# *In Vitro* Microcorm Formation in Saffron (*Crocus sativus* L.)

W. Raja, G. Zaffer, S.A. Wani
Division of Plant Breeding & Genetics
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir
Shalimar Srinagar–191121
India

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**Astract**

**In-vitro microcorm production has been obtained by culturing leaf segments of saffron on MS medium containing BA (4.0 mg $l^{-1}$) + NAA (0.50 mg $l^{-1}$) + 9 % sucrose. Maximum explant survival was observed when leaf was used as explants source (48.90 %) and incubated in MS medium supplemented with BA (1.0 mg $l^{-1}$) and 2,4-D (1.0 mg $l^{-1}$). Similar results were obtained for establishment as well as for callusing of initiating cultures of leaf explants. Maximum proliferation of established cultures (56.30) was got with BAP + NAA (2.0 mg $l^{-1}$ + 0.50 mg $l^{-1}$). Plant regeneration through somatic embryogenesis using regenerable embryogenic calli was also obtained from leaf explants cultured in MS medium containing 1.0 mg$l^{-1}$ and 1.0 mg $l^{-1}$ 2,4-d. Somatic embryo formation was attained to the tune of 8.66 embryos per culture by using BA+2,4-D (2.25 mg $l^{-1}$ + 0.10 mg $l^{-1}$) in MS medium. Matured embryos germinated after incubation on MS medium containing 20.0 mg $l^{-1}$ of GA3 plus 2.0 mg $l^{-1}$ of ABA for five days. An average of 10.82 shoots per explant of proliferated culture was obtained when MS medium was supplemented with BAP (2.0 mg $l^{-1}$) + NAA (0.50 mg $l^{-1}$).**

## INTRODUCTION

Saffron (*Crocus sativus* L.) has been cultivated for its stigmas, which not only comprise a highly valued spice but also have various therapeutic uses (Sampathu et al., 1984). The saffron plant is a geophyte and propagated by vegetative reproduction through the formation of daughter corms from the mother corm. The autotriploid nature of the species renders improvement by breeding very difficult (Basker and Negbi, 1989). Application of tissue culture techniques for the propagation and genetic improvement of the saffron has been emphasized recently (Ilahi et al., 1987; Isa and Ogasawara, 1988). Regeneration has been described from corm-derived callus cultures via somatic embryogenesis (George et al., 1992; Ahuja et al., 1994), organogenesis and protoplasts (Isa and Ogasawara, 1990) but with low frequencies of normal plant formation. The present study was undertaken for successful regeneration of shoots via organogenesis and somatic embryogenesis using leaf segments as explant source.

## MATERIALS AND METHODS

Healthy leaves obtained from the corms collected from saffron growing fields of Pampore (Kashmir) and were sterilized after treating them with sodium hypochlorite for 8-10 minutes. Callus cultures were established by culturing the leaf segments on different media compositions such as Murashige and Skoog (MS) 1962 [$M_1$], Gamborg's B5 1968 [$M_2$], Nitsch and Nitsch N6 , 1972 [$M_3$] and MS liquid medium [$M_4$] supplemented 2,4-D (1.0 mg$l^{-1}$) and BA (1.0 mg$l^{-1}$) and NAA (0.50

$mgl^{-1}$) individually and in combination the calli thus obtained were maintained and multiplied through periodic subculturing on to same media composition. Based on histological observations white translucent granular embryogenci calli was transferred to different media compositions M1,M2,M3 and M4 supplemented with BA (2.25 $mgl^{-1}$), 2, 4-D (0.10 $mgl^{-1}$), NAA (0.25 $mgl^{-1}$) and their combinations.

The cultures were incubated at 15±1 °C and were given 16h/8h (light/dark) photoperiodic treatment. Somatic embryos developed were transferred to half strength MS medium supplemented with ABA (1.09-1.75, 2.0, 2.75 mg $l^{-1}$) and BAP (0.50 mg $l^{-1}$) for maturation. Matured embryos were then transferred to MS medium containing different concentrations of $GA_3$ for germination. Germinated embryos were sub-cultured in MS medium supplemented with BA (2.75 $mgl^{-1}$) plus NAA (0.50 $mgl^{-1}$) for plantlet development.

For microcorm differentiation and development excised shoots from callus and somatic embryos were placed on MS basal medium (Half-strength) supplemented with BA+ NAA and with varying levels of sucrose.

Statistical analysis of data was carried out using completely randomized design. Interpretation of result was carried out on the basis of critical difference at (0.05) between means.

## RESULTS AND DISCUSSION

### Induction of Callus and Somatic Embryo

Leaf segments cultured on various media i.e. Murashige and Skoog 1962 (MS), Gamborg's B5 1968, Nitsch and Nitsch N6, 1972 and MS Liquid medium in combination with BA, NAA and 2,4-D at concentrations of 1.0, 0.50 and 1.0 $mgl^{-1}$ (Table 1), respectively became swollen and generally differentiated and developed white translucent callus after 14-15 days of culture. Maximum callusing was got with MS full strength medium containing BA (1.0 mg $l^{-1}$) + 2,4-D (1.0 $mgl^{-1}$). Within 20-25 days of culture, the globular embryos were formed directly on the surface of embryogenic callus. When the explants of primary somatic embryos were cut into fragments and cultured on same induction medium secondary somatic embryos were induced within two weeks.

Among the various media tested in combination with different concentrations of BA, NAA and 2, 4-D, the percentage of explants responded for somatic embryogenesis was found to be maximum of 8.66 somatic embryos when MS medium was supplemented with BA (2.25 mg $l^{-1}$) + 2, 4-D (0.10 $mg1^{-1}$) (Table 2).

### Plantlet Formation from Callus and Somatic Embryos

Highest number of shoots per explant of proliferated cultures was achieved when MS full strength medium was supplemented with BAP (2.0 $mgl^{-1}$) plus NAA (0.5 $mgl^{-1}$). The calli embryogenic form leaf explants containing globular embryos were transferred for maturation and germination medium containing half strength MS medium supplemented with ABA (1.75 $mgl^{-1}$) + BAP (0.5 $mgl^{-1}$) and $GA_3$ at different concentrations. Maximum number of embryoids which matured and germinated occurred when ABA (1.75 $mgl^{-1}$) + BAP (0.5 $mgl^{-1}$) + $GA_3$ (20 $mgl^{-1}$) was used (Table 3).

After 30 days of embryo germination, the plantlets were obtained. Elongated shoots uniform in size (5-8 cm) from callus cultures and somatic embryos when excised and incubated with MS medium containing BA (4.0 $mgl^{-1}$) + NAA (0.50 $mgl^{-1}$) with different concentration of Sucrose, the appearance of maximum number of small globular micro-corm was observed on the basal portion of the shoots after 8-10

weeks when the half strength MS medium was supplemented with BA (4.0 $mgl^{-1}$) + NAA (0.50 $mgl^{-1}$) + 9 % sucrose (Table 4).

In the present investigation the results on organogenesis and somatic embryogenesis have shown that auxin such as 2,4-D along with cytokinin BA are essential for somatic embryogenesis from leaf explants of saffron (*Crocus sativus* L.) the auxin or auxin in combination with cytokinins used in the medium can greatly influence the frequency of induction and also on maturation of somatic embryos.

In-vitro induction of embryogenesis in Iridaceous genera was reported earlier by Wang et al., 1990, Kim and Kang, 1992; Bach, 1992; Ahuja et al., 1994; Jehan et al., 1994. Ahuja et al. (1994) described somatic embryogenesis from bulblets derived callus and derived callus and found embryoids after maturation resulted in regeneration of complete plantlets on media containing $GA_3$. Regeneration protocol with help in the development and rapid clonal propagation of novel plant material.

**Literature Cited**


Ahuja, A., Kaul, S., Ram, G. and Kaul, B.L. 1994. Somatic embryogenesis and regeneration of Plantlets in saffron. *Crocus sativus* L. Ind. J. Exp. Biol. 32:135-140.

Bach. A. 1992. Induction of somatic embryogenesis and regeneration in *Fressia hybrida* cultures. Folia Hortic. 4:11-21.

Basker, D. and Negbi, M. 1983. Uses of Saffron. Econ. Bot. 37:228-236.

Gamborg, O.L., Miller R.A. and Ojima, K. 1968. Nutrients requirements of suspension culture of soybean root cell. Exp Cell. Res. 50:151-158.

George, P.S., Viswanath, S., Ravishankar, G.A. and Venkataraman, L.V. 1992. Tissue culture of saffron, *Crocus sativus* L.: Somatic embryogenesis and shoot regeneration. Food Biotech. 6:217-223.

Ilahi, I., Jabeen, M. and Firdous, N. 1987. Morphogenesis with saffron tissue culture. J. Plant Physiol. 128:227-232.

Isa T & Ogasawara T 1990. Regeneration from the protoplasts of saffron (*Crocus sativus* L.) Japan Journal of Breeding. 40 : 153-158.

Isa T and Ogasawara T 1988. Efficient regeneration from callus of saffron (*Crocus sativus* L.). Japan J. Breed. 38: 371-374

Jehan, H.D., Courtois, D., Ehret, C., Lerch, K. and Petiard, V. 1994. Plant regeneration of *Iris pallida* L, and *Iris germanica* L. via somatic embryogenesis from leaves apices and young flowers. Plant cell Rep. 13(12):671-675.

Kim, K.W. and Kang, M.S. 1992. Somatic embryogenesis and plant regeneration from Gladiolus callus *in vitro*. J. Korean Soc. Hortic. Sci. 33(1):87-94.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bio-assay with tobbacco tissue cultures. Physiol. Plant 15:473-497.

Nisch, J.P. 1972. Haploid plant from pollen. Z. Pflanzenzucht 67:3-8.

Sampathu, S.R., Shivashanker, S. and Lewis, Y. 1984. Saffron (*Crocus sativus* L.) Cultivation, processing, chemistry and standardization. In: Furuya TE (ed) Crit. Rev. Food Sci. Nut. 20:123-157.

Wang, L., Haung, B.C., He, M.Y. and Hao, S. 1990. Somatic embrogenesis and its hormonal regulation in tissue cultures of *Fressia refreta*, L. Ann. Bot. 65(3):271-76.

Table 1. Influence of media and growth regulator regime on callusing* (%) of initiating cultures in Leaf explants of saffron

| Growth regulator (mg $l^{-1}$) | Media | | | | Mean |
|---|---|---|---|---|---|
| | $M_1$ | $M_2$ | $M_3$ | $M_4$ | |
| BA (1.0) | 44.13 | 39.25 | 40.24 | 42.19 | 41.45 |
| | (41.62) | (38.79) | (39.37) | (40.50) | (40.07) |
| 2,4-D (1.0) | 54.74 | 49.87 | 51.80 | 52.77 | 52.29 |
| | (47.72) | (44.92) | (46.03) | (46.58) | (46.31) |
| NAA (0. 50) | 34.14 | 29.70 | 30.85 | 32.00 | 31.67 |
| | (35.75) | (33.02) | (33.73) | (34.44) | (34.24) |
| BA (1.0) + 2,4-D (1.0) | 75.56 | 65.44 | 67.95 | 70.66 | 69.90 |
| | (60.37) | (53.99) | (55.52) | (57.20) | (56.77) |
| BA (1.0) + NAA (0.50) | 57.77 | 54.74 | 55.73 | 56.75 | 56.24 |
| | (49.47) | (47.72) | (48.29) | (48.87) | (48.59) |
| BA (1.0) + 2, 4 D (1.0) | 46.77 | 42.19 | 44.13 | 45.08 | 44.54 |
| + NAA (0.50) | (43.14) | (40.50) | (41.62) | (42.17) | (41.86) |
| Mean | 52.18 | 46.86 | 48.45 | 49.90 | |
| | (46.34) | (43.16) | (44.09) | (44.96) | |

| Effect | LSD ( $p \leq 0.05$) | S.E difference |
|---|---|---|
| Main effect | | |
| Growth regulator(GR) | 0.38 | 0.19 |
| Media | 0.31 | 0.15 |
| Interaction effect | | |
| GR x Media | 0.76 | 0.38 |

$M_1$ = MS (Murashige and Skoog,1962)
$M_2$ = Nitsch and Nitsch ($N_6$. 1972)
$M_3$ = Gamborg's B5 1968
$M_4$ = MS (Liquid)

** Data in parenthesis are transformed values ($\sin^{-1}\sqrt{p}$)*

Table 2. Influence of different treatments on formation of somatic embryos per culture

| Growth regulators ($mg\ l^{-1}$) | Concentration ($mg\ l^{-1}$) | Media | | | | Mean |
|---|---|---|---|---|---|---|
| | | $M_1$ | $M_2$ | $M_3$ | $M_4$ | |
| BA | 2.25 | 5.33 | 5.00 | 0.00 | 0.00 | 2.58 |
| BA + NAA | 2.25 + 0.25 | 6.66 | 5.66 | 0.00 | 0.00 | 3.08 |
| NAA | 0.25 | 2.33 | 0.00 | 0.00 | 0.00 | 0.58 |
| BA + 2,4-D | 2.25 + 0.10 | 8.66 | 8.33 | 7.00 | 6.66 | 7.66 |
| 2, 4-D + NAA | 0.25 + 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2, 4-D | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean | | 3.83 | 3.16 | 1.16 | 1.11 | |

| Effect | LSD ( $p \leq 0.05$) | S.E difference |
|---|---|---|
| Main effect | | |
| Growth regulator (GR) | 0.07 | 0.03 |
| Media | 0.06 | 0.03 |
| Interaction effect | | |
| GR x Media | 0.15 | 0.07 |

Table 3. Effect of ABA, BAP and $GA_3$ on germination (%) of somatic embryos*

| $GA_3$ ($mg\ l^{-1}$) | ABA + BAP ($mg\ l^{-1}$) | | | | Mean |
|---|---|---|---|---|---|
| | 1.0 + 0.50 | 1.75 + 0.50 | 2 + 0.50 | 2.75 + 0.50 | |
| 5 | 58.80 | 62.40 | 42.30 | 41.20 | 51.17 |
| | (50.06) | (52.17) | (40.57) | (39.93) | (45.68) |
| 10 | 62.40 | 72.40 | 54.40 | 49.88 | 59.77 |
| | (52.17) | (58.30) | (62.40) | (44.93) | (50.73) |
| 20 | 69.80 | 82.00 | 62.40 | 61.20 | 68.85 |
| | (56.66) | (64.89) | (52.18) | (51.47) | (56.30) |
| 25 | 69.80 | 81.20 | 72.20 | 61.40 | 71.15 |
| | (56.66) | (64.31) | (58.18) | (51.58) | (57.68) |
| Mean | 65.20 | 74.50 | 57.82 | 53.42 | |
| | (53.89) | (59.92) | (49.61) | (46.98) | |

| Effect | LSD ( $p \leq 0.05$) | S.E difference |
|---|---|---|
| Main effect | | |
| $GA_3$ | 0.35 | 0.17 |
| ABA | 0.35 | 0.17 |
| Interaction effect | | |
| $GA_3$ x ABA | 0.70 | 0.34 |

* *Data in parenthesis are transformed values ($\sin^{-1}\sqrt{p}$)*

Table 4. Effect of MS basal medium salts strength on number of micro corm formation *in vitro*

| Basal medium | BA + NAA (mg $l^{-1}$) | Number of shoots incubated | Shoots with corms after 8-10 weeks |
|---|---|---|---|
| MS | 0 | 20 | 0 |
| MS | 4.00+.50 | 20 | 6 |
| MS (½ strength) | 4.00 + 0.50 | 20 | 7 |
| MS (½ strength) with 6% sucrose | 4.00 + 0.50 | 20 | 9 |
| Ms (½ strength) with 9% sucrose | 4.00 + 0.50 | 20 | 14 |
| MS (½ strength) with 12% sucrose | 4.00 + 0.50 | 20 | 8 |
| Mean | | | 7.33 |

| Effect | LSD ( $p \leq 0.05$) | S.E difference |
|---|---|---|
| Main effect | | |
| Media | 2.12 | 0.95 |
| Shoots with corms | 0.86 | 0.38 |
| Interaction effect | | |
| Media x Shoots with corms | 2.12 | 0.95 |