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INDUCTION OF CALLUS FROM ROOT EXPLANTS OF *Capparis spinosa* L.

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ABSTRACT

***Capparis spinosa* L. of the family Capparidaceae is an important medicinal shrub. The plant, although bears several medicinal properties, even though it has not been domesticated and are found in wild habitat. In the present work attempts have been made to initiate callus from the root explants, because both the roots and fruits bear several secondary metabolites, which are being used by different pharmaceutical companies. Root explants prepared from the healthy roots, were cultured on Murashige and Skoog (1962) basal medium, supplemented with different, concentrations and combinations of growth regulators such as 2,4-D and NAA either alone or with BAP or KN for induction of callus. Among the six different concentrations of 2,4-D, maximum percentage of response at 1.5 mg/l, 2,4-D alone was 46.73. However, highest percentage of response for callusing in the root explants was 92.54 in MS + 1.0 mg/l 2,4-D + 0.5 mg/l BAP. This was followed by the percentage of response among the explants, inoculated in MS + 1.5 mg/l 2,4-D + 0.5 mg/l BAP, where it was 72.46. The similar concentration of 2,4-D that is 1.0 mg/l 2,4-D + 0.5 mg/l KN however gave the highest percentage which was 68.74 only. It was further observed that MS + 1.0 mg/l NAA + 0.5 mg/l BAP induced calli only in 58.64% of the explants. Likewise MS + 1.0 mg/l NAA + 0.5 mg/l KN gave only 48.38 percent responses. It was further noted that lower concentration of 2,4-D that is 0.25 mg/l alone there was no induction of callus in the explants. Therefore, in the present work, 2,4-D was found superior than NAA, with respect to callus induction in the root explants of *Capparis spinosa* L.**

KEYWORDS: Callus, Root Explants, *Capparis spinosa*, Growth Regulators, Medicinal Plant, Secondary Metabolites

Abbreviation: MS - Murashige and Skoog, 2,4-D - 2,4, dichlorophenoxy acetic acid, NAA - Naphthalene acetic acid, BAP - 6, Benzyl amino purine, KN – Kinetin.

Capparis spinosa L. is a wild medicinal plant. The roots and fruits bear, several secondary metabolites which are being used in the formulation of different drugs by the different pharmaceutical companies. In the present work attempt has been made to induce callus on the root explants in culture conditions. Subsequently these calli may be used for different purpose. Tissue culture experiments for callus induction have been done by different workers. First of all Skoog (1955) was capable to culture for longer period when the medium was supplemented with kinetin. He recommended after induction the subculture of callus in the same medium should be fortified with kinetin also. Kelkar and Krishnamurthy (1998) cultured *Piper colubrium* and from its different explants such as, stem, petiole, leaf and roots could regenerate adventitious roots *in vitro*. Kusakari *et al*; (2000), cultured roots of *Bupleurum falcatum* L. and reported enhanced production of saikosaponin by the same, where the sugar concentration was changed. Vicur *et al*; (2000) cultured roots of *Populus tremula* and were capable to enhanced bud generation from this explant. Reddy *et al*; (2001), cultured leaf of *Coleus forkohlii* and regenerated calli from it. These calli were subcultured and differentiated multiple shoots for mass propagation. Nath and Burago hin, (2003) reported *in vitro* propagation through culture of shoot tips of *Centella asiatica*. Sharma and Wakhlu (2003) cultured callus of *Heracleum candicans* and through organogenesis developed plantlets.

Kuruppusamy *et al*; (2006) reported micropropagation of *Vanasushava pedata* an endangered medicinal plant. Thomas and Maseena (2006), callus induction and plant regeneration in *Cardiospermum helicacabum* Linn an important medicinal plant. Balaraju *et al*; (2009) performed tissue culture experiments for micropropagation of *Vitex agnus castus* a valuable medicinal plant. Yadav *et al*; (2009) cultured leaf segment, internodes and root segment of *Populus deltoids*. They were capable in direct regeneration of plantlets from these explants. Irvani *et al*; (2010) performed tissue cultures studies of *Dorem ammoniacum*, an endangered medicinal plant. They were capable to induce calli on different explants. These calli were used for plant regeneration. Meena *et al*; (2010), used epicotyls explants of ethnomedicinally important *Caesalpinia bonduc* L. They induced calli from the above explants and used this for different secondary metabolite production. Srivastva *et al*; (2010) cultured callus of *Lantana camera* L. and was capable to observed accumulation of betulinic, oleanic, and urcolic acids. Such findings are of immense value.

Shasthree (2010) cultured leaves and stem as explants of *Citrullus colosynthis* Schard, an endangered medicinal plant and was capable to generate multiple

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shoots on it. Tan *et al*; (2010) induced callus from different explants of *Centella asiatica* and observed effect of different plant growth regulators on callus, cell suspension culture and cell line selection for high rate of flavonoid production. Vijayan *et al*; (2010) reported advancement in the production of secondary metabolites through plant tissue culture, and proposed that in this way the medicinal plants shall be conserved in nature.

Kumar *et al*; (2012) cultured roots of *Caesalpinia bonduc* a threatened medicinal plants of Western Ghat. They were capable to regenerate plantlets directly and via callogenesis *in vitro*. Ikeuchi *et al*; (2013) described all the factors, responsible for induction of callus or its repression on different explants in the culture conditions. Ahmad *et al*; (2014) did experiments to initiate callus *in vitro* from different explants of *Celosia argentea* an important medicinal plant. These calli were utilized for different applications. Niratkar *et al*; (2014) induced callus form leaf explants of *Spilanthes acmela*, Murr: an endangered medicinal plant. They were capable to successfully regenerate plantlets from the above calli. Meenakshi (2015) performed tissue culture study of *Catharathes roseus* and initiated calli on different explants. The dry mass of these calli was used for the extraction of secondary metabolites. Ritika (2015) induced callus on leaf and internodal explants of *Bacopa monnieri*. The dry biomass was used for the extraction and characterization of secondary metabolite Bacoside-A, an important ingredient of the capsule memory plus.

Varporn Veraplakoru (2016), performed tissue culture study for the callus induction and micropropagation of *Lantana camera* L. an important medicinal plant that is found growing in wild population. Elangomathavan *et al*; (2017) reported high efficient protocol for callus induction and regeneration of plantlets in a medicinally important plant *Orthosipnon staminens*.

MATERIALS AND METHODS

Freshly collected roots of *Capparis spinosa* was surface sterilized under running tap water, followed by treatment with 0.1% $HgCl_2$ for 5 minutes. It was rinsed with glass distilled water to remove even a trace of chemical from the surface. The explants were further treated with 0.5% Bavistin a systemic fungicide for 5 min and was rinsed thrice. Such materials were preserved at low temperature wrapped in pre-sterilized and moist cloth. Ingredients of MS basal medium were dissolved in 500 ml glass distilled water.

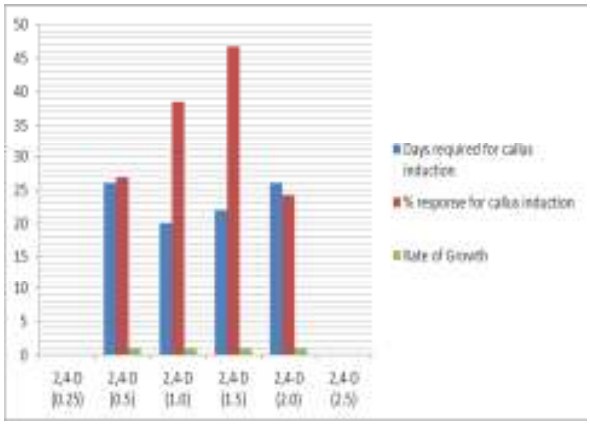
30g of sucrose was properly dissolved in it. The growth regulators were added separately either alone or in combination. Here 2,4-D and NAA were taken at 0.25 – 2.5 mg/l while BAP and KN at 0.5 mg/l concentration. After adding these growth regulators the pH of the medium was adjusted to 5.8, with the help of 1 NHCl or KOH drops. In another flask 8 gm of agar was dissolved in 500 ml distilled water by heating them slowly. Both the above solutions were mixed together so that the volume became 1000 ml. 40 ml of the above medium was taken in 250 CC culture flasks. The mouth was closed with cotton plugs wrapped with muslin cloth. All the plugs were covered with the aluminum foil to avoid wetting during autoclaving. Autoclaving was done at 15 lb pressure for 20 min. All the culture flasks were stored for 2 days and then used for inoculation. Inoculation of the explants was done under aseptic condition of Laminar flow chamber. All the culture flasks inoculated with explants were incubated in the culture room at 26 ± 1^0C with relative humidity varying from 68-70%. Observation was made on an alternate day and culture showing contaminations were discarded after autoclaving. The observations were made with respect to percentage of response for callus induction, time taken for callusing, growth rate of the calli, etc.. The data have been represented by the graph 1-5.

RESULT AND DISCUSSION

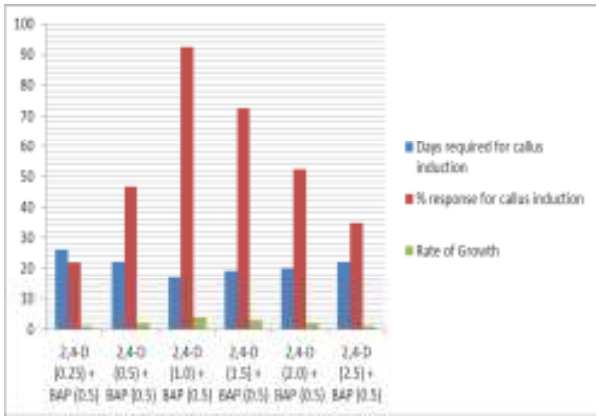
Results obtained in the present work have been represented by the graph 1 to graph 5. Perusal of the data clearly indicate that lower concentration of 2,4-D that is 0.25 mg/l had no impact on callusing, MS + 1.5 mg/l 2,4-D alone could induce callus in 46.72 percent of the explants. However, when 0.5 mg/l BAP was added, callus was induced even in MS + 0.25 mg/l 2,4-D medium. Highest percentage of callus induction was observed in MS + 1.0 mg/l 2,4-D + 0.5 mg/l BAP which was 92.45. Increasing concentration of 2,4-D had no promising impact. Similarly, at similar concentration of 2,4-D + 0.5 mg/l NAA the percentage of response was reduced to 68.74. It is clear from the above graph no.1, that 2,4-D alone either at lower or at higher concentration or when 0.5 mg/l was added to different concentrations of 2,4-D, percentage of response for callus induction was not encouraging. This was true for KN also (graph no.3). In both the combinations that is NAA + BAP (graph no.4) or NAA + KN (graph no.5), there were lower percentage of response than 2,4-D + BAP or 2,4-D + KN. So 1.0 mg/l 2,4-D + 0.5 mg/l BAP was found most suitable combination and for concentration than the other combinations and concentration (graph no.2). Minimum

period 17 days, for induction, and maximum growth rate of calli were also obtained among the explants where higher percentage of response was found.

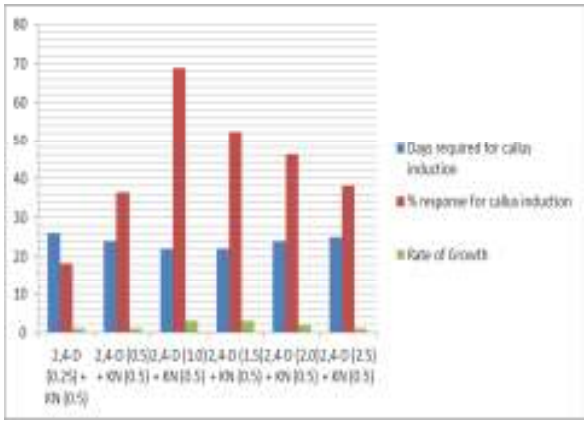
Growth rate of the calli was also noted. From the table it is clear that excellent growth rate denoted by 0 – 4, was observed in the calli initiated on the explants inoculated in the medium MS + 1.0 mg/l 2,4-D + 0.5 mg/l BAP. While better growth rate was noted among the calli induced and the explants inoculated in the medium MS + 1.5 mg/l 2,4-D + 0.5 mg/l BAP or Kinetin. (Graph no.1-5)



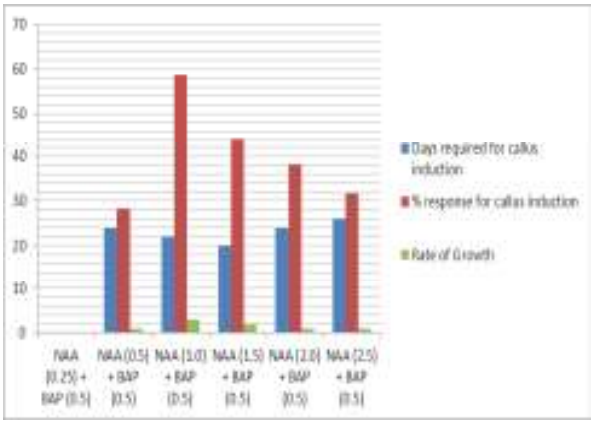
Graph 1: Showing days required for callus induction, % response for callus induction and rate of growth on different concentrations of 2,4-D alone.



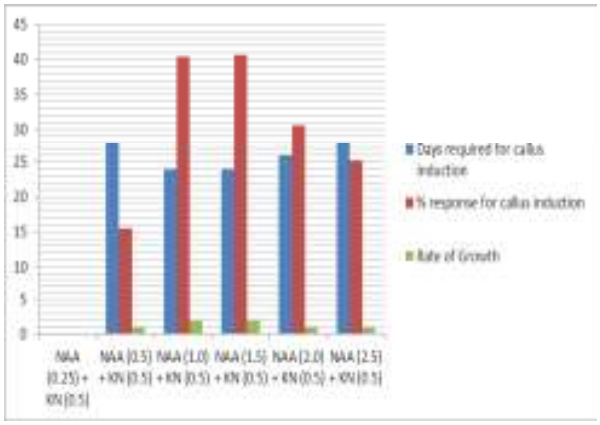
Graph 2: Showing days required for callus induction, % response for callus induction and rate of growth on different concentrations of 2,4-D + BAP (0.5)



Graph 3: Showing days required for callus induction, % response for callus induction and rate of growth on different concentrations of 2,4-D + KN (0.5)



Graph 4: Showing days required for callus induction, % response for callus induction and rate of growth on different concentrations of NAA + BAP (0.5)



Graph 5: Showing days required for callus induction, % response for callus induction and rate of growth on different concentrations of NAA + KN (0.5)

Callus induction on different explants of medicinal plants have been done by different workers- such as Ahmad *et al*; (2010) in *Ruta graveolens* L; Baluprakash *et al*; (2011) in *Exacum wightianum* Arn. An endemic medicinal plant of Western Ghats; Niratker *et al*; (2014) in *Spilanthes acmella* Murr: an endangered medicinal plant; Sen *et al*; (2014) in *Achyranthes aspera* a high value medicinal plant. Tirupathy *et al*; (2014) in *Tephrosia hookeriana*, an important medicinal plant. Elaleem *et al*; (2015) in *Ricinus communis* L; Teshome and Feyissa (2015) in *Glinus lotoids* (L.) an important medicinal plant. Zamini *et al*; (2016), in *Valeriana officinalis*; Borpuzari and Borthakur (2016) in *Plumbago rosea*, Varaporn Veraplakorn (2016) in *Lantana camera* L. a medicinal plant.

Findings of the present work are in agreement with the findings of the above workers. Therefore, it may be concluded that among the growth regulators auxin 2,4-D along with BAP is most suitable combination for induction of callus in different explants of medicinal plants in general and the root explants in particular. Elaleem *et al*; (2015) observed that 1.0 mg/l 2,4-D induced callusing in root explants of *Ricinus* which was 100%, findings of the present work therefore, corroborate with the above finding as here also 1 mg/l 2,4-D gave the maximum percentage of callusing in the root explants of *Capparis spinosa*.

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