SEASONAL ROOTING AND NODULATION RESPONSE IN *DESMODIUM ELEGANS* - AS INFLUENCED BY EXOGENOUS AUXIN APPLICATIONS

D.S. Chauhan, B.P. Bhatt & N.P. Todaria

Department of Forestry, H.N.B. Garhwal University, Srinagar Garhwal - 246174, U.P., India

Received January 1995

CHAUHAN, D.S., BHATT, B.P. & TODARIA, N.P. 1996. Seasonal rooting and nodulation response in *Desmodium elegans* - as influenced by exogenous auxin applications. Exogenous application of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthaleneacetic acid (NAA), and dichlorophenoxyacetic acid (2,4-D) resulted in significant rooting responses for branch cuttings of *Desmodium elegans*. Compared to November plantings, irrespective of growth regulators, cuttings treated and planted in February to August proved better for rooting. The number of sprouted cuttings, number of root-shoots per plant, root-shoot length per plant and root-shoot dry weight per plant were highest for cuttings planted in February as compared to other months. In seasonal stimuli responsible for nodule formation in rooted branch cuttings, it was observed that the winter set of branch cuttings did not exhibit nodulation although there was moderate rooting response, whereas cuttings planted in the rest of the seasons showed moderate nodulation per cutting. Nitrogen percentage was comparatively higher in IAA and IBA treated branch cuttings.

Key words: *Desmodium elegans* - nodulation - rooting response - seasonal sprouting - vegetative propogation

CHAUHAN, D.S., BHATT, B.P. & TODARIA, N.P. 1996. Pengakaran bermusim dan tindakbalas penodulan *Desmodium elegans* - di bawah pengaruh auksin eksogen. Penggunaan eksogen indola-3-asid asetik (IAA), indola-3-asid butirik (IBA), asid naftalenasetik (NAA), dan asid diklorofenoksiasetik (2,4-D) menyebabkan tindakbalas pengakaran yang ketara ke atas keratan dahan *Desmodium elegans*. Berbanding dengan penanaman pada bulan November, tanpa mengambil kira pengatur pertumbuhan, keratan yang dirawat dan ditanam dalam bulan Februari hingga Ogos menunjukkan pengakaran yang lebih baik. Bilangan keratan yang berpucuk, bilangan sulur akar sepokok, panjang sulur akar sepokok dan berat kering sulur akar sepokok adalah paling tinggi bagi keratan yang ditanam di bulan Februari berbanding dengan bulan-bulan yang lain. Di dalam rangsangan bermusim yang bertanggungjawab bagi pembentukan nodul di dalam keratan dahan berakar, diperhatikan bahawa keratan dahan di musim sejuk tidak mempamerkan penodulan walaupun terdapat tindakbalas pengakaran yang sederhana, manakala keratan-keratan yang ditanam di musim-musim yang lain menunjukkan penodulan sederhana bagi setiap keratan. Peratus nitrogen adalah lebih tinggi secara perbandingan di dalam keratan dahan yang dirawat dengan IAA dan IBA.

Introduction

In the Garhwal hills of northern India, *Desmodium elegans* shows considerable potential as a multipurpose species. The species occurs naturally in oak and spruce forests at an altitude ranging from 1300 to 2600 m. *Desmodium elegans* is a nitrogen fixing deciduous species in the family Fabaceae and the sub-family Papilionaceae (locally known as chamlei). Due to its multiferious nature, introduction of this species under agroforestry systems would have tremendous scope for biomass allocation and enrichment of soil fertility. Farmers in this region are accustomed to combining trees with agricultural crops. As a result of continuous cropping and tree harvesting, however, the nutrient status of their soils tends to be poor. Not only could *D. elegans* help address soil fertility problems but the tree's small size makes it suitable for planting as a hedge on field bunds to help control soil erosion (Todaria *et al.* 1993). Farmers harvest these small trees for fuel wood and fodder so extensively that it is very difficult to get desired amount of seeds for sexual propagation; therefore, we are concerned to develop its mass multiplication through vegetative means.

We have undertaken an intensive programme of conservation of genotype of various multipurpose tree species of Garhwal Himalaya (Bhatt 1991, Bhatt & Badoni 1993, Bhatt & Todaria 1990, 1991, 1993a,b, Chauhan *et al.* 1993). This study reports on the vegetative propagation and nodulation response of *D. elegans* as influenced by growth regulators and seasons.

Materials and methods

The experiment was performed in the experimental garden of the Department of Forestry, HNB Garhwal University, Srinagar, Garhwal (elevation 550 m; 78' 48°E , 30 - 31° N). Branch cuttings were obtained from juvenile and healthy field growing plants at four different seasons of a year, i.e. spring (February), summer (May), rainy (August) and winter (November). In each season, branch cuttings were collected from the same parent trees and harvested at a length of 20 - 25 cm; the thick basal portion (5 cm) of each cutting was dipped into an aqueous solution of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), napthaleneacetic acid (NAA) or dichlorophenoxyacetic acid (2,4-D) at 100, 200 and 500 ppm (mg l^{-1}) for 24 h. Ten branch cuttings for each treatment were used in each season. At each harvest, a set of ten branch cuttings treated with double distilled water was used as control. Treated cuttings were planted in pots filled with garden soil and river sand in a 1:1 ratio. Small quantities of farmyard manure were also added, and pots were watered regularly.

The sprouting response of cuttings was recorded at 30, 60 and 90 days after planting. However, observations on rooting and nodulation were made only after 90 days when cuttings were uprooted.

Nitrogen content in root nodules was estimated by the method of Iswaran (1980) as follows:

Preparation of the sample solution: 100 mg of dried nodules were transferred into a conical flask and 3 ml of concentrate sulphuric acid (H_2SO_4) (GR) were added. The plant material was maintained in H_2SO_4 for 5-6 h at approximately 100 °C until the whole solution turned white in colour. Then 2 ml of 30% hydrogen peroxide (H_2O_2) were added and the total volume was adjusted to 50 ml with distilled water. The solution was filtered, and the filtrate taken as the sample solution.

Spectrophotometric estimation of nitrogen

To 0.5 ml of the sample solution, 0.5 ml of 10% sodium silicate ($NaSiO_2$), 0.5 ml of 10% sodium hydroxide (NaOH) solution and 1.0 ml of Nessler's reagent were added and the total volume was adjusted to 25 ml with distilled water. Finally it was scanned in the spectrophotometer to determine the optical density (O.D.) at 440 nm. Nitrogen percentage was calculated when measured optical density was multiplied by 5.

A one-way analysis of variance (*F*-test) was conducted to test growth regulator treatment effects on the various rooting attributes. Similarly *t*-test was computed between treatments for root-shoot attributes in the month of February.

Results

Seasonal sprouting response of *D. elegans* showed that profused sprouting was induced in February by exogenous treatments of IAA, IBA, NAA and 2,4-D but these too were comparable with the control (Table 1). Moderate sprouting was recorded in the November harvest of plantings, whereas the August and May sets of plantings ranked in between these two extremes in terms of sprouting response (Tables 2- 4).

Cuttings planted in February showed the best rooting response as compared to other seasons. However, the rooting response of treated branch cuttings was not significently different from untreated ones. Very low concentrations of growth regulators induced rooting in the May and August sets of branch cuttings; however, in the winter set branch cuttings all the growth regulators induced rooting (Tables 1-4).

A large number of branch cuttings showed rooting success with exogenous treatments of IAA or IBA but the response was different. While the increase in the concentration of IAA and IBA from 100 to 200 ppm induced rooting in a larger number of cuttings, further increase in the concentration of IBA to 500 ppm had an adverse effect. Similarly, an increase in the concentration of NAA resulted in a lower number of rooted cuttings, but in winter, a different picture emerged. By increasing the concentration of IAA or IBA an increase in the number of rooted cuttings was obtained. For NAA treated cuttings, increasing the concentration of treatment resulted in rooting in a larger number of cuttings, but only for NAA concentrations up to 200 ppm.

In February root number per cutting was similar in all the treatments including the control. However, a lower number of roots per cutting was recorded in May and August for IBA, IAA or NAA treatments including the control, but it increased again in winter (Tables 2 - 4). Comparing the root number per plant within the treatments in one season, no significant differences were recorded. On average dry weights of shoot and root per cutting were also recorded higher (0.5 - 1.8 g $plant^{-1}$) in spring than in other seasons, irrespective of growth regulator concentrations. Branch cuttings rooted in the winter season ranked after the February planting in dry matter partitioning.

Table 1. Root- shoot attributes of branch cuttings (± standard error) of *D.elegans* as influenced by exogenous auxin applications in the month of February

Parameter	Treatment												
	Control	IAA 100 ppm	IAA 200 ppm	IAA 500 ppm	IBA 100 ppm	IBA 200 ppm	IBA 500 ppm	NAA 100 ppm	NAA 200 ppm	NAA 500 ppm	2,4-D 100 ppm	2,4-D 200 ppm	2,4-D 500 ppm
Shoot no. per cutting	ac 3.8 ± 0.4	ad 3.3 ± 1.03	af 3.8 ± 0.38	ah 3.4 ± 0.34	cfh 4.6 ± 0.75	cfh 4.4 ± 0.54	d 2.6 ± 0.70	cdfhi 4.0 ± 0.71	cfh 4.7 ± 0.65	cfgh 4.0 ± 0.71	dgh 3.3 ± 0.63	e 2.0 ± 0.7	dghi 4.0 ± 0.0
Root no. per cutting	abc 15.2 ± 1.4	de 11.3 ± 1.96	g 14.6 ± 1.9	af 13.1 ± 1.52	ah 14.6 ± 0.98	dh 13.2 ± 2.03	ai 16.8 ± 5.35	bdh 13.0 ± 2.65	bf 14.3 ± 0.85	bfgi 17.0 ± 0.82	7.0 ± 1.87	e 10.0 ± 2.01	cfgi 17.0 ± 3.01
Root/shoot ratio	ab 0.31 ± 0.00	c 0.43 ± 0.00	d 0.32 ± 0.00	c 0.35 ± 0.00	d 0.32 ± 0.00	ac 0.32 ± 0.00	f 0.78 ± 0.00	f 0.28 ± 0.00	c 0.34 ± 0.00	ac 0.93 ± 0.00	be 0.25 ± 0.00	0.35 ± 0.00	0.19 ± 0.00
Root nodule per cutting	abc 7.5 ± 0.65	eadc 9.5 ± 1.2	cf 6.2 ± 1.2	b 5.5 ± 0.72	d 10.2 ± 2.19	ef 7.3 ± 1.04	g 3.3 ± 0.48	h 4.5 ± 0.47	i 6.0 ± 2.23	gh 7.3 ± 1.11	i 5.0 ± 1.2	h 5.5 ± 0.50	ec 9.0 ± 3.01
Nitrogen percentage (%)	0.25 ± 0.05	a 0.42 ± 0.06	b 0.37 ± 0.07	ab 0.38 ± 0.08	ab 0.39 ± 0.06	0.47 ± 0.04	c 0.20 ± 0.03	d 0.07 ± 0.01	cd 0.18 ± 0.02	cde 0.17 ± 0.03	d 0.07 ± 0.07	0.10 ± 0.02	c 0.14 ± 0.03

Means followed by same letter for each growth parameter are not significantly ($p < 0.05$) different.

There were significant differences in nodulation per cutting among the several treatments as well as seasons. The numbers of root nodules per cutting were higher in May and August as compared to February (Tables 2 and 3) and nodulation was not observed in winter (Table 4). Nodulation per cutting was higher in treated cuttings than in untreated ones irrespective of the season. Lower concentrations of IAA or IBA were best suited not only for inducing higher number of root nodules per cutting but also for inducing higher nitrogen percentages in them. However, on average, nitrogen percentage was higher in February than in May and August plantings (Table 1).

Table 2. Root- shoot attributes of branch cuttings (± standard error) of *D.elegans* as influenced by exogenous auxin applications in the month of May

Parameter	Treatments						
	Control	IAA 200 ppm	IAA 500 ppm	IBA 200 ppm	IBA 500 ppm	2,4-D 100 ppm	2,4-D 200 ppm
Shoot no. per cutting	4.00 ± 0.00	3.0 ± 0.00	4.0 ± 0.00	1.50 ± 0.50	4.70 ± 0.33	4.00 ± 0.58	3.50 ± 1.26
Root no. per cutting	6.50 ± 1.00	7.50 ± 0.50	5.50 ± 2.51	5.50 ± 0.50	9.00 ± 0.58	9.70 ± 3.72	4.00 ± 2.04
Root/shoot ratio	0.11 ± 0.00	0.06 ± 0.00	0.14 ± 0.00	0.34 ± 0.00	0.16 ± 0.00	0.13 ± 0.00	0.19 ± 0.00
Root nodule per cutting	7.50 ± 2.51	10.00 ± 2.00	11.0 ± 5.52	9.0 ± 3.00	8.30 ± 1.76	6.30 ± 0.67	7.50 ± 0.50
Nitrogen percentage (%)	0.12 ± 0.05	0.32 ± 0.01	0.24 ± 0.01	0.39 ± 0.06	0.21 ± 0.07	0.09 ± 0.01	0.05 ± 0.01

Discussion

The results of the present investigation and those from others for rooting response of various species (Puri & Shamet 1988, Bhatt 1991, Bhatt & Todaria 1991) indicate a wide variation in the rooting ability of cuttings, and an inconsistent effect of the different concentrations of the various auxins tested.

Analysis of variance "*F*" computed for various growth parameters showed that there was a significant ($p< 0.05$ and $p<0.01$) effect of auxin application on root-shoot attributes. However, the present results suggest that no general conclusion can be made about the effect of a particular auxin, or of any treatment as such on vegetative propagation. The new Duncan's Multiple range test applied to root - shoot attributes showed that the effect of auxin application changed with the season. However, exogenous applications of various concentrations of IBA or IAA induced a significant ($p<0.05$) increase in root-shoot number shoot length, root-shoot dry weight and root nodules as compared to NAA or 2,4-D, including the control. The overall assessment was that season, besides growth regulators, played a crucial role in adventitious root formation in *D. elegans.* Spring season as well as winter season was found most suitable for mass multiplication through vegetative

Table 3. Root-shoot attributes of branch cuttings (± standard error) of *D.elegans* as influenced by auxin applications in the month of August

Parameter	Treatment								
	IAA 100 ppm	IAA 500 ppm	IBA 100 ppm	IBA 200 ppm	IBA 500 ppm	NAA 100ppm	NAA 500 ppm	2,4-D 200 ppm	2,4-D 500 ppm
Shoot no. per cutting	2.70 ± 0.68	3.00 ± 1.00	3.00 ± 1.00	4.00 ± 0.00	3.30 ± 1.20	5.00 ± 0.00	2.00 ± 1.00	4.00 ± 0.00	4.00 ± 0.00
Root no. per cutting	4.30 ± 1.34	9.00 ± 1.00	12.30 ± 2.34	9.00 ± 0.00	15.70 ± 2.03	0.00 ± 0.00	7.00 ± 4.01	8.00 ± 0.00	9.00 ± 0.00
Root/shoot ratio	0.25 ± 0.00	0.33 ± 0.00	0.33 ± 0.00	0.30 ± 0.00	0.36 ± 0.00	0.00 ± 0.00	0.35 ± 0.00	0.13 ± 0.00	0.29 ± 0.00
Root nodules per cutting	14.50 ± 3.51	7.50 ± 0.50	13.30 ± 4.18	14.00 ± 0.00	13.50 ± 1.50	0.00 ± 0.00	16.50 ± 0.00	9.01 ± 0.00	15.00 ± 0.00
Nitrogen percentage (%)	0.27 ± 0.08	0.17 ± 0.03	0.22 ± 0.01	0.31 ± 0.09	0.37 ± 0.03	0.00 ± 0.00	0.24 ± 0.02	0.15 ± 0.25	0.05 ± 0.07

Table 4. Root- shoot attributes of branch cuttings (± standard error) of *D.elegans* as influenced by exogenous auxin applications in the month of November

Parameter	Treatment											
	Control	IAA 100 ppm	IAA 200 ppm	IAA 500 ppm	IBA 100 ppm	IBA 200 ppm	IBA 500 ppm	NAA 100 ppm	NAA 200 ppm	NAA 500 ppm	2,4-D 100 ppm	2,4-D 500 ppm
Shoot no. per cutting	4.00 ± 0.00	6.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.82	3.80 ± 0.48	4.00 ± 0.10	3.30 ± 0.53	3.40 ± 0.88	3.00 ± 0.52	3.00 ± 0.58	2.40 ± 0.00	2.30 ± 0.43
Root no. per cutting	9.50 ± 1.50	18.00 ± 0.00	8.50 ± 1.50	11.80 ± 2.02	21.30 ± 1.79	16.00 ± 5.32	13.00 ± 1.81	6.00 ± 2.52	11.30 ± 3.74	11.00 ± 5.01	5.00 ± 0.00	3.00 ± 0.00
Root/shoot ratio	0.97 ± 0.00	0.48 ± 0.00	0.18 ± 0.00	0.49 ± 0.00	0.28 ± 0.00	0.30 ± 0.00	0.27 ± 0.00	0.76 ± 0.00	0.90 ± 0.00	0.39 ± 0.00	0.37 ± 0.00	0.60 ± 0.00

means in *D. elegans.* This may be due to mobilization of reserve food materials under low temperature (Wallace & Harrison 1977). *Desmodium elegans* is naturally distributed at high altitudes which favour best rooting at low temperature. During November until the last week of January and then early February to May, the average air temperatures at the experimental site were 19.0 °C and 26.3 °C respectively, which were found suitable for root initiation.

Table 5. Analysis of variance (*F*- test) for various growth parameters recorded for *Desmodium elegens* in the month of February and November

Parameter	*F*-values	
	February	November
Sprout no.	6.2**	3.9**
Root no.	4.7**	9.8**
Shoot length	6.5**	2.4*
Root length	2.1*	12.3**
Root dry weight	3.6**	5.5**
Shoot dry weight	8.9**	21.3**
Root nodules	7.4*	-

** Significent at 1%,
* Significent at 5%,
Nodulation was not recorded in winter planting.

Although seasonal rooting response in cuttings is basically a function of auxin produced and cambial activity, it is also governed by growth regulator treatments. A balance between auxin and nutrition is necessary for the purpose (Nanda & Dhaliwal 1974). In February planting, branch cuttings had (data not shown here) sufficient reserve food materials and thus could root more rapidly even in the control. Interestingly in this species, the results for non-treated cuttings were comparable with the treated ones. Similarly, at the end of growing season, i.e. September-October, sufficient food accumulates in the branches and, therefore, rooting was also observed in the winter season. Hence, it can be concluded that seasonal stimuli play an active role in root induction in *D. elegans,* besides exogenous auxin treatments.

As far as nodulation in this species is concerned, nodulation per plant was recorded in February and was higher in May and August plantings. However, the winter set of stem cuttings did not show nodulation although there was profused rooting. Lack of nodulation in winter may be due to the low temperature at the experimental site. Various other works have shown that nodulation in legumes is seriously retarded at low temperature (Jones *et al.* 1971, Hely *et al.* 1980), and 20-30 °C is the most suitable temperature for nodulation in temperate region (Gibson & Jordan 1983). Apart from this fact, altitude also effects nodulation. Sharma and Purohit (1978) have shown that nodulation per plant decreased with increase in altitude. We recorded the highest values of nodules per cutting in the

May and August and the lowest in the February plantings. On the other hand, nitrogen percentage was higher in the February planted cuttings than in those of May and August. Moreover, branch cuttings treated with IBA or IAA had comparatively higher nitrogen percentages then the branch cuttings treated with the other growth regulators, suggesting that growth regulators affect both nodulation and nitrogen percentage.

Acknowledgement

The third author (N.P. Todaria) gratefully acknowledges the financial support from the Ministry of Environment and Forests, Government of India, New Delhi.

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