

Withania somnifera(L)Dunal (Ashwaganda) an Indian medicinal herb: *In vitro* Micropropagation from meristem explants.

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ABSTRACT

From the past few years herbal medicines and their extracts are gaining recognition as they are effective, affordable, no side effects and remedy for acute as well as chronic diseases. It is important to conserve genetic diversity of medicinal plant resources by developing efficient methods for micropropagation. For rapid multiplication and production of plants; tissue, cell and organ culture techniques with cost effective protocols is required. Successful *In-vitro* meristem tip culture of the magical Indian medicinal herb *Withania somnifera* is the objective of present study.

INTRODUCTION

Medicinal plants are an integral component of research and development in the pharmaceutical industry. They constitute nearly 70 % of the basis of modern pharmaceutical products including 25 % of drugs derived from different plants and many others are synthetic analogues built on prototype compounds isolated from them (Malik et.al, 2012). Ayurvedic and herbal medicines are the foundation for primary health care because of the better acceptability with the human body and less side effects (Kamboj, 2000). *Withania somnifera*(L.)Dunal is a member of the family Solanaceae commonly known as Ashwagandha. Its root part rich in alkaloids (withanine) (Majumdar, 1955), which are valuable constituents in traditional ayurvedic drug preparations against many diseases viz., hiccup, female disorders, cough, rheumatism and dropsy (Valizadeh and Valizadeh, 2009, Valizadeh and Valizadeh, 2011 Kiritkar and Basu, 1975). It is a potential medicinal plant that has been used medicinally in the treatment of tuberculosis, rheumatism, inflammatory conditions, cardiac diseases, and it is used as a general tonic, anti-stress drug and as an antitumor, antibiotic, anticonvulsant and central nervous system (CNS)-depressant agent besides being an ingredient of several ayurvedic preparations (Uma Devi et al., 1993; Kandil et al., 1994; Tripathi et al., 1996; Mishra et al., 2000). The traditional use of 'Ashwagandha' was to increase energy, youthful vigor, endurance, strength, health, nurture the time elements of the body, increase vital fluids, muscle fat, blood, lymph, and semen and cell production. It helps counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature aging emaciation, debility, and convalescence and muscle tension. It helps invigorate the body by rejuvenating the reproductive organs, just as a tree is invigorated by feeding the roots (Verma and Kumar, 2011). It is also useful as anodyne, bactericide, contraceptive and spasmolytic (Asthana and Raina, 1989). Recently *Withania somnifera* is also used to exhibit the development of tolerance and dependence on chronic use of various psychotropic drugs. The major biochemical compounds of Indian ginseng are steroidal alkaloids and steroidal lactones in a class of compounds named withanolides. The biological activities of withanolides, especially of the dominant withanolide A and with a ferin A, have been studied extensively and, more recently, activity (Kulkarni et.al, 1996 and Kulkarni et.al, 2000). Many plants propagated by vegetative means contaminated with microorganism like bacteria, fungi and viruses which may affect the quality of the medicine. Due to wide use of herbs for naturopathy and scientific research, it is essential to adopt alternative methods for rapid multiplication. Thus the systematic micropropagation of *Withania somnifera* is of great significance for future programmes on quality enhancement of the crop. Micropropagation provides a fast and dependable method for production of a large number of uniform plantlets in a short time. Moreover, the plant

multiplication can continue throughout the year irrespective of season and the stocks of germ plasm can be maintained for many years (Malik, 2007). Previously Murab et.al. (2014) done *Withania somnifera* culture by nodal explants, meristem tissue are fast growing; so to get the disease free and rapid plantlet of *Withania somnifera*, *In-vitro* Micropropagation from meristem explants was the objective of the present study.

MATERIALS AND METHODS

Collection and sterilization of seeds

Seeds of *Withania somnifera* were procured from local nurseries. The seeds were surface sterilized with 0.1% HgCl_2 and repeatedly washed in sterile distilled water followed by treatment with 0.4% of ascorbic acid for 30 minutes and rinsed 4-5 times with distilled water (Ishnava, 2012).

Germination of seeds, seedling development and explants preparation

Seed were inoculated on MS medium containing 30g/l sucrose and 8.0 g/l of purified agar for germination of seed minus the growth hormones. The pH of the half strength MS medium was adjusted to 5.7 before autoclaving, and then autoclaved at 1.06 kg/cm² and at 121°C for 20 min. shoot tips measuring 1-2 mm and leaf section measuring 2x2 mm were excised and inoculated in MS medium containing different levels of growth hormones, 30g/l sucrose and 8.0 g/l of purified agar. Inoculated cultures were sealed with Para film and were kept for incubation at 25± 2 under 1250 lux fluorescence light for 16 hrs of photoperiod. The seed culture produces plantlets within 15- 20 days and these were sub-cultured on the same concentration of the medium to produce multiple shoots (Murashige and Skoog, 1962).

Plant growth regulators

The preliminary experiments were conducted using two auxins (2, 4-D and IBA) and cytokinins (BAP and Kinetin) either alone or in combinations in varying concentrations (Table 1).

Micropropagation

The basal nutrient medium containing MS salts and vitamins was used with BAP (6-benzylaminopurine) and KN (kinetin). In the first experiment, the effects of BAP and KN were examined individually at the concentrations of 0.5–3.0mg/l and in the second experiment, BAP combined with KN and subculture at every two weeks to the same medium. The number of shoot buds was recorded after five weeks of culture. To test their rooting capacity of the shoots were transferred on to MS media fortified with different concentrations IBA (1.0–8.0mg/l). The rooting i.e., frequency of rooting(%), root number per shoot and root length(cm) were noted after two weeks of culture.

Table 1. Response of meristem tip cultured on MS medium supplemented with different growth regulator in varying concentrations.

S.No.	Culture Media	Direct shoot Formation	Root Formation	Callus formation
1	MS 00	+	+	-
2	MS 0.2 BAP	++	+	+
3	MS 0.3 BAP	+	++	+
4	MS 0.5 BAP	++	++	+
5	MS 1BAP 0.1IBA	++	+	+
6	MS 1BAP 0.2IBA	+++	++	++
7	MS 1.5BAP 0.1IBA	+++	+	+
8	MS 1.5BAP 0.2IBA	++++	++	+++
9	MS 1.5BAP 0.4IBA	++++	+++	+++
10	MS 2BAP 0.2IBA	+++	++	++++
11	MS 3BAP 0.1IBA	+	++	++
12	MS 1.5 2,4-D, 0.1 KN	++	++	++
13	MS 1.5 2,4D, 0.2	++	+	+

	KN			
14	MS 2 2,4D, 0.2KN	+	+	++++
15	MS 3 2,4D, 0.3KN	+	+	+
16	MS 2 2, 4-D.5KN	+	+	+

Response: Very high > 80% (++++); High (+++); Moderate 30-60%(++); Low<30%(+)

Plantlet regeneration was found to be direct as a stepwise process starting from callus induction, callus proliferation, morphogenesis, followed by plantlet regeneration.

DISCUSSION

Micropropagation technique, an effective methods to conserve microorganism free *Withania somnifera* under tissue culture conditions (Gita et.al, 2003, Gita and Grover, 1999). Sivanesan, 2007 and Sivanesan and Murugesan, 2005, 2008 has developed an efficient protocol of *Withania somnifera* micropropagation, standardized efficient and time saving. In the present study meristem explants of *Withania somnifera* were cultured on MS medium fortified with different concentrations of auxins and cytokinin (alone as well as with varying combinations) to establish an efficient protocol for propagation of *Withania somnifera*. It was observed that combinations of cytokinins and auxins (BAP and IBA) in MS medium was most suitable for shoot multiplication in its presence, shoot buds were differentiated directly from the cotyledon explants and with in six weeks of culture, innumerable propagules were developed in clusters showing high sprouting ability, followed by another responding group of culture media i.e. 1.5BAP 0.2IBA and 2,4D, Kinetin. Least regeneration was obtained in media combination 1.5BAP 0.4IBA. It was also observed that the best combination for root initiation was with the combination of 1.5 BAP + 0.4 IBA. Combinations of cytokinins and auxins (2BAP + 0.2IBA) led to direct morphogenesis. Culture media supplemented with lower concentration of BAP alone or in combination with lower concentration of auxins led to direct shoot proliferation. Higher percentage of callusing was observed in culture medium supplemented with either 2,4-D or Kinetin(KN).

In conclusion, the outlined procedure offers a potential system for conservation and mass propagation of *Withania somnifera* from cotyledon explants. In the present investigation 2BAP + 0.2IBA on MS media is more effective for shoot multiplication for both cultivars of *Withania*. It was also observed that the best combination for root initiation was with the combination of 1.5 BAP + 0.4 IBA.

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