In vitro Propagation of Chrysanthemum morifolium Using Shoot Tip and Nodal Segment as Explant

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ABSTRACT

The large-scale multiplication of Chrysanthemum morifolium through in vitro propagation by using shoot tip and nodal segment as explants needs a designed protocol. Main objective of the present work is to find out the optimum condition for minimum contamination of explants and its rapid multiplication. The procedure includes surface sterilization of explants to remove most of the surface contaminants, such as bacteria and fungi with a suitable sterilizing agent and suitable aseptic conditions for rapid growth of explants. Among various agents, mercuric chloride (HgCl₂) is highly antimicrobial, which acts against both fungi and bacteria. The explants are treated with various conc. of HgCl₂ for different time periods in order to find out the optimum condition for minimum contamination. Explants with 0.1% of HgCl₂ for 7 min. of surface sterilization showed the best results in terms of decontamination. Among the explants, shoot tips were the most responsive towards sterilization followed by nodal segments. Indole 3 Acetic Acid (IAA) and 6-Benzylaminopurine (BAP) were used as plant bio-regulator for shoot induction in this experiment. MS media supplemented with Indole 3 Acetic Acid (IAA) and 1.3 Mg/l of 6-Benzylaminopurine (BAP), showed promising results for regeneration of shoot as compared to other concentrations and combinations.

Keywords: Chrysanthemum morifolium, Explants, in vitro, Sterilization, Tissue culture, IAA, BAP.

INTRODUCTION

Among various medicinal herbs Chrysanthemum morifolium is one of the non-toxic, biocompatible and eco-friendly herbs. The flowering plants of the genus Chrysanthemum belongs to family Asteraceae. Most of the species of this genus originate from East Asia and the center of diversity is in China. The genus Chrysanthemum was named by Carolus Linnaeus from two Greek words, 'Chrys', which means golden (the color of the original flowers), and 'anthemon', meaning flowers. Chrysanthemums are one of the pretiest varieties of perennial herbs that start blooming early in the autumn seasons. This plant is also known to as favorite flower for the month of November. The flowers of this plant bloom in various forms, shape and sizes. The flowers may be daisy-like, decorative, pompons or buttons and are available in a wide array of colours - from white, to yellow and gold, pink, orange, bronze, deep red, maroon, violet and purple. Some Chrysanthemums are having a mix of two and even more colours but yellow is the most common color among all. Some species has stronger fragrance and aroma than others; the best species is said smells like the Dryobalanops aromatica.

Tissue culture studies on Chrysanthemum were first initiated by Morel & Martin in 1952. For virus free plants they have used meristem tip culture. Now tissue culture is a necessary first step in the production of virus indexed Chrysanthemums. It serves as a nuclear stock for the delivery of high quality cuttings¹. The plant is generally propagated using suckers and terminal cuttings. However, this approach is insufficient to get fast multiplication rate, as these conventional propagating methods are very slow and time consuming. Secondly, cuttings obtained frequently from mother plants could be subjected to any virus contamination and degeneration, thereby increasing production costs². These problems have been solved by...
applying micro propagation methods. Micro propagation and other in vitro techniques have been used for plants which present particular problems in conventional horticulture. Caboni et al., reported the use of biotechnological approaches to improve horticultural crop production. The regeneration of plants from tissue culture is significant and necessary constituent of biotechnological research. High rate of plant regeneration from the invitro cultured tissue is a pre-requisite for victorious application of tissue culture techniques for crop improvement. It is achievable now to get a huge number of plants from one explants in vitro. A decade ago, the protocols for rapid true to type, disease-free propagation has been developed in Chrysanthemum through bud/shoot proliferation.

In tissue culture, the use of plant growth regulators play a vital role in influencing different plant processes comprising mostly of growth, differentiation and development. The presence of a cytokinin is almost always helpful, and is often all that is necessary, optimum rates of shoot initiation generally occur with the combinations of auxins and cytokinin. The presence of auxin in defined combinations with cytokinins in the culture medium is also necessary to obtain adventitious shoot formation. Chrysanthemum has been cultivated for more than 2000 years and today, it is the world’s second most economically important floricultural crop following the rose. It has become one of the first commercial targets for Micro propagation for its large scale production due to high popularity and demand. Development of Chrysanthemum is also a positive impact on the economy in rural areas, particularly the increased income of farmers and communities involved in its development. In Indonesia, the demand for Chrysanthemum increased 25% per year, even by the year 2003 the market demand increased 31.62%. Material was brought to the laboratory and washed from the tissue culture centre, OUAT, BBSR. Then the collected material was brought to the laboratory and washed thoroughly with running tap water for 30 min. shoot tip and 1 cm long nodal segments (containing a single node) were prepared, by using forceps and scalpel.

Sterilization
Sterilization is a procedure for elimination of microbes. To make the explants free from contamination, surface sterilization of shoot tip and nodal explants were conducted with 0.01% of mercury chloride for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 minutes with 1% of NaOCl for 1 min to establish maximum contaminant free cultures. The excised explants were dipped in laboline for 30 mins following bavistin for 20 mins. Immediately explants were rinsed with double distilled water twice. Shoot tip and Nodal segments were then surface sterilized with 0.1% of HgCl2 for 1 min to 10 mins, following 1% sodium hypochlorite for 1 min. In the laminar air flow. Shoot tip and nodal cuttings were finally washed with sterilized distilled water for several times so as to lower the toxic effects of HgCl2. After sterilization each segment was divided by a longitudinal cut of shoot tip and nodal explants so that each piece received shoot tip cuttings and a half of the nodal portion.

Shoots multiplication
Explants were cultured on solidified MS media with agar (6 gm/l) and its pH was adjusted to 5.7 by using 0.1 HC1 or NaOH. Before autoclaving at 121°C for 30 min. On cooling of the media, explants were cultured in Murashige and Skoog (1962) media containing different concentrations of auxins and cytokinins. The shoot tip and nodal explants are cultured in MS medium supplemented with 1% of IAA and different concentration (0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3 and 1.4 mg/ml) of BAP for shoot initiation. Two explants were cultured in each cultured bottle. Each treatment was replicated three times the bottles were covered with autoclaved caps after culturing, the cultured bottles were incubated for 16 h daily light of fluorescent, Philip white tubes with intensified 1000LUX, at 25 ± 1°C temperature.

RESULTS
The result shows that the highest percentage of healthy and contaminant free explants were established when the explants were treated with 0.01% of HgCl2 for 7 minutes in case of shoot tip culture and 8 for nodal culture. Shoot initiation started very soon within 8 days from Shoot tip explants and 10 days in Nodal segment explants in M10 medium (MS + 1.3 mg/l BAP plus 1% IAA).

DISCUSSION
The present study has main objective to identify better explants type, standard surface sterilization procedure and plant bioregulator concentration for shoot induction to produce large number of disease free explants. From the experiment the shoot tip explants responded very quickly for shoot induction as well as highest percentage of infection free plant, where as the nodal segments took more time for shoot regeneration.
Table 1: Effect of surface sterilization on shoot tip and nodal segment of *Chrysanthemum morifolium*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatments 0.01% of HgCl₂ and varying in time duration of treatment</th>
<th>Fungal %</th>
<th>Bacterial %</th>
<th>Death %</th>
<th>Contamination free %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot tip</td>
<td>Nodal segment</td>
<td>Shoot tip</td>
<td>Nodal segment</td>
<td>Shoot tip</td>
</tr>
<tr>
<td>T1</td>
<td>Tap water</td>
<td>92</td>
<td>90</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>T2</td>
<td>0.01% HgCl₂ for 1 min</td>
<td>85</td>
<td>83.5</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>T3</td>
<td>0.01% HgCl₂ for 2 min</td>
<td>81</td>
<td>79.5</td>
<td>0.5</td>
<td>5.5</td>
</tr>
<tr>
<td>T4</td>
<td>0.01% HgCl₂ for 3 min</td>
<td>75</td>
<td>74</td>
<td>0.8</td>
<td>4</td>
</tr>
<tr>
<td>T5</td>
<td>0.01% HgCl₂ for 4 min</td>
<td>59.3</td>
<td>57</td>
<td>1.2</td>
<td>6.8</td>
</tr>
<tr>
<td>T6</td>
<td>0.01% HgCl₂ for 5 min</td>
<td>56.5</td>
<td>53</td>
<td>1.5</td>
<td>5.5</td>
</tr>
<tr>
<td>T7</td>
<td>0.01% HgCl₂ for 6 min</td>
<td>47</td>
<td>48</td>
<td>2</td>
<td>9.5</td>
</tr>
<tr>
<td>T8</td>
<td>0.01% HgCl₂ for 7 min</td>
<td>38.4</td>
<td>40</td>
<td>2.4</td>
<td>12</td>
</tr>
<tr>
<td>T9</td>
<td>0.01% HgCl₂ for 8 min</td>
<td>51.5</td>
<td>36</td>
<td>6</td>
<td>11.5</td>
</tr>
<tr>
<td>T10</td>
<td>0.01% HgCl₂ for 9 min</td>
<td>57.8</td>
<td>32</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>T11</td>
<td>0.01% HgCl₂ for 10 min</td>
<td>40</td>
<td>35</td>
<td>25</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Figure 1. Effect of surface sterilization of shoot tip

Figure 2. Effect of surface sterilization of the nodal segment
**Figure 3:** Effect of plant bioregulator for shoot induction in *in vitro* condition

**Figure 4:** (a). inoculated plant (b). Explant showing infection  (C). Dead explant (d). shoot induction from shoot tip explant (e). shoot induction from nodal explant.
It was found that the highest percentage (56%) of healthy and contaminant free explants were established when they were treated with 0.01% of mercuric chloride for 7 minutes in case of shoot tip culture where as in nodal explants it shows 50% in 8 minutes. In the present study the shoot tip segment shows good response followed by nodal segments. Among all the explants T8 shows highest percentage (56%) of regeneration of shoots. Culture medium containing 1.3 mg/l of BAP shows shoot initiation within 8 days in case of shoot tip culture Followed by nodal segments within 10 days. After shoot regeneration, auxins of different concentration were used for root initiation. Here, it is observed that the concentration of cytokinin has prominent role in shoot proliferation as well as the concentration of auxin in the media has prominent role in root induction.

CONCLUSION

The efficient in vitro propagation system of Chrysanthemum morifolium describe here for the production of healthy mother stock plants, from which healthy cuttings could be taken for further propagation in the nurseries. The highest percentage of regeneration (56%) in M8 medium was obtained by surface sterilization of shoot tip explants in 0.01% of HgCl₂ for 7 minutes, while nodal explants shows highest percentage of regeneration (50%) in M8 medium when sterilized in .01% of HgCl₂ for 8 minute. Auxin/cytokinin balance is required to obtain adventitious shoot induction as well in plant regeneration. Both growth regulators (IAA and BAP) show their significant effect on the regeneration of Chrysanthemum. Plantlets using shoot tip and nodal explants culture. When their combinations were tested, MS media supplemented with lower concentrations of IAA (1.0 mg/l) and intermediate concentrations of BAP (1.3mg/l) shows far better results regarding regeneration of Chrysanthemum plantlets as compared to other combinations. The results of the experiment indicate that Chrysanthemum can be multiplied in large scale through in vitro propagation. Farmers face many difficulties while raising Chrysanthemum in nursery in open field condition, due to the climatic condition prevailing in that particular area, pest and disease incidence. This study clearly indicates that the above problems can be overcome by in vitro propagation of Chrysanthemum and production of healthy plant for better floriculture. To the best of our knowledge, this is a better report on successful plantlets regeneration from Chrysanthemum shoot tip and nodal explants at all. Such research activities could also lead to the development of rapid multiplication of new genotypes, selection of somaclonal and induced variants, preservation of germplasm and long-term storage.

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REFERENCES


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