- Short communication

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In vitro Plant Regeneration of *Withania somnifera*

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An efficient protocol was developed for large scale propagation using seed as an explant in *Withania somnifera.* MS supplemented with BAP at 0.6 mg/l with 0.4 mg/l IAA was found to be most effective in initiating multiple shoots at the rate of ten per explant. By repeated subculturing, a high frequency of shoot multiplication was established. About 90% rooting was achieved with 0.4 mg/l of IBA and 0.4 mg/l IAA. Micropropagated plants were hardened in half strength of MS and then established in (1 : 1) sand and soil mixture.

Withania somnifera is known by common names such as Winter cherry, Indian ginseng, belonging to Solanaceae, mainly cultivated in a limited area of Mandsore district of M.P. However, it is widely distributed throughout the dry regions of India up to an altitude of 2000 m in Himalayas. *Withania somnifera* contains flavonoids and many active ingredients of the class withanolides. So far 12 alkaloids, 35 withanolides and several sitoindosides have been isolated from this plant species and thoroughly studied for its medicinal properties. Much of the pharmacological activity of *Withania somnifera* has been attributed to two main withanolides, withferin A and withanolide.

Withaferin A is the most important of the withanolide isolated from *Withania somnifera*, to which the curative properties of the leaves are attributed (Uma and Akagi 1996, Mohan 2004). Studies over two decades indicate that withanolide has anti-inflammatory, anti tumor, antistress (Archana and Namasivayam1999), antioxidant, mind boosting, rejuvenating and antimicrobial properties (Jaffer and Jawad 1988). Withanolides also account for multiple medicinal applications. In addition to the alkaloids, the roots are reported to contain starch, reducing sugars, hentriacontane, glycosides, dulcitol, and withaniol.

Withania somnifera can be propagated both by sexual and asexual method. Seed propagation, however is not always satisfactory, since the heterogenetically the strain produces a great deal of variation. Again multiplication through cuttings give rise to less ramified plants and is consequently less productive than plants obtained from seeds. The requirement of *Withania somnifera* has sharply raised due to its popularity owing to a large scale unrestricted exploitation. This

medicinally important plant species has been depleted from their natural habitat and is now included in the list of threatened species by The International Union for Conservation of Nature and Natural Resources (Kavidra et al. 2000). Keeping this fact in view, the present investigation was carried out for *in vitro* regeneration of plantlets followed by successful *ex vitro* establishment of regenerated plants (Sen and Sharma 1991).

Plants were collected from the Botanical garden of the Department of Botany, ST. Thomas College, Bhilai and were grown in the experimental garden. Seeds of the plants were collected from garden and were washed thoroughly with running tap water for 10 min and then washed with distilled water and then surface sterilized with 0.1% (w/v) mercuric chloride solution for five min followed by three times washing in sterilized distilled water to remove traces of mercuric chloride.

For initiation of multiplication, MS basal medium supplemented with different concentrations of cytokinin, BAP (0.1 - 1.0 mg/l), Kn (0.1 - 1.0 mg/l) in combination with auxin, IAA (0.4 mg/l) was used. The pH of the medium was adjusted to 5.8 before the addition of 0.8% (w/v) agar. All cultures were maintained under continuous 16 h dark/light period for four weeks.

For further elongation and multiplication of regenerated shoots, the primary shoots formed *in vitro* were separated aseptically and cultured in MS supplemented with BAP (0.5 - 1.0 mg/l) and IAA (0.1 - 1.0 mg/l). All cultures were maintained at 16 h photoperiod. Subsequent subcultures were placed on MS medium.

For root induction, excised shoots with three or more leaves were transferred to MS basal medium fortified with IAA (0.2 - 0.6 mg/l) and IBA (0.2 - 0.6 mg/l).

Plantlets with well-developed roots were removed from culture medium and transferred to half strength of MS basal medium after gentle washing with sterilized double distilled water and carefully transferred to earthen pots containing sand and soil in 1 : 1 ratio. These were watered with half strength of MS medium. Plantlets were maintained in controlled condition and after two weeks transferred outside under the full sun.

Shoot formation was observed from seed explants to trigger its inherent regeneration capacity. Cytokinins were incorporated into the medium in combination with auxin, IAA (0.4 mg/l). Two different cytokinins *viz.* BAP (0.1 - 1.0 mg/l) were tested for shoot bud induction (Table 1). The highest percentage of plant regeneration (10%), i.e., 9.9 shoots per culture (Fig. 1A) were found in BAP supplemented medium. Ray and Jha (2001) grew shoot tips on MS supplemented with BA (1.0 mg/l). Shoot induction was found to be 10.0 microshoots per explant.

The shoot bud regeneration frequency gradually increased up to (0.6 mg/l) of BAP; with further increase there was a sharp reduction in the number of shoots. However, (0.2 mg/l) BAP and (0.4 mg/l) IAA and (0.2 mg/l) Kn and (0.4 mg/l) IAA combinations produced lowest percentage of regeneration (20 and 10, respectively). However, Furmanowa (2001) *Withania somnifera* plantlets were produced *in vitro* from the shoot-tip of aseptically germinated seedlings. Culture conditions were optimized using different plant growth regulators, which gave rise to shoots from a single bud.

Table 1. Effect of different cytokinins in combination with IAA (0.4 mg/l) on shoot multiplication of *Withania somnifera* using seed as explants on MS medium after four weeks.

Plant growth regulators (mg/l)		Percentage response	Number of shoots/explant
BAP	**IAA**		
0	0	0	00
0.1	0.4	20	2.1 ± 0.4
0.2	0.4	60	5.8 ± 0.2
0.4	0.4	75	7.4 ± 1.1
0.6	0.4	90	9.9 ± 0.1
0.8	0.4	80	8.1 ± 0.3
1.0	0.4	70	7.6 ± 0.2
Kn	**IAA**		
0.1	0.4	10	1.1 ± 1.3
0.2	0.4	50	6.1 ± 0.5
0.4	0.4	70	7.4 ± 0.7
0.6	0.4	85	8.9 ± 1.1
0.8	0.4	70	7.5 ± 0.9
1.0	0.4	65	6.9 ± 0.4

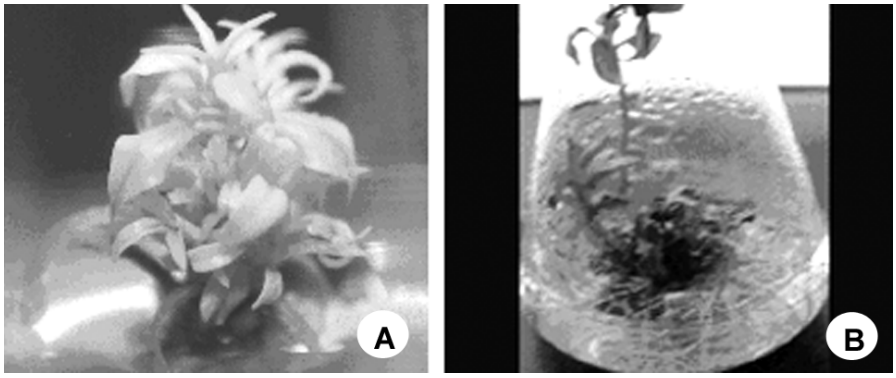

Fig. 1. (A) Adventitious shoot induction from seed as explants. (B) Adventitious root induction from regenerated *Withania somnifera* plant.

Among the Kn and IAA combination Kn (0.6 mg/l) and IAA (0.4 mg/l) combination developed 85% shoots. In present study, BAP was found to be the

best cytokinins for shoot regeneration as well as more number of shoots than Kn. Blakesley and Constantine (1992) also reported uptake and metabolism of BAP.

The elongated shoots were separated and transferred for root formation. Before transferring the shootlets in rooting medium were kept in a hormone free medium containing MS basal medium. These treatments showed better elongation. For rooting, elongated shoots were transferred to medium containing MS basal medium with IAA and IBA either individually or in combination (Table 2).

Table 2. Effect of various auxins on root formation in *Withania somnifera* on MS after four weeks.

Auxin conc. (mg/l)	Percentage rooting	Number of roots/shoots	Basal callus
IAA			
0	0	0	-
0.2	20	1.05 ± 0.3	+
0.4	35	1.90 ± 0.1	++
0.6	40	2.85 ± 0.2	++
IBA			
0.2	40	1.20 ± 0.1	-
0.4	55	2.10 ± 0.4	-
0.6	60	2.85 ± 0.2	-
IAA + IBA			
0.2 + 0.4	40	1.20 ± 0.1	-
0.4 + 0.4	85	5.10 ± 0.2	-
0.6 + 0.4	70	3.80 ± 2.75	+
0.2 + 0.6	60	2.75 ± 0.1	+
0.4 + 0.6	70	3.25 ± 0.4	-
0.6 + 0.6	50	2.01 ± 0.2	+

Different concentrations of IAA or IBA (0.2 - 0.6 mg/l) were tried for root induction. In only IAA incorporated medium, roots were formed from basal callus; roots were thick, fragile and short. Roots were growing long and thin in IBA supplemented medium of the two auxins used. IBA was found to be the best as this supplement yielded the highest number of roots. The rooting response was found to be best when both the auxins were used in combination (Fig. 1B), 35% rooting was noticed when medium supplemented with IAA (0.4 mg/l) and IBA (0.4 mg/l) produced average number of roots (5 roots/shoot, Table 2).

Plantlets with fully expanded leaflets and well developed roots were removed from culture tubes and washed thoroughly to remove all traces of agar; initially these plants were successfully transferred to sand and soil in 1 : 1 ratio and kept in a controlled condition for one month. The Micropropa-gated plants survived and grew normally following transfer to soil. There was no detectable variation among potted plants with respect to morphological and growth

characteristics. The protocol described here could be highly useful for large scale micropropagation of this important medicinal plant.

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