Micropropagation Of *Prosopis Cineraria* (L.) Druce – A Multipurpose Desert Tree

Surender Kumar And Narender Singh

Department Of Botany, Kurukshetra University Kurukshetra (India)
E.mail: suren_kr@yahoo.co.in

ABSTRACT
A protocol has been developed for micropropagation of a multipurpose desert tree species *Prosopis cineraria* under *in vitro* conditions. Nodal explants from mature plant of *Prosopis cineraria* were taken and cultured after surface sterilization. One to multiple shoots were induced on Murashige and Skoog's (1962) medium supplemented with various cytokinins and auxins individually and in various combinations. 5.0 mg/l BAP and Kinetin were found to be effective individually. 5.0 mg/l BAP +1.0 mg/l IAA responded batter than all other media combinations. Rooting of *in vitro* regenerated shoots (>2.5 cm long) was achieved on half strength MS medium supplemented with 3.0 and 5.0 mg/l IBA. *In vitro* regenerated plantlets were transferred in pots containing sterilized sand and vermiculites (1:1). After four weeks plantlets were acclimatized to field conditions successfully with 60% survival rate. The survived plants grew normally. [Researcher. 2009;1(3):28-32]. (ISSN: 1553-9865).

Key words: Micropropagation, Nodal explants, *Prosopis cineraria*, Axillary shoots.

Micropropagation is an important area of plant biotechnology. Many leguminous trees have been micropropagated namely *Acasia* (Skolmen and Mapes,1976,)*Albizzia lebbeck*(Gharyal and Mahaswari,1981),Upadhayay and Chandra,1983),*Dalbergia* (Mukhopadhayay,MohanRam,1981)*Leucaena leucocephala* (Dhawan and Bhojwani,1985, Nangia and Singh,1996),*Prosopis juliflora* (Nandwani and Ramawat,1991),*Prosopis laviegata* (Gonzalvez *et al*.,2007). *Prosopis cineraria* is a versatile species commanly known as Jhand or Khezri. *Prosopis* species are the dominant species in Indian desert. *Prosopis cineraria* has a very good economic importance in arid regions and is assumed to treat snake bite and scorpion stings. Green pods of this plant are used as food. This species is highly drought tolerant and can withstand in the area having 50mm rainfall annually(Bhandari,1978).

MATERIALS AND METHODS

The nodal explants (approximately 1-1.3 cm long) were obtained from a mature tree growing in ,Kurukshetra university,Kurukshetra.The nodal segments were kept under running tap water for 1 hr followed by treatment with a commercial liquid detergent Tween 80(1%) and then were surface sterilized in 90%ethyl alcohol(2 min) followed by mercuric chloride 0.1%(4-5) minute. Thereafter nodal segment were washed several time in double distilled sterilized water. These nodal segments were inoculated in MS medium supplemented with various growth regulators individually and in combinations.

MS basal medium (Murashige and Skoog,1962) was used for present investigation. MS medium supplemented with Various growth regulators individually (auxins i.e.IAA, NAA, 2,4-D and IBA) and (cytokinins i.e. BAP and kinetins) and in combinations(Kn+IAA, Kn+NAA and BAP+IAA,BAP+NAA) were tried for shoot regeneration and callus induction. The pH of the media was adjusted to 5.6 followed by addition of 0.5W/V agar prior to autoclaving at 120^0C at 1.06 KPa for 15 minutes for the purpose of sterilization. All the culture conditions were maintained at 25 ± 1^0C, with 16 hrs light and 8 hrs dark period with 60% relative humidity.

Observations like callus formation, growth of callus, number days taken for bud break, percentage of bud break and number of shoots regenerated per explants were recorded regularly. A mean of 15 replicates was taken per treatment.

RESULTS

Axillary shoot formation: Direct shoots regeneration was observed in all media fortified with various auxins and cytokinins. M S basal medium without growth regulators served as the control. This medium could produce only one shoot per explant. Supplementation of cytokinins gave better results than auxins in present investigation. Among cytokinins 5.0 mg/l BAP and Kn resulted better in terms of period

required for bud break, percent bud break, number of shoots regenerated per explant and length of regenerated shoots (Table1).

Among auxins 1.0mg/l IAA and NAA gave better results. The rate of percent bud break and number of shoot regenerated per explant media supplemented with these hormones was maximum(Table-2.).

Excised nodal segments were also cultured on MS medium fortified with different cytokinins (BAP and kinetin) and auxins (IAA, NAA and 2,4-D) in various combination(Table3.). As the nodal explants cultured on media with 1.0mg/l NAA and 1.0mg/l IAA individually responded better, these concentrations were taken for further study in combination with cytokinins. The combination of Kn with IAA and NAA gave better results as compared to their individual treatments. Supplementation of NAA in place of IAA with kinetin did not make much difference. Substitution of BAP in place of Kn resulted better in terms of percent bud break, number of days required for bud break and multiple shoots formation. The explants cultured on MS medium fortified with 5.0mg/l BAP+1.0 mg/l IAA responded best among all media used in combinations.

Callus regeneration: Simultaneously, callus formation was noticed in media fortified with various concentrations of kinetin and BAP. Highest percentage of callus induction was observed in the media supplemented with 5.0 mg/l Kn. Among auxins, the medium supplemented with 1.5mg/l IAA supported highest (60%) percent of callus induction. Callus so produced was greenish, white and fragile . However there was not reported any correlation between concentration of different auxins and percentage of callus formation. A combined effect of cytokinins (BAP and kinetins) and auxins (IAA, NAA and 2,4-D) was also studied for callus formation. Callus formation was noticed in all media supplemented with the combination of auxins (IAA,NAA and 2,4-D) and Cytokinins (BAP and Kinetin).MS medium fortified with 5.0mg/l BAP+ 1.0mg/l NAA resulted in the hundred percent callus formation after 10.5 days of inoculation **.**

Rooting of *in vitro* regenerated shoots

Root formation was not achieved on half strength and full strength MS medium devoid of growth regulators (Table.4.).Among all treatments of NAA (0.5, 1.0, 2.0, 2.5, 3.0 and 5.0 mg/l) used, 3.0 mg/l NAA was found effective in regeneration of roots. IBA proved better as compared to NAA .Rooting was observed in media fortified with 2.0, 2.5, 3.0, 5.0 mg/l IBA. The medium supplemented with 5.0 mg/l IBA was found most suitable for rooting because it regenerated roots in least time (15-19 days) Fig5.

Hardening of Plantlets – *In vitro* regenrated complete plantlets were implanted in pots having sterile soil and vermiculites (1:1). The plantlets were irrigated with half strength MS salt solution. High humidity was maintained for initial 15 days with the help of polythene bags and thereafter, these pots were exposed to natural conditions for 3-4 hours daily in an attempt to acclimatize the plantlets to natural conditions.(Fig.4.58-4.61). After a month these plants were shifted to glasshouses where they grew normally with 60% survival rate. After six weeks of glass house period, the plants were transfered to fields. The survived plants grew normally.

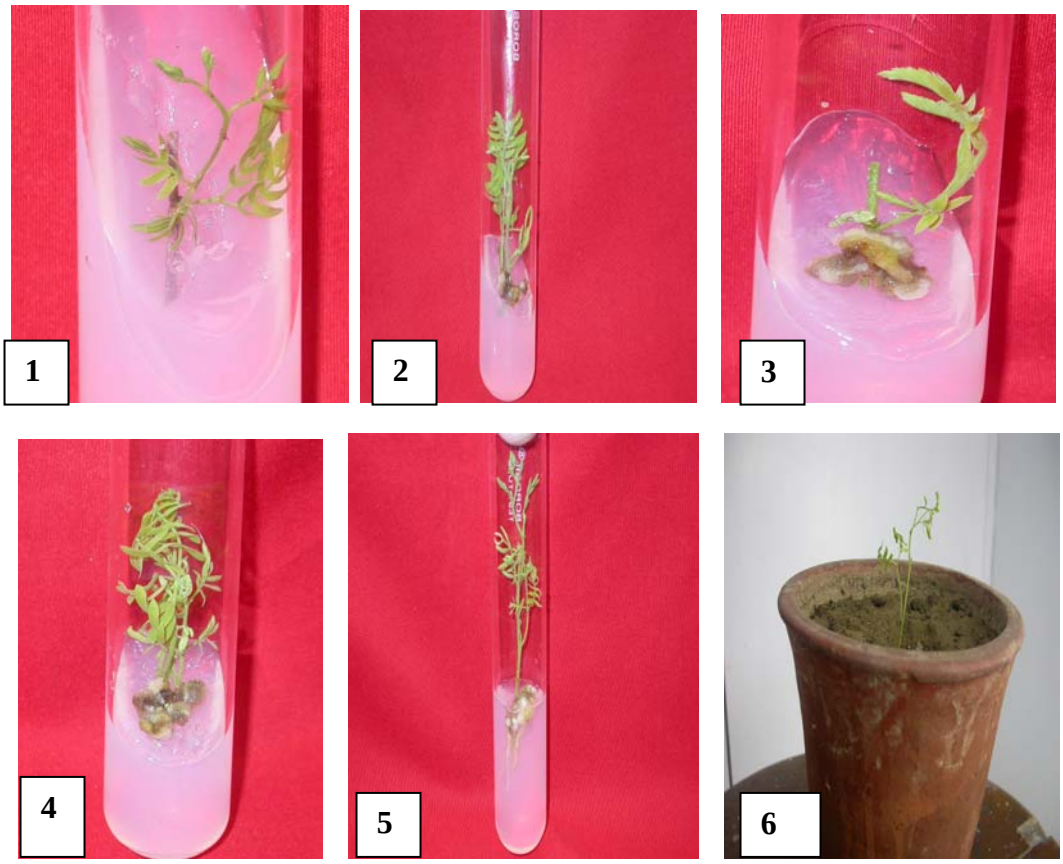

Fig.1 Shoot regeneration from nodal explant on MS +1.0 mg/l Kn.
Fig.2 Callus growth and shoots proliferation on MS+5.0 mg/l BAP.
Fig.3 Development of shoot and callus formation on MS+1 mg/l IAA.
Fig.4 Induction of multiple shoots and callus formation from nodal explant on MS + 5.0mg/l BAP+1.0 mg IAA.
Fig.5 Root formation from *in vitro* grown shoots on half strength MS + 3.0 mg/l IBA.
Fig.6 Establishment of *in vitro* grown plantlets under *in vivo* conditions.

Discussion

In present study shoot regeneration was reported without growth regulators but the percentage of shoot regeneration was less as compared to shoots regenerated by various media supplemented with different concentration of cytokinins (BAP and kinetin). Similar observation were made by Paal *et al.* (1981) and Cavallini and Lupi (1987).

BAP responded best for shoot formation .Other leguminous trees species where BAP induced shoot multiplication has been reported are *Acacia koa* (Skolmen and Mapes,1976),*Dalbergia* sissoo(Mukhopadhayay and Mohan Ram,1981,*Albizzia lebbeck*(Upadhayay and Chandra,1983) *Leucaena leucocephala* (Dhawan and Bhojwani,1985, Nangia and Singh,1996), *Prosopis juliflora*(Nandwani and Ramawat,1991),*Prosopis laevegata* (Gonzalez,et al.,2007)).Higher concentration of auxins did not supported better results as compared lower concentration of the same. Among all treatments of auxins, IAA was found to be more effective as compared to NAA and 2,4-D as also reported by Sudha Devi and Natraja (1987) in *Dalbergia latifolia*. In present investigation Combination of BAP and IAA proved better than other media tried. However Goyal and Arya(1979) reported that Kn with IAA proved better for shoot multiplication in *Prosopis cineraria*. Combination of BAP and IAA considerably enhanced shoot bud differentiation on nodal explantssimilar results were reported by Nandwani and Ramawat(1991) in

Prosopis juliflora. The development of axillary shoots from the nodal explants was accompanied by basal callusing of the explants. However this remained undifferentiated. Same type of observation have been made by Dhawan and Bhojwani(1985), Nandwani and Ramawat(1991) working with *Leucaena leucocephala* and *Prosopis juliflora* respectively. This may be due to production of endogenous auxins from the damaged cells of cut surface which triggered the cell division as found in *Ornithogallum* (Hussey,1976) when active division was observed in cut ends of the tissue. Root formation was observed on half strength MS medium supplemented IBA and NAA as reported by Nandwani and Ramawat in *Prosopis juliflora*(1991). The mixture of sterile soil and vermiculite in the ratio of 1:1 was used to acclimatize the plantlets with newly formed roots in Plastic pots. Similar soil composition i.e. Soil and sand was used to acclimatize *Dalbergia latifolia* (Raghwaswamy *et al*.,(1992)

Thus the present investigation has resulted in the establishment of a reliable and reproducible protocol of this important tree species. It could be used for mass multiplication as well as for the conservation of germplasm.

REFERECES-

Bhandari,M.M.1978. Flora of Indian Desert.Scientific Pub.Jodhpur.

Cavalinii,A.and Lupi,M.1987.Cytological study of callus and regeneration of plant of sunflower (*Helianthus annus* L.).Plant Breed.,99:203-208.

Dhawan,A. and Bhojwani,S.S.1985.*In vitro* vegetative propagation of *leucaena leucocephala* (Lam.) de wit.Plant Cell Report.,4:315-318.

Gharyal,P.K. and Maheswari,S.C.1981.*In vitro* differentiation of somatic embryoids in a leguminous tree *Albizzia lebbeck* L.Naturewissenschschaften., 68:379-380.

Gonzalez,B.,Orozco-Villafuerte,J,Cruz-Sosa,F..,Chavez-Avilla,V.M. and Vernon- Carter, E.J.2007. Conal propagation of mesquite tree (*Prosopis laevigata* Humb. And Bonpl. Ex willd. M.C. Johnston).I. via cotyledonary nodes. s*In vitro* Cell.Dev.Biol.Plant.,43:260-266.

Goyal,Y. and Arya,H.C.1984.Clonal propagation of *Prosopis cineraria* Linn. through tissue culture and differentiation. J.Indian Bot. Soc.,58:61.

Hussey,G.1976.Planlets regeneration from callus and parent tissue in *Ornithogalum thyrsoides*.J. Exp.Bot.,27:375-382.

Mukhopadhayay,A. and Mohanram Ram,H.Y.1981.Regeneration of plantlets from excised roots of *Dalbergia sissoo*.Indian J. Exp.Biol.,19:113-119.

Murashige,T. and Skoog,F.1962.A revised medium for rapid growth and bioassay with tissue culture. Physiologia Pl.,15:483-487.

Nandwani,D. and Ramawat,K.G.1991.Callus culture and plantlets formation from nodal explnt of *prosopis juliflora*(Swartz) DC. Indian J. Exp. Biol.,29:523-527.

Nangia, S. and Singh, N.1996. Micropropagation of *Leucaena leucocephala* (Lam.) de Wit.Annl. Biol., 12(1):82-85.

Paal, H.A.,Kurnik, E. and Szabo,L.1981.Planlets regeneration from *in vitro* shoots tip culture of sunflower.Noveniterms.**,**30:201-208.

Raghwaswamy,B.N., Himabindu, K. and Sita,C.H.1992. *In vitro* propagation of elite rosewood (*Dalbergia latifolia* Roxb.).Plant Cell Report.,11:126-131.

Skolmen, R.G. and Mapes,M.O.1976.*Acacia koa* gray plantlets from somatic callus tissue.J.Hered., 67:114-115.

Sudha Devi,A.M. and Natraja,K.1987. *In vitro* regeneration and establishment of plantlets in stem cultures of *Dalbergia latifolia* Roxb. Indian Forester.,113:501-506.

Upadhayay, S. and Chandra, N.1983. Shoot and plantlets formation in organ and callus cultures of *Albizzia lebbeck* Benth. Ann.Bot.,52:421-424.

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