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RESEARCH ARTICLE

EFFECT OF GROWTH REGULATOR ON *IN VITRO* ORGANO GENESIS OF *PROSOPIS CINERARIA* (L.) DRUCE

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ABSTRACT

Prosopis cineraria L. is a species of flowering tree in the pea family, Fabaceae. It is the state tree of Rajasthan (India). Due to its economic importance in state Rajasthan has drawn attention towards it. In the present study, the propagation of a multipurpose tree species *Prosopis cineraria* under *in vitro* condition. Binodal explant from the mature plant of *Prosopis cineraria* was taken and cultured after surface sterilization. The explants were cultured on MS medium supplemented with various concentration of cytokinins and auxins individually and in various combination (1.0–3.0 mg/l) BAP, Kn, 2,4-D. Maximum percentage of callus induction were recorded at 2.5mg/l 2,4-D with 0.5mg/l Kn. Callus is directly sub cultured on MS medium having different concentration of auxins and cytokinin:(0.5 – 5.0 mg/l) BAP, Kn, α-NAA and 2,4-D. Maximum shoot induction was observed on 2.0mg/l BAP with 1.0mg/l α-NAA.

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INTRODUCTION

Rajasthan (India), *Prosopis cineraria* are grown in an agro forestry setting in conjunction with millet. *P. cineraria* are grown in an agro forestry setting in conjunction with millet. It is an established introduced species in part of Southeast Asia, including Indonesia. In India, many Rajasthani families use the green and unripe pods (sangri) in preparation of curries and pickles. The bark of *Prosopis cineraria* have been used traditionally in the treatment of respiratory diseases like asthma, cough, bronchitis and gastrointestinal ailments (Janbaz *et al.,* 2012). The aqueous extract of *Prosopis cineraria* leaves exerts antidepressant-like effect and skeletal muscle relaxant activity (George *et al.,* 2012).Microprapagation is an important tool in plant biotechnology. *Prosopis*genus tree have been micropropagated like *Prosopisjuliflora* (Nandwani and Ramawat, 1991), *Prosopislaviegata* (Gonzalvez *et al.,* 2007). *P. cineraria* has a very good economic value in arid region and assumed to treat pain, High cholesterol level, Diabetes, Anaemia, Kidney, Liver disorders and snake bite. Due to the economic value of *P. cineraria* has been taken for micropropagation. The present study we report the establishment of callus culture from explant with different cytokinins and auxins along and in different combination.

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MATERIAL AND METHODS

Binodal explant of *Prosopis cineraria*were obtained from the selected genotype through Achrol Locality near NIMS University campus, Jaipur India. Binodal explants were pretreated with tween-20 for 15 minutes and washed under distilled water. Binodal explants were surface disinfected by ethanol (90%) for 3 minutes followed by mercuric chloride (0.1%) for minutes and then washed with sterilized distilled water upto 4-5 times in laminar air flow. Finally binodal explants were transferred into Murashige and Skoog (1962) medium (MS) containing 0.8% agar and 3% sucrose with growth regulators in semisolid and MS medium with 3% sucrose with growth regulator having filter paper bridge in liquid.

Culture Media

MS medium was used throughout the investigation. Various growth regulators:BAP (0.5-3.0mg/l), Kn (0.5-3.0mg/l) and 2,4-D (0.5-3.0mg/l), IBA (0.5-3.0mg/l) were used for regeneration studies at a range of concentration. The pH of medium was adjusted to 5.8 with 0.01 N KOH and 0.01 N HCl before autoclaving at 121°C for 20 minutes.

Culture Conditions

Binodal explants were placed upright and adaxial, respectively, 1 in each culture tube (125x25mm, Borosil) or

Erlenmayerflask containing 20ml and 40ml culture medium, respectively. Culture vessels were incubated under fluorescent and incandescent lights with 16 hr illumination (2500lux) at 26±1°C temperature and 55% (±5%) relative humidity. Each treatment consisted of 24-30 replicates, and cultures for initiation of callus and multiplication and elongation were grown for at least 3 passages of 6 weeks.

Shoot Regeneration Medium

Binodal axillary buds were cultured on MS medium in liquid form supplemented with different combinations and concentrations of BAP (0.5-5.0mg/l), Kn (0.5-5.0mg/l) and 2,4-D (0.5-5.0mg/l), α-NAA (0.5-5.0mg/l) for callus and shoot proliferation. The optimization of media was then followed by a test in modified MS media were used with varying concentration. After ten weeks of inoculation, the regenerated shoots were then studied morphologically and effects of calcium on growth and differentiation were evaluated.

RESULT AND DISCUSSION

Medium composition has its impact on *in vitro* responses so the change and modification in medium composition also leads to different results in plant tissue culture. In present study three basal media *viz*. MS, SH and B_5 were investigated for induction of buds from binodal explants of *P. cineraria* MS medium was reported the best among all media taken in account. The importance of MS medium and its significance for *in vitro* response has been advocated by many earlier reports (Hossain *et al.,* 2008; Ibrahim *et al.,* 2008; Anabzhagan *et al.,*2010). Initiation and establishment of organogenesis from binodal explants and callogenesis was achieved in *Prosopis cineraria*, a woody medicinal tree. Different explants *viz*. leaf, apical bud and binodal segments were tested for their response of auxillary bud induction (Figure no. 1).

Figure 1.Incubation of Binodal explant for callus induction. Data recorded after 1 week of incubation

The nature of explants has its impact over the results of plant tissue culture (Siwach *et al.,* 2011). Initiation of callus in different concentration of auxins and cytokinis resulted about 35 % binodal explants producing callus. Optimum response on MS medium containing kinetin and 2,4-D (Graph 1& Figure no. 2). Callus grows fast on phenoxy acid containing media. The axillary of binodal explants were inoculated for callus indction and proliferation on MS mediun supplemented with 2,4-D (1.0-3.0mg/l) and Kn (0.5-3.0mg/l) along and in different combination. The explant were maintained in both light and dark alternately upto 3 weeks. The induction of callus was observed best over 2,4-D (2.5mg/l) with Kn (0.5mg/l) from binodal explant. Binodal segments were recorded as best explants of *Prosopiscineraria* which showed the best shoot induction response (Figure no. 3).

Figure 2. Effect of Kn and 2,4-D in different combination supplemented in MS medium for callus induction. Data were recorded after 3 weeks of culture

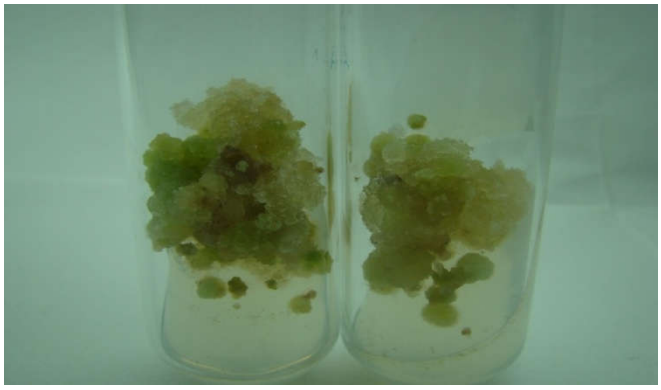


Figure 3.Effect of Kn and 2,4-D supplemented in MS medium for callus multiplication. Data recorded after 4 weeks

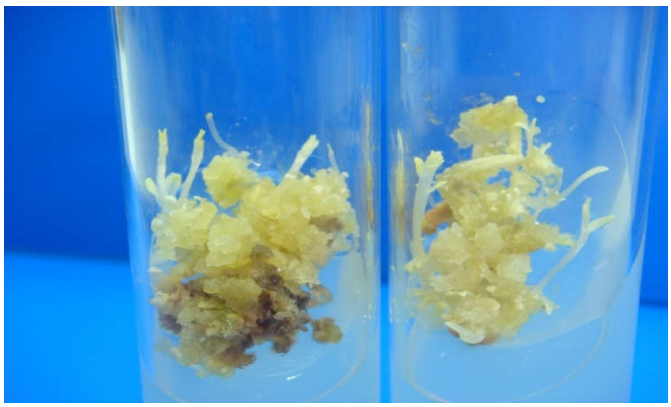
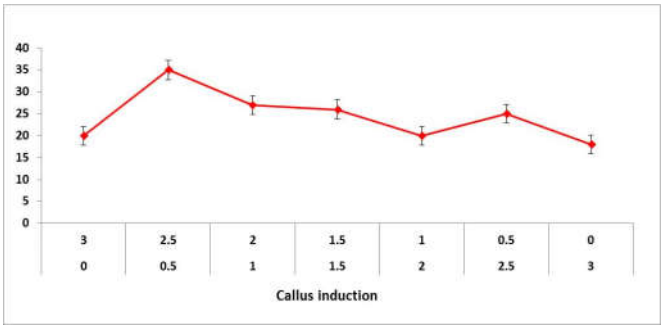


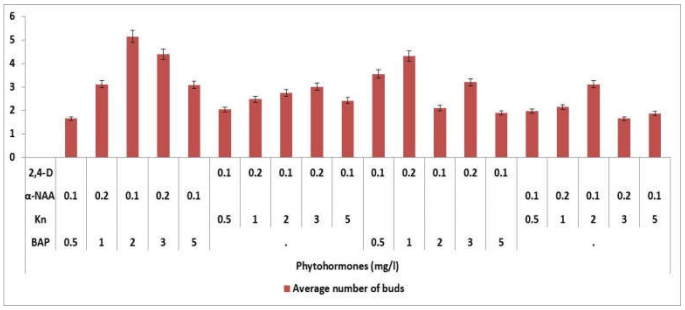
Figure 4. Effect of BAP (0.5-5.0mg/l)+α-NAA (0.1mg/l)+$CaCl_2.2H_2O$ combination supplemented in MS medium for shoot proliferation

Apical bud and leaf explants showed poor response comparatively. Nodal segments were reported earlier as the best explant source for enhanced organ multiplication in a number of medicinal plants (Anand and Jeyachandran, 2004; Kalidass *et al.,* 2008) was in concordance with our findings of present study. Sivram and Mukundan (2003) used apex, nodal segment and leaf explant. Debnath, (2008) and Tamura *et al.* (1984) correlated the formation of multiple shoots with the size

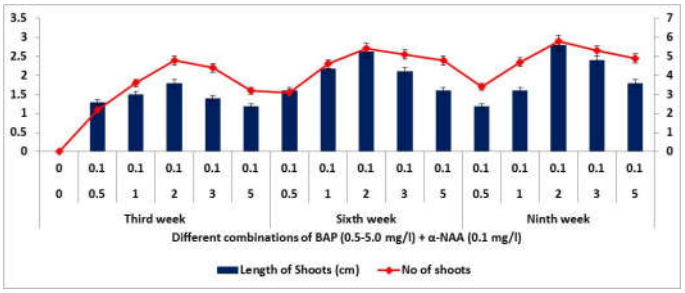
of binodal segment and the number of leaf primordia which is also supported by our findings and those of many other researchers (Ferreira and Handro, 1988; Nepovím and Vanek, 1998; Sivram and Mukundan, 2003; Patel and Shah, 2009).



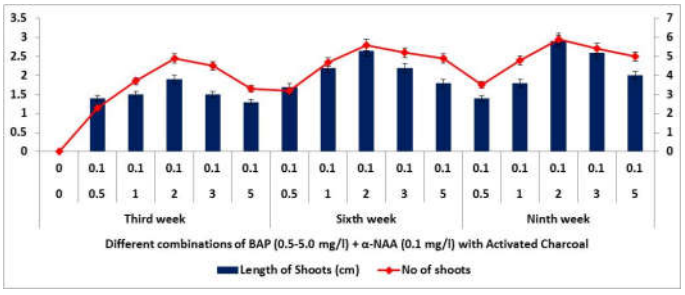
Graph 1. Effect of Kn and 2,4-D in different combination supplemented in MS medium for callus induction. Data were recorded after 4 weeks of culture



Graph 2. Effect of different cytokinins and auxins combination supplemented in MS medium on callus for shoot induction. Data were recorded after 4 weeks of culture

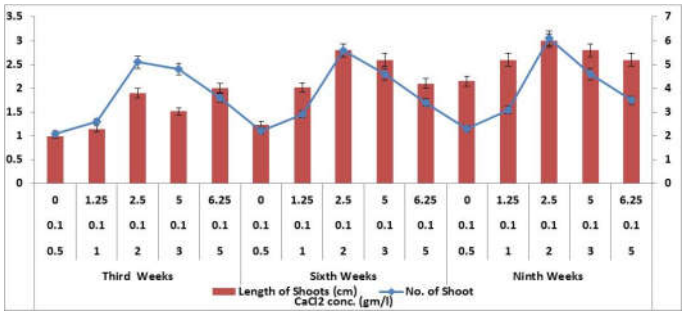


Graph 3. Effect of BAP (0.5-5.0mg/l)+α-NAA (0.1mg/l) combination supplemented in MS medium for shoot proliferation



Graph 4. Effect of BAP (0.5-5.0mg/l)+α-NAA (0.1mg/l) with activated charcoal supplemented in MS medium for shoot proliferation

Kornilova and Kalashnikova, (1997) and Das *et al.,*(2011) in their report stated that the shoot tip explants gave quicker shoot induction than binodal segment and auxillary bud explants.



Graph 5. Effect of BAP (0.5-5.0mg/l)+α-NAA (0.1mg/l) with $CaCl_2.2H_2O$ (0-6.25gm/l) supplemented in MS medium for shoot multiplication

This cultured callus was directly transferred for organogenesis and the medium containing MS medium liquid with filter bridge having 0.5-5.0mg/l BAP and Kn in combination with 2,4-D (0.1-0.2mg/l) and α-NAA (0.1-0.2mg/l). The best effect were observed with BAP (2.0mg/l) with IAA (0.1mg/l) which shows proved that MS medium supplemented with BAP (0.5-5.0mg/l)+α-NAA (0.1-0.2mg/l) in which BAP with 0.1mg/l α-NAA gives best result of shoot induction from callus (Graph 2). BAP (2.0mg/l) with 0.1mg/l α-NAA gives number of shoots and length of shoots 4.8 and 1.8cm respectively, whereas BAP (2.0mg/l) with 0.1mg/l α-NAA gives 5.8 number of shoots and 2.8cm length of shoots and data recorded after ninth weeks (Graph 3). Different parameters influence the initial stage of micro propagation. Micropropagation of plant has played an important role in the production of healthy, disease-free plants and in the rapid multiplication of plants (Ahmadian *et al.,* 2013). Two different auxins*viz*. 2,4-D and α-NAA were used in present investigation at different concentrations in various combinations. Cytokinins are the major class of growth regulators responsible for *in vitro* callus induction (Skoog, 1971). Two different cytokinins*viz*. 2,4-D and Kn were used in present investigation at different concentrations alone as well as in various combinations with auxins. Effect of activated charcoal 1% along with BAP (0.5-5.0mg/l)+α-NAA (0.1mg/l) supplemented in basal MS medium was studied. Significant effect was observed over shoot formation. The best result was given by BAP (2.0mg/l)+α-NAA (0.1mg/l) with 1% activated charcoal having number of shoots 4.9, 5.6, 5.9 and length of shoots 1.9cm, 2.64cm, 2.9cm in third weeks, sixth weeks and in ninth weeks respectively (Graph 4).These results showed similarity with the reports of (Debnath *et al.,* 2008). Their study reported that MS medium supplemented with BAP and α-NAA in combination were found to be most effective in initiation of multiple shoot proliferation. These results were comparable with previous finding where BAP was reported as best hormone for organogenesis in *Prosopis cineraria* (Debnath, 2008). The present investigation was in accordance with (Ahmed *et al.,* 2000, Mitra and Pal, 2007). These results were comparable with previous finding where BAP was reported as best hormone for shoot formation in *Prosopis cineraria* (Ali *et al.,* 2010). The impact of calcium was studied for the multiplication of shoots *Prosopis cineraria* at same interval of time period as taken for shoot induction and it`s proliferation. The basal MS medium supplemented with BAP (0.5-5.0mg/l)+α-NAA (0.1mg/l)+$CaCl_2.2H_2O$ (0-6.25 gm/l) combination was used in different concentrations and number of multiplied shoots as well as their length was observed. In third weeks after culturing the combination MS (BAP 2.0mg/l)+(α-NAA 0.1mg/l)+$CaCl_2.2H_2O$ (2gm/l) showed

maximum number, 5.1 and length of shoots, 1.9cm was showed with $CaCl_2.2H_2O$ (2gm/l). In the sixth weeks again MS (BAP 2.0mg/l)+(α-NAA 0.1mg/l)+$CaCl_2.2H_2O$ (2gm/l) concentration showed highest number of shoots, 5.6 and length of shoots, 2.8cm. In the ninth weeks again it was found that MS (BAP 2.0mg/l)+(α-NAA 0.1mg/l)+($CaCl_2.2H_2O$ 2.0gm/l) concentration showed highest number of shoots, 6.1 and length of shoots; 3.0cm (Graph 5 & Figure no. 4). These results showed a co-ordination with (Sen and Batra *et al.,* 2011) they reported that there was no morphological changes and growth when *Phyllanthusamarus* explants were cultured on MS medium devoid of calcium sources. It was found that media with higher $CaCl_2.2H_2O$ content was much better for plant *in vitro* regeneration (Kumar *et al.,* 2017). In present study the calcium were reported with significant effect on multiplication of shoots when compared with plant growth regulator BAP and α-NAA combination supplemented in MS medium. Calcium sources $CaCl_2.2H_2O$ (2.5gm/l) along with α-NAA (0.1 mg/l) and BAP (2.0mg/l) were observed for their impact on the initiation and multiplication of shoots on MS media. The use of α-NAA and BAP for initiation and multiplication in MS media was in concordance with (Ahmed *et al.,* 2007, Rafiq *et al.,* 2007).

Conclusion

Thus is concluded that MS medium is appropriate for *in vitro* culture and callus initiating medium on 2,4-D and Kn. The multiplication of callus on same medium of first stage media and initiation of organogenesis on MS medium with BAP and α-NAA and same media found precise for proliferation of shoots.

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