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Evaluation Effect of Organs Culture of Poinsettia Under *In vitro* Condition

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Astract: In order to study the effects of organs culture (stem, leaf, half stem and petiol cutting) *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, IAA (0.1 mg/L), sucrose (3%) and agar (75%). The results show that organs culture on size of callus, bud differentiation and number of bud callus in poinsettia had a significant difference in 1 % probability level. The highest size of callus (1.27 mm), bud differentiation (22.88) and number of bud callus (1.29) in micro cuttings of poinsettia were obtained with application half stem cutting. The lowest size of callus, bud differentiation and number of bud callus in micro cuttings of poinsettia were obtained with application leaf and petiol cutting. Result of analysis of liner show that between size of callus, bud differentiation and number of bud callus in micro cuttings of poinsettia a positive and very significant correlation.

Key words: Poinsettia · *In vitro* culture · Organs culture · Callus

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].

This study has been conducted to find the best organs culture on poinsettia (Euphorbia pulcherrima wild) under *in vitro* condition.

MATERIALS AND METHODS

In order to study the effects of organs culture (stem, leaf, half stem and petiol cutting) *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 poinsettia of Ecks point

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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. organs culture (stem, leaf, half stem and petiol cutting) 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 0.1 mg/L indole-3-acetic acid (IAA). pH was adjusted to 5.8 before adding 3% (w/v) sucrose and 75% (w/v) agar. After culturing the erlens of explants were to gross chamber for cullus formation. The darkness and 22-25°C prepared in gross chamber.

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), organs culture on size of callus in poinsettia had a significant difference in 1 % probability level. The highest size of callus in poinsettia was obtained with application half stem cutting (1.27 mm) (Table 2). The lowest size of callus in poinsettia was obtained with application petiol cutting (Table 2). Results of variation analysis show that (Table 1), organs culture on bud differentiation of poinsettia had a significant difference in 1 % probability level. The highest bud differentiation of poinsettia was obtained with half stem cutting (22.88) (Table 2). The lowest bud differentiation of poinsettia were obtained with application leaf and petiol cutting (Table 2). Results of variation analysis show that (Table 1), organs culture on number of bud callus in poinsettia had a significant difference in 1 % probability level. The highest number of bud callus in poinsettia was obtained with half stem cutting (1.29) (Table 2). The lowest number of bud callus in poinsettia were obtained with application leaf and petiol cutting (Table 2). Result of analysis of liner (Table 3) show that between size of callus, bud differentiation and number of bud callus in poinsettia a positive and very significant correlation.

Micropropagation 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 are 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

Table 1: Analysis of variance effect of organs culture of poinsettia under *in vitro* condition

Sours of variance	DF	Size of Callus	Bud differentiation	Number of Bud Callus
Replication	3	0.045	0.86	0.005
Organs Culture	3	0.058**	465.42**	1.904**
Error	9	0.0001	0.417	0.002
C.V %		1.5	8.32	6.96

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

Table 2: Comparison of means effect of organs culture of poinsettia under *in vitro* condition

Treatment	Size of Callus (mm)	Bud differentiation	Number of Bud Callus
Stem Cutting	1.18B	8.16B	1.08B
Leaf Cutting	1.07C	0C	0C
Half Stem Cutting	1.27A	22.88A	1.29A
Petiol Cutting	0.99D	0C	0C

Means followed by the same letter in the same column are not significantly different at the 5% probability level by Duncan test

Table 3: Correlation of size of callus, bud differentiation and number of bud callus in poinsettia

Treatment	Size of Callus	Bud differentiation	Number of Bud Callus
Percent of Rooting	1		
Root Number	0.72**	1	
Root Length	0.74**	0.89**	1

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

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 [11, 12].

In vitro propagation through meristem culture is the best possible means of virus elimination and produces a large numbers of plants in a short span of time. It is a powerful tool for large-scale propagation of horticultural crops including pot plants. The term 'meristem culture' specifically means that a meristem with no leaf primordia or at most 1-2 leaf primordial which are excised and cultured. The pathway of regeneration undergoes several steps. Starting with an isolated explant, with de-differentiation followed by re-differentiation and organization into meristematic centres. Upon further induction the cells can form unipolar structures i.e. organogenesis, or bipolar structures called somatic embryogenesis. The organization into morphogenetic patterns can take place directly on the isolated explant or can be expressed only after callus formation, which is called indirect morphogenesis. When shoots are developed directly from leaf or stem explants it refers to direct morphogenesis. Micropropagation is an alternative method of vegetative propagation, which is well suited for the multiplication of elite clones. It is accomplished by several means, i.e., multiplication of shoots from different explants such as shoot tips or axillary buds or direct formation of adventitious shoots or somatic embryos from tissues, organs or zygotic embryos. The first significant use of plant tissue culture in ornamental was made during 1920s when orchid seeds were germinated under laboratory conditions [13]. Micropropagation 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 are 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 micropropagation [12].

The cell suspension obtained from the stem callus of *Euphorbia esula* on basal medium containing a reduced oxidized nitrogen ratio of 33:67 under the fluorescent lights. Roots and shoots formed and then acclimatized [14]. The organogenesis in *Euphorbia esula* was studied by Davis and Olson [15] IAA induced the roots very effectively, high concentrations of IAA, NAA, 2,4-D promoted callusing. High or low concentrations of picloram reduced the efficiency of rooting. Martin *et al.* [16] reported the influence of auxins in direct *in vitro* morphogenesis of mesophyll cells of *Euphorbia nivulia*, Kn reduced the rate of morphogenesis, where as BAP induced somatic embryogenesis. The combination of BA with NAA and IAA had positive effect on morphogenesis. IBA (13.3 μM) induced shooting and half MS + IBA (2.46μM) suitable for rooting; BA (4.44μM) +2,4-D (2.26μM) were optimum for somatic embryogenesis of proximal explant. GA3 supplementation to half MS medium resulted in the conversion of embryos into plantlets with survival rate of 90%.

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