7	19.6	4,970,000	25.7	3,991	–	–	–
C. junghuhniana Pondicherry (coast)	1.1	47.2	1,310,000	51.7	2,949	–	–	–

Table 3 Contribution of *C. equisetifolia* provenances to the Orchard gene pool

Pondicherry – *C. junghuhniana*			Contribution (%)		Panampally – *C. junghuhniana*			Contribution (%)	
Country	No. of Prov.	No. of trees	Female	Male	Country	No. of Prov.	No. of trees	Female	Male
Land races									
India	5	62	30.4	28.7	India	9	40	38.8	21.5
Kenya	3	44	15.8	29.0	Kenya	3	29	42.8	37.1
China	3	32	6.1	0.1	China	5	40	5.4	12.5
Vietnam	1	5	0	10.6	Vietnam	2	11	4.5	5.7
Total	12	143	52.3	68.4		19	120	91.5	76.8
Natural									
Solomon Is	2	27	9.8	3.8	Solomon Is	1	4	0.3	1.5
Benin	1	6	0.8	2.4	Philippines	1	6	0	5.4
Thailand	1	16	8.6	2.8	Thailand	4	33	6.5	11.6
Malaysia	3	15	6.7	0	Malaysia	2	15	0.2	1.6
PNG	1	7	2.3	0.4	PNG	1	12	1.5	3.1
Guam	1	2	0.3	0					
Australia	2	39	19.2	22.2					
Total	11	112	47.7	31.6		9	70	8.5	23.2

Prov. = Provenances. Same as in Table 4.

residual effects remaining after fitting the model. Analysis was performed using the ANOVA directive in the statistical software package Genstat Release 9 (VSN International Ltd, United Kingdom).

3 Results

3.1 Fertility variation in seed orchards

The percentage of fertile trees, proportion of different sexes and the mean fecundity of the orchard trees are given in Table 2. The proportion of male and female trees in *C. equisetifolia* orchards did not vary much between the coastal and inland locations. There were around 50% female and 25% male trees in both sites and 13-15% trees were either monoecious or non-flowering. The pattern was quite different in *C. junghuhniana* since almost 55% trees in the inland site were not flowering compared to just over 1% non-flowering trees in the coastal site. Both species had greater fecundity (producing 1.3 to 1.6 times more fruits and the 3 times more male flowers since the inflorescences were longer) in the inland sites than in the coastal sites. The monoecious *C. equisetifolia* trees however had greater male fecundity in the coastal site even though the female fecundity was almost similar in both sites. In *C. equisetifolia* the contribution from land races, especially those from India and Kenya was substantially higher in the inland location. In the coastal site the representation from natural provenances was also high that their collective female fecundity was on par with that of the land races (Table 3). In *C. junghuhniana*, fruit production in both orchards was almost entirely by the Timor seedlots. The orchards however differed in the pollen contribution from the Kenyan land race. Whereas more than 70% of the male contribution in the inland site was from the Kenyan seedlot, this land race contributed only 11% of the male flowers in the coastal site (Table 4).

Table 4 Contribution of *C. junghuhniana* provenances to the Orchard gene pool

Pondicherry – *C. junghuhniana*			Contribution (%)		Panampally – *C. junghuhniana*			Contribution (%)	
Country	No. of Prov.	No. of trees	Female	Male	Country	No. of Prov.	No. of trees	Female	Male
Land races									
Kenya	6	22	0.9	11.1	Kenya	6	48	0	71.5
Natural									
Timor	6	239	99.1	87.9	Timor	6	163	100	25.5
Java	2	8	0	1	Java	2	3	0	3
Total	14	269	100	100		14	214	100	100

Orchards in the coastal region had similar values for sibling coefficient ($\Psi < 3$) and relative population size with 34-36% of the trees contributing equally to the gene pool in both species. Since fertility variation was very high in the inland site, though 28% of *C. junghuhniana* trees contributed effectively to random mating, fertility variation resulted in a predicted deviation of 7 times from panmixis. The inland *C. equisetifolia* orchard also had a high sibling coefficient ($\Psi = 5.8$) value and less than half the number of effectively contributing trees (17%) as that of the coastal site.

Table 5 Estimates of fertility variation and relative effective population size in four seedling seed orchards of Casuarina

Trait	*C. equisetifolia* Karunya	*C. equisetifolia* Pondicherry	*C. junghuhniana* Panampally	*C. junghuhniana* Pondicherry
N	190	255	214	269
N_r	0.17	0.36	0.28	0.34
Ψ	5.83	2.79	7.06	2.97

N = number of trees in orchard, N_r = relative effective population size and Ψ = Sibling coefficient.

3.2 Genetic gain trials

The orchard seedlots had good survival (Table 6) in both trial locations (88-95%) on par with the control seedlots of *C. equisetifolia* namely the natural Thailand seedlot (86% and 96%) and the local CSO seedlot (91% and 95%). The orchard seedlots did not differ significantly from each other as well as the controls in growth and conical wood volume at the inland site. At the coastal site, *C. junghuhniana* seedlots had higher conical wood volume compared to the control. The Thailand seedlot of *C. equisetifolia* also showed good diameter growth at the coastal site. *C. equisetifolia* seedlots from the seedling orchards and the CSO (control) had in general higher fecundity and better stem straightness than *C. junghuhniana* and the natural Thailand seedlot of *C.*

equisetifolia. The *C. junghuhniana* seedlot from the coastal orchard had comparatively better stem straightness than that from the inland orchard. Fecundity was higher at the inland trial location compared to the coastal site. Fecundity of the orchard seedlots at the inland location was on par or higher than that of the domesticated CSO seedlot *C. equisetifolia*. *C. junghuhniana* had low fecundity at the coastal site whereas the Thailand *C. equisetifolia* seedlot had poor fecundity at both the trial sites.

Table 6 Performance of Casuarina seedlots at two trial sites (36 months)

Trial location	Orchard seedlot *	Survival (%)	Ht 36 (m)	DBH 36 (cm)	Conical volume (m^3)	Axis persistence (1-6)	Stem straightness (1-4)	Fruits $tree^{-1}$
Pondicherry	CE Pon	91.8	8.54	7.45	0.012	5.34	2.89	114
	CE Kar	92.7	8.57	7.17	0.01	5.27	2.95	89
	CJ Pon	95.1	8.72	8.49	0.02	5.35	2.25	15
	CJ Pan	91.8	9.12	8.75	0.02	5.22	1.96	0
	CE Thai	96.3	9.09	8.05	0.016	5.38	2.64	0
	CE CSO	95.1	8.54	7.35	0.012	5.23	3.05	149
	P	0.45	0.078	<0.001	<0.001	0.278	<0.001	<0.001
	SE	2.8	0.30	0.25	0.002	0.08	0.09	35.7
Karunya	CE Pon	91.3	10.37	6.79	0.012	4.92	2.88	701
	CE Kar	90.8	10.02	6.31	0.01	4.98	3.05	380
	CJ Pon	87.8	9.96	6.75	0.01	5.04	2.81	276
	CJ Pan	88.3	10.20	7.16	0.013	4.91	2.56	193
	CE Thai	85.7	9.64	6.73	0.013	4.94	2.63	27
	CE CSO#	90.8	9.97	6.52	0.01	4.94	2.96	402
	P	0.59	0.42	0.17	0.681	0.72	<0.001	<0.001
	SE	3.63	0.34	0.30	0.002	0.09	0.09	134

* Thailand seedlot of *C. equisetifolia*; # Seedlot from clonal seed orchard of *C. equisetifolia* land race.

4 Discussion

Land races of *C. equisetifolia* from India and Kenya have high fertility and fecundity compared to the newly introduced natural seedlots. This is particularly evident in the inland orchard. The fertility difference is comparatively low and the overall fecundity is also less in the coastal orchard. The situation is slightly different in *C. junghuhniana* since there is a clear difference in adaptability of the Timor and Kenyan seedlots to the two sites. The Kenyan land race of *C. junghuhniana* contributes significantly to the gene pool at the inland trial site but not at the coastal site; that too as a male parent only. Kamalakannan *et al*. (2009) studied the molecular diversity of the orchard progeny in the genetic gain trials. This study clearly showed that the diversity of the coastal *C. equisetifolia* orchard progeny was substantially higher than that of the inland orchard. This is mainly because of the high contribution of the land races in the inland orchard. Though there was big difference in the number of fertile trees in the two *C. junghuhniana* orchards, the molecular diversity was only marginally lower in the inland orchard since most of the male trees were from the Kenyan land race (Kamalakannan *et al*., 2009). This shows that the seedlots emanating from the inland and coastal orchards are quite different even though it is not manifested in their growth. The diversity of the progeny from the local land race CSO was at least three times lower than that of the coastal *C. equisetifolia* orchard but the growth was not significantly different. With one generation of domestication there has been an overall improvement in the fecundity of the orchard progeny in the inland trial site but it is quite probable that the orchard seedlots are quite different in their genetic composition as there would be a higher representation of the land race genes in the inland orchard. It is quite interesting to note that even though the proportion of sexes in the two *C. equisetifolia* orchards were more or less similar the high fecundity of the land races and high fertility variation (as indicated by the high sibling coefficient) has resulted in substantial reduction in genetic diversity in progeny of the inland orchard. The situation is further compounded by the high fecundity in the seed crops of *C. equisetifolia* orchards in the next generation. There could be a gradual influx of the land race genes in orchards raised in inland locations.

Seedling seed orchards after thinning are expected to yield adequate quantity of improved seed and enhance the productivity of plantations. Though suitable provenances are often identified in provenance tests at various sites, very little information is generally collected on the fertility of natural provenances or the suitability of sites for getting adequate flowering in the newly introduced natural seedlots. Progeny originating from orchards of the same seed origin could vary depending on the flowering status and the fertility variation between trees. Excessive fertility of a few trees can lead to relatedness among progeny. Loss of diversity occurs from increase in coancestry levels in the orchard as a result of variable flowering among trees. Poor and irregular flowering is often observed in orchards that are not located on good flowering sites. If there are not many flowering trees in orchards, a low effective to actual population size would lead to loss of diversity in seed crop (Varghese *et al.*, 2004).

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Improved Cone and Seed Handling Procedures for *Casuarina equisetifolia*

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Abstract Industrial plantations are gradually displacing wood from natural forests. The success of such plantation programmes, among other things, hinges on a continuous supply of high quality seeds for the production of the desired quantity of seedlings in nurseries or for successful stand establishment by direct sowing out in the field. To ensure that only the best quality seed is marketed, seed quality control programmes are becoming increasingly important. The genetic quality of seed and the level of gene diversity within a seedlot show discrepancies primarily due to the broad genetic base of seedlots. This accounts for wide variations in the level of maturity at time of collection and in the degree of dormancy that is present in many species of woody plants. The greatest early losses in seed quality result from collecting cones before seeds are fully mature. In addition, in the tropics, seed collection is manual and not equipment driven hence the chances of product quality and efficiency decreasing are very high. In addition, bulking of cones from orchards brings in an assortment of cones of varied sizes which has also found to have a bearing on seed quality. A study was conducted to determine if cone characteristics and seed quality in Casuarina vary by cone size, since a better understanding of this will be useful during the harvesting process and will provide indicators for quality of the seedlots. Cones of *Casuarina equisetifolia* collected from single tree collections were graded into large, medium, small and ungraded cones and seeds extracted. The cones showed variations in relation to size, weight and germination percent, large cones exhibiting higher percentage of germination followed by ungraded, medium and small cones. The cones were cut into 3 parts namely upper, middle and lower portion and time taken for release of seeds studied. Upper portion exhibited faster release and higher percentage of germination followed by middle portion and lower portion. Bulked cones could be subjected to grading based on size followed by segregation of seeds based on time release to obtain quality seeds in the species.

1 Introduction

Trees begin as seeds and successful tree planting depends on the health status and genetic quality of the seeds. Seed must be available in adequate quantities and be able to germinate well. In many cases the seed may not be healthy or fresh enough. However, reproductive abnormalities like parthenocarpy hinder development of seeds and thereby reduce germination. Physiological quality is a measure of the potential performance of a seedlot under optimal conditions. Seed purity is also of prime importance in fixing seed quality. Impurities in seedlots exclusive of non-seed materials would include dead, shriveled, immature and pre-germinated seeds. In India, casuarinas are mainly propagated through seeds. There is an ever increasing demand for casuarinas seeds as small farmers prefer seedlings for planting. Hence large quantities of seed need to be handled for raising plantations. The germination performance of *Casuarina equisetifolia* seedlots is reported to be low, 40-50%, even when freshly collected (Anandalakshmi *et al*., 2001; Umarani and Vanangamudi, 2002). Reasons for low germination in fresh seeds have been attributed to various external and internal factors like research on temperature (El-lakany and Shepherd, 1983), light (Badran and El-lakany, 1978; Halos, 1983), cone traits (Mahadevan *et al*., 1999), age of trees (Raghavan, 1947) and season of collection (El-lakany *et al*., 1989) have been conducted to improve germination. In the past, several attempts have been made to upgrade the germination performance of *C. equisetifolia* seeds. The South Dakota seed blower was used to grade *C. equisetifolia* seeds by density and the result showed that high-density seeds, retained at the highest air-speed setting, had a better germination and vigour (Maideen *et al*., 1990). Attempts made to grade seeds by size (seed mass) have resulted in better germination for larger (167 mg/100 seeds) than smaller (105 mg/100 seeds) seeds (Umarani *et al*., 1997), and specific gravity separator yielded better germination for heavier seeds (Umarani and Vanangamudi, 2002), which in turn showed significant correlation with progeny growth (Mahadevan *et al*., 1999). The present study aims to (i) study the biometric characters of cones and seeds all-through its fruiting season, (ii) understand the seed development process in the cones during maturation, and (iii) arrive at a relationship between season, maturity and cone size to obtain high germination in *C. equisetifolia*.

2 Materials and Methods

2.1 Seed materials

2.1.1 Bulked

Cones collected from February to June, 2009 from a seedling seed orchard of *C. equisetifolia* at Panampalli, Kerala, India were bulked, dried under sun for two days to release the seeds by shaking. Weight of 100 seeds was determined in eight replications (International Seed Testing Association, 2003). This material was termed as bulk throughout the study.

2.1.2 Seedlots

Cones were collected from five randomly selected trees in the seed orchard and seedlot numbers were given serially for each tree. Seed weight was determined for each seedlot by weighing samples of 100 seeds in eight replications (International Seed Testing Association, 2003). The material was termed as seedlots throughout the study.

2.2 Sorting based on size

Cones collected in February were maintained as pre-samples, March-April as peak-samples, and May-June as post-samples to study the exact period of maturity in the species. The pre-, peak- and post-samples of 25 cones collected from bulk and seedlots were assessed for morphological characters like length, breadth, roundness and perimeter. Image analyzer (Leica Quantimet 500 + or QWin) was used for measurements. The length of the bulk cones ranged from1.3 cm to 5 cm. Based on the length, the cones were grouped into small (1.3-2.5 cm), medium (2.5-3.7 cm) and large (> 3.7 cm). The cones collected in the peak season were cut into three portions, (upper, middle and lower) using a sharp blade. The cut cones were then maintained separately.

2.3 Cone and seed data

The cones, namely bulk, seedlots, and the cut portions, were shade dried and when they dehisced the seeds were separated. The number of seeds per cone and 100 seed weight were recorded. Four replications each with 100 seeds were sown on germination papers in Petri dishes that were kept in germination room maintained at 25 ± 3℃, 95 ± 2% relative humidity and a photoperiod of 12/12 h light/dark. Initiation of germination was noticed after 4 days and final count was taken after 21 days.

2.4 Statistical analysis

The data were analyzed by one-way or two-way analysis of variance (ANOVA). Prior to statistical analyses, variables were checked for normality and transformed when necessary. Correlation analysis was performed to examine the relationship between cone size and seed traits.

3 Results and Discussion

Cone traits were studied for bulk and seedlots across phases (Table 1). Cones collected during the peak season in both lots were significantly varying in the parameters namely length, breadth, perimeter and roundness over the other phases. And between the bulk and seedlots, the latter showed significantly larger cones during the various phases over the bulk. Seedlots showed increased seed weight and germination per cent over the bulk seeds despite the bulk being higher in number (Table 1). The number of seeds in the bulk during the peak phase was very high; nonetheless, the seed weight (145.86 mg) did not show any significant variation over the pre phases. This suggests that the seeds may have been ill filled or empty. Seed number, therefore, is not an apt measure; rather, seed weight could be a reliable measure of the germinability of the seeds.

In the case of casuarinas, cone collection can be made from single trees throughout its flowering period, graded based on size, and seeds collected. Following this, seeds can be bulked. The bulk were sorted into big, medium and small cones and measured across phases (Table 2). Though sorting was initially carried out based on length, the cones could also be sorted based on breadth and roundness, which were also significantly distinguishing the clones. Perimeter was the highest for medium cones than the big cones suggesting more surface area for accommodating the seeds. In the sorted cones, the highest number of seeds was observed in the small cones during the peak phase. However, the seeds had < 10% germination. In the case of large cones, though the number of seeds was only 50% that of the small cones, the germination per cent was higher. This indicates poor filling of the seeds, and when such cones contribute to the bulk, the overall per cent of the bulk tends to decrease. Seed weight was also highest in the big cones, which showed high germination per cent in all the phases compared to the medium and small cones. Seeds from seedlots could thus initially be sorted into large, medium and small, following which they could be pooled to form a bulk.

Seeds from big cones showed the highest germination when collected from the upper portions of the cone. However, seeds at the middle and lower portions, despite being heavier, showed low germination. Medium cones also exhibited similar pattern, however, the number of seeds produced during the peak phase were higher than the large cones. In the case of small cones, despite having large number of seeds, the seed weight was low (i.e. small seed size), and germination was very poor in all the three phases. In all the cases, maximum seed production was observed during the peak season with the big cones showing the highest germination.

Seedlots of *C. equisetifolia* are composed of a large quantity of empty and shrivelled seeds with substantial inter-seedlot variability (Sivakumar *et al.*, 2007). The average germination of bulk seeds recorded is less than 50% (Anandalakshmi *et al.*, 2001; Umarani and Vanangamudi, 2002) which is also consistent with the present study. The variation in germination of bulk and seedlots is partly related to the variation in seed weight among seeds which has fairly good positive relationship between these two characters. This is consistent with previous studies where an increase in germination of *C. equisetifolia* with increasing seed weight has been observed (Umarani and Vanangamudi, 2002). Another feature which was observed was that seedlots showed increased germination over the bulk. This could be attributed to the inter seedlot variability which shows pronounced effects on bulking if cones are not collected during the right seasons and mixed in appropriate proportions.

Another observation in this study is that not just seed weight alone is a good indicator of germination performance; rather the size of the cones and phase of collection also affect germinability of seeds. During size grading, the diameter of the cone plays major role when compared to the length. Seedlots maintained with identity showed variation in size of the cones, number of seeds produced and the germination per cent suggesting that cones could be collected separately from single trees, graded based on diameter/roundness and then bulked which would give better germination results. In the different sized cones studied, there were a relatively high proportion of non germinable seeds, but the percentages were much higher than those observed by Sivakumar *et al.* (2007).

Petroleum flotation has been reported to be a feasible technique to upgrade the germination of *C. equisetifolia* seeds, and its efficiency is influenced by wing surface area, seed density and wing quotient of filled and empty seeds (Sivakumar *et al.*, 2007). Improvement in germination following grading and separation of empty and filled seeds was distinct, however, few seedlots still could not be separated, thus leaving scope for further improvement in seed germination and separation efficiency.

Maternal factors, such as position of the seed in the fruit/tree and the age of the mother plant during seed maturation, also have an influence on seed germination (Wulff, 1995), which partly explains the observed variation in germination between the seeds collected from different portions of the cones. The presence of a large fraction of empty/dead seeds in small cones suggests the need for developing methods for enhancing the production of filled seeds in seed orchards or seed production areas through precision silviculture. It is thus recommended that selection of cones by length and diameter could be followed for individual tree collections. Once this is done, the seedlot identities are to be maintained, seeds collected and there after separated by density grading for increased germination.

Correlation studies to identify relationship between the cone and seed traits and germination (Table 3) revealed that broader cones with increased perimeter gave seeds with better germination. However, there was no significant correlation between number of seeds and germination.

Thus, it can be seen from the present study that there is copious seed production in *C. equisetifolia* however; emptiness or abortion of seeds is observed. To overcome this, cultural treatments such as nitrogen fertilization (Graham, 1986), root pruning, spacing or stand density (Shearer and

Table 1 Summary of cone and seed traits in different samples

Samples	Phases	Length (cm)		Breadth (cm)		Perimeter (cm^2)		Roundness		100 seed weight (mg)		Number per cone	Germination (%)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	Mean	SD
Bulk	Pre	2.469b	0.45	1.731b	0.12	7.391b	0.80	1.386b	0.034	120.31a	26.86	14c	30.83b	8.47
	Peak	2.998a	0.56	2.103a	0.20	8.984a	1.22	1.683a	0.084	145.86a	9.83	87a	48.34a	3.51
	Post	2.062c	0.37	1.447c	0.10	6.174d	0.65	1.158b	0.024	99.28b	12.12	20b	21.81c	7.36
Seedlots	Pre	5.006b	1.21	1.936b	1.01	8.619b	1.05	1.551a	0.039	220.00b	35.10	18b	33.25c	7.88
	Peak	6.508a	1.58	2.517a	1.31	9.198a	0.90	1.684a	0.068	272.20a	36.70	27a	49.75a	4.96
	Post	5.423c	1.31	2.098b	1.09	6.015d	1.01	1.508a	0.054	205.60c	30.20	19b	41.80b	4.14

Table 2 Summary of cone and seed traits in sorted cones

Samples	Phases	Length (cm)		Breadth (cm)		Perimeter (cm^2)		Roundness		100 seed weight (mg)		Number per cone	Germination (%)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	Mean	SD
Big	Pre	2.898b	0.19	1.669b	0.04	7.208b	0.08	1.390b	0.11	150.89b	26.56	38c	16.18c	3.47
	Peak	3.478a	0.22	2.003a	0.05	8.650a	0.09	1.667a	0.13	181.07a	31.87	147a	31.06a	8.77
	Post	3.135c	0.15	1.391c	0.03	6.007c	0.06	1.158c	0.09	125.74b	22.13	50c	10.54c	1.98
Medium	Pre	2.508a	0.05	1.868b	0.04	8.267e	0.28	1.419b	0.04	109.56b	9.67	34c	7.19a	1.62
	Peak	2.415a	0.07	2.335a	0.06	10.334a	0.35	1.773a	0.04	136.94a	12.09	168a	9.07a	1.77
	Post	2.090b	0.04	1.557b	0.04	6.889c	0.23	1.182c	0.03	87.64c	7.74	54c	6.22a	1.56
Small	Pre	2.000b	0.18	1.657b	0.10	6.697b	0.57	1.351b	0.03	100.49a	12.00	30d	7.46a	1.20
	Peak	2.380a	0.22	1.972a	0.12	7.969a	0.68	1.607a	0.04	119.58a	14.28	335a	8.21a	1.09
	Post	1.681c	0.15	1.393c	0.09	5.627b	0.48	1.135c	0.03	84.44b	10.08	47d	5.05a	1.01

Table 3 Pearson's Correlation co-efficients of various cone and seed traits in relation to germination percentage

	Length (cm)	Breadth (cm)	Perimeter (cm^2)	Roundness	Number per cone
Pearson's Correlation co-efficients	1.0000	−0.9508*	0.9806**	0.9536**	0.7309

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

Schmidt, 1987) as well as heat and drought treatments (Philipson, 1995), which have been shown to improve floral induction and subsequent seed production in other species (e. g., *Larix* species) could be tested. Production of cones and filled seeds has been achieved using girdling alone or in combination with application of gibberellins. Such works could be tried out to determine the best treatment that enhances the production of filled seeds of *C. equisetifolia.*

4 Conclusions

Casuarina equisetifolia produces large quantity of seeds, however many are non-germinable. Big cones produce heavier seeds which have good germination. Smaller cones produce large number of seeds, which have relatively low seed weight, and poor germination. Bulking of cones should be done only after ascertaining the time of collection and following grading. Cones should be maintained as single tree collections until bulking to obtain higher proportion of germinable seeds. Once seeds are collected, they should be separated to floaters and sinkers for enhanced recovery of viable seeds. Improvement in cone production could be brought about by adopting appropriate cultural treatments.

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Species and Provenance Trials of Casuarinaceae in Raoping, Guangdong

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Abstract A screening test was performed on six species of two genera *Casuarina* and *Allocasuarina* in Raoping county, Guangdong province. Significant differences in height, diameter at breast height, stem straightness and survival rate were observed. *A. littoralis*, *A. huegeliana* and *C. obesa* showed poor performance. *C. glauca* had moderate performance. *C. cunninghamiana*, *C. collina*, and local *C. equisetifolia* had better growth and adaptation. In addition, we investigated eight provenances of *C. cunninghamiana* and nine provenances of *C. glauca*. Northern provenances of *C. cunninghamiana* outperformed southern provenances in height, diameter, stem straightness and survival. Significant differences in height and diameter at breast height were observed among the provenances of *C. glauca*, but no significant differences in stem straightness and survival rate were detected.

1 Introduction

Trees of the family Casuarinaceae originate in the Oceania, the Pacific and Southeast Asia. To date, a total of 96 species have been described and the majority of them are adaptable to various ecological environments. Most of the species are characterized by drought tolerance, saline-alkali tolerance and having early rapid growth. Some species have been widely used for coastal shelterbelt construction (Wang, 1983). Casuarina has evolved many geographical provenances with rich genetic diversity during the long-term process of evolution. E1-lakany and Shepherd (1983) were among the earliest scientists conducting research on *Casuarina* provenances which led to international cooperation in provenance trials. Currently, *C. equisetifolia*, *C. cunninghamiana* and *C. glauca* have been widely introduced and cultivated in Hainan, Guangdong and Fujian provinces and other tropical and subtropical regions of China (Guo *et al.*, 2003). However, *C. collina*, *C. obesa*, *A. littoralis* and *A. huegeliana* have not been widely cultivated in China.

The germplasm resources should be comprehensively collected, conserved and studied to achieve the sustainable development and utilization of casuarina plantation. Tree species and provenance tests have many advantages, such as low investment cost, short to medium term, and easy to translate into productivity. It is an effective method of tree improvement.

2 Materials and Methods

2.1 Test site

The test was carried out in Dacheng, Raoping, Chaozhou city of Guangdong province. It is located in a subtropical region with maritime monsoon climate, sunny days and rainfall. Mean annual rainfall ranges from 1,300 mm to 1,800 mm. The rainy season is from April to September, accounting for 80% of total annual rainfall. The annual mean temperature is 21-22℃. The mean temperature of the coldest month (January) is 12.8-14.1℃, and the hottest month (July) 28.1℃. Strong oceanic winds, tropical storms, typhoons and heavy rains are the main severe weather at that time. In this study, we selected coastal sand belt for trial planting.

2.2 Plant materials

The test materials consisted of 27 provenances which belonged to six species from two genera, *Casuarina* and *Allocasuarina*. Seed, collected directly from the open-pollinated natural forests, was provided by the Australian Tree Seed Centre. The control in this test was locally grown *C. equisetifolia* which has already been planted widely in Dongshan, Fujian province. The origins of all provenances are presented in Table 1.

Table 1 Origins of provenances of *Casuarina* and *Allocasuarina* species

Species	Seedlot No.	Origin	Latitude (S)	Longitude (E)	Altitude (m)
C. collina	19110	Plum Village, New Caledonia	22°17′	166°12′	0
C. cunninghamiana	13521	North Queensland, QLD	N/A	N/A	N/A
C. cunninghamiana	13516	West Normanby River, QLD	15°46′	144°59′	110
C. cunninghamiana	15574	Clairview, QLD	22°04′	149°30′	10
C. cunninghamiana	13520	120km N Marlborough, QLD	22°21′	149°06′	140
C. cunninghamiana	13508	1km E Of Augathella, QLD	25°47′	146°36′	370
C. cunninghamiana	15601	32.6km Wsw Glen Innes, NSW	29°50′	151°36′	1,000
C. cunninghamiana	15002	Blaxlands Ck Grafton, NSW	29°50′	152°53′	140
C. cunninghamiana	15004	14km W Singleton, NSW	32°34′	151°01′	140
C. glauca	15941	Burrum Heads, QLD	25°12′	152°37′	1
C. glauca	15218	Caloundra, QLD	26°48′	153°09′	5
C. glauca	15938	Yuragir NP, NSW	29°52′	153°15′	2
C. glauca	13987	Coffs Harbour, NSW	30°18′	153°08′	1
C. glauca	13142	Dawson River E Of Taree, NSW	31°54′	152°29′	15
C. glauca	13128	E Of Singleton, NSW	32°32′	151°17′	90
C. glauca	13143	Mangrove Creek, NSW	33°23′	151°09′	20
C. glauca	15930	Jervis Bay, NSW	35°08′	150°38′	1
C. glauca	13146	Tuross Lake N Bodalla, NSW	36°02′	150°05′	20
C. obesa	13892	31.5k NWPaynes-Find, WA	29°05′	117°27′	300
C. obesa	15394	Lime Lake S Of Wagin, WA	33°25′	117°22′	300
A. huegeliana	13163	21km W Of York, WA	31°53′	116°34′	300
A. huegeliana	13171	40km W Of Narembeen, WA	32°05′	118°50′	380
A. littoralis	13876	Gordon And Chili Creeks, QLD	12°42′	143°20′	80
A. littoralis	13376	Near Julatten, QLD	16°36′	145°20′	400
A. littoralis	13551	Atherton (Plantation), QLD	17°17′	145°27′	800
A. littoralis	13133	Ne Gympie, QLD	25°57′	152°56′	50
A. littoralis	13991	NthStradbroke Island, QLD	27°30′	153°25′	10
C. equisetifolia	Control (CK)	Dongshan, Fujian, China	23°68′	117°30′	3

2.3 Experimental design and measure indexes

The experimental design was a completely randomized block with 7 replicates and 6 trees per plot. The distance between rows was 2 m, and that within rows was also 2 m. The planting holes were 40 cm × 40 cm × 40 cm. A total of 150 g of superphosphate was put into each hole prior to planting. Growth indicators, including tree height (H, m), diameter at breast height (DBH, cm), stem straightness (SS), survival percentage (%) were routinely observed after they were planted.

Stem straightness was divided into four classes: 4 = straight trees without bends; 3 = almost straight; 2 = 1-2 bends; and 1 = more than 2 bends.

2.4 Statistical analysis

Analysis of variance was carried out using SAS software (Huang and Xie, 2001). Tree height, crown width, DBH and stem straightness were analysed based on individual tree data. Survival rate of provenance was analysed based on block average values.

The statistical model was $Y = \mu + B_i + C_j + E_k + F_{ijkl}$, where μ = population mean, B_i = block effect, C_j = species effect, E_k = provenance effect and F_{ijkl} = error effect. The data of survival rate and stem straightness were transformed when conducting the variance analysis. Least significant difference (LSD) was used to compare differences between treatment means.

Species and provenance screening method was used with the weighted score, $Y_i = \sum X_i \times W_i / M_i$. Y_i is the comprehensive score. W_i is the weight of each trait. X_i is the value of each trait. M_i is the average of each trait in the population. In this test, the weigh of survival rate, height, DBH and stem straightness was 0.3, 0.3, 0.2 and 0.2 respectively.

3 Results and Discussion

3.1 Species differences in growth traits

Data analysis showed that tree height, DBH, stem straightness and survival rate differed significantly ($P < 0.01$) among tree species (Table 2). This provides basis and proof for species selection. Significant differences were also observed between the two genus groups in terms of stem straightness and survival rate, suggesting that stem straightness and survival of casuarina are not only controlled by genetic factors, but also affected by the external environment.

Table 2 Analysis of variance of *casuarina* species and provenance trials at six-year-old

Source	Tree height (m)		DBH (cm)		Stem straightness		Survival (%)	
	Degree of freedom	F value	Degree of freedom	F value	Degree of freedom	F value	Degree of freedom	F value
Block	6	2.47 *	6	0.71	6	7.80 **	6	3.92 **
Species	6	80.75 **	6	78.77 **	6	15.98 **	6	21.97 **
Provenance	21	10.97 **	21	8.59 **	21	7.29 **	21	3.04 **
Block × Species	32	1.01 ns	32	0.94 ns	32	1.85 **	–	
Block × Provenance	105	1.38 *	105	1.18 ns	105	1.26 *	–	
Error	498		498		498		155	

* and ** indicated significant difference at 0.05 and 0.01 levels, ns indicates no significant difference.

Tree height and DBH are important indicators for tree species evaluation. The control (local *C. equisetifolia* from Dongshan) was the best in the trial with 6-year average height of 9.29 m and DBH of 8.21 cm. Among the newly introduced species *C. cunninghamiana* was the best (mean height 5.69 m and mean DBH 6.88 cm) followed in order of magnitude by *C. collina*, *C. glauca*, *C. obesa*, *A. huegeliana* and *A. littoralis* (Table 3). It is clear that all *Casuarina* species outperformed *Allocasuarina* species.

Table 3 Differences of casuarina species/provenances at six-year-old

Species	Seedlot No.	H(m)	DHB(cm)	SS	S (%)	CS
C. collina	19110	5.37	5.88	2.65	80.9	1.22
C. cunninghamiana	13521	6.55	8.43	3.33	95.2	1.53
	13516	6.13	7.15	3.13	92.9	1.43
	15574	7.44	8.99	3.29	80.9	1.54
	13520	5.91	6.96	2.64	85.7	1.32
	13508	4.15	5.24	1.67	64.3	0.95
	15601	3.46	4.17	1.63	64.3	0.86
	15002	5.03	6.25	2.25	76.2	1.16
	15004	5.89	6.49	2.08	61.9	1.13
	Mean	5.57	6.71	2.50	77.7	1.24
C. glauca	15941	4.66	5.13	1.95	70.0	1.03
	15218	4.20	4.59	2.00	76.2	1.02
	15938	5.42	6.37	1.97	69.0	1.13
	13987	3.85	4.03	1.64	52.4	0.82
	13142	4.03	4.60	2.19	64.3	0.96
	13128	4.08	4.16	2.23	53.1	0.89
	13143	3.83	3.97	1.88	59.5	0.87
	15930	3.50	3.65	1.82	54.8	0.81
	13146	3.36	3.03	1.72	76.2	0.88
	Mean	4.10	4.39	1.93	63.9	0.93
C. obesa	13892	6.50	6.80	4.00	4.2	1.06
	15394	3.55	3.22	2.22	64.3	0.88
	Mean	5.03	5.01	3.11	34.3	0.97
A. huegeliana	13163	3.42	3.84	1.72	60.0	0.83
	13171	3.09	2.93	1.72	42.9	0.68
	Mean	3.26	3.39	1.72	51.5	0.76
A. littoralis	13876	2.77	2.27	2.35	47.6	0.72
	13376	2.23	1.04	2.00	28.6	0.51
	13551	2.44	1.52	2.33	42.9	0.64
	13133	2.69	1.78	2.36	33.3	0.62
	13991	2.53	1.00	2.00	16.7	0.46
	Mean	2.53	1.52	2.21	33.8	0.59
C. equisetifolia	Control (CK)	9.29	8.21	3.29	16.7	1.30
Grand Mean		4.48	4.70	2.29	58.39	0.97
LSD ($P=0.05$)		1.15	1.69	0.71	23.66	

H = tree height; SS = stem straightness; S = survival; CS = comprehensive score.

In terms of stem straightness, the control local *C. equisetifolia* was also the best, with an average score of 3.29, which was followed by *C. collina* (2.65) and *C. cunninghamiana* (2.59), *C. obesa* (2.29), *A. littoralis* (2.25), *C. glauca* (1.93) and *A. huegeliana* (1.72).

Survival rate is an indicator of ecological adaptability which can measure the adaptability of species and provenances. The results showed that *C. collina*, *C. cunninghamiana* and *C. glauca* recorded high survival rates of 63.7-81%. *A. huegeliana*, *A. littoralis* and *C. obesa* had survival rate less than 50%. It is interesting to see local *C. equisetifolia* survived least at 26.7% (Table 3). The variations described above have a huge potential and yield-increasing effect. Therefore, the introduction of casuarina species for selection can provide useful results.

Fast growth rate and good adaptability of casuarinas make them popular tree species for coastal shelterbelts. We conducted the comprehensive score by weighted scoring method for survival rate, tree height, DBH and stem straightness. Results showed that the control *C. equisetifolia*, *C. cunninghamiana* and *C. collina* had the highest scores, viz. 1.35, 1.26 and 1.22 respectively (Table 3). The top ranking of *C. equisetifolia* confirms the suitability of this species for large-scale planting in coastal areas of South China. This experiment also reveals that *C. cunninghamiana* and *C. collina* are worthy of further investigation for Guangdong conditions.

Table 3 showed that *C. glauca*, *C. obesa*, *A. littoralis* and *A. huegeliana* displayed slower growth rate, poor stem form and lower survival rate (less than 50%). The results thus suggest that these species are less suitable to the test site conditions in Guangdong.

3.2 Provenance differences in growth traits

Variance analysis of 28 provenances demonstrated significant difference between different provenances in tree height, DBH, stem straightness, and survival rate ($P < 0.01$) (Table 2). Growth performance and the comprehensive score in Table 3 indicated more than four-fold differences between the best and the worst provenances in tree height, DBH and survival rate. The average tree height, DHB, stem straightness and survival rate of 28 provenances were 4.48 m, 4.70 cm, 2.29 and 58.39% respectively. The LSD ($P = 0.05$) for tree height, DHB, stem straightness and survival rate were 1.15 m, 1.69 cm, 0.71 and 23.66% respectively (Table 3).

Based on the comprehensive score as the initial evaluation of the provenances, the top three were 13521, 15574 and 13516, all belong to *C. cunninghamiana* (Table 3). They all grew rapidly; had high survival rate and adaptability; their comprehensive score was greater than 1.4. Their average tree height at 6 years was 6.55 m, 7.44 m and 6.13 m, respectively. Average DBH was 8.43, 8.99 and 7.15 cm, respectively. Average stem straightness was 3.33, 3.29 and 3.13, respectively. Average survival rate was 95.2%, 80.9% and 92.9%, respectively (Table 3).

Other provenances which displayed good performance were 13520, local control, 19110, 15002, 15004, 15938, 13892, 15941 and 15218. Their comprehensive scores were all above 1.0, which was greater than the overall average.

The provenances which showed poor performances were 13876, 13171, 13551, 13133, 13376 and 13991. Their average tree height was only 2.62 m, accounting for 56.9% of the overall average; their average DBH was only 1.76 cm, accounting for 34.6% of the overall average, and their average survival rate was 35.3%, accounting for 59.7% of the overall average (Table 3).

Further analysis was performed on *C. cunninghamiana* and *Ccasuarina glauca* seedlots to determine variation between provenances within species. There were significant differences in all growth parameters among the eight provenances of *C. cunninghamiana* while significant differences in height and DBH only were found among *C. glauca* provenances (Table 3).

There was a clear evidence of geographic variation in *C. cunninghamiana*. Provenances from more northern latitudes performed better than those from southern latitudes. In general, Queensland provenances grew faster, had better stem form and higher survival than provenances from NSW (Table 3). NSW provenances are distributed in subtropical to warm temperate regions and are thus less adaptable to the test site than tropical Queensland provenances.

Casuarina glauca also displayed variation between provenances in height and DBH, and, to a lesser extent, in stem straightness and survival. The best provenance was 15938 with average tree height of 5.42 m and average DBH of 6.37 cm. The worst provenance was 13146 with an average tree height of 3.36 m and average DBH of 3.03 cm (Table 3).

The provenances 15574, 13521, 13516 and 13520 of *C. cunninghamiana* had a greater DBH and tree height, better stem straightness and higher survival rate than the provenance mean (Table 3). Provenances 15574 and 13521 were by far the best two. Under 25% of selection pressure, these two provenances had better performance. Provenances 15938, 15941 and 15218 of *C. glauca* exceeded the trial mean in the growth parameters (Table 3), and provenance 15938 was significantly superior to others of the same species.

4 Conclusions

In this study *Casuarina* species were found to be more adaptable than *Allocasuarina* species in Raoping county of Guangdong province. This conclusion is however subject to further verification because these species have wide natural distribution area and the participations are just one very small part of provenances. It is beneficial to utilize and develop *C. cunninghamiana* and *C. collina* in Guangdong coastal areas because they have good growth and better adaptation. Phenotypically superior individual trees of the best performing provenances should be selected as candidate plus trees for further genetic improvement programme. The development will also increase the genetic diversity of coastal protection forests.

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Effects of Medium Components and Storage Conditions on Pollen Germination of *Casuarina equisetifolia* under *in vitro* Conditions

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Abstract The optimal concentrations of sucrose and boric acid on pollen germination of *Casuarina equisetifolia* were studied under *in vitro* culture conditions. In addition, the effects of storage temperature and storage time on pollen germination rates were explored. The results showed that 15% sucrose was the optimal concentration for *in vitro* pollen germination based on the highest germination rate. Adding boric acid to germinating media with 15% sucrose significantly improved the germination rate, and 250 mg kg^{-1} was found to be optimal boric acid concentration. Low temperature storage was more effective to maintain viability of pollen. Pollens lost viability rapidly when stored at room temperature. The morphological differences between fresh pollen and stored pollen were presented using scanning electron microscope (SEM), and cause of these changes was explained.

1 Introduction

The Casuarinaceae is a family of dicotyledons widely distributed in SE Asia, Australia and Pacific Islands. Within the Casuarinaceae there are four genera (*Allocasuarina*, *Casuarina*, *Ceuthostoma* and *Gymnostoma*) and about 96 species, including monoecious or dioecious shrubs and trees (Johnson and Wilson, 1989), which extends their natural distribution from sea level to 3,000 m altitude (Midgley *et al*., 1983). Among the four genera, the genus of *Casuarina* L. Johnson is commercially cultivated in many tropicaland subtropical regions around the world. *Casuarina* species (mainly *C. equisetifolia*, *C. cunninghamiana* and *C. glauca*) are nitrogen-fixing trees of considerable social, economic and environmental importance in southern China (Pinyopusarerk *et al*., 2004). Their roles in sand stabilization, wind protection, rehabilitation of depauperate soils, provision of timber and fuelwood and as a component of agro-forestry systems have been recognized. In recent years, however, many casuarina plantations in southern part of China have been affected by diseases, insect pests, typhoons and drought. The sustainable ecological and economic benefits of casuarina plantations have been seriously jeopardized. Therefore, there are urgent needs to carry out genetic improvement programme of casuarina, and to select new favorable varieties or clones for practical uses. In order to resolve some difficulties in cross breeding such as asynchronous flowering period of parents and long distant-transportation of pollen, studies on pollen storage and viability test are necessary.

The most important factors affecting pollen viability are temperature, humidity and oxygen (Wang and Lu, 2002). The most important factors affecting pollen viability are temperature, humidity and oxygen (Wang and Lu, 2002). Most of plant pollens can maintain viability for long time under low temperature (-20℃) or extreme low temperature (-196 to -80℃) in liquid nitrogen (Heslop-Harrison and Heslop-Harrison, 1970). Conditions of low humidity, temperature and oxygen concentration decrease respiration and enzymatic activities of pollen and consequently help maintain viability of pollen.

According to published reports, *in vitro* pollen germination method is a rapid and reliable quantitative method to determine pollen viability, andhas been used extensively in various plants (Gudin *et al*., 1991). The *in vitro* conditions required for pollen germination vary between species, but in general the optimum temperatures range from 20℃ to 30℃, and the medium components include boron, calcium and carbon source such as sucrose, lactose or raffinose (Stanley and Linskens, 1974). *In vitro* pollen germination of *Betula alnoides* which related taxonomically to casuarina reveals the optimal germinating temperature, sucrose concentration and boric acid concentration are 3℃, 15%, and 200 mg kg^{-1}, respectively (Cheng and Zeng, 2002). However, there is no report on viability test and storage method of *C. equisetifolia* pollen.

In this paper, *in vitro* germination method was used to study the effects of sucrose and boric acid concentrations on germination of *C. equisetifolia* pollen, and the effects of storage temperatures and time on pollen germinability. Optimal sucrose and boric acid concentrations, and appropriate storage temperature and time for pollen storage and transportation were then determined. Morphological differences between fresh pollen and stored pollen were presented using scanning electron microscope (SEM), and the cause of these changes was explained.

2 Materials and Methods

2.1 Pollen collection and storage

A male tree growing at Chihu State-owned Forest Farm of Huian county, Fujian province, China was selected. Branches with male inflorescences which were about to shed pollen were collected and cultured in tap water in April 2009. The pollen was shaken off on to a blank paper. Pollen was collected in small capped vials, and stored at room temperature (25℃), 4℃ and -20℃ respectively with a view to determine the pollen germinability under various storage temperatures.

2.2 Experimental methods

2.2.1 Experimental design

Three experiments were conducted to determine the optimal sucrose concentration and the optimal boric acid concentration on pollen germination under *in vitro* condition. Germinability of pollen stored under different temperatures was determined at 3, 7, 15 and 30 days after storage.

Experiment 1: Six sucrose concentrations were investigated, namely 0%, 5%, 10%, 15%, 20% and 30%, to determine the optimal concentration in germinating media, no boric acid was added.

Experiment 2: Using the optimal sucrose concentration achieved in Experiment 1, nine boric acid concentrations were investigated, namely 0, 50, 100, 150, 200, 250, 300, 400, 500 mg kg^{-1}, to determine the optimal boric acid concentration in germinating media.

Experiment 3: Pollen viability was measured using *in vitro* germination method after pollen had been stored at 25℃, 4℃ and -20℃ for 3 days, 7 days, 15 days and 30 days. The optimal sucrose and boric concentrations attained in Experiments 1 and 2 were used as germinating media.

In these three experiments, various components were dissolved in de-ionized water, pH value was adjusted to 6.8 using pH meter, and the media were solidified with 1% agar. Three replicates were arranged within each experiment.

2.2.2 *In vitro* pollen germination on germinating media

Two drops of germinating media were placed on a glass slide and a sample of pollen was inoculated. Slides with medium and pollen were then placed in the Petri dishes lined moist filter paper thus serving as germination chambers. An evenly spread of pollen on the surface of the germinating media was achieved by gently tapping the nylon hairbrush loaded with pollen grains.

A pollen grain was considered to have germinated when the length of the germinated pollen tube was equal to or longer than the diameter of the pollen (Luza *et al*., 1987). Counts were made at random in three fields using light microscopy after 24 hours germinating period, three slides per treatments were examined. The final percentage germination was defined as:

Germination rate (%) = (number of germinated pollen per field/total number of pollen per field) × 100

2.2.3 Observation of scanning electron microscope (SEM)

To detect differences between fresh pollen and pollen stored for 30 days at 4℃, a S-530 type SEM was used to observe their morphological differences, and micrographs were taken. Pollen grains were sputter-coated using a IB-5 sputter-coater prior to SEM observation.

3 Results

3.1 Effects of sucrose concentration on pollen germination

There were significant effects of different sucrose concentrations on pollen germination rate (Fig. 1), based on the analysis of variance (ANOVA) and Duncan's multiple range test at 5% level using SAS software package. The pollen reached highest germination rate (7.0%) when sucrose concentration of germinating media was 15%, and the germination rate of pollen was only 1.2% when no sucrose was added to germinating media. The 15% sucrose is considered the optimal concentration for pollen germination of *C. equisetifolia*.

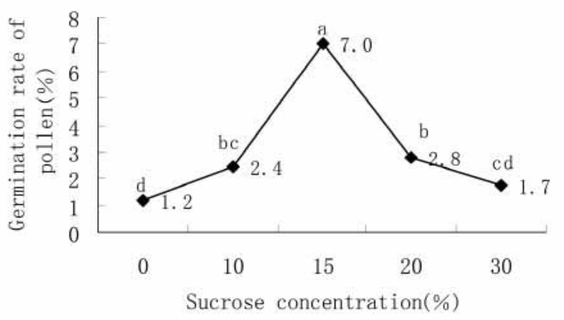


Fig. 1 Effect of sucrose on pollen germination rate of *C. equisetifolia*

3.2 Effects of boric acid on pollen germination

Marked effects of boric acid concentration on pollen germination were found (Fig. 2). The highest germination rate reached 13.2% when boric acid concentration of the germinating media was 250 mg kg^{-1}, while it was only 7.0% when boric acid was not added. Within the range 0-250 mg kg^{-1} of boric acid concentrations in germinating media, pollen germination rate increased with increasing concentration of boric acid, but as soon as the boric acid concentrations exceeded 250 mg kg^{-1}, pollen germination rate decreased significantly, implying that excessive boric acid would restrain pollen germination. The 250 mg kg^{-1} was determined as the optimal boric acid concentration for germinating media of *C. equisetifolia* pollen.

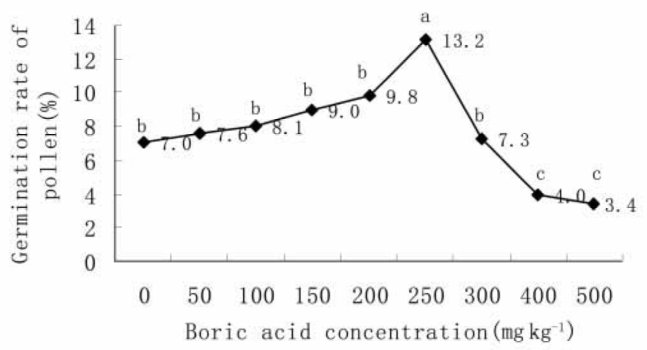


Fig. 2　Effect of boric acid on pollen germination rate of *C. equisetifolia*

3.3　Effects of storage temperatures and time on pollen viability

Both storage temperature and storage time had significant effects on pollen viability. Viability was highest when pollen was stored at -20℃ temperature and lowest when stored at room temperature (Fig. 3). The results were consistent throughout the storage duration from 3 days to 30 days.

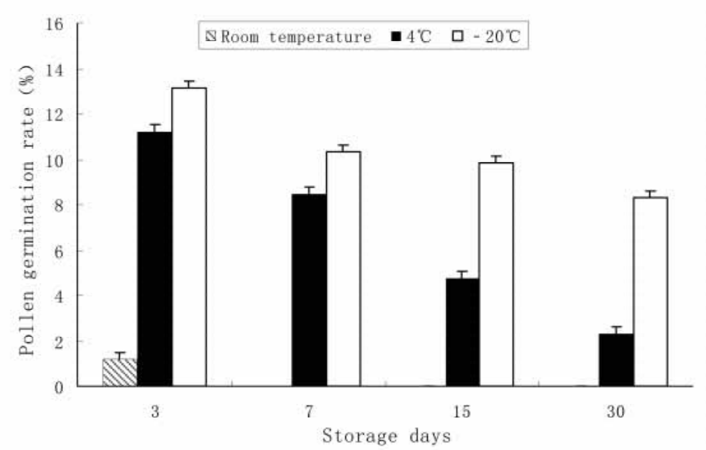


Fig. 3　Effects of storage temperature and time on pollen germination rate of *C. equisetifolia*

Germination rates of pollen declined with the extension of storage time, irrespective of storage temperatures. The decline was faster when pollen was stored at 25℃ than at 4℃ and - 20℃. As shown in Fig. 3, the germination rate of pollen stored at 25℃ for 3 days was 0.8%, and no germination was observed beyond 3 days. Under 4℃ storage temperature, the germination rates were 11.2, 8.5, 4.8 and 2.3% when pollen was stored for 3, 7, 15, and 30 days respectively. Under

- 20℃ storage temperature, the germination rates 13.2, 10.3, 9.9 and 8.3% for pollen stored for 3, 7, 15, and 30 days respectively. These results indicated that casuarina pollen lost viability rapidly when stored at 25℃ temperature. Pollen stored - 20℃ maintained the viability effectively for 30 days.

Fig. 4 Pollen germination on germinating medium

3.4 Morphological and content differences between fresh and stored pollen

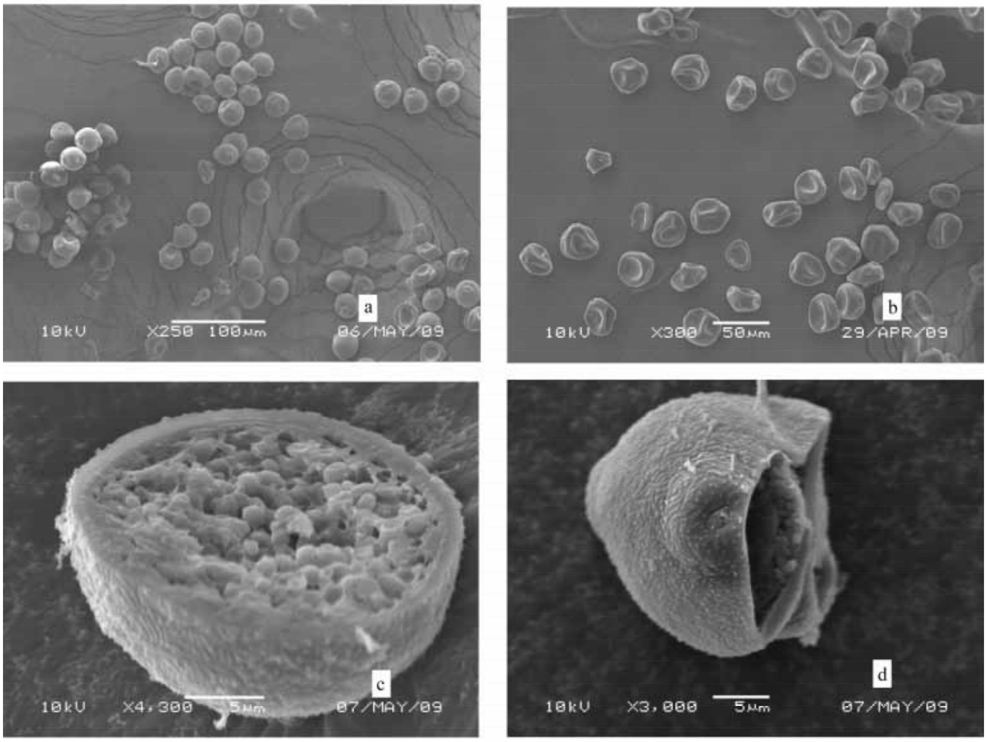
Using scanning electron microscope, it was possible to see the change in morphological characteristics between fresh pollens and those stored for 30 days at 4℃. A high proportion of fresh pollen grains remained turgid (Fig. 5a), whereas most of the stored pollen grains were found to be constrictive and deformed (Fig. 5b). Sections of pollen grains showed that there were abundant contents in fresh pollen (Fig. 5c). In contrast, little content was found in the stored pollen grain (Fig. 5d).

4 Discussion

Sugar and boron are the basic components of germinating media of plant pollen (Yistra *et al*., 1998; Wang *et al.*, 2003). Sugar not only supplies energy for pollen germination, but also adjusts osmotic potential of pollen grains, making them possible to germinate on media. However, the optimal concentrations of sucrose and boric acid in pollen germination of different plants vary considerably. For example, the optimal sucrose concentration of *Trifolium pralense* pollen germination reached 40% (Kendall, 1967), and optimal boric acid concentration was 50 mg kg^{-1}. In contrast, the optimal sucrose and boric acid concentrations for *Borago officinalis* pollen germination were 20% and 100 mg kg^{-1} respectively (Montaner *et al.*, 2003). In this study the optimal sucrose and boric acid concentrations of *C. equisetifolia* pollen germination were found to be 15% and 200 mg kg^{-1} respectively. Taxonomically *Casuarina* is closely related to *B. alnoides*, but further studies are needed to determine the similarity in the conditions of pollen germination of both species.

The effects of storage temperature on pollen viability were clearly demonstrated in this study. Casuarina pollen lost viability very quickly under room temperature of 25℃ after 3 days storage. Storage temperature of 4℃ could maintain germination reasonably well for up to 7 days. Where longer storage is necessary it is recommended to store casuarina pollen at - 20℃.

The viability of *C. equisetifolia* pollen is low compared to other plant species, such as 57.4% in *Trifolium pratense* (Nurhan *et al.*, 2003) and 73% in *Arachis hypogaea* (Kakani *et al.*, 2002).



a: Freshly collected pollen morphology of *C. equisetifolia* under SEM, Bar = 100 μm; b: Stored pollen morphology of *C. equisetifolia* under SEM, Bar = 50 μm; c: The cross-section of freshly collected pollen grain which associated with the presence of ample contents, Bar = 5 μm; d: The cross-section of stored pollen grain which associated with the absence of contents, Bar = 5 μm.

Fig. 5 Pollen morphology of *C. equisetifolia*

Such low viability is however compensated by the enormous amount of pollen grains produced by male inflorescences of *C. equisetifolia*. There are approximately 850-1,500 pollen grains per anther, and more than 100 individual male flowers per inflorescence, giving a total of about 85,000-150,000 pollen grains per inflorescence (Primack, 1985), thus ensuring successful pollination.

It was observed that there were always a percentage of collapsed pollen grains in either fresh or stored pollens. The proportion of collapsed pollen grains increased markedly after storing for 30 days at 4℃, most of pollen grains had shrunk and deformed. The cross-sections of pollen grains of *Carya illinoensis* stored in liquid nitrogen for 10 years showed that viable pollen grains had complete cell membrane structures and evenly distributed contents, but collapsed cell membrane structures and little contents were observed in non-viable pollen grains (Sparks and Yates, 2002). The result which was consistent with that achieved in casuarina pollen can explain why casuarina pollen grains shrank and deformed after losing viabilities.

Acknowledgments

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Preliminary Report on Cold Tolerance of Species/Provenances of Casuarina Seedlings

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Abstract Under different conditions of low temperature, seedlings of three *Casuarina* species: *C. cunninghamiana*, *C. equisetifolia* and *C. glauca* were treated, and their electrical conductivity and percentage of electrolyte leakage determined. The results showed that electrical conductivity is not suitable for evaluating capability of cold resistance, but percentage of electrolyte leakage is suitable. The temperature at corner point of logistic curve expressing relationship between percentage of electrolyte leakage and temperature can be regarded as semi-lethal temperature, which assessed quantitatively capability of cold resistance, offering scientific basis for selecting cold-resistant species of casuarinas.

1 Introduction

Casuarinas are important fast-growing tree species for afforestation and protection forest in the northern tropical region and between mid-subtropical and south subtropical zone of China (Zhong *et al*., 2005). Low-temperature damage is the limiting factor of introduction and cultivation expansion of casuarinas. At present, studies on low-temperature damage to casuarinas have not been reported. Research on freezing injury of casuarinas will help more successful cultivation of these species.

Membrane is most sensitive to low-temperature. Many studies show that cell injury caused by low temperature is mainly damage of membrane system. Membrane damage caused by low-temperature stress increases membrane permeability and electrolyte leak of protoplasts. In this paper, low-temperature treatments were carried out, and electrical conductivity of casuarinas were determined. The author proposed that the temperature at which electrolyte leakage rate is 50% could be regarded as semi-lethal temperature, offering theoretical basis for selecting cold-tolerant species of casuarinas.

2 Materials and Methods

2.1 Materials

The tested species and provenances of *Casuarina* are shown in Table 1. There were one seedlot each of *C. glauca* and *C. equisetifolia*, and three seedlots of *C. cunninghamiana* (seedlot 14997, 15004 and 15600). The seedlings of *C. glauca*, *C. equisetifolia* and *C. cunninghamiana* (14997) were 1 year old, and those of *C. cunninghamiana* 15004 and 15600 were 2 years old. The seeds were provided by CSIRO Australian Tree Seed Centre (ATSC).

Table 1 The tested species and provenances of *Casuarina*

No.	Seedlot No.	Species	Locality	Latitude (S)	Longitude (E)	Altitude (m)
1	15932	*C. glauca*	Royal NP, NSW, Australia	34°05′	151°05′	1
2	18142	*C. equisetifolia*	Kilifi, Kenya	03°38′	39°51′	20
3	14997	*C. cunninghamiana*	Lachlan River Cowra, NSW, Australia	33°53′	148°44′	330
4	15004	*C. cunninghamiana*	14 km W Singleton, NSW, Australia	32°34′	151°01′	140
5	15600	*C. cunninghamiana*	17.5 km ESE Armidale, NSW, Australia	30°36′	151°48′	900

2.2 Methods

2.2.1 Low-temperature treatment

Normal green twigs were selected. They were rinsed in distilled water for 15 min, and then dried with filter paper. These twigs were cut into about 6 mm segments and mixed together. After that, each sample weighed 0.2 g was put in a test tube, and placed in a refrigerated cycling water container (Heto, Denmark) for 1 h at temperature of - 1℃, - 4℃, - 7℃, - 10° C and - 13℃, respectively. There were 3 replicates for each treatment.

2.2.2 Electrical conductivity

After the cold treatments, the test tubes were removed and 5 ml distilled water added. They were infiltrated in vacuum for 15 min and oscillated, then stored at 25℃ for 3h and oscillated again. Electrical conductivity was measured by DDS-11A digital electrical conductivity metre, and then the tubes were put into boiling water bath for 15 min. After the tubes had cooled down electrical conductivity was measured again. Percentage of electrolyte leakage was calculated using the following formula.

Electrolyte leakage (%) = (conductivity after cold treatment/conductivity after boiling treatment) × 100

2.2.3 Data processing and analysis

Based on the electrical conductivity after cold treatment and electrical conductivity after boiling treatment, the percentage of electrolyte leakage was calculated. Regression analysis was applied. The temperature at corner point could express semi-lethal temperature at which electrolyte leak rate is 50% by electrolyte leak rate with the logistic equation. SPSS statistical analysis software was used to fit curve model.

3 Results

3.1 Change in electrical conductivity of twig

Changes in electrical conductivity after the different low-temperature treatments are shown in Fig. 1.

Fig. 1 shows that electrical conductivity of twig of *Casuarina* species increased with the decrease in temperature. The stress intensity increased but the increasing level was different between different species. From - 10℃ to - 1℃, electrical conductivity of *C. glauca* and *C. equisetifolia* increased, but conductivity decreased from - 13℃ to - 10℃. For these two species, - 10℃ was the temperature at corner point.

For three provenances of *C. cunninghamiana*, the conductivity increased with the decrease in temperature from - 7℃ to - 1℃. This was followed by a slight decline from - 10℃ to - 7℃, and increase again from - 13℃ to - 10℃. Therefore, cold tolerance of seedlings can not be directly

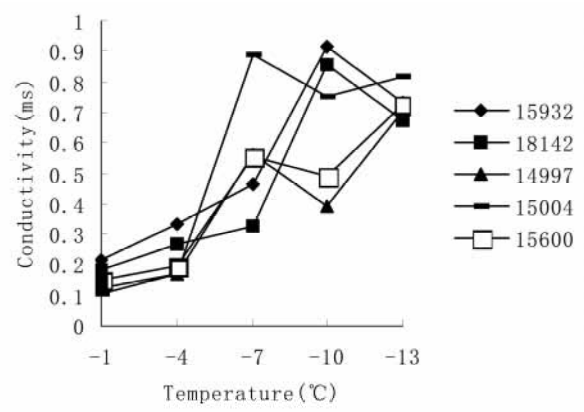

Fig. 1 Change in electrical conductivity of twig after subjecting to different low-temperature treatments

determined by the size or increase range of conductivity of twig.

3.2 Changes in electrolyte leakage percentage under the different low-temperature stresses

Percentage of electrolyte leakage is an index expressing membrane permeability. Percentage of electrolyte leakage of the samples under different low-temperature treatments are shown in Fig. 2.

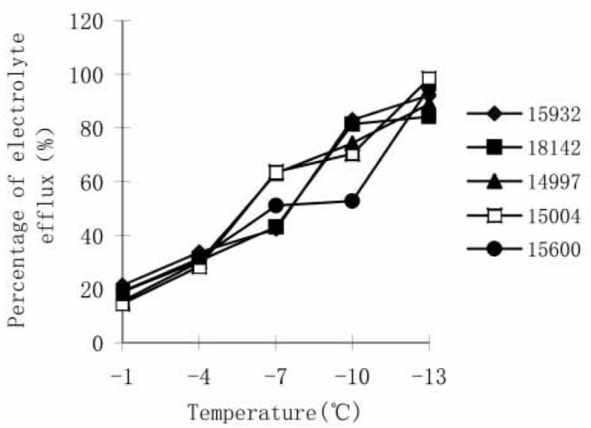

Fig. 2 Percentage of electrolyte leakage of different *Casuarina* species under different low-temperature treatments

As can be seen in Fig. 2, percentage of electrolyte leakage increased with the decrease in temperature for all three *Casuarina* species. Increase in percentage of electrolyte leakage resulted from the damage of membrane permeability caused by low temperature. All three species showed similar percentage of electrolyte leakage at - 13℃ indicating that these species could not endure this temperature. At - 7℃ and - 10℃, there were differences in the percentage of electrolyte leakage between species though most species could endure this temperature range. Thus, regression analysis between the relative conductivity and temperature in the condition of low- temperature stress was necessary to identify the temperature at corner point.

3.3 Regression analysis between relative conductivity and temperature under low- temperature stress

Curve equation of relative percentage of electrolyte leakage and temperature under low- temperature stress was fitted by regression analysis as shown in Table 2.

Table 2 Curve equation of percentage of electrolyte leakage and temperature under low-temperature stress and the temperature at corner point of three *Casuarina* species

No.	Seedlot No.	Curve equation	Correlation coefficient	Temperature at corner point (℃)
1	15932	$Y = 1/(1/100 + 0.069867 \times 1.388761^x)$	0.97	-5.92
2	18142	$Y = 1/(1/100 + 0.065551 \times 1.330372^x)$	0.97	-6.59
3	14997	$Y = 1/(1/100 + 0.072744 \times 1.372313^x)$	0.99	-6.27
4	15004	$Y = 1/(1/100 + 0.134697 \times 1.572683^x)$	0.95	-5.74
5	15600	$Y = 1/(1/100 + 0.081384 \times 1.384188^x)$	0.91	-6.45

The curves between relative percentage of electrolyte leakage and temperature could be fitted with the Logistic curve equation ($Y = 1/(1/U + b_0 \times b_1{}^X)$, where Y is relative percentage of electrolyte leakage, U is the maximum, set at 100, b_0 and b_1 are calculated values from equations, and x is temperature). F values and relationship tests showed that the fitted value of the samples had reached a significant level. The calculated temperature at corner point was the semi-lethal temperature, which could be used for quantitative analysis on cold tolerance.

In general, the temperature at corner point could be ranked in the order *C. equisetifolia* < *C. cunninghamiana* < *C. glauca*, and for the three provenances of *C. cunninghamiana*, seedlot 15004 < seedlot 14997 < seedlot 15600. These results suggested differences in cold tolerance among *Casuarina* species and provenances.

3.4 Changes of conductivity under the different low-temperature treatments

The samples of *C. cunninhgamiana* seedlot 14997 were treated in the same low temperature (- 7℃) for 1h, 2h and 4h, respectively, in order to study the effects of low-temperature duration on cold tolerance. The results (Table 3) showed that with low-temperature duration extending, the changes in percentage of electrolyte leakage and electrolyte were not consistent, indicating that percentage of electrolyte leakage is not correlated to short duration.

Table 3 Electrolyte and percentage of electrolyte leakage of *C. cunninghamiana* seedlot 14997

Duration	Electrolyte (ms)	Percentage of electrolyte leakage(%)
1 h	0.562	63.19
2 h	0.235	27.25
4 h	0.263	34.27

4 Discussion and Conclusion

Generally, it is difficult to express degree of cold injury by electrical conductivity under the different low-temperature treatments, and the percentage of electrolyte leakage is more accurate (Liao, 1997; Li and Wang, 2002). This study suggests that percentage of electrolyte leakage can be used to express the degree of freezing injury of casuarinas. Liao (1997) obtained similar results in a study with eight *Eucalyptus* species.

Temperature and moisture are the main ecological factors affecting cold tolerance of plant. Through coupling the percentage of electrolyte efflux with the Logistic equation, the temperature at corner point could be used to express semi-lethal temperature, Percentage of electrolyte leakage could better explain semi-lethal temperature of *Casuarina* species.

Percentage of electrolyte leakage is an index expressing membrane permeability. Through calculating percentage of electrolyte leakage under the low-temperature treatments, the authors found that percentage of electrolyte leakage is suitable to express cold-tolerant capability of the three *Casuarina* species used in this study.

From the above results, percentage of electrolyte leakage could better express cold-tolerant capability. The temperature at corner point of *C. equisetifolia* seedlot 18142 is - 6.59℃, which is the most cold-tolerant, and the temperature near corner point of *C. cunninghamiana* seedlot 15004 is -5.74℃. This range is consistent with the observation of freezing injury of many tree species in recent years. The present study has some theoretical and practical value.

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Nitrogen Fixation/Mycorrhiza/Symbiosis/Biotechnology/Genetics

Molecular Basis of *Casuarina/Frankia* Symbiosis

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Abstract Our group has concentrated on the molecular study of the plant genes involved in the interaction between *Frankia* and *Casuarina glauca*. Tools for the functional analysis of candidate genes have been developed; they include genetic transformation procedures based on *Agrobacterium tumefaciens* and *A. rhizogenes*, gene silencing by RNA interference, and microarrays. ESTs and expression analyses from roots and nodules have been compared, providing insights in the genes expressed in the actinorhizal nodules. We recently showed that the casuarina receptor-like kinase gene *SymRK* is a vital component of the genetic basis for both plant-fungal and plant-bacterial endosymbioses and is conserved between legumes and actinorhiza-forming fagales. All together, with the new tools now available, our understanding of the molecular mechanisms of *C. glauca* has considerably increased in the past few years.

1 Introduction

Actinorhizal root nodules result from the interaction between a nitrogen-fixing actinomycete called *Frankia* and roots of dicotyledonous plants belonging to eight plant families and 25 genera (Benson and Silvester, 1993). Actinorhizal plants share common features; with the exception of *Datisca*, which has herbaceous shoots, they are perennial dicots and include woody shrubs and trees such as *Alnus* (alder), *Elaeagnus* (autumn olive), *Hippophae* (sea buckthorn) and *Casuarina* (beef wood). Most actinorhizal plants are capable of high rates of nitrogen fixation comparable to those found in legumes. As a consequence, these plants are able to grow in poor and disturbed soils and are important elements in plant communities worldwide. In addition, some actinorhizal species can grow well under a range of environmental stresses such as high salinity, heavy metal and extreme pH. This facility for adaptation has drawn great interest to actinorhizal plants, particularly to several species of Casuarinaceae such as *Casuarina glauca*, which can be used for fuelwood, agroforestry, and land reclamation in the tropics and subtropics.

The basic knowledge of the symbiotic association between *Frankia* and actinorhizal plants is still poorly understood, although it offers striking differences with the *Rhizobium*-legume symbiosis (Obertello *et al.*, 2003; Vessey *et al.*, 2005). *Frankia* is a filamentous, branching, Gram-positive actinomycete, whereas rhizobia are Gram-negative unicellular bacteria. *Frankia* can interact with a diverse group of dicotyledonous plants; whereas rhizobia only enter symbiosis with plants from the legume family and with one non-legume *Parasponia*. In actinorhizal plants, the formation of the nodule primordia takes place in the root pericycle and the nodule consists of multiple lobes, each representing a modified lateral root without a root cap and with infected cells present in the cortex. In indeterminate nodules formed on roots of temperate legumes, the nodule primordium starts in the root inner cortex and determinate nodule primordia are formed in the root outer cortex of tropical and subtropical legumes. Legume root nodules represent stem-like structures with peripheral vascular bundles and infected cells in the central tissue, whereas actinorhizal nodules conserve the structure of a lateral root with a central vascular bundle and peripheral infected cortical tissue.

The molecular understanding of regulatory events in actinorhizal nodulation is mainly limited by the microsymbiont *Frankia*; this actinomycete is characterized by slow growth rate, high G + C DNA content and the lack of a genetic transformation system (Lavire and Cournoyer, 2003). So far, investigations to detect any DNA sequences homologous to the *nod* genes in the *Frankia* genome have failed. However, in the past decade, some progress has been made in the knowledge of the plant genes that are expressed at different stages of actinorhizal nodule differentiation. Differential screening of nodule cDNA libraries with root and nodule cDNA has resulted in the isolation of a number of nodule-specific or nodule-enhanced plant genes in several actinorhizal plants including *Alnus*, *Datisca*, *Eleagnus* and *Casuarina* (for reviews see Obertello *et al*., 2003; Vessey *et al*., 2005).

In this short review we summarize recent advances on molecular biology of *Casuarina/Frankia* symbioses.

2 Tools for Functional Analysis of Symbiotic Genes: Gene Transfer Based on *Agrobacterium rhizogenes* and *A. tumefaciens*

The natural susceptibility of members of the Casuarinaceae family to *A. tumefaciens* was used to develop a gene transfer procedure for *C. glauca* (Smouni *et al*., 2002). Epicotyls fragments of 2 cm in length were excised from 45-day-old plantlets and cocultivated with the disarmed strain C58C1 (pGV2260; pBIN 19). After selection on kanamycin, one to three transgenic calli were observed on 26% of the epicotyls. Integration of the transgenes was further confirmed by PCR and Southern blot analyses. Transgenic plants were regenerated in approximately nine to ten months for *C. glauca*. The nodulation efficiency was found to be similar in transgenic Casuarinaceae and in non-transformed control plants inoculated by *Frankia*, and the transgenic nodules fixed nitrogen at the same rate as those of the non transformed control nodules. This transgenic approach has contributed to the characterization of two symbiotic genes from *C. glauca*, *Cgenod* 40, a homolog of early nodulin gene *enod*40 from legumes (Santi *et al*., 2003), and *cg*12, an actinorhizal symbiotic gene encoding a subtilisin-like serine protease (subtilases) (Svistoonoff *et al*., 2003).

A rapid procedure for producing composite plants of *C. glauca* is also available. It relies on the induction of a hairy root system by *A. rhizogenes* while the aerial part of the plant remains untransformed. Young seedlings of *C. glauca* were wounded on the hypocotyl and inoculated with *A. rhizogenes* A4RS containing the chosen binary vector. After two weeks, highly branched roots exhibiting rapid growth were observed at the inoculation site. The normal root system was removed at the stem base, and the composite plant decontaminated with cefotaxime. Cotransformation with the wild-type T-DNA and the T-DNA from the binary vector was observed in about 50% of *C. glauca* hairy roots. After inoculation with *Frankia*, nodulation was observed on 40% of the transformed roots. Using this "composite plant" approach, the pattern of expression conferred by the promoter region of a symbiotic gene can be studied in both roots and nodules of *C. glauca* within about 4 months (Diouf *et al.*, 1998).

These composite plants are currently used to dissect symbiotic gene function by RNAi gene silencing. To demonstrate the potential of this approach for actinorhizal plants, two RNAi silencing vectors directed against the β-glucuronidase (GUS) gene were introduced into transgenic plants of *Casuarina* stably transformed by the GUS gene under the control of the 35S promoter. Our data established that the reporter gene was efficiently silenced in both roots and actinorhizal nodules of composite plants (Gherbi *et al.*, 2008b).

3 Genomics of *Casuarina-Frankia* Symbiosis

Our group has developed the first genomic platform to identify new genes involved in the symbiotic process between *Frankia* and *C. glauca.* A total of 15,000 unigenes were obtained from cDNA libraries corresponding to mRNA extracted from (1) young nodules induced by *Frankia* and (2) non-infected roots. Unigenes were classified into functional categories. As expected, several nodule cluster sequences corresponded to proteins previously described as actinorhizal nodulins (*i. e.* hemoglobin, metallothioneins, subtilisin, rubisco activase, saccharose synthase, glycine and histidine rich proteins). In order to explore the early events of *C. glauca* - *Frankia* symbiosis, a substractive hybridisation library (SSH) was also constructed with roots sampled 4 days after infection. SSH sequences were validated and annotated revealing a large proportion of ESTs implicated in defence, cell wall structure and gene expression. More recently, unigenes were used to design microarrays. Expression analysis has led to the identification of few up regulated genes during the early steps of nodule development (Hocher *et al.*, in preparation).

In order to test the usefulness of transciptomics data to identify symbiotic genes, genes were selected to verify nodule-specific and /or nodule-enhanced expression by Quantitative Real-Time RT-PCR (qRT-PCR) on the basis of their putative involvement in nodule development and/or functioning. Differential expression was observed between roots and nodules for genes coding for flavonoid biosynthesis enzymes, thus suggesting a possible role of flavonoids in actinorhizal nodule development (Hocher *et al.*, 2006). Experiments are now in progress to characterize more precisely the expression of these genes at different stages of *C. glauca* nodule differentiation.

4 *SymRK* Defines a Common Genetic Basis for Plant Root Endosymbioses

Although several genetic components of the host-symbiont interaction have been identified in legumes; the genetic basis of actinorhizal symbiosis is still unknown. We demonstrated that the receptor like-kinase gene *SymRK*, which is required for nodulation in legumes, is also necessary for actinorhizae formation in *C. glauca*. This indicated that both types of nodulation symbiosis share genetic components. We have also shown that *SymRK* is involved in AM formation in *C. glauca* and can restore both nodulation and AM symbiosis in *Lotus japonicus SymRK* mutants. Taken together, our results demonstrate that *SymRK* has a key role for both plant-fungal and plant-bacterial endosymbioses and is conserved between legumes and actinorhizal plants (Gherbi *et al.*, 2008a).

5 Conclusions

In the past decade, considerable advances have been made in the identification and characterization of the plant genes involved in the development and functioning of actinorhizal nodules. However, in comparison with the progress achieved in the molecular dissectioning of the communication between *Rhizobium* bacteria and legumes our understanding of the early steps of the interaction between *Frankia* and the actinorhizal plants lags well behind.

The genetic transformation procedures and RNA interference technology developed in the Casuarinaceae family now make it possible to perform functional analysis of the actinorhizal symbiotic genes. Moreover, in the next years, emerging genomics tools (454 and solexa technology) may help investigate the early communication between the actinomycete and the host plant.

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Genetic Transformation of Casuarinaceae Trees

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Abstract Actinorhizal species are non-leguminous perennial plants belonging to eight angiosperm families. They are able to form root nodules as a result of the infection by a nitrogen-fixing actinomycete called *Frankia*. Using the biological vectors *Agrobacterium rhizogenes* and *A. tumefaciens*, gene transfer has been successful in *Casuarina glauca*. Transgenic plants proved to be valuable tools for exploring the molecular mechanisms resulting from the infection process of actinorhizal plants by *Frankia* and should contribute in the future to the goal of engineering Casuarinaceae for enhanced performance and better adaptation to biotic and abiotic stress.

1 Introduction

Long breeding cycles, large size, high levels of heterozygosity, and the economics of producing and evaluating large segregating populations of trees are some of the difficulties encountered in breeding forest trees. Genetic engineering offers prospects for generating novel forest tree genotypes at an accelerated rate. One major advantage of this approach over conventional breeding is that only the characteristics of interest are inserted into the recipient plant while the original genetic framework remains unchanged. Although genetic engineering in trees is still in its infancy, several studies have clearly established its potential for introducing novel genetic characters, such as herbicide tolerance, insect resistance, or modifying lignin content. Casuarina plantations are faced with a number of problems including diseases and pests. Developing gene transfer techniques in Casuarinaceae trees is therefore a major issue to contribute to the genetic improvement of these valuable tropical tree species. Besides, it is an important tool for the basic molecular knowledge of the symbiosis established between *Frankia* and *Casuarina*.

2 Gene Ttransfer Based on *Agrobacterium tumefaciens*

In order to successfully regenerate transgenic plants using the natural *A. tumefaciens* gene transfer system, a number of parameters has to be fulfilled (Gelvin, 2000): (1) the virulence of the *Agrobacterium* strain should permit the transfer of the T-DNA into the wounded plant cells; (2) the transformed cells should be efficiently selected among the population of non-transformed cells; and (3) the transformed cells should be regenerated into plants.

The natural susceptibility of members of the Casuarinaceae family to *A. tumefaciens* was used to develop a gene transfer procedure for *C. glauca* (Smouni *et al*., 2002). Epicotyls fragments of 2 cm in length were excised from 45-day-old plantlets and cocultivated with the disarmed strain C58C1 (pGV2260; pBIN19). After selection on kanamycin, one to three transgenic calli were observed on 26% of the epicotyls. Integration of the transgenes was further confirmed by PCR and Southern blot analyses. Transgenic plants were regenerated in approximately nine to ten months for *C. glauca*. The nodulation efficiency was found to be similar in transgenic Casuarinaceae and in non-transformed control plants inoculated by *Frankia*, and the transgenic nodules fixed nitrogen at the same rate as those of the non-transformed control nodules. This transformation procedure has two major advantages: the kanamycin selection is efficient and there are very few escapes; and only one medium is required for both bud differentiation and shoot elongation. The same approach is currently in progress to achieve the genetic transformation of *C. equisetifolia* and *C. cunninghamiana* (RITF and IFGTB, unpublished data).

This transgenic approach has contributed to the characterization of several symbiotic genes from *C. glauca*: *Cgenod*40, a homolog of early nodilin gene *enod*40 from legumes (Santi *et al*., 2002), *CgMT*1, a metallothionein-like gene of type 1 (Laplaze *et al*., 2002), *Cg*12, an actinorhizal symbiotic gene encoding a subtilisin-like serine protease (subtilases) (Svistoonoff *et al*., 2003), the auxin influx transporter gene *CgAUX*1 (Péret *et al*., 2007) and *CgSYMRK*, a leucine-rich-repeat receptor kinase gene required for nodulation and mycorrhization (Gherbi *et al*., 2008a).

3 Fast *Agrobacterium rhizogenes*-based Transformation System of *Casuarina glauca*

A rapid procedure for producing composite plants of *C. glauca* is also available (Diouf *et al*., 1995; Gherbi *et al*., 2008b). It relies on the induction of a hairy root system by *A. rhizogenes* while the aerial part of the plant remains untransformed. Young seedlings of *C. glauca* were wounded on the hypocotyl and inoculated with *A. rhizogenes* A4RS containing the chosen binary vector. After two weeks, highly branched roots exhibiting rapid growth were observed at the inoculation site. The normal root system was removed at the stem base, and the composite plant decontaminated with cefotaxime. Cotransformation with the wild-type T-DNA and the T-DNA from the binary vector was observed in about 50% of *C. glauca* hairy roots. After inoculation with *Frankia*, nodulation was observed on 40% of the transformed roots. Using this "composite plant" approach, the pattern of expression conferred by the promoter region of a symbiotic gene can be studied in both roots and nodules of *C. glauca* within about 4 months (Franche *et al*., 1998).

These composite plants are currently used to dissect symbiotic gene function by RNAi gene silencing. To demonstrate the potential of this approach for actinorhizal plants, two RNAi silencing vectors directed against the β-glucuronidase (GUS) gene were introduced into transgenic plants of *Casuarina* stably transformed by the GUS gene under the control of the 35S promoter. Our data established that the reporter gene was efficiently silenced in both roots and actinorhizal nodules of composite plants (Gherbi *et al*., 2008b).

4 Gene Transfer for the Improvement of Casuarinaceae

Gene transfer procedures based on *A. tumefaciens* and knowledge of gene expression conferred by heterologous promoters such as the 35S in Casuarinaceae (Smouni *et al*., 2002; Obertello *et al*., 2005) pave the way for genetic engineering these tropical trees. Strategies can be developed to engineer *Casuarina* to resist major pathogens such as *Rhizoctonia solani*, and insect pests such as *Lymantria xylina* (Diouf *et al*., 2008). Transgenic trees that are more tolerant to adverse edaphic conditions such as salt and drought would also be very valuable in tropical regions. Other aspects may include modification of lignin content and/or composition to obtain trees that are more suitable for industrial uses. Paper production has more than tripled in the last 35 years and the paper industry suffers from the high cost of removing lignin from cellulose, which also has a negative environmental impact. Another goal linked to lignin modification could be to prevent casuarina wood from splitting when it dries. This is currently a major drawback for the use of casuarina wood for the manufacture of furniture.

No transgenic *Casuarina* trees have been planted in the field. Additional information on the stability of transgene expression in field-grown Casuarinaceae exposed to changing environments is thus needed to determine the real potential of genetic engineering for the introduction of valuable new traits in this tropical tree family. Furthermore, to prevent an uncontrolled escape into the environment, efforts should be made to obtain sterile transgenic Casuarinaceae trees that do not form fertile pollen or seeds.

5 Conclusions and Future Challenges

Actinorhizal plants input of fixed nitrogen on a global scale is enormous; they contribute to 15% of symbiotic nitrogen fixation (Franche *et al*., 2009). Casuarinaceae species are largely distributed and contribute to maintain/rehabilitate marginal lands, as well as to provide incomes to smallholders of various tropical and sub tropical countries. Understanding the development and functioning of the actinorhizal nodules is thus an important challenge. In the past decade, molecular tools have been developed and considerable advances have been made in the identification and characterization of genes implicated in actinorhizal symbiosis (Laplaze *et al*., 2008). The genetic transformation procedures developed for Casuarinaceae make it possible to perform functional analysis of the isolated symbiotic genes and offers numerous prospects for generating novel forest tree genotypes at an accelerated rate.

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Beneficial Microbial Interaction for the Sustainable Growth and Biomass of *Casuarina equisetifolia* in Farm Forestry

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Abstract A survey of the beneficial microbial association in the rhizosphere of *Casuarina equisetifolia* was undertaken in Coimbatore district of Tamil Nadu in India. Assessment in the rhizosphere soil of *C. equisetifolia* showed the association with different species of AM fungi belonging to the four genera *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora*. *Glomus* was the dominant genus of AM fungi found in Casuarina plantations. Root nodulation in trees (72.8%) was recorded in the sandy clay loam soil and the average nodule size ranged between 5 and 6.8 cm in diameter. An experiment was also conducted to study the growth, biomass nutrient distribution and nutrient cycling of *C. equisetifolia* in the farm forestry. Trees were inoculated with a combination of different bioinoculants such as *Azospirillum brasilense*, phosphate solubilising bacterium (PSB), AM fungi (*G. fasciculatum*) and *Frankia*, along with compost in farmland. Growth, biomass, nutrient distribution, nutrient uptake and nutrient return through litter were determined 36 months after planting. Maximum height, girth at breast height and total biomass were obtained in the combined application of *Azospirillum*, phosphobacterium, AM fungi and *Frankia* with compost. Nutrient uptake and nutrient recycling potential was also higher in trees treated with bioinoculation + compost. Nutrient accumulation in the various parts of standing tree was in the order of nitrogen > calcium > potassium > magnesium > phosphorus. Nutrient return through litter was in the order of calcium > nitrogen > potassium > magnesium > phosphorus.

1 Introduction

Farmers from Orissa, Andhra Pradesh, Karnataka, and Tamil Nadu in the southern part of India have developed large-scale cultivation of Casuarina for agro-forestry and farm-forestry. For the sustainable development of Casuarina in farm-forestry, there is a need to exploit the beneficial role of microorganisms including AM fungi, *Frankia*, *Azospirillum* and phosphobacterium in field conditions. Hence, the present study had three main objectives. The first objective was to identify beneficial microbial and AM fungal spore diversity, and *Frankia* nodulation in the rhizosphere of *C.*

equisetifolia. The second objective was to provide information on the growth and productivity of Casuarina tree species growing in farm forestry. The third objective was to find out suitable bio-inoculation methods and to estimate above ground biomass and carbon sequestration of these plantations inoculated with biofertilizers (*Frankia*, arbuscular mycorrhiza (AM), *Azospirillum*, phosphobacterium) in farm forestry plantations.

2 Materials and Methods

2.1 Study area

The study was conducted in the farm land on the bank of the Noiyal River in Coimbatore, Tamil Nadu, India (latitude 11°02′ N, longitude 76°58′ E, elevation 409 m asl, mean annual precipitation 450-650 mm). Soil type is sandy clay loam (sand 63.6%; silt 26.8%; clay 9.6%) with a pH of 7.1. Organic content of the soil is 1.75%. The nitrogen, phosphorus, potassium, calcium and magnesium contents are 0.55%, 0.11%, 0.47%, 0.71% and 0.36% respectively.

2.2 Experimental design, treatments and planting

Six-month-old *C. equisetifolia* seedlings were planted in 30 cm × 30 cm × 30 cm pits at a spacing of 1 m ×1 m. The experiment was set up in a completely randomized block design (CRBD) with 4 treatments and 3 replications (each treatment block contains 36 plants). A total of 432 plants were included in the treatments T1 to T4 described below. In between each treatment block, we raised a bund and 5 trees were kept to reduce the microbial contamination. Treatments include T1-Bioinoculants (*Frankia*, *Arbuscular Mycorrhiza* (AM), *Azospirillum*, phosphobacterium), T2-Compost (Agricultural waste compost, 500 g tree^{-1}), T3- Bioinoculants + compost and T4-Control.

After planting, the field was kept free from weeds through periodic hand weeding and hoeing every two months during the study period. Watering was done twice a month during summer season.

2.3 Biometric observations

2.3.1 Growth and biomass

Total tree height and girth at breast height (GBH) 1.3 m above the ground were measured. In each plantation the height of ten trees per species was measured 3 years after planting. Biomass was estimated at the end of the third year. Random sampling method was adopted to estimate the biomass. Biomass components such as foliage, branch, stem, bark and root were separated, and fresh weight estimated. Plant samples were oven dried at 80℃ and taken for dry weight estimation and nutrient analysis.

2.3.2 Litter fall determination

The amount of litter fall was estimated by collecting the litter in each treatment block. Periodically collected litter was brought to the laboratory and oven drying at 70℃ to a constant weight (Rajendran and Devaraj, 2004).

2.4 Nutrient analysis

Plant samples were taken for the biochemical analysis. Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) were determined by the method as described by Jackson (1973).

2.5 Nutrient cycling

Nutrient uptake, nutrient retain and nutrient return through litter of trees were calculated by multiplying the nutrient percentage concentration with biomass of the tree components and total litter production.

2.6 Statistical analysis

The data were statistically analyzed by analysis of variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test (DMRT, $P<0.05$) (Duncan, 1955).

3 Results

3.1 Initial and final microbial population, AM fungal status and *Frankia* nodulation in rhizosphere of *C. equisetifolia*

The analysis of rhizosphere soil of *C. equisetifolia* showed that the association of different genus of AM fungi belonging to *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora*. *Glomus* as the dominant genus of AM fungi was found in Casuarina plantation grown in sandy clay loam at Coimbatore district of Tamil Nadu in India. The AM spore population ranges from 40 g to 100 g of dry soil. Mycorrhizal infection was observed in 88% of the trees. Maximum *Frankia* root nodules were observed in 0-30 cm-soil depth in all the sites. Root nodulation in trees (72.8-88%) was recorded in the sandy clay loam soil and the average diameter of the nodules was 5-6.8 cm. The microbial populations of actinomycetes, *Azospirillum* and phosphobacterium were 6×10^3, 7×10^4 and 13×10^4 cells g^{-1} of soil respectively.

At the end of the field experiment, an increase in microbial population of actinomycetes, *Azospirillum* and phosphobacterium (45×10^4, 57×10^4 and 79×10^4 cells g^{-1} of dry soil respectively) was observed in the trees treated with the combination of bioinoculants and compost (T3). Similarly, 100% mycorrhizal infection was seen in trees treated with bioinoculants and compost + bioinoculants in farm forestry plantation (Table 1).

3.2 Height and GBH growth

The results showed that Casuarina trees treated with combination of bioinoculation with compost (T3) recorded maximum height, with a 5% increase over the control at 36 months after planting (Table 2). Similarly, combined inoculation of bioinoculation with compost (T3) recorded maximum GBH, with a 24% increase over the control.

3.3 Biomass and nutrient accumulation

The highest total biomass and nutrient content was obtained with the combined application of bioinoculants (*Azospirillum*, phosphobacterium, AM, *Frankia*) with compost (T3). Biomass was increased by 59% in T3, 28% in T2 and 21% in T1 over control trees biomass (Table 2). Among all the nutrients, N content was found greater than K, Ca, Mg and P. Nitrogen content was the highest (174.500 g^{-1} $plant^{-1}$) in trees treated with bioinoculants + organic manure (Table 3).

3.4 Litter production and nutrient return through litter

Litter production and nutrient return were the highest in trees treated with Bioinoculants with compost (T3) and reached 0.946 kg $tree^{-1}$ after 36 months. Trees treated with the T2 compost produced litter 0.939 kg $tree^{-1}$. Nutrient return was in the order: calcium > nitrogen > potassium > magnesium > phosphorus (Table 4).

Table 1 Assessment of microbial population in three-year-old *Casuarina equisetifolia* treated with bioinoculants and compost

Treatment	pH	*Actinomycetes* cfu g^{-1} of dry soil	*Azospirillum* cfu g^{-1} of dry soil	phosphobacterium cfu g^{-1} of dry soil	AM spore /100 g of dry soil	AM infection	*Frankia* nodulated trees (%)
T1	7.1 a	25×10^4 c	33×10^4 c	44×10^4 b	93 c	100 b	100 b
T2	7.2 a	12×10^4 b	28×10^4 b	34×10^4 a	73 b	82 a	82 a
T3	7.1 a	45×10^4 d	57×10^4 d	79×10^4 c	98 c	100 b	100 b
T4	7.1 a	9×10^4 a	17×10^4 a	31×10^4 a	69 a	89 a	86 a

cfu (colony forming units). Means followed by a common letter(s) in the same column are not significantly different at the 5 % level by DMRT.

Treatments: T1 Bioinoculants (*Azospirillum*, phosphobacterium, Arbuscular mycorrhizae (AM), *Frankia*); T2 Compost (Agricultural waste compost, 500 g $tree^{-1}$); T3 Bioinoculants + Compost; T4 Control.

Table 2 Growth and biomass of different components in three years old *Casuarina equisetifolia* treated with bioinoculants and compost in farm forestry

Treatment	GBH (cm)	Height (m)	Stem weight (kg)	Bark weight (kg)	Branch and twig weight (kg)	Needle weight (kg)	Total agb weight (kg)	Root weight (kg)	Root nodule (kg)	Total bgb (kg)	Total biomass (kg)
T1	17.50 a	10.80 ab	7.754 b	1.378 b	1.742 b	3.528 b	14.402 b	2.435 b	0.113 b	2.548 b	16.950 b
T2	19.41 b	10.66 ab	8.240 c	1.446 b	1.893 bc	3.810 bc	15.389 c	2.509 b	0.029 a	2.538 b	17.927 c
T3	21.23 c	11.18 c	11.499 d	1.842 c	1.921 c	4.027 c	19.289 d	2.741 c	0.127 b	2.868 c	22.157 d
T4	17.04 a	10.55 a	6.611 a	1.102 a	1.372 a	2.862 a	11.947a	1.953 a	0.019 a	1.972 a	13.919 a

agb (above ground biomass) and bgb (below ground biomass). Treatments are similar to those described in Table 1.

Table 3 Nutrient accumulation in various plant parts and total nutrient content of three years old *Casuarina equisetifolia* treated with bioinoculants and compost in farm forestry (g tree^{-1})

Nutrient (g tree^{-1})	Treatment	Stem	Bark	Twig & branch	Needle	Above ground	Root	Nodule	Below ground	Total nutrient content
N	T1	38.14b	9.00b	16.20b	79.04b	142.38b	13.26b	0.84c	14.60b	156.48b
	T2	43.32c	9.26b	16.52b	85.46c	153.56c	16.90c	0.30a	17.20c	170.76c
	T3	62.62d	13.60d	16.80b	63.52a	156.56d	17.00c	0.96d	17.96cd	174.50d
	T4	31.58a	7.14a	12.16a	64.18a	115.06a	11.00a	0.47b	11.47a	126.53a
P	T1	7.00b	1.00a	2.00b	8.00b	18.00b	3.00b	0.23a	3.23b	21.23b
	T2	8.20c	1.60b	1.80b	9.00c	20.60c	3.60b	0.23a	3.83b	24.43c
	T3	13.80d	2.66b	2.56c	10.00d	29.02d	4.80c	0.34b	5.14c	34.14d
	T4	5.00a	1.00a	1.00a	4.60a	11.60a	1.00a	0.23a	1.23a	12.83a
K	T1	47.80b	9.12b	12.04b	31.42b	100.38b	13.60b	0.60c	14.20b	114.58b
	T2	51.06c	9.62b	11.26b	33.34c	105.28b	15.50c	0.36b	15.86c	121.14c
	T3	70.64d	12.30c	14.70c	35.88d	133.52c	16.92cd	0.64c	17.56d	151.08d
	T4	39.70a	7.14a	8.32a	24.82a	79.98a	7.58a	0.27a	7.85a	87.83a
Ca	T1	38.20b	8.72b	8.62b	54.62b	110.16b	8.60a	0.84b	9.44a	119.96b
	T2	40.74c	9.46b	9.10b	58.22bc	117.53c	9.48a	0.20a	9.68a	127.20c
	T3	57.70d	13.44c	9.20b	57.18bc	137.52d	9.60a	1.48c	11.08b	148.68d
	T4	31.72a	7.12a	6.52a	40.72a	86.08a	9.00a	0.40a	9.40a	95.48a
Mg	T1	18.40a	2.12b	4.10b	13.48b	38.10a	5.70bc	0.10a	5.30bc	43.40b
	T2	19.70a	5.60d	7.34c	15.38c	48.02b	4.16b	0.60a	4.76ab	52.78c
	T3	27.50b	3.10c	4.68b	15.26c	50.54c	6.24d	0.74b	6.98c	57.52d
	T4	22.96a	1.56a	2.28a	10.42a	37.22a	2.72a	0.46a	2.76a	39.98a

Means followed by a common letter (s) in the same column within a nutrient are not significantly different at the 5% level by DMRT. Treatments are similar to those described in Table 1.

Table 4 Litter production (kg tree^{-1}) and nutrient return (g tree^{-1}) through litter in one to three years old *Casuarina equisetifolia* treated with bioinoculants and compost in the farm land

Treatment	Litter production (kg tree^{-1})				Nutrient return (g tree^{-1}) through litter				
	1 year	2 years	3 years	Total	N	P	K	Ca	Mg
T1	0.017a	0.648a	0.868b	1.533b	25.754b	2.913a	5.825a	29.127b	4.446b
T2	0.030b	0.680b	0.939c	1.649c	28.033b	2.968c	6.596b	32.155c	4.947c
T3	0.048c	0.788c	0.946c	1.782c	30.473b	3.564b	7.484c	35.284b	5.524d
T4	0.016a	0.610a	0.790a	1.416a	23.647a	2.690b	5.644a	26.337a	4.248a

Treatments are similar to those described in Table 1.

3.5 Nutrient cycling

Based on the above data, nutrient uptake, nutrient retain and nutrient return through litter of trees were calculated (Table 5). The values were the highest in trees treated with combined application of bioinoculants (*Azospirillum*, phosphobacterium, AM, *Frankia*) with compost (T3).

Table 5 Nutrient cycling of three years old *Casuarina equisetifolia* inoculated with bioinoculants and compost in the farm land

Treatments	Nutrient cycling	Nutrients (g $tree^{-1}$)				
		N	P	K	Ca	Mg
T1	Retained	156.480	21.230	114.580	119.960	43.400
	Return	25.754	2.913	5.825	29.127	4.446
	Uptake	182.234	24.143	120.405	149.087	47.846
T2	Retained	170.760	24.430	121.140	127.200	52.780
	Return	28.033	2.968	6.596	32.155	4.947
	Uptake	198.793	27.398	127.736	159.355	57.727
T3	Retained	174.500	34.140	151.080	148.680	57.520
	Return	30.473	3.564	7.484	35.284	5.524
	Uptake	204.973	37.704	158.564	183.964	63.044
T4	Retained	126.530	12.830	87.830	95.480	39.980
	Return	23.647	2.690	5.644	26.337	4.248
	Uptake	150.177	15.520	93.474	121.817	44.228

Treatments are similar to those described in Table 1.

4 Discussion

In the present study, we established the association of *C. equisetifolia* with different species of AM fungi belonging to four genera of *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora*. *Glomus* was found to be the dominant genus of AM fungi in *C. equisetifolia* plantation, which is in agreement with our earlier report (Rajendran *et al*., 1999). While comparing the growth of Casuarina in various climatic zones of different countries, trees attained 204.8 cm in height and 3.6 cm in diameter after two years under arid conditions in Egypt (El-lakany, 1983). The height of 1, 2 and 3 years old Casuarina was respectively found to be 1.6, 2.2, and 2.5 m in the Philippines (Halos, 1983). In the present study excellent growth has been observed since the height of *C. equisetifolia* ranged from 10.8 m to 11.18 m and GBH was 17.5 cm to 21.23 cm at three years after planting. The growth might be due to the application of suitable biofertilizers with organic manure, regular irrigation, and intensive care of the experimental field.

In the present study, 1.501 kg $tree^{-1}$ litter production was recorded in three years. Similarly, the litter production of 4.873 t ha^{-1} yr^{-1} was estimated in 3-year-old *C. equisetifolia* in the East Coast District, Tamil Nadu, India (Rajendran, 2001). Litter accumulation in 3.5-year-old *C. equisetifolia* plantation in Puerto Rio was 16.2 t ha^{-1} yr^{-1} (Lugo *et al*., 1990).

5 Conclusions

This study is a holistic approach of sustainable utilization of bio-resources and management with locally available resources in cultivation of Casuarina. It is inferred that under appropriate management, the use of efficient biofertilizers co-inoculated with organic compost lead to an increased growth and biomass of Casuarina in tropical conditions. Therefore, it is recommended to use simultaneously bioinoculants and compost for higher biomass production and nutrient cycling.

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Iron Stress Induces Activated Oxygen and Antioxidant Defenses in Nodules of *Casuarina cunninghamiana*

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Abstract Iron is one of the most important elements affecting plant growth, nodule formation and nitrogen fixation. The effect of iron deficiency was explored in this study by propagating *Casuarina cunninghamiana* seedlings in water culture jars containing Hoagland's solution prepared with different iron concentrations (0-20 μM) in the form of Fe-EDTA. Results showed that severe Fe-deficiency induced significant reduction in nodulation and plant growth. The uninoculated and non-nodulated seedlings showed typical iron deficiency symptoms with reduction in plant biomass and chlorophyll content. Iron deficiency also affected the activity of some antioxidant enzymes, such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in both nodule and shoot tissues of seedlings. All these enzymes showed decrease in their activities under severe iron deficiency, except superoxide dismutase which showed increased activities. A non-enzymatic antioxidant (glutathione) also showed a significant increase in activity under severe Fe-deficiency. Augmentation of the lipid peroxidation level was highly correlated with iron stress. The presence of high levels of enzymatic and non-enzymatic antioxidants in nodule and the shoot tissues of inoculated seedlings may reflect the importance of inoculation as a defense mechanism under iron-stress conditions.

1 Introduction

Of all micronutrients, plants require iron in the largest amounts. Iron is accumulated in the ferric (Fe^{3+}) or ferrous (Fe^{2+}) ion forms, although the latter is more common due to its greater solubility. In plants, iron serves many important functions. It acts as a catalytic group for many redox enzymes required for chlorophyll biosynthesis, is a constituent of several oxidase enzymes, such as catalase and peroxidase, and plays a role in energy transfer. It is also important for nitrogen fixing plants where it plays a key role in nodule intiation and in the synthesis of iron-containing proteins necessary for nitrogen fixation process.

Iron deficiencies invariably lead to simultaneous loss of chlorophyll and degradation of chloroplast structure as well as other metabolic functions including symbiotic relationship and N_2 fixation process (Lesueur and Diem, 1997). Iron deficiencies are common because of the tendency of Fe^{3+} to form insoluble hydrous oxides ($Fe_2O_3 \cdot 3H_2O_3$) at biologically relevant high pH. In Egypt, most cultivated or newly reclaimed soils are characterized by an alkaline pH and low iron availability

(Elskhiry, 2000). Increased knowledge on how a plant/endymbiont symbiosis responds to different soil character is essential to evaluate its effectiveness and potential for successful induction into other areas, especially those of low fertility to meet the demand of new agricultural lands.

Physiological research of leguminous and non-leguminous N_2-fixing plants reported the presence of antioxidants in their nodules as a defense mechanism against iron defficiency (Matamoros *et al.*, 2003). Antioxidant enzymes detected in root nodule extracts of these plants may indicate the striking defense role of endosymbiont (O'Hara, 2003). Meanwhile, Travares *et al*. (2003) detected and identified antioxidant enzymes in a pure culture of N_2 fixing endosymbiont (*Frankia*, the endosymbiont in actinorhizal plants).

Our rsearch aims to explain the reasons for the high adaptability of inoculated *Casuarina* plants versus uninoculated ones under stress conditions (Mansour and Megahed, 2002). We investigated the role of *Frankia* inoculation and nodule formation for protecting casuarina plants against oxidative stress as a result of iron deficiency which frequently occurs in newly reclaimed Egyptian soils (Elskhiry, 2000).

2 Materials and Methods

2.1 *Frankia* strain and growth conditions

The *Frankia* strain ISS6 isolated from root nodules of *Casuarina cunninghamiana* was used in this study. It has the ability to re-infect its host and produce effective root nodules along the root system. *Frankia* strain ISS6 was propagated at 30℃ for 2-3 weeks in defined B medium, supplemented with 2 μM NH_4Cl. Hyphae were harvested, washed with sterile distilled water and then homogenized. After homogenization, the hyphae were ready for inoculation.

2.2 Plant material and propagation

Seeds of *C. cunninghamiana* were collected from growing trees in the campus of Suez Canal University, Ismailia, Egypt. Seeds were surface sterilized and propagated in flats containing acid-washed sand. After germination, seedlings were fertilized once a week with 1/4-strength Hoagland's solution. The young seedlings were transferred to water-culture jars containing Hoagland's solution lacking nitrogen with different iron concentrations depending on the treatment. To investigate the role of *Frankia*, one set of casuarina seedlings was inoculated with *Frankia* strain ISS6 and a second uninoculated set was used as control. Three iron concentrations (0, 10, 15 μM) were tested together with standard concentration, normally 20 μM. For each treatment, five jars each containing three seedlings were used. The experiment was run for 9 weeks and plants were harvested at four time intervals to evaluate the effect of iron stress on the seedlings. The first harvest was at 3 weeks post inoculation and each subsequent harvest was performed every 2 weeks thereafter.

2.3 Infectivity and nodule formation

To test the effect of iron deficiency on the infectivity of *Frankia* strain ISS6, 15 seedlings per each treatment were inoculated, each seedling received 0.01 packed cell volume (PCV) of *Frankia* cells. Inoculated seedlings were transferred to the water culture jars containing prepared testing Fe-nitrogen free-Hoagland's solution. Nodule formation was recorded at regular intervals and the first nodule formed was recorded. Nodulation percentage and number of nodules per seedling were also recorded at the end of each harvesting time.

2.4 Plant growth and biomass measurements

To assess the effect of iron deficiency on plant growth and biomass, plant height and plant dry weight were measured at each harvesting time.

2.5 Chlorophyll determination

Chlorophyll (a and b) content of shoot system of *C. cunninghamiana* plants was quantified by spectrophotometry according to Lichtenthaler (1987).

2.6 Defense mechanism and enzyme activities

In order to measure oxidative damage as a result of iron deficiency and to determine the response of plants to such stress conditions, the level of antioxidant enzymes, such as catalase (Aebi, 1983), peroxidase (Malik and Singh, 1980) and superoxide dismutase (Giannopolitis and Ries, 1977) were evaluated in shoot and nodule tissues. Non-enzymatic antioxidant (glutathione) and oxidative damage to lipids (lipid peroxidation) were also measured.

2.7 Statistical analyses

One-way analysis of variance (ANOVA) and *t*-test were used to determine significant differences among treatments at $P < 0.05$ significant level. Minitab software release 13.1 was used (Minitab Inc., Stat College, PA, USA).

3 Results

3.1 Effect of Fe on symbiotic performance and nodulation percentage

Upon infection with *Frankia* strain ISS6, iron deficiency was found to drastically affect infectivity, nodule formation and number of nodules per plant. Nodulation was delayed with no iron and the first nodule was observed after seven weeks of inoculation. In contrast, nodulation was faster (two weeks after inoculation) when iron was added at 15 and 20 μM (Table 1). The percentage of nodulated plants achieved by the end of the experiment with no iron addition was significantly lower (38%) compared to nodulated plants grown in Hoagland medium supplemented with iron (all 100%).

Table 1 Effect of iron concentration on nodulation of *C. cunninghamiana* seedlings

Iron concentration (μM)	Appearance date for 1st nodule (day)	Nodulation percent (%)			Number of nodules plant^{-1}		
		Time after inoculation (week)			Time after inoculation (week)		
		3	5	9	3	5	9
0	40	0.0	0.0	38.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.25
10	19	44.0	85.0	100.0	2.0 ± 0.19	2.0 ± 0.10	3.26 ± 0.7
15	14	56.0	95.0	100.0	2.0 ± 0.10	2..2 ± 0.11	4.06 ± 0.15
20	14	57.0	100.0	100.0	3.0 ± 0.10	3.1 ± 0.09	5.2 ± 0.17

Means are significant different at $P < 0.05$ within columns except between 10 and 15 μM iron concentrations where no significant differences were recorded.

3.2 Plant growth and biomass

Growth of *C. cunninghamiana* was significantly affected by iron concentrations (Table 2). Inoculation with *Frankia* strain ISS6 produced healthier plants and significantly greater measured parameters than those of uninoculated plants. An increase in plant height and dry weight correlated positively with different iron concentrations; iron at 20 μM concentration was clearly the best.

Inoculated seedlings were able to overcome the stress condition of iron deficiency (Table 2).

Table 2 Effect of iron concentrations on growth and chlorophyll concentration of *C. cunninghamiana* seedlings at 9 weeks after inoculation

Iron concentration (μM)	Plant height (cm)		Plant dry weight (mg plant^{-1})		Chlorophyll concentration (mg g^{-1} plant^{-1})	
	1	2	1	2	1	2
0	5.0 ±0.5	7.5 ±0.1	76.68 ±1.89	121.38 ±13.2	9.95 ±0.6	18.60 ±0.8
10	7.1 ±0.06	10.5 ±0.6	97.77 ±15.05	179.17 ±11.02	13.92 ±0.4	38.00 ±6.8
15	7.6 ±0.9	17.6 ±0.13	132.98 ±15.08	199.2 ±19.9	23.73 ±1.4	60.90 ±12.5
20	7.9 ±0.07	25.1 ±0.22	147.28 ±14.8	471.08 ±21.8	40.85 ±2.9	96.00 ±9.8

Values are mean ±SE for 15 plants; 1 = un-inoculated seedlings; 2 = inoculated seedlings.

3.3 Iron and chlorophyll content

Seedlings grown in the absence of iron developed typical Fe-deficiency symptoms, especially in the tips of the growing stem (Table 2). However, these symptoms disappeared in inoculated plants after nodule development, 6 weeks after inoculation.

3.4 Oxidative damage and defense mechanisms

Iron stress led to significant reduction in most enzymatic activities involved in the removal of H_2O_2. Uninoculated seedlings showed a continuous decrease in catalase activity as the seedlings became older verses toinoculated seedlings, which recorded a highly significant continuous increase in catalase activity and reached the highest by the end of the experiment (data not shown). Peroxidase activity, another direct scavenger of H_2O_2, was markedly affected and recorded low levels for uninoculated seedlings; although significant increases in its level were recorded in shoot and nodule tissues of inoculated plants as a result of iron deficiency (data not shown). The elevated activity of peroxidase in shoots and root nodules of inoculated seedlings may reflect the metabolic interaction between the two partners and the importance of inoculation. In response to iron-stress conditions, superoxide dismutase (SOD) was highly active and showed a different response pattern of activity in comparison to catalase activity (Fig. 1). Nodulated seedlings (shoot and nodule tissues) showed significantly higher levels of SOD activity that continued to increase with plant age. For nonenzymatic antioxidant, *Casuarina* nodules recorded higher glutathione concentration than other tissues of inoculated seedlings and uninoculated ones as well, even when these seedlings were grown with 20 μM of iron (Fig. 2).

3.5 Oxidant damage

The content of lipid peroxide (malondialdehyde) was used as a marker of free radical damage in plant tissues of uninoculated and inoculated plants as well as in nodule tissues (Fig. 3). Withholding iron led to increase of 180 to 256% in the content of lipid peroxide of uninoculated seedlings versus inoculated ones . For nodule tissues, lipid peroxide content was 56% less with iron limitation than those of uninoculated seedlings (Fig. 3).

4 Discussion

The symbiotic relation between *Casuarina* roots and its endosymbiont *Frankia* strain ISS6 exposed to iron deficiency showed delay in nodule development and less infective propagules as evidenced by

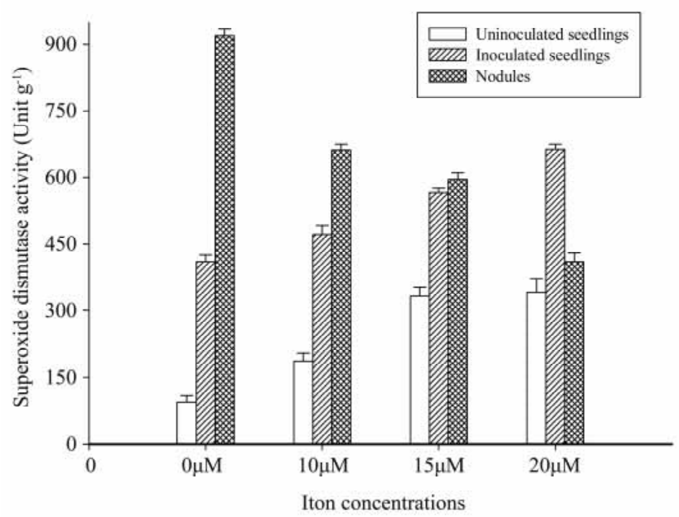

Fig. 1 **Activity of superoxide dismutase in nodule and shoot tissues of inoculated versus uninoculated seedlings subjected to different iron concentrations at 9 weeks after inoculation**

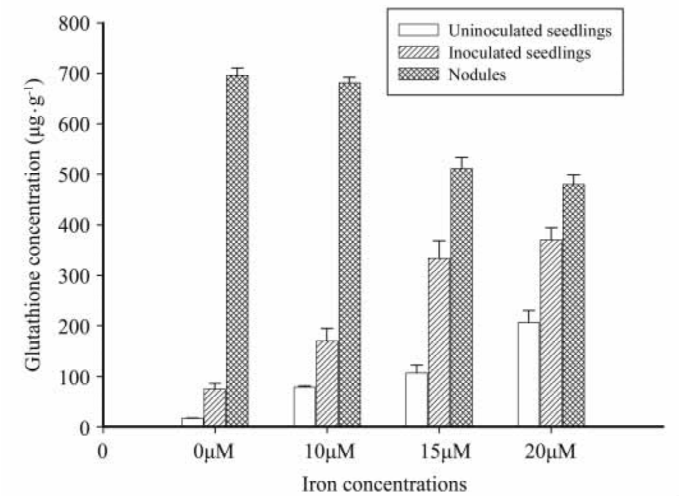

Fig. 2 **Glutathione concentrations detected in shoot tissues of uninoculated and inoculated seedlings as well as in nodule tissues of seedlings grown at different iron concentrations at 9 weeks after inoculation**

consistent reduction in nodulation and number of nodules developed per seedlings. The lower number of nodules observed may result from fewer infection events and/or larger number of arrested infections due to the deficiency of iron (Lesueur and Diem, 1997). Therefore, iron limitation affects *Casuarina*- *Frankia* symbiosis. Meanwhile, successful inoculation with endosymbiont strengthens seedling growth and seedling biomass. The same results have been reported by O'Hara (2003). Moreover, seedling growth and biomass were 50% lower than those observed for seedlings grown in a normal iron dose (20 μM). Nodulated seedlings grown under the same stress conditions showed better growth but plant height and dry weight did not exceed respectively 74% and 81% of those

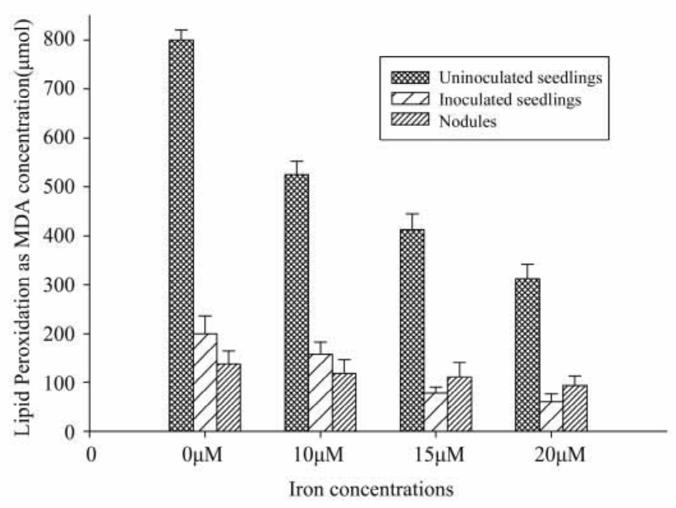

Fig. 3 Levels of lipid peroxidation in nodule and shoot tissues of inoculated versus uninoculated seedlings grown under different iron concentrations at 9 weeks after inoculation

observed for uninoculated seedlings grown in normal iron dose. These results confirm the importance of inoculation for growth of *Casuarina* species.

Chlorophyll content was reduced under iron deficency; this may be ascribed to well-known Fe requirements for the formation of precursors of the chlorophyll (Ranieri *et al*., 2001). However, the recovery in chlorophyll content recorded after nodule development may indicate the role of both partners, through nodule formation, to overcome such stress through siderophore formation and fix more atmospheric nitrogen accompanied by a large increase in carbon fixation. This explanation is supported by data obtained by Lopez-Millan *et al*. (2000) that iron deficiency induces large increase in carbon fixation and oxygen consumption, both favorable conditions for N_2-fixing symbioses.

Plant cells contain an impressive array of antioxidant metabolites and enzymes that scavenge the compounds that form the most aggressive reactive oxygen system (ROS), thus protecting cells from oxidative damage. The main sources of ROS in plant as well as microbial cell are respiration, photosynthesis and N_2 fixation. In addition, ROS is produced at high rates when plants/microbe are exposed to abiotic, biotic or xenobiotic stress. In our study both plants and microbe were exposed to abiotic stress resulting from the iron deficiency. Since iron is essential for many plant/microbe functions, its deficiency induces oxidative stress (Ranieri *et al*., 2001; Mohamed and Aly, 2004). The present study confirms the induction of oxidative stress in casuarina plants resulting from iron depletion; plants suffered from oxidative damage, as indicated by high lipid peroxide content and effects on CAT and POD by inducing a reduction in their activities in detoxification processes. Similarly, Ranieri *et al*. (2001) found a marked fall in the activity of these enzymes in sunflower plants as a consequence of iron depletion.

Restoration of enzyme activities (CAT and POD) in plant tissues after nodule formation was recorded over 9 weeks after inoculation. The elevated level of these enzymes under iron-deficient conditions may suggest a role of nodule formation in protecting plants of increasing production of adequate antioxidant defenses. This suggestion was strengthened by lipid peroxidation levels that were significantly lower compared to uninoculated plants.

In this study, the superoxide dismutase (SOD) represents a primary line of defense against the superoxide radical and derived reactive oxygen species that may result from iron deficiency, since it was detected at high activity levels. Therefore, SOD performs important antioxidant function (Matamoros *et al*., 2003). The high activity of superoxide dismutase in the absence of iron gives more evidence for the presence of different classes of SOD, which differed by the metals in their active sites. Catalase is highly inhibited by iron deficient conditions since iron is one of the main constituent of this enzyme. Therefore, the other metal SODs could be active and react with superoxide radicals resulting from oxidative stress, suggesting a compensation between activities of different metallo-types of SOD under stress conditions (Rubio *et al*., 2001). This explanation is also strengthened by the data obtained by Matamoros *et al.* (2003). Moreover, high levels of SOD, coincided with an increase in ascorbate peroxidase (APX) activity (our laboratory, data not shown). Consequently, overproduced SOD, particularly in nodule tissues, can reduce oxidative damage through the enhancement of other detoxifying enzymes, especially nonhaem-containing peroxidase enzyme (Gupta *et al*., 1993).

Another antioxidant, detected at high concentration was glutathione (GSH) which is critical for monitoring the operation of the ascorbate-GSH cycle required indirectly for nitrogen fixation (Matamoros *et al.*, 2010). The presence of glutathione in high concentration in nodule tissues strengthens its role against toxic oxygen species and reinforces the sites of its synthesis. Enhanced production of GSH in nodule tissues compared to those of plants (940% and 130% of inoculated plant tissues grown at zero and 20 μM Fe respectively) may also support the view that some GSH might have originated in the endophyte, which may become partly broken during extraction due to the different important roles of GSH other than as an antioxidant (Matamoros *et al*., 2003).

In conclusion, iron deficiency induces an oxidative stress in *C. cunninghamiana* plants, as evidenced by high levels of lipid peroxidation. Inoculation with a compatible *Frankia* strain can compensate this stress through formation of nodules which showed elevated concentrations of antioxidants. Enzymatic and non-enzymatic antioxidants detected under iron stress play a role as a defense mechanism in which activity of SOD and levels of GSH are more pronounced.

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Growth Response of *Casuarina junghuhniana* to Indigenous *Frankia*, Arbuscular Mycorrhizal Fungi and Phosphobacterium under Nursery Conditions

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Abstract An experiment was conducted to determine the influence of indigenous Arbuscular mycorrhizal (AM) fungi, phosphobacterium (PSB) and root nodule bacteria (*Frankia*) individually as well as combinations on the growth response of *Casuarina junghuhniana* seedlings in sterilized soil under nursery conditions. Indigenous AM fungi *Glomus geosporum* and phosphobacterium (PSB) (*Pseudomonas fluorescens*) were isolated from the rhizosphere of matured trees of *C. junghuhniana*. The spores of *G. geosporum* were multiplied in the roots of *Vigna unguiculata* and PSB was multiplied in Pikovskya medium. Similarly *Frankia* was isolated from the root nodules of *C. junghuhniana* and cultured in P medium. These microbial cultures were used to inoculate the seedlings of *C. junghuhniana* individually and in combination. The results showed that co-inoculation of *Frankia*, AM fungi and PSB increased the growth, bio mass, root nodule numbers, root nodule biomass and nutrient uptake of *C. junghuhniana* seedlings. In overall, the effect of co-inoculation on parameters was stronger than single inoculation. The growth and biomass were increased 3 folds in *Frankia*, AM fungi and PSB inoculated seedlings rather than un inoculated control seedlings. From this study it was deduced that co-inoculation with these microbial symbionts in seedlings of *C. junghuhniana* facilitated early symbiotic associations and thereby improved the seedlings quality.

1 Introduction

Casuarina junghuhniana is a native tree of the eastern part of Indonesia (east Java, Bali and parts of the Lesser Sunda archipelago) occurring from near sea level to 3,100 m (Pinyopusarerk and House, 1993). It is widely cultivated in farm lands particularly in South India and usually yields 120-140 tonnes ha^{-1} in 4 years rotation. The wood is highly suitable for firewood and charcoal production, and also as pulpwood for paper making. The energy from the charcoal is 34,500 kJ kg^{-1} and considered as the highest among the firewood species (Orwa *et al.*, 2009). It is good shelter species and suitable for windbreak. Its service also extends to rehabilitating degraded soils worldwide. For these utilities there is a high demand for *C. junghuhniana* seedlings. However, increased costs of seedling production and environmental concern have led to the development of alternative strategies for seedling production of *C. junghuhniana* in nurseries. These include the use of soil microorganisms such as AM fungi and other beneficial microbes which may reduce the amount of fertilizer input through increasing the efficiency of nutrient availability or uptake. *C. junghuhniana* fixes atmospheric nitrogen by nodulation with actinomycete bacteria of the genus *Frankia*. It has AM fungi that further enhance its adaptability in poor soils. Among microbial inoculants AM fungi are important microbes of soil that form symbiotic association in *C. junghuhniana*. Similarly PSB present in the rhizosphere fraction are also immensely important as they have been reported to *convert* the phosphate present in the soil from unavailable to an available-to-the plant form. These microbial inoculants are used in many studies related to growth enhancement of *C. equisetifolia* (Karthikeyan *et al*., 2009). In this present study these microbial inoculants were used for growth enhancement of *C. junghuhniana* under nursery conditions. Further, more than one inoculation of microbial inoculants may stimulate plant growth better than inoculation with either organism singly (Kim *et al*., 1998). Therefore the individual and combinations of AM fungi, PSB and *Frankia* were used for improvement of growth and nutrient uptake in *C. junghuhniana*.

2 Materials and Methods

The seeds of *C. junghuhniana* were collected from mature trees at Panampally, Kerala, India. The seeds were directly sown in the nursery beds containing pure sand with sufficient water spray. Ten-day-old seedlings were transplanted to polythene bags (10 cm × 14 cm) containing sand and soil (1 : 1 v/v).

2.1 Isolation and culture of AM fungi

The AM fungi *Glomus geosporum* (Nicol. & Gerd.) Walker were isolated from the rhizosphere of *C. junghuhniana* by the method of Gerdemann and Nicolson (1963) and identified with Schenck and Perez manual (1987). The freshly collected *G. geosporum* spores were then multiplied and maintained in sterile media (alfisol: sand) with *Vigna unguiculata* (as a host) under laboratory conditions at 21-23℃ and 40-45% relative humidity (RH) for three months in clay pots.

2.2 Isolation of phosphobacterium (PSB)

Three 0.1 ml aliquots of soil diluted in sterile water (10^{-6}-10^{-3}) were spread on the standard perspective media for dilution plate counts for PSB. The PSB medium Pikovoskya (Sundara Rao and Sinha,1963) composition for a litre of distilled water was prepared as follows: 0.5 g yeast extract, 10 g Dextrose, 5.0 g Calcium phosphate, 0.5 g ammonium sulphate, 0.2 g KCl, 0.1 g $MgSO_4$, 0.001 g $MnSO_4$, 0.0001 g $FeSO_4$, and 15.0 g agar. The plates were incubated at 37℃ for 3-5 days. The colonies in PSB medium that formed a clear zone on the medium were noticed. The PSB were identified as *Pseudomonas fluorescens* based on yellowish green fluorescence of isolated colonies under ultra violet light (Karthikeyan *et al*., 2009).

2.3 Isolation of *Frankia*

The root nodules were collected from a field grown *C. junghuhniana* and stored in frozen condition at - 4℃ for 10 days. Afterwards, the nodules were surface sterilized with 30% H_2O_2 and kept in shaker for 30-40 minutes. Under aseptic conditions the nodules were rinsed in sterile water and 0.2 gram of nodule was ground manually in sterile mortar and pestle. Then, the nodule solutions were centrifuged at 1,000 rpm for 20 minutes and the supernatant was filtered through Whatman No. 1 filter paper. The suspension was then plated in P media and incubated at 25℃ for 3-4 weeks. One litre of P medium was prepared as follows (Shipton and Burgraff, 1983): 10 g $CaCl_2 \cdot 2H_2O$, 20 g $MgSO_4$, 0.46 g Propionic acid, 0.15 g H_3BO_3, 0.15 g $ZnSO_4 \cdot 7H_2O$, 0.45 g $MnSO_4 \cdot H_2O$, 0.004 g $CuSO_4 \cdot 5H_2O$, 0.028 g $Na_2MoO_4 \cdot 2H_2O$, 0.009 g $CoCl_2 \cdot 6H_2O$, 0.04 g Biotin, 100 g K_2HPO_4, 67 g $NaH_2PO \cdot 2H_2O$, 0.1 g FeNa EDTA, and 8 g agar. The pH of the medium was adjusted to 6.8. After 21-30 days of incubation the *Frankia* growth was observed as white spongy colonies on P media plates. These colonies were transferred into P media broth for mass multiplication.

2.4 Inoculation of *G. geosporum*, *Frankia* and PSB

The AM fungal inoculum of *G. geosporum* along with soil from pot cultures of *V. unguiculata* comprising mycorrhizal roots, soil hyphae and spores was used for inoculations. 10 g of inoculum at the rate of 1,367 propagules g^{-1} of inoculum (Karthikeyan *et al*., 2005) was placed 5cm below the soil surface of polythene bag of seedlings. Thereafter the seedlings were maintained under nursery conditions for 2 minutes and watered regularly. 15 ml of inoculum of PSB cultured in nutrient medium broth and 10 ml of *Frankia* culture in P media broth were applied to each seedlings of *C. junghuhniana* individually and in combination with AM fungi. Hence 8 treatments were applied along with a control as follows (i) Control, (ii) *Frankia*, (iii) AM fungi, (iv) PSB, (v) *Frankia* + AM fungi, (vi) *Frankia* + PSB (vi) AM fungi + PSB and (vii) *Frankia* + AM fungi + PSB. *Each treatment* was applied to 5 seedlings per plot and there *were* 15 *replicate* plots arranged in a randomized block design and maintained under green house conditions at 31.6℃ (±2.8℃) and 72% (±5.2%) relative humidity.

2.5 Harvest and assessment of seedlings

Thirty days after inoculation 5 replicates of seedlings were harvested destructively with their root system intact. The root length, shoot length, number of branches and collar diameter of each seedling were measured. Similarly the remaining replicates of seedlings were harvested at 60 days and 90 days after inoculation for growth measurements.

2.6 Tissue nutrient analysis

Constant weight (10 g) of cladophylls of *C. junghuhniana* treated with beneficial microbial inoculants was collected for tissue nutrient analysis. After drying in a hot air oven at 80℃ for 72 hr the cladophylls were assessed for nutrient contents of N, P and K. The total N was determined on kjeltec auto analyzer (1030), P determination was done by Vanadomolybdate Phosphoric yellow colour method and K content was determined by flame photo meter (Jackson, 1971).

2.7 Seedling quality index (SQI)

The seedling quality index of microbial inoculants inoculated seedlings was calculated according to Dickson *et al*. (1960) by using the following formula: SQI = [Total dry weight]/[(Height/Root collar diameter) + (Shoot dry weight/Root dry weighty)], where height and diameter were in cm and dry weight was in g $plant^{-1}$.

2.8 Statistical analysis

Each measured variable in the nursery experiment was subjected to analysis of variance and means were separated using Duncan's Multiple Range Test (SPSS ver. 10).

3 Results

3.1 Growth and biomass

Single and combined microbial inoculants inoculated seedlings showed their growth and biomass increased over un-inoculated control seedlings 30 days after inoculation. However, the number of branches did not increase over a 30-day period. Sixty days after inoculation the seedlings showed significant growth and biomass production after dual and triple microbial inoculants. Ninety days after inoculation it was observed that the growth and biomass production were increased 3 folds in *Frankia* + AMF + PSB inoculated seedlings over control (Table 1). The R/S ratio was significantly lower in this combination compared to single and dual inoculations. Number of nodules, nodule biomass, lateral roots and collar diameter were also significantly ($P < 0.05$) higher in *Frankia* + AM fungi + PSB inoculated seedlings compared to control and other treatments (Table 1).

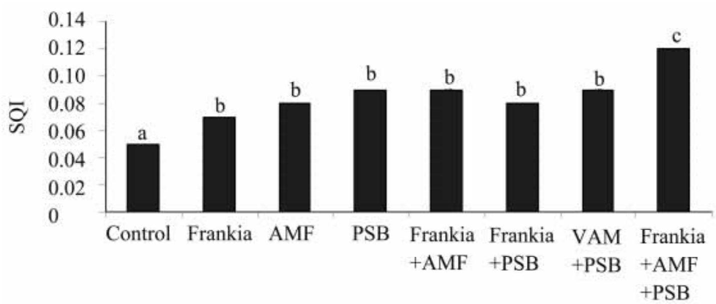
Table 1 Growth and biomass of *C. junghuhniana* under nursery conditions (90 DAI)

Sl. No.	Treatments	Collar diameter (cm)	Shoot length (cm)	Root length (cm)	Shoot dry weight ($g\ plant^{-1}$)	Root dry weight ($g\ plant^{-1}$)	No. of lateral roots $plant^{-1}$	No. of branches $plant^{-1}$	No of nodules $plant^{-1}$	Nodule biomass ($mg\ plant^{-1}$)	R/S Ratio
1	Control	0.803 a	71.12 a	22.10 a	4.233 a	2.111 a	11.65 a	4.23 a	–	–	0.49 c
2	*Frankia*	1.255 b	112.34 b	41.88 b	6.336 b	2.665 a	19.25 b	8.86 b	6.45 a	9.4 a	0.42 b
3	AMF	1.537 b	111.28 b	41.86 b	6.422b	2.265 a	18.93 b	8.78 b	–	–	0.35 b
4	PSB	1.567 b	118.24 b	42.22 b	7.321bc	2.698 b	18.96 b	8.65 b	–	–	0.36 b
5	*Frankia* + AMF	1.553 b	156.22 bc	44.56 b	9.124 c	3.358 b	21.69 b	9.84 b	8.34 b	11.2 b	0.36 b
6	*Frankia* + PSB	1.558 b	163.35 c	44.34 b	9.004 c	3.457 b	22.54 b	9.25 b	8.45 b	11.8 b	0.38 b
7	VAM + PSB	1.567 b	161.42 bc	43.40 b	9.052 c	3.205 b	22.84 b	9.47 b	–	–	0.35 b
8	*Frankia* + AMF + PSB	1.989 c	210.88 d	65.05 c	13.623 d	4.229 c	28.85 c	12.32 c	16.57c	20.5 c	0.31 a

Data was mean of 5 replicates; Means followed by same letters are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test; DAI = days after inoculation.

3.2 Seedling quality index

Seedling quality index was higher in the seedlings inoculated with *Frankia*, AM fungi and PSB either individually or in combination than in control seedlings (Fig. 1).

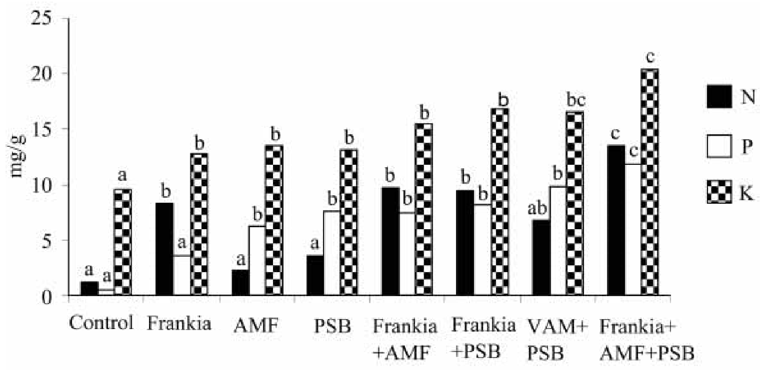


DAI: Days after inoculation

Fig. 1 Seedling quality Index (SQI) in *C. junghuhniana* inoculated with bioinoculants (90 DAI)

3.3 Nutrient status

Significant increase of N, P and K content were observed in these microbes inoculated seedlings over control seedlings. N, P and K contents were higher in *Frankia* + AM fungi + PSB inoculated seedlings than in control and other treatments (Fig. 2).



Means followed by same letters are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

Fig. 2 Nutrient status of *C. junghuhniana* seedlings inoculated with *Frankia*, AM fungi and PSB under nursery condition (90 DAI)

4 Discussion

This study has clearly shown that AM/PSB inoculation can improve the plant growth through increased P uptake whereas *Frankia* inoculation increased N uptake. Co-inoculation of AM and *Frankia* results in positive effect on *C. junghuhniana* growth through improvements in uptake of nutrients. Similar results were reported for dual root symbionts (AM + *Rhizobium* sp.) in legume species (Karthikeyan and Muthukumar, 2006). PSB are also immensely important as they have been reported to increase uptake of P by converting insoluble form to soluble ones (Sperber, 1998). They produce organic acids, phosphatases, chelating compounds and mineral acids that play a vital role in P solubilization (Singh *et al.*, 1989). The seedlings of *C. junghuhniana* inoculated with PSB showed better performance than the un-inoculated control seedlings. Previous study has already shown that growth, N and P accumulation in *C. equisetifolia* seedlings were enhanced by inoculation with effective *Frankia*, AM and PSB (Karthikeyan *et al.*, 2009). In *C. junghuhniana* the microbes *Frankia* + AM + PSB inoculated seedlings showed significantly improved growth, biomass and nutrient uptake which is the reason the combined inoculation always promote the plant growth in multiple ways particularly P and N uptake. Furthermore, multiple inoculations of *Frankia* + AM + PSB stimulated better activity of plants and these microbial inoculants interacted with each other to improve soil nutrients too (Karthikeyan *et al.*, 2009). Nodule number and nodule biomass were higher when *Frankia* was mixed with other microbial inoculants. This may be due to higher amount of nodular tissue concentration of hemoglobin and acetylene which were increased by AM and PSB (Daft and El Giahmi, 1975). An increased K status in microbial inoculants inoculated seedlings indicates K may act as enzyme activator that stimulates nodule activity (Barea *et al.*, 1987). Seedlings of *C. junghuhniana* inoculated with *Frankia*, AM and PSB resulted in decreased root to shoot ratio by increasing above ground production and possibly by reducing the need for below ground production since the function of nutrient acquisition is taken over by those beneficial microbes (Karthikeyan, 2005). The increased quality index of these microbial inoculants inoculated *C. junghuhniana* seedlings indicates the benefits of growth improvement and nutrient uptake derived by the seedlings through *Frankia*, AMF and PSB.

5 Conclusions

In this study the indigenous AM, *Frankia* and PSB increased the shoot and root growth in *C. junghuhniana* by providing essential levels of P and N under nursery conditions. It was understood that due consideration should be given to beneficial and essential microbes such as *Frankia*, AM fungi and PSB for the growth and nutrient improvement of *C. junghuhniana* in nursery practices.

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Characterization of Endomycorrhizal Strains from *Casuarina equisetifolia* to be Used for Reforestation and Rehabilitation of Degraded Ecosystems in Senegal

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Abstract In recent decades, the loss of ecosystem productivity has been accelerated in the Sahelian zone. This decrease is caused by human and natural effects, which result in deforestation and degradation of tropical lands. It has been demonstrated that fast-growing trees such as Australian species could promote the rehabilitation of degraded West African lands. Similarly, *Casuarina equisetifolia* an Australian native tree was established in Senegal. Casuarina plantation plays an important role in stabilizing coastal dunes, helping to protect adjacent agricultural areas by acting as a windbreak. *C. equisetifolia* develops nitrogen-fixing nodules in association with the soil actinomycete *Frankia* and a mycorrhizal symbiosis in association with arbuscular mycorrhizal fungi (AMF). It has been previously demonstrated that actinorhizal and AMF symbiosis enhance *Casuarina* species development. The symbiotic relationship between AMF and *Casuarina* species improves phosphate and nitrogen uptake. Fungal symbionts significantly improve the development of *C. equisetifolia*. The aim of this study was to assess the effects of fungal strains collected in Australia, the *C. equisetifolia* origin zone and strains collected in Senegal, where *C. equisetifolia* was introduced. *C. equisetifolia* plants were inoculated with Senegalese, Australian strains and/or *Frankia* bacteria *CcI*3. After growing in the greenhouse, plants were harvested and parameters like shoot and root biomass, and arbuscular mycorrhizal colonization were assessed. In parallel, we analysed the expression of marker genes encoding a mycorrhizal phosphate transporter, an hemoglobin gene, a marker gene involved in *Frankia* infection and a marker gene encoding the nitrogenase. We evaluated the activity of mycorrhiza using histochemical techniques. Our results showed that Senegalese strains have a positive effect on *C. equisetifolia* growth and this correlates with an increased expression of symbiotic marker genes and with increased metabolic

activity. Our work will help to select the most efficient fungal strains to be used for the rehabilitation of Senegalese soils.

1 Introduction

In West Africa, ecosystem fertility decreases every year. The degradation of the ecosystem is generally due to natural and/or anthropological effects. To restore soil fertility in these areas, applications of chemical fertilizers are often used. However, these chemical products are expensive and not ecofriendly. An alternative strategy to increase soil fertility is the use of symbiotic fungi and bacteria. These microorganisms improve the plant growth by enhancing the uptake of nutrients and water. Several Australian trees have been introduced to West Africa. They are characterized by early rapid growth even on poor and disturbed soils. It has been demonstrated that Australian species could promote the rehabilitation of degraded ecosystems. In Senegal, *C. equisetifolia* was established in the Niayes region through a reforestation programme that began in 1948. This plantation is localized in the North littoral of Senegal between Dakar (west of Senegal) and St Louis (north of Senegal). It plays an important role in stabilizing coastal dunes, helping to protect adjacent agricultural areas by acting as a windbreak. The Niayes region production contributes to about 65% of the national vegetable production (Ba, 1991). The rehabilitation programme of Sahelian zone was performed using tree species able to achieve a symbiotic relationship with mycorrhizal fungi and nitrogen-fixing bacteria. *C. equisetifolia* forms a symbiosis with ectomycorrhizal (EM) and arbuscular mycorrhizal fungi (AMF). Our study was carried out with arbuscular mycorrhizal fungi, which are obligate biotrophs being unculturable in the absence of plants host (Declerck *et al.*, 2005). AMF symbiosis is a symbiotic relationship between the plant and the fungus of the Glomeromycota phyllum (Schüssler *et al.*, 2001). This symbiosis is the most widespread and the oldest association dates back to the Devonian (Smith and Read, 2008).

AMF are associated with the roots of about 80% plant species. As a result of this symbiosis, the fungi deliver inorganic phosphate (Pi), nitrogen and other micronutrients, in turn they receive carbohydrates from plant (Smith and Read, 2008). AMF may consume up 20% of total net plant photosynthetic carbon (Jacobson and Rosendahl, 1990). In addition to their biological fertilizer role, AMF increase plants production and also confer resistance against abiotic and biotic stresses (Evelin *et al.*, 2009; Li *et al.*, 2010). Duponnois *et al.* (2003) demonstrated that the inoculation of *C. glauca* and *Allocasuarina verticilata* with *Glomus intraradices* increased plant shoot biomass 1.8 and 2.6 times respectively. *Casuarina* also forms a symbiotic relationship with the soil actinomycete *Frankia*. With their nitrogen-fixing ability *Casuarina* trees increase the nitrogen content in soil. Forrester *et al.* (2006) demonstrated that *Casuarina* contributes to N uptake and increases indirectly the amount of nitrogen available to non-fixing trees planted in mixed plantations. Similar results were obtained by Dommergues and Subba-Rao (2000), who have shown that the total biomass of non nitrogen-fixing *Eucalyptus robusta* was higher when planted in mixture with *C. equisetifolia* than when planted as pure species. In an attempt to restore degraded ecosystems in Senegal, *C. equisetifolia* was introduced in the Niayes region.

The aim of this work is to study the effects of Australian and Senegalese fungal inoculation on *C. equisetifolia* growth and characterize the basis of differences obtained by using molecular and histochemical markers.

2 Material and Methods

2.1 Plant, fungi and bacteria

Seeds of *C. equisetifolia* were collected in Notto Gouye Diama (north of Thies, Senegal). Australian fungi were collected in Queensland under casuarina stand and Senegalese fungi were collected under Bel-Air casuarina plantation (Dakar). Fungal inoculum was prepared by extracting spores from a maize trap over 3-month period. A sandy sterile soil was inoculated with the trap soil containing propagules. For the actinorhizal symbiosis, plants were inoculated with the *Frankia CcI*3. The experiment was carried out in greenhouse. After 6 months period, 8 plants for each treatment were harvested and parameters like shoot and root biomass and mycorrhizal colonization were assessed. Histochemical activities and molecular analyses were carried out with plant growing in greenhouse during 4 months using 3 pooled plants.

2.2 Histochemical activities

After harvested, viable mycorrhiza roots were gently washed with tap water. Three plants were pooled to evaluate the metabolic activity in mycorrhizal roots using histochemical techniques. All metabolically active AMF structures (Hyphae, vesicle, spores and arbuscules) were evaluated in roots by staining with Nitro Blue Tetrazolium (NBT) as described by Vierheilig *et al.* (2001; 2005). In order to evaluate the senescence of mycorrhizal structures, roots were stained with the Diaminobenzidine (DAB) as described by Vierheilig *et al.* (2001). The intensities of metabolically active AMF structures and clumped structures were assessed using Trouvelot method (Trouvelot *et al.*, 1986).

2.3 Expression of marker genes

Expression of molecular markers was used to compare the effect of Australian and Senegalese fungi using the half part of the 3 pooled plants and 3 replicates were carried out to analyse the expression of marker genes.

For each symbiosis, a marker gene was isolated. For the mycorrhizal symbiosis, we isolated the *CePT*4 gene encoded a phosphate transporter specifically expressed in mycorrhizal roots. As markers of the actinorhizal symbiosis we isolated *Ce*12, an ortholog of *Cg*12, a marker gene expressed specifically during *Frankia* infection (Svistoonoff *et al.*, 2003) and *CeHB*, an ortholog of *CgHb*, a symbiotic hemoglobin gene expressed in nodules (Jacobsen-Lyon *et al.*, 1995). Finally, we used the *Frankia* gene *NifH* encoding the nitrogenase (Normand *et al.*, 2007).

As an internal control gene, we isolated a gene coding an ubiquitin which is expressed constitutively. After harvesting, plant material was immediately frozen in liquid nitrogen and stored at - 80℃. The total RNA of mycorrhizal and nodulated roots was extracted using the ultracentrifugation method based on sedimentation of RNA through the cesium chloride as described by Chirgwin *et al.* (1979). RNA was purified using the Turbo DNAse- free™ to remove contaminating genomic DNA (Udvardi *et al.*, 2008). The reverse transcriptase and the Real-Time PCR were carried out as described by Gherbi *et al.* (2008). The results were standardized with *CeUbi* expression level (Hocher *et al.*, 2006).

3 Results

Our results showed that inoculation with Australian and Senegalese fungal strains significantly stimulated *C. equisetifolia* growth and mycorrhizal colonization in greenhouse conditions. The histochemical studies carried out with the mycorrhizal roots to evaluate the metabolic activity using enzyme such as succinate dehydrogenese (SDH) showed metabolically AMF structures active within roots. These observations are only possible when the roots were stained with the Nitro Blue Tetrazolium which reacts with the succinate dehydrogenase enzyme. Stronger metabolic activity in AMF was found within mycorrhizal plants roots inoculated with Senegalese fungi. Staining with the Diaminobenzidine allowed the visualization of clumped structures. A higher DAB activity was observed in roots which contained the lowest SDH metabolic activity AMF. In parallel, qPCR analysis showed a higher expression level of *CePT*4 in plants inoculated with Senegalese fungi compared to those inoculated with Australian fungi. The expression of marker gene involved in actinorhizal symbiosis showed that expression of *Ce*12, *CeHB* and *Nif* marker genes involved in the actinorhizal symbiosis was up regulated in mycorrhizal roots inoculated with Australian fungus. Co-inoculation with fungi and bacteria increased *Ce*12 and *Nif* expression in roots.

4 Discussion

Our study showed that inoculation with mycorrhizal fungi increased *C. equisetifolia* growth. This effect could be attributed to the fungus strains which improve nutrient uptake and directly increase *Casuarina* growth (Seddas *et al.*, 2009). Our results showed that the fungi inoculation increased the shoot biomass. Similar results were obtained by Rajendran and Devaraj (2004) who demonstrated that the inoculation with AMF increased the biomass and the growth of *C. equisetifolia*. This positive effect is probably due to the enhancement of shoot branching. The important branching could be attributed to an effectiveness of the mycorrhizal symbiosis which enhances the amount of nutrient uptakes by the fungus. It is well known that AMF with their phosphatase enzyme activities can enhance Pi availability for roots. Soluble Pi is directly absorbed and transported to the periarbuscular membrane by fungi before being released to the plant through a plant specific phosphate transporter.

The positive effect of fungal strains on *C. equisetifolia* development is also correlated with an increased metabolic activity in mycorrhizal roots staining with SDH. SDH is a tricarboxylic acid cycle enzyme present in AMF cycle and it reacts with the Nitro Blue Tretrazolium resulting in insoluble dark blue-purple formazan clearly distinguished in roots (Vierheillig *et al.*, 2005). Inoculation with Senegalese fungi showed the highest metabolic activity in roots; due to the presence of many viable arbuscules. It has been demonstrated that the amount of living arbuscules could reflect a surface area for nutrient exchange between living symbionts and symbiotic efficiency (Smith and Dickson, 1991). The efficiency of mycorrhizal symbiosis in plants inoculated with Senegalese fungi is also probably linked with the low number of senescent structures within the roots. In opposite, a higher DAB activity was observed in plants inoculated with Australian fungi and Vierheilig *et al.* (2001) demonstrated that their clumped structures are collapsed arbuscules detected through the presence of H_2O_2. Alexander *et al*. (1988) and Blee and Anderson (1996) have demonstrated that H_2O_2 induction occurred in the later stages of arbuscule life cycle when they degenerate. These results could explain the basis of differences obtained between plants inoculated either with Senegalese or Australian strains. Our results also suggest that the inoculation with Australian fungi and *CcI*3 results in an earlier arbuscule development which would cause their rapid degeneration compared to plants inoculated only with Australian fungi.

Since *CePT*4 encodes a symbiotic phosphate transporter, the higher *CePT*4 expression in mycorrhizal roots inoculated with Senegalese fungi would results in a higher level of Pi which is linked to the effectiveness of mycorrhizal symbiosis. In *Medicago truncatula*, this phosphate transporter is localized in the periarbuscular membrane and its expression is probably coordinated to arbuscular development and degeneration (Harrison *et al.*, 2002; Pumplin and Harrison, 2009). It has been demonstrated that optimal Pi absorption occurs in mature arbuscules (Pumplin and Harrison, 2009). In *C. equisetifolia*, *CePT*4 is also probably a marker of functional symbiosis and allows a continuous arbuscule development. Javot *et al*. (2007) showed that without this mycorrhiza specific phosphate transporter, arbuscules died prematurely. Recent studies showed that, the arbuscule lifetime is influenced by their ability to deliver phosphate (Javot *et al.* , 2007). The mutation of *MtPT*4 results in a premature degradation of arbuscules (Javot *et al.*, 2007) demonstrating a correlation between *MtPT*4 expression and the metabolic activity in roots. The post regulation of *CePT*4 expression obtained in mycorrhizal roots with Australian fungi, corresponding to a lower metabolic activity and a higher DAB activity within these roots may be related to a weaker effectiveness of the mycorrhizal symbiosis.

The expression levels of the molecular markers involved in actinorhizal symbiosis is higher in mycorrhizal roots inoculated with Australian fungus compared with roots inoculated with Senegalese fungi. Since the fungal inoculum was collected under *Casuarina* natural stand, inoculum from Australia (the native region) may be associated with more efficient bacteria. The efficiency of these *Frankia* induce a better actinorhizal symbiosis development and hence increase the expression of molecular markers involved in this symbiosis like *Ce*12, *CeHb* and *NifH*.

Despite these positive effects, the best *C. equisetifolia* development occurred in plants inoculated with Senegalese fungi. This result suggests that under the conditions tested the enhancement of the mycorrhizal symbiosis has a stronger effect on plant growth than the enhancement of the actinorhizal symbiosis.

5 Conclusion

Our results show that Senegalese fungi have higher positive effect on *C. equisetifiolia* growth probably because these strains are already adapted to the local conditions. The marker genes isolated allow study on the molecular basis of growth enhancement by AMF or *Frankia*.

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Development of an *in silico* Gene Bank for Plant Abiotic Stresses: Towards Its Utilization for Molecular Analysis of Salt Tolerant and Susceptible *Casuarina equisetifolia* Clones

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Abstract Worldwide, abiotic stresses represent the main cause of crop loss. Molecular marker and genetic engineering approaches are increasingly being used for breeding varieties with improved productivity in stressed sites. However, application of these techniques by Casuarina breeders requires understanding of the genetic determinants of salt tolerance. Gene isolation efforts in model systems have resulted in the identification of a large number of these polygenic determinants. Comparative genomics provide cost effective approaches for identification of gene homologues from Casuarina, a species widely planted in the coastal areas of tropics. To provide a one-point resource of gene sequences implicated in stress tolerance, a database of gene sequences implicated in abiotic stress tolerance was developed. The nucleic acid and protein sequence information for cellular signaling components, transcription factors, transporters, and those involved in protein and membrane protection were downloaded and analyzed for identification of conserved regions and deducing PCR primers that could be tested for isolation of gene homologues from salt tolerant tree species. The gene information for transcription factors and transporters were uploaded into a prototype WAMP environment. After validation, the comprehensive database will be web-enabled for access and editing by researchers. The database, together with the EST resource for nodules and non-nodulated roots of *C. glauca* developed at the Institut de Recherche pour le Développement (IRD), France, would facilitate identification of genes implicated in salt tolerance in *Casuarina equisetifolia*. Eighty-five clones of *C. equisetifolia* were assessed for their salt stress response and highly tolerant and susceptible clones were identified. While the most susceptible clones had a shoot to root ratio of sodium ranging from 1.5 to 3.6, in the tolerant clones it was

lesser than or around one indicating that the parameters could be considered as an important marker for screening of salt tolerant casuarina clones. These clones will be used for differential transcriptome analysis under salt stress.

1 Introduction

Salt tolerance is a polygenic trait. The determinants of salt stress tolerance, classified as "effector" molecules that are directly involved in stress adaptation, and "regulatory" molecules that control the amount and timing of effector molecules, and the genes implicated in these functions have been extensively reviewed (Nelson *et al.*, 1998; Hasegawa *et al.*, 2000; Grover *et al.*, 2001; Wang *et al.*, 2003; Bartels and Sunkar, 2005; Munns and Tester, 2008). The abundance of sequence information of transcripts implicated in abiotic stress responses in different plant species necessitates integration of available DNA and protein sequence information for enabling exhaustive bioinformatic analyses. Currently available websites on abiotic stresses include the *Generation Challenge Programme - Comparative Plant Stress Responsive Gene Catalogue* comprising protein sequence datasets (Wanchana *et al.*, 2008) and the *Plant Environment Stress Transcript Database* comprising transcript information from 16 crop species (Balaji *et al.*, 2006). Here we describe the development of an *in silico* gene bank specifically for nucleic acid and protein sequences implicated in abiotic stress tolerance in all plant species for which data is available. Wide variation in salt stress response has been reported in *Casuarina equisetifolia* clones. Eighty-five clones of *C. equisetifolia* were assessed, to identify highly salt tolerant and highly susceptible clones that differed in their shoot to root sodium ratio. These will be subject to differential transcriptome analysis for salt tolerance in Casuarina.

2 Materials and Methods

2.1 Retrieval of gene information and sequence analysis

Nucleic acid and protein sequence information for cellular signaling components, transcription factors, transporters (sodium transporters, potassium transporters, calcium transporters, proton transporters, water transporters), and those involved in protein and membrane protection (molecular chaperones, dimethyl sulfonium compounds, polyols, proteins-osmotin, ROS scavenging membrane fluidity) were downloaded into Excel spread sheets from NCBI website. ClustalW (Thompson *et al.*, 1994), the online multiple sequence alignment tool, was used for identification of conserved regions and deducing the relationship between the different species. Online software PriFi, which works with an alignment of DNA sequences from phylogenetically related species to provide a list of degenerate primer pairs that have a maximal probability of amplifying orthologous sequences in other phylogenetically related species, was used (Fredslund *et al.*, 2005).

2.2 Database development

Tables and their relationships were created for all the sequences downloaded after data normalization. Gene information was organized in Excel spread sheets for both amino acid and nucleic acid sequences with fields including gene name, locus name, sequence length, molecular type, modification date, IFGTB update, definition, accession number, version, GI, database source, taxonomic class, reference, authors, consortium, title, journal, pubmed, comment, method, IFGTB remark, features, gene, CDS, STS, miscellaneous, mRNA sequence, wet lab primers, product length, primer references, conserved regions, *in silico* primers, product length, RNAi targets, promoter, specific conserved elements, stress conditions, information about the function of the sequence. Gene information of transcription factors and transporters were used for the development of prototype *in silico* gene bank for plant abiotic stresses in WAMP environment.

2.3 Screening of salt tolerant *C. equisetifolia* clones

To identify *C. equisetifolia* clones substantially differing in their salt stress response, 85 clones obtained from the Casuarina germplasm collection of the Institute of Forest Genetics and Tree Breeding, Coimbatore, India (Jayaraj and Savio, 1998) were screened for their salt tolerance in hydroponic condition. Nine-month-old ramets rooted in vermiculite were used for the experiment. Each clone was represented by 28 ramets, of which 20 ramets were subjected to salt stress while 8 ramets were used as control. The clones were allowed to grow in Hoagland solution for two months after which the salt concentration was gradually increased at an increment of 50 mM each week from 50 mM to 500 mM. The clones were then left in same concentration for one month after which the salt concentration was increased to 550 mM. Data on the number of survived ramets and the number of needles were taken after the clones were subjected to a NaCl concentration of 200 mM, 400 mM and 500 mM. The percent difference in the number of needles in each clone relative to the control plants when the salt concentration was increased from 200 mM (4^{th} week) to 500 mM (10^{th} week) was calculated using the formula $[(B\text{-}A)/A \times 100]_{control}\text{-}[(B\text{-}A)/A \times 100]_{treated}$, where B is the average number of needles present in the clone after 10^{th} week and A is the average number of needles present in the clone after 4^{th} week following the start of the experiment. Needles were also collected after 200 mM, 400 mM and 550 mM NaCl treatment for sodium and potassium analysis by flame photometry. Sodium and potassium analyses were also carried out in roots after 550 mM NaCl treatment.

3 Results

3.1 Retrieval of gene information and sequence analysis

A total of 2,377 nucleotide sequences and 2,520 amino acid sequences were downloaded from NCBI (Sayers *et al*., 2010), Swiss-prot (Boeckmann *et al*., 2003) and Plant Transcription Factor database (Perez *et al*., 2009). The sequences were analyzed for identifying conserved regions. Based on the results of multiple sequence alignments, PriFi was used to design PCR primers for the phylogenetically related species.

3.2 Development of database

The total number of fields for nucleic acid and protein sequence information for the genes were 48 and 30 respectively. These fields were subdivided into 11 nucleotide and 4 protein subtables viz, Gene-NA 1 to 11 and Gene-AA 1 to 4 respectively. The "gene name" field common to all tables was used as a primary key. The database with information for transcription factors and transporters in WAMP environment using phpMyAdmin, dreamweaver software was used to develop the web pages. PHP scripts were included in the site to facilitate retrieval of information for sodium antiporter genes.

3.3 Screening for salt tolerant and susceptible clones of *C. equisetifolia*

Eighty five clones were screened for their salt stress response in hydroponic condition. The survival percentage (Fig. 1) and visual observation on the overall health of the plants were used to identify 17 highly salt tolerant and 14 susceptible clones. These 31 identified clones were analyzed for percent difference in the number of needles during salt stress (data not shown), and sodium and potassium content in needles and roots (Fig. 2 and Fig. 3). These data along with visual observation on the overall health of the plants were used to identify 5 most salt tolerant (TNIPT-4, TNKBM-407, APKKD-10, APVSP-14 and TNMT-2) and 6 most susceptible clones (PYN, JKCE-8, APVJM-33, TNPP-4, TNVM-3 and TNPV-2).

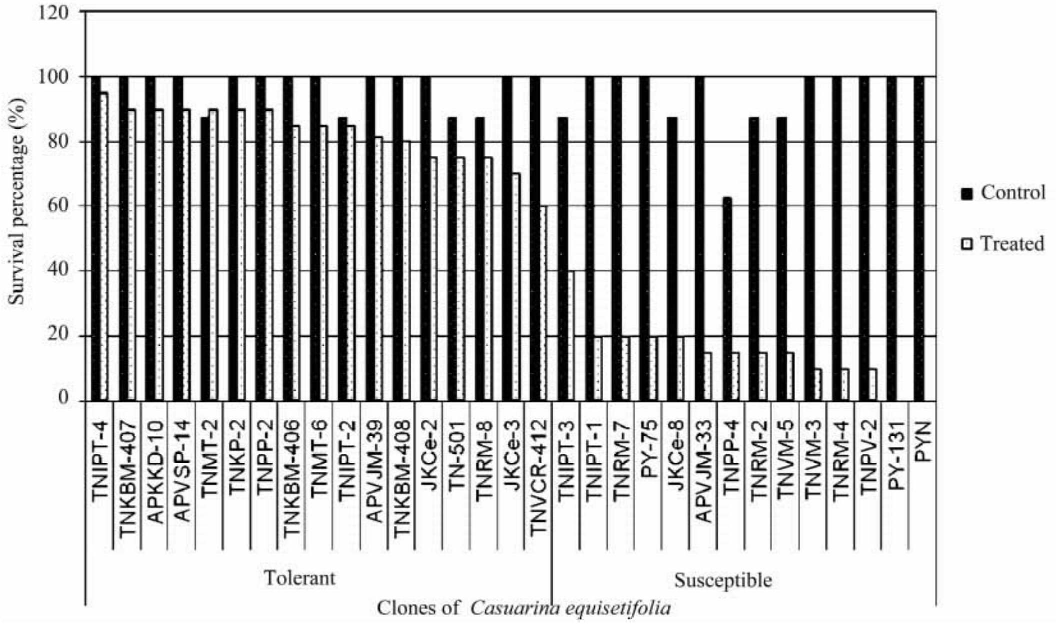

Fig. 1 Survival percentage of *C. equisetifolia* clones after 500 mM NaCl treatment

3.4 Effect of NaCl treatment on sodium concentration in shoots and roots

Analysis of sodium and potassium content in needles and roots collected after 550 mM NaCl treatment in the 31 clones (Fig. 2) revealed that the susceptible clones tended to accumulate more sodium in the shoots than in the roots in contrast to tolerant clones where in the shoot to root sodium was lesser than or around one.

4 Discussion

4.1 Database development

The database being developed is intended to be a one-point resource for molecular breeders for accessing information on protein and nucleotide sequences of different abiotic stress tolerance conferring genes. This database of abiotic stress tolerance would complement the EST database developed for nodules and non-nodulated roots of *C. glauca* at the Institut de Recherche pour le Développement (IRD), France (Hocher *et al*., 2006), for facilitating identification of gene homologues related to salt tolerance in *C. equisetifolia*. Differential transcript and functional analysis of these genes using the composite transgenic approaches (Gherbi *et al*., 2008) would provide information on the major genes contributing to salt tolerance in *C. equisetifolia*. The information would, therefore, provide inputs for development of candidate gene based markers in *C. equisetifolia*.

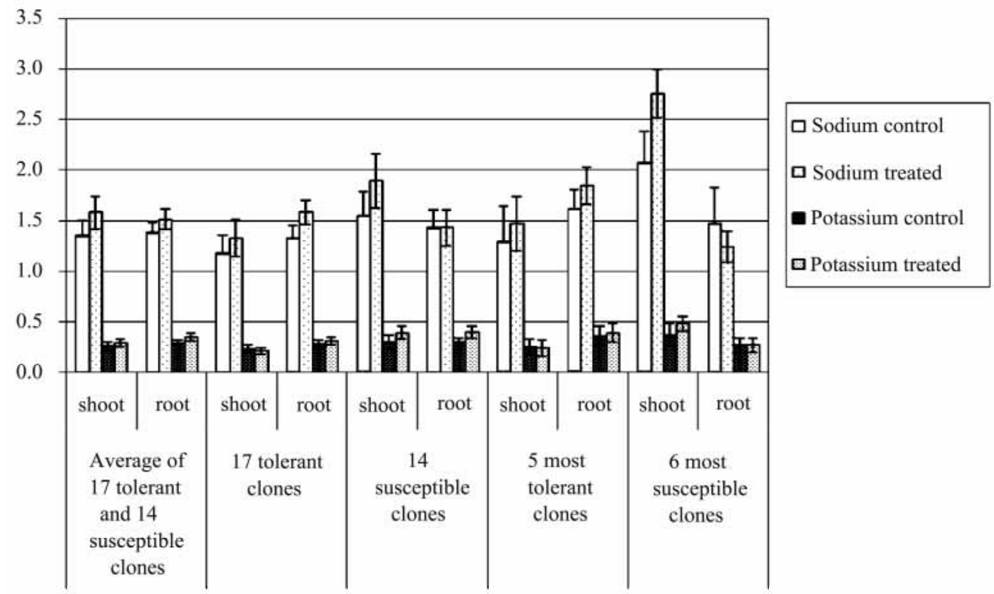

Fig. 2 The average sodium and potassium percentage in the shoots and roots of identified tolerant and susceptible *C. equisetifolia* clones at 550 mM NaCl treatment

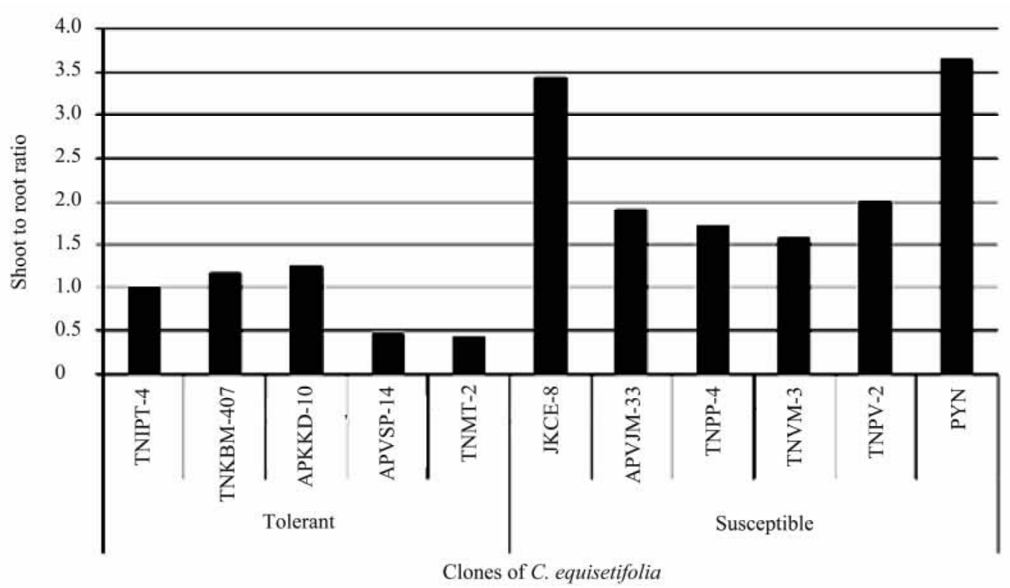

Fig. 3 The shoot to root ratio of sodium content of selected tolerant and susceptible *C. equisetifolia* clones

4.2 Effect of salt on survival of *C. equisetifolia*

Wide variation in the response of *C. equisetifolia* clones to salt stress has been observed (Balasubramanian, 2001). Further screening of the vegetative propagules from these clones at 340 mM NaCl stress (under hydroponic condition) identified a Casuarina clone able to survive for 30 days, while other clones failed to survive beyond 8 days. Altered protein profiles under salt stress were observed (Tripathi *et al.*, 2001). In the present study, 85 clones were assessed for their salt stress response and 17 highly tolerant and 14 highly susceptible clones were identified based on the percentage of survival and visual observations. The percent difference in the number of needles in each clone when the salt concentration was increased from 200 mM to 500 mM, the percent decrease in the needles calculated by comparing the difference in the number of needles in the treated and control plants (data not shown), the overall health of the plants and the sodium content in needles and roots were used to identify 5 most salt tolerant and 6 most susceptible clones (Fig. 3). The most susceptible clone PYN wilted at a salt concentration of 400 mM, while the most tolerant clone TNIPT-4 survived for 45 days post 550 mM NaCl stress. The tolerant clones could be good candidate clones for further testing in flooded coastal saline areas.

4.3 Effect of salt on sodium concentration in shoots and roots

In this study swelling of older needles was observed in few of the identified resistant as well as susceptible clones. No red phylloclades were observed. However, Dutt *et al.* (1991) reported that salinity stress caused higher levels of Na^+ accumulation in the older red phylloclades. Salt tolerance in Casuarina species have been attributed to a more prolonged intake of ions by older needles via the transpiration stream, reduced mobility into younger tissues (Greenway, 1962), greater compartmentation in the older tissues, or efficient retranslocation (Aswathappa and Bachelard, 1986). The sodium and potassium contents in shoots and roots were estimated (Fig. 2). When compared to the susceptible clones, the resistant clones accumulated lesser sodium in shoots and higher sodium in the roots. While the shoot to root sodium content was less than or close to one in resistant clones, in the case of susceptible clones, shoots accumulated 0.5 to 2.6 times more sodium than in the roots (equivalent to shoot to root ratio of 1.5 to 3.6) (Fig. 3). Studies by Clemens *et al.* (1983) and Aswathappa and Bachelard (1986) have also shown that highly salt tolerant species like *C. equisetifolia* and *C. glauca* exclude both Na^+ and Cl^- from their shoots. The species (*C. inophloia*, *C. stricta*, *C. instata* and *C. decaimeana*) that accumulated the highest concentrations of Na^+ and Cl^- in the shoot tip were those that suffered the greatest reductions in growth, shoot tip chlorosis and/or death, while more tolerant species like *C. equisetifolia* and *C. glauca* showed higher levels of Na^+ and Cl^- in the roots (Clemens *et al.*, 1983). Thus, sodium transporter homologues involved in xylem unloading, vacuolar compartmentation, Na^+ efflux and Na^+ influx may have significant roles in imparting salt tolerance in Casuarina. However, in this study, shoot to root ratio greater than 1.4 was observed in 4 of the 17 tolerant clones viz, TNKP-2 (1.4), TNPP-2 (1.6), TNIPT-2 (1.4) and TNKBM-408 (1.4). Similarly, shoot to root ratio lesser than 1 was observed in 6 of the 14 susceptible clones TNIPT-1 (0.9), PY-75 (0.4), TNRM-2 (0.6), TNVM-5 (0.9), TNRM-4 (0.9) and PY-131 (0.3). The tolerance or susceptibility in these clones may be attributed to other genetic determinants. The most tolerant and most susceptible clones identified in this study will be used for identification of sodium transporter gene homologues and the other genetic determinants contributing to salt tolerance.

5 Conclusions

The database on plant abiotic stresses would provide a ready reference of gene sequences implicated in stress tolerance, their conserved motifs, and consensus primer sequences for use in gene isolation and marker assisted selection in Casuarinas. The database after validation and testing would be hosted on the web and would complement the EST resource of *C. glauca* developed at the IRD. The study identified 5 most salt tolerant and 6 most susceptible clones that would serve as a base material for differential expression studies for identification of gene homologues, and development of molecular marker approaches. The identified tolerant clones could be good candidates for further testing in flooded coastal saline tracts. The higher root to shoot ratio of sodium could be considered as one of the markers for screening of salt tolerance in *C. equisetifolia*.

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Genetic Diversity of *Casuarina equisetifolia* Provenances

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Abstract *Casuarina equisetifolia* is an important tree species in the tropical and subtropical zones of Asia, the Pacific and Africa. In this study, 220 individuals of *C. equisetifolia* from seven native provenances and eleven introduced provenances (land races) were analyzed to assess the genetic variation and structure using amplified fragment length polymorphism (AFLP) markers. A total of 465 bands were obtained by eight primer pairs, among which 153 were polymorphic. The mean NEI's gene diversity $H = 0.2113$ calculated for the 18 provenances and the total gene diversity $H_T = 0.4065$ calculated for native provenances suggested abundant variation within provenances and species. The genetic information provided important implications for future conservation and breeding programmes of *C. equisetifolia*.

1 Introduction

Casuarina equisetifolia (L. Johnson) has two subspecies: subsp. *incana* and subsp. *equisetifolia* (Wilson and Johnson, 1989). The former occurs exclusively along the coast of Queensland and northern New South Wales of Australia and in Vanuatu. The latter has a wide distribution from throughout Malesia to northern Australia, Melanesia, and Polynesia. As a fast-growing and nitrogen-fixing tree of social, economic and environmental importance, it is commonly used in agroforestry systems, for soil stabilization, in coastal protection and rehabilitation. Though a littoral species in natural habitats it is well adapted to inland area, and has been introduced into Asia (especially China, India and Vietnam), East and West Africa, the United States of America, the Caribbean and some Middle East countries for more than a century. Provenances of *C. equisetifolia* exhibit considerable variation in growth characteristics, and grow on a variety of sites with very different annual precipitation and climatic conditions. Marked variation in growth and branching habit between different provenances, as well as individual trees within provenances, has been observed (Pinyopusarerk and House, 1993; Pinyopusarerk and Williams, 2000; Pinyopusarerk *et al*., 2004). However, very little information is currently available on the genetic variation of this species based on molecular assessment.

Information on the magnitude of genetic variation within a species is an integral part of conservation and improvement of genetic resources. Amplified fragment length polymorphism (AFLP), which can be used to analyze large numbers of loci distributed throughout the genome, has proven a powerful tool in the assessment of genetic variation both within and among plant provenances without detailed prior knowledge of DNA sequences (Brown *et al*., 1990; Vos *et al*., 1995; Jones *et al*., 1998). AFLP markers have been successfully used for analyzing genetic variation and genetic structure (Janssen *et al*., 1996; Barrett *et al*., 1998; Xue *et al*., 2005; Stefenon *et al*., 2006).

The main goal of this study was to survey the levels and patterns of genetic variation of *C. equisetifolia* provenances using AFLP markers. Furthermore, we compared the genetic diversity between native and introduced provenances. This work provides valuable information for further genetic conservation and breeding programme for *C. equisetifolia*.

2 Materials and Methods

2.1 Plant materials and DNA extraction

A total of 220 individual trees belonging to 18 provenances and land races were collected from an international provenance trial of *C. equisetifolia* subsp. *equisetifolia* established at Zhangzhou in Fujian province of China. Introduced provenances (land races) were included in the samples in order to determine the variation and genetic information for future management of these provenances since they have been identified as potential provenances in the south of China (Zhong *et al*., 2001). The geographic and ecological parameters of provenances are shown in Table 1. Genomic DNA of each tree was extracted from fresh branchlets using a modified CTAB extraction (Doyle, 1991).

Table 1 Information of 18 provenances of *Casuarina equisetifolia* subsp. *equisetifolia* investigated for AFLP variation

Pro. No.	CSIRO Seedlot No.	Location	Sample size	Latitude	Longitude	Altitude (m)
Natural provenances						
1	18153	Ela Beach, Papua New Guinea	15	09°05′ S	148°17′ E	10
2	18008	Darwin, Northern Territory, Australia	10	12°25′ S	130°50′ E	20
3	18357	Narra, Palawan, Philippines	10	09°19′ N	118°29′ E	10
4	18154	Aklan, Panay Island, Philippines	10	11°31′ N	122°30′ E	30
5	18348	Kuantan, Pehang, Malaysia	15	06°30′ S	99°45′ E	30
6	18298	Had Chao Mai, Trang, Thailand	10	07°33′ N	100°37′ E	2
7	18297	Ban Kam Phuam, Ranong, Thailand	15	09°21′ N	98°27′ E	10
Introduced provenances						
8	Local	Dongshan, Fujian, China	12	23°40′ N	117°28′ E	4
9	18128	Non Nuoc, Vietnam	10	16°06′ N	106°20′ E	2
10	18086	Hai Thinh, Ha Nam Ninh, Vietnam	13	20°22′ N	106°21′ E	1
11	18152	Ninh Chu, Ninh Thuan, Vietnam	10	11°33′ N	108°59′ E	2
12	18015	Chandipur, Balasore, Orissa, India	15	21°30′ N	86°54′ E	2
13	18014	Balukhanda, Orissa, India	15	19°50′ N	85°53′ E	10
14	18120	Chengai Anna, Tamil Nadu, India	10	12°36′ N	79°48′ E	50
15	18119	Rameswaram, TamilNadu, India	10	09°15′ N	79°20′ E	5
16	18288	Madagama, Sri Lanka	15	08°06′ S	80°15′ E	80
17	18287	Hambantota, Sri Lanka	10	06°08′ N	81°07′ E	16
18	18355	Cotonou, Benin	15	06°23′ S	02°13′ E	8

2.2 AFLP analysis

AFLP analysis was carried out by the method of Vos *et al*. (1995) with some modifications. Approximately 300 ng of total genomic DNA per samples were digested with *Eco*RI and *Mse*I and then ligated with *Eco*RI adaptor and *Mse*I adaptor. Of the total 64 primer combinations with three selective bases screened in a preliminary test, eight primer combinations (Table 2) found to give good amplifications were selected for further study. Following the selective amplification, the reaction products were mixed with an equal volume of loading buffer (98% formamide, 10 mm EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). The mixtures were denatured for 5 min at 94℃ then directly placed on ice. About 6 μl of the mixtures were run in 6% (v/v) polyacrylamide gels with a DNA sequencer. The gels were silver stained using the protocol published by Streiff *et al*. (1998).

Table 2 Number of fragments, number of polymorphic fragments, percentage of polymorphic fragments, and size of fragments for each primer pair used for AFLP analysis in *Casuarina equisetifolia*

Primer pairs	Number of fragments	Number of polymorphic fragments	Percentage of polymorphic fragments	Size of fragments (bp)
M-CAC/E-ACT	45	19	42.22	75-600
M-CTA/E-AAG	80	24	30	50-620
M-CTA/E-ACA	65	23	35.38	115-820
M-CTC/E-AAC	60	25	41.67	40-530
M-CTC/E-ACT	50	20	40	40-375
M-CTG/E-AAG	48	22	45.83	100-400
M-CTT/E-ACC	64	9	14.06	40-680
M-CTT/E-AGC	53	11	20.75	102-410
Total	465	153		
Average	58.13	19.13	32.90	

2.3 Genetic analysis

Each AFLP band of unambiguous pattern was given a score of 1 for presence or 0 for absence across all the polymorphic loci to create a binary matrix. POPGENE, version 1.31 (Yeh *et al*., 1999) was used to calculate genetic parameters within the 18 sampled provenances, including the number of polymorphic loci (*NP*), percentage of polymorphic loci (*P*), observed number of alleles per locus (*N*a), effective number of alleles per locus (*N*e), gene diversity (*H*) (Nei, 1973), Shannon's information index (*I*) (Lewontin, 1972). The total gene diversity (H_T) within species and within provenances (H_S) were calculated only for native provenances assuming Hardy-Weinberg equilibrium.

3 Results

The amplified results by each primer pair were shown in Table 2. Eight primer pairs revealed a total of 465 DNA fragments ranging from 40 bp to 820 bp, among which 153 (32.90%) of these bands were polymorphic loci either among or within provenances. Primer pairs were significantly different in their capacity to detect polymorphisms.

The genetic parameters for each of 18 *C. equisetifolia* provenances and land races summarized in Table 3 indicated a tendency for higher genetic variation of the Ela Beach native provenance in Papua New Guinea and Balukhanda, Orissa introduced provenance in India than other seed sources.

The local Dongshan, Fujian provenance showed very low level of variation (P = 13.07% and H = 0.0472). The overall mean gene diversity (H) was 0.2113 and Shannon information index was 0.3109. The POPGENE analyses for native provenances showed an abundant diversity ($H_T = 0.4065 \pm 0.0128$) at the species level, partitioning which $H_S = 0.2139 \pm 0.0093$ distributed within provenances and the rest among provenances.

Table 3 Genetic variation parameters of *Casuarina equisetifolia* provenances detected by AFLP markers

Pro. No.	*NP*	*P*	*N*a	*N*e	*H*	*I*
1	117	76.47	1.7647	1.4569	0.2669	0.3990
2	62	40.52	1.4052	1.2651	0.1533	0.2263
3	87	56.86	1.5686	1.3701	0.2125	0.3140
4	91	59.48	1.5948	1.4481	0.2466	0.3568
5	87	56.86	1.5686	1.4328	0.2369	0.3411
6	72	47.06	1.4706	1.3066	0.1737	0.2561
7	104	67.97	1.6797	1.3515	0.2077	0.3175
8	20	13.07	1.1307	1.0817	0.0472	0.0701
9	85	55.56	1.5556	1.4030	0.2248	0.3266
10	92	60.13	1.6013	1.3307	0.1972	0.2986
11	91	59.48	1.5948	1.4258	0.2359	0.3430
12	99	64.71	1.6471	1.4026	0.2328	0.3458
13	105	68.63	1.6863	1.5186	0.2854	0.4121
14	81	52.94	1.5294	1.3909	0.2186	0.3173
15	80	52.29	1.5229	1.3261	0.1871	0.2774
16	93	60.78	1.6078	1.4113	0.2355	0.3460
17	97	63.40	1.6340	1.4316	0.2457	0.3609
18	78	50.89	1.5098	1.3475	0.1963	0.2878
Mean	85.61	55.95	1.5596	1.3723	0.2113	0.3109

The mean values were calculated across provenances. *NP*, number of polymorphic loci; *P*, percentage of polymorphic loci; *N*a, observed number of alleles per locus; *N*e, effective number of alleles per locus; *H*, NEI's gene diversity; *I*, Shannon's information index.

4 Discussion

This study revealed that a large amount of genetic variation in *C. equisetifolia* at provenance level (both native and introduced sources) and at species level. The only exception was local land race from Dongshan, Fujian. The mean gene diversity (0.2113) was higher than 0.1473 reported by Ho *et al*. (2002) for 12 *C. equisetifolia* provenances assessed by RAPD. This may be attributed to the different provenances used in the two studies and more gene loci be detected by AFLP markers. The average Shannon's information index (0.3109) and the total gene diversity (0.4065) were also higher than many other plants such as *Picea asperata* (0.227 and 0.237, respectively), based on AFLP (Xue *et al*., 2005).

The present results showed genetic variation among provenances (46.07%) was a little higher than Ho's study (39.28%) for 12 *C. equisetifolia* provenances. However, there was a similar tendency of high proportion of total variation among provenances tested by the two different markers. Such results are in contrast with expectations for woody, perennial, predominantly outcrossed species, which maintain most variations within provenances (Hamrick and Godt, 1990). However, this may be due to the small sample size in the study. High variation among provenances was also found in another tropical tree, e. g. 43% in *Tectona grandis* (Shrestha *et al*., 2005) by AFLP. Higher variations among provenances (51.53%) have been detected in *Syringa oblate* (Ming and Gu, 2006), *Moringa oleifera* Lam. (59%, Muluvi *et al*., 1999) and *Euterpe edulis* Mart. (57%, Cardoso *et al*., 2000) based on AFLP. The presence of such a high genetic variation among provenances could be attributed to the features of the wide distribution range of this species and the habitats where the sampled provenances occur. Its wide distribution is scattered through many islands, and therefore geographical isolation may occur.

It is interesting to find out that an introduced provenance Balukhanda, Orissa, India recorded the highest in genetic variation, reflecting the important implications on the introductions into this site. Multiple seed sources could have been collected to establish this plantation. Similarly, presumption that introduced provenances may have been derived from multiple seed sources can be seen from the higher variation (two Orissa provenances in India, two Sri Lanka provenances) than some native provenances.

5 Conclusions

Using AFLP markers this study showed abundant variation in *C. equisetifolia* within provenances and species. The genetic information provided important implications for future conservation and breeding programmes of *C. equisetifolia*.

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In vitro Studies in *Casuarina* Species

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Abstract Research and development work was carried out on *Casuarina equisetifolia* and *C. junghuhniana. C. equisetifolia* studies were carried out on development of micropropagation protocol, mycorrhizal association with tissue culture raised propagules and morphogenetic potential of different explants *in vitro*. A micropropagation protocol for rapid multiplication has been standardized using explants from mature trees over 30 years old. Extensive work has been carried out on standardizing the season of collection, type of explants, sterilization treatment, basal media and growth regulators. Axillary proliferation and elongation of cladodes have been obtained. These cladodes were rooted and plantlets were successfully hardened. Morphogenetic studies were carried out using different explants such as immature fruits, male flowers (catkins), seeds, seedling explants and young needles on various basal media with or without plant growth regulators. Successful callusing has been obtained from seeds, seedling explants and young needles. The callus developed from young needles formed cladodes. Work done on the mycorrhizal association with tissue culture raised plants in *C. equisetifolia* using mycorrhizal infected roots (MIR) entrapped arbuscular mycorrhizae fungi (AMF) in calcium alginate and inoculation of local *Frankia* strain have resulted in the increased plant survival and higher biomass. It has been concluded that *Glomus mosseae* is the efficient AMF strain for better survival rate of micropropagated plants. Further studies on micronutrient assimilation have revealed increased elemental uptake resulting in increased survival and better performance of plants. In *C. junghuhniana* work on *in vitro* germination of seeds and axillary bud proliferation was successfully carried out. Further studies on standardising a micropropagation protocol for this species are in progress.

1 Introduction

In recent times casuarinas are emerging as evergreen multipurpose agro-forestry species providing a wide range of social, economic and environmental benefits. *Casuarina equisetifolia* is cultivated widely in many countries accounting for about 1.4% of tree plantations of the casuarinas (FAO, 1995). It is widely used for sand stabilization and rehabilitation as shelter belts. The distribution, biology, economic importance and ecological benefits of *Casuarina* species have been reviewed (Parotta, 1993; Zhong *et al*., 2010). In India, major effort is directed towards production of improved planting stocks through selection of superior performers from plantation/provenances and their vegetative propagations (Yashoda *et al*., 2004).

In vitro propagation using tissue culture method is important for the rapid multiplication of selected clones/superior trees. Tissue culture has facilitated propagation of hybrids and difficult-to-root clones of *Casuarina* species. *In vitro* regeneration is of significance for genetic transformation studies. Tissue culture studies have been carried out in casuarinas since 1980s (Duhoux *et al*., 1986; Abo El-Nil, 1987). Genetic transformation studies have also been reported (Franche *et al*.,1994; Le *et al*., 1996). The explants used were from epicotyls and root of seedlings, mature male inflorescence and female flower buds. Protocol for micropropagation of mature tree derived explants has also been developed (Rupali Seth *et al*., 2006; Kendurkar *et al*., 2007).

In this paper an attempt has been made to the work done on (i) development of micropropagation protocol for selected superior clones from mature tree derived explants (ii) regeneration studies using young branchlets as explants from five-year-old trees, and (iii) mycorrhizal association with tissue culture raised propagules of *C. equisetifolia* at our laboratory.

C. junghuhniana is a suitable species for planting as windbreak, reclamation of degraded soils, especially landslide-prone soils. It is emerging as a species of choice by smallholder farmers in. India. However, *in vitro* studies have not been reported in this species. This paper reports the studies on *in vitro* germination of seeds and establishment of shoot cultures that were carried out in *C. junghuhniana*.

2 Materials and Methods

2.1 Development of micropropagation protocol

2.1.1 Plant material collection

Fresh green healthy twigs from 30 years old *C. equisetifolia* and *C. junghuhniana* trees were collected from different locations in Panchgani (Maharashtra), Umargaon Forest Department (Maharashtra), National Chemical Laboratory, Pune (Maharashtra) and in Dandeli (Karnataka), respectively. The plant material was collected throughout the year to standardise the best season. The plant material was processed and sterilized using the protocol reported earlier by Rupali Seth *et al*. (2006).

2.1.2 Establishment, elongation, multiplication and rooting of cultures

Different types of basal media were used (Murashige and Skoog's media (MS), 1962; Nitsch 1969; Gamborg's media (B5), 1968; Gupta and Durzan media (DCR), 1985; Woody Plant Medium (WPM), 1980) either alone or in combination with kinetin and benzyl adenine (BA). Various rooting hormones like naphthalene acetic acid (NAA) and indole butyric acid (IBA) were tested for rooting of elongated branchlets. The rooted plants were transplanted successfully, hardened in soil mixtures as reported earlier by Rupali Seth *et al*. (2006).

2.2 Studies of *C. junghuhniana in vitro* germination

The seeds were collected at Dandeli Karnataka; surface sterilized and inoculated on MS media full and half strength, Gupta and Durzan media full and half strength. The culture tubes were incubated in dark and shifted to light after emergence of plumule. Nodal explants were collected at Dandeli Karnataka, surface sterilized and inoculated on different basal media with or without growth regulators.

2.3 Regeneration studies of *C. equisetifolia*

Young branchlets of *C. equisetifolia* were collected from five-year-old trees growing in NCL and Pune garden. They were surface sterilized using protocol reported earlier by Rupali Seth *et al.* (2006). The segments were transferred on callus induction medium, mainly MS medium supplemented with 0.54, 1.07, 1.61, 2.14, 2.69 μM NAA, 0.45, 0.90, 1.35, 1.81, 1.26 μM 2, 4 D and 0.44, 0.88, 1.33, 1.77, 2.22, μM BA.

The callus was shifted to various media containing different concentrations of BA. The callus was further transferred to MS and DCR medium with lower concentrations of BA and NAA for regeneration of shoots.

2.4 Studies on mycorrhizal association

Tissue culture raised plants with fully developed cladodes and roots having uniform growth were selected for mycorrhizal association.

The AMF spores isolated from rhizospheric soil of native *C. equisetifolia* plants were mass multiplied on *Eleusine coracana* roots. The AM fungi colonized roots were further used as inoculum. The root inoculum was surface sterilized as described by Boody *et al.* (1999). The root pieces were encapsulated in calcium-alginate using the method described by Declerck *et al.* (1999). The tissue culture raised plants were transplanted in the plastic bags containing sterile sand and MIR entrapped Ca-alginate beads. The control set was inoculated only in sterile sand. After 30 days the plants were transferred to polybags containing 10 gm of mass multiplied AM fungi inoculum (root and rhizospheric soil) in autoclaved sand : soil (3 : 1) mixture. This inoculum also contained native *Frankia* infected roots of *C. equisetifolia* plants. The morphological observations were noted at 60 and 90 days. Samples from these plants have been tested for elements like iron, copper, zinc and magnesium by ICP-spectrometric method.

3 Results and Discussion

3.1 Development of micropropagation protocol

Extensive studies carried out on standardizing season of explant collection, type of explants, sterilization treatment, basal media and growth regulators, have resulted in development of micropropagation protocol for rapid multiplication of *C. equisetifolia* using explants from mature trees over 30 years old. It was observed that March to May was the best period to obtain higher sprouting response accompanied with low microbial infestation.

Among different media tested DCR basal medium was more effective in establishment and axillary bud proliferation. BA (Benzyl adenine) at 1 mg L^{-1} resulted in obtaining higher axillary bud sprouting. IBA 2 to 4 mg L^{-1} pulse treatment for 48 h resulted in higher percentage of root formation, in micropropagated shoots. The rooted plants could be successfully transplanted to soil. The plant material collected from the National Chemical Laboratory gave best response.

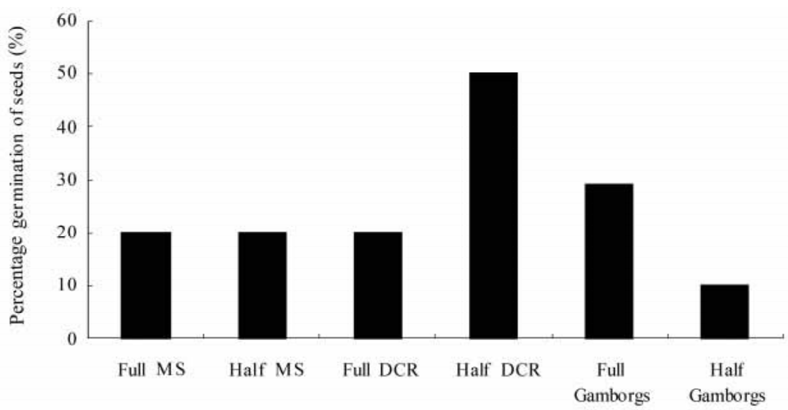
3.2 Regeneration studies in *C. equisetifolia*

Young branchlets from 5-year-old trees formed globular callus on MS basal media supplemented with NAA, 2-4 D and BA. Callus induction was slow on medium containing NAA or 2, 4 D whereas rapid induction and growth of callus was obtained on medium containing BA only. This callus on transfer to MS supplemented with NAA and BA turned hard globular and after several passages on this medium. Regeneration of shoots was obtained on half strength DCR supplemented with BAP.

3.3 Mycorrhizal association

Studies carried out on tissue culture raised plants of *Casuarina* using MIR entrapped AM fungi in Ca-alginate and local *Frankia* strain resulted in higher plant survival, growth enhancement by 2-3 folds and increase in biomass (Prabhu *et al.*, 2007). The elemental uptake studies revealed an increase in elemental uptake.

Studies on standardizing sterilization method and basal media for *in vitro* germination of seeds have shown that highest percentage of germination was obtained on half DCR medium (up to 50%) followed by full Gamborg's medium 30% germination (Fig. 1). The preliminary work on establishment of cultures using explants from mature *C. junghuhniana* has resulted in successful axillary bud sprouting and *in vitro* proliferation. Half DCR medium was found to be best for highest sprouting response (42%) (Fig. 2).



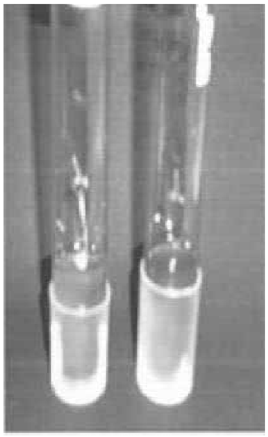


Fig. 1 Effect of basal medium on germination of seeds of *Casuarina junghuhniana*

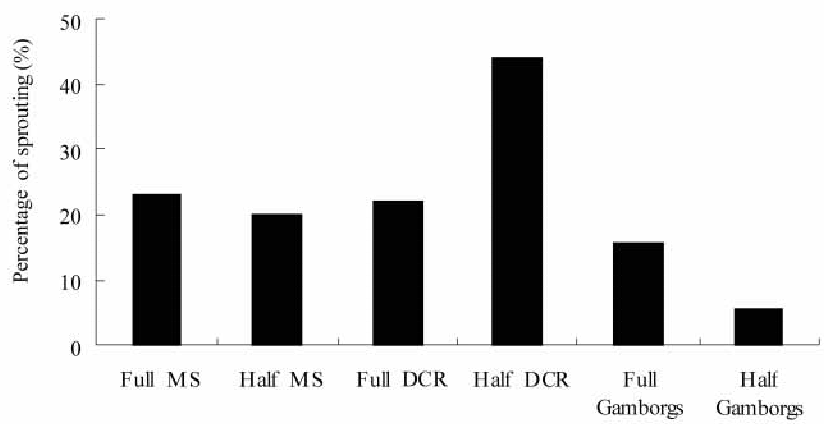


Fig. 2 Effect of basal medium on axillary bud sprouting of *Casuarina junghuhniana*

4 Conclusions

This paper reports successful development of micropropagation protocol for multiplication of *C. equisetifolia* mature trees over 30 years old. The increased plant survival, biomass, and elemental uptake in tissue culture raised propagules were obtained in association with Ca-alginate entrapped *Glomus mossae* and *Frankia*. The protocol can further be extended for the propagation of selected clones of *C. equisetifolia* with required modifications. The protocol needs to be fine tuned for other casuarina species.

Regeneration of shoots from calli emerging from young branchlets of *C. equisetifolia* over 5 years old is an important study which will form the basis for genetic transformation studies in this recalcitrant species. However, the protocol takes a long time from callus to shoot regeneration (9 months). Therefore extensive studies are needed to refine this protocol for improvement of regeneration efficiency.

In *C. junghuhniana* which is fast coming up as a species of choice for large scale plantations, studies on *in vitro* germination and successful establishment of shoot cultures using explants from mature trees are important in view of developing micropropagation protocol for this species. As far our knowledge goes this is the first report on tissue culture studies on *C. junghuhniana*.

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Genetic Transformation of *Casuarina equisetifolia* by *Agrobacterium tumefaciens*

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Abstract Different seedlots of *Casuarina equisetifolia* and media compositions were studied to improve callus regeneration and genetic transformation. The explants included epicotyl fragments from 45-day-old seedlings and the biological vector to achieve gene transfer was based on the disarmed *Agrobacterium tumefaciens* strain C58C1 (pGV2260) containing the β-glucuronidase (GUS) or green fluorescent protein (GFP) reporter genes under the control of the constitutive 35S promoter. Following a selection on kanamycin, GUS or GFP expression was observed in *C. equisetifolia* calli and young shoots, thus suggesting a successful transfer of the reporter genes into the host plant.

1 Introduction

The family Casuarinaceae contains 4 genera and 96 species native from Australia to the islands of the Pacific and to Southeast Asia (Wilson and Johnson, 1989). Casuarinaceae includes important tree species with good potential for afforestation in subtropical and tropical areas (Midgley *et al*., 1983). *Casuarina equisetifolia* has been extensively cultivated in the inland and coastal areas of southern China (Zhong and Bai, 1996; Zhong and Zhang, 2003). Several *Casuarina* species have been *in vitro* regenerated, including *C. equisetifolia* (Duhoux *et al*., 1986), *C. glauca* (Le *et al*., 1996; Santi *et al*., 2003) and *Allocasuarina verticillata* (Cao *et al*., 1990; Franche *et al*., 1997). The natural susceptibility of members of the Casuarinaceae family to *Agrobacterium tumefaciens* has previously been used to develop gene transfer procedures for both *A. verticillata* (Franche *et al*., 1997) and *C. glauca* (Diouf *et al*., 1995; Franche *et al*., 1998; Smouni *et al*., 2002). A number of physiological and environmental factors, such as the presence of plant phenolic compounds, sugars, pH, temperature and osmoprotectant compounds, influence the induction of virulence genes and, consequently, the efficiency of T-DNA transfer from *A. tumefaciens* to the wounded plant cells. Previous experiments performed with *C. glauca* showed that optimal transformation rates were obtained when epicotyls from 45-day-old seedlings were co-cultivated for three days at pH 5.6 with the *A. tumefaciens* strain C58C1 (pGV2260) (Le *et al*., 1996).

In this study we report the induction of organogenic calli from epicotyls of *C. equisetifolia* and the genetic transformation of this actinorhizal species by *A. tumefaciens*. Calli and young shoots expressing the reporter genes GUS or GFP were obtained. These preliminary data pave the way for the genetic transformation of this environmentally important tree species.

2 Material and Methods

2.1 Seed source

The seeds of *C. equisetifolia* were collected in four locations: HA4, Huian Forest Farm, Fujian, China; HA3, Huian, Fujian, China; TH5, native forest in Thailand; and DS5, Dongshan, Fujian, China.

2.2 Disinfection and seedling production

Chemical scarification was carried out by soaking the seeds in concentrated H_2SO_4 (98%) for 2.5 min and subsequently rinsing with tap water for 30 min. The scarified seeds were then disinfected with 5% calcium hypochlorite for 35 min, rinsed 3 times with sterile distilled water, and germinated in Petri dishes containing H medium solidified with 8 g L^{-1} Difco-Bacto agar as previously described (Le *et al*., 1996).

2.3 Experimental design

Four experiments, referred as Exp. 1-4, were carried out in the laboratory of Institut de Recherche pour le Développement (IRD), Montpellier, France. Plant material was grown in a chamber at 27 ± 1℃ with a 16-h photoperiod (45-60 μmol m^{-2} s^{-1}), and growth media were renewed every 3 weeks.

The objectives of the experiments were the followings: Exp. 1: Callus induction on epicotyls from the four seedlots of *C. equisetifolia* grown on MSC (half-strength MS salts and NN vitamins) (Murashige and Skoog, 1962; Nitsch and Nitsch, 1969; Le *et al*., 1996), treatments were described in Table 1; Exp. 2: Effect of 6-benzylaminopurine (BA), naphthaleneacetic acid (NAA) and $AgNO_3$ on the organogenic potential of the calli induced from epicotyls, treatments were described in Table 2 with seedlot H 4; Exp. 3: Genetic transformation by *A. tumefaciens* of the four *C. equisetifolia* seedlots, treatments were described in Table 4; Exp. 4: Genetic transformation of *C. equisetifolia* seedlot HA4 by *A. tumefaciens* containing the binary vectors pBIN-35S-GUS-INT or pBIN-35S-GFP, treatments were described in Table 5.

Experiments were carried out in Petri dishes (10 cm in diameter) containing 12-32 epicotyl segments per petri dish, and epicotyl segments were around 1.2-1.5 cm in length. In the Exp. 3, 1688 epicotyl segments from the four *C. equisetifolia* seedlots were genetically transformed with C58C1 (pGV2260) containing pBIN-35S-GUS-INT (Table 4). Exp. 4 included 298 epicotyl segments cocultivated with C58C1(pGV2260; pBIN-35S-GFP5ER) and 354 explants genetically transformed with C58C1(pGV2260; pBIN-35S-GUS-INT) (Table 5). In the Exp. 3 and Exp. 4, selection of transgenic cells was achieved with the addition of 25 mg L^{-1} kanamycin in the nutrient medium for two weeks; kanamycin concentration was then raised to 50 mg L^{-1} as previously described (Le *et al*., 1996; Franche *et al*., 1997). The MSC nutrient medium consisted half-strength MS medium salts (Murashige and Skoog, 1962), NN medium (Nitsch and Nitsch, 1969) vitamins, and 30 g L^{-1} sucrose.

2.4 Data collection and treatment

All data were collected 2 months after culture. Calli with a diameter of at least 3 mm were counted Exp. 1 and Exp. 2. CN refers to "callus number" and the callus induction rate was calculated as follows: (%) = 100 ×(segment number with at least 1 callus /total segments per replicate(Petri dish), in order to get standard and comparable data. Each replicate will get a figure to use for Analysis of Variance. Additional observations for Exp. 1 and Exp. 2 included TCS (top callus size, mm), BCS (basal callus size, mm), BN (bud number), RN (root number) and RL (root length, mm) and the CS (mean callus size per segment, mm) was calculated as follows SQRT(TCS^2 + BCS^2). In Exp. 3 and Exp. 4, we observed indexes based on per Petri dish: KCN = kanamycin resistant callus number; ESN = epicotyl segment number with at least one callus; TSN = total segment number per Petri dish. The genetic transformation efficiency was determined as (GTE,%), with GTE_1 = (KCN/TSN) × 100%, and GTE_2 = (ESN/TSN) × 100%. All the count number data was exchanged by SQRT(x) (note: x means that CN, KCN, ESN or TSN in the experiments) and genetic transformation efficiency data was exchanged by ARSIN (SQRT (GTE/200))/ 3.1415926 ×2 ×90 before running GLM model for Analysis of Variation, and Duncan's Multiple Range (DMR) Test.

3 Results

3.1 *In vitro* culture

Significant differences were observed in callus induction among the four *C. equisetifolia* seedlots ($P < 0.01$) (Table 1). Seedlot HA4 gave the best results for callus induction with a rate of 97.8% and seedlot DS5 reacted poorly to callus induction with 10.7% of calli induced on the epicotyls. Seedlots HA3 and TH5 gave an intermediary response with respectively 48.8% and 54.2% of callus induction.

Table 1 Callus induction rate (%) on epicotyls fragments excised from four different seedlots in the Exp. 1

Design and treatment			Mean callus induction rate (%) at 2 months culture
Seedlot No.	Replicate (No. of PD)	Number of epicotyl segments	
HA4	6	101	97.8[a] ± 1.4
HA3	4	61	48.8[b] ±21.9
TH5	9	185	54.2 [b] ±12.3
DS5	3	39	10.7[c] ± 3.1

Data were recorded two months after callus induction. PD = Petri dishes. Data was the means of 3-9 repetitions ± STD (Standard Error). Means with the same letter are not significantly different ($P < 0.01$), according to Duncan's Multiple Range Test.

3.2 Effect of BA, NAA and $AgNO_3$ on regeneration

Table 3 gives the details of the Duncan's Multiple Range Test for the 7 indexes. There were no significant differences in basal callus number and callus size among the 5 treatments R1-R5 described in Table 1 ($P < 0.05$ or $P < 0.01$); on the opposite, there were significant differences in top callus number, size, colour, and organogenic potential (bud number, root number and root length) among the 5 treatments ($P < 0.05$ or $P < 0.01$). Concerning the top callus, the R4 and R5 media was significantly more favourable to callus growth than the other media. In the R2 treatment, induced calli exhibited a green colour that was a positive indication of the organogenic potential. The reported data suggest that the growth regulators concentrations and the addition of $AgNO_3$ in the R2 treatment could significantly improve buds regeneration from *C. equisetifolia* callus, whereas the R5 treatment induces rhizogenesis (increase in root number and root length). However, a major problem encountered in these experiments was the low efficiency of shoot differentiation and the poor shoot growth.

Table 2 Media tested for callus induction and *in vitro* regeneration of *Casuarina equisetifolia.* seedlot H4 in the Exp. 2

Treatment	Time (weeks)		
	0	3	6
R1	BA0.5 + NAA 0.1	BA0.5 + NAA 0.1	BA0.5 + NAA 0.1
R2	BA0.5 + NAA0.01	BA0.5 + NAA 0.01	BA0.5 + NAA 0.01
R3	BA0.5 + NAA 0.1	BA0.5 + NAA 0.01	BA0.5 + NAA 0.01
R4	BA0.5 + NAA 0.1	BA0.5 + NAA 0.1 + $AgNO_3$ 3.0	BA0.5 + NAA 0.1
R5	BA0.5 + NAA 0.1	BA0.5 + NAA 0.01 + $AgNO_3$ 3.0	BA0.5 + NAA 0.01

Concentrations are indicated in mg L^{-1}.

Table 3 Effect of different concentrations of growth regulators and silver nitrate on the organogenic potential of *C. equisetifolia* seedlot H4 calli in the Exp. 2

Treatment	Duncan's Multiple Rang Test for callus, bud and root mean values at 2 months after treatment						
	MCN ± STD	TCS ± STD	BCS ± STD	CS ± STD	BN ± STD	RN ± STD	RL ± STD
R1	1.5^{a} ±0.1	0.9^{b} ±0.2	4.3^{a} ±0.3	4.5^{b} ±0.3	0.0^{b} ±0.0	0.3^{b} ±0.1	1.9^{b} ±0.9
R2	1.6^{a} ±0.1	1.4^{ab} ±0.3	4.3^{a} ±0.3	4.9^{ab} ±0.3	0.4^{a} ±0.2	0.4^{b} ±0.1	3.6^{b} ±0.9
R3	1.4^{a} ±0.1	1.2^{b} ±0.3	4.5^{a} ±0.3	4.9^{ab} ±0.3	0.0^{b} ±0.0	0.5^{b} ±0.1	3.4^{b} ±1.1
R4	1.7^{a} ±0.1	2.1^{a} ±0.3	4.5^{a} ±0.2	5.4^{a} ±0.3	0.0^{b} ±0.0	0.4^{b} ±0.1	2.5^{b} ±0.9
R5	1.5^{a} ±0.1	1.6^{ab} ±0.3	5.0^{a} ±0.3	5.7^{a} ±0.3	0.3^{ab} ±0.2	1.2^{a} ±0.3	14.8^{a} ±5.0
Sign. level	0.05	0.05	0.05	0.05	0.01	0.01	0.01

BA, NAA and $AgNO_3$ concentrations are provided in mg L^{-1}. Means with the same letter are not significantly different; Data was mean ± STD(Standard Error). The 8 indexes are CN (callus number), TCS (top callus size, mm), BCS(basal callus size, mm), BN(bud number), RN(root number), RL(root length, mm) and CS (mean callus size per segment, mm).

3.3 Genetic transformation by *A. tumefaciens* of four *C. equisetifolia* seedlots

For the Exp. 3, Table 4 shows the mean values of the data obtained after genetic transformation of the epicotyl fragments with C58C1(pGV2260; pBIN-pBIN-35S-GUS-INT). Positive controls (PC) included epicotyl fragments without contact with agrobacterium, grown on the organogenic medium MSC. Negative control (NC) included explants without contact with *Agrobacterium*, grown on the nutrient medium MSC containing the antibiotic kanamycin; no callus growth was expected in these conditions. Results in Table 4 indicate that there were significant differences in KCN, ESN, TSN and GTE among the seedlots HA3, HA4, TH5 and DS5, and among treatments ($P < 0.01$), but no significant differences among replicates.

Table 4 Genetic transformation efficiency by the strain C58C1 (pGV2260; pBIN19-35S-GUSINT) observed among the four *C. equisetifolia* seedlots in the Exp. 3

Seedlot	Treat.	Repl. (PD)	No. of segments	TSN	KCN	ESN	GTE_1, %	GTE_2, %
	GUS	18	466	15.3 ±2.3	19.0 ±4.6	14.25 ±2.6	121.2 ±14.3	92.58 ±5.1
HA3	PC	4	61	25.9 ±1.1	–	2.06 ±0.32	–	–
	NC	2	34	17.0 ±1.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	GUS	26	548	16.8 ±1.1	23.5 ±1.1	16.3 ±1.2	142.5 ±10.5	97.0 ±1.4
HA4	PC	6	101	21.1 ±0.8	–	2.9 ±0.3	–	–
	NC	2	45	22.5 ±1.5	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
	GUS	22	510	20.4 ±1.4	12.5 ±3.5	9.7 ±2.5	63.2 ±17.9	48.8 ±12.3
TH5	PC	10	204	24.0 ±0.9	–	2.1 ±0.3	–	–
	NC	4	80	20.0 ±0.7	0.3 ±0.3	0.3 ±0.3	1.3 ±1.3	1.3 ±1.3
	GUS	5	164	19.5 ±3.5	2.0 ±1.0	2.0 ±1.00	9.7 ±3.4	9.7 ±3.4
DS5	PC	2	39	32.8 ±4.8	–	0.0 ±0.00	–	–
	NC	2	38	19.0 ±1.0	0.0 ±0.0	0.0 ±0.00	0.0 ±0.0	0.0 ±0.0

GUS: ß-glucuronidase reporter gene; PC: postive control; NC: negative control. TSN = total mean segment number per petri dish; KCN = Km resistant callus number; ESN = epicotyl segment number with at least one callus; and calculated the genetic transformation efficiency (GTE, %), GTE_1 = (KCN/TSN) × 100%, GTE_2 = (ESN/TSN) × 100%. Data was mean ± STD.

3.4 GFP and GUS gene expression in the seedlot HA4

As reported in Table 5 in the Exp. 4, the efficiency of genetic transformation was similar using the *A. tumefaciens* strain containing either the binary vector pBIN-35S-GUS-INT or the vector pBIN-pBIN-35S-GFP. GFP expression was observed under UV light in the kanamycin resistant calli and blue indogo colour was seen in the antibiotic resistant calli and young shoots obtained after the genetic transformation with the vector containing the ß-glucuronidase gene. No callus growth was observed for the negative control.

Table 5 Genetic transformation of the seedlot HA4 by the *A. tumefaciens* strains C58C1 (pGV2260; pBIN19-35S-GUSINT) and C58C1 (pGV2260; pBIN19-35S-GFP) in the Exp. 4

Genes	Repl. (PD)	No. of segments	TSN	KCN	ESN	GTE_1, %	GTE_2, %
PC	4	61	15.3 ±0.6	–	14.8 ±0.6	–	–
GFP	14	298	21.3 ±0.7	5.1 ±0.6	4.8 ±0.5	24.4^{a} ±2.7	22.7^{a} ±2.4
GUS	18	354	19.7 ±0.9	3.2 ±0.4	3.1 ±0.4	16.5^{a} ±2.0	16.0^{a} ±2.0
NC	2	45	22.5 ±1.5	0.0 ±0.0	0.0 ±0.0	0.0^{b} ±0.0	0.0^{b} ±0.0

PC: postive control; GFP = green fluorescent protein reporter gene; GUS: β-glucuronidase reporter gene; NC: negative control. TSN = total mean segment number per petri dish; KCN = Km resistant callus number; ESN = epicotyl segment number with at least one callus; and calculated genetic transformation efficiency (GTE, %), GTE_1 = (KCN/TSN) × 100%, GTE_2 = (ESN/TSN) × 100%. Data was mean ± STD.

4 Discussion

In this study, we reported the successful callus induction on epicotyls of *C. equisetifolia*, and we obtained GUS and GFP gene expression in calli and young shoots after genetic transformation with *A. tumefaciens*. In other Casuarina species, the use hormones has proven to be effective for plant regeneration (Duhoux *et al*., 1986; Le *et al*., 1996; Parthiban *et al*., 1996; Surendran *et al*., 1996).

In our study, the type, concentration, and combination of hormones were shown to play an important role in stimulating regeneration in *C. equisetifolia*. Data from the literature demonstrate that plant growth regulators can greatly affect callus formation and subsequent bud regeneration (Shen *et al*., 2009). In our study, adventitious bud regeneration was improved with the addition of the $AgNO_3$; this finding was consistent with previous reports on regeneration of other plants (Aditi *et al*., 2008). We also established that the genotype was important for *C. equisetifolia* callus induction. This is in agreement with numerous reports indicating that the genotype is one of the key factors for plant regeneration (Liu and Pijut, 2008).

Following genetic transformation with the strain C58C1 (pGV2260), we obtained potentially transformed calli and young shoots of *C. equisetifolia* that were kanamycin resistant and expressed the reporter genes GFP and GUS under the control of the 35S promoter. To further establish the gene transfer into *C. equisetifolia*, these preliminary data have to be confirmed by a molecular analysis based on Southern hybridization. In addition, *in vitro* culture procedures still have to be improved in order to obtain rooted transgenic plants of *C. equisetifolia*. The accumulation of phenolic compounds in the calli is probably one of the major factors limting the organogenic potential of *C. equisetifolia*.

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Silviculture

Dynamics of Soil Heterotrophic Respiration of *Casuarina equisetifolia* Plantation at Different Ages in Southeastern Coast of China

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Abstract Soil heterotrophic respiration (R_h) is of great importance in estimating net ecosystem production (NEP), and crucial to understand the interactions between ecosystem process and global climate change. In three different growth stages of coastal plantation of *Casuarina equisetifolia*, R_h were separated by trenching method and measured using an LI-8100 automated soil CO_2 flux system from May 2006 to April 2007 in Huian county, Fujian province, Southeast of China. Seasonal dynamic of R_h featured a single peak, with the maximum in June or July and the trough in December or January. Regression analysis showed that soil temperature (at a depth of 5 cm) and surface soil moisture (at a depth of 0-20 cm) were the major drivers of R_h. In addition, regression equation - $R = a \times e^{bT} \times W^c$ could better predict R_h than using respective independent variable. R_h rates were the highest in middle-age plantation, followed by the mature plantation and the young-age plantation. With the increase in plantation age, the Q_{10} value of R_h based the 5 cm soil temperature increased.

1 Introduction

As the main output way of soil carbon pool and the key process of carbon cycle, soil respiration (soil surface CO_2 flux, R_s) has received much attention in the last decades. R_s are mainly composed of two components, autotrophic respiration (R_a) of plant roots and heterotrophic respiration (R_h) of microorganisms and soil animals (Kuzyakov and Larionova, 2005). R_h is the main process of net loss of soil carbon in terrestrial ecosystems. Separating and quantifying R_a and R_h are of great importance, and the prerequisite for accurately estimating the soil carbon pool content (Hanson *et al*., 2000) and the carbon balance of an ecosystem (Bond-Lamberty *et al*., 2004).

Present studies in the world about R_h of forest ecosystems focus on the forest communities inland (Ngao *et al*., 2007; Saurette *et al*., 2008), rarely little R_h research for coastal forest ecosystem (Tyree *et al*., 2006), and there was no report about R_h of coastal forest ecosystem in China. Coastal plantations play an important role in preventing wind, sand stabilisation and improving the local environment. Casuarinas are extensively planted in southern China, especially in Hainan, Fujian, Guangdong and Zhejiang provinces. Zhong and Zhang (2003) reported that there are 300,000 ha of *Casuarina equisetifolia* plantations in China in 2003. To date, studies about the influence factors of R_h in forest communities focus upon temperature and moisture, the extent and mechanism of the effect of forest age on R_s and on its two major components were not well known (Saurette *et al*., 2008).

Consequently, reinforcing research on R_h in different forest ages is of great significance for fully exploring the driving mechanism of R_s and accurately estimating the CO_2 emission of forest soil and predicting the potential carbon sink of forest ecosystem.

In this paper, *in situ* R_h rates of *C. equisetifolia* coastal protective plantations at different ages in Fujian province, China were measured. The questions addressed were: (1) characteristics of seasonal dynamics of R_h; and (2) effects of temperature, soil water content and forest age on R_h.

2 Materials and Methods

2.1 Site description

The experimental sites are situated at Chihu state-owned shelter forest farm at Huian county in Fujian province, China (24°55′N, 118°55′E). The altitude is 5-400 m above-sea-level with an average slope of < 10°. The climate is maritime monsoon with a mean annual air temperature of 19.8℃. Annual mean precipitation is 1,000-1,500 mm with annual mean evaporation of 2,000 mm. The typical soil is Homogeneity Aeolian sand with low nutrients. The forest communities are dominated by pure *C. equisetifolia* plantations with few shrubs and grasses.

In our study, three forest ecosystems were chosen with 6-year-old plantation as the young age (*P*6), 17-year-old plantation as the middle age (*P*17) and 31-year-old plantation as the mature (*P*31). The three sample plots representing three age classes were 20 m × 20 m in size. Before the soil respiration rates were monitored, the forest community characteristics and soil physiochemical properties of three plots were investigated as follows (Table 1).

Table 1 Forest community characteristics and soil physiochemical properties of three *Casuarina equisetifolia* plantations

Forest type	Community characteristics			Soil physiochemical properties (0-20cm)				
	density (trees ha^{-1})	Average DBH (cm)	Average tree height (m)	pH	Soil bulk density ($g\ cm^{-3}$)	SOC ($g\ kg^{-1}$)	TN ($mg\ kg^{-1}$)	C/N
*P*6	2,500	5.5	9.5	5.47 ± 0.08	1.22 ± 0.05	7.14 ± 1.14	72.72 ± 3.81	98.21 ± 5.62
*P*17	2,202	12.2	10.8	5.24 ± 0.05	1.24 ± 0.05	12.00 ± 1.65	76.90 ± 4.12	156.05 ± 7.73
*P*31	1,962	15.1	13.7	5.10 ± 0.05	1.37 ± 0.09	12.43 ± 1.69	81.15 ± 4.26	153.27 ± 7.32

2.2 Measurement methods

Three plots (1 m × 1 m, excluding roots) as the R_h points were randomly established in each plantation. Trench was dug along the plot perimeter to exclude plant roots. Around each edge of the plot, the soil was excavated to 100 cm, and then the plot was surrounded with double-layer thick plastic film, and the ditch filled. We excavated the ditches in July 2005, and measured R_s and R_h in May 2006 to ensure the CO_2 emission at the plot came from the decomposition of soil organic matter by microorganisms.

R_h rates were measured every month from May 2006 to April 2007, and twelve days for each forest type in total, mostly in the middle of the month. Measurements were at hourly intervals throughout a daytime from 8:00 am to 6:00 pm. Every measurement lasted for 2 min with 3 repetitions. In addition, four observations on daily variations (beginning at 8:00 am in the morning and ending at 8:00 am of the next morning) were carried out for each forest site in July 2006, October 2006, January 2007 and April 2007, respectively.

R_h was measured using an LI-8100 Automated Soil CO_2 Flux System (LiCor Inc., Lincoln, NE, USA) equipped with a short-term chamber. A PVC soil collar (inner diameter 20.4 cm × 7.5 cm tall) was inserted into the soil at 5 cm deep in May 2006 in every R_h point, and left in the same locations throughout the study period. Meanwhile, the studied plots were kept free of living plants during the study period by removing all living vegetations aboveground without disturbing the litter.

Simultaneously, air temperature (T_a), soil surface temperature (T_0), soil temperature at 5 cm depth (T_5) and soil water content were monitored by a DHM2 ventilated thermometer, convoluted tubule geo-temperature table, the temperature probe belong to the LI-8100 and drying method, respectively.

2.3 Data analysis and modeling

Daily average flux of R_h: According to the 24 h daily variations in the four days in July 2006, October 2006, January 2007 and April 2007, we found that the effluxes measured during 10:00 – 12:00 in the morning could basically represent the daily average flux of R_h. Therefore, in this study, we used the R_h rates during 10:00-12:00 in the morning to represent the R_h daily average flux in the other eight months.

The relationship between R_h and temperature (T) was simulated with an exponential equation and Q_{10} value (defined as the increase in respiration rate per 10℃ increase in temperature).

$R_h = ae^{bT}$, $Q_{10} = e^{10b}$, where a is the basic respiratory (the respiration rate when temperature is at 0℃), and b is temperature sensitive coefficient.

The relationship between R_h and soil surface water content (W, volume to volume, 0-20 cm) was simulated with a linear equation.
$R = cW + d$, where c, d are regression coefficients.

Correlation analysis showed that R_h had a better correlation with T_5 than with T_a and T_0. So the dependence of T_5 and soil water content (W) to R_h were analyzed by a nonlinear regression model as follows.

$R = a \times e^{bT} \times W^c$, where T is soil temperature at 5 cm depth, W was soil water content, and b, c are regression coefficients.

The statistical analyses were carried out using SPSS 13.0 (SPSS Inc., 2001). Relationships between R_H and T_a, T_0, T_5 and W, and the differences among the three forest types were analyzed one-way ANOVA. Two-factor regression analyses were made under the nonlinear module. Graphs were prepared by Excel (Microsoft Corp., 2003).

3 Results

3.1 Seasonal dynamics of R_h

The mean R_h rate (μmol CO_2 m^{-2} s^{-1}, mean ± SE) was ranked as: *P*17 (2.76 ± 0.085) > *P*31 (2.17 ± 0.055) > *P*6 (2.00 ± 0.039). Seasonal dynamics of R_h featured a similar single peak curve during the studying period for the three plantation ages (Fig. 1). In March-April, soil CO_2 efflux stayed at a low level, and then increased slowly because of the increase in microbial activity resulted by the rise of temperature and the improvement of soil water content, reaching the maximum in June (for *P*17) or July (for *P*6 and *P*31) and began to decrease gradually to the minimum in December (for *P*6 and *P*17) or January (*P*31). The coefficients of seasonal variation in one year were 23.68%, 36.96% and 30.46% for *P*6, *P*17 and *P*31, respectively.

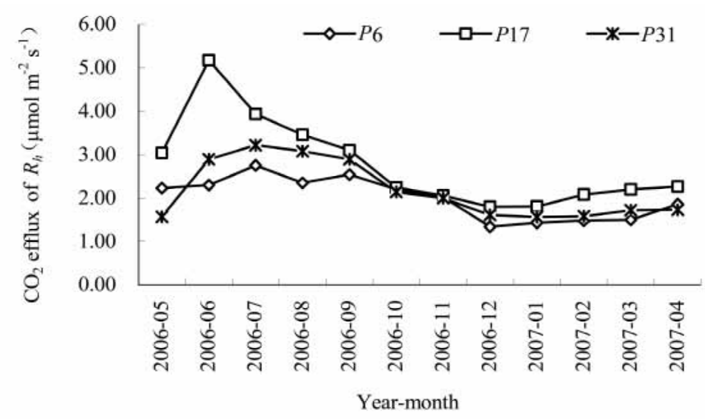

Fig. 1 Seasonal variations of R_h rates for the 3 plantations

3.2 Models of R_h to temperature and soil surface water

The extent of temperature influence on R_h was different among the three temperature factors (Table 2). The R_h showed no significant correlation with T_a and T_0 except the relationship between T_a and R_h in the young-age plantation. However, R_h had significant correlation with T_5 in all three plantation ages ($P < 0.05$). T_5 could account for 51.73% to 62.87% of variations in R_h.

Table 2 Models of R_h to temperature and soil surface water

Forest type	$R = ae^{bT}$			$R = aW + b$	$R = a \times e^{bT} \times W^c$			
	T_a	T_0	T_5					
	R^2	R^2	R^2	R^2	a	b	c	R^2
*P*6	0.5309 ($P = 0.082$)	0.5908 ($P = 0.116$)	0.6287 ($P = 0.000$)	0.5926 ($P = 0.000$)	1.973	0.014	0.114	0.689 ($P = 0.000$)
*P*17	0.3239 ($P = 0.002$)	0.4510 ($P = 0.067$)	0.5173 ($P = 0.000$)	0.7865 ($P = 0.003$)	3.731	0.020	0.422	0.919 ($P = 0.000$)
*P*31	0.4750 ($P = 0.157$)	0.5806 ($P = 0.719$)	0.6230 ($P = 0.000$)	0.8207 ($P = 0.001$)	2.262	0.038	0.552	0.901 ($P = 0.000$)

All models were tested at the 0.05 significance level (two-tailed). The temperature indicator in the two-factor model was based T_5.

In addition, the Q_{10} values of R_h based T_5 were 1.39, 1.44 and 1.95 for *P*6, *P*17 and *P*31, respectively (Table 3), which showed that the temperature sensitivity of microbial respiration improved with the increase of forest age.

Table 3 Q_{10} value of R_h to T_5

Forest type	$R = ae^{bT}$	Q_{10}
$P6$	$0.9614e^{0.0329T}$	1.39
$P17$	$1.0698e^{0.0365T}$	1.44
$P31$	$0.5458e^{0.0667T}$	1.95

The linear models showed significant correlations between R_h rate and soil surface water content (Table 2). Soil water content could account for 59.26% to 82.07% of variations in R_h for the three plantation ages, which indicated clearly that the effect of moister on microbial respiration was larger than that of temperature because of the sandy soil in coastal plantations.

The biological processes in R_s can be considerably influenced by soil temperature and moister, and their interactions correlated to R_s must be considered, and so R_h, which was confirmed in our study. The two-factor regression model could better predict R_h than using respective independent variable, and the R^2 values were 0.689, 0.919 and 0.901 for $P6$, $P17$ and $P31$, respectively.

3.3 Differences in R_h characteristics among the three plantation ages

The R_h rates among the three plantations ranked $P17 > P31 > P6$. Further study by multiple comparisons in SPSS, at the 0.05 significant level, a significant difference of R_h rates was found between $P6$ and $P17$ ($P = 0.018$), but not between $P17$ and $P31$ ($P = 0.060$) and between $P6$ and $P31$ ($P = 0.593$).

Seasonal dynamics of R_h rates featured a similar single peak curve, and there were no obvious differences of seasonality of R_h rates among the three plantations.

4 Discussion

4.1 Seasonal dynamics and controlling factors of R_h

In most cases, temperature is the main controlling factor of R_h in an ecosystem (Davidson *et al.*, 2000). When soil water condition is not favourable, R_h is no longer controlled by temperature, and is significantly correlated to soil water content (Raich and Mora, 2005). In our study, the air temperature and soil temperature were maintained at a relatively high level and with low variation, but soil water content of the coastal sandy was very low with an average of 4.33% during the study period, and was the restrictive factor to R_h. Therefore, R_h had a better relationship with soil moister than temperature and the maximum of R_h appeared in June or July which had better soil moister conditions than in August. However August had the highest air temperature.

4.2 The effect of forest age on R_h

There are limited published reports on differences in R_h among plantation ages. Saurette *et al.* (2008) studied the R_h in a chronosequence of hybrid poplar plantations that were 4, 6, 8, and 13 years old in Canada, and no significant differences of R_h rates among plantation ages were observed. Our results showed that in *C. equisetifolia*, there were significant differences in R_h rates among the three plantations with the highest R_h rate in middle-age plantation ($P17$). The differences in R_h rates among different forest communities which had similar climatic conditions and soil environment are mainly driven by different quantity and quality of respiratory substrates (Yang *et al.*, 2005). The three *C. equisetifolia* plantations had similar meteorological conditions and soil physiochemical properties; especially there were no significant differences in soil organic carbon contents with low background levels, and low contribution of soil mineral respiration to soil heterotrophic respiration. Moreover, the difference in litter quality among different *C. equisetifolia* plantations was not obvious. Consequently, we hypothesized that the differences in R_h rates among different ages in *C. equisetifolia* plantations were caused by different litter quantities (especially different quantities of fresh litter). Of course, this hypothesis must be tested in future.

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Tannins and Nutrient Dynamics, and Nutrient Resorption of *Casuarina equisetifolia* Branchlets in Pure and Mixed Forests

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Abstract Seasonal changes in tannins and nutrient contents in mature, senescent branchlets, and litter of *Casuarina equisetifolia* in pure and mixed forests were studied to evaluate the possible nutrient conservation strategies of the species under nutrient limitation. Total phenolics and extractable condensed tannins in mature branchlets were significantly higher than those in senescent branchlets and litter both in pure and mixed forests. Total phenolic contents of the litter were significantly lower in wet season than dry season both in pure and mixed forests. Leaching would be responsible for the loss of total phenolics of litter in wet season. N and P concentrations decreased significantly during branchlet senescence; N and P concentrations changed with season and forest type. N : P ratios of mature branchlets were greater than 16, indicating both pure and mixed forests were P-limited; phosphorus resorption efficiency (PRE) was significantly higher than nitrogen resorption efficiency (NRE) both in pure and mixed forests, and PRE in mixed forest was higher than that in pure forest. Seasonal changes in tannins and nutrient contents in branchlets and litter of *C. equisetifolia* in pure and mixed forests will provide a better understanding of nutrient conservation strategies in *C. equisetifolia* ecosystem.

1 Introduction

Tannins known as the group of phenolic compounds are significant plant secondary metabolites (Kandil *et al.*, 2004). Because tannins are complex and energetically costly molecules to synthesize, their widespread occurrence and abundance suggests that tannins play an important role in plant function and evolution (Cates and Rhoades, 1977; Zucker, 1983).

In most terrestrial ecosystems, nitrogen and phosphorus availability limit plant growth (Güsewell, 2004). Nutrients may be used more efficiently in nutrient-poor sites, and this efficient nutrient use could be important for the maintenance of the standing biomass at such sites (Aerts, 1995).

Despite the widespread planting and known ecological and physiological properties of *Casuarina equisetifolia*, there is scant information about the possible nutrient conservation strategies of *C.*

equisetifolia forest under nutrient limitation. In this study, we determined: (1) whether tannins and nutrient (N and P) concentrations of branchlets followed a similar seasonal pattern in pure and mixed forests with the higher concentrations during the wet season than the dry season? (2) whether mature branchlets had the higher tannins and nutrient concentrations than senescent branchlets in pure and mixed forests? (3) whether nutrient resorption responded to nutrient limitation?

2 Materials and Methods

2.1 Materials

In November 2008 (dry season) and July 2009 (wet season), two forest types: a mixed forest of *C. equisetifolia* and *Acacia crassicarpa* and a pure forest of *C. equisetifolia* were chosen at Chishan Forestry Centre of Dongshan county, Fujian province, China. The pure forest of *C. equisetifolia* was planted in 1992. The coverage of dense forest was 0.7, tree density was 1,425 trees ha^{-1}, and canopy height was 13.08 m, respectively. The mixed forest of *C. equisetifolia* and *A. crassicarpa* was also planted in 1992. The coverage of dense forest was 0.85, tree density was 1,125 trees ha^{-1} (525 trees ha^{-1} for *C. equisetifolia* and 600 trees ha^{-1} for *A. crassicarpa*). The canopy height of *C. equisetifolia* and *A. crassicarpa* averaged 7.75 m and 14.69 m, respectively.

Thirty trees with similar height and growth conditions of each forest type were selected for branchlet sampling. The development stages of branchlets (leaves) were demarcated into two stages, i. e. mature branchlets (fully developed, usually 15-25 cm long and dark green in color) and senescent branchlets (old branchlets, white or grey in color). The litter was collected randomly from the surface at each site.

2.2 Chemical analyses

Procedures described by Lin *et al.* (2006) were used to determine total phenolics (TP), extractable condensed tannins (ECT), protein bound condensed tannins (PBCT), and fibre bound condensed tannins (FBCT) and protein precipitation capacity (PPC). TP were measured with the Prussian blue method (Graham, 1992), ECT, PBCT and FBCT were assayed by the butanol-HCl method, using purified tannins from *C. equisetifolia* branchlets as the standard. Total condensed tannin (TCT) content was calculated by adding the respective quantities of ECT, PBCT and FBCT (Terrill *et al.*, 1992).

Plant samples were digested with sulfuric acid and hydrogen peroxide. The N concentrations of plant samples were determined by the microKjeldahl method (Yoshida *et al.*, 1972), while the P concentrations were determined by acorbic acid-antimony reducing phosphate colorimetric method (Nanjing Institute of Soil Science, 1978).

2.3 Calculations

Resorption efficiency (*RE*) was calculated as the percentage of *N* or *P* recovered from the senescing leaves (Aerts, 1996; Killingbeck, 1996):

$RE\ (\%) = (A_1 - A_2)/A_1 \times 100$, where A_1 is *N* or *P* concentration in MB; A_2 is *N* or *P* concentrations in SB.

2.4 Statistical analysis

All analyses were performed by SPSS13.0 for Windows.

3 Results

3.1 Seasonal changes in total phenolics, condensed tannin contents in mature, senescent branchlets and branchlet litter in pure and mixed forests

In dry season, total phenolic contents in pure and mixed forests followed the order: mature branchlets > senescent branchlets ≈ branchlet litter. In wet season, total phenolic contents followed the decreasing trend: mature branchlets > senescent branchlets > branchlet litter (Fig. 1a, b).

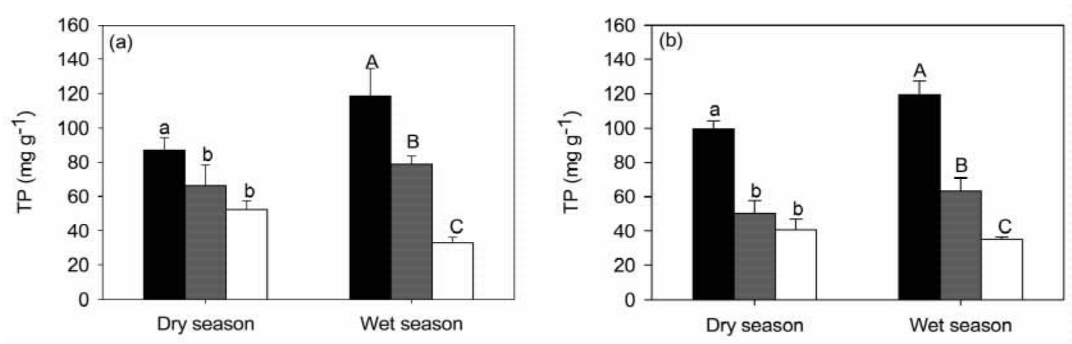

Fig. 1 Contents of total phenolics as a purified tannin standard at the different stages of branchlets and litter of *C. equisetifolia* in pure (a) and mixed (b) forests. Symbols are: black bars for mature branchlets, shaded bars for senescent branchlets, and white bars for litter

In pure and mixed forests, total phenolic contents of mature branchlets were significantly higher in wet season than in dry season ($P < 0.05$).

The ECT contents in mature branchlets of *C. equisetifolia* were significantly higher than those in senescent branchlets and litter in two forest types (Table 1). The PBCT contents increased during branchlet senescence; the FBCT remained relatively stable during branchlet senescence; TCT contents changed slightly during branchlet senescence, except for a significant decrease in mixed forest in dry season.

Table 1 Seasonal changes in ECT, PBCT, FBCT, and TCT contents of *C. equisetifolia* branchlets at different stages in pure and mixed forests

Condensed tannins		Mixed forest		Pure forest	
		Dry season (November)	Wet season (July)	Dry season (November)	Wet season (July)
ECT ($mg\ g^{-1}$)	MB	119.55(10.32)A	104.44(19.16)A	140.92(22.31)A	105.89(13.00)A
	SB	55.17(14.45)B	39.98(5.25)B	95.60(19.16)B	63.96(11.76)B
	BL	50.40(4.81)B	30.28(5.46)B	64.07(7.87)C	22.59(5.59)C
PBCT ($mg\ g^{-1}$)	MB	9.84(1.25)B	16.66(2.63)B	8.13(0.84)B	14.37(2.22)C
	SB	29.06(5.36)A	54.54(2.38)A	21.46(4.89)A	47.37(7.82)B
	BL	33.50(4.16)A	61.15(7.04)A	26.59(2.18)A	61.11(2.04)A
FBCT ($mg\ g^{-1}$)	MB	14.65(1.65)A	6.57(0.88)B	16.54(3.55)A	6.54(0.41)A
	SB	13.50(2.47)A	8.76(0.77)A	12.14(1.77)A	6.63(0.39)A
	BL	14.44(6.13)A	7.43(2.10)AB	11.79(2.34)A	8.08(1.89)A

(continued)

Condensed tannins		Mixed forest		Pure forest	
		Dry season (November)	Wet season (July)	Dry season (November)	Wet season (July)
TCT (mg g^{-1})	MB	144.04(10.04)A	127.67(21.22)A	165.59(26.16)A	126.80(14.20)A
	SB	97.73(12.56)B	103.28(2.56)A	129.20(23.17)AB	117.97(15.91)A
	BL	98.34(7.81)B	98.85(11.19)A	102.46(5.56)A	91.79(7.80)B

Different capital letters are significant differences at $P < 0.05$ levels; The values in the parentheses are SD of the mean, n = 4. MB: mature branchlets; SB: senescent branchlets; BL: litter branchlets.

PPC changed with season and senescence. PPC decreased during branchlet senescence, except for that in mixed forest in dry season (Fig. 2). PPC in mature branchlets was significantly higher in wet season than in dry season (Fig. 2a, b).

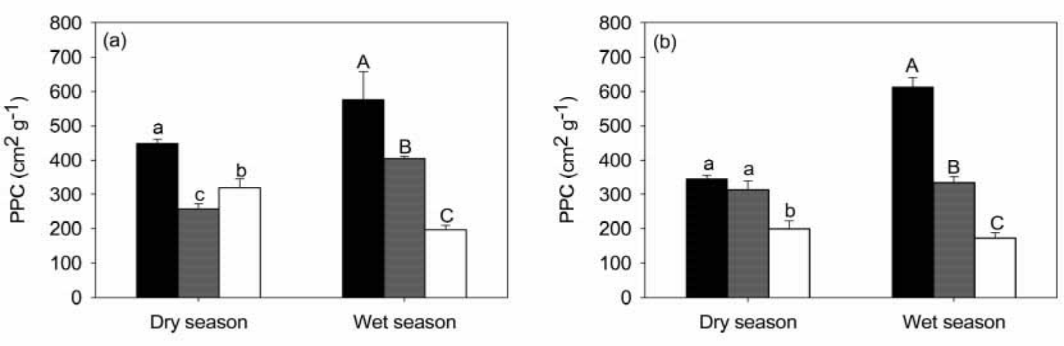

Fig. 2 Changes in protein precipitation capacity (PPC) at the different stages of branchlets and litter of *C. equisetifolia* in pure (a) and mixed (b) forests. Symbols as described in Fig. 1

3.2 Seasonal changes in N and P concentrations, N : P ratio and nutrient resorption in pure and mixed forests

N and P concentrations changed with season and forest type (Table 2). In both pure and mixed forests, N concentrations in mature and senescent branchlets were significantly higher in dry season than in wet season. P concentrations in mature and senescent branchlets in pure forest were obviously higher than those in mixed forest.

The N : P ratios in mature branchlets in mixed forest were higher than those in pure forest, and were all above 16. In the two forest types, N : P ratios increased during branchlet senescence.

Phosphorus resorption efficiency (PRE) was significantly higher than nitrogen resorption efficiency (NRE) in pure and mixed forests (Table 2). NRE in pure forest was close to that in mixed forest. However, PRE in mixed forest was significantly higher than that in pure forest.

4 Discussion

Total phenolics (TP) and extractable condensed tannins (ECT) contents in mature branchlets were higher than those in senescent branchlets and litter for *C. equisetifolia* in pure and mixed forests. This may reflect an increase in bound or nonextractable phenolics with time, and there have been suggestions of an increase in polymerization and binding to cell walls with senescence. The increase in PBCT contents during senescence (Table 1) supported these suggestions. Some phenols are

Table 2 Seasonal changes in N, P concentrations, N∶P ratios in mature, senescent branchlets, and litter, and resorption efficiency of N and P (NRE and PRE) in pure and mixed forests

Variable		Mixed forest		Pure forest	
		Dry season (November)	Wet season (July)	Dry season (November)	Wet season (July)
N ($mg\ g^{-1}$)	MB	19.46(0.31)A	16.99(0.50)A	21.10(0.66)A	17.51(0.40)A
	SB	11.19(0.66)B	8.12(0.43)B	12.24(1.14)B	8.91(0.49)B
	BL	10.03(0.55)C	7.36(0.41)C	11.20(0.88)B	8.56(0.82)B
P ($mg\ g^{-1}$)	MB	0.69(0.02)A	0.63(0.02)A	1.17(0.02)A	1.07(0.02)A
	SB	0.12(0.01)C	0.14(0.01)C	0.59(0.04)B	0.46(0.02)B
	BL	0.18(0.02)B	0.17(0.02)B	0.51(0.01)C	0.46(0.03)B
N∶P ratio	MB	28.23(0.97)C	26.63(0.66)C	18.06(0.55)A	16.32(0.42)B
	SB	95.08(11.29)A	58.17(5.81)A	20.90(2.10)A	19.23(1.41)A
	BL	55.36(5.75)B	43.19(6.94)B	21.87(2.03)A	18.54(1.29)A
NRE (%)		42.50(3.55)b	52.25(1.74)a	41.94(5.50)b	49.15(2.35)a
PRE (%)		82.79(1.92)a	77.75(2.29)b	49.77(3.05)d	56.75(2.37)c

Different capital letters are significant differences at $P < 0.05$ levels; For NRE and PRE at each site, means with different small letters are significant differences at $P < 0.05$ levels. MB: mature branchlets; SB: senescent branchlets; LB: litter branchlets.

leached by rainfall and translocation from the leaves of trees may be another possible mechanism (Harborne, 1997).

Seasonal changes in leaf chemistry reflect changing demands for carbohydrates and nutrients resulting from normal growth and differentiation processes (Wareing, 1959; Moorby and Wareing, 1963). *C. equisetifolia* growth is active in wet season with the relatively high soil water availability, which will attribute to enhancement of growth and the accumulation of total phenolics in mature branchlets. However, ECT contents in mature branchlets did not increase in wet season. The changes in contents do not necessarily reflect the quantitative allocation of tannins to the leaves because of rapid turnover of labile compounds (Kleiner *et al*., 1999) and because the contents are affected by concomitant changes in proportions of other components of the leaves such as structural leaf components (Koricheva, 1999).

In both pure and mixed forests, N and P concentrations in mature branchlets were significantly higher in dry season than in wet season. N and P concentrations were diluted by branchlets mass accumulation during the wet season when *C. equisetifolia* grew rapidly. Meanwhile, portions of N and P were allocated to other plant parts, such as fruits. There were no significant differences in the N content of mature branchlets in two forest types through the season, reflecting a nitrogen-fixing effect of *C. equisetifolia*. P concentrations in mature branchlets in pure forest were higher than those in mixed forest. NRE were basically below 50% in two forest types, while PRE were almost above 50%, especially was about 80% in mixed forest. PRE in mixed forest was significantly higher than that in pure forest (Table 2) reflecting mixed forest had stronger nutrient conservation than pure forest.

Acknowledgments

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Branchlet N and P Concentrations, N : P Ratios and Nutrient Resorption in *Casuarina equisetifolia* Protection Forest in Southeastern China

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Abstract Changes in N and P concentrations were studied in branchlets of *Casuarina equisetifolia* trees grown across a coastal gradient at Chishan Forestry Centre of Dongshan County (Fujian province, China). Results showed that N and P concentrations at various stages of branchlets followed the same order across the coastal gradient: young branchlets > mature branchlets > senescent branchlets. N : P ratio of young branchlets was the lowest, and increased with maturity and senescence. N and P concentrations, N : P ratio of young and mature branchlets were not affected by coastal gradient whereas senescent branchlets were significantly affected. Phosphorus resorption efficiency was significantly higher than nitrogen resorption efficiency among corresponding coastal gradients in response to P deficiency (high N : P ratio), but nutrient resorption efficiency was influenced by coastal gradients and decreased under stressed environments.

1 Introduction

Casuarina equisetifolia is a nitrogen-fixing tree of considerable social, economic, and environmental importance in tropical/subtropical littoral zones of Asia, the Pacific, and Africa. Despite the widespread planting and known ecological and physiological properties of *C. equisetifolia*, very little has been done to understand its nutrient conservation strategies under stressful environments (e. g. arid, nutrient limitation, and strong wind stress) in the coast.

Nitrogen to phosphorus (N : P) ratios have been applied to identify thresholds of nutrient limitation (Güsewell and Koerselman, 2002). Retranslocation from senescing leaves is the process by which plants withdraw nutrients from these leaves, making them available for later investment in new structure (Aerts, 1996). The process of retranslocation is closely associated with leaf senescence and conservation of nutrients, and is an important mechanism enabling plants to maintain growth at nutrient-poor sites (Lodhiyal and Lodhiyal, 2003). Nutrients may be used more efficiently at nutrient-poor sites, and this efficient nutrient use could be important for the survival of individuals under such conditions (Birk and Vitousek, 1986). However, some researchers reported that high RE is not an important adaptation to low nutrient status, but a characteristic of most plant species with contrasting life histories (Miao, 2004).

In this study, we ask: (1) whether there are changes in nitrogen and phosphorus concentrations and N∶P ratios during development and senescence of branchlets; (2) whether there is difference in resorption efficiency of phosphorus and nitrogen during senescence of branchlets; (3) whether resorption efficiency of phosphorus and nitrogen changes with a coastal gradient from seaward to inland? To answer these questions, a field investigation of *C. equisetifolia* was conducted at Chishan Forestry Centre of Dongshan county, Fujian province, China.

2 Materials and Methods

2.1 Study site

The study was carried out at Chishan Forestry Centre of Dongshan county (23°40′N, 117°18′E), Fujian province, China. The climate of the region belongs to southern subtropical maritime monsoon climate, with annual temperature ranging from 3.8℃ to 36.6℃. Mean annual precipitation and evaporation are 1,103.8 mm and 2,027.9 mm, respectively. The rainy season is from March to October, and the dry season is from November to February. The soils are coastal sandy, barren, with a pH ranging from 4.1 to 4.4.

A coastal gradient for the field investigation was established with 6 sites selected at every 100 m from seaward to inland. The *C. equisetifolia* plantations were artificial, pure forests which were planted in 1989 and 1992. The coverage of dense forest was 0.5-0.8, tree density was about 15 trees 100 m^{-2} and canopy height ranged from 4 to 20 m. The understory consisted of *Cajanus cajan*, *Spinifex littoreus* and *Verbena negando*.

2.2 Sample collection

In March 2007, 20 trees similar in height and growth conditions at each site were chosen and two branches of each tree were randomly selected from the upper crown for branchlet sampling. The development stages of branchlets (leaves) were demarcated into three stages, i.e. young branchlets (newly-emerged, usually shorter than 5 cm in length at the top of branch, light green in color), mature branchlets (fully developed, usually 15-25 cm long and dark green in color) and senescent branchlets (old branchlets, white or grey in color). Branchlets damaged by insects and disease or mechanical factors were avoided. All samples were taken to the laboratory immediately after sampling and cleaned with distilled water.

Soil samples (at 20 cm deep) at each site were collected using auger. Soil samples were air-dried in the laboratory and then passed through a 2 mm mesh sieve to remove stones and large roots before oven-drying at 65℃. The oven-dried samples were then crushed and passed either through a 0.25 mm sieve for determination of total N and P or through a 2 mm mesh sieve for measurement of pH. Soil pH was measured with an Orion 3 Star pH meter (Thermo Electron Corporation, USA) in a 1∶1 mixture of soil and CO_2-free water which had been agitated for 1 min.

2.3 Chemical analyses

Plant samples were digested with sulfuric acid and hydrogen peroxide. The N contents of plant samples were determined by the micro Kjeldahl method (Yoshida *et al*., 1972), while the P contents were determined by acorbic acid-antimony reducing phosphate colorimetric method (Nanjing Institute of Soil Science, 1978).

2.4 Calculations

Resorption efficiency (RE) was calculated as the percentage of N or P recovered from the senescing leaves (Aerts, 1996; Killingbeck, 1996):

RE (%) = $(A_1 - A_2)/A_1 \times 100$, where A_1 is N or P concentration in mature branchlets; A_2 is N or P concentration in senescent branchlets.

2.5 Statistical analysis

Mean and standard deviation values of triplicate samples were calculated. A one-way analysis of variance (ANOVA) was performed with the developmental stage as the treatment factor. The Student-Newman-Keuls multiple comparison method was used to test significant differences among the developmental stages. All analyses were performed by SPSS 11.0 for Windows.

3 Results

3.1 Nitrogen and phosphorus concentrations

N and P concentrations, which were highest in young branchlets, lowest in senescent branchlets and moderate in mature branchlets, decreased during the development and senescence of *C. equisetifolia* branchlets (Fig. 1). The N and P concentrations decreased with senescence, indicating that N and P were translocated out of senescing branchlets to the other parts of plant during the senescence. N and P concentrations of young and mature branchlets at site 1 to 3 were not significantly different from those at site 4 to 6, while the N and P concentrations of senescent branchlets at site 1 to 3 were significantly higher than those at site 4 to 6, respectively (Fig. 1).

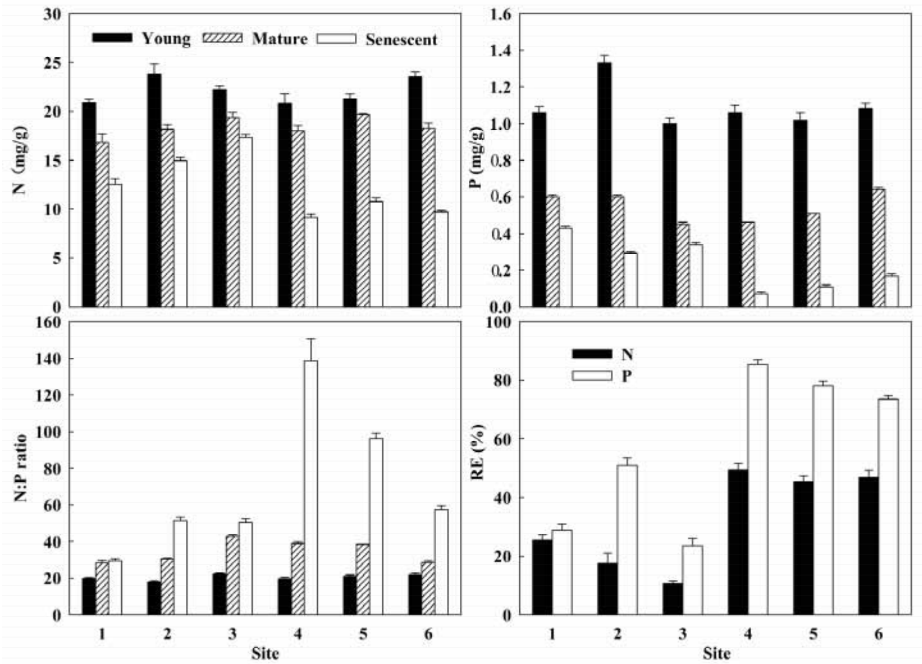

Fig. 1 N, P concentrations, N : P ratios in young, mature and senescent branchlets, and resorption efficiency of N and P (NRE and PRE) across a coastal gradient

3.2 N : P ratio

The N : P ratio was the lowest in the young branchlets and increased with maturity and senescence across the entire coastal gradient (Fig. 1). The N : P ratios of the young branchlets were from 17.90 ± 0.63 to 22.26 ± 0.45, while those of the mature and senescent branchlets were all above 25. The N : P ratios for young and mature branchlets at site 1 to 3 were not significantly different from those at site 4 to 6, whereas the N : P ratios of senescent branchlets at site 1 to 3 were significantly lower than those at site 4 to 6, respectively (Fig. 1).

3.3 Nitrogen and phosphorus resorption efficiency

Phosphorus resorption efficiency was significantly higher than nitrogen resorption efficiency across the entire coastal gradient except that no significant difference between PRE and NRE was found at site 1 (Fig. 1). NREs or PREs at site 1 to 3 were significantly lower than those at site 4 to 6 with PREs were all higher than 70% at site 4 to 6.

4 Discussion

Nitrogen and phosphorus resorption efficiency (NRE and PRE) varied with sites. PRE was significantly higher than NRE among corresponding coastal gradients except for site 1. Plant species adapted to P-limited sites tend to resorb P more efficiently than N (Wright and Westoby, 2003). A significant correlation was found between PRE and NRE, thus suggesting that N and P resorption was coupled.

Thresholds of foliar N : P ratios were found to be < 14 for nitrogen limitation and > 16 for phosphorus limitation (Güsewell and Koerselman, 2002). According to the N : P ratios of mature branchlets (> 25) in our study, nitrogen was less limiting than phosphorus, consequently N availability varied less than P availability, NRE was lower, and N resorption was mostly incomplete. Killingbeck (1996) modified the concepts of nutrient resorption efficiency and proposed the use of "resorption proficiency", which is the absolute level to which nutrients are reduced in senescing leaves. Obviously, P resorption proficiency was significantly higher than N resorption proficiency (Fig. 1). NREs or PREs at gradients 1 to 3 sites were basically lower than 30% whereas their N : P ratios of mature branchlets were still high. Plants growing on infertile soils do not retranslocate a greater fraction of nutrients from senescing leaves, for example, RE is independent of status of individuals (Birk and Vitousek, 1986). Escudero *et al.* (1992) showed that leaf longevity was far more important as a nutrient conservation mechanism than high resorption efficiency. Environmental stress (i.e. strong wind) at gradients 1 to 3 sites strongly influenced NRE or PRE.

Contrary to an earlier study (Yuan *et al.*, 2005) which showed that a significant linear correlation was found between N concentrations in senescing and in green leaves, this study did not reveal the significant linear relationship between N, P concentrations in senescent and in mature branchlets (Table 1). This phenomenon may contribute to different species in different study. There was a significant linear correlation between N and P concentrations in the senescent branchlets, which is consistent with previous study (Huang *et al.*, 2007), but no such significant relationship was found in the mature branchlets (Table 1). Nutrient resorption efficiency was not related to the nutrient concentrations or N : P ratios of mature branchlets, but was found to be a particular strong relationship with those of senescent branchlets (Table 1).

Table 1 Relationships between N and P, NRE and N, PRE and P, NRE and N∶P, PRE and N∶P in mature and senescent branchlets

Y-X	Equation	n	*r*	*P*
N_m-N_s	$Y = 0.07x + 17.508$	18	0.198	0.432
P_m-P_s	$Y = 0.141x + 0.51$	18	0.253	0.311
P_m-N_m	$Y = -0.032x + 1.128$	18	−0.461	0.054
P_s-N_s	$Y = 0.032x - 0.157$	18	0.723	<0.01
NRE-N_m	$Y = 0.811x + 17.602$	18	0.055	0.830
NRE-N_s	$Y = -5.081x + 95.463$	18	−0.967	<0.001
PRE-P_m	$Y = -2.595x + 58.156$	18	−0.008	0.976
PRE-P_s	$Y = -180.109x + 99.079$	18	−0.961	<0.001
NRE-N_m∶P_m	$Y = -0.173x + 38.475$	18	−0.065	0.799
NRE-N_s∶P_s	$Y = 0.299x + 11.584$	18	0.692	<0.01
PRE-N_m∶P_m	$Y = 0.171x + 50.843$	18	0.041	0.872
PRE-N_s∶P_s	$Y = 0.542x + 18.79$	18	0.803	<0.001

N_m or P_m corresponds to N and P concentrations in mature branchlet;
N_s or P_s corresponds to N and P concentrations in senescent branchlet.

The significant negative correlation between NRE and N concentration of senescent branchlets in our study (Table 1) is consistent with that reported in previous study (Yuan *et al*., 2005) whereas NRE is not correlated with either N concentration or N∶P in MB, which is consistent with earlier reports (Aerts, 1996; Huang *et al*., 2007). This could result from a partial control of leaf N resorption over leaf N concentration by providing a large source of osmotically active substances in the senescent leaves (Aerts, 1996). PRE exhibited significant linear correlation with P concentration in senescent branchlets, but no correlation was found with either P concentration or N∶P in mature branchlets, which is similar to previous study (Huang *et al*., 2007).

In conclusion, nutrient resorption is an important strategy for *C. equisetifolia* under coastal environments (i.e. arid, nutrient limitation). N∶P ratio is a good variable for indicating nutrient limitation in *C. equisetifolia* ecosystem. N∶P ratios of mature branchlets were all above 25; phosphorus resorption efficiency was significantly higher than nitrogen resorption efficiency among corresponding coastal gradients, but nutrient resorption efficiency was influenced by coastal gradients and decreased under stressed environments.

Acknowledgments

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Biomass Assessment of *Casuarina equisetifolia* Shelterbelt on Coastal Sandy Land in Guangdong Province

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Abstract Two different age groups of *Casuarina equisetifolia* plantations in Donghai Island were investigated in order to develop biomass prediction models and to compare biomass accumulation status at two ages. A total of 69 trees were measured, 6 trees were harvested to measure diameters and fresh weight at designated height intervals, such as bole, branch, foliage, and root was measured in the field. The main objective is to explore the relationship between fresh weight of different component and the measured variables including diameters and height, and to build functions for estimation of biomass, and further to examine the difference of two stands in biomass accumulation. Logarithmic transformations were used in biomass estimation procedures with a linearity assumption in regression analysis after transformation. The functions for each component and total biomass were built with R squares ranging from 0.664 to 0.944. T-test was used to examine whether the biomass in 18-year stand was significantly different from that in 40-year stand. The result showed that the latter had much less biomass allocated in each component. However, the percentage of different component was quite stable. It implies that over matured *Casuarina* species in Guangdong coastal area needs facilitated regeneration to keep sustainable growth.

1 Introduction

Casuarina equisetifolia is suitable for planting as shelterbelt along sandy coastal areas in the tropical and subtropical regions of China. In the early 1950s, this species was introduced to the southeastern coastal area of China (Xie *et al*., 2008) with the aim to mitigate natural disaster brought by typhoons, to improve the ecological environment, and to provide additional raw material resource to the local economy. The species has been widely planted along the coastal areas of Guangdong province as windbreaks.

This report presents the results of a study to determine the biomass productivity of the species in Guangdong. Better understanding of biomass productivity of the species will allow better utilization as raw material and for use in shelterbelts.

Different methods have been developed for biomass estimation. Rajendran and Devaraj (2004), Zhang *et al*. (2007), and Xie *et al*. (2008) developed techniques to directly estimate field biomass of casuarina species. Regression models of biomass on tree measurements have been developed Verwust (1991) and Li *et al*. (2009).

This study was conducted to develop regression models of biomass productivity to measurements of trees in two age classes of *C. equisetifolia* planted along the coastal region of Guangdong.

2 Materials and Methods

2.1 Study site

The study sites are located in the Donghai Island (20°55′-21°05′ N latitude, 110°15′-110°32′ E longitude) in Zhanjiang city of Guangdong province. The coastal area of Donghai Island supports southern subtropical dry deciduous forests. The climate is subtropical and influenced by monsoon conditions with dry winter and hot humid summer. The mean monthly temperature varies from 11.8℃ (January) to 32.2℃ (July), and the annual rainfall averages 1,361 mm of which 78% occurs in the rainy season (May-September). The sites have predominantly sandy soil.

Two *C. equisetifolia* plantation stands aged 18 years and 40 years were selected for this study. It is noted that the seeds of *C. equisetifolia* are seldom found germinated in the plantations in Donghai Island. Thus, natural regeneration of this species on the island is generally non-existent.

2.2 Field sampling

After an initial survey of the plantation stands, 6 sample plots (10 m × 10 m each) were established randomly within the plantations. Height and diameter at breast height (DBH) of all trees in the sample plots were measured. The average DBH of trees within each sample plot was used to identify a representative tree for each sample plot. The six sample trees were harvested in January 2010. The harvested above ground parts of trees were separated into bole (2 m segments), branches and foliage. Roots of each harvested tree were also excavated. The fresh weights of every part of the sample trees were measured in the field.

The diameters of each sample trees were recorded at 1.3 m from the ground (DBH) and every 2 m interval, such as, 3.6 m, 5.6m and so on to the top of the tree (BJFU, 1990).

2.3 Statistical analysis

SPSS16.0 was used to explore the relationships between fresh biomass weights of foliage, branch, bole, root and total biomass against measured variables, such as DBH, diameters at different heights, tree height. Different regression methods such as enter, stepwise, remove, backward, and forward were tried to find the best one with 95% confidence interval chosen for each relationship.

3 Results

3.1 Biomass prediction models for *C. equisetifolia*

The 18-year-old casuarina plantation had an average height of 18 m with 1,800 trees ha^{-1}, while the 40-year-old stand had an average height of 16 m with 500 trees ha^{-1}. Other than having a considerably lower stand density, the basal area in the 40-year-old stand was also smaller (18.4 m^2 ha^{-1}) than that of the 18-year-old stand (34.2 m^2 ha^{-1}).

The diameters at different height, tree height, and fresh weight for foliage, branches, bole, root separately, and total biomass were used to explore their correlations.

Regression analysis was used to build models of relationships between biomass estimates and the field measured variables after taking the natural logarithms of both dependent and independent factors.

A total of 10 model equations were made based on field measurements and biomass estimates. The correlation (R^2) ranged from 0.664 to 0.994.

Results indicate that DBH can be used to predict the total biomass of even aged *C. equisetifolia* plantations (R^2 = 0.910) for both 18-yearold trees and 40-year-old trees (Table 1).

Table 1　Models for prediction biomass of *Casuarina equisetifolia*

Equation *	R^2	Sig.
LnBt = 0.831 + 1.779Ln DBH	0.910	0.003
LnBt = −1.274 + 1.890Ln DBH + 0.621LnH	0.942	0.014
LnBl = −3.663 + 2.451Ln DBH	0.827	0.012
LnBl = −3.097 + 2.421Ln DBH − 0.167LnH	0.828	0.072
LnBb = −2.324 + 2.091Ln DBH	0.732	0.030
LnBb = −3.231 + 2.139Ln DBH + 0.268LnH	0.736	0.136
LnBs = 0.705 + 1.659Ln DBH	0.871	0.007
LnBs = −2.336 + 1.820Ln DBH + 0.897LnH	0.944	0.013
LnBr = −0.737 + 1.754Ln DBH	0.664	0.048
LnBr = −1.639 + 1.801Ln DBH + 0.266LnH	0.669	0.191

* In the above equations, Bt for total biomass, Bl for fresh weight in kg of foliage, Bb for that of branch, Bs for bole, Br for root, DBH for diameter at breast height, and H for tree height.

3.2　Stand biomass estimation

The above equations deduced from the measurement based on the harvested representative trees were used to calculate the biomass for each sample plot. The two different aged sample plots showed different fresh weight both in total amount and in different components.

Results showed that the total biomass in 18-year-old stand was higher (5,399 ± 174 kg) compared to that in 40-year-old stand (2,646 ± 450.9 kg). The proportional biomass of each component part of the trees in the two age classes varied from 10.0% and 10.8% for branches, 7.1% and 8.5% for foliage, and 19.5% and 19.4% for roots. The bole contributed 63.7% and 62.1% of the total biomass in both age classes (Table 2).

4　Discussion

Both DBH and height could be used to estimate biomass of *C. equisetifolia.* The equations deduced from the representative trees were comparable to other studies.

Of the total biomass, more than half was contributed by the bole component (62-64%), followed by roots (19%), Branches (10-11%) and foliage (7-9%). For the same species, Srivastava (1995) obtained similar percentage of each component in a 5-year-old stand though using a different method.

Although sandy soil has very low nutrient content, estimates indicate *C. equisetifolia* produces biomass at 29.99 t $ha^{-1}yr^{-1}$ in the 18-year-old plantations at the study site. This is higher than that

Table 2 Biomass estimation for both 18-year and 40-year *Casuarina* stands (10 m × 10 m)

Component	*Casuarina* stand		Percentage of each component	
	18-year (kg)	40-year (kg)	18-year	40-year
Foliage	393.6 ± 12.0	256.9 ± 64.4	7.1 ± 0.1	8.5 ± 0.9
Branch	548.9 ± 16.9	306.8 ± 63.1	10.0 ± 0.1	10.8 ± 0.6
Bole	3,410.4 ± 112.3	1,590.2 ± 251.2	63.7 ± 0.1	62.1 ± 1.8
Root	1,049.9 ± 34.0	509.2 ± 85.4	19.5 ± 0.0	19.4 ± 0.1
Total	5,399.0 ± 174.0	2,646.0 ± 450.9	100	100

Values in the table were Mean ± std. deviation.

Table 3 Biomass comparison for both 18-year and 40-year *Casuarina* stands

Component	T-value	DF	Sig. (2-tailed)	Mean difference	Std. error difference	95% Confidence interval of the difference	
						Lower	Upper
Foliage	3.615	4	0.022	136.7	37.8	31.7	241.6
Branch	6.424	4	0.003	242.1	37.7	137.5	346.8
Bole	11.459	4	0.000	1,820.2	158.8	1,379.1	2,261.2
Root	10.187	4	0.001	540.8	53.1	393.4	688.1
Total	9.868	4	0.001	2,753.4	279.0	1,978.6	3,528.1

of 8-12 t ha^{-1} yr^{-1} in dry forests and comparable to 13-28 t ha^{-1} yr^{-1} in wet tropical forests (Srivastava, 1995). It shows that *C. equisetifolia* has strong ecological adaptability to the coastal dry sandy environment. The over matured 40-year-old plantations had much less rate of biomass accumulation (6.62 t ha^{-1} yr^{-1}), due to much less number of tree remained in the stand, even though the percentage of foliage was similar to younger stand.

No evidence of successful natural regeneration was observed for this exotic species along Guangdong coastal area. It is inferred that as the plantation grows older, biomass accumulation rate decreases. As such, some measures may be required to further boost biomass accumulation and maintain healthy shelterbelt forest conditions for better protection against strong typhoon winds. Most plantation grounds are normally covered with thick layers of dried needles which may have prohibited seed germination. Controlled ground burning can help promote seed germination but such practice must be carried out with great care.

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Suitable Clones of *Casuarina equisetifolia* for Sodic Soils in India

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Abstract Salt affected soils are defined as soils that have been adversely modified for the growth of most plants by the presence of soluble salts, exchangeable sodium or both. Soil amelioration and management strategies have to be essentially worked out for bringing such lands under cultivation and the economics of the reclamation process attracts considerable attention. The role of trees in amelioration of degraded soils is well understood. *Casuarina equisetifolia* is identified as one among the fast growing salt tolerant tree species with substantial intraspecific variation. A field experiment was established at Tiruchirapalli, Tamil Nadu, India to exploit the variation in this species with reference to sodic salt tolerance. Sodicity, the presence of a high proportion of sodium ions relative to other cations in a soil, directly reduces plant yield through its effect on soil properties. High levels of sodium in surface soils cause increased crusting and decreased water entry. In subsoil, sodicity reduces soil water storage, aeration and leads to increased soil strength. Susceptibility to erosion under rainfall impact or flowing water is increased in sodium affected soils. Explicit inter clonal variation with respect to growth and physiological traits was observed in the present study. Among the 73 clones, two proved their intolerance and died within six months of growth. Eight clones exhibited superior growth characteristics at age 3 years. Instantaneous water use efficiency (WUE), measured as the ratio of net photosynthesis to transpiration, varied from 0.19 μmol mmol^{-1} to 1.59 μmol mmol^{-1} and two clones registered significantly superior values when compared to the other clones. Clones 31 and 18 possessed excellent water use efficiency coupled with superior growth traits. However, no correlation was noticed between productivity and WUE in general.

1 Introduction

Salinity and sodicity are the major factors restricting the economic and efficient utilization of the available land resources (Tiwari *et al*., 1994). Salt affected soils occur mostly in regions of an arid or semiarid climate where leaching and transportation of salts to streams or the oceans is not complete as in high rainfall areas. About 7.3 million ha of India's land area is afflicted with the twin problems of alkalinity and salinity (Central Soil Salinity Research Institute, 2010). The problem is compounded by the relatively low salt tolerance of most crop plants. Soil amelioration and management strategies have to be essentially worked out for bringing such lands under cultivation. The role of trees in amelioration of degraded soils is well understood. Identification of economically important tree species which will not only survive, but also grow and yield satisfactorily on problem soils is of utmost importance in the forestry sector.

Casuarina equisetifolia L. is a species of socio-economic and environmental importance. It is grown from Australia in the east to Africa in the west due to its importance in the livelihood of people. In India, it is extensively grown in the southern peninsular region as a cash crop (Kondas, 1983). Casuarina species have been the farmers' favourite as they fit well in an agrarian ecosystem. Short gestation period, periodic returns in the form of pruned branches, ability to improve soil fertility and ready marketability are the major attractions for this species. Planting for reclaiming mined, salt affected and wind-prone areas are some of the popular uses of casuarina (Gurumurthi, 2001). Its usefulness in environmental protection has been fully realized after the tsunami and is now a major component in any coastal afforestation programme in India (Nicodemus, 2007). Though *C. equisetifolia* is identified as one among the fast growing salt tolerant tree species, substantial intraspecific variation in salt tolerance exists (Allen *et al*., 1994) and there is an urgent need to identify the salt tolerant genotypes which can be used to reclaim salt-damaged forests (Allen *et al*., 1993).

2 Materials and Methods

A field experiment was established at Anbil Dharmalingam Agricultural College and Research Institute (Tamil Nadu Agricultural University), Tiruchirapalli, India to screen salt tolerant clones of *C. equisetifolia*. The field is located between 10°45′N latitude and 78°36′E longitude at an altitude of 85 m asl. The area receives a mean annual rainfall of 870 mm from North-East monsoon. The soil is sodic with pH 9.2, exchangeable sodium percentage (ESP) 28.5 and electrical conductivity (EC) 0.24 dSm^{-1}. The details of the experimental plot are given below. The clones were originally selected from Chengalpet, Chidambaram and Tiruchandur in Tamil Nadu (Kumar and Gurumurthi, 1996; Balasubramanian, 1999).

Number of clones: 73
Statistical design: Randomized Complete Block Design
Number of replications: 3
Number of ramets used per clone: 4
Spacing: 2 m × 2 m

Data on biometric traits like total height, collar diameter (CDM) and diameter at breast height (DBH) were recorded at quarterly intervals. The product of square of DBH and total height was used as a surrogate for total tree volume (Elliott *et al*., 2002; Luna and Singh, 2007) and expressed in cm^3 (volume index). Observations on various physiological characteristics including net photosynthesis rate, stomatal conductance, intercellular CO_2 concentration and transpiration were also collected using a Portable Photosynthesis System, LiCor, 6200 (Licor, USA). Instantaneous water use efficiency (WUE) was estimated as the ratio of net photosynthesis rate to transpiration. The data were subjected to analysis of variance (ANOVA) for RCBD. The significance of difference among treatment means was tested by 'F' test and the means were compared using Duncan's Multiple Range Test (DMRT).

3 Results and Discussion

Salt tolerance can be measured by a number of criteria. Survival at high salt concentration has been the fundamental selection criterion for agriculture crops. However, if we consider the mechanism that plants used for survival, they may not be the same ones that are necessary for the maintenance of high growth rate at moderate salt stress (Shannon, 1984). Many halophytes withstand high salt stress by such strategies as temporary dormancy, increased succulence, or shortening the growing season (Levitt, 1972). Dormancy is not compatible with high yields and increasing succulence contributes nothing to dry weight. Determination of growth or yield response under salt stress is another method of measuring salt tolerance (Shannon, 1984). The results discussed below were derived from the growth/physiological data recorded at age 3 years. Clone 24 recorded the maximum value for volume index (23,010.69 cm^3) and 15 other clones were found on par with it (Table 1). Eight clones in particular (24, 31, 64, 26, 70, 52, 23 and 13) exhibited superior growth. The minimum value (283.74 cm^3) for volume index was recorded by clone 48. Fifty-five clones were found on par with it. Two clones (08 and 44) could not withstand the salt stress and died in all the three replications within six months. Sodicity, the presence of a high proportion of sodium ions relative to other cations in a soil, directly reduces plant yield through its effect on soil properties. High levels of sodium in surface soils cause increased crusting and decreased water entry. In subsoil, sodicity reduces soil water storage, aeration and leads to increased soil strength. Susceptibility to erosion under rainfall impact or flowing water is increased in sodium affected soils (Shaw *et al*., 1995). Large differences in tolerance to salt are found within many plant species (Greenway, 1973; El-lakany and Luard, 1982; Allen *et al*., 1994; Reddy *et al*., 2001).

Table 1 Volume index and water use efficiency of casuarina clones

Clone No.	Volume Index (cm^3)	WUE ($\mu mol\ mmol^{-1}$)	Clone No.	Volume Index (cm^3)	WUE ($\mu mol\ mmol^{-1}$)
1	12,983.80 a-e	0.22 r-s	38	8,555.57 b-g	1.20 b-c
2	9,089.22 b-g	0.35 n-s	39	7,712.49 b-g	0.33 o-s
3	11,986.78 a-g	0.74 e-o	40	3,557.57 b-g	0.39 m-s
4	11,766.31 a-g	0.39 m-s	41	2,955.87 b-g	0.65 h-q
5	5,968.71 b-g	0.69 g-p	42	4,916.76 b-g	1.10 b-g
6	10,039.21 b-g	0.65 h-q	43	552.05 f-g	0.63 i-r
7	5,355.29 b-g	0.36 n-s	44	Did not survive	–
8	Did not survive	–	45	2,594.46 c-g	0.64 h-q
9	6,048.89 b-g	1.14 b-e	46	8,381.49 b-g	0.83 c-l
10	5,096.54 b-g	0.72 f-p	47	6,722.48 b-g	0.76 e-n
11	12,859.46 a-e	0.22 r-s	48	283.74 g	0.76 e-n
12	7,656.46 b-g	0.27 q-s	49	6,323.90 b-g	0.72 g-p
13	13,193.31 a-d	0.19 s	50	8,101.57 b-g	0.43 l-s
14	5,740.00 b-g	1.29 a-b	51	9,175.84 b-g	0.42 l-s
15	6,015.14 b-g	0.60 i-s	52	13,589.45 a-d	0.47 k-s
16	11,829.39 a-g	0.50 k-s	53	7,202.56 b-g	0.88 c-k
17	12,282.48 a-f	0.32 p-s	54	8,775.42 b-g	0.83 c-l
18	12,982.80 a-e	1.12 b-f	55	8,774.26 b-g	1.18 b-d
19	11,481.96 b-g	0.43 l-s	56	11,568.49 b-g	0.43 l-s
20	5,082.72 b-g	0.20 s	57	4,285.81 b-g	0.43 l-s
21	3,670.12 b-g	0.53 k-s	58	1,112.17 e-g	0.43 l-s
22	3,520.21 b-g	0.95 b-j	59	5,890.45 b-g	0.58 j-s

(continued)

Clone No.	Volume Index (cm^3)	WUE (μmol $mmol^{-1}$)	Clone No.	Volume Index (cm^3)	WUE (μmol $mmol^{-1}$)
23	13,319.07 a-d	0.36 n-s	60	7,642.47 b-g	1.12 b-f
24	23,010.69 a	0.97 b-i	61	4,500.64 b-g	0.38 m-s
25	4,073.58 b-g	0.79 d-m	62	4,027.47 b-g	0.48 k-s
26	13,977.46 a-c	0.88 c-k	63	1,849.52 d-g	0.57 j-s
27	9,089.42 b-g	0.40 m-s	64	14,690.72 a-b	0.76 e-n
28	8,112.96 b-g	0.66 h-q	65	2,115.09 c-g	0.43 l-s
29	12,880.20 a-e	0.51 k-s	66	3,895.36 b-g	0.53 k-s
30	7,381.09 b-g	0.70 g-p	67	2,017.12 d-g	0.83 c-l
31	14,807.17 a-b	1.59 a	68	4,999.26 b-g	1.03 b-h
32	10,898.65 b-g	0.73 e-p	69	10,704.99 b-g	1.04 b-h
33	3,999.17 b-g	0.65 h-q	70	13,650.15 a-d	0.39 m-s
34	10,067.60 b-g	0.19 s	71	9,716.93 b-g	0.37 m-s
35	7,018.09 b-g	0.20 s	72	9,742.83 b-g	0.47 k-s
36	3,385.41 b-g	0.82 c-l	73	4,468.78 b-g	0.76 e-n
37	7,255.52 b-g	0.54 j-s			

Means with the same letter in column do not differ significantly as per Duncan's Multiple Range test at $P < 0.05$ level of significance.

The ratio of the net photosynthesis rate to transpiration is known as instantaneous water use efficiency (Petite *et al.*, 2000) and it ranged from 0.19 μmol $mmol^{-1}$ in clone 13 to 1.59 μmol $mmol^{-1}$ in clone 31 (Table 1). Higher the value better the efficiency to divert water for photosynthesis than transpiration. Clones 31 and 18 possessed excellent water use efficiency (WUE) coupled with superior growth traits. Clone 24, the top ranking clone with respect to growth traits, estimated a value of 0.97 μmol $mmol^{-1}$ for WUE. However, no correlation ($r = 0.03$) was noticed between productivity and WUE in general.

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Environmental Amelioration and Wood Utilization

Casuarina equisetifolia - a Promising Species for Green Belt Project of Coastal and Off-shore Islands of Bangladesh

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Abstract Bangladesh is one of the coastal marginal countries of the Bay of Bengal possessing an area of 47,211 km^2 as coastal area (32% of the country's total area) with a population of 35 million (28% of the country's total population). The entire coastal zone of Bangladesh (710 km long) is prone to violent storm and tropical cyclones during pre-monsoon and post-monsoon seasons. The coastal livelihoods are facing serious consequences of global climate change and sea level rise including permanent inundation of huge land masses along the coastline. *Casuarina equisetifolia* (Jhau), an indigenous species of Chittagong coast is a promising species in the coastal plantation programmes of sandy beaches of the coast and off-shore islands. After the devastating cyclone of 1991 in Bangladesh when more than 10,000 people died, a plantation programme of *Casuarina* was taken in sandy beaches of an off-shore island Kutubdia. The *C. equisetifolia* plantation was successful and stabilizes the sandy beaches from erosion and protecting the livelihoods from frequent tidal surges and storms. The paper describes the vulnerability of the island and the prospects of *Casuarina* plantations for mitigating the risks of future climate change disasters in Kutubdia Island.

1 Introduction

The coast of Bangladesh includes off-shore islands, mudflats, chars and new accretions which are low lying and very flat. The coastline is approximately 710 km long and the coastal zone covers about 47,211 km^2 (32% of the country's total area) with a population of 35 million, i.e. 28% of the country's total population (Islam, 2007). The coastal areas is susceptible to variety of natural hazards including flood, cyclone, storm surge, tornado, coastal erosion and landslides, and causing immense sufferings and damage to people, property and the environment. Because of the funnel shaped coast of the Bay of Bengal, the country very often becomes the landing ground of cyclones formed in the Bay of Bengal. Except for natural mangroves, the long shoreline of Bangladesh was without tree cover until the beginning of the regular mangrove afforestation programmes in the mid sixties (Siddiqi and Khan, 2004). Being exposed to direct wind and wave action, the life and property of the coastal population is always at risk. Experiences showed that a permanent green belt along the shoreline and in the near shore and offshore islands would considerably reduce the losses incurred from the frequent cyclones and tidal surges (Siddiqi, 2001; Tanaka, 2009).

Coastal afforestation with mangrove species on barren coastal areas in Bangladesh was initiated in 1966 to protect life and property of the coastal people from cyclones and tidal surges (Saenger and Siddiqi, 1993). However, industrial raw material and fuelwood production, conservation of coastal ecosystem and the environment, protection of wildlife and aquatic resources, agricultural land protection against salt intrusion, tourism, poverty reduction and enhancing the land accretion and establishment were later added to the programme. During the last four decades, the Forest Department has successfully implemented massive plantation programmes and has established some 172,000 ha of mangrove plantations scattered over coastal areas and off-shore islands of the country (Hossain *et al.*, 2008).

Sonneratia apetala is a pioneer tree species widely used in the coastal plantation programmes followed by *Avicennia* species. However, in the coastal sandy beaches, *Casuarina equisetifolia* appeared as the only suitable tree species (Hossain *et al.*, 1998). Alhough the species is indigenous in the coasts of Bangladesh (Troup, 1921), it is the preferred plantation species in the sandy beaches, off-shore islands, roadside and sometimes in coastal homesteads of the country. This paper provides a description of the coastal vulnerability of Kutubdia Island of Bangladesh and the prospects of *Casuarina* plantings in the sandy beaches on this island.

2 Vulnerability of the Coastal Zone and Off-shore Islands of Bangladesh

The coastal areas of the country have been suffered from tropical cyclones and storm surges at regular intervals over the past century. During 1900-2001, the rate of tropical cyclones and storm surges has risen significantly along with their frequency and intensity. About 53 damaging cyclones were reported in the coastal areas of Bangladesh from 1793 to 2009 causing about one million human casualties, death of animals, and destroying millions of houses, agricultural crops, coastal embankments and mangrove forests (Hossain, 2009).

3 Embankments for the Protection of Coastal Lands and Off-shore Islands

Government initiates construction of embankments, dykes and other allied structures and annual maintenance in association with the Water Development Board in order to protect the life and resources from cyclone, tidal surges and coastal erosion. Bangladesh Water Development Board has built over 4,000 km of embankments to provide safeguard against the intrusion of saline water and devastation associated with repeated attacks of tidal surge and cyclonic storms. However, severe bank erosion problems occur frequently in the embankments of the coastal belt and off-shore islands.

4 Study Area

Kutubdia, a small off-shore island 3 km from Bangladesh mainland is situated in the Cox's Bazar district with an area of 32 km^2 which originally was 155 km^2. The island is bounded by the Bay of Bengal on the north, west and south and Kutubdia channel on the east. Population is approximately 114,000. The Island is very vulnerable to cyclones and tidal surges and more than 10,000 people from this Island died in 1991. Many lost their assets, houses and making many people to environmental refugees. Experiences show that where forests existed they mitigated the losses in comparison to areas where mangroves and beach forests were absent. Not only that, people also took shelter in the trees during cyclone and tidal surges.

5 The Need of the Establishment of a Green Belt in Coastal Areas of Kutubdia

A 50 - 200 m wide belt of well-established mangrove or non-mangrove plantation plays a vital protective role in a system of coastal defense including saving the embankment and habitations from cyclone and storm surges (Annonymous, 1994). It is proved that a permanent green belt along the coastline and surrounding islands were able to considerably reduce the losses incurred from the frequent cyclones and tidal surges (Sajjaduzzaman *et al.*, 2005). Many *Casuarina* shelterbelts established to protect coasts from cyclones, tsunami and other coastal hazards in India, Sri Lanka and Thailand were found effective against the 2004 Indian Ocean tsunami (Forbes and Broadhead, 2007). They also provide wood and wood fuels to local people, harbor the biodiversity and increase the marine fish populations (Islam, 2007). The Forest Department initiated the coastal plantations in this island in 1968 and a total of 667 ha mangrove plantations (*S. apetala* and *A. officinalis*) and 118 ha of *Casuarina* plantations have been established.

6 Suitable Plantation Species in the Kutubdia Coasts

Kutubdia has limited areas for plantation. The south and west of the coast consist of sandy soil, whereas the eastern boundary is silty-clay. The mature charlands of the eastern part are converted to salt bed, quite unsuitable for mangrove plantations, whereas, the accreted charlands are only suitable for coastal afforestation only (Siddiqi, 2001). In the muddy charlands of the south-west coast, the suitable species are *S. apetala* and *A. officinalis* and *C. equisetifolia*.

7 *C. equisetifolia*, a Suitable Species for Sandy Beaches of Kutubdia

C. equisetifolia is indigenous in the coasts of Chittagong - Cox's Bazar (Troup, 1921) and preferred for firewood, poles and posts. The species is also listed as a national priority species of the Forest Department plantation programmes in Bangladesh (Islam, 2003). Besides coastal plantations, this species is also recommended for agroforestry systems in Bangladesh (Jashimuddin *et al.*, 2006), and reclamation of unstable coastal ecosystems (Pinyopusarerk *et al.*, 2004). The wood of *C. equisetifolia* has been used for firewood, boat building, house construction and furniture making (Puri *et al.*, 1994; Nicodemus *et al.*, 1996). Since the earlier *Casuarina* plantations in Bangladesh are becoming mature for utilization, the high density wood of could also be used as a structural timber (Chowdhury *et al.*, 2009).

Bangladesh has a prospect for the expansion of *Casuarina* plantations throughout the country, particularly in the stabilization of coastal sand dunes, off-shore islands and coastal embankments. Seedlings may be raised in polybag of 22 cm × 15 cm in size (Bhuiyan *et al.*, 2000), whereas, Hossain *et al.* (1998) recommended 30.5 cm × 15 cm size polybag for raising vigorous and quality seedlings. Plantations are established on the onset of monsoon in May - June with a spacing of 2 m × 2 m. In the sandy beaches, the seedlings need support of bamboo sticks. Weeding is nor needed as the beaches are generally free of ground vegetation.

The survival, height and diameter growth of *C. equisetifolia* plantations in the sandy beaches of Kutubdia Island are shown in Table 1. Older plantations (17-19 years old) are totally damaged by frequent cyclones and later from illegal cutting by local people. Younger plantations are promising and survival ranges from 65% to 95%. Ten-year-old plantation attained an average height of 15.3 m and diameter of 14.6 cm. The result supports the growth performance of *C. equisetifolia* in similar trial plantations in degraded hilly areas of the country (Hossain and Khan, 2005).

Table 1 Growth of *C. equisetifolia* in the sandy beaches of Kutubdia Island

Location	Plantation area (ha)	Age (year)	Ht (m)	DBH (cm)	Survival (%)
North Dhurong	2.0	19	–	–	Damaged
Matbar para	6.0	18	–	–	Damaged
Pillar para	10.0	17	–	–	Damaged
Matbarpara	10.0	10	15.3	14.6	85
Kudiartek	5.0	9	–	–	Damaged
Matbarpara	5.0	9	15.0	13.8	78
Madhya Kayerbill	5.0	6	11.4	11.0	88
Ali Fakir Dail	5.0	5	10.8	9.1	65
Pillar para	15.0	4	7.2	6.8	65
North Kayerbill	15.0	3	4.5	5.1	86
Gilachari	20.0	2	3.6	3.1	89
Gilachari	20.0	1	0.9	–	95

C. equisetifolia was highly adaptable to moderate saline zones due to its outstanding growth performance in both inner and outer slopes of embankments (Nandy *et al*., 2002). However, poor growth was found in high saline zones of eastern coastal areas.

Homestead forests in Bangladesh are rich in tree resources supplying about 70% of all wood consumed and 90% of all fuelwood and bamboo of the country (Abedin *et al*., 1990). Similar to some common homestead tree species, *Casuarina* is also planted in institute compounds, roadsides and homesteads. Though the growth performance is not consistent (Table 2), the species is a promising plantation species in coastal sandy beaches, avenue and roadside plantations in Bangladesh.

Table 2 Survival percent and growth of *C. equisetifolia* in different plantations of Bangladesh

Plantation site	Age (year)	Survival (%)	Ht (m)	DBH (cm)	References
Teknaf	3	86	5.9	6.4	Field data
Sitakunda	3	92	5.9	6.2	BFRI
Teknaf	4	88	8.6	9.3	Field data
Rangabali	4	85	8.43	3.82	Serajuddoula *et al*., 1995
Char Kukri Mukri	6	65	6.54	6.6	Siddiqi and Khan, 2004
CU campus	7	92	13.6	15.3	Hossain and Khan, 2005
CU campus	15	82	24.3	25.0	IFESCU campus
CU campus	15	71	21.5	23.7	RMPS Centre
CU South Hill	19	67	17.5	18.2	Field data
North of Arts Faculty	22	58	21.3	18.5	Field data
Shamsun Nahar Hall	24	68	30.3	36.4	Field data

8 *Casuarina* Provenance Trial in Bangladesh

A field trial of 11 provenances of *C. cunninghamiana* from CSIRO, Australia along with a local *C. equisetifolia* seedlot was established in Madhupur, Tangail in 1988 (Hossain *et al.*, 1995). Four-year results showed that the local *C. equisetifolia* provenance had better growth and development in comparison to the provenances of *C. cunninghamiana* (Table 3).

Table 3 Growth of 11 provenances of *C. cunninghamiana* and one local *C. equisetifolia* at 4 years of age in Bangladesh

Seedlot-Locality	Seedling Ht (cm) at planting	Survival (%)	Ht (m)	DBH (cm)
13508 – 1km E of Augathella, Qld	57	85	3.09	2.8
13511 – 26 km SE of Mt Morgan, Qld	64	88	2.97	2.9
13512 – NW Chartes Towers, Qld	67	86	3.37	3.4
13513 – 36 km NE of Oasis, Qld	58	88	4.33	3.7
13711 – Petford, Qld	60	90	3.12	3.7
13515 – 9 km SE of Mareeba, Qld	60	88	3.74	3.3
13516 – West Normanby Rover, Qld	57	95	2.81	2.9
13517 – Annan R, NE Helenvale, Qld	62	88	3.29	2.8
13518 – 8 km S of Mt Molloy, Qld	61	80	2.95	2.8
13519 – 9 km N Rollingstone, Qld	54	88	3.27	3.4
15574 – Clarview, Qld	54	84	3.04	2.6
290 – *C. equisetifolia* from Cox's Bazar, Bangladesh	23	97	4.86	4.0

9 Stabilization of Sandy Beaches

Pioneer colonizing species in the sandy beaches of Kutubdia are *Ipomoea pes-caprae*, *Panicum repens*, *Clerodendrum infortunatum* and *Vitex negundo* that are not able to protect the coast from the damages of cyclone and tidal surges. An experimental *Casuarina* plantation was established in 1989 on the west coast and initial results showed the success of the plantation on the sandy beaches. Until 2009, an area of about 118 ha casuarina plantation had been established in the open exposed sandy beaches only (Table 1). After the establishment of *Casuarina* plantations, sand stabilization is significant and landless people and fishermen are moving towards the plantation site. This shelterbelt acts as a first line of defense against the effect of cyclonic storms and winds. Local people also collect the litters and the fallen twigs for cooking as there is a scarcity of fuelwood.

10 Conclusions

Beach erosion as a result of stronger and higher tides, cyclones and storm surges is gradually diminishing the western and southern coast of Kutubdia Island. Once a 155 km^2 island, it has been reduced to about 32 km^2 within a century, and the islanders are convinced that the future natural disasters and sea level rise make the island more vulnerable. Global warming is sounding the death knell for low-lying coastal islands, like Kutubdia which is only 2-3 m asl. The rising sea will also increase tidal forces and exacerbate erosion. The exposed area of the western coast needs immediate vegetation cover to reduce the damage from cyclones and storm surges. *C. equisetifolia* is the potential tree species in the sandy beaches of the Island and also in the coastal areas of the country, because the species can be used as windbreaks and protect erosion along the exposed seashores of Bangladesh.

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Casuarinas in the Philippines: Land Rehabilitation, Climate Change Adaptation and Industry Use

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Abstract The paper examines how casuarinas have contributed to Philippine land rehabilitation efforts since the 1980s specifically in coastlines, sand dunes, mined-out areas and areas affected by energy production. Among the three known casuarinas naturally occurring in the Philippines, viz. *Casuarina equisetifolia*, *Gymnostoma rumphianum* (syn *C. rumphiana*) and *G. nobile* (syn *C. sumatrana*), the first two species have been more widely used in combination with various site amelioration treatments to improve success particularly during abnormally dry periods and in very adverse site conditions. *C. equisetifolia* has been found to grow well along coastal as well as inland areas. An investigation is being conducted to verify the identity of trees recently found growing at 1,500 m asl in the Philippines. It associates well with native vegetation and can establish itself in old exotic plantations. Casuarinas have been used along with other popular mine rehabilitation species such as *Pinus kesiya*, *Acacia auriculiformis*, *A. mangium* and *Alstonia macrophylla*. The role of casuarinas in supporting the country's climate-change adaptation strategy and in green energy production programme is highlighted. Although casuarinas have been mostly used for environmental and rehabilitation purposes since the 1980s, its use in sustainable plantation timber production is anticipated with the recent submission of the new Sustainable Forest Ecosystems Management Act 2010.

1 Introduction

Early in the 1500s, prior to the coming of the Spaniards, the country boasted of 90% (approximately 27 M ha) pristine tropical forests cover (Garrity *et al.*, 1993). By the year 2000 almost 97% of the original cover (Acosta, 2004) have been lost. Deforestation rates fluctuate yearly but averaged at 150,000 ha annually (Rebugio *et al.*, 2005) while reforestation averaged only at 49,513 ha year^{-1} (Forest Management Bureau Statistics, 2008). Deforestation was attributed to flawed implementation of selective logging system, forest fires, and unsustainable upland agriculture aggravated by massive upland migration and poverty brought about by poor governance (ineffective forest policies and corruption).

The government introduced regional reforestation projects in 1910 with anticipation that the private sector would take over forest management near or upon maturity of the resource. With a fast growing population, and faced by the impacts of global climate change, the country is struggling to regain much of its lost forest cover.

The Philippine Atmospheric, Geophysical, Astronomical Services (PAGASA, 2010) has predicted that hot temperature and heavy precipitation will continue to become more frequent during the next decades. Significant increase in rainfall is very likely in most parts of Luzon and Visayas during June to July, while a decreasing trend is likely in Mindanao. An overall reduction in rainfall in 9 months of every year is expected in most parts of the country. More than 64.7 million of the country's population live along 855 coastal municipalities and right in the brunt of potential sea level rises. And more than 20 million people are living in now upland areas made unstable by the lack of forest cover. In both situations casuarinas can potentially help alleviate the negative impacts.

This paper describes the current uses of casuarinas in the Philippines and how the species can help in rapid restoration of forest cover in degraded lands in view of the impacts of global climate change. The paper outlines practical strategies to increase the utilization of casuarinas in land rehabilitation and in developing community-based forest industries.

2 Casuarinas in the Philippines

Casuarinas are found growing from coastlines to as high as 1,500 m asl in some parts of the Philippines archipelago. There are three identified *Casuarina* species in the Philippines, namely *Casuarina equisetifolia*, *Gymnostoma rumphianum* (syn *C. rumphiana*) and *G. nobile* (syn *C. sumatrana*). *C. equisetifolia* grows up to 50 m in height while *G. rumphianum* and *G. nobile* are smaller trees up to 10 m in height.

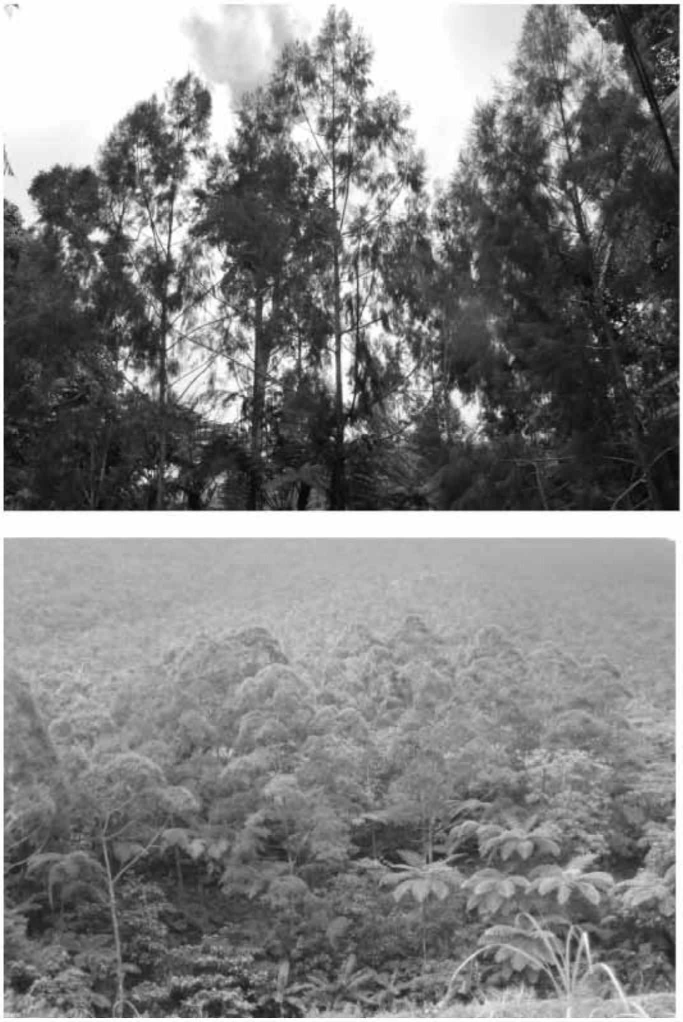
C. equisetifolia is most common and widely planted. It is found along beaches and coastlines beyond the upper tidal limits as pure stands or in association with *Terminalia catappa*, *Erythrina orientalis*, *Thespesia populnea*, *T. populneoides*, *Talipariti tiliaceum*, *Calophyllum inophyllum*, *Millettia pinnata*, *Heliotropium foertherianum* and *Barringtonia asiatica* (Fernando *et al*., 2008). An investigation is being conducted to verify the identity of trees, believed to be *C. equisetifolia*, recently found growing at 800 to 1,500 m asl in Mindanao (see Fig. 1).

G. rumphianum is found at 680 m asl and as high as >1,000 m asl (Fernando *et al*., 2008). *G. nobile* is found in ultramafic rock formations at 200-500 m asl (Fernando *et al*., 2008). The natural habitats of these species indicate the unique ability to grow even in adverse sites, making them highly resilient and suitable for land rehabilitation particularly in changing climate conditions.

Casuarinas have been included in many field trials in the Philippines to develop silvicultural techniques since the 1970s. Casuarina plantation establishment and tree improvement work started in 1981. Two field trials of *C. equisetifolia* were established in 1981 to test the performance of 5 local provenances in two sites in Northeastern Luzon in 1981 (Halos S. C. and Tumaliuan B., pers. comm.). The 5 provenances included in the trials were Los Baños (Laguna), San Fabian (Pangasinan), Candelaria (Zambales), San Jose (Mindoro) and Infanta (Quezon). Local investigation on phenology, fecundity and vegetative propagation was conducted during the same period.

These trials were then followed by the establishment of a much broader international provenance

A



B

Fig. 1 ***Casuarina equisetifolia*** **in upland forest at elevations approximately 1, 500 m a. s. l. (A); *Gymnostoma rumphianum* in forest at elevations approximately 600 m asl (B) (Photo: MA Galang)**

trials in 1993. This trial was established in Atimonan, Quezon. Poor survival at the site led to the abandonment of the trial.

Results of a two-year assessment of a field trial with 28 provenances of *C. junghuhniana* in San Remigio, Antique indicated Mt. Lamore (Lombok, Indonesia) had the biggest average height growth (52.08 cm) while Mt. Kawi (East Java, Indonesia) had the smallest average height growth (25.33 cm). Kwai Mission (Tanga, Tanzania) had the biggest diameter growth (4.83 cm) while Mt. Argopuro (East Java, Indonesia) had the smallest mean diameter growth (2.06 cm). Average survival was about 65% (Lustica, 1998 unpublished report).

A trial revealed the excellent growth performance of *C. equisetifolia* as well as *Acacia auriculiformis*, *A. mangium* and *Samanea saman* in volcanic-ash or lahar laden sites around Mt. Pinatubo in Central Luzon. Inoculation with mycorrhiza and soil amelioration with organic matter, pre-planting with pioneer herbaceous creepers and grasses, and supplemental watering aided in survival and growth in lahar areas (Castillo, 1996; ERDB, 2010).

3 Land Rehabilitation with Casuarinas in the Philippines

Casuarinas are among the top ten species used in land rehabilitation efforts during the last 50 years (Forest Management Bureau, 2000). Casuarinas mainly provide fuelwood for households, bakeries, tobacco flue curing and for a period was being looked at as a real alternative to the then failing *Leucaena leucocephala* plantation programme that would have fueled the rural dendrothermal power generation programme since the 1980s. It was projected then that the Philippines would need about 2.19 million m^3 of fuelwood for rural communities and to fuel rural power generation in 1990 alone (Halos, 1983).

Degradation of the land and ecosystems vital to an archipelagic environment like the Philippines lowered the emphasis on meeting the wood production requirement of a growing population. In line with the priority of that time the Forest Research Institute (FORI) was given new priorities and was reorganized to what is currently known as the Ecosystems Research and Development Bureau (ERDB) in 1986. More recent and upcoming legislations (for example, the Sustainable Forest Ecosystems Management Act of 2010) are signaling a new set of national priorities directed towards sustainable management of forest land and coastal areas in view of the country's climate change adaptation strategy and in providing livelihood opportunities from short- and medium-rotation tree farming.

For the last 25 years, therefore, casuarinas played a very important role in land rehabilitation and less to meet timber production requirements. The following case studies are presented to describe the important role of casuarinas in land rehabilitation in the Philippines.

Case* 1. *Casuarina in stabilisation of moving sand dunes

Moving sand dunes have been a problem along the northwest coast of Luzon Island. Moving sand dunes bury agricultural land, including coastal villages. These sand dunes have no organic matter, 75.1 mg P kg^{-1}, 35 mg K kg^{-1}, pH of 6.4, sandy soil extending to a depth of about 1.5 m. Annual rainfall in the area averages 1,850 mm with peaks at about 4 months.

Field trials were conducted since the 1970s to test and select endemic beach-type tree species, and to develop practical site amelioration techniques that would stop or reduce the impacts of moving sand dunes. The beach-type trees included in the field trials were *C. equisetifolia*, *T. catappa*, *B. asiatica* and *C. inophyllum*. Site amelioration techniques included (a) addition of top soil and animal manure, (b) mulching with banana bracts, and (c) mulching with hay (dried rice stalks soaked in water). Watering was conducted during the very dry period of the first year until the third year at a reducing rate. Fertilizers (ammonium sulfate and superphosphate) were applied a month after planting. Wind and flood erosion exposed roots of planted seedlings or fully cover newly planted seedlings. No other soil erosion control measures were employed on the trial. No pest and disease management measure was applied on the seedling weakened by the arid conditions.

Survival of trees in that trial was very low at only 20% which was mainly *C. equisetifolia* (SFF, 1989). This result prompted the wide planting of *C. equisetifolia* in semi-arid regions across the Philippines. Casuarina plantations along the semi-arid regions of northwestern Luzon have also created real livelihood opportunity for surrounding rural communities.

Case* 2. *Casuarina in mine rehabilitation

About 30% (9 million ha) of the Philippines land area is geologically prospective for metallic minerals. Estimates indicate the value of these minerals to be US $840 billion. This is roughly equivalent to ten times the country's annual GDP and 15 times its total foreign debt (Senate Economic Planning Office, 2005).

Despite the projected enormous economic benefit, the environmental cost of mining can likewise be staggering if not properly managed. The ever contentious economic benefit versus environmental costs debate over development activities opens enormous opportunities for restoration science to address.

The unique ability of casuarinas to survive, grow and develop in adverse site conditions e. g. mined-out sites, tailings pond makes the species a natural choice for mine rehabilitation. Many species of casuarinas have exhibited this versatile site adaptability as proven in several studies (El-lakany, 1983; Gauthier *et al*., 1985; Lugo *et al*., 1990; Sellstedt and Geoffrey, 1990; Ndiaye *et al*., 1993; Srivastava, 1995; Warren and Zou, 2002; Cadiz, 2005; Cadiz, 2006; Sasidharan *et al*., 2007).

C. equisetifolia is being used in broad-scale rehabilitation in at least eight mining companies in the Philippines based on a recent survey conducted by the Ecosystems Research and Development Bureau (2010). The survey compiled the current practices involving casuarinas among other species from seed collection, seed processing and storage, planting stock propagation, field planting, and plantation management.

Case* 3. *Casuarina supporting green energy production fields

Electricity generation has grown importance in the Philippines during the last 25 years while the country's hydro-electric power plants have started to age and have become inadequate to current demand. Natural gas-fired power plants are being promoted along with geothermal power generators. Geothermal energy provides 27% of the Philippines' total electricity production generated in power plants. The Philippines is the second biggest user of geothermal energy in the world. Geothermal power plants are on the islands Luzon, Negros, Mindanao and Leyte. The use of geothermal energy can minimize the use of fossil fuels and thus help mitigate global warming.

However, the fluids drawn under the earth carry a mixture of gases, notably carbon dioxide (CO_2), hydrogen sulfide (H_2S), methane (CH_4) and ammonia (NH_3). These pollutants could contribute to global warming, acid rain, and noxious smells if released into the air. Most of these geothermal production fields in the Philippines are located in upland watersheds which require clearing portions of forests. With plans to expand the country's geothermal energy programme up to 3,100 MW within the next decade, more forests will have to give way.

The heat and air pollution generated by geothermal plants have been observed to kill native plants and trees around them. Establishing *C. equisetifolia* and/or *G. rumphianum* buffer zones around geothermal power plants have been found to be effective in protecting native vegetation.

Despite the upgrade in power generation in the country during the last 25 years, electricity supplies in remote rural areas are still lacking. Proposals for village size dendro-thermal energy generators have been in the pipeline since the 1980, but the lack of sustainable supply of fuelwood have left the proposal on the side. Attention on fuelwood production strategies are being revived as the community-based approach to forest resources management takes into effect. Technical support to fuelwood production, as well as to support timber production for village-level livelihood programmes, is anticipated to accelerate planting of village fuelwood plantations of which *C. equisetifolia* is anticipated to be one of the dominant species of choice.

4 Conclusion: Enhancing the Role of Casuarinas in the Philippines

The main use of casuarinas in the Philippines remains mainly to date for land rehabilitation. Developing an industry around casuarinas was not given much attention during the last decades also due to availability of other alternative species and other government priorities.

The potentials of casuarinas as bioremediation material against air and water pollutions from traffic, mines, industry and urban sewerage are now being investigated by scientists at the University of the Philippines science community.

Casuarinas are expected to be included among the top priority species for the upcoming national tree plantations programme, which will form part of the national strategy for climate change adaptation and to lift people's living standards in rural communities. The anticipated role of casuarinas in the new tree plantation programme targets the development of short-rotation community plantations that will produce raw materials for small diameter timber industries.

The renewed interest on casuarinas in the Philippines will require a thorough assessment of the available genetic materials. Understanding the status of the native populations of casuarinas is essential to evaluate the "species and genetic capital" of the country.

Hastening technology adoption and innovations that will optimize the use even of short-rotation or small-diameter trees of casuarinas are imperative to promote its wide-spread planting among small-holder tree farms as well as by corporate tree farmers. New products and markets should be explored and developed to make casuarinas competitive in the domestic and international markets.

A systematic collection of seed throughout the Philippine archipelago is now being organized under the collaboration between the DENR-ERDB and the UPLB College of Forestry and Natural Resources. It is anticipated that this collection of seed will come in time for the second international casuarina trials being coordinated by CSIRO Australia and partners in Southeast Asia.

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A Review on Wind Protection Efficiency and Wind Resistance of Coastal *Casuarina equisetifolia* Shelterbelt in Southern China

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Abstracts The successful role of shelterbelt is measured by its ability to protect damage from strong wind or in other word - wind protection efficiency (WPE). Wind protection efficiency is expressed as the percentage of the difference in the wind speed between open field (windward side) and behind shelterbelt (leeward side). This paper reviews wind protection efficiency of *Casuarina equisetifolia* shelterbelt in relation to the height, width, interval and density of shelterbelt, and mixed planting models based on the research conducted in southern China. It is concluded that:

(1) WPE increases with increasing wind speed. When wind speed is less than 2 m s^{-1} maximum WPE is 3%. When the wind speed is higher than 10 m s^{-1} maximum WPE reaches 52%.

(2) Shelterbelt height influences WPE. WPE of 15 m shelterbelt height is greater than WPE of 10 m shelterbelt height. The effective wind protection distance is 20-35 times of the height of the shelterbelt, i.e. if shelterbelt is 10 m high the protection distance is up to 350 m.

(3) Narrow shelterbelt is better than wide shelterbelt. The most effective shelterbelt width is 8-28 m. However, shelterbelt width does not have a direct influence on the WPE. WPE is depended on the ratio of shelterbelt width to shelterbelt height. Shelterbelt width will have little impact on the WPE if the ratio is less than 5. In addition if the ratio is greater than 5 wind protection distance will decrease.

(4) Small interval (gap) between shelterbelts is better than large interval. It is a good strategy to keep the interval within the wind protection distance. The best interval is 150-200 m.

(5) Stand density of shelterbelt affects WPE. 1,750-2,500 stems ha^{-1} is effective.

(6) Age of casuarina stand can affect WPE. Middle-age stand of 15 years is most effective. Over mature stand (35 years old) is less effective in wind protection.

(7) Mixed species planting improves WPE and reduces risk of insects and diseases.

1 Introduction

As a suitable tree species for coastal shelterbelt protection, *Casuarina equisetifolia* was introduced to China in the early 1950s and has since been planted extensively in southern provinces of Fujian, Guangdong, Guangxi, Zhejiang, Hainan, Taiwan and Yunnan. Introduction trials have been carried out in southern China by the Chinese Academy of Forestry (Wang *et al.*, 1992; Zhong *et al.*, 2005). At present, the total plantation area is about 0.3 million ha (Zhong *et al.*, 2005).

C. equisetifolia has significant ecological benefits in southern China (Luo, 2002; Lin *et al.*, 2003; Zeng, 2005). Its wind protection efficiency is especially remarkable. This paper gives an overview of wind protection efficiency and wind resistance of coastal *C. equisetifolia* shelterbelts in southern China.

2 Wind Protection Efficiency (WPE) of Casuarina Shelterbelts

In coastal area, wind often becomes a disaster for people and crops, especially during the typhoon season when wind speed can reach 50 m s^{-1}. High-speed winds often destroy crops and houses. Casuarina trees have tall stem and wide crown with needle-like foliage, and thus can withstand winds and subsequently decrease wind speed. Therefore, casuarina shelterbelts have good wind protection efficiency.

WPE is expressed as percentage of the difference in the wind speed between open field (f) and behind shelterbelt (n) or leeward side of the shelterbelt.

$$WPE = (W_f - W_n)/W_f \times 100$$

Thus higher percentage indicates higher wind protection efficiency of the shelterbelt. There are many factors affecting the level of WPE including height and width of shelterbelt, structure of shelterbelt, and age of the trees.

2.1 Effect of shelterbelt height on WPE

Shelterbelt height significantly influences WPE. Table 1 shows that the WPE of 15 m high shelterbelt is better than that of 10 m high shelterbelt. When WPE was compared at the point of distance 5, 10 and 15 times of shelterbelt height (5H, 10H and 15H respectively) the wind velocity was much lower behind the 15 m shelterbelt than the 10 m shelterbelt. Both heights, however, showed a consistent decline in the WPE with increasing distance from the shelterbelt.

Table 1 Changes of wind speed passing through two different height shelterbelts (modified from Lin *et al.*, 1998)

Shelterbelt height (m)	Open field (m s^{-1})	5H		10H		15H	
		Velocity (m s^{-1})	WPE (%)	Velocity (m s^{-1})	WPE (%)	Velocity (m s^{-1})	WPE (%)
15	4.30	0.86	80	1.12	74	1.70	60
10	5.70	2.02	65	3.41	40	4.63	19

With regard to wind protection distance, higher shelterbelt is more efficient than lower shelterbelt. Of the 10 m height, WPE reduced by 25% from 5H to 10H, and 21% from 10H to 15H. According to this trend, the wind speed at 20H would be about the same at the open field. For the 15 m height this would occur at 35H. Thus the wind protection distance of 15 m high shelterbelt is much greater than that of the 10 m high shelterbelt.

2.2 Effect of shelterbelt width on WPE

Shelterbelt width does not appear to directly affect WPE, but rather the ratio of shelterbelt width and height. Zheng *et al.* (2008) examined three different shelterbelt width and the results showed that the shelterbelt whose width is 0.5 time its height had the minimum wind speed at 4 H. The shelterbelt whose width is 0.5 to 2 times its height had the minimum wind speed at 3-4 H. The WPE values of three shelterbelts were almost the same (45%). The trend was that WPE would decrease gradually if the shelterbelt width was increased. Thus narrow belt would have better wind protection than wider belt. Based on several studies, the most effective shelterbelt width is 8-28 m.

2.3 Structure of shelterbelt and WPE

Shelterbelt structure is a comprehensive structure that consists of tree crown; under canopy; shelterbelt width; vertical section of stand (optical porosity); distribution of stems, branches and leaves; tree density; and degree of ventilation. WPE of different shelterbelt structures varies greatly because of spatial distribution of all parts of trees. There are three main structure types: sparse structure, closed structure and ventilated structure. Generally, sparse structure had the best WPE, and ventilated structure had the least WPE. Zheng *et al.* (2008) reported that the average WPE within 20 H distance from shelterbelt of sparse structure, closed structure and ventilated structure was 37.5, 32.3 and 16.7% respectively.

Stand density is a key parameter of forest structure. Based on effective protection distance of shelterbelt, Zhu (1992) worked out that ventilation coefficient was 0.5- 0.6 for best shelterbelt structure. According to this parameter, Ye *et al.* (2000) recommended the suitable density for best shelterbelt structure at 1,755-2,505 stems ha^{-1}. Experiments indicated that WPE of shelterbelt with 2,205 stems ha^{-1} was 2.4% greater than that of 3,375 stems ha^{-1}, and 15.7% greater than that of 875 stems ha^{-1} (Ye and Wang, 2008). WPE of shelterbelt with 2,505 stems ha^{-1} was 6.35% greater than that of 1,845 stems ha^{-1}, and 9.96% greater than that of 1,755 stems per ha (Lin, 2004). According to these studies, 2,250 stems ha^{-1} is considered the best planting density for *C. equisetifolia* shelterbelt.

2.4 Shelterbelt interval and WPE

Proper shelterbelt interval increases WPE. If the interval distance is less than the wind protection distance, the next shelterbelts will continue to reduce the wind speed until the wind becomes very weak. If the interval distance is more than the wind protection distance, the wind speed will remain the same as the open field speed.

Table 2 shows that short interval between shelterbelts gives higher WPE than longer interval (Zheng *et al.*, 2008). Huang *et al.* (2003) drew a conclusion that the best interval was 150-200 m, considering multi-protection benefits including WPE.

Table 2 Wind protection efficiency of two different interval shelterbelts (Zheng *et al.*, 2008)

Shelterbelt No.	Height (m)	Interval (m)		Field wind speed ($m\ s^{-1}$)	WPE (%)
		Main belt	Assistant belt		
1	12.5	150	100	3.5	39.3
2	12.9	200	150	4.2	26.8

2.5 Age of stand and WPE

Shelterbelts of different growth stage have different WPE because of differences in height, structure and density. Lin (2004) found that WPE increased with the age of the stand from 15 years to 30 years old, and WPE decreased when age of the stand increased from 30 years to 35 years. WPE recorded for 15, 25, 30 and 35 years old stands were 10.53, 17.09, 19.06 and 5.57% respectively. However in another study, Wang *et al*. (2008) found WPE (based on decrease in wind speeds) increased with the age of the stands from 15 years to 35 years between 5H and 25H distance (Table 3).

Table 3 Wind protection efficiency of different growth stage shelterbelts (Wang *et al.*, 2008)

Stand age (year)	Height (m)	Density (stems ha^{-1})	Wind speed ($m\ s^{-1}$)						
			Field	In belt	5H	10H	15H	20H	25H
15	11.61	1,534	7.0	5.0	4.5	3.0	3.5	4.8	6.0
30	13.71	1,350	7.0	5.6	5.0	3.4	4.0	5.0	6.2
35	13.35	1,209	7.0	6.3	5.7	4.3	5.2	6.0	6.8

2.6 Mixed planting, interplanting and WPE

Besides *C. equisetifolia*, there are many shelterbelts planted with other species such as acacia, eucalypt and pine in southern China. Single species tends to be more susceptible to damage by insects and diseases. Mixed species planting is a good way to minimize this problem. WPE of casuarina and acacia mixed forest was found to be highest (80%), followed by casuarina and pine mixed forest (74.6%), casuarina and eucalypt mixed forest (63.8%), and pure casuarina forest (54.4%) (Su, 2004). In an 8-year-old shelterbelt, Ye *et al*. (2008) also found that WPE of pure *C. equisetifolia* or pure pine shelterbelt was less than that of mixed-species shelterbelts.

Seven shelterbelt models were qualitatively analyzed and evaluated by Yue *et al.* (2009) using hierarchical process. WPE of casuarina and acacia mixed model was the best, followed by casuarina and fruit tree mixed model, but the latter had weak stabilisation and stress resistance because of damage by insects and diseases. Li (2007) found that casuarina and pine mixed planting by block was more efficient than mixed planting by rows. Mixed-species shelterbelts decreased wind velocity by 88-98%.

In fact, casuarina and acacia mixed forest not only provided the best WPE, but also had the benefit of improving soil fertility (You, 2005). Interplanting hybrid *Eucalyptus grandis* × *E. urophylla* in gaps of *C. equisetifolia* shelterbelt helps form an uneven-aged forest, and improve WPE (Liao, 2007). Zhou (2007) and Wu (2009) also found similar results.

3 Wind Resistance of Casuarina Shelterbelts

In construction of coastal protection shelterbelts, we not only consider the WPE, but also consider wind resistance. Wind resistance of casuarina shelterbelt has a close relationship to characteristics of tree. Chen *et al*. (1999) reported that Typhoon No. 9615 which occurred on September 9, 1999 with wind speed of 48-57 $m\ s^{-1}$ caused severe damage to shelterbelts in the coast of Zhanjiang. After the storm, wind damage to *C. equisetifolia* forest was investigated. Results showed that sample plots with more and thicker lateral roots had fewer damages. Sample plots with taller trees, wide crown and greater height and diameter ratio had more severe damages.

Casuarina trees raised from seed have a well developed taproot and are more wind resistant than those raised by cuttings. However, seedling plantations have higher rate of broken stems by strong wind. Cutting plantations have less broken stems but higher rate of wind throw.

Wind resistance has a close relationship to age of casuarina stands. Wind resistance of a 30-year-old shelterbelt was found to be the best. Its damage ratio was 12% lower than that of a 15-year-old shelterbelt, 10% lower than that of a 25-year-old shelterbelt, and 8% lower than that of a 35-year-old shelterbelt (Lin, 2004).

Shelterbelts with low height and high ventilation coefficient have good wind resistance. Shelterbelts with varying tree height (shorter trees in front) also have good wind resistance. But the latter has shorter wind protection distance (Dexin and Zhu, 1998).

Narrow row and wide spacing within row of shelterbelt plantations have been reported to enhance wind resistance (Xu and Lao, 1984; Chen, 1997; Chen *et al*., 1999).

Compared to *E. exserta* and *Hevea brasiliensis*, wind resistance of *C. equisetifolia* was moderate. *E. exserta* was the best and *H. brasiliensis* was the worst (Yang and Feng, 2007).

4 Conclusions

The effectiveness of coastal shelterbelt is measured by its ability to protect and resist against winds. There are many factors affecting wind protection efficiency including height and width of shelterbelt, interval between shelterbelts, stand density, age of stand, and structure of shelterbelt.

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Important Roles of Casuarina in Coastal Shelterbelt Construction in Hainan Province

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Abstract This paper gives an overview of casuarina planting in Hainan. Casuarinas are a very important group of species for this island province which faces frequent typhoons every year. Casuarinas play important roles in environmental protection and supporting economic development. The effort of government on stabilization of the moving sand is described. There is a decline in plantation productivity which may be due to very few clones being used. The need for genetic improvement of casuarinas is emphasized.

1 Background

1.1 Natural geography and social economy

Hainan lies in the south of China with an area of 340,000 km^2. It is the only tropical island province of China. Its coast line is over 1,528 km, of which 876 km is sandy soil land, 250 km is soil land, and 402 km is rocky land. Hainan Island lies in the northern part of tropical regions in the world. It belongs to tropical monsoon climate with high temperature and humility, clear raining and dry season, no frost, and mean annual rainfall 1,500-2,000 mm. The population is 8.5 million of which the majority is Han (7.2 million). The other 1.3 million belong to Li and Miao. In 2009, Hainan GDP was 1.466×10^{12} RMB (1 USD = 6.7 RMB).

1.2 Forest

The forest area in Hainan is about 1,993,000 ha or 58.5% of the island's total area. Of this 651,000 ha are natural forest and 1,342,000 ha are plantations. The plantations include species such as rubber (600,000 ha), eucalypt (180,000 ha), betel nut palm (130,000 ha), mango (100,000) ha, acacia (65,000 ha), casuarina (50,000 ha) and 217,000 ha of economic crops, e.g. litchi, coconut, longan and jack fruit.

Casuarina is mainly distributed along coastal area as windbreak shelterbelt, which plays important role as key tree species against typhoons, desertification, and improvement of livelihoods. For the last three years, over 10,000 ha of casuarina plantations were established with a few clones (i.e. Bao 9 and Dong 2). In the coastal front line, plantations established from seedlings are preferred as they tend to resist the strong winds better than plantations established from cuttings.

2 Casuarina Introduction and Sand Dune Management

2.1 Status of land desertification of Hainan before rehabilitation

There are 63, 000 ha of coastal sandy land in Hainan province, which are distributed in more than 80 towns in 12 counties. Up to 3 million people live in these areas. It is well known that coastal areas are economically and socially developed regions in Hainan with many important industries, agriculture-based industries and tourist resorts. However, before 1955, most of the 63, 000 ha coastal lands were moving sand dunes due to very scarce vegetative cover. It was hard for people to survive in such poor living conditions. According to available data, the sand dunes in Fengpo town of Wenchang city moved inward 1, 600 m during 1, 749-1, 949, an average of 8 m per year. About 180 ha of agricultural land were affected or destroyed each year. Extremely poor living conditions led to many infectious diseases, such as tuberculosis.

2.2 Achievements of moving sand control and stabilization

In order to improve the poor ecological environment, two forest farms were established in 1955 in east and west of Hainan Island with a main task to stabilize and rehabilitate the moving sand. Based on a series of surveys and experiments, casuarina (*Casuarina equisetifolia*) was found to be an excellent species for forestation on sandy land. Moving sand stabilization techniques by establishing casuarina plantations was extended rapidly. By 1980s, more than 50, 000 ha of casuarina coastal shelterbelt were established. In Wenchang, mixed plantations of casuarina and coconut were innovatively established to enhance protection efficiency, landscape and economic benefits. Up to now, a total of 7, 000 ha of mixed plantation have been accomplished, and subsequently the renowned Dongjiao coconut plantation holiday resort was built. Furthermore, coconut industry was rapidly developed following the mixed plantation construction that at present coconut processing industry has become the pillar industry of Dongjiao in Wenchang city. As a result of successful introduction of casuarina, moving sand dunes have been stabilized and rehabilitated. About 1, 100 km "green wall" has been constructed along the coast line of Hainan Island, and livelihoods of local people improved substantially.

2.3 Excessive exploitation leading to destruction of coastal shelterbelt

In 1985, ilmenite ($FeTiO_3$) and Zircon ($ZrSiO_4$) minerals were found under casuarina coastal shelterbelt in Hainan which led to excessive and uncontrolled exploitation and consequently serious destruction of the coastal shelterbelt. In the early 1990s, large area of coastal shelterbelt was cleared as a result of tourism and real estate development. In the late 1990s, fish and shrimp farming was expanded rapidly further causing severe loss of coastal shelterbelts. The protection efficiency of coastal shelterbelt against typhoons has been adversely affected. Based on a survey in 2005, the total length of coastal shelterbelts was reduced from 1,100 km to 799 km of which only 260 km (32%) is considered to meet the national standard. Up to 230 km coast line was completely without shelterbelt protection. The total area of casuarina plantations was decreased to 40, 000 ha. Ecological disaster once again occurred. Since 1990, there have been 14 typhoons and 21 tropical storms in Hainan, causing 36 dead, 260, 000 houses completely destroyed and 510, 000 houses seriously damaged. The direct economic loss is estimated at 28.6 billion RMB.

2.4 Rehabilitation and reconstruction of coastal shelterbelt

In 2007, the government issued a decision for complete rehabilitation and reconstruction of coastal shelterbelt within 3-5 years. In the decision, 0.25 billion RMB has been approved to establish or regenerate 10,000 ha of coastal shelterbelt, and 1,200 ha fish ponds would be returned to coastal shelterbelt. Many measures have been taken to ensure the implementation, such as liability contracts between provincial government and local governments were signed. Construction progress is periodically encouraged and inspected by provincial government. Local governments are also committed to coastal shelterbelt construction. At the end of 2009, 10,000 ha of coastal shelterbelt (mainly casuarina plantations) were established, and 1,000 ha fish ponds have been returned to coastal shelterbelt. The area of casuarina plantations has reached 50,000 ha, and previously broken coastal shelterbelt has been restored. For the effective protection of coastal shelterbelt, the government has issued a "Regulation on construction and protection of coastal shelterbelt in Hainan". Many special staff have been employed to protect and manage the coastal shelterbelt.

2.5 Industry development in relation to casuarina

Casuarina plays important roles not only in protecting life and property security of coastal residents, but also in promoting economic development of Hainan and improving income of farmers.

2.5.1 Yield and economic output of casuarina plantations

Yield and economic output of casuarina plantations are increased considerably using techniques such as tree breeding and selection, clonal propagation by root cutting, etc. Currently, there are 10,000 ha short-rotation and fast-growing casuarina plantations in Hainan. The highest timber volume recorded is 160 m^3 ha^{-1}, average MAI is 15 m^3 ha^{-1}, and average output is 4,500 RMB ha^{-1}. Since 1986, the amount of cuttings produced by water culture has increased consistently (Table 1). Based on available data, 12 million of cuttings were produced all around Hainan in 2009, which could meet the seedling demand for 5,400 ha casuarina plantations.

Table 1 Casuarina seedling production in Hainan

Year	No. of propagation sites	Yield (millions)	Price (RMB $cutting^{-1}$)	Price (RMB $seedling^{-1}$)	Regions of planting	Producer
1986	2	2	0.03	0.12	Wenchang	Forest farms and research institution
1990	2	6	0.03	0.12	Hainan	Forest farms and farmers
2000	3	8	0.05	0.18	Hainan	Forest farms and farmers
2009	3	12	0.05	0.25	Hainan	Forest farms and farmers

2.5.2 Export of casuarina wood chip

Since 1996, casuarina wood chips have been exported in large quantities to Korea, and the export amount increased steadily from 30,000 tonnes in 1996 to 70,000 tonnes in 2007, earning 7 million USD in revenue. The amount of export has declined to 20,000 per year from 2008 due to increase in domestic consumption. The main local buyer is the Asia Pulp and Paper in Hainan (Table 2).

Table 2 Export amount of casuarina wood chips in 1996-2009

Year	Country	Export amount (tonne)	Price (USD $tonne^{-1}$)
1996	South Korea	30,000	98
2000	South Korea	40,000	96
2005	South Korea	50,000	100

(continued)

Year	Country	Export amount (tonne)	Price (USD $tonne^{-1}$)
2006	South Korea	60,000	100
2007	South Korea	70,000	100
2008	South Korea	20,000	100
2009	South Korea	20,000	100

2.5.3 Diverse uses of casuarina wood products

Prior to 1990, casuarina wood in Hainan was mainly used as fuelwood, for charcoal production, as sheet material for boat building and general construction timber. Casuarina timber is very suitable for building fishing boat. Since 1996, casuarina wood has been increasingly used for paper pulp, and from 2001, rotary-cut veneers are produced from casuarina logs by a local plyboard factory (Table 3).

Table 3 Changes in the use of casuarina wood in Hainan

Type of use	1980 (%)	1990 (%)	2000 (%)
Fuel and charcoal wood	80	60	20
Sheet material	10	20	10
Construction timber	10	20	10
Wood chips and paper pulp	0	0	50
Rotary-cut veneer	0	0	10

3 Main Problems of Casuarina Plantations in Hainan

3.1 Low-quality casuarina stands

According to the surveyed data obtained in 2008, there were 43,000 ha of casuarina plantations in Hainan, for a total timber volume of 1,290,000 m^3, with an average timber volume of 30 m^3 ha^{-1}. This can be broken down into different age classes as follows:

- Sapling plantations 12,400 ha, total timber volume 143,000 m^3, average volume 11.5 m^3 ha^{-1}.
- Middle-age plantations 14,400 ha, total timber volume 408,000 m^3, average volume 28.3 m^3 ha^{-1}.
- Mature plantations 8,200 ha, total timber volume 377,000 m^3, average volume 46 m^3 ha^{-1}.
- Over-mature plantations 8,000 ha, total timber volume 377,000 m^3, average timber volume 47.1 m^3 ha^{-1}.

In general, the timber volume of casuarina plantations per unit area is very low. There are plausible reasons: (1) loss caused by frequent typhoons; (2) damage caused by human activities; and (3) degradation in productivity of casuarina clones.

3.2 Simple structure of casuarinas stands

More than 80% of the casuarinas plantations in Hainan are pure, even-aged forests. The simple structure, low biological diversity, and lack of natural regeneration have contributed to low protection efficiency against typhoons and storms.

3.3 Lack of seed orchards

Currently, only small number of clones (Bao 9, Dong 2, Weng 10, A 8 and A 13) is being cultivated. It is estimated that clone Bao 9 alone covers up to 90% of the total casuarina plantation area. Although Hainan forestry agencies such as the Hainan Forest Institute has initiated a breeding programme and clonal selection. Plantations from seedlings are also important to enhance genetic diversity and improve resistance against typhoons. However, there are no seed orchards or seed production stands in Hainan that can be used to collect good quality seed for plantation establishment. Only recently has a progeny trial/seedling seed orchard of *C. equisetifolia* been planted in Hainan by the Research Institute of Tropical Forestry. It will be several more years before seed collection can be made.

4 Recommendations for Further Development

4.1 Quality enhancement of coastal shelterbelt

To improve quality of coastal shelterbelt which is usually dominated by casuarina species, more mixed-species planting should be considered. Mixed species may be between casuarina and coconut, casuarina and *Calophyllum inophyllum*, casuarina and *Acacia confusa*, and casuarina and *A. auriculiformis*. Mixed species planting will lead to more stable coastal shelterbelt and promote natural regeneration capacity.

4.2 Speeding up development of fast-growing and high-yielding casuarina plantations

Focus should be transferred gradually from stabilization of moving sands to timber industry development. Selection of superior clones with fast-growing and strong adversity resistance should be stressed. Meanwhile, diversifying use of casuarina timber should be developed to improve the timber price, and subsequently increase income of farmers.

4.3 Establishment of germ plasma bank of casuarina

Based on a thorough survey on available casuarina germplasm resources in Hainan, plus trees (both male and female trees) should be selected. Seeds or root cuttings of plus trees are then collected to establish seed orchards or seed production stands. These stands can supply large quantity of superior seed for fast-growing and high-yield casuarinas plantations. However, this is a short-term approach to quickly obtain genetically improved seed and capture genetic gain. For long-term sustainability, there is a need to start the genetic improvement programme by importing new genetic material from broad genetic base to set up breeding populations which include progeny/seedling seed orchards. The progeny trials already established by the Research Institute of Tropical Forestry will be useful for selection and breeding to meet long term objective.

Acknowledgment

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Application of Diffused Reflectance Near Infrared Spectroscopy for Evaluation of Kraft Pulp Yield and Lignin in *Casuarina equisetifolia* and *C. junghuhniana*

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Abstract *Casuarina equisetifolia* is widely raised in coastal regions of India as a pulp wood species. *C. junghuhniana* is a recent introduction and is being evaluated for its pulp traits. Conventional methods of measuring kraft pulp yield (KPY) and lignin are laborious and quick estimation of KPY and lignin from core samples is necessary. To accomplish this we developed NIR (Near Infrared) calibration models for screening trees for KPY and lignin. The KPY calibration model has a correlation coefficient (R) 0.97, root mean square error of estimation (RMSEE) of 0.19 and residual prediction deviation (RPD) of 4.3. The R value for lignin model (0.97) and RPD (4.18) are comparable to that of KPY model whereas the RMSEE (0.7) is higher. The calibration statistics indicate that these two models are suitable for screening lignin and KPY in *C. equisetifolia* and *C. junghuhniana*. Using these models NIR predictions were made for KPY and lignin in *C. equisetifolia* at different heights for 10 trees. KPY was highest at 30% of tree height and lignin showed a reverse trend.

1 Introduction

The paper industry in India uses casuarina wood raised mostly in the coastal regions of India to meet the growing demand of raw material. There is however not much information available on pulp yield variation in casuarina. Conventional methods of estimation of pulp yield and lignin are quite laborious and large number of trees cannot be screened in a short time. *Casuarina equisetifolia* is traditionally grown in agroforestry systems for a variety of uses like windbreaks, firewood, charcoal, poles and pulp wood. *C. junghuhniana* is a recent introduction and in the early stages of domestication. It is thus important to screen this species for pulp yield before selection to suit each end use. Wood variation in *C. equisetifolia* was investigated within a tree (Chowdhury *et al*., 2009), across different sites (Varghese and Sivaramakrishna, 1996) and for pulp yield (Kandeel *et al*., 1982). These investigations were based on a few trees from each site and may not represent the entire variation within a population. A rapid method to screen large number of samples is needed to breed for pulp yield. Near Infrared Spectroscopy (NIRS) has been widely used for rapid measurement of pulp wood traits in eucalypts (Birkett and Gambino, 1989; Michel and Schimleck, 1998;

Terdwongworakul *et al.*, 2005). This paper describes the development of NIRS models for rapid estimation of kraft pulp yield (KPY) and lignin (klason) in *C. junghuhniana* and *C. equisetifolia*.

2 Materials and Methods

Ten trees each of *C. junghuhniana* and *C. equisetifolia* were sampled from two-year-old plantations in two locations (Mettupalayam - 11°30′N, 76°95′E and Ongole - 15°33′N, 80°02′E) in southern India. Core samples were extracted from each tree at breast height. Disc samples were collected at 10, 30, 50 and 70% height from each *C. equisetifolia* tree for the study. For NIRS studies the wood discs were debarked, converted into chips, force air dried at 50℃, milled and the material that passed through 60 mesh sieves was used. Lignin estimation was done on extracted material prepared as per Tappi method T-264 (Tappi, 2007).

Kraft pulp yield (KPY) was estimated in a rotary digester. The conditions used for digestion are: weight of OD chips, 1.0 kg; NaOH/Na_2S liquor ratio, 1 : 2.8; sulphidity, 22%; active alkali, 16%. The temperature programme for the cook was: ambient to 98℃, 55-60 min; 98℃ to 165℃, 45-55 min; time at 165℃, 90 min. After digestion the pulp was separated, washed and refined in a disc refiner. The pulp was extracted using a hydro extractor, separated and weighed. Acid insoluble lignin was estimated as per the Tappi method T-222 (Tappi, 2006).

2.1 Near infrared spectroscopy

NIR measurements were made with a Bruker Multi Purpose Analyzer (MPA) (Bruker Optics, Ettlingen, Germany) using the integrating sphere accessory over the range 12,800-3,600 cm^{-1} at 2 cm^{-1} resolution. Wood meal was placed in quartz vials for analysis. The average value of three measurements was used for each sample.

Spectral processing and calibrations were performed using the Bruker QUANT software package within OPUS version 6 (Bruker Optik, 2006). Calibration models were developed using partial linear regression analysis (PLR). The spectra were processed using a straight line subtraction for lignin and no spectral processing for KPY calibration. Cross validations and external set validations were performed on the calibration models. Root mean square error of cross validation (RMSECV), correlation co-efficient (R), co-efficient of estimation (R^2) and residual prediction deviation (RPD) were used to assess the performance of the calibration. For screening studies RPD value greater than 2.5 is preferred.

NIRS calibration models developed for *E. camaldulensis* (Ramadevi *et al.*, 2010) was checked for its suitability in casuarina. It was found that the prediction error for KPY was higher (±5%) than that for Lignin (± 2%). Hence Lignin estimation in casuarina was done using eucalyptus calibration model where as a separate calibration model was developed for KPY.

3 Results and Discussion

A calibration model for KPY was developed with 18 core samples comprising 9 trees each of *C. junghuhniana* and *C. equisetifolia.* The wave numbers used for KPY estimation are 9,998-7,478 and 6,102-4,598 cm^{-1}. The cross validation plot is represented in Fig. 1. The statistical data of partial linear regression analysis for calibration and cross validation are presented in Table 1. The values obtained for R (0.97), RMSEE (0.19) and RPD (4.3) for the current calibration plot are better than that reported for *E. camaldulensis* (R = 0.93, root mean square error of estimation - RMSEE = 1.25 and RPD = 2.79) (Ramadevi *et al.*, 2010). The reason could be that the eucalyptus model was developed with multiple eucalyptus species unlike the casuarina model which is done with 2 species having less variation in two geographic locations or due to wood structure and composition or both. The validity of the plot was evaluated by external calibration with 20 samples of *Casuarina* (10 each for *C. junghuhniana* and *C. equisetifolia*) collected from the same locations. The prediction error against each clone is presented in Fig. 2. The prediction error for 95% of the samples tested is within ±2% except one sample with 2.2% error, which needs to be further investigated. The KPY data shown in Table 2 indicate that the variation in KPY values between the two *Casuarina* species is marginal. Rajesh *et al*. (2009) estimated 50.2% KPY in *Casuarina* (without bark) by conventional pulping process. In the current study KPY values estimated ranged from 47% to 51.7%.

Table 1 NIR Calibration Statistics-Statistical results of partial linear regression analysis

Parameter	Data range	Pretreatment	Wavelength region	Calibration			Prediction			
				RMSEE	R	RPD	RMSECV	* RPD	* R	* Bias
KPY	47-50%	No spectral processing	9998-7478 6102-4598	0.19	0.97	4.3	0.3	2.03	0.87	0.03
Lignin-Acid insoluble	19.9-34%	Straight line Subtraction	7749-6935 6522-5863 4964-4008	0.7	0.97	4.18	0.77	3.55	0.96	0.02

RMSEE: Root mean square error of estimation, R: Correlation coefficient, RPD: Residual prediction deviation, RMSECV: Root mean square error of cross validation. * For cross validation.

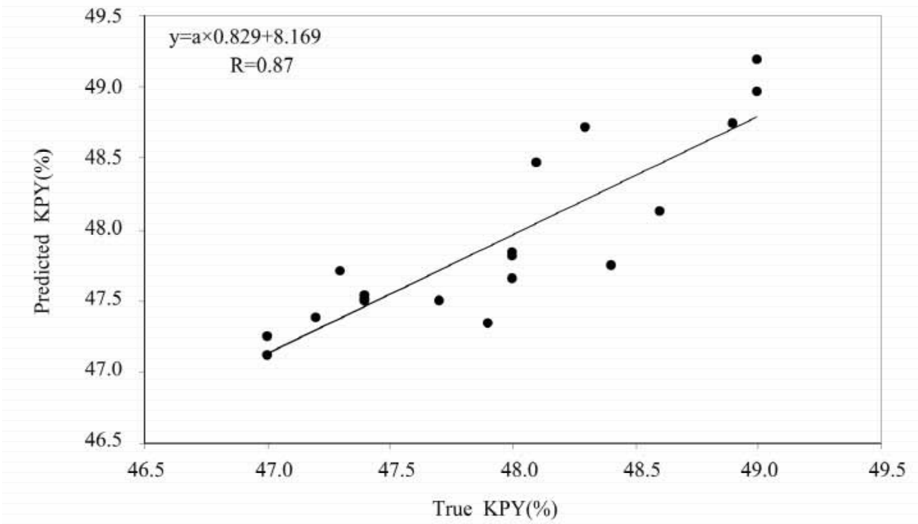

Fig. 1 Cross validation plot for KPY - *C. equisetifolia* and *C. junghuhniana*

Wave number selected for estimation of lignin are 7,749-6,935, 6,522-5,863, 4,964-4,008 cm^{-1}. Cross validation plot for lignin is shown in Fig. 3 and the statistics are presented in Table 1. The calibration model used for lignin measurement in *Casuarina* was developed with 50 samples of *E. camaldulensis* (Ramadevi *et al*., 2010). The validity of the model for *Casuarina* was tested with nine casuarina samples. The prediction error ranges from −1.8% to 0.7% (Fig. 4). The calibration model will be expanded with additional samples of diverse material of *C. junghuhniana* and *C. equisetifolia* to improve accuracy of estimation. The suitability of the lignin calibration model

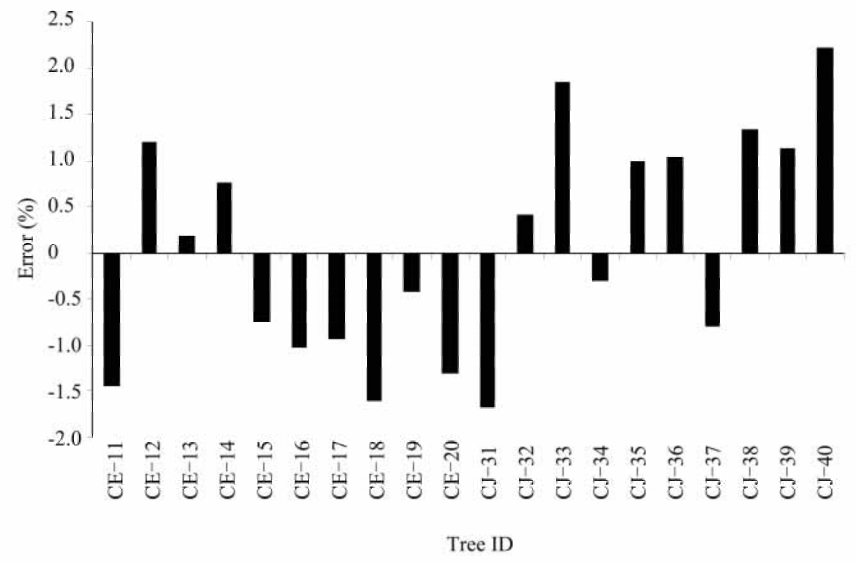

Fig. 2 External validation - *C. equisetifolia* and *C. junghuhniana*

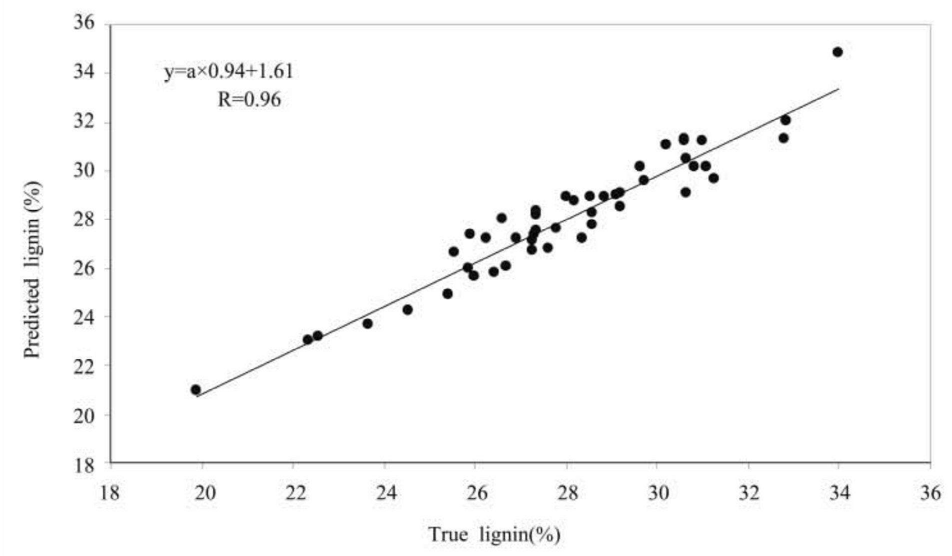

Fig. 3 Cross validation plot for lignin - *E. camaldulensis* and *E. pellita*

developed for eucalyptus may be due to wavelength selection used or similarity with respect to lignin in both.

Wood samples collected from *C. equisetifolia* at different heights (10, 30, 50 and 70% of tree height) in 10 trees (2 years old) were evaluated for lignin and KPY using NIR (Tables 2 and 3). The data indicate that average KPY values range from 47.4% to 50.2%. A uniform trend is observed in KPY values at different positions within a tree (10, 30, 50 and 70% of tree height). KPY is marginally higher at 30% height than at 50% and 70% height except in the case of sample CE-16 where the values at 50% height are nearly the same as that at 30%. The KPY value at 70%

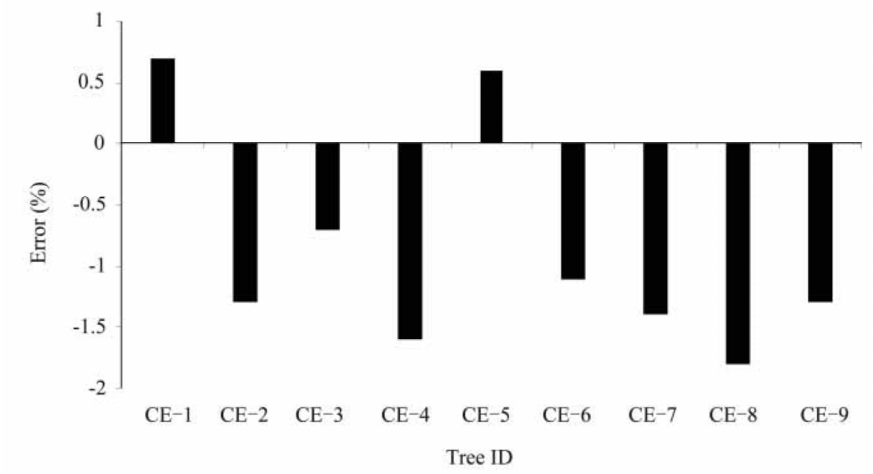

Fig. 4 Prediction error for external validation of lignin - *C. equisetifolia*

Table 2 KPY at different tree height positions in *C. equisetifolia*

Tree ID	KPY				
	10% of Height	30% of Height	50% of Height	70% of Height	Average %
CE-11	51.5	48.5	49.0	47.0	49.0
CE-12	51.6	46.0	46.6	45.5	47.4
CE-13	50.9	51.0	48.6	47.0	49.4
CE-14	52.6	48.8	48.4	47.5	49.2
CE-15	50.9	49.2	48.6	49.5	49.6
CE-16	50.5	50.8	49.6	51.1	50.5
CE-17	50.2	49.1	49.0	49.3	49.4
CE-18	50.6	48.5	49.4	44.1	48.2
CE-19	50.2	50.9	49.3	49.2	49.9
CE-20	51.7	50.2	49.3	49.5	50.2

Table 3 Klason lignin at different tree height positions in *C. equisetifolia*

Tree ID	KPY				
	10% of Height	30% of Height	50% of Height	70% of Height	Average %
CE-11	19.5	21.0	20.9	20.7	20.5
CE-12	21.3	21.7	23.6	20.9	21.9
CE-13	23.5	23.2	24.8	23.3	23.7
CE-14	21.4	24.3	22.8	21.9	22.6
CE-15	22.8	24.1	25.1	23.8	24.0
CE-16	22.6	23.6	24.7	22.1	23.3
CE-17	22.4	23.8	23.2	22.4	22.9
CE-18	22.7	22.9	21.9	–	22.5
CE-19	24.1	24.3	24.3	24.3	24.2
CE-20	21.5	24.1	23.7	24.3	23.4

height is approximately the same or lower than that at 50% tree height. Variation in KPY between different tree positions (30% to 70% height) ranges from 0.9% to 6.1%, whereas it is only 0-2.8% in lignin. The lower variation in lignin within a tree may be linked to the stability of this trait in the tree. The lower average lignin content (20.51 ± 3.2) observed in the present study compared to the reported value (26.32 ± 3.71) by Mahmood (1993) for *C. equisetifolia* wood may be attributed to the differences in age of the tree and site.

4 Conclusion

The calibration models can be used to quickly estimate pulp yield and lignin content in large number of trees in both species of *Casuarina*. Core samples are quite handy for non destructive estimation of the whole tree pulp yield (since variation within tree is marginal) which can be used as an important criteria for selecting trees in a breeding programme. The models will be expanded further with more samples from different locations to reduce prediction error (< 1.0%), improve precision and ruggedness of the estimation.

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Pests and Diseases

Current Research on Main Casuarina Diseases and Insects in China

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Abstract Bacterial wilt (*Ralstonia solanacearum*) is a major disease widely affecting casuarina trees. More than ten fast-growing and disease-resistant clones were selected and three have been cultivated extensively in Fujian and Guangdong provinces. *Anoplophora chinensis* and *Lymantria xylina* are major insect pests in casuarina forests. In order to control insects of casuarina trees, five provenances developing sticky paste of *Beauveria bassiana* for trunk borer were screened out. Effectiveness of *Melia azedarach*'s trap on *A. chinensis* was tested. Good results were obtained on biocontrol of *L. xylina* by means of *B. bassiana* and nucleopolyhedrosis virus (NPV). Future research should focus on (1) occurring characteristics of major diseases and insect pests of casuarina trees, comprehensive prevention and control measures; (2) development and application of effective low toxic biological pesticides; (3) further screening of resistant clones of casuarina trees; and (4) propagation techniques. Meanwhile, integrated-control model forest is to be built with existing scientific and technological achievements.

1 Introduction

Casuarina trees are pioneer, localized on sandy soils along the coast of southeastern China which includes Fujian, Guangdong, Guangxi, Hainan, Zhejiang and Taiwan provinces. However, poor ecological conditions and poor management lead to degraded casuarina forests and subsequently create favorable conditions for diseases and insect pests in casuarina trees. Symptoms include top blight, broken trunk and windfall of trees. In some areas, tree shelter has become too sparse with survival less than 50%, and gradually lowering shelter efficiency. This paper summarizes current research on major diseases and insects pests of casuarina trees in China and suggests strategies for further studies.

2 Diseases of Casuarina Trees

Main diseases of casuarina trees found in China include bacterial wilt (*Ralstonia* (*Pseudomonas*) *solanacearum*), hexenbesen (mycoplasma-like organism and bacterium-like organism), canker (*Phomopsis* sp.), powdery mildew (*Oidium* sp.) and cataplexy (*Rhizoctonia* sp.) of plantlets. Of these, bacterial wilt is the most serious (He, 2007a), and the main focus for discussion here.

2.1 Research on casuarina bacterial wilt in China

Bacterial wilt of casuarina trees is caused by *R. solanacearum*. It was first found in 1964 on Hailing island in Yangjiang county, Guangdong province (Liang and Wang, 1982), and later found in Hainan, Fujian and Guangxi provinces (Xu, 1980; Liang and Cen, 1982; Gao *et al*., 1987; He, 1998). The interaction of its pathogenesis and the meteorological factors, tree species, tree ages and management techniques, and resistant mechanism have been studied (Xie *et al*., 1980; Liang and Chen, 1982; Liang and Chen, 1984; Guo and Liang, 1985; Gao *et al*., 1987; Xie, 1991; Wang *et al*., 1997; Luo *et al*., 1998; Xue *et al*., 1999; Ye *et al*.,1999; Luo *et al*., 2002).

2.2 Selection of disease-resistant casuarina clones

Bacterial wilt is a vascular bundle disease mainly infecting via root, and is difficult to prevent and cure using chemical agents and general approaches in forest operation. Selection and cultivation of disease-resistant clones are considered better solution. More than ten clones with fast growth and disease resistant characteristics were selected by South China Agricultural University and Fujian Academy of Forestry, three of which have been applied (Chen *et al*., 2003). Liang and Chen (1984) screened clones with fast growth, straight and upright trunk and good disease resistance for application in field. Disease-resistance of 201 progeny clones of *C. equisetifolia* was screened, and clones with fast growth and high vegetative propagation rates were selected (Ke *et al*., 1994).

The results of cross-inoulation test between *C. equisetifolia* clones and bacterial wilt revealed that horizontal resistance (no race specialty) and vertical resistance (race specialty) co-existed in the pathological system of *C. equisetifolia* and bacterial wilt, and the dominant one was horizontal resistance (Chen and Zhang, 1995; Wang, 1997). Wang (1996) found disease resistance of *C. equisetifolia* clones to bacterial wilt was influenced by plant materials, inoculation condition and disease pathogenic classes. It was suggested that moderate concentration of bacterial wilt fluid as substrates to inoculate on rooting casuarina cutting should be a reliable and quick method to detect casuarina disease-resistant clones.

2.3 Interaction of *Frankia* and disease resistance

Important characteristics of tree species of Casuarinaceae family are the ability to form root nodule, have symbiotic association system with *Frankia* actinomycetes and bear the active nitrogen fixation function. Some studies revealed that strong disease resistant clones had 2-4 times nodulation numbers and nitrogen fixation activity, compared with those with weak disease resistance (Zheng, 1996). *Frankia* can also improve the capacity of nitrogen fixation of casuarina trees plantlets and prevent bacterial wilt (Kang, 1999).

3 Insect Pests of Casuarina Trees

Insects pests of casuarina trees include *Anoplophora chinensis* Forster, *Zeuzera multistrigata* Moore, *Arbela bailbarana* Mats., *Lymantria xylina* Swinhoe, *Chondracris rosea* De Geer, *Stauropus alternus* Walker, *Euzophera batangensis* Caraadia, *Poophilus coastalis* Waiker and *Icerya purchasi* Maskell. Among them, *A. chinensis* and *L. xylina* are the most serious of all (He, 2007b).

3.1 *Anoplophora chinensis*

3.1.1 Biological characteristics of *A. chinensis*

Anoplophora chinensis is the most serious trunk-boring insect of casuarina trees (Chen, 1983; Huang, 1991; Zhong, *et al*., 1993; Xu, 1997). Each generation cycle is one year though some may produce two generations cycles in three years. Larvae feed in the trunks and are mostly concentrated at the base of the trunks up to 40 cm above ground. The hazardous period lasts at least ten months. As a result, the damaged trees may die in about one year after the attack. Mortality in new shelter forests may reach 67%, especially at sites with high moisture.

3.1.2 Prevention and cure techniques of *A. chinensis*

By artificial inoculation of larvae in glasshouse and field experiments, resistant clones C38, C39, C40, C43 and C44 have been selected and cultivated along the coast of Fujian (Ding *et al*., 1996; Huang *et al*., 1999b). Meanwhile, the investigation on morphology and distribution of vessels, rays and fibers of wood tissues of resistant clones in combination with the analysis of the fundamental nutrition and chemical compounds in timber, including soluble sugar, free amino acids, tannin, total nitrogen and phenols, were studied as mechanism of insect resistance (Huang *et al*., 1999a).

Resistance and growth of casuarina trees in mixture with other tree species were better than those of pure casuarina forests because properly retain of litter and other ground covers in the forests can ameliorate small ecological environment and enhance productivity of forests or increase the species and numbers of natural enemy of the insects. The parasitism percentage of *A. fukutaiin* in the casuarina forests with abundant ground covers was 15.5% higher than those forests with bare ground at Huian Forest Farm in Fujian (He *et al*., 2000).

Fungi paste consisting of waste molasses, sweet potato powder and *Beauveria bassiana* spores at 100:10:1 ratio was found to be effective in the control *A. chinensis*; death rate of larvae reached 77% (Huang *et al*., 1990a; 1997; Liu *et al*., 1999). Eight nematodes species and two application methods were used to control *A. chinensis* in plantation and the best infection strain was *S. f. Beijing* with mean infection of 86.65% (Huang *et al*., 1990b).

Studies indicated that *M. azedarach* bears strong attraction to the imagoes of *A. chinensis*. The straight distance of attraction can reach more than 200 m, and peak of attraction is around June and July. The percentage of trees in the stand with larvae after trapping can be reduced by more than 20% (Huang *et al*., 2001).

As the hazardous area of *A. chinensis* is mainly concentrated at the base of the trunks, some artificial treatments can be applied. For example, from the last ten days of May to the first ten days of August, it is suggested to crush the eggs of *A. chinensis* where there is evidence of frass near the trunk. This method is convenient, non-polluted and effective.

3.2 *Lymantria xylina*

Lymantria xylina is a dominant leaf-feeding insect on casuarina trees in Fujian, Guangdong, Zhejiang and Taiwan provinces. Each generation cycle is one year and the hazardous period during larval stages is from March to June. Generally, mature forests are more seriously damaged than younger forests. Pure casuarina forests were damaged seriously probably due to lack of species diversity. *C. glauca* and *C. cunninghamiana* were found to be more resistant to *L. xylina* than *C. equisetifolia* (Li *et al*., 1981; Zhang *et al*., 1985). The egg mass, larvae and pupa of *L. xylina* gather in the casuarina shelter forest and the larvae are spread in the forest in the manner of aggregation (Chen *et al*., 1993).

3.2.1 Biological prevention and cure of *L. xylina*

Lymantria xylina nucleopolyhedrosis virus can cause epidemic disease of *L. xylina* and as a result, it can be used as a biological control agent. Much of the work has been done in Fujian province. In the middle and last ten days of May, larvae of 4-6 stages are about 85%, which is suitable period to control *L. xylina* using the virus. The success rate in the forest is up to 84.7% and the effective time last 3 to 5 years (Chen *et al*., 1987; Xie *et al*., 1987; He *et al*., 1991). Primary studies regarding the cytoplasmic polyhedrosis virus and granulosis virus of *L. xylina* have also been reported (Chen, 1987; Chen and Zhang, 1995).

High virulence *B. bassiana* strains against *L. xylina* has been obtained in Fujian through isolation and culture of *B. bassiana* agents. Sustainable control of the insect pests in the stands is achieved by using these agents. *B. bassiana* Bb-tz was selected and manufactured to spore suspension in Taiwan. The suspension applied to larvae 3-5 different stages was reported to result in 83% of attacking rate after 7days (Cai *et al*., 2001).

In a control comparative experiment by Huang(2000), *B. bassiana*(Bb), *L. xylina* nucleopolyhedrosis virus (LxNPV), *Bacillus thuringiensis* and deltamethrin powder mixed in different ratio were applied to *L. xylina* in glasshouse and field. The control effects of the mixed powder of *B. bassiana*, the nuclear polyhedrosis virus (2×10^6 PIB g^{-1}) for *L. xylina*, *B. thuringiensis* ($140 \times 10^8 g^{-1}$) and 0.05% of deltamethrin with 2 : 1 : 1 : 1 proportion were most effective. The average effects reached 92.1% and the mixed-powder control possessed the evident synergism as compared with the 4 single-powder control.

3.2.2 Other methods

Mixing various tree species together, retaining vegetative cover in forest and strengthening management can improve resistance of forests to insect damage. Other methods like picking and destroying egg mass on the deadwood and trapping imagoes by black-light lamps can also be applied. Insecticide can control larvae at 3-4 different stages, however, it is difficult to apply in large area of forests, and also causes pollution.

4 Conclusions

How to control the diseases and insect pests is important to successful management of shelter forest along the coast. Biological control is the trend among insect pest management. With regard to the current situation of serious insect pests in casuarina shelter forests, it is necessary to study forecast of insect pests, develop biological pesticide and effective low toxic insecticide, and develop biological agents which promotes tree growth and safe. The development and application of biological pesticide should steadily impel. Moreover, specialized and fungi strains and bacterial agents with strong virulence against pest insects, such as *A. chinensis* and *L. xylina* should be screened and prepared continually.

The variety of insect or disease resistant casuarina clones is still limited, and with the wide spread of single clones, insects will adapt to these clones and thus increasing hazardous levels. Breeding for resistance is another efficient way to control casuarina plantation pests. Studies of casuarina resistant clones also need to continue.

Acknowledgments

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Guam Ironwood (*Casuarina equisetifolia*) Tree Decline Conference and Follow-up

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Abstract Ironwood trees (*Casuarina equisetifolia*) on the island of Guam are in the midst of a decline that was first noticed in 2002 by a local farmer. The trees at that site were less than 10 years old and planted in single-row windbreaks of several hundred trees. Less than 5 trees were characterized as wilted with the following symptoms: acropetal progression of chlorosis, tip-burn of lower branchlets giving the tree a singed appearance, and tree death within 6 months. Roughly 15 trees had symptoms of decline, which included internal wood discoloration, thinning of branches and tree death after several years. By 2005, Ironwood Tree Decline (IWTD) was widespread on Guam. In January 2009, a five day IWTD conference was held with participants from Guam and off-island. The six off-island experts and other participants visited healthy and declined tree sites, collected samples, and reviewed research related to *C. equisetifolia* production worldwide and its growth on Guam. Participants concluded that a complex of biotic and abiotic factors were responsible for the decline and advanced the theory that an opportunistic conk producing fungus like *Ganoderma* in association with wounding could explain the majority of Guam's declining trees. Among the abiotic factors are typhoons and lawn equipment that damage roots, branches, or the boles of trees. Insects that may play a role in IWTD are termites and a newly discovered eulophid wasp which forms galls in branchlet tips. In subsequent post-conference analyses, at least five conk-forming species have been found fruiting on Guam's ironwood trees. Of those, *Ganoderma* and *Phellinus* are most likely playing a role in IWTD. By applying various modelling techniques to a set of IWTD predictors, it was concluded that the presence of basidiocarps, termites, and improper

tree care were significant explanatory variables for the decline.

1 Introduction

The ironwood tree (*Casuarina equisetifolia*) has grown on Guam for thousands of years (Athens and Ward, 2004). Ironwood Tree Decline (IWTD) was first noticed in 2002 by a local farmer and reported to the University of Guam Cooperative Extension. At the time, the impact was negligible as Donnegan *et al*. (2004) reported the non-occurrence of primary damage types in their survey of Guam's estimated 115,924 trees that were greater than 12.7 cm in diameter at breast height (DBH). By 2005, IWTD was widespread (Campora, 2005). Based on ironwood tree surveys conducted in 2008 and 2009, an estimated 51% of Guam's trees greater than 12.7 cm DBH are showing symptoms of decline.

This paper gives a historical account of decline on Guam and reports on the findings of a 5-day IWTD conference held in January 2009 together with results from subsequent research (Mersha *et al*., 2009; Mersha *et al.*, 2010a; Mersha *et al*., 2010b; Schlub, 2010; Schlub *et al*., 2010).

2 Materials and Methods

Conference participants and attendees included administrators, researchers, students, the general public, and six off-island experts. Fourteen sites were visited during the 5-day conference period where samples in the form of branches, cross-sections (roots, trunks and branches) and basidiocarps (conks) were collected and brought to the laboratory at the University of Guam's science building.

Ironwood Tree Decline (IWTD) was assessed through visual monitoring, destructive sampling, and GPS assisted surveys. Photographs of 44 randomly selected trees with varying levels of decline were categorized into saplings to small trees (DBH$\leq$ 32 cm) or large trees (DBH $>$ 32 cm). These were then visually catalogued based on a five-scale decline severity (DS) rating. Percent loss of tree vigour or percent decline (PD) and percent bare branches (PBB) were determined by analyzing the photographs. Cross-sections of 5 small and 3 large tree trunks and of branch trunk intersections from 34 small and 26 large trees were examined for evidence of discoloration or wood rot. Four to five branches from randomly selected trees were removed (30 cm from branch tip) and growth parameters measured. The branch sections were stripped and branches and branchlets (needles) weighed. Cones were counted, weighed, and placed in 20 cm diameter Petri dishes on the laboratory bench (temperature 24-25℃ and 50-55% relative humidity) to promote seed release.

For each tree and site, explanatory variables of decline were measured including tree diameter, fire damage, typhoon damage, presence or absence of termites, presence or absence of conks, and various geographical or cultural conditions. A total of 1,398 trees at 38 sites was surveyed for decline from October 2008 to June 2009 (Survey I). From July 2009 to December 2009, a follow up survey of the original trees was conducted (Survey II). This survey was expanded to include additional characteristics as well as 29 additional trees and 6 sites.

Survey II data was examined to identify those tree and site variables that may aid in predicting IWTD, while addressing the data structure issue of cluster sampling at various sites. Logistic and multinomial modelling techniques were applied to the survey data. The logistic model was determined to be the best.

3 Results

Percent bare branches (PBB) and percent decline (PD) increased progressively as decline severity (DS) increased from 0 to 4. The difference between PBB and PD was negligible (Fig. 1). The weight differences of branchlets were not significant (ANOVA using Tukey's test) between DS 0 and DS 1 or DS 2 and DS 3 (23 and 25 grams and 15 and 12 grams respectively). DS 4 was considerably worse with a 95.3% loss in weight when compared to branchlets of DS 0 trees.

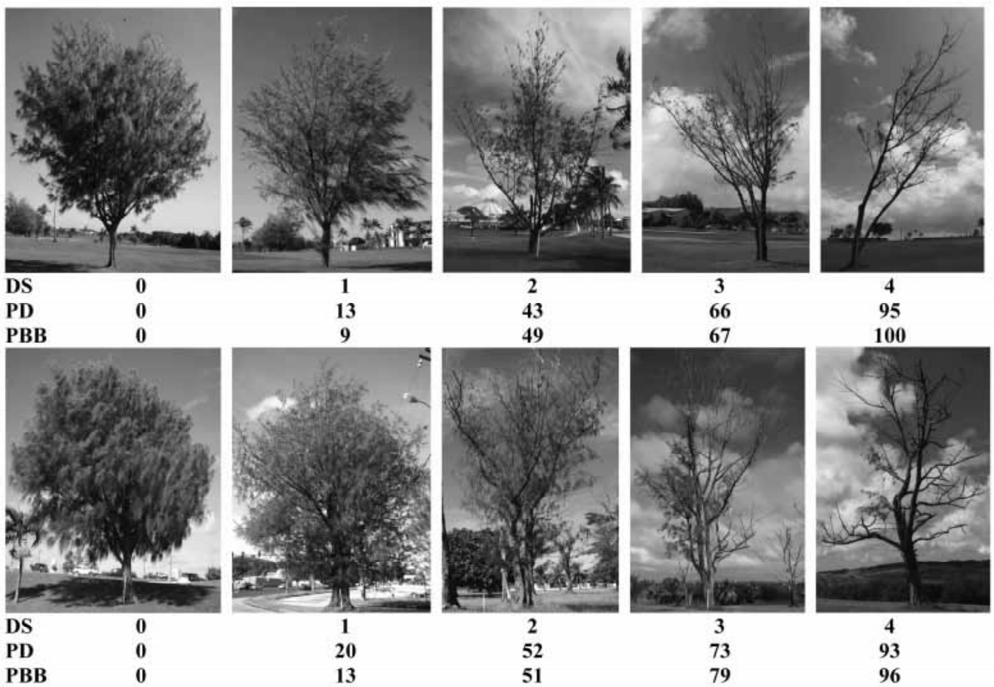

Fig. 1 Representative photographs of small (above) and large (below) solitary trees from across Guam depicting a five-level decline severity scale (DS), percent decline (PD), and percent bare branches (PBB)

The proportions of the wasp-damaged branchlets were 0.07, 0.08, 0.20, 0.49 and 0.38 for DS 0, 1, 2, 3 and 4 trees, respectively. The average lengths of branchlets (with and without galls) that formed in bundles were 13.06, 14.04, 13.70, 10.62 and 6.67 cm for DS 0, 1, 2, 3 and 4, respectively.

Percent germination of seeds was not significantly affected by the extent of decline (ANOVA, P = 0.138). Percent seed germination of 31.3, 30.4, 29.5, 44.7 and 24.6% were recorded for trees at DS 0, 1, 2, 3 and 4, respectively. However, germination was significantly affected (one-way ANOVA using Tukey's test, P = 0.041) by sowing medium; Sunshine mix (37.5%) and Sunshine mix plus *Frankia* nodules (36.1%) were better than the clay soil brought from the root area of ironwood trees (22.8%).

Internal discolouration due to wood rotting fungi was consistent with decline. The proportion of discolouration in cross-sectional areas taken from the branch junction of large branches of declining trees ranged from 80% to 100% and discolouration was present at all DS levels. For small declining trees it consistently appeared only for DS levels 3 and 4 trees. In healthy trees, discolouration did not appear in small tree branch cross-sections but was occasionally observed from those of large trees, due to mature heartwood. There was a clear, consistent gradient of discolouration within the tree trunk of declining trees (Fig. 2). Linear functions derived from the average proportion of discoloured wood at each sampling distance describe well the actual acropetal wood discoloration gradients recorded within small and large trees (Fig. 2). Wood rotting fungi that produce conks are known to cause the internal discolouration and white soft rot commonly found in DS 3 level trees (Fig. 2). The importance of these fungi in decline is also supported by the fact that the percentage of trees with conks increased with IWTD: 2, 18, 35, 47, and 66% for DS 0, 1, 2, 3, and 4 level trees respectively.

Throughout the course of the surveys, when lower tree branches were visually accessible, flower types were noted and the tree was categorized. Guam's ironwood population was comprised of 80% monoecious and 13% dioecious trees of which 3% were males. The remaining 7% were non-flowering trees during the surveys. As a result of the survey II, it was determined that IWTD largely remained the same at 9 sites, had increased at 17 sites and had decreased at 12 sites (Fig. 3). Apart from the longitude and latitude variables for their spatial effects, the remaining 13 predictive variables were evaluated for fitness through the application of several model selection algorithms. The final logistic model was reduced to six variables: conk, human intervention, termites, tree stand density, latitude, and longitude (Schlub *et al.*, 2010). Level of human intervention was rated as slight, moderate, or high based on the level of landscape maintenance at a tree site. Tree sites that were unattended were rated as slight. Those located in parks and cemeteries were rated as moderate and those located on golf courses and campuses were rated as high.

4 Discussion

From the results of the logistic model, the most significant variables that could explain the ironwood tree's state of health are the presence of basidiocarps, level of human intervention, or the presence of termites. However, an alternative cumulative logit model using all five DS classes had some lack of fit. Some reasons could be that assumptions on proportional odds were not met. Possible ways to improve the cumulative model include adding more explanatory variables or to move away from the cumulative model to a multinomial model where the decline severity index is not considered ordinal (Schlub, 2010; Schlub *et al.*, 2010).

The five-level visual DS rating (Fig. 1) was helpful in monitoring the spatial and temporal dynamics of IWTD on Guam and could potentially be useful for the neighboring islands. At least 5 basidiocarp genera have been identified on Guam's ironwood trees: *Ganoderma*, *Favolus*, *Phellinus*, *Pycnoporus* and *Sarcodon.* Of those, *Ganoderma* and *Phellinus* are most likely playing a role in the IWTD. The prevalent *Ganoderma* sp. appears to be a part of the *G. australe* species complex. When dead and dying trees were excavated and examined, the origin of infection by a conk producing fungus could often be traced to mechanical damage or by root contact with infected trees. The rate of spread by root contact appears to be 6 to 8 m in 5 years. All other pathogens being considered as components of IWTD on Guam are *Botryosphaeria*, *Fusarium*, *Pestalotia*, and yet unidentified fungal and bacterial cultures.

Insects are believed to play a supporting role in IWTD by reducing tree vigour and creating wounds that serve as ports of entry for tree pathogens. Though two of Guam's invasive scarab beetles, *Protaetia pryeri* (Janson) and *P. orientalis* (Gory and Percheron) have been shown to be associated

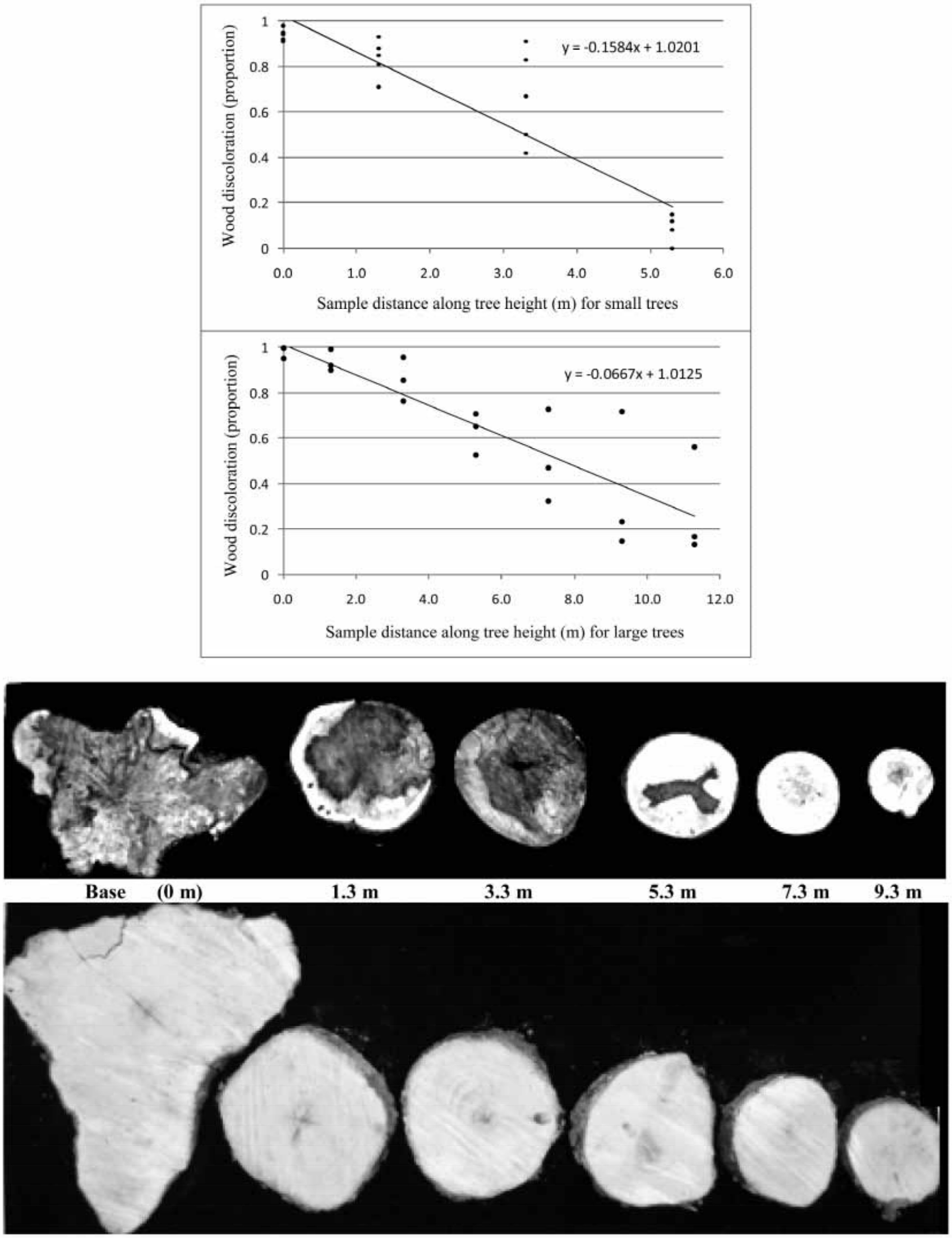

Fig. 2 **Proportion of wood discoloration in trunk cross-sections fitted to a linear decay function for small (upper) and large (lower) trees and trunk cross-sections from two small trees, one declined (top) and one healthy (bottom)**

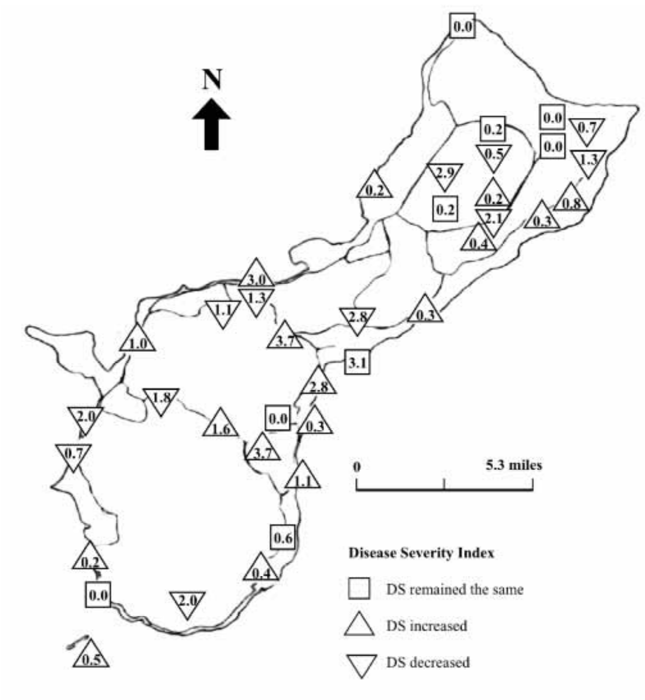

Fig. 3 Means of decline severity (DS) found at sites during Survey II (July to December 2009). Values in comparison to Survey I (October 2008 to June 2009) remained nearly the same (square), increased (up-triangle) or decreased (down-triangle)

with ironwood trees, they do not appear to be correlated with IWTD (Campora, 2005). Most of the severely declining trees (DS 2 to 4) on Guam were heavily infested with termites. From past entomological surveys and reports, colonies of *Nasutitermes* sp. and *Microtermes* sp. were found feeding on dead ironwood trees (Moore, A., personal communication). The Philippine milk termite *Coptotermes gestroi* was responsible for killing ironwood trees transplanted onto a new golf course (Yudin, L. S., personal communication). A species of wasp reared from branchlet tip galls was identified as belonging to the genus *Selitrichodes* (Eulophidae: Tetrastichinae) by John LaSalle, CSIRO, Australia. Damage to branchlet tips by this wasp is correlated with decline severity in restricted locations on the island; however, future studies are needed to confirm whether the wasp is responsible for any significant impact on the health of ironwood trees on Guam (Mersha *et al.*, 2009).

Preliminary survey results, as presented in this paper, indicate IWTD is associated partly with the level of human intervention. Data collection on variables like edaphic characteristics, typhoon history, grassland fires and soil available water are still underway. The two major typhoons, Chata'an in July 2002 and Pongsona in December 2002, accompanied by an intervening drought were mentioned at the IWTD conference as possible contributing factors to IWTD (Guard, C. and Lander, M., personal communications).

5 Conclusions

Ironwood trees, like all trees, have a natural finite life span within various ecosystems and under various abiotic and biotic pressures; however, Guam's trees are dying at rates that far exceed the norm for the region. What is happening to Guam's ironwood trees fits the classic definition of tree decline: symptoms are nonspecific in nature; tree health gradually deteriorates leading to tree death over a course of several years; and the decline is attributed to a complex of environmental and non-infectious and infectious agents. However, Guam's trees deviate from the classic model, where large trees are more prone to decline.

Though visual assessment of a tree's canopy and branchlet biomass are good indicators of decline, they fail to separate the various physiological and environmental factors that may also be responsible for poor growth; whereas, the detection of wood discoloration in the trunk and to a lesser extent in the branches does provide such insight. A good indicator of decline is the appearance of discoloration that does not conform to those of heartwood and other natural causes. From preliminary reports, it appears that IWTD does exist in pockets on the nearby island of Rota approximately 80 km from Guam but not on the island of Saipan which is 200 km away. Unlike Guam, Saipan has some large healthy trees with basal conks between their buttressing roots, thereby providing evidence that wood rotting basidiomycetes alone are not always responsible for IWTD.

There are several implications that can be drawn from this study. First, the strong biotic association with decline indicates that decline may be reduced or eliminated with measures that preclude favorable conditions for termites and wood rotting fungi. Second, due to the slow progression and general sporadic nature of decline, the biotic component of decline is not likely to be highly virulent; therefore, decline on Guam could be reduced substantially by the introduction of new cultivars of *C. equisetifolia*. However, if nothing is changed, decline will likely continue on Guam. Third, Guam's current management and cultural practices need to be revised to avoid promoting decline by creating wounds from improper use of grass trimmers and lawnmowers or by spreading infectious agents on pruning tools. The bases of *Casuarina* trees should be protected against scarring from fire. Tree density should be maintained at a level that is appropriate for the age and size of the tree so that competition does not become too great between neighbouring *Casuarina* trees. Storm damaged limbs and branches should be removed to prevent termites and wood rotting fungi from becoming established in the tree. Trees broken from typhoons should be felled by excavation instead of sawing where their colonization by a wood rotting fungus could possibly lead to infecting the root systems of neighbouring healthy trees.

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Short Communications

Role of Defense-Related Genes in the Early Stages of the Actinorhizal Symbiosis

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The soil actinomycete *Frankia* has the ability to establish a nitrogen-fixing symbiosis on the roots of actinorhizal plants. The symbiotic process involves a complex interplay between the host and its symbiont, and results in a drastic alteration of the expression of the host actinorhizal genes. Within the framework of a bilateral project PESSOA (Portugal/France), the role of defense related genes was investigated during the symbiotic interaction between the tropical tree *Casuarina glauca* and the *Frankia* strain *CcI*3.

Transcriptomic and proteomic analyses are in progress to reveal the molecular mechanisms of the plant response upon *Frankia* infection. Gene expression profiles during the early stages the symbiotic process have been analyzed by means of a cDNA array including 15,000 non-redundant Expressed Sequenced Tags. A number of genes involved in the defense response to pathogens and other stresses were found to be up-regulated. A proteomic analysis is also being developed and will contribute to additional knowledge about root differentiation during nodule development.

Together with the global 《omic》 approaches, several candidate genes have been chosen for functional analysis, based on their putative link in defense response. These genes were identified among cDNAs from young nodules of *C. glauca*. Two of them, *CgChi*3 (encoding a class III chitinase) and *CgHin*1 (encoding a homologue of hairpin inducing protein) were expressed preferentially in nodules as compared to uninoculated control roots and leaves. Their expression was further regulated by salicylic acid and wounding. To shed light on the functions of these genes, promoter studies in *C. glauca* and *Lotus japonicus*, mutant complementation analysis in Arabidopsis and the production and characterization of the encoded proteins are in progress. RNA interference based on hairpin constructs introduced via *Agrobacterium rhizogenes* will also provide further data on the putative role of these defense-related genes during infection and nodule ontogenesis in *C. glauca*.

All these data should contribute to significant insights in the communication that is required to establish a peaceful symbiotic relationship between *Frankia* and its actinorhizal host.

Casuarinaceae for Soil Rehabilitation in Algeria

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Casuarinaceae trees are fast-growing multipurpose species which do not require chemical fertilizers due to their symbiotic association with the nitrogen-fixing actinomycete *Frankia* and with mycorrhizal fungi that contribute to improve phosphorous and water acquisition by the root system. Casuarinaceae trees can grow in difficult sites, colonize eroded lands and improve their fertility, allowing the subsequent growth of more demanding plant species. Therefore, these trees have been increasingly used for reforestation and reclamation of degraded lands in tropical and subtropical areas.

In Algeria, sand mining activities are developed to fulfill the need for building construction. These activities have indeed a negative impact on the environment due to the destruction of natural ecosystems through removal of soil and vegetation. The restoration of mined land includes ecosystem reconstruction via reestablishment of the capability of the land to capture and retain fundamental resources.

The objective of the project that is currently being developed between Montpellier and Oran is to evaluate the potential of Casuarinaceae trees for rehabilitation of the degraded areas due to intensive sand extraction in the region of Mostaganem (Société des Carrières de l'Ouest). Some Casuarinaceae species such as *Casuarina equisetifolia* have already been introduced in Algeria. So far, to our knowledge, no data are available concerning the identification of symbionts assocciated with *Casuarina* in Algeria.

The Casuarinaceae species that will be the most appropriate for these degraded sandy and salty areas will be identified. The production of casuarina trees for land reclamation will imply the inoculation with suitable *Frankia* and mycorrhizal strains. Their effectiveness for nitrogen and phosphorous acquisition will be evaluated together with their ability to persist in the soil after planting. Contribution of *Casuarina* to soil fertilization will be determined.

Comparative Analysis of the Infection Process in *Casuarina glauca* and *Discaria trinervis*

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Two basic patterns of nodule initiation are found among actinorhizal plants. The first one involves initial entry by the microsymbiont as an intra-cellular penetration through curled root hair; this mode of infection is observed in *Casuarina glauca*. The second pattern involves an initial phase of inter-cellular colonisation of the root tissue, with neither root-hair involvement nor intracellular penetration until the nodule primordium stage. Three of the eight host families are nodulated via the root-hair infection pathway, whereas the other five actinorhizal families initiate nodules via intercellular colonization. The actinorhizal shrub *Discaria trinervis* that grows in Patagonia belongs to this second category.

Molecular analysis of the infection process in *C. glauca* has led to the identification of several marker genes that are expressed during the early stages of the symbiotic interaction, when root hairs become infected by the actinomycetal hyphae. These include the subtilase gene *Cg*12, the auxin influx-carrier gene *CgAUX*1 and *CgDMI*3, a calcium-calmodulin dependent kinase gene. Besides, the *MtENOD*11 promoter from the realy nodulin gene of *Medicago truncatula* was also found to drive reporter gene expression in root hairs of *C. glauca* infected by *Frankia CcI*3.

In order to compare the intracellular and intercellular infection pathways in the two actiorhizal plants *C. glauca* and *D. trinervis*, a procedure for rapid functional analysis of symbiotic genes in *Discaria* was tested using the biological vector *Agrobacterium rhizogenes*. Two different strains of *A. rhizogenes*, A4RS and ARqua1, carrying a 35S-GFP construct were used on *Discaria*. Transgenic roots expressing the reporter gene GFP were obtained for both strains with transformation efficiencies up to 80%. Roots obtained with ARqua1 were more similar to wild-type roots and were easier to nodulate compared to A4RS.

Genetic transformation of *Discaria* was then achieved with the constructs MtENOD11-GUS, Cg12-GUS and CgAUX1-GUS. Preliminary data indicate that CgAUX1-GUS was found to retain its non-symbiotic pattern of expression but did not respond to *Frankia* infection. On the opposite, MtENOD11-GUS exhibited a similar pattern of expression during the symbiotic process in *D. trinervis* as compared to the expression observed in roots of *M. truncatula* upon infection by Rhizobium.

These data pave the way for the introduction of gene constructs that will contribute to decipher the early steps of the association with *Frankia*. This work was financially supported by the Argentinian-French bilateral Project ECOS Sud No. A07B02.

Genetic Diversity of *Frankia* in Fujian Province, China

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There are large number of actinorhizal plants distributed in coastal area and mountains in Fujian province, China. The aim of this study was to describe the genetic diversity of *Frankia* strains symbiotically associated with several species of *Casuarina*, *Myrica*, *Alnus* and *Elaeagnus* in Fujian. Genomic DNA was extracted from *Frankia* strains and used as template in PCR with primers targeting two DNA regions, one in ribosomal operon, and the other in *nif* D-K gene. PCR products were then digested by using a set of restriction endonucleases to generate the restriction fragment length polymorphism patterns. Except two strains nodulating *M. rubra* and a strain infecting *E. oldhami*, 17 *Frankia* strains had an amplified fragment of *rrn* region in 16S-23S rDNA intergenetic spacer (IGS), 12 *Frankia* strains nodulating *C. equisetifolia*, *C. cunninghamiana* and *C. glauca* living in six geographical origins had high homogeneity and were assigned to one group by cluster analysis, five strains from *M. rubra* and *A. cremastogym* were closely related and fell to the other group. All 25 *Frankia* strains showed a single copy of IGS *nif*D-K and generated 8 PCR-RFLP patterns while clustering into three different groups. *Frankia* strains nodulating three species of *Casuarina* in seven different sites had a high degree of genetic similarity to cluster one group. Isolates infecting *M. rubra*, *A. cremastogym* and *A. formosana* were closely related and belonged to the other cluster. *Frankia* from *E. oldhami* formed a distinct different cluster. These results demonstrated considerable genetic diversity among *Frankia* microsymbionts in Fujian.

Casuarina obesa - A Species for Saline Land

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Casuarina obesa is an indigenous species in Western Australia. It often occurs as a small tree or shrub 3 to 12 m tall. It has adapted a natural tolerance to waterlogged and saline soils, usually in river valleys and lakebeds. This species is a 'pioneer' and will colonize degraded agricultural land where it occurs in adjacent areas or where introduced in revegetation projects.
There is an opportunity to develop commercial crops for saline land in Western Australia. Four million ha of this type of land is available, a resource that is increasing in area each year due to past clearing of woodland and forest for agricultural commodities. This saline land has become a serious degradation issue. Perennial grasses such as *Pucinella* sp. and shrubs *Atriplex* sp. in conjunction with drain construction have been the main methods of containment. Integrated revegetation of the catchments with deep-rooted perennials is recognized as one method to control the problem.

The *Casuarina obesa* Working Group, a sub-committee of Timber 2020, obtained funds in 2005 to carry out investigative work managed by the Centre of Excellence in Natural Resource Management based at the University of Western Australia, Albany campus. The study was aimed at providing information for a wood product enterprise to create commercial opportunity for land managers and owners for regional economic development. Perennials may influence the decline of local saline watertables under and adjacent to towns and infrastructure as an additional benefit.

Further genetic development is required to increase the wood yield per hectare. Evaluation of the biomass energy production from this *Casuarina* is ongoing in 2010 for wood pellets and direct power production.

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Cold-tolerant Selection and Effect of Low Temperature on Physiological Index of *Casuarina glauca* Clones

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Cold-tolerant clones of *Casuarina glauca* survived after low temperature (-6 – -5℃) stress. The scion multiplication garden management techniques were summarized as thrice picking and thrice cutting of *C. glauca* in a year in Hangzhou Bay area. The key technologies on cutting propagation including choice of shoot cuttings, substrate ratio, moisture and temperature management were tested and summarized. The survival rate of *C. glauca* cuttings reached 90% or more by application of the management technology. The physiological characterization of two cold-resistant clones and control at different low-temperature treatments were tested. The results show that the relative conductivity, soluble protein content, malondialdehyde (MDA) content content and superoxidase dismutase (SOD) activity of all clones increased with the decrease in temperature. The relative conductivity of two cold- resistant clones of *C. glauca* was lower than that of the control, while the soluble protein content, MDA content and SOD activity were higher than that of the control. The relative conductivity, the soluble protein content, MDA content and SOD activity can be used as screening index for the cold tolerance identification of *C. glauca*.

The Role of Casuarinas in Environmental Amelioration in China

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Casuarina was first introduced to China in 1897, and has since been grown along the coast from Zhejiang to Guangxi. The more commonly planted species are *Casuarina equisetifolia*, *C. cunninghamiana* and *C. glauca*.

The coastline of mainland China is about 18,000 km and islands costline is 14,000 km. Casuarinas are major species for coastal windbreaks that they are known as the "green great wall" in China. These windbreaks resist typhoons, stabilize moving sands and protect farmlands. They provide wood and fuel wood and improve the livelihood of more than ten million people.

The current total area of plantation is about 300,000 ha. Casuarinas are also grown in the inland area. Casuarinas are planted on the "four side", i. e. house side, village side, road side and water side.

Casuarinas are pioneer species for afforestation and improve soil fertility. The litter return to soil is about 14.17 tonne ha^{-1} $year^{-1}$. Plantations of *Eucalyptus* and *Acacia* are more successful on sites formerly planted with casuarinas.

Community Forestry - Great Southern Region Western Australia

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In the Great Southern region of Western Australia where there were thriving forestry family communities, logging native forest timbers Karri (*Eucalyptus diversiflora*) and Jarrah (*E. marginata*) coupled with the global trend to reduce native forest logging mean we now have a complex system of plantation forest growing and management. Forestry has become planted forests on large tracts of agricultural land cleared only around 50-100 years ago in this region.

It has been a challenge for the community to adopt the change. Not only has the industry become more technically advanced, it is multi-functional. In order to make it work, partnering with government, industry, landowners, environmentalists and the wider community has been a significant factor in enhancing the changing face of forestry. Instead of an elite family entity, forestry has become all encompassing, engaging with a broad range of stakeholders, addressing place, time, culture, economics, politics and the environment.

Timber 2020 is a community organization focusing on commercial forestry development. The group has focused its attention on salt land farm forestry. *Casuarina obesa* has proven to be an excellent species in addressing the rising saline water table which has made land unproductive for broad-acre crop and pasture production.

The region covered by Timber 2020 is 38,917 km^2 with a rainfall distribution of 1,500 mm in the west to 350 mm in the east. Soil types range from duplex sand over clay to acidic quartz sands which gives us a wide range of options for species especially casuarinas.

With an estimated population of around 60,000 people in the region, our major challenge for our communities is distance, lack of infrastructure and the cost of transport.

The opportunities ahead for ourselves and our children are endless. The value of information exchange, communication and networking enhances partnership participation towards a common goal, this conference being one of them.

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