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In Vitro Propagation of *Chrysanthemum morifolium* Ramat Using Stem Nodal Explants

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Abstract: This study was conducted to investigate the effect of some growth regulators on *in vitro* shoot regeneration, multiplication and rooting of *Chrysanthemum morifolium* to develop a protocol for its mass propagation. Nodal explants of *C. morifolium* were cultured on MS basal medium supplemented with the cytokinins BAP and kinetin, both at 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 mg/l, for shoot regeneration. The regenerated shoots were separated from the nodal explants and cultured on MS medium supplemented with BAP and kinetin, both at 0.0, 0.1, 0.2, 0.5 mg/l for shoot multiplication. The shoots were then cultured on MS medium supplemented with 0.0, 0.1, 0.2, 0.5 mg/l of IBA for rooting. Twenty explants were used per treatment. The percentage of responded explants, average number of shoots per explant, length of shoot, percentage of rooted shoots, number of roots per shoot and length of root were determined. The concentration of 0.1 mg/l of both BAP and kinetin showed the best response with regard to shoot regeneration and multiplication giving the highest number of shoots per explant. Cytokinin–free medium gave significantly the highest length of shoot. All IBA treatments gave 100% rooting. However, MS+ 0.5 mg/l IBA resulted in significantly the highest number of roots per shoot, and IBA–free medium resulted in significantly the highest root length.

Key words: *In vitro* Propagation; *Chrysanthemum*; Benzylaminopurine; Kinetin; Nodal explants

INTRODUCTION

Chrysanthemum is one of the most popular ornamental flowers in the world. It is the third most important cut flower in the world after rose and carnation, It can be used as a cut flower, a potted flowering plant and a bedding plant. It is important not only for its outstanding aesthetic beauty and long lasting capability but also for its good potential for marketing as cut flowers and potted plants in many countries (Karim *et al*. 2002). Chrysanthemum is propagated vegetatively, either through root suckers or terminal cuttings, but this method is too slow to be commercially practiced. Due to the high popularity and demand for chrysanthemum it became one of the first commercial targets for micropropagation (Levin *et al*. 1988). Ben-Jaacov and Langhans (1972) described *in vitro* Chrysanthemum micropropagation from shoot tips and shoot-initiated callus. Karim *et al*. (2002) developed a protocol for direct regeneration, multiplication and rooting under *in vitro* conditions of Chrysanthemum using nodal segments and shoot tips as explants.

Clonal multiplication through tissue culture produces a large number of plants in a short time that are uniform, vigorous and available at any time for greenhouse planting. It is possible now to obtain a large number of plants from one explant through *in vitro* propagation (Bajaj 1992). Chrysanthemum is one of the flower crops imported by some Sudanese companies dealing with cut flowers and potted plants. Growers and research workers face great difficulties in obtaining planting material of this plant which is not locally available and hence mother plants have to be imported from abroad. The objective of this study was to investigate the effect of some growth regulators on *in vitro* shoot regeneration, multiplication and rooting of *C.morifolium* to develop a protocol for mass propagation of this species.

MATERIALS AND METHODS

This research was carried out at the tissue culture laboratory of the Sudan University of Science and Technology at Shambat, Sudan. *C. morfolium* plants (about 4 months old) were obtained from Dal Company for Agricultural services - Sudan. Single nodal explants were taken from

these plants. Explants were first washed intensively with tap-water for 30 minutes, dipped in ascorbic acid for 1 hour, soaked in 70% (v/v) ethanol for a few seconds and then immersed in a 20% (v/v) clorox (2% sodium hypochlorite) to which 1-2 drops of tween 80 per 100 ml of solution were added for 30 minutes and rinsed three times with sterile distilled water .

All media used in this study were based on Murashige and Skoog (MS) basal medium (Murashige and Skoog 1962).The pH of all media was adjusted to 5.6 using 1 N HCl or 1 N NaOH prior to autoclaving. Twenty five ml of medium were distributed in GA7 containers for the experiment of regeneration and multiplication and in 25X150 mm culture tubes covered with cotton and aluminum foil for the experiment of rooting and sterilized in the autoclave at 1.05 kg/cm^2 and 121°C for 30 minutes. The cultures were grown at 25 ± 1°C with 16 h cool white fluorescent light at photon flux density of 13.5 µmol m^{-2} s^{-1}. The explants were cultured on MS medium supplemented with Benzylaminopurine (BAP) and Kinetin at 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 mg/l for shoot regeneration and BAP and kinetin at 0.0, 0.1, 0.2, 0.5 mg/l for shoot multiplication. For rooting of *in vitro* regenerated shoots, the shoots were cultured on MS medium supplemented with different concentrations (0.0, 0.1, 0.2, 0.5 mg/l) of Indole-3- butyric acid (IBA). A completely randomized design with 5 replications was used and 4 GA7 containers represented one replicate. After six weeks of culture, shoot regeneration percentage (percentage of explants responded), average number of shoots per explant, length of shoot, percentage of rooted shoots, number of roots per shoot and length of root were recorded. The collected data were analyzed using the Statistics 9 computer program. Duncan's Multiple Range Test at the 5% level of probability was used to compare means.

RESULTS AND DISCUSSION

Effect of different concentrations of BAP and kinetin on shoot regeneration and multiplication

Tables 1 and 2 illustrate the influence of BAP and kinetin concentrations on shoot regeneration on nodal explants and Tables 3 and 4 show the influence of BAP and kinetin on *in vitro* multiplication of detached shoot explants of *C. morfolium*. The best response to shoot regeneration (percentage of explants responded), the highest number of shoots⁄ nodal explants and the highest multiplication rate (number of shoots⁄ *in vitro* shoot explants) was obtained on the concentration MS + 0.1 mg⁄l of both BAP and kinetin. Karim *et al.* (2002) found that 1.0 mg/l of both BAP and kinetin was the best concentration for shoot regeneration on nodal explants of *C. morifolium.* Gul (2001) reported that in *chrysanthemum,* maximum shoot regeneration was observed in stem nodal segments at 0.5 mg/l BAP.

Table 1. Influence of BAP concentration on shoot regeneration on nodal explants of C. *morifolium* cultured for 6 weeks on MS basal medium ((data is a mean of 20 explants)

BAP concentration (mg\l)	Explants responded (%)	Number of shoots/ explant
0	25	1.00 ab
0.1	70	2.40 a
0.2	45	1.80 ab
0.5	45	1.80 ab
1	40	1.60 ab
2	25	1.60 ab
5	15	0.60 b
10	10	0.40 b

Means within a column, followed by the same letter (s) are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table 2. Influence of Kinetin concentration on shoot regeneration on nodal explants of *C. morifolium* cultured for 6 weeks on MS basal medium ((data is a mean of 20 explants)

Kinetin concentration(mg\l)	**Explants responded (%)**	**Number of shoots/explant**
0	25	1.00 a
0.1	60	2.30 a
0.2	55	2.00 a
0.5	40	1.60 a
1	30	1.20 a
2	25	1.00 a
5	20	o.90 a
10	15	0.80 a

Means within a column followed by the same letter (s) are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table 3. Influence of BAP concentration on *in vitro* multiplication of *C. morifolium* shoot explants cultured for 6 weeks on MS basal medium (data is a mean of 20 explants)

BAP concentration(mg/l)	**Number of shoots*/in vitro* shoot explant**	**Length of shoot (cm)**
0	2.40b	3.11a
0.1	8.00a	2.18b
0.2	7.80a	1.38c
0.5	7.60a	1.68bc

Means within a columns followed by the same letter (s) are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Table 4. Influence of kinetin concentration on *in vitro* multiplication of *C. morifolium* shoot explants cultured for 6 weeks on MS basal medium (data is a mean of 20 explants)

Kinetin concentration (mg\l)	Number of shoots/ *In vitro* shoot explant	Length of shoot (cm)
0	2.40b	3.12a
0.1	7.00a	1.88b
0.2	4.60ab	1.48b
0.5	4.40ab	1.06b

Means, within a column, followed by the same letter (s) are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

Khan *et al.* (1994) observed shoot proliferations on MS medium supplemented with 0.5 and 1.0 mg/l BAP. At 2.0 mg/l BAP, shoot regeneration was higher but they were compact and stunted in growth. Increased levels of BAP in the medium increased the number of *chrysanthemum* shoots but suppressed their growth (Singh and Arora 1995). Proliferation and formation of chrysanthemum plantlets was the best when the medium was supplemented with 0.8 mg/l kinetin (Rout *et al.* 1996). Gul (2001) stated that multiple shoot formation occurred when shoots were sub-cultured on the basal medium containing various levels of BAP (1.0 - 4.0 mg/l). Chrysanthemum shoots transferred onto solid MS medium containing 0.2 mg/l BAP, increased about 4-folds in mass after 30 days of subculture (Furuya 1999). Different responses of chrysanthemum explants to different concentrations of BAP and kinetin with respect to shoot regeneration and multiplication might be attributed to genetic differences. Hence, variable results were obtained including our own. Prasad *et al.* (1983) reported that the rate of shoot multiplication is genotypic dependent in *C. morifolium*.

Increasing cytokinin concentration led to decrease of shoot length, and the highest length of shoot was given by cytokinin–free medium (Tables 3 and 4). Similar result was obtained by Kharrazi *et al.* (2011) and Brar *et al.* (1995) in their study on *in vitro* propagation of carnation (*Diathus caryophyllus* L.).

Effect of Indole-3- butyric acid (IBA) on rooting of *in vitro* shoots of *Chrysanthemum morifolium*

Table 5 illustrates the response of *C. morifolium* shoots to different concentrations of IBA (0.0, 0.1, 0.2, 0.5 mg\l). All IBA treatments gave 100% rooting. There was a significant difference between the different concentrations of IBA in number and length of roots. The highest number of roots was obtained at 0.5 mg/L IBA and was not significantly different from 0.2 mg/l IBA. This result is in agreement with that of several workers (Khan *et al.* 1994; Hoque and Fatema 1995; Hoque *et al.* 1998; Faisal and Amin 2000; Sarker and Shaheen 2001; Karim *et al.* 2002; Waseem, *et.al*. 2011) who reported *in vitro* rooting of *Chrysanthemum morifolium* shoots on media with 0.2 mg\l IBA.

As the IBA concentration increased, root length decreased. Root length was significantly low at 0.5 mg/l of IBA, and IBA–free medium resulted in significantly the highest root length. Similar results were obtained on *in vitro* rooting of the banana cultivar Tanduk (Elhory *et al.* 2009).

The results of the present study indicate that culturing nodal explants on MS medium supplemented with BAP or kinetin at 0.1mg/ l for shoot regeneration and multiplication and with 0.5 mg/l IBA for rooting of regenerated shoots is quite sufficient for *in vitro* production of *C. morifolium* plantlets.

Table 5. Effect of IBA concentration on rooting of *C. morifolium* shoots cultured for 6 weeks on MS basal medium (data is a mean of 20 explants)

IBA Concentration (mg\l)	Rooted shoots (%)	Number of roots/ shoot	Length of root (cm)
0	100	3.54c	1.26a
0.1	100	15.64b	1.14a
0.2	100	16.52ab	1.04a
0.5	100	20.32a	0.52b

Means, within a column, followed by the same letter (s) are not significantly different at P = 0.05, according to Duncan's Multiple Range Test.

REFERENCES

Bajaj, Y.P.S. (1992). A suggested method for *in vitro* long term storage at 4°C of Chrysanthemum and petunia germplasm. *Plant Tissue Culture* 3, 57-58.

Ben-Jaacov, J. and Langhans, R.W. (1972). Rapid multiplication of chrysanthemum plant by stem tip proliferation. *HortScience* 7, 289-290.

Brar, M.S.; Al-khayri, M. and Klingaman, G.L. (1995). Effect of thidiazuron and benzylaminopurine on *in vitro* shoot proliferation of carnation (*Diathus caryophyllus* L.). *Proceedings of Arkansas Academy of Science* 49, 30-33.

Elhory, S.M.A.; Aziz, M.A.; Rashid, A.A. and Yunus, A.G. (2009). Prolific plant regeneration through organogenesis from scalps of *Musa* sp. cv. Tanduk. *African Journal of Biotechnology* 8 (22), 6208-6213.

Faisal, S.M. and Amin, M.S. (2000). Rapid multiplication of two chrysanthemum cultivars through *in vitro* shoot tip culture. *Plant Tissue Culture* 10(2), 131-136.

Furuya, H. (1999). Development of rapid mass propagation of chrysanthemum by shoot tip culture for superior mother plants cv. 'Shuho no Chikara'. *Bulletin of the Hiroshima Prefectural Agriculture Research Center* 67, 33-40.

Gul, A. (2001). *Micropropagation of* Chrysanthemum. M.Sc. thesis Department of Botany, University of Peshawar, Pakistan.

Hoque, M.l. and Fatema, M. (1995). *In vitro* multiple shoot regeneration in *Chrysanthemum morifolium* Ramat. *Plant Tissue Culture* 5(2), 153-162.

Hoque, M.I.; Jaham, M.T. and Sarker, R.H. (1998). *In vitro* shoot Regeneration and *ex vitro* rooting in *Chrysanthemum morifolium* Ramat. *Plant Tissue Culture* 8 (1), 157-164.

Karim, M.Z.; Amin, M.N.; Asad, Z.U.; Islam, S.; Hossin, F. and Alam, R. (2002). Rapid multiplication of *Chrysanthemum morifolium* through *in vitro* culture. *Pakistan Journal of Biological Sciences* 5(11), 1170-1172.

Khan, M.A. Khanam, D.; Ara, K.A. and Hossaib, A.K.M. (1994). *In vitro* plant regeneration in *Chrysanthemum morifolium* Ramat. *Plant Tissue Culture* 4(1), 53-57.

Kharrazi1, M.; Nemati, H.; Tehranifar, A.; Bagheri, A. and Sharifi, A. (2011). *In vitro* Culture of Carnation (*Dianthus caryophyllus* L.) Focusing on the Problem of Vitrification. *Journal of Biological and Environmental Sciences* 5 (13), 1-6.

Levin, R.; Gaha, V.; Tal, B.; Hirsch, S.; Denola, D. and Vasil, I. (1988). Automated plant tissue culture for mass propagation. *Biotechnology* 6, 1035-1040.

Murashige. T, and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarium* 15, 473-479.

Prasad, R.N.; Sharma, A.K. and Chaturvedi, H.C. (1983). Clonal multiplication of *Chrysanthemum morifolium* "Otome zakura" in long-term culture. *Bangaladesh Journal of Botany* 12, 96–102.

Rout, G.R.; Palai, S.K.; Panday, P. and Das, P. (1996). Direct plant regeneration of *Chrysanthemum morifolium* Ramat. cv. Deep Pink: influence of explant source, age of explants, culture environment, carbohydrates, nutritional factors and hormone regime. *Proceedings of the National Academy of Sciences, India* 67, 57–66.

Sarker, R.H. and Shaheen, I. (2001). *In vitro* propagation of *Chrysanthemum morifolium* Ramat. through callus culture. *Plant Tissues Culture* 11(1), 85-91.

Singh, K. and Arora, J.S. (1995). *In vitro* multiplication of *Chrysanthemum morifolium* Ramat (V. Riot.). *Ornamental Horticulture* 2 (1-2), 63-68.

Waseem, K.; Jilani, M.S.; Khan, M.S.; Kiran, M. and Khan, G. (2011). Efficient *in vitro* regeneration of chrysanthemum (*Chrysanthemum morifolium* Ramat) plantlets from nodal segments. *African Journal of Biotechnology* 10 (8), 1477-1484.

الإكثار النسيجي لنبات الأراولا باستخدام العقد الساقية

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المستخلص: أجريت هذه الدراسة لبحث تأثير بعض منظمات النمو على إستجابة نبات الأراولا للزراعة النسيجية من حيث تكون السيقان وتضاعفها وتجذيرها بغرض تطوير تقنية للاكثار الدقيق لهذا النبات. زرعت العقد الساقية في وسط موراشيجي وسكوج (MS) المحتوي على السايتوكينينات بنزايل أمينوبيورين (BAP) والكاينتين (Kinetin) كل بتركيز 0.0 ، 0.1 ، 0.2 ، 0.5 ، 1.0 ، 2.0 ، 5.0 ، 10.0 مليجرام/ لتر لتكوين الأفرع . فصلت الأفرع المتكونة وزرعت في وسط MS المحتوي على السايتوكينينات BAP و Kinetin كل بتركيز 0.0 ، 0.1 ، 0.2 ، 0.5 مليجرام/ لتر بغرض تضاعف الأفرع . زرعت الأفرع بعد ذلك في وسط MS المحتوي على الأوكسين حمض الاندول بيوتيرك (IBA) بتراكيز 0.0 ، 0.1 ، 0.2 ، 0.5 مليجرام/ لتر بغرض التجذير . تم استخدام 20 عقدة ساقية لكل معاملة . جمعت بيانات عن نسبة العقد الساقية التي استجابت (كونت أفرع) ، متوسط عدد الأفرع في العقدة ، طول الفرع ، نسبة الأفرع المجذرة ، عدد الجذور في الفرع وطول الجذر . أعطى التركيز 0.1 مليجرام/ لتر من كل من BAP و Kinetin أفضل إستجابة لتكوين السيقان وتضاعفها معطياً أعلى عدد من السيقان في العقدة الساقية . أعطى الوسط الخالي من السايتوكينين أعلي طول للسيقان . أعطت كل تراكيز IBA نسبة تجذير 100% لكن التركيز 0.5 مليجرام/ لتر أعطي أعلي عدد من الجذور في الفرع . أعطى الوسط الخالي من IBA أعلى طول للجذور .