

## Discussion

Our analyses indicate that both location and location  $\times$  clone effects contribute little to the variation in fifth-year height observed in our trials. General conclusions cannot be drawn from the above results, as our two sites were separated by only 40 km and were visually quite similar. However, our results are encouraging in that our intuitive concept of site similarity seems biologically justified. Also, our results support the conclusion of SHELBOURNE (1974); clones are reasonably well characterized by measurements on few ramets.

Omission of cuttings damaged during any phase of field testing from further analyses is strongly supported by our results. Justification for such an omission is obtained from the substantial reduction in location, location  $\times$  clone and error components of variance, and from the increases in clone and clone-mean heritability that result from analyzing only undamaged cuttings. Resultant clone-mean heritability is the ultimate criterion for deciding whether to include or omit damaged plants, as it is linearly related to genetic gain. For our trials, omission of all damaged cuttings results in an 18% improvement in clone-mean heritability (and thus for gain) over the case in which all cuttings are included. The above recommendation should not be accepted uncritically for all trials, as clone-mean heritability depends both on the variance introduced by damaged plants and on the number of observations (ramets) per clone remaining after omission of damaged cuttings. When damage introduces little variation, or is so widespread as to eliminate most of the available observations, omission may not be justified. Further, when damage is genetically correlated with the trait of interest (i.e. frost damage and height), elimination of damaged trees may bias selection.

As indicated above, the observed reduction in the location  $\times$  clone component of variance due to omission of damaged cuttings creates a dilemma: the biological factors

that cause such an interaction appear to be associated with damage. If location  $\times$  clone component of variance due to omission of damaged cuttings creates a dilemma: the biological factors that cause such an interaction appear to be associated with damage. If location  $\times$  clone variation is caused by rank changes across locations, omission of damaged cuttings may bias selections. For our case, analysis of fifth-year height transformed to a log (10) scale gave essentially the same results as for untransformed values, with the important exception that interaction variance was no longer associated with damage classification. This result suggests that location  $\times$  clone interactions result from changes in variance or scale across locations, rather than changes in rank.

Regardless of the source of the interactions in our study, interaction variance is a small proportion of the total; and omission of damaged cuttings is probably justified. Our results indicate opportunities for improvement of clonal selection methods and demonstrate the value of early damage assessment as a tool in Norway spruce clonal selection programs.

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# Inheritance and Gain in a Half-Diallel Cross Among Loblolly Pines Selected for Resistance to Fusiform Rust

By E. R. SLUDER

Research Plant Geneticist, Southeastern Forest Experiment  
Station, Macon, Georgia 31020, U.S.A.<sup>1)</sup>

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## Summary

Ten seed orchard clones of loblolly pine (*Pinus taeda* L.) were selected for resistance to fusiform rust based on progeny test performance. These 10 clones were crossed in a half-diallel and the resulting progenies planted in an area of high rust hazard. Inheritance and genetic gains were estimated at age 10. In the field test, a standard check lot and a seed orchard bulk check lot each averaged 1.5% rust-free. The 45 progenies in the half-diallel averaged 15.8% rust-free. Five of the 10 clones had good breeding values for rust resistance. The 10 crosses among these five clones averaged 35.8% rust-free. Family heritability of rust-

free percentage was 0.69. The two stages of selection done—selecting 10 clones then the best five of them—produced a genetic gain in rust-free percentage of 28.1. Gains in other traits were not large but greater gains in them should occur when individuals within families are selected. Selection and breeding should be quite effective in developing fusiform-rust resistance in loblolly pine.

**Key words:** *Pinus taeda*, *Cronartium quercuum* f. sp. *fusiforme*, breeding.

## Zusammenfassung

Zehn Samenplantagen-Klone von *Pinus taeda* L. wurden auf der Grundlage ihrer Leistung in einer Nachkommen-schaftsprüfung als resistent gegen *Cronartium quercuum*

<sup>1)</sup> In cooperation with Georgia Forestry Commission, Macon, Ga. and Georgia Kraft Company, Rome, Ga.

f. sp. *fusiforme* selektiert. Diese zehn Klone wurden in einem halben Diallel gekreuzt und die daraus resultierenden Nachkommenschaften wurden in einem Gebiet mit hohem Rostisiko ausgepflanzt. Vererbung und genetische Gewinne wurden im Alter 10 geschätzt. Im Feldversuch waren eine Standard- und eine Samenplantagenmischprobe im Mittel zu jeweils 1,5% rostfrei. Die 45 Nachkommenschaften im Halbdiallel zeigten zum 15,8% Rostfreiheit. Fünf der zehn Klone hatten gute Züchtungswerte für Rostresistenz. Die zehn Kreuzungen zwischen diesen 5 Klonen waren im Mittel zu 35,8% rostfrei. Die Familienheritabilität der rostfreien Anteile betrug 0,69. Die zwei Stadien der durchgeführten Selektion — Selektion der 10 Klone, dann der 5 besten davon — führten zu einem genetischen Gewinn beim rostfreien Anteil von 28,1. Gewinne bei anderen Merkmalen waren nicht groß, aber größere Gewinne könnten bei ihnen erzielt werden, wenn Einzelbäume innerhalb der Familien selektiert würden. Selektion und Züchtung wären durchaus ganz wirkungsvoll, um *Cronartium fusiforme*-Resistenz bei *Pinus taeda* L. zu entwickeln.

### Introduction

The loblolly (*Pinus taeda* L.) and slash (*P. elliottii* ENGELM. var. *elliottii*) pine forests of the southern United States are being converted to plantations as the natural stands are harvested. The incidence of fusiform rust (*Cronartium quercuum* (BERK.) MIYABE ex SHIRAI f. sp. *fusiforme*) has persistently increased in those plantations relative to natural stands of both pine species over approximately the last 30 years. Severe economic losses to the fungus are occurring (CZABATOR 1971, SCHMIDT *et al.* 1974, PHELPS 1974, PHELPS and CHELLEMAN 1975, POWERS *et al.* 1974, 1975; HOLLIS and SCHMIDT 1977; SLUDER 1977a, DINUS and SCHMIDT 1977).

Strategies for reduction of losses from fusiform rust in pine plantations have been formulated (DINUS and SCHMIDT 1977). Some recommended practices are to protect seedlings in the nursery with systematic fungicides and to treat planting stock with systemics to protect seedlings during the critical first 1 or 2 years in the field (KELLEY 1980, 1985; ROWAN 1982a, 1984; SKOLLER *et al.* 1983). Such treatments, however, leave the young trees vulnerable to infection for a few succeeding years, and no fungicide has yet been found effective for eradicating galls on trees (ROWAN 1982b).

An alternative practice for reducing the impact of fusiform rust in pine plantations is to use genetically resistant species or resistant varieties of susceptible species as planting stock. Slash and loblolly pines vary in resistance both among and within geographic sources (BLAIR 1970, KINLOCH and STONECYPHER 1969, KRAUS 1967, GRIGGS and WALINSHAW 1982, GODDARD *et al.* 1975, SKOLLER *et al.* 1983, SLUDER 1980, WELLS and SWITZER 1971, WELLS and WAKELY 1966). Resistance to fusiform rust should therefore be strongly emphasized in tree improvement programs with these two species.

Many of the 133 clones in the Georgia Forestry Commission's loblolly pine seed orchards have been progeny tested in central Georgia where the incidence of fusiform rust in plantations is very high, giving a rigorous test for resistance (PHELPS 1974, SOHN and GODDARD 1979). Fifth-year rust incidence data from some of the progeny tests were used to select 10 clones whose progenies had above-average resistance to infection. To study inheritance of the rust resistance trait and to evaluate the 10 clones for use in developing a rust-resistant strain of loblolly pine, the clones were crossed in all possible combinations except selfs and reciprocals (half-diallel cross). This report presents the 10-year results from that half-diallel cross. Five-year results were reported earlier (SLUDER 1981).

Table 1. — Loblolly pine clones used in the study and the superiorities of their progenies in rust resistance at age 5 in the original progeny-test plantations. <sup>a</sup> /

Clone number	G.F.C. serial number	Progeny superiority	
		Percent	SD
1	617	75.8	2.79
2	518	39.2	1.13
3	541	71.9	1.51
4	600	67.2	1.22
5	603	74.1	.96
6	520	50.2	3.45
7	542	50.9	1.46
8	578	80.5	5.78
9	566	47.9	1.93
10	582	80.9	2.34
Mean		63.9	1.85

G.F.C. = Georgia Forestry Commission.

SD = Standard deviations.

<sup>a</sup>/ The resistance trait was the percentage of rust-free trees for four plantations and the number of galls per tree for two plantations.

### Materials and Methods

Data from six progeny-test plantations were used to select the 10 clones used in the study. In these plantations, progenies of the selected clones averaged 1.85 standard deviations above their respective plantation means in rust resistance, for a mean superiority of 64% (Table 1). These progenies were in the upper 10%.

The clones were arranged in a half-diallel cross according to flowering phenology (SLUDER 1977b). Clone number 1 flowered the earliest and clone number 10 flowered the latest. Pollen from the earlier flowering clones was used to pollinate the later flowering ones. In this way, all 45 crosses were made in one season without using stored pollen. Pollinations were made in 1972.

Seeds collected in 1973 were stratified and planted in 7.6-cm square peat pots in a greenhouse. Five replications were used in the greenhouse, four of which were transferred to the field in June 1974. The planting site was a disked field in Houston County, Georgia. The field planting design had four blocks with 16-tree square randomized plots and a spacing of 2.5 by 2.5 m. Included in each block were the 45 crosses and three check lots. The check lots were a standard Georgia Crop Improvement Association (GCIA) lot, a bulk seed orchard lot, and a commercial collection lot, all started in the greenhouse along with the 45 crosses. Seedling survival at age 3 years averaged 88.7%.

Traits assessed at age 10 years were survival, height, d.b.h., volume per tree, percentage rust-free (cumulative to age 10), and stem straightness. Plot mean data were subjected to standard randomized block and to diallel analysis of variance. Individual tree data were subjected only to the diallel analysis (Table 2). General combining abilities (GCA) and breeding values (twice the GCA plus the diallel general

Table 2. — Expected mean squares for the diallel analysis of variance, individual tree basis.

Source	df	EMS <sup>a</sup>
Rep (R)	3	$\sigma_w^2 + 11.58\sigma_p^2 + 0.57\sigma_{sca}^2 + 1.09\sigma_{gca}^2 + 480.56\sigma_p^2$
Crosses (C)	44	$\sigma_w^2 + 11.16\sigma_p^2 + 42.70\sigma_c^2$
GCA	9	$\sigma_w^2 + 11.62\sigma_p^2 + 44.35\sigma_{sca}^2 + 341.51\sigma_{gca}^2$
SCA	35	$\sigma_w^2 + 11.07\sigma_p^2 + 42.27\sigma_{sca}^2$
RxC (plot)	132	$\sigma_w^2 + 10.51\sigma_p^2$
Within plot	1745	$\sigma_w^2$

<sup>a</sup>/ A computer procedure known as SAS, Type I, was used to estimate EMS coefficients for this analysis of unbalanced data.

mean) of the clones were calculated. Variance components were calculated and used to estimate family and individual tree heritabilities.

Heritability equations were:

$$h^2_f = \sigma^2_{gca} \div (\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_e)$$

$$h^2_i = 4\sigma^2_{gca} \div (\sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_p + \sigma^2_w)$$

$h^2_f$  = family heritability, with variance components from plot mean data

$h^2_i$  = individual tree heritability, variance components from individual tree data

$\sigma^2_{gca}$  = general combining ability variance

$\sigma^2_{sca}$  = specific combining ability variance

$\sigma^2_e$  = error variance, plot means data

$\sigma^2_p$  = plot variance, individual tree data

$\sigma^2_w$  = within plot variance, individual tree data

The genetic correlation between mean height and rust-free percentage at age 10 was calculated as follows:

$$r_{g,XY} = \text{Cov}_{gca,XY} \div \sqrt{(\sigma^2_{gca}X)(\sigma^2_{gca}Y)}$$

where:

$r_{g,XY}$  = genetic correlation between X (mean height) and Y (rust-free) percentage

$\text{Cov}_{gca,XY}$  = covariance of the general combining abilities of X and Y

$\sigma^2_{gca}X$  = general combining ability variance of X

$\sigma^2_{gca}Y$  = general combining ability variance of Y

### Results

Standard analysis of variance of plot mean data showed highly significant (0.01) differences among the 45 crosses and three check lots in height, volume per tree, and rust-free percentage, and significant (0.05) differences in d.b.h. Differences were nonsignificant for survival and stem straightness index. Among the 48 seed lots, ranges of the data were: survival, 53 to 89%; height, 5.73 to 8.11 m; d.b.h., 10.5 to 14.6 cm; tree volume, 0.021 to 0.048 m<sup>3</sup>; rust-free 0 to 51%; and stem straightness index, 2.36 to 3.55. The diallel mean was better than the GCIA check lot in all traits except stem straightness (Table 3).

In the diallel analyses, plot mean data from the 45 crosses showed highly significant variation in general combining abilities (GCA) of the parents in all traits except survival (Table 4). Variation in specific combining abilities (SCA) was nonsignificant for all traits assessed. With individual tree data, GCA variation was highly significant for height, d.b.h., and volume per tree and significant for stem straightness but, as with plot mean data, all SCA effects were nonsignificant. The replication  $\times$  crosses interaction was highly significant for the four traits assessed on an individual tree basis. Variance components for SCA were considerably less than those for GCA for all traits except survival.

Breeding values of the 10 clones varied from less than zero to 55 for percentage rust-free to age 10 (Table 5). Breeding values for survival were not calculated because the GCA variance component for that trait was zero, leav-

Table 3. — Tenth-year means for the check lots and the diallel.

Lot	Traits					
	Survival	Height	D.b.h.	Volume	Rust-free	Stem straightness <sup>a/</sup>
	percent	m	cm	m <sup>3</sup>	percent	
Orchard	53.1	7.15	12.4	0.036	1.5	2.36
GCIA	68.7	6.84	11.6	.028	1.5	3.08
Commercial	57.8	6.64	11.2	.027	8.3	2.43
Diallel mean	71.6	7.12	12.3	.034	15.8	2.94

<sup>a/</sup> An index varying from 1 (straight) to 6 (most crooked).

Table 4. — Mean squares and significance tests for the diallel analysis of plot mean and individual tree data.

Source	Trait					
	Survival	Height	D.b.h.	Vol./tree $\times 10^2$	Rust-free	Stem straightness <sup>a/</sup>
	Plot mean					
Replication	486.08*	8.95**	13.70**	0.0877**	155.81NS	2.68**
GCA	137.36NS	2.80**	5.71**	.0270**	3024.33**	.55**
SCA	170.91NS	0.53NS	1.25NS	.0056NS	163.15NS	.18NS
Error	146.68	0.40	1.48	.0060	106.43	.23
	Individual tree					
Replication (R)	95.07**	134.06**	0.9676**			31.41**
Crosses (C)	10.43**	18.96**	.0950**			2.63NS
GCA	29.12**	52.05**	.2547**			5.44**
SCA	5.63NS	10.45NS	.0539 NS			1.91NS
R $\times$ C (plot)	3.86**	11.48**	.0478**			2.01**
Within plot	1.21	7.22	.0253			0.41

<sup>a/</sup> An index varying from 1 (straight) to 6 (most crooked).

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

NS Nonsignificant.

Table 5. — Breeding values of the 10 clones in the half-diallel, as estimated from 10-year family mean data.

Clone	Trait					
	Height	D.b.h.	Vol./tree	Rust-free	Stem straightness <sup>a/</sup>	
	m	cm	m <sup>3</sup>	percent		
1	7.56	11.9	0.0339	- 1.85	3.33	
2 <sup>b/</sup>	6.76	11.1	.0267	4.94	2.96	
3 <sup>b/</sup>	7.63	13.0	.0387	28.30	2.83	
4 <sup>b/</sup>	7.93	12.9	.0394	24.47	3.22	
5 <sup>b/</sup>	7.13	12.4	.0331	0.23	2.92	
6 <sup>b/</sup>	7.30	13.4	.0400	28.41	2.66	
7 <sup>b/</sup>	6.12	11.9	.0273	- 1.54	3.29	
8 <sup>b/</sup>	6.38	11.4	.0278	25.43	2.61	
9 <sup>b/</sup>	6.85	11.7	.0299	- 4.34	2.89	
10 <sup>b/</sup>	7.62	13.4	.0420	55.05	2.68	
Mean						
All Clones <sup>b/</sup>	7.13	12.3	.0339	15.91	2.94	
Five best <sup>b/</sup>	7.37	12.8	.0376	32.33	2.80	

<sup>a/</sup> An index varying from 1 (straight) to 6 (most crooked).

<sup>b/</sup> Clones selected as best for rust-free percentage.

Table 6. — Family and individual tree heritabilities estimated from 10-year data.

Basis of heritability	Trait					
	Survival	Height	D.b.h.	Vol./tree	Rust-free	Stem straightness <sup>a/</sup>
Family	0.00	0.35	0.27	0.31	0.69	0.17
Individual		.18	.06	.08		.07

<sup>a/</sup> An index varying from 1 (straight) to 6 (most crooked).

ing the diallel general mean as the expected breeding value for each clone. The five clones best in rust-free percentage had a mean breeding value for that trait that was twice as high as the mean of all 10 clones. In other traits, means for these five clones were also greater than the diallel means.

The heritability of rust-free percentage was 0.69 (Table 6). Family-basis heritabilities for other traits were considerably lower than this, and individual tree heritabilities lower still. At age 5, the heritability of rust-free percentage was 0.62 (SLUDER 1981). Also at age 5, the heritability of number of galls per tree was 0.56 on a family mean basis and 0.24 on an individual tree basis. The genetic correlation between percentage rust-free and average height was 0.30 at age 5 and 0.57 at age 10.

Within the half-diallel, there were 10 crosses among the five clones selected for their good breeding values for rust resistance. The progenies from these 10 crosses averaged 35.8% rust-free to age 10 (Table 7). The best rust-free phe-

Table 7. — Rust-free percentages at age 10 of the 10 crosses among the 5 selected clones.

Male clone	Female clone			
	4	6	8	10
	-----Percent-----			
3	28.9	24.7	33.0	44.4
4		35.0	24.3	45.9
6			29.2	41.6
8				51.1
Mean = 35.8				

Table 8. — Genetic gains from selection at the three stages involved in this study of inheritance and gain in loblolly pine in central Georgia.

Selection stage	Trait					
	Survival	Height	D.b.h.	Vol/tree	Rust-free	Stem straightness <sup>a/</sup>
	Percent	m	cm	m <sup>3</sup>	Percent	
Original	- 5.6	0.31	0.8	0.0080	0	0.72
10 clones	18.5	-.03	-.1	-.0017	14.3	-.58
5 clones	0	-.03	-.1	-.0007	13.8	-.01
Total	12.9	.31	.8	.0070	28.1	.13
-----Percent of base-----						
Original	- 8.15	4.53	6.90	28.98	0	23.38
10 clones	38.84	-0.42	-0.82	-4.77	953.33	-24.58
5 clones	0	0.42	0.81	2.06	87.34	-0.34
Total	26.69	4.53	6.89	26.27	1040.67	-1.54

<sup>a/</sup> An index varying from 1 (straight) to 6 (most crooked).

notype in each of these 10 progenies will be selected for further breeding. Fifteen crosses among unrelated individuals will be possible with these selections.

The data in Table 7 are the result of selection at three stages: the original selection that went into establishing the seed orchard, selection of the 10 clones used in the half-diallel (Table 1), and selection of the five clones whose progenies are listed in Table 7. Genetic gains from each of these stages of selection can be estimated.

For the original selection, the gain can be estimated as the mean of the orchard bulk lot less the mean of the GCIA standard check lot. For the next stage, selection of the 10 clones, the gain is the diallel mean less the mean of the orchard bulk lot. These differences are the selection differentials and for these first two stages are also the realized genetic gains, since reproduction of the selected phenotypes has been accomplished. The third-stage selection differentials are the means of the 10 families from crosses among the five selected clones less the diallel means. These selection differentials must be multiplied by family heritability estimates to arrive at genetic gains.

As expected, the gains in rust-free percentage in the second and third stages were far greater than those in other traits, since rust resistance was the only trait selected for in those stages (Table 8). Fortunately, no serious losses in other traits were incurred at any stage except the loss in stem straightness in the second stage.

Another selection stage to be done is selection of individual trees within families. Since the trees to be selected will be rust-free, the selection differential for rust-free percentage will be quite high at that stage ( $100 - 35.8 = 64.2$ ). This process, therefore, should add a significant amount to the gains in rust resistance already produced by the previous stages of selection. There is no direct way to estimate this gain, but if the trait had as high an individual tree heritability as did galls per tree at age five ( $h^2 = 0.24$ ),

the expected gain in rust-free percentage would be  $64.2 \times 0.24 = 15.4$ . This is about the same as the gain produced by each of the two previous stages (Table 8).

## Discussion

The breeding values for percentage rust-free of the 10 clones used in this study divided sharply into five moderately resistant and five susceptible clones (Table 5). There is no obvious reason why this happened. All 10 clones had shown approximately the same degree of resistance in the original progeny tests. It may be that selecting among loblolly pines with a moderate range of rust resistance will be somewhat unpredictable. On a more positive note, the five clones that showed up well in the half-diallel have now shown resistance in two mating schemes and two progeny tests. The degree of resistance which they carry should be stable and useful.

The positive genetic correlation between height and rust-free percentage indicates that these two traits can be improved in loblolly pine in any manner convenient to the breeder. Improvement in either trait will have no negative effect on improvement in the other, but rather there may be some mutual enhancement. It is probable that the positive genetic correlation may be produced largely by greater height growth in trees with noninfected stems compared with those with infected stems (SLUDER 1977a), so the enhancement may be in one direction only.

Mechanisms of resistance to fusiform rust have been studied to some extent (KINLOCH 1982), but the number of genes involved and their mode of inheritance are not well understood. Apparently there is more than one mechanism of resistance and a few to several genes may be involved in each mechanism. Until these details are better known, it is possible that strong selection pressure in loblolly pine could fix some favorable genes as well as lose some which could later be needed to meet changes in virulence in the rust pathogen. Maintaining a broad genetic base will help avoid loss of valuable genes while selecting and breeding for rust resistance.

The test site for this study has probably the most severe rust hazard in Georgia and probably as severe as anywhere in the Southeast. The level of resistance reflected by the data in Table 5 therefore is a big step toward the level of resistance needed in loblolly pine to be planted in areas under high hazard from fusiform rust. Further breeding with the five selected clones (Table 7) and other genotypes of equal or better genetic value promises to be effective in increasing the level of resistance to fusiform rust in loblolly pine.

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## The in vitro Proliferation of Forest Trees

### 1. *Dalbergia sissoo* Roxb. ex Dc

By B. SUWAL, A. KARKI<sup>1)</sup> and S. B. RAJBHANDARY<sup>2)</sup>

Tissue culture Laboratory, Royal Botanical Garden,  
Godawary, Kathmandu, Nepal

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#### Abstract

Multiple shoots were induced on cotyledonary node culture of *Dalbergia sissoo* in the presence of benzylaminopurine (BAP) at 1 mg/l and naphthaleneacetic acid (NAA) at 0.1 mg/l. These shoots continued to proliferate at a sustained rate of 10–15 microshoots over two years of 8 weekly subcultures in the basic medium, with the supplement of BAP at 0.25 mg/l. Such microshoots rooted readily in non-sterile sand beds with subsequent successful field establishment.

**Key words:** *Dalbergia sissoo*, cotyledonary culture, multiple shoots non-sterile rooting, field establishment.

#### Zusammenfassung

Bei Kotyledonen-Nodien-Kulturen von *Dalbergia sissoo* wurden durch die Anwendung von Benzylaminopurin (BAP) (1 mg/l) und Naphthalenessigsäure (NAA) (0,1 mg/l) multiple Sprosse induziert. Diese Sprosse pflanzten sich weiterhin mit einer ununterbrochenen Rate von 10–15 Sprossen über 2 Jahre fort, wobei die Subkulturen 8 Wochen dauern, bei Verwendung des Basismediums unter Zugabe von 0,25 mg/l BAP. Solche Mikrosprosse wurden in nicht sterilem Sand leicht bewurzelt und erfolgreich in Feldversuche ausgebracht.

Present address:

<sup>1)</sup> Department of Botany, University of Nottingham, School of Biological Sciences, University Park, Nottingham, U. K.

<sup>2)</sup> Department of Medicinal Plants, Thapathali, Kathmandu, Nepal

#### Introduction

*Dalbergia sissoo* is a multipurpose native species which is extensively used in afforestation in the Nepal Terai for production of fodder, fuel and timber. Since there is considerable phenotypic variation between trees in provenance trials of *D. sissoo* raised from seed, genetic improvement will depend on the selection of individual trees with superior characteristics and propagation of these by clonal means. However, cloning of superior material through the use of conventional vegetative cuttings resulted in uneven growth, as only tip-cuttings gave rise to straight-growing plants, whereas branch cuttings produced trees which retained their characteristics as branches (plagiotrophic growth) (K. WHITE, personal communication). Moreover only 25 percent of the cuttings were successfully rooted (SINGH, 1982). An alternative to vegetative propagation by cuttings is to use the tissue culture method of multiplying clones using meristem or shoot-tips or buds as the explants.

To develop a process for cloning elite candidate trees of *Dalbergia sissoo* it was thought convenient first to devise a method of producing tissue culture plants using explants from seedlings grown in culture. Once a successful proliferation medium has been established it can then easily be tested for cloning superior individuals through meristem or shoot tip or bud culture.

We report here the shoot proliferation, followed by non-sterile rooting of these shoots, and subsequently field estab-