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PROJECT

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Acacia mangium clone n. 5: field comparison of *in vitro* plantlets against open pollinated seedlings.

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Introduction

This experiment was established with the main objective of evaluating two propagation method (tissue culture and seeds) in *Acacia mangium*. The hypothesis to test was that clonal propagation of superior trees gives more homogeneous and better performing material than seeds obtained by the same trees, which contain an unknown male contribution.

In 1995, The Plant Biotech Laboratory (PBL) selected in the PISP's *A. mangium* seed orchards (Tiagau) few superior trees to be propagated by tissue culture in the PBL. In 1996, the production of *in vitro* plantlets from one of these trees, the clone n. 5, was sufficient to establish a field trial. The results of the second assessment, one year and four months after plantation, are reported here.

Material and methods

The experiment consisted of two treatments: *in vitro* plantlets of clone n. 5 (T1); open pollinated seedlings from clone n. 5 (T2). The two treatments have been planted (November 1, 1996) in a randomised complete block design with three repetitions (R1, R2, R3), at a spacing of 3 x 3 meters. Around the trial, a two line buffer (B) has been planted with seedlings obtained from a non selected seed bulk. The map of the trial is given in Figure 1.

At this assessment, the following characters have been measured:

1) DBH (diameter at breast height), in cm
2) Height, in cm
3) Branching (note: 0=no branching, 1= aerial branching, 2= branching from the base)
4) Straightness (note: 0=straight, 1=slightly crocked, 2= very crocked)

The trial has been assessed twice, once on the July 1, 1997, and later on March 5, 1998. The analysis of the first assessment has been distributed earlier (Bacilieri, 1997). The second assessment has been analysed as follows:

1) A two-factors (repetitions and treatments) analyse of variance was performed to compare the diameter and height of T1 and T2 over the trial;
2) The treatments' ranking for diameter and height was tested with a Duncan's test;
3) The variation between plants within treatments was studied by a Bartlett's test;

4) The other two characters (branching and straightness), consisting of a qualitative coding, was studied by a non-parametric analysis (Wilcoxon χ^2 test).

5) Diameter and height data of both T1 and T2 have then been individually compared with the internal buffer line B1 by means of a Student T-test. In fact, even if the non selected material was only planted in the buffer and not in the experimental design, the comparison among selected and not selected material might be interesting, provided appropriate cautions are taken during the interpretation.

For details of the statistical analysis please refer to Sokal & Rohlf (1981) and to the SAS documentation (SAS Institute, 1988).

Results

The analysis of variance showed that there were not significant differences between treatments or blocks neither for diameter nor for height (Table 1). The significant interaction (repetition*treatment) was just due to the fact that one treatment (T1) was superior to the other (T2) in one repetition (R2) but not in the other two (R1 and R3; not shown); however, because the main effects were not significant, this has to be attributed to the low number of trees within the experimental unit rather than to a real interaction.

The ranking of the treatments showed a slightly better performances of seedlings as compared to micro-cuttings; however the Duncan's test revealed (in concordance with the analysis of variance) that the differences in ranking was not significant (Table 2).

TABLE 1. Analysis of variance for the RBC design. Treatments: micro-cuttings versus seedlings. Measured characters: diameter and height

Dependent Variable: Diameter

Source	*DF*	*Sum of squares*	*Mean square*	*F Value*	*Pr > F*
Model	5	40.794	8.158	2.48	0.0436
Error	51	167.658	3.287	--	--
Repetition	2	1.275	0.537	0.19	0.8243
Treatment	1	6.628	5.528	2.02	0.1617
*Rep*Treat*	2	32.125	15.052	4.89	0.0114

Dependent Variable: Height

Source	*DF*	*Sum of squares*	*Mean square*	*F Value*	*Pr > F*
Model	5	134003.377	26800.575	2.45	0.0456
Error	51	557047.500	10922.500	--	--
Repetition	2	29745.536	14872.768	1.35	0.2654
Treatment	1	11214.858	11214.858	1.03	0.3157
*Rep*Treat*	2	88735.009	44367.505	4.06	0.0231

TABLE 2. Average and ranking for diameter and height between micro cuttings and seedlings. Duncan test for ranking.

Dependent Variable: Diameter

Treatment	*Number of trees*	*Mean*	*Critical Range*	*Duncan Grouping*
Seedlings	30	8.087	0.965	A
Micro cuttings	27	7.404	0.965	A

Dependent Variable: Height

Treatment	*Number of trees*	*Mean*	*Critical Range*	*Duncan Grouping*
Seedlings	30	820.50	55.65	A
Micro cuttings	27	792.41	55.65	A

As for the other two measured characters, straightness and branching, the non-parametric test of differences (Wilcoxon χ^2 test) showed that, also in this case, there were no significant differences between treatments or blocks (data not shown).

The hypothesis of a lower variation in micro-cuttings (that have all the same genotype) as compared to seedlings (that have different genotypes) could not be validated by this experiment (Bartlett test, Table 3). In fact, micro-cuttings were only slightly more homogeneous in terms of height growth, but sligthly less homogeneous in diameter, as compared to seedlings. The fact that the differences were not significant has more to be attributed to the small size of the experiment than to a true lack of difference. A larger experiment is needed to definitely validate or reject this hypothesis.

TABLE 3. Bartlett's test of the hypothesis of a difference in the variance between treatments.

Dependent Variable: Diameter

Treatment	*Number of trees*	*Variance*	*Chi square* χ^2	$Pr>\chi^2$
Seedlings	30	3.39	0.266	0.501
Micro cuttings	27	4.03		

Dependent Variable: Height

Treatment	*Number of trees*	*Variance*	*Chi square* χ^2	$Pr>\chi^2$
Seedlings	30	14087.78	1.099	0.294
Micro cuttings	27	10005.25		

Outside of the plan of the experiment, we compared the growth of the (vegetative or sexual) progenies of clone n. 5 with the bulk of seedlings used for the most internal line buffer. In our other Acacia trials (Seed Orchards, Tiagau), the buffer usually performed better than the inner treatments, mainly because these plants have more space and more light. However, in this experiment the bulk of seedlings in the buffer grew considerably slower than the progenies of clone n. 5. Even if this comparison was not included as a main treatment, we can assume with some degree of confidence that clone n. 5 is a true superior genotype.

Table 3. T-test of the differences between micro cuttings, seedlings of clone n. 5 and seedlings from unselected bulk (buffer).

Comparison micro-cuttings / bulk of seedlings.

Variable: Diameter	*Number of trees*	*Mean*	*Std Dev.*	*T-test*	*DF*	*Prob > T*
Micro cuttings	27	7.404	1.713	1.818	47	0.0754
Seedlings (unselec. Bulk)	22	6.341	2.280			
Variable: Height	*Number of trees*	*Mean*	*Std Dev.*	*T-test*	*DF*	*Prob > T*
Micro cuttings	27	792.407	122.422	2.123	47	0.039
Seedlings (unselec. Bulk)	22	712.272	141.722			

Comparison seedlings clone n. 5 / bulk of seedlings.

Variable: Diameter	*Number of trees*	*Mean*	*Std Dev.*	*T-test*	*DF*	*Prob > T*
Micro cuttings	30	8.085	2.003	2.928	50	0.005
Seedlings (unselec. Bulk)	22	6.341	2.280			
Variable: Height	*Number of trees*	*Mean*	*Std Dev.*	*T-test*	*DF*	*Prob > T*
Micro cuttings	30	820.500	100.029	3.231	50	0.002
Seedlings (unselec. Bulk)	22	712.272	141.722			

Conclusion

At one year and four months after plantation, we could not observe any significant difference among micro-cuttings or seedlings of *A. mangium* clone n. 5. Possible explanations for this are:

1. maternal effects, tha are quite common in forest trees. Maternal effects make a progeny more similar to the maternal than to the paternal parent tree. Most of the times, these are due to the aploid genotype of chloroplasts and mithochondria, that are generally maternally inherited, and also to the quantity of reserves that the maternal plant has been able to build and store in the seeds. Maternal effects usually disappear with the ageing of the plant. In later assessments, we will probably be able to see a more clear difference.
2. the small size of the experiment, both in term of number of plants and of number of maternal trees (only one, clone n. 5). At the period of the experiment, other superior trees have failed to propagate, mainly because of a slower reactivity to tissue culture. As these problems will be overcome, a larger experiment can be established.
3. A high sensibility of *A. mangium* to environmental effects. This has to be considered when thinking of propagation strategy. Again, a larger experiment will help to better evaluate the genotype and environment effect on *A. mangium* growth.

Figure 1. Map of the Acacia trial near the Luasong river: Comparison of *in vitro* plantlets (clones) against seedlings (open pollinated progeny) of clone n. 5 (*A. mangium*)

Planting date 1/11/96 File:c:\cirad\acacia\am5\amclon5.doc

Spacing 3m by 3m.

Line/col number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	B	B	B	B	B	B	B	B	B	B	B	B	B	B
2	B	B1	B1	B1	B1	B1	B1	B1	B1	B1	B1	B1	B1	B
3	B	B1	R1 T1	R1 T1	R1 T1	R1 T1	R1 T1	R1 T2	R1 T2	R1 T2	R1 T2	R1 T2	B1	B
4	B	B1	R1 T1	R1 T1	R1 T1	R1 T1	R1 T1	R1 T2	R1 T2	R1 T2	R1 T2	R1 T2	B1	B
5	B	B1	R2 T2	R2 T2	R2 T2	R2 T2	R2 T2	R2 T1	R2 T1	R2 T1	R2 T1	R2 T1	B1	B
6	B	B1	R2 T2	R2 T2	R2 T2	R2 T2	R2 T2	R2 T1	R2 T1	R2 T1	R2 T1	R2 T1	B1	B
7	B	B1	R3 T1	R3 T1	R3 T1	R3 T1	R3 T1	R3 T2	R3 T2	R3 T2	R3 T2	R3 T2	B1	B
8	B	B1	R3 T1	R3 T1	R3 T1	R3 T1	R3 T1	R3 T2	R3 T2	R3 T2	R3 T2	R3 T2	B1	B
9	B	B1	B1	B1	B1	B1	B1	B1	B1	B1	B1	B1	B1	B
10	B	B	B	B	B	B	B	B	B	B	B	B	B	B

Nursery

Luasong river

B and B1 = buffer lines, 80 seedlings
T1 = clone 5 from the lab: 30 plantlets
T2 = seedlings (OP seeds collected on clone 5)
R1, **R2** and **R3** = repetition 1, 2 and 3