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Standard Review

Micropropagation of genus *Crocus* - a review

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Micropropagation has increasingly become a valuable tool assisting breeders to release new species and cultivars into the market more rapidly. Here we review the progress made in Genus *Crocus* of Family Iridaceae, and highlight the potential for future expansion in this field.

Key words: *Crocus*, micropropagation, iridaceae

INTRODUCTION

Crocus species are members of the family *Iridaceae*. The plants in this family are herbs with rhizomes, corms or bulbs. The family *Iridaceae* embraces about 60 genera and 1,500 species. The genus *Crocus* includes native species from Europe, North Africa and temperate Asia, and is especially well represented in arid countries of south-eastern Europe and Western and Central Asia. Among the 85 species belonging to the genus *Crocus*, *C. sativus* L. (Saffron) is the most fascinating and intriguing species (Fernandez, 2004). The domesticated saffron (*C. sativus*), a fall-flowering perennial plant is a sterile triploid mutant of the eastern Mediterranean fall-flowering *Crocus cartwrightianus* (Deo, 2003). The stigmas are dried for use in medicine, food seasoning and coloring for centuries and are characterized as the most expensive spice by weight. Saffron contains more than 150 volatile and aroma-yielding compounds. It also has many nonvolatile active components, many of which are carotenoids, including zeaxanthin, lycopene, and various α- and β-carotenes. However, saffron's golden yellow-orange colour is primarily the result of α-crocin (Abdullaev, 2002).

Saffron is propagated by corms as the flowers are sterile and fail to produce viable seeds. A corm survives for only one season, producing up to ten "cormlets" that eventually give rise to new plants (Deo, 2003). Therefore, reproduction is human dependent; the corms must be manually dug up, broken apart and replanted. The natural propagation rate of most geophytes including saffron is relatively low.

Besides conventional methods of propagation, *in vitro* cultural methods contribute importantly for the propagation of many important and economic plants (references?). Conventional propagation methods are very slow and propagation by tissue culture represents an important potential to effectively propagate it. In recent years, there is increasing interest to exploit tissue culture and genetic engineering techniques for propagation and genetic improvement of saffron.

The micropropagation of *Crocus* has been reviewed by Plessner and Ziv (1999) and Bagheri and Vesal (2006). What follows is a brief overview including the most recent literature (Table 1). 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 IM 2, 4-D. Using a similar medium, Ilahi et al. (1987) produced callus on corm explants that differentiated buds.

Shoot development on corm explantswas promoted by cytokinins (kinetin or zeatin, 14 - 56 IM) and 2, 4-D (4.5 IM), while corm formation and growth was promoted by ethylene exposure (35 mM, Plessner et al., 1990). Floral organs of four spring-flowering *Crocus* species were investigated for their competence to produce callus by Choob et al. (1994). *C. sieberi* and *C. vernus* exhibited low response whereas *C. chrysanthus* and *C. aureus* had a higher success rate. Ovary wall explants gave the best response, with stigma and style-type structures regenerating from the explants (Choob et al., 1994). Corms of *C. sativus* were induced on shoot explants and from callus and occurred optimally at 10℃ with 3% sucrose under a 16 h photoperiod (Milyaeva et al., 1995). Ovary explants were used for direct shoot regeneration with 53.7 IM NAA and 4.44 IM BA (Bhagyalakshmi, 1999). Under continuous

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Table 1. Summary of Genus *Crocus* micropropagation.

Genus	Species/cultivar	Explant type[a]	Factors[b]	Response[c]	Ex vitro?	References
Crocus	*almehensis*	C, I	H	C, S	?	Ebrahimzadeh et al. (1996)
	aureus	S, I, Ov, A	H, L	C, Mo	No	Choob et al. (1994)
	cancellatus	S	H	C, P, S, O	No	Karamian and Ebrahimzadeh, 2001
	cancellatus	C, I	H	C, S	?	Ebrahimzadeh et al. (1996)
	cancellatus	S	H, L	C, S, O	Yes	Karamian (2004)
	caspius	S	H, L	C, S, O	Yes	Karamian (2004)
	chrysanthus	S, I, Ov, A	H, L	C, Mo	No	Choob et al. (1994)
	gilanicus	C, I	H	C, S	?	Ebrahimzadeh et al. (1996)
	michelsonii	S	H, L	C, S, O	Yes	Karamian (2004)
	sativus	C	H	C, S	No	Ding et al. (1979)
	sativus	C	H	C, S	No	Ilahi et al. (1987)
	sativus	Ov, stigma	H	Stigma	no	Sano and Himeno (1987)
	sativus	C	H, Ex	S	No	Homes et al. (1987)
	sativus	Ov	H	Parthenocarpic fruit	no	Chichiricco and Caiola (1987)
	sativus	C	H, T	S, R, O	No	Plessner et al. (1990)
	sativus	A, Ov	H	Stigma	No	Sarma et al. (1991)
	sativus	C, L, I, A	H, L, T	C, Se, S, O, R Y	Yes	Milyaeva et al. (1995)
	sativus	Ov	H, L, T, M	S, O	No	Bhagyalakshmi (1999)
	sativus	C	H, L, T, M	C	No	Chen et al. (2003)
	sativus	Style	H, T, Ex Age	F	No	Jun et al. (2007)
	sativus	L	H	C, Se, S, O	No	Raja et al. (2007)
	sativus	S	H, L	C, P	No	Darvishi et al. (2007)
	sativus	S	H	S	No	Majourhay et al. (2007)
	sativus	C	H, T, L	C, Se, S, R, O	Yes	Sheibani et al. (2007)
	sativus	C, S, I, Ov, F, Stigma	Decontamination, H	O, S, Se	No	Karaog˘lu et al. (2007)
	sativus	S, C	H, L	Se	No	Bla´zquez et al. (2004a, b)
	sativus	Callus	C, R, TI	Se	No	Bla´zquez et al. (2004a, b)
	sieberi	S, I, Ov, A	H, L	C, Mo	No	Choob et al. (1994)
	speciosus	C, I	H	C, S	?	Ebrahimzadeh et al. (1996)
	vernus	S, I, Ov, A	H, L	C, Mo	No	Choob et al. (1994)

[a]Explant type: A, Anthers; B, Bulb; C, Corm; F, Flowers; Hy, Hypocotyl; I, Inflorescence; L, Leaf; M, Meristem; Ov, Ovaries; P, Plantlets; R, Root; S, Shoot; TS, Twin scales
[b]Factors: Ac, Activated charcoal; Ag, Agar (type or concentration); An, Antibiotics; C, Carbohydrate (level or type); Ex, Explant (type, orientation or age); GM, Genetic modification; H, Hormones (level or type); L, Light; M, Medium (strength or composition); R, Growth retardant; T, Temperature; TI, Temporary immersion
[c]Response: C, Callus; F, Flowering; GM, Genetic modification; Me, Meristemoid formation; Mo, Morphogenesis; O, Storage organ formation; P, Protoplasts; R, Roots; S, Shoots and shoots.

Under continuous darkness, many shoot primordial were formed. These elongated when placed in the light, and formed normal plantlets with corms (Bhagyalakshmi, 1999). Similar results were obtained with the same hormones by Karaoglu et al. (2007). Leaf explants produced callus that regenerated somatic embryos and plantlets when cultured on 10 IM BA and 0.5 IM 2, 4-D (Raja et al., 2007). These were used for microcorm induction which was promoted by a half-strength MS medium plus 9% sucrose (Raja et al., 2007). Culture of immature styles in media containing 26.8 IM NAA and 31.1 IM BA in the dark resulted in flowering of explants (Jun et al., 2007). Using apical meristems, Majourhay et al. (2007) investigated the ability of different cytokinins to induce shoot formation. They found that 22 IM BA produced greater and more vigorous shoots than either 2-isopentenyladenine (2iP) or thidiazuron (Majourhay et al., 2007). One reason for initiating *in vitro* cultures of *C. sativus* is for the commercial potential to produce crocin, safranal and picrocrocin (the flavour and colouring distinctive to saffron) less expensively than conventional means (that is, through manual harvesting of styles). To this end, stigmas

and ovaries have been used as explants to induce morphogenesis and growth of additional stigma-like structures that produce the desired chemical constituents. Successful stigma and ovary formation has been attained when cultured on media containing BA (4.4 - 22.2 IM) and kinetin (4.7 - 23.3 IM, Sano and Himeno; 1987). Style and perianth explants produced stigma-like structures that proliferated forming up to 100 structures per explant (Ebrahimzadeh Karamian, 2000). However, micropropagation may not be feasible for saffron production since stigmas produced using this method, showed much lower levels of crocin (Sarma et al., 1991). One possibility is to rapidly increase callus biomass with 1.1 IM NAA and 4.4 IM BA, then subculture onto media with 1.1 IM IAA and 2.2 IM BA for crocin production (Chen et al., 2003). This two-stage culture system significantly increased production of crocin compared to the one-stage system. Since *C. sativus* is a triploid and cannot produce seeds. Chichiricco and Caiola (1987) investigated the possibility of producing fruit and seeds *in vitro*. The ability to induce parthenogenesis was independent of both the stage of ovary excision and pollination. BA (4.4 IM), gibberellic acid (GA3, 2.9 IM) and 2,4-D (4.4 IM) promoted ovary growth, while abscisic acid (ABA, 3.8 IM) inhibited parthenocarpic fruit development (Chichiricco and Caiola, 1987). Embryogenic callus of *C. sativus* was initiated from shoot meristems with 17.8 IM kinetin and 4.4 IM 2,4-D and used for protoplast culture by Karamian and Ebrahimzadeh (2001). Highest growth rate and cell division occurred in Ca-alginate beads with a ‘nurse culture’ containing 4.4 IM 2, 4-D, 8.9 IM kinetin and 0.57 mM ascorbic acid in the dark. Colonies formed and produced embryogenic callus that regenerated somatic embryos and plantlets (Karamian and Ebrahimzadeh, 2001). The production of non-embryogenic and embryogenic callus for the purposes of protoplast formation was investigated by Darvishi et al. (2004). Non-embryogenic callus formed best on media containing 10.8 IM NAA and 8.9 IM BA, while embryogenic callus formed most frequently with 4.5 IM 2, 4-D and 4.4 IM BA (Darvishi et al., 2004). Somatic embryogenesis without an intervening protoplast culture stage was accomplished for three other *Crocus* species (*C. cancellatus*, *C. caspius*, and *C. michelsonii*) incorporating 21.5 IM NAA and 17.8 IM BA or 4.5 IM 2,4-D and 18.6 IM kinetin (Karamian, 2004) in the media. In some cases, thidiazuron can be as effective as other cytokinins for inducing somatic embryogenesis. The use of liquid culture in a temporary immersion system increased callus weight four times compared to those grown on solid media (Blazquez et al., 2004b). Regeneration of callus into somatic embryos on solid media was improved by addition of 2.4 IM jasmonic acid (Blazquez et al. 2004b). Recently, Blazquez et al. (2004a) showed a link between type, occurrence and expression of antioxidant enzymes (superoxide dismutases and catalase) and the stage of somatic embryogenesis, suggesting these could act as markers of embryogenesis.

Conclusion

In vitro cultural methods contribute importantly for the propagation of many important and economic plants as the rate of conventional propagation methods is very slow. Saffron is propagated by corms as the flowers are sterile and fail to produce viable seeds. Besides, the rate of natural propagation of most geophytes including saffron is relatively low. Tissue culture represents an important potential for its propagation over conventional methods

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