Date palm improvement with innovative technologies

S. Mohan Jain, Department of Agricultural Sciences, University of Helsinki, PL-27, Helsinki, Finland. Email: mohan.jain@helsinki.fi

Introduction

Date palm, *Phoenix dactylifera* L., a monocotyledonous angiosperm diploid species belongs to the Arecaceae family. This perennial and dioecious species is the cornerstone of the economy in many date palm producing countries in North Africa and the Middle East and provides nutrition, food security, and raw materials to the food industry; and is considered as a tree of life. It can be propagated by seeds and the progeny is highly heterogeneous and often produces poor fruit quality. Propagation by offshoots is a routine practice. Date palm trees provide sustainable agro-ecosystems in harsh dry environment, and create 'microclimate' that allows to develop agriculture with multiple cropping systems. It provides fruit, fuel, fiber and shade for other essential cover crops. Dates are highly nutritious, a source of sugar, minerals, and vitamins; consumed as fresh or dried, and various products derived from dates.

Sustainable date palm production faces new challenges - industrialization, loss of gene pool, and climate change. There are several climate-change factors including abiotic and biotic stress, increase in UV-B radiation level, global warming etc. Global warming may hamper overall agriculture production due to the appearance of new insect pests and diseases and some existing ones may disappear. Conservations of genetic resources along with the applications of conventional breeding in combination with the biotechnological tools are essential for date palm production.

Red palm weevil (*Rhynchophorus ferrugineus*) is a major challenge to date palm cultivation worldwide. So far, pheromone trapping is used to control them. The Bayoud disease caused by *Fusarium spp.*, a major disease devastates date palm production. Other biotic constraints like phytoplasmas diseases like lethal yellowing and Al-Wijam need attention as well. Utmost attention is needed in developing date palm varieties harboring resistance to these biotic agents, tolerant to drought, salinity, and high temperature and the development of multiple cropping systems in date palm oasis.

In vitro culture

In vitro culture techniques such as somatic embryogenesis and organogenesis have been effectively used for large-scale plant multiplication of horticultural crops and forest trees. Plant multiplication via organogenesis is routinely followed in commercial laboratories worldwide especially in ornamental plant industries and also to some extent in fruits and cash crops like coffee, sugarcane etc. The cost of plant production is generally high due to labor and electricity, which reduces the profit margin. Most of the date palm micropropagation commercial laboratories are operating in countries with low labor cost. The performance of *in vitro* propagated plantlets seems to be improving in terms of yield and early flowering. No major variation was detected in comparative analyses of fruit quality of micropropagated and conventionally-propagated plants. The results clearly indicated that *in vitro*-grown date palm are quite uniform in terms of fruit quality and physical properties. However field performance of somatic seedlings of date palm showed that the plants started bearing fruits within 4 years of field planting of small plants with a leaf length of 100 cm and 1.5 cm diameter at the base. Fruit from the tissue culture-derived plants, cultivar Barhee, was indistinguishable from the fruits of plants originated from offshoots. These results certainly justify the commercial scale of micropropagation procedures of somatic embryogenesis to provide rapid, cost-effective means of obtaining elite date palm planting material. However, this approach has a major bottleneck in that the plant multiplication rate is highly genotypic dependent, and may require modification of culture medium, depending on the genotype. Some of the major advantages of micropropagation are year-round availability of plants, quality control, rapid production of plants of elite cultivars and cold storage of elite genetic material.

Embryo rescue

Embryo rescue technique is carried out by the removal of a zygotic embryo from the seed and planting in a sterile nutrient culture medium. This technique has been used in several crops to produce new hybrids e.g. triticale; used for haploid production by making intergeneric and interspecific crosses, e.g. wheat and oat, *Hordeum vulgare* and *H. bulbosum*. It is used to save embryos that fail to develop naturally in interspecific or intergeneric hybridization where defective endosperms are common. Excised embryos cultured *in vitro*, under suitable basal nutrient culture media, usually germinate immediately. Embryo rescue was successful in reducing the date palm height from a cross between a dwarf palm species *Phoenix pusilla* and cultivated selected *P. dactylifera* cultivars. This is the first report on reducing the plant height in date palm by embryo rescue, and opens the way to genetically improve date palm in a short time.

Protoplasts

There are very few reports on date palm protoplast work. During 2007, callus formation from protoplasts in cvs. Deglet Noor and Takerboucht, Berhee and Zaghloul was produced. So far, critical steps of plant regeneration from recalcitrant date palm protoplasts have been accomplished. The use of feeder layer was the main factor for inducing cell divisions as well as subsequent microcallus and callus formation. However, plant regeneration from protoplast callus have yet to be accomplished before this technology can further be used for producing somatic hybrids. Another major application of protoplast technique is to genetic transformation of date palm by introducing useful genes, e.g. disease resistant, fruit quality, plant height and others. This approach would enable the selection of resistant cultivars and cultivars with excellent fruit quality through field trials, and then combining both traits in one cultivar through conventional (crossbreeding) or somatic hybridization. Also resistance genes can be taken from a cultivar or species with high resistance level to a particular disease through asymmetric somatic cell hybridization, partial genome transfer from donor to the recipient parent. By this approach, virus resistant plants have been produced by fusing protoplasts of *Solanum brevidens* and *S. tuberosum*; herbicide resistance in *Solanum nigrum* and *S. tuberosum*, and *S. nigrum* and *Lycopersicon esculentum*.

Somaclonal variation

Somaclonal variation can become an important component of date palm breeding in which variation regenerated from somatic cells can be used for the introduction of new agronomic, tolerance or quality traits; has a real advantage in widening the genetic basis of this species, relying more or less solely on vegetative propagation. Variation in the somaclones has often been associated with changes in chromosome numbers and/or structure, punctual mutations or DNA methylation or other epigenetic events. Somaclonal variation is undesirable from an industrial production stand point of view but may provide an enrichment of the genes pool. Its frequency depends, among others, on the genotype and the length of the proliferation process. Rapid shoot proliferation was achieved from various parts of the plant including shoot tips, stem cuttings, auxiliary buds and roots. However the selection of the genotype and the number of sub-culture cycles helpful in limiting the appearance of somaclones after the step of plant regeneration. Many off-type plants and abnormal dwarf phenotypes with low fruit sets as well as vitiated multi-carpel fruits are observed among the *in vitro*-propagated date palm tree population. These phenotypes are not always detectable at seedling stages and often become apparent a few years after planting. However, the technological advances and the development of molecular markers have made it possible, in recent years, to early and accurately detect these variants and eliminate them for the mass production.

In vitro conservation and cryopreservation of germplasm

Cryopreservation is widely used for long-term storage of *in vitro* cultures of genetic material under ultralow temperatures, usually at -196^{o}C in the liquid nitrogen This method preserves contamination-free material and prevents somaclonal variation. Since date palm *in vitro* culture has been worked out for plant regeneration, several groups have been engaged in cryo-storage of date palm tissues such as shoot tips, nodular cultures, callus, and somatic embryogenic cultures. Cryo-protectant treatment is given before plunging the tissue in the liquid nitrogen for preventing ice crystal formation in the tissue in order to avoid any damage to the tissue that may adversely affect plant regeneration upon thawing of cryo-stored material. The common cryoprotects are polyehthylglycol (PEG), glucose, and dimethylsulfoxide (DMSO). In date palm, somatic embryo growth remains normal when treated with cryo-protectant mixture of glycerol and sucrose. The growth rate or germination rate of somatic embryos should remain normal after the cryopreservation and that would reflect any adverse impact of various treatments during the following the protocol.

Mutation induction

The exploitation of genetic variability is essential for the development of new cultivars. Genetic variability can be induced by chemical and physical mutagens, T-DNA insertional mutagenesis, and tissue culture-derived variation or somaclonal variation. The most common physical mutagen used is gamma radiation. Induced mutations are random changes in the nuclear DNA or cytoplasmic organ, resulting in chromosomal or genomic mutations that enable plant breeders to select useful mutants such as disease resistant, high yield etc. First of all, gamma irradiation breaks DNA into small fragments and secondly DNA starts a repair mechanism. During this second step, new variations develop or mutations occur. Mutation induction in date palm is feasible now due to a reliable plant regeneration system via somatic embryogenesis and organogenesis. Several putative date palm mutants were isolated that showing resistance to Bayoud disease, caused by *Fusarium oxysporum albinidis,* in Morocco and Algeria, which are being under the field trials in Algeria. So far putative mutants are growing well in hot spots in the field.

Genetic transformation

Since date palm is more or less like a food crop and feeds people and serves as nutrition security, genetically engineered date palm would be able to generate disease and pest resistant plants by over expression of bio pesticide and antifungal. Genetic engineering would assist in reducing time scale in developing new cultivars only when precisely single trait genes to be expressed without altering the remaining genetic makeup. Primarily two different strategies have been used, which are T-DNA delivery with *Agrobacterium tumefaciens* and direct gene transfer with particle bombardment. For date palm, *Agrobacterium*-mediated transformation used GUS (β-glucuronidase) as a reporter gene, which is easy to assay. So far, no conclusive report is available on the expression of economically-important genes in date palm to the present.

Molecular markers

Molecular markers based on simple sequence repeats and single nucleotide polymorphisms, are playing an increasingly important role in plant variety identification, germplasm resource collection and breeding activities. The major types of DNA markers are described and the resources available to the date palm community are identified. In general, the molecular marker resources for date palm are somewhat limited. However, most of the available DNA marker types have been used on some material, mostly to cluster date palm varieties into related groups. The development of a series of sequenced tagged sites (probably based in SSRs) will supply resources needed for the screening of collections to reduce the number of samples kept in germplasm banks. They will also add impetus to identifying markers linked to the various disease-resistant genes. With the steady increase in the sequencing resources, SNPs will also become more useful but the relative costs of SNP and SSR analyses may well determine which of the two-marker systems becomes most widely used. It is undoubted that the collection of many high polymorphism information content SSR primer pairs and validated SNPs will provide the tools for phylogenetic analyses as well as germplasm conservation. However, once genomic regions associated with important characteristics such as disease resistance, taste and post-harvest stability, the sequencing of these regions and the identification of the actual bases for these characteristics can be incorporated into the breeding and improvement programs. The identification of off-types arising in tissue culture propagation and the complete genome sequencing of normal and off-type individuals will lead to the identification of both markers for assessing off-type individuals in the regenerated plants as well as the 'mutations' responsible for these off phenotypes.

Genomics

Genomics is carried out to study the whole genome of an organism, which is the sum total of DNA molecules harbouring all genes of an organism. It is performed to study all the genes of a given cell, tissue and organism; DNA (genome) as well as RNA (transcriptome), and protein (proteome) in the context of a regulatory network as well across taxa (evolution). The field includes intensive efforts to determine the entire DNA sequence of various organisms and to construct a genetic map, using large-scale sequencing technology, to generate massive, adequate and high-quality data, by using bioinformatics tools for assembly, annotation and in-depth analysis. A major branch of genomics is still focused on sequencing the genomes of various species.

Date palm as a bio-energy source

Today the whole of humanity is very much dependent on fossil energy for routine daily life. The surge in the industrialization in the developing world has enhanced fossil energy consumption. The rise in fossil fuel energy consumption in the transportation sector, together with other gaseous pollutants, is adversely influencing world climatic conditions. Renewable energy is the alternate source to the fossil fuel energy, which can be termed as *green energy* or *bio-energy* or *plant-based energy*. Bio-fuel is gradually replacing petroleum in the transport sector, which is a blend of petrol and bio-ethanol or bio-diesel and fossil diesel. Date palm could become a major source of producing bio-ethanol, since its fruits have a high percentage of carbohydrates (total sugars 44-88%). Millions of date palm trees are grown in the Middle East, North Africa, and South Asia, and they provide food and nutrition to millions of people, and could also become a major source of bio-energy. In Algeria alone, the estimated number date palm trees is over 10 million, and production increased from 302,993 mt in 1997 to 526,921 mt in 2007. The over production of date palm fruits, however, could lead to price reduction and loss of farmer's income. These problems can be overcome by using date palm for bio-ethanol production

Recommendations

1. Establishment of date palm germplasm website, describing passport of each collection, and identify trait specific markers
2. Development of Postharvest storage technologies, e.g. improvement of shelf-life, and link it with marketing strategies
3. Genetic improvement of date palm varieties for sustainable production with innovative technologies, e.g. molecular marker assisted selection and breeding, functional genomics, identification of useful genes
4. Use embryo rescue method for date palm hybrid production in a short time, by crossing two date palm varieties or interspecific crossing
5. Fine tuning of somatic embryogenesis and organogenesis protocols for large-scale date palm plantlet production. Date palm somatic embryos must be individually separated and follow maturation and germination into plantlets
6. Determine genetic fidelity of in vitro plants with molecular markers together with morphological traits
7. Establish doubled haploid production for producing pure line homozygous lines
8. Protoplast fusion for developing somatic hybrids, e.g. asymmetric and symmetric somatic hybrids, e.g. transfer of partial genome from the donor
9. In vitro mutagenesis for the isolation of useful mutants, e.g. setting up of fine selection, and use for selection against fungal toxin or salinity
10. Genetic improvement of date palm against the climate change, and use model systems for the prediction date palm sustainable production, effect on soil quality, water availability, changes in the soil microbial activity. Future development of date palm varieties should have multiple traits to withstand onslaught of climate change.
11. Use of date palm as a source of bio-energy- food, feed and bio-energy, and thereby date palm cultivated land could be expanded by increasing date palm plantations.
12. Agro-forestry could be encouraged for small land owners in order to get additional income.
13. Use of bioreactors for large-scale shoot production and somatic embryos
14. Targeting induced local lesions in genomes (TILLING) for improving date palm, a reverse genetics approach
15. Sex determination of date palm at the early stage of tree development is needed to discriminate between productive female and non-productive male trees in the nursery before transplanting them to the field
16. Red palm weevil continues to remain a challenge to the date palm growers. In order to control this menace different strategies should be followed: development of transgenic baculovirus containing neurotoxin gene and firefly gene; use of attractant together with a neurotoxin chemical to a quick kill of RPW; identify specific chemicals produced by date palm that attract RPW to damage trees. Sterile insect technique has not been successful in RPW control, still radiation induced changes could be made in RPW influencing their mating behaviour or feed habits.
17. Mutation-based functional genomics (MFG) could also be promoted in date palm even though 2 different groups have already initiated date palm genomics.
18. Transgenic approach could be used for a specific transgene transfer, gene pyramiding for enhancing abiotic and biotic stress tolerance level, enhancing shelf-life of fruits, and improvement of nutrition of fruits.