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THE EFFECT OF SEASON ON *IN VITRO* ESTABLISHMENT OF *DALBERGIA SISSOO* ROXB.

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ABSTRACT

Our study confined to observe the effect of season of explant collection on *in vitro* survival and rooting. *Dalbergia sissoo* is an important timber yielding species in India. From last decade, heavy mortality rate and slow propagation has threatened its existence. So rapid multiplication is the urgent need of hour. Season of explant collection plays a crucial role for shoot proliferation and establishment of cultures. Nodal explants were excised from five superior clones of *Dalbergia sissoo* and cultured *in vitro* using MS media supplemented with auxin and cytokinin. Explants were collected in two seasons i.e., S1 (April to June) and S2 (July to September). Maximum survival, bud response and rooting were observed in explants collected in the months of April to June. A significant variation of explant collection season was observed in establishment and rooting of cultures. Explants collected in month of July to September exhibited maximum contamination.

Keywords: *In vitro*, Clone, Season, Contamination, Survival.

INTRODUCTION

Dalbergia sissoo Roxb., commonly known as Shisham, belonging to the family Leguminosae, is one of the most important timber species because of its strength and durability (Thirunavoukkarasu *et al*., 2010). It is also reported that it is useful to cure diseases like gonorrhea, ulcers, dysentery, stomach troubles, skin diseases, blood diseases, nausea, nose disorders *etc*. (Bharath *et al*., 2013).

Heavy demand in timber industry, high mortality rate and deforestation are the major threats to the existence of *D. sissoo* (Sahu *et al*., 2014). During last few decades, high mortality rate in Shisham was observed. Thus, the multiplication at high rate is urgently required. The propagation of Shisham through conventional methods can be challenging due to issues like seed dormancy and slow growth. Although, the *in vitro* method of propagation offers promising alternative, still the season of explant collection can significantly impact the success of *in vitro* regeneration. The multiplication of *D. sissoo* through *in vitro* technique can enhance the rate of plantlet production (Awais *et al*., 2022).

The propagation through *in vitro* techniques is significantly influenced by several factors (Nanda and Anand, 1970; Hartmann *et al*., 1997). Season of explant collection has the positive influence on the survival rate for *in vitro* propagation of tree species (Uniyal *et al*., 1995, Chhajer and Kalia, 2017). Seasonal effect on explant viability and contamination rate was also noticed on *Pinus sylvestris* by Hohtola (1988). The seasonal effect of explant collection on the *in vitro* establishment of Shisham has been a subject of considerable research interest due to the importance of this plant in the timber industry. The seasonal effect of explant collection plays a crucial role in successful *in vitro* plant regeneration of *D. sissoo* (Nautiyal *et al*., 2022). The current work is to find out the seasonal effect of explant collection on the *in vitro* establishment of *D. sissoo*.

MATERIALS AND METHODS

The experiments were carried out at Plant Physiology Discipline of Forest Research Institute, Dehradun, India. *In vitro* experiments were carried out by aseptically culturing the nodal explants on MS media supplemented with plant growth regulators. The observations on bud response, survival rate and rooting were recorded in two seasons (SI-April to June & SII-July to September).

Collection Plant material and explant preparation

Five clones (C14, C46, C59, C86 and C114) of *D. sissoo* from diverse geographical regions were selected, based on their superior characters which were already established in Vegetative Multiplication Garden by Plant Physiology Discipline, Botany Division, situated at Forest Research Institute, Dehradun (Uttarakhand). C14 clone was collected from Haridwar, C46 from Gonda, U.P., C59 from Ambala, Haryana, C86 from Shergarh, Rajasthan, C114 from Hatauda, Nepal. Nodal explants were prepared from freshly collected plant material. The size of the explant ranged from 2.5 to 3.0 cm. Explants were treated with 0.1% Bavistin for 5 minutes. (Plate-1). After washing with distilled water, surface sterilization was carried out with different concentrations (0.05%, 0.10% and 0.15%) of $HgCl_2$ in laminar air flow cabinet. Lastly explants were rinsed with autoclaved double distilled water 3-4 times. For establishment of cultures, 24 replicates were used per treatment and each of them was repeated three times.

Plate-1: Surface Sterilization of explants

Establishment of cultures and induction of shoots

Surface sterilized explants were inoculated vertically in semisolid MS media supplemented with various concentrations of auxin (NAA) and cytokinin (BAP). All the cultures were incubated in a culture room and maintained at 26±1° C for 16 hours in light (illuminated by 40 watt cool white fluorescent tubes, 1200 lux) and for 8 hours in dark. After 20 days of inoculation data on survival rate was recorded.

In vitro shoot multiplication

The *in vitro* regenerated shoots were multiplied by repeated transfer (3-4 subculture cycles) of mother explants to fresh media. This is the most critical stage, which determines the success of micro propagation system. For multiplication of cultures, MS medium was supplemented with cytokinin. Multiplication rate was recorded in respect to gain in number of shoots per subculture cycle. (Plate-2)

Plate-2: Shoot proliferation

In vitro rooting of shoots

For *in-vitro* rooting micro shoots were excised and transferred to rooting medium (1/2 strength MS medium with 2% Sucrose and 0.7% agar). The medium was supplemented with auxin (IBA 0.5-1.5 mg/l). (Plate-3).

Plate-3: *In vitro* rooting

RESULTS

Seasonal effect on establishment

Nodal explants of five clones of *Dalbergia sissoo* showed great variability in respect of seasons. Bud break was achieved in 7-8 days in season I. (Fig.-1c). Bud response was also higher (67.5%) in season I, while in season II, bud response was lower (42.5%). Survival percentage ranged from 33.3% to 65%. In season I, 65% explants survived and in season II, minimum survival percentage was noticed. (Fig.-1a). Contamination rate was also found to be affected by season of explant collection as cultures were contaminated maximum in rainy season and minimum in winter months. Contamination percentage ranged from 29.1% to 43.3% being minimum in season I. (Fig.-1a). Maximum number of bud (2.4), shoot (1.6) and their length (2.19 cm) were observed in season I. (Fig.-1b). Statistical analysis revealed that variation among seasons was highly significant at 0.1% level for survival percentage. Bud response and contamination

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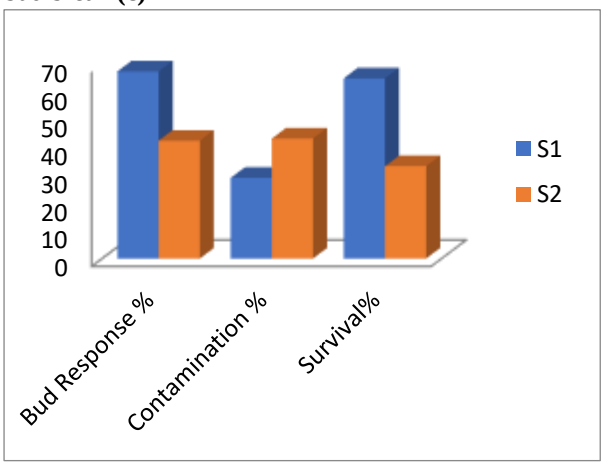
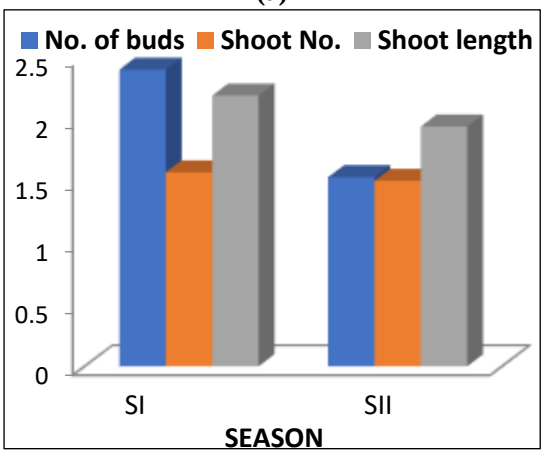
percentage were significant at 1% level for seasons. (Table-1).

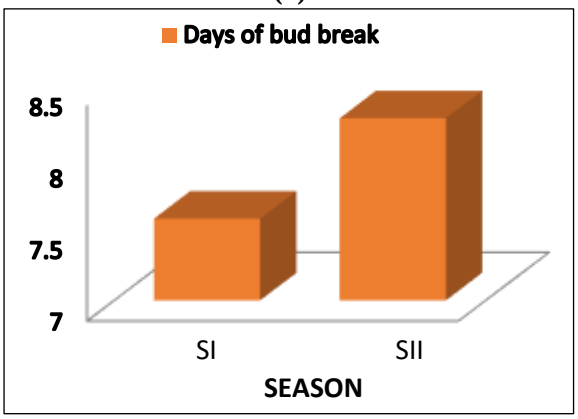
Figure-1: Effect of season on culture establishment- percent survival, bud response and contamination **(a),** numbers of buds, shoot and shoot length **(b),** days of bud break **(c)**



(a)

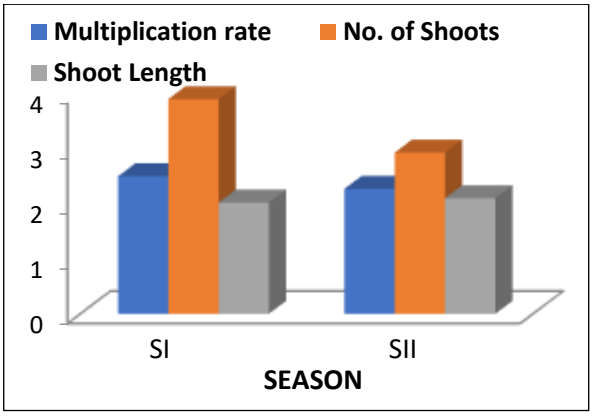


(b)



(c)

Figure-2: Seasonal variation on *in vitro* shoot multiplication



Seasonal effect on shoot multiplication

Multiplication rate

Analysis of variance revealed that the variation among season was insignificant for multiplication rate (Table-2). Maximum multiplication rate (2.5) was observed in season I and minimum (2.2) in season II (Fig.-2).

Mean number of shoots

Weak significant variation was seen at 5% level for number of shoots. (Table-2). Maximum number of shoots (3.9) was observed in season I and minimum number (2.9) of shoots was seen in season II (Fig.-2).

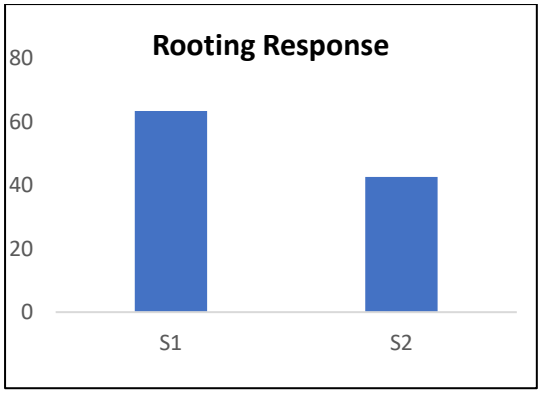
Shoot length

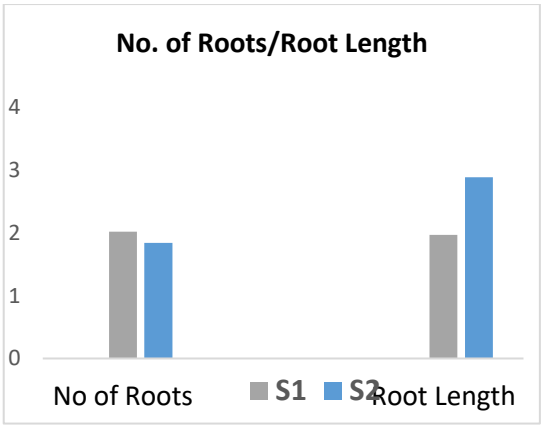
Maximum mean shoot length (2.22 cm) was reported in season I (Fig.-2). Analysis of variance revealed that the variation among season was insignificant for shoot length (Table-2).

Seasonal effect on rooting

Maximum rooting percentage (63.4%) was observed in season I. (Fig.-3a). Maximum (2.02) number of roots was obtained in season I, while maximum (2.89 cm) root length was attained in season II. (Fig.-3b). Statistical analysis revealed that highly significant seasonal variation (0.1%) was found for rooting percentage and root length. Number of roots showed insignificant seasonal variation. (Table-3).

Figure-3: Seasonal effect on *in vitro* rooting- rooting response % (a), number of roots and root length (b)





(a) **(b)**

Table-1: ANOVA for culture establishment (survival%, bud response % & contamination %)

Source of Variation	df	Mean sum of square		
		Survival %	Bud response %	Contamination %
Season	1	5008.61***	3122.5**	1002.53**
Critical difference				
Season		8.7	16.18	8.9

Table-2: ANOVA for *in vitro* shoot multiplication

	df	Mean sum of square		
		Multiplication rate	Increase in no. of shoots	Shoot length
Season	1	N.S.	14.017*	N.S.
Critical difference				
Season		-	0.772	-

Table-3: Results of ANOVA for *in vitro* rooting

Source of Variation	df	Mean sum of square		
		Rooting response %	Root no.	Root length
Season	1	828.56***	N.S.	6.0768***
Critical difference				
Season		2.51	-	0.2459

* Significant at 5% level, ** significant at 1% *** Significant at 0.1% level, NS-Non significant

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DISCUSSION

Seasonal variations in sprouting response for *in vitro* multiplication was observed in *Gmelina arborea* by Thakar and Bhargava (1999). In their work, the bud response declined in the months of July-September. Similar observations were noticed for *Dalbergia sissoo* in the current work. In our studies, the maximum bud response (67.5%) and survival (65%) was obtained in the season of April to June.

Seasonal influences on *in vitro* bud break in *Dendrocalamus hamiltonii* Arn. Ex Munro was seen by Singh *et al.* (2012). Early summer was the best period for explant collection for *Dendrocalamus hamiltonii*. In Shisham, same months proved to be the most suitable for the maximum gain of plantlets. Strong seasonal variation in *in vitro* plant regeneration frequency was seen in immature scutella of two different genotypes of Barley (Sharma *et al*., 2005). For *in vitro* multiplication of five superior clones of *D. sissoo*, a significant variation in bud response was reported. Hence, our results corroborate with the above work done on Barley.

Capacity of differentiation of explants depends very much upon their physiological status which is susceptible to fluctuations during different seasons as illustrated in our studies.

Similar seasonal fluctuations on morphogenetic response in *in vitro* conditions were observed in *Fagus sylvatica* (Bakshi and Ahuja, 1996). The axillary bud response appeared to depend on the physiological state of the donor plant. The bud response declined after May.

Significant variation in the response of explants excised and cultured during different months was recorded in earlier studies (Prasad and Chaturvedi, 1988).

Bud response was observed maximum in summer by Evers *et al*. (1988) in many woody species e.g., *Alnus, Salix, Quercus, Populus, Ulnus, Pseudotsuga, Platanus, etc*.

Kumar *et al.* (2006) observed marked seasonal variations in morphogenic response of nodal explants in *in vitro* conditions in *Holarrhena antidysenterica* (L.) with maximum response in May- July, gradually declining and finally dropping to zero from Oct.-Feb. Seasonal variations in micropropagation of giant bamboo and in relation to season rainfall pattern was observed by Ramanayake and Yakandawala (1997).

The similar results were observed in some genotypes of *Dalbergia sissoo* Roxb. The maximum *in vitro* shoot regeneration was obtained in the month of May using nodal explants (Kalia *et al.*, 2004). Although, they observed seasonal fluctuations in the morphogenetic response on the same species, but the genotypes were different. However, in our work, the effect of season on different genotypes of *Dalbergia sissoo* was analyzed and similar results were seen. In the present case, organogenesis is induced in five other clones of *D. sissoo* representing five various geographical regions. The month of April and May proved to be the best period for explants collection and culture in our studies as the explants readily established under *in vitro* conditions with subsequent shoot growth which is in consonance to the earlier findings of Arya *et al.* (2005) in *Dalbergia sissoo.*

Lower response of explants in present study during rainy season (July-Sept.) was observed. Among both seasons selected, maximum explants successfully established in April to June months.

Seasonal effect for explant collection on *in vitro* culture establishment revealed significant variations for *in vitro* establishment of *D. sissoo* which were discernible among two seasons. April to June months of explant collection proved to be the best time of explant collection for *in vitro* establishment as well as rooting on MS medium. This work will be helpful by selecting the best time of explant collection to maximize the plantlet production.

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Conflict of interest

The authors do not have any conflict of interests.

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