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PRODUCTION OF SYNTHETIC SEEDS FROM NODAL SEGMENTS OF *SOLANUM NIGRUM*

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ABSTRACT

Production of synthetic seeds is an efficient technology for the delivery of cloned plantlets as compared to traditional micropropagation. The study presented here describes an efficient protocol for the production of synthetic seeds in *Solanum nigrum*, an important medicinal plant. Artificial seeds were produced by encapsulating nodal segments of *S. nigrum* in calcium alginate gel. 3% (w/v) sodium alginate and 100 mM $CaCl_2 \cdot 2H_2O$ were found most suitable for encapsulation of nodal segments. Maximum number of multiple shoots were produced in Murashige and Skoog medium supplemented with thiadzuron (3.0 µM) + naphthalene acetic acid (1.0 µM). The encapsulated nodal segments were able to successfully regenerate after 60 days when stored at 4 °C. The shoots so regenerated were successfully rooted using half strength liquid MS medium supplemented with .5 µM Indole butyric acid and were acclimated to greenhouse conditions.

KEYWORDS | *Solanum*, artificial seeds, microshoots

INTRODUCTION

Solanum nigrum L. is an important medicinal plant belonging to the family Solanaceae. The plant has been used as hepatoprotective agent in traditional Indian medicine. Fruits of the plant have also been used as an antioxidant and cancer chemo-preventive material (Son et al. 2003). *S. nigrum* has been extensively used traditionally to treat various ailments such as pain, inflammation and fever (Acharya et al. 2006; Zakaria et al. 2006). Though propagation is through seeds, this method is season dependent (Verma et al. 2010) and therefore is unreliable.

Indiscriminate overexploitation of the plant for its medicinal uses might lead to the reduction in population. Therefore it is imperative that alternative methods of germplasm conservation is devised. Production of artificial seeds through encapsulation of vegetative propagules, offers an alternative method of conservation and germplasm exchange of this medicinally important plant. Though, earlier studies have reported the tissue propagation methods for this important medicinal plants (Hassaneen et al. 2000; Shahzad et al. 1999), studies on synthetic seed production for *S. nigrum* is very limited.

The present study reports, an efficient protocol for the production of artificial seeds through encapsulation of nodal segments derived from sterile seedlings of *S.nigrum*. To the best of our knowledge, a study of this nature has not been performed earlier.

MATERIALS AND METHODS

Seeds of *S.nigrum* was procured from Tamil Nadu Agricultural University,Coimbatore, India. The seeds were washed in running tap water, followed by surface sterilization in 70% ethanol for 2 to 3 minutes. The seeds were then rinsed using distilled water and sterilized for 15 min in sodium hypochlorite (1:1) and then rinsed in sterile distilled water. The sterilized seeds were aseptically inoculated on Murashige and Skoog (1962) solid medium. Nodal explants (1-2mm) were excised from the sterile plantlets and were used for encapsulation.

For encapsulation of the nodes, 3% (m/v) of sodium alginate in MS basal medium and 1 % (m/v) calcium chloride ($CaCl_2$) were steam-sterilized for 20 min under 1.2 kg cm^{-2} pressure at 120 °C after adjusting the pH of the solution to 5.8. Nodes were mixed with sodium alginate solution (at 25 ± 2 °C) and were subsequently dropped individually using a wide mouthed glass dropper into calcium chloride solution. The beads were allowed to form on a gyratory shaker at 80 rpm. Finally,the beads were recovered by decanting $CaCl_2$ solution and were washed thrice with sterilized double distilled water. The encapsulated nodal segments were kept in Petri dishes sealed with parafilm and stored in the laboratory fridge at 4 °C before being transferred for regeneration, Remya et al. (2013).

The beads containing a single node were aseptically inoculated in test tubes containing MS medium supplemented with various concentrations of Thiadzuron (1 to 3 µM) and NAA (0.5 to 1.5 µM) for regeneration of microshoots. The cultures were incubated at 25 ± 2 °C under 12h photoperiod. The number of shoots produced per encapsulated node were recorded.

Regenerated shoots from callus and encapsulated nodes were transferred to rooting medium

which consisted of MS liquid medium supplemented with different concentrations of IBA (0.2 to 1 µM).

Healthy plantlets with approximately 5cm long shoots and well developed long tap roots were removed from the culture flasks, washed and transferred to small plastic pots filled with sterilized coir pith and soil. The plantlets were initially maintained under laboratory conditions and were gradually exposed to natural environmental conditions.

Each experiment was repeated thrice

RESULTS AND DISCUSSIO

The nodal segments were successfully encapsulated in calcium alginate beads. Table 1 shows the effect of different growth hormones on the regeneration of multiple shoots from the encapsulated nodes of *S.nigrum*.

In the present study we found that the encapsulated nodes retained their viability to germinate even after 60 days when stored at 4 °C. These findings are based on earlier reports by Mohanraj et al. 2010; Remya et al. 2013, that storage at 4 °C is effective for longterm preservation of artificial seeds. Determining the optimal temperature for the longterm storage of artificial seeds is imperative for the conservation and transport of useful germplasm resources; an essential part of biodiversity conservation.

Maximum number of multiple shoots were produced in MS medium supplemented with thiadzuron (TDZ) (3.0 µM) + naphthalene acetic acid (NAA) (1.0 µM). Thiadzuron has been reported be just as effective as combined auxins (NAA) and cytokinin (BAP) in evoking morphogenic responses (Hutchinson et al. 2014)

Table 2 shows the effect of different concentrations of IBA on the induction of roots from the microshoots of *S.nigrum*.

Half strength liquid MS medium supplemented with .5 µM IBA was effective in root induction from the microshoots of *S. nigrum.* IBA has been reported to be beneficial for root induction during in vitro propagation of *S.nigrum* using nodal explants (Padmapriya et al. 2011).

The hardened plantlets were successfully transplanted into field where they grew normally without any morphological variations.

CONCLUSIONS

The present study describes a simple and efficient protocol for the production of synthetic seeds of *S.nigrum* directly using the nodal segments from aseptic seedlings. The protocol presented could be used as an alternative method for germplasm conservation of this valuable medicinal plant.

TABLE 1 EFFECT OF DIFFERENT GROWTH HORMONES ON THE REGENERATION OF MULTIPLE SHOOTS FROM THE ENCAPSULATED NODES OF *S.NIGRUM*

Growth regulators [µM]	Number of shoots(+SE)	Time (days)
TDZ (1)+ NAA(1)	6.47 + 0.65	34.78 + 0.32
TDZ (2)+ NAA(1)	8.32 + 0.23	29.92 + 0.21
TDZ (3)+ NAA(1)	12.32 + 0.14	23.56 + 0.37
TDZ (1)+ NAA(1.5)	5.43 + 0.32	33.45 + 0.87
TDZ (2)+ NAA(1.5)	8.21 + 0.11	31.62 + 0.76
TDZ (3)+ NAA(1.5)	10.65 + 0.18	26.21 + 0.92
TDZ (1)+ NAA(.5)	4.57 + 0. 21	30.86 + 0.72
TDZ (2)+ NAA(.5)	7.68 + 0.54	31.71 + 0.72
TDZ (3)+ NAA(.5)	9.43 + 0.98	28.93 + 0.23

TABLE 2 EFFECT OF DIFFERENT CONCENTRATIONS OF IBA ON THE INDUCTION OF ROOTS FROM THE MICROSHOOTS OF *S.NIGRUM*

1/2MS + IBA [µM]	Root length (cm) (+ SE)
0.2	7.5 + 0.82
0.5	9.3 + 0.66
0.7	7.9 + 0.86
1	6.4 + 0. 73

REFERENCES

1. Acharya, E. and Pokhrel,B. 2006. Ethno-medicinal plants used by Bantar of Bhaudaha, Morang, Nepal. Our Nature. 4: 96-103.
2. Hassanein, A.M., Soltan, D.M. 2000. Solanum nigrum is a model system in plant tissue and protoplast cultures. Biologia Plantaum. 43, 501–509.
3. Hutchinson,M.J., Onamu,R., Kipkosgei,L. and Obukosia,S.D. 2014. Effect of Thidiazuron, NAA and BAP on in vitro propagation of Alstroemeria aurantiacaCV. 'Rosita' from shoot tip explants. Journal of Agriculture, Science and Technology. 16(2)
4. Mohanraj, R., Ananthan, R. and Bai, V.N. 2009.Production and storage of synthetic seeds in Coelogyne breviscapa Lindl. Asian J Biotechnol 1:124–128
5. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant. 15: 473-497.
6. Padmapriya, H. Karthikeyan, A.V.P., Jahir Hussain, G., Karthi, C. and Velayutham, P. 2011. An efficient protocol for in vitro propagation of Solanum nigrum L. from nodal explants. Journal of Agricultural Technology. 7(4):1063-1073.
7. Remya, M., Bai, V.N. and Mutharaian, V.N. 2013. In vitro regeneration of Aristolochia tagala and production of artificial seeds. Biologia Plantarum.57 (2): 210-218.
8. Shahzad, A., Hasan, H. and Siddiqui, S.A., 1999. Callus induction and regeneration in Solanum nigrum L. In vitro. Phytomorphology 49, 215–220.
9. Son, Y.O., Kim, J., Lim, J.C., Chung, Y., Chung, G.H. and Lee, J.C., 2003. Ripe fruits of Solanum nigrum L. inhibits cell growth and induces apoptosis in MCF-7 cells. Food Chem. Toxicol. 41, 1421–1428.
10. Zakaria, Z.A., Gopalan, H.K., Zainal, H., et al. Antinociceptive, anti-inflammatory and antipyretic effects of Solanum nigrum chloroform extract in animal models. Yakugaku Zasshi 2006;126:1171-1178.