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In vitro Production of Pathogen-free Plantlets via Meristem Culture in Saffron (*Crocus sativus* L.)

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Abstract: *Crocus sativus* L. is a triploid and sterile crop that propagates vegetatively by corm. Tissue culture is a useful method for large scale production of pathogen-free plants. In this study, in order to determine the best hormone treatment for nonembryogenic and embryogenic calli induction, apical meristem of young corms derived from saffron (*Crocus sativus* L.) were cultured in solid LS medium supplemented with different concentration of two auxin (NAA and 2,4-D) and three cytokinin (BAP, Kin and 2ip) at 22°C in darkness. Statistical analysis using Kruskal-Wallis Test showed that treatment containing 2 mg L^{-1} NAA and BAP with highest Mean Rank had the best effect on induction of nonembryogenic callus and treatment containing 1 mg L^{-1} 2,4-D and BAP had the best effect on induction of embryogenic callus. Careful selection of embryogenic regions and transferring them into glassware with 2 cm width and 10 cm height resulted in notable growth of embryogenic calli. Sequential subcultures of embryogenic calli in same medium resulted in maturation of embryos, production of leaf organs and neoformed corms.

Key words: Triploid, nonembryogenic callus, embryogenic callus

INTRODUCTION

Crocus sativus L. is a triploid and sterile crop that propagates vegetatively by corm. Disease factors such as fungi, bacteria and viruses that exist in corms usually result in decrease of growth and flowering in this plant. Tissue culture is a useful method for large scale production of pathogen-free plants (Ding *et al*., 1981; Chrungoo *et al*., 1987). Reported that in LS medium containing NAA and BAP induction of nonembryogenic calli were highest (Ahuja *et al*., 1994; Homes *et al*., 1987). Showed that synthetic auxins such as 2,4-D in low concentration were essential for embryogenesis in some monocots (Plessner *et al*., 1990; Vasil and Vasil, 1992). Ebrahimzadeh *et al*. (2000) used meristem culture of saffron in LS medium containing different concentration of NAA, 2, 4-D and Kin. They observed different stages of embryogenesis and plantlet formation. In this study, effect of different hormone treatments on nonemberyogenic and embryogenic callus induction and plantlet formation were studied.

MATERIALS AND METHODS

Materials and sterilization: *Crocus sativus* L. corms were collected from the Province of Mashhad, Iran in June 2004. In order to sterilize the corms, after removing the fibers, were rinsed in running tap water for 30 min, then dipped in 70% ethanol. They surface-sterilized in 2 ‰ $HgCl_2$ for 30 min and rinsed 3 times in sterile water. Apical meristem of corms together with little part of the corm tissue attached to them, were removed as explant. In order to prevent browning of the tissue before culture, explants were soaked for 10 min in 1% ascorbic acid.

Medium and culture condition: Sterilized explants were cultured on LS (Linsmaier and Skoog, 1965) medium containing 3% sucrose and different concentration of two auxin: NAA (0, 5, 10, 15 mg L^{-1}) and 2,4-D(0, 1, 2, 10 mg L^{-1}) and three cytokinin: BAP, Kin, 2ip (0, 1, 2 mg L^{-1}). All media were solidified by 0.7% agar after adjusting the pH to 5.7 and autoclaved for 15 min at 1.2 kg cm^{-2}. Cultures were incubated at 22±2°C in darkness. All cultures were subcultured every 1 month in the same medium. The experiment was repeated four times using three explants per treatment. Data obtained from tissue culture experiments were statistically analyzed using non-parametric Kruskal-Wallis Test with SPSS program.

Proliferation of embryogenic calli: Precise selection of embryogenic calli which were compact with shining

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globular regions and transferring them to glassware with 2 cm width and 10 cm height carried out due to proliferation and maturation of embryogenic calli. This investigation performed from July 2004 until May 2005 in the Agricultural Biotechnology Research Institute of Iran (ABRI).

RESULTS AND DISCUSSION

Induction of nonembryogenic and embryogenic calli: After 4.5 month of culture, nonembryogenic calli formed from apical meristem on LS medium containing different growth regulators. Results obtained from statistical analysis using non-parametric Kruskal-Wallis Test indicated that there was significant difference among treatments at 0.01 probability level from view of nonembryogenic and embryogenic callus induction. (Table 1, Fig. 1 and 2).

The treatment containing 2 mg L^{-1} NAA and 2 mg L^{-1} BAP had the highest Mean Rank of nonembryogenic callus induction (Fig. 3a). This result is similar to the Ahuja *et al.* (1994) study on *C. sativus*. LS medium containing 2 mg L^{-1} 2,4-D and 1 mg L^{-1} Kin is in secondary importance which is corresponded to George *et al.* (1992) study. After 6 month of cultures, the highest Mean Rank of embryogenic callus induction was recorded with LS media contained 1 mg L^{-1} 2,4-D and BAP (Fig. 3b). This result is similar to Sano and Himeno (1987) and Vasil and Vasil, (1992) studies. During initial stages of callus development, white to yellowish and soft calli grew from cultured explants, which after 4 transfers were visually distinguishable to be of two types: off-white, friable and nodular embryogenic calli and yellowish and soft nonembryogenic calli. This asymmetric development that resulted in nonembryogenic and embryogenic callus formation (Fig. 3c) is distinguishable property of numbers of monocots (Vasil *et al.*, 1988). Careful selection of embryogenic regions and transferring them into glassware with 2 cm width and 10 cm height resulted in notable growth of embryogenic calli (Fig. 3d). As noted embryo formation *in vitro* condition is a stress response, hence limitation of this glassware in supplying sufficient space for growth and nutrient exchange, increased embryogenesis (Williamson *et al.*, 1985; Morris *et al.*, 1990). Sequential subcultures of embryogenic calli in same medium resulted in maturation of embryos, production of leaf organs and neoformed corms (Fig. 3e-h).

Table 1: The result of Kruskal-Wallis Test in measured characters

	Embryogenic callus	Nonembryogenic callus
Chi-square	584/80	356/21
df	71	71
Asymp. Sig	0/000	0/000

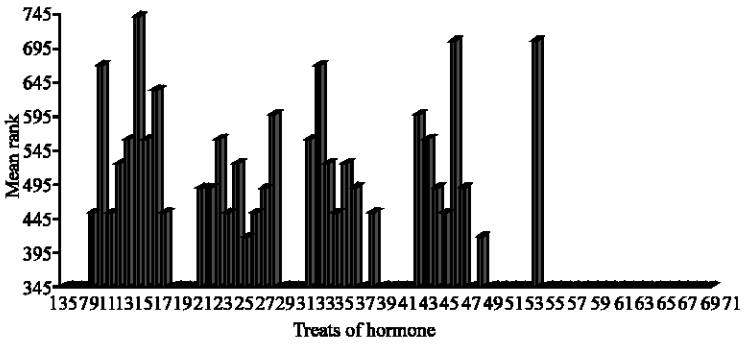

Fig. 1: Ranking of different hormone treatments effects in nonembryogenic callus induction

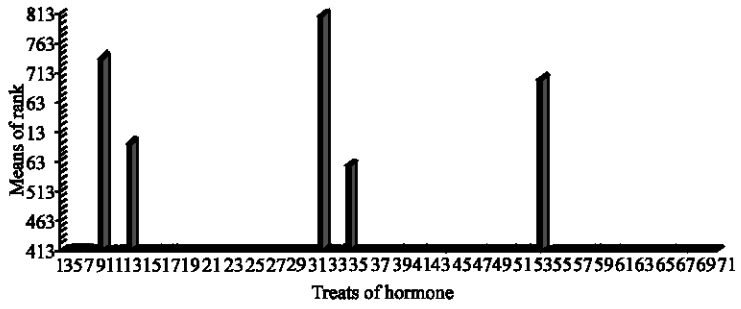

Fig. 2: Ranking of different hormone treatments effects in embryogenic callus induction

Fig. 3: a-h) Induction of nonembryogenic and embryogenic callus and plantlet formation via somatic embryogenesis in *C. sativus*. (a) Nonembryogenic callus. (b) Embryogenic callus. (c) Asymmetric growth of calli. (d) Transferring of embryogenic callus into glassware with 2 cm width and 10 cm height. (e) Maturation of embryos. (f-g) Germination of embryos and (h) Plantlet with corm

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