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ORIGINAL ARTICLES

Comparative effects of BAP and NAA on explant development of micropropagated saffron (*Crocus sativus* L) corms.

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ABSTRACT

In vitro propagation of saffron is considered to be an efficient alternative method for large-scale propagation of pathogen free corms. The shoots initiated from the Upper parts of the corm and Sprout on the corms maintained at room temperature were excised and used as explants. The scales were removed and the explants were washed with running tap water for 30 minutes and surface sterilized with 90% ethanol for 3 minutes. different types of corm explants (Sprout, upper and lower parts of the corm tissue) were cultured on Murashige and Skoog medium to find out the best explant type cultured to induce the highest explant development. Different concentrations of BAP (6-benzylaminopurine) at the rates of 0.0, 2.0, 4.0 and 6.0 mg/L and NAA (naphthalene-acetic acid) at the concentration were tested. Data indicated that Upper parts surpassed Sprout and lower parts corm explants in explant development. Also, lower parts of the corm surpassed both upper parts and Sprout explants in callus initiation. On the other hand, Lower part of the corm surpassed both Upper part of the corm and Sprout explants in callus initiation. Higher BAP concentration (6.0 mg/L) enhanced callus production. However, BAP at the rate of 4.0 mg/L enhanced shoot length, number of shoots and callus initiation. The best root length, number of roots and callus initiation were obtained when 4.0 mg/L NAA was added to the medium. Also, NAA at the rate of 6.0 mg/L enhanced callus production.

Key words: Saffron (*Crocus sativus* L), *In vitro* propagation, Sprout, callus production, BAP (6-Benzylaminopurine), NAA(α-Naphthalene acetic acid), MS (Murashige and Skoog media).

Introduction

Saffron (*Crocus sativus* L.) is a perennial, triploid and genetically sterile plant. 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).Conventional methods are insufficient: therefore, saffron cultivation needs efficient mass production of pathogen-free corms. Micropropagation of saffron has therefore been advocated as the best alternative for its propagation (George *et al.* 1992; Ahuja *et al*. 1994).

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).

The explant develops sprouts, shoots and mini corms when inoculated on Murashige and Skoog (MS) medium supplemented with different concentration of BA, NAA, sucrose and paclobutazol or CCC (Raja *et al*., 2007; Salwee *et al*., 2011). The effect of various concentration of TDZ,BA and 2,4-D on somatic embryogenesis from 5 different types of corm explants terminal or axillary buds, upper or lower parts of the corm tissue and terminal buds(Sheibani *et al* 2007; Rajabpoor *et al*.,2007).

Complete plantlets with well developed root system and corm formation were obtained on transferring germinated embryos to half strength MS medium supplemented with BAP ($5x10^{-6}$M) + NAA (5×10^{-6}) of saffron, *crocus sativus* L. (Ebrahimzadeh *et al.,* 1996).

Sharafzadeh and Khosh Khui (2004) produced microcorms on corm explants and leaves were regenerated by subculturing of microcorms. Homes *et al*., (1987) produced microcorms on corm explants. Ovary wall explants gave the best response, with stigma and style-type structures regenerating from the explants, (Choob *et*

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al., 1994). Corms of *C. sativus* were induced on shoot explants and from callus (Nehvi *et al.,* 2007). Style and perianth explants produced stigma-like structures (Ebrahimzadeh and Karamian, 2000).

Embryogenic callus of *C. sativus* was initiated from shoot meristems by Karamian and Ebrahimzadeh (2001). Non-embryogenic and embryogenic callus were produced by Darvishi *et al*., (2007).

The corms with adventitious shoots were rooted in medium without growth regulators and were able to generate dormant microcorms in vitro (Parray *et al.,* 2010).

In the present study, effect of various concentrations of BAP and NAA on saffron somatic embryogenesis induction from different types of corm explants (Sprout, upper and lower parts of the corm tissue) was investigated.

Materials and Methods

This study was carried out at the Biotechnology laboratory of Pomology Department, National Research Center during the period from 2009 to 2011. The shoots initiated from the Upper parts of the corm and Sprout on the corms maintained at room temperature were excised and used as explants. The scales were removed and the explants were washed with running tap water for 30 minutes and surface sterilized with 90% ethanol for 3 minutes and then 0.2% (w/v) $HgCl_2$ solution for 10 minutes and finally the explants were rinsed with sterile distilled water 3 times for 15 min, then sterilized with 20% Clorox (commercial bleach) with 0.1% tween-20 for 15 min then, washed with sterilized distilled water 3 times for 15 min each. The prepared explants were cultured on MS medium (Murashige and skoog, 1962) supplemented with 100 mg/L myoinstol, 0.5 mg/L BA (benzyl adenine), 0.1 mg/L IBA (Indol 3-butyricacid), 30 g/L sucrose and 7 g/L Difico-Bacto agar which was considered as basal medium. The pH of the medium was adjusted at 5.8 and autoclaved at 121°C and 1.5 $Ib/inch^2$ for 25 min. All the cultures incubated in the culture room under controlled conditions, where temperature was maintained at 25 $\pm$ 2°C and kept under dark conditions until explants initiation was occurred then transferred to light intensity of about 2500 Lux with white fluorescent lamps. The photo period was 16 hr light and 8 hr dark. Thus, the following experiment was carried out.

1- Effect of explants type:

Sprout, upper and lower parts of the corm as explants were tested to select the best explant type cultured to induce the highest explant development.

2- Effect of different concentrations of BAP and NAA:

Upper parts of the corm explants cultured on Murashige and Skoog medium were supplemented with BAP (6-benzylaminopurine) at the rates of 0.0, 2.0, 4.0 and 6.0 mg/L and NAA (naphthalene-acetic acid) at the concentration that induce the highest growth parameters.

Data and calculations:

Scores were given for necrosis (determined as dead cells or parts of the plantlets), callus (volume), growth (referred for the plantlets growth) and greening (determined as the degree of keeping the explant with original green color of the degree of green leaves). These scores were given as follow:

Negative results=1, below average=2, average=3, above average=4 and excellent=5 according to (pottino 1981).

Also, shoot and root length (cm), number of shoots and roots (number) were included.

The treatments used in this study were arranged in a complete randomized block design and replicated 5 times for each treatment. Results of the measured parameters were subjected to computerized statistical analysis using MSTAT package for analysis of variance (ANOVA) and means of treatments were compared using LSD at 0.05 according to (Snedecor and Cochron 1980).

Results and Discussion

Effect of explants type:

Figure (1) and Photo (1) show the effect of explant type on its development, such as shoot initiation, callus initiation and greening parameters of saffron (*Crocus sativus* L). It is clear that Upper part of the corm explants gave highly significant increase in explant development (5.00), followed by Sprout (3.33) as compared with Lower part of the corm (2.67). Mean while, Lower part of the corm explants significantly increased callus initiation production comparing to the others. However, significant increase in shoot initiation production was obtained when Sprout

explants was used (4.33). Also Upper part of the corm explants was the best explant type as it significantly had reduced necrosis (1.00), and increased explant development (5.00), and greening in comparison with Sprout and Lower part of the corm explants.

Generally Upper part of the corm surpassed Sprout and Lower part of the corm explants in explanted development and greening parameters. On the other hand, Lower part of the corm surpassed both Upper part of the corm and Sprout explants in callus initiation. These results assured the findings of plessner *et al.* 1990, they found that various plant tissue have been used for regeneration and induction of somatic embryogenesis in saffron including terminal bud.

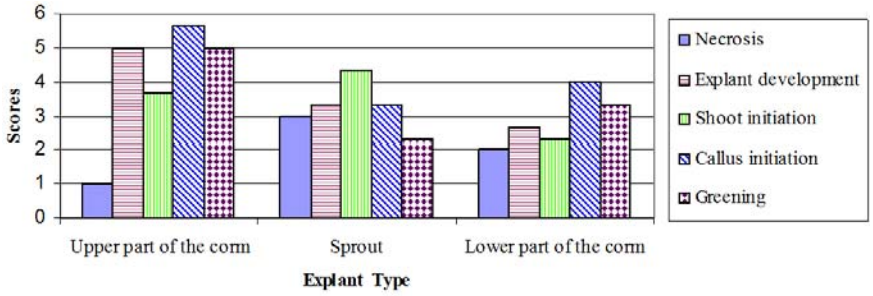

Fig. 1: Effect of explant type on explant development, shoot intiation, callus initiation and greening parameters of saffron (Crocus sativus L.).

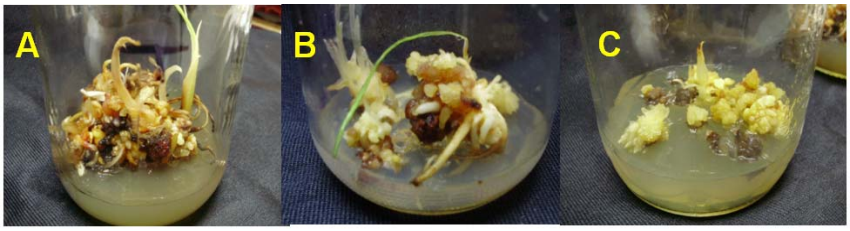

Photo 1: Effect of explant type on explant development.
A: Upper part of the corm B: Sprout C: Lower part of the corm

Effect of BAP concentrations:

Data in Figure (2) show the effect of different concentrations of BAP on shoot length, number of shoots, callus initiation and callus production of saffron (*Crocus sativus* L). It is clear that the concentrations of BAP (4.00 mg/L) had significantly superior effect on shoot length (6.00), number of shoots (10.00), and callus initiation (4.83) in comparison with the other BAP concentrations used. Meanwhile, callus production was significantly increased, it was (5.00), when the concentration of BAP (6.00 mg/L) was used as compared with the other treatments (0.0, 2.00 and 4.00mg/L). Moreover, the concentration of BAP (2.0 mg/L) significantly decreased necrosis. Thus, the previous results indicate that BAP at low concentration (2.00 mg/L) slightly enhanced callus production compared to control. Mean while BAP at 4.0 mg/L increased shoot length, number of shoots and callus initiation. These results are in harmony with the findings of piqueras and Fernandez (2004) reported that BAP was the most efficient cytokinin for shoot proliferation from saffron meristems.

Effect of NAA concentrations:

Data presented in Figure (3) and photo (3) show that supplementing the medium with (4.0 mg/L) of NAA significantly increased root length (9.33 cm) followed by using (2.00 and 6.00 mg/L) as compared with the control. However, number of roots, callus initiation significantly increased when the concentration of NAA (4.00 mg/L) was used. Whereas callus production was the highest (5.00 Score) when the concentration 6 mg/L was added

Meanwhile, using lower NAA concentrations (0.00 and 2.00 mg/L significantly decreased necrosis in comparison to the higher concentrations (4.00 and 6.00 mg/L). Generally, these results indicate that NAA at high concentration (4.00 mg/L) enhanced root length and number of roots. These results are in harmony with the findings of Sarma *et al*. (1990) reported in vitro production of stigma like structures from stigma explants of

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Crocus sativus L. MS medium supplemented with NAA (10 mg dm^{-2}) and BA (1mg dm^{-3}) induced the optimum response. NAA was found to be an important additive to achieve a good response.

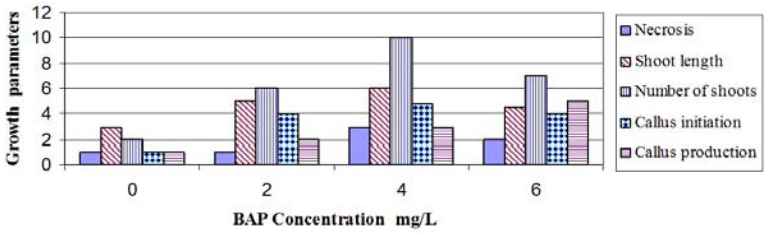

Fig. 2: Effect of different concentrations of BAP on shoot length, number of shoot, callus initiation and callus production of saffron (*Crocus sativus* L.).

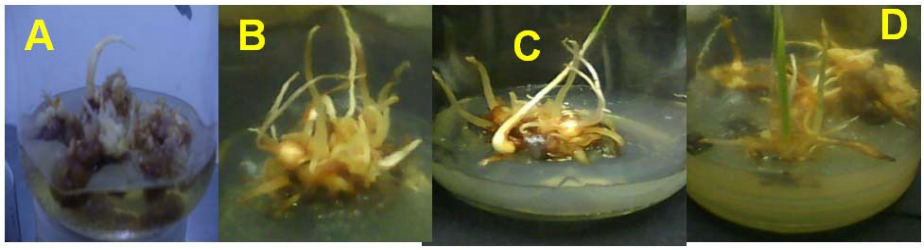

Photo 2: Effect of different concentrations of BAP on shoot length, number of shoots, callus initiation and callus production of saffron (*Crocus sativus* L.).

A: 0.00 mg/L BAP (control) **B**: 2.00 mg/L BAP

C: 4.00 mg/L BAP **D**: 6.00 mg/L BAP

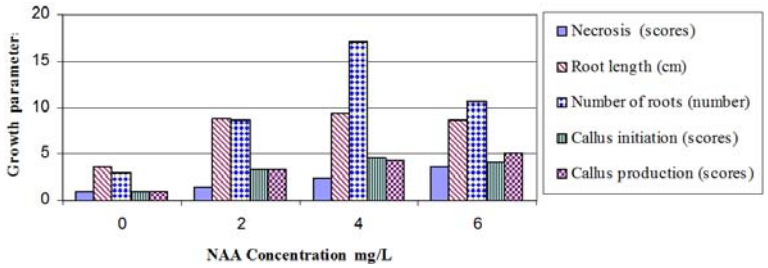

Fig. 3: Effect of different concentrations of NAA on root length, number of roots, callus initiation and callus production of saffron (*Crocus sativus* L.).

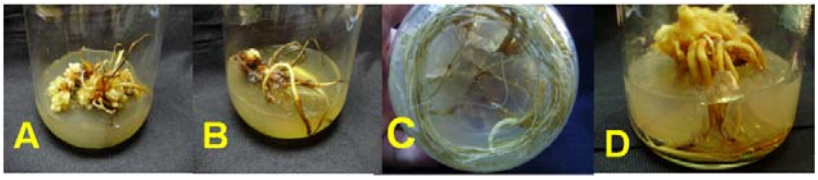

Photo 3: Effect of different concentrations of NAA on root length, number of roots, callus initiation and callus production of saffron (*Crocus sativus* L.).

A: 0.00 mg/L NAA (control) **B**: 2.00 mg/L NAA

C: 4.00 mg/L NAA **D**: 6.00 mg/L NAA

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