Review article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

# *In Vitro* Regeneration of *Chrysanthemum Morifolium* CV White through Petal Explants

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## ABSTRACT

*Chrysanthemum morifolium* is an ornamental flower. The tissue culture of chrysanthemum is well known, but the use of petal tissue in regeneration is less undertaken, most likely due to the risk of chimerism.In the present research, *Chrysanthemum morifolium* cv. White petals were taken as explant to study indirect organogenesis in which optimization of media was done for callus induction and its growth. Although, callus could be formed in all tested media (MS-I, MS-II, MS-III and MS-IV) but the highest callus induction was in MS-II i.e. MS supplemented with NAA (0.5 mg/l) and kinetin (10.0 mg/l). The callus, which showed ++ and +++ growth status, was chosen for standardizing the regeneration media. Among different regeneration media (i.e. R1, R2 and R3), the best result of multiple shoot number (4.79) on callus and of callus responded to shoot organogenesis was highest in R2, which contains of MS fortified with Kinetin (10.0 mg/l) and NAA (0.5 mg/l). This shows that initial media for callus induction determines the healthy growth status of the callus for further its organogenesis. Regenerated shoots successfully elongated and formed roots on MS medium

**KEYWORDS:** Callus formation, Explant, Organogenesis, Petals, plant growth regulators (PGR), Regeneration

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## INTRODUCTION:

Chrysanthemums are one of the most important ornamentals in the cut flower, flowering potted plant and herbaceous perennial markets worldwide.[1]Chrysanthemum cultivars are generally propagated by vegetative cuttings or suckers.[2-3] However, this conventional approach of shoot cutting is very slow.[2] Therefore efficient tissue culture methods for regeneration will need to be developed, especially if genetic transformation via Agrobacterium is to be attempted.[4]*In vitro* shoot regeneration in chrysanthemum through induction of adventitious shoots is affected by the PGRs interaction, the kind of explant and plant genotype.[5-6]

Chrysanthemum petals are amongst the candidate sources for organogenesis.[5] Regeneration from petals has been achieved in chrysanthemum by a number of researchers using different cultivars and various plant growth regulator (PGR) combinations or concentrations.[7-14] PGRs provide a proportional stimulus that allows regulation of the cell cycle for cell division induction and specialization during plant development.[15]

Park et al. (2007) achieved the highest adventitious shoot regeneration frequency by culturing petal explants on the Murashige and Skoog medium (MS) (1962) supplemented with 57.0 µM IAA, 44.0 µM BAP and 0.4 µM kinetin.[11,16]Song et al. (2011) observed significant differences in frequency of regeneration among different cultivars when grown on media supplemented with various plant growth regulators.[12] Among different surveyed explants in their research, petals exhibited the highest frequencies of shoot organogenesis and mean number of shoots per explant. Shoot formation or adventitious organogenesis is preferable due to retaining clonal fidelity as numerous floricultural crops are cultivars that are propagated for unique traits.[11]

Efficient protocols for a given cultivar may not be suitable for the other ones. Thus, it is necessary to develop appropriate protocols for regeneration of various commercial cultivars.[12]This study was implemented to scrutinize the effect of different PGR combinations on shoot organogenesis from petal explants in two chrysanthemum cultivars in order to introduce.

Notwithstanding, plant regeneration is strongly dependent on biotic and abiotic factors, such as the type and age of the explants, source, genotype, medium composition, growth regulators, environmental stress, gelling agent, dark, light period, sucrose concentration, and their interactions.[17-18]Adventitious shoot regeneration from petal explants of chrysanthemums has also been reported.[5,

[17]Yet, despite extensive research on plant regeneration of chrysanthemums, there are still many problems. One distinct problem is that the adaptation capacity of one protocol of cultivars is not identical to other cultivars.

Moreover, no detailed information is currently available on media manipulation for efficient indirect organogenesis from petals. Therefore, the present study investigated the optimum suitable media for plant regeneration using petal explants from *Chrysanthemum morifolium*cv. White.

## MATERIAL AND METHODS:

This work is part of project work done in fulfilment of Master Degree. The explants were from field. Petals were rinsed thoroughly with tap water and sterile distilled water. Collected explants were washed with a solution containing 3-4 drops of liquid detergent Teepol. Thereafter, the detergent was completely drained out from the explants by 3-4 washings with vigorous shaking by hand. For surface sterilization of explants to treatment were given Bavistein (0.1%) +8HQC(200 ppm) for 3-4 hours and was shaken regularly. Thereafter, the solution was completely drained out from the explants by 3-4 washing with vigorous shaking by hand. Second treatment of mercuric chloride (0.1%) for 3-4 minutes and washed again sterile distilled water.

To screen out the most suitable media for callus induction in *Chrysantheummorifolium* cv. White, petals were injured with scalpel and cultured for callus induction in on different media in Macro strength, and concentration of NAA. The media used for callus induction from petals are listed in table 1.

**Table 1: Media used for callus induction**

| Media Code | Macro Strength | Kinetin (mg/l) | NAA (mg/l) |
|---|---|---|---|
| MS-I | Half | 10 | 0.1 |
| MS-II | Full | 10 | 0.1 |
| MS-III | Full | 10 | 0.5 |
| MS-IV | Half | 10 | 0.5 |

### Regeneration Studies

To find out the most responsive regeneration media on *Chrysantheummorifium* cv. White for shoot organogenesis on callus. Promising freshly induced callus showing ++ and +++ degree were

transferred to three regeneration media which was MS medium with full Macro strength supplemented with different concentration and combination of Plants growth regulators as mentioned in table 2.

**Table 2: Regeneration media used for shoot organogenesis**

| Media Code | Treatment | |
|---|---|---|
| | Kinetin(mg/l) | NAA (mg/l) |
| R-I | 10 | 0.1 |
| R-II | 10 | 0.5 |
| R-III | 10 | 1.0 |

After one week of culture, callus induction response, shoot induction percentage (number of explants with visible shoots/ total number of explants) and average number of shoots per callus were recorded for each treatment. During this period, regenerated shoots were separated from callus and transferred to MS medium for further elongation and rooting.

## RESULT AND DISCUSSION:

Callus could be formed in all tested media. Callus induction initiated after 5-7 days after culture inoculated callus induction initiated earliest on MS-II.The percentage callusing on petals of *Chrysanthemum morifolium* cv. White of on different media is shown in Table. It was observed that the percentage callusing after 7 days was highest in MS-III that containing MS supplemented with NAA (0.5 mg/l) and Kinetin (10 mg/l) followed by MS-II . MS-II contains MS supplemented with NAA (0.1 mg/l) and kinetin (10 mg/l). In MS-I and MS-IV, more number of cultures dried. Moreover, duration required for callus induction was much shorter in MS-III (as compared to MS-I and MS-IV).

Observation for nature and response of callus growth on different callus induction media were taken after 14 (Table 4) and 21 days (Table 5). Callus growth was more vigorous in MS-III followed by MS-II.

After 21 days all callus were inoculated on the most responsive media (MS-III) for further callus proliferation. Calluses showing ++ and +++ were inoculated on different regeneration media. Among different regeneration media, the most responsive media was $R_2$ (Table 7 )that is MS supplemented with NAA (0.5 mg/l) and kinetin (10 mg/l) the highest number of callus showing shoot organogenesis in $R_2$ was 48.48 ±4.29 and the multiple microshoot number was 4.79.(Fig. 1d)

Micro shoots originated on callus were very tiny. They were not suitable for rooting. For further shoot elongation media and rooting that is MS supplemented with BAP (0.1 mg/l) and $GA_3$ (0.5 mg/l) after 10 days, micro shoot increased in 1.5 to 2.0 cm in length (Fig. 1e). Regenerated plants were further kept acclimatized (Fig. 1f)

**Table 3: Effect of different media on percentage callus induction on petals in *Chrysanthemummorifolium* cv. White after seven days**

| Media Code | Treatment | | | No. of Petal Culture | Callusing(%)±SE |
|---|---|---|---|---|---|
| | Macro strength | Kinetin (mg/l) | NAA (mg/l) | | |
| MS-I | Half | 10 | 0.1 | 20 | 55.00± 4.09 |
| MS-II | Full | 10 | 0.1 | 20 | 75.00± 4.07 |
| MS-III | Full | 10 | 0.5 | 20 | 88.33± 2.38 |
| MS-IV | Half | 10 | 0.5 | 20 | 63.33± 2.35 |

CD at 5 % level (p 0.05)

**Table 4: Effect of different media on response of callus growth after 14 days**

| Media Code | Treatment | | | Degree of Callus | | | | |
|---|---|---|---|---|---|---|---|---|
| | Macro strength | Kinetin (mg/l) | NAA (mg/l) | Very Few | Few | + | ++ | +++ |
| MS-I | Half | 10 | 0.1 | 9.33 | 1.67 | - | - | - |
| MS-II | Full | 10 | 0.1 | 9.67 | 5.33 | - | - | - |
| MS-III | Full | 10 | 0.5 | 6.66 | 10.33 | - | - | - |
| MS-IV | Half | 10 | 0.5 | 8.33 | 4.33 | - | - | - |

**Table 5: Effect of different media on response of callus growth after 21 days**

| Media Code | Treatment | | | Degree of Callus | | | | |
|---|---|---|---|---|---|---|---|---|
| | Macro strength | Kinetin (mg/l) | NAA (mg/l) | Very Few | Few | + | ++ | +++ |
| MS-I | Half | 10 | 0.1 | 4.33 | 5.33 | - | - | - |
| MS-II | Full | 10 | 0.1 | 2.67 | 6.33 | 7.67 | - | - |
| MS-III | Full | 10 | 0.5 | 1.33 | 4.33 | 10.33 | 2.0 | - |
| MS-IV | Half | 10 | 0.5 | 5.33 | 4.67 | 1.33 | - | - |

**Table 6: Effect of different media on response of callus growth after 35 days**

| Media Code | Treatment | | | Degree of Callus | | | |
|---|---|---|---|---|---|---|---|
| | Macro strength | Kinetin (mg/l) | NAA (mg/l) | Few | + | ++ | +++ |
| MS-I | Half | 10 | 0.1 | 4.33 | 3.33 | 1.66 | - |
| MS-II | Full | 10 | 0.1 | 1.33 | 3.12 | 4.33 | 3.33 |
| MS-III | Full | 10 | 0.5 | 0.66 | 2.37 | 6.00 | 5.67 |
| MS-IV | Half | 10 | 0.5 | 1.66 | 4.32 | 3.67 | - |

**Table 7: Effect of different regeneration media on callus for its shoot organogenesis**

| Media Code | Kinetin (mg/l) | NAA (mg/l) | Callus showing shoot organogenesis (%) | Mean No. of shoots/callus |
|---|---|---|---|---|
| $R_1$ | 10 | 0.1 | 36.39± 3.06 | 2.37 |
| $R_2$ | 10 | 0.5 | 48.48± 3.29 | 4.79 |
| $R_3$ | 10 | 1.0 | 30.30±2.43 | 1.20 |

CD at 5 % level (p 0.05)

Little has been published on techniques of *in vitro* plant regeneration from petal explants in *Chrysantheum morifolium*. Calli could be induced in all tested media ((Table 3). Callus formation from petals was observed 5-7 days after culture initiation. Both kinetin and NAA together are necessary to induce callus formation. Number of culture dying in MS-I is much it evaluates role of macronutrient for callus induction and further its organogenesis.

Results suggested that MS-II and MS-III with full strength of macronutrients had higher percentage of callusing from petals i.e. 75.00± 4.07&88.33± 2.38 respectively, while in MS-I and MS-IV which has half strength of macronutrient showed lower percentage of callusing from petals 55.00± 4.09 and 63.33± 2.35 respectively.

Observation at day 14 mentioned in Table 4 also indicated that strength of macronutrient not only affects callus induction initiation also affected callus growth. In MS-III 10.33 petals was showing few degree of callus while in MS-I, only 1.67 petals exhibited few degree of callus growth. Significantlythe mineral composition of plant tissue particularly Ca, Mg and K plays a fundamental role in tissue and growth response *in vitro* showed variable callogenesis and callus growth in *Chrysanthemum Morifolium*.[19]

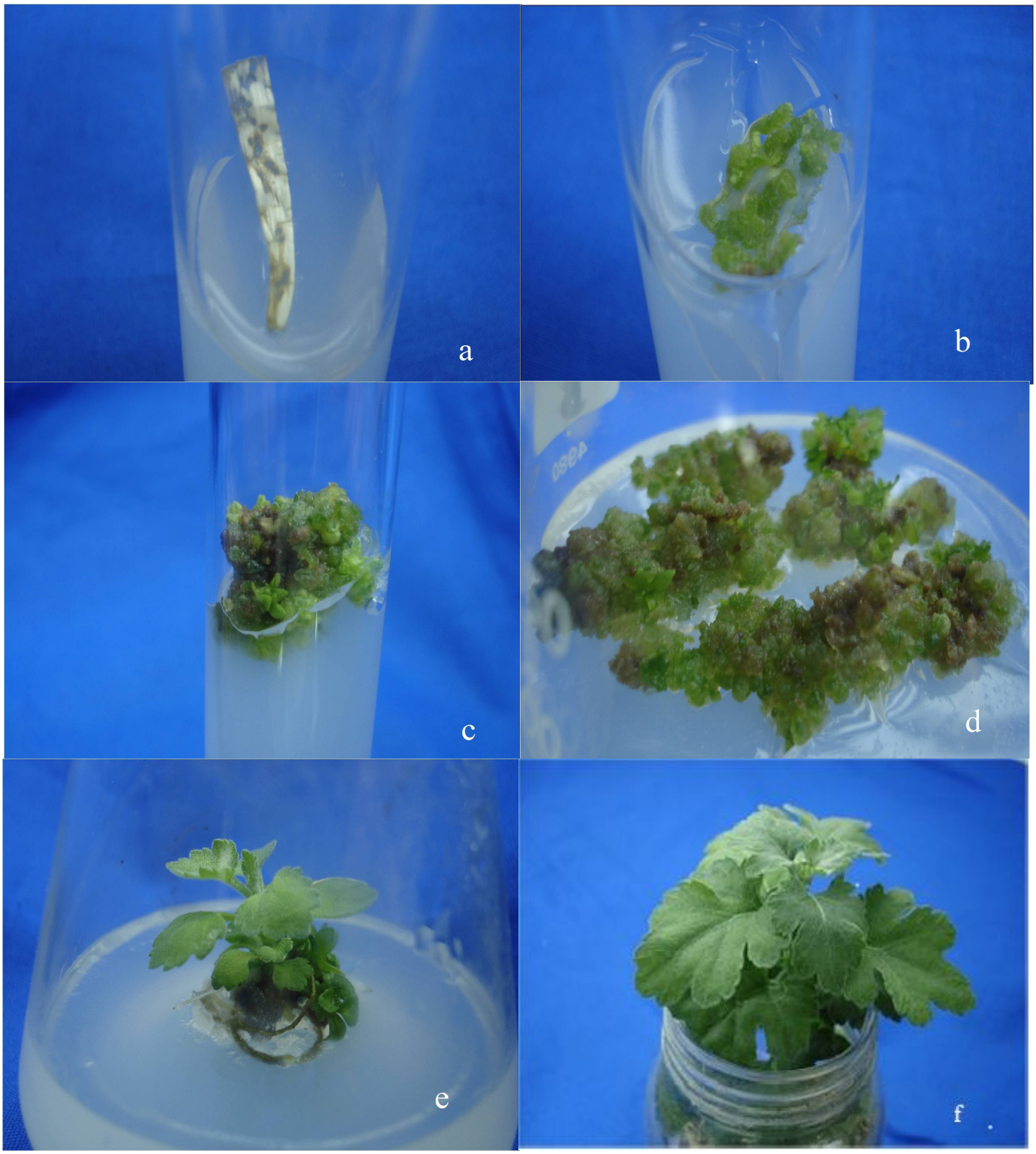


Figure 1: Successful *in vitro* culture of chrysanthemum from petal explants a. Petal inoculation b. Few Callus induction on petal explant c. Vigorous callus +++ in MS-II media d. Micro-shootsregeneration from calluses e. Shoot elongation and rooting initiation g. Regenerated acclimatized plant from petal

The efficiency of callus induction and callus growth rate has been reported to be partly genotype dependent.[20]

In table 5 and table 6 presented callus growth status at day 21 and day 35, respectively in different MS media. Vigorous Callus growth ++ and +++ were observed in MS-II and MS-III media on day 35(Table 7).

Petals showed different callus growth status in same media. For instance, In MS-III, 5.67 callus with vigorous +++ degree growth, 6.00 ++ degree callus and 2.37 few degree of callus on day 35 (Table 6). It may be due to different physiological, biochemical and development stages of petals. Plant cells normally inherit the same genetic information however, the morphogenesis response vary due to difference in spatial and temporal distribution of cells and their physiological, biochemical and development stage.[21]

Moreover, as illustrated in table 7, range of callus differentiation into shoots varied noticeably among different treatments, which indicates that the ability of fresh callus to differentiate into plantlets not only depends on the cultivar, but also relies on the hormone level of the induction medium.[20]

It should be uttered that type of regeneration not only depends on the choice of cytokinin, but also attributes to the chrysanthemum genotype.[12]Abilities to uptake and metabolize PGRs might also be different among various chrysanthemum genotypes.[5]Rademaker and de Jong (1990) reported that type of cultivar in comparison to the medium type had a greater effect on regeneration.[22]

Microshoots originated on callus were very tiny. There were not suitable for rooting. For further shoot elongation media, Fig. e, that is MS supplemented with BAP (0.1 mg/l) and $GA_3$ (0.5 mg/l). Gibberellins are known for inducing stem elongation in a number of crops.

## CONCLUSION:

The *in vitro* regeneration of chrysanthemum is notably genotype-dependent. This study provides a protocol for a single cultivar, but the reader is cautioned that the protocol may not be effective for other cultivars. Nonetheless, the experimental design and preliminary data for this cultivar will hopefully spur new and expanded research on other cultivars.

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