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AN ATTEMPT TO PROPAGATE AND CONSERVE *WITHANIA SOMNIFERA* (L). DUNAL: A POLYPHARMACEUTICAL PLANT THROUGH *IN VITRO* CULTURE

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ABSTRACT

The present study was conducted to establish a simple and effective protocol for plant propagation and conservation and by using shoot tip of *Withania somnifera* (Dunal). The plant is one of the important medicinal cash crop in many states of India. It is widely used in the treatment of inflammatory conditions, in tumor, tuberculosis, rheumatism and is beneficial to improve rejuvenating effect. In *In Vivo* condition the germination of seed is very poor. Regeneration protocol for multiple shoots development from shoot tips explants were established. Shoots were initiated directly from shoot tips and maximum of 11.3 ± 0.28 shoots/explants were achieved, in MS medium containing 2 mg BAP/L, $AdSo_4$ 50 mg/L and citric acid 1 mg/L. MS media supplemented with 2 mg/L IBA was most suitable for rooting. The rooted plantlets were successfully established in field.

KEYWORDS: Sterilization, *Withania somnifera*, Multiplication, Explants, Mercuric Chloride

INTRODUCTION

Herbal medicines are still the mainstay of about 75.80% of the world population for primary health care because of the better acceptability with the human body and less side effects (Kamboj, 2000). *Withania somnifera* commonly known as Ashwagandha, is mentioned as an important ancient Ayurvedic drug of India. It is described as an herbal tonic and health food in Vedas and considered as Indian Ginseng in traditional Indian system of medicine (Singh et al., 2001). It is generally used as anti-inflammatory, anticancer, anti stress, and immune modulator, adaptogenic, central nervous system, endocrine and cardio-vascular activities respectively (Bhattacharya et al., 1997a; Rai, et al., 2003; Ahmad, et al., 2005; Mohanty, et al., 2004)

It is also useful as abortificient amoebicide, anodyne, bactericide, contra-ceptive and spasmodic (Asthana and Raina, 1989). The active principles as *Withanine, somnef-erine, Withanine, somenine* and tropine along with withaferine accumulates in roots as secondary metabolites which are of pharma-ceutical interest necessitates uprooting of the whole plant for harnessing the compounds *which* brings the plant under threatened category. Rapid multiplication of *Withania somnifera* by tissue culture techniques can help to solve these problems.

The present investigation was made out to explore the possibilities for developing a protocol for induction and establ-ishment of plant regeneration from shoot multiplication from *in vivo* explants. Through *in vitro* micro-propagation it is possible to produce a large number of plantlets within a very short time period.

MATERIALS AND METHODS

Collection of Seeds

Seeds of *Withania somnifera* were collected from Birsa Agricultural University, Kanke, Ranchi. Seeds were

maintained at room temp since they have a long viability period of three years. Seeds were then sown in soil to obtain *in vivo* plants. Shoot tips from these *in vivo* grown plants were used as explants.

Sterilization of Explants

Shoot tips were washed thoroughly under running tap water for 15 mins followed by treatment of 0.1% Bavistin for 10 minutes and rinsed 3 times with double distilled water. The explants were then surface sterilized with an aqueous solution of 0.10% mercuric chloride for 10 mins. Proper removal of $HgCl_2$ from the explants is very important and therefore the explants were rinsed three times with autoclaved distilled water.

Tissue Culture Medium and Plant Growth Regulators

To identify the most suitable medium for shoot multiplication, MS ready media (Himedia Pvt. Ltd.) fortified with various concentration and combination of auxin, cytokinines, $AdSo_4$ and citric acid were used. The growth regulators were dissolved in their respective solvents and diluted with double distilled water. The medium was gelled with 0.8% agar (W/L). Prior to autoclaving the pH of medium adjusted at 5.6 – 5.8 by using 1N NaOH or 1 N HCl solutions and then autoclaved at around 121^0C at 16 lbs pressure for 16 mins. Media without growth regulators served as a control.

Direct Shoot Multiplication and Subculture

The shoot tip explants from *in vivo* grown plants were after sterilization were individually inoculated in MS medium supplemented with various concentration and combinations ofhormones to check the effect on multiple shooting.

Following hormonal regime was checked for shoot mulplication

- MS + no hormone (control)
- MS + BAP (.5; 1, 2, 3 mg/L)
- MS + BAP 2 mg/L + IAA (.25, .50, 1, 2 mg/L)
- MS + IAA .5 mg/L + kn (.25, .50, 1, 2 mg/L)
- MS + BAP 2 mg/L + Ads (25, 50, 75, 100 mg/L)
- MS + BAP 2 mg/L + Ads 50 mg/L + Citric acid (.5, 1, 2,3 mg/L).

Shoot tip explants with emerging shoots were sub cultured with the same composition at 28 days intervals.

Root Induction and Acclimatization of Regenerated Plants

In vitro regenerated shoots with height of 2-3 inches and above were excised from explants and transferred to MS medium containing NAA, IAA and IBA indivi-dually. MS medium without growth regulator served as control for all experiments. Culture were maintained under white fluore-scent light at a photon flux of 35 μ $mol/m^2/S^{-1}$ for a 16 h light/8 h dark photoperiod at 25 ± 2ºC.

The rooted plants were removed from culture vessels and washed properly in running tap water for remove agar. The number of roots developed per explants were counted and planted in plastic pots containing sterile mixture of soil, sand and cow dung {1:2:1 (v/v)}. The plantlets were maintained in 80% relative humidity after 3 to 4 weeks, well grown plants were transferred to field.

Statistical Analysis

Each experiment consisted 10 explants and each experiment was repeated 3 times. Mean standard deviation (SD) and standard error (SE) was calculated for significant effect.

RESULTS AND DISCUSSIONS

According to vakeswaran and krishnasamy(2003), seed viability of *withania somnifera* is very low in *in vivo* condition. In present study the shoot tips explants were inoculated in different concentration and combination of BAP, Kn, IAA, $AdSO_4$, and citric acid. 20 concentrations of hormones were tested. Kulkani et al 2000, stated that nodal explants formed multiple shooting on MS medium fortified with 0.1to 0.5mg/L BAP but in present study BAP alone in 0.5,1.0,2.0,3.0mg/l were tested and found that 2.0mg/l BAP gives best result 46.6% with 3.9±0.20 shoots/explants (Figure 1A). After that effect of IAA along with BAP was tested 2 mg/L BAP with varying concentration of IAA (.25 mg/L, .5 mg, 1 mg, 2 mg/L) was checked and found that 2 mg/L BAP along with .5 mg IAA was best in them giving 7.4 ± 17 shoots / explants. (Figure 1B) Then effect of .5 mg/L IAA along with Kn (.5 – 3 mg/L) was checked for shoot multiplication. It was found that Kn 1 mg/L along with .5 mg/L IAA gives best result in these combination showing 5.2±0.31shoots/explants. (Figure 1C)

Table 1: Shoot Multiplication from Shoot Tip Explants following 28 Days Culture on MS Medium Fortified with Different Growth Regulator

BAP	IAA	Kn	Ads	C.A	Cultures Producing Shoots (%)	No. of Shoots/Explants
0.5					20.0	2.1 ± 0.16
1.0					26.7	2.9 ± 0.34
2.0					46.6	3.9 ± 0.20
3.0					23.3	2.8 ± 0.31
2.0	0.25				36.7	3.6 ± 0.32
2.0	0.50				53.3	6.4 ± 0.49
2.0	1.0				40.0	3.3 ± 0.10
2.0	2.0				23.3	2.5 ± 0.33
	0.5	0.25			33.3	4.2 ± 0.29
	0.5	0.50			46.7	5.9 ± 0.15
	0.5	1.0			57.0	8.4 ± 0.30
	0.5	2.0			31.0	3.7 ± 0.13
2.0			25.0		43.3	4.9 ± 0.17
2.0			50.0		76.7	7.1 ± 0.18
2.0			75.0		66.6	5.8 ± 0.11
2.0			100.0		43.3	4.1 ± 0.05
2.0			50.0	0.5	66.7	7.9 ± 0.15
2.0			50.0	1.0	**86.6**	**11.3 ± 0.28**
2.0			50.0	2.0	70.0	7.2 ± 0.41
2.0			50.0	3.0	61 0	6.3±0.15

After that effect of adenine sulfate was checked on multiple shooting Different concentrations (25.0, 50.0, 75.0, 100.0 mg/l) of Ads combined with BAP 2mg/l and found that 2.0mg/l BAP with 50.0 mg/l Ads resulted 76.7% with 7.1 ± 0.18 shoots/explants. (Figure 1D) Then 2mg BAP, 50 $AdSO_4$ with diff concentration of citric acid (.5, 1, 2, 3 mg/L) was tested and found that 2 mg BAP with 50 mg $AdSO_4$ along with 1 mg/L citric acid was found best

concentration and combination giving 11.3 ± .28 shoots/ explants. (Figure 1E) Thus out of 20 concentrations chocked it found that 2mg/l BAP, 50 mg/l Ads, along with 1mg/l citric acid was best for shoot multiplication in *withania somnifera.*

MS medium without supplying any auxin failed to induce roots in excised shoots even after 4 weeks. Out of 3 different auxins IAA, IBA, NAA tested, best result recorded with 1.0 mg/l IBA. 0.5, 1.0, 1.5, 2.0 mg/L IAA, IBA, NAA was supplemented individually to the media. In IAA 1.5mg/l gave 56.7% response with 4.10 ± 0.18 roots/shoot (Figure 1F). In NAA 0.5 mg/l gave 33.3% response with 3.2 ± 0.22 roots/shoot (Figure 1G). While 1.0 mg/l IBA responded maximum 93.3% with 8.2 ± 0.12 roots/shoot. (Figure 1H)

Finally the well rooted plants obtaining the height (5.0 – 6.0 cm) transferred to plastic cups for hardening. (Figure 1I) The plants were acclimatized in plant growth chamber maintained high humidity with initiation of new leaf primordial the plants transferred to pots in green house. About 85% survival was recorded in field. (Figure 1J).

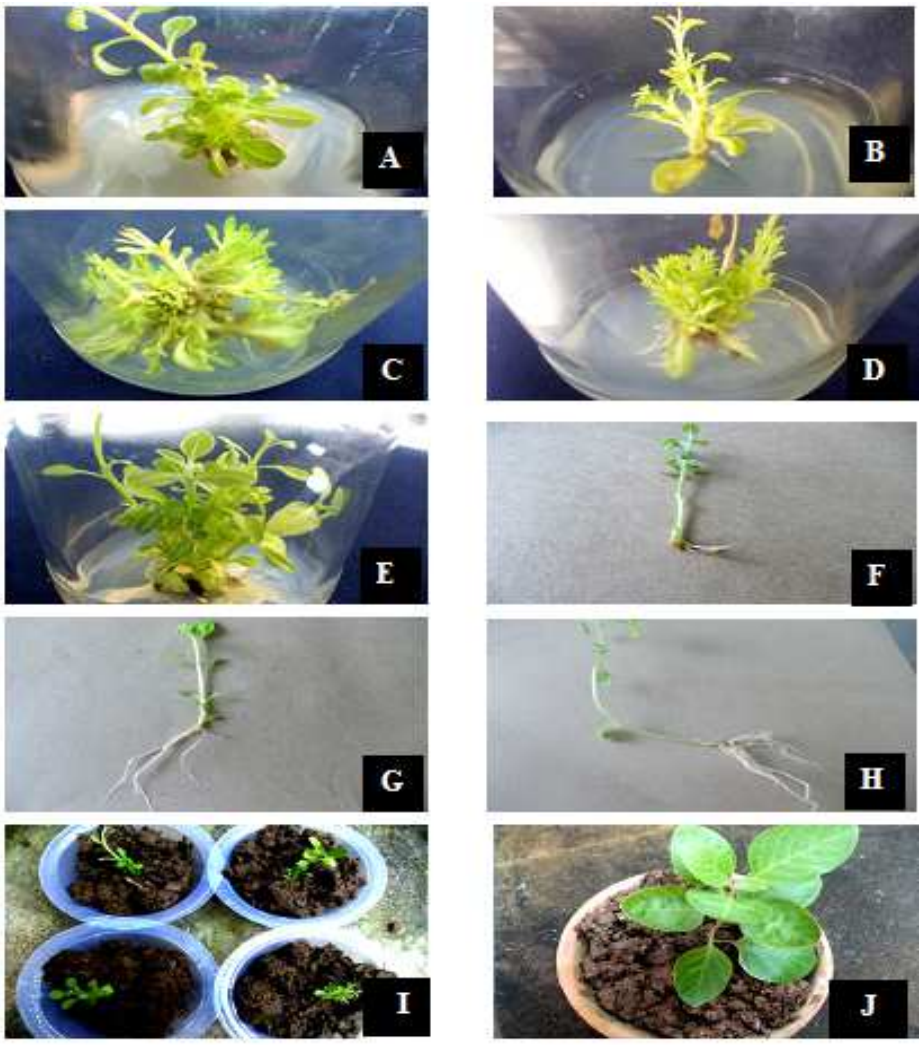

Figure 1: A-E:-Effect of Different Concentration and Combination of Hormones on Shoot Multiplication after 4 Weeks (A) Shoot Multiplication on 2 mg/L.BAP (B) 2mg/L BAP +.5mg/L IAA (C) .5mg/L IAA+1mg/L Kn (D) 2mg/L BAP+ 50mg/L Ads (E) 2mg/L BAP+ 50mg/L Ads +1mg/L Citric Acid. F-H-Effect of Different Auxines on Rooting (F) .5mg/L NAA (G) 1mg/L IAA (H) 1mg/L IBA (I) Transfer of *in vitro* Grown Plants to Sterilized Soil (J) Plants Transferred to Pots Containing Garden Soil and Vermicompost

Table 2: Effect of Different Auxins on Rooting of *in vitro* Derived Shoots of *Withania somnifera*

Plant Growth Regulator mg/L	Cultures Producing Root (%)	No. of Root / Explant
IAA		
0.5	40.0	2.6 ± 0.30
1.0	43.3	3.3 ± 0.09
1.5	56.7	4.1± 0.18
2.0	36.7	3.1 ± 0.40
IBA		
0.5	60.0	2.9 ± 0.05
1.0	93.3	**8.2 ± 0.12**
1.5	66.7	3.2 ± 0.08
2.0	50.0	2.8 ± 0.20
NAA		
0.5	33.3	3.2 ± 0.22
1.0	30.0	2.4 ± 0.12
1.5	23.3	2.1 ± 0.10
2.0	16.7	1.6 ± 0.33

CONCLUSIONS

Medicinal plants are the most important source of life saving drug for majority of the world's population (Padmavati et al., 2005). In this view, herbal business of plants has increased several folds in recent years. About 35 constituents have been isolated and characterized from its roots and leaves (Agarwal et al., 2000). Due to accumulation of secondary metabolites in roots, necessitates uprooting and killing of whole plant for harnessing the compounds which bring this plant under threatened category. IUCN included *Withania somnifera* in list of threatened species (Kavidra et al., 2000). Therefore for germplasm conservation and to meet the increasing demand, *in vitro* propagation in very important

The protocol reported herein for *In vitro* plant regeneration of *Withania somnifera* via shoot multiplication will considerably facilitate large scale propagation and conservation of this poly pharmaceutical plant species.

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REFERENCES

1. Agarwal, R., Diwanay, S., Patki, P. and Patwardhan, B. (1999). Studies of immundomodulatory activity of *Withania somnifera* in experimental immune inflammation. J. Ethnoph. 67 (2): 27 – 35.
2. Ahmad, M.S., Saleem, A. S., Ahmed, M.A., Ansari, S. Yousuf and Hoda, M.N. (2005). Neuroprotectine effect of *Wthania somnifera* on 6-hydroxydopamine induced parkin-sonism in rats. Hum. and Exp. Toxicol. 24: 137 – 147.
3. Asthana, R. and Raina, M. K. (1989). Pharmacology of *Withania somnifera* (L.) Dunal. Ind. Drugs 26: 199 – 205.
4. Bhattacharya, S.K., Satyan, K. S. and Chakarbarti, A. (1997a). Effect of Trasina, an Ayurvedic herbal formulation on paracentric is let superoxide dismutase activity in hyperglycamic rats. Ind. J. of Exp. Bio. 35: 297 – 299.

5. Davis, L., Girija, K. and Kuttan, G. (2000). Immundo-modulatory activity of Ahwagandha J. Ethnopharma. 71: 193 – 200.

6. Kamboj, V. P. (2000). Herbal medicine. Curr. Sci. 78 (1): 35 – 39.

7. Kavidra, N. T., Neelesh, C.S., Vaibhav, T. and Brahma, D. (2000). Micropropagation of *Centella asiatica* (L.). A valuable medicinal herb. Plant Cell Tiss. Org. Cult. 62: 175 – 179.

8. Kulkarni AA, Thengane, Krishnamurthy KV (2000). Direct shoot regeneration from node, internode, hypocotyls and embryo explantsof withania somnifera. Plant Cell Tissue Org. Cult. 62: 203-209.

9. Padmavati, B., Rath, C.P., Rao, R. A. and Singh, P.R. (2005). Roots of *Withania somnifera* inhibit forest-omach and skin carcino-genesis in Mice CAM. 2 (2): 99 – 105.

10. Rout J. R., Sahoo S., and Das R., (2011). An attempt to conserve Withania somnifera (L.) Dunal-Ahighly essential medicinal plant, through in vitro callus culture Pak. J. Bot., 43(4):1837-1842.

11. Rai, D.G., Bhatia, T. Sen and Palit, G. (2003). Antistress effect of Ginkgo *biloba* and panax ginseng a comparative study. Journal of Pharmacological Sciences. 93: 458 – 64.

12. Thomas, T.D. and Philip, B. (2005). Thidia zuron induced high frequency shoot organogenesis from leaf derived callus of medicinal climbers Tylop-hora indica (Burm, F.) Merrill. *In vitro* cell Dev. Biol. Plant 41: 124 – 128.

13. Thomas, T.D. and Shankar, S. (2009). Multiple shoot induction and callus regeneration in *Sarcostoma brevis-tigma* Wight and Arnett a rare medicinal plant. Plant bio-techhol. Rep. 3: 67 – 74.

14. Vakeswaran, V. and Krishnasamy, V. (2003). Improvement in storability of Ashwagandha (*Withania somnifera*) seeds through pre-storage treatments by triggering their physio-logical and biochemical properties seed technology. 25: 203.