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Evaluation Effect of Temperature Management on Rooting in Micro Cuttings of Poinsettia

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Astract: In order to study the effects of temperature levels (8, 10, 15, 20, 27 and 30°C) *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 temperature levels on percent of rooting, root number and root length in micro cuttings of poinsettia had a significant difference in 1 % probability level. The highest percent of rooting (100 %), root number (4.67) and root length (20.65 mm) in micro cuttings of poinsettia were obtained with application 27°C temperature. The lowest percent of rooting, root number and root length in micro cuttings of poinsettia were obtained with application 8°C and 10°C temperature. Result of analysis of liner (Table 3) show that between percent of rooting, root number and root length a positive and very significant correlation.

Key words: Poinsettia · *in vitro* culture · Temperature · Rooting

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 rooting in micro cuttings of poinsettia (Euphorbia pulcherrima wild) with application MS medium levels and IBA hormone levels.

MATERIALS AND METHODS

In order to study the effects of temperature levels (8, 10, 15, 20, 27 and 30°C) *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 cultivar was used for

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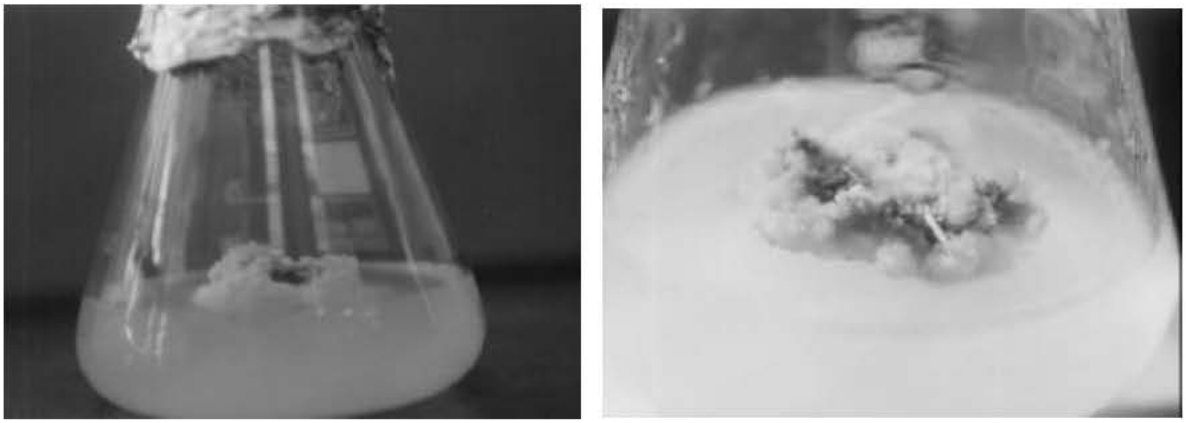

Fig. 1: Formation of shoot and root after *in vitro* culture on MS basal medium

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 5mg/L Indole-3-Butyric Acid (IBA). (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 photoperiod at temperature management (8, 10, 15, 20, 27 and 30°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), temperature levels on percent of rooting of poinsettia had a significant difference in 1 % probability level. The highest percent of rooting in micro cuttings of poinsettia was obtained with application 27°C temperature (100 %) (Table 2). The lowest percent of rooting in micro cuttings of poinsettia were obtained with application 8°C and 10°C temperature (Table 2). Results of variation analysis show that (Table 1), temperature levels on root number of poinsettia had a significant difference in 1 % probability level. The highest root number in micro cuttings of poinsettia were obtained with application 27°C (4.67) and 30°C temperature (4.78) (Table 2). The lowest root number in micro cuttings of poinsettia were obtained with application 8°C and 10°C temperature (Table 2). Results of variation analysis show that (Table 1), temperature levels on root length of poinsettia had a significant difference in 1 % probability level. The highest root length in micro cuttings of poinsettia was obtained with application 27°C temperature (20.65 mm) (Table 2). The lowest root length in micro cuttings of poinsettia were obtained with application 8°C and 10°C temperature (Table 2). Result of analysis of liner (Table 3) show that between percent of rooting, root number and root length 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 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]. Rooting the shoots produced *in vitro*, or micro cuttings, has been achieved through *in vitro* and *ex vitro*, or non-sterile, conditions [13]. In some cases, micro cuttings root better *in vitro* environments. *in vitro* rooting was superior to *ex vitro* rooting for *Prunus* x 'Hally Jolivette' [14]. Also, in some cases *in vitro*, it is beneficial to make changes to the medium.

Table 1: Analysis of variance effect of temperature levels on percent of rooting, root number and root length of poinsettia

Sours of variance	df	Percent of Rooting	Root Number	Root Length
Replication	3	65.746	0.119	2.387
Temperature levels	5	8289.542**	17.917**	350.895**
Error	15	7.422	0.016	0.314
C.V %		4.93	5.38	5.32

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

Table 2: Comparison of means effect of temperature levels on percent of rooting, root number and root length of poinsettia

Treatment	Percent of Rooting (%)	Root Number (-)	Root Length (mm)
Temperature= 8°C	0E	0D	0D
Temperature= 10°C	0E	0D	0D
Temperature= 15°C	56.39D	2.15C	7.16C
Temperature= 20°C	79.38C	2.51B	17.92B
Temperature= 27°C	100A	4.67A	20.65A
Temperature= 30°C	95.89B	4.78A	17.49B

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 percent of rooting, root number and root length

Treatment	Percent of Rooting	Root Number	Root Length
Percent of Rooting	1		
Root Number	0.96**	1	
Root Length	0.97**	0.92**	1

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

Dijkshoorn-Dekker [15] studied the influence of light and temperature on propagation profile of F. benjamina. Variability has been reported in different chrysanthemum cultivars through physical or chemical mutagenesis or low temperature tolerant mutants [16]. By induced mutations a wide range mutants can be isolated including abiotic and biotic stresses. Preil *et al.* [17] developed low temperature tolerant mutants of E. pulcherrima and Dendranthema from irradiated cell suspension cultures by using X-irradiation (15 and 20 Gy). Euphorbia mutants adapted better at low temperature in the greenhouse as compared to the parental cultivar. The Dendranthema mutants flowered 7-10 days earlier than the original variety. Most of the low-temperature tolerant mutants were obtained by single step selection procedure [18, 19]. Sakai [20] first reported the survival of plant tissues after exposure to ultra low temperature-196°C and the significance of using DMSO as a cryoprotectant [21]. Effect of altered temperature on plant regeneration frequencies in stamen culture of rubber trees was studied by Wang *et al.* [22]. Wang and Chen [23] studied the effects of temperature on stamen culture and somatic plant regeneration frequencies in stamen culture of rubber tree. The optimum temperature for the growth of endosperm reported as 25°C and the pH was 5.0 for *Ricinus communis* [24].

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