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IN VITRO MULTIPLE SHOOT INDUCTION FROM SHOOT TIP EXPLANTS OF ASHWAGANDA – AN IMPORTANT MEDICINAL PLANT

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ABSTRACT : Attempt was made to standardize the regeneration protocol. Medium was optimized for shoot organogenesis and rooting of Ashwaganda (*Withania somnifera* (L) Dunal). *In vitro* regeneration of shoot buds were achieved from shoot tip explants cultured on MS medium supplemented with BAP, KN and BAP+KN. Regeneration and multiplication rate was high in MS medium containing BAP(2mg/l) along with KN(2mg/l). The shoot buds were isolated from multiple shoot clusters and cultured on MS medium supplemented with different concentrations of indole-3-butyric acid(IBA) for root induction. Higher degree of rooting was obtained on MS with IBA(5mg/l). Rooted plantlets were hardened and successfully established using soilrite and transplanted in earthen pots and were showed 80-90% survival during transplantation.

Key words: Ashwaganda, BAP, KN, MS medium, shoot tip.

INTRODUCTION

Medicinal plants are an important source of compounds for the pharmaceutical industry and traditional medicine. About 80% of the population living in developing countries still use traditional medicines derived from plants for their primary health care needs (1,2). *Withania somnifera* (L) Dunal is a member of the family Solanaceae commonly known as Ashwaganda. Its root part rich in alkaloids (withanine) (3) which are valuable constitutes in many traditional Ayurvedic drug preparations against many diseases viz., hiccup, female disorders, cough, rheumatism and dropsy (4). Besides roots, the other parts of this plant also useful for the treatment of inflammatory conditions, tuberculosis and exhibits excellent antitumor and antibacterial activities (5,6). Due to the indiscriminate collection of huge amount of this plant by local herbalists, Ayurvedic and Unany companies, this plant species is on the verge of extinction. In conventional systems seeds are generally utilized for multiplication and production. This method has some disadvantages such as short viability period, low rate of germination and high risk of catching various diseases. In order to facilitate the development of plant biotechnology based cultivar improvement for this crop, considerable effort has been devoted in developing and optimizing efficient *in vitro* regeneration protocols for rapid mass propagation of this species to meet up the commercial need and also for protecting the genetic erosion. Shoot meristem multiplication is generally used for producing virus free material and maintaining germplasm via cryopreservation (7). Several micropropagation protocols of Ashwaganda have been reported from shoot tip explants (8-14). However, the rate of plant regeneration per explant is not sufficiently high to be practical application. So, it is necessary to establish reliable regeneration systems for Ashwaganda developed for commercial purposes. In this study we developed an efficient protocol for *in vitro* micropropagation of *Withania somnifera* (L) Dunal through direct organogenesis and for subsequent multiplication by using shoot tip explants.

MATERIALS AND METHODS

Explant preparation

Seeds of *Withania somnifera* (L) Dunal were collected from Herbal Garden of Botany Department, Andhra University, Visakhapatnam (India) and were surface sterilized with 0.1% $HgCl_2$ and repeatedly washed in sterile distilled water. The seeds were than inoculated in glass container with 50ml of half strength MS medium (15) for germination. The shoot tip explants were derived from 30 days old seedlings grown *in vitro* transferring on to culture media.

Micropropagation

The basal nutrient medium containing MS salts and vitamins was used with BAP(6-benzyl aminopurine) 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.

In vitro conditions

All media were supplemented with 3% sucrose and 0.8% agar, the P^H of the media was adjusted to 5.8 with 1N NaOH or 1N HCl prior to autoclaving. The cultures were maintained at 25±2^{0}C temperature with a 16 hour photoperiod under an illumination of 20 m mol $m^{-2}s^{-1}$ photosynthetic photon flux density provided by cool-white fluorescent light.

Acclimatization

Plants with roots were transferred during two weeks, after washing of the agar with distilled water and to pots with a mixture of soilrite (1:1). Potted plantlets were covered with transparent polythene membrane to ensure high humidity and watered every three days with half strength MS salts solution for two weeks in order to acclimatize plants to field conditions. After two weeks the acclimatized plants were transferred to pots containing normal garden soil and maintained in greenhouse under natural day length conditions.

Statistical analysis

Experiments were set up in Randomized Block Design (RBD) and each experiment was replicated thrice. Observations recorded on the percentage of response, number of shoots per explant, number of roots per shoot and root length. Mean and standard errors were carried out for each treatment.

RESULTS AND DISCUSSION

During the present research investigation, multiple shoots were directly induced when shoot tips inoculated on MS medium supplemented with BAP(0.5-3.0mg/l) and KN(0.5-3.0mg/l) alone or in combination (BAP+KN) while, the explants cultured on MS medium without growth regulators failed to induce multiple shoots but each explant developed into single shoot (Table 1). Among the three combinations tested viz., (MS+BAP, MS+KN and MS+BAP+KN), the MS medium fortified with BAP+KN treated explants achieved higher response (shooting and shoot number per explant) than those treated with BAP and KN alone. Stimulation of multiple shoots formation by MS medium containing BAP+KN has been reported in several explants of *Withania* (11,12,16,17). From our study it was clear that BAP(2mg/l)+KN(2mg/l) was significantly more effective for inducing shoot organogenesis (100 and 54.0±0.20) shooting(%) and shoot number per explant respectively (Fig 1a, band c).

The number of regenerated shoots, which is found to be in better with results obtained by Sivanesan (11) and Satyajit and Santi Lata (14) in *Withania somnifera* (L) Dunal. In many plant species, micropropagation requires two media viz., (propagation medium and shoot elongation medium) making the micropropagation procedures cumbersome and uneconomical (18), in our present work, shoot multiplication and subsequent elongation were achieved on the same medium. The elongated shoots were excised and implanted on MS medium supplemented with different combinations of IBA (1-8mg/l) for rooting (Table 2). The optimum rooting efficiency of *in vitro* raised shoots (64.0±0.22), root number per shoot (12.8±0.16) and root length(cm) (11.0±0.05) were achieved on MS medium fortified with IBA (5mg/l) (Fig 1d). These results are in accord with findings from previous studies (17,19,20). The regenerated plants showed 80-90% survival during hardening and acclimatization and there were no observable differences between the parent plant and *in vitro* raised plants.

Table 1: Effect of BAP and KN on shoot tip explants of *Withania somnifera* (L) Dunal.

Plant growth regulators (mg/l)		Shooting (%)	Shoot No./explant
BAP	KN		
0.0	0.0	100.0±0.00	1.0±0.00
0.5	0.0	56.0±0.15	10.6±0.04
1.0	0.0	63.0±0.19	16.9±0.10
1.5	0.0	71.0±0.08	22.0±0.08
2.0	0.0	92.8±0.24	26.3±0.14
2.5	0.0	80.6±0.04	18.0±0.08
3.0	0.0	69.4±0.16	10.2±0.16
0.0	0.5	42.0±0.06	6.8±0.11
0.0	1.0	55.0±0.14	10.2±0.06
0.0	1.5	62.5±0.11	13.8±0.16
0.0	2.0	75.0±0.19	16.2±0.08
0.0	2.5	82.0±0.24	22.4±0.13
0.0	3.0	63.0±0.12	15.0±0.22
0.5	0.5	68.0±0.08	29.0±0.16
1.0	1.0	74.5±0.20	32.0±0.08
1.5	1.5	86.0±0.16	39.8±0.13
2.0	2.0	100.0±0.00	54.0±0.20
2.5	2.5	88.0±0.10	42.0±0.16
3.0	3.0	71.0±0.22	28.6±0.11

Table 2: Effect of IBA on root induction in *Withania somnifera* (L) Dunal *in vitro* raised shoots

Plant growth regulators (mg/l)	Rooting (%)	Root No./shoot	Root length (cm)
IBA			
0.0	00.0±0.00	0.0±0.00	0.0±0.00
1.0	12.0±0.20	2.0±0.06	1.8±0.05
2.0	14.0±0.08	2.2±0.02	3.6±0.04
3.0	19.0±0.10	3.6±0.11	4.2±0.03
4.0	25.0±0.19	6.4±0.08	5.8±0.03
5.0	64.0±0.22	12.8±0.16	11.0±0.05
6.0	40.0±0.09	8.0±0.04	8.2±0.09
7.0	29.0±0.16	6.8±0.15	6.3±0.06
8.0	16.0±0.24	5.6±0.05	5.0±0.10

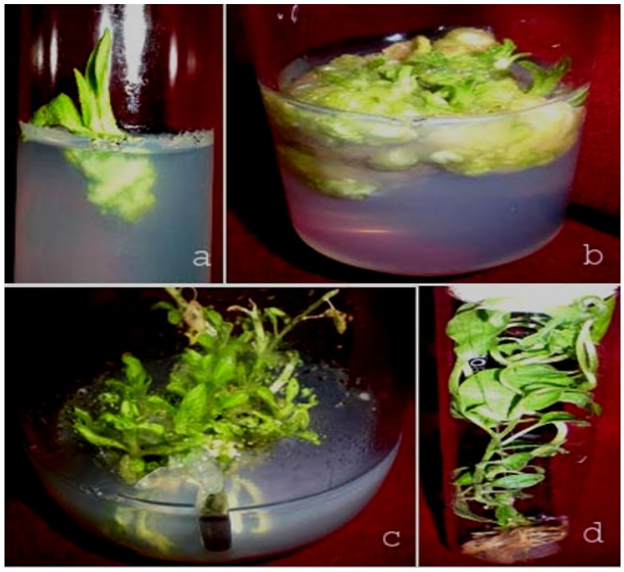

Fig 1(a-d): multiple shoot induction and plant regeneration from shoot tip explants of *Withania somnifera* (L) Dunal. a: inoculated shoot tip; b: initiation of multiple micro shoots on MS+BAP(2mg/l)+KN(2mg/l); c: proliferated multiple shoot cluster on MS+BAP(2mg/l)+KN(2mg/l) ; d; rooted plant on MS+IBA(5mg/l).

CONCLUSIONS

From our investigation, it is evident that MS medium containing BAP+KN combinations are the best suited for inducing multiple shoots and IBA(5mg/l)for rooting. In conclusion, this communication describes an efficient rapid propagation system of *Withania somnifera* (L) Dunal.

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