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Full Length Research Paper

Direct shoot organogenesis from petiole and leaf discs of *Withania somnifera* (L.) Dunal

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An efficient and reproducible procedure is described for direct shoot regeneration using petiole and leaf explants of *Withania somnifera* (L.) . The shoots were mainly induced from the distal end of the petiole, whereas in leaf explants, shoot regeneration was initiated from the basal part and wounded tissue. The regeneration medium that induced the highest numbers of shoots in the petiole and leaf explants was Murashige and Skoog (MS) medium supplemented with 2 mg/l N^6-benzyladenine (BA) alone or with 0.1 mg/l a-naphthalene acetic acid (NAA). The frequency of shoot regeneration was greatly influenced by the type of explant, the carbon source, the orientation of the explant, and the basal medium used in the regeneration medium. Explants produced shoot buds and adventitious shoots within four weeks. Histological analysis of the regenerating shoots showed that the shoot buds emerged from sub epidermal parenchymal cells, with no intermediate callus formation. Plantlets were rooted on MS alone or MS containing different concentrations of 3-indolebutyric acid (IBA). The addition of 1 mg/l IBA to the medium was most effective in inducing root formation. The regenerated plantlets were acclimatized in the greenhouse and successfully transferred to the field, with a 90% survival rate. The acclimatized plants showed normal flowering and were not morphologically different from the seed-derived mother plants.

Key words: Histology, medicinal plant, plant growth regulator, plant regeneration, *Withania somnifera.*

INTRODUCTION

Withania somnifera (L.) Dunal (Solanaceae), popularly called “winter cherry”, is widely distributed in eastern Asia, Africa, and Australia (Kulkarni and Dhir, 2008; Sivanesan and Murugesan, 2008). It has long been used in traditional medicine in India (Singh et al., 2001). Extracts from this herb have also been used for centuries by some indigenous people of South Africa to treat sexually transmitted infections and asthma, and as an anti-inflammatory agent (Van Wyk et al., 1997) . Its leaves, bark, and roots have been used as an aborti-facient amoebicide, bactericide, and contraceptive (Devi, 1996), and they have adaptogenic (Bhattacharya and Muruganandam, 2003), lipid peroxidation, antistress, immunomodulatory (Dhuley, 1998) and antioxidant activities (Panda and Kar, 1997). Other studies have demonstrated that *W. somnifera* is also an inhibitor of angiogenesis and thus is protective against certain types of cancers (Devi et al., 1992; Mohan et al., 2004) . Roles for *W. somnifera* have also been suggested in neuro-transmission, accounting for its activity in various central nervous systems (CNS)-related disorders (Tohda et al. 2005).

Propagation of *W. somnifera* is primarily via seeds (Kattimani and Reddy, 1999). However, conventional

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Abbreviations: SEM, Scanning electron microscope**; MS,** Murashige and Skoog; **IBA,** indole-3-butyric acid; **BA,** N^6-benzyladenine**; TDZ,** thidiazuron**; NAA,** -naphthalene acetic acid; **2,4-D,** 2,4-dichlorophenoxy acetic acid; **FAA,** formalin-aceto-alcohol; **CNS,** central nervous systems.

propagation of this plant by seeds is not reliable and is inadequate to meet commercial demands because of the low viability of the stored seeds (Siddique et al., 2004) and low seed germination rates (Vakeswaran and Krishnasamy, 2003). Therefore, the development of an efficient regeneration system is desirable for this species.

There have been few reports of shoot regeneration in *W. somnifera* (Baburaj and Gunasekaran, 1995; Manickam et al., 2000; Kulkarni et al., 2000; Rani et al., 2003; Siddique et al., 2004; Sivanesan and Murugesan, 2008; Dewir et al., 2010). Most of these reports have described indirect regeneration via callus induction. To the best of our knowledge, there is no published report describing the regeneration of *W. somnifera* via direct adventitious shoots from petiole explants.

Therefore, the objectives of this study are to develop a high frequency direct regeneration system for *W. somnifera* using leaf and petiole explants and to compare explants responses to different hormone concentrations, as well as to determine the optimal media and culture conditions for genetic transformation.

MATERIALS AND METHODS

Seed germination and explants preparation

Seeds of *W. somnifera* were provided by the Bioherb Research Institute, Kangwon National University, Korea. The explants were washed thoroughly under running water for 40 min. They were then disinfected in 70% ethanol for 1 min, rinsed with sterile distilled water, and then soaked in a solution of 5% (v/v) sodium hypochlorite plus tween 20 for 15 min. This was followed by six washes with sterile distilled water. The surface-sterilized seeds were planted in Murashige and Skoog (MS) medium and then incubated at 25 – 27°C under a 16 h photoperiod (36 mol m^{-2} s^{-1} light intensity provided by cool white fluorescent bulbs). After seed germination, the plantlets were maintained in half-strength MS medium. *In vitro* grown seedlings (4 – 5 weeks after germination) were used as the sources of the explants.

Culture conditions

To examine the effects of the culture medium on shoot regeneration, different basal media were examined, including MS(Murashige and Skoog, 1962), B5 (Gamborg et al., 1968), and SH media (Schenk and Hildebrandt, 1972). The effects of the carbohydrate source (sucrose, maltose, or fructose at 10, 20, or 30 g/l) were also evaluated by adding these components individually to the medium designed for the induction of shoots from leaf and petiole explants. The pH of all media was adjusted to 5.8 before the addition of 8 g/l plant agar, and the media were then autoclaved for 20 min at 120°C.

Plant regeneration

Aseptically excised leaves and petioles explants (0.5 or 0.7 cm^2, respectively) from 4 – 6-week -old seedlings were cultured on sterile MS medium fortified with 0 – 2 mg/l a-naphthalene acetic acid (NAA) alone and or in combination with 0 – 2 mg/l N^6-benzyladenine (BA) or 0 – 2 mg/l thidiazuron (TDZ) for adventitious shoot induction. Fifteen to thirty (15 – 30) explants were used per treatment, and each experiment was repeated at least three times.

The percentage of shoot initiation and the average number of shoots per explant were scored after four weeks. Adventitious shoots were excised and transferred onto MS medium for elongation.

Rooting and plant acclimatization

After four weeks, the healthy elongated shoots of 2.0 – 2.5 cm in length with at least two expanded leaves were excised from the explants and transferred to rooting medium consisting of MS basal medium supplemented with different concentrations of indole-3-butyric acid (IBA), 30 g/l sucrose, and 8 g/l plant agar. All cultures were maintained at 25 – 27°C under a 16 h photoperiod (36 mol m^{-2} s^{-1} light intensity provided by cool white fluorescent bulbs). The experiment was repeated three times with 12 shoots per replicate. The percentage of root initiation and the average number of roots per explant were scored after four weeks. For plantlet acclimatization, the regenerated plantlets with well-developed roots were gently washed in tap water to remove any attached medium and then transferred to 100 × 85 mm diameter plastic pots containing a mixture of sterilized bed soil and perlite (3:1, respectively), and soaked with half -strength MS salts. Each pot was covered with a polythene bag to maintain high humidity, and the pots were placed in a glasshouse under a 16 h photoperiod with reduced light intensity (30 mol m^{-2} s^{-1}) at 25 – 27°C. After two weeks, the humidity in the sealed pots was reduced by punching holes in the bags. The polythene bags were gradually opened after six weeks, and the plantlets were moved to the shade (70%). They were then acclimatized to greenhouse conditions and moved to the field after one month. Data on the percentage survival of the plants were obtained after six weeks.

Scanning electron microscopy (SEM)

Samples were fixed in 3% glutaraldehyde and washed in 0.05 M potassium phosphate buffer (pH 7.0). The samples were dehydrated through a graded series of ethanol solutions at 10 min intervals. The samples were dried to critical point in liquid carbon monoxide for 10 min. After vacuum drying, the samples were fixed to aluminum stubs with double-sided tape and coated with platinum using an ion-sputter apparatus (Hitachi E-1010). The samples were then examined at an accelerating voltage of 15 kV in a field emission scanning electron microscope (Hitachi S-4300 FESEM) at different resolutions and magnifications.

Histological analysis

During the culture period, samples were periodically taken and fixed in formalin-aceto-alcohol (FAA) solution (70% ethanol, 5% glacial acetic acid, and 5% formaldehyde) for 24 h at room temperature. After fixation, the samples were dehydrated through a graded series of butanol solutions and then embedded in paraffin. For histological analysis, the tissue was sectioned to 7 µm with a microtome (Uchida, Japan), stained with hematoxylin solution for 3 min, examined under a light microscope (Olympus BX 50), and photographed with an Olympus C4040 Zoom camera.

Statistical analysis

Each treatment consisted of 15 – 30 explants. All the experiments were repeated at least three times. The data shown represent the mean ± SD. The data were statistically analyzed using the one-way analysis of variance (ANOVA) and significant differences between the means were assessed by Duncan's multiple comparison tests at $P < 0.05$.

Table 1. Effect of different plant growth regulator on adventitious shoot regeneration from mature leaves and petiole explants of *W. somnifera* observed after 4 weeks.

Plant growth regulator (mg/l)			Leaf explants			Petiole explants		
NAA	BA	TDZ	Callus formation (%)	No. of roots/explant	No. of shoots/ explant	Callus formation (%)	No. of roots/ explant	No. of shoots/ explant
0.0	1.0	-	76.67^{b}	0.00^{a}	0.00^{a}	84.00^{e}	0.00^{a}	0.00^{a}
0.0	2.0	-	83.33^{d}	0.00^{a}	23.00 ± 2.94^{f}	92.00^{e}	2.67 ± 1.20^{b}	3.67 ± 1.25^{e}
0.1	0.1	-	100.00^{h}	0.33 ± 0.47^{a}	0.00^{a}	90.00^{e}	0.00^{a}	0.00^{a}
0.1	1.0	-	100.00^{h}	0.00^{a}	9.67 ± 3.86^{c}	90.90^{e}	0.00^{a}	0.00^{a}
0.1	2.0	-	100.00^{h}	0.00^{a}	17.67 ± 3.40^{e}	91.00^{e}	3.00 ± 0.58^{c}	2.67 ± 1.70^{c}
1.0	0.1	-	100.00^{h}	7.067 ± 2.62^{e}	0.00^{a}	100.00^{i}	0.00^{a}	0.00^{a}
1.0	1.0	-	93.33^{f}	3.00 ± 1.63^{c}	0.00^{a}	96.00^{fg}	0.00^{a}	0.00^{a}
1.0	2.0	-	96.67^{g}	0.00^{a}	0.00^{a}	97.57^{gh}	0.00^{a}	0.00^{a}
2.0	0.1	-	100.00^{h}	12.00 ± 2.45^{f}	0.00^{a}	100.00^{i}	0.00^{a}	0.00^{a}
2.0	1.0	-	80.00^{c}	0.00^{a}	0.00^{a}	84.43^{c}	0.00^{a}	0.00^{a}
2.0	2.0	-	100.00^{h}	1.33 ± 0.47^{b}	0.00^{a}	100.00^{i}	0.00^{a}	0.00^{a}
0.1	-	0.1	76.67^{fb}	0.00^{a}	1.33 ± 0.47^{b}	78.33^{b}	0.00^{a}	0.00^{a}
0.1	-	1.0	93.33^{f}	0.00^{a}	12.67 ± 2.06^{d}	75.67^{a}	3.33 ± 1.33^{d}	3.29 ± 1.25^{d}
0.1	-	2.0	100.00^{h}	0.00^{a}	16.67 ± 2.87^{e}	85.67^{cd}	0.00^{a}	1.67 ± 0.47^{b}
1.0	-	0.1	90.00^{e}	3.67 ± 1.70^{d}	0.00^{a}	100.00^{i}	0.00^{a}	0.00^{a}
1.0	-	1.0	100.00^{h}	0.00^{a}	0.00^{a}	100.00^{i}	0.00^{a}	0.00^{a}
1.0	-	2.0	93.33^{f}	0.00^{a}	0.00^{a}	100.00^{i}	0.00^{a}	0.00^{a}
2.0	-	0.1	83.33^{d}	0.00^{a}	0.00^{a}	95.00^{f}	0.00^{a}	0.00^{a}
2.0	-	1.0	83.33^{d}	0.00^{a}	0.00^{a}	98.00^{hi}	0.00^{a}	0.00^{a}
2.0	-	2.0	100.00^{h}	0.00^{a}	0.00^{a}	100.00^{i}	0.00^{a}	0.00^{a}

The data represent the mean number of shoots per explant ± standard deviation (SD) of three independent experiments. Data having the same letter in a column were not significantly differerent by Duncan's multiple range test ($P < 0.05$).

RESULTS

Direct shoot regeneration

In the present study, direct shoot regeneration was achieved by culturing petiole and leaf explants of *W. somnifera*. To investigate the effects of plant growth regulators on direct adventitious shoot regeneration, the petiole and leaf explants were inoculated into shoot induction medium supplemented with different concentrations of auxins and cytokinins (Table 1). In this study, the types and concentrations of plant growth regulators and the type and orientation of the explant significantly influenced the frequency of shoot regeneration.

Shoot initiation occurred at the cut ends and margins of the leaf explants within two weeks of inoculation in culture medium, without callus formation. Adventitious shoot buds first appeared as small knob-like structures on the leaf bases and later developed into new plants (Figure 1A - B). The distal part of the leaf segment was less regenerative than the segments closer to the petiole. Among the cytokinins tested, BA gave the best results in terms of the frequency of shoot bud induction (Table 1). MS medium containing BA alone, and or in combination with NAA, was superior to that containing TDZ in combination with NAA for shoot induction. MS medium supplemented with 2 mg/l BA produced the best results, with the highest number of shoots per explant (23.00 ±2.94). In the absence of BA or TDZ, a low

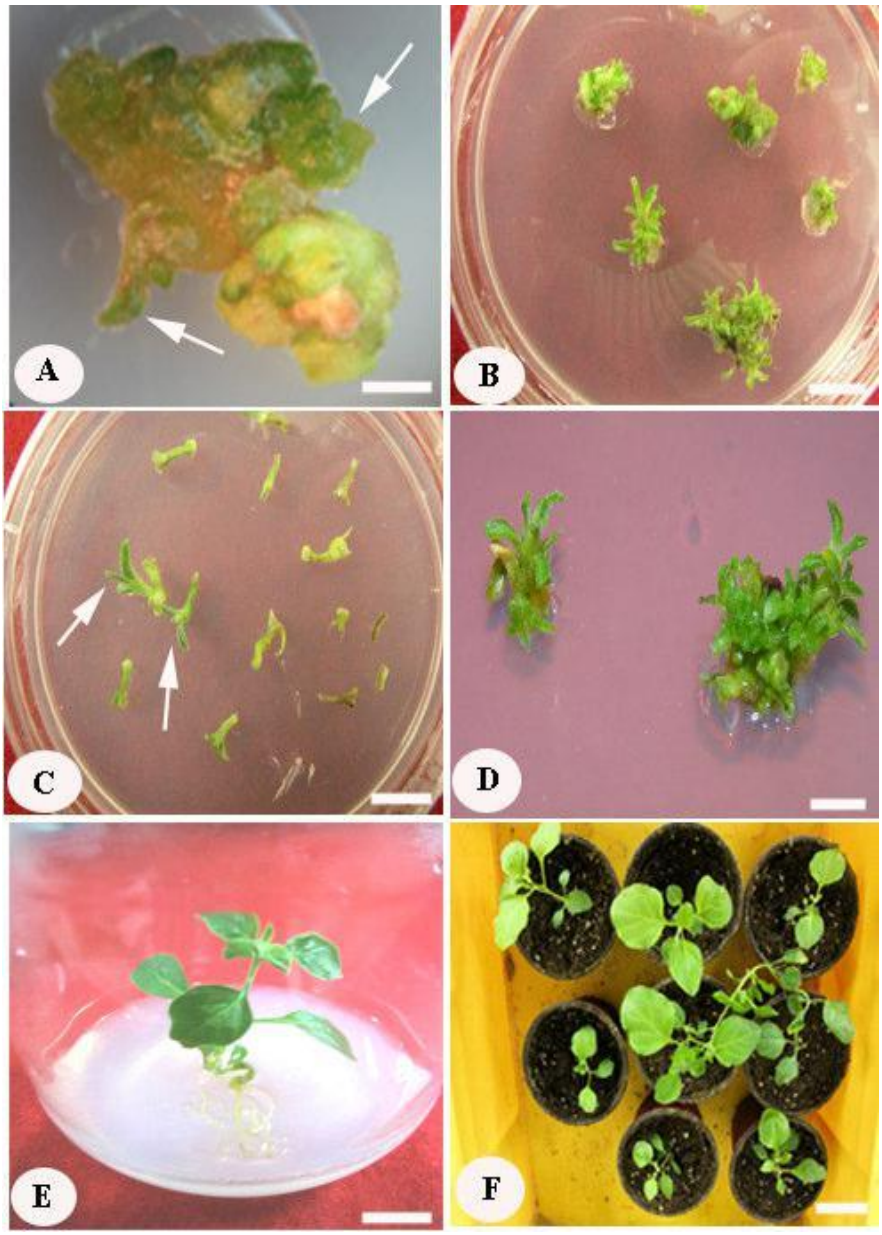

Figure 1. Direct induction of adventitious shoots and plant regeneration via petiole and leaves from *in vitro* plantlet of *W. somnifera*. (A) Emergence of multiple shoot buds from the cutting edges of a leaves explants after 3 weeks in regeneration medium (bar = 300 µm); (B) high frequency of regeneration of multiple shoots from the leaves explants after 5 weeks in regeneration medium (bar = 0.5 cm); (C) regeneration of shoots from the petiole explants after 4 weeks in regeneration medium (bar = 0.5 cm); (D) well developed elongated shoots after 4 weeks on elongation medium (bar = 0.5 cm); (E) *in vitro* rooting of regenerated shoot on MS supplemented with 1 mg/l IBA for 30 days (bar = 0.5 cm); (F) a well rooted plantlet growing in the green house (bar = 1 cm).

frequency of shoot regeneration was observed, indicating that these plant growth regulators are important in shoot regeneration in *W. somnifera.* In contrast, adventitious shoots formed predominantly at the distal ends of the petiole explants (Figure 1C). The numbers of shoots per explants from the petioles were significantly lower (3.67 ± 1.25) than those from the leaf explants. As in the leaf explants, low concentrations of NAA were necessary for direct shoot regeneration and the formation of multiple shoots, whereas higher levels of NAA led to a decline in the shoot numbers. The efficiency of direct shoot regeneration increased with increasing BA concentrations

Table 2. Effect of carbohydrate source and concentration on the number of shoots per explant of *W. somnifera* in regeneration medium.

Carbohydrate source	Concentration (%)	Shoot length (mm)	No. of shoot initiation (%)
Sucrose	1	4.03 ± 0.35^{c}	72.00^{c}
	2	4.67 ± 0.10^{d}	81.00^{d}
	3	4.97 ± 0.15^{d}	95.00^{e}
Maltose	1	1.50 ± 0.03^{a}	3.00^{a}
	2	2.33 ± 0.03^{b}	5.00^{ab}
	3	4.00 ± 0.36^{c}	7.00^{b}
Fructose	1	2.34 ± 0.11^{b}	2.00^{a}
	2	1.33 ± 0.10^{a}	4.00^{ab}
	3	1.26 ± 0.15^{a}	5.00^{ab}

Each value represents the mean ± standard deviation (SD) of three independent experiments with 30 explants per treatment. Data having the same letter in a column were not significantly differed by Duncan's multiple range test ($P < 0.05$). Culture medium was supplemented with 2.0 mg/l BA.

up to 2 mg/l.

Lower BA concentrations (1.0 mg/l) reduced both the number of explants that formed shoots and the number of shoots per explant. There was a considerable increase in increase in shoot number on medium containing low NAA concentrations (0.1 mg/l) whenever it was added to the medium in combination with TDZ (2.0 mg/l). Shoot bud induction occurred when the adaxial surface of the leaf explant was in contact with the medium.

Effects of the carbohydrate source on organogenesis

It is well documented that the carbohydrate requirements of plants depend on the stage of culture and may differ with species. Observations of the shoot regeneration responses of petiole and leaf explants on media containing different carbohydrates revealed that 3% sucrose was the most effective for shoot development, with moderate shoot lengths (4.97 ± 0.15 mm) and healthy shoots. Concentrations of 2 and 1% sucrose in the regeneration medium significantly reduced the frequency of shoot regeneration (Table 2) . The reduced frequency of shoot regeneration was even more pronounced in maltose and fructose supplemented media, and the shoots were not healthy.

Effects of the basal medium on organogenesis

To investigate the effects of the basal medium composition on the induction of direct adventitious shoots, *W. somnifera* petiole and leaf explants were cultured on MS, SH, or B5 medium containing 2 mg/l BA. Among the different basal media tested, MS medium supplemented with 2 mg/l BA was the most effective (23.00 ± 2.94 shoots/explant) for shoot development (Figure 3). Explants on B5 and SH media showed lower average numbers of shoots per explant (2.45 ± 0.47 and 3.33 ± 1.63, respectively).

SEM observations of shoot regeneration

Additional evidence for the direct formation of shoots from leaf and petiole explants was provided by SEM images taken after 8– 23 days of culture. The images showed the regeneration of several globular structures in the basal regions of the leaf explants and at the distal ends of the petiole explants (Figure 2A). They consisted of small cells surrounded by extracellular matrix. After two weeks of culture in MS medium supplemented with 2 mg/l BA, these structures developed into primordial shoots after undergoing several divisions (Figure 2B). After three weeks of culture, distinct regenerated shoots were observed, with at least two leaf primordia per regenerated bud (Figure 2C).

Histological analysis

Histological analysis of the regenerating shoots was performed on explants at different stages of development and showed that the shoot buds had emerged from epidermal parenchymal cells, with no intermediate callus formation (Figure 2D). After 10 days of culture, the shoot buds showed well-developed leaf primordia and apical meristems (Figure 2E). The meristematic cells were much smaller than the surrounding cells, which consisted of closely arranged and highly cytoplasmic cells. After four weeks of culture, numerous adventitious buds had developed on the explants, with distinct epidermal regions and differentiated vascular tissue (Figure 2F).

Shoot elongation and *in vitro* rooting of shoots

Healthy regenerated shoots were excised from the explant

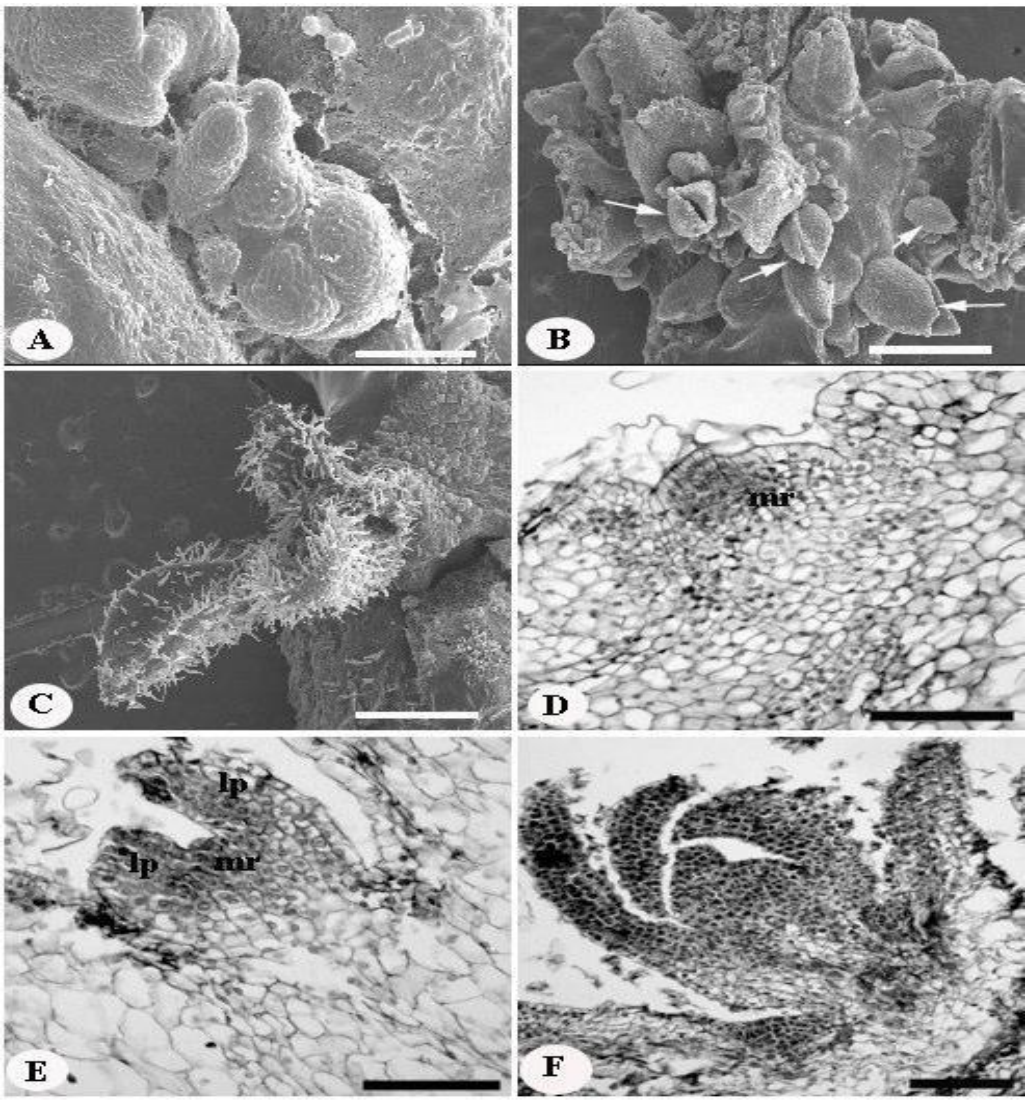

Figure 2. Histological analysis of *W. somnifera.* (A) Early stage of bud primordia from a meristematic dome (bar = 30 µm); (B) SEM image showing multiple shoot-bud regeneration from leaf explant after 12 days of culture (bar = 1mm); (C) regenerated young plant with hairy leaf (arrow head) on the surface of leaf explant on regeneration medium after 23 days of culture (bar = 1mm); (D) explant section showing meristematic region after two weeks of culture (bar = 20 µm); (E) longitudinal section of bud connected to leaf explant showing leaf primordia and the shoot apical meristem (bar = 20 µm); (F) longitudinal section of adventitious shoots from petiole explants with differentiated primary leaves and apical meristems after 23 days of culture (bar = 20 µm).

explant cultures and transferred into elongation medium (data not shown). Efficient shoot elongation occurred when half-strength MS medium was used. Well-developed and elongated shoots of 1.5 – 2.0 cm were excised from the shoot cultures and transferred into rooting medium with or without plant growth regulators. Root initiation occurred within eight days, and the cultures produced roots after four weeks on rooting medium, at rates of 6.7
– 100%, depending on the treatment. However, the root lengths showed only minor differences in media containing the various auxins used for root induction (Table 3). The best rooting was observed with 1 mg/l IBA, on which 100% of the regenerated shoots developed roots (Figure 1E) with an average number of 7.99 ± 0.81 roots per shoot. Profuse callusing was observed, together with the roots, with all the other IBA treatments.

Acclimatization

Healthy rooted plantlets were removed from the medium after eight weeks, transferred to pots containing a 1:1

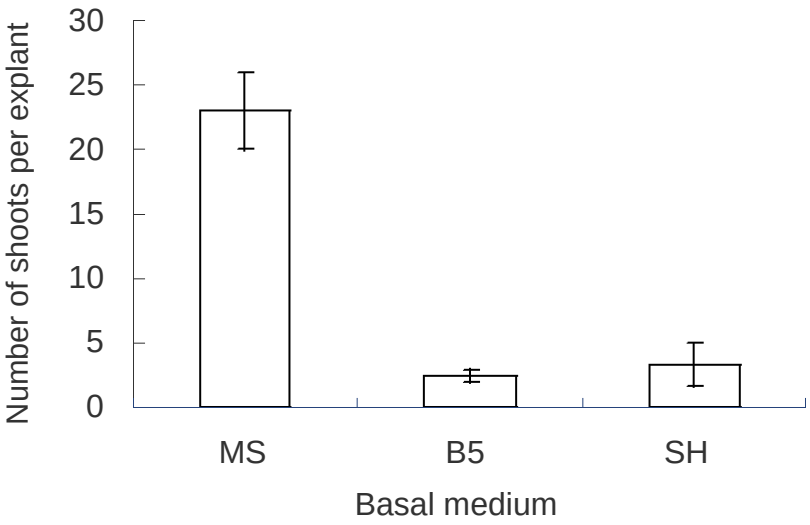

Figure 3. The effect of basal culture media MS, SH, B5, on *W. somnifera* shoot regeneration evaluated after 4 weeks. Each value represents the mean ± standard deviation (SD) of three independent experiments with 30 explants per treatment. Data having the same letter in a column were not significantly different by Duncan's multiple range test ($P < 0.05$). Culture medium was supplemented with 2.0 mg/l BA.

Table 3. Effect of different concentrations of IBA on rooting of the shoots in *W. somnifera* after 4 weeks in culture.

Medium	Formation of root (%)	No. of root/explant	Root length (cm)	Callusing
MS	20.00^{c}	2.33 ± 0.30^{d}	10.00 ± 1.00^{c}	No
½ MS	6.34^{a}	2.03 ± 0.82^{cd}	6.89 ± 0.84^{bc}	No
MS	10.33^{b}	1.18 ± 0.47^{ab}	5.83 ± 0.76^{a}	No
¼ MS	9.00^{ab}	0.44 ± 0.14^{a}	5.42 ± 0.83^{a}	No
MS+ IBA 1 mg/l)	100.00^{f}	7.99 ± 0.81^{g}	11.96 ± 0.79^{d}	No
MS+ IBA 2 (mg/l)	27.27^{d}	5.23 ± 0.75^{e}	8.09 ± 0.59^{b}	Callusing
MS+ IBA 3 (mg/l)	62.17^{e}	5.42 ± 0.83^{f}	11.07 ± 1.01^{cd}	Callusing

Each value represents the mean number ± Standard deviation (SD) of three independent experiments with 30 explants per treatment. Data having the same letter in a column were not significantly differed by Duncan's multiple range test (P < 0.05).

Mixture of vermiculite and perlite, and covered with polyethylene sheets to prevent water loss during the initial stages of plant growth. The rooted plants were transplanted into a growth chamber for one week and transferred to a greenhouse with a 14 h light/10 h dark period at 25 ± 1°C. More than 90% of the plants survived when transferred into pots. The acclimatized plantlets grew normally, showing no morphological variations in the field (Figure 1F).

DISCUSSION

This report presents a reproducible and effective method of shoot regeneration directly from *in vitro* cultured petioles and leaves of *W. somnifera*. Rapid regeneration via adventitious bud formation, avoiding callus production is the system of choice because it minimizes the risk of chimeras and somaclonal variation. Moreover, the *in vitro* regeneration of adventitious shoots is an essential component of most methods of genetic transformation (Mertens et al., 1996). The direct regeneration of shoots from petiole segments has been reported in potato (Yee et al., 2001) and *Heracleum candicans* (Sharma et al., 2001). To the best of our knowledge, there has been no previous report of the direct regeneration of multiple shoots from petiole segments of *W. somnifera*.

In this study, the types and concentrations of the plant growth regulators added to the medium had a significant effect on shoot induction. The presence of cytokinins was important for direct shoot regeneration from leaf explants. In the absence of cytokinins, NAA-containing medium produced calluses with profuse roots but failed to regenerate shoots. The frequency of shoot regeneration and the

number of shoots per explant decreased with decreasing BA or TDZ concentrations.

The regeneration medium containing BA alone, or in combination with NAA, produced the highest shoot regeneration rates in terms of the number of shoots per explant and the shoot length. BAP induced shoot regeneration from the leaf explants have also been reported in other species of Solanaceae like in *Solanum tuberosum* (Abd Elaleem et al., 2009), in *Solanum melongena* (Kanna and Jayabalan, 2010), in *Capsicum frutescens* (Kumar et al., 2007). In contrast, the presence of TDZ in combination with NAA reduced the number of regenerated shoots, even though several studies have reported the efficacy and improved regeneration obtained when TDZ was used in the regeneration of plant species (Lane et al. 1998; Leblay et al. 1991).

The frequency of shoot regeneration was lower from petioles than from leaves. Similar observation was also made in *Capsicum* spp. (Venkataiah and Subhash, 2001), indicating that the leaf explants was more amenable to regeneration of adventitious shoots than petiole explants. Regeneration from petioles always occurred from the distal part of the explants. This may be explained by the increased density of the vascular tissue, and thus the levels of phytohormones and metabolites, near the patio-lar region of the explants (Welander and Maheswaran, 1992). Another reason for this phenomenon could be the basipetal transport of endogenous auxins and/or carbohydrates (Dubois and de Vries, 1995). In this study, the addition of low NAA and BA concentrations was important for high-frequency direct shoot regeneration from petiole explants. Like leaf explants, petioles failed to regenerate when the concentration of NAA was increased to 2.0 mg/l with any concentration of BA. Shoot regeneration was initiated from either or both of the cut ends of the petiole, resulting in fewer shoots per explant. In contrast, the number of shoots per explant was higher in leaf explants. The difference in the number of shoots formed from leaf and petiole explants may result from differences in the regeneration potential of the two explants, attributable to the physiological state, age, and cellular differentiation of the constituent cells (Murashige, 1974).

The type of basal salts in the regeneration medium affected the regeneration efficiency. The percentage of regeneration and the number of shoots per explant were higher on MS medium than on SH or B5 medium. The type and concentration of the carbon source in the induction medium were other important determinants of the regeneration response. In this study, 3% sucrose was most effective in supporting the regeneration of shoots from leaf and petiole explants. Sucrose is an important source of carbohydrate in the culture medium as well as acting as an osmoticum (Hartmann et al., 1997), and also stimulates growth as a result of more negative water potential in the medium (Lipavska and Verugdenhil, 1996; Riek et al. 1997).

Conclusion

The present study reports a simple and efficient regeneration of plantlets from petiole and leaf explants of *W. somnifera*. BA alone or with 0.1 mg/l NAA was more effective on inducing direct shoot from petiole and leaf explants. We have observed that the leaf explants produce more shoots than the petiole explants. Subculture of these shoots onto MS medium with 1 mg/l IBA gave rise to plantlets of normal appearance. The rapid *in vitro* propagation of *W. somnifera* by this method may be used in the large scale propagation, *ex situ* conservation and genetic transformation.

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