Influence of media on *in vitro* root regeneration and micropropagation of *Chrysanthemum morifolium* Ramat cv. Hwiparam

Soo Cheon Chae

Department of Horticultural Science, College of Industrial Sciences, Kongju National University, Daehoe-ri, Yesan-kun, Chungnam, 340-720, Korea
scchae@kongju.ac.kr

Abstract: To investigate improved root organogenesis and micropropagation in *Chrysanthemum morifolium* Ramat cv. Hwiparam, we established an efficient protocol using different media and concentrations. Stem explants were cultured on 3 full strength basal MS (Murashige and Skoog 1962), SH (Schenk & Hildebrandt 1976) and B5 (Gamborg et., al 1972) medium. Then, the best medium for root regeneration was investigated at 5 different concentrations (1/4, 1/2. 1, and 2 strength). The best type of medium for root regeneration and growth was SH medium). The results showed that half strength of SH (1/2SH) is the best condition for the number of root per explant (4.3) and root length (31.4 mm).
[Chae SC. **Influence of media on *in vitro* root regeneration and micropropagation of *Chrysanthemum morifolium* Ramat cv. Hwiparam.** *Life Sci J* 2014;11(9):797-799]. (ISSN:1097-8135). http://www.lifesciencesite.com. 120

Keywords: *Chrysanthemum morifolium*, media, micropropagation, root organogenesis

1. Introduction

Chrysanthemum morifolium is a perennial flowering plant from Asteraceae family. It is native to Asia and northeastern Europe and most species originate from East Asia and the center of diversity is in China. There are a lot of horticultural varieties and cultivars. *Chrysanthemum morifolium* is an important ornamental crop for cut flower and pot plant in Korea (Lee et al., 2003; Xie et al., 2012; Liang et al., 2014).

Micropropagation through plant tissue culture has tremendous potential for the production of clonal plants in a short period. Many researchers have reported for plant regeneration from different plant organs of *C. morifolium*. Recently, extensive research has been conducted on the development of shoot organogenesis and plant regeneration from petal (Xue et al., 2004; Nahid et al., 2007), leaf (Xue et al., 2003), and stem (Lu et al., 1990) explants cultures of *C. morifolium* for improved micropropagation system.

In vitro rooting of *C. morifolium* is one of the most important factors governing the growth and micropropation. Therefore, this study was conducted to find out the suitable medium and *in vitro* condition for rapid root proliferation from young stem explants of *C. morifolium.*

2. Material and Methods

2.1. Plant materials

Young shoots were collected from 1-years-old plants of *Chrysanthemum morifolium* cv. Hwiparam. growing in the green house of the Kongju National University, Daejeon, Korea. For establishment of *in vitro* shoot cultures, the leaves from young shoots were eliminated and cutted around 5 cm in length. These explants were washed with tap water for 5–10 minutes and were surface sterilized with 70% (v/v) ethanol for 30 sec and 1% sodium hypochlorite solution for 10 min. The explants were then rinsed thoroughly with sterilized distilled water and were incubated on 50 mL of hormone free MS (Murashige and Skoog, 1962) basal medium in Magenta box under the light condition. The basal medium consisted of mineral salts and vitamins supplement together with 30 g/L of sucrose and 8 g/L of Phytagar as a solidifying agent. The pH of the medium was adjusted to 5.8 before adding the Phytagar and sterilized by autoclaving at 121°C for 20 minutes. After 4 weeks cultured elongated shoots were obtained and maintained under controlled environmental conditions until used.

2.2. In vitro rooting using different media

Seven shoot explants were cultured in each magenta box containing 50 ml of hormone free full strength basal MS (Murashige and Skoog 1962), SH (Schenk & Hildebrandt 1976) and B5 (Gamborg et.,al 1972) medium respectively. These basal media, consisted of salts and vitamins, were solidified with 0.3 % Gelrite and supplemented with 3% (w/v) sucrose. The pH of the medium was adjusted to 5.8 prior to the addition of Gelrite and autoclaved at 121°C with the pressure of 1.1 kg cm^{-2} for 20 min. Cultures were then incubated at 25±1°C with a 16- hr photoperiod per day under the standard cool and white florescent tubes. All experiments were carried out in triplicate. Rooting efficiency, average number of root per explant and root length were measured at 4 weeks after incubation.

2.3. Determination of SH medium concentration

The most suitable medium was selected from the preliminary experiment. Then, root regeneration was determined by using 4 different concentration ($^1/_4$SH, $^1/_2$ SH. SH and 2SH) of the medium. About 1~2 cm of seven shoot segments were cultured on the respective medium. The same procedure for sterilizing the medium and culture conditions, mentioned in the first experiment, was applied.

2.4. Determination of SH medium concentration

Regenerated plantlets were maintained continuously under the *in vitro* condition until the roots become well developed. After 4 weeks, the rooted plants were then transferred to the pots containing autoclaved vermiculite soil. Immediately after transplantation, the plants along with the pots were covered with moist polythene bag to prevent from desiccation. To reduce sudden shock, the pots were kept in a growth-controlled room for 7-15 days. After two to three days, the polythene bags were gradually perforated to expose the plants to natural environment. The bags were then completely removed after 10-15 days when the plantlets appeared to be self-supportive. At that stage, the plantlets were placed in natural environment for 3-10 hours daily. Finally, after 15-20 days, they were transferred to a greenhouse condition.

2.5. Statistical analysis

Data for the 30 tested leaf explants are expressed as mean ± standard deviation.

3. Results

For better in vitro root regeneration, we used different media to investigate the efficiency of root organogenesis in *Chrysanthemum morifolium* cv. Hwiparam. The type of medium has been found to play an important role in the root regeneration of *C. morifolium*. Roots started developing one week after the explants were cultured on different basal media. At this stage, different medium has no significant effect on the rooting efficiency. Number of roots per explant and root length were found to be significantly different after three weeks of culture.

Among the media used in this study, SH medium induced the highest root regeneration and growth. The highest root growth was observed on SH medium supplemented with 30 g/L of sucrose, resulting in 100% regeneration frequency with the largest number of roots (3.9) and the longest roots (27.3 mm) in each explant (Table 1). Therefore, SH medium was selected as a suitable basal medium for the root development and growth of *C. morifolium*.

In a study on the effect of different SH medium concentration on root regeneration of the excised stem culture of *C. morifolium*, stem explants were grown for three weeks on basal media ($^1/_4$SH, $^1/_2$ SH. SH and 2SH). The results showed that half strength of SH (1/2SH) is the best condition for the number of root per explant (4.3) and root length (31.4 mm) eventhough it follows after SH medium in terms of regeneration frequency (Table 2).

4. Conclusions

Our results demonstrated that the selection of medium was essential for improvement of in vitro root regeneration and micropropagation of *C. morifolium*. We found that the best type of medium for regeneration and root growth was half strength of SH (1/2SH). Further study is needed on root regeneration of *C. morifolium*. by using other different concentration and combination of plant hormones for advanced root regeneration.

Table 1. Effect of different media on root regeneration and growth from excised stem of *Chrysanthemum morifolium* after three weeks of *in vitro* culture.

Medium	Regeneration frequency * (%)	No.of root/explant**	Root length[a] (mm)
B5	95	3.5 ± 0.3	23.5 ± 2.6
MS	90	2.8 ± 0.4	19.8 ± 2.4
SH	100	3.9 ± 0.4	27.3 ± 3.1

* Regeneration frequency (%) = No. of explant with root differentiation / all explants x 100
**From 30 stem explants tested.
[a]Values represent the mean ± standard deviation of 30 roots.

Table 2. Effect of different concentration of SH media on root regeneration and growth from excised stem of *Chrysanthemum morifolium* after three weeks of *in vitro* culture.

Medium	Regeneration frequency* (%)	No.of root/explant**	Root length[a] (cm)
1/4 SH	100	3.5 ± 0.3	24.7 ± 2.4
1/2 SH	100	4.3 ± 0.4	31.4 ± 3.3
SH	100	3.9 ± 0.4	27.3 ± 3.1
2SH	90	3.2 ± 0.3	22.7 ± 2.4

* Regeneration frequency (%) = No. of explant with root differentiation / all explants x 100
**From 30 stem explants tested.
[a]Values represent the mean ± standard deviation of 30 roots.

Acknowledgements:

This work was supported by the research grant of the Kongju National University in 2013.

Corresponding Authors:
Dr. Soo Cheon Chae
Department of Horticultural Science, College of Industrial Sciences, Kongju National University, Daehoe-ri, Yesan-kun, Chungnam, 340-720, Korea
E-mail: scchae@kongju.ac.kr

References

1. Gamborg OL, Murashige T, Thorpe TA, Vasil IK . Plant Tissue Culture Media. In Vitro. 1976; 12: 473-478.
2. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiol. Plant. 1962; 15: 473-497.
3. Lee JH, Goudriaan J, Challa H. Using the expolinear growth equation for modelling crop growth in year-round cut chrysanthemum. Ann Bot. 2003; 92(5): 697-708.
4. Liang F, Hu C, He Z, Pan Y. An arabinogalactan from flowers of *Chrysanthemum morifolium*: structural and bioactivity studies. Carbohydr Res. 2014; 387: 37-41.
5. Lu CY, Nugent G, Wardley T. Efficient, direct plant regeneration from stem segments of chrysanthemum (*Chrysanthemum morifolium* Ramat. cv. Royal Purple). Plant Cell Rep. 1990; 8(12): 733-736.
6. Nahid JS, Shyamali S, Kazumi H. High frequency shoot regeneration from petal explants of *Chrysanthemum morifolium* Ramat. in vitro. Pak J Biol Sci. 2007;10(19): 3356-3361.
7. Schenk RU, Hildebrandt AC. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 1972; 50: 199-204.
8. Xie YY, Qu JL, Wang QL, Wang Y, Yoshikawa M, Yuan D. Comparative evaluation of cultivars of *Chrysanthemum morifolium* flowers by HPLC-DAD-ESI/MS analysis and antiallergic assay. J Agric Food Chem. 2012; 60(51):12574-12583.
9. Xue JP, Chang W, Zhang AM, Sheng W. Studies on petal tissue culture of the traditional Chinese medicine *Chrysanthemum morifolium*. Zhongguo Zhong Yao Za Zhi. 2004; 29(3): 211-21410.
10. Xue JP, Yu M, Zhang AM. Studies on callus induced from leaves and plantlets regeneration of the traditional Chinese medicine *Chrysanthemum morifolium*. Zhongguo Zhong Yao Za Zhi. 2003; 28(3): 213-216.

8/28/2014