ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

In vitro propagation method of *Withania somnifera* by tissue culture technique.

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Manuscript Info

Manuscript History:

Received: 12 April 2014
Final Accepted: 23 May 2014
Published Online: June 2014

Key words:
In vitro, propagation, growth regulators, *Withania somnifera*.

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Abstract

The experiment has been conducted to achieve a method for producing multiple shoots from sterilized shoot-tips and obtaining a lot of seedlings of ever green shrub *Withania somnifera*, family Solanaceae as well as studying the effect of different culture media composition on shoots formation. Our results revealed that the level of 2.0 mg/l BAP+0.1 mg/l NAA increased the number of *W. somnifera* shoot/explant and the increasing of BAP concentration to 3.0 mg/l significantly decreased number of shoot/explant to about 50%. On the other hand the tallest shoot length which derived from the treatment of 2.0 mg/l BAP+ 1.0 mg/l NAA was a significant increment compared with other concentrations of growth regulators. The concentration of 1.0 mg/l Kin+0.1 mg/l NAA gave the shortest shoots compared with the other treatments including the control and the difference was significant.

INTRODUCTION

Plants in addition to their aesthetic value constitute the major natural source of the food we eat, the air we breathe and the medicine to cure our many ills. Recently, the utilization of medicinal plants as a natural source of drugs is being increasingly encouraged. Consequently, medicinal plants have been targeted for uncontrolled collection and destruction as a result of urbanization, overgrazing, pollution and expansion of cultivated areas. Plant secondary metabolism gives rise to the formation of a vast array of chemically complex compounds, many of which are commercially important. Many problems are associated with the production and marketing of such compounds as well as the supply of raw materials can be erratic due to several reasons. So, it may become critical to develop an alternative source of important therapeutically natural products. Plant cell culture provides an environmentally friendly, renewable alternative for secondary metabolite supply.

Withania somnifera (L.) Dunal, commonly called Indian ginseng is a member of the family Solanaceae, growing up to a height of 30-150 cm. It considered as an important medicinal plant in the Ayurvedic and indigenous medicinal system of India. The drug extracted from roots is used for the treatment of tuberculosis, rheumatism, inflammatory conditions, cardiac, bacterial diseases and as a general tonic (Asthana and Raina, 1989 and Furmanova et al., 1999). The major biochemical compounds of Indian ginseng are steroidal alkaloids and steroidal lactones in a class of compounds named withanolides (Ray and Gupta 1994). The biological activities of withanolides, especially of the dominant withanolide A and withaferin A, have been studied extensively and, more recently, have been shown to have anti-cancerous activity (Jayaprakasam et al., 2003 and Ichikawa et al. 2006). In 2009, Mirjalili et al. presented an overview of the chemical structures of triterpenoid components in *W. somnifera* extracts and their biological activity, focusing on two novel activities, tumor inhibition and antiangiogenic properties of withaferin A and the effects of withanolide A on Alzheimer's disease. Little attention has been given to micropropagation method

of *W. somnifera*. Therefore, the aim of this research paper was to study the effect of different culture media composition for enhancing shoot proliferation and elongation.

MATERIALS AND METHODS

This study was carried out in the tissue culture laboratory of Vegetable and Ornamental Department, Faculty of Agriculture, Mansoura University, Egypt.

Sterilization method:

Young shoot-tips of *W. somnifera* were subjected to surface sterilization by washing in tap water containing soap and small drops of tween 20, 1 min. in ethanol 70%, washed with sterile distilled water and immersed in 30% commercial Clorox solution (1% Sodium hypochlorite) for 15 min. Shoot-tips were washed three times with sterile distilled water in laminar air flow hood to remove the residuals.

Culture media:

Sterilized shoot-tips were cultured in full strength of Murashige and Skoog (1962) (MS) medium supplemented with Benzylamino purine (BAP) at 1.0, 2.0, and 3.0 mg/l and Kinetin (Kin.) at 1.0, 2.0, and 3.0 mg/l combined with 0.1 mg/l Naphthalene acetic acid (NAA). Agar was used at 8g/L, and sucrose at 30 g/L. The media were distributed into clean jars. Each of which contained 30 ml of nutrient media. The media was adjusted to pH 6.2 before autoclaving for 15 min. at 121 ºC, 1.5 kg/cm^3. All treatments were incubated in the growth chamber at 26±2 ºC and exposed to 16 hr. light/day photoperiod under constant fluorescent light of 1500 Lux.

Experimental treatments:

The experiment consisted of seven treatments; each treatment included ten jars (each contained three explants). The following combination treatments were carried out:

1- 0.0 mg/l BAP or Kin. + 0.0 mg/l NAA (control).
2- 1.0 mg/l BAP + 0.1 mg/l NAA.
3- 2.0 mg/l BAP + 0.1 mg/l NAA.
4- 3.0 mg/l BAP + 0.1 mg/l NAA.
5- 1.0 mg/l Kin. + 0.1 mg/l NAA.
6- 2.0 mg/l Kin. + 0.1 mg/l NAA.
7- 3.0 mg/l Kin. + 0.1 mg/l NAA.

Experimental design and statistical analysis:

A complete randomize design was used throughout the research. The obtained data were subjected to analysis of variance and the treatment means were compared using L.S.D. test as outlined by Snedecor and Cochran (1975).

Data recorded:

The following data were recorded after 3 weeks from culturing on media for:

1- Average length of shoots (cm).

2- Average number of shoots per explant.

RESULTS AND DISCUSSION

a- Number of *W. somnifera* shoots:

Data represented in Table 1 and Fig. (1) illustrate the significant enhancing effect of different concentrations of BAP, kinetin and NAA on shoot production of *W. somnifera*. Most of the treatments significantly increased number of shoots per explant than the control. The highest number of shoots (2.57) was obtained as a result of modifying MS medium with 2.0 mg/l BAP + 0.1 mg/l NAA. Increasing BAP concentration to 3.0 mg/l significantly decreased number of shoot per explant to about 50%.

This result might be explained by the assumption that the externally applied cytokinin BAP at 2.0 mg/l and auxin NAA at 0.1 mg/l achieved an internal hormonal balance that induces the highest cell differentiation into vegetative organ. This enhancing effect could be ascribed to the stimulating effect of these concentrations of BAP as a cytokinin and NAA as an auxin on cell division on the expense of cell enlargement. Moreover, cytokinins have been shown to activate RNA synthesis and to stimulate protein synthesis and enzyme activity. On the other hand, the results indicated that, in *W. somnifera*, the presence of kinetin was not effective as BAP to induce shoot formation.

Our results were supported by Sen and Sharma (1991) who reported that the most effective cytokinin in promoting *W. somnifera* shoot proliferation from shoot tips was BA, shoot multiplication rates were higher at greater BA concentration; kinetin was less effective than BA in inducing shoot multiplication.

Table (1): The effect of different concentrations of BAP, kinetin and NAA on shoot production of *Withania somnifera*.

Treatments	Number of shoots /explant	Shoot length/ explant (cm)
0.0 mg/l BAP + 0.0 mg/l NAA (control)	**0.0**	**0.67**
1.0 mg/l BAP + 0.1 mg/l NAA	**0.71**	**1.21**
2.0 mg/l BAP + 0.1 mg/l NAA	**2.57**	**3.09**
3.0 mg/l BAP + 0.1 mg/l NAA	**1.29**	**1.57**
1.0 mg/l Kin.+ 0.1mg/l NAA	**0.14**	**0.96**
2.0 mg/l Kin.+ 0.1 mg/l NAA	**1.14**	**1.19**
3.0 mg/l Kin.+ 0.1 mg/l NAA	**0.86**	**1.20**
L.S.D at 5%	**0.84**	**0.59**

b- Shoot length (cm) of *W. somnifera*:

As shown in the same table and figure, there was a significant increment between shoot length of (3.09 cm) which derived from the concentration of 2.0 mg/l BAP + 0.1 mg/l NAA and other concentrations of growth regulators. On the other hand the concentration of 1.0 mg/l kin. +0.1 mg/l NAA gave the shortest shoots (0.96 cm) compared with the other treatments including the control and the difference was significant. A significant difference in shoot length was noticed between the concentrations of 1.0 mg/l BAP + 0.1 mg/l NAA, 2.0 mg/l kin. + 0.1 mg/l NAA and 3.0 mg/l kin. + 0.1 mg/l NAA which gave the same shoot length (1.2cm) and the concentration of 2.0 mg/l BAP + 0.1mg/l NAA. Our results disagree with Saritha and Naidu (2007) who observed in *W. somnifera* that kinetin 2.0 mg/l and NAA 0.1 mg/l gives a considerable shoot elongation.

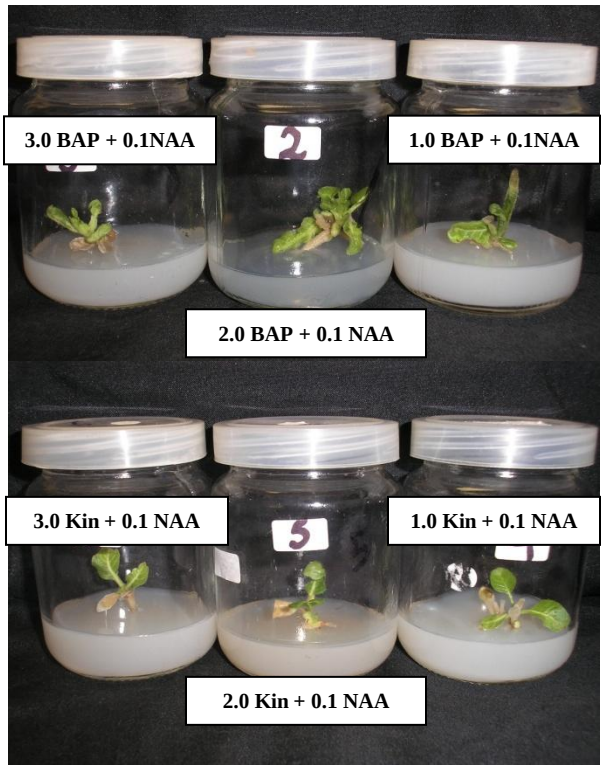

Fig. (1): Effect of different concentrations of BAP, kinetin and NAA on shoot production of *Withania somnifera*.

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