4. RESULTS

4.1 Morphological characters assessment of mother trees and seeds germination

The morphological characteristics of each selected mother tree were recorded as shown in Table 4.1. Categories of mother trees were classified according to diameter breast height (Dbh) measurements because this is relatively easier to measure than other parameters (*e.g.* height). There were three categories namely A (Dbh > 0.6m), B (Dbh <0.6m but > 0.4m) and C (Dbh < 0.4m).

A. excelsa seeds are known for their recalcitrant feature (Kijkar , 1992). Therefore, after seeds were collected from each category of mother trees from the field, they were sown immediately (within a week) in the potting trays. Most of the seeds germinated within a month. The percent seed germination was recorded every week (Table 4.2). Percent germination of seeds from each seedlot of the mother trees ranged from 71% to 98%. Seeds from category A mother trees were all achieved above 80% germination rate. Some values of the final percent germination were found to be lower than the previous recorded figure due to the death of the seedling plants.

Table 4.1: Morphological characteristics of selected mother trees

Tree Number	Dbh (m)	Height (m)	Volume (m^3)	Canopy diameter (m)	Tree form	Crown shape
A1	0.713	35.1	9.81	13.32	Straight	Oval
A2	0.612	34.1	7.02	9.31	Straight up to 6m then bent	Oval
A3	0.703	29.1	7.91	14.05	Straight	Oval to oblong
A4	0.864	42.2	17.32	11.48	Straight	Oval
B1	0.445	24.3	2.53	9.34	Straight	Oblong
B2	0.435	20.9	2.28	5.23	Straight	Oblong
B3	0.435	15.2	1.58	6.44	Bend in one direction	Oval to oblong
B4	0.464	34.5	4.08	7.63	Straight	Oval
C1	0.397	30.4	2.63	8.29	Straight	Oblong
C2	0.287	27.5	1.25	7.34	Straight up to 10m then bent	Oblong
C3	0.238	10.4	0.32	4.76	Straight	Oval to oblong
C4	0.347	26.7	1.77	7.34	Straight	Oval
C5	0.380	21.3	1.69	3.83	Straight	Oval

Table 4.2 : Percent germination of seeds from each category of mother trees

Tree Number	Sample size of seeds	Time from seeds sowing			
		1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK
A1	196	7.0	95.5	95.5	98.0
A2	168	3.0	82.0	83.5	84.0
A3	183	10.0	90.5	91.5	91.5
A4	161	4.5	80.5	80.5	80.5
B1	86	2.0	93.0	96.0	96.0
B2	165	1.5	73.0	73.5	73.5
B3	186	9.0	91.5	91.5	93.0
B4	141	4.6	68.0	71.6	71.6
C1	147	10.0	82.5	84.0	82.5
C2	192	3.1	85.7	85.7	87.7
C3	172	3.0	82.0	86.0	86.0
C4	157	5.5	77.5	78.0	78.5
C5	89	11.8	73.9	75.6	74.8

4.2 Morphological character assessment of seedling plants

Mean distribution for heights of the seedling plants at 3 and 6 months growth from seeds of the 13 mother trees are presented in Figure 4.1 and 4.2 respectively. After 3 months of growth, the highest mean height ($x = 36.2 \pm 8.1$cm) was attained in C4 progeny plants (Figure 4.1). Individual seedlings with heights equal or greater than 45 cm were considered as seedlings at the upper end of the distribution curve. The highest percent of these seedlings were found in A3 group followed by C4 group (Table 4.3). The results did not display any obvious trend that taller seedlings were occurred more frequently at a particular category of seedlings at this stage.

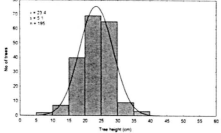
After 6 months of growth, the highest mean height achieved was 70.5±18.5 cm in A3 progeny plants (Figure 4.2). Seedlings with heights equal or greater than 90 cm were taken as upper end of distribution for height. The highest percent of taller seedlings were found in A3 followed by B3 (see Table 4.3). Similar to 3 months old seedlings, no trend was observed for the 6 months old seedlings.

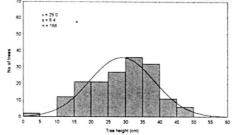
The number of leaf nodes represents number of leaflets each progeny seedling may have. Since higher numbers of leaf node result in a higher photosynthetic rate and thus may lead to bigger size of plants, the number of leaf nodes for each progeny seedling was investigated. The distribution curves in Figure 4.3 show that all 3-month old seedlings had an average of 5 leaf nodes. However, after 6 months growth, the highest mean number of leaf nodes attained was 11, in C1 category (Figure 4.4). Most of the seedlings in C1 category were found to have 10 or greater

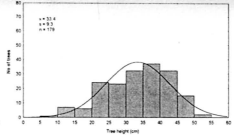
number of leaf nodes. After 3 months, seedlings with equal or greater than 8 leaf nodes were found mostly in C5 group (3.9%) (see Table 4.3). However, 6 months old seedlings with larger number of leaf nodes (*i.e.* equal or greater than 90 cm) were found in B2 group (7.8 %). No obvious trend was observed in frequency of occurrence of seedlings with high number of leaf nodes in a particular tree category.

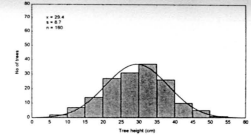
Table 4.3 : Percent of seedlings at extreme upper end of height and number of leaf node distribution in each tree group over 3 months and 6 months growth period

Tree Group	3 months			6 months		
	Sample size	Tree height (%)	Number of leaf nodes (%)	Sample size	Tree height (%)	Number of leaf nodes (%)
A1	196	0.0	0.0	196	0.0	0.0
A2	164	3.6	0.6	164	7.8	2.4
A3	175	9.1	3.4	175	10.8	7.4
A4	156	3.8	1.9	156	3.1	0.6
B1	80	0.0	0.0	80	7.5	0.0
B2	164	0.0	0.0	164	0.6	7.8
B3	181	0.5	0.0	181	9.6	5.3
B4	132	2.3	0.0	132	6.8	4.5
C1	146	0.7	0.0	143	0.0	2.1
C2	187	0.0	0.0	187	0.0	0.0
C3	168	3.6	0.0	168	0.6	0.0
C4	154	7.1	0.6	154	0.6	0.6
C5	76	5.3	3.9	76	6.1	3.6

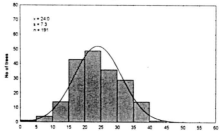




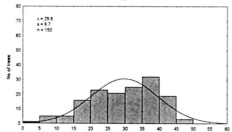




B1



B2



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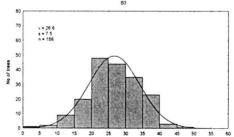
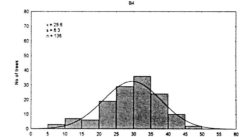
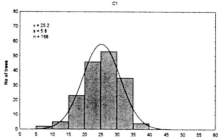


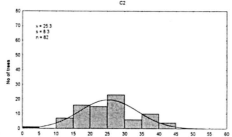
B4



C1



C2



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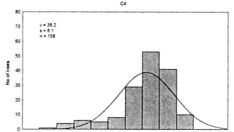
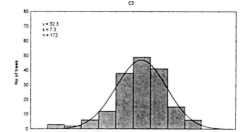


C3



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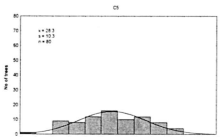
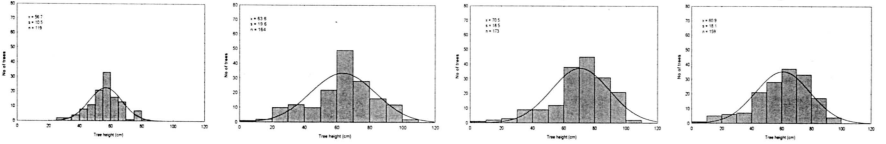
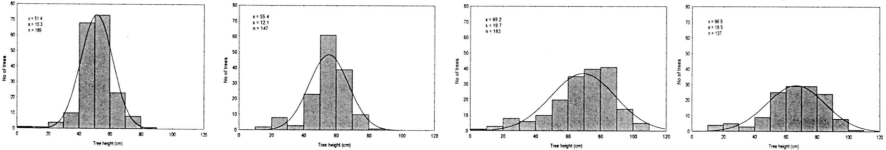
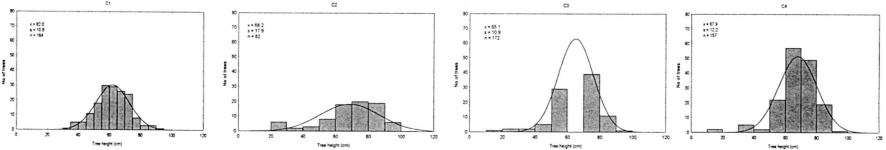
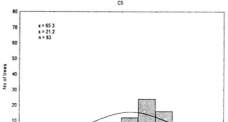


Where x – mean height
s – standard deviation
n – total number of seedlings

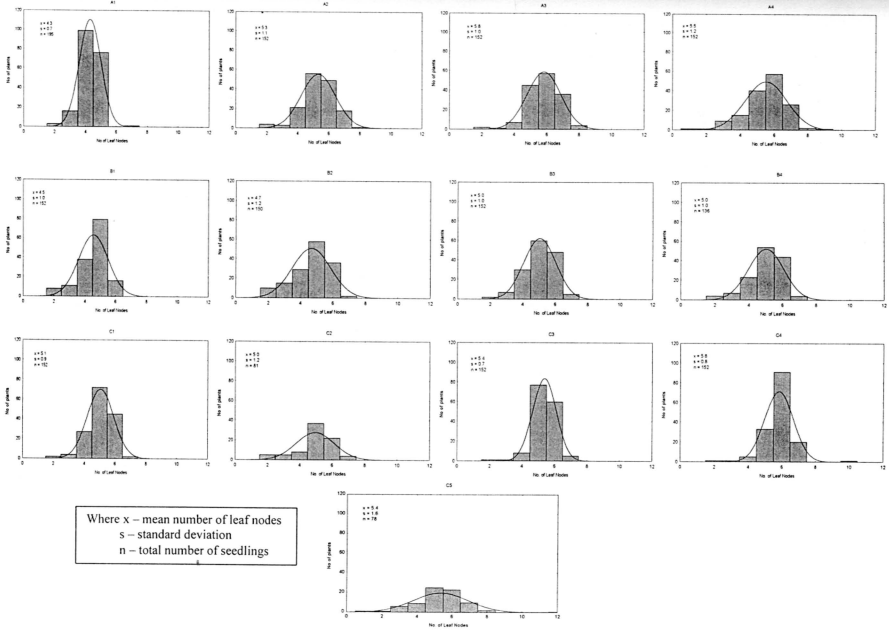




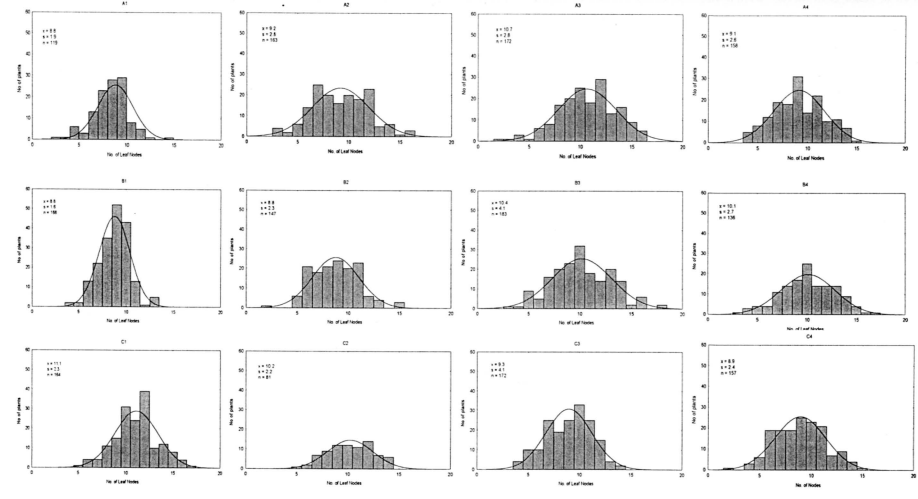




Where x – mean height
s – standard deviation
n – total number of seedlings



Where x – mean number of leaf nodes
s – standard deviation
n – total number of seedlings



Where x – mean number of leaf nodes
s – standard deviation
n – total number of seedlings

4.3 Morphological character assessment of plants in the field

After growing *A. excelsa* seedlings in the nursery for six months (see Plate 2), they were planted in the experimental plot in a randomised complete block design with three blocks with fixed effect. Each progeny tree group consisted of fifty trees in each block. Since some progeny tree group had less than 100 trees (*e.g.* B1 and C5), only two blocks were formed with these groups. As newly planted seedlings needed a certain period of time to adjust to the new environment, measurements were made six months after the seedlings were planted. From June 1998 to December 1999, a total of seven measurements were recorded at 3-month intervals. The parameters measured periodically were Dbh (diameter breast height) and total height. The different growth stages of *A. excelsa* trees in the field are shown in Plate 3. Large variations in size were observed among progeny trees in the population (Plate 4).

4.3.1 Dbh

Mean distribution curves for Dbh in block 1 (Figure 4.5 to 4.7) shows that after 1 year of growth in the field, mean Dbh in A category progeny trees was higher than B or C except for B1. B1 had the highest mean Dbh of 1.9 cm. However, the differences in mean Dbh was not so obvious among various category groups in block 2 and block 3. The trend of growth continued until the trees were two and a half years old. The highest mean Dbh achieved at this stage was 8.43 cm in B1 category. The number of trees found at the upper range of distribution curve was investigated. This is to assess individual trees in order to select high performance

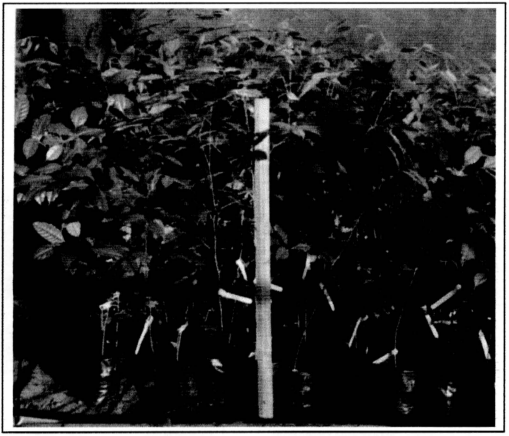


Plate 2 : Six months old *A. excelsa* seedlings ready to be transplanted to the experimental field. Note that the length of the ruler is 3 m.

6 months

9 months

1 year

1.5 years

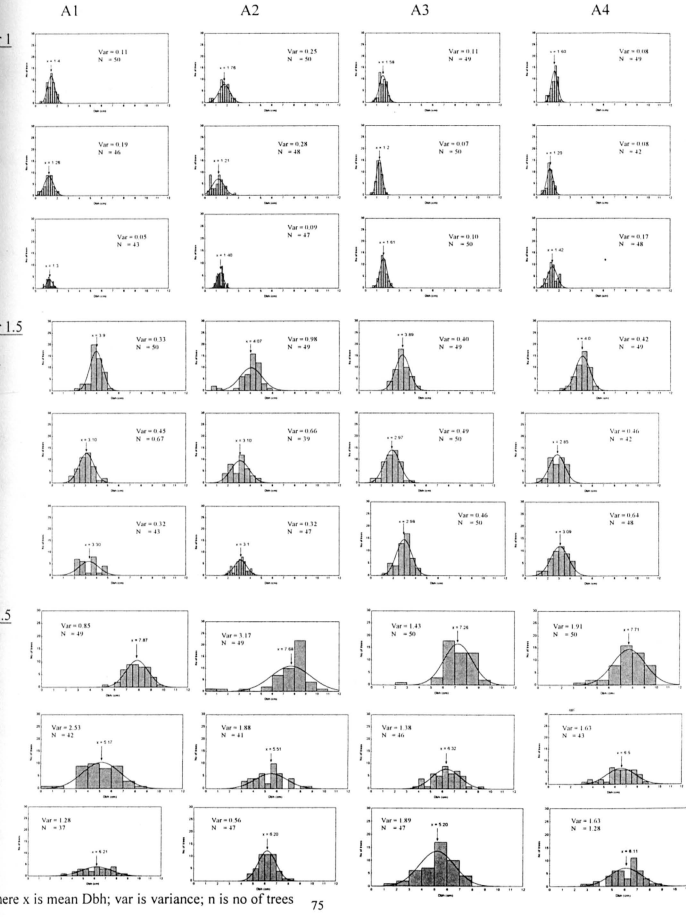
2 years

2.5 years

Plate 3 : Progression growth of *A. excelsa* trees in the field over the period of 2.5 years

Plate 4 : Variation of sizes in the population. Arrows indicate small trees

Figure 4.5 : Mean Dbh distribution for each progeny tree group A in different blocks over 2.5 years



here x is mean Dbh; var is variance; n is no of trees

Figure 4.6 : Mean Dbh distribution for each progeny tree group B in different blocks over 2.5 years

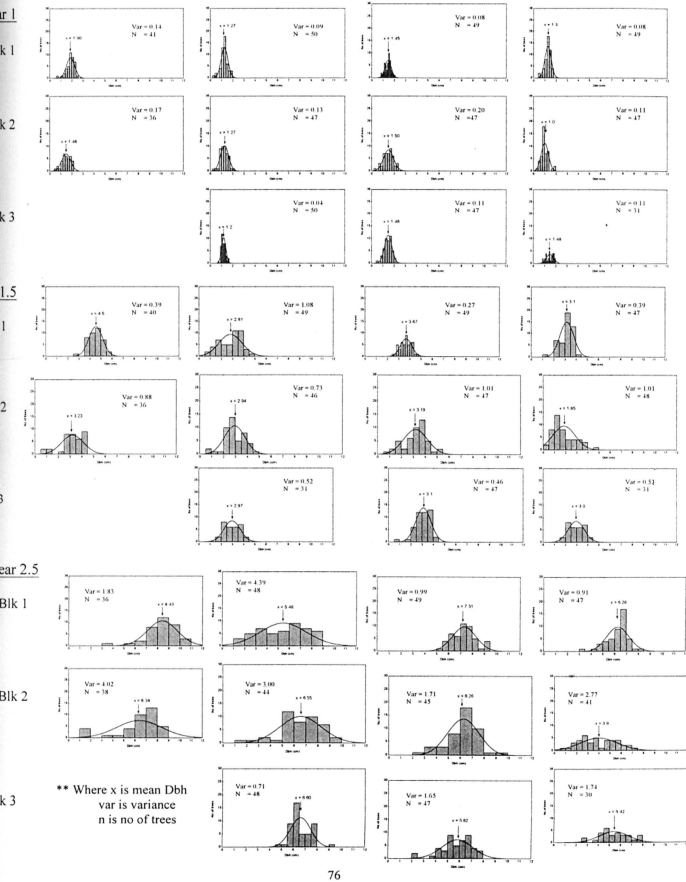
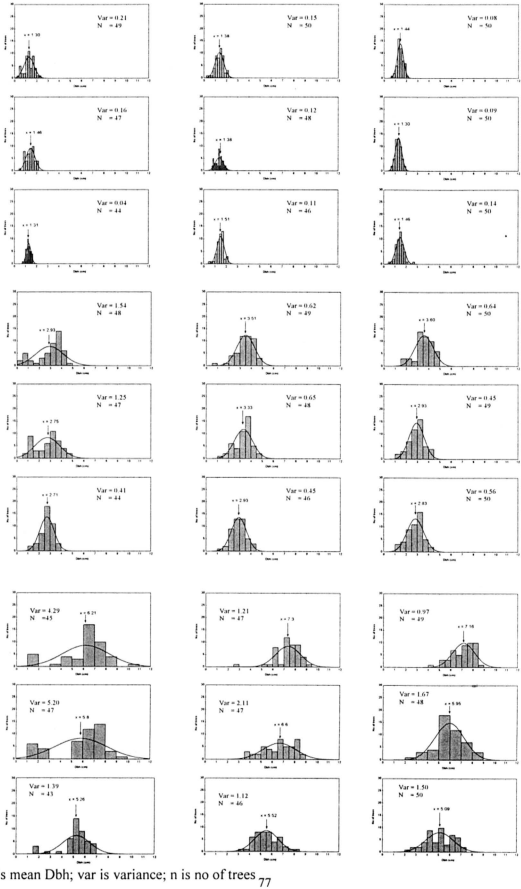
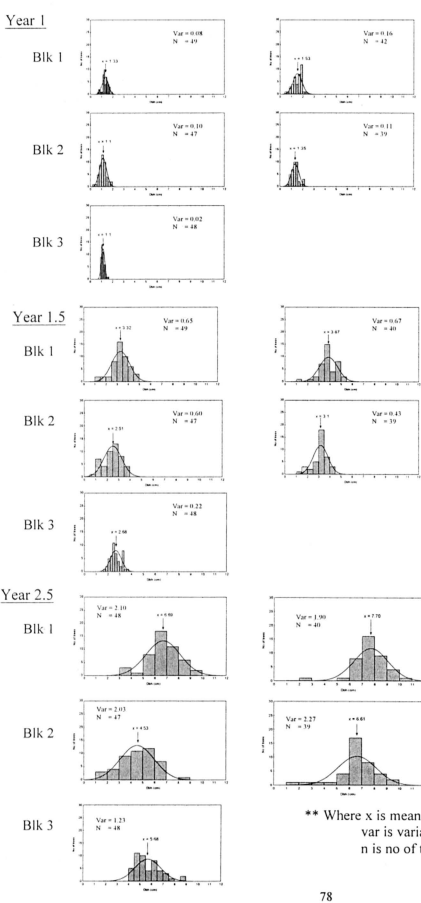


Figure 4.7 : Mean Dbh distribution for each progeny tree group C in different blocks over 2.5 years



** Where x is mean Dbh; var is variance; n is no of trees

Continue from Figure 4.7



** Where x is mean Dbh
var is variance
n is no of trees

trees based on Dbh and tree height at selected stages of growth. After 1 year of growth in the field, number of trees with Dbh equal or greater than 2 cm were considered as candidate superior trees. In block 1, candidate superior trees were found in all tree groups in A category that ranges from 2% to 32% (Table 4.4). B1 has the highest percentage of candidate superior trees (41.4%). In block 2, the highest percentage of candidate superior trees was also found in B1 (13.9%) whilst A4 has the highest percentage of candidate superior trees at 12.5 in block 3. At 1.5 years, candidate superior trees are trees with Dbh equal or greater than 4.5 cm. In block 1, candidate superior trees were found in all 3 categories with the highest number in B1 (45%). While the highest percentage of candidate superior trees was found in 6.4% in block 2, no candidate superior tree was found in block 3. Similar trends were observed after the trees were 2.5 years in the field whereby trees with Dbh equal or greater than 9 cm were considered as candidate superior trees. The highest percentage of candidate superior trees was found in B1 (27.8%) and C5 (2.6%) in block 1 and 2 respectively. No candidate superior tree was found in block 3.

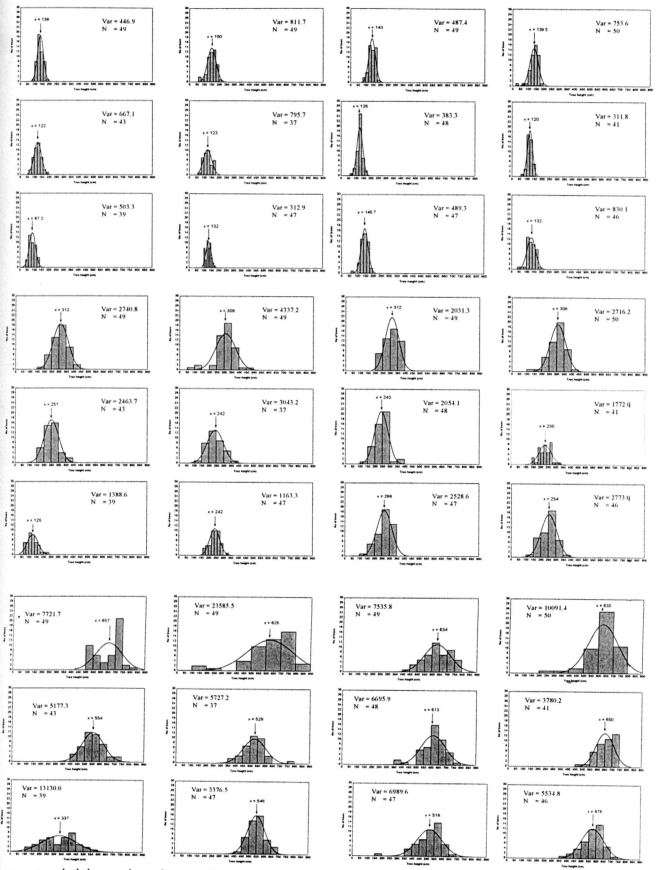
Table 4.4 : Number of trees at the upper range of Dbh distribution curve after 1, 1.5 or 2.5 years of growth in percentage

	Block 1			Block 2			Block 3		
	Yr 1	Yr 1.5	Yr 2.5	Yr 1	Yr 1.5	Yr 2.5	Yr 1	Yr 1.5	Yr 2.5
A1	8.0	18.0	8.2	4.3	4.6	0.0	0.0	0.0	0.0
A2	32.0	36.7	12.2	4.2	5.1	0.0	4.2	0.0	0.0
A3	8.0	18.4	4.0	0.0	2.0	2.2	12.0	0.0	0.0
A4	2.0	24.5	20.0	0.0	0.0	0.0	12.5	0.0	0.0
B1	41.4	45.0	27.8	13.9	0.0	0.0	-	-	-
B2	0.0	2.0	0.0	2.1	2.2	4.5	0.0	0.0	0.0
B3	2.0	4.1	4.1	12.8	6.4	2.2	4.2	0.0	0.0
B4	0.0	2.1	0.0	2.1	2.1	0.0	3.2	0.0	0.0
C1	4.1	2.1	0.0	8.5	4.2	2.1	0.0	0.0	0.0
C2	6.0	8.2	2.1	4.2	4.2	2.1	8.7	0.0	0.0
C3	2.0	10.0	0.0	2.0	0.0	0.0	4.0	0.0	0.0
C4	0.0	6.1	4.2	0.0	0.0	0.0	0.0	0.0	0.0
C5	7.1	17.5	10.0	7.7	0.0	2.5	-	-	-

4.3.2 Total height

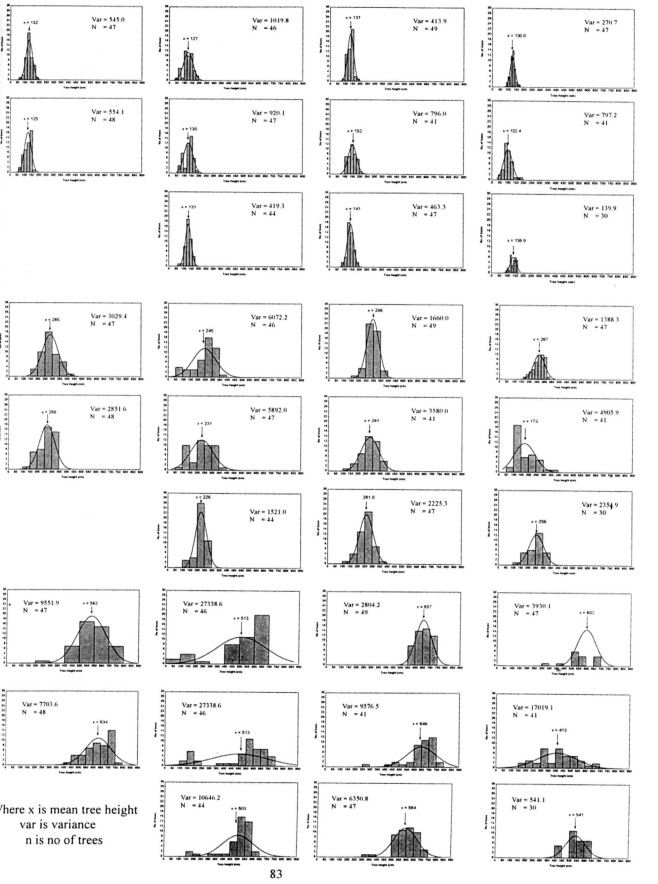
A similar trend was also found in the distribution of tree height (Figure 4.8 to 4.10) whereby the Dbh for A category progeny trees was relatively higher than for B or C category over the two and a half years growth period. To assess the performance of trees based on tree height, after 1 year of growth in the field, trees with total height equal or greater than 170 cm were considered as candidate superior trees. In block 1, the highest number of candidate superior trees was found in B1 (36.1%). B3 (29.3%) and A3 (14.9%) have the highest number of candidate superior trees in block 2 and block 3 respectively (see Table 4.5). After 1.5 years of growth, trees with total height equal or greater than 380 cm were considered as candidate superior trees. Again, B1 is the tree group with the highest number of candidate superior tree at 36.1% in block 1. In block 2, the highest number of candidate superior trees was found in B3 (4.9%). No candidate superior tree was found in block 3. After 2.5 years of growth, trees with total height equal or greater than 750 cm were considered as candidate superior trees. At this stage, A2 has the highest number of candidate superior trees (18.4%) in block 1. Candidate superior trees were found in all tree groups in A category. In block 2, B2 has the highest number of candidate superior trees (7%). No candidate superior trees were found in block 3.

re 4.8 : Mean tree height distribution for each progeny tree group A in different blocks over 2.5 years



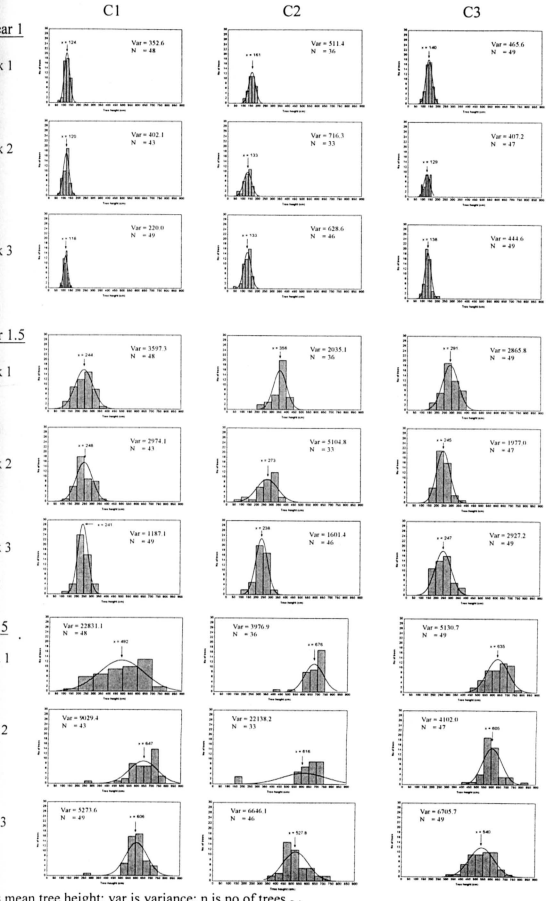
mean tree height; var is variance; n is no of trees

4.9 : Mean tree height distribution for each progeny tree group B in different blocks over 2.5 years



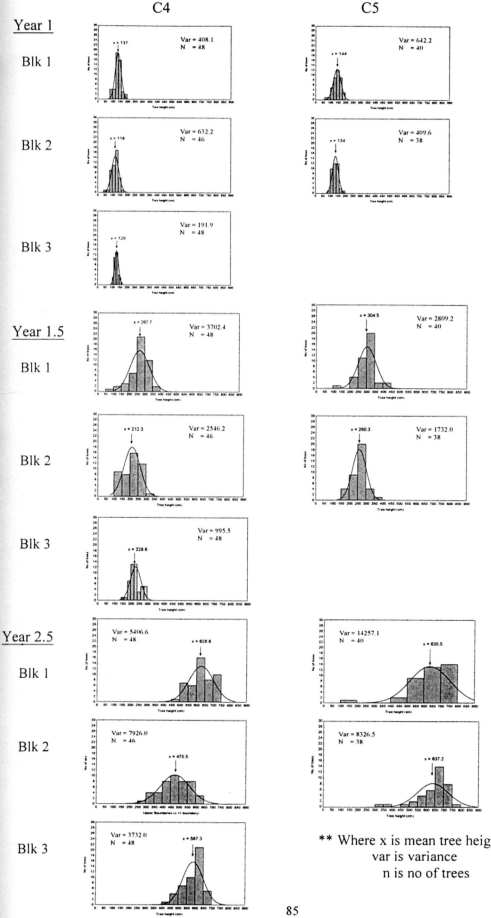
Where x is mean tree height
var is variance
n is no of trees

4.10 : Mean tree height distribution for each progeny tree group C in different blocks over 2.5 years



mean tree height; var is variance; n is no of trees

Continue from Figure 4.10



** Where x is mean tree height
var is variance
n is no of trees

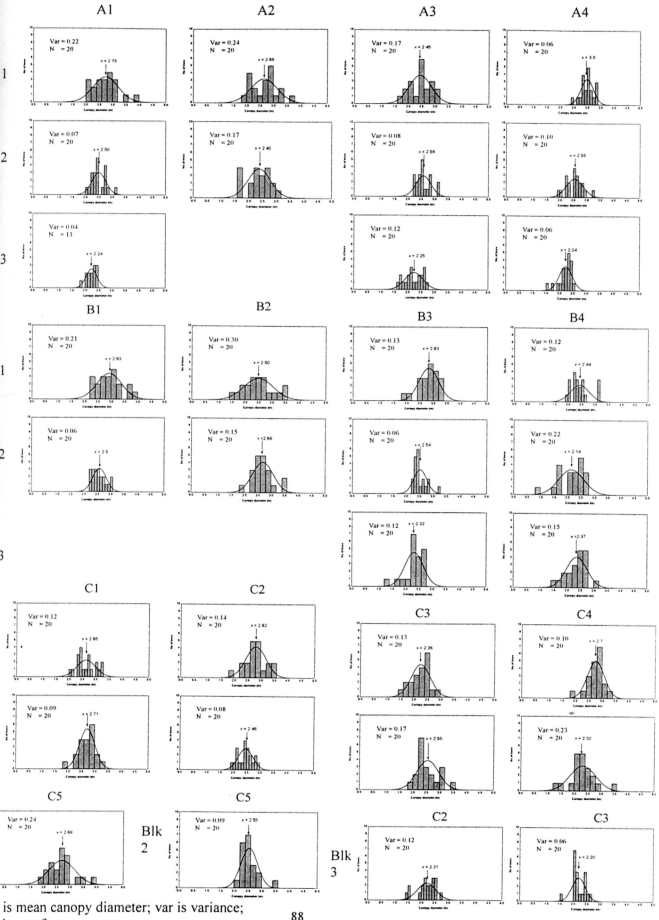
Table 4.5 : Number of trees at the upper range of tree height distribution curve after 1, 1.5 and 2.5 years of growth in percentage

	Block 1			Block 2			Block 3		
	Yr 1	Yr 1.5	Yr 2.5	Yr 1	Yr 1.5	Yr 2.5	Yr 1	Yr 1.5	Yr 2.5
A1	8.2	10.2	8.2	2.3	0.0	0.0	0.0	0.0	0.0
A2	24.5	8.2	18.4	5.4	2.7	2.7	2.1	0.0	0.0
A3	12.2	6.1	10.2	2.1	0.0	2.1	14.9	0.0	0.0
A4	4.0	10.0	6.0	0.0	0.0	0.0	8.7	0.0	0.0
B1	36.1	36.1	0.0	3.0	0.0	0.0	-	-	-
B2	0.0	0.0	0.0	0.0	0.0	7.0	0.0	0.0	0.0
B3	4.1	2.0	0.0	29.3	4.9	4.9	10.6	0.0	0.0
B4	2.1	0.0	0.0	2.4	2.4	0.0	0.0	0.0	0.0
C1	4.3	0.0	0.0	4.2	0.0	0.0	2.3	0.0	0.0
C2	8.5	2.1	0.0	0.0	0.0	2.1	2.2	0.0	0.0
C3	6.1	6.1	2.1	0.0	0.0	0.0	8.2	0.0	0.0
C4	6.2	0.0	0.0	2.2	0.0	0.0	0.0	0.0	0.0
C5	10.0	7.5	10.0	5.3	0.0	2.6	-	-	-

4.3.3 Number of nodes and canopy diameter

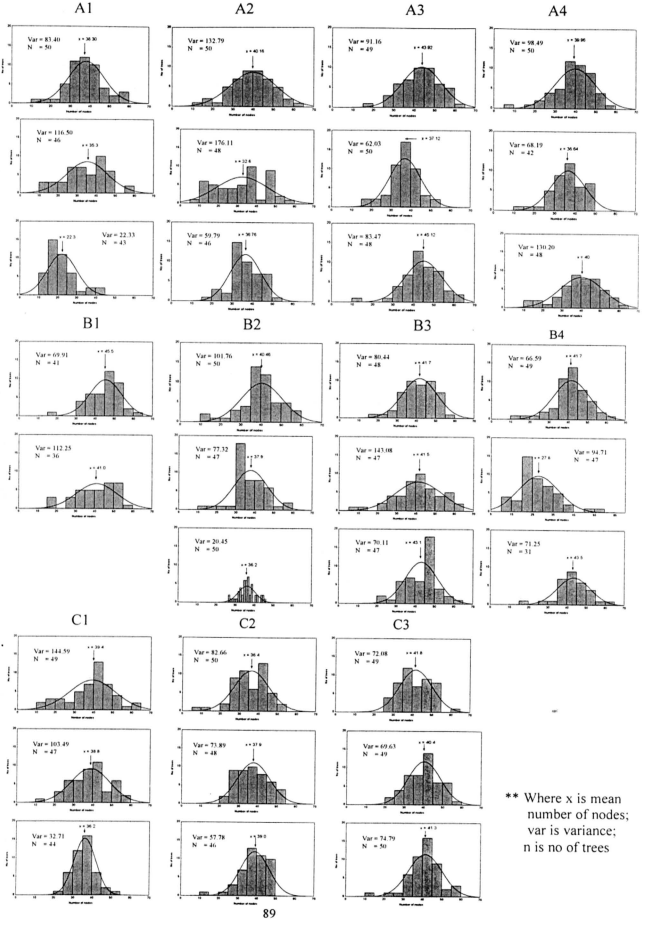
The measurements of two characters namely nodes and canopy diameter were taken. The counting of the number of nodes for each progeny tree was performed after the tree was one year old. The canopy diameter was measured when the progeny trees were two years old. Only 20 trees from each progeny group were randomly chosen for the measurements of canopy diameter as it was very tedious to perform the measurements for each tree. The distribution curves for canopy diameter (Figure 4.11) show that mean canopy diameter of some progeny groups is distinctly larger. For example, mean canopy diameter of A4 is 3 m compared to 2.26 m in C3 in block 1. However, no obvious trend exists in different tree progeny group categories. Similarly, no obvious trend was found in the mean number of nodes measured among various tree progeny groups (Figure 4.12).

gure 4.11 : Mean canopy diameter (m) distribution for each progeny tree group in different blocks

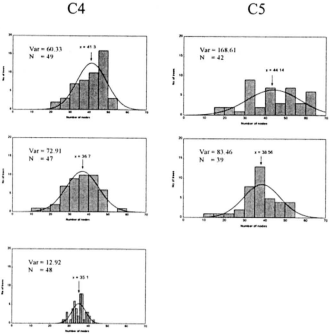


is mean canopy diameter; var is variance;
is no of trees

igure 4.12 : Mean number of nodes distribution for each progeny tree group in different blocks



Continue from Figure 4.12



** Where x is mean number of nodes;
var is variance;
n is no of trees

4.4 Analysis of variance (ANOVA) on quantitative characters

Quantitative characters related to growth included diameter breast height (Dbh) and total height. The mean growth parameters were calculated from final measurement taken when the trees were two and a half years old in the field. Increment of mean growth parameters was calculated over an annual growth period. Only block 1 and 2 progeny tree measurements were included in the analysis because some progeny groups had a smaller sample size and therefore could not be planted in block 3. Analysis of variance shows that differences between blocks are highly significant. Other quantitative characters used in the analysis were canopy diameter and number of nodes.

4.4.1 Diameter breast height (Dbh)

When the progeny trees were one year old in the field, a narrow range of variability in mean Dbh was obtained among the progeny trees. As the trees grow older (*i.e.* two and a half years old when the measurements were made), differences in Dbh measurements were apparent. As a result, wider range of variability in mean Dbh was shown (see Figure 4.5 to 4.7). There is not much difference in variability in range of variance between block 1 and 2, and among progeny groups as reflected by the sum of squares obtained in Table 4.6.

Table 4.6 shows that the difference in mean Dbh was not significant among different progeny trees grouped according to various sizes or categories and mean Dbh of

progeny trees from various groups. However, mean Dbh between block 1 and 2 was shown to be highly significant ($p < 0.0001$).

Table 4.7 shows that mean Dbh of progeny trees obtained ranges from 6.89 cm in category DC to 8.55 cm in category DB. Overall, mean Dbh of progeny trees from A category was higher than B or C category. However there is no difference between the mean Dbh of B and C category progeny trees. A similar trend was observed in mean Dbh increment of progeny trees. The results show that mother trees of large mean Dbh do not necessary produce large sizes progeny trees. However, there is a trend showing that large mother trees tend to produce progenies with larger mean overall sizes. For example, the mother trees selected for category A have a mean Dbh much higher than those selected from categories B or C (0.28m and 0.39m respectively), the mean Dbh of the progeny trees from category A is larger than from categories B or C. While difference in mean Dbh of mother trees between category B and C is relatively small (0.11m) and no significant difference in mean Dbh of progeny trees was obtained.

Within the A category progeny trees, A4 has the largest mean Dbh of 8.39 cm (Table 4.7). In B category, the highest mean Dbh was achieved by B1 (8.55 cm). The results of C category progeny trees shows that C5 group attained the highest mean Dbh of 8.39 cm. The largest Dbh in A category mother tree is A4 and in B category, B4 mother tree has the largest Dbh while in C category C1 mother tree has the largest Dbh.

Table 4.6 : ANOVA on mean Dbh

Source	df	Type III Sum of Square (SS)	Mean SS	F Value	Pr > F
MODEL	25	761.166	30.447	15.59	0.0001
BLK	1	285.027	285.027	145.98	0.0001
Prog	12	275.954	22.996	1.42	NS
Category	2	25.545	12.773	0.82	NS
Parent (Category)	10	242.729	24.273	1.49	NS
BLK*Prog	12	194.736	16.228	8.31	0.0001
BLK*Category	2	31.343	15.671	8.03	0.0003
BLK*Parent(Category)	10	163.233	16.323	8.36	0.0001
ERROR	1125	2196.603	1.952		
TOTAL	1150	2957.770			

Where NS represents non significant difference at 5 % probability level

Table 4.7 : Diameter breast height (Dbh)

Tree category	Dbh of mother tree (m)	Mean Dbh of progeny trees (cm)	Mean Dbh increment of progeny trees (cm)
DA1	0.71	7.80	6.38
DA2	0.61	7.99	6.35
DA3	0.70	8.13	6.72
DA4	0.86	8.39	6.98
Mean	**0.72**	**8.08**	**6.61**
DB1	0.44	8.55	6.84
DB2	0.43	7.21	5.90
DB3	0.43	8.01	6.51
DB4	0.46	7.13	5.78
Mean	**0.44**	**7.67**	**6.22**
DC1	0.42	7.68	6.18
DC2	0.29	8.05	6.64
DC3	0.24	7.47	6.10
DC4	0.35	6.89	5.65
DC5	0.38	8.39	6.92
Mean	**0.33**	**7.67**	**6.28**
Overall Mean	**0.49**	**7.80**	**6.37**
S^2		1.95	1.52
C.V.		17.92	19.36

4.4.2 Total height

For mean tree height measurements, the range of variability increases as the trees reaches two and a half years old (Figure 4.8 to 4.10). At two and a half years old, wide ranges of variability can be observed among progeny tree group. For example, in block 2, the variance obtained for tree group C2 is 22138.2 as compared to variance for tree group C3, which is 4102. This is reflected in the relatively larger sum of square obtained for among progeny groups in Table 4.8.

Analysis by ANOVA (Table 4.8) shows that the difference in mean total height among progeny groups between block 1 and 2 was highly significant ($p<0.0001$). When progeny trees were categorized according to sizes of Dbh as in 4.4.1, the mean total height of progeny trees was found to be no significant difference among the three categories.

There is a trend showing that the overall progeny trees in category A are taller than those in category B and progeny trees in category B are taller than C (Table 4.9). The mean total height increment of progeny trees in category A is displaying similar trend.

As in Dbh (see section 4.4.1), similar trend was observed within all the three category progeny trees. While the highest mean total height was achieved by B1 (at 599.8 cm) in progeny trees, the tallest mother tree was found to be A4 at a height of 42.2 m.

Table 4.8 : ANOVA on mean total height

Source	Df	Type III Sum of Square (SS) x 10^4	Mean SS $X10^4$	F Value	Pr > F
MODEL	25	266.03	10.64	13.75	0.0001
REP	1	32.38	32.38	41.84	0.0001
Prog	12	125.24	10.44	1.17	NS
Category	2	11.07	5.54	1.41	NS
Parent (Category)	10	113.58	11.36	1.15	NS
REP*Prog	12	107.20	8.93	11.54	0.0001
REP*Category	2	7.85	3.92	5.07	0.0064
REP*Parent(Category)	10	98.48	9.85	12.73	0.0001
ERROR	1142	883.69	0.77		
TOTAL	1167	1149.73			

Where NS represents non significant difference at 5 % probability level

Table 4.9 : Total height based on Dbh sizes

Tree category	Total height of mother tree (m)	Mean total height of progeny trees (cm)	Mean total height increment of progeny trees (cm)
DA1	35.1	577.6	416.8
DA2	34.1	545.6	379.7
DA3	29.1	576.4	417.2
DA4	42.2	588.8	434.6
Mean	**35.1**	**572.7**	**412.8**
DB1	24.3	599.8	420.4
DB2	20.9	523.9	379.7
DB3	15.2	598.4	427.8
DB4	34.5	537.5	385.8
Mean	**23.7**	**562.2**	**402.1**
DC1	30.4	486.7	337.6
DC2	27.5	571.1	415.0
DC3	10.4	576.2	418.7
DC4	26.7	521.9	374.3
DC5	21.3	584.6	421.8
Mean	**23.3**	**547.5**	**393.2**
Overall Mean	**27.1**	**559.8**	**401.9**
S^2		1.9	1.5
C.V.		17.9	19.4

When progeny trees were categorized according to the total heights of mother trees, the mean total heights of the progeny trees in each category changes as shown in Table 4.10. Progeny trees in category HC was found to be taller than those in categories HA and HB. While progeny trees in category HA are taller than HB. Therefore the order of arrangement according to mean total height in progeny trees becomes HC > HA > HB. The mean total height increment of progeny trees for categories HC and HA is higher than HB.

For total height measurement, there is no trend showing that taller trees will produce taller progeny trees. In fact Table 4.10 shows that mean total heights of progenies from shorter mother trees (*i.e.* HC2 and HC4) was higher (599.8cm and 598.4cm respectively).

Table 4.10 : Total height

Tree category	Total height of mother tree (m)	Mean total height of progeny trees (cm)	Mean total height increment of progeny trees (cm)
HA1	35.1	577.6	416.8
HA2	34.1	545.6	379.7
HA3	34.5	537.5	385.8
HA4	42.2	588.8	434.6
Mean	**36.5**	**562.5**	**404.6**
HB1	29.1	576.5	417.1
HB2	30.4	486.7	337.6
HB3	27.5	571.1	415.0
HB4	26.7	521.9	374.2
Mean	**28.4**	**539.9**	**386.8**
HC1	20.9	523.9	379.7
HC2	24.3	599.8	420.4
HC3	10.4	576.2	418.7
HC4	15.2	598.4	427.8
HC5	21.3	584.6	421.8
Mean	**18.4**	**574.9**	**413.0**
Overall Mean	**27.1**	**559.8**	**401.9**
S^2		7738.1	5135.5
C.V.		15.7	17.8

4.4.3 Tree Volume

ANOVA table (Table 4.11) shows that mean tree volume varied significantly ($p<0.0001$) among different groups of progeny trees between block 1 and 2. However, the mean tree volume of progeny trees categorized according to section 4.4.1 based on sizes of Dbh does not vary significantly among the three categories.

Even though the mean volume of progeny trees is significantly higher in category A than B or C, the larger progeny trees (B1 with mean tree volumn = 23257.8cm^3) were found in category B (Table 4.12). A similar trend was observed in mean volume increment of progeny trees.

Similar to Dbh assessment, the mean volume of mother tree in A category is much higher than B and C categories (112834.1m^3 and 127554.2m^3 respectively) compared to the difference between B and C (14720.1m^3). The mother trees from category A also produce a larger mean volume of progeny trees than B or C categories. Similar trend was also observed in volume within all the three progeny tree categories as in Dbh (see section 4.4.1).

Table 4.11 : ANOVA on mean tree volume

Source	Df	Type III Sum of Square (SS) x10^6	Mean SS X10^6	F Value	Pr > F
MODEL	25	22443.7	897.7	18.95	0.0001
BLK	1	7855.7	7855.7	165.78	0.0001
Prog	12	7828.3	652.3	1.17	NS
Category	2	616.2	308.1	0.47	NS
Parent (Category)	10	7039.6	703.9	1.31	NS
BLK*Prog	12	6682.1	556.8	11.75	0.0001
BLK*Category	2	1323.8	661.9	13.97	0.0001
BLK*Parent(Category)	10	5354.9	535.5	11.30	0.0001
ERROR	1124	53261.0	47.4		
TOTAL	1149	75704.7			

Where NS represents non significant difference at 5 % probability level

Table 4.12 : Tree volume

Tree category	Volume of mother tree (m^3)	Mean volume of progeny trees (cm^3)	Mean volume increment of progeny trees (cm^3)
A1	140162.7	18724.5	18394.6
A2	100323.7	19260.6	18893.5
A3	112966.5	19418.1	19217.6
A4	247449.2	20682.4	20375.2
Mean	**150225.5**	**19524.4**	**19227.4**
B1	36118.6	23257.8	22739.6
B2	32509.7	15456.1	15218.4
B3	22592.7	19350.7	19119.9
B4	58344.7	14032.2	13780.3
Mean	**37391.4**	**17662.2**	**17361.0**
C1	41523.3	16873.1	16646.3
C2	17792.7	19121.0	18814.3
C3	4627.4	16768.0	16481.2
C4	25253.2	13542.1	13322.9
C5	24159.8	21180.6	20833.2
Mean	**22671.3**	**17375.2**	**17100.7**
Overall Mean	**66448.0**	**18141.3**	**17851.1**
S^2 (x10^7)		4.739	4.563
C.V.		37.94	37.84

4.4.4 Number of nodes

Large variance of the mean number of nodes was obtained which is shown by the wide distribution curve in Figure 4.12. As a result large sum of square were obtained in both block and progeny components (Table 4.13).

Table 4.13 shows that no significant difference in mean number of nodes was found among different progeny groups and when the progeny trees were grouped into categories according to the sizes of their respective mother trees. Table 4.14 shows that mean number of nodes ranged from 38 to 44. The highest mean number of nodes was in category B.

4.4.5 Canopy diameter

Wider range of variability in mean canopy diameter were obtained among progeny trees in Block 1 as compared to block 2 and 3 (Figure 4.11). Within each block, the distribution of variance in each progeny group was wide as shown in relatively high sum of square obtained in Table 4.15.

Table 4.15 shows that there is no difference in mean canopy diameter among the progeny groups and for progeny trees from the three categories. Table 4.16 shows that mean canopy diameter of the progeny groups range from 2.4 m to 2.8 m.

Table 4.13 : ANOVA on mean number of nodes

Source	Df	Type III Sum of Square (SS)	Mean SS	F Value	Pr > F
MODEL	25	7693.743	307.750	3.56	0.0001
BLK	1	3349.730	3349.730	38.78	0.0001
Prog	12	3204.847	267.070	2.35	0.0867
Size	2	1315.599	657.800	13.86	0.0879
Parent (Size)	10	2021.944	202.194	1.64	0.2319
BLK*Prog	12	1363.237	113.603	1.32	0.2033
BLK*Size	2	94.952	47.476	0.55	0.5773
BLK*Parent(Size)	10	1231.352	123.135	1.43	0.1633
ERROR	1152	99510.820	86.381		
TOTAL	1177	107204.564			

Table 4.14 : Mean number of nodes

Tree category	Mean number of nodes
A1	38.2
A2	39.5
A3	40.5
A4	38.4
Mean	**39.2**
B1	44.0
B2	40.0
B3	41.6
B4	41.4
Mean	**41.6**
C1	40.2
C2	37.8
C3	41.3
C4	39.1
C5	42.0
Mean	**40.0**
Overall Mean	**40.2**
S^2	86.4
C.V.	23.1

Table 4.15 : ANOVA on mean canopy diameter

Source	Df	Type III Sum of Square (SS)	Mean SS	F Value	Pr > F
MODEL	25	16.411	0.656	4.50	0.0001
BLK	1	2.794	2.794	19.17	0.0001
Prog	12	6.716	0.560	0.97	NS
Size	2	0.124	0.062	0.68	NS
Parent (Size)	10	6.592	0.659	0.98	NS
BLK*Prog	12	6.901	0.575	3.94	0.0001
BLK*Size	2	0.182	0.091	0.62	0.5366
BLK*Parent(Size)	10	6.719	0.672	4.61	0.0001
ERROR	494	72.013	0.146		
TOTAL	519	88.424			

Where NS represents non significant difference at 5 % probability

Table 4.16 : Mean canopy diameter

Tree category	Mean canopy diameter (m)
A1	2.63
A2	2.53
A3	2.52
A4	2.78
Mean	**2.61**
B1	2.76
B2	2.59
B3	2.69
B4	2.40
Mean	**2.61**
C1	2.68
C2	2.64
C3	2.39
C4	2.56
C5	2.62
Mean	**2.58**
Overall Mean	**2.60**
S^2	0.15
C.V.	14.68

4.5 Assessment on qualitative characters

Qualitative characters of *A. excelsa* include bending or straightness of tree trunk, time to production of first branching whorl (early or late branching habit) and forking of tree trunk were investigated.

4.5.1 Bending or straightness of tree trunk

Most of the progeny trees have straight trunks. However, a small number of progeny trees exhibit a kink on their trunk that caused them to bend (Plate 5). Table 4.17 shows the number of bent progeny trees in each progeny tree group from block 1 and 2. Progeny tree group with the highest number of bent progeny trees occurred in C4 followed by A2 and A3. Progeny tree group with the least number of bent trees was B1.

4.5.2 Early or late branching habits

All *A. excelsa* trees produce several whorls of branches as they mature. The first whorl of branches was observed after the tree was one year in the field (Plate 6). However, not all trees produced the first whorl of branches at the same time. The trees that produced the first whorl of branches after growing one and a half years in the field were therefore recorded. Table 4.18 shows the number of one and a half years old progeny trees in block 1 and 2 that produced early first branching whorl. The highest number of progeny trees with early branching habit was recorded in

Plate 5 : Arrow indicates bending of tree trunk

Plate 6 : (A) Arrows indicate growth of first whorl of branches
(B) A close up view of first whorl of branches

Table 4.17 : Number of bent trees in different progeny tree groups

Progeny tree group	Number of tree samples	Number of trees with bending tree trunk	Ratio of bending : straightness
A1	91	7	1 : 13
A2	85	16	1 : 5.3
A3	98	13	1 : 7.5
A4	92	8	1 : 11.5
B1	72	3	1 : 24
B2	93	9	1 : 10.3
B3	98	11	1 : 8.9
B4	94	4	1 : 23.5
C1	91	5	1 : 18.2
C2	95	8	1 : 11.9
C3	96	8	1 : 12
C4	94	22	1 : 4.3
C5	79	10	1 : 7.9

Table 4.18 : Number of progeny trees with early branching habits

Progeny tree group	Number of tree samples	Number of trees with early branching habit	Ratio of early : late
A1	91	30	1 : 3
A2	85	15	1 : 5.7
A3	98	26	1 : 3.8
A4	92	33	1 : 2.8
B1	72	35	1 : 2.1
B2	93	13	1 : 7.2
B3	98	37	1 : 2.6
B4	94	24	1 : 3.9
C1	91	25	1 : 3.6
C2	95	16	1 : 5.9
C3	96	38	1 : 2.5
C4	94	18	1 : 5.2
C5	79	31	1 : 7.9

progeny tree group C3. However, B1 has the lowest ratio of early to late branching habits of progeny tree groups because of smaller number of tree samples available.

4.5.3 Forking

Some progeny trees forked at the lower part of the tree trunk and were recorded for analysis (Plate 7). Table 4.19 shows that the tree group with the highest number (*i.e.* 6) of forked trees is C1. Most of the tree groups have at least one forked tree except for B3 and C5.

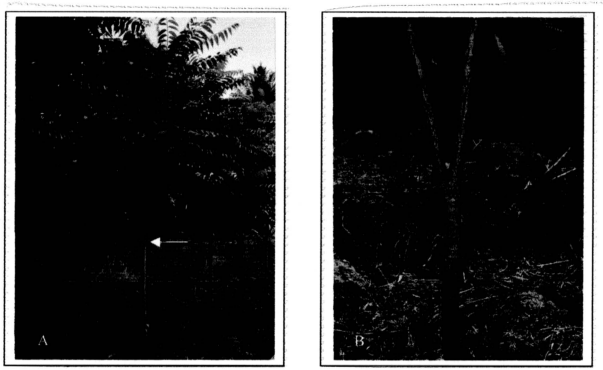


Plate 7 : (A) Arrow indicates forking of the tree at tree trunk (B) A close up view of the forked tree

Table 4.19 : Number of trees with forking habit in various progeny tree groups

Progeny tree group	Number of tree samples	Number of trees with forking habit	Ratio of fork : no fork
A1	91	3	1 : 30.3
A2	85	4	1 : 20.3
A3	98	2	1 : 49
A4	92	2	1 : 46
B1	72	2	1 : 36
B2	91	1	1 : 91
B3	98	0	-
B4	94	4	1 : 23.5
C1	93	6	1 : 15.5
C2	95	4	1 : 23.8
C3	96	1	1 : 96
C4	94	1	1 : 94
C5	79	0	-

4.6 Correlations

Phenotypic correlations were estimated among growth related traits (Table 4.20). Mother trees are poorly correlated with their respective progeny trees among all the growth related traits under study. However, when the progeny trees are grouped into various categories, the mean tree volume of the mother trees are highly correlated to the progeny trees (r^2 = 0.99). Significant ($p \leq 0.05$) correlations were observed between Dbh and total height (r^2 = 0.48), and total height and volume (r^2=0.536) among the progeny trees. As expected, phenotypic correlation between Dbh and tree volume is high (r^2 = 0.965), suggesting that Dbh can be used as an indicator of the relevant commercial trait *i.e.* final tree volume growth of the tree.

Table 4.20 : Correlations of growth related traits among different progeny groups (below diagonal) and categories (above diagonal). The values in diagonal are correlations between mother and different progeny groups, and categories (in parentheses)

	Dbh	Total height	Tree volume
Dbh	0.14 (0.92)	0.03	0.98
Total height	0.48**	0.03 (0.16)	0.00
Tree volume	0.96**	0.54**	0.08 (0.99)**

** correlations are significant at $p < 0.05$

Correlation was estimated between the size (*i.e.* volume) of the tree and the number of nodes, and canopy diameter. The result in Table 4.21 shows that tree volume of the progeny trees is poorly correlated to the number of nodes. However, significant ($p \leq 0.05$) correlations were observed between tree volume and canopy diameter ($r^2 = 0.38$) of the progeny trees.

Table 4.21 : Correlation between tree volume and indirect growth related traits among different progeny tree groups

	Number of nodes	Canopy diameter
Tree Volume	0.08	0.38**

** correlations are significant at $p < 0.05$

4.7 Variance component analysis

In this analysis, Dbh, total height and tree volume was used to estimate variations among progeny groups categorized according to sizes of their respective mother trees. All factors were assumed to be random for the estimation of variance components, so the selected phenotypic responses were assumed to have a common normal distribution with mean μ and a common variance. The model for the variance components of the phenotypic variance, $\sigma^2_{phenotypic}$, of either Dbh, total height or tree volume is represented as follows

$\sigma^2_{phenotypicy} = \sigma^2_e + \sigma^2_{ap} + \sigma^2_{bp}$

where σ^2_e is the component of error variance

σ^2_{ap} is among progeny groups variance

σ^2_{bp} is variance due to interaction effects between block and progeny groups

The expected mean square and sources of variance were represented in Table 4.22.

Table 4.22 : Expected mean square (EMS) and mean square (MS) values of various sources of variance

Sources of variance	Df	Expected MS	MS (Dbh)	MS ($x10^4$) (Total height)	MS ($x10^6$) (Tree volume)
Progeny	12	$\sigma^2_e+50\sigma^2_{bp}+100\sigma^2_{ap}$	22.996	10.44	652.3
BLK * Prog	12	$\sigma^2_e + 50\sigma^2_{bp}$	16.228	8.93	556.8
Error	a	σ^2_e	1.952	0.77	47.4

Where a = 1125 for MS (Dbh)
a = 1142 for MS (Total height)
a = 1124 for MS (Tree volume)

Table 4.23 : Variance components of selected growth related traits. Estimated variance components are in parentheses.

	Dbh	Total height (x 10^4)	Tree volume (x 10^6)
σ^2_{ap}	22.996 (0.068)	10.44 (0.02)	652.3 (1.0)
σ^2_{bp}	16.228 (0.286)	8.93 (0.16)	556.8 (10.2)
σ^2_e	1.952	0.77	47.4

Table 4.23 shows that major sources of variation of total variance components were contributed by the variance among progeny groups (σ_{ap}) and its interaction with block effect (σ_{bp}).

4.8 Heritability

Heritability was estimated as

$$h^2 = \sigma^2_{ap} / \sigma^2_{phenotypic}$$

For half-sib population

$$rh^2 = \sigma^2_{ap} / \sigma^2_{phenotypic}$$

where r is the coefficient of relationship of each tree with each other tree in the same progeny group. r is ¼ for strict half-sibs (Hartl, 1989). However, the outcrossing estimate did not support that the *A. excelsa* population is strictly half-sibs (Table 4.32). .Therefore r needed to be adjusted for inbreeding and a coefficient of 1/3 has been recommended.

Hence, heritability for half-sibs was estimated as

$$h^2 = 3 (\sigma^2_{ap} / \sigma^2_{phenotypic})$$

$$= 3 (\sigma^2_{ap} /(\sigma^2_e + \sigma^2_{ap} + \sigma^2_{bp}))$$

The estimated value of narrow-sense heritability for tree volume is 0.05. And the values of heritability for total height and Dbh were estimated to be 0.06 and 0.09 respectively.

4.9 Selection of plus trees

Plus trees from Replicate 1 and 2 were selected from 10% of the upper extreme distribution curves of Dbh, height and tree volume. Therefore, at this growth stage, a tree is considered to be a plus tree if it has achieved a total tree volume equal to or greater than 3.5 m^3. If selection is based on Dbh, then a tree with Dbh equal to or greater than 9 cm will be selected. While in terms of height, selection will be made on trees with heights equal to or greater than 0.8 m.

The occurrence of plus tree in terms of volume growth occurred most frequently in B1 progeny trees (Table 4.24). Trees with large Dbh also occurred most frequently in B1. When different tree categories are considered, A category is found to have the highest frequency of plus trees occurrence with at least one plus tree in each tree group. It was interesting to find that plus trees (in terms of volume) occurred more frequently in progeny tree groups (*e.g.* B1 and C5) with lower number of progeny trees correspond to the availability of fewer seeds from their respective mother trees.

Among the traits under study, the values of Dbh could be a useful criterion for the selection of plus trees. The reason being that Dbh is a relatively stable trait and easier to measure as compared to other traits such as height. In addition, Dbh is highly

correlated to tree volume (see Table 4.20) which means that selection for plus trees based on Dbh indirectly selected for trees with large tree volume. However, if the value of Dbh or tree volume is 5% below the selection criteria mentioned above, the value of other traits such as height will be taken into considerations when selecting plus trees if they are above the selection criteria.

Table 4.24 : Frequency of plus tree occurrence in various progeny tree group

Tree category	Number of trees	Frequency of occurrence of plus trees (%)		
		Dbh	Height	Tree Volume
A1	93	5.4	1.1	3.2
A2	92	7.6	3.3	5.4
A3	96	4.2	0	1
A4	93	10.8	0	5.4
A		**28**	**4.4**	**15**
B1	74	18.9	0	9.5
B2	92	3.3	0	2.2
B3	94	4.3	2.1	1.1
B4	90	0	0	0
B		**26.5**	**2.1**	**12.8**
C1	92	2.2	0	0
C2	96	2.1	0	0
C3	99	0	1	0
C4	97	2.1	0	1
C5	79	10.1	1.3	7.6
C		**16.5**	**2.3**	**8.6**

4.10 Molecular markers analysis

4.10.1 Estimation of chromosome number and DNA content

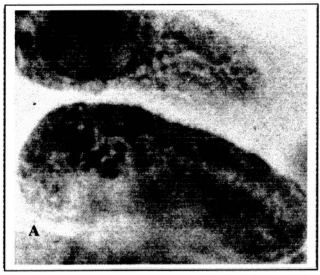
The pictures in Plate 8 show the appearance of chromosomes in a cell of *A. excelsa* root tip. From different planes of view, as shown by the three different pictures (A, B and C), the number of chromosomes was estimated to be 28. This is similar to the number recorded by Bowden (1945) in *Melia azedarach* (another name for *A. excelsa*). An *A. excelsa in vitro* plantlet was sent to Dr K.Arumuganathan in University of Nebraska for DNA content analysis using flow cytometry method. The DNA content was found to be 2.75×10^8 bp per haploid genome.

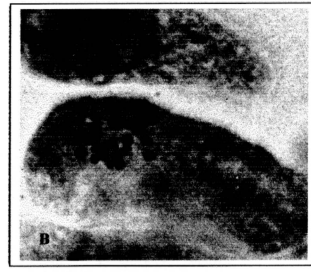
4.10.2 AFLP marker technology

AFLP marker analysis of *A. excelsa* DNA samples was carried out to provide more genetic information on the different progeny tree groups. A large number of AFLP polymorphic markers were generated to enable more in depth study on genetic variability among the progeny tree groups. Also with the dendrogram, better selection of plus trees for hybridisation can be carried out in future breeding programs.

4.10.2.1 Selection of primer pairs

DNA samples were extracted from leaf samples of *A. excelsa* trees collected from the field using a modified CTAB method as outlined in Appendix 2. The purity and concentration of each DNA sample was checked by electrophoresing each extracted DNA sample in a 1.2% agarose gel (Figure 4.13). All DNA samples were then digested





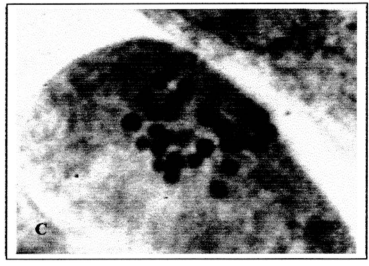


Plate 8 : Chromosomes in an *A. excelsa* cell of squash root tip. Pictures A & B, magnification : 10 x 100. Picture C, magnification : 100 x 100 (2N = 28)

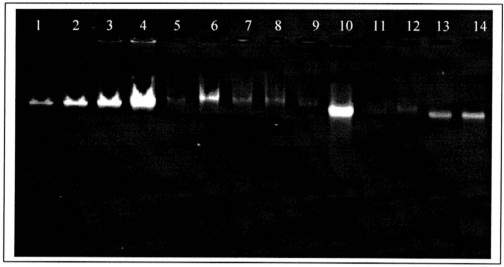


Figure 4.13 : Genomic DNA extracted from *A. excelsa* leaves. Lane 1: 25 ng λ DNA; Lane 2: 50 ng λ DNA; Lane 3: 100 ng λ DNA; Lane 4: 250 ng λ DNA; Lanes 5 to 14: DNA samples

with two restriction enzymes namely, *Eco*RI and *Mse*I. To ensure that the DNA was fully digested, all the digested DNA samples were checked in 1.2 % agarose gel. A continuous smear along the length of the gel indicated that the DNA sample had been fully digested (Figure 4.14). The digested DNA samples were then ligated with adaptors specific for the two restriction enzymes. Preamplification of all digested DNA fragments was carried out using *Eco*RI and *Mse*I-primers with single nucleotide as follows :

	CORE	ENZ	EXT
EcoR I	5'-GACTGCGTACC	AATTC	-3'
Mse I	5'-GATGAGTCCTGAG	TAA	-3'

Preamplification reaction was carried out to reduce the background smear in the fingerprinting pattern and to lower the level of mismatch amplification products in subsequent selective amplification reactions (refer to Vos P *et al.*, 1995). The preamplification reactions were checked by 1.2 % agarose gel elctrophoresis (Figure 4.15). The selective amplification reaction was then achieved through the use of *Eco*RI and *Mse*I primers which included three extra nucleotides at the 3' end of the primer sequence. Each selective amplification reaction was checked by 1.2% agarose gel electrophoresis before being loaded onto a polyacrylamide gel in a 377 sequencer (Figure 4.16).

A panel of 16 of the selective primers, consisting 8 each for *Eco*RI and *Mse*I primers (Appendix 3) were tested in various combinations resulting in 64 *Eco*RI-*Mse*I primer pair combinations. Primer pairs, which produced the highest number of clear

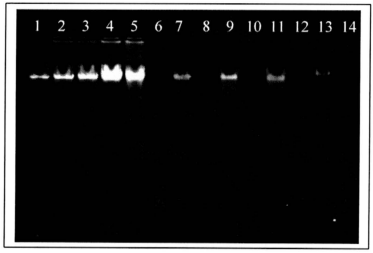


Figure 4.14 : Genomic DNA digested with *Eco*RI and *Mse*I. Lane 1: 25 ng λDNA; Lane 2: 50 ng λDNA; Lane 3: 100 ng λDNA; Lane 4: 250 ng λDNA; Lanes 5,7,9,11 and 13: DNA samples from *A. excelsa* leaves; Lanes 6,8,10,12 and 14: fully digested DNA samples of lanes 5,7,9,11 and 13 respectively

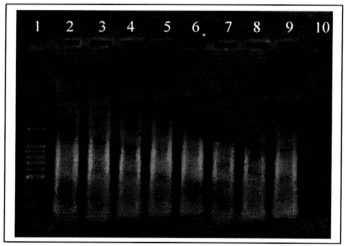


Figure 4.15 : Preamplification reactions. Lane 1: 100 bp DNA ladder; Lanes 2-9: preamplification reaction samples; Lane 10: a negative control with water instead of DNA template added

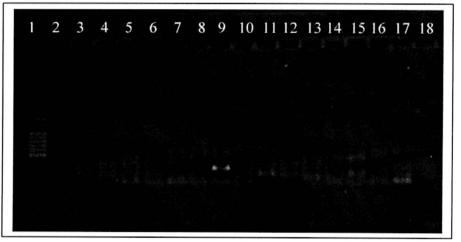


Figure 4.16 : Selective amplification reaction. Lane 1: 100 bp ladder; Lane 2 to 17 : selective amplification reaction samples. Lane 18 : a negative control with water instead of DNA template added

polymorphic bands, were selected for further studies. Figure 4.17 shows an AFLP gel profile with 64 primer-pair combinations using the A4 mother plant DNA as template. There were three colors (*i.e.* blue, green and yellow) available in the fluorescent labeled AFLP *Eco*RI primers to be used for the laser scanning. In order to optimise the number of gel runs in the 377 sequencer, a combination of 3 different colors was chosen per loading lane. This factor needed to be taken into consideration when selecting the *Eco*RI primer. From the GeneScan fragment analysis, the number of scorable bands for each primer-pair combination was recorded (Table 4.25). The shaded areas represent the primer-pair combinations which were selected. A high number of bands occurred when E-ACT or E-AGG primers were used. Other primer-pairs that produced a relatively large number of bands (e.g. E-ACA + M-CAT and E-AAG + M-CAG) were also selected so that fragments could be amplified from various parts of the genome.

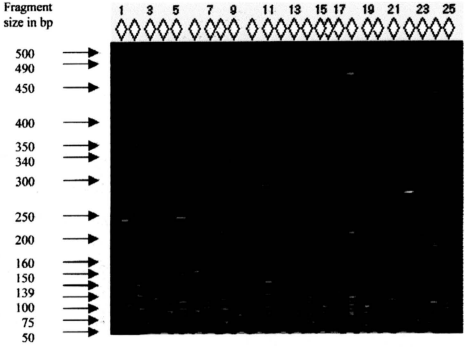


Figure 4.17 : An AFLP gel profile showing testing of 64 primer-pair combinations using A4 mother plant as DNA template

Table 4.25 : Number of bands obtained for each primer-pair combination

	E-ACT *B	E-ACG *G	E-AGG *G	E-ACA *B	E-AAG *G	E-AGC *Y	E-AAC *Y	E-ACC *Y
M-CAA	28	3	34	14	3	3	0	5
M-CAC	36	17	28	12	19	2	1	7
M-CAT	34	9	41	32	4	0	2	6
M-CTC	33	6	34	17	4	0	15	5
M-CTG	35	5	30	17	4	3	5	11
M-CTA	26	1	42	18	0	0	0	5
M-CTT	41	4	54	0	2	0	0	15
M-CAG	15	1	46	11	27	0	5	9

*B – Blue color fluorescent label
*G – Green color fluorescent label
*Y – Yellow color fluorescent label

The selected primer-pairs from Table 4.25 were screened for further selection based on the number of polymorphic bands obtained. Polymorphic bands were scored by comparing the fingerprinting pattern obtained using A1, A2, B3 and B4 mother plant DNA samples as template. An AFLP gel profile is shown in Figure 4.18. A total of six primer-pair combinations were selected as shown in Table 4.26.

Table 4.26 : Number of polymorphic bands scored with various selected primer-pair combinations

	E-ACT	E-AGG	E-ACA	E-AAG	E-AAC	E-ACC
M-CAC	16					
M-CAT		22	13			6
M-CTC					7	15
M-CTG	27					11
M-CTA		22	16			
M-CTT	20	22				8
M-CAG		28		6		

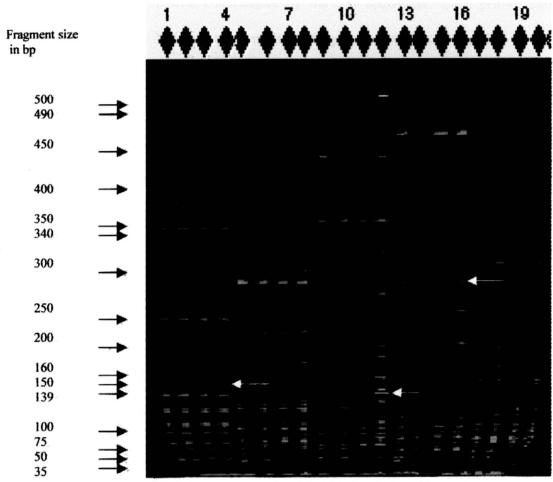


Figure 4.18 : An AFLP gel profile showing band pattern generated by amplification of 4 mother tree DNA template using various primer-pairs. The templates are Lanes 1, 5, 9, 13 and 17 : A1 ; Lanes 2, 6, 10, 14 and 18 : A2 ; Lanes 3, 7, 11, 15 and 19 : B3; Lanes 4, 8,12,16 and 20 : B4. Some of the polymorphic bands are shown by the white arrows.

4.10.3 Genetic variability

Six selected AFLP primer-pair combinations (Table 4.26) produced a total of 1250 scorable AFLP markers detectable by the 377 sequencer. Each primer combination produced about 200 scoreable amplified fragments between 50 and 500 bp in size. The sequence of each selected primer-pair is shown in Table 4.27.

The number of polymorphic fragments per primer pair combination obtained ranged from 16 to 60 which added up to a total of 204 markers. About 16.3% of AFLP fragments were polymorphic.

Table 4.27 : Primer names and sequence for six selective amplified fragment length polymorphism primer combinations.

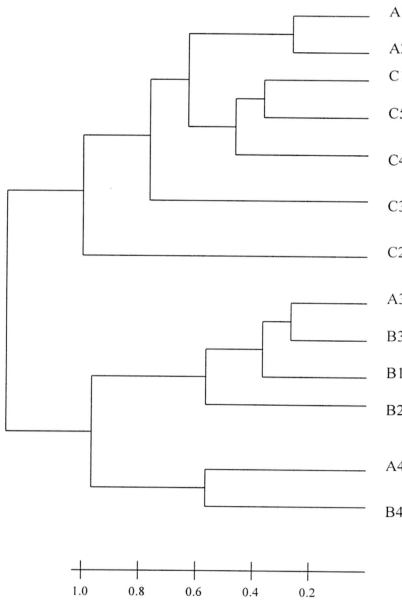
Primer pairs	Sequence
EcoRI + 3-AGG MseI + 3-CAT	5'-GACTGCGTACCAATTC/AGG-3' 5'-GATGAGTCCTGAGTAA/CAT-3'
EcoRI + 3-ACA MseI + 3-CTA	5'-GACTGCGTACCAATTC/ACA-3' 5'-GATGAGTCCTGAGTAA/CTA-3'
EcoRI + 3-ACC MseI + 3-CTC	5'-GACTGCGTACCAATTC/ACC-3' 5'-GATGAGTCCTGAGTAA/CTC-3'
EcoRI + 3-ACT MseI + 3-CTG	5'-GACTGCGTACCAATTC/ACT-3' 5'-GATGAGTCCTGAGTAA/CTG-3'
EcoRI + 3-ACC MseI + 3-CTG	5'-GACTGCGTACCAATTC/ACC-3' 5'-GATGAGTCCTGAGTAA/CTG-3'
EcoRI + 3-AGG MseI + 3-CAG	5'-GACTGCGTACCAATTC/AGG-3' 5'-GATGAGTCCTGAGTAA/CAG-3'

The relationship among mother trees was examined with a similarity matrix established based on the frequencies of shared fragments as shown in Table 4.28. The estimated values ranged from 0.35 between A4 and C2 to 0.91 between A1 and A2, and A3 and B3. As expected, since all mother trees originated from the same source, most of the values (approximately 67 %) obtained are above 0.60 indicating relatively high genetic homogeneity of the mother tree population. However, as the mother trees were planted from seeds, some genetic variabilities were observed. The dendrogram in Figure 4.19 shows the relationship among various mother trees. The trees from categories B and C were grouped into two clusters, which indicated that they were different from each other. Category A trees exhibited large differences and scattered between the two clusters.

Table 4.28 : Similarity matrix among mother trees based on Nei's estimate of similarity coefficient

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	C5
A1	1.00												
A2	0.91	1.00											
A3	0.79	0.77	1.00										
A4	0.73	0.71	0.75	1.00									
B1	0.73	0.71	0.82	0.71	1.00								
B2	0.61	0.61	0.78	0.62	0.85	1.00							
B3	0.76	0.74	0.91	0.74	0.90	0.82	1.00						
B4	0.66	0.66	0.72	0.79	0.65	0.56	0.69	1.00					
C1	0.83	0.88	0.71	0.63	0.68	0.56	0.69	0.59	1.00				
C2	0.53	0.61	0.45	0.35	0.40	0.40	0.41	0.38	0.68	1.00			
C3	0.65	0.68	0.49	0.46	0.52	0.42	0.51	0.41	0.77	0.62	1.00		
C4	0.76	0.78	0.64	0.56	0.58	0.49	0.59	0.49	0.85	0.67	0.84	1.00	
C5	0.76	0.82	0.64	0.56	0.62	0.54	0.62	0.56	0.89	0.74	0.76	0.83	1.00

Figure 4.19: Dendrogram of mother tree populations generated by Unweighted Pair Group Method using Arithmetic Average (UPGMA)



An examination of the similarity matrix values among the progeny trees (see Table 4.29) shows that all the estimated values are above 0.60 indicating that the progeny trees are closely related to one another. Higher similarity values were found among the progeny tree populations than among mother tree populations probably due to a high degree of selfing within the mother tree population. Further examination of the relationships among progeny trees with dendrogram (Figure 4.20) revealed that progeny trees in the same category group tend to cluster together. However, some progeny trees (example A3 and A2) were found to scatter in various clusters.

4.10.4 Estimation of outcrossing rate

Selfing or inbreeding in plants can be due to hybridization taking place within the same plant (*i.e.* between pollens and ovules from the same plant) for the monoecious species or crossing between closely related genotypes of plants within the same species. Since *A. excelsa* is a monoecious species and the flower is hermaphrodite, a measure of outcrossing rate is the proportion of progeny that do not perform selfing within the same plant.

As the primer-pair combination *Eco*RI + 3AGG and *Mse*I + 3CAG (for sequence of primers, refer to Table 4.27) produced the highest number of polymorphic bands, it was used in the estimation of outcrossing rate.

Outcrossing events can readily be identified if the maternal plant has a homozygous null genotype, *i.e.* they do not have the AFLP marker so that outcrossed offspring that have the dominant allele can be directly counted. However, when using dominant AFLP markers, the homozygous genotype for the dominant-allele "band presence" (+/+) and heterozygous genotype (+/-) cannot be differentiated from each other. Therefore for outcrossing estimation, the more informative markers are those where the dominant allele ("band presence") is at low frequency in the population, increasing the probability that the maternal plants are homozygous for "band absence".

Allele frequencies for the dominant "band-presence" allele and their standard errors were estimated by MLDT (multilocus estimation of outcrossing with dominant markers). A χ^2 statistic to test the conformity of marker loci to the mixed-mating model indicated that the number of observed progeny individuals for each genotype class from each maternal genotype departed from the expected numbers, occurred with sixteen AFLP markers (marker loci marked with *** in Table 4.30).

Maternal genotypes obtained from the AFLP assay were compared with potential maternal genotypes inferred based on the dominant marker-allele frequency in the progeny array (MLDT) (Table 4.31). Deviations of maternal genotypes obtained from AFLP assay to that inferred by MLDT were estimated. The variability was found to be ranging from 22% to 52%.

Table 4.30 : Estimates of AFLP marker allele frequencies (+), their respective standard deviations (σ) and χ^2 statistics for agreement with the mixed-mating model

AFLP marker locus	Frequency (+)	σ	χ^2
E3M8_52	0.034	0.043	4.17***
E3M8_71	0.092	0.056	0.55
E3M8_83	0.051	0.052	4.04***
E3M8_91	0.047	0.031	0.18***
E3M8_112	0.030	0.019	1.13***
E3M8_118	0.054	0.036	0.43
E3M8_126	0.273	0.058	1.02
E3M8_132	0.161	0.212	6.20***
E3M8_135	0.216	0.097	2.34
E3M8_145	0.037	0.026	0.00***
E3M8_152	0.037	0.024	0.00***
E3M8_158	0.111	0.037	0.17
E3M8_161	0.025	0.032	0.16***
E3M8_164	0.031	0.030	0.80***
E3M8_170	0.108	0.045	0.34***
E3M8_173	0.074	0.043	0.04***
E3M8_186	0.092	0.038	0.00
E3M8_202	0.074	0.128	6.76
E3M8_213	0.096	0.046	2.00***
E3M8_255	0.092	0.043	0.79
E3M8_267	0.325	0.065	0.49
E3M8_281	0.112	0.063	0.13***
E3M8_308	0.115	0.077	2.51
E3M8_331	0.048	0.039	1.46***
E3M8_336	0.114	0.032	1.73
E3M8_390	0.028	0.015	0.97***
E3M8_473	0.277	0.240	3.01***

*** Marker locus with significant deviation at the 0.01 level

Table 4.31: Maternal genotype for the 6 families determined by AFLP assay (in parentheses) or inferred by MLDT. Genotype 1: homozygous for "band presence" allele (+/+); genotype 2 : heterozygous (+/); genotype 3 : homozygous (-/-); genotype 0 : either genotype 1 or 2

AFLP Marker Locus	O.P. Family					
	1	2	3	4	5	6
E3M8_52	2 (3)	3 (3)	3 (0)	3 (3)	3 (3)	3 (3)
E3M8_71	3 (3)	3 (0)	3 (3)	2 (3)	3 (0)	3 (3)
E3M8_83	2 (0)	2 (0)	3 (0)	3 (3)	3 (3)	3 (3)
E3M8_91	3 (3)	3 (3)	3 (3)	3 (3)	2 (3)	2 (3)
E3M8_112	3 (3)	3 (3)	3 (0)	3 (3)	3 (0)	2 (3)
E3M8_118	3 (3)	1 (3)	2 (0)	2 (3)	3 (3)	1 (3)
E3M8_126	3 (3)	3 (3)	2 (0)	3 (3)	2 (3)	3 (0)
E3M8_132	3 (3)	3 (0)	3 (3)	3 (3)	3 (0)	3 (0)
E3M8_135	2 (0)	2 (0)	3 (0)	3 (3)	2 (3)	3 (0)
E3M8_145	3 (0)	2 (3)	2 (3)	2 (3)	3 (3)	3 (3)
E3M8_152	3 (3)	3 (3)	3 (3)	3 (3)	2 (3)	3 (3)
E3M8_158	3 (0)	3 (3)	3 (3)	3 (3)	2 (3)	3 (0)
E3M8_161	3 (3)	2 (3)	3 (3)	3 (0)	2 (3)	2 (3)
E3M8_164	3 (0)	2 (3)	2 (3)	3 (3)	2 (3)	2 (3)
E3M8_170	3 (3)	2 (3)	3 (3)	3 (3)	3 (3)	2 (0)
E3M8_173	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)
E3M8_186	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)
E3M8_202	3 (3)	3 (3)	3 (3)	3 (0)	3 (0)	3 (3)
E3M8_213	3 (3)	3 (3)	2 (3)	2 (0)	3 (3)	3 (0)
E3M8_255	3 (3)	2 (3)	3 (3)	3 (0)	3 (3)	3 (3)
E3M8_267	2 (0)	3 (3)	3 (3)	3 (3)	3 (3)	3 (0)
E3M8_281	3 (0)	3 (3)	3 (3)	3 (3)	3 (3)	3 (0)
E3M8_308	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)
E3M8_331	3 (0)	3 (3)	3 (3)	1 (3)	2 (3)	3 (3)
E3M8_336	3 (3)	3 (3)	3 (3)	2 (3)	3 (3)	1 (3)
E3M8_390	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)
E3M8_473	3 (3)	3 (0)	3 (0)	3 (3)	3 (3)	3 (0)
Deviation (%)	6/27 =22	9/27 = 33	9/27 = 33	8/27 = 30	12/27 = 44	14/27 = 52

The estimates of multilocus outcrossing rates (t_m) and single-locus outcrossing rates (t_s) obtained from MLDT clearly indicate that outcrossing is predominant in the *A. excelsa* open-pollinated breeding population. The estimation of multilocus outcrossing rate was around 81% which did not differ significantly from the single-locus estimates (Table 4.32).

Table 4.32 : Estimates of multilocus outcrossing rates (t_m) single-locus outcrossing rate (t_s) and fixation index (F)

$t_m \pm SE$	t_s	t_m-t_s	F
0.810± 0.086	0.685± 0.088	0.125± 0.033	0.034± 0.048

4.10.5 Assessment of molecular markers linked to morphological traits

Attempts were made to find molecular markers linked to the morphological traits recorded in section 4.4 and 4.5. Since *A. excelsa* population in the experimental plot is half-sib with no information on the paternal parent, finding the markers by segregation analysis was not possible. Moreover, AFLP markers are dominant which means that heterozygous loci could not be differentiated. However, an advantage of AFLP technology is that a large number of polymorphic markers can be generated which would increase the chances of finding the markers linked to the designated morphological trait. In this study, a strategy has been improvised which used a total of twenty-one selected primers (as listed in Table 4.34) to screen the selected *A. excelsa* DNA samples from the population.

4.10.5.1 Implementation of the proposed strategy

First, to maximize the chances of finding the marker(s), progeny tree group with the largest variance was selected for screening. Since correlation between Dbh and volume was found to be relatively high (see section 4.6), Dbh was selected as a morphological trait for marker linkage analysis. The calculated variances for various progeny tree groups in replicate 1 and 2 were presented in Table 4.33. Although C1 has the highest variance in replicate 1 and B2 has the highest variance in replicate 2, B2 was chosen as the tree group for screening because it has the overall largest average variance.

Table 4.33 : Variances of various progeny tree groups

Progeny tree group	Variance in Replicate 1	Variance in Replicate 2	Average variance
A1	0.845	2.533	1.689
A2	3.168	1.879	2.524
A3	1.431	1.377	1.404
A4	1.910	1.627	1.769
B1	1.827	4.200	3.014
B2	4.294	5.203	4.749
B3	0.991	1.707	1.349
B4	0.907	2.768	1.838
C1	4.391	3.000	3.696
C2	1.214	2.111	1.663
C3	0.968	1.667	1.318
C4	2.097	2.033	2.065
C5	1.896	2.274	2.085

To identify segregating markers, DNA samples from all the mother trees were screened with the twenty-one primer-pairs listed in Table 4.34. The rare markers occurring in B2 were selected *i.e.* bands that only found in B2, or B2 plus one other mother tree. A total of 103 rare markers were obtained. The seventeen primer-pairs that produced the rare markers in mother trees were then used for screening five random samples of B2 progeny trees (Table 4.34). If the selected rare markers from the first screening were found to be a rare marker in the second screening (*i.e.* the markers only found in one B2 sample or none at all), they were classified as segregating markers. These markers were then used for further screening of selected B2 progeny samples with eleven selected primer-pairs (Table 4.34). A total of 35 segregating markers were found.

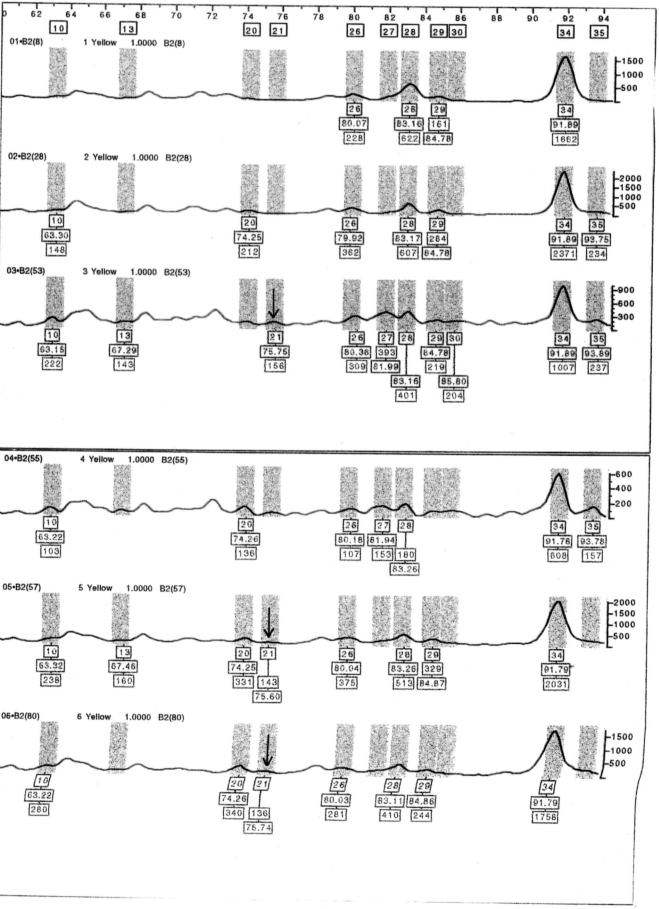
Table 4.34 : Number of segregating markers for B2 obtained by each primer-pair combination

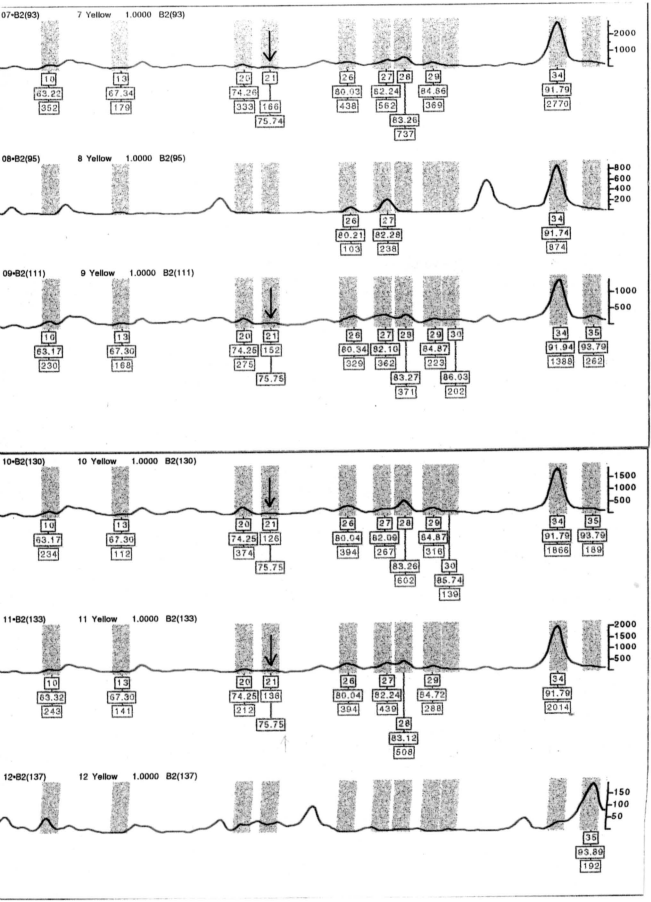
	Eco RI Primer	Mse I Primer	(a) Number of markers found rarely in mother tree population	(b) Number of segregating markers (from a) found rarely in progeny tree population
1	E-AAC	M-CTC	3	3
2	E-AAG	M-CTA	4	2
3	E-AAG	M-CAG	0	0
4	E-ACA	M-CAT	6	2
5	E-ACA	M-CTA	13	1
6	E-ACA	M-CTG	1	0
7	E-ACC	M-CTT	2	0
8	E-ACC	M-CTG	4	4
9	E-ACC	M-CTA	6	5
10	E-ACC	M-CTC	3	3
11	E-ACC	M-CAT	5	0
12	E-ACT	M-CAT	0	0
13	E-ACT	M-CTG	18	9
14	E-ACT	M-CAC	11	1
15	E-ACT	M-CTT	4	0
16	E-AGC	M-CTC	0	0
17	E-AGG	M-CTA	6	0
18	E-AGG	M-CAG	4	1
19	E-AGG	M-CTG	0	0
20	E-AGG	M-CTT	4	4
21	E-AGG	M-CAT	9	0
Total number of markers			103	35

- Sequence of E- is 5'-GACTGCGTACCAATTC-3'
  and M- is 5'-GATGAGTCCTGAGTAA-3'
- Shaded areas are primer-pairs that produced segregating markers

The segregating markers were used for screening B2 samples from extreme Dbh measurements with the eleven selected primer-pairs. Seven progeny trees with the largest Dbh and five progeny trees with the smallest Dbh from B2 tree group were selected for initial screening by AFLP assay. Band scoring was converted by the Genotyper software into binary code 1 (band presence) or 0 (band absent). Based on these data, putative markers that were present or absent in only one extreme phenotypic group of progeny trees were selected. Initial screening showed that one putative marker with a fragment length of approximately 75.5 bp amplified by primer-pair combination E-AAC and M-CTC was postulated to have linkage with Dbh. This marker was found to occur in all of the progeny trees with large Dbh but in none of the progeny trees with small Dbh (Figure 4.21). The presence of the marker was indicated by the peak shown by an arrow. Further screening using the same primer-pair combination was thus carried out to include a larger number of samples. However, the trend did not persist indicating that linkage disequilibrium was not established between the marker and the small Dbh trait.

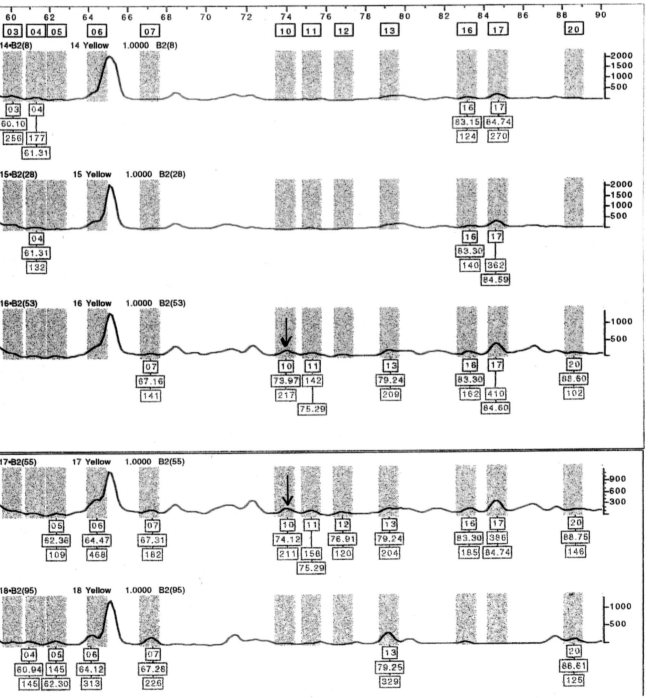
Figure 4.21 : Candidate molecular marker linked to Dbh using B2 DNA samples. Fragment size ~ 75.5 bp (see arrows and green boxes) occurs only in trees with large Dbh *i.e.* B2(53), B2(57), B2(80), B2(93), B2(111), B2(130) and B2(133); but not in trees with small Dbh *i.e.* B2(8), B2(28), B2(55), B2(95) and B2(137)




07-B2(93)
7 Yellow 1.0000 B2(93)
2000
1000
10
83.22
352
13
67.34
179
20
74.26
333
21
166
75.74
26
80.03
438
27
82.24
562
28
83.26
737
29
84.56
369
34
91.79
2770
08-B2(95)
8 Yellow 1.0000 B2(95)
800
600
400
200
26
80.21
103
27
82.28
238
34
91.74
974
09-B2(111)
9 Yellow 1.0000 B2(111)
1000
500
10
83.17
230
13
67.30
168
20
74.25
279
21
152
75.75
26
80.34
329
27
82.10
362
28
83.27
371
29
84.87
223
30
86.03
202
34
91.94
1388
35
93.79
262
10-B2(130)
10 Yellow 1.0000 B2(130)
1500
1000
500
10
83.17
234
13
67.30
112
20
74.25
374
21
126
75.75
26
80.04
394
27
82.09
267
28
83.26
602
29
84.87
316
30
85.74
139
34
91.79
1866
35
93.79
189
11-B2(133)
11 Yellow 1.0000 B2(133)
2000
1500
1000
500
10
83.32
243
13
67.30
141
20
74.25
212
21
138
75.75
26
80.04
394
27
82.24
439
28
83.12
508
29
84.72
288
34
91.79
2014
12-B2(137)
12 Yellow 1.0000 B2(137)
150
100
50
35
93.89
192

A small number of progeny trees were found to fork at the middle or lower level of the tree trunk. Since B2 has the highest number of forked trees (Table 4.19), it was also selected to screen for markers related to forking pattern of the tree. Based on the segregating markers, preliminary screening was carried out using the eleven selected primer-pairs (Table 4.34) on four unforked and three forked progeny tree DNA samples (Figure 4.22). One putative marker was found at approximately 74 bp when amplified by the same primer-pair (E-AAC and M-CTC). All the unforked progeny trees have the marker while there was none detected in the forked trees. Further screening of thirty B2 progeny samples included six forked progeny trees in B2 and twenty-four unforked trees were carried out. However, the 74 bp fragment did not amplify from some of the unforked trees (e.g. B2(13) and B2(14)) (Figure 4.23). The loss of the fragment could be due to recombination during meiosis indicating that the marker is not tightly linked to the forked trait.

Figure 4.22 : Candidate molecular marker linked to forking characteristics using B2 DNA samples. Fragment size ~ 74 bp (see arrows and green boxes) occurs only in unforked trees *i.e.* B2(53), B2(55), B2(130) and B2(137) but not in forked trees *i.e.* B2(8), B2(28) and B2(95)



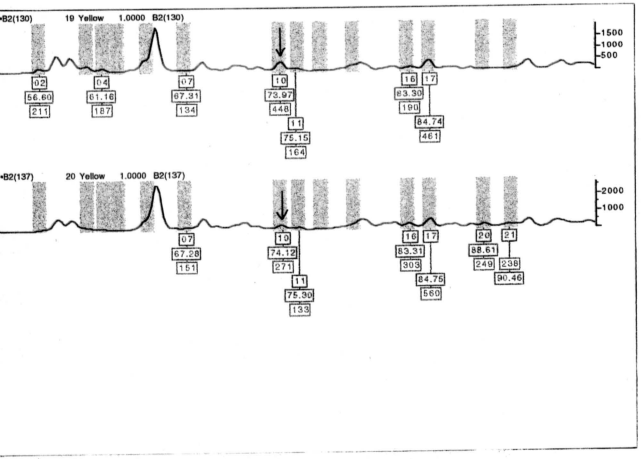
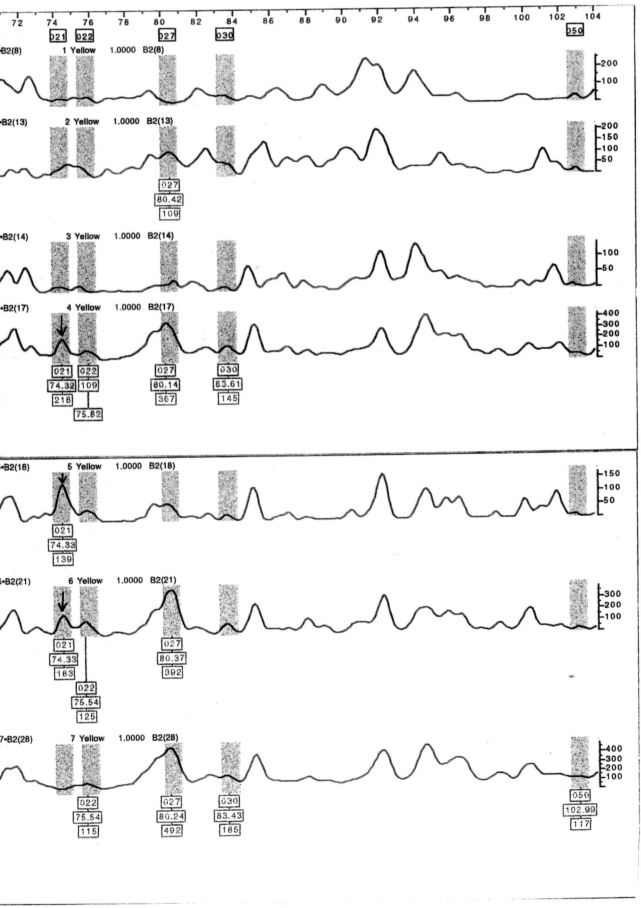
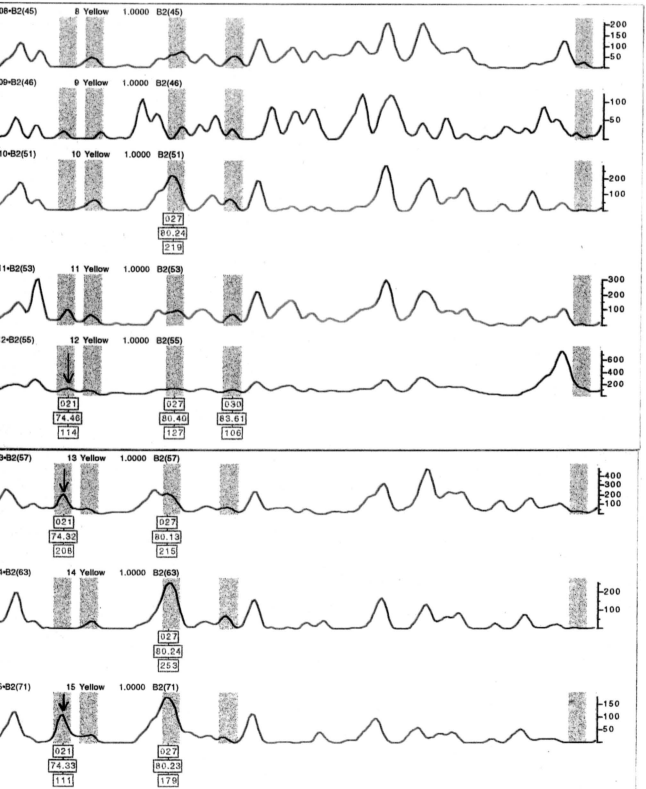
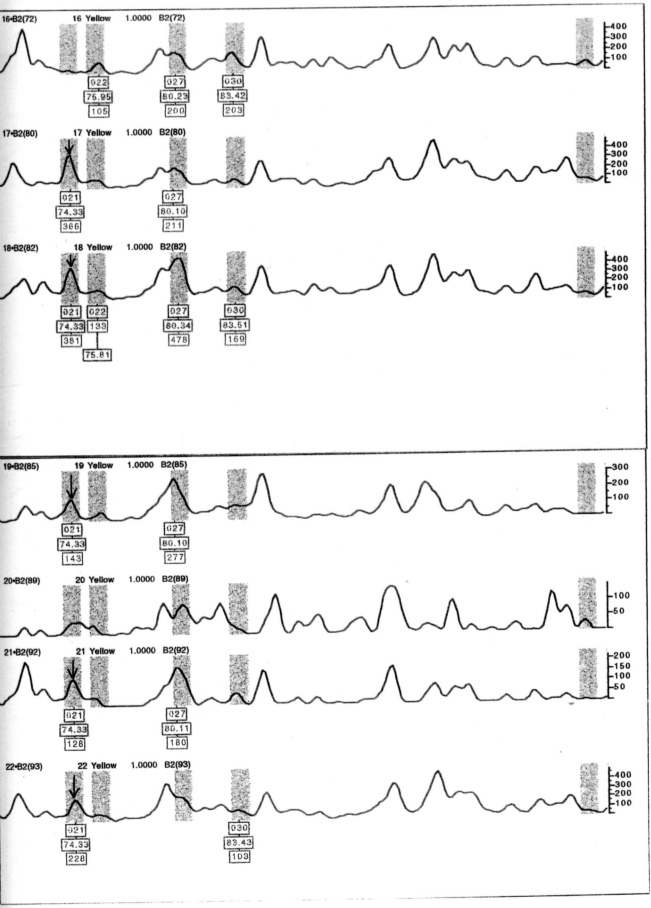
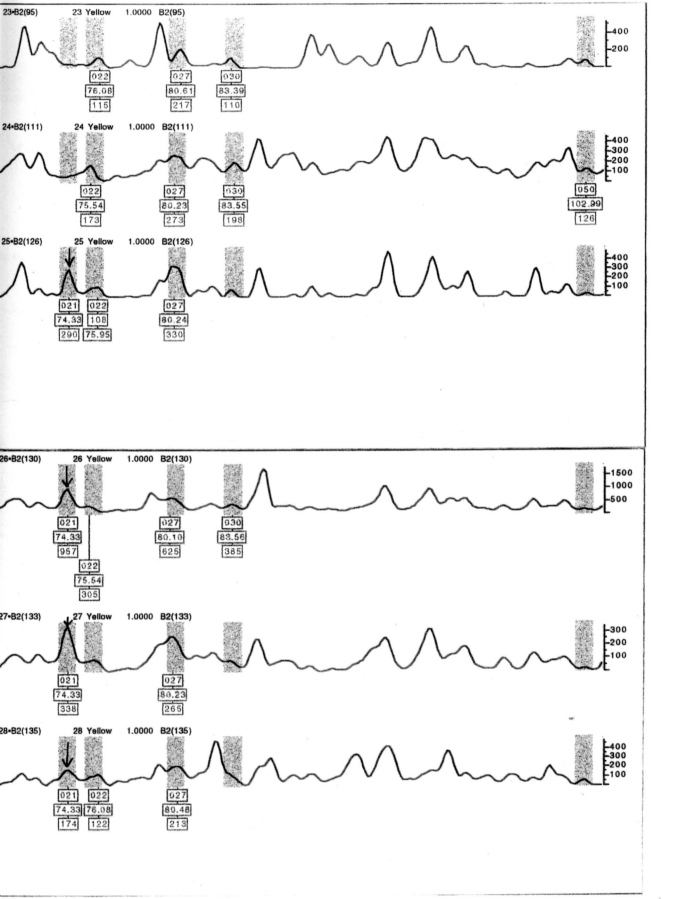

•B2(130)
10 Yellow 1.0000 B2(130)
1500
1000
500
02
56.60
211
04
61.16
187
07
67.31
134
10
73.97
448
11
75.15
164
16
83.30
190
17
84.74
461
•B2(137)
20 Yellow 1.0000 B2(137)
2000
1000
07
67.28
151
10
74.12
271
11
75.30
133
16
83.31
303
17
84.75
560
20
88.61
249
21
238
90.46

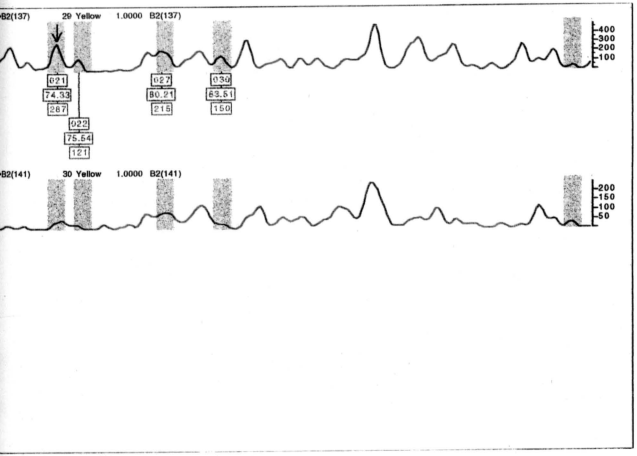
Figure 4.23 : Screening of 30 B2 DNA samples for fragment size ~ 74 bp (see arrows and green boxes). B2(8), B2(28), B2(72), B2(82), B2(89) and B2(95) – forked trees. The rest – unforked trees




08•B2(45)
8 Yellow 1.0000 B2(45)
200
150
100
50
09•B2(46)
9 Yellow 1.0000 B2(46)
100
50
10•B2(51)
10 Yellow 1.0000 B2(51)
200
100
027
80.24
219
11•B2(53)
11 Yellow 1.0000 B2(53)
300
200
100
12•B2(55)
12 Yellow 1.0000 B2(55)
600
400
200
021
74.46
114
027
80.40
127
030
83.61
106
13•B2(57)
13 Yellow 1.0000 B2(57)
400
300
200
100
021
74.32
208
027
80.13
215
14•B2(63)
14 Yellow 1.0000 B2(63)
200
100
027
80.24
253
15•B2(71)
15 Yellow 1.0000 B2(71)
150
100
50
021
74.33
111
027
80.23
179

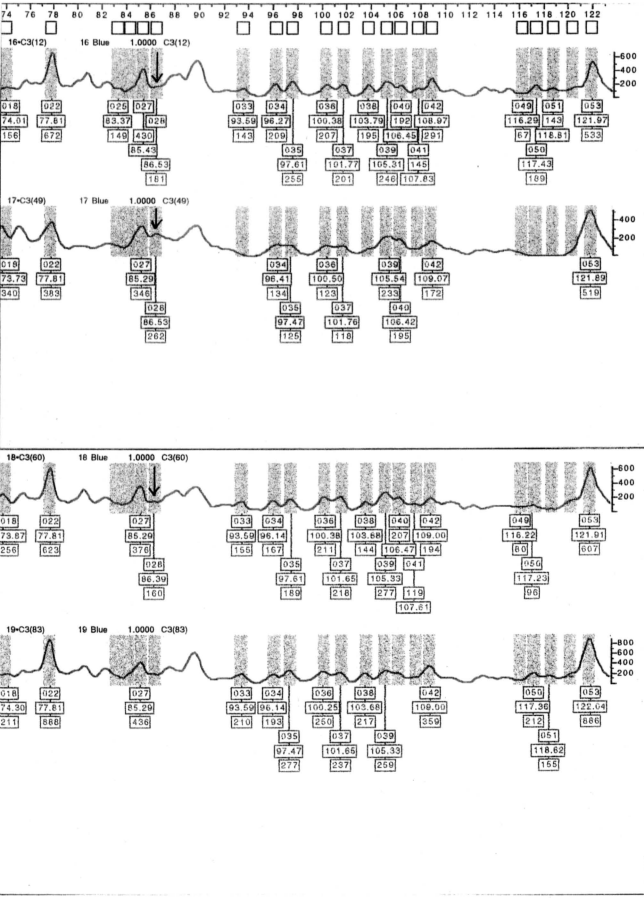

16-B2(72)
16 Yellow 1.0000 B2(72)
022
75.95
105
027
80.23
200
030
83.42
203
400
300
200
100
17-B2(80)
17 Yellow 1.0000 B2(80)
021
74.33
366
027
80.10
211
400
300
200
100
18-B2(82)
18 Yellow 1.0000 B2(82)
021
74.33
351
022
133
75.81
027
80.34
478
030
83.51
169
400
300
200
100
19-B2(85)
19 Yellow 1.0000 B2(85)
021
74.33
143
027
80.10
277
300
200
100
20-B2(89)
20 Yellow 1.0000 B2(89)
100
50
21-B2(92)
21 Yellow 1.0000 B2(92)
021
74.33
128
027
80.11
180
200
150
100
50
22-B2(93)
22 Yellow 1.0000 B2(93)
021
74.33
228
030
83.43
103
400
300
200
100

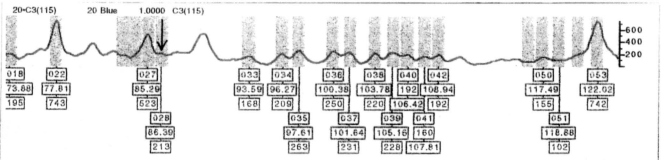

23-B2(95)
23 Yellow 1.0000 B2(95)
400
200
022
76.68
115
027
80.61
217
030
83.39
110
24-B2(111)
24 Yellow 1.0000 B2(111)
400
300
200
100
022
75.54
173
027
80.23
273
030
83.55
198
050
102.99
126
25-B2(126)
25 Yellow 1.0000 B2(126)
400
300
200
100
021
74.33
290
022
75.95
108
027
80.24
330
26-B2(130)
26 Yellow 1.0000 B2(130)
1500
1000
500
021
74.33
957
022
75.54
308
027
80.10
625
030
83.56
385
27-B2(133)
27 Yellow 1.0000 B2(133)
300
200
100
021
74.33
338
027
80.23
265
28-B2(135)
28 Yellow 1.0000 B2(135)
400
300
200
100
021
74.33
174
022
76.08
122
027
80.48
213

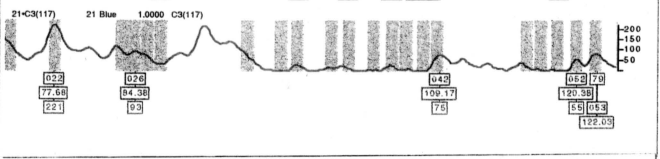

B2(137)
29 Yellow
1.0000 B2(137)
400
300
200
100
021
74.33
287
022
75.54
121
027
80.71
215
030
83.51
150
B2(141)
30 Yellow
1.0000 B2(141)
200
150
100
50

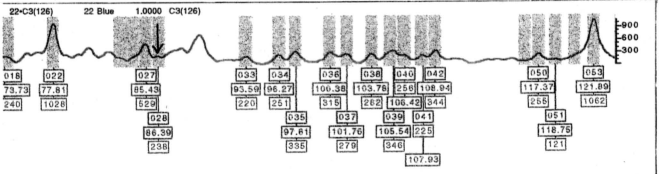
Another trait that was used for marker linkage analysis is the early branching characteristic. Since C3 has the largest number of progeny trees (*i.e.* 38 see Table 4.18) with early branching trait, it was selected for further study. Similar method was used to find segregating markers to screen the C3 progeny trees. A total of 27 segregating markers were obtained and twelve primer-pairs were selected (Table 4.35). Initial screening by the twelve selected primer-pairs was carried out on six progeny trees with early branching characteristics and four progeny trees that produced first whorl of branches at a later date. The result showed that an approximately 86.5 bp fragment amplified by primer-pair E-ACT and M-CAC appeared in all early branching trees (Figure 4.24). However, the fragment was absent in all samples of later branching trees except for sample C3(164). Further screening including seven other progeny tree DNA samples with early branching trait and four with non-early branching trees were carried out. The result shows that the putative marker (86.5 bp) was not consistently linked to the early branching trait (Figure 4.25).

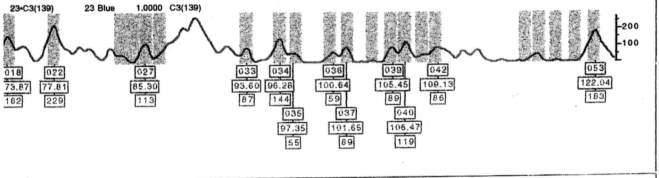
Figure 4.24 : Candidate molecular marker (fragment size ~ 86.5 bp – see arrows and green boxes) linked to early branching using C3 DNA samples. Early branching trees are C3(12), C3(49), C3(60), C3(115), C3(126) and C3(156) Non-early branching trees are C3(83), C3(117), C3(139) and C3(164)

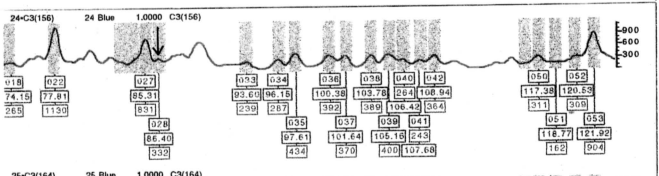



20•C3(115)
20 Blue 1.0000 C3(115)
600
400
200
018
73.88
195
022
77.81
743
027
85.29
523
028
86.39
213
033
93.59
168
034
96.27
209
035
97.61
263
036
100.38
250
037
101.64
251
038
103.78
220
039
105.18
228
040
106.42
192
041
107.81
160
042
108.94
192
050
117.49
155
051
118.88
102
053
122.92
742


21•C3(117)
21 Blue 1.0000 C3(117)
200
150
100
50
022
77.68
221
026
84.38
93
042
109.17
75
053
120.38
55
79
053
122.03


22•C3(126)
22 Blue 1.0000 C3(126)
900
600
300
018
79.73
240
022
77.81
1026
027
85.43
529
028
86.39
238
033
93.59
220
034
96.27
251
035
97.61
336
036
100.38
315
037
101.76
279
038
103.78
262
039
105.54
346
040
106.42
225
041
107.93
042
108.94
344
050
117.37
258
051
118.78
121
053
121.89
1062


23•C3(139)
23 Blue 1.0000 C3(139)
200
100
018
79.87
162
022
77.81
229
027
85.30
113
033
93.60
87
034
96.28
144
035
97.35
55
036
100.64
59
037
101.65
89
039
105.45
89
040
106.47
119
042
108.13
86
053
122.04
183


24•C3(156)
24 Blue 1.0000 C3(156)
900
600
300
018
74.15
265
022
77.81
1130
027
85.31
831
028
86.40
332
033
93.60
239
034
96.15
267
035
97.61
434
036
100.38
392
037
101.64
370
038
103.78
389
039
105.16
400
040
106.42
264
041
107.68
243
042
108.94
364
050
117.38
311
051
118.77
162
052
120.53
309
053
121.92
904

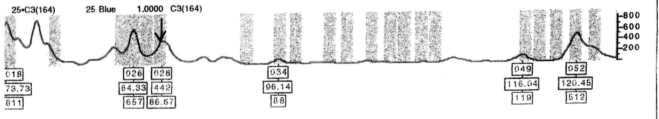
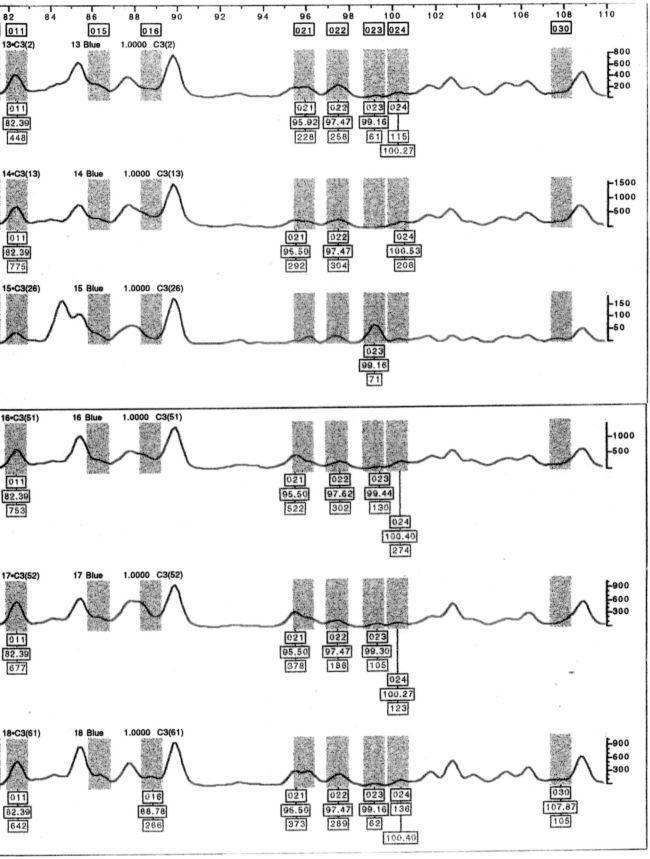
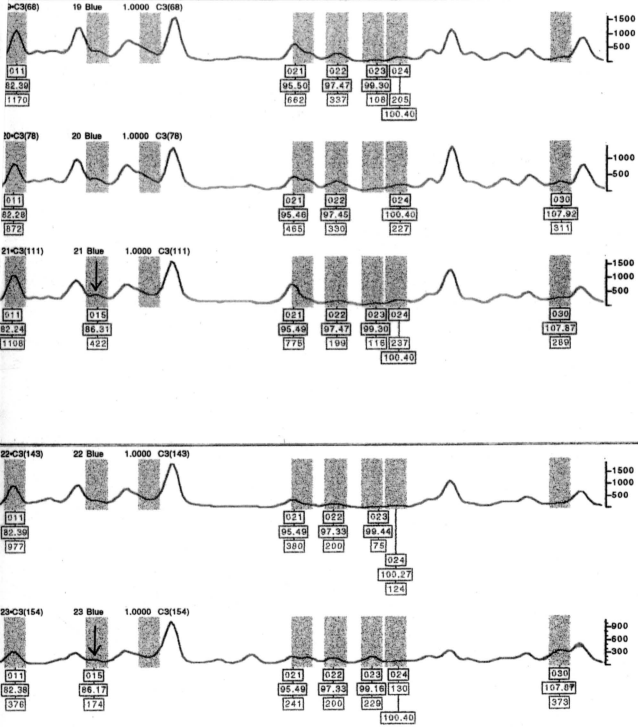

25•C3(164)
25 Blue 1.0000 C3(164)
800
600
400
200
018
75.73
611
026
84.39
657
028
86.67
442
034
96.14
88
049
116.04
119
052
120.45
512

Figure 4.25 : Screening of other C3 DNA samples. Early branching trees are C3(2), C3(13), C3(51),C3(61), C3(68), C3(143) and C3(154) Non-early branching trees are C3(26), C3(52), C3(78) and C3(111)

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1500
1000
500
011
82.39
1170
021
95.50
662
022
97.47
357
023
99.30
108
024
205
100.40
20 Blue 1.0000 C3(78)
1000
500
011
82.28
872
021
95.46
465
022
97.45
350
024
100.40
227
030
107.92
311
21 Blue 1.0000 C3(111)
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1000
500
011
82.24
1108
015
86.31
422
021
95.49
775
022
97.47
199
023
99.30
116
024
237
100.40
030
107.87
289
22 Blue 1.0000 C3(143)
1500
1000
500
011
82.39
977
021
95.49
380
022
97.33
200
023
99.44
75
024
100.27
124
23 Blue 1.0000 C3(154)
900
600
300
011
82.38
376
015
86.17
174
021
95.49
241
022
97.33
200
023
99.16
229
024
130
100.40
030
107.87
373

Table 4.35 : Number of segregating markers for C3 obtained by each primer-pair combination

	Eco RI Primer	Mse I Primer	(a) Number of markers found rarely in mother tree population	(b) Number of segregating markers (from a) found rarely in progeny tree population
1	E-AAC	M-CTC	3	1
2	E-AAG	M-CTA	4	0
3	E-AAG	M-CAG	0	0
4	E-ACA	M-CAT	6	2
5	E-ACA	M-CTA	13	4
6	E-ACA	M-CTG	1	0
7	E-ACC	M-CTT	2	1
8	E-ACC	M-CTG	4	0
9	E-ACC	M-CTA	6	2
10	E-ACC	M-CTC	3	0
11	E-ACC	M-CAT	5	2
12	E-ACT	M-CAT	0	0
13	E-ACT	M-CTG	18	2
14	E-ACT	M-CAC	11	3
15	E-ACT	M-CTT	4	1
16	E-AGC	M-CTC	0	0
17	E-AGG	M-CTA	6	2
18	E-AGG	M-CAG	4	0
19	E-AGG	M-CTG	0	0
20	E-AGG	M-CTT	4	4
21	E-AGG	M-CAT	9	3
Total number of markers			103	27

- Sequence of E- is 5'-GACTGCGTACCAATTC-3'
  and M- is 5'-GATGAGTCCTGAGTAA-3'
- Shaded areas are primer-pairs that produced segregating markers

4.11 Micropropagation

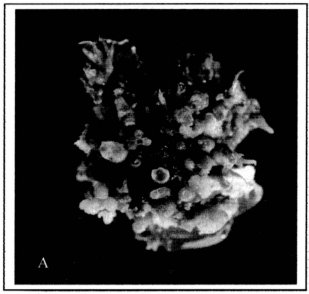
Clonal propagation provides the advantage of retaining the genetic fidelity of source plant. Plants with desirable morphological traits controlled by genetic factors can therefore be mass multiplied through micropropagation. This is an effective method of mass producing selected plus trees (section 4.10) in an *A. excelsa* improvement program. In this study, experiments have been carried out to induce shoot production from two sources of explant namely *in vitro* shoots and leaf cuttings. The shoots were cultured in MS medium supplemented with various concentrations of NAA or IBA to induce root formation. Trials were then carried out to wean out the successfully rooted shoots.

4.11.1 Introduction of axenic shoot tips

20% or four shoot-tip explants survived after sterilization treatment as outlined in section 3.3.3. The shoots were subcultured by nodal cuttings on MS medium supplemented with 2 mgL^{-1} BAP as recommended by Nor Aini Ab S. *et. al.* (1998). The multiplied shoots were used as a source of explants in the subsequent experiments.

4.11.2 Induction of buds and shoots from shoot explants

Shoot tips excised from *in vitro* shoot explants of *A. excelsa* were cultured onto MS medium supplemented with various concentrations of BAP and kinetin. Buds were formed directly from the base of the shoots after 2 weeks of culture (see Plate 9). Some buds developed into shoots.



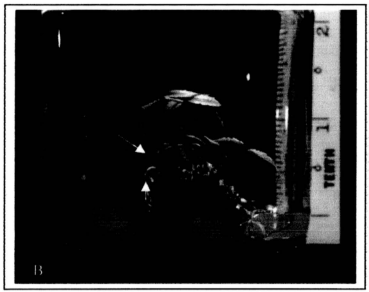


Plate 9 : (A) Shoots have been removed to expose the growth of buds at the base. (B) Arrows indicate some of the buds developed into shoots

Analysis of results shows that mean number of buds and shoots developed from *in vitro* shoot explants varied significantly between MS medium supplemented with various concentrations of BAP ($p<0.05$, $n = 20$) (Appendix 4). The highest mean number of buds was recorded in MS medium supplemented with 1 mgL^{-1} BAP. However, the number of shoots developed from the buds was significantly higher on MS medium supplemented with 0.5 mgL^{-1} BAP than other BAP concentrations (Table 4.36). There were no buds or shoots formed on the control medium (MS medium alone).

The mean number of buds and shoots formed in MS medium supplemented with kinetin were relatively low in all concentrations. The best performance was recorded in MS + 0.1 mgL^{-1} kinetin. (Table 4.36). Analysis of variance showed that there was no significantly different mean number of buds and shoots formed in MS medium supplemented with various concentrations of kinetin (Appendix 5). Higher mean number of buds and shoots were obtained in MS medium supplemented with BAP than with kinetin.

Table 4.36 : Shoots and buds development from shoots cultured on various concentrations of BAP and kinetin

Cytokinin	Concentration (mgL^{-1})	Mean number of buds ± s.d.	Mean number of shoots ± s.d.
BAP	0 (control)	0	0
	0.1	4.9 ± 3.4	1.1 ± 1.0
	0.2	4.9 ± 3.0	1.7 ± 1.0
	0.5	6.3 ± 2.4	2.1 ± 0.9
	1	7.8 ± 2.2	1.9 ± 1.0
	2	5.6 ± 3.2	1.7 ± 0.8
Kinetin	0 (control)	0	0
	0.1	2.1 ± 1.4	1.5 ± 0.9
	0.2	1.7 ± 1.0	1.4 ± 0.9
	1	1.4 ± 0.9	1.2 ± 0.7
	2	1.3 ± 1.2	1.2 ± 0.5
	5	1.3 ± 1.0	1.1 ± 0.6

4.11.3 *In vitro* culture of *A. excelsa* from leaf explant

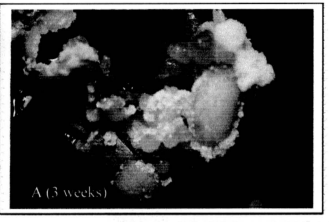
Previous work done by J. P. Eeswara *et. al.* (1998) on *Azadirachta indica* showed that shoots can be initiated from leaf explants. The procedure was modified for the micropropagation of *A. excelsa.*

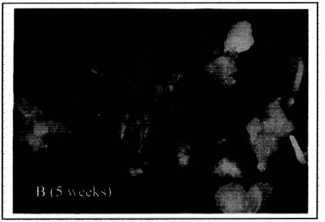
4.11.3.1 Induction of shoots from leaf explants

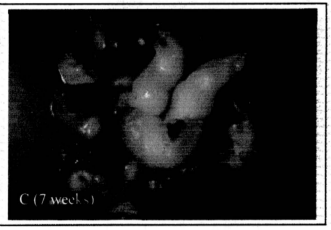
MS medium supplemented with various concentrations of BAP, kinetin and adenine sulphate were used to initiate shoots from leaf cuttings. The leaf cuttings were obtained from *in vitro* shoots. The mean number of leaves that induced callus formation and the mean number of shoots formed were recorded after 12 weeks of culture.

Formation of callus was observed on the cut edges of leaf pieces after 3 weeks of incubation in continuous darkness. The leaves curled but did not expand. The calli were friable and white in color then turned green upon exposure to light. Some of the calli grew into globular shaped structures that eventually became primordial shoots after 5 weeks of culture. (see Plate 10)

Analysis of results shows that a significantly higher mean number of shoots was formed on MS medium supplemented with 2 mgL^{-1} BAP, 1.2 mgL^{-1} kinetin and 6 mgL^{-1} adenine sulphate ($p<0.001$, $n=10$). An average of up to 5 primordial shoots per explant was obtained after 12 weeks of culture (Table 4.37). The highest mean number of leaves that formed callus was recorded at 80 % with the application of 1 mgL^{-1} BAP, 0.8 mgL^{-1} kinetin and 6 mgL^{-1} adenine sulphate.







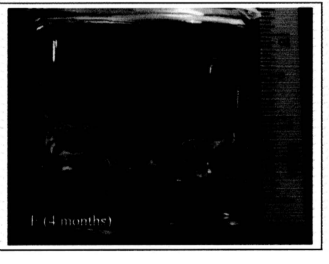


Plate 10 : Induction of shoots from leaf cuttings - (A) Development of callus on the edges of leaf cutting. (B) Some protruding structures start to form (C) Formation of primordial shoots (D) Formation of true leaves (E) Development of primordial shoots into shoots
Time from leaf culture is shown in parenthesis.

Table 4.37 : Callus and shoot formation from leaf explants cultured on various concentrations of BAP, kinetin and adenine sulphate (after 12 weeks of culture)

BAP (mgL^{-1})	Kinetin (mgL^{-1})	Adenine sulphate (mgL^{-1})	Mean number of leaves formed callus (%)	Mean number of primordial shoots formed ± s.d.
0.5	0.4	6	35	2.3± 1.2
0.5	0.4	8	50	2.5± 1.1
0.5	0.8	6	40	3.1± 1.4
0.5	0.8	8	50	4.0± 2.6
0.5	1.2	6	35	2.2± 0.8
0.5	1.2	8	35	2.5± 1.4
1	0.4	6	65	1.9± 1.2
1	0.4	8	35	3.3± 1.8
1	0.8	6	80	1.5± 0.7
1	0.8	8	50	2.0± 1.3
1	1.2	6	40	3.3± 1.4
1	1.2	8	60	2.4± 1.8
2	0.4	6	75	4.3± 2.3
2	0.4	8	40	2.2± 1.5
2	0.8	6	50	3.4± 1.6
2	0.8	8	45	2.7± 1.6
2	1.2	6	25	5.0± 2.5
2	1.2	8	40	2.3± 1.2
Control	0	0	0	0

4.11.3.2 Shoot development

The primordial shoots from 4.11.3.1 were transferred to MS medium supplemented with various concentrations of BAP for shoot development. Various concentrations of magnesium sulphate were tested because application of higher concentrations has been shown to produce better shoot growth in *A. excelsa* (Dr Jinil Malaji, personal communications).

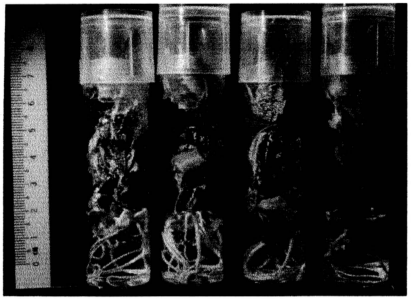
Overall, the mean number of primordial shoots which became shoots upon culture on various media was low. The highest mean number of shoots obtained was 2.5 at medium with 1 mgL^{-1} BAP and 12.5 mgL^{-1} magnesium sulphate (Table 4.38).

4.11.4 Effect of NAA and IBA on root induction

Experiments were set up to induce roots from shoots obtained in section 4.11.3.1 and 4.11.3.2 (Plate 11). Analysis of results shows that the percentage of shoots rooted is not significant with the application of different concentrations of NAA or IBA. The highest percent of shoots formed roots on the medium supplemented with 10 mgL^{-1} BAP after 4 weeks. Higher percentage of shoots formed roots earlier on the medium supplemented with NAA than with IBA (Figure 4.26).

Table 4.38 : Shoots developed from primordial shoots cultured on various concentrations of BAP and magnesium sulphate

BAP (mgL^{-1})	MgSO4.7H2O (mgL^{-1})	Mean number of shoots developed ± s.d.
0	0	0
0	5	0
0	12.5	0
0	20	0
0.5	0	0.5± 0.7
0.5	5	0
0.5	12.5	1.5± 2.1
0.5	20	1.0± 1.4
1	0	0.5± 0.7
1	5	1.0± 1.4
1	12.5	2.5± 0.7
1	20	2.0± 1.4
2	0	0.5± 0.7
2	5	0.5± 0.7
2	12.5	2.0± 1.4
2	20	1.5± 0.7
5	0	1.5± 2.1
5	5	0
5	12.5	1.0± 1.4
5	20	0

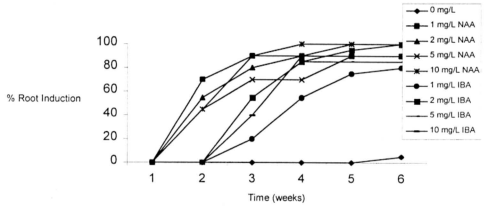


IBA (mgL^{-1}) 10 5 2 1

NAA (mgL^{-1}) 10 5 2 1

Plate 11 : Comparison of different concentrations of IBA and NAA on root induction after 6 weeks of growth

Figure 4.26: Roots formation with the application of NAA or IBA



While the number of roots produced is not significant between different concentrations of NAA (Appendix 6), the results indicate that low concentration of NAA (1 mgL^{-1}) can induce high number of roots production (Table 4.39). For MS medium supplemented with IBA, results of analysis show that higher concentrations of IBA produced significantly higher numbers of roots ($p<0.001$, $n=10$) (Appendix 6). Higher numbers of roots were produced on media supplemented with NAA than IBA. No roots were produced on control medium (MS medium alone).

Table 4.39 : Mean number of roots produced from *in vitro* shoots cultured on various concentrations of NAA or IBA

Auxins	Concentrations (mgL^{-1})	Mean number of roots ± s.d.
NAA	1	9.5 ± 2.6
	2	9.5 ± 4.4
	5	11.1 ± 4.1
	10	10.2 ± 3.9
IBA	1	3.0 ± 2.2
	2	6.4 ± 2.8
	5	7.7 ± 3.9
	10	9.3 ± 1.5
control	0	0

4.11.5 Weaning of plantlets

Rooted plantlets were taken out from tissue culture vessels. The roots were washed under running tap water to remove traces of agar. The plantlets were then planted in a potting tray half filled with wet crushed Jiffy material and placed in a 30 °C growth chamber.

Plate 12 shows a rooted plantlet ready to be weaned out in a potting tray. Observations showed that 100 % of the rooted plantlets survived by the end of first month. All of them had developed a healthy young shoot.

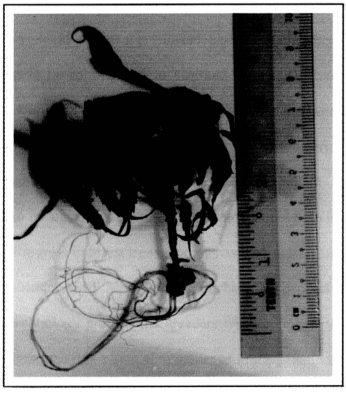


Plate 12 : Formation of *in vitro* plantlet ready for weaning