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In vitro regeneration of Crocus sativus L.

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Abstract For *in vitro* regeneration of saffron, the effects of 2,4-D (2,4-dichlorophenoxyacetic acid) and BAP (6-benzylaminopurine) were tested initially. It was observed that 1 mg/L 2,4-D and 1 mg/L BAP combination was favorable for direct organogenesis and 0,25 mg/L 2,4-D and 1 mg/L BAP combination was superior for indirect organogenesis. Frequency regeneration by in vitro indirect organogenesis was very low. Callus induction rate was 5%. Not all fragments produced embryogenic callus, moreover, many embryos failed to develop. The best results were obtained by direct organogenesis. All tested individuals had regeneration ability. The number of regenerants per explant varied between 0 and 7.

Key words

saffron; *in vitro* micropropagation; direct organogenesis; indirect organogenesis

Saffron, *Crocus sativus* L., is an important crop cultivated as the source of its spice for at least 3,500 years. It is a spice derived from the flower of the saffron (*Crocus sativus*), a species of *Crocus* in the family Iridaceae. Dried stigmas of saffron flowers compose the most expensive spice which has been valuable since ancient times for its odoriferous, coloring, and medicinal properties (1). It is considered to be the highest priced spice in the world (2). Saffron has been also used as a drug to treat various human health conditions such as coughs, stomach disorders, colic, insomnia, asthma and cardiovascular disorders (2,3). Some researchers also indicated that saffron might be a potential anticancer agent (3, 4, 5, 6).

Within the last few decades, an increasing number of bulbous and cormous monocotyledons have been successfully cultured. Tissue culture technology was greatly influenced by the demand of rapid multiplication and clonal propagation of slow-growing monocots. Comparatively, bulbous and cormous monocotyledons are regarded as difficult *in vitro* material. Recently there are many attemps to in vitro propagation of Crocus (7, 8, 9, 10, 11; 12)

The aim of this study was *in vitro* micropropagation of saffron (*Crocus sativus* L.) using direct and indirect organogenesis.

Biological Material and Methods

For the initiation of in vitro cultures were used corms fragments and buds. The corms were first thoroughly washed under tap water and immersed in 70% ethanol for 2 min, and then in 5% sodium hypochlorite with 0.1% Tween 20, for 10 min. The corms were cut into segments and were once again sterilized by immersion in 70% ethanol for 10 sec., and then in 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 3 min, followed by four washes with sterile distilled water. Fragments were inoculated on Murashige - Skoog (MS) media added with appropriate phytohormones, solidified with agar and using instruments and sterile Petri dishes (table 1). We studied 10 individuals. Four explants were cultured on each sterile Petri dish (90 mm diameter) containing 20 ml of medium sealed with Parafilm (four explants x four Petri dishes per each individuals). We used two experimental variants: V1 was used to induce direct organogenesis (adventitious shoot formation by using apical and lateral buds of saffron) and V2 variant to induce indirect organogenesis (callus formation by using corm parts other than buds). A general structure of saffron and organs are shown in Figure 1.

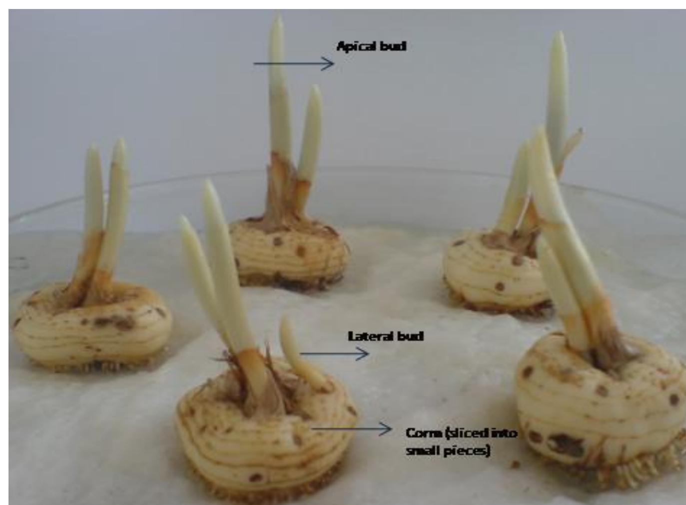

Fig. 1: Explant source

Culture media for the two experimental variants used in this experiment had the same plant growth regulators composition as the combination used in the work of (13) . The media were adjusted to pH 5.8 before sterilization. All culture media were autoclaved at 121^0 C, 1 bar, for 30 min. Cultures were incubated at 25 ± 1^0C under low light intensity and 16 h photoperiod. The regenerants were maintained in aseptic conditions for further growth. Developed plants were transferred to pots and grown in growth chamber under a 16 h photoperiod. In vitro regenerated plants were transferred to pots nutrient substrate for acclimatization in the growth chamber.

Table 1

In vitro culture conditions for inducing organogenesis

Explant	Culture media	Hormonal balance	
corms fragments	Murashige – Skoog (MS)	V_1 direct organogenesis 1mg/l 2,4 D + 1 mg/l BAP	V_2 indirect organogenesis 0,25 mg/l 2,4 D + 1 mg/l BAP

Results and Discussions

Frequency regeneration by in vitro indirect organogenesis was very low. Callus induction rate was 5%. Not all fragments produced embryogenic callus, moreover, many embryos failed to develop. The experiment was continued only through direct organogenesis. The number of regenerants per explant varied between 0 and 7 (table 2).

Table 2

The number of regenerants per explant

No. Crt.	Hormonal balance	Explants/Genotype	Regenerants/explant $\bar{x} \pm s_{\bar{x}}$
	1	2	3
1	V1	16	2,6±0,5
	V2	16	-
2	V1	16	2,8±0,5
	V2	16	-
3	V1	16	2,6±0,5
	V2	16	1±0,1
4	V1	16	3±0,6
	V2	16	0,8±0,1
5	V1	16	2,3±0,4

	1	2	3
	V2	16	1,3±0,2
6	V1	16	2,8±0,5
	V2	16	1,1±0,2
7	V1	16	2,5±0,5
	V2	16	-
8	V1	16	2,2±0,4
	V2	16	-
9	V1	16	2,7±0,5
	V2	16	-
10	V1	16	2,3±0,4
	V2	16	-

Results are expressed as mean ± standard error (SE).

Around 20 plantlets were produced through direct somatic embryogenesis. Plants were transferred to peat and grown in growth chamber for acclimatization. All tested individuals had regeneration ability (Fig. 2).

Fig. 2: Direct organogenesis: new shoots and cormlets

The micropropagation of *Crocus* has been reviewed by Plessner and Ziv (1999) and Bagheri and Vesal (2006). Ding et al. (1979, 1981) were first to report the successful tissue culture of *Crocus*. They successfully regenerated callus and intact plantlets from corm explants when the culture media contained indole- 3- acetic acid (IAA) and 2, 4-D. Later, Homes et al. (1987) observed microcorms forming on 1/8th corm explants. These regenerated shoots when cultured on a medium with 9 lM 2, 4 D. Using a similar medium, Ilahi et al. (1987) produced callus on corm explants that differentiated buds. Schenk and Hildebrandt (1972) reported the importance of medium composition and techniques for induction and growth of monocotyledonous and dicotyledonous plants in cell culture. They found that a high level of auxin-type growth regulating substances generally favored cell cultures of monocotyledonous plants, while low levels of cytokinin were essential for most dicotyledonous cell cultures.

Conclusions

Frequency regeneration by in vitro indirect organogenesis was very low. Callus induction rate was 5%. Not all fragments produced embryogenic callus, moreover, many embryos failed to develop. The best results were obtained by direct organogenesis. All tested individuals had regeneration ability. The number of regenerants per explant varied between 0 and 7.

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