IN VITRO MICROPROPAGATION OF MEDICINALLY IMPORTANT ROOTS AND AXILLARY BUD COMBINATION

SMITA SHARMA, MUKESH.CHANDRA.SHARMA[*a], D. V. KOHLI[a]
Department of Chemistry Yadhunath Mahavidyalya Bhind (M.P) 477001 India
[a]Department of Pharmaceutical Sciences Dr.H.S.Gour.University Sagar (M.P) 470003 India
E-mail: mukesh2206@rediffmail.com

In vitro micro-propagation of medicinally important plant was achieved for the generation of multiple shoots and roots of *Withania somnifera*. Shoots were induced from the axillary buds of *Withania somnifera* on Murashige and Skoog's (MS) basal media supplemented with different concentrations of BAP (0.10 mg/L, 0.15 mg/L, 0.20 mg/L, 0.25 mg/L, 0.30 mg/L) along with GA_3. Axillary bud explants showed initiation of shoots within 5-6 days of transfer, with optimum concentration of 0.25 mg/L and it is found to be most effective for multiple shoot generation within minimum time period. The generated shoots were successfully rooted on MS basal medium supplemented with NAA (optimum concentration – 0.5 mg/L) along with GA_3 and the plantlets acclimatized in soil.

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1. Introduction

Withania somnifera (L.) or Ashwagandha or Winter cherry belongs to family *Solanaceae.* It grows widely in all dry parts as a stoutshrub. It is a well known for years as an important drug in the ayurvedic literature. All the parts of the plants have shown remarkable importance in the field of pharmacology. The active pharmacological components of Withania somnifera are steroidal lactones of the withanolide type. Several chemotypes have been found differing in their withanolide content. The principal withanolide in Indian *Withania somnifera* are withaferin A and withanolide D. Both leaves and roots of the plant are used as the drug and steroidal lactones occur in both parts. The total alkaloid content in the root of the Indian types has been reported to vary between 0.13 and 0.31%. Roots of the plant show antitumour and radio sensitizing effects in animal models. It also possesses antistress, immunomodulatory, antioxidant and antibacterial activity. The present study was undertaken to develop a method of high multiplication rate using axillary buds using different concentrations of growth regulators in short period of time[1-3]. The present study was focussed on optimizing the Plant Tissue Culture technique for high multiplication rate (generation of multiple shoots and roots) from explant and enhancing the production of rare and endangered medicinally important plant species [4-6].

2. Experimental work

2.1 Collection of plants

Ex plant of *Withania somnifera* was collected from Indore.

2.2 Surface sterilization

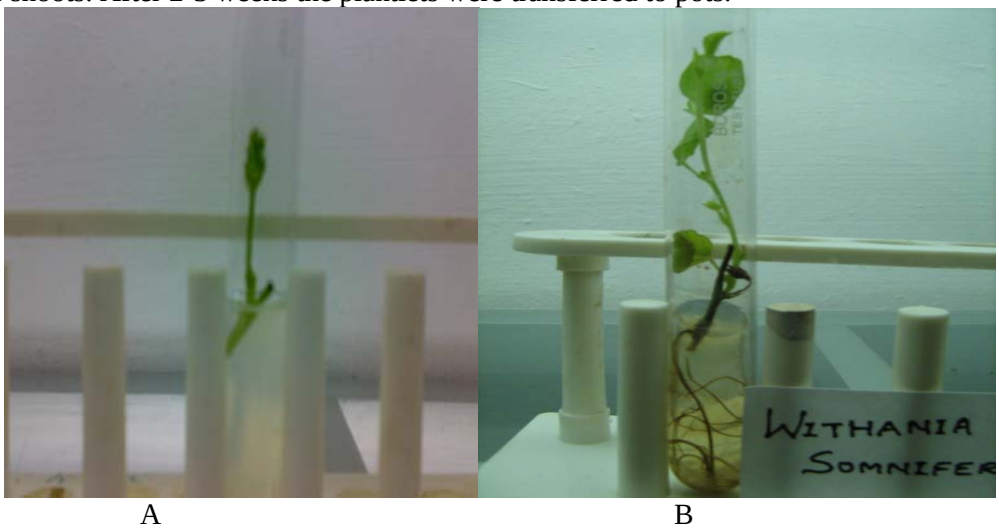
Axillary buds were excised from the plants and washed thoroughly with tap water, immersed in 1% mild detergent (cleaning agent) for 2-3 min. and washed thoroughly with distilled water. Subsequently, these were surface sterilized with 0.1% mercuric chloride solution for 1-2 min. and again washed well in distilled water to remove the traces of mercuric chloride.

2.3 Regeneration of in vitro plants

MS medium supplemented with different hormonal concentration of BAP (Benzyl amino-purine) along with GA_3 (Gibberelin) were used for the study. The media were congealed with agar and sucrose was used as a source of carbohydrate. The pH of the medium was adjusted to 5.7 using 0.1 N NaOH and 0.1 N HCl solutions before autoclaving. The culture tubes of molten media were autoclaved at 121° C for 15 min. The explants were inoculated carefully (under laminar air flow) on MS medium containing different hormone concentration[1, 2, 7]. All culture tubes were incubated at 25±2° C, 55±5% relative humidity; under a 16 h photoperiod (light and dark regime is created in growth chamber). The regenerated shoots were aseptically excised and subcultured on MS medium supplemented with NAA (Naphthalene acetic acid) along with GA_3. Rooted plantlets were taken out from culture vessels. The roots were gently washed with sterile distilled water to remove the adhering agar. The regenerated plantlets were transferred to pots containing a mixture of soil and sand (3:1) under controlled conditions of temperature and humidity.

3. Results and discussion

The explants of *Withania somnifera* cultured on MS basal medium with varying hormonal concentration were observed regularly for 3-4 weeks for their development by measuring their length. Shoots started generating after 5-6 d of inoculation and the best results (optimum conc. for maximum shoot generation in minimum time) were obtained with the BAP conc. 0.25 mg/L (BAP is a cytokinin). Cytokinins have major role in the initiation of the shoots. Other concentrations showed initiation after some time (0.20 mg/L BAP), but less effective responses were obtained. The healthy shoot buds were separated and transferred onto rooting medium supplemented with optimum concentration of 0.5 mg/L NAA (NAA is a auxin). Auxin have major role in the initiation of the roots. The roots developed at the base of the shoots. After 2-3 weeks the plantlets were transferred to pots.



A B

Fig. 1. In vitro micropropagation of Withania somnifera from axillary bud: A – Explant of Withania somnifera; B – New plantlet developed from explant.

4. Conclusion

From this study, it is concluded that multiple shoots of *Withania somnifera* were developed from axillary bud explants on MS medium supplemented with BAP at optimum concentration (0.25 mg/L) and rooted on MS medium supplemented with NAA at optimum concentration (0.5 mg/L). This study aims to develop a standard protocol to initiate multiple shoot and root culture of plant that may provide a good source of pharmacologically active plant constituents.

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