Journal of Medicinal Plants Studies
www.PlantsJournal.com

**ISSN 2320-3862**
JMPS 2016; 4(3): 136-139

Received: 20-03-2016
Accepted: 22-04-2016

**Naveen Gaurav**
Assistant Professor Department of Biotechnology, S G R R P G College Dehradun, U.K.

**AP Singh**
Professor, Department of Botany Govt. P.G. Science College, Rewa, M.P.

**Abhishekh Srivastava**
Assistant Professor, Department of Botany Govt. S.V. College Teonthar, M.P.

**Arun Kumar**
Assistant Professor, Department of Biotechnology, S G R R P G College Dehradun, U.K.

**Deepak Kumar**
M.Sc, Department of Biotechnology, S G R R P G College Dehradun, U.K.

**Komal**
M.Sc, Department of Biotechnology, S G R R P G College Dehradun, U.K.

**Hira Singh Gariya**
B.Sc Biotechnology, Department of Biotechnology, SGRRPG College Dehradun, U.K.

**Correspondence**
**Naveen Gaurav**
Assistant Professor Department of Biotechnology, S G R R P G College Dehradun, U.K.

# Morphology of callus, shoots, roots and leafs of *Withania somnifera* (Cultivated and Wild) *in vitro* tissue culture *conditions* with different hormones concentration

**Naveen Gaurav, AP Singh, Abhishekh Srivastava, Arun Kumar, Deepak Kumar, Komal, Hira Singh Gariya**

**Abstract**
The present and recent study highlights the advantages of biotechnological involvement in ashwagandha plants in order to overcome the presence of plant-to-plant chemically variable or different to be used for the high or large-scale production of the drug at low cost which will be affordable levels. Callus culture were started from shoot tip, leaf and root explants (excised plant pieces) on MS medium also known as Murashige and Skoog (1962) [8] medium containing 2,4-D,NAA and IBA. Shoot culture were started from callus with conc. of 2.0 mg/l Kinetin + 1.0 mg/l BAP while root culture were started from shoots with conc. of 0.5 mg/l BAP + 1.0 mg/l IBA.Shoot cultures were regenerated or reform by these three explants and its generation capacity, its length and morphology were determined. Plant tissue culture is based on the reason or fact that many of the different plant cells have the tendency or capacity to regenerate reform and proliferate a whole plant due to totipotency. Single cells, plant cells without cell walls k/a protoplasts, pieces of leaves explants, or less commonly roots explants can maximally be used to produce a new variant plant than its parent plant on culture media (mostly MS) given the required nutrients supplements and plant hormones.

**Keywords:** Biotechnological involvement, ashwagandha, chemically variable, Murashige and Skoog, callus culture, shoot cultures, root culture etc

## 1. Introduction
Modern plant tissue culture is practiced under aseptic or favourable conditions under filtered air. Living plant materials which was taken from the environment or free space are being naturally contaminated or will show contamination on their external surfaces (and sometimes interiors) with pathogenic microorganisms, so surface sterilization of initial materials (explants) in chemical solutions (usually Sodium or calcium hypochlorite or mercuric chloride) is done. Mercuric chloride is mostly used as a plant sterilant now a days, unless other sterilizing agents which require for removal of contamination are dispose found to be least effective, as it is very dangerous and harmful to use, and is very difficult to dispose of (Aniel, *et al.,* 2011) [1]. Explants are then generally placed or maintained on the upper surface of a solidified culture medium (normally MS) (Pawar & Maheshwari, 2004) [11], but during some cases it will be inoculate directly into a liquid nutrient medium, normally when cell suspension cultures are required. Solid nutrient and liquid nutrient media usually consists of hormones. Solidified media are prepared or produced from the liquid media with the use of a gelling or solidified agent, generally a purified agar. The constituents of the solid medium or the requirement of the plant growth hormones and the nitrogenous source (nitrate/ammonium salts and amino acids) have produces morphological effects of the grown tissues that from the initial explants. For example, high concentration of auxin responsible for the high multiplication of roots whereas an excess cytokinin may yield shoots. A balance concentration of auxin and cytokinin will often generate an unorganised and undifferentiated growth of cells, i.e. callus (Sabir, *et al.,* 2008) [14], but the morphology of the outgrowth depends on the different plant varieties as well as the suitable medium composition (Joshi & Padhya, 2010) [5]. As cultures grow, explants transferred to new media (sub-cultured) to allow for growth or to change the morphology of the culture.
Gita Rani and Avinash, (2003) [4] observed or reported the callus induction or cultivation from

the hypocotyls, root and cotyledonary leaf segments of Ashwagandha grown on MS medium provided with various different concentrations and combinations of the 2,4-di-chlorophenoxyacetic acid (2,4-D) and Kinetin plant growth harmone. Maximum callusing (near about 100%) was observed with root and cotyledonary leaf explants segments grown on Murashige and Skoog medium provided with a combination of 2,4-di-chlorophenoxyacetic acid (2,4-D) and Kinetin plant growth harmone

Gita Rani and Avinash (2003) [4] also told that or suggested that the frequency of the formation of root was comparatively lower or become slow with the higher concentrations of Indole 3- butyric acid (IBA) either alone or in combination with indole 3- acetic acid (IAA) or NAA and with all concentrations of indole 3- acetic acid (IAA) alone in Ashwagandha.

## 2. Material and methods

### Medium Used For Tissue Culture

Tissue Culture for *in vitro* growth and regeneration of Ashwagandha (Senthil, *et al.*, 2015) was the standard with MS medium (Murashige and Skoog, 1962) [8] having macronutrient salts, micronutrient salts, vitamins, Fe-EDTA, 0.01%(w/v) myo-inositol along with 3%(w/v) sucrose (Naveen Gaurav, *et al.*, 2015, 2016) [9, 10]. The media composition is listed as follows:-

**For 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 preparation 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). Suitable amount of plant growth regulators or phytohormones were added to the MS medium and were completely dissolved by continuously mixing on magnetic shaker stirrer. Final required volume was maintained with the help of double distilled water. The pH was adjusted to 5.7-5.8 by using of NaOH or HCl. For the preparation of solid medium 0.6-0.8% (w\v) agar powder was dissolved by heat (Naveen Gaurav, *et al.*, 2015, 2016) [9, 10].

### 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. which is make contamination less by washing or dipping in 70% ethanol followed by burner flaming and then cooling in sterilized water at regular intervals while using (Naveen Gaurav, *et al.*, 2015, 2016) [9, 10].

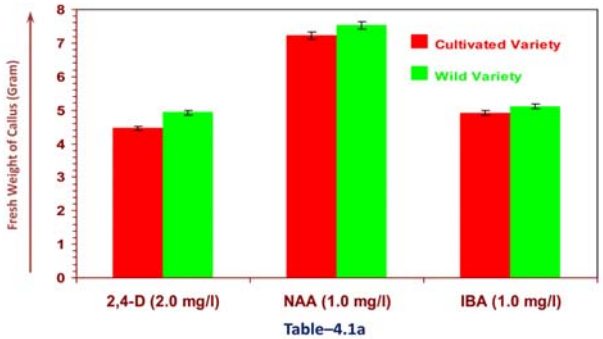
## 3. Results

*In vitro* condition fresh, dry and frequency of formation of callus maximum observed with MS medium supplemented with 2.0mg/l 2,4 D, 1.0mg/l NAA and 1.0mg/l IBA separately but frequency of formation of fresh and dry callus slightly higher in wild variety than cultivated variety of explants of *Withania* result has shown in table 4.1.

**Table 3.1:** Morphology of callus in *in vitro* tissue culture conditions with different Auxins (max. conc.):-

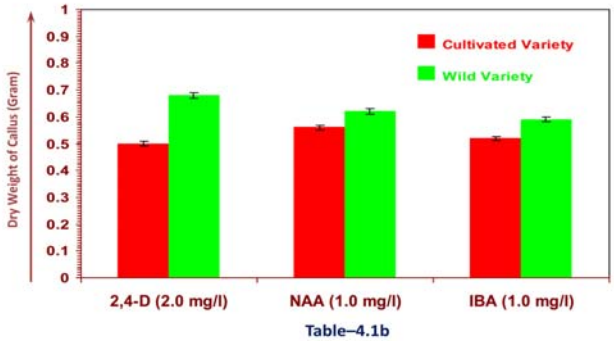
| S. No. | Conc. of auxins (mg/l) | Cultivated Variety | | | Wild Variety | | |
|---|---|---|---|---|---|---|---|
| | | Fresh Weight (g) | Dry Weight (g) | Frequency of callus formation (%) | Fresh Weight (g) | Dry Weight (g) | Frequency of callus formation (%) |
| 1. | 2.0(mg/l) 2,4-D | 4.46 ±0.067 | 0.50 ±0.010 | 90 ±1.71 | 4.92 ±0.07 | 0.68 ±0.019 | 92 ±1.75 |
| 2. | 1.0 (mg/l) NAA | 7.23 ±0.202 | 0.56 ±0.016 | 100 ±2.80 | 7.52 ±0.21 | 0.62 ±0.014 | 99 ±2.77 |
| 3. | 1.0 (mg/l) IBA | 4.91 ±0.083 | 0.52 ±0.011 | 85 ±1.45 | 5.11 ±0.08 | 0.59 ±0.011 | 86 ±1.38 |

(Mean [+ or -] Standard error).




Table–4.1a

Bar diagram showing effect of formation of callus in vitro tissue culture conditions with different auxins(max. conc.) of cultivated & wild variety of *Withania somnifera*




Table–4.1b

Bar diagram showing effect of formation of callus in vitro tissue culture conditions with different auxins(max. conc.) of cultivated & wild variety of *Withania somnifera*

**Table 3.2:** Morphology of qualitative traits of *Withania somnifera in vitro* Culture conditions (MS shoot induction media) with conc. of 2.0 mg/l Kinetin + 1.0 mg/l BAP.

| S. No. | Days | Cultivated Variety | | | Wild Variety | | |
|---|---|---|---|---|---|---|---|
| | | Growth of shoots (cms) | Growth of roots (cms) | Size of leafs (cms ) | Growth of shoots (cms) | Growth of roots (cms) | Size of leafs(cms) |
| 1. | 15 | 0.40 ±0.004 | – | 0.50 ±0.01 | 0.50 ±0.007 | – | 0.75 ±0.01 |
| 2. | 25 | 4.00 ±0.064 | – | 1.00 ±0.02 | 6.00 ±0.126 | – | 1.50 ±0.03 |
| 3. | 35 | 6.00 ±0.108 | – | 2.00 ±0.04 | 8.00 ±0.184 | – | 2.90 ±0.07 |
| 4. | 45 | 9.00 ±0.225 | – | 3.00 ±0.08 | 10.00 ±0.280 | – | 3.50 ±0.10 |

Data represents average Mean [+ or -] Standard error

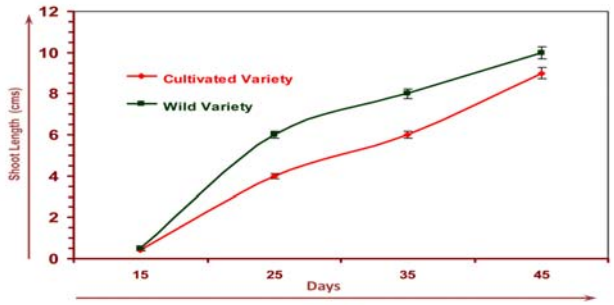



Table-4.2a

Line diagram showing growth of shoots (cms) of Cultivated & Wild variety of *Withania somnifera* in vitro culture conditions (shoot induction media) with conc. of 2.00 mg/l Kinetin + 1.0 mg/l BAP

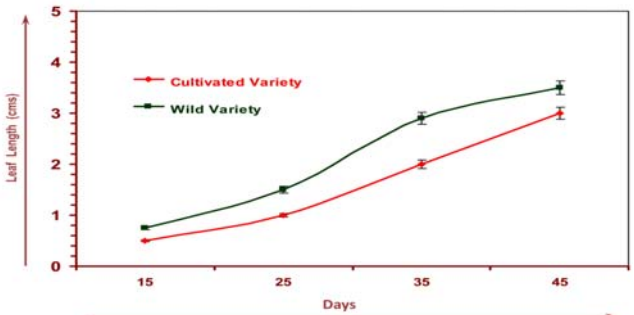



Table-4.2b

Line diagram showing growth of leafs (cms) of Cultivated & Wild variety of *Withania somnifera* in vitro culture conditions (shoot induction media) with conc. of 2.00 mg/l Kinetin + 1.0 mg/l BAP

*In vitro* condition from the above experiment in MS basal medium proof that the formation of shoot from callus in shoot induction medium is higher than wild variety than cultivated variety. Result is shown in table 4.2.

**Table 3.3:** Morphology of qualitative traits of *Withania somnifera* in *in vitro* Culture conditions (root induction media) conc. of 0.5 mg/l BAP + 1.0 mg/l IBA

| S. No. | Days | Cultivated Variety | | | Wild Variety | | |
|---|---|---|---|---|---|---|---|
| | | Growth of shoots (cms) | Growth of roots (cms) | Size of leafs(cms) | Growth of shoots (cms) | Growth of roots (cms) | Size of leafs (cms) |
| 1. | 15 | 1.20 ±0.01 | 0.30 ±0.003 | 0.45 ±0.005 | 1.50 ±0.02 | 0.40 ±0.006 | 0.50 ±0.007 |
| 2. | 25 | 4.50 ±0.07 | 4.00 ±0.064 | 2.20 ±0.035 | 5.00 ±0.09 | 4.50 ±0.081 | 3.00 ±0.054 |
| 3. | 35 | 8.60 ±0.18 | 7.10 ±0.149 | 5.10 ±0.107 | 10.00 ±0.25 | 7.50 ±0.188 | 5.60 ±0.140 |
| 4. | 45 | 9.20 ±0.21 | 7.50 ±0.173 | 5.50 ±0.127 | 13.00 ±0.36 | 8.50 ±0.238 | 6.00 ±0.168 |

(Mean [+ or -] Standard error).

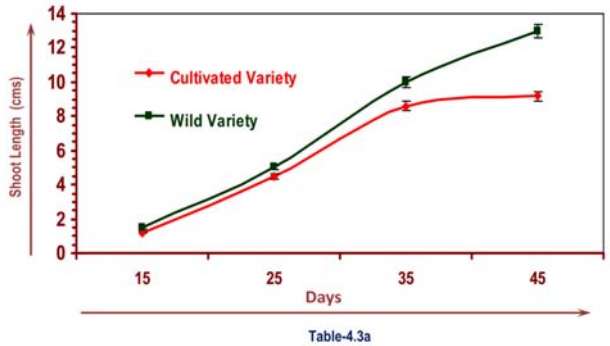



Table-4.3a

Line diagram showing growth of shoots (cms) of Cultivated & Wild variety of *Withania somnifera* in vitro culture conditions (root induction media) conc. of 0.5 mg/l BAP + 1.00 mg/l IBA

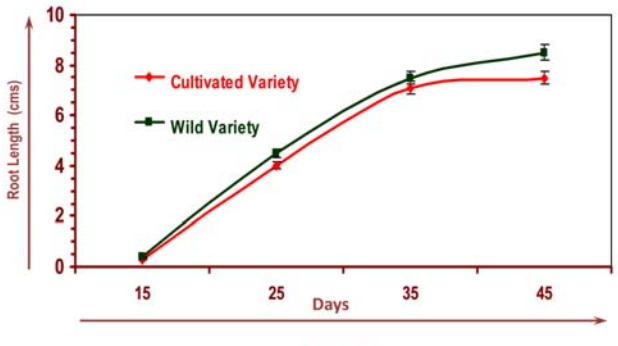



Table-4.3b

Line diagram showing growth of root (cms) of Cultivated & Wild variety of *Withania somnifera* in natural habitat in vitro culture conditions (root induction media) conc. of 0.5 mg/l BAP + 1.00 mg/l IBA

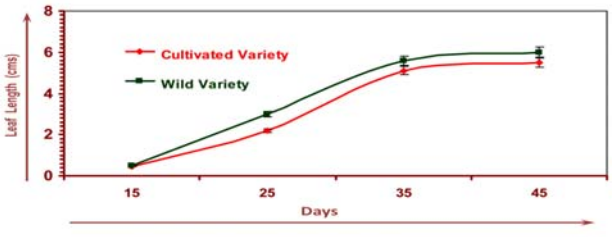



Table-4.3c

Line diagram showing growth of leafs (cms) of Cultivated & Wild variety of *Withania somnifera* in natural habitat in vitro culture conditions (root induction media) conc. of 0.5 mg/l BAP + 1.00 mg/l IBA

In above experimental analysis in MS basal medium proved that when plant grow in root induction medium the size and growth of complete plant (leaf, stem and root) of wild variety is more than cultivated variety result has shown in table 4.3.

## 4. Discussion

At recent days there are a large number of reports contained regarding in lab propagation of *Withania somnifera* L. Dunal by applying various pieces of tissue known as explants such as shoot tip explants (Sen and sharma, 1991), nodal segments explants (Kulkarni, *et al.*, 2000) [7], axillary meristem explants (Roja, *et al.*, 1991) [13], axillary shoot explants, and hypocotyls segments and root segment explants (Rani and Grover, 1999) [12].

Chitturi, *et al.*, (2010) [2] established a protocol for *in vitro* production of Ashwagandha plants by which callus cultures of Ashwagandha from leaves were established or reported on growing nutritional MS (Murashige and Skoog) media having Kin and sugar sucrose (3% w/v). For growth kinetics MS media placed with Kinetin (0.1 mg/l) and sugar sucrose (3% w/v) without the use of the agar which was found to be very effective with good success and responsible for the maintenance and initiation of the suspension cultures from the calli without any contamination.

Kulkarni, *et al.*, (1996) [6] 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.

The presence of 1 mg/l Indole 3-butyric acid to the nutrient medium was highly efficient in inducing or originating root formation. The regenerated or reformed (plantlets small plant) were acclimatized and grow in the greenhouse environment and successfully or efficiently transferred to the field, with a high survival rate. The acclimatized and survived plants possessed normal flowering and were not morphologically distinct from the seed-derived mother plants (Ghimire, *et al.*, 2010) [3].

## 5. References


1. Aniel KO, Jyothirmayee G, Subba TS. Multiple Shoot Regeneration from Nodal Explants of Ashwagandha (*Withania somnifera* (L.) Dunal Asian. J. Exp. Biol. Sci. 2011; 2(4):636-640.
2. Chitturi D, Venisetty RK, Molmoori RK, Kokate CK, Apte SS. Enhanced Bioproduction of Withaferin A from Suspension Cultures of *Withania somnifera*; Annals of Biological Research, 2010; 1(2):77-86.
3. Ghimire BK, Eun SS, Eun HK, Kabir L, Chang YY, Min CI. Direct shoot organogenesis from petiole and leaf discsof Withania somnifera (L.) Dunal, 2010.
4. Gita Rani, Arora S, Nagpal A. Direct Rhizogenesis from *in vitro* Leaves of *Withania somnifera* (L.) Dunal. J of Herbs, Spices and Medicinal Plants. 2003; 10(3):47-53.
5. Joshi AG, Padhya MA. Shoot regeneration from leaf explants of *Withania somnifera* (L.) Dunal. Notulae Scientia Biologicae, 2010; 2(1):163-168.
6. Kulkarni AA, Thengane SR, Krishnamurthy KV. Direct *in vitro* regeneration of leaf explants of *Withania somnifera* (L.) Dunal. Plant Sci, 1996; 119:163-168.
7. Kulkarni AA, Thengane SR, Krishnamurthy KV. Direct shoot regeneration from node, internode, hypocotyls and embryo explants of Witahnia somnifera L. Plant Cell Tiss. Org. Cult, 2000; 62:203-209.
8. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue culture, Physiol plant, 1962; 48:473-497.
9. Naveen G, Arun K, Preeti J, Deepak K, Chauhan UK, Singh AP. *In Vitro* Callusing and Effect of Growth Regulators on *in vitro* Propagated Withania (Cultivated & Wild) through Cotyledonary Leafs. International journal of current science research. 2015; 1(5):85-94. ISSN: 2454-5422.
10. Naveen G, Singh AP, Aviral M, Hira SG, Bhoora *In Vitro* Tissue Culture from Nodal Segments of Wild Traits of *Withania somnifera L.* International Journal for Innovative Research in Science & Technology. 2016; 2(9):78-81. ISSN (online): 2349-6010.
11. Pawar PK, Maheshwari VL. Agrobacterium rhizomes mediated hairty root induction in two medicinally important members of family Solanaceae. Indian Journal of Biotechnology. 2004; 3:414-417.
12. Rani G, Grover IS. *In vitro* callus induction and regeneration studies in *Withania somnifera*. Journal of Plant Cell Tissue and Organ Culture. 1999; 57(1):23-27.
13. Roja G, Heble MR, Sipahimalani AT. Tissue cultures of *Withania somnifera*: morphogenesis and withanolide synthesis. Phytotherapy Research, 1991; 5:185-87.
14. Sabir F, Sangwan NS, Chaurasiya ND, Misra LN, Sangwan RS. *In vitro* withanolide production by *Withania somnifera* L. cultures. Physiol Planta, 2008; 63:409-412.
15. Senthil KM, Vinoth KD, Saravana KA, Aslam A, Shajahan A. The Phytochemical Constituents of *Withania somnifera* and Withania obtusifolia by GCMS Analysis. International Journal of Pharmacognosy and Phytochemical Research. 2011; 3(3):31-34.