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RESEARCH ARTICLE

Tissue Culture Studies in *Withania somnifera* (L.) Dunal

Haribabu Narra

SPDM Arts, SBB and SHD Commerce and SMA Science College, Shirpur, Dist Dhule Maharashtra-425405

Corresponding Author : haribabuspdm@gmail.com

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ABSTRACT

Withania somnifera is an important medicinal herb that has been widely used for the treatment of different clinical conditions. The overall medicinal properties of *Withania somnifera* make it a viable therapeutic agent for addressing anxiety, cancer, microbial infection, immunomodulation, and neurodegenerative disorders. Biochemical constituents of *Withania somnifera* like withanolide A, withanolide D, withaferin A and withaniamides play an important role in its pharmacological properties. Proteins like *Withania somnifera* glycoprotein and lectin like-protein possess potent therapeutic properties like antimicrobial, anti-snake venom poison and antimicrobial. In this review, we have tried to present different pharmacological properties associated with different extract preparations, phytochemical constituents and protein component of *Withania somnifera*. Future insights in this direction have also been highlighted.

Keywords: Immunomodulation, Neurodegenerative disorders, Anti-microbial, Withanolides, Withaferin, Glycoprotein.

INTRODUCTION

Withania somnifera (L.) Dunal commonly known as Ashwagandha belongs to family Solanaceae is high in medicinal value i.e. contains secondary metabolites which are antibacterial, anti-inflammatory and control against infection (Jaffer et al., 1988). Roots and leaves are used in a number of preparations for their anti-inflammatory, antitumor, immunosuppressive and antioxidant properties besides for promoting vigour and stamina (Devi et al., 1992; Kulkarni et al., 2000; Furmanova et al., 2001).

The requirement of *Withania somnifera* has sharply risen due to its popularity owing to a large scale unrestricted exploitation. According to red list of threatened species, 44 plant species are critically endangered, 113 endangered and 87 vulnerable. *W. Somnifera* proved to be 99.75% of the endangered medicinal plant (Siddique et al., 2005). *W. somnifera* has been deplete d from its natural habitat and is now included in the list of threatened species by the International Union for Conservation of Nature and Natural Resources (Kavidra et al., 2000; Supe et al., 2006).

In present work during the research in vitro protocol for rapid regeneration of *Withania somnifera* practiced and establishment of plants in the field conditions were done. Propagation use by seed, but seed viability is limited to more than one year (Roja and Heble, 1991) reported, callus formation from explants medicinally important plant species has been depleted from their natural habitat and is now included in the list of threatened species by The International Union for Conservation of Nature and Natural Resources (Kavidra et al. 2000).

The present work deals with in vitro plant growth of *Withania somnifera* through tissue culture for propagation and ex-situ conservation. Regenerated plants after acclimatization were transferred to soil to soil and they showed >80% survival. Weeds free germination of *Withania* is more effective and beneficiary through tissue culture method. Control use of fertilizers in cultivated crops at the time of germination may be effect the quality of plant and seeds. The rapid multiplication of *W. somnifera* by tissue culture techniques can help to solve these problems and the benefits are extensive in the agricultural world.

MATERIAL AND METHODS

Plant explants of *Withania somnifera* (L.) Dunal were collected from the Green house of Plant Biotechnology Unit, West Bengal State Council of Science & Technology at Salt Lake, Kolkata India.

In vitro propagation

Experiments were carried out with *Withania somnifera* to standardize the best media and three different explants were tested, viz. apical bud, nodal bud and leaf to observe the better toti-potency and response. The basal medium in which the explants exhibit regeneration was further supplemented with various concentrations and combinations of growth regulators viz., cytokinin, auxin and Gibberellins, depending on the type of experiments carried out to study their effects on in vitro response of different explants. Basal media devoid of growth regulators was used as control for all the tissue culture experiments.

Effect of Diverse Explants in Governing In Vitro Culture Response

Various explants such as apical, nodal segments and leaves were tested for understanding in vitro response in the nutrient media. Among the 3 explants, the apical bud explants gave the best results and were used for further experiments. The table shows a comparative studies of the three explants used for *W. somnifera*. The media used was MS and various hormone combinations and concentrations were used to select the best media suitable for each explant.

The percentage of culture response, the number of days required for the cultures to establish and the percentages of plantlet formation in regeneration medium was noted in all the explants.

Apical /Nodal/ Leaf Explants

Surface sterilization was standardized with $HgCl_2$ at varying concentrations and time duration. Among the different concentrations so far tested the best concentration was 0.1% $HgCl_2$ (w/v) for 10 min. Further washing was made in both the explants in 70% ethyl alcohol as well as for nodal explants (80%) after $HgCl_2$ treatment for protection from fungal contamination. Finally the explants were washed at least 3-5 times with sterile glass distilled water for 5 min each. All experiments were in growth chambers at 25±2° C with a 16 h ours photoperiod, provided by 40 Watt white fluorescent lamps (Philips, Kolkata, India) as per standardized protocols. All experiments were done in twice and each experiment at least has twenty replicas.

Inoculation, Incubation and Sub culture:

Shoots (8-9 cm long) with sufficient rooting were transferred to ½ strength MS liquid medium with filter paper raft support for hardening, for two weeks. These well-developed rooted plantlets were then transferred to

sterile vermiculite and covered with poly bags for 2 weeks to retain moisture. They were irrigated with ¼ strength MS medium without sucrose and kept less than 16 hr light and 25±2°C. Further they were transferred to soil and normal growth of the potted plants was kept in room condition for 1 week.

OBSERVATIONS & RESULTS

This is an effort to develop an efficient in vitro tissue culture protocol to obtain maximum plantlet regeneration that has a tremendous importance in ex situ conservation of biodiversity. The formulation of the best nutrient media, selection of right explants and role of plant growth regulators either in single or in combination is a prerequisite for exploitation of tissue culture technology in ex situ conservation strategies. Keeping this background in mind a series of experimentation has been carried out in a medicinally important plant, *W. somnifera*.

Table 1: Effect of Auxins and Cytokinins on shoot regeneration from callus.

Callus derived from	Concentration of PGRs (mg/L)		Number of shoots /culture	Regeneration of shoots (%)
	NAA	BAP		
Cotyledonary leaf	0.5	1.0	--	--
		1.5	--	--
		2.0	--	--
		2.5	--	--
		3.0	--	--
Shoot tip	0.5	1.0	2	20
		1.5	5	45
		2.0	7	60
		2.5	11	90
		3.0	3	40

Values represents as mean on five replicates and percentage based on two experiments each of five replicates.

The good development of callus type was observed in the MS media supplemented with BAP (2mg/l) for best regeneration, IBA (2mg/l) for root formation and BAP (1.5) + IAA (1.5mg/l) for multiple shoot formation, when MS supplemented with GA3 it was found the best media for seed germination (0.5mg/l) as well as shoot elongation (0.3mg/l) and MS +2, 4-D (0.5) + Kinetin (0.2mg/l) was media for maximum callus induction. Following the total study starting from seed germination up to hardening the most interesting part of study was found to be the regeneration. It was very promising in all the media tried and especially in the presence of BAP (Benzyl Amino Purine). It was observed from all the replicas that the regeneration was seen in callus of about age ranging from 4-6 months. Only the solid green callus and not the friable one showed regeneration, with the brown portions showing maximum frequency over the green portions (Table 2).

Table 2: Effect of Cytokinins (BAP and KIN) on induction of shoot

Explant	Concentration of plant growth regulators (PGRs)(mg/L)	No. of shoots/ Explant	Shoot induction (%)
	BAP		
Shoot tip	1.0	07	50
	2.0	15	98
	3.0	05	45
	4.0	01	10
	5.0	-	-
	KIN		
	1.0	05	45
	2.0	10	82
	3.0	04	40
	4.0	01	10
	5.0	-	-

In Vitro Organogenesis, Regeneration and Propagation

Shoot tips grown on MS medium supplemented with BA (1 mg/l) induced 10.0 micro shoots per explants and shoot cultures accumulated withaferin A (0.04 %) and withanolide D (0.06%). Supplementation of MS solid agar medium with 1.0 mg BA/L and 4% sucrose enhanced accumulation of both withaferin A (0.16%) and withanolide D (0.08 %). MS liquid medium containing 1.0 mg BA/L and 10% coconut milk favoured a maximum increase in biomass (27 fold), induced microshoots (37.6) as well as accumulation of withaferin-A (0.14%).

In addition, its propagation by conventional methods takes a long time. Now, it is an important technique for the production of economically valuable biochemical's, including enzymes, flavonoids, pigments, vitamins etc. *Withania somnifera* is medicinally important crop of agriculture field but effects of weeds are suppressing the growth and rate of germination of this plant in cultivated and agriculture field. Weeds are suppressing the rate germination of Ashwagandha and uses of the weedicides and fertilizers are very expensive and costly. For now there is no doubt about the role of tissue culture in improving agricultural production. The plant tissue culture is desirable in order to satisfy production demands, which has been developed for the mass propagation of medicinal plants were supported by (Thomas and Philip, 2005; Thomas and Shankar, 2009), the conservation of particular and endangered species (Nagesh, 2008; Offord and Tyler,

2009), and the bioactive compounds sources (Wadegaonkar et al., 2006).

DISCUSSION

Tissue culture in Ashwagandha will be for mass production of planting materials, production of virus-free plants, plant breeding purposes, conservation, and multiplication of crops and livestock. Furthermore research may be implemented for phyto-chemical analysis of Ashwagandha for active ingredients by tissue culture method.

Furthermore this standardized callus induction and proliferation protocol might be used in further research for mass propagation of *W. somnifera* via indirect regeneration methods. The production of *W. somnifera* roots through conventional methods of cultivation (seed) is less than the requirements due to several reasons, such as poor yield, poor viability of seeds, susceptibility of the seeds and seedlings to fungal infections like seedling mortality and blight, leaf blight, seed rotting etc (Misra et al., 1997). Plant tissue culture is an integral part of molecular approaches to plant improvement and acts as an intermediary whereby advances made by the molecular biologists in gene isolation and modification are transferred to plant cells. Efficient protocol for initiation of callus of *W. somnifera* using stem explants. This protocol might be useful for the production and isolation of metabolites in callus culture, because the natural propagation of *W. somnifera* is time taking because of long germination period and low levels of seed germination.

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PLATE I : Initiation of Shoots

PLATE II : Multiple Shoot Formation