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Full Length Research Paper

Ex vitro shoot regeneration and lateral buds of freshly harvested saffron corms

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After formation of the replacement corms, the saffron leaves wither and the apical buds enter dormancy, which is released after-ripening at 23 to 30°C. This study aimed to break this dormancy and reports successful *ex vitro* shoot regeneration from saffron corms, just after harvesting by pulse treating for 30, 60, 90, 120 and 150 min with 50 mg/L Indole acetic acid (IAA) or 50 mg/L IAA + 10 mg/L Thidiazuron (TDZ). All corms were cultured in organic matter rich soil contained in plastic sieve trays in greenhouse with day time temperature in range of 9 to 15°C and night temperature in range of 5 to 8°C during experimentation with relative humidity of 50 to 60%. After 2 to 3 weeks, the pulse treated corms regenerated multiple number of shoots, which increased to 5-6 shoots per corm after 10 weeks of culture; showing statistically different and superior regeneration on 50 mg/L IAA + 10 mg/L TDZ pulse treated corms compared to IAA pulse treated corms. This protocol will help to increase and improve flowering for an extended period of time.

Key words: *Ex vitro*, shoot regeneration, lateral buds, saffron, multiplication.

INTRODUCTION

The domesticated saffron (*Crocus sativus* L.), belonging to the Iridaceae family; is a perennial sterile auto-triploid mutant; which is cultivated for its stigmas since time immemorial (Karaoglu et al., 2007). The saffron plant is a geophyte and is propagated by vegetative reproduction through the formation of daughter corms from the mother corm. The stigmas of saffron are used as important high valued spice and are characterized as the most expensive spice by weight unit. They are also used for various therapeutic purposes in medicine, food seasoning and coloring (Sampathu et al., 1984; Karaoglu et al., 2007). 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 produced in a very few countries of the world and invariably involves traditional labour intensive methods, which contribute to its very high price. Autotriploid nature of the species renders improvement by breeding very difficult (Basker and Negbi, 1989). Saffron has been produced and exported from Turkey until the 19th century.

However, due to a number of socio-economic and technical problems, now the cultivation of saffron has been limited to a few villages in the Safranbolu District of Karabuk province of Turkey (Karaoglu et al., 2007). 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). It is common observance that after the formation of the replacement corms, the saffron leaves wither and the apical buds enter dormancy (Saiedian et al., 2007). This dormancy is released by high summer temperature and sprouting occurs only after a period of after-ripening obtained by curing bulbs at 23 to 30°C. Therefore, reproduction is human dependent; the corms must be manually dug up, broken apart and replanted. The natural propagation rate of most geophytes (Sevimay et al., 2005; Parmaksiz and Khawar, 2006; Ozel and Khawar, 2007; Aasim et al., 2008; Ozel et al., 2008), including saffron is relatively low (Karaoglu et al., 2007).

The aim of the study was to develop a successful *ex*

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vitro shoot regeneration from terminal and lateral buds of 6 to 7cm circumference saffron corms; soon after harvest by pulse treatment with 50 mg/L IAA or 50 mg/L IAA + 10 mg/L TDZ in the greenhouse to find if they induce differentiation to form shoots.

MATERIALS AND METHODS

Whole corms of *C. sativus* L. (6 to 7 cm bulb circumference) were collected of Iranian origin were collected from the Department of Field Crops, Faculty of Agriculture, Ankara University, Turkey during the month of September after harvest of floral stigmas. The corms were packed in craft paper bags and lifted to store for 3 to 4 days. Thereafter, they were pulse treated for 30, 60, 90, 120 and 150 min with 50 mg/L Indole acetic acid (IAA) or 50 mg/L Indole acetic acid and 10 mg/L Thidiazuron (TDZ) for 30, 60, 90, 120 and 150 min.

The pulse treated corms were planted in equivalent of 15 kg of thoroughly mixed soil containing clay, sand and organic matter (1:2:1). Optimal pH of the soil was 7 to 7.5. The soil was passed through an 8-mm sieve in 20 L sieved plastic trays in greenhouse of the Department of Field Crops, Faculty of Agriculture, Ankara University, Turkey before use. Large pieces (>~2 cm) of plant or other residues in the organic matter, if any, were removed by hand. Corms were watered with tap water until they reached field capacity. The day time temperature of greenhouse ranged 9 to 15°C and night time temperature ranged 5 to 8°C during experimentation in October to November, 2009, with relative humidity of 50 to 60%.

Observations and statistical analysis

Each treatment was replicated 6 times with 5 explants per replication (6 replications × 5 corms = 30 corms). Data was analyzed with one way ANOVA using SPSS 16.0 and the post hoc tests were performed using Duncan's multiple range test. All data given in percentages were subjected to arcsine transformation before statistical analysis (Snedecor and Cochran, 1967).

RESULTS

The results showed high frequency of dormancy release and bud regeneration in all corms pulse treated with 50 mg/L IAA and 50 mg/L IAA + 10 mg/L TDZ (Table 1). New bud regeneration frequency ranged 93.33 to 100% on 50 mg/L IAA treated corms and 100% on 50 mg/L IAA+ 10 mg/L TDZ treated corms after 3 weeks of culture. The results showed showed maximum of 4.35 shoot buds per corm after 150 min of 50 mg/L IAA treatment (Figures 1a and b). Development of termainal bud was faster and dominant on 50 mg/L IAA+ 10 mg/L TDZ treated corms (Figure 2a). The remaining buds developed only once the terminal bud developed fully bearing green leaves (Figures 2a and b). Consequently, this affected number of shoots per corm from the newly developing buds. Number of shoots per corm from 50 mg/L IAA pulse treated bulblets ranged 1.67 to 4.33. The results showed consistent and significant ($p< 0.05$) increase in the number of shoots per corm as the duration of pulse tratment increased from 30 to 150 min. Pulse treatment with 50 mg/L IAA+ 10 mg/L TDZ had more significant ($p< 0.05$) effects on the release of bulb dormancy. It showed sharp increase in the number of shoots per treated corm as the duration of pulse treatment increased. Minimum number of 2.87 shoots were recorded upon 30 min pulse treatement; which increased to 4.87, 5.07 and 9.27 shoots per corm on 90, 120 and 150 min pulse treatment respectively.

The length of leaves is an important consideration that can significantly affect growth of corms and subsequently flowering of the corms. Conversion of buds into leaves was significantly ($p< 0.05$) low on IAA pulse treated corms compared to 50 mg/L IAA+ 10 mg/L TDZ treated corms. It was expected that due to low number of shoot buds that was converted into leaves on IAA pulse treated corms; the leaves will grow more vigorously due to decreased competiton. However, it did not happen and the length of leaves per corm on IAA pulse treated corms remained significantly ($p< 0.05$) less compared to longer leaves on 50 mg/L IAA+ 10 mg/L TDZ treated corms. The results showed that 50 mg/L IAA + 10 mg/L TDZ pulse treatment had positive effect on development of leaves, which were longer with range of 23.83 to 26.27 cm compared to the leaves developed on IAA pulse treated corms with length range of 14.20 to 17.57 cm. Least length of 23.83 cm on 50 mg/L IAA+ 10 mg/L TDZ pulse treated leaves was sharply different from the longest leaves recorded on 50 mg/L IAA pulse treated leaves. The longest leaves of 26.27 cm were recorded on 150 min pulse treatment.

Each increase in the duration of 50 mg/L IAA pulse treatment was found to be associated with linear increase in the length of leaves on corms; which ranged 14.20 to 17.57 cm. It was interesting to note that longer periods of pulse treatment were associated with longer leaves on IAA pulse treatment. Although, duration of either, IAA or 50 mg/L IAA+ 10 mg/L TDZ pulse treatment in minutes had no effect on leaf width; yet 50 mg/L IAA+ 10 mg/L TDZ pulse treated corms induced significantly ($p<0.05$) broader leaves compared to leaves on corms pulse treated with 50 mg/L IAA only. Irrespective of the growth regulator used in pulse treatment, a sharp difference was observed in leaf width on IAA and 50 mg/L IAA+ 10 mg/L TDZ pulse treated corms. The leave width ranged from 1.33 to 1.67 mm on 50 mg/L IAA pulse treated corms and from 1.33 to 3.33 mm on 50 mg/L IAA+ 10 mg/L TDZ pulse treated corms. Moreover, irrespective of IAA or 50 mg/L IAA+ 10 mg/L TDZ pulse treatment, leave width increased with every increase in the pulse duration. Stem diameter showed sharp increase when any of the 50 mg/L IAA pulse treated corms were compared with 50 mg/L IAA+ 10 mg/L TDZ pulse treated corms. They showed stem diameter of 0.74 to 0.82 cm on IAA pulse treated corms and 1.02 to 1.06 cm on 50 mg/L IAA+ 10 mg/L TDZ pulse treated corms.

Table 1. Effects of pulse treatment of saffron corms with 50 mg/L IAA, and 50 mg/L IAA+ 10 mg/L TDZ for different duration of time on frequency of shoot regeneration, number of shoots per explants, ahoot length and stem diameter.

Duration (min) of pulse treatment	Frequency (%) of shoot regeneration		Number of shoots per corm		leaf length (cm)		Leaf width (mm)		Stem diameter (cm)	
	IAA	IAA+TDZ	IAA	IAA+TDZ	IAA	IAA+TDZ	IAA	IAA+TDZ	IAA	IAA+TDZ
30	93.33	100.00	1.67^{b*B**}	2.87^{cdA}	14.20^{Db}	23.83^{cdA}	1.33^{A}	1.33^{A}	0.74^{B}	1.02^{A}
60	93.33	100.00	1.73^{Bb}	3.80^{bcA}	14.80^{cdB}	24.27^{cA}	1.50^{B}	1.67^{A}	0.80^{B}	1.03^{A}
90	93.33	100.00	1.80^{abB}	4.87^{abA}	15.00^{bcdB}	24.73^{bcA}	1.50^{B}	2.00^{A}	0.80^{B}	1.05^{A}
120	93.33	100.00	2.87^{abB}	5.07^{abA}	15.63^{bcdB}	25.33^{abA}	1.50^{B}	2.67^{A}	0.81^{B}	1.05^{A}
150	100.00	100.00	4.33^{aB}	9.27^{aA}	17.57^{abcdB}	26.27^{Aa}	1.67^{B}	3.33^{A}	0.82^{B}	1.06^{A}

*Values within a column followed by different small letters are significantly different at 0.05 level of significance using Duncan's test. **Values within a row followed by different capital letters are significantly different at 0.05 level of significance using Duncan's test. Each value is the mean of 30 corms.

Figure 1. Effects of 50 mg/L IAA pulse treated saffron corms on shoot regeneration (a) pulse treated corms with main shoot and newly regenerated shoots (b) pulse treated corms growing in the greenhouse. Bar Figure 1a = 0.7 cm, Figure 1b = 7 cm.

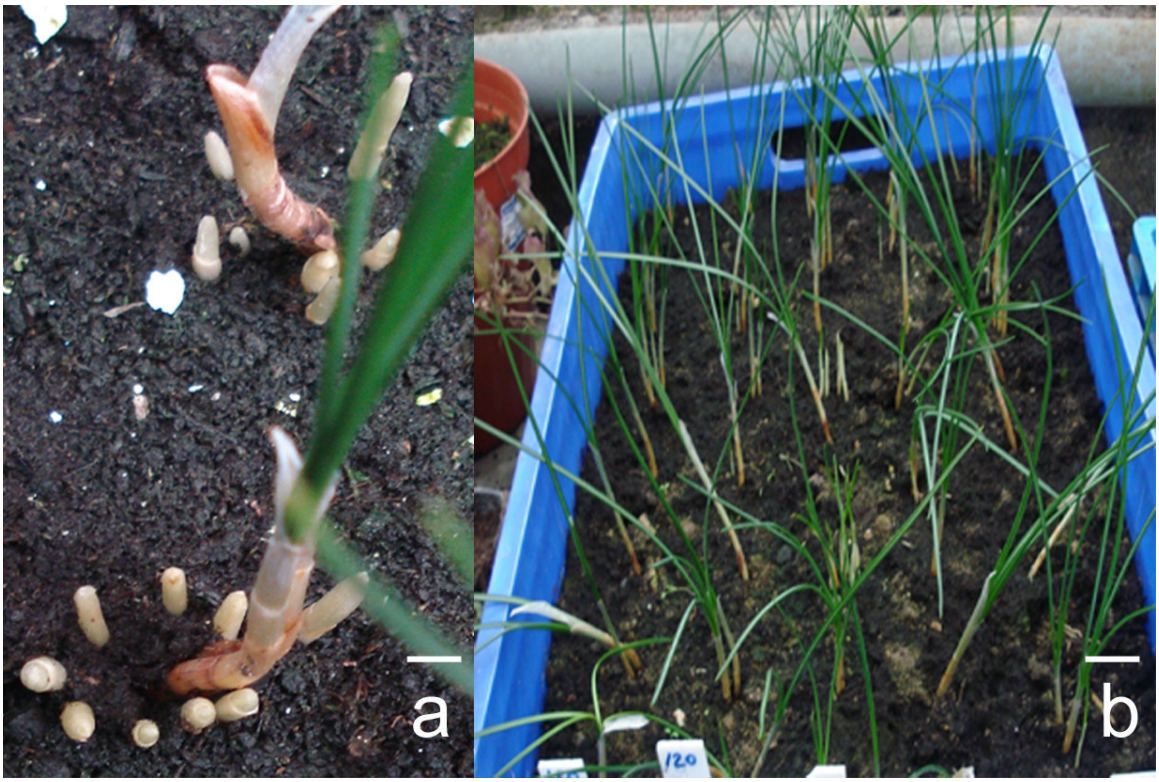

Figure 2. Effects of 50 mg/L IAA+ 10 mg/L TDZ pulse treated saffron corms on shoot regeneration (a) pulse treated corms with main shoot and newly regenerated shoots (b) pulse treated corms growing in the greenhouse. Bar Figure 2a = 1cm, Figure 2b = 5 cm.

DISCUSSION

The saffron is a sterile geophyte that reproduces only vegetatively by its annual replacement corms. Among the 85 species belonging to the genus *Crocus*, *C. sativus* (domesticated Saffron) is the most fascinating and intriguing sterile fall-flowering perennial plant species (Fernández, 2004), which survive for only one season, producing up to ten "cormlets" that eventually give rise to new plants (Deo, 2003) for the following season. There is no previous study that explain role of growth regulators on the regeneration of saffron corms under *ex vitro* conditions. There is need to develop *ex vitro* protocols for breaking corm dormancy for increased saffron production. Under various conditions examined in this study, it was found that pulse treatment with IAA and 50 mg/L IAA+ 10 mg/L TDZ for different durations of time had positive effects on corm regeneration. However; IAA +TDZ pulse treatment was more favorable compared to IAA pulse treatment. Activity of 50 mg/L IAA+ 10 mg/L TDZ was significantly higher ($p< 0.05$) and about two to threefold stronger compared to IAA in production of number of shoots per corm. The activity was about 2 folds in leaf elongation, leaf width and had considerable impact on stem diameters. The results of this study show that IAA or 50 mg/L IAA+ 10 mg/L TDZ induced some metabolites in the juvenile saffron corms that led to the dormancy break and increased production of green leaves. Similarly, Plessner et al. (1989) found that plant growth regulators, particularly zeatin, and 2,4-D were essential for regular development of isolated saffron buds and enhanced bud development on intact corms *in vitro*. Ding et al. (1979, 1981) also successfully regenerated callus and intact plantlets from corm explants on culture media containing indole- 3- acetic acid (IAA) and 2, 4-D. Similarly, Homes et al. (1987) also observed microcorms formation on 1/8th corm explants under *in vitro* conditions under the influence of 9 µM 2, 4-D.

Cell division seem to be regulated by the joint action of auxins and cytokinins, each of which appear to influence different phases of the the cell cycle. Auxins exert and effect on DNA replication, while cytokinin seem to exert some control over the events leading to mitosis (Pasternak et al., 2000). Therefore, auxins might be considered as “inducers” of the cell cycle, while cytokinins might behave more as its promoter (Wood et al., 1990). Normal cell divisions require synchrony between the S phase and cell division, suggesting that auxin and cytokinin levelsin cultures need to be carefully matched. Late replication of DNA in cell cultures has been advanced as one cause of chromosome rearrangement (Lee and Phillips, 1988).

Conclusion

In vitro propagation is a viable alternative for large-scale propagation, which enables rapid multiplication of plants. However, success and cost of micropropagation mainly relies on the percent of plantlet survival in field conditions. *In vitro* propagation has been reported by many workers; however, the results are often irreproducible or the time of propagation is too long to make the procedures unfit for large scale propagation of saffron (Ding et al., 1979, 1981; Plessner et al., 1989; Karaoglu et al., 2007). The aforementioned study shows that *ex vitro* cultural methods could also be effectively used to propagate saffron and contribute importantly for its fast propagation and acclimatization in a single step, helping in saving time and reduction of cost.

Moreover, this non conventional novel approach can contribute importantly for multiplication of saffron corms out of place and season. It is supposed that this will also help in the increased harvest of saffron flowers and consequently stigmas. This may help in production and popularization of this important plant in nontraditional areas of saffron production throughout the world.

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