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Research Articles

In vitro propagation of *Withania somnifera* (L.) Dunal from shoot tip and nodal explants

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Abstract

Withania somnifera L. called as "Ashwagantha" in India, is an important medicinal plant equivalent to ginseng. It is used as stimulant, aphrodisiac, diuretic and tonic. For mass multiplication, shoot tip and nodal explants were inoculated in the Murashige and Skoog's medium fortified with various growth regulators such as 6-benzyl aminopurine, indole-3-butric acid, ∝-naphthalene acetic acid, kinetin and gibberellic acid. Nodal explants responded better than the shoot tip explants and gave maximum shoots on BAP + Kn + NAA (0.5 to 2.0 mg/l) supplemented medium. Shoots transferred into the rooting medium containing NAA 0.5 mg/l and IBA 1 mg/l gave good result (80%). The rooted plantlets were hardened under green house and transferred to the field.

Keywords: explants, *in vitro* propagation, *Withania somnifera*.

INTRODUCTION

Plants are the most important source of medicinal and chemical compounds. Collection of medicinal plants on a mass scale from the natural habitats leads to depletion of plant resources. Micropropagation is of special use for the conservation of these valuable genotypes (Abhyankar and Chinchanikar, 1996), with shoot culture, which is often utilized to maintain clonal fidelity, would be of special advantage.

Withania somnifera (L.) Dunal (Solanaceae), commonly known as "Ashwagandha" or Indian ginseng, is a valuable medicinal plant frequently used in the Indian system of medicine with a wide spectrum of biological activity. It is a component of nearly hundred different preparations/formulations of Ayurveda/Unani of which many of them are already available commercially (Anonymous, 1976).

Ashwagandha is characterized by the presence of steroidal lactones (commonly called Withanolides), alkaloids and flavonoids. Hence,it is prescribed for the treatment of common diseases of respiratory and reproductive tracts, skin diseases, diabetes, gastrointestinal disorders, rheumatism and epilepsy and in improving general health (Govindaraju *et al.*, 2003). "Ashwagantha" is one of the endangered medicinal plants of North-Eastern Karnataka, India (Tripathi *et al.*, 1996). The root extract of the plants is widely used as tonic for curing numerous ailments. The present paper reports an efficient micropropagation system for generating a large number of plants directly from shoot tip and nodal explants of *Withania somnifera* which would form a strategy in the conservation of this important medicinal plant.

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MATERIALS AND METHODS

Berries of *Withania somnifera* growing in and around Thanjavur, Tamil Nadu, India were collected, sun dried and the seeds were used to raise seedlings in the Botanical garden of A.V.V.M. Sri Pushpam College, Poondi, Thanjavur district,Tamil Nadu, India.

Explants were selected from one-year old field grown and *in vitro* raised seedlings. Nodal and shoot tips explants were used for direct regeneration on MS medium (Murashige and Skoog, 1962).

Explants were surface sterilized by washing initially under running tap water, then with liquid soap (Teepol), for few minutes and with double distilled water and then treated with 0.1% mercuric chloride solution (w/v) for 5 minutes. They were rinsed with repeated changes of sterile water to remove all traces of mercuric chloride.

The shoot tip and nodal explants were inoculated by inserting their cut ends in the MS medium supplemented with 0.5, 1.0, 1.5 and 2.0 mg/l of BAP or Kn individually or along with NAA to induce multiple shoots. The medium contained 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH was adjusted to 5.6 and autoclaved at 121°C, 15 lb pressure for 15 minutes. The cultures were maintained at 25 ± 2°C under the light intensity of 3000 lux provided by cool-white fluorescent lamps.

Shoots initiated from both the explants were excised after 30 days and cultured on MS medium, supplemented with 0.5, 1.0, 1.5 and 2.0 mg/l of gibberellic acid (GA_3), for shoot proliferation and elongation. The shoots (5-6 cm long) bearing atleast 4-5 internodes were excised from the mass of proliferated shoots and

transferred to the rooting medium containing 0.5, 1.0, 1.5 and 2.0 mg/l of either indole butyric acid (IBA) or ∝-naphthalene acetic acid (NAA). Rooted plantlets were transferred to polycups containing sterile soil and vermiculite (1:1), and covered with plastic bags to maintain 85-92% humidity. Subsequently, the plantlets were transferred to green house after one month. The plantlets were planted in the soil after one month period of hardening. Experiments were set up in completely randomized block design. Ten cultures were raised for each treatment and all experiments were conducted thrice. Data on number of shoots, shoot length and number of roots and root length were collected.

RESULTS AND DISCUSSION

MS medium supplemented with different concentrations of BAP/Kn in combination with NAA resulted in initiation of shoots from nodal and shoot tip explants (Table 1) (Figs.1c & 1d). Maximum number of multiple shoots were induced in MS medium supplemented with 1.5 mg/l BAP (Fig.1e) when compared to other and higher concentrations used. Hence,it is suggested that this optimum concentration of BAP promotes multiple shoot induction. Similar reports were also obtained with the cultures of *Phyllanthus amarus* (Ghanti *et al.*, 2004) and *Celastrus paniculatus* (Nair and Seeni, 2001). The higher concentrations of BAP inhibited the formation of shoots, and even when the shoots so formed were short and thick (Fig.1a). Such thick rosette type of shoot formation was recorded when higher concentrations of BAP was used in the case of *Melissa officinalis* (Tavares *et al.*, 1996).

Table 1. Efficacy of MS medium fortified with different growth regulators on shoot tip and nodal explants of *W. somnifera* after 4 weeks of culture

Growth regulator (mg/l)	% of response	Mean no. of shoots from shoot tip	Mean no. of shoot from nodal explants
BAP			
0.5	60	4.3 ± 0.3	5.2 ± 0.1
1.0	80	5.3 ± 0.5	6.1 ± 0.5
1.5	100	6.7 ± 0.2	7.2 ± 0.3
2.0	100	5.6 ± 0.4	5.5 ± 0.4
KN			
0.5	80	3.2 ± 0.7	3.0 ± 0.1
1.0	90	5.1 ± 0.3	4.2 ± 0.3
1.5	100	6.2 ± 0.4	7.4 ± 0.6
2.0	100	8.2 ± 0.3	9.1 ± 0.8
BAP + Kn (each 1 mg/l)	100	12.3 ± 0.4	10.3 ± 0.4
BAP + NAA (each 1 mg/l)	100	1.42 ± 0.6	14.6 ± 0.3
BAP + Kn + NAA (each 1 mg/l)	100	16.4 ± 0.8	18.2 ± 0.6

Values represents mean ± standard deviation of 10 replicates per treatment in three repeated experiments.

Multiple shoots were also induced from shoot tip and nodal explants on MS medium supplemented with different concentrations of Kn (0.5–2.0 mg/l). The number of shoots and shoot length were higher on the medium containing 2.0 mg/l. The higher concentrations of Kn inhibited the shoot formation from the shoot explants (Fig.1b).

Two cytokinins namely BAP and Kn were used with auxin (NAA) on the medium, which induced maximum number of shoot initiation. Combinations of BAP, Kn and NAA (each 1 mg) gave maximum response in the induction of more number (Fig.1f) of shoots than the individual cytokinins. Similar finding had also been reported by Vadawale *et al.* (2006) in *Vitex negundo*.

The dwarf shoots subcultured on MS medium supplemented with GA_3 (2 mg/l) showed maximum elongation. For root induction, plantlets were transferred to MS medium supplemented with different concentrations of IBA and NAA (Table 2). Number of roots per explant and root length were more on the medium containing IBA(1 mg/l) and NAA(0.5 mg/l) (Fig.1g). The number of roots and root length decreased, when the concentrations of IBA and NAA were increased. IBA proved slightly superior to NAA in terms of root induction. The influence of IBA on enhanced root formation had also been reported in the case of *Phyllanthus amarus* (Ghanti *et al.*, 2004), *Centella asiatica* (Banerjee, 1999) and *Phyllanthus caroliniensis* (Catapan *et al.*, 2000).

Table 2. Effect of auxins on root induction from *in vitro* raised shoot of *W. somnifera* after 4 weeks of culture.

Growth regulators (mg/l)	% of response	Mean no. of roots / shoot	Mean root length (cm)
IBA (0.5) + NAA (0.5)	60	8.42 ± 0.81	6.78 ± 1.05
IBA (1.0) + NAA (0.5)	100	12.30 ± 0.71	10.56 ± 0.97
IBA (1.5) + NAA (0.5)	80	10.56 ± 0.83	4.78 ± 0.83
IBA (2.0) + NAA (0.5)	60	7.58 ± 0.97	3.89 ± 1.05

Values represents means ± standard deviation of 10 replicates per treatment in three repeated experiments.

Rooted plantlets when transferred to polycups containing sterile soil and vermiculite (1:1) (Fig.1h) got well acclimatized and exhibited 90% survivability when transferred to green house. Thus this proves that this present protocol could successfully be used for large scale clonal propagation without any seasonal constraint. Moreover shoot tip and nodal explants were able to give rise to 16.4 and 18.2 shoots per explant, respectively. Such superior shoot cultures could be a better source for getting steroidal lactones compounds, and can also be used for genetic transformation studies through *Agrobacterium*.

Fig. 1a

Fig. 1b

Fig. 1c

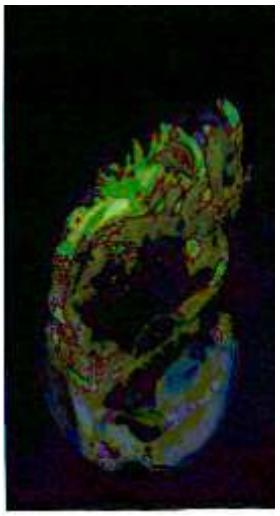

Fig. 1d

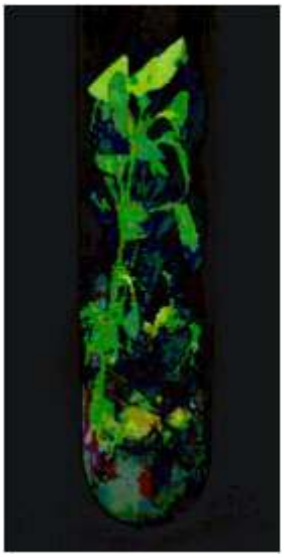

Fig. 1e

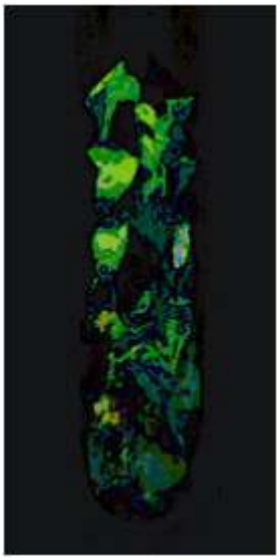

Fig. 1f

Fig. 1g

Fig. 1h

Fig.1. Micropropagation of *W. somnifera*

a) Shoot initiation from the shoot tip explant on MS medium containing 2 mg/l BAP after two weeks
b) Nodal explant culture on MS medium supplemented with 2 mg/l Kn after one week.
c) Multiple shoot regeneration was formed from nodal explant culture on MS medium with BAP + NAA + Kn (each 1 mg/l) after two weeks.
d) Multiple shoot regeneration was formed from shoot tip explant culture on MS medium containing BAP + NAA + Kn (each 1 mg/l) after two weeks.
e) Long shoots formation from shoot tip explant culture on MS medium containing 1.5 mg/l BAP after five weeks.
f) Six week-old culture showing emergence of multiple shoots from nodal explant on MS medium supplemented with BAP + NAA + Kn (each 1 mg/l).
g) Direct rooting from regenerated shoots on MS medium containing 0.5 mg/l NAA and 1.5 mg/l IBA after three weeks of culture.
h) Hardened plant in poly cups containing sterile soil and vermiculite.

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