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IN VITRO PROPAGATION AND PRODUCTION OF QUALITATIVE TRAITS OF CULTIVATED VARIETY OF *WITHANIA SOMNIFERA L.* FROM CALLUS OF EMBRYONIC COTYLEDON EXPLANTS IN B5 MEDIUM

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ABSTRACT

Withania somnifera is a member of family Solanaceae is one of the most important and effective Ayurvedic and medicinal herb in different region of India. It is widely and preferlly used in the curing of many diseases and disorders like inflammatory conditions, rheumatism, different types of tumor which also responsible to cause cancer, tuberculosis or in reproductive system to become young again or to decrease the sterility, cure nervous system related diseases and it is very important and beneficial to improve the vitality. The world wide interest in Ashwagandha and the large demand for its dry roots give a great opportunity to propagate this plant on commercial scale. Now present price for its roots is very attractive, Ashwagandha crop provides most economically returns in compare to other traditional crop plants. In India Ashwagandha is commercially propagated as rain fed crop plant in Rajasthan mainly in Kota district and Bhanpura, Manasa, Neemuch, Jawad tehsils of Mandsaur district of Madhya Pradesh and Kur-nool, Prakasam, Anantapur, Mahabubnagar and Warangal districts of Andhra Pradesh. Cultivation has been occurred at few areas in Karnataka.

KEYWORDS: *Withania Somnifera,* Solanaceae, Curing, Diseases, Improve, Vitality, Roots, Rain Fed Crop Etc

INTRODUCTION

Leafs as well as Cotyledonary excised small explants of Ashwagandha were responsible and introduced to evaluate the effect of various growth regulators upon the *in vitro* micro-propagation of direct shoot and root initiation processes. Explants were applied to generate callus, shoot and root regeneration. Ashwagandha is an straight, strong, erect, perennial shrub, evergreen and belong to a member of Solanaceae family, it is a widely used most important medicinal plant which is greatly useful in the treatment of inflammatory, anti-tumor agent (Devi, *et.al.,* 1993). Ashwagandha product is very well known for many years as an essential drug in Ayurvedic literature. Roots of the plant *Withania somnifera* generally exhibit antioxidant, immunomodulatory and haematopoietic properties (Mishra, *et.al.,* 2000). Roots of Ashwagandha mostly used in Unani and Ayurveda medicines. Roots are very important and used as important medicines for hiccups, bronchitis, several female disorders, dropsy, rheumatism, lung inflammation, stomach and skin diseases. The ingredients in medicines are also used as for treating sexual weakness in males and disability (Joshi and Padhya, 2010). As per the record of red list of extinct species, fourty four (44) species of plants are skillfully endangered, 113 endangered and

87 vulnerable. *W. somnifera* variety of Ashwagandha is proved and included to be 99.75% of the endangered and extinct medicinally important plant (Siddique, *et.al.,* 2005). As over rate of harvesting of Ashwagandha that medicinal plant root is going and moving to be extinction condition in the region of Southern India (Manickam, *et.al.,* 2000). The agile pharmacetical substances of *ashwagandha* components are various withanolides a Steroidal lactones with rings having ergostane framework and various important alkaloids (Elsakka, *et.al.,* 1990; Gavande, *et.al.,* 2015). The active important substances of local Indian ashwagandha are normally withanolide-D and withaferin-A, both are generally present in leaves, stems and roots of the plant in high quantity, are used as a source of drugs. In the root of the Indian Ashwagandha the total alkaloid volume has been reported that varies between 0.13% to 0.31% so this plant have antitumor and radio sensitizing consequences in animal models (Sharma, *et.al.,* 2009). Ashwagandha herb also possesses anti-stress properties, immunomodulatory properties, anti-oxidant properties and antibacterial properties (Devi, *et.al.,* 1992; Devi, *et.al.,* 1993).

MATERIAL AND METHODS

Specimen Collections

The seeds with embroys of the cultivated type of ashwagandha or *Withania* were obtained from the local nurseries. The Agriculture Research Station (ARS) Gulbarg produces the large stocks of Ashwagandha. The seeds taken is washed carefully and properly with the running tap water for 1-2 min and surface will be sterilized with the 70 % ethanol initially followed solution of mercuric chloride (0.1 % w/v) for 3-5 min and thoroughly rinsed 3-5 times in sterile double distilled water to remove the excess or traces of mercuric chloride and absorbed in double distilled water for four to six hours.

Chemicals

All chemicals were mostly of Himedia, India and Sigma, USA and some of the chemical were also obtained from SRL, Qualigens and E. Merck, India.

Preparation of B5 Medium

Added 23.23 grams weight of dehydrated medium in sterilized 600ml of double distilled water and to wash or clean the media vial by suitable and small quantity of double distilled water to remove out the traces of powder. Apply constant gentle animation to the solution in a proper way till the powder dissolves completely. Add heat stable supplements to obtain after autoclaving. Maintain the obtain pH of the medium by using 1N HCl/1N NaOH/1N KOH. Make up the final volume of media to one litre (1000ml) with continuous adding distilled water. Sterilize the medium or make the medium free from contamination by the process of autoclaving at 15 lbs or 121ºC for 15 minutes. Then cool the autoclaved medium to 45ºC prior addition of the filter sterilized heat sensitive supplements. Store the prepared medium at 2-8ºC away from direct light.

Medium and Glassware Sterilization

All the tissue culture media and vessels were steam sterilized by autoclaving at 15psi (1.04 kg/cm2) pressure at 121ºC for 20 min. thermolabile substance were sterilized separately filtration (0.22um Millipore) then added to the autoclaved media when it was cooled at 40-45ºC and mixed thoroughly. The media were then dispensed into autoclave culture tubes of radiations sterilized Petri dishes at allot to solidify. The glassware the solutions biodegradable detergent (labolene, India) and rinsed with double distilled water, over dried at 80ºC for 2 hours, followed by most heat sterilization

the instrument used for tissue culture, viz. forceps, needles, scalpels, spatula etc.

Commonly Used Planted Growth Regulators

Substances (PGR's)	Solvent	Stock Concentration	Sterilization	Storage Conditions
1AA	1N Na OH	5 mg/1	F	O^0C
1BA	1N Na OH	0.5 mg/1	CA	O^0C
BAP	1N Na OH	20 mg/1	CA	O^0C
ZET	1N Na OH	4 mg/1	F	O^0C
NAA	1N Na OH	2 mg/1	CA	4^0C
GA3	70% ethanol	1 mg/1	CA	4^0C

CA= Co-autoclavable with other media components

F= Filter sterilization with 0.22 micro Millipore filter

Medium and Glassware Sterilization

All the tissue culture medium, glasswares equipments and vessels were subjected to steam sterilization through autoclave at 15psi or 1.04 kg/cm2 pressure 121ºC for 20 min. thermolabile substance were sterilized separately filtration (0.22um Millipore) then added to the autoclaved media when it was cooled at 40-45ºC and mixed thoroughly. The media were then dispensed into autoclave culture tubes of radiations sterilized Petri dishes at allot to solidify.

RESULTS

Table 4.1: Effect of Different Cytokinins (With Different Concentration) Alone on *in Vitro* Induction of Multiple Shoots in B5 Medium Derived from Embryonic CotyledonExplants of Ashwagandha or *Withania Somnifera* (Cultivated)

S No.	Cytokinins	Conc. (Mg/L)	Explants (No.)	Shoots/ Culture (%)
01	BAP	1.0	88	23.66±0.26
		2.0	90	33.66±0.57
		3.0	85	56.33±1.58
		4.0	82	53.33±1.33
		5.0	80	50.33±1.11
02	Kinetin	1.0	71	33.66±0.61
		2.0	75	44.33±0.84
		3.0	67	30.33±0.49
		4.0	63	28.33±0.42
		5.0	58	25.33±0.33

(Mean [+ or -] Standard error

Table 4.2: Effect of Different Cytokinins (With Different Concentration) alone *in Vitro* Induction of Multiple Shoots in B5 Medium in Combination with BAP and Kn Derived from Embryonic Cotyledon Explants of Ashwagandha or *Withania Somnifera* (Cultivated)

S No.	Explants	Cytokinins (mg/l)		Shoots/ Culture (%)
		Kinetin	BAP	
01	90	2.00	1.00	65.66±1.18
02	90	2.00	1.00	77.11±1.93
03	90	2.00	1.00	46.00±0.60

(Mean [+ or -] Standard error).

Table 4.3: Effect of BAP/IBA Ratio in B5 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	44±0.79
2.	50	0.50	2.00	56±1.40
3.	50	0.50	3.00	35±0.46

(Mean [+ or -] Standard error).

Table 4.4: Hardening Frequency *in Vitro* (B5- Medium) 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	7	6	20±0.36
2.	0.5	2.00	30	1 : 2 : 2	10	9	30±0.75
3.	0.5	3.00	30	1 : 2 : 2	4	3	10±0.13

(Mean [+ or -] Standard error).

Fully mature embryos from cotyledonary leaves (embryonic leaves) of two *Withania* cultivars (cultivated) were cultured or micro-propagated in MS medium in various fortifications of various growth regulators. During my present experiments much of the plantlets regenerated in an indirect manner that mean plantlets originated or formed via callus formation. Callus initiation was observed or examined after 5-7 days of culture from the most of the *in vitro* cultured cut edges from the cotyledonary explants.

Shoot Multiplication in Cotyledon Explants

B5 culture media with Kn and BAP alone shows maximum formation of shoot from callus with concentration of 3mg/l BAP and 2mg/l Kn while maximum frequency of shoot initiation in cultivated variety takes placed with BAP as shown in table 4.1.

In cultivated variety of *Withania* shoot initiation from callus is takes placed in B5 medium having both Kn and BAP. Maximum frequency of shoot formation takes placed in B5 medium supplemented with 2mg/l Kn and 1mg/l BAP as shown in table 4.2.

Root Multiplication in Cotyledon Explants

When the well developed shoots forms it has been transferred to root induction media it may be contains 0.5mg/l BAP + 1mg/l IBA. After three to four weeks shoots are forms from the callus. Results has been shown as follows in table 4.3. In cultivated variety of explants rooting initiate d with high auxin to cytokinin ratio. In B5 medium maximum rooting takes placed with 0.5mg/l BAP and 2mg/l IBA as result occurs in table 4.3.

Hardening

For hardening-off, 7 to 8 weeks old rooted shoots were withdrawed from the culture flacks and washed properly to remove agar on plant part and then transfer to pot having vermi compost fertilizer, sand and red soil with the ratio of

1:2:2 and transferred to the mist house for regular acclimatization and after successful acclimatization (after 2-months successfully grown plants) it is transferred to greenhouse. Highest hardening and surviving frequency also shown in table 4.4.

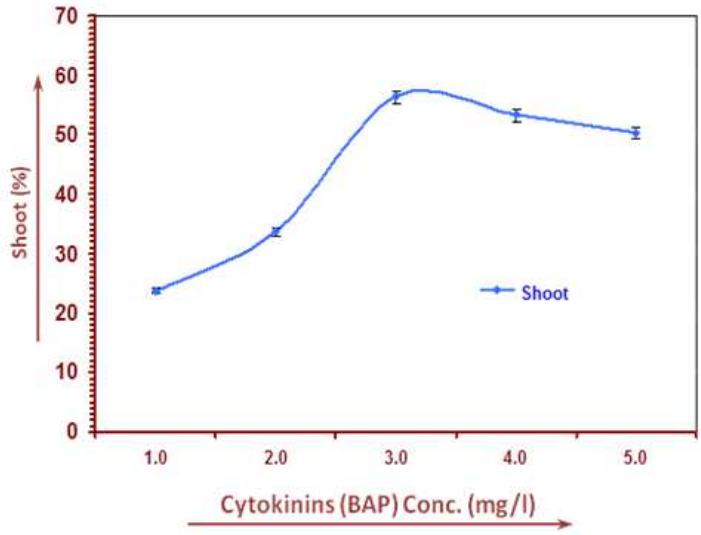

Table-4.1a

Line diagram showing effect of cytokinins (BAP) alone on *in vitro* induction of multiple shoots in B5 medium of *Withania somnifera* (Cultivated)

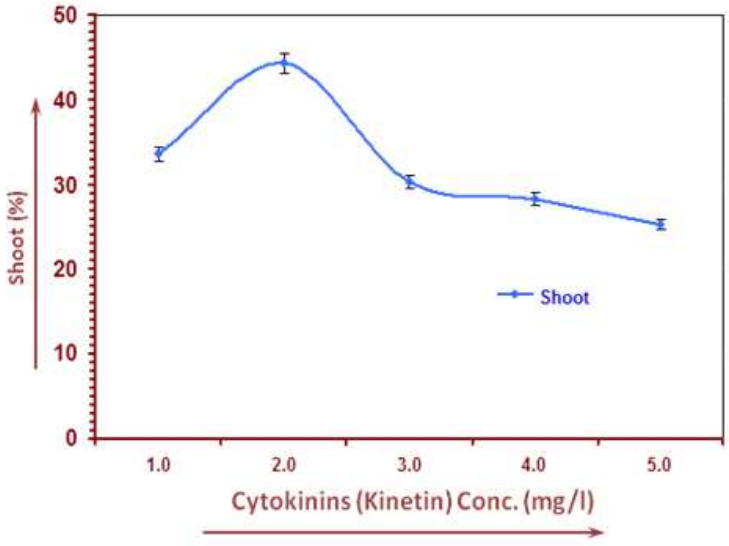

Table-4.1b

Line diagram showing effect of cytokinins (Kinetin) alone on *in vitro* induction of multiple shoots in B5 medium of *Withania somnifera* (Cultivated)

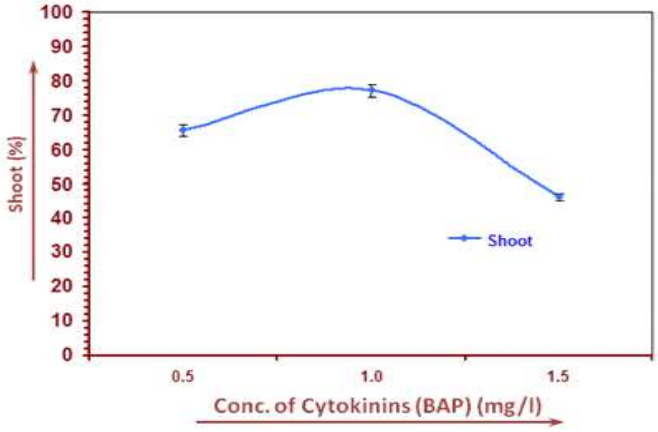

Table-4.2

Line diagram showing effect of cytokinins on in vitro induction of multiple shoots in B5 medium in combination with BAP and Kn of *Withania somnifera* (Cultivated)

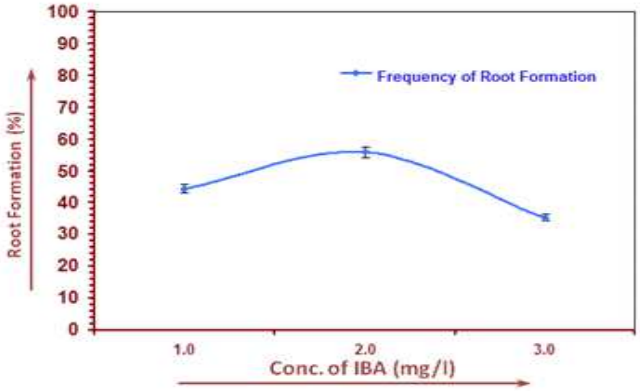

Table-4.3

Line diagram showing effect of combination of BAP and IBA ratio in B5 medium on induction of roots from multiple shoots of *Withania somnifera* (Cultivated)

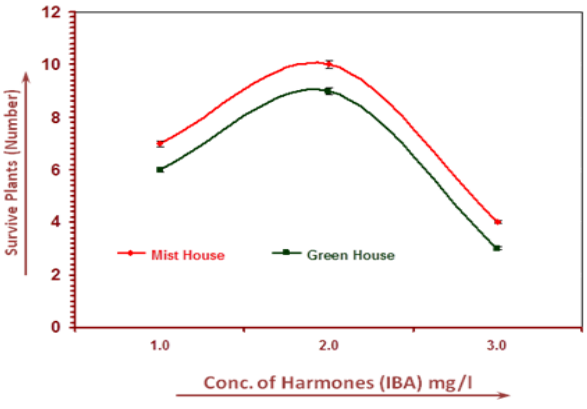

Table-4.4

Line diagram showing Hardening frequency *in vitro* (B5 Medium) plants (Cultivated) in mist and green house

DISCUSSIONS

In India Ashwagandha is commercially propagated as rain fed crop plant in Rajasthan mainly in Kota district and Bhanpura, Manasa, Neemuch, Jawad tehsils of Mandsaur district of Madhya Pradesh and Kur-nool, Prakasam, Anantapur, Mahabubnagar and Warangal districts of Andhra Pradesh. Cultivation has been occurred at few areas in Karnataka (Kattimani, *et.al.,* 1999; Kattimani, *et.al.,* 2001; Rajeswara, *et.al.,* 2006). In Madhya Pradesh, farmers normally do not apply any fertilizers. Various experimental results and conclusion have also proved that the root of Ashwagandha yield is not affected by fertilizer in Madhya Pradesh. So therefore, organically grown Ashwagandha plants are having a good demand in both international and national indian markets (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.

After planting or propagated medicinally important *Withania* plants are ready for harvesting in 135-180 days but in some regions of about 150-180 days old medicinal *Withania* crop 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 and 7-10 cm roots are stored having various medicinal importance. Berries (fruits) are hand plucked and crushed by applying pressure, seeds released dried and stored for the next future crop (Rajeswara, *et.al.,* 2006; Sastry and Rajeswara, 2007; Singh and Kumar, 1998).

CONCULSIONS

Various important value added medicinal components obtain from *Withania somnifera* crop includes root extract (Chatterjee, *et.al.,* 2009), herbal beer, root powder, capsules with extract, alkaloids, steroid and traditional medicinal value added drugs made by *Withania*. But the difficulties associated with *Withania somnifera* for its commercial and conventional cultivation, normally it takes very long germination periods for seed and its whole strains important productivity. *In vitro* micro-propagation of *Withania* (Darwesh, *et.al.,* 2014) introduces various excised pieces such as auxiliary highly division phase meristems (Roja and Heble, 1991), cotyledonary leaves (embryonic leaves), stem or shoot tips (Sen and Sharma, 1991), auxiliary or apical shoot, auxiliary tip leaves, root (small), hypocotyls (Rani and Grover,1999) has been demonstrated.

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REFERENCES

1. Chatterjee, S., Shatakshi, S., Asna, K., Niharika, S., Sangwan, R.S., Om, P.S., Raja, B., Khetrapal, C.L., Chaudhry, S., Raghuvanshi, N., Mathur, R., Gautam, L. 2009, In Vitro Studies On Withania Somnifera (L) Dunal: Effect Of Different Media, Explants, Carbon Sources, Salt Levels And Phytochemical Analysis; Biochem. Cell. Arch. *Vol*. 9, No. 1, pp. 51-55.
2. Darwesh., Hadeer, Y. A., Abd El-Kafie., Omaima, M., Hamza, A.M., Gohar, A.A., Sedki., Mahasen, A. 2014, *In vitro* propagation method of *Withania somnifera* by tissue culture technique, International Journal of Advanced Research (2014), *Vol*ume 2, Issue 6, 1018-1021
3. Devi, P.U. and Sharada, A.C. 1992, In vivo growth inhibitory effect of *Withania somnifera* (Ashwagandha) on a

transplantable mouse tumor, Sarcoma 180. Indian Journal of Experimental Biology 30(3): 169-172.

4. Devi, P.U., Sharada, A.C., Solomon, F.E. 1993, Antitumor and radiosensitizing effects of *Withania somnifera* (Ashwagandha) on a transplantable mouse tumor, sarcoma-180. Indian Journal of Experimental Biology 31 (7), 607–672.
5. Elsakka, M., Grigorescu, E., Stanescu, U., Stănescu, U., Dorneanu, V. 1990, New data referring to chemistry of *Withania somnifera* species. Rev Med Chir Soc Med Nat Iasi 94:385–387.
6. Gavande, K., Dr. Kirti J., Dr. Bharti Jain, Rakesh M. 2015, Comprehensive Report on Phytochemistry and Pharmacological Prominence of *Withania somnifera*, UK Journal of Pharmaceutical and Biosciences *Vol*. 3(2), 15-23.
7. Joshi, A.G., Padhya, M.A. 2010, Shoot regeneration from leaf explants of *Withania somnifera* (L.) Dunal. Notulae Scientia Biologicae., 2(1) : 163-168.
8. Kattimani, K.N., Reddy, Y.N., Rajeswara Rao, B.R. 1999, Seed Sci. Technol., 27(2), 483-488.
9. Kattimani, K.N., Reddy, Y.N., Rajeswara Rao, B.R. 2001, J. Med. Arom. Pl. Sci., 22(4A) and 23(1A), 221–223.
10. Manickam. V.S., Elango, R., Antonysami, R. 2000, Regeneration of Indian ginseng plantlets from stem callus. Plant Cell Tissue Org. Cult. 62:181-185.
11. Mishra, L.C., Singh, B.B., Dagenais, S. 2000, Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A Review. Altern Med. Rev. 5(4), 334-346.
12. Rajeswara Rao, B.R., Patel, R.P., Rajput D.K. 2006, Maps Dew, 2(2), 20-21.
13. Rani, G., Grover, I.S. 1999, *In vitro* callus induction and regeneration studies in *Withania somnifera*, Journal of Plant Cell Tissue and Organ Culture 57(1): 23-27.
14. Roja, G., Heble, M.R., Sipahimalani, A.T. 1991, Tissue cultures of *Withania somnifera*: morphogenesis and withanolide synthesis. Phytotherapy Research. 1991; 5:185-87.
15. Sastry, K.P., Rajeswara Rao, B.R. 2007, Maps Dew, 3(2), 9-13
16. Sen, J., Sharma, A.K. 1991, Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. Plant Cell Tissue Organ Culture, 26: 71-73.
17. Sharma, S., Sharma, M.C., Kohli, A.D.V. (2009), In Vitro Micropropagation of Medicinally Important Roots And Axillary Bud Combination; Journal of Optoelectronic And Biomedical Materials; *Vol.* 1, No. 4, December 2009, P. 379-381.
18. Siddique, N.A., Bari, M.A., Shahnewaz, S., Rahman, M.H., Hasan, M.R., Khan, M.S.I., Islam, M.S. 2005, Plant regeneration of *Withania somnifera* (L.) Dunal (Ashwagandha) from nodal segments derived callus an endangered medicinal plant in Bangladesh. Journal of Biological Sciences 4(2) : 219-223.
19. Singh, S., Kumar, S. 1998, *Withania Somnifera:* The Indian Ginseng Ashwagandha, 293.