Study Effect of Cytokinin Hormone Management on Shoot Length of Poinsettia under In-Vitro Condition

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ABSTRACT: In order to study the effects of cytokinin hormone management in vitro culture of poinsettia (Euphorbia pulcherrima wild), an experiment as factorial in RCBD with three replications was conducted during 2010 at Research Laboratory of Faculty of Agriculture, Lahijan University in Iran. Factors were cytokinin hormone types (A1= Adenin (An), A2= benzyladenine (BA), A3= Kinetin (Kn) and A4= 6-(γ,γ-dimethylallylamine)purine (2ip)), and Auxin hormones levels (L1=0.01, L2=0.1, L3=1 and L4=2, L5= 3, L6=4 and L7= 5 mg/L). The culture bed were contained MS, sucrose (3%) and agar (75%). The culture bed were contained MS, sucrose (3%) and agar (75%). The results show Interaction effect of cytokinin hormone types and cytokinin hormone levels on shoot length in vitro culture of poinsettia had a significant difference in 1%. Comparison of mean between interaction cytokinin hormone types and cytokinin hormone levels show that, the highest shoot length of poinsettia were obtained from C4L6 (26.23 mm) and C4L7 (26.93 mm) treatment (23.39 mm) and the lowest shoot length of poinsettia were obtained from C1L2 (1.67 mm) treatment.

Key words: Poinsettia, In vitro culture, Cytokinin types, Cytokinin levels, Shoot length.

INTRODUCTION

Several compounds, such as alkaloids, flavonoids, lignans, phenols, and terpenes with potential beneficial therapeutic action against hepatitis B, nefrolitiasis, and painful disorders have been isolated and identified from a great variety of Phyllanthus species (Calixto et al., 1998). The main constraint for plant production of many Phyllanthus species is the low seed germination rate and slow plant growth (Unander et al., 1995). Therefore, there is an urgent need to develop micropropagation, callus, and root culture systems to provide plant material to foster studies on plant production, phytochemical, and pharmacologic analysis and to investigate the application of biotechnologic approaches for germplasm conservation. According to the pharmacologic importance we have optimized in vitro culture systems of P. tenellus, P. corcovadensis, P. niruri, P. fraternus, P. caroliniensis, P. urinaria, and P. stipulatus(Catapan et al., 2002). Both in vitro and ex vitro morphogenic responses of different species are very much genotype-dependent. The pharmacologic screening carried out with callus extracts of these species demonstrates potent analgesic properties against neurogenic and inflammatory pain (Catapan et al., 2000). Sterols and triterpenes such as stigmasterol and glochidone are responsible for the antinociceptive effect (Krogh et al., 1999).

The family Euphorbiaceae comprises nearly 322 genera and 8910 species (Bingtao et al., 2008). 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 (Rajesh Kondamudi et al., 2008). Major components of Euphorbia latex are sterols, terpenoids vitamins and insecticides and anti cancer drugs (Rani et al., 2002; Martin et al., 2005).

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 (MS) (Murashige and skoog, 1962; Daniel Lineberger, 2009).
In vitro culture is one of the key tools of plant biotechnology that exploits the totipotency nature of plant cells, a concept proposed by Haberlandt (1902) and unequivocally demonstrated, for the first time, by Steward et al. (1985). Tissue culture is alternatively called cell, tissue and organ culture through in vitro condition (Debergh and Read, 1991). 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, micro propagation 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 (IAEA-TECDOC, 2004).

This study has been conducted to find the best cytokinin hormone types and cytokinin hormone levels on micro cuttings of poinsettia (Euphorbia pulcherrima wild) under in vitro condition.

**MATERIALS AND METHODS**

In order to study the effects of cytokinin hormone management in vitro culture of poinsettia (Euphorbia pulcherrima wild), an experiment as factorial in RCBD with three replications was conducted during 2010 at Research Laboratory of Faculty of Agriculture, Lahijan University in Iran. Factors were cytokinin hormone types (A1= Adenin (An), A2= benzyladenine (BA), A3= Kinetin (Kn) and A4= 6-(γ,γ-dimethylallylamine)purine (2ip)), and Auxin hormones levels (L1=0.01, L2=0.1, L3=1 and L4=2, L5= 3, L6=4 and L7= 5 mg/L). 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. Shoot tips 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 (Murashige and skoog, 1962) basal supplemented with different cytokinin hormone types and levels. PH was adjusted to 5.8 before adding 3% (w/v) sucrose and 75% (w/v) agar. After culturing the buds put in illuminated condition of 16 h light and 8 h darkness with temperature 25-27˚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.

**RESULTS AND DISCUSSION**

Results of variation analysis show that (Table 1), cytokinin hormone types on shoot length in vitro culture of poinsettia had a significant difference in 1 % probability level. The highest shoot length in micro cuttings of poinsettia was obtained with application 2ip hormone (14.76 mm) (Figure 1). The lowest shoot length in micro cuttings of poinsettia was obtained with application a hormone (3.30 mm) (Figure 1). Results of variation analysis show that (Table 1), cytokinin hormone levels on shoot length in vitro culture of poinsettia had a significant difference in 1 % probability level. The highest shoot length in micro cuttings of poinsettia was obtained with application 4 mg/L cytokinin hormone (14.87 mm) (Figure 2). The lowest shoot length in micro cuttings of poinsettia was obtained with application 0.1 mg/L cytokinin hormone (4.75 mm) (Figure 2). Interaction effect of cytokinin hormone types and cytokinin hormone levels on shoot length in vitro culture of poinsettia had a significant difference in 1% (Table 1). Comparison of mean between interaction cytokinin hormone types and cytokinin hormone levels show that (Figure 3), the highest shoot length of poinsettia were obtained from C4L6 (26.23 mm) and C4L7 (26.93 mm) treatment (23.39 mm) and the lowest root number of poinsettia were obtained from C1L2 (1.67 mm) treatment.

Micro propagation generally involves four distinct stages: initiation of cultures, shoot multiplication, rooting of in vitro grown shoots, and acclimatization. The first stage: culture initiation depends on explant type or the physiological stage of the donor plant at the time of excision. Explants from actively growing shoots generally used for mass scale multiplication. The second stage: shoot multiplication is crucial and achieved by using Plant Growth Regulators i.e. auxin and cytokinin. The third stage: the elongated shoots, derived from the multiplication stage, are subsequently rooted either ex vitro or in vitro. In some cases, the highest root induction occurs from excised shoots in the liquid medium when compared with semi-solid medium. The fourth stage: acclimatization of in vitro grown plants is an important step in micro propagation (Rajesh Kondamudi et al., 2009; Rani et al., 2002). 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 (Rout et al., 2006; Smart, 2008).

Nodes and shoots of *Mallotus repandus* cultured on MS + BA, 2ip (6- (γ, γ- dimethylallylamine) purine), Kn and BA (4.44μM) induced shoots, where as the roots were induced with NAA (32.23μM), the regenerated plants were acclimatized properly and then analyzed chemically to compare with mother plants (Kaewsawan et al., 2005). The nodal explants of *Euphorbia pulcherrima*, cultured on MS + NAA, 2,4-D, Kn and 2ip generated the embryogenic callus which on subculture to MS medium supplemented with 2ip (9.8μM) and NAA (0.54μM) gave somatic embryoids. The embryoids germinated successfully and were acclimatized with high survival rate (Jasrai et al., 2003). Results of study effect of 2ip hormone Concentrations (0, 0.31, 0.62, 1.25, 2.5 5 μM) In vitro culture of Phyllanthus stipulatus (Euphorbiaceae) show that, the highest shoot length of poinsettia was obtained with application 0.31 μM 2ip Concentration (Catapan et al., 2001).

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<th>Table 1. Analysis of variance effect of auxin hormone management of poinsettia under in vitro condition</th>
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<td><strong>Sours of variance</strong></td>
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** and * respectively significant in 1% and 5% area

![Figure 1. The effect of auxin hormone types on shoot length in vitro culture of poinsettia](image)
Figure 2. The effect of auxin hormone levels on shoot length in vitro culture of poinsettia

Figure 3. The effect of auxin hormone types and levels on shoot length in vitro culture of poinsettia

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Partial fulfillment of the requirements for the designation as UNDERGRADUATE RESEARCH SCHOLAR1-35.


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