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Vegetative Propagation of *Albizia amara* through Stem Cuttings and Biochemical Status during Rooting

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ABSTRACT

Albizia amara is one of the species used widely in different agroforestry systems in the semi arid region particularly under silvipastoral systems. The present investigation revealed that IBA was more effective compared to NAA in inducing rooting in the stem cuttings of this species. The best treatment for inducing rooting was IBA 200 ppm. The biochemical status of the stem cuttings during different stages of rooting play an important role in the success rate for that species. The C/N ratio is very crucial factor in initial stages and higher the ratio higher is the success rate. The total sugar reduced with the rooting as it was utilized as a source of energy during root initiation.

Key Words : *Albiza amara, biochemical changes, vegetative propagation*

1. INTRODUCTION

The choice of a species is very important for developing any agroforestry model. *Albizia amara* is one of the species used widely in different agroforestry systems in the semi arid region particularly under silvipastoral systems (Roy et al., 1998; Rai et al., 1999). The *A. amara* belongs to family Papilionaceae and produce an excellent fuel with high calorific value (Troup, 1921) even in rainfed situations, it is highly drought resistant fuelwood species. The wood is lustrous and gives smooth finish. Although the propagation of the species through seed is easy but for establishing seed orchards and maintaining genetic purity of elite trees propagation of any species through vegetative methods is essential. Very few reports are available in literature regarding vegetative propagation of *A. amara* (Handa et al., 2001). In this regard, an attempt has been made to study the influence of season and donor plant on vegetative propagation of the species.

2. MATERIALS AND METHODS

The experiment was conducted in the nursery of National Research Centre for Agroforestry, Jhansi, situated between latitude 24° 11' - 26° 27' N and longitude 78° 17' - 81° 34' E at about 275 m above mean sea level.

2.1 Rooting Studies

The cuttings were taken from the mature trees (more than 10 years old) during spring(February), summer (April) and rainy seasons (July) during 2000 and 2001. The stem cuttings were 20 cm in length so as to include three-four nodes and 0.5 to 1.5 cm in diameter. These cuttings were treated with different concentrations (100, 200, 400, 600, 800 and 1000 ppm) of Indole-3- Butyric Acid (IBA) and (ii) Napthalene Acetic Acid (NAA) for 12 hours. Stem cuttings were dipped in distilled water for the same period were taken as control. After treatment with growth regulators the stem cuttings were planted in polybags in complete randomised design with three replications. Each treatment has 20 cuttings.

2.2 Biochemical and Nutrients Analysis

2.2.1 Collection of Materials

In order to estimate the level of total sugar, starch, carbohydrates, C/N ratio, a random sample of five basal segments measure 2.5 cm length were collected at the time of cutting plantation, just at the root initiation stage and root completion. These segments were chopped and dried in an oven at 80°C. Oven dried samples were grinded and stored. For analysis of peroxidase, fresh samples were collected from random samples of 10 basal segments at 0 day and just at the root initiation stage and were stored in refrigerator at 0° C before using them for estimation.

2.2.2 Alcoholic Extraction for Analysis of Carbohydrate and Phenols

Oven dried sample weighing one gram was boiled in a water bath with10 ml of 80% Ethanol for about 10 minutes. After cooling, the supernatant was decanted and the alcoholic extract was filtered through Whatman No. -1 filter paper and the final volume was made to 25 ml with alcohol. This extract was used for the estimation of total sugar and residue was used for the determination of starch.

2.2.3 Estimation of Sugars

Total sugars were estimated with phenol - sulphuric acid method. One ml of the alcoholic extract was taken in a test tube to which one ml of 5 per cent phenol solution was added. Five ml concentrated sulphuric acid was added to this by allowing the chemicals to run down by the sides of the tube and mix thoroughly. Thereafter the

Table. 1. Effect of different growth regulator treatments on sprouting and rooting of *A. amara*

Concentration (ppm)	Sprouting percent			Rooting percent			Shoot length (cm)			Root length (cm)		
	IBA	NAA	Mean	IBA	NAA	Mean	IBA	NAA	Mean	IBA	NAA	Mean
100	71.33	59.33	65.33	51.33	41.33	46.33	19.60	20.30	19.95	9.20	9.30	9.25
200	93.33	69.66	81.49	81.66	53.66	67.66	24.30	17.50	20.90	16.20	9.60	12.90
400	71.66	75.33	73.49	63.33	57.66	60.49	31.50	25.40	28.45	21.50	14.50	18.00
600	51.33	43.66	47.49	41.66	28.66	35.16	27.50	16.30	21.90	15.20	11.50	13.35
800	39.66	36.33	37.99	16.66	9.33	12.99	16.20	9.20	12.70	9.60	6.30	7.95
1000	11.33	16.33	13.83	0.00	0.00	0.00	11.50	7.50	9.50	0.00	0.00	0.00
Mean	56.44	50.10		42.44	31.77		21.76	16.03		11.95	8.53	
Control	43.33			16.99			8.50			5.80		

CD (5%) : Sprouting : Concentration : 7.33; Growth regulator : 4.36; Growth regulator x concentration : 6.21
Rooting : Concentration : 12.45; Growth regulator : 5.32; Growth regulator x concentration : 4.67
Shoot length : Concentration : 3.25; Growth regulator : 0.89; Growth regulator x concentration : 3.62
Root length : Concentration : 2.71; Growth regulator : 1.67; Growth regulator x concentration : 3.01

tube was cooled under the running tap water. The intensity of the yellow orange colour was measured at 490 nm wavelength with a Spectronic - 20 Colorimeter. The amount of sugar was calculated by using a standard curve prepared from glucose and expressed in per cent on dry weight basis.

2.2.4 *Estimation of Starch*

The residue was hydrolyzed with 5 ml of distilled water and 6 ml of 52 % per chloric acid. The contents were shaken for thirty minute and filtered after adding 20 ml of distilled water and finally the volume was made to 100 ml. The sugars in the sample were determined by phenol - sulphuric acid method and the starch content was calculated by using the conversion factor 0.9 to convert the value of glucose to starch. Total carbohydrate content was calculated by adding total sugar and starch percentage and expressed on dry weight basis.

2.2.5 *Peroxidase Activity*

To three ml of 0.05 M Pyrogallol solution prepared in 0.1 M phosphate buffer (pH 6.0), 0.1 ml of the enzymes extract was added in to a cuvette. The absorbance was adjusted at 0 at 420 nm in colorimeter. The change in absorbance was measure at 20 second interval for three minutes. The average change in absorbance per 20 second between 40 and 160 seconds was calculated to plot peroxidase activity. Peroxidase activity was expressed in change in absorbance per minute per mg protein (^{a}420/min/mg protein).

2.2.6 *Carbohydrate/Nitrogen(C/N) Ratio*

The Nitrogen was estimated by MicroKjeldhal method (Jackson, 1973). The C/N ratio was calculated by dividing carbohydrate with nitrogen percentage for the corresponding sample.

3. RESULTS AND DISCUSSION

3.1 Rooting Studies

The data recorded on the rooting and related parameters of the species are presented in Table 1. The data obtained for this species revealed that the maximum sprouting, rooting, shoot length and root length were obtained in the cuttings treated with IBA (56.44 %; 42.44 %; 21.76 cm and 11.95 cm, respectively) compared to NAA (50.10 %; 31.77 %; 16.03 cm and 8.53 cm, respectively), clearly exhibited the supremacy of IBA over NAA in the overall success rate for rooting and related parameters in this species. The results showed that IBA is significantly superior over NAA for all the parameters. However, both the growth regulators observed to be statistically superior compared to control for all the parameters studied.

The results exhibited significant differences among the different concentrations of the growth regulators. The maximum sprouting(81.49 %) was recorded for 200ppm concentration, which differs significantly compared to all other concentrations. The minimum sprouting was recorded with 1000ppm (13.83 %), which differed significantly from all other treatments. The rooting results exhibited that maximum rooting (67.66 %)was achieved with the treatment of 200ppm. The cuttings treated with 1000ppm concentration failed to root and 800 ppm concentration resulted in only 12.99% success for rooting. All these treatments were significantly different from each other. The data for shoot and root length observed the

similar pattern and maximum value for shoot and root length were recorded with 400 ppm (28.45 and 18.00 cm, respectively), which were significantly superior compared to all other treatments. The minimum shoot and root length (9.5and 0.0cm, respectively) were recorded for 1000ppm, which was significantly lower over other treatments.

The growth regulator and concentration interaction studies exhibited that IBA 200 ppm treatment recorded maximum value for sprouting (93.33%) and rooting (81.66%) and differed significantly from all other treatments. The maximum values for shoot and root length were recorded with IBA 400 ppm (31.50 and 21.50 cm, respectively). This treatment differed significantly from all other treatments. The minimum values for sprouting (11.33%), rooting (0.0 %), shoot length (7.5cm) and root length (0.0cm) were recorded with IBA 1000 ppm, IBA & NAA 1000 ppm , NAA 1000 ppm and IBA & NAA 1000 ppm, respectively .These treatments differed significantly compared to other treatments.

In the present studies increased rooting percentage compared to control may be attributed to the fact that auxins play multifarious roles related to the division and elongation of meristem, differentiation of cambial initials into root primordia and the mobilisation of reserve food materials by enhancing the activity of hydrolysing enzymes. Role of auxins in enhancing callusing and rooting has been reported by Nanda et al., (1974) and Loach (1988). According to Kralik and Sebanek (1983) auxin treatment cause considerable cell elongation and proliferation in the cortex, phloem and cambium and results in breakage of continuous sclerenchymatous rings. According to Haissig (1986), auxins induce hydrolysis and mobilisation of nutritional factors to the site of application, promoting thereby, root initiation.

3.2 Biochemical Analysis

The changes occurring in the status of biochemicals within the stem cuttings at different stages of rooting revealed that the starch percentage which was higher initially at the time of planting (2.23 %) of stem cuttings reduced at the initiation of rooting(2.09 %) and further reduced at the completion of rooting (2.01 %) and sugar content which was 1.67 per cent initially increased to 1.72 at root initiation required due to more energy requirements for rooting and reduced to 1.49 percent at completion of rooting as it was consumed during rooting. The starch percentage reduced as it was used as energy source. The C/N ratio reduced from initial value of 18.1 to 14.0 at the completion of rooting. The higher C/N ratio is essential for initiating rooting in the species and once rooting is initiated the ratio declines due to consumption of carbohydrates as food material during rooting. Similarly, the peroxidase enzyme activity is higher at initial stage 0.75 and resulted in root initiation and reduced to 0.61 at the completion of rooting.

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