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Evaluation of the Effect of BA Hormone Levels of Poinsettia under *In vitro* Culture Condition

[1]*Sirous Bidarigh,* [2]*Abdollah Hatamzadeh and* [3]*Ebrahim Azarpour*

[1,3]Department of Agriculture, Lahijan Branch,
Islamic Azad University, Lahijan, P.O. Box 1616, Iran
[2]Department of Agriculture, Guilan Branch, Guilan, Iran

Astract: In order to study the effects of BA hormone levels (0, 0,01, 0.1, 3, 5 and 10 mg/L) in vitro culture of poinsettia (Euphorbia pulcherrima wild), an experiment as RCBD with four replications was conducted during 2010 at Research Laboratory of Faculty of Agriculture, Lahijan University in Iran. The culture bed were contained MS, sucrose (3%) and agar (75%). The results show that BA hormone levels on shoot length in vitro culture of poinsettia had a significant difference in 1 % probability level. The highest shoot length in vitro culture of poinsettia was obtained with application 3 mg/L BA hormone (8.57 mm). The lowest shoot length in vitro culture of tea was obtained with application 0 mg/L BA hormone (4.28 mm).

Key words: Poinsettia · *In vitro* culture · BA hormone · Shoot length

INTRODUCTION

The family Euphorbiaceae comprises nearly 322 genera and 8910 species [1] many of which have their own economic value and hence contribute to the floristic wealth of tropical and subtropical countries of the world. The family comprises a number of endemic and endangered taxa. However the *in vitro* studies are confined only to a few genera of aesthetic, medicinal, timber yielding, rubber yielding, dye yielding, cottage industries, ornamental and food crops like *Acalypha, Baliospermum, Codiaeum, Cleistanthus, Croton, Euphorbia, Emblica, Eryngium, Excoecaria, Givotia, Glochidion, Hevea, Jatropha, Mallotus, Manihot, Phyllanthus, Putranjiva, Ricinus, Sapium* and *Uapaca* [2]. Major components of *Euphorbia* latex are sterols, terpenoids vitamins and insecticides and anti cancer drugs [3, 4].

A factor that must be considered when propagating a plant species *in vitro* is the type of medium to use. The medium is comprised of basal salts and essential nutrients that a plant requires for proper growth and development. *In vitro* culture techniques involving the use of high- and low-salt media, such as Murashige and Skoog [5, 6] (MS).

In vitro culture is one of the key tools of plant biotechnology that exploits the totipotency nature of plantcells, a concept proposed by Haberlandt [7] and unequivocally demonstrated, for the first time, by Steward *et al*. [8]. Tissue culture is alternatively called cell, tissue and organ culture through in vitro condition [9]. It can be employed for large-scale propagation of disease free clones and gene pool conservation. Ornamental industry has applied immensely in vitro propagation approach for large-scale plant multiplication of elite superior varieties. As a result, hundreds of plant tissue culture laboratories have come up worldwide, especially in the developing countries due to cheap labour costs. However, micropropagation technology is more costly than conventional propagation methods and unit cost per plant becomes unaffordable compelling to adopt strategies to cut down the production cost for lowering the cost per plant [10].

The objective of the present research was to enhance the development of a culture method of poinsettia (*Euphorbia pulcherrima* wild) with application BA hormone levels.

MATERIALS AND METHODS

In order to study the effects of benzyladenine (BA) hormone levels (0, 0,01, 0.1, 3, 5 and 10 mg/L) in vitro culture of poinsettia (Euphorbia pulcherrima wild), an experiment as RCBD with four replications was conducted during 2010 at Research Laboratory of Faculty of

Corresponding Author: Sirous Bidarigh, Department of Agriculture, Lahijan Branch,
Islamic Azad University, Iran. P.O. Box 1616. E-mail: bidarig@yahoo.com.

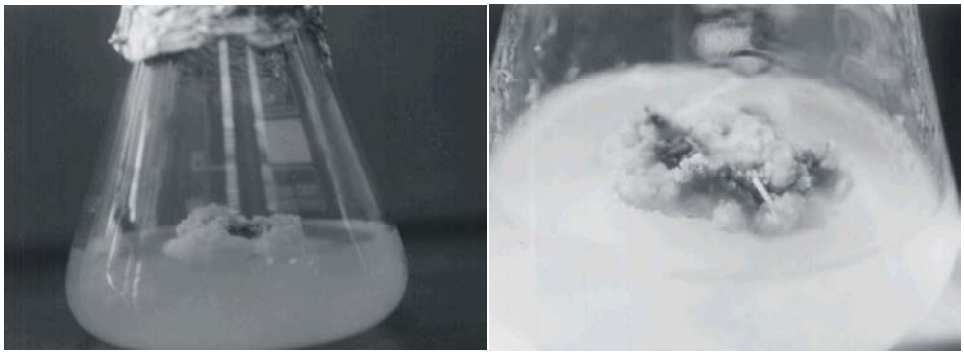

Fig. 1: Formation of shoot and root after in vitro culture on MS basal medium

Agriculture, Lahijan University in Iran. The poinsettia of Ecks point cultivar was used for doing the experiments. The plants were pot flowers and were propagated through cutting. All plants were five years-old and their length didn't 30 cm because of repeated pruning. Shoots tip samples were washed with tap water and surface sterilized in a drop of liquid detergent for 1 min, followed by three rinses in sterile distilled water. Then, they were re-sterilized with 10% etilic alchol for 30s and with 30% sodium hypochlorite for 10 min, followed by three rinses in sterile distilled water, all under laminar flow. Discs of ca. 0.5 cm2 diameter were cut from the leaves and were cultured on MS [5] basal supplemented with BA hormone levels. (Figure 1). PH was adjusted to 5.8 before adding 3% (w/v) sucrose and 75% (w/v) agar. The cultures were maintained with a 16 h light and 8 h darkness at 27±3°C.

Data analyses were analyzed by using SAS software. The Duncan's multiple range tests was used to compare the means at %5 of significant.

Table 1: Analysis of variance effect of BA hormone levels on shoot length in vitro culture of poinsettia

Sours of variance	DF	Shoot length
Replication	3	0.73
BA hormone levels	5	14.62**
Error	15	0.013
C.V %		2

** and * respectively significant in 1% and 5% area.

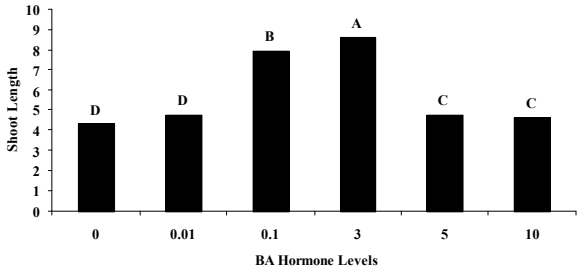

Fig. 2: The effect of BA hormone levels on shoot length in vitro culture of poinsettia

RESULTS AND DISCUSSION

Results of variation analysis show that (Table 1), BA hormone levels on shoot length in vitro culture of poinsettia had a significant difference in 1 % probability level. The highest shoot length in vitro culture of poinsettia was obtained with application 3mg/L BA hormone (8.57 mm). The lowest shoot length in vitro culture of tea was obtained with application 0 mg/L BA hormone (4.28 mm) (Figure 2).

In plant tissue cultures, cytokinin is required for callus growth (an undifferentiated, tumor-like mass of cells) and ratio of cytokinin to auxin is important to determine the fate of the callus. Moreover, cytokinin is known to promote the light-induced formation of chlorophyll and conversion of etioplasts to chloroplasts [11]. benzyladenine (BA), a synthetic cytokinins, may regulate cell cycle and cell division, stimulate auxiliary and adventitious shoot proliferation, regulate differentiation and inhibit root formation [12]. Benzyladenine (BA) as a cytokinin is generally used in plant cell culture at a concentration range of 0.1-10 mg/L. Cytokinins stimulate Cdk-A activity in the G1-S transition and in the G2-M phases, regulate cell cycle progression partly by inducing CycD3/1 transcription. When BA is added in appropriate concentrations, it may regulate cell cycle and cell division, stimulate auxiliary and adventitious shoot proliferation, regulate differentiation, inhibit root formation, activate RNA and protein synthesis and enzyme activity, delay senescence and promote nutrient uptake and cause greening process [11, 13]. Shoot buds from the axillary nodes and leaf segments of non-toxic *Jatropha curcas* were cultured on MS + Kn (2.3-46.5µM), BA (2.2-44.4µM) and TDZ (2.3-45.4µM) individually, the leaf segments were cultured on MS+ BA

(8.9µM) + IBA (2.5µM) and got adventitious shoots [14]. The shoot tips of *Glochidion multiloculaire* produced multiple shoots when cultured on MS + BA (1.0 mg/l) and IAA (1.0 mg/l) callus derived from the leaf and stem explants on a medium containing 2,4-D (0.5-2.0 mg/l) produced shoot buds when transferred to MS + BA (1.0-2.0 mg/l) + CM (Coconut milk, 10% v/v) the rooting combination was MS + IBA (1.0 mg/l) and were acclimatized [15].

The tips of cristate lateral shoots of *Euphorbia pugniformis* were cultured on the MS + sucrose 2% and NAA 0.1 mgl-1 and BA, gave cristate shoots of both forms. While 20% gave normal shoots. The rooting combination for cristate shoots was MS+ IBA (0.5mgl-1), 95-100% of rooted shoots were acclimatized *ex vitro*. Quite a lot of cristates reverted into the normal form. Effect of MS nitrogen salts had a considerable effect on the cristate form shoot reversion [16].

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