

# MICROPROPAGATION OF A MEDICINAL PLANT WITHANIA SOMNIFERA L. DUNAL BY SHOOT BUD CULTURE

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## KEY WORDS

*In vitro* culture  
Withania somnifera  
Multiple shoot  
Induction

## Received on :

12.09.2010

## Accepted on :

17.01.2011

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## ABSTRACT

An efficient protocol was devised for rapid *in vitro* propagation of *Withania somnifera* through shoot bud culture. The proliferating auxiliary shoot was isolated from nodal explants of field grown *Withania somnifera* established on Murashige and Skoogs medium (MS) and Gamborg B5 medium supplemented with six concentrations of Benzyl amino purine (BAP), Naphthalene acetic acid (NAA), Kinetin(Kn) and 2,4-Dichlorophenoxy acetic acid (2, 4-D). After 30 days of culture of *Withania somnifera*, the maximum numbers of shoots was produced on MS medium supplemented with 1.0mg/L BAP. Each of the explants had developed more than two shoots per nodes, while in six concentrations each of kinetin, NAA and 2, 4-D developed either two or less than two shoots/explants. The study revealed highest frequency of shoot formation and maximum number of shoots per explants in MS medium supplemented with 1mg/L BAP than the other growth hormone. Key Words: *In vitro* culture, *Withania somnifera*, multiple shoot induction.

## INTRODUCTION

The medical world has posed many complex challenges in recent times. Thus, there is an urgent demand of an integrated and pluralistic approach towards health care to cope up the situation effectively. In this respect, interest in scientific approaches to use ayurveda in the past one decade has greatly increased. A way of obtaining genuine crude drug by large scale destruction of natural habitat due to population pressure and over-exploitation, have become a major threat to important bioresources (Nath and Buragohain, 2005). Medicinal plants play a key role in world health care systems. The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medicinal treatments (Ballabh and Chaurasia, 2007), however, overexploitation of medicinal plants for obtaining drug has made a great challenge to the existence of these plants, therefore, there is a need of a technology for fast regeneration of such plants. In this pursuit shoot bud culture technique may become useful.

In light of above knowledge presented study was conducted in a medicinal plant Ashwagandha, a traditional Indian (ayurvedic) medicinal herb widely known as "Indian ginseng". The scientific name of this herb is *Withania somnifera*, also known as winter cherry or Dunal, belongs to family solanaceae. It has been an important herb in the ayurvedic and indigenous medical systems for over 3000 years. It is a small woody shrub grows about two feet in height. It is commercially cultivated in Madhya Pradesh (Saleeby, 2006). The roots are the main portion of the plant used therapeutically

(Weiner and Weiner, 1994). Ashwagandha is reported to have anti-carcinogenic effects in animal and cell cultures by decreasing the expression of nuclear factor-kappa B, suppressing intercellular tumor necrosis factor, and potentiating apoptotic signalling in cancerous cell lines (Ichikawa *et al.*, 2006). The roots of *Withania somnifera* contain several alkaloids, withanolides, a few flavanoids and reducing sugars (Ganzera *et al.*, 2003). The active compounds reported in *W. somnifera* include withaferin A, withasomniferin-A, sitoindosides VII-X, 5-dehydroxywithanolide-R, 2,3-dihydrowithaferin-A, withanoside I-VII, physagulin D (Jayaprakasam *et al.*, 2003).

Ashwagandha is considered to be one of the best rejuvenating agents in Ayurveda. Its roots, seeds and leaves are used in Ayurvedic and Unani medicines. It is reported to have antitumor, radiosensitizer, antistressor, immunomodulatory, anti-inflammatory and antibacterial effects (Umadevi, 1996; Archana and Namasivayam, 1999). Ashwagandha has been employed for numerous conditions in traditional Asian therapies, and for additional disorders in contemporary herbal practice. A major traditional use of the herb is in "balancing life forces," which may be regarded as an adaptogenic or anti-stress tonic effect. Thus, ashwagandha is considered to be a general promoter of health, or a "rasayana" that promotes rejuvenation according to traditional Ayurvedic practice (Mathew, 2007).

*In vitro* propagation technology has sound and extensive potential for commercial rapid multiplication of medicinal plants and horticultural crops because it is a quick method,

allows round the year propagation of identical plants, and produces plants free from diseases (Banerjee and Shrivastava, 2006). Shoot bud culture has been successfully employed for the conservation of medicinal crop genetic resource, particularly with those crops which are vegetatively propagated (Indrayan *et al.*, 2004).

Development of viable micro propagation protocol will be important for ex-situ conservation and sustainable utilization of selected species. Therefore this paper describes rapid and efficient propagation of *Withania somnifera* which is very useful medicinal plant using shoot bud culture for providing a better source for continuous supply of plants in manufacturing of drugs. The present study was done to establish a reproducible protocol for mass propagation and preservation of valuable medicinal plant *Withania somnifera*.

## MATERIALS AND METHODS

Actively growing and healthy shoot material of *W. somnifera*, with three to four nodes were collected from mature plants growing in the Botanical garden of the college. After removing the leaves, the shoots were thoroughly washed under running tap water for 20 minute and were treated with detergent labolene (Merck, India) for 5 minute followed by distilled water. The shoots were surface sterilized with 0.1% (w/v) mercuric chloride for 10 minutes and rinsed with sterile distilled water for 5-6 times. The shoots were cut into pieces of 0.5-1.0 cm size containing a single node with dormant auxiliary bud and inoculated to establishment medium like MS and B5 medium to study the suitability of medium for shoot induction and effect of different concentration of BAP, kinetin and NAA independently on explants establishment. Established micro shoots were cut and placed vertically on rooting medium. Full strength and half strength MS medium was used for rooting. Hardening of in vitro regenerated plant was done in two stages. The first stage was consist of liquid hardening for 3-4 weeks, followed by transfer in root trainers containing pre-sterile sand, soil rite and soil (2:1:1). Plantlets with well-developed adventitious root systems were washed with sterile distilled

water and dipped in 0.2% (w/v) solution of fungicide bavistin (BASF, India) for 30 minutes, later, plantlets were dipped in IBA solution for 10 minutes and planted in the root trainers containing sterile sand, soil rite and garden soil (2:1:1). Humidity was maintained by covering each pot with transparent polythene bags.

## RESULTS AND DISCUSSION

The segments showed bud break response on both the basal mediums (Table 1 and 2). For *W.somnifera*, the formulation of Murashige and Skoog (1962) basal medium was found more suitable than B5 medium for establishment of nodal explants. Shoots per explants, average shoot length and nodes per shoot were more on MS medium as compared to B5 medium. The MS medium supplemented with 1.0mg/L BAP was found to be the most suitable medium for in vitro establishment of nodal explants. On this medium, the nodal segments showed bud break 100% while,  $2.6 \pm 0.2$  shoots per explants,  $4.8 \pm 0.4$ cm average shoot length and  $5.2 \pm 0.2$  nodes per shoot was noted after 30 days of incubation (Table 1). B5 medium supplemented with BAP exhibited the nodal segments induced bud break 80-100%,  $1.0 \pm 0.08$  shoots per explants,  $1.8 \pm 0.02$ cm average shoot length and  $2.0 \pm 0.18$  nodes per shoot after 30 days of incubation (Table 2). When Nodal segments were inoculated on MS medium supplemented with varying concentration of NAA showed 80-100% bud break. The maximum number of shoot per explant, average shoot length and nodes per shoot were recorded  $1.3 \pm 0.1$ ,  $3.0 \pm 0.2$  and  $4.8 \pm 0.2$  respectively on MS medium with 1.0mg/L NAA (Table 3). Higher concentrations of NAA in MS medium had reduced bud break percentage, shoots per explants, average shoot length and nodes per shoot. When MS medium was supplemented with kinetin, 100% bud break was recorded at all the concentrations. Maximum values for shoot number per explants ( $1.0 \pm 0.00$ ), average shoot length ( $3.0 \pm 0.1$ ) and nodes per shoot ( $4.8 \pm 0.2$ ) were recorded with 0.50mg/l kinetin (Table 4). The different concentrations of BAP in MS medium had influenced shoot number, shoot length and node number. Cultures raised from nodal explants of *W.somnifera* ex-

**Table 1: Effect of MS medium supplemented with different concentrations of BAP on auxiliary shoot growth from nodal explant 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 (%)
0.00	$1.2 \pm 0.2$	$0.3 \pm 0.2$	Nil	Nil	50
0.10	$1.5 \pm 0.1$	$0.6 \pm 0.1$	$1.0 \pm 0.1$	$7.2 \pm 0.9$	80
0.25	$1.5 \pm 0.2$	$2.2 \pm 0.2$	$3.2 \pm 0.3$	$12.9 \pm 0.4$	100
0.50	$2.2 \pm 0.3$	$2.3 \pm 0.4$	$2.9 \pm 0.3$	$13.2 \pm 0.2$	90
0.75	$2.2 \pm 1.0$	$3.5 \pm 0.4$	$3.0 \pm 0.2$	$17.3 \pm 1.8$	100
1.0	$2.6 \pm 0.2$	$4.8 \pm 0.4$	$5.2 \pm 0.2$	$30.2 \pm 1.5$	100

**Table 2: Effect of B5 medium supplemented with different concentrations of BAP on auxiliary shoot growth from nodal explants of *W. 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	$0.6 \pm 0.08$	$0.6 \pm 0.09$	$1.3 \pm 0.02$	$1.2 \pm 0.16$	80
0.50	$0.8 \pm 0.08$	$1.0 \pm 0.08$	$1.6 \pm 0.17$	$1.0 \pm 0.18$	80
0.75	$0.6 \pm 0.09$	$0.8 \pm 0.18$	$1.2 \pm 0.06$	$1.0 \pm 0.09$	80
1.00	$1.0 \pm 0.08$	$1.8 \pm 0.20$	$2.0 \pm 0.18$	$2.2 \pm 0.16$	100
1.50	$0.8 \pm 0.10$	$0.6 \pm 0.10$	$0.0 \pm 0.00$	$0.0 \pm 0.00$	80

**Table 3: Effect of MS medium supplemented with different concentrations of NAA on auxiliary shoot growth from nodal explant of *Withania somnifera* after 30 days of incubation**

Growth Regulator conc. (NAA) (mg/L)	Shoots/Explant	Average Shoot Length (cm)	Nodes/Shoot	Leaves/Shoot	Bud Break (%)
0.10	1.0 ± 0.00	0.8 ± 0.02	1.0 ± 0.1	6.2 ± 0.8	100
0.25	1.0 ± 0.0	1.2 ± 0.1	2.2 ± 0.1	9.3 ± 0.4	100
0.50	1.0 ± 0.0	1.5 ± 0.8	2.8 ± 0.2	16.2 ± 1.0	100
0.75	1.2 ± 0.1	2.0 ± 0.6	4.0 ± 0.3	17.6 ± 2.4	80
1.00	1.3 ± 0.1	3.0 ± 0.2	4.8 ± 0.2	20.2 ± 1.6	100
1.50	1.2 ± 0.1	2.0 ± 0.1	2.2 ± 0.2	13.8 ± 2.0	80

**Table 4: Effect of MS medium supplemented with different concentrations of Kinetin on auxiliary shoot growth from nodal explant of *Withania somnifera* after 30 days of incubation**

Growth Regulator conc. (Kn) (mg/L)	Shoots/Explant	Average Shoot Length (cm)	Nodes/Shoot	Leaves/Shoot	Bud Break (%)
0.10	1.0 ± 0.0	2.2 ± 0.1	4.1 ± 0.1	17.2 ± 0.2	100
0.25	1.0 ± 0.0	2.3 ± 0.1	4.0 ± 0.2	18.3 ± 1.3	100
0.50	1.0 ± 0.0	3.0 ± 0.1	4.8 ± 0.2	22.2 ± 1.2	100
0.75	1.0 ± 0.0	2.8 ± 0.1	4.1 ± 0.2	20.2 ± 1.2	100
1.00	1.0 ± 0.0	1.8 ± 0.09	4.0 ± 0.3	18.3 ± 1.8	100
1.50	1.0 ± 0.0	1.3 ± 0.06	2.3 ± 0.1	14.4 ± 0.6	100

All the values are mean ± standard deviation

hibited maximum number of shoots production on MS medium supplemented with 1.0mg/L BAP. These explants were developed greater than 2 shoots per nodes, while in different concentrations of Kn, NAA and BAP had developed either two or less than two shoots/explant. In *W. somnifera*, explants showed low percentage of shoot bud induction on cytokinin free MS medium, but addition of BAP in MS medium had increased shoot bud induction percentage, shoot length and number of nodes. Direct shoot regeneration from nodal explants has been reported earlier on MS medium with 27.2mg/L Ads + 2.46 mg/L IBA (Kumar and Bhavanandan, 1988; Selvakumar et al., 2001). Similarly, Verma et al., (2002) had reported rapid propagation of *Plumbago zeylanica* with a maximum of four multiple shoots per nodal segments with 8.87 mg/L BAP and 0.49mg/L IBA. In the present study the shoots of *W.somnifera* produced with BAP were longer and more uniform in appearance than those produced in kinetin and NAA and 1.0mg/L of BAP was found suitable for an establishment of explants.

## ACKNOWLEDGEMENT

The authors are thankful to the H.O.D., E.R.R.Autonomous Science. P.G. College, Bilaspur, for providing the laboratory facilities.

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