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In-Vitro Micropropagation through Cotyledonary nodal segments in *Prosopis cineraria L.*

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ABSTRACT

A Protocol has been developed for micropropagation of a multipurpose tree of desert i.e *Prosopis cineraria L* under aseptic conditions. Cotyledonary nodal segments (1 to 1.5 cm) were taken from 15 to 20 days old aseptically grown seedlings. 15-20 shoot buds were induced on Murashige and Skoog (1962) medium supplemented with various cytokinins and auxins individually and in various combinations. Best response was observed in 2.0 mg/l BAP added singly and 2.0 mg/l BAP and 0.05 mgl IBA in combinations. Rooting on *in vitro* regenerated roots was achieved on MS medium supplemented with 2.0 mg/l IBA. In vitro regenerated plantlets were successfully transferred to pots under field conditions.

Keywords: Micropropagation, Cotyledonary node, *Prosopis cineraria.*L

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INTRODUCTION

Prosopis cineraria (Khejari) is a multipurpose tree of desert in Western Rajasthan and is regarded as the backbone of rural economy. It provides food, fodder, fruit, firewood, timber, livestock feed, vegetable, construction and fencing material, medicine, gum and shade. The species is highly drought tolerant and can withstand in the area having 50mm rainfall annually [1]. *Prosopis juliflora* [10] *Prosopis laniegata* [5] have been successfully micropropagated.The available literature reveals that no substantial research work on micropropagation of this economically important plant is reported. hence further attempts have been made in this direction with the aim to develop a reproducible protocol in order to grow this plant in field. More ever this plant species has become endangered due to its overexploitation and it needs an immediate attention of the tissue culturists and biotechnologists for its survival.

MATERIALS AND METHODS

Seeds of *Prosopis cineraria* were procured from CAZRI, Jodhpur. Seeds were scarified with a pair of scissors on the end opposite to hilum and surface sterilized with 0.1% mercuric chloride for 5-7 min and then rinsed in autoclaved distilled water 3-4 times and were innoculated in half strength Murashige and Skoog medium. The cotyledonary node explants were excised from 15-20 days old aseptically grown seedlings. The explants were innoculated on MS medium supplemented with various auxins (2, 4-D, 2, 4 5-T, NAA, IAA, IBA, Picloram and cytokinins (BAP, Kn) added singly (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) or in combinations of each other. To overcome the problem of browning of explants due to oxidation of polyphenols a variety of pretrement measures were adopted to obtain healthy cultures. Aseptic manipulations were done in a Laminar air flow cabinet provided with UV lights. All cultures were incubated in a culture chamber or BOD incubator. The temperature of the chamber was maintained at 26+- 2^0C using air conditioners. Light intensity (1200 Lux) was provided from fluorescent tubes (40 Watt) and incandescent bulbs (40 Watts). A photoperiod of 16 hours and 8 hours darkness was maintained with the help of timer.

Observations were taken fortnightly. Number of shoots/roots formed was recorded 45 days after inoculation. The standard error of the arithmetic mean was calculated for individual treatment using the following formula. The pH of the medium was adjusted to 5.6 and autoclaving was done at 1.05kg/cm2 for 20 minutes.

S.E. = ±√ x2/n(n-1) (Snedecor 1956)

Where S.E. Standard error
x = Deviation from mean
n = number of replicates

Histological studies were carried out where serial sections (10-12 um) were cut with the help of rotary microtome stained in safranine and mounted in DPX.

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RESULTS

Codyledonary node formed cream friable callus on MS medium supplemented with auxins. Best callusing was observed on 2, 4-D (2.0 mg/l). Moderate callus was formed on 2, 4, 5-T (5.0 mg/l). White friable callus along with some roots was formed on NAA (1.0 mg/l) and IAA (2.0 mg/l), while at 2.0 mg/l IBA only root formation was observed. On 1.0-5.0 Mg/l Kn shoot buds were formed but very few in number (2-3) and on all other concentrations of growth substances (1.0 - 5.0 mg/l) swelling or traces of callus formation was observed.

Table 1. Number of shoot buds proliferating per cotyledonary node segment on BAP (2.0 mg/) in combination with different auxins.

BAP (2.0 mg/l)	Number of shoot buds + (% Response) Auxin (mg/l)				
2,4-D	0.005	2.4	±	0.2 2	(60)
	0.05	3.5	±	0.31	(60)
	0.5	2.4	±	0.43	(50)
NAA	0.005	5.9	±	0.31	(80)
	0.05	11.4	±	0.29	(90)
	0.5	5.3	±	0.25	(80)
IAA	0.005	6.1	±	0.67	(70)
	0.05	8.7	±	0.24	(90)
	0.5	5.7	±	0.25	(80)
IBA	0.005	7.4	±	0.14	(90)
	0.05	14. 7.9	±	0.38	(90)
	0.5		±	0.43	(80)

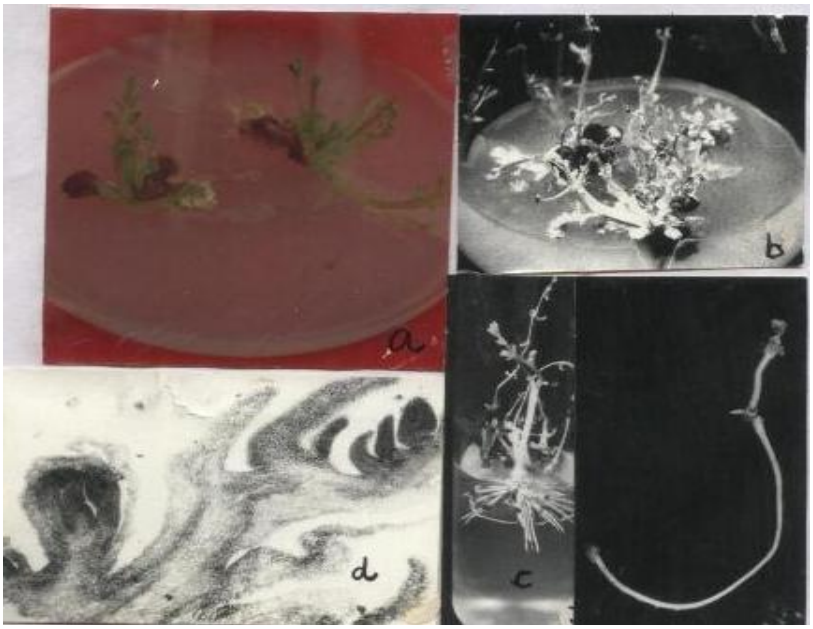

Figure 1. Regeneration of shoot buds and plantlet formation in cotyledonary node cultures of *Prosopis cineraria* A) Shoot bud formation from cotyledonary node cultured on MS medium with 2.0 mg/l BAP, B) Elongated shoots on further sub-culture, C) Rooted shoots on MS + IBA (2.0mg/l) fortified with 2.0 mg/l phytagel D) Histological section of cotyledonary node showing shoot bud

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Best induction of shoot buds was observed at 2.0 mg/l BAP(Fig. 1a) Therefore keeping 2.0 mg/l BAP constant MS medium was supplemented with lower concentrations of auxins IBA, IAA, NAA, 2, 4-D (0.005, 0.05 and 0.5 mg/l). Best response was observed at 0.05 mg/l IBA and 2.0 mg/l BAP where 15-20 shoot buds were formed without any callus formation (Table 1)(Fig 1b). In other combinations also shoot buds were formed but their number and percentage declined.

Rooting was observed on MS medium supplemented with 3% sucrose, 2.0 gm/l phytagel and 2.0 mg/l IBA (Fig 1c) where tap to fibrous roots were formed. The root system formed was well developed and vigorious with root hari and no intermediate callus formation was seen.

In vitro regenerated plantlets well transferred to pots having sterile soil and vermiculite (1:1). Polythene bags were kept over the plants to maintain humidity initially and thereafter the pots were transferred to natural conditions. Histological studies revealed the differentiation of shoot buds in basipetal sequence and also precocious proliferation of lateral buds (Fig 1 d).

DISCUSSION

In vitro establishment of explants of woody trees is difficult due to oxidation of polyphenols from the explants and its leaching in to the medium which results in browning of the explant.

BAP responded best for the formation of shoot buds. Similar results are recorded in other leguminous trees like *Acacia koa* [12] *Dalbergra sissoo* [8] *Albizzia lebbeck* [14] *Leuceaena leucocephala* [4], *Prosopis juliflora* [10]. *Prosopis laevegata* [5]. IBA among auxins was found more effective as compared to NAA and 2, 4-D. However Surendra Kumar and Narendra Singh (2009) reported IAA to be effective in *Prosopis cineraria* and Sudha Devi and Natraja 1987 in *Dalbergia latifolia*. In the present investigation BAP along with IBA was found best for shoot proliferation. Similar results are reported by Sharma et al.1997However Goyal and Arya 1979 reported kn with IAA proved better for shoot multiplication in *Prosopis cineraria.*

Root formation was observed on MS medium supplemented with 2.0 mg/l IBA. The mixture of sterile soil and vermiculite in the ratio of 1:1 was used to acclimatize the plantlets with namely formed roots in plastic pots. Similar soil composition i.e. soil and sand was used to acclimatize *Dalbergia sissoo* (Raghuwaswamy et al 1992).

Thus the present investigation has resulted in the establishment of a reliable and reproducible protocol of *Prosopis cineraria* an important tree species which could be further used for the conservation of germplasm.

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