

A note on the micropropagation of endangered medicinal plant, *Withania somnifera*

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ABSTRACT

From the beginning of human civilization, plants have been used in medicine and drugs to alleviate human sufferings. The traditional system of medicine is so engrained in our culture that over 75% of Indian population depends on this indigenous system for relief. With such a huge section of an ever-increasing population relying on herbal remedies, coupled with the fact that most of the medicinal plants are getting extinct day by day with their rich sources decreasing at an alarming rate, it becomes imperative to find alternative growth and culture method which can serve economical as well as conservational. *In vitro* plant regeneration is a biotechnological tool that offers a potential solution to this problem as it provides, a means of putting the plants on to the market at lower and affordable prices at the same time conserving the nature, *Withania somnifera* is one such wonderful plant which has tremendous potential in the use of several ailments such as Parkinson's, memory loss and heart diseases. It is also being used as an antioxidant, antivenom, anti-inflammatory and antitumor agent, However its over use is making this plant disappear fast, hence micropropagation of *Withania somnifera* (Solanaceae) collected locally was tried. For initiation of multiplication, MS medium supplemented with different concentrations of BAP and IAA were used. Cultures were maintained at 16hrs photoperiod. Satisfactory growth was obtained with axillary cotyledonary shoots growing significantly.

Key words: Micropropagation, *Withania somnifera* endangered medicinal plant.

Plants have been an important source of medicine for thousands of years. The World Health Organization estimates that up to 80% of people still rely on herbal remedies for their health care. The demand for medicinal plants is therefore very high leading to their over-exploitation from the wild. There are various medicinal plants which are known to have several medicinal properties since ancient times. Among these medicinal plants, *Withania somnifera* (Solanaceae) is such a wonderful herb having great potential for the treatment of several maladies. *Withania somnifera*, also known as ashwagandha, Indian ginseng and winter cherry, has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. The roots and leaves of the plant are categorized as rasayans, which are reputed to promote health and longevity by augmenting defence against disease, arresting the ageing process, revitalizing the body in debilitated conditions (Agrawal and Paridhavi, 2007).

Historically the plant has been used as an antioxidant, adaptogen, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers,

bacterial infection, venom toxins and senile dementia. Clinical trials and animal research support the use of *Withania somnifera* for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson's disease. Recently it has been also used to inhibit the development of tolerance and dependence of chronic use of various psychotropic drugs (Sharma *et al.*, 1985).

Though India has a rich biodiversity, the burgeoning demand of *Withania somnifera* due to its enormous medical properties, is putting heavy strain on the existing resources, hence it is increasingly being threatened in its natural habitat. Plant *in vitro* regeneration is a biotechnological tool that offers a potential solution to the problem of medicinal plants decimation in India and other countries. Hence, the micropropagation of *W. somnifera* was attempted in our tissue culture laboratory.

Withania somnifera were collected from the vicinity of Bhopal after proper identification and explants were taken from the leaves, hypocotyls, and axillary nodal segments. Explants were washed thoroughly with running tap water for 10 min and then washed with distilled water and then surface

sterilized with 0.1% (w/v) mercuric chloride solution for five minutes followed by three times washing in sterilized distilled water to remove traces of mercuric chloride. For initiation of multiplication, M.S basal medium supplemented with different concentrations of cytokinin, BAP (0.1-1.0mg/l). Kn (0.1-1.0mg/l) in combination with Auxin, IAA (0.4mg/l) was used (Ray and Jha,2001).

The pH of the medium was adjusted to 5.8 before addition of 0.8(w/v) agar. All cultures were maintained under continuous 16h dark/light period for four weeks. For further elongation and multiplication of regenerated shoots, the primary shoots formed *invitro* were separated aseptically and cultured in M.S supplemented with BAP (0.5-1.0mg/l) and IAA (0.1-1.0mg/l). Subsequent subcultures were placed on MS medium. For root induction, excised shoots with three or more leaves were transferred to MS basal medium fortified with IAA (0.2-0.6mg/l) and IBA (0.2-0.6mg/l). Plantlets with well developed roots were removed from culture medium and transferred to half strength of MS basal medium after gentle washing with sterilized double distilled water and carefully transferred to earthen pots containing sand and soil in 1:1 ratio. Plantlets were maintained in controlled condition and after two weeks transformed outside under the full sun (Sen and Sharma,1991).

The *invitro* propagation of wonder herb *Withania somnifera* was found to be successful as significant regeneration of shoots occurred from *Withania somnifera* axillary nodal explants cultured on M.S.media containing various carbon sources also supplemented with varying concentrations of BAP, IBA and other important plant hormones. A satisfactory shoot proliferation from axillary nodal explants was obtained on 3% (w/v) of sucrose. The number of roots and root length was found to be satisfactory. IBA was found effective for root

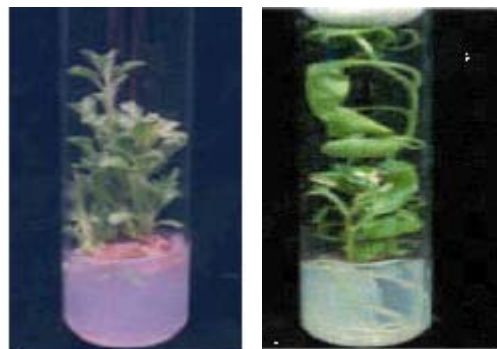


Fig. 1: Showing explant regeneration; *Withania somnifera*
(a) shoot induction (b) root induction

induction. All micro propagated plants were free from external defects.

In the present study, attempt to micro propagate the plant of *Withania somnifera*, using commercially available well known, Murashige and Skoog (MS) culture medium supplemented with CaCl_2 and varying concentrations of BAP along, with IBA was done. The micro propagation proceeded successfully in the conditions of keeping temperature $25 \pm 2^\circ\text{C}$, relative humidity 55-60% and 16 hours light and 8 hours dark period alternatively. It was shown that appropriate concentration of IBA, BAP and Auxin gave significant growth. It took 23 days for axial shoot formation and 52 days for root regeneration. Thus it can be concluded that the choice of culture medium, concentration of growth regulators and their ratios, culture conditions and the parts of plant taken as explant are the factors, which regulate of micro propagation of medicinal plants. Micro propagated plants can be used to supplement the natural stock of plants in wild populations as well as provide a ready supply to the herbal medicinal trade.

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