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Cultivation Experiment: Take Dendranthema × Grandiflorum as an Example

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Abstract

Chrysanthemum is locally known as 'Gul e Daoudi' . It is an ornamental plant used both as a cut flower and potted plant for its aesthetic beauty and long lasting presence. A biotechnological susbtitute to produce large quantities of healthy plants in a less time duration is in-vitro micropropagation of healthy explants and meristems into disease free and identical progenies. In this study shoot multiplication was attained on optimized medium. Proliferation of axillary shoots and growth was obtained from the nodal portion of the mature plants in vitro. MS medium along with different concentrations of the BAP was tested. Best response towards highest mean number of shoots proliferation was achieved on ½ MS media along with 4.0mg/l BAP. Highest mean length of shoots was recorded on ½ MS media containing 1mg/l BAP. Highest mean number of leaves were produced on ½ MS media along with 3.0 mg/l BAP. Rooting was easily achieved on simple ½ MS media. The present research verified large scale production of highly demanded purple chrysanthemum ornamental plants with high commercial value through cost effective method.

Keywords

Chrysanthemum Grandiflorum; BAP; Multiplication; ½ MS Media; Cost Effective; In Vitro Axiallry Root.

1. Introduction

Floriculture has attained great interest and recognition in the global market and has marked a robust annual growth rate of 7% in 2019 (Sánchez & Antequera, 2017). Global market of cut flowers, pot plants and ornamental plants is increasing gradually. Flowers are nature's best gift, which are used in our daily life occasions and now becoming as a fast emerging business. Currently horticulture industry have became as one of the most popular business and growing as commercial and financial livelihood in agriculture. A massive extent of cut flowers and house plants are sold on daily basis over the world. Inside the conditions of contruction value Netherlands, Japan, Italy, Germany and Canada are the substantial producers and traders of cut flowers and ornamental plants while Germany, US, France and UK are the primary buyers. It has underlying the traditional field crop cultivation due to increased units per return (Shamoon et al., 2020).

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The word chrysanthemum is arised from the Greek words 'chrysos' (gold) and 'anthemon' or 'anthos' (flower) (Anderson, 2007). There are two types of chrysanthemum viz, spray type and standard type. Standard type of chrysanthemum is usually grown for the production of cut flower as well as ornamental flowering plants for presentation and decoration. The spray type of chrysanthemums has genetic incapability and mostly grown for loose flower production. Flowers of spray type varieties are mostly suitable for making vases, garlands, and bracelets, as well as for glorifying purpose. They are available in a wide variety of shapes and sizes, with several flower colors including yellow, pink, purple, green, red, and, along with many other colors (Rao et al., 2015).Chrysanthemum is a genus includes about 41 species, and are largely found in East Asia, mainly in China, Korea, and Japan. China is thought to be the center of species diversity. This also contributes to high regional demand for cut flowers. Increasing governments interest in the adoption of floriculture as an industry will result in rapid growth of the floriculture market, and results in increasing demand for cut flowers and ornamental plants (Sharma & Chauhan, 2008). Chrysanthemum is also known as a 'Gul e Daoudi' is an ornamental plant used both as a cut flower and potted plant for its aesthetic and long lasting presence. Chrysanthemum (Chrysanthemum sp.) is associated to Asteraceae family which is a highly ravishing and appealing blooming plant, having above 2000 members globally reported (Singh, 2017). Vegetative propagation of Chrysanthemum is carried out using individual stems and shoots which is time consuming and prone to environmental factors and diseases. This refrains from timely fulfilment of floriculture demand of cut flowers like Chrysanthemums (Guillard et al., 2018). A biotechnological susbtitute to get large quantities of healthy plants in less time is the in-vitro isolation and micropropagation of healthy explants and meristems into disease free and identical progenies. Now a days tissue culture is an essential tool for creating new chrysanthemum cultivars on large scale without any effect of external environment in limited time and space (Jamal Uddin et al., 2015). Chrysanthemums that have the abilities to grow in rockwool with required nutrients 1 or 3 times/day produced potential developement (Kishimoto et al., 2004). Mutation breeding is the most common type of chrysanthemum breeding and it has a positive impact in chrysanthemum biotechnology (Vishnevetsky & Meyerowitz, 2002). Common practice for propagation of suckers and terminal cuttings, inappropriate for wide scale production of Chrysanthemums. Plant tissue culture technology can assist farmers due to its assurance of season-independent mass production of the selected variety. This technique is so far utilized to study large extent propagation of Chrysanthemums through distinct renwel progression (Purnobasuki et al., 2014).

The researchers are investigating with having practical approaches to produce Chrysanthemums of choice with least investments (Minas, 2007; Sahu & Sahu, 2013). As a photosensitive crop, Chrysanthemum essentially requires long days for vegetative growth and short days for flowering. Flowering and growth of chrysanthemums are largely controlled by light and temperature. Hence, the performance of genotypes depends on region, weather and growing orders (Thakur et al., 2018). Consequently, increasing the demands there is a need to produce Chrysanthemum plants having worthwhile system wholesale production where the growers can purchase the variety of their option with least expenditures, hence guarantee good income to the farmers. Regarding other reports for in vitro regeneration of Chrysanthemums from different explants via organogenesis (Rao et al., 2015; Purnobasuki et al., 2014; Murashige & Skoog, 1962). somatic embryogenesis (Thompson & Wang, 2002; Imtiaz et al., 2019) and callus culture has been reopted sucessfully. (Kishimoto et al., 2004; Misra & Chaturvedi, 1984). On the other hand tissue culture is also called cell, tissue and organ culture through in vitro condition. In most studies, direct and sustained shoot formation was recorded on MS medium supplemented with high concentrations of phytoharmones (cytokinin and auxin). It has been studied that differnces in concentrations have vital effects on the growth of crysanthemum, lower concentration of auxin is suitable for shoot regeneration, on the other hand higher

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concentration could easily produce calli but shoots formation was very poor (Song et al., 2011). Previous findings attributed that, increasing the concentration of BAP from 2 to 4mg/l resulted in decreased average number of shoots and the mean shoot length (Waseem et al., 2011). This deterrent result has been revealed that undesirable embryogenic effect of BAP on protein synthesis (van Staden & Crouch, 1996). This research seeks to explore responses of culture and media conditions accompanied with varied cytokinin concentrations to optimize an effective and reliable protocol for in vitro initiation and multiplication of Chrysanthemums, to produce disease free, healthy, and homogeneous plants to target socio-economic benefits. The aim of the current research was to evolve a protocol for least cost micropropagation of a speculative valuable artistic Chrysanthemum that will give vast production with minimal economic cost.

2. Material and Methods

2.1. Plant Material

The mother plant of purple chrysanthemum was purchased from a commercial nursery and kept in the greenhouse of Fuping pilot Base. Nutrition and watering are done on time to keep the plants thriving and healthy.

2.2. Initiation of Explants

Internodal segments were employed as the starting medium for the micro propagation. Explants were cleaned by liquid detergent (commercial max liquid) atleast five minutes followed by washing with runing tap water. Further sterilization was achieved with 20% bleach treatment for 20 min. Thereafter, 2-3 cm long inter-nodal segments 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).

2.3. Culture Multiplication and Rooting

After 25 days of initiation, shoots of chrysanthemum were separated from initiated cultures and internodal segments were transferred on 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 Cº under 16/8 hrs (light/dark) photoperiod for one month.

2.4. Acclimatization

For acclimatization in-vitro grown plantlets were shifted in-to polythene bags filled with sandy soil without any fertilizer. After one month plantlets with developed roots and shoots were shifted to the green house in pots containing 1:2 combination of farm yard manure and garden soil.

3. Results

3.1. Initiation and Multiplication:

After successful initiation of chrysanthemum on ½ MS medium (Fig 1. A and B). The medium containing 4mg/l BAP gave highly significant ($p = 0.00002$) highest number of shoots (6) as compare to the other medium combinations (Table 1). While the highest number of leaves (19) were observed on medium containing 3mg/l BAP having insignificance level of ($p = 0.1461$) as shown in (Table 2). Whereas medium containing 1mg/l BAP revealed statistically highly significant ($p = 0.0004$) highest length of shoots (4.7cm) were differentiate to the other medium combinations as shown in (Table 3).

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Table 1. In vitro shoot proliferation with highest number of shoots growth from the nodal explant of Dendranthema × grandiflorum on ½ MS medium with combination of cytokinin after 4-8 weeks.

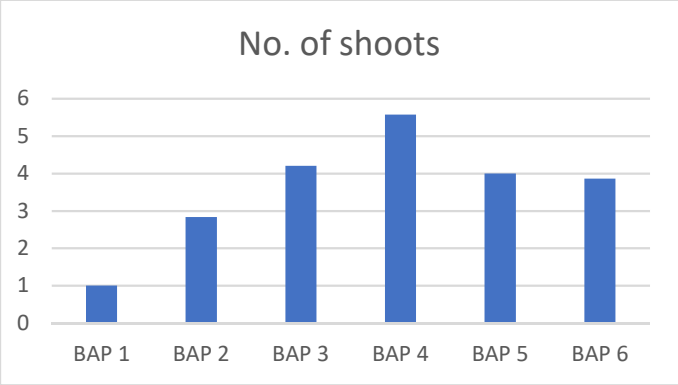


Table 2. In vitro shoot proliferation with highest number of leaves growth from the nodal explant of Dendranthema × grandiflorum on ½ MS medium with combination of cytokinin after 4-8 weeks.

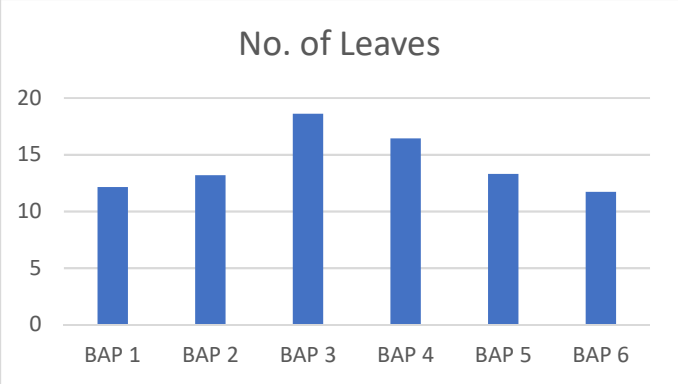
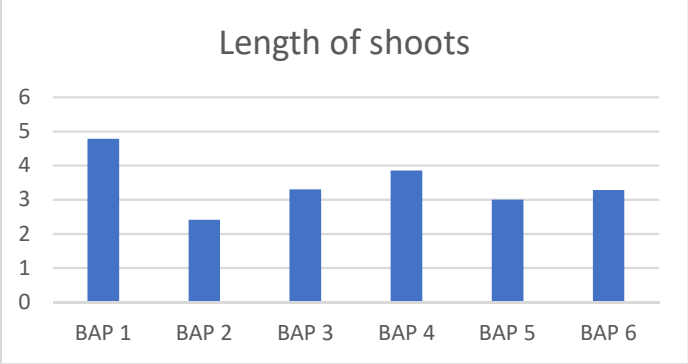


Table 3. In vitro shoot proliferation with highest length of shoots growth from the nodal explant of Dendranthema × grandiflorum on ½ MS medium with combination of cytokinin after 4-8 weeks.



3.2. Acclimatization:

The purple crysanthemum (Dendranthema × grandiflorum) plants were successfully acclimatized in the green house. The potting mix contained 1:2 ratio of farmyard manure with garden soil (Fig 1.G).

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Figure 1. (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 incorporate of ½ MS medium along with activated charcoal. (E) Multiplication of chrysanthemum along with rooting. (F) Acclimatization stage of chrysanthemum plantlets.(G) Gown up flowering purple chrysanthemum plant.

4. Discussion

In this study, a protocol was established to determine the optimum growth of chrysanthemum in less time period with less expenditure of resources. 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 diffrent sorts and concentrations of BAP used. Among 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) (Table 1). Highest mean number of leaves were achieved on ½ MS media with 3.0 mg/l BAP (Table 2). During the process of multiple shoot regeneration of 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) (Table 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 for the development of in vitro shoots has also been reported like other plants like Rosmarinus officinalis, Arachis hypogaea and Atropa beladona ((Misra & Chaturvedi, 1984; Mhatre et al., 1984; Imtiaz et al., 2019). Results of he present study indicates that using internodal segment of explant is good for the multiplication of Chrysanthemum in length on large scale with ½ MS medium boost with BAP. Optimum concentrations of BAP gave potential growth of crysanthemums as comprae to higher concentration which is responsbile to decreased regeneration rate (Waseem et al. 2009; Karim et al., 2002). After getting this successful multiple propagation results of chrysanthemum in short time 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. It is particularly important to optimize the culture growth and development environment for successful propagation (Zhen et al., 2015). It is concluded from the present study findings that optimised protocol can be used for rapid profit-oriented propagation of Chrysanthemums to get overseas trade and also to meet the needs of regional demand with least investments.

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