IN VITRO MICROPROPAGATION OF POINSETTIA (EUPHORBIA PULCHERRIMA WILLD.)

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ABSTRACT

A rapid, efficient and reproducible propagation protocol was developed for initiation, multiplication and root formation of poinsettia (Euphorbia pulcherrima Willd.) through the culture of single nodal segments on basal MS medium. The results showed that the best cultures were established on solid MS medium supplemented with (١,٠+٠,٥) from both BA and NAA respectively after being surface disinfested in ٧٠٪ ethyl alcohol for ٣٠ seconds, followed by immersion in mercuric chloride (٠,١٪) for five minutes. The best shoots multiplication was achieved with the combination treatment ١,٠+١,٠ mg/l of BA and NAA (٦,٥٦ shoots/ explant) as compared with the use of BA or Kinetin separately. The rooting percent (١٠٠٪) was recorded by adding ٠,٢ and ٠,٣ mg/l IBA. IBA performance was better than IAA in producing higher number of roots by giving ٦,٦٢ roots/ explant on shoots cultured on solid MS medium supplemented with ٠,٢ mg/l IBA. A high survival rate (٩٠٪) was found with poinsettia plantlets gradually acclimatized into the out-air conditions.

Key words: Poinsettia, Euphorbia pulcherrima Willd., micropropagation, in vitro

INTRODUCTION

Poinsettia plants (Euphorbia pulcherrima Willd) belong to the family Euphorbiaceous and the genus Euphorbia consisting of close to ٢٠٠٠ species (Ecke et al., ٢٠٠٤). In abroad, poinsettia is one of the most popular houseplants seen during the Christmas time. It has brilliant colored bracts ranging from scarlet, crimson, yellow to red and white. The ability of these spectacular bracts to remain fresh and intact for three to four months adds to its demand as an ornamental plant. This plant is native of Central America (Jasrai et al., ٢٠٠٣). It is considered as a fast growing plant and as one of the most common growing shrubs throughout the world. Global production of poinsettia has exceeded hundreds of millions and is still expanding, indicating it's economic and market potential for the floral industry (Clarke, ٢٠٠٨). In Iraq, poinsettia is considered as an outdoor ornamental plant.

The classical propagation methods of poinsettia are usually done by cuttings and seeds which have several limitations. Propagation by seeds is difficult since seeds lose their viability upon storage.

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Furthermore, plants originating from seeds have genetic variability which is not preferred in producing uniform plants for market demand. Propagation through cuttings has drawbacks because it is seasonal and cuttings take one to two months to form roots (Jasrai et al., ٢٠٠٣). So, these propagation methods of poinsettia failed to meet the high demand for quality plants during the winter holiday market.

Several studies on propagation of E. pulcherrima have been reported (Nataraja et al., ١٩٧٣; Marcos et al., ٢٠٠٨). For instance, Castellanos et al. (٢٠١٠) used stem nodal explants cultured on semi-solid MS basald medium supplemented with BAP and NAA to develop shoots from adventitious axillary buds after ٧ weeks of culture. Rooting of in vitro regenerated shoots was achieved in semi-solid MS-based medium containing IAA. This research aimed to develop an effective and efficient in vitro propagation and proliferation protocol for poinsettia. As well as for reduction in the cost of production and rapid multiplication and rooting by determining the most optimal types and concentrations of plant growth regulators as medium constituents which is one of the most important aspects of successful in vitro

culture technique. Rapid multiplication of elite clones, the production of pathogen-free plants and more rapid introduction of novel cultivars with desirable traits, represent important driving forces in the poinsettia industry. In recent years, different strategies have been adopted to micro propagate poinsettia, which could assist breeders to meet consumer demands (Marcos, ٢٠٠٨). The development of reliable in vitro regeneration procedures is likely to play a crucial role in future production systems.

MATERIALS AND METHODS

This investigation was carried out in Plant Tissue Culture laboratory of the Horticulture Department, School of Plant Production, Faculty of Agriculture and Forestry, University of Duhok, Iraq during the period from April, ٢٠١٠ to October, ٢٠١٠. Single nodal segments of poinsettia were excised from healthy plants grown in the greenhouse. The explants were surface disinfested by soaking them in ٧٠٪ ethyl alcohol for ٣٠ seconds, followed by mercuric chloride (٠,١٪) for five minutes. Finally, they were rinsed in sterile distilled water thrice for ٥ minutes. Nodes were inoculated into MS medium (Murashige and Skoog, ١٩٦٢) containing ١٠٠ mg/l inositol, ٣٠ g/l sucrose, ٧ g/l agar and supplemented with ٢ mg/l BA. The pH of the medium was adjusted to ٥,٧. Each culture test tube (١٨ cm X ٢٠٠ mm) containing ٢٠ ml of the medium, were autoclaved at a temperature ١٢١°C at ١,٠٥ kg/ $m^{٢}$ pressure for ٢٠ minutes.

Two weeks after the explants inoculation, the successfully established cultures were sub cultured into multiplication MS medium containing either BA at (٠, ٠,٥, ١,٠ and ١,٥) mg/l, Kinetin at (٠, ٠,٥, ١,٠ and ١,٥) mg/l or BA+NAA at (٠,٥+٠,٥, ١,٠+٠,٥, ١,٥+٠,٥, ٠,٥+١,٠, ١,٠+١,٠+ and ١,٥+١,٠) mg/l respectively. Another experiment was arranged at this stage to test the effects of the combination of sucrose and BA by investigating different combinations (٢٠ g/l sucrose+ ٠, ٠,٥ or ١,٠) mg/l BA and ٣٠ g/l sucrose(+ ٠, ٠,٥ or ١,٠ mg/l BA). Explants of ٠,٥ to ١ cm long were taken from the previous multiplication stage experiment.

The shoots produced at multiplication stage were sub cultured into MS rooting medium supplemented with either IBA or IAA at (٠, ٠,١, ٠,٢ and ٠,٣) mg/l separately. The cultures were incubated at ٢٥°C under ١٦ hrs daily exposures to ١٠٠٠ lux of cool white light. Observations for both multiplication and rooting stages were recorded after ٦ weeks of culture period.

The experiments were designed as a factorial complete randomized design (CRD). The comparison between means was carried out according to Duncan's multiple range test (P < ٠,٠٥). Finally, for acclimatization stage, a group of successfully rooted plantlets were removed from culture test tubes and their roots were washed with distilled water and immersed in Benlate fungicide (٠,١٪ for ١٠ minutes) (Toma, ٢٠٠٩). They were transferred to pots containing a steam sterilized soil mix (peatmoss+ loam+ Styrofoam ١:١:٠,٥, v:v:v) under tightly controlled atmosphere of the greenhouse.

RESULTS AND DISCUSSION

The disinfestations procedure adopted at the current study was highly effective and successful in producing healthy clean cultures at the initiation stage. Figure (١) shows the response percent of the cultured explants in MS medium supplemented with different growth regulators concentrations. The highest response (١٠٠٪) was achieved from the both combinations of BA+NAA, (١,٥+٠,٥ and ٠,٥+١,٠) mg/l respectively which were significantly higher than the rest of treatments except the both combinations of BA+NAA, (١,٠+٠,٥) and ١,٠+١,٠ mg/l. Since that, one can choose the lower significant concentration (١,٠+٠,٥) to reduce the costs of nutrient media as excessive accumulation of BA and NAA can be avoided. These results declared that auxin (NAA) together with cytokinin (BA), were involved in growth

initiation. Cell division seems to be regulated by the joint action of auxins and cytokinins, each of which appears to influence different phases of cell cycle and explants growth (George et al., ٢٠٠٨).

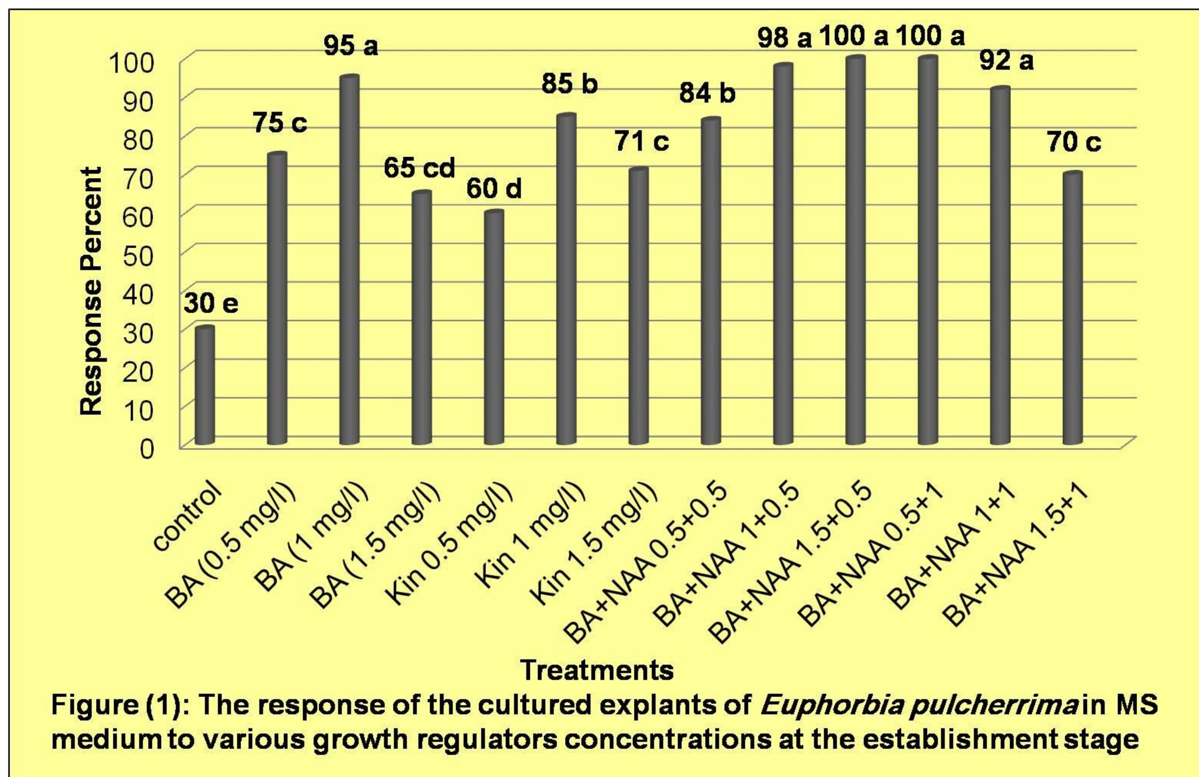

Figure (1): The response of the cultured explants of *Euphorbia pulcherrima* in MS medium to various growth regulators concentrations at the establishment stage

The successfully established explants were sub cultured into the multiplication stage to investigate the ability of different concentrations of BA and Kinetin and different combinations between BA and NAA to induce shoot multiplication of poinsettia explants. Table (١) shows that the highest number of shoots and leaves (٦,٥٦ shoots and ١٥,٦٨ leaves per explants) were recorded for the combination treatment between BA and NAA (١,٠+١,٠ mg/l) which were significantly higher the rest of treatments except the ١,٥+٠,٥ (BA+NAA) in the case of number of shoots. While the longest shoots (٥,١٢ cm) were found for ١,٠ mg/l BA treatment. The least multiplication parameters were recorded for the control treatment. These results are in agreement with those published by Castellanos et al. ;(٢٠١٠).

Table (١): The effects of different concentrations of BA, Kinetin and NAA in MS medium on the multiplication stage of Euphorbia pulcherrima explants after six weeks in culture

Treatments	Number of shoots/ explant*	Mean length of shoots (cm)	Number of leaves/ explant
Control	١,٦٢ f	٢,٠٢ e	٣,١٢ h
BA (mg/l)			

٠,٥	٣,١٠ e	٤,٢٨ b	٤,٦٦ g
١,٠	٤,٠٨ d	٥,١٢ a	٥.٤٨ f
١,٥	٣,٠٦ e	٣,٧٢ c	٤,٣٢ g
Kinetin (mg/l)			
٠,٥	٣,٦٠ d	٢,٦٨ d	٤,٨٠ g
١,٠	٣,٣٠ de	٤,١٦ b	٤,٥٠ g
١,٥	٢,٩٢ e	٣,١٨ c	٣,٤٤ h
BA+NAA (mg/l)			
٠,٥+٠,٥	٥,١٢ bc	٣,٤٨ c	١٣,٢٨ c
١,٠+٠,٥	٥,٨٠ b	٣,٨٢ b	١٤,١٦ b
١,٥+٠,٥	٦,٤٢ a	٤,١٢ b	١٣,٥٠ c
٠,٥+١,٠	٤,٣٠ d	٣.٤٢ c	١١,٢٤ de
١,٠+١,٠	٦,٥٦ a	٤,٠٨ bc	١٥,٦٨ a
١,٥+١,٠	٥,٤٤ b	٣,٥٢ c	١٢,٠٨ d

*Different letters within columns represent significant differences according to Duncan’s multiple range test at ٥٪ level.

In another test at the multiplication stage, different combinations between sucrose and BA were investigated. Table (٢) shows that the highest multiplication parameters (٧,٤ shoots/ explants, ٥,٧ cm as the longest mean of shoots and a ٩,٧٢ leaves/ explants) were recorded while adding ٣٠ g/l sucrose in combination with ١,٠ mg/l BA which were significantly higher as compared with other treatments. Generally, the in vitro growth and development increase with the increased sugar concentration (Goodwin, ١٩٦٦). Sucrose is usually added to culture media in plant tissue and cell culture as a source of energy to replace the carbon which the plant normally fixes from the atmosphere by photosynthesis, moreover, regulating the osmotic pressure in the nutrient medium (George, ٢٠٠٨). Cytokinin represented here by BA was effective in combination with sucrose. The reasons behind the positive role of BA on multiplication stage might be due to cytokinins great role in releasing lateral buds from the dominance of terminal buds without the need to remove the apical bud by promoting the formation of xylem tissues of buds that facilitate the transfer of water and nutrients leading to lateral bud growth (Mohammed and Al-Younis, ١٩٩١).

Table (٢): The effects of different combinations of sucrose and BA in MS medium on the multiplication stage of Euphorbia pulcherrima explants after six weeks in culture

Sucrose+ BA (mg/l)	Number of shoots/ explants*	Mean length of shoots (cm)	Number of leaves/ explants
Control	١,١٢ f	٠,٨٢ e	٢,٨٨ e
٢٠+٠	٣,١٨ e	١,٧٦ d	٧,٧٦ d
٢٠+٠,٥	٤,١٠ d	٣,٣٢ c	٨,١٠ d
٢٠+١	٥,٢٠ c	٤,٤٠ b	٨,٦٤ d
٣٠+٠	٤,٥٤ d	٣,١٠ c	٩,٧٢ c
٣٠+٠,٥	٦,٣٠ b	٥,١٠ ab	١١,٥٠ b
٣٠+١	٧,٤٠ a	٥,٧٠ a	١٩,٧٤ a

*Different letters within columns represent significant differences according to Duncan’s multiple range test at ٥٪ level.

At rooting stage of poinsettia micro propagation, both auxins IBA and IAA were investigated at different concentrations. Figure (٢) reveals that the highest rooting percentage (١٠٠٪) was recorded for both ٠,٢ and ٠,٣ mg/l IBA whereas the least rooting percentage (٦٦٪) was found for the control. The

addition of IAA at both ٠,١ and ٠,٢ mg/l also gave high rooting percentages estimated at ٩٦ and ٩٠٪ respectively.

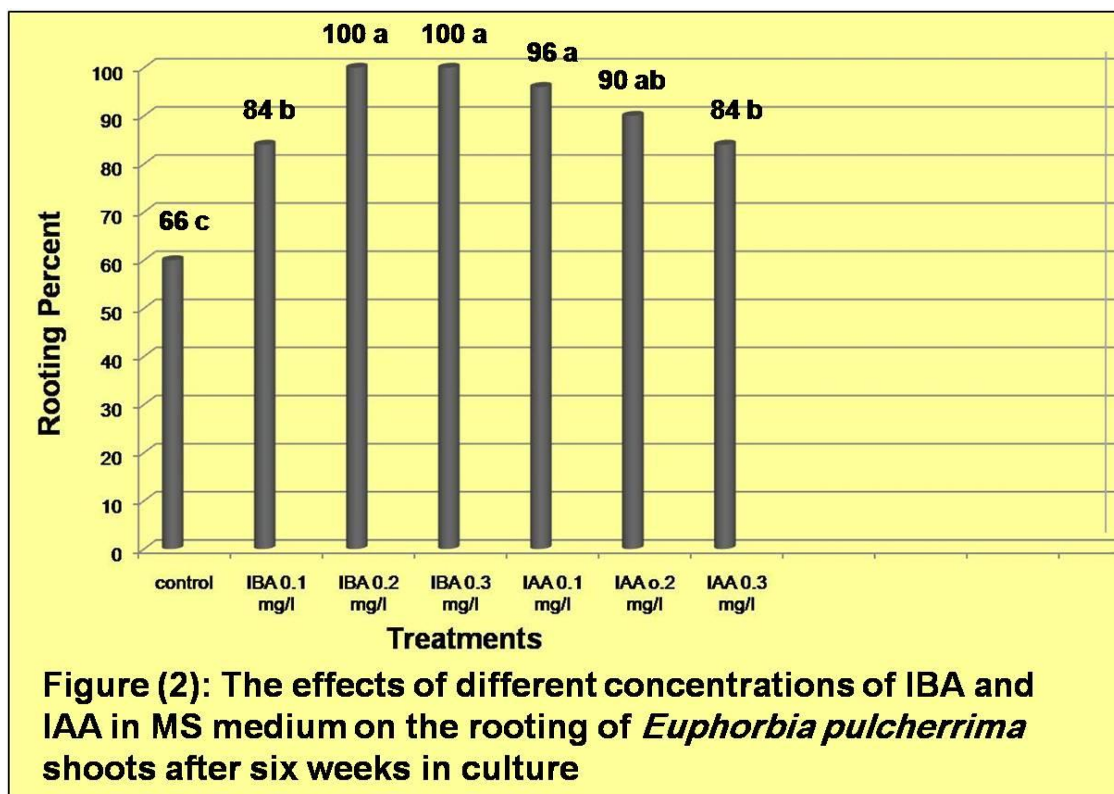

Figure (2): The effects of different concentrations of IBA and IAA in MS medium on the rooting of *Euphorbia pulcherrima* shoots after six weeks in culture

The results of the effects of different concentrations of IBA and IAA on the number of roots per explants and on the mean length of roots are shown in Table (٣). The highest number of roots per explants (٦,٦٢) was achieved while using ٠,٢ mg/l IBA which was significantly higher than the other treatments. Whereas the longest roots (٤,٤٦ cm) were found while adding ٠,٣ mg/l IBA which showed significant differences only when compared with the auxin-free treatment (control). It can be concluded here that ٠,٢ mg/l IBA was the best treatment among the tested concentrations.

Table (٣): The effects of addition IBA and IAA in MS medium on the rooting of Euphorbia pulcherrima shoots after six weeks in culture

Treatments (mg/l)		Number of roots/ explant*	Mean length of roots (cm)
Control		٣,٣٠ d	٢,٧٦ b
IBA	٠,١	٥,١٦ b	٣,٣٢ ab
	٠,٢	٦,٦٢ a	٣,٨٢ a
	٠,٣	٥,٤٤ b	٤,٤٦ a
IAA	٠,١	٣,٧٤ d	٣,٠٠ ab
	٠,٢	٤,٣٤ c	٣,٧٦ a
	٠,٣	٤,٠٦ c	٤,١٠ a

*Different letters within columns represent significant differences according to Duncan’s multiple range test at ٥٪ level.

Rooting results obtained in this investigation confirmed the need for auxins for poinsettia adventitious root formation. These results indicated that the presence of auxins had positive influences on rhizogenesis in poinsettia in vitro. In addition, the more effective auxin in the rooting was IBA followed

by IAA (Table, ٣). Such differences in the potency of auxin in inducing rooting might attributed to the structure of the auxins under study, the endogenous hormone level, as well as the genetic makeup of species under consideration (Karhu and Zimmerman, ١٩٩٣).

Figure (٣) shows the different developmental stages of poinsettia micro propagation including establishment stage (A), shoot multiplication stage (B), root formation stage (C) and acclimatization or hardening-off stage (D).

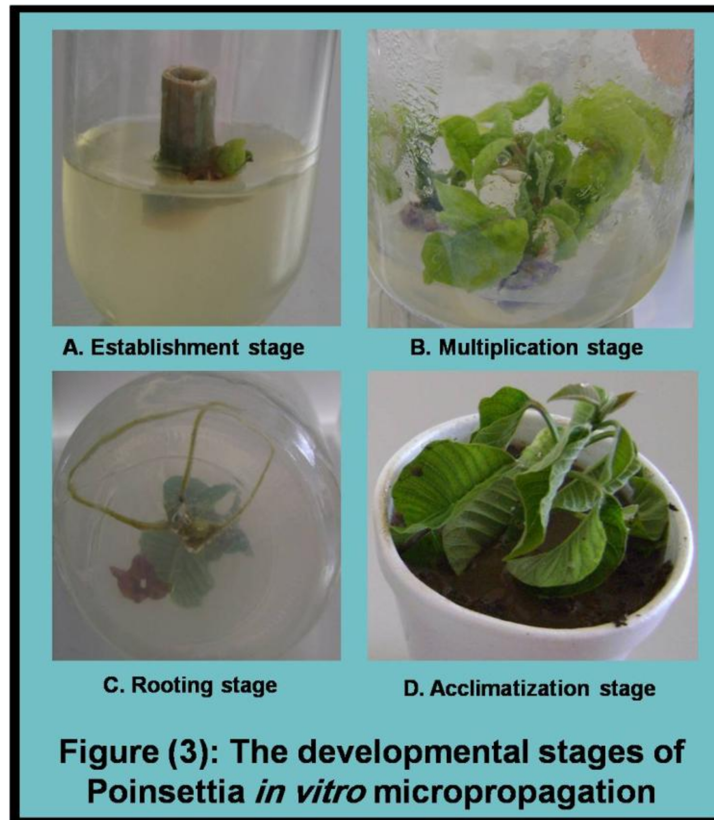

Figure (3): The developmental stages of Poinsettia *in vitro* micropropagation

Well developed plantlets were successfully transplanted to the out-air conditions with ٩٠٪ survival showing normal features without any morphological variation. The results achieved here, demonstrate successful and reproducible colonial propagation protocol of Euphorbia pulcherrima Willd. leading to commercial mass production.

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الإكثار الدقيق خارج الجسم الحي لنباتات بنت القنصل
(Euphorbia pulcherrima Willd.)

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الملخص

خلال هذه الدراسة تم التوصل إلى طريقة سريعة وكفوءة وقابلة للتطبيق للإكثار الدقيق لنباتات بنت القنصل Euphorbia pulcherrima Willd.) بوساطة زراعة عقد ساقية مفردة في الوسط الغذائي MS الصلب. أظهرت النتائج بأن أفضل المزارع النسيجية تم تثبيتها في الوسط الغذائي المزود بكل من BA و NAA بتركيز (٠،١+ ٠،٥) ملغم/ لتر على التوالي بعد تعقيم الأجزاء النباتية سطحياً بغمرها في محلول الكحول الأثيلي (٧٠%) لمدة ٣٠ ثانية ومن ثم في كلوريد الزئبق (٠،١%) لمدة خمس دقائق. أما أفضل تضاعف خضري للأفرع المزروعة حصل في معاملة التداخل بين (١،٠+) ١،٠ ملغم/ لتر من كل من BA و NAA عند مقارنتها مع استخدام كل من BA والكاينتين كلٌ على حدا. تحققت أعلى نسبة تجذير (١٠٠%) من خلال إضافة (٠،٢ و ٠،٣) ملغم/ لتر من IBA. كما وُجد إن أداء IBA كان أكثر كفاءة من IAA وخاصة في إعطاء أكبر عدد من الجذور مسجلاً ٦،٦٢ جذر/ جزء نباتي على الأفرع المزروعة في وسط MS الصلب المزود بـ٠،٢ ملغم/ لتر IBA. في مرحلة الأقلمة، بلغت نسبة بقاء الشتلات الناتجة ٩٠% عند نقلها إلى ظروف البيت الزجاجي.