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Full Length Research Paper

Influence of Culture Media and Explant Origin on the Micropropagation Efficiency of *Dalbergia sissoo* Roxb.: A Key Multipurpose Forest Species

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Attempts were made to evaluate the suitable explant and media type for micropropagation of *Dalbergia sissoo* from axillary bud explants of epicormic and coppice shoots origin. Murashige and Skoog (MS) medium and Woody Plant medium (WP) supplemented with N^6 –benzyl adenine (BA) either alone or in combination with indole-3-acetic acid (IAA) were used for plantlet production. Auxilary buds of coppice shoots origin responded better than of epicormic shoot origin. Thus, the axillary buds of coppice shoot origin exhibited maximum response (84.6 %) on the medium with MS + 6.6 ∝M BA + 1.14 ∝M IAA where an average of 8.38 shoots/explant with 5.87 cm shoot length/shoot was recorded. MS medium was found to be superior to WP medium in terms of shoot numbers and shoot length. Rooting of *in vitro* shootlets was achieved on half strength MS medium supplemented with either IAA or indole-3-butyricacid (IBA). Rooting was best in the medium supplemented with 7.5 ∝M IBA. Rooted plantlets were acclimatized with 70 % survival rate.

Keywords: *Dalbergia sissoo,* axillary bud, epicormic shoots, coppice shoots, rooting.

INTRODUCTION

Over the years the pressure on forest and tree-based resources have tremendously increased resulting in wide-scale felling of trees and depletion of natural forest cover to a level which poses serious environmental consequences for the very subsistence of human population. Therefore it is imperative to augment propagation and breeding techniques of forest/tree species to foster environmental improvement and thus, meet the market demand of wood and wood products. For many years plant tissue culture propagation methods involving regeneration of plantlets from existing meristem MS- Murashige and Skoog IBA- indole-3-butyric acid IAA- indole-3-acetic acid or from adventitious meristem, have been developed and discussed as topics of primarily academic interest.

However, in recent times perspectives have changed and spectacular progress has been achieved on successful commercial-scale exploitation of tissue culture techniques to forest tree improvement (De Gyves et al., 2007; Rathore et al., 2007). *Dalbergia sissoo* Roxb., commonly known as *Sissoo* (family: Fabaceae), is a moderately fast-growing multipurpose deciduous tree. Because of its wide range of economic and ecological uses the species is widely planted in its natural habitat and also elsewhere as an exotic (Tewari, 1994). Sissoo is best known internationally as a premier timber species, primarily used for durable furniture making with excellent finishing colour and smoothness. Due to high caloric content the wood is as an excellent source of fuelwood and charcoal. It is valued for its ability to increase soil fertility through fixation of atmospheric nitrogen and is commonly planted as an ornamental, windbreak and shade tree.

Since tree species are out breeders there is a large amount of genetic variation in any seed raised population

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Abbreviations:

BA- N^6-benzyl adenine

(Jones and Van Standen, 1997). Cloning of mature trees is generally preferred over seed raised trees population (Bonga, 1987). There are a few reports on *in vitro* propagation of *D. sissoo*, but most of the authors used seedling explants such as cotyledonary nodes (Pradhan et al., 1998), zygotic embryo (Chand and Singh, 2005) and cotyledons (Singh and Chand, 2010). A major detriment in using such seedling explants is that the superiority of the explants is unknown. The objectives of the current study therefore were to investigate the potential of mature explant on *in vitro* regenerative potential of *D. sissoo*. In order to ascertain the suitable explants for cloning of elite lines of *D. sissoo*, we used two types of explant originated from epicormic and coppice shoots. Several micropropagation techniques such as somatic embryogenesis, organogenesis, and axillary shoot proliferation have been applied for *in vitro* propagation of woody plants. Among these methods, axillary shoot proliferation is the most widely used for clonal propagation. We report here the effects of explant types and culture media on the plant regeneration potential of axillary bud explants of *D. sissoo*.

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MATERIALS AND METHODS

Study site and source materials

The study was undertaken at the Institute of Minerals and Materials Technology, Bhubaneswar, India, situated at 20^0 17 45" N latitude and 85^0 49 15" E longitudes. Out of the total annual rainfall of 1500 mm, 75 % is generally recorded between July and September. The mean temperature of the region varies from a maximum of 42 - 44 ^{0}C in April-May to a minimum of 12^0C in December. Explants for the *in vitro* culture experiments were collected from the selected stock plants of *D. sissoo* maintained in the experimental garden of the Institute.

Stem nodes of *D. sissoo* were collected from actively growing shoots from epicormic and coppiced tree trunk. The explants were thoroughly washed in running tap water using 5% v/v aqueous solution of Labolene (Qualigen, India), followed by ringing 5 to 6 times in double distilled water. The explants were surface sterilized with 0.1% w/v aqueous solution of $HgCl_2$ for 3 to 4 minutes and then rinsed 4 to 5 times with autoclaved double distilled water. The surface sterilized nodal explants were further trimmed (0.8-1.2 cm) before inoculating onto nutrient media. Surface sterilization and trimming operations were performed in a laminar air flow cabinet (Thermadyne, India).

Nutrient media preparation and culture conditions

The formulation of Murashige and Skoog (1962) basal medium (MS) and Woody Plant (WP) medium (Mc Cown and Lloyd, 1981) were used throughout this study. All basal salts, sucrose and agar were procured from Qualigens Fine Chemicals, India. For shoot induction, the media were further supplemented with growth regulators such as BA (6-benzyl adenine; 2.2-8.8 ∝M) and IAA (indol-3-acetic acid; 0.57-1.14 ∝M) either alone or in combination. For root induction, half-strength MS basal medium supplemented with either of IAA (2.85-11.4 ∝M) or IBA (indole-3-butyric acid; 2.45-9.8 ∝M) were used. Growth regulators (BA, IAA and IBA) were bought from Sigma Chemical Co., St. Louis, USA. The pH of media was adjusted to 5.8 prior to gelling with 0.8% (w/v) agar (Qualigen, India), and then autoclaved at 121° C and 104 k Pa for 15 min. Depending on the requirement, media were dispensed into either 150 mm X 25 mm test tubes (15 ml/tube) or in 250 ml Erlenmeyer flasks (50 ml/ flask). All the cultures were incubated in the culture room maintained at 25 ± 2 °C under 16/8 h light/dark cycle, 45 ∝mol m^{-2} s^{-1} irradiance level provided by cool white fluorescent tubes (Philips, India) with 55 – 60 % RH.

Acclimatization

Plantlets obtained after *in vitro* rooting were carefully removed from the agar medium, washed thoroughly in water to remove agar. After dipping the roots in 0.5 g l^{-1} fungicide solution (Bavistin, BASF, India) for 1 min., the plantlets were transferred to pots containing sterilized vermiculite (TAMIN, India). The potted plantlets were maintained in a temperature controlled environmental chamber for 3 weeks.

Statistical analysis

For shoot regeneration each treatment consisted of 13 replicates and repeated thrice. Data on percent response and the number of shoots per explant were determined after a period of 7 weeks following culture initiation. For root induction each treatment consisted of 15 replicates and repeated thrice. Data on percent rooting and root number were determined after 5 weeks of culture. All experimental data were subjected to analysis of variance (ANOVA) for a completely randomized design (CRD). Duncan's new multiple range test (DMRT) (Gomez and Gomez, 1984) was used to separate the means to determine significant effects.

RESULTS

Cultures response to media type

Effects of MS and WP media on the mean shoot induction rate of axillary buds reared from epicormic and coppice shoots are presented in Table 1 and 2. Both the media supported shoot induction but the cultures grown on MS medium exhibited better response than the cultures grown in WP Medium. Analysis of the data revealed that when axillary buds were cultured in MS medium supplemented with growth regulators, maximum response of about 85 % was achieved with average shoot number of 6.62 and 8.38 from epicormic and coppice shoot explants, respectively (Table 1 and 2). On the other, when axillary buds from epicormic and coppice shoots were reared on WP medium supplemented with growth regulators, a maximum response of 76.9 % and 84.6 % with an average shoot number of 4.62 and 6.69 respectively was observed (Table 1 and 2).

Response of nodal explants derived from epicormic shoots

Nodal explants from epicormic shoots grown on MS medium and supplemented with BA (6.6 ∝M) and IAA

Table 1. Influence of different levels of BA and IAA on shoot development of *D. sissoo* axillary buds derived from epicormic shoots.

Basal medium + Growth regulators (∝M)		% Response	Days to shoot initiation	Mean shoot numbers	Mean shoot length (cm)
1) MS medium					
BA	**IAA**				
0	0	-	-	-	-
2.2	0	30.8	14-16	0.46 g	0.55 g
4.4	0	53.8	14-16	0.92 efg	0.82 eg
6.6	0	61.5	14-16	1.38 defg	1.05 eg
8.8	0	53.8	14-16	1.31 defg	0.86 eg
2.2	0.57	30.8	14-16	1.08 efg	1.18 eg
4.4	0.57	61.5	12-15	2.15 cdefg	2.13 deg
6.6	0.57	69.2	12-15	3.23 bcd	2.52 cde
8.8	0.57	61.5	12-15	2.62 cdef	2.18 deg
2.2	1.14	53.8	12-15	2.69 cde	3.45 bcd
4.4	1.14	61.5	12-15	4.00 bc	3.98 abc
6.6	1.14	84.6	12-15	6.62 a	5.42 a
8.8	1.14	69.2	12-15	4.92 b	4.45 ab
2) Woody Plants Medium					
BA	**IAA**				
0	0	-	-	-	-
2.2	0	23.07	16-18	0.38 d	0.42 e
4.4	0	46.1	16-18	0.77 d	0.69 e
6.6	0	53.8	16-18	1.00 d	0.86 e
8.8	0	46.1	16-18	0.85 d	0.78 e
2.2	0.57	30.8	16-18	0.92 d	0.88 e
4.4	0.57	53.8	15-17	1.69 d	1.87 de
6.6	0.57	61.5	15-17	1.92 cd	2.15 cde
8.8	0.57	53.8	15-17	1.85 cd	1.94 de
2.2	1.14	46.1	15-17	2.08 cd	3.02 abcd
4.4	1.14	61.5	15-17	3.54 bc	3.88 abc
6.6	1.14	76.9	15-17	4.62 a	4.69 a
8.8	1.14	61.5	15-17	3.92 ab	4.00 ab

1.Data pooled from three independent experiments each with 13 replicates per treatment.
2.Data collected after 7 weeks of culture.
* Mean values within column followed by the same letter are not significantly different ($p \leq 0.05$; Duncan's New Multiple Range Test).

(1.14 ∝M) showed a maximum response of 84.6 %. Maximum number of shoot (6.62) and the highest shoot length of (5.42 cm) were also observed in the same combination of culture condition. The next best response (69.9 %) was obtained in the combination of MS medium + 8.8 ∝M BA + 1.14 ∝M IAA, where an average number of 4.92 shoots/explant with a mean shoot length of 4.45 cm was recorded (Table 1). In case of the explants cultured on WP medium highest response of 76.9 % was achieved in the medium supplemented with BA (6.6 ∝M) and IAA (1.14 ∝M) where the average number of shoots produced was 4.62/explant with a mean shoot length of 4.69 cm. Explants cultured in both the media types supplemented with BA alone exhibited poor response producing lesser number of shoots. Results indicate that by increasing BA concentration shoot bud formation from the explants increased up to 6.6 ∝M, showing a positive correlation between BA and shoot number after which it starts declining with further increase in BA concentration (Table 1). Callusing of explant base was a common feature on both the media (MS and WP). The main problem faced was the exudation of phenolic substances immediately after 3rd day of culture followed by browning of explants. Transfer of explants onto fresh medium on every 3rd day of the culture could control this problem to some extent. Usually three transfers were enough to stop such exudation.

Response of nodal explants derived from coppice shoots

Nodal explants originated from coppiced shoots exhibited better shoot growth with increased number of

Table 2. Influence of different levels of BA and IAA on shoot development of *D. sissoo* axillary buds derived from coppice shoots.

Basal medium + Growth regulators (∝M)		% Response	Days to shoot initiation	Mean shoot numbers	Mean shoot length (cm)
1) MS medium					
BA	**IAA**				
0	0	-	-	-	-
2.2	0	30.8	10-12	0.85 ef	0.64 f
4.4	0	53.8	10-12	1.23 ef	1.04 ef
6.6	0	76.9	10-12	2.38 def	2.02 def
8.8	0	69.2	10-12	1.46 ef	1.12 ef
2.2	0.57	30.8	9-12	1.46 ef	1.25 ef
4.4	0.57	61.5	9-12	2.31 def	2.12 def
6.6	0.57	76.9	9-12	4.85 bc	3.56 bcd
8.8	0.57	69.2	9-12	3.08 bcde	2.51 cdef
2.2	1.14	53.8	8-10	3.54 bcde	3.02 bcde
4.4	1.14	61.5	8-10	4.08 bcd	4.30 abc
6.6	1.14	84.6	8-10	8.38 a	5.87 a
8.8	1.14	69.2	8-10	5.00 b	4.56 ab
2) Woody Plants Medium					
BA	**IAA**				
0	0	-	-	-	-
2.2	0	30.8	12-14	0.54 f	0.33 f
4.4	0	38.5	12-14	0.69 f	0.72 f
6.6	0	76.9	12-14	1.62 def	1.18 def
8.8	0	38.5	12-14	0.77 f	0.72 f
2.2	0.57	30.8	12-14	1.15 ef	0.79 f
4.4	0.57	61.5	12-14	1.69 def	1.73 def
6.6	0.57	76.9	12-14	4.15 bc	2.72 bcd
8.8	0.57	69.2	12-14	2.38 cdef	1.56 def
2.2	1.14	53.8	12-14	3.00 bcde	2.48 bcde
4.4	1.14	61.5	10-12	3.46 bcd	3.92 abc
6.6	1.14	84.6	10-12	6.69 a	5.02 a
8.8	1.14	69.2	10-12	4.46 b	4.10 ab

1.Data pooled from three independent experiments each with 13 replicates per treatment.
2.Data collected after 7 weeks of culture.
* Mean values within column followed by the same letter are not significantly different ($p \leq 0.05$; Duncan's New Multiple Range Test).

shoots/explant in comparison to nodal explants of epicormic shoots. Bud break (Figure 1a) was first noticed after 8 days of culture and at the end of 12th day all the treatment showed bud break (Table 2). During the first 2 weeks of establishment, axillary bud development was followed by callus initiation at the base of the explant (Figure 1b). Hence after the third week the calli were trimmed off from the explants and the explants with the growing shoots were sub-cultured on to the same medium composition. Shoot elongation was noticed after one week of sub-culture. The optimum response was achieved when the MS medium had BA (6.6 ∝M) + IAA (1.14 ∝M), where about 84.6 % culture responded and the maximum number of shoots per explant was 8.38 with a mean shoot length of 5.87 cm (Table 2). The next best response was observed in the medium composition of MS + BA (8.8 ∝M) + IAA (1.14 ∝M) in which an average of 5.0 shoots per explant with a mean shoot length of 4.56 cm was recorded. The results further revealed that BA alone at lower concentration was not effective. Axillary buds showed a maximum response of 84.6 % with an average number of 6.69 shoots/explant and mean shoot length of 5.02 cm when cultured on the medium composition of WP + BA 6.6 ∝M + IAA 1.14 ∝M. Further, with increase in concentration of BA beyond 1.5 mg L^{-1} there was a distinct decline in percent response as well as number of shoots produced per explants (Table 2). After 7 weeks of culture the shoots attained about 4-5

Table 3. Influence of different levels of IAA and IBA on rooting response of *in vitro* generated shoots from axillary bud culture of epicormic and coppice shoot origin

½ MS + Growth regulators (∝M)		% Response	Days to root initiation	Mean root numbers	Mean root length (cm)
IAA	**IBA**	**Shootlets derived from epicormic nodal culture**			
0	0	-	-	-	-
2.85	0	-		-	-
5.7	0	33.33	12-14	0.5 a	0.98 b
8.55	0	33.33	12-14	0.7 a	1.15 b
11.4	0	53.33	12-14	1.0 a	1.74 ab
0	2.45	33.33	10-12	0.6 a	1.07 b
0	4.9	46.67	10-12	0.9 a	1.59 ab
0	7.35	66.67	10-12	1.3 a	2.40 a
0	9.8	53.33	10-12	0.8 a	1.39 ab
IAA	**IBA**	**Shootlets derived from coppice shoots nodal culture**			
0	0	-	-	-	-
2.85	0	13.3	12-14	0.2 f	0.31 e
5.7	0	40	12-14	0.8 e	1.21 cde
8.55	0	60	10-12	1.4 c	1.88 abcd
11.4	0	60	9-10	1.1 d	1.41 bcd
0	2.45	40	8-10	0.9 de	1.39 bcde
0	4.9	60	8-10	1.7 b	2.62 ab
0	7.35	80	8-10	2.4 a	3.06 a
0	9.8	66.67	8-10	1.5 bc	2.24 abc

1. Data pooled from three independent experiments each with 15 replicates per treatment.
2. Data collected after 5 weeks of culture.

*Mean values within column followed by the same letter are not significantly different ($p \leq 0.05$; Duncan's New Multiple Range Test).

cm long (Figure 1c); such shoots were reared and transferred to root induction medium.

In vitro rooting

Root induction studies were carried out in MS medium. Shootlets of epicormic origin when cultured in the medium containing half-strength MS basal salts supplemented with 7.35 ∝M IBA showed a maximum of 66.67 % response. (Table 3). Depending upon the auxin concentration root initiation was noticed between 10-14 days after culture. The earliest response was in the IBA supplemented media where root initiation was observed after 10 days of inoculation. Root initiation proceeded with the formation of callus at the bottom from where root numbers varying between 1and 3 were produced per explant. Mean root numbers and root length were better when the cultures were maintained in the medium with a composition of half-strength MS basal salts supplemented with 7.35 ∝M IBA where an average number of 1.3 roots/shootlet with a mean length of 2.40 cm was recorded. IAA at 11.4 ∝M gave a maximum of 53.33 % response with an average root numbers of 1.0 and a mean root length of 1.74 cm (Table 3).

Shoots originated from the coppice shoot nodal culture showed comparatively better response than the shootlets originated from epicormic shoots. A maximum of 80 % rooting was recorded in the medium supplemented with 7.35 ∝M IBA followed by 66.67 0% response in the medium supplemented with 9.8 ∝M IBA (Table 3). Root initiation occurred between 8-14 days of culture. Roots varying from 1 to 4 in numbers were produced from each shootlet (Fig 1d). Maximum number of roots per explant was high in the medium supplemented with 7.35 ∝M IBA where an average of 2.4 root numbers with a mean root length of 3.06 cm was obtained. Cultures in the IAA supplement medium produced a maximum of 1.4 roots when the shoots cultured on 8.55 ∝M concentration of IAA. The excised shoots did not show any sign of root development in an auxin-free half strength MS medium even after 6 weeks of culture. Rooted plantlets were successfully acclimatized (Figure 1e) and maintained under a shade house for future use.

DISCUSSIONS

In the present study, two types of media viz MS and WP were used for identifying suitable medium for optimal response. We observed that cultures were more responsive in MS basal medium than in WP medium. In

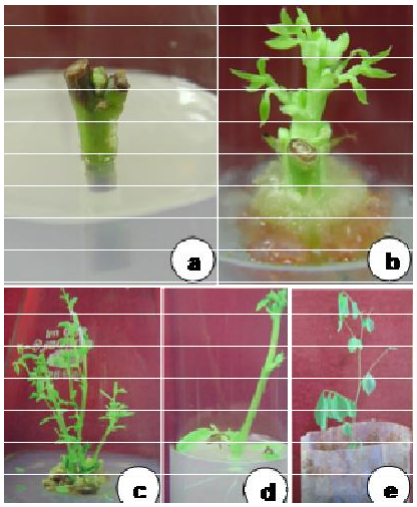

Figure 1. Plant regeneration from axillary bud (coppice shoot origin) culture of *Dalbergia sissoo* Roxb.

Captions to Figure 1

a. Axillary bud of coppice shoot origin cultured on MS + 6.6 ∝M BA + 1.14 ∝M IAA showing bud break on 8th day of culture.
b. Axillary bud of coppice shoot origin cultured on MS + 6.6 ∝M BA + 1.14 ∝M IAA showing shoot development and callus formation at the end of 2 week culture period.
c. Well developed shoots on the same medium after 7 weeks of culture.
d. Microshoot rooted on ½ MS + 7.35 ∝M at the end of 5 week culture.
e. An acclimatized plant ready for out planting.

general, tissue growth and the quality of morphogenetic responses are strongly influenced by the type and concentrations of nutrients present in the culture media (Niedz and Evens, 2007). Effect of different macronutrient formulations on number and growth of *in vitro* cultured explants was investigated by many workers (Cohen, 1995; Rout et al., 1998; Thirunavoukkarasu and Debata, 2002). Mc Cown and Sellmer (1987) reported that some species give analogous response in all media while others show preference for a specific medium for explant establishment and growth. The most widely used culture medium is MS medium, because most plants respond to it favorably. It is classified as a high salt medium in comparison to many other formulations, with high levels of nitrogen, potassium and some of the micronutrients, particularly boron and manganese (Cohen, 1995).

It has been shown previously that explant juvenility is an essential condition for the successful *in vitro* culture of woody adult plants (George, 1993). Multiplication of shoot bud explants of both tropical and temperate trees is easier if the explants are derived from germinated seedlings and said to be juvenile in nature. But multiplication of trees through seeds is not a satisfactory means of conserving the characteristics of a desired clone because of strong heterozygosity of trees (Das and Mitra, 1990). To avoid such a situation explants were collected from newly formed shoots in the coppiced area of the mature plants (Roy et al., 1992). The results obtained in the present study clearly indicated that the response of nodal explants was dependent on the origin of the explants. Nodal explants obtained from coppice shoots were more responsive than the explants obtained from the epicormic shoots. This is obvious in the present investigation that the nodal explants of coppice shoot origin showed an overall response of around 57 % as against 53 % response in the nodal explants of epicormic origin indicating that coppice shoots are more juvenile than the epicormic shoots. In many hardwood species the stumps that remain mature, after trees are felled often produce juvenile sprouts which provide ideal explants for micropropagation (Menzies, 1992). This is in corroborated in the results obtained in the present investigation that a maximum response was achieved when the explants were of coppice shoot origin.

Low response in the explants of epicormic origin is due to exudation of pheonolic compounds from the cultured explants, which was comparatively less in the coppice shoots explants. This is in accordance with the results obtained in species like *Eucalyptus tereticornis, E. camaldulensis* and *Tectona grandis* (Das and Mitra,

1990; Gill and Gill, 1994; Devi et al., 1994). An important factor observed in the present investigation was that the nodal explants that were slightly tender and having active buds showed quicker and high rate of multiplication compared to hard nodal explants with brownish buds, which showed no sign of growth. A similar response was also reported in mulberry species (Chitra and Padmaja, 1999; Oka and Ohyama, 1975).

Frequency of shoot formation and further development were greatly influenced by the presence of auxin and cytokinin in the medium. We observed that MS medium without any growth regulator failed to elicit regeneration, but addition of BA proved to be significantly useful in multiple shoot production in axillary buds of *D. sissoo.* Although a number of cytokinins have been used for shoot induction studies, BA found to be the most ideal cytokinin as observed by many workers (Arumugam and Rao, 1996; Purohit and Dave, 1996; Pradhan et al., 1998; Nayak et al., 2007; Behera et al., 2008). In contrast to BA-enriched medium alone, we observed an improved response in the media enriched with BA and IAA. Cultures with such media composition increased the number of shoots per explant by fourfold. The requirement of growth hormones as a supplement for obtaining optimal response for sprouting and further shoot differentiation is well documented in other tree species such as *Aegle marmelose* (Hossain et al., 1995; Nayak et al., 2007), *Gmelina arborea* (Thirunavoukkarasu and Debata, 1998; Behera et al., 2008), *Khaya senegalensis* (Danthu et al., 2003) and *Acacia senegal* (Kaur et al., 1998).

Auxin supplement is an essential factor for obtaining rooted shoots in the present investigation. An auxin free medium did not support rooting on the cultured micro shoots of *D. Sissoo*. Concentration of auxin in the medium was found to be the critical factor in producing healthy roots. It was observed that low concentration of IAA (2.85 ∝M) on the shootlets of nodal explants origin completely failed to induce rooting. In comparison to IAA, IBA even at low concentration (2.45 ∝M) produced better rooting response. Pradhan et al. (1998) reported that the rooting of *in vitro* shootlets of *D. latifolia* was better at 9.8 ∝M of IBA. Ndoye et al. (2003) observed the highest rooting response at 19.6 ∝M concentration of IBA while working in *Balanites aegyptiaca*. However, in the present study IBA, even at 9.8 ∝M concentrations IBA showed inhibitory effect in all the experiments. It may also be worthwhile to mention that rooting of *in vitro* propagated shoots was achieved on the half strength MS medium supplemented with IAA or IBA. Initially full strength MS medium along with the auxins IAA and IBA was tried which proved to be ineffective so the strength was reduced to half strength. Our present results are in agreement with previous *in vitro* studies on *Acacia seyal*, where shootlets produced longer and normal roots on the half-strength MS medium while cultures on full-strength MS medium produced shorter and abnormal roots (Al-Wasel, 2000). Also there was a significant effect of the origin of explant on rooting of the culture generated shootlets. Thus about 42 % of the *in vitro* shootlets originated from coppice shoots and 32 % from the epicormic shoots responded for rooting respectively. Plants successfully rooted were acclimatized and established under greenhouse condition. The *in vitro* propagation protocols developed in the present study, thus, can be effectively utilized for producing elite planting materials of *D. sissoo*.

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