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INVITRO REGENERATION IN *NARAVELIA ZEYLANICA* (L.) DC.

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Abstract
Naravelia zeylanica is a highly medicinal plant belonging to the family Rannunculaceae. Nodal and internodal explants were inoculated on MS medium supplemented with varying concentrations of auxins and cytokinins. Maximum number of shoots was observed on MS medium supplemented with 0.5 mg/l TDZ. Callus derived from the internodal explants showed indirect regeneration.

Key words: *Naravelia zeylanica , Invitro regeneration* , Organogenesis

Introduction

In India, over 7,500 species of plants are estimated to be consumed by 4,635 ethnic communities for health care needs. Over 1,700 species of plants are fully documented in terms of their biological properties and over 10,000 herbal drug formulations are recommended for a range of health conditions (Shankar *et al*., 1997). *In vitro* culture helps in clonal propagation and conservation of germplasm . *Naravelia zeylanica* (L.) DC. or *Atragene zeylanica* Linn. (Sivarajan and Balachandran, 1958) includes about fifty genera and about 2000 species (Cronquist, 1981). The plant is useful on vitiated conditions of pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds and ulcers. The roots and stems have a strong penetrating smell (Warrier et al., 1995). In Kerala *Naravelia zeylanica* is used as a source of the drug for intestinal worms, skin disease, leprosy, toothache and headache (Sivarajan and Balachandran 1958).

Materials and Methods

Nodes, internodes were used as explants .The explants were washed thoroughly under running tap water for 30 minutes, followed by washing in 10% labolene. Then treated with 0.1% Mercuric Chloride ($HgCl_2$) (w/v). The sterilized explants were washed using autoclaved double distilled water. The cultures were maintained at a temperature of 25 ± 1°C and incubated at 16 hour photoperiod at a light intensity of 2500 lux from cool fluorescent tubes. For shoot multiplication MS medium was supplemented with 0.1,0.5, 1, 2,4 or 5mg/l BAP/Kinetin/NAA/IAA alone or in combination with 0.5, 1.0 or 5mg/l BA or Kin. 0.1, 0.5,0.7,1,or 2mg/l TDZ alone was also supplemented with MS medium for shoot multiplication. For rooting 0.5 mg/l NAA was used. The rooted plantlets were transferred initially to ¼ th strength basal liquid medium supported by filter paper bridges. After 14 days of hardening, they were then planted into autoclaved vermiculite in plastic cups. Humidity was maintained by covering the plants with polythene bags for a few days. Plants with new leaves were then transferred to pots containing soil.Internodal explants were used for callus induction.Callus obtained from internodal explants were sub cultured on MS medium supplemented with BA or Kin in combination with IAA or NAA to assess the regeneration potential of callus.

Observation

Nodal segments segments showed shoot initiation after 15- 20 days of inoculation Nodal explants inoculated on MS medium containing 5.0 mg/l BA produced 4 shoots .Shoot multiplication decreased when the concentration of BA was increased to 6 mg/l.The multiple shoots obtained from the BA containing medium were sub cultured in MS basal medium for further growth . These plants showed elongation in the basal medium. Kin had no effect on multiple shoot induction.TDZ was found to have an effect on multiple shoot induction. 0.5 mg/l TDZ induced 6 shoots from a single nodal explant. The number of shoots increased to 9 after thirdsubculture. BA and TDZ (1.0mg/l each) produced four shoots from the nodal explants. Further increase in the concentration of BA decreased number of shoots.

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Minimum number of shoots was observed from the nodal segments on MS medium with BA and TDZ (0.5: 1.0 mg/l). Auxins alone in MS medium showed no shoot multiplication. BA in combination with 2,4 -D (5.0:0.1 mg/l) induced 4 shoots from a single node . Nodal explants on MS medium with 5 mg/l Kin and 0.5-mg/l 2,4-D produced 5 shoots. Nodal explants inoculated on MS medium containing BA and IAA (0.5: 1.0mg/l), produced 5 shoots. Kin in combinations with IAA (0.5:1.0 mg/l) produced 4 shoots from nodal explants.

Internodes inoculated on MS medium supplemented with 2,4-D produced light brown, friable and sticky callus after 30 days. Internodal explants on 1mg/l NAA produced pale green callus. MS medium supplemented with IAA showed poor callus proliferation. In IAA maximum callus proliferation was obtained in 0.5 mg/l and the callus was brownish, friable. MS medium containing 2,4-D in combination with BA produced callus. The explants on MS medium supplemented with 2,4-D (2mg/l) and BA (0.1mg/l) produced moderate callus proliferation in this combination callus morphology was green compact. In this combination the callus morphology varied from brownish yellow to green compact. Minimum amount of brownish yellow callus was produced from internodal explants on MS medium with 1 mg/l 2,4-D and 0.1 mg/l BA . 2,4-D in combination with Kin showed callus proliferation. Lower and higher concentration of 2,4-D along with different concentrations of Kin produced more callusing. The callus morphology varied from pale brown friable to dark green compact. 2,4-D in combination with Kin showed callus proliferation. Lower and higher concentration of 2,4-D along with different concentrations of Kin produced more callusing. The callus morphology varied from pale brown friable to dark green compact. Internodal explants inoculated on MS medium augmented with IAA and BA in combination induced callus proliferation.. In this combination callus morphology varied from yellowish green compact to green compact. IAA in combination with Kin also induced callus proliferation. Lower and higher concentration of IAA along with different concentrations of Kin produced more callusing. The callus morphology varied from friable pale brown yellow to dark green compact.

In vitro regenerated plants having a length of 2-6 cm were transferred to rooting medium for efficient rooting. Of the two auxins tried such as IAA or NAA tried (0.1, 0.5 or1 mg/l), 0.5 mg/l NAA was found to be more effective for rooting. In this concentration, a maximum number of 6 white elongated roots were developed from the base of shoots. Plantlets with suitable length and enough roots were selected for field transplantation. The plantlets were transferred to ¼ strength basal liquid medium for hardening. They showed 80 % survival rate. The survived plantlets were transferred to plastic cup containing vermiculate after 14 days. Humidity around the plantlet was maintained by covering plastic cups with polythene.

Discussion

Tissue culture methods have been successfully employed for large-scale multiplication of a number of woody plants (Yogesh et al., 1999). Higher concentrations of BA promoted multiple shoot induction from nodal explants . This type of response of BA was also observed in *Asparagus maritimus (*Stajner et al., 2002*)* and *Piper longum* (Soniya and Das*, 2002)*. TDZ played an important role by providing maximum number of shoots, these results are in concomitant with the result of adventitious shoot regeneration from cotyledons of white ash (Bates et al., 1992). Nodal explants inoculated on BA in combination with 2,4-D induced multiple shoot induction. This result is contrary to the result of *Picea omorika* (Budimir and Vujicic, 1992) . As per the report of Sudha and Seeni (1994) in *Adhatoda beddomei* response of nodal explant was increased by BA and IAA combinations in the medium. BA and IAA in combination also promoted induction of multiple shoots. Multiple shoot formation was also noticed from nodal explants inoculated on MS medium containing BA and IAA in *Aegle marmelos* (Ajithkumar and Seeni, 1998). This synergestic effect of NAA and BA combinations was also observed in European linden (Sarvosa and Darkovic , 2002) and in *Echinacea purpurea* (Koroch et al., 2002). Internodal explants was found to be most suitable for the initiation of callus. A similar observation was made by Debnath *et a*l. (2001) in *Lathyrus japonicus* in which leaf segments gave better callusing. In the present study the explants inoculated on MS medium with 2 mg/l NAA produced green semi friable

callus. Callus proliferation was also maximum in this medium. Lower and higher concentrations of NAA showed less callusing. The combination of BA and NAA was most effective for high shoot regeneration frequencies in *Echinacea purpurea* (Koroch et al., 2002). According to Yodanova et al., (2002), when intra specific hybrids were cultured on MS medium containing BA and NAA in combination, showed regeneration of shoots.

The *in vitro* regenerated shoots were rooted when transferred to MS medium supplemented with 0.5 mg/l NAA. This was also observed in *Olea europaea* (Cozza,1997), peach (Hammerschlag et al., 1987). According to Abraham and Nair (2001), in *Chlorophytum* plantlets that have been cultured *in vitro* are generally susceptible to desiccation on transplanting to soil. Available data confirms the presence of this alkaloid in many members of ranunculaceae family (Iwasa et al., 1993). However, presence of berberine in *Naraveliazeylanica* is not reported. The Rf value of berberine were calculated as 0.07 (Harbone). Its detection in the present study material suggests it as a valuable source for this medicinally important compound.

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