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Somatic Embryogenesis and Plant Regeneration from Poinsettia (*Euphorbia pulcherrima* L.) Stem Explants

Hee-Sung Park†

Department of Plant Breeding, Catholic University of Taegu-Hyosung, Kyungsan, Kyungbuk 712-702, Korea

Abstract

Conditions for somatic embryogenesis and plant regeneration from stem tissues of *Euphorbia pulcherrima* were established. Explants from leaf, petiole, stem were examined for their embryogenesis on MS solid medium supplemented with plant growth hormones in combination at various concentrations. From leaf or petiole explants, callus was induced well but never proceeded to the embryonic stage in our expermental conditions. From stem explants, however, multiple shoots following callus induction emerged in about 6 to 8 weeks on MS agar medium supplemented with 1.5 mg/L of benzyladenine. Upon transfer, roots were developed on hormone-free MS solid medium.

Key words : *Euphorbia pulcherrima, stem tissue culture, somatic embryogenesis*

Introduction

Poinsettia (*Euphorbia pulcherrima* L.), as a multi-flowered ornamental plant, is very popular throughout the year, especially welcome during christmas season[1]. Attempts to establish new plants with improved characteristics have been continuously excercised to many economically valuable plant species by the method of traditional breeding or somatic embryogenic tissue culture. The latter is understood to be more convenient and easily practicable even though a disadvantage of genetic variation observed from *in vitro* cultured cells, tissues and regenerated plants shoud be overcome. Classical breeding efforts have stabilized new poinsettia cultivars especially in terms of varied colours of bracts (modified leaves) from red to white. For the improvement of poinsettia cultivars by tissue culture method, however, little has been reported so far ; this may be due to difficulties in direct somatic embryogenesis from poinsettia explants even though embryonic liquid culture using callus derived from shoot tips was reported for large-scale plant propagation and confirmed for their genetic stability[2,3]. For feasibility in this study, poinsettia regeneration via direct somatic embryogenesis has been tested for different parts of explants of poinsettia such as petiole, stem and leaf. Poinsettia was purchased from a local plant farm and maintained in the green house condition. For plant tissue culture, leaves-attached stem materials in length of 5 to 10 cm were collected in a beaker containing sterile water and left overnight for the removal of white milky sap from the cutting sites. The samples were vigorously agitated in detergent-added sterile water for 10 min and then washed with sterile water several times. For surface sterilization, the washed explants were immersed in 70% ethanol for 1 to 2 min, rinsed with sterile distilled water, immersed in 5% com-

† Corresponding author

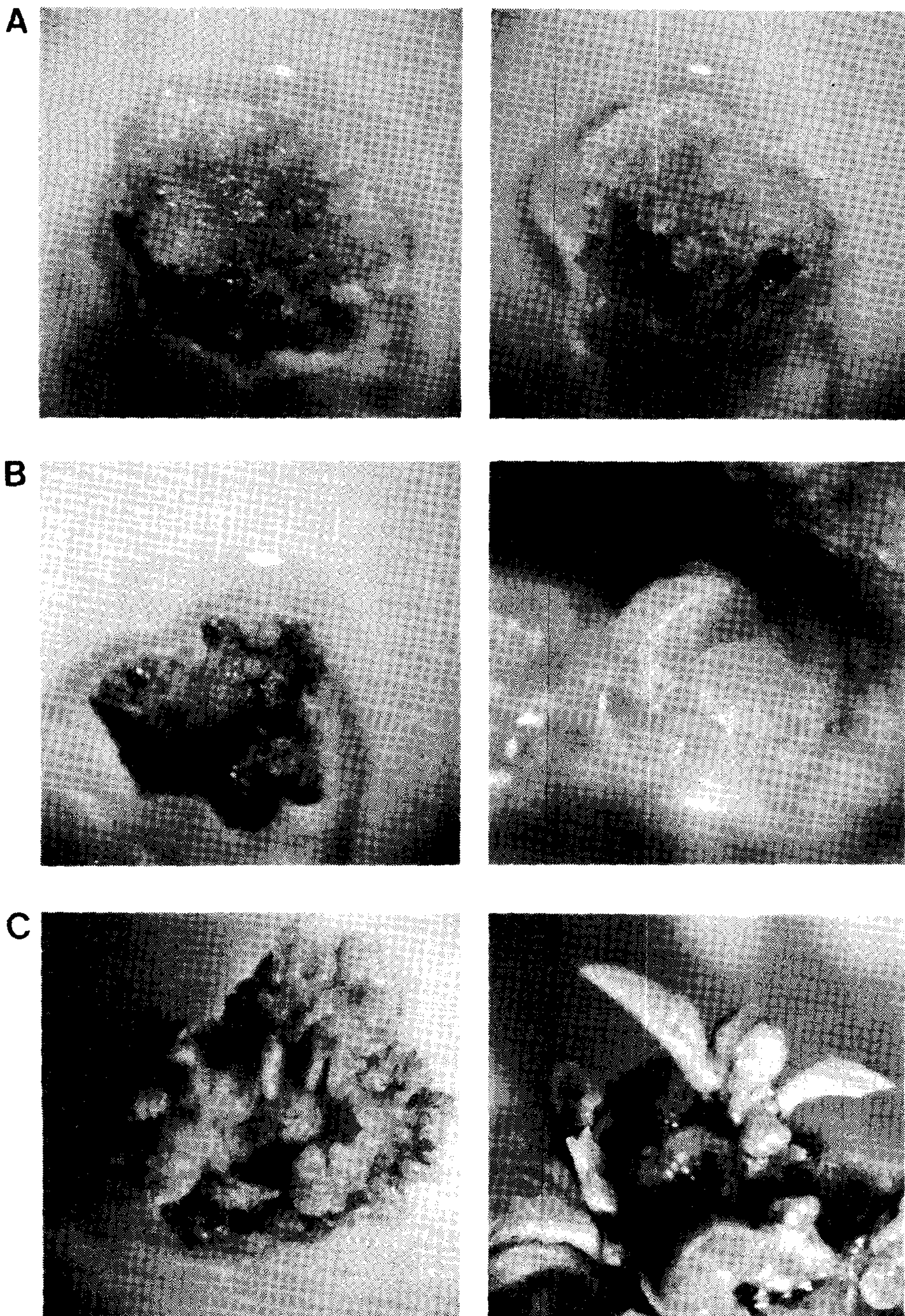

Fig. 1. Multiple shoot formation from poinsettia stem explants.

mercial bleach (Yuhanrox) solution for 15 min, and finally rinsed with steril water several times. The plant materials were cut into 5 mm-length tissues of leaf, stem from internode (SFI) or from nodal section (SFN), or petiole. These explants were tested for their somatic embryogenesis in the condition of MS agar medium[4] supplemented with plant growth hormones (PGH) such as benzyladenine (BA), 2-isopentenyl adenine (2-iP), 2, 4-dichlorophenoxyacetic acid (2,4-D), or naphthaleneacetic acid (NAA). As a cytokinin, BA or 2-iP was supplemented in the range from 1.0 to 2.5 mg/L ; as an auxin, 2,4-D or NAA in the range from 0 to 1.0 mg/L. PGH was combined to add to the MS medium as follows ; BA or 2-iP alone, BA+2,4-D or NAA, 2-iP+2,4-D or NAA. From most leaf, SFI or petiole explants, callus was formed well when supplied with high cytokinin (1.5−2.5 mg/L) in combination with low auxin (0.1−0.5 mg/L), but failed to show embryonic organogenesis during the period of following 16 to 20 week subculture. Hairy root structures could be observed if leaf explants were placed in the conditions supplied with low cytokinin (<1.5 mg/L) in combination with high auxin (>0.5 mg/L). Therefore, under the experimental conditions tested, simultaneous supplement of cytokinin and auxin was concluded to be undesirable for somatic embryogenesis of poinsettia explants. Next, BA or 2-iP alone (0.5−2.5 mg/L) was tested for each type of explants, and the best results could be obtained from stem explants treated with BA at the concentration of 1.5 mg/L. The result is shown in Fig. 1. Following callus emergence (Fig. 1-A) in about 4 to 6 weeks from stem explants, multiple embryonic structures (1-B) were emerged through 6 to 8 weeks, and finally developed into mature leaf structures (1-C). The frequency of shoot formation from stem explants was less than 5% of total number of stem explants tested. Other types of explants under the same PGH conditions never developed embryonic structures. SFN explants were usually shown to develop normal shoots due to their axillary buds under the condition of high cytokinin/low auxin or cytokinin alone in the media. Interestingly, callus induced under the condition supplemented with BA and 2,4-D (1.5 mg/L and 0.1 mg/L, respectively) was developed after 8 to 10 weeks into multiply-emerged embryo-like structures. This may suggest an alternatively practicable way for somatic embryogenesis.

In summary, the regenerated poinsettia plants were developed by tissue culture from stem explants on MS medium supplied with 1.5 mg/L of BA. Even though the regeneration frequency was not high, this established condition may first open an easy access to poinsettia breeding industry besides embryogenesis from shoot-tip derived suspension cultures[2,3].

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초록 : 포인세티아 줄기조직배양에 의한 재분화체 제조.

박희성†
(대구효성가톨릭대학교 식물육종학과)

포인세티아식물체의 줄기조직을 이용한 재분화조건을 확립하였다. MS배지에 종류 및 농도별 식물성장조절제를 첨가하여 포인세티아의 잎, 줄기, 엽병조직으로부터의 배구조의 발생을 조사하였다. 잎과 엽병조직에서는 callus의 형성은 실험조건에서 매우 활발하였으나 배구조로의 발달은 전혀 이루어지지 않았다. 줄기조직의 경우에서는 1.5 mg/L의 BA가 첨가되는 경우 6~8주 정도의 경과 후 엽초의 발생이 관찰되었다. 이들을 식물생장조절제를 무첨가한 MS고체배지로 이동시 뿌리의 발달이 관찰되었다.