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Effect of BAP/IBA ratio in MS medium on induction of roots from multiple shoots and differentiation study of hardening frequency of cultivated and wild traits of *Withania somnifera L.*

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Abstract

Withania somnifera is an important medicinal plant and utilized by all over the world in pharmaceutical industry or in medicine production. Generally Ashwagandha propagates vegetatively with in its natural or exceptable state, but propagation rate is too slow or too late to meet demands of high quality planting material for the useful commercial cultivation. In the past twenty years *in vitro* tissue culture technology has been successfully used in commercial settings to produce disease free plants, mass propagation and to conserve and protect endangered plants. This *in vitro* tissue culture technique is now being used globally for the multiplication of medicinally important plant species and also responsible to produces large numbers of secondary metabolites. In present years, the interests in using these techniques for rapid and large scale propagation of highly valuable medicinally plants have been significantly increases. It is a potent technique for mass multiplication which is known to be efficient for conservation of threatened plant species. After the production of shoots from a culture, they may be cutted off plantlets and rooted or transplanted with auxin and cytokinin to produce which when became mature, can be transferred or to potting soil or hardening in field for the further rapid growth in the greenhouse as normal plants.

Key words: Medicinal plant, pharmaceutical industry, ashwagandha, commercial cultivation, *in vitro* tissue culture, secondary metabolites etc.

Introduction

Withania somnifera (L.) Dunal (Family: Solanaceae, commonly known as Ashwagandha, English name: Winter cherry) is an important perennial plant species with immense therapeutic uses in traditional as well as modern system of medicine (Datta *et al* 2010). Due to restorative property of roots, the species is also known as 'Indian Ginseng' (Tripathi *et al* 1996; Andallu & Radhika 2000; Winters 2006; Kumar *et al* 2010). The Indian Himalayan region (BHR), one of the richest reservoirs of biological diversity in the world, is undergoing irrational extraction of wild, medicinal herbs, thus endangering many of its high value gene stock. *Withonia somnifera L*. (Dunal) is a member of solanaceae, also known for thousands of years by Ayurvedic practitioners. *Withania somnifera* root contains flavonoids, alkaloids, steroid and many active functional ingredients (Kulkarni and Dhir 2007). *Withania somnifera* consists of very high concentration of secondary metabolites that can be also known as bioreactors like steroidal lactones, alkaloids and flavonoides, which have effective properties and they used in ninety commercially Ayurvedic formulations (Sreerekha *et al* 2004). *Withania sominfera* are propagated in northern western region of Madhaya Pradesh in India, on about 400 ha (Khare 1996; Thapliyal & Thapliyal 2001). But the risks of fungal infections are very high in these plants. *Withania sominifera* having small white flowers mainly in rainy and winter seasons that can be develop into fruit during the winter seasons. Plants products can be obtained from the roots, leaves, and branches, by using many different biological techniques. *Withania* which is also known as Ashwagandha having effective property can also used in blends and supplements which are designed to show many multiple effects. 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).

Material and Methods

In vitro regeneration is the basic technique of growing plant cell (s) tissue, organs on artificial nutrient media under controlled tissue culture conditions to achieve various objectives of multiplication, for development of plant system as bioreactors for the production of value added compounds. *In vitro* regeneration of cultivated *Withania* has been a subject of research because of the very high commercial and economic value of the crop and its amenability for further improvement via genetic manipulation (Murashige and Skoog 1962).

MS media, four stock solutions were prepared as follows:

Stock I	macronutrients	10x
Stock II	micronutrients	100x
Stock III	Fe-EDTA	100x
Stock IV	Vit. and AA	100x

MS medium anticipation can be done by taking the volume of stock-I, II, III and IV in two-third volume of demineralized double distilled water followed by the addition of myo-inositol (0.01%w\v) and sucrose (3%w\v). Required amount of plant growth regulators [PGR] were added to the medium and were completely dissolved by continuously mixing on magnetic stirrer. Final required volume was perpetuate with the help of double distilled water. The pH was adjusted to 5.7-5.8 by using of HCl or NaOH. For the preparation of solid medium 0.6-0.8% (w\v) agar powder was dissolved by heat (Naveen Gaurav *et al* 2015).

Medium and glassware sterilization

All the tissue culture media and vessels were steam sterilized by autoclaving at 15psi (1.04 kg/cm2) pressure at 121^0C for 20 min. Thermolabile substance were sterilized separately by filtration (0.22µm Millipore) then added to the autoclaved media when it was cooled at 40-45^0C and blend thoroughly. The media were then dispensed into autoclave culture tubes of radiations sterilized allot to solidify (Naveen Gaurav *et al* 2015).

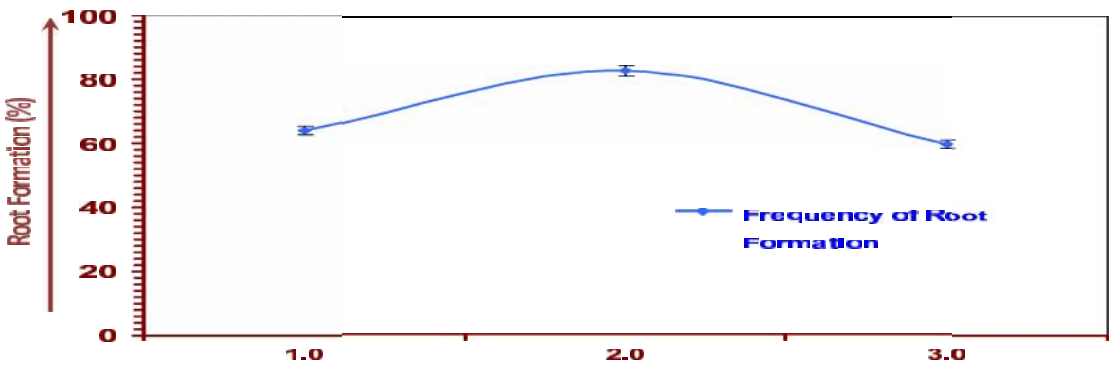
The application of 3.0-5.0 t/ha of vermicompost and 10-15 t/ha of farmyard manure along with biofertilizers such as phosphorus solubilizing bacteria and some growth hormones are recommended for high root yields on a sustainable basis hardening. Alternatively by the use of 60-65 kg urea, single superphosphate and 50 kg of potash/ha may be applied; K and P at planting and nitrogen in splits at 30 and 60 days after planting (Rajeswara *et al* 2006). It is generally denoted as a rain fed crop plant. Once in 15-20 days light irrigations encourage good and efficient crop growth promotion with high root yield.

Results

Table 4.1: Effect of BAP/IBA ratio in MS medium on induction of roots from multiple shoots of *Withania somnifera* (Cultivated)

S.No.	Multiple Shoot nos.	Conc. of BAP/IBA (mg/l)		Frequency of roots formation (%)
		BAP	IBA	
1.	50	0.50	1.00	64±1.28
2.	50	0.50	2.00	83±2.08
3.	50	0.50	3.00	60±0.90

(Mean [+ or -] Standard error).



Fig–4.1

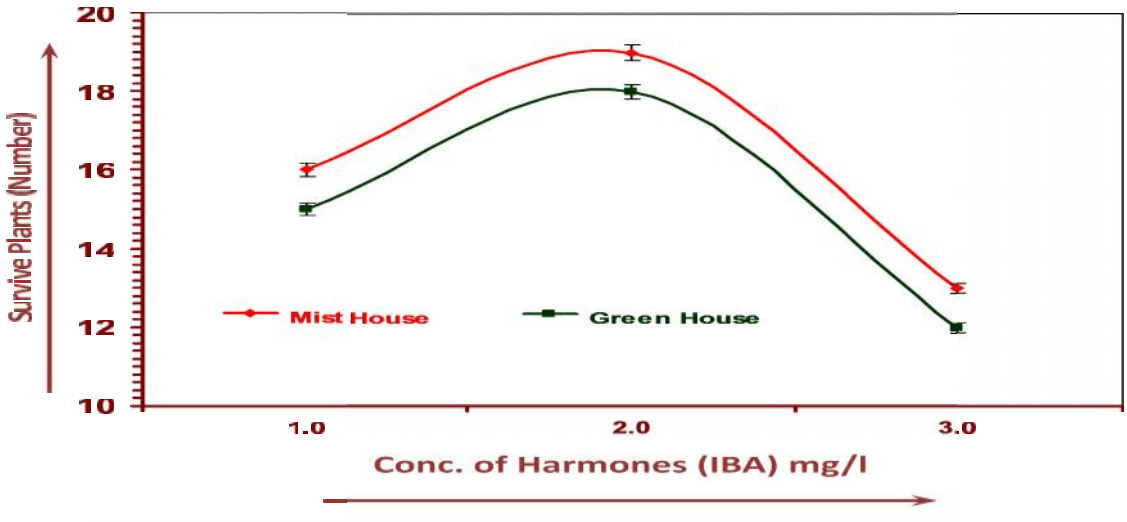
Line diagram showing effect of combination of BAP and IBA ratio in MS medium on induction of roots from multiple shoots of *Withania somnifera* (Cultivated)

In cultivated variety of explants rooting initiated with high auxin to cytokinin ratio. In MS medium maximum rooting takes placed with 0.50 mg/l BAP and 2.00 mg/l IBA as result occurs in table **4.1.** When the well developed shoots forms it has been transferred to root induction media it may be contains BAP + IBA . After three to four weeks shoots are forms from the callus gives rooting. Results has been shown as follows in table **4.1**.

Table 4.2: Hardening frequency *in vitro* (MS Media) plants (Cultivated) in mist and green house:-

S. No.	Conc. of Harmones (mg/l)		Nos. of rooted plants	Small pots containing vermin compost: red sand: red soil	Nos. of survive plants in mist house	Nos. of survive plants in green house	Hardening frequency (%)
	BAP	IBA					
1.	0.5	1.00	30	1 : 2 : 2	16	15	50±0.90
2.	0.5	2.00	30	1 : 2 : 2	19	18	60±1.50
3.	0.5	3.00	30	1 : 2 : 2	13	12	40±0.52

(Mean [+ or -] Standard error).



Fig–4.2

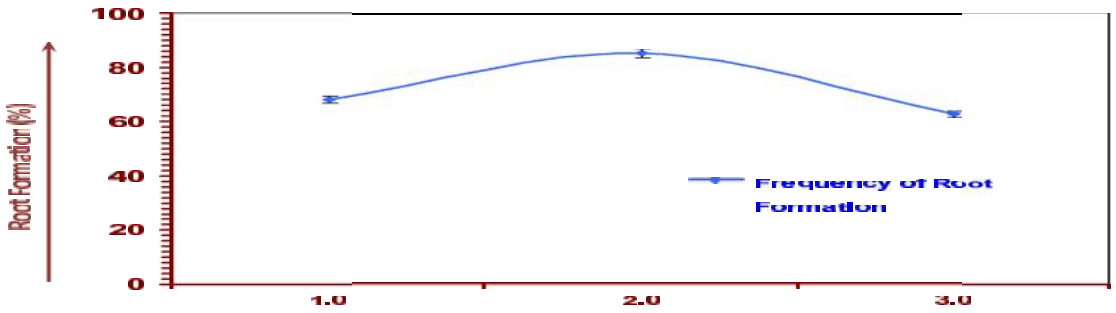
Line diagram showing Hardening frequency *in vitro* (MS Medium) plants (Cultivated) in mist and green house

For hardening-off, 7 to 8 weeks old rooted shoots were withdrawed from the culture flacks. After giving the washing treatment to Agar with water the rooted plantlets obtained from shoots were transferred to bags of polythene or small pots that contains vermi compost fertilizer, red soil and sand in the mixture ratio of 1:2:2 and transferred in a mist house for acclimatization. After successful acclimatization in the mist house for 2-months successfully grown plants are transferred to greenhouse. The plantlets were prosperously planted in the field. Highest hardening and surviving frequency also appears in those *in vitro* plants, which was rooted in MS supplemented with 2mg/l IBA and 0.5mg/l BAP as shown in table **4.2**.

Table 4.3: Effect of BAP/IBA ratio on induction of roots from multiple shoots of *Withania somnifera* (Wild)

S.No.	Multiple Shoot nos.	Conc. of BAP/IBA (mg/l)		Frequency of roots formation
		BAP	IBA	
1.	50	0.50	1.00	68±1.224
2.	50	0.50	2.00	85±2.125
3.	50	0.50	3.00	63±0.819

(Mean [+ or -] Standard error).



Fig–4.3

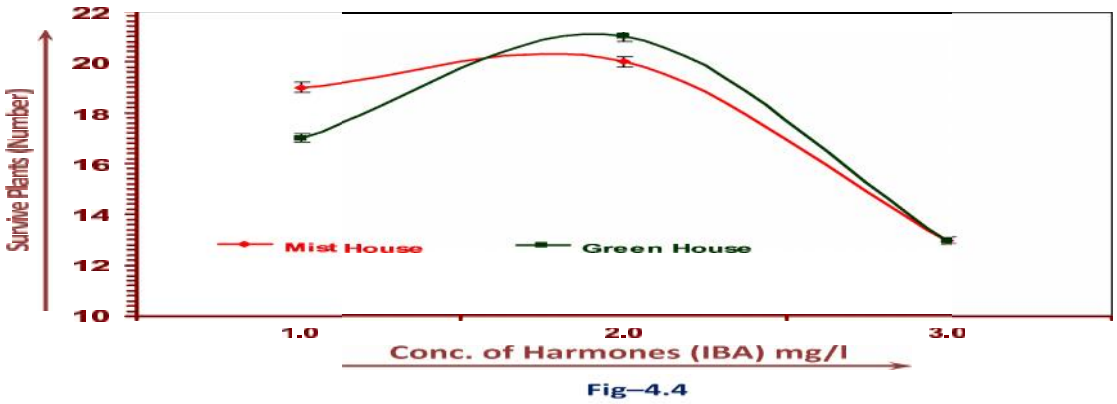
Line diagram showing effect of combination of BAP and IBA ratio in MS medium on induction of roots from multiple shoots of *Withania somnifera* (Wild)

When the well developed shoots forms it has been transferred to root induction media it may be contains BAP + IBA . After three to four weeks shoots are forms from the callus gives rooting. In wild variety of explants rooting initiated with high auxin to cytokinin ratio. In MS medium maximum rooting takes placed with 0.5mg/l BAP and 2mg/l IBA as result occurs in table **4.3**.

Table 4.4: Hardening frequency in *in vitro* plants (wild) in mist and green house:-

S. No.	Conc. of Harmones (mg/l)		Nos. of rooted plants	Small pots containing vermin compost: red sand: red soil	Nos. of survive plants in mist house	Nos. of survive plants in green house	Hardening frequency (%)
	BAP	IBA					
1.	0.5	1.0	30	1 : 2 : 2	19	17	55.66±1.00
2.	0.5	2.0	30	1 : 2 : 2	20	21	70.00±1.75
3.	0.5	3.0	30	1 : 2 : 2	13	13	40.33±0.52

(Mean [+ or -] Standard error).



Fig–4.4

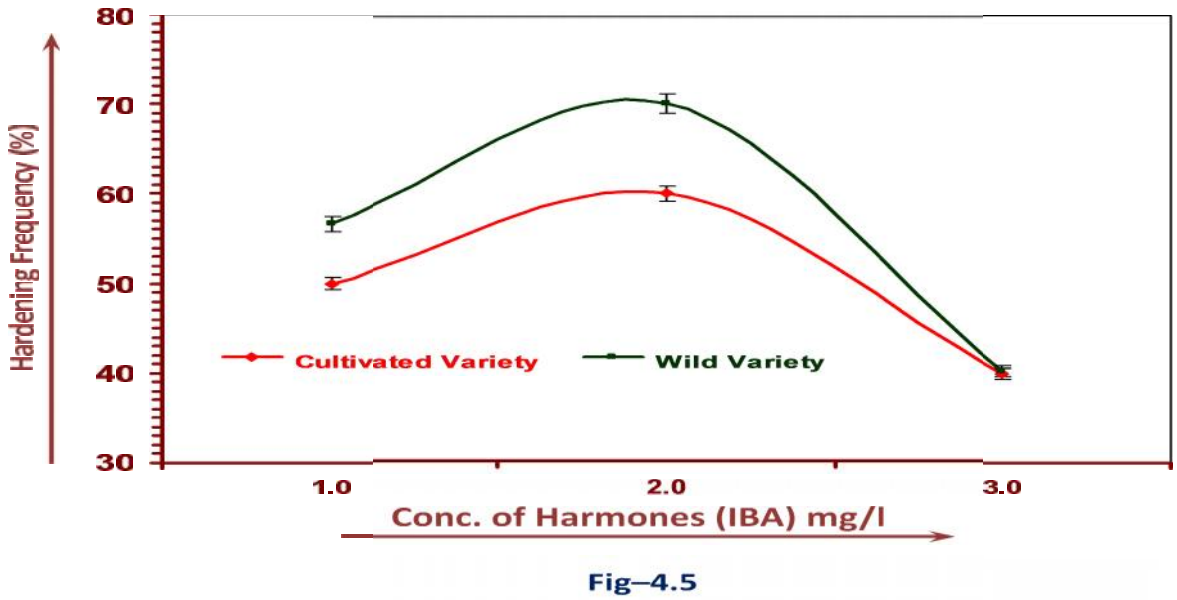
Line diagram showing Hardening frequency *in vitro* (MS medium) plants *Withania somnifera* (Wild) in mist and green house

Highest hardening and surviving frequency also appears in those *in vitro* plants, which was rooted in MS medium supplemented with 2mg/l IBA and 0.5mg/l BAP as shown in table **4.4**.

Table 4.5: Differentiation study of hardening frequencies in wild and cultivated traits of *Withania somnifera* plants which grow in MS- medium:

S.No.	Harmones Conc.(mg/l)		Nos. of rooted plants	Hardening frequency (%)	
	BAP	IBA		Cultivated	Wild
1.	0.50	1.00	30	50±0.90	55.66±1.00
2.	0.50	2.00	30	60±1.50	70.00±1.75
3.	0.50	3.00	30	40±0.52	40.33±0.52

(Mean [+ or -] Standard error).



Fig–4.5

Line diagram showing Hardening frequency in cultivated and wild traits of *Withania somnifera* grows in MS medium

In above result it is proved that the rooting frequency maximum occurs in wild trait and hardening frequency in wild variety is higher than cultivated variety of *Withania* result as shown in table Highest hardening and surviving frequency also appears in those *in vitro* plants, which was rooted in MS medium supplemented with 2mg/l IBA and 0.5mg/l BAP as shown in table **4.5**. After planting Ashwagandha plant is ready for harvest 140-180 in days while in some areas 150-180 days old crop plant is harvested. Maturity of the crop identify by drying out of leaves and reddening of fruits. The

whole plant is pulled out and separates the roots. The roots are washed out with water and cleaned then it either excise into 7-10 cm or whole plant roots are stored. Berries (fruits) are hand plucked and crushed by applying pressure, seeds released dried and stored for the next future crop.

Discussion

Kulkarni *et al* (1996) shows the direct or regular shoot formation from leaf explants or the pieces of tissue and organs of *in vitro* grown seedlings using MS medium containing IAA (3-acetic acid) and BA (6-benzyladenine) in Ashwagandha. After the production of shoots from a culture, they may be cutted off plantlets and rooted or transplanted with auxin to produce which when became mature, can be transferred or to potting soil or hardening in field for the further rapid growth in the greenhouse as normal plants.

Patel & Krishnamurthy, (2013) did work on the micro-popagation of *Withania* explants with increased regeneration potential raised through *in vitro* viability and germination of seeds of two different genotypes of Ashwagandha. Regenerated plantlets were produced successfully or safely in the field region after the primary and secondary hardening.

Higher percentage of *in vitro* morphogenic response was exhibited by explants mature embryo followed by explants mature cotyledon leaves. Cotyledonary leaves are generally produces callus in callus induction media and after several sub culturing its produces shoots and roots in shoot and root induction media. Regenerated plantlets were obtained successfully in the field after hardening (Jhankare *et al* 2011). In above result it is proved that the rooting frequency maximum occurs in wild trait and hardening frequency in wild variety is higher than cultivated variety of *Withania.*

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References

Andallu B and Radhika B 2000 Hypoglycemic diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera* Dunal) root. Ind. J. Exp. Bio., 38: 607-609

Datta AK, Das A, Bhattacharya A, Mukherjee S, Ghosh BK 2010 An Overview on *Withania somnifera* (L.) Dunal – The ‘Indian ginseng’. Med Aro Pl Sci Biotech. 5: 1-15

Jhankare A, Tiwari G, Tripathi MK, Baghel BS, Tiwari S 2011 Plant regeneration from mature cotyledon, embryo and hypocotyl explants of *Withania somnifera* (L.) Dunal. J. Agricultural Tech. 7(4): 1023-1035 Available online http://www.ijat-aatsea.com ISSN 1686-9141

Khare MN 1996 Methods to test seeds for associated fungi, Indian Phytopath. 49(4), 319-328

Kulkarni AA, Thengane SR, Krishnamurthy KV 1996 Direct *in vitro* regeneration of leaf explants of *Withania somnifera* (L.) Dunal. Plant Sci 119:163–168

Kulkarni S K, Dhir A. 2007 *Withania somnifera*: An Indian ginseng,Prog Neuropsychopharmacol Biol Psychiatry, (2007) (in press)

Kumar V, Aggarwal NK, Singh BP. 2000 Influence of P- solubilizing analogue resistant mutants of Azotobacter chroococcumon yield and quality parameters of Helianthus annus. Folia Microbiol 45:347-352

Murashige T, and Skoog F. 1962 "A revised medium for rapid growth and bioassay with tobacco tissue culture", Physiol plant, 48, pp. 473-497

Naveen G, Arun Kumar, Preeti Juyal, Deepak Kumar, Chauhan UK, Singh AP 2015 *In Vitro* Callusing And Effect Of Growth Regulators On *In Vitro* Propagated *Withania* (cultivated & wild) through Cotyledonary Leafs. 1(5). pp 85-94. IJCSR; ISSN: 2454-5422

Patel P, Krishnamurthy R 2013 Feasibility studies on *in vitro* mass-scale propagation of Indian Ashwagandha (*Withania somnifera*) cultivars for commercial purpose; Journal of Pharmacognosy and Phytochemistry; *Vol*. 2 No. 2; pp-168

Rajeswara R, Rajput DK, Nagarju G, Adinarayan G 2012 Opportunities and Challenges in the Cultivation of Aswagandha, ISSN: 0976-884X & E-ISSN: 0976-8858, Volume 3, Issue 2, 2012, pp.-88-91

Singh B, Saxena AK, Chandan BK, Gupta DK, Bhutani KK, Anand 2001 Adaptogenic activity of a novel withanolide-free aqueous fraction from the roots of *Withania somnifera*. Dun Phytother Res. 2001; 15(4): 311-318

Sreerekha MV, Patel KV, Bhatnagar R, Sriram S 2004 Distribution of total Withanoloides in various plant part of Ashwagandha (*Withania somnifera*) accession as influenced by light and dark reaction cycle, J. Medicinal & Aromatic Plant Sci. 26: 681-683

Thapliyal M, Thapliyal RC 2001 Recent Advances in research on seed technology of Medicinal plants Indian Scenario, J. Medicinal & Aromatic Plant Sci. 27: 230-237

Tripathy AK, Shukla YN, Kumar S 1996 Ashwagandha [*Withania somnifera* Dunal (Solanaceae)]: A status report. J. Medicinal & Aromatic Plant Sci. 18:46-62

Winters M 2006 Ancient medicine, modern use: *Withania somnifera* and its potential role in integrative oncology; Altern Med Rev.; 11(4): 269-77