Micropropagation Of Commercially Important Ornamental Plants

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ABSTRACT

Micropropagation is the practice of rapidly multiplying stock plant material to produce many progeny plants, using modern plant tissue culture methods. Micropropagation is used to multiply plants that have commercial importance on a large scale in cheaper cost. This technique is also used for those plants which have been genetically modified or bred through conventional plant breeding methods. It is also used to provide enough plantlets for planting from a stock plant which does not produce seeds or does not respond well to vegetative reproduction. Ornamental plants are always in demand on both national and international level due to their aesthetic beauty. In Pakistan, even many nurseries are importing ornamental plants like Orchids, Lily, Chrysanthemums, Anthurium, Song of Thai, Fern, Tricolor, Tulsi, Furcaria, Croton, Imported Aloe vera species, Rubber plant, Dracaena, Duranta and many plants which can be produced locally in lower price by establishing tissue culture and acclimatization facility. In department of biotechnology, Jinnah University for women we have won and successfully executed HEC-TDF project of Rs 9.918 million. The outcome of this project is the commercialization of three valued ornamental plants like Orchids, Lily and Chrysanthemums. The Plant tissue culture facility is now fully established and functional in the Jinnah University as it was demanded by HEC team in their visit. Facility is fulfilling the needs of graduate and post graduate practical's and thesis research. Other advantages include a continuous commercial chain for development of plants on demand either of agricultural or ornamental value.

INTRODUCTION

Micropropagation is used to multiply plants that have commercial importance on a large scale in cheaper cost. This technique is also used for those plants which have been genetically modified or bred through conventional plant breeding methods. It is also used to provide enough plantlets for planting from a stock plant which does not produce seeds or does not respond well to vegetative reproduction. In case of this project plant tissue culture technique can be effectively used to develop ornamental plants and sale them into the market to earn profit. This project had provided baseline facilities for plant tissue culture laboratory which is also helpful in graduate and postgraduate teaching and research.

Research and propagation of ornamental plants is a continuous process due to their commercial value. Orchids occupy top position among all flowering plants and are valued for cut flower production and as potted plants, often with a very high price in the international market. Among many reports of orchids, one is efficient in vitro plant regeneration protocol from protocorms of Dendrobium aqueum developed by (Selvaraju, P., 2015). Micropropagation of Dendrobium signatum Rchb.f was reported by (Rattana & Sangchanjiradet, 2017). An efficient protocol for propagation of Dendrobium aggregatum using the axenic immature seeds, derived from green capsule, was developed by (Vijaya, 2012), this study seems to be very practical because pods are collections of thousands of seeds which will germinate to form at least hundreds of plants. Methodology for development of orchid seed pods is reported many times like asymbiotic seed germination and in vitro

propagation of Cattleya trianae Linden & Reichb.f (Orchidaceae) by (Contreras, 2017). Anthurium (Anthurium spp.) is an ornamental that is widely appreciated around the world, primarily for its showy and colorful spadix. Many studies are available on micropropagation of anthurium from different explants like (Jaime, 2015). A review summarizes all the research that has been conducted with ornamental ferns using in vitro techniques at different phases in their life cycle, starting from spores, to gametophytes and sporophytes (Ferna´ndez, 2003) and in vitro propagation of bird's nest fern (asplenium nidus) from spores by (Khan, 2008). Lilium is one of the leading cut flowers all over the world. It ranks seventh among the cut flowers in the world in terms of auction turnover, and is a very popular pot plant as well; the study was conducted by (Khan, 2010) on cultivation of lilies (Lilium regale) from healthy, juvenile bulbs. Croton (Codiaeum variegatum) with its amazing colors and leathery leaves is regarded as a beautiful foliage plant. The report was published on in vitro micropropagation of croton by (Nasib, 2007).Callus culture of Chrysanthemum is also reported by (Nasri et al.,2018).

Dieffenbachia species are popular foliage potted plants used in interior escapes of homes, offices, and malls throughout the world. In vitro micropropagation of Dieffenbachia plant was reviewed by (Elsheikh, 2013). In recent years, propagation of numerous ornamental plants by tissue culture has become an accepted commercial practice. Current production techniques for In vitro propagation of plants have allowed for strong and continued growth within the micropropagation industry. This project is based on the plant tissue culture facility development, production and commercializing of commercially important ornamental plants like Orchids, Lily and Chrysanthemums. Green houses are agreed to purchase Jinnah University plants and sale them to consumers.

The optimization of White Chrysanthemum rooting, shooting and acclimatization is discussed here.

MATERIAL AND METHODS

Plant Material

Mother plant of white chrysanthemum was purchased from commercial nursery and kept in the green house of Department of Biotechnology, Jinnah University for Women, Karachi, Pakistan. Nutrients and watering was done on time for vigor growth and health.

Initiation of explants

Internodal segments were employed as the starting medium for the micro propagation. Explants were cleaned by liquid detergent (commercial max liquid) at least five minutes followed by washing with running tap water. Further sterilization was achieved with 20% bleach treatment for 20 min. Thereafter, the 2-3 cm long segments of inter-nodal were subjected to thorough washing (3-4 times) under laminar flow cabinet by using autoclaved distilled water to vanish the remains of sterilant. For culture initiation, purified explants were inoculated on ½ MS medium (Murashige & Skoog, 1962).

Culture multiplication and rooting

After 25 days of initiation, shoots of chrysanthemum were separated from initiated cultures and transferred on growth regulator half strength MS basal medium along with different concentrations of BAP 0, 1, 2, 3, 4 mg/l for *in vitro* multiplication. Rooting was easily obtained on ½ MS media along with 0.5g/l activated charcoal in 25 days. The cultures were maintained in controlled condition at 25±2 C^{o} under 16/8 hrs (light/dark) photoperiod for the one month.

Acclimatization

For acclimatization *in-vitro* grown plantlets were shifted in-to polythene bags filled with sandy soil without any fertilizer. The root and shoot developed plantlets were shifted to green house in pots containing 1:2 combination of farm yard manure and garden soil.

RESULTS

Initiation and multiplication

After successful initiation of chrysanthemum on ½ MS medium, shoots and leaves were grown (Fig **1**. A and B). The medium containing 4mg/l BAP was highly significant ($p = 0.0000221$) with highest number of shoots (6) as compared to the other medium combinations (Graph **1**). While the highest number of leaves (19) were observed on medium containing 3mg/l BAP having significance level of ($p = 0.1461$) as shown in (Graph **2**). Whereas medium containing 1mg/l BAP revealed statistically highly notable ($p = 0.000414166$) highest length of shoots (4.7cm) were differentiate to the other medium combinations as shown in (Graph **3**).

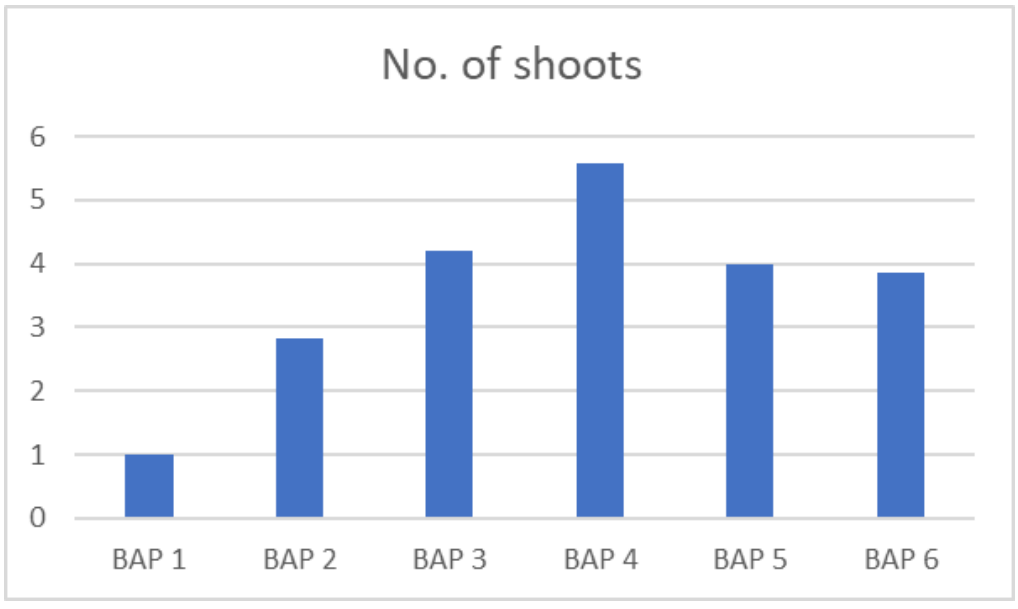
Acclimatization

The white chrysanthemum (Dendranthema × grandiflorum) plants were successfully acclimatized in the green house.

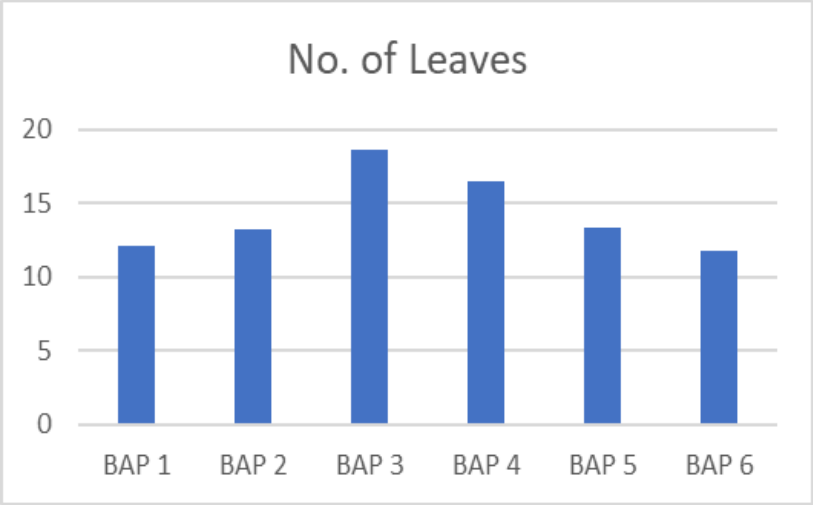
DISCUSSION

In this study, a protocol was established to determine the optimum growth of chrysanthemum in less time period with less expenditure. For this purpose, internodal segments of mother plant were selected as a source material for the micro propagation. Different concentrations of BAP were employed in ½ MS media for shoot and leaves regeneration. The shoot length was also notably affected by different concentrations of phytohormones. Axillary shoot growth and proliferation from mature plant's nodal segments and in vitro upraised shoots initiation were exceptionally controlled by different sorts and concentrations of BAP used. Between varying concentrations, ideal retaliation towards shoot proliferation, highest no of shoots were obtained on ½ MS media along with 4.0mg/l BAP (Fig 1. A,B) (Graph 1). Highest mean number of leaves were achieved on ½ MS media with 3.0 mg/l BAP (Graph 2). During working process of multiple shoot regeneration for chrysanthemum (Fig 1. C). For nodal explant, in a superior of axillary shoot proliferation was obtained on medium carrying 1.0 mg/l of the cytokinin (BAP) (Graph 3). BAP was proved to be most effective cytokinin. Previously reported that ½ MS media amplified with BAP was sufficient for several species and vascular plant species for in vitro propagation. Precedence of BAP over further cytokinins in constructing in vitro shoots has also been reported. Other plants like Rosmarinus officinalis, Arachis hypogaea and Atropa beladona (Misra & Chaturvedi, 1984; Mhatre et al., 1984; Imtiaz et al., 2019). The present study results indicate that using internodal segment of explant is good to the multiplication of Chrysanthemum at length on large scale with ½ MS medium boost with BAP. Optimum concentrations of BAP gave potential growth of chrysanthemum as compare to higher concentration which is responsible to decreased regeneration rate. (Waseem et al. 2009; Karim et al., 2002). After getting this successful multiple propagation results of chrysanthemum on short time of period. Acclimatization process was completed in one month (Fig 1. F). Plants were transferred from the lab to soil and used micro nutrient spray of MS (Murashige & Skoog, 1962) time to time after one month of acclimatization process to provide the continuous flourish growth of plants. Therefore, it is very important to optimize the culture growth and development environment for successful propagation (Zhen et al., 2015). In contrast the other findings, It is concluded from the present findings of study that optimised protocol can be used for rapid profit-oriented propagation of Chrysanthemum to get overseas trade and also to meet the needs of regional demand with least investments.

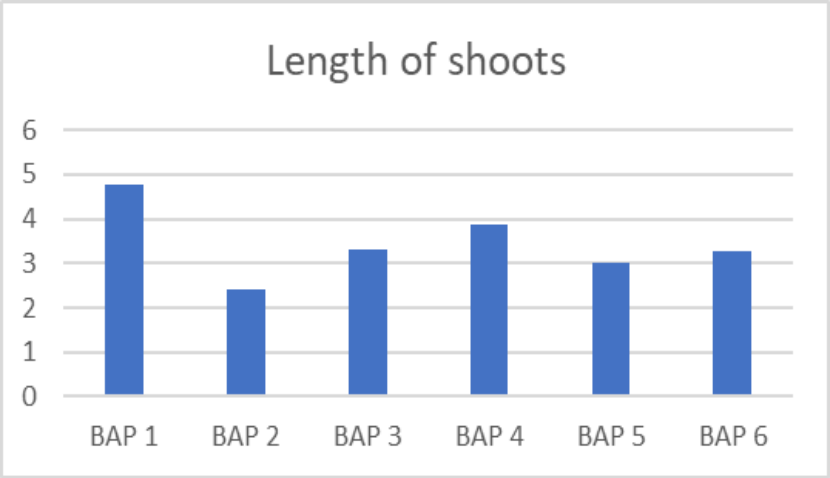
Figure1. (A). Shoots initiation of chrysanthemum after 25 days. (B). Growth of shoots. (C). Multiplication of chrysanthemum shoots. (D). Growth of roots on root initiation medium incorporates of ½ MS medium along with activated charcoal. (E). Multiplication of chrysanthemum along with rooting. (F). Acclimatization stage of chrysanthemum plantlets.



Graph1. In vitro shoot proliferation with highest number of shoots growth from the nodal explants of Dendranthema × grandiflorum on ½ MS medium with combination of cytokinin after 4-8 weeks.



Graph 2. In vitro shoot proliferation with highest number of leaves growth from the nodal explants of Dendranthema × grandiflorum on ½ MS medium with combination of cytokinin after 4-8 weeks.



Graph3. In vitro shoot proliferation with highest length of shoots growth from the nodal explants of Dendranthema × grandiflorum on ½ MS medium with combination of cytokinin after 4-8 weeks.

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