**In Vitro Propagation of Chrysanthemum: an Overview on its Utility in Mutagenesis and Genetic Transformation Techniques**

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Submission: March 29, 2018; Published: April 25, 2018

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### Abstract

Chrysanthemum morifolium within Asteracea family which is one of the most important cut flower and pot species. Callus tissue is genetically unstable and regeneration via this tissue is associated with the occurrence of somaclonal variation or chimera structure. The use of ray florets and leafy tissues for the in vitro propagation of *C. morifolium* for shoot regeneration (Direct and indirect) or somatic embryogenesis is of great importance for the mutagenic breeding programs and genetic transformation of this species. Cytokinin and auxin hormones have been effective in shoot regeneration and creating calluses in *C. morifolium* species. The ability to regenerate shoots from callus is essential for establishing a successful plant culture system as well as in vitro mutagenesis. In this mini review, induction of embryogenic callus and direct organogenesis and importance of these methods in mutagenesis and genetic transformation are described.

**Keywords:** Tissue culture; Embryogenic callus; Organogenesis; Mutation; Somaclonal variation

**Abbreviations:** PGRs: Plant Growth Regulators; BAP: N6-benzylaminopurine; NAA: a-Naphthalene Acetic Acid; IBA: Indole-3-Butryic Acid; IAA: Indole Acetic Acid; 2,4-D: 2,4 Dichloro Phenoxo Acetic Acid; MS: Murashige and Skoog (1'92) Medium

### Introduction

*Chrysanthemum morifolium* species belong to the Asteraceae family and is one of the most important cut flower and pot species on the market that traditionally propagated by root suckers or terminal cuttings [1]. These methods are very slow processes, and the risk of transmission of the virus and other diseases is very high [2]. Tissue culture techniques can improve the efficiency of plant propagation processes and as well as facilitates the rapid replication and development of superior genotypes [3]. There are numerous reports on the in vitro regeneration of chrysanthemum, which chiefly have been regenerated through the passage of the callus phase [4-6].

Previous studies have examined the impact of various Chrysanthemum explant types such as leaf [7], pedicle [8], protoplast [9], shoot bud [10] and stem [11] on callus induction and organogenesis. The regeneration of shoots by organogenesis is one of the main methods *in vitro* propagation of chrysanthemum and many other plant species [7,9,11]. Efficient direct organogenesis of Chrysanthemum using leaf explants was achieved using a higher concentration of cytokinin than of auxin [12,13]. It seemed that the endogenous level of cytokines contained in leaves of Chrysanthemum is too low to induce shoot regeneration. D’jong et al. [14] reported regeneration of adventitious shoots from leaves of *in vitro* grown chrysanthemum (*Dendranthema grandiflora* Tzvel.) without passing through the callus phase.

Production of regenerated plant through indirect organogenesis is one possible method to help to Chrysanthemum genetically modified (Figure 1). Furthermore, callus production is also a useful tool in genetic improvement of Chrysanthemum species in order to introduce useful genes or producing new cultivars [6,15].

Regeneration efficiency in chrysanthemum is reacted by the PGRs interaction, plant genotype and types of explant [16,17]. Diversity in adventitious shoot regeneration of various cultivars
has been reported in chrysanthemum cultivars [4-5,9,18,19]. Song JY, et al. [5] reported significant differences in percentage of regeneration among various cultivars when grown on media supplemented with different concentrations of auxin and cytokinin. Likewise, a cytokinin and auxin combination has been reported to be important for efficient shoot organogenesis for chrysanthemum [10,20,21].

In most studies, direct shoot formation were observed in MS medium supplemented with high concentrations of cytokinin and lower of auxin. Direct organogenesis requires the re-initiation of cell division by the use of PGRS [22]. Waseem K, et al. [23] reported that the lower concentration of auxin is apt for shoot regeneration in chrysanthemum. Against, concentrations of higher could easily produce calli but shoots formation is very poor. Based on previous studies, increasing the concentration of BAP from 2 to 4mg L-1 resulted in a decrease in the average number of shoots and the mean shoot length. This disincentive effect has been attributed to a Embryogenic undesirable influence of BAP on protein synthesis [24].

**Callus induction**

In previous studies, the explants were unable to produce callus on MS medium without growth regulators. Callus induction was observed only when the MS medium containing auxin compounds (2,4-D, NAA, IBA and IAA) was prepared alone in combination with cytokinin [1,2,5,15,16,25]. Therefore, adding the auxin compounds to the medium is essential for callus induction [26,27]. Both cytokinin and auxin have been effective in creating calluses in chrysanthemum species (Figure 1). The rate of callus production increased with increasing concentration 2,4-D (2-4mg L-1) and then decreased with higher concentrations. With further increase in the concentration of 2,4-D, the callus turned brownish. Thomas & Maseena [28] reported that, a range of 2,4-D concentrations (0.1-2.0mg L-1) is necessary for embryogenic callus formation from leaf and nodal explants. Obukosia SD, et al. [29] reported that the MS medium containing 2mg L-1 L 2,4-D was a favorable media for induction of callus in Chrysanthemum plants.

**Induction of somatic embryos**

Developing somatic embryogenic culture systems with reliable regeneration capacity from ornamental plants is a prerequisite for mass propagation and their genetic improvement. The process can be induced in tissue cultures of chrysanthemum either directly from the epidermal cells of explants [26] or indirectly via intervening callus [30,31]. Somatic embryogenesis was observed by adding BAP in the presence of 2,4-D (Figure 1). In addition, the ratios between 2,4-D and BAP concentrations were significantly associated with the percentages of somatic embryogenesis and number of somatic embryos. Shinoyama H, et al. [30] reported combination of 2mg L-12,4-D and 2mg.L-1 kinetin was found to be as optimal concentration for somatic embryos induction. Also, Tymoszuk A, et al. [32], using ray florets, concluded that 85% of the explants were able to produce embryos, and in each ray florets about 5.70 somatic embryos were placed on the MS medium containing 4mg L-1of 2,4-D and 1mg L-1of kinetin. Indirect somatic embryogenesis has been reported in many other chrysanthemus genotypes [25,26,30,31]. With regard to the previous results, the use of embryogenesis in mutagenic breeding programs could be very useful.

**Regeneration of embryogenic callus**

![Figure 1: Different stages of callus induction, somatic embryos and plant regeneration and direct organogenesis in Chrysanthemum](image_url)
The ability to regenerate shoots from callus is essential for establishing a successful plant culture system as well as in vitro mutagenesis (Figure 1). The findings show that induction of embryogenesis and plant regeneration are significantly related to genotypes [5,33]. Cytokinins play an important role in the regeneration of shoot from callus in vitro conditions [34]. Auxins and cytokinins are an important factor in the induction of embryogenesis due to inclusive and effective interventions in cell cycle and cell division [35].

### Importance of tissue culture in the production of new cultivars

Tissue culture is an essential tool for creating new chrysanthemum cultivars [1]. The most common type of chrysanthemum breeding is mutation breeding. Mutagenesis has had a positive influence in chrysanthemum biotechnology [36-38], leading to the production of novel flower colours, modified architecture and leaf chimeras, some of which have been stably propagated. Callus tissue is genetically unstable and regeneration via this tissue is associated with the occurrence of somaclonal variation. Ray florets explants are a useful source for the formation of adventitious shoots or somatic embryogenesis in creating novel cultivars of chrysanthemum [1,2,39]. In addition, leaf explants may be a useful source of new variation in the plant, which may be due to the somaclonal variation or chimera structure in regenerated plants [40].

### Conclusion

Auxin/cytokinin balance is required to obtain adventitious shoot induction as well in embryogenic callus induction and plant regeneration. Optimization of in vitro propagation of Chrysanthemum morifolium by direct organogenesis and embryogenic callus induction can be used for genetic transformation and for further in vitro mutagenesis investigations.

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DOI: 10.19080/ARTOAJ.2018.15.555962