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Rapid in Vitro Plant Regeneration from Nodal Explants of *Withania somnifera* (L.) Dunal: a Valuable Medicinal Plant

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Abstract: ***Withania somnifera (L.) Dunal is high demanding valuable medicinal plants which have pharmaceuticals properties, known for its with anolides. Nodal segment explants of W. somnifera when cultured on MS (Murashige and skoog's, 1969) medium horizontally containing different concentration(1-3mg/l) of 6-benzylaminopurine (BAP), kinetin (Kn) and thidiazuron (TDZ) produced multiple shoots on both axillary ends of explants when kept horizontally in the medium, within 3 weeks of culture incubation. Maximum multiple shoots was found in MS medium supplemented with (2.0mg/l) BAP as it induced an average of 21 ± 0.0.57 shoots per explants and the maximum shoot length (5.24 ± 0.20) cm. Successful in vitro rooting was induced from cut end of the microshoots when placed on half-strength MS medium supplemented with (5.0mg/l) Indole-3-butyric acid (IBA). The regenerated shoots with well developed root system were successfully acclimatized and established in pots containing soil:leaf manure (3:1) and grown under greenhouse conditions with 94% survival rate.***

Keywords: *Withania somnifera*, BAP, nodal explants, in vitro tissue culture, plant regeneration

1. Introduction

Withania somnifera(L.) Dunal also known as Ashwagandha, Indian ginseng, winter cherry belongs to the family of solanaceae. This plant is highly reputed as it holds medicinal properties and also considered a major plant in Ayurveda. The major biochemical constituents of Ashwaganda are steroidal alkaloids and steroidal lactones in a class of constituents called withanolides viz. withanolide A, withanolide D, withanone, withaferin, sitoindosides, withanosides and also flavanoids and sterols as secondary metabolites (Atta and Dur, 1993; Chatterjee et al., 2010; Chen et al., 2011; Sharma et al., 2011; Chaturvedi et al., 2012). Due to presence of withanolides roots and leaves of *W.somnifera* are used in a number of preparations for their anti-inflammatory, antitumour, immunosuppressive and antioxidant properties besides for promoting vigour and stamina (Devi et al., 1992, Kulkarni et al., 2000 and Furmanowa et al., 2001). Propagation of this plant is by seed but the viability of seed exist for a year only (Roja et al., 1991). Conventional method of its propagation takes a long time. The increase in demand of this plant is growing day by day. Therefore, present study was undertaken by vitro tissue culture that can be used for mass propagation of this plant to satisfy its demand in a lesser time and for its conservation.

2. Material and Method

Plant Material

Young nodal explants were collected from CSIR-National Botanical Research Institute, Lucknow, India. The nodal explants were excised into 0.5-1.0 cm in length and were thoroughly washed in running tap water. The explants were taken into laminar flow for further sterilization procedure. The explants were disinfected with 70% alcohol for 30 sec. and 0.1% (w/v) $HgCl_2$ for 25 min followed by 4-5 rinsed by sterile distilled water in order to remove traces of chemicals from surface of nodal explants. After surface sterilization, both the ends of the explants were finely chopped with the help of forcep in order to remove the injured dead cells which were exposed to chemicals. Inoculated nodal segments were kept in 16/8 h light/dark period at 25 ± 2ºC.

Media Preparation

MS (Murashige and skoog's, 1969) medium was used with 3%(w/v) sucrose (Himedia, India).The medium was supplemented with different cytokinins of different concentration. The pH of all the media was adjusted to 5.8 before adding agar and autoclaved at 121ºC for 20 min. All the media were solidified with 0.8% (w v^{-1}) agar. Cultures were incubated at 25 ± 2ºC under 3 klux light through fluorescent tubes for 16-h light: 8-h dark photo-cycle at a light intensity of 50-60 µmol m^{-2} s^{-1}. All the phytohormones used for the preparation of media were from M/s Sigma Aldrich, USA.

Shoot Induction

The surface sterilized nodal explants were inoculated on the culture medium. *In vitro* shoot induction from nodal explants of *Withania somnifera* on MS medium supplemented with various concentrations of of 6-benzylaminopurine (BAP), kinetin (Kn) and thidiazuron (TDZ) are shown in Table 1. After 2 weeks of culture incubation percentage response was calculated and after 3 weeks, number of shoots per explants was counted. Each experiment was replicated 5 times.

Table 1: Effect of MS medium supplemented with different concentrations of plant growth regulators (PGR) on multiple shoot induction in nodal explants of *W.somnifera*

PGR treatment	*Conc.(mg/l)*	*% shoot induction*	*Av. No. of shoots/explants ±S.E.*	*Av. Length of shoots(cm)±S.E.*
BAP	1.0	90	12±0.57	3.30±0.10
	2.0	100	21±0.57	5.24±0.20
	3.0	95	14±0.57	4.32±0.18
Kn	1.0	80	7.6±0.33	2.16±0.13
	2.0	85	7.0±0.63	2.17±0.08
	3.0	72	6.3±0.66	2.20±0.12
TDZ	1.0	65	8±0.57	1.41±0.20
	2.0	32	7.6±0.33	1.57±0.29
	3.0	40	7.3±0.66	1.09±0.05

Root Induction

Well grown shoots of 2 to 3 cm in length with 5-6 leaves were transferred to root induction medium comprising of MS medium with Indolebutyric acid (5mg/l)IBA. The plantlets with well developed roots were transferred to sterilized soilrite poured into plastic disposable glass and irrigated with sterilized water. These plantlets were grown at 25 ± 2ºC under 16/8 h light/dark period for 3 weeks hardening and then transferred to earthen pots (120 x100 mm) filled with soil:leaf manure (3:1) to grow further.

Statistical Analysis

Each experiment was repeated five times. The values of data are mean ± standard error of three replicates.

3. Result and Discussion

In vitro tissue culture is an important biotechnological tool that is used for rapid production of disease free plants in order to conserve the germplasm of rare, endangered and medicinal plants.

Withania somnifera is an endangered medicinal plant (Kanungo and Sahoo, 2011; Patel and Krishnamurthy 2013) which has been categorized in the list of endangered species by International Union for Conservation of Nature and Natural Resources (Kavidra et al., 2000; Supe et al., 2006). Therefore it was need to establish a protocol for propagation of this highly valuable medicinal plant to meet the increasing demand. Hence, present study deals with in vitro tissue culture of *Withania somnifera* for its mass propagation because very small pieces of plant tissue organs are used as starting vegetative tissues in addition to less time and irrespective of seasonal condition where as conventional method is a long duration method. *Withania somnifera* plant was efficiently regenerated from nodal explants.

Establishment of Aseptic Nodal Explants

Nodal explants have been found superior to other explants as reported in *Jatropha curcas* **(**Maharana et al., 2011), *Aristolochia indica* L. (Pattar et al., 2012), *Mentha piperita* (Sujana et al., 2011) therefore nodal explants were taken in present study. Nodal explants from field-grown plants of *Withania somnifera* were successfully sterilized and cultured on MS medium supplemented with different types and concentrations of cytokinins.

Shoot Multiplication and Proliferation

The effects of different cytokinins on nodal explants were evaluated (Table 1). The emergence of direct shoot buds was observed within 2 weeks after culture incubation of nodal explants from both axils of the nodal explants (Figure 1). In an initial experiment it was observed that nodal explants has shown no sign of bud break in plant growth regulator free medium similar to *Andrographis neesiana (*Karuppusamy et al., 2010). Hence cytokinin was important to induce regeneration from nodal explants. Among different cytokinins used, BAP with optimal concentration of 2mg/l was effective in inducing direct multiple shoots. Highest number of multiple shoots (21 shoots) and maximum average length of shoots (5.24cm) was obtained at (2mg/l) BAP (Table 1).As shown in table 1 (3mg/l)BAP induces 14 shoots per explants with an average height of 4.32cm and 12 shoots per explants of an average height of 3.30 cm was obtained at (1mg/l)BAP. The number of shoots that developed on MS medium supplemented with Kn and TDZ were less with stunted growth and decreases eventually with increase in concentration respectively. The stimulating effect of BAP on formation of multiple shoots has been reported earlier in several medicinal plants (Pattar et al., 2012; Sujana et al., 2011; Singh et al., 2009; pandey et al., 2013)

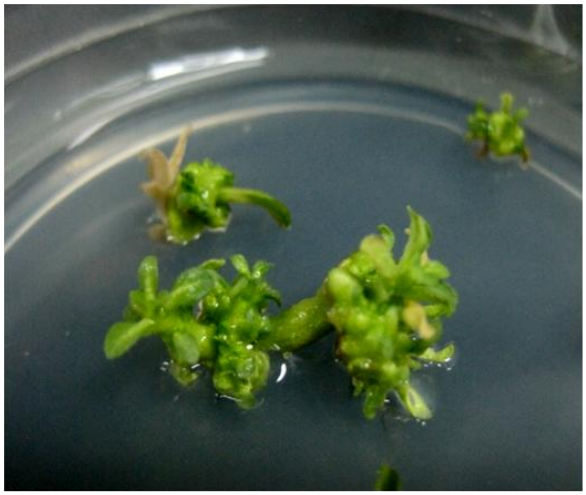

Figure 1: In vitro regeneration of shoots from nodal explants in *W. somnifera* in MS medium supplemented with 2.0 mg/l BAP.

Rooting

The roots were directly induced from the base of shoot in MS medium supplemented with (5mg/l) IBA (Valizadeh and Valizadeh, 2009; Joshi and Padhya 2010; Logesh et al., 2010; Sharma et al., 2010) without an intervening callus (Figure 2).

Figure 2: Rooting of plantlet in the presence of 5 mg/l IBA

Acclimatization

As shown in figure 3, the plantlet was successfully acclimatized and there was phenotypically no difference.

Figure 3: In vitro-raised plant of *W. somnifera* growing in pot.

4. Conclusion

The present protocol for In vitro regeneration of *Withania somnifera* L. reported here can be used to make this plant for biotechnological applications, pharmaceutical usages, germplasm conservation, commercial cultivation in addition to production of secondary metabolites.

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