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ORIGINAL ARTICLE **OPEN ACCESS**

In Vitro Plant Propagation of *Withania somnifera* by using Shoot Culture

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ABSTRACT

In the present work in vitro propagation of a multipurpose medicinal plant, Withania somnifera was done. Direct regeneration of nodal explants and their multiplication have been optimized using cytokinin BAP (0.5-5.0 mg/l) and combination of BAP (0.5 mg/l) + IAA (1.0-3.0 mg/l) respectively. MS media with nodal explants supplemented with BAP (2.0 mg/l) produced maximum average number of shoots (2±0.48) and average shoot length was found to be 2.8±0.26 cm. Best initiated shoots then sub cultured for shoot multiplication, an improved shoot multiplication in terms of average number of shoots (5.3±0.41) and average shoot length 6.5±0.12 cm was observed on MS media in combination with BAP (0.5 mg/l) + IAA (1.5 mg/l). Regenerated plantlets were successfully transferred to greenhouse condition.

Key word*:Withania somnifera, Ashwagandha, Micropropagation, Nodal Explant, BAP, IAA*

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INTRODUCTION

Plants in addition to their aesthetic value constitute the major natural source of the food we eat, the air we breathe and the medicine to cure our many ills. Recently, the utilization of medicinal plants as a natural source of drugs is being increasingly encouraged. Consequently, medicinal plants have been targeted for uncontrolled collection and destruction as a result of urbanization, overgrazing, pollution and expansion of cultivated areas. Plant secondary metabolism gives rise to the formation of a vast array of chemically complex compounds, many of which are commercially important. Many problems are associated with the production and marketing of such compounds as well as the supply of raw materials can be erratic due to several reasons. So, it may become critical to develop an alternative source of important therapeutically natural products. Plant cell culture provides an environmentally friendly, renewable alternative for secondary metabolite supply [17].

Application of biotechnology for conservation of important plant species has been given priority under circumstances, in particular when many valuable plant genetic resources are getting decimated rapidly from natural flora [10]. Herbal medicines are still the mainstay of about 75-80% of the world population for primary health care because of the better acceptability with the human body and less side effects [5]. Many studies revealed that cultivation of medicinal plants especially high value medicinal plants is creating new dimension in the field of agriculture. Indian herbal industry is at blooming stage. However, cultivation of medicinal plant is not easy. It is a challenging task because very little knowledge of seed biology. Efforts have not been made to search elite specimen and their propagation. *Withania sominifer*a is a green shrub found throughout the drier parts in India, Baluchistan, Pakistan, Afghanistan, Shri Lanka, Congo, South Africa, Egypt, Morocco, and Jordan. In India, it is widely grown in the provinces of Madhya Pradesh, Uttar Pradesh, plains of Punjab and northwestern parts of the India like Gujarat and Rajasthan *Withania sominifera* (L.)Dunal, commonly called Indian ginseng is a member of the family Solanaceae, growing up to a height of 30-150 cm. It is considered as important medicinal plant in the Ayurvedic and indigenous medicinal system of India. It has many medicinal properties like anti-inflammatory, anticancer, anti-stress, anti-ageing, immune-modular, adaptogenic and shows the free radical scavenging activity It is used for treatment of tuberculosis, rheumatism, inflammatory conditions and cardiac diseases. It is also useful as abortificient, amoebicide, anodyne, bactericide, contraceptive and spasmolytic. The roots are also used as sedative for senile debility and for the prevention and inhibition of Alzheimer's disease [20, 22, 3, 18].

Many earlier studies have reported *in vitro* propagation of Ashwagandha by using different explants, such as shoot tips [14, 2, 16, 17], axillary bud [15], hypocotyl [8], cotyledon [11], leaf [6, 9], seed [21], cotyledonary leaf segments [13], callus of leaf [1], shoot tip and root [19] and the nodal areas [11].
The present study was done to determine the effect of growth hormones on shoots initiation, multiplication, and hardening of Ashwagandha to standardize the micro propagation technique in Ashwagandha, as very less literature available for plant regeneration through nodal explant.

MATERIAL AND METHODS

The plant material of Ashwagandha was collected in plastic bags from NarainCollege Botanical Garden, Shikohabad. Young nodal segments were selected as explants and were washed under running tap water for 15 minutes. Later immersed in 1% tween-20 for 1 minute and washed with sterile double distilled water for 2-3 times. The primarily surface sterilized nodal segments then rinsed in 70% ethanol for 1 minute under laminar air flow hood and washed with sterile double distilled water for 2-3 times. Finally rinsed with 0.1% mercuric chloride (HgCl2) for 7-8 minutes and washed with sterile double distilled water for 4-5 times to remove all the surfactants. One by one explants were placed on the filter paper to soak up the extra water. The nodal explants were then cut from both ends. Finally with the help of forcep the explants were inoculated on the surface of the MS media in such a way that ¾ part of the nodal explants would be in contact with MS media. The culture bottles containing explants inoculated on MS media supplemented with respective different conc. of BAP and was incubated at 25±3°C under white fluorescent light (2000 lux) for 16/8 hours light and dark conditions. In total 6 weeks of initiation period one subculture was done in 3rdweek and data for average shoot number per explants and average shoot length was recorded at the end. The best initiated grown shoots were then transplanted on MS media supplemented with different concentrations of BAP and IAA. Again, the data for average shoot number per explant and average shoot length was recorded after 6 weeks of incubation period. Each treatment was repeated trice and statistical analysis were done by calculating the standard error (SE) for the treatments.

RESULTS AND DISCUSSION

During initiation of explants, it has been observed that nodal explants cultured on MS media devoid of growth hormone (Cytokinin) failed to induce the initiation of explants (Table 1). Out of all concentrations of BAP (0.5-5.0 mg/l) used for shoot initiation BAP (4.0 mg/l) promoted the shoot initiation i.e., average number of shoot (2±0.48) and average length of shoot (2.8±0.26cm) (Fig-1. A & B). Darwesh *et al.,* [3] also found similar results i.e., Number of shoot 2.57 and shoot length/explants 3.09 but with hormonal concentration as 2.0 mg/l BAP and 0.1 mg/l IAA.

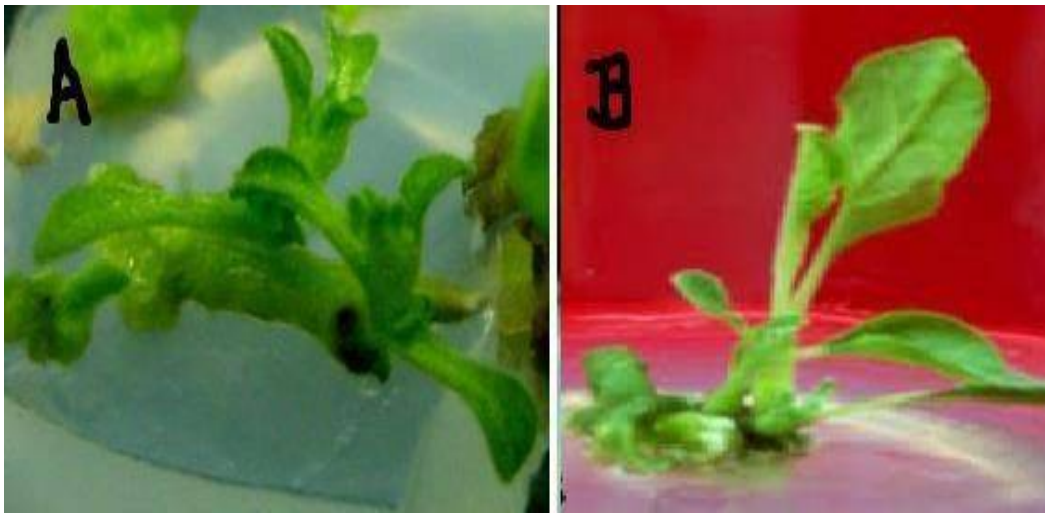

Fig:- 1. (A) & (B)- Shoot initiation from nodal explant.

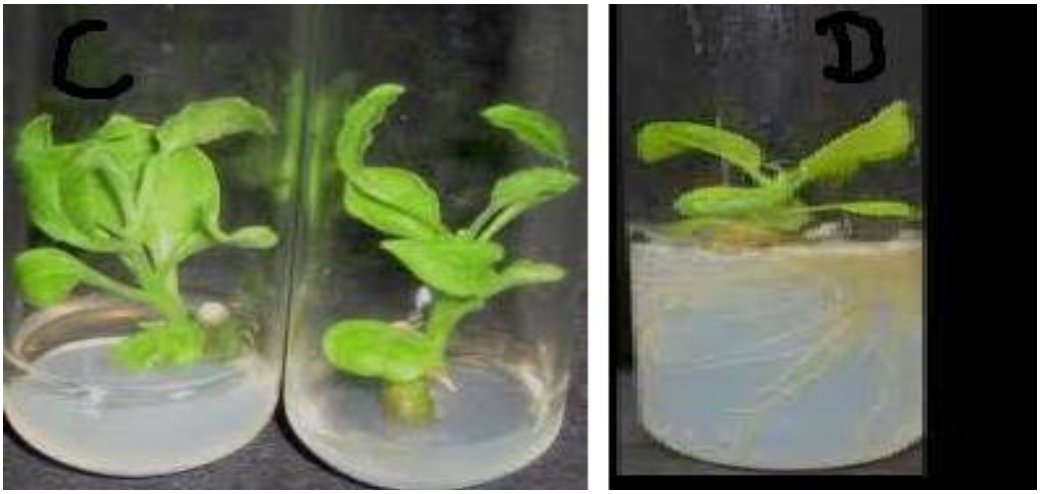

Fig:- 2. (C) & (D)- Shoot multiplication

Prominent *in vitro* response (average shoot number 5.3±0.41 and average shoot length 6.5±0.12cm) was observed of best initiated shoots cultured on MS media augmented with BAP (0.5 mg/l) + IAA (1.5 mg/l) (Table 2& Fig- 2). Increasing concentration of BAP and IAA resulted in gradually increased in *In vitro* response of shoots, while further increased concentration was found to be directly proportional to poor response of the shoot multiplication. Rani *et al*., [14] observed the different concentration of IAA and BAP were showing the best result for shoot elongation and direct shoot regeneration at 0.5 mg/l BAP with 3.0 mg/l and 2.0 mg/l IAA. Similar results were found by Rout *et al.,* [18] when different growth hormone tested in augmentation with MS media for shoot elongation. 2.0 mg/l BAP with 1.0 mg/l IAA was found to be best eliciting 82.3% shoot induction with highest shoot number 4.8 shoot /callus and shoot length was 4.3 cm.

Table 1: Effect of BAP on shoot initiation from nodal explants

Sr. No.	MS Medium + BAP (mg/l)	No. of shoot / explants	No. of shoot length(cm)/explants
1.	MS medium+0.5	_	_
2.	MS Medium+1.5	1 ±0.29	1 ±0.27
3.	MS Medium+2.5	1.3 ±0.06	1.6 ±0.44
4.	MS Medium+3.5	1 ±0.21	1.4 ±0.26
5.	MS Medium+4.0	2 ±0.48	2.8 ±0.26
6.	MS Medium+4.5	1.3 ±0.12	1 ±0.21
7.	MS Medium+5.0	1.3 ±0.42	1.2 ±0.12

The production of Ashwagandha roots through conventional methods of cultivation (seed) is less than the requirement due to numerous reasons viz. poor yield, takes long time, poor viability of seeds, susceptibility of the seeds and seedlings to fungal infections like seedling mortality and blight, leaf blight, seed rotting [12]. This medicinally significant plant species has been depleted from its natural habitat and is now included in the list of endangered species [6] by the International Union for Conservation of Nature and Natural Resources [7, 21].

Table 3: Effect of different concentrations of BAP+IAA on shoot multiplication and elongation.

Sr.no.	BAP+IAA (mg/l)	No. of shoot/explant	Shoot length (cm)
1.	0.5+1.0	1.0±1.20	2.0±0.24
2.	1.0+1.0	2.15±1.24	2.24±0.22
3.	1.5+1.0	1.25±1.20	2.36±0.29
4.	2.0+1.5	1.32±1.15	2.42±0.71
5.	2.5+1.5	1.37±0.59	3.20±0.32
6.	3.0+1.5	2.0±0.30	3.60±0.40

The rapid multiplication of Ashwagandha by tissue culture techniques can help to solve these problems and the benefits are extensive in the agricultural world. *In vitro* propagation of Ashwagandha has been achieved by using nodal segment as explant. The explant was initiated on MS media supplemented with different concentration of BAP (0.5 to 4.0 mg/l) resulted in best response on BAP (2.0mg/l) produced in terms of average number of shoots 2±0.37 and average shoot length was 2.8±0.15cm. The best initiated

shoots then transferred for multiplication on MS medium supplemented different concentration of BAP in combination with IAA. After six weeks of incubation BAP and IAA (0.5mg/l+ 1.5mg/l) produced maximum average number of shoots 5.3±0.41 and average shoot length 6.5±0.12cm. It was concluded from this study that plant regeneration from nodal explant of *W. somnifera* offered a great potential in agriculture and this in genetic transformation of this important species. The protocol can be exploited for *in vitro* generating new genetic variability and production of bioactive constituents from the plant.

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