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Title: Utilisation of wastewater for fuel and fodder production and environmental and social benefits in semi-arid, peri-urban zones of sub-Saharan Africa.

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Key words: peri-urban, wastewater recycling, irrigation, fodder, fuelwood, microsymbionts

Editor: Julia Wilson

Coordinator's overview

As previously noted, there have been some serious delays arising from partners' cash-flow difficulties related to the purchase of items for water treatment and irrigation. Partners have worked hard to overcome these difficulties, and the project is now getting back on track. Nevertheless these delays will have an impact on the delivery of the project unless an extension to the project can be negotiated.

Partners are working hard to collect good data in all areas. Training and exchanges of staff between partners are providing valuable new experiences. The annual meeting provided an excellent venue for discussion and all partners benefited.

Partner 1: CEH

Work package 3: Tree Water–use and soil water status

CEH staff (Julia Wilson and Bob Munro) spent 2 weeks training IER staff in Mali during April – May 2005. They provided hands-on training, with information sheets on setup and use of sapflow and meterological equipment, including use of data loggers and data processing, together with use of soil moisture equipment and use of scanners and software for leaf area determination. Equipment had previously been in use in Niger and some small repairs were required. Some changes have been made to the methods used for measuring leaf area to take advantage of free windows software which is now available (previous software was expensive and required knowledge of DOS). Most repairs were conducted in Mali, but some cables required new connectors and these were returned to the UK for fitting.

Workpackage 4: Microsymbionts and N fixation

The irrigation experiment was set up in the glasshouse in October 2004. Irrigation treatments were applied from January 2005 onwards, with 250ml of a modified Ingestad's nutrient solution or water applied once per week to each pot. The Ingestad's nutrient solution was modified to contain increased levels of N (132 mg/l - x2 normal) Zn (1.46 mg/l – x100 normal) and Cu (0.157 mg/l – x 10 normal). From March 2005 onwards applications were increased to 500ml per week.

Monthly non-destructive measurements (stem diameter) have been made (Figure 1). These show that growth slowed over the winter months, but from March 2005 onwards, growth of all inoculated trees began to accelerate, whereas growth of uninoculated trees was slow. Growth of *Leucaena leucocephala* and *Khaya senegalensis* was similar for both fungal inoculants (*Glomus mosseae* and *Glomus etunicatum*). Growth of *Senna siamea* inoculated with *G. mosseae* was greater than those inoculated with *G. etunicatum*. By May 2005, there were signs that growth of all inoculated trees was being stimulated by the irrigation treatment.
In April 2005, significant leaf fall was observed for all *S. siamea* trees inoculated with *G. etunicatum*, but not for those inoculated with *G.mosseae* (Figure 4). Leaf samples were removed from these trees for foliar nutrient analysis.

Annual meeting

The annual project meeting was held in Ouagadougou in May 2005. All partners were represented.

Overall progress

Lab and training work are proceeding as planned. Development of simple irrigation model (wp3) cannot commence until suitable water use data is available from the irrigated plots.

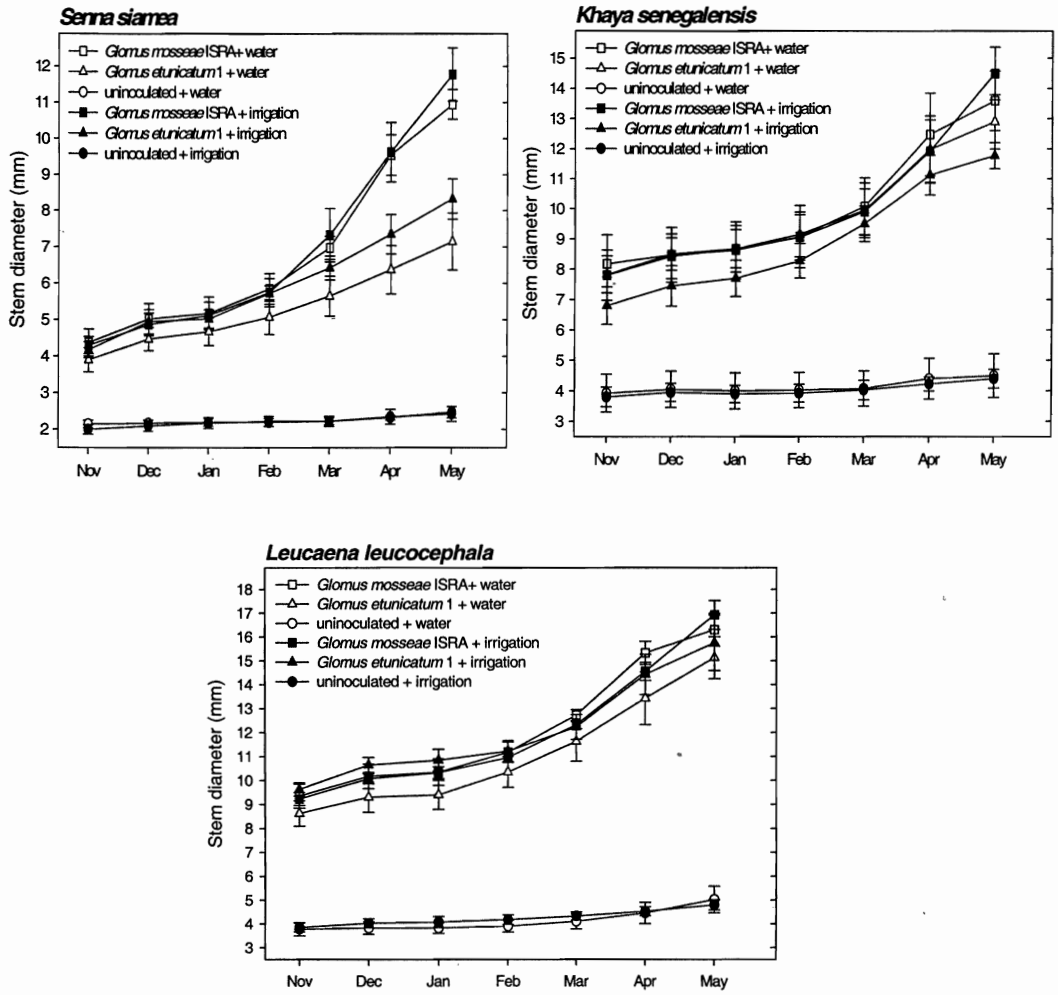

Figure 1. Growth of inoculated and uninoculated trees after application of irrigation treatment.

Figure 2 *Senna siamea* trees inoculated with *Glomus etunicatum* (front) and *Glomus mosseae* (back).

Partner 2: Institut d'Economie Rurale, Bamako, Mali

Work package 1: Water treatment and Irrigation

All the materials concerning the irrigation system have been bought and are already present in the experimental site. As mentioned in the previous report, the irrigation system is functioning in Mali since April 2004, so the main works were the maintenance of the system and the equipments. But some works have been performed during this reporting period which are: digging planting holes for the experiment 1 and 2, and reinforcement of the second embankment with clayey soil. The following works still have to be performed: management of artificial water basin (this work should be done preferably during the rainy season); a small dam on Minimana drain should be constructed.

Work package 2: Tree growth and Management

The activities concern mainly nursery works for the 2 main experiments: Nursery works involve filling pots, construction of stands to keep potted nursery plants off the ground (to prevent cross-contamination), transportation and installation of pots on the stands, sowing seeds, regular watering, and follow up of seedling growth.

Experiment 1:

Objective: To compare the growth of tree species in different countries under irrigated field conditions in Burkina Faso, Niger and Mali.

Species used in Mali are: *Gliricidia sepium*, *Acacia angustissima*, *Leuceana leucocephala* and *Kaya senegalensis*.

Data such as seed germination, percentage of survival, seedlings diameter and height, phonological observations have been collected.

Experiment 2

Objective: to do a quick screening of other species. Species used in Mali: *Acacia crassicarpa*, *Acacia mangium*, *Acacia auriculiformis*, *Leucaena leucocephala*, *Gliricidia sepium*, *Calliandra calothyrsus, Acacia angustissima*, *Acacia senegal*, *Pterocarpus lucens* and *Khaya senegalensis*.

The same data concerning experiment1 have been also collected in this trial.

The planting of these species in the two experiments will occur during the last week of May.

Work package 3: Tree Water–use and soil water status

Researchers from CEH (Julia Wilson and Bob Munro) have spent two weeks in Mali providing training on sap flow, moisture and meteorological measurements. Some of the data (sap flow) collected during the training were shown during the third coordination meeting held in Burkina Faso.

Work package 4: Microsymbionts and N-fixation

Mycorrhizal inoculum was sent to us by Partner 4 from INERA Burkina Faso. This mixed inoculum is used by all the countries. The biotic ingredients of this inoculum are: Endomycorrhizal (VAM) spores; minimum 40 spores/cc of blended *Glomus brasilianum*, *Glomus clarum*, *Glomus deserticola*, *Glomus intraradices*, *Glomus monosporus*, *Glomus mosseae* and *Gigaspora margarita*.

Work package 6: Soil and Plant nutrition

A detailed report has been provided in the second annual report.

Work package 7: Planting stock quality
Assessments will be done on the screening experiment set up at Sotuba. It was agreed during the meeting held in Ouagadougou to assess shoot: root ratio, the sturdiness quotient and Dickson's quality index before planting the seedlings in the two experiments.

Work package 5 & 8: Socio economic surveys, and Pest monitoring
These are the responsibility of Partner 3 in Mali.

Coordination meeting: Mr Daouda Sidibe and Dr Khalifa Traoré represented IER at the annual meeting.

Problems encountered:
We are still anxious about the second payment because of the amount of remaining work. We are seriously concerned about the following problems: labour (planting, watering, cares of equipments and infrastructures), technical staff salaries, and researcher's field trips.

Partner 3: University of Bamako, Mali

WP2: Economics

According to the 2004 annual meeting's recommendations, Youssouf Cissé who is responsible of this workpackage has performed questionnaires. On the basis of these, investigation on costs and sociological aspects of existing production chains will start on the end of May 2005.

WP 4 : Microsymbiont and N2 fixation

Nodulation and tree growth was determined in nursery on plants intended for outplanting in Experiment 1 at Minimana.

Tree species	Number of nodules	Nodule Weight (mg)	Height (cm)	Collar (Ø cm)
Acacia angustissima	**52,50a**	**185,3a**	**37,65**	0,302
Gliricidia sepium	39,15**a**	137,2**b**	25,47	**0,500**
Leucaena leucocephala	13,91**b**	99,1**c**	33,30	0,425

Acacia angustissima performed the best in terms of nodulation and height growth. This result confirms previous results obtained in the nursery at Sotuba station during the first year of the project.

Molecular characterization. DNA was extracted from nodules collected using plants in experiment 1 and 2. DNA quality was checked using gel electrophoresis.
PCR of rDNA IGS 16S-23S was performed in our lab. Molecular studies are still in progress.

Work package 6: Plant and soil nutrition

Irrigated wastewater analysis are performed in Laboratoire de la Qualité des Eaux au Mali.

Soil and plant nutrient analysis will be done on the field trials at Minimana.

Work package 8: Pest monitoring and management

The objective is to define the risks of attack by pests or diseases caused on exotic tree species under irrigated conditions.
In the nursery, three months after sowing, observations were made on plant shoots. Nematodes were investigated in roots and soil collected from nursery (experiments 1 and 2) and field before transplanting.
Primary main results are as follows:
- shoots are healthy and a good colour, no pest attacks are evident,
- no phytophageous nematodes were observed on the roots,

After the meeting in Burkina Faso, researchers who are charged with this workpackage in each country may harmonize methodology on irrigated field experiment to carry out all pest management and monotoring studies.

Outline plan until the end of Year 2005

Activities	Months							
	May	June	July	Aug	Sept	Oct	Nov	Dec
WP- Economics	x	x	x					
WP- Pest management		x	x	x	x	x	x	
WP- Microsymbiont & N2 Fixation	x							
Inoculum potential studies			x		x		x	
Molecular Characterization	x	x	x	x	x	x	x	x
Training		x	x	x	x	x	x	x
Reports	x							x

Meeting

Dr Inamoud I YATTARA has attended to the 3rd meeting of Ubenefit Project held from 3rd to 5nd in Ougadougou in Burkina Faso. Our socioeconomist Youssouf CISSE was unable to attend the 3rd meeting in Burkina Faso, because of other commitments.

Problems encountered:

There is a need to improve information exchange between partners to ensure better coordination of activities.

Some activities were delayed due to financial problems (delays between submission of cost statements and receipt of payments)

Partner 4: INERA Burkina

Wp 1. Wastewater treatment and irrigation

Installation of the wastewater purification system

In its previous report, INERA mentioned that the construction of the wastewater treatment equipment was delayed due to technical and administrative difficulties. During the last six-months period, a concerted effort by all local actors allowed overcoming of the major problems encountered. At present, the installation of the wastewater purification system is being completed, and there are good indications that this equipment will be functioning by June.

Wp 2. Tree growth and management

Planting stock quality

(preliminary experiments as recommended at the 2nd annual meeting)

Materials and methods

Plants were raised in containers of two different volumes in a nursery in Ouagadougou, Burkina Faso, West Africa (12°22' N and 1°30' W and at an altitude of 306 m.a.s.l). Three treatments comprising a single inoculation with *Rhizobium* alone (T1), a single inoculation with Mycorrhizas alone (T2) and a double inoculation with both *Rhizobium* and Mycorrhizas (T3) were applied, with seedlings without inoculation serving as controls (T0). The design was a split plot design with single seedling as the experimental unit. Three months after the beginning of the experiment, morphological parameters of seedlings were measured and used to derive Sturdiness quotient, Quality index and Shoot:root ratio. These indices were further used to appreciate the quality of the seedlings.

Results

The results showed that when the Sturdiness quotient and shoot:root ratio decreased, the seedling quality is better for harsh conditions. In reverse this is the case when the Dickon's quality index increases. Based on these indexes, we may conclude that *Afzelia africana, Gliricidia sepium*, and the two *Leuceana* are the best when raised in big pots and double inoculated (Table 1). Such conclusion needs to be confirmed by transferring the plants into irrigated conditions to ascertain whether these indices should be interpreted similarly for harsh and irrigated conditions.

Table 1: Planting stock quality assessed by Shoot:Root ratio, Sturdiness Quotient and Dickson's Quality Index for five introduced and one local species in Burkina Faso, West Africa. T0 = control; T1 = inoculation with rhizobium, T2 = inoculation with mycorrhizas; T3 = double inoculation with rhizobim and mycorrhizas

Parameters	Species	Pot size	T0	T1	T2	T3	Mean
Sturdiness Quotient	Acacia angustissima	Big	8.7	4.0	6.3	7.4	6.7
	Acacia angustissima	Small	10.3	9.6	7.9	8.7	9.2
	Acacia mangium	Big	7.4	15.9	6.4	6.4	9.3
	Acacia mangium	Small	5.6	6.8	7.0	6.7	6.5
	Afzelia Africana	Big	3.0	4.0	3.7	3.9	3.7
	Afzelia Africana	Small	3.9	2.8	3.8	3.4	3.5
	Gliricida sepium	Big	2.4	2.2	2.4	3.0	2.5
	Gliricida sepium	Small	2.2	2.7	2.8	2.5	2.6
	Leucaena hybrid	Big	5.1	4.6	5.1	6.9	5.4
	Leucaena hybrid	Small	5.7	6.2	6.2	6.7	6.2
	Leucaena leucocephala	Big	5.2	5.0	5.1	5.7	5.2

	Leucaena leucocephala	Small	6.0	5.7	6.7	5.3	5.9
Dickson's Quality Index	Acacia angustissima	Big	0.010	0.026	0.027	0.028	0.022
	Acacia angustissima	Small	0.008	0.009	0.009	0.013	0.010
	Acacia mangium	Big	0.016	0.018	0.031	0.037	0.025
	Acacia mangium	Small	0.028	0.032	0.021	0.021	0.025
	Afzelia Africana	Big	0.777	0.559	0.583	0.781	0.681
	Afzelia Africana	Small	0.167	0.487	0.241	0.248	0.291
	Gliricida sepium	Big	0.309	0.605	0.366	0.478	0.439
	Gliricida sepium	Small	0.256	0.218	0.295	0.268	0.260
	Leucaena hybrid	Big	0.109	0.112	0.131	0.246	0.149
	Leucaena hybrid	Small	0.080	0.061	0.116	0.102	0.090
	Leucaena leucocephala	Big	0.158	0.138	0.131	0.133	0.140
	Leucaena leucocephala	Small	0.153	0.112	0.091	0.082	0.107
Shoot:Root ratio	Acacia angustissima	Big	2.6	2.3	3.5	2.1	2.7
	Acacia angustissima	Small	1.6	1.9	2.1	2.8	2.1
	Acacia mangium	Big	4.6	6.3	5.1	5.8	5.4
	Acacia mangium	Small	2.8	3.3	2.3	1.8	2.5
	Afzelia Africana	Big	1.2	1.3	1.2	1.1	1.2
	Afzelia Africana	Small	2.3	1.7	1.2	1.4	1.6
	Gliricida sepium	Big	1.5	1.3	1.2	1.7	1.4
	Gliricida sepium	Small	1.7	1.6	1.7	1.5	1.6
	Leucaena hybrid	Big	1.4	2.2	1.1	1.7	1.6
	Leucaena hybrid	Small	1.6	1.6	1.6	1.7	1.6
	Leucaena leucocephala	Big	1.2	1.5	1.3	1.3	1.3
	Leucaena leucocephala	Small	2.0	1.5	1.7	3.8	2.3

Wp 4. Microsymbionts and N-fixation

INERA provided the mycorrhizal inoculants to all the partners (Mali and Niger).

Wp 5. Economics

INERA contributed to the elaboration of questionnaire for the socio-economic studies.

Other activities

INERA organised the 3rd annual meeting held in Ouagadougou (3-5 May 2005).
All partners, except the CPS team, attended this meeting.

Future plans

- Nursery activities will start at the end of May so to allow starting field trials in the next 2-3 months.
- Socio-economic studies also will be completed during the next 6 months period according to the agreed questionnaire.
- A technician will be trained in strains tracing (Molecular biology) by IRD in Dakar.

Partner 5: University Abdou Moumouni, Niger

INTRODUCTION

During this first semester of the third year of the UBENEFIT project, the sap flow and soil moisture measurements were carried out. The nursery for the second experiment has also began. However the establishment of the functioning waste water treatment and irrigation system was late due to the delay in the second payment of the project.

WP 1. Water Irrigation And Treatment

The construction of the station for water purification was completed in 2004. We are in discussion with SOPAM, a company from Burkina for ordering 2 pumps (1 pump for waste water and the second one for irrigation). In addition, contacts were also established with another company, SEHI-SENEGAL for irrigation system. The tree functioning irrigation system will be ready at the middle of June 2005.

WP 2 and 4. Tree Growth, Management, And Microsymbionts

The nursery work for the second experiment began at the end of March 2005. Seeds of five species were used: *Acacia angustissima, A. crassicarpa, A. seyal, Gliricidia sepium and Leuceana leucocephala.* Seeds were disinfected with alcohol 95° than rinsed with sterilized water. After that, seed were treated with sulfuric acid at different times according to the species: *Leuceana leucocephala :* 15 mn; *Acacia angustissima* : 20 mn; *A. crassicarpa* : 25 mn; *A. seyal :* 30 mn.
Than seeds of all species were soaked in sterilized water during 8 hours and sown. The germination occurred after 2 days for *Acacia angustissima, A. seyal, Gliricidia sepium and Leuceana leucocephala* and 4 days for *A. crassicarpa.*

Thereafter, seeds were transferred in pots and inoculated with mycorrhiza on April 11, 2005. The inoculation with rhizobia strains (4 ml of inoculums/tree) was done on May 5, 2005 with the following inoculum strains: *Leuceana leucocephala :* LDK4; *Acacia angustissima* : 11c + ORS 324; *A. crassicarpa* : 11c; *A. seyal :* ORS 3324; *Gliricidia sepium :* GSK4.
Plants in pots were protected against insects with a mosquito net.

WP 3. Tree water use and soil water status

The variation of sap flow according to the species and the weather conditions has also been studied during this semester. Six (6) sets of sap flow measurements were conducted for the eleven (11) species studied in the first experiment (2004). After nursery, plants of these species were transferred in field conditions and watered with tap water. Sap flow of one plant of each species was studied during 10 days (1 day without power). Data of sap flow and weather were daily collected. Plants identified for this study were previously watered with waste water (6 liter/tree) during one week and during the sap flow measurements. In the same time, the soil water status was also determined using a profile probe.

WP 5. Economics And Quality Of Produce

A revised version of the socio economic questionnaires was sent to the other partners after the coordination meeting held in Bamako. But no feedback was recorded from the colleagues about this version of questionnaire.
At the meeting held in Ouagadougou from May 3-4, 2005, Mrs Germaine Ibro, who is coordinating this work package, has registered the comments of her socio economic colleagues. Thereafter she elaborated a final version of questionnaires. These

questionnaires are related to different aspects of wood production and commercialization such as : production and commercialization of wood; wood energy timber: questionnaire to wood cutters, wood sellers, and wood dealers. Utilization of energy source by householders; Description of actual system of fuel wood production In Niger, the socio economic investigation was began in April for two (2) axis of wood supply for Niamey city : axis Torodi to Niamey and axis Dosso to Niamey.

WP 6. Soil And Plant Nutrition

In order to determine the nutrient status of irrigation sites, soil samples where collected on May 21, 2005. These samples will be analyzed at the soils lab of ICRISAT for nutrient such as C, N, P, K, Na, Mg, Ca.

Participation To The Third Coordination Meeting At Ouaga

Mrs Germaine Ibro and Mr Sanoussi Atta attended to the third coordination meeting held in Ouagadougou (Burkina Faso) from 3 to 4 May, 2005. During this meeting, some preliminary results of sap flow measurements and soil water status were presented. In addition, the participants were also informed in the progress registered in Niger in the different work packages of the Project.

PROBLEMS AND DIFFICULTIES

The main problem was the delay in the second payment of the project. Therefore the lack of money slowed our activities, and moreover prevented the ordering of the irrigation equipment.

PLAN OF ACTIVITIES

-The socio-economics investigation will be conducted in the 3 main axis for fire wood supply of Niamey city by using the questionnaires;
- The establishment of the functioning waste water treatment and irrigation system;
- The continuation of the nursery and the transfer of plants to field conditions.

Partner 6 IRD, France / Senegal

Work package 4 (Microsymbionts and Nitrogen-Fixation)
Development of molecular techniques

Nodules of woody legumes were previously obtained by inoculation with their corresponding strains: Aust 13C for *A. mangium*, Aust 13C for *A. crassicarapa*, Aust 11C for *A. auriculiformis*, mixture of CIRAD 300, 301 and 302 for *A. senegal*, LDK4 for *Leucaena leucocephala*. DNA of the tree nodules was obtained by the improved DNA extraction method (Guanidine Thiocyanate 0.0005 M and 15 min incubation at 65°C) described in the previous report.

To test the quality of extracted DNA for PCR amplification, two 16S-23S rDNA IGS prokaryotic specific primer FGPS1490-72 (5'-TGCGGCTGGATCCCCTCCTT-3') (Normand *et al.*, 1996) and FGPL132-38 (5'-CCGGGTTTCCCCATTCGG-3') (Normand *et al.*, 1992) designed from conserved regions of rrs and rrl sequences of *Frankia* sp. were used for PCR amplification of 16S-23S rDNA IGS region. PCR was carried out in 25 μl reaction volume containing 50 ng of pure total DNA extract, one dried bead (Ready-to-Go PCR beads, Pharmacia Biotech) containing 1.5 U of *Taq* polymerase, 10 mM Tris-HCl, (pH 9 at room temperature), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP and 2.5 μl of each primer (1 μM). PCR amplification was performed in a GeneAmp PCR System 2400 (Perkin Elmer) thermal cycler adjusted to the following temperature profile: initial denaturation at 95°C for 5 min; 35 amplification cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min; and final extension at 72°C for 7 min.

The extracted DNA was amplified. However, PCR yielded multiple IGS PCR products (Figure 1).

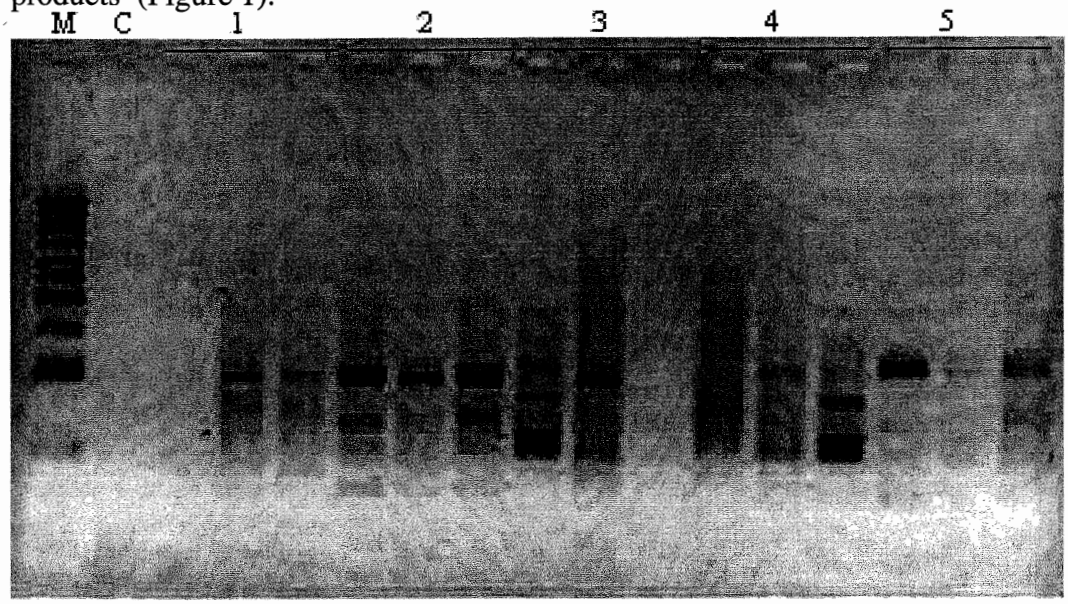

Figure 1. Gel electrophoresis of amplified 16S-23S rDNA obtained on individual crushed nodules of *A. mangium* (1), *A. crassicarapa* (2), *A. auriculiformis* (3), *A. senegal* (4), *Leucaena leucocephala* (5) .
Lane M, 1 kb size marker (Pharmacia Biotech); C, PCR control

The bands around 1000 bp size correspond to the sizes of amplified 16S-23S rDNA IGS product of inoculated strains (data not shown), whereas the lower bands could correspond to non-specifically primers binding. This can be due to high DNA quantity used for PCR or to not quite stringent PCR conditions (annealing at 55°C). There brought random amplification of some DNA portions. Further studies will be performed on more diluted DNA and at more stringent annealing temperature (for example, 57-58°C).

DNA probes design for inoculants

16S-23S rDNA IGS region of selected rhizobia strains Aust 11 C and Aust 13 C were completely sequenced. The sequences of the strains GsK4 and LdK4 were partially sequenced. A complete sequencing will be finished later. The sequences of Aust 11C and 13C were used for development of strain-specific DNA probes. The specificity of the probes was checked by alignments of their sequences with those available in laboratory and in international GenBank by using the algorithm BLAST (Altschul *et al.*, 1997). The designed specific probes are presented in Table 1.

Table 1. Designed probes

Probe	Target strain	Sequence 5' - 3'	% G+C[a]	l[b]
Aust 13C	Aust 13C	CGCTTGTTCATCGCGGCTCATCG	61	23
Aust 11C	Aust 11C	GGTGAGCGGGTTGTAAATGATCCC	54	24

[a]G/C content of the oligonucleotide;
[b]l is the length of the oligonucleotide

The probe Aust 13C is being actually in synthesis with a 5', 3' and internal Dig-labeling. The specific hybridization conditions will be determined empirically by probe hybridization on strain DNA on the basis of calculated hybridization temperature as a starting point. The best stringent conditions will be tested further on DNA extracted from crushed nodules that have been formed by the corresponding strain.

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