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Effects of Different Concentrations of 2,4-D and BAP on Somatic Embryogenesis Induction in Saffron (*Crocus sativus* L.)

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Abstract: To optimize an *in vitro* protocol for propagation of saffron through somatic embryogenesis, effects of various concentrations of 2,4-D (0, 0.25, 0.5, 1, 2, 4 and 8 mg L^{-1}) in combination with BAP (0, 0.25, 0.5, 1, 2, 4 and 8 mg L^{-1}) were studied. Surface-sterilized corms were cut transversally into equal portions and the upper or lower parts were used separately as explants. All treatments were maintained in the darkness at 24±2 ℃. After 70 days, the first globular embryos were observed and the number of embryos on each explant reached to its maximum 3 months after culture. Statistical analysis showed that there were significant differences between treatments regarding the number of embryos induced on each explant. The most effective treatment was 2.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP for both types of explant (inducing 6.5±1.3 and 35.95±4.9 embryos on each explant for the upper and lower parts, respectively). The average percentages of explants showing embryogenic response were 33.3 and 93.3% for the upper and the lower part of corm tissue respectively in this treatment. Complementary studies are in progress to optimize maturation and germination stages of these somatic embryos.

Key words: Saffron (*Crocus sativus* L.), somatic embryogenesis, *in vitro*, propagation, cormogenesis

INTRODUCTION

Saffron (*Crocus sativus* L.) is a perennial, triploid and genetically sterile plant which is propagated vegetatively by corm. This plant was the first spice that cultivated by human and now it is known as one of the most expensive and precious cultivated plants. (Sedighara, 2003). While annual production of saffron is estimated to be 205 tons in the world, Iran is said to produce 80% of this, i.e., 160 tons (Fernandez, 2004). Saffron is a strategic plant in Iran and has many socio-economical advantages such as exportation and income revenue, creating job opportunities, avoiding migration of villagers to cities etc. These make it very special and unique among other crops in some eastern and central parts of Iran (Kafi *et al*., 2002).

Traditionally, saffron is propagated vegetatively by planting the young cormlets annually formed at the top of the mother corm. From the point of quality and quantity views the best saffron yield produce when the old corms are replaced once every 4-5 years (Sadeghi *et al*., 2003). But after 7-8 years, different pathogenic agents (bacterial, viral and fungal) can infect the corms and reduce saffron production. Using these infected corms in new plantation areas will spread the infections widely. So, under these conditions, productions of pathogen-free plant materials would not be easily possible. Considering mentioned problems, using tissue culture and micropropagation techniques for mass propagation of pathogen-free saffron corms can be very advantageous (Bagheri and Vesal, 2003).

In vitro propagation protocols have been developed for many species through somatic embryogenesis using different kind of explants. Propagation by this method is one of the efficient ways for proliferation of desirable plants (Jimenez and Thomas, 2005). Somatic embryogenesis can be the most promising technique for *in vitro* plants propagation. One outstanding characteristic of somatic embryogenesis is the continuation of growth and development of many embryos. Somatic embryogenesis can be induced from both somatic and zygotic tissues. Auxin is one of the most important factors for induction of somatic embryogenesis both in monocots and dicots. Among auxins, 2,4-D can affect the embryo induction by its direct or indirect influence on metabolism of phytohormones which are involved in this phenomenon

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(Raemarkers *et al*., 1995). Furthermore, 2,4-D can act as a stress factor for changing direction of cell differentiation to embryogenesis (Feher *et al*., 2003). Somatic embryogenesis can be induced using a mixture of cytokinin and auxin too. To induce somatic embryogenesis, a balance of auxin and cytokinin is important. BAP is one of the cytokinins commonly used in combination with other growth regulators (Gaj, 2004).

There are some reports describing *in vitro* propagation of saffron through regeneration system (Georg *et al*., 1992; Radjabian, 1992; Blazquez *et al*., 2004; Karamian, 2004). However, there are not many reports or a detailed protocol published on somatic embryogenesis induction of saffron. In this research, various concentrations of 2,4-D and BAP were applied on corm tissue explants of saffron to induce somatic embryogenesis. Then the induced somatic embryos were used for cormlet production. Experiments on optimizing the medium for obtaining maximum somatic embryos and also the best culture medium for their maturation and cormlet production are being conducted in the second step.

MATERIALS AND METHODS

Plant materials (corms) were collected in September 2005 from a 4-year old saffron farm in Torbat e Heydaryeh (Khorasan province). The scales were removed and the corms were washed with tap water for 30 min. For surface sterilization; 70% ethanol was used for 1 min, followed by 20% sodium hypochlorite solution for 15 min and washing 3 times with sterile distilled water. After that, 0.15% (w/v) mercuric chloride solution was used for 7 min, then washing again with sterile distilled water for 3 times. Very thin layer from the surface of the corms was removed to eliminate dead or damaged cells caused by mercuric chloride. In order to investigate the effect of different types of explant, each corm was divided into the upper and the lower parts by cross-sectioning. These two kind of explants were cultured on MS basal medium containing different concentrations of 2,4-D and BAP as described in Table 1.

Cultures were maintained under dark conditions in a growth chamber at 24±2°C and monitored weekly under stereo microscope. Numbers of globular embryos induced on each explant were counted every 4 weeks after culture. All treatments had at least 4 replications, each containing 4 explants. Data were analyzed as a factorial experiment based on completely randomized design using SPSS Software, Ver. 12.

Table 1: Treatments of various concentrations of 2,4-D and BAP

	$D_{0.25}$-B_8	$D_{0.5}$-B_4	D_1-B_2	D_2-B_1	D_1-B_1	D_4-$B_{0.5}$	D_8-$B_{0.25}$
2,4-D mg L^{-1}	0.25	0.5	1	2	1	4	8
BAP mg L^{-1}	8	4	2	1	1	0.5	0.25

RESULTS AND DISCUSSION

After 70 days, the first globular embryos were observed and the number of embryos on each explant reached to its maximum 3 months after culture. The embryos were recognized as creamy-light-brown structures and could be separated easily by tip of forceps (Fig. 1). Statistical analysis of data in term of percentage of embryogenic response and number of embryos on each explant showed significant differences among treatments regarding the type of explants, 2,4-D and BAP concentrations and their interaction effects (Table 2). Explants from the lower part of the corm tissue, without consideration of growth regulators showed more embryogenic response and produced more embryos compared to the upper part (Fig. 1a, b and Fig. 2). The greatest embryogenic response (93.3%) was observed from the lower part explants using a concentration of 2 mg L^{-1} 2,4-D and 1 mg L^{-1} BAP (D_2-B_1) (Fig. 2a). The maximum number of embryos (35.95 globular embryos on each explant) was obtained on D_1-B_2 treatment (containing 2 mg L^{-1} 2,4-D and 1 mg L^{-1} BAP) again from the lower part explants (Fig. 2b).

It seems that these combinations provided more suitable conditions for progression of totipotant somatic cells in to somatic embryos. Increasing the concentration of auxin more than 2 mg L^{-1} showed a reverse effect on embryogenesis which is different from the results reported by Karamian (2004) who observed the highest embryogenesis induction at 4 mg L^{-1} 2,4-D and 1 mg L^{-1} BAP or from Radjabian (1992) who achieved embryogenesis using 4 mg L^{-1} NAA and 1 mg L^{-1} BAP.

Different responses observed from the upper and the lower parts of the corm to embryogenesis can be possibly attributed to polar transportation of auxin and the existence of a gradient level of free auxin (Cook *et al*., 1993). It has also been observed that polar transport of auxin is essential for the establishment of bilateral symmetry during embryogenesis in dicotyledonous somatic (Schiavone and Cooke, 1987) and zygotic (Liuc *et al*., 1993) embryos and more recently it was also demonstrated for monocotyledonous zygotic embryos (Fisher and Neuthaus, 1996). For this gradient to be established, relatively high levels of free IAA may be necessary in the competent tissue.

In the present research, lower part segment of the corm tissue was found to be more embryogenic responsive compared to the upper part segment and produced the highest globular embryos per each explant when cultured on MS medium containing 2 mg L^{-1} 2,4-D and 1 mg L^{-1} BAP and was considered as the best treatment for induction of somatic embryogenesis.

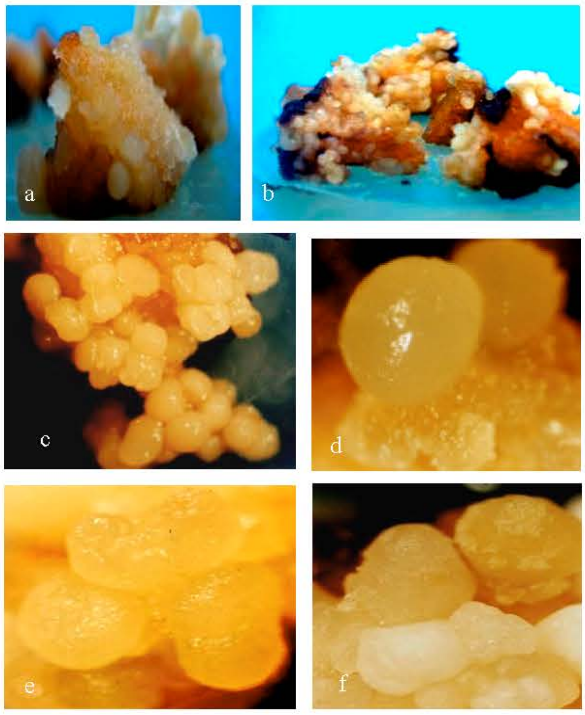

Fig. 1: Induction of somatic embryos in saffron using combination of 2,4-D and BAP: a. Globular embryos developed from the upper part explant after 3 months of culture. b. Globular embryos developed from the lower part explant after 3 months of culture, c. Globular embryos on the lower part explant (closer view). d. An individual globular embryo. e and f. Somatic embryos apparently developing to a bipolar stage

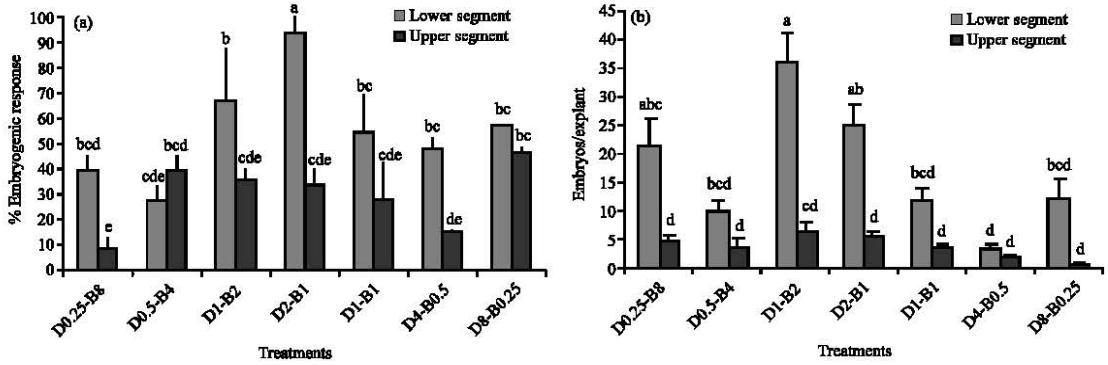

Fig. 2: Effects of various concentrations of 2,4-D and BAP and the upper or lower part segments of saffron corm tissue on percentage of embryogenic response (a) and number of embryos formed on each explant (b), 3 months after culture. Mean values with the same letters are not significantly different

Table 2: Effects of various concentrations of 2,4-D combined with BAP and explant types on percentage of embryogenic response and number of somatic embryos induced on each explant after 3 months of culture

	% Embryogenic response		No. embryos/explant	
	df	MS	df	MS
2,4-D and BAP Conc.	6	1164.68**	6	587.6**
Explant types	1	6832.98**	1	3668.3**
Conc.*explant	6	722.41*	6	327.2*
Error	28	241.22	95	147.6
Total	41		108	

*, **Significant at 0.05 and 0.01 probability, respectively

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REFERENCES

Bagheri, A. and S. Vesal, 2003. Saffron improvement in Iran, development and barriers. Tertian National Symposium on Saffron. Mashhad, Iran 2-3 Dec., 2003, pp: 1-10.

Blazquez, S., A. Piqueras, M.D. Serna, J.L. Casas and J.A. Fernandez, 2004. Somatic embryogenesis in saffron: Optimisation through temporary immersion and polyamine metabolism. Acta Hortic., 650: 269-276.

Cook, T.J., R.H. Racusen and J.D. Cohen, 1993. The role of auxin in plant embryogenesis. Plant Cell, 5: 1494-1495.

Feher, A., T.P. Pastenak and D. Dudits, 2003. Transition of somatic plant cells to an embryogenic state. Plant Cell, Tiss. Org. Cult., 74: 201-228.

Fernandez, J.A., 2004. Biology, biotechnology and biomedicine of saffron. Re. Res. Devel. Plant Sci., 2: 127-159.

Fisher, C. and G. Neuthaus, 1996. Influence of auxin on the establishment of bilateral symmetry in monocots. Plant J., 59: 659-669.

Gaj, M.D., 2004. Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana* Heynt. Plant Growth Regul., 43: 27-47.

Georg, P.S. S. Visvanaths, G.A. Ravishankar and L.V. Venkataraman, 1992. Tissue culture of saffron (*Crocus sativus* L.): somatic embryogenesis and shoot regeneration. Food Biotech., 6: 217-223.

Jimenez, V.M. and C. Thomas, 2005. Participation of plant hormones in determination and progression of somatic embryogenesis. Plant Cell Monog., 30: 103-118.

Kafi, M., M.H. Rashed, A. Kochake and A. Molapheyayi, 2002. Saffron Production, Technology, Processing. Ferdosi University Mashhad Publication, Iran.

Karamian, R., 2004. Plantlet regeneration via somatic embryogenesis in four species of *Crocus*. Acta Hortic., 650: 253-259.

Liuc, M., Z.H. Xu and N.H. Chua, 1993. Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell, 5: 621-630.

Radjabian, T., 1992. Study of saffron pigment in plants produced using tissue culture. M.Sc. Thesis, Tehran University. Iran.

Raemakers, G.J.J.M., E. Jacobsen and P.G.F. Visser, 1995. Secondary somatic embryogenesis and application in plant breeding. Euphytica, 31: 93-107.

Sadeghi, B. A. Aghamiri and A. Negari, 2003. Effect of summer irrigation on saffron production. Tertian National Symposium on Saffron, Mashhad, Iran 2-3 Dec., pp: 171-172.

Schiavone, F.M. and T.J. Cooke, 1987. A geometric analysis of somatic embryo formation in carrot cell cultures. Can. J. Bot., 63: 1573.

Sedighara, P., 2003. Medical characteristics of saffron. Tertian National Symposium on Saffron, Mashhad, Iran 2-3 Dec., 2003, pp: 368-382.