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Clonal Propagation of *Dalbergia sissoo* Roxb. by Softwood Nodal Cuttings: Effects of Genotypes, Application of IBA and Position of Cuttings on Shoots

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Summary

Softwood nodal shoot cuttings were collected from the hedge garden at New Forest campus of Forest Research Institute, Dehra Dun. The cuttings were rooted in mist chamber to investigate the effect of IBA application and position of cuttings on shoots on rooting ability in different clones of *Dalbergia sissoo* Roxb. As compared to the 0% (control) and 0.1%; 0.2% IBA showed maximum adventitious root formation. Overall percent rooting, percent sprouting, mean number of shoots and their length, mean number of roots and their length increased with increasing concentration of IBA. Among different positions of cuttings within the shoots, the best rooting response was recorded in the middle part followed lower and upper part. The findings exhibited significant interclonal variation regarding adventitious rooting and maximum response was observed in C42 (Gonda, Uttar Pradesh) clone. Interactive effect of clone C42 and middle position significantly increased percent sprouting while; interactive effects of middle position and 0.2% IBA treatment significantly increased percent rooting. It is concluded that the selection of genotypes with good rooting ability with respect to shoot position in hedged plants and concentration of IBA can be made to produce high quality planting stock material to start clonal forestry programme of *D. sissoo.*

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Key words: Dalbergia sissoo, IBA, position on shoots, clone, nodal cuttings, rooting.

Introduction

Dalbergia sissoo Roxb. commonly known as shisham is an important multipurpose tree species of Northern India. Traditionally, plantations of this species are raised through seedling resulting in large variation in growth, form, irregular seed setting and disease prone trees. In order to reduce plant variability and at the same time ensure increased productivity, the development of vegetative/clonal propagation techniques is required. For the production of high quality timber and faster tree growth, it is essential to start by selecting superior clones/trees from which the stem shoot cuttings are to be taken. Vegetative propagation of shisham and other tree species through stem cuttings is markedly affected by several factors (NANDA, 1970; HARTMANN et al., 1997). Several studies have demonstrated variation in rooting ability within genotypes on the same locations due to natural genetic variation (HAINES et al., 1992; HUSEN and PAL, 2003a). Positions from which cuttings are taken also influence the overall quality, its ability to root and subsequent growth habit (HARTMANN et al., 1997; ZAKARIA and ONG, 1982; TCHOUNDJEU and LEAKEY, 1996; HUSEN and PAL, 2003b). Adventitious root formation in juvenile

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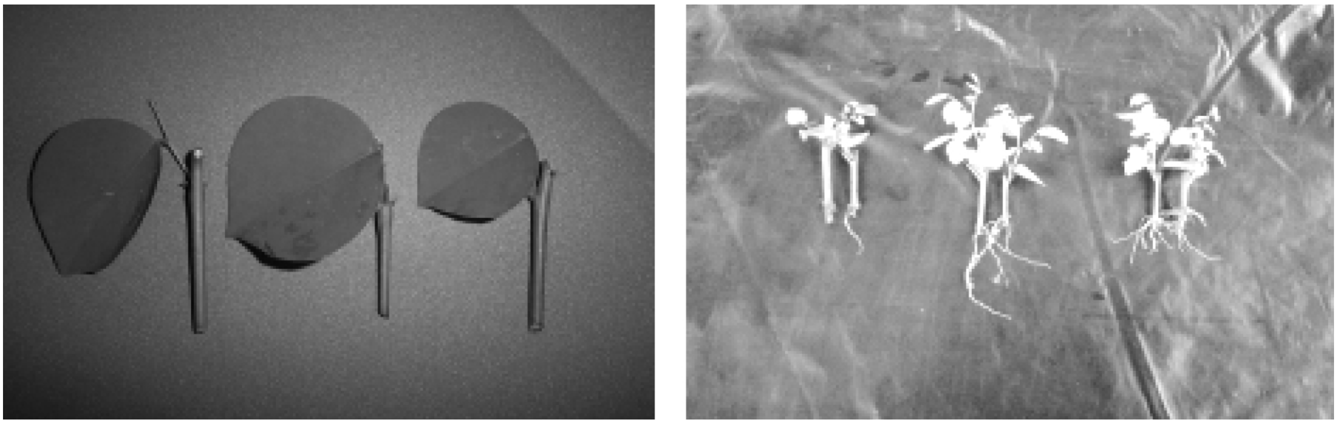

Figure 1. – Clonal Propagation of *Dalbergia sissoo.*

cuttings, which are obtained from shisham hedge gardens, is the most promising in terms of economics, ease and simplicity of the method for mass production of quality planting stock. Although successful vegetative propagation has been achieved in shisham (PAL, 1988), no information is currently available on the factors influencing rooting ability. Therefore, the present study was conducted to (1) compare rooting potential of cuttings collected from different clones, (2) the effect of position on rooting and (3) test the effects of applying a root-inducing powder i.e., indole-3-butyric acid (IBA) to enhance root initiation.

Materials and Methods

Selection of clones

Five different clones of *Dalbergia sissoo* namely C10 (Haridwar, Uttaranchal), C41 (Gonda, Uttar Pradesh), C42 (Gonda, Uttar Pradesh), C66 (Shergarh, Rajasthan), and C84 (Hanumangarh, Rajasthan) were selected for rooting trial.

Collection and propagation of plant material

Root suckers 50 cm long and 10–15 mm diameter were directly dug out from the selected candidate plus trees (CPTs) at various regions as mentioned earlier. They were wrapped in moist peat moss and brought to the laboratory in wet sacks. The root suckers from different CPTs were treated with 0.10 percent Bavistin (BASF India Ltd. Mumbai) for 10–15 minutes and placed horizontally in the nursery beds at New Forest Campus, Forest Research Institute (FRI), Dehra Dun in year 1998. Regular hedging was done yearly to maintain them as hedges and juvenile shoots were collected for multiplication. However, hedging in this experiment was done in the month of February, 2000 at 30 cm height and the upper portion of the coppiced stump was coated with chaupatia past, comprising mixture of 1 gm copper carbonate and 1 gm red led in 1 litre blue copper to avoid infection.

Preparation of mono-nodal softwood cuttings

The shoots were collected after eight week of hedging in April, 2000 and mono-nodal leafy, softwood cuttings were prepared. Total length of the cutting was approximately 4 cm (*Figure 1*). Twenty five cuttings were used per replicate and 3 replicates were maintained per treatment. Further, details of experimental design and statistical analysis are given under forthcoming paragraphs of statistical analysis.

Treatments

The cuttings were subjected to the following treatments: – a) position: shoots with 10–14 internodes were collected from five different clones. Each shoots were divided into three portions viz., upper, middle and lower; b) clones: as previously mentioned, the shoot cuttings were taken from hedge garden of five clones of *D. sissoo* namely C10, C41, C42, C66 and C84. The shoots emerged in hedge garden, FRI were made into cuttings and used for experimentation; c) dipping the basal ends into IBA. Treatments of IBA were included application of 0.2%, 0.1% and 0% (untreated control) concentrations by basal dip method. The IBA was applied in its powder formulation, which also contain 0.05 percent Bavistin. The control cuttings similarly treated with talcum powder containing Bavistin only.

Planting

After treatments the cuttings were planted in plastic trays containing vermiculite (pH 7.0) which was presoaked for 24 hours in tap water before filling the trays to allow it to absorb the water. The trays were kept into a mist chamber under misting duration of 1 hour intervals and on delay of 60 seconds and off delay of 30 minutes. Inside the mist chamber, the relative humidity was maintained at 85 ± 2% and maximum and minimum day/night temperature at 32 ± 10°C to 26 ± 10°C respectively.

Observation on rooting response

Rooting and sprouting on cuttings occur with 3–4 weeks after planting. However, after 30 days, the cuttings were carefully removed from the rooting medium and observation were recorded on percent rooting, percent sprouting, mean number of shoots and their length (in cm) per cutting and mean number of roots and their length (in cm) per cutting.

Statistical analysis

A factorial completely randomized design (CRD) was used for the statistical analysis of the data. Because the percentage

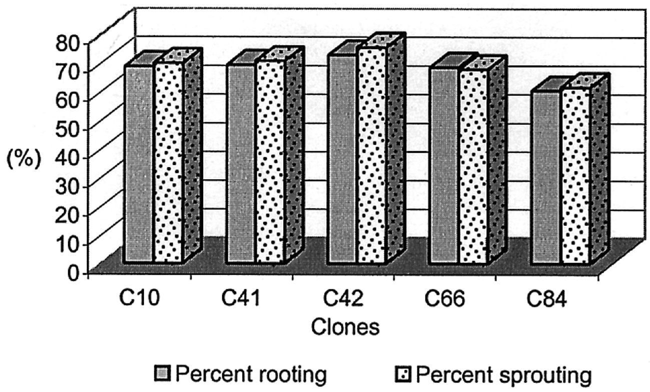

Figure 2. – Clonal variation in percent rooting and percent sprouting.

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Table 1. – Effect of IBA treatment, clone, position and their interaction on rooting and sprouting response of shoot cuttings taken from hedged plant of *Dalbergia sissoo*.

Parameters	Position	Treatment of IBA	Clone C10	C41	C42	C66	C84	Mean of position x treatment
Mean number of shoots per cutting	Upper	0 ppm	*1.77*	*1.86*	*2.33*	*2.97*	*2.95*	2.38♦
		1000 ppm	*3.21*	*2.52*	*3.19*	*3.83*	*3.67*	3.28♦
		2000 ppm	*3.57*	*3.04*	*4.11*	*4.70*	*4.67*	4.01♦
	Mean of position x clone →		2.84	2.47	3.21	3.83	3.76	3.22#
	Middle	0 ppm	*2.29*	*1.99*	*3.18*	*3.17*	*3.00*	2.72♦
		1000 ppm	*3.34*	*2.50*	*4.05*	*3.99*	*3.83*	3.54♦
		2000 ppm	*4.04*	*4.27*	*5.27*	*4.84*	*4.71*	4.62♦
	Mean of position x clone →		3.22	2.92	4.17	4.00	3.85	3.63#
	Lower	0 ppm	*2.59*	*1.95*	*3.47*	*3.09*	*2.98*	2.81♦
		1000 ppm	*2.54*	*2.74*	*4.50*	*3.85*	*3.67*	3.46♦
		2000 ppm	*3.10*	*3.17*	*4.99*	*4.77*	*4.66*	4.12♦
	Mean of position x clone →		2.74	2.62	4.32	3.88	3.77	3.47#
	Treatment of IBA x clone →	0 ppm	2.22	1.93	2.99	3.07	2.98	2.63⬍
		1000 ppm	3.03	2.58	3.91	3.89	3.72	3.43⬍
		2000 ppm	3.57	3.49	4.79	4.75	4.68	4.26⬍
	Mean of clone →		2.93	2.67	3.89	3.90	3.79	
Mean length of shoot per cutting (in cm)	Upper	0 ppm	*1.99*	*2.64*	*2.17*	*2.33*	*2.24*	2.27♦
		1000 ppm	*3.85*	*3.85*	*5.67*	*3.67*	*3.70*	4.15♦
		2000 ppm	*5.20*	*4.50*	*6.17*	*5.00*	*4.10*	4.99♦
	Mean of position x clone →		3.68	3.66	4.67	3.67	3.35	3.80#
	Middle	0 ppm	*3.41*	*2.33*	*2.33*	*2.43*	*3.30*	2.76♦
		1000 ppm	*4.57*	*3.50*	*5.00*	*4.33*	*4.67*	4.41♦
		2000 ppm	*6.05*	*5.17*	*5.50*	*5.00*	*5.07*	5.36♦
	Mean of position x clone →		4.67	3.67	4.28	3.92	4.34	4.18#
	Lower	0 ppm	*2.28*	*2.33*	*2.60*	*2.50*	*2.33*	2.40♦
		1000 ppm	*4.41*	*3.02*	*3.67*	*3.83*	*3.83*	3.75♦
		2000 ppm	*5.82*	*3.89*	*5.17*	*4.67*	*4.33*	4.78♦
	Mean of position x clone →		4.17	3.08	3.81	3.67	3.50	3.65#
	Treatment of IBA x clone →	0 ppm	2.56	2.43	2.37	2.42	2.62	2.48⬍
		1000 ppm	4.28	3.45	4.78	3.94	4.07	4.10⬍
		2000 ppm	5.69	4.52	5.61	4.89	4.50	5.04⬍
	Mean of clone →		4.17	3.47	4.25	3.75	3.73	
Mean number of roots per cutting	Upper	0 ppm	*2.73*	*2.16*	*3.33*	*3.33*	*3.40*	2.99♦
		1000 ppm	*6.29*	*7.07*	*6.00*	*7.00*	*5.73*	6.41♦
		2000 ppm	*9.33*	*9.83*	*8.69*	*9.67*	*9.07*	9.32♦
	Mean of position x clone →		6.12	6.36	6.01	6.67	6.07	6.24#
	Middle	0 ppm	*3.45*	*3.74*	*4.67*	*4.50*	*4.67*	4.20♦
		1000 ppm	*7.18*	*8.42*	*8.67*	*8.20*	*7.67*	8.02♦
		2000 ppm	*10.57*	*11.53*	*10.00*	*8.76*	*9.85*	10.14♦
	Mean of position x clone →		5.07	7.89	7.78	7.15	7.39	7.46#
		0 ppm	*3.15*	*2.67*	*3.67*	*4.00*	*3.67*	3.43♦
		1000 ppm	*7.07*	*6.51*	*8.02*	*7.33*	*8.67*	7.52♦
		2000 ppm	*9.60*	*8.76*	*9.51*	*9.07*	*9.74*	9.34♦
	Mean of position x clone →		6.60	5.98	7.07	6.80	7.35	6.76#
	Treatment of IBA x clone →	0 ppm	3.11	2.86	3.89	3.94	3.91	3.54⬍
		1000 ppm	6.85	7.33	7.56	7.51	7.35	7.32⬍
		2000 ppm	9.83	10.04	4.90	9.16	9.55	9.59⬍
	Mean of clone →		6.59	6.74	6.95	6.87	6.94	
Mean length of root per cutting (in cm)	Upper	0 ppm	*2.54*	*3.03*	*3.03*	*3.07*	*2.67*	2.87♦
		1000 ppm	*4.33*	*4.50*	*4.74*	*4.69*	*4.33*	4.52♦
		2000 ppm	*5.77*	*5.64*	*6.17*	*6.24*	*5.38*	5.84♦
	Mean of position x clone →		4.21	4.39	4.65	4.67	4.12	4.41#
	Middle	0 ppm	*3.06*	*3.11*	*3.68*	*2.87*	*3.00*	3.14♦
		1000 ppm	*4.69*	*5.00*	*4.88*	*4.35*	*4.83*	4.75♦
		2000 ppm	*5.94*	*8.00*	*5.80*	*5.98*	*5.67*	6.28♦
	Mean of position x clone →		5.57	5.37	4.79	4.40	4.50	4.72#
		0 ppm	*3.04*	*3.00*	*3.07*	*3.33*	*2.83*	3.05♦
		1000 ppm	*5.39*	*5.16*	*4.69*	*5.00*	*4.29*	4.93♦
		2000 ppm	*6.32*	*6.83*	*6.24*	*6.48*	*5.50*	6.27♦
	Mean of position x clone →		4.92	5.00	4.67	4.94	4.24	4.75#
	Treatment of IBA x clone →	0 ppm	2.88	3.04	3.26	3.09	2.83	3.02⬍
		1000 ppm	4.88	4.8	4.77	4.68	5.52	4.73⬍
		2000 ppm	6.01	6.82	6.07	6.23	5.51	6.13⬍
	Mean of clone →		4.56	4.92	4.70	4.67	4.29	

Mean of position, ⬍ Mean of IBA treatment, ♦ Interactive mean of position and IBA treatment, and italic number represents three way interaction mean of position, clone and IBA treatment.

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Table 2. – Analysis of variance for the effect of clone, position, treatment of IBA and their interactions on rooting and sprouting parameters of *Dalbergia sissoo.*

Source of variation	df	Mean sum of square					
		Percent rooting	Percent sprouting	Mean number of shoots per cutting	Mean length of shoot per cutting	Mean number of roots per cutting	Mean length of root per cutting
Clone	4	563.22*	697.32*	9.44*	2.92*	0.61	1.43
Position	2	926.77*	659.83*	1.88	3.35*	16.67*	1.64
IBA treatment	2	2128.82*	3068.44*	29.39*	75.57*	42.15*	109.07*
Clone x Position	8	15.36	53.61*	0.62	1.09	1.85	0.67
Clone x IBA treatment	8	20.23	8.17	0.10	1.14	1.81	0.51
Position x IBA treatment	4	82.31*	66.81	0.38	0.28	1.21	0.12
Clone x Position x IBA treatment	16	42.18	39.54	0.19	0.43	0.80	0.37
Error	90	20.64	19.40	0.82	0.57	2.49	0.69
Critical difference at P< 0.05 level							
Clone		2.42	2.35	0.48	0.40	-	-
Position		1.87	1.82	-	0.31	0.65	-
IBA treatment		1.87	1.82	0.37	0.31	0.65	0.34
Clone x Position		-	4.07	-	-	-	-
Clone x IBA treatment		-	-	-	-	-	-
Position x IBA treatment		3.25	-	-	-	-	-
Clone x Position x IBA treatment		-	-	-	-	-	-

data are based upon a binomial response and some mean percentage lie outside the stable variance range of 30 to 70 percent, all percentage data were transformed to arcsine $\sqrt{p}$ following the method of ANDERSON and MCLEAN (1974). All other analyses were performed on untransformed data. Analysis of variance (ANOVA) procedures were used to test for significant treatment effects of the treatments on each variable measured (*Table 2*). In the ANOVA, the value for each replication was estimated based on all available cuttings and subjected to the following model:

$$Y_{ijk} = \mu + p_i + c_j + t_k + (pc)_{ij} + (pt)_{ik} + (ct)_{jk} + (pct)_{ijk} + e_{ijk}$$

Where, i = 1...3, j = 1...5 and k =1...3
- μ = overall mean
- Y_{ijk} = value of position i, clone j and IBA treatment k
- p_i = effect of position i
- c_j = effect of clone j
- t_k = effect of IBA treatment k
- $(pc)_{ij}$ = interaction effect of position i and clone j
- $(pt)_{ik}$ = interaction effect of position i and IBA treatment k
- $(ct)_{jk}$ = interaction effect clone j and IBA treatment k
- $(pct)_{ijk}$ = interaction effect position i, clone j and IBA treatment k
- e_{ijk} = random error related to Y_{ijk}

Results

Effect of clone

Significant clonal variation was observed for the percent rooting, percent sprouting, mean number of shoots and their length per cutting at P < 0.05 level (*Table 1* and *2*). Maximum percent rooting (72.63%) and percent sprouting (75.23%) was observed on the cuttings from the C42 clone while minimum in clone C84 (*Figure 2*). More shoots were produced on the cuttings from the C66 clone than other clones (*Table 1*). Cuttings of C42 clone produced longer shoots (4.25 cm) followed by C10 (4.17 cm), C66 (3.75 cm), C84 (3.73 cm) and C41 (3.47 cm) clones (*Table 1*).

Effect of position

The position of shoot, from which cuttings were taken, on the donor hedged plant had a significant effect on the percent rooting, percent spouting, mean length of shoot per cutting and mean number of roots per cutting (*Table 1*). The cuttings taken from the middle part of the shoots showed the highest percent rooting and percent sprouting followed by lower and upper part (*Figure 3*). However, mean length of shoot per cutting was higher in middle part followed by upper and lower part (*Table 1*). Further, the highest number of roots was recorded in middle part followed by lower and upper part (*Table 1*).

Effect of IBA treatment

All the rooting and sprouting parameters shows significant variation at P < 0.05 level (*Table 2*). Increasing the concentration of IBA increased percent rooting, percent sprouting, mean number of shoots and their length per cutting; and mean number of roots and their length per cutting (*Figure 3* and *Table 2*). Therefore, the highest response was with its 2000 ppm concentration followed by 1000 ppm and the lowest in control (0 ppm) cutting was recorded for all the studied parameters.

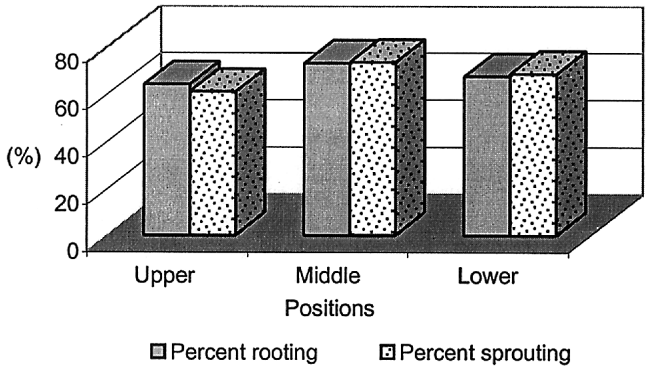

Figure 3. – Effect of position of shoot on percent rooting and percent sprouting.

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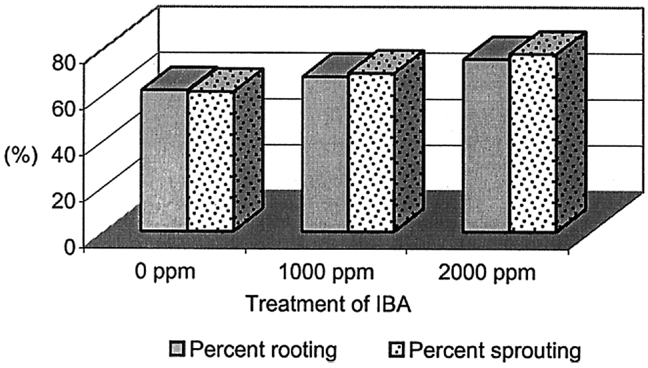

Figure 4. – Effect of IBA treatment on percent rooting and percent sprouting.

Interactive effects

The combined effects of clone and position was significant at P < 0.05 level for percent sprouting only (*Table 2*). Shoot cuttings taken from the middle part of the clone C42 exhibited the highest (80.34%) percent sprouting. While, the lowest (58.89%) was recorded in cuttings taken from the upper part of the clone C84 (*Figure 5*). Interactive effects of position and IBA treatment exhibited significant variation at P < 0.05 level for percent rooting only (*Table 2*). The percentage of adventitious root formation was the highest (82.74%) in middle part of the cuttings that's were treated with 0.2% IBA than in any other combination. While, the lowest response (56.99%) was recorded with control (0%) x upper part of the shoot cuttings (*Figure 6*). Further, it was observed that the two factors (clone x IBA treatment) and three factors (clone x position x IBA treatment) interaction effects on the studied rooting and sprouting parameters did not exhibited any significant variation at P < 0.05 level (*Table 1* and *2*).

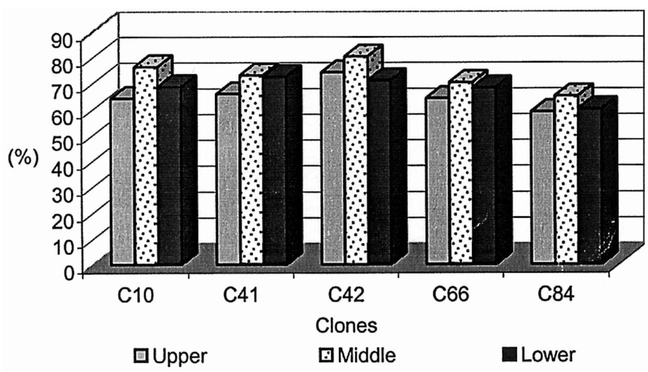

Figure 5. – Interactive effects of position and clone on percent sprouting.

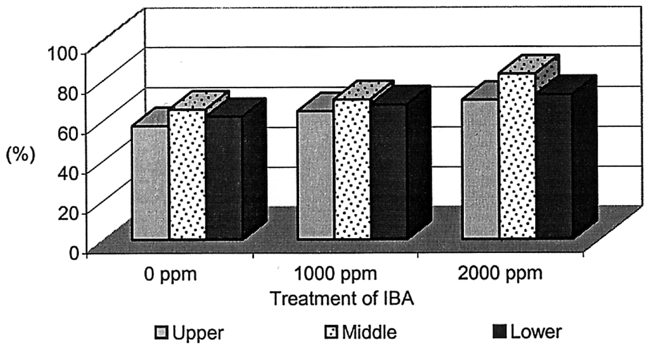

Figure 6. – Interactive effects of position and IBA treatment on percent sprouting.

Discussion

The findings clearly show that variation do exit with respect to rooting ability in the different genotypes of *D. sissoo.* Overall, clone C42 (Gonda, Uttar Pradesh) was the best genotype and maximum rooting and sprouting in cuttings taken from the middle part of the shoots were observed. Significant clonal variation in rooting behaviour was also reported earlier in various tree species (Snow, 1939; Hains et al., 1992; Husen and Pal, 2003a). Clonal differences in rooting ability of tree cuttings (Haissig and Riemenschneider, 1988; Pounders and Foster, 1992) and root and shoot growth (Zobel and Talbert, 1984) are not uncommon in both angiosperms and gymnosperms (Cunningham, 1986). However, the reason for clonal variation are not fully understood, and may (Nanda and Anand, 1970) or may not (Greenwood et al., 1976) be attributed to different concentration of endogenous auxins.

Differences existed in the position of shoot on hedged donor plants for rooting ability of the cuttings. Maximum rooting and sprouting percentage was observed in the cuttings taken from middle part followed by those taken from basal and minimum in the cuttings taken form the upper part. However, mean length of shoot per cutting was more in the cuttings taken from the same position but followed by upper and lower part. Similar results for percent rooting and percent sprouting were found in *Tectona grandis* (Husen and Pal, 2003b). Although, in most of tree species rooting ability of cuttings has been reported to increase from apical to basal part of the crown and of the shoots which has been attributed to accumulation of carbohydrates at the base of shoot (Hartmann et al., 1997), there are many deviation from this general trend. For example, in *Triplochiton scleroxylon*, rooting percentage of cuttings form different node positions was found to decline basipetally (Leakey and Mohammed, 1985). Further, cuttings originating from the apical position of shoot of *Milicia excelsa* (Ofori et al., 1997) *T. scleroxylon* (Leakey, 1983) and *Nauclea diderrichii* (Matin, 1989) displayed higher rooting percentages than those taken from the basal portions. But the results of this study indicate that cuttings of shoots originating from the middle part of shisham displayed higher rooting percentage. It is evident from these findings that optimal branch position for the best rooting response varies with the plant species, and the position effect on rooting may be caused by variation in the physiological status of shoot tissues with the position of branch on stock plant resulting in occurrence of gradients along the stem axis in the cellular activity, in the level of assimilates and growth regulators, and in the level of lignification etc.

Effect of IBA on rooting and sprouting ability of the cuttings also varied with position of the shoot and the concentration of IBA. Increasing the concentration of IBA increased rooting and sprouting of cuttings, which, were also taken form the middle part while it was comparatively less effective in cuttings taken from the other position. Similar findings were recorded in *T. grandis* (Husen and Pal, 2003b). Although, the effect of various auxins (IBA or NAA) in promoting rooting of cuttings is well known (Nanda, 1970; Hartmann et al., 1997; Husen and Mishra, 2001; Husen and Pal, 2003b and c; Husen, 2003) but very little information is available on the effectiveness of auxins in relation to the branch position, especially when the cuttings were taken from the hedged garden.

Overall, the findings of this investigation suggest that selection of genotypes with good rooting ability with respect to shoot position in hedged plants and concentration of IBA can be made to produce high quality planting stock material to start clonal forestry programme of *D. sissoo.*

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Sexual Asymmetry based on Flowering Assessment in a Clonal Seed Orchard of *Pinus densiflora*

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Abstract

Two equations were formulated in order to estimate the degree of sexual asymmetry for monoecious species. The concepts of the equations were formulated on the basis of the effective population numbers of female and male parents [i.e, $A_s^{(v)}$], and the differences of relative frequency between genders [i.e., $A_s^{(x)}$]. These equations were applied to estimate the degree of sexual asymmetry based on the empirical data of flowering assessment in a clonal seed orchard of *Pinus densiflora.* The yearly variation in the production of female and male strobili was found. The effective population numbers at gamete levels (v_f and v_m) and clone level (v_b) varied among 8 observation years. Both $A_s^{(x)}$ and $A_s^{(v)}$ were negatively correlated with effective numbers at gamete and clone levels. Averages of female and male strobilus production and estimates of sexual asymmetry were negatively correlated but the correlation was not significant. The correlations among effective number of clone (v_b), arithmetic mean of female and male effective numbers (v_a) and estimate of sexual asymmetry [$A_s^{(x)}$] were strong and significant. Relatively larger difference between v_b and v_a were found when higher level of sexual asymmetry were observed.

Key words: sexual asymmetry, effective number, strobilus production, fertility, coefficient of variation.

Introduction

Pinus densiflora Sieb. et Zucc. is one of the important tree species from the aspects of utilization of forest resources and the management of forest ecosystem in Korea. The breeding program for this species has been performed since 1959 with a selection of plus trees and an establishment of 99 ha seed orchards (HAN *et al.,* 2001a).

The main goals for establishing and managing seed orchards are the massive production of genetically improved seeds and the maintenance of genetic diversity present in seed orchards. Tree breeders want to maintain identical allelic structures between parental and offspring in seed orchard populations. However, this option can be expected only in the case of population under panmictic equilibrium. From this point of view, the maintenance of random mating among clones is one of the cru-

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