academicJournals

Vol. 13(26), pp. 2637-2640, 25 June, 2014
DOI: 10.5897/AJB2013.12831
Article Number: C95E74645608
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Short Communication

In vitro development and regeneration of microcorms in saffron (*Crocus sativus* L)

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Receive 23 May, 2013; Accepted 25 February, 2014

Saffron (*Crocus sativus* L.) being triploid in nature is propagated by vegetative means through corms. The natural propagation rate of such plant species is relatively low; therefore an *in vitro* multiplication technique like micropropagation has been used as an alternative method of propagation for saffron. In the present investigation, apical bud explants were cultured on different nutrient media supplemented with various concentrations of plant growth regulators. Micro-corm formation was observed on all the media combinations. Maximum number (10) and weight (1.54 g) of microcorms developed were observed on MS media supplemented with 6-benzyl amino purine (BAP) (2 mg/L) + naphthalene acetic acid (NAA) (0.5 mg/L) + paclobutrazol (1.5 mg/L). Cultural conditions under light or in dark did not affect the corm formation and growth. Shoot and root regeneration was observed in the microcorms developed under *in-vitro* conditions. Maximum number of shoot (11.6) and length of shoots (11.4 cm) was also observed on MS media supplemented with NAA (21.6 µM) + BAP (22.2 µM). Maximum number of roots (11) and length of roots (11.4 cm) were obtained on G-5 media containing NAA (21.6 µM) + BAP (22.2 µM). The above observations will be useful as the base to make a possible road way for production of quality planting material in saffron.

Key words: Saffron, growth regulators, micropropagation, apical bud.

INTRODUCTION

Crocus sativus L. (*Iridaceae*) is cultivated in few countries of the world for its highly valued stigmatic lobes. Saffron being triploid (2n = 3x = 24) is sterile and is propagated vegetatively through corms. A corm survives for only one season, producing up to ten "cormlets" that eventually give rise to new plants (Deo, 2003). Corm production is a rate limiting factor in saffron propagation as rate of generation of daughter corms under natural conditions is low (Chahota et al., 2003) which results in limited availability of propagating material for field cultivation. Micropropagation is very good alternative for quality planting material/seed production and large scale multiplication of disease free saffron (Ascough et al., 2009). The successful tissue culture protocol was developed in saffron by several workers (Sharma et al., 2008, Mir et al., 2010). Microcorm production under *in-vitro* conditions shows promise with respect to rate of multiplication and number of microcorms produced in saffron (Darvishi et al., 2007). Regeneration has been described from corm-derived callus cultures via somatic

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Table 1. *In-vitro* corm multiplication in saffron from apical buds.

Medium	BAP mg L^{-1}	NAA mg L^{-1}	Paclobutrazol mg L^{-1}	Number of microcorms	Weight of corm (g)
MS	1.0	0.1	1.0	$8.4^{abc} \pm 0.56$	$0.96^{bc} \pm 0.12$
	1.0	0.5	1.5	$8.4^{abc} \pm 0.25$	$0.6^{cd} \pm 0.35$
	2.0	0.5	1.5	$10.2^{a} \pm 0.40$	$1.54^{a} \pm 0.11$
	2.0	1.0	2.0	$7.6^{bc} \pm 0.25$	$0.48^{cd} \pm 0.02$
G-5	1.0	0.1	1.0	$6.2^{c} \pm 0.48$	$0.72^{cd} \pm 0.10$
	1.0	0.5	1.5	$7.2^{bc} \pm 0.18$	$0.48^{cd} \pm 0.02$
	2.0	0.5	1.5	$8.8^{ab} \pm 0.36$	$1.34^{ab} \pm 0.11$
	2.0	1.0	2.0	$6.0^{c} \pm 0.38$	$0.34^{d} \pm 0.02$

Means followed by the same letter within the columns are not significantly different (P= 0.05) using Duncan's multiple range test.

Figure 1. *In-vitro* corm multiplication in saffron.

embryogenesis, organogenesis and protoplasts (Demeter et al., 2010; Mir et al., 2010) but with low frequencies of normal plant formation. The present study was undertaken for efficient corm multiplication and *in-vitro* regeneration in saffron.

MATERIALS AND METHODS

Explants

The present experiment has been carried out during 2010-2012 at Biotechnology Laboratory of Central Institute of Temperate Horticulture, Rangreth, Srinagar. Saffron corms were grown under controlled conditions in polyhouse of CITH, Srinagar Farm. The bulbs were harvested just before the onset of flowering and apical buds were removed and used for microcorm production and regeneration experiment. Explants were thoroughly washed under running tap water and sterilized by dipping in 70% ethanol for 3-4 min followed by surface sterilization with 0.1% $HgCl_2$ for 10 min and rinsed 5 times with sterile distilled water.

Culture conditions

Basal media employed were (Murashige and Skoog, 1962) and G-5 Gamborg et al., 1968), each at pH 5.8 and with 0.9 or 1% agar. Phytohormones were used at different concentrations. BA (2.22, 22.2, 4.44 and 44.4 µM) and NAA (10.8, 16.2, 21.6 and 27.0 µM) in combinations were used for regeneration and BA (0.5, 1.0, 1.5 and 2.0 mg/L) and NAA (0.1 and 0.5 mg/L) were used for corm multiplication. After preparing the media explants (apical buds) were cultured in glass tubes (90 × 25 mm) and jam bottles (500 ml). Cultures were maintained at 25 ± 1°C under 16/8 h (light/darkness) photoperiod with a light intensity of approximately 4000 lux.

Microcorm formation and regeneration

Cultures were sub-cultured and transferred to fresh media after every 4 weeks. Observations with respect to shoot length, number of shoots, root length, number of roots, number of corms and weight of corms were taken after every six weeks. This study was carried out as a factorial experiment based on completely randomized design (CRD) with 48 treatments in 5 replications.

Statistical analysis

Each treatment was replicated 5 times and observations in stages of development were recorded periodically. The data was analyzed by comparing means using one way analysis of variance (ANOVA) and the significance was determined by Duncan's Multiple Range Test using SAS (v 9.2).

RESULTS AND DISCUSSION

Corm multiplication

Maximum number (10) and weight (1.54 g) of microcorms developed were observed on MS media supplemented with 2 mg/L BAP + 0.5 mg/L NAA + 1.5 mg/L paclobutrazol followed by 8.8 average number of microcorms with 1.34 g average weight on G-5 media supplemented with 2 mg/L BAP + 0.5 mg/L NAA + 1.5 mg/L paclobutrazol (Table 1, Figure 1). Cultural conditions under light or in dark did not affect the corm formation and growth. Our results showed further

Table 2. *In-vitro* shoot and root regeneration in saffron from apical buds.

Medium	NAA (µM)	BA (µM)	Shoot length (cm)	Number of shoots	Root length (cm)	Number of roots
MS	27	44.4	$10.8^{ab} \pm 0.53$	$10^{ab} \pm 0.57$	$7.6^{c} \pm 0.43$	$7.4^{bc} \pm 0.53$
	21.6	22.2	$11.4^{a} \pm 0.51$	$11.6^{a} \pm 0.64$	$10^{ab} \pm 0.38$	$9.8^{ab} \pm 0.43$
	16.2	4.4	$10.0^{abc} \pm 0.35$	$8.8^{abc} \pm 0.69$	$8.4^{bc} \pm 0.20$	$7^{bc} \pm 0.35$
	10.8	2.22	$6.8^{de} \pm 0.43$	$6.6^{bc} \pm 0.56$	$7.2^{c} \pm 0.18$	$5.8^{c} \pm 0.36$
G-5	27	44.4	$8.4^{bcd} \pm 0.25$	$8^{abc} \pm 0.57$	$10.2^{ab} \pm 0.43$	$11.0^{a} \pm 0.89$
	21.6	22.2	$10.4^{abc} \pm 0.37$	$10^{ab} \pm 0.52$	$11.40^{a} \pm 0.46$	$11.4^{a} \pm 0.53$
	16.2	4.4	$08^{cde} \pm 0.35$	$6.8^{bc} \pm 0.55$	$10.0^{ab} \pm 0.22$	$8.8^{abc} \pm 0.53$
	10.8	2.22	$5.6^{e} \pm 0.37$	$5.4^{c} \pm 0.49$	$8.8^{bc} \pm 0.18$	$7.4^{bc} \pm 0.37$

Means followed by the same letter within the columns are not significantly different (P= 0.05) using Duncan's multiple range test.

Figure 2. *In-vitro* shoot (a & b) and root (c & d) regeneration.

improvement over the protocols developed earlier (Mir et al., 2010). *In vitro* micro-corm production of saffron has been obtained by culturing leaf segments and apical buds (Sharma et al., 2008), shoot explants (Milyaeva et al., 1995) and ovary explants (Raja et al., 2007). Microcorm formation from apical bud takes only eight months under *in-vitro* conditions as against 22 months under field conditions.

Shoot and root regeneration

Apical buds started sprouting within 10 days of incubation on culture media; however, only those cultured at appropriate concentrations of NAA and BA produce multiple shoots. Maximum number of shoot (11.6) and length of shoots (11.4 cm) was also observed on MS media supplemented with 21.6 µM NAA + 22.2 µM BAP (Table 2, Figure 2). In the present investigation, the results on shoot regeneration revealed that NAA and BA are essential for shoot regeneration of saffron (*Crocus sativus* L.).

The apical buds inducing multiple shoots (11.6) with length of 11.4 cm was obtained in our studies were higher than that reported earlier from corms (Chauhan et al., 1999), isolated buds (Ascough et al., 2009), ovaries (Demeter et al., 2010) or apical buds (Sharma et al., 2008).

Auxins in combination with cytokinins can greatly influence the frequency of regeneration (Raja et al., 2007; Abbas and Qaiser, 2012; Sivanesan and Jeong, 2012).

Majourhay et al. (2007) investigated the ability of different cytokinins to induce shoot formation. Shoot development on corm explants was promoted by cytokinins while corm formation and growth was promoted by ethylene exposure (Plessner et al., 1990). Maximum number of roots (11) and length of roots (11.4 cm) were obtained on G-5 media containing NAA (21.6 µM) + BAP (22.2 µM). Our results on root multiplication and elongation are better than that reported earlier from corms (Sharma et al., 2008; Raja et al., 2007). The above observations will be useful as the base to make a possible road way for production of quality planting material in saffron. *In-vitro* regeneration in saffron was reported earlier by Mir et al. (2010); Devi et al. (2011); Ahouran et al. (2012); Cavusoglu et al. (2013); Sivanesan et al. (2014).

Our results on micro-corm production and multiplication promise to bridge the gap between land availability for saffron cultivation and availability of quality saffron planting material and regeneration protocol, with help in the development and rapid clonal propagation of novel saffron plant material.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Abbas H, Qaiser M (2012). *In vitro* response of *Ruellia bracteolata* to different growth hormones - an attempt to conserve an endangered species. Plant Cell Tiss. Organ Culture 44(2):791-794.

Ahouran M, Hosseini R, Zarghami R (2012). Corms as a source of explants for the successful clonal propagation of Crocus cancellatus. J. Crop Sci. Biotechnol. 15(1):47-51.

Ascough GD, Erwin JE, Staden J (2009). Micropropagation of *Iridaceae*-a review. Plant Cell Tissue and Organ Culture 97:1-19.

Cavusoglu A, Sulusoglu M, Erkal S (2013). Plant regeneration and corm formation of saffron (*Crocus sativus* L.) *in vitro.* Res. J. Biotechnol. 8(12):128-133.

Chahota RK, Dhiman KC, Rana SS, Singh M (2003). Efficacy of different propagating methods for higher daughter corm production in saffron (*Crocus sativus* L.). Indian Perfumer 47:155-158.

Chauhan RS, Sharma TR, Chahota RK, Singh BM (1999). *In vitro* cormlet production from micro-propagated shoots of saffron (*Crocus sativus* L.). Indian Perfumer 43:150-155.

Darvishi E, Zarghami R, Mishani CA, Omidi M (2007). Effects of different hormone treatments on non-embryogenic and embryogenic callus induction and time-term enzyme treatments on number and viability of isolated protoplasts in saffron. (*Crocus sativus* L.). Acta Horti. 739:279-284.

Demeter Z, Gyula Suranyi V, Molnar A, Sramko G, Beyer D, Konya Z (2010). Somatic embryogenesis and regeneration from shoot primordia of *Crocus heuffelianus*. Plant Cell Tiss. Organ Culture 100:349-353.

Deo B (2003). Growing Saffron – The World's Most Expensive Spice, Crop & Food Research (New Zealand Institute for Crop & Food Research), no. 20, http://www.crop.cri.nz/home/productsservices/publications/broadsheets/020 Saffron.pdf.

Devi K, Sharma M, Sing M, Ahuja PS (2011). *In vitro* cormlet production and growth evaluation under greenhouse conditions in saffron (*Crocus sativus* L.) -A commercially important crop. Eng. Life Sci. 11:189-194.

Gamborg O, Miller R, Ojima K (1968). Nutrient requirement suspensions cultures of soybean root cells. Exp. Cell Res. 50:151-158.

Majourhay K, Fernandez JA, Martınez-Gomez P, Piqueras A (2007). Enhanced plantlet regeneration from cultured meristems in sprouting buds of saffron corms. Acta Horti. 739:275-278.

Milyaeva EL, Azizbekova NS, Komarova EN, Akhundova DD (1995). *In vitro* formation of regenerant corms of Saffron (*Crocus sativus* L.). Russian Journal of Pl. Physio. 42:112-119.

Mir JI, Ahmed N, Rashid R, Wani SH, Itoo H, Sheikh MA (2010). Corm multiplication and development of stigma like structures under *In vitro* conditions in saffron. In*:* Proceedings of National Symposium on Botanical Researches- Present Scenario. Department of Botany, Punjabi University, Patiala. 143:18-19

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15:473-497.

Plessner O, Ziv M, Negbi M (1990). *In vitro* corm production in the saffron crocus (*Crocus sativus* L.). Plant Cell Tiss. Organ Culture 20:89-94.

Raja W, Zaffer G, Wani SA (2007). *In vitro* microcorm formation in saffron (*Crocus sativus* L.) Acta Horti. 739:291-296

Sharma KD, Rathour R, Sharma R, Goel S, Sharma TR, Singh BM (2008). *In vitro* cormlet development in *Crocus sativus*. Biologia Plantarum 52:709-712.

Sivanesan I, Jana S, Jeong BR (2014). *In-vitro* shoot regeneration and microcorm development in *Crocus vernus* (L.) hill. Pak. J. Bot. 46(2):693-697.

Sivanesan I, Jeong BR (2012). Identification of somaclonal variants in proliferating shoot cultures of *Senecio cruentus*cv. Tokyo Daruma. Plant Cell Tiss. Organ Culture 111:247-253.