Journal of Tropical Forest Science 1 (3): 263 - 267

GROWTH PERFORMANCE AND CHROMOSOME COUNT OF *ACACIA MANGIUM* PLANTLETS DERIVED FROM CULTURED NODAL EXPLANTS

Darus bin Haji Ahmad

Forest Research Institute Malaysia, 52109 Kuala Lumpur, Malaysia.

Received January 1989.

DARUS BIN HAJI AHMAD. 1989. Growth performance and chromosome count of *Acacia mangium* plantlets derived from cultured nodal explants. Clonal propagation of *Acacia mangium* by nodal cultures produced normal diploid plantlets. The chromosome number of plantlets (2n = 26) was similar to that of normal germinated seedlings. However, the growth rate of these plantlets was somewhat slower compared to that of germinated seedlings. The average heights of the micropropagated plantlets and germinated seedlings at 12 months after potting were 46.5 and 81.6 *cm*, respectively.

Key words: *Acacia mangium* - nodal culture - growth performance - chromosome count.

Introduction

Acacia mangium, a fast growing tropical hardwood species from the Australia-Papua New Guinea region (Anonymous 1983), is being planted on a wide scale in Malaysia. In consequence, an intensive program of research on the species is being conducted. Among the studies, the artificial propagation potential of *A. mangium* as well as its cytological characteristics were examined.

Plants derived from cultured tissues are known to develop alterations in the chromosomes. For example, Murata and Orton (1982) found hypodiploid, diploid, hyperdiploid, hypotetraploid and tetraploid cells in six-month-old suspension cultures of *Apium graveolens*. Preil (1985) states that somaclonal variations are frequently observed when plants are propagated vegetatively *in vitro* for a long time.

It has also been further shown that abnormal chromosome numbers resulting from callus cultures do cause slow growth in seedling populations, example *Betula pendula* (Cameron 1984).

In view of the above, I examined the chromosome numbers and growth performance of *A. mangium* plantlets derived from cultured nodal explants. The results are reported here.

Materials and methods

For growth performance study, ten germinated seedlings and ten plantlets derived from cultured nodal explants of eight-month-old seedlings were potted into plastic pots (7.5 x 7.5 x 6.2 *cm*) containing a 50:50 mixture of Irish sphagnum peat moss and vermiculite. These seedlings and plantlets were placed in a greenhouse in which the ranges of the day and night temperatures were 18°*C* to 25°*C* and 15°*C* to 19°*C*, respectively. The plants received an 18 hour photoperiod at a light intensity of about 40000-50000 *lux* from mercury vapour lamps. The relative humidity was between 55 to 69%. Plantlets and germinated seedlings were watered daily and fertilised twice a week with Bio-Plant Fertilizer (N:P:K ratio 5.2: 5.2: 6.0), starting one month after planting. The plant height was measured from root collar, above the potting medium surface to the shoot apex. The height measurement was made every two months for one year. The results at one year between the two planting stocks were analysed using the t-test.

For the cytological study, ten six-month-old plantlets were randomly selected from the population. The young roots were taken at 09:00 *h* and cut at 0.5 *cm* behind the root-tips and washed in running water to remove the potting medium. Root-tips were first pretreated in a solution of 0.05% colchicine and 0.03% (w/v) 8-hydroxyquinoline for 2 *h* at room temperature. The root-tips were subsequently treated with freshly prepared fixative (10 parts absolute alcohol, 2 parts chloroform, 2 parts glacial acetic acid and 1 part formalin) for 2.5 *h* and then washed lightly with 70% ethanol before immersing into 5M HCl for 10 *min* at 60°*C*. The root-tips were then transferred into Feulgen solution for 30 to 40 *min*. For squashing, a stained root-tip was taken out and placed on a glass slide. The dark portion of each root-tip was cut to about 1 *mm* long and then treated with a drop of aceto-carmine. A cover slip was placed on top of the cut root-tip and gently pressed to squash the root-tip and to spread the cells.

The number of chromosomes per cell was recorded at 1250 magnification using a Wild stereo microscope fitted with transmitted Light Base III. Ten chromosome counts were made from each plantlet. The same method was used to count the chromosome number for germinated seedlings.

Results

Growth performance

Figure 1 compares the cumulative growth of plantlets derived from nodal cultures of eight-month-old seedlings with that of potted germinated seedlings over a one year period. The germinated seedlings grew faster than micropropagated plantlets. The average height of micropropagated plantlets and germinated seedlings at 12 months after planting were 46.5 and 81.6 *cm* respectively, which was highly significant (t-test; $p = 0.01$).

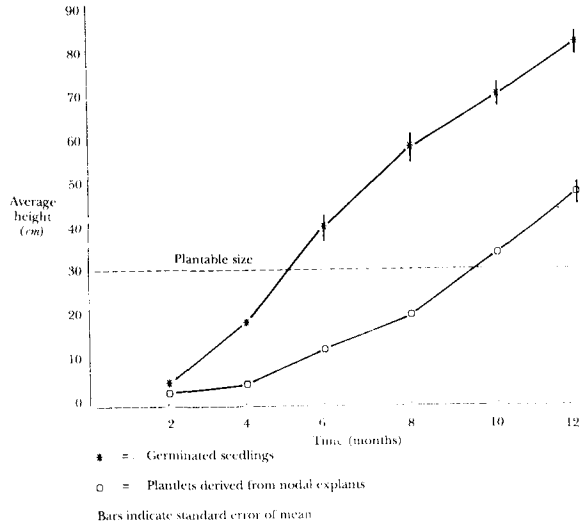

Figure 1. The cumulative growth of plantlets derived from nodal cultures in comparison to germinated seedlings (n=10)

Chromosome count

Figure 2 illustrates the frequency of chromosome number of six-month-old plantlets derived from nodal cultures and one-month-old germinated seedlings. The results show that the stock plants derived from nodal cultures had normal diploid (2n=26) chromosome complements similar to that of germinated seedlings (Figure 3).

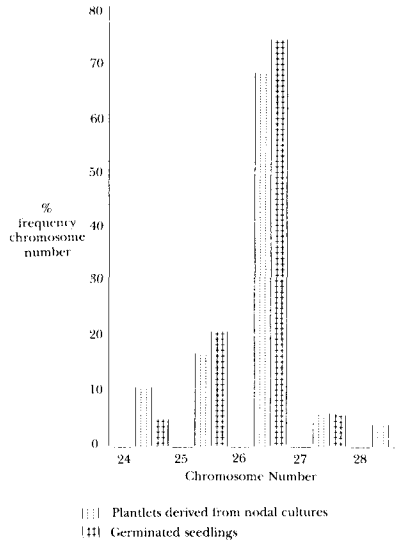

Figure 2. Frequencies of chromosome number of plantlets derived from nodal cultures and germinated seedlings (n=100)

Figure 3. A photograph (1250x) of mitotic chromosome in root cells of *Acacia mangium* germinated seedlings

Discussion and conclusions

A. mangium seedlings potted into 12 x 22 *cm* black polythene bags containing 75% forest top-soil and 25% river sand were ready for field planting at about four months after potting (Darus 1983). These seedlings had a minimum height of 30 *cm*. In the present experiment, germinated seedlings which were potted into smaller pots and a different potting mixture required about five to six months to achieve 30 *cm* in height, and plantlets derived from cultured nodal explants of eight-month-old seedlings needed more time to reach that plantable height (30 *cm*). The slower growth of plantlets might be due to poorer rooting systems compared to that of germinated seedlings (Darus 1988). At the early stage of root formation, developed shoots do not have direct vascular connections, and this would affect the uptake of water and nutrients by rooted plantlet. Another possible factor causing the slower growth of plantlets could be the transplantation shock during transfer from the humid condition in misted rooting chambers to the greenhouse which had slightly different environmental conditions, humidity in particular.

Root-tips and the Feulgen and aceto-carmine double staining method has made the counting of *A. mangium* chromosomes possible. Although the chromosome counts from plantlets derived from nodal cultures and germinated seedlings varied from 2n = 24 to 2n = 28, the most frequent chromosome number was 2n = 26. This variation was probably due to counting error especially when chromosomes did not lie in the same plane: the number counted was sometimes lower because of overlapping chromosomes and sometimes higher because of counting the same chromosome twice (Cameron 1984).

In conclusion, the growth of plantlets derived from cultured nodal explants of eight-month-old *A. mangium* seedlings was slower in comparison to that of germinated seedlings. The chromosome number of these plantlets was however the normal diploid chromosome complement, similar to that of normal seedlings.

Acknowledgements

I am greatly indebted to S. Thompson and A. Pirrie (University of Aberdeen) for their invaluable guidance and encouragement during the course of this study. Thanks are also extended to two anonymous reviewers and Wan Razali (FRIM) for their criticisms of an earlier draft of the manuscript.

References

ANONYMOUS. 1983. *Mangium and other fast growing species for the humid tropics.* National Academy Press, Washington, D.C.

CAMERON, A.D. 1984. *Clonal propagation of Betula pendula Ruth. and Pinus sylvestris L: By stem cuttings and tissue cultures.* Unpublished Ph.D. thesis, University of Aberdeen.

DARUS, H.A. 1983. Nursery techniques for *Gmelina arborea, Acacia mangium, Albizia falcataria* and *Eucalyptus* species at the FRI nursery, Kepong, *FRI Report* No. 36: 1-14.

DARUS, H.A. 1988. *Vegetative propagation of Acacia mangium by stem cuttings and tissue culture techniques.* Unpublished Ph. D. thesis, University of Aberdeen.

MURATA, M. & ORTON, T.J. 1982. Analysis of karyotypic in suspension cultures of celery. Pp. 435-436 in Fujiwara, A. (ed.). *Plant Tissue Culture.* The Japanese Association of Plant Tissue Culture, Tokyo.

FREIL, W. 1985. *In vitro* propagation and breeding of ornamental plants: Advantages and disadvantages of variability. Pp. 377-398 in Horn, W., Jensen, C.J., Odenbach, W. & Schieder, O. (eds.). *Genetic Manipulation in Plant Breeding,* 1986. Walter de Gruyter & Co., Berlin, New York.