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Direct Multiple Shoot Regeneration from Shoot Tip and Nodal Explants of *Solanum Nigrum* L. A Medicinal Herb

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Abstract

In vitro multiple shoot regeneration of *Solanum nigrum* L., an Indian medicinal plant was accomplished on MS medium utilizing shoot tip and nodal explants. Direct multiple shoots differentiated within 6 weeks when explants were cultured on MS medium containing BAP (1.0 – 5.0 mg/l) and KIN (1.0 – 5.0 mg/l) individually. Among various concentrations of cytokinins tested, maximum number of multiple shoots was obtained on MS medium supplemented with BAP (1.0 mg/l) from shoot tip (20.4 ± 0.22) and MS medium supplemented with BAP (3.0 mg/l) from nodal explants (8.4 ± 0.22). The *in vitro* regenerated shoots were rooted (8.4 ± 0.16 roots per shoot) on MS medium supplemented with NAA (1.0 mg/l) within 2-3 weeks of culture and the regenerated plantlets could be successfully established in soil where they grow normally.

Keywords: BAP, Black nightshade, Micropropagation, NAA, Solanaceae.

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INTRODUCTION

Solanum nigrum L. (Family: Solanaceae) commonly known as black nightshade is an herbaceous annual plant. It has been utilized as a general promoter of health in medicine (Jain *et al*., 2011). The plant is effective in the treatment of cirrhosis of the liver (Lin *et al*., 2008). The plant is also credited with emollient, diuretic, antiseptic and laxative properties (Kiritikar and Basu, 1935, Jain, 1968). *Solanum nigrum* was found to possess lot of medicinal properties including anti-tumour, liverfibrosis inhibitory activity, hepatoprotective activity, antiulcer activity, etc., (Jian Lia *et al*., 2008, Jain *et al*., 2011). This can be also utilized for bioremediation of land having heavy metal contamination (Shuhe Wei *et al*., 2006).

Micropropagation via shoot culture, often utilized to maintain clonal fiedility, would be a special advantage in this case (Franca *et al*., 1995). Most of the secondary metabolites start to accumulate when the proper organs are regenerated from the cultured cells. Production of these compounds in cultured cells requires decoupling of biochemical differentiation from morphological differentiation, which has so far been successful. This situation makes organ cultures a favored option. Shoot cultures have been considered appropriate when the target secondary metabolites are produced in aerial parts of the plant (Saito and Mizukami, 2002). Hence, the purpose of this study was to develop in vitro propagation methods from shoot tip and nodal explants of *Solanum nigrum*. Few preliminary studies on *Solanum nigrum*, with a limited success, have been reported on in vitro regeneration of the plants. Amzad Basha Kolar *et al*., (2008) reported that highest frequency of multiple shoots was obtained from nodal explants on MS containing 6.0 mg/l BAP and 0.5 mg/l IAA. Direct organogenesis and in vitro flowering was obtained in *Solanum nigrum* by Venugopal *et al*., (2005). The highest frequency and number of multiple shoots were obtained from leaf and nodal explants on MS medium supplemented with benzyladenine and IAA. Sathish *et al*., (2010) reported the production of synthetic seeds from *Solanum nigrum* by using in vitro proliferated shoot tip explants. In vitro regeneration of Solonum nigrum with enhanced solasodine production was achieved by using leaf explants on MS medium fortified with BAP (2.0 mg/l) and KN (1.5 mg/l) (Bhat *et al*., 2010). In vitro regeneration of *Solanum nigrum* with a high power of alkaloid accumulation was achieved on MS-basal medium containing BA and NAA (0.5 mg/ml each). A series of in vitro and in vivo plants were successfully produced and chemical analysis revealed contents of glycoalkaloids higher than those reported for intact field plants (Hanan *et al*., 2010).

The regeneration technique has to be improved in order to use this system for effective clonal propagation; supplying these plants for phytopharamceutical industries for the production of phytopharmaceuticals at large scale level and genetic improvement of the plant through transformation. As a first step towards establishing a system to achieve this goal, we report a reliable and efficient protocol for shoot regeneration of *Solanum nigrum* using different explants such as shoot tip and node.

MATERIALS AND METHODS

The plantlets were collected from Botanical Garden of Muthayammal College of Arts and Science, Rasipuram. The shoot tip and nodal explants were washed thoroughly under running tap water for 10 min followed by treatment with solution of 0.1% bavistin (fungicide) for 1 min and thereafter washed thoroughly under running tap water for 15 min to completely remove the fungicide. The explants were then transferred to the sterile hood and surface disinfected with 0.1%(w/v) $HgCl_2$ for different time intervals (3-8 min) and finally rinsed with sterile distilled water for 3-4 times. The explants were blotted dry before inoculation. The explants were then trimmed at both the ends prior to inoculation. MS medium fortified with various concentrations of cytokinins such as BAP (1.0 mg/l - 5.0 mg/l) and KIN (1.0 mg/l - 5.0 mg/l) were investigated for to optimize hormonal requirements for multiple shoot induction from shoot tip and nodal ex-

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plants. The effect of auxins on multiple shoot induction was tested by using BAP (1.0 mg/l) and NAA, IAA (0.1 – 1.0 mg/l) individually. Single disinfected explants were culture on MS media (Murashige and Skoog, 1962) basal medium supplemented with 100 mg/l myo-inositol and 3% w/v sucrose. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.7±0.1 using 1N HCl or 1N NaOH before adding 0.8 % agar (Himedia Mumbai). The medium was dispensed into culture tubes and was subsequently autoclaved under 105 kPa at a temperature at 121°C for 15 min. The explants were implanted vertically on the culture medium (test tubes [150 cm x 25 mm] containing 15 ml medium) and plugged tightly with non-absorbent cotton. All the cultures were incubated at 25±2°C under 16 h photoperiod of 45-50 µmol m^{-2} S^{-1} irradiance provided by cool white fluorescent tubes (Philips, India) and with 55-60% relative humidity. All subsequent subculture was done at 4 week intervals. For rooting, single shoot was excised and transferred individually to MS medium containing NAA (0.1-1.0 mg/l). Each and every experiment was performed with 10 replicates and repeated twice. For hardening, the rooted plants were transferred to plastic vessels containing sterilized sand and vermiculture (1:1) and maintained in the same culture conditions. They were covered with polythene bags. After 12 days polythene bags were removed and these plantlets were placed under shade in the laboratory for 3 weeks and finally established in the field.

RESULTS

Multiple shoot development was observed directly from shoot tip and nodal explants on MS medium fortified with different concentrations (1.0 – 5.0 mg/l) of BAP and KIN individually. Of the various concentrations (1.0 – 5.0 mg/l) of BAP tested for multiple shoot induction from shoot tip, 1.0 mg/l produced the maximum number (20.4 ± 0.22) of shoots per explants with the maximum percentage of response (79.8%). Shoot tip explants cultured on MS medium supplemented with KIN (3.0 mg/l) produced the maximum number of shoots (12.6 ± 0.68) per explants (Table 1, Fig. 1). The nodal explants produced the maximum number (8.4 ± 0.22 per explants) of shoots on MS medium fortified with BAP (3.0 mg/l). Nodal explants cultured on MS medium supplemented with 5.0 mg/l of KIN produced 7.0 ± 0.83 shoots per explants (Table 1, Fig. 1). The explants cultured on MS medium supplemented with BAP (1.0 mg/l) and different concentrations (0.1 – 1.0 mg/l) of auxins (NAA, IAA) individually showed only callus formation. The regenerated shoots transferred individually into MS solid medium containing NAA (0.1- 1.0 mg/l) for rooting. The root initiation was observed from 0.3 to 1.0 mg/l concentration. The maximum number of roots (8.4 ± 0.16) was observed in MS medium supplemented with NAA (1.0 mg/l) (Fig. 1, Table 2). *In vitro* raised plantlets resumed normal growth was transferred to plastic cups filled with sand and soil (1:1) and developed healthy leaves 2 weeks after transplantation. The plantlets transferred to the field where they grew normally.

DISCUSSION

Raising the demand for wild source herbal source, herbal drugs has abetted over the exploitation of medicinal plants, leading to cumulative and sustainable use of forest wealth. The importance of conservation of genetic resources cannot be over emphasized. The maintenance of living material by traditional method is expensive, laborious and risky. Clonal propagation through tissue cultures offers an alternative to vegetative practices used in the past and has the potential to provide high multiplication of uniform genotypes, resulting in short term gains (Sacha L. Beck *et al*., 1998). Beta 2-solamargine , solamargine , solanoside and degalactotigonin are the important secondary metabolites of *Solanum nigrum*. The shoot tip and nodal culture could be a valuable technique for the production of these secondary metabolites in large scale (Sen and Sharma, 1999).

In the present study on MS basal medium the explants (shoot tip and node) shriveled within 3 weeks after emergence of 2-3 leaves without forming the multiple shoot. Similar results have

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also reported in Ocimum sanctum (Girija *et al*., 2006). These findings suggested that endogenous levels of hormones present in these explants are not sufficient to sustain their growth in the basal medium. MS medium supplemented with BAP or Kin induced multiple shoots from both the shoot tip and nodal explants. However, MS medium supplemented with BAP (1.0 mg/l) was found to produce the maximum number multiple shoots than the KIN or BAP in combination with NAA or 2,4-D individually. The BAP is the most efficient cytokinin in promoting adventitious shoot formation in many plants (Pirek, 1987). BA was superior to KIN in inducing high frequency shoot regeneration in many numbers of plants (Devendra *et al*., 2010; Malek *et al*., 2010; Johnson and Manickam, 2003; Johnson *et al*., 2004; Johsnon *et al*., 2007). Combination of auxin and cytokinin favored shoot bud differentiation in many plants (Sudha *et al*., 2005; Sanjaya Rathore *et al*., 2005). In contrast in the present study, when the explants cultured on MS medium supplemented with BAP and NAA/2, 4-D individually showed only callus formation without multiple shoot induction. This may be due to the fact that requirement of cytokinin and auxin depends on the endogenous levels these substances in the tissues used for the culture (Gupta, 1998). Shoot tip explants were found to be an excellent explants source to induce direct organogenesis than nodal explants in *Solanum nigrum*. The shoot tips are better than nodal segment for multiple shoot production because of the higher cytokinin to auxin ratio present in the shoot tip. Similarly the shoot tip was found to be the superior explants for micropropagation in many number of plants, for example Cannabis sativa (Ren Wang *et al*., 2009); Boehmeria nivea (L) Gaud (Sut *et al*., 2004), Ocimum sanctum (Girija *et al*., 2006), Alternanthera sessils (Wesely *et al*., 2011), Lippia nodiflora (Evelyne Priya, S and Ravindhran, R., 2011), Cicer arietinum (Islam *et al*., 1995) and Stevia rebaudiana Bert., (Arpita Das *et al*., 2011). The percentage of shoots forming roots and the number of roots per shoot significantly varied depending on concentrations of NAA. The maximum number of roots was obtained in medium containing 1.0 mg/ l NAA. Root development was; however, slow at lower concentrations of NAA. Jabeen *et al*., (2005) reported that NAA was a more effective rooting agent for *Solanum nigrum*. On the other hand Sundari *et al*. (2010) observed only 3-4 roots on MS medium supplemented with combination of IAA (5.58 µM) IBA (4.92 µM). NAA was also found to promote rooting in many numbers of plants (Gyana Ranjan Rout, 2004; Mohammad Anis *et al*., 2003; Kambaska Kumar Behera and Santilata Sahoo, 2009; Andrew Riseman and Siva Chennareddy, 2004).

In the present study the effective multiple shoot regeneration for maximum number of shoots was accomplished on MS medium supplemented with BAP (1.0 mg/l) from shoot tip explants of *Solanum nigrum* and using this procedure the plants can be regenerated on a large scale under in vitro conditions in a short span of time. The protocol standardized here could be used to isolate medicinally important secondary metabolite from the multiple shoots and this protocol would also have importance in genetic transformation of this medicinally important plant.

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Tables

Table 1. Effect of various concentrations of BAP and kinetin individually on multiple shoot induction from shoot tip and node.

MS medium + Cytokinin Concentration (mg/l)	Number of multiple shoots from shoot tip ± SE	Number of multiple shoots from node ± SE
MS alone	0.0 ± 0.00	0.0 ± 0.00
MS + BAP (1.0 mg/l)	20.4 ± 0.22	5.6 ± 0.30
MS + BAP (3.0 mg/l)	14.2 ± 0.32	8.4 ± 0.22
MS + BAP (5.0 mg/l)	10.6 ± 0.47	3.2 ± 0.24
MS + KN (1.0 mg/l)	6.7 ± 0.39	2.0 ± 0.25
MS + KN (3.0 mg/l)	12.6 ± 0.68	3.4 ± 0.45
MS + KN (5.0 mg/l)	2.4 ± 0.45	7.0 ± 0.83

Table 2. Effect of NAA on rooting on *in vitro* derived shootlets of Solanum nigrum

Concentration of NAA	No. of roots per shoot ± SE
0.1 mg/l	0.0 ± 0.00
0.2 mg/l	0.0 ± 0.00
0.3 mg/l	0.5 ± 0.22
0.4 mg/l	1.5 ± 0.40
0.5 mg/l	2.5 ± 0.45
0.6 mg/l	3.6 ± 0.70
0.7 mg/l	4.3 ± 0.26
0.8 mg/l	5.3 ± 0.61
0.9 mg/l	6.0 ± 0.69
1.0 mg/l	8.4 ± 0.16

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Figures

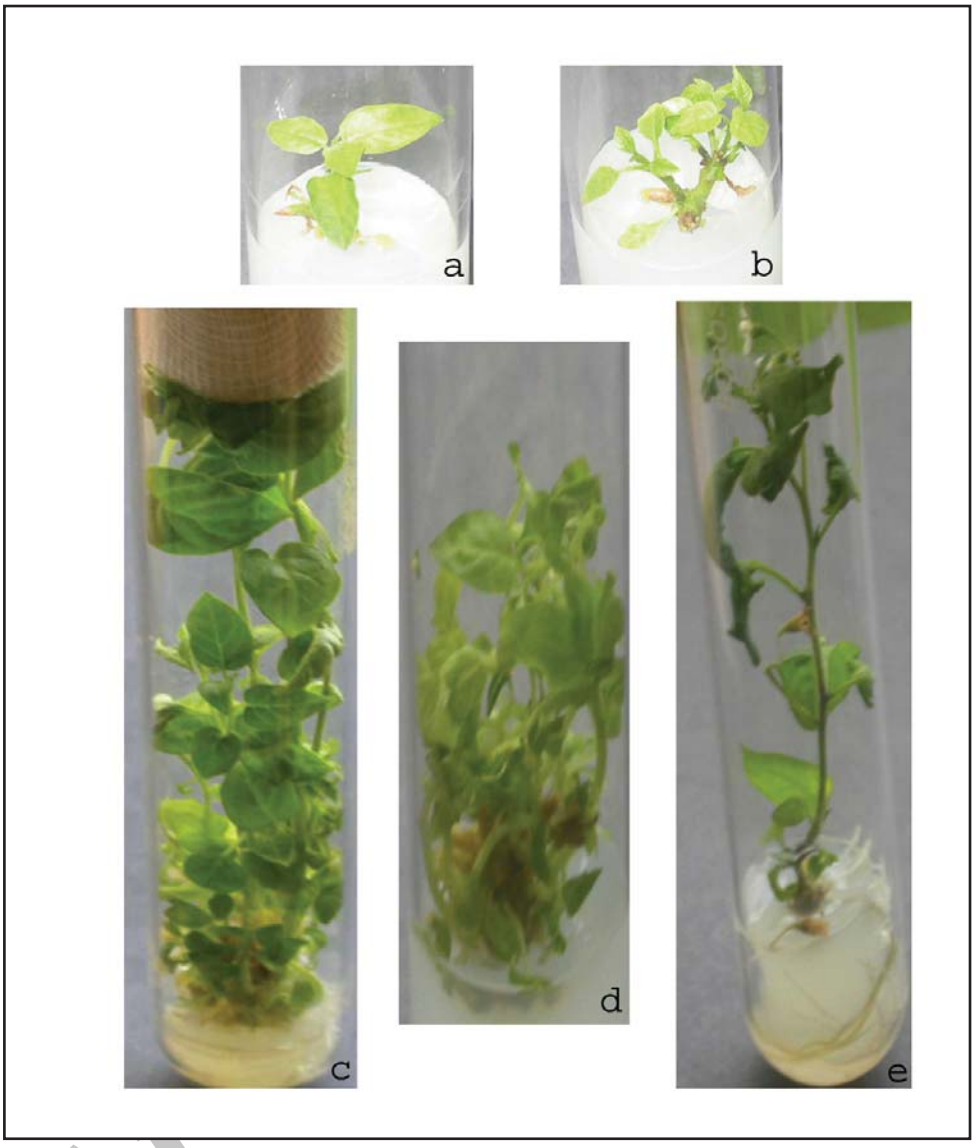

Fig. 1. Micropropagation of *Solanum nigrum*
a. Multiple shoot induction from shoot tip explant,
b. Multiple shoot induction from nodal explant,
c. Multiple shoot proliferation from shoot tip explant,
d. Multiple shoot proliferation from nodal explant,
e. Rooting

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