

In vitro multiplication and phyto-chemical analysis of a valuable medicinal plant *Withania somnifera* L. Dunal

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Abstract

Withania somnifera, a popular Indian medicinal plant has long been used commonly in ayurvedic system of medicine. All the parts of the plants have shown remarkable importance in the field of pharmacology. The present paper gives an account of information on *in-vitro* micro propagation of medicinally important plant for the generation of multiple shoots and roots of *Withania somnifera* and its phyto-chemical activities. Shoots were induced from the axillary buds of *Withania somnifera* on Murashige and Skoogs's (MS) basal media supplemented with different concentrations of BAP (0.10mg/l, 0.25mg/l, 0.50mg/l, 0.75mg/l, 1.00mg/l). Axillary bud explants showed initiation of shoots with the concentrations of 1mg/l and it is found to be most effective for multiple shoot generation within minimum time period. The leaf, stem and root of *Withania somnifera* were analyzed for phyto-chemical screening. The micro propagated plants were transplanted to pots for hardening.

Key words: *In-vitro* Multiplication; MS medium; *Withania somnifera*; Shoot bud culture; Benzyl Amino Purine; Axillary.

Introduction

In the past two decades plant tissue culture technology has been successfully used in commercial settings to produce pathogen free plants, mass propagation and to conserve endangered plants (Fay, 1992). These techniques are now being used globally for the multiplication of medicinally important plant species and also monitoring of their secondary metabolites. In recent years, the interests in using these techniques for rapid and large scale propagation of highly valuable medicinally plants have been significantly increases (Sahoo *et al.*, 1997). It is a potent technique for mass multiplication which is known to be efficient for conservation of threatened plant species (Bapat *et al.*, 2008).

Withania somnifera or Ashwagandha or winter cherry belongs to family Solanaceae having enormous medicinal and aromatic properties. It is a well known for years as an important drug in the ayurvedic literature. All the parts of the plants have shown remarkable importance in the field of pharmacology. The active pharmacological

components of *Withania somnifera* are steroidal lactones of the withanolide type. The principal withanolide in Indian *Withania somnifera* are withaferin A and withanolide D. Both leaves and roots of the plant are used as the drug and steroidal lactones occur in both parts. The total alkaloid content in the root of the Indian types has been reported to vary between 0.13 and 0.31%. Biological assays label the plant as having the properties against different diseases e.g. leprosy, nervous disorders, disease of respiratory and reproductive tract, venereal disorders, rheumatism, inflammation, psoriasis, bronchitis, asthma, consumption, ulcers, scabies, insomnia, carbuncles, epilepsy, diabetics etc. (Tripathy *et al.*, 1996; Kirtikar and Basu, 2001). *In vitro* propagation is an effective means for rapid multiplication of species in which conventional methods have limitations (Siddique and Anis, 2007). The present study describes *in vitro* propagation of *Withania somnifera* through shoot bud culture as an alternative method to achieve a higher rate of shoots multiplication and regeneration of tissue culture plants.

Material and methods

Collection and sterilization of explants

Explants were collected from the plant of *Withania somnifera* growing in botanical garden, govt. E.R.R. Auto. Sci. P.G. College, Bilaspur (C.G.). The explants obtained from various sources viz. leaf, inter-nodal segment, shoot segment were used for callus induction. Healthy shoot materials of *Withania somnifera* with 3 to 4 nodes were collected. Gathered leaves and shoots were washed under running tap water for 20 minutes. Then explants were treated with detergent labolene for 5 minutes then distilled water and sterilized with 0.1% HgCl_2 for 10 minute then with sterile distilled water for 5-6 times. Then shoots were cut into pieces 0.5-1.0 cm and inoculated to MS medium.

Shoot multiplication

The explants were cultured on Murashige and Skoog media fortified with different concentrations and combinations of plant growth regulators. The pH of the medium was adjusted to 5.7 using before autoclaving at 121°C for 20 minutes. Shoot segments and nodal segments were aseptically excised from field grown mature plant and inoculated on MS medium containing different concentrations of BAP. All cultures were maintained at 16 hour photoperiod with 3000 Lux light intensity at $25\pm 2^\circ\text{C}$.

Phyto-chemical screening

The leaf, stem and root extract of *Withania somnifera* were analyzed for the presence of glycosides, steroids, phenolic compounds and flavonoids. One gram of sample was weighed and dissolved with various solvents such as ethanol, methanol and water. Then the sample was allowed to stay overnight for 24 hours. After overnight incubation the sample was filtered by Whatman filter paper, the filtrate was centrifuged at 25,000rpm for 10 minutes, and the supernatant was used for Phyto-chemical screening.

Test for Glycosides

About 1ml of extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1 ml of concentrated sulphuric acid. A brown ring obtained indicated the presence of glycosides.

Test for Steroids

To the 0.5 ml of filtrate, 2ml acetic anhydride was added followed by an addition of 3ml concentrated sulphuric acid. Blue green ring indicate the presence of Steroids.

Test for Phenolics

To the 2ml of extract, 1ml of 1% ferric chloride was added, blue or green colour indicates the presence of phenolics.

Test for Flavonoids

To one ml of the extract, a few drops of dilute sodium hydroxide were added. An intense yellow colour was produced in the plant extract, which become colour-less on addition of a few drops of dilute acid indicates the presence of flavonoids.

Hardening and acclimatization

The plantlets developed *in-vitro* were taken out from the rooting medium and washed thoroughly but delicately to remove adhering agar. The plantlets were then transferred to pots containing a mixture of sterilized sand:soilrite:garden soil (2:1:1), and then these pots were incubated in growth chamber for their hardening and acclimatization for about 2-3 weeks. Plants were then transferred to earthen pots containing garden soil and watered with tap water.

Result and discussion

In order to establish an efficient *in-vitro* micro propagation system for *Withania somnifera* from nodal explant, stem nodal segments were incubated on Murashige and Skoog's basal medium (1962) supplemented with varying levels of BAP. Callus initiation was observed after 15-20 days of culture from the cut ends of leaves, which probably allows more absorption of nutrients leading to rapid cell division and subsequent callus formation. Callus

showed a different response according to the growth regulator used.

Effect of basal medium

Table 1. Effects of different level of BAP (in MS medium) on axillary shoot growth from nodal explants of *Withania somnifera* after 30 days of incubation

Conc. (BAP)	Shoots/explants	Average shoot length (cm)	Nodes /shoots	Leaves /shoot	Bud Break
0.00	1.2±0.1	0.4±0.2	0.7±0.1	1.2±0.4	40
0.10	1.4±0.1	0.6±0.1	1.0±0.1	1.8±0.4	40
0.25	2.0±0.2	2.2±0.2	1.2±0.1	5.2±0.4	100
0.50	2.1±0.1	2.3±0.2	2.6±0.2	10.6±0.4	100
0.75	2.4±0.2	3.4±0.4	2.8±0.3	16.2±1.9	100
1.00	3.0±0.2	6.8±0.4	6.0±0.3	22.4±1.6	100

Table 2. Effects of different levels of BAP (in B5 medium) on axillary shoot growth from nodal explants of *Withania somnifera* after 30 days of incubation

Growth Regulator conc. (BAP) (mg/l)	Shoots/Explant	Average Shoot Length (cm)	Nodes/ Shoot	Leaves/ Shoot	Bud Break (%)
0.25	0.6±0.08	0.6±0.09	1.3±0.02	1.2±0.16	80
0.50	0.8±0.09	1.0±0.08	1.6±0.18	1.0±0.18	80
0.75	0.6±0.09	0.8±0.18	1.2±0.06	1.0±0.09	80
1.00	1.0±0.02	2.0±0.20	3.0±0.18	2.2±0.16	100
1.50	0.8±0.10	0.6±0.10	0.0±0.00	0.0±0.00	80

Table 3. Presence of phytochemicals in root, stem and leaf of *Withania somnifera* when extracted with ethanol

Chemical compounds	Root	Stem	Leaf
Flavonoids	+	-	+
Steroids	+	+	-
Glycosides	-	+	+
Phenols	+	-	+

The nodal segments showed bud break response on both the basal mediums (Table-1&2). The MS medium fortified with BAP induced bud break in 100% explants whereas B₅ medium supplemented with BAP induced bud break in 80-100% of explants. The nodal segments produced 3.0±0.2 shoots per explant on MS medium as compared to 1.0±0.02 on B₅ medium. The shoots elongated from nodal explants on different media showed variation in shoot length. The average shoot length and nodes per shoot were 6.8 ± 0.4 and 6.0±0.3 respectively on MS medium while on B₅ medium the average shoot length and nodes per shoot were found to be 2.0±0.22 and 3.0±0.14 respectively.

Effect of BAP on shoot bud induction

The nodal segments showed 100% bud break, shoots per explant 3.0+0.2, average shoot length 6.8+0.4 and nodes per shoot 6.0+0.3 on MS medium with 1.0 mg/l BAP, while shoot tips were used as explants, no response was observed. The caulogenic potential of the nodal explants increased with increase in the level of BAP from 0.1mg/l to 1.0mg/l at concentrations above 1.0mg/l. Moreover, a decrease in the caulogenic potential is suggesting that lower concentration of BAP promote the multiple shoot induction. Similar results were also reported by other workers in *Phyllanthus carolinensis* (Catapan *et al.*, 2000), *Phyllanthus amarus* (Bhattacharya and Bhattacharya, 2001), *Coleus forskohlii* (Dube *et al.*, 2011), *Celastrus paniculatus* (Laxshami and Seeni, 2001).

Test of phyto-chemicals

Root, stem and leaf of *W. somnifera* exhibited differences in the presence of secondary cell metabolites. Different plant parts were extracted with ethanol and methanol and the results were compared in the Table-3&4:-

- Flavonoids, glycosides and phenols were present in leaf while steroid was present in both root and stem when extracted with ethanol.
- When extracted with methanol flavonoids, steroids, glycosides and phenols were present in leaf, steroid was present in roots and glycosides and phenols were present in stem. Preliminary phyto-chemical screening of plant extract has been reported in several medicinal plants (Amerjothy *et al.*, 2007). In the present study, the different parts of the *Withania somnifera* contain flavonoides, glycosides, steroids and phenolic compounds were analyzed.

Table 4. Presence of secondary metabolites in root, stem and leaf of *Withania somnifera* when extracted with methanol

Chemical compounds	Root	Stem	Leaf
Flavonoids	-	-	+
Steroids	+	-	+
Glycosides	-	+	+
Phenols	-	+	+

Conclusion

From the present investigation we have developed a promising method for an efficient regeneration from nodal explants of *Withania somnifera* using BAP. Phytochemical analysis of different part of the plant was also performed and the results were compared. The different parts of the *Withania somnifera* contain flavonoides, glycosides, steroids and phenolic compounds were analysed. The protocol could be useful for large scale production of single genotypes and provides a possible system towards genetic improvement of the plant. Rapid multiplication approach could be a viable option in domestication and commercial cultivation of *Withania somnifera*.

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