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EFFECTS OF CORM DIPPING IN SALICYLIC ACID OR POTASSIUM NITRATE ON GROWTH, FLOWERING, AND QUALITY OF SAFFRON

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ABSTRACT

The present research was conducted to find influence of salicylic acid (SA) at 0.5, 1, and 2 mM and potassium nitrate (KNO_3) at 250, 500, and 1 000 ppm on reproductive and vegetative parameters, and color characteristics of saffron under field condition. The results indicated the highest *a*, *L*, and chroma and the lowest *b* at 2 mM SA treatment. The highest flowering appearance rate and membrane stability index and the lowest electrolyte leakage were observed at 2 mM SA and to lesser extent at 1000 ppm KNO_3. The highest flower number, corolla dry weight, and stigma dry weight was shown in plants treated with SA. Chlorophyll a content and Fv/Fm value increased with the application of 2 mM SA. The data indicated the effectiveness of SA on the growth of saffron.

Key word: saffron, corm, salicylic acid, color characteristics, Fv/Fm

INTRODUCTION

Crocus sativus or saffron is an economically important crop, the world's highest priced spice (Rubio-Moraga et al. 2010). About 90% of its production is from Iran (Fallahi et al. 2014). For the past 5,000 years, farmers have selected plants with the best stigma quality including accumulation of carotenoids (Rubio-Moraga et al. 2009). This plant is adapted to overcome a dry period in the form of an underground dormant corm (Molina et al. 2005). Shortly afterwards, flower morphogenesis takes place and all the flowers are already differentiated by the end of August (Molina et al. 2005). Sprouting is onset at the end of October. Almost every sprouting bud produces a corm, so factors related to phytohormone and sugar signals affecting sprouting are highly important for corm and flower production (Chrungoo 1992). Corm dormancy is strongly associated with abscisic acid (ABA) (Ahrazem et al. 2012), and apical bud sprouting needs gibberellins (GAs) after dormancy cessation (Farooq & Koul 1983). Salicylic acid (SA) is a hormone-like substance that influences many physiological processes in plants such as seed germination, stomatal conductivity, transpiration, glycolysis, heat production, flowering, and fruit yield (Kang et al. 2003). It probably acts as an endogenous signaling molecule in connection with flowering and thereby influences yield (Klessig & Malamy 1994). Rubio-Moraga et al. (2014) found the highest level of SA in apical and axillary buds of saffron. Foliar applications of SA or its precursors (such as benzoic acid) have been shown to affect flower induction and flower numbers in several species of *Lemna* (for example *Lemna gibba* and *Lemna paucicostata*) and the related genus *Spirodela* (Larqué-Saavedra & Martin-Mex 2007; Wada & Takeno 2013).

Potassium nitrate as a nutritional source plays an important role in reproductive growth stage of plants. It stimulates flowering and increases the yield of flowering plants (Karagüzel et al. 1999). Rojas and Leal (1997) found that the application of potassium nitrate to the mango tree caused a slight

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flowering promotion. Yeshitela et al. (2004) observed that potassium nitrate promoted the initiation of buds for vegetative growth in non-inductive temperature conditions. Sritontip et al. (2005) showed that on *Dimocarpus longan*, the highest percentage of leaf flushing, the least time required for terminal bud break, the highest efficiency of photosystem II (PSII), and net carbon dioxide (CO_2) assimilation rate were obtained with a potassium nitrate treatment compared with potassium chlorate, sodium hypochlorite, and thiourea.

According to our knowledge, there are few reports about the effect of SA and KNO_3 on saffron plant. The results of a pot experiment on corm dipping of saffron in SA showed that the concentration of 1 mM was the best treatment for increasing the antioxidant activity and crocin content in stigmas (Tajik et al. 2015). Jabbari et al. (2017) also observed an enhancement in saffron growth and yield when the corms were pre-soaked in SA (2 mM) and KNO_3 (1000 mg dm^{-3}). For the extension of previous study, the aim of our experiment was to evaluate the effect of dipping of dormant corms in SA or KNO_3 solutions on reproductive and vegetative features, and on color characteristics of saffron.

MATERIALS AND METHODS

Plant material and treatments

To study the effects of saffron corm dipping in KNO_3 and SA during dormancy on quantitative and qualitative characteristics, an experiment was performed in the Research Station of Saffron (32°52'N and 52°12'E, 1381 m altitude), University of Birjand, South Khorassan, Iran, during 2015–2016 growing season, using popular landrace Sarayan. Climate condition is dry and warm with a mean temperature of 17 °C and precipitation of 121 mm. Similar dormant saffron corms of 6–8 g were dipped in July for 6 h in solutions as follow: potassium nitrate (250, 500, and 1000 ppm), salicylic acid (0.5, 1, and 2 mM) and in distilled water (control). To inhibit any disease development, a Benlate fungicide (methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate) was added to each solution at 100 ppm. After dipping, corm surfaces were dried and then planted in the field, on 1 × 2 m^2 plots, 12 cm depth, and at density of 100 corm m^{-2}. The seven experimental treatments were arranged in a randomized complete block design with four replications (plots) for each level of KNO_3 or SA and control. On each plot 200 corms were planted; accordingly, in each treatment 800 corms was observed. Some chemical and physical soil characteristics are presented in Table 1. All conventional farm management including irrigation, fertilization, and weeding were done as needed; however, no herbicides or insecticides were applied.

Table 1. Physical and chemical characteristics of soil (0–30 cm depth)

Chemical characters				Physical characters			
electrical conductivity ($dS \cdot m^{-1}$)	pH	SAR*	potassium (mg dm^{-3})	texture	silt (%)	sand (%)	clay (%)
2.4	8.1	1.15	1.3	loam	18.0	60.9	21.1

* sodium adsorption ratio

Flowering characteristics

After breaking dormancy and growth initiation in November, developed flowers were calculated in each day and then harvested to transport to Horticultural Laboratory for further evaluation. The number of flowers opened per day (flowering rate) was calculated using equation 1 (Koocheki et al. 2016). Flower lengths were assessed using a digital vernier caliper. Fresh corolla and stigma were separated from all flowers per each plot, in the morning, dried daily in a drying cabinet at 80 °C for 1 h, weighted again with 0.0001 accuracy, and dry weight data were expressed in grams.

$$\text{Flowering rate} = \sum_{i=1}^{n} \frac{NF}{DAFF} \qquad \text{Equation 1}$$

NF is the number of flowers in each harvesting date, DAFF is the number of days after the first flowering, and n is the harvesting date.

Color measurement

In each plot, 300 flowers were used for color measurement. Color of stigma powder was evaluated using a colorimeter (TES 135, Shenzhen Youfu Tools Co., Ltd., Taiwan). The results were expressed as Hunter

color values of *L*, *a*, and *b*, where *L* was used to denote lightness, *a* to denote redness and greenness, and *b* to denote yellowness and blueness. In addition, color intensity (Chroma) and Hue angle were calculated using equations 2 and 3 (McGuire 1992):

$$\text{Chroma} = (a^2 + b^2)^{0.5} \qquad \text{Equation 2}$$

$$\text{Hue angle} = tg^{-1}(\frac{b}{a}) \qquad \text{Equation 3}$$

Vegetative characteristics

Leaf appearing rate was calculated based on equation 4, which was first developed by Koocheki et al. (2016). Each day, new leaves were recorded to find leaf number per plant. In saffron, lower leaf appearing rate during flowering phase is appropriate, because if leaves appear before or simultaneously with flowers, the flower picking becomes difficult.

$$\text{LAR} = \sum_{i=1}^{n} \frac{\text{NLA}}{\text{DHID}} \qquad \text{Equation 4}$$

where LAR is the leaf appearance rate, NLA is the number of leaves appearing on day n, DHID is the distance of day n since the first irrigation date, and n is the day.

For calculation of membrane stability, leaf segments were cut out at random, washed 3 times with distilled water in order to remove surface contaminants, and then placed individually in stoppered vials containing 10 ml of distilled water. Consequently, they were incubated at room temperature (25 ºC) on a shaker (100 × g) for 24 h to measure electrical conductivity (EC) of the solution (EC1). Then the same vials with leaf samples were placed in an autoclave at 120 ºC for 20 min and the second measurement of conductivity (EC2) was done after cooling the solution to room temperature (Kordi et al. 2013). Finally, the electrolyte leakage (EL) was calculated using equation 5 and membrane stability index (MSI) was calculated using equation 6. Lower EL and higher MSI indicate lower damage to the cell membrane and thus more tolerance of plant to environmental stresses.

$$\text{Electrolyte leakage} = \frac{\text{EC1}}{\text{EC2}} \qquad \text{Equation 5}$$

$$\text{MSI} = 100\text{-EL} \qquad \text{Equation 6}$$

Chlorophyll content and chlorophyll fluorescence

Total chlorophyll and chlorophyll a and b contents were determined by the method of Arnon (1949) using 0.5 g of fresh leaf. Chlorophyll fluorescence was measured on the attached and dark-adapted leaf of each plant (the area of leaf located in fluorometer = 4 cm^2) using an MINI PAM fluorometer (WALZ, Effeltrich, Germany) according to the protocol of Genty et al. (1989). Leaves (three leaves in each plot) were kept for 30 min in the dark-adapted state using light-exclusion clips. At this state, all reaction centers and electron carriers of PSII are re-oxidized, which is necessary for the rapid induction of fluorescence. Under such condition, non-photochemical quenching (qN) is relaxed to its minimum value (Zhang & Xu 2003). Low-intensity modulated light (< 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was used to measure the minimum fluorescence (F0). The maximum fluorescence (Fm) was obtained by 0.3 s pulses of saturating light of 20 000 Hz. The maximum photochemical efficiency of PSII, Fv/Fm, was calculated according to Kitajima and Butler (1975), where Fv=Fm-F0.

Statistical analysis

This experiment was set up in a complete randomized block design, with seven treatments and four replications, each treatment consisted of 4 plots (800 plants). We assumed that all the measured data came from normal (Gaussian) data distribution even if it was not always true, especially in the case of fluorescence measurements (Lazár & Nauš 1998, Lazár et al. 2006). Statistical analysis of data was carried out using analysis of variance (ANOVA) procedure on GENSTAT. The averages were compared with Fisher-protected LSD at 5% level.

RESULTS AND DISCUSSION

Applied treatments influenced almost all analyzed traits (Table 2).

Flower characteristics

The lowest flower appearing rate was observed in control, compared with others, and the application of both treatments was useful. The results indicated an increasing trend of this variable as KNO_3 and SA levels increased. The highest flowering rate was observed at 1000 ppm KNO_3 and 2 mM SA (Table 3). Rubio-Moraga et al. (2014) indicated the highest

level of SA in apical and axillary buds of saffron followed by the basal plate, which may be essential for future flowering. Martínez et al. (2004) suggested that SA treatment accelerates the transition from the vegetative to reproductive phases and is a regulator of flowering time in plants, which may be the reason of sooner flowering in our experiment. Jin et al. (2008) also stated that SA is known to promote flowering in plants. The application of 500 and 1000 ppm of KNO_3 did not affect the flower number compared with control; however, application of SA at 2 mM level resulted in higher value of this variable in comparison to the control and KNO_3 treatments, although no significant difference among SA levels were found. Flower length was not affected by applied treatments (Table 3).

Table 2. Mean squares of flowering and vegetative traits under different levels of SA and KNO_3

Source of variation	Df	Flowering rate	Flower number	Flower length	Corolla dry weight	Stigma dry weight
Block	3	0.2734	0.1513	9.33	0.00005489	4.146
Treatments	6	4.5847**	1.4431**	11.41	0.00052881**	3.046**
Residual	18	0.1149	0.2361	37.43	0.00009458	4.524
Total	27					

Source of variation	Df	Leaf appearing rate	Corm leaf number	Cormel leaf number	Electrolyte leakage	Membrane stability index
Block	3	0.32947	0.702	3.6667	154.79	154.79
Treatments	6	4.49463**	5.238*	0.9881	143.92*	143.92*
Residual	18	0.05899	1.397	0.9722	56.10	56.10
Total	27					

Source of variation	Df	*A*	*b*	*L*	Hue	Chroma
Block	3	1.341	24.580	20.020	61.540	17.620
Treatments	6	32.555**	49.280**	188.730*	32.750	78.600**
Residual	18	2.757	14.560	55.410	21.210	14.100
Total	27					

Source of variation	Df	Chlorophyll a	Chlorophyll b	Chlorophyll a/ Chlorophyll b	Fv/Fm
Block	3	0.0013109	0.0002491	0.757	0.02253
Treatments	6	0.0131970**	0.0011275*	6.161*	0.02374*
Residual	18	0.0009190	0.0003437	2.020	0.02048
Total	27				

** and * values are statistically significant at 1% and 5%, respectively

Table 3. Effect of different levels of SA and KNO_3 on flowering characteristics of saffron

Treatment		Flowering rate (day^{-1})	Mean flower number per plant	Flower length (mm)	Corolla dry weight ($g \cdot plant^{-1}$)	Stigma dry weight ($mg \cdot plant^{-1}$)
Control (distilled water)	0	1.52 d	2.55 b	69.8 a	0.088 c	4.2 c
KNO_3	250 ppm	2.51 c	1.35 c	68.4 a	0.090 c	5.0 bc
	500 ppm	3.47 b	2.15 b	71.6 a	0.085 c	4.1 bc
	1000 ppm	4.39 a	2.10 b	71.4 a	0.093 bc	5.3 b
Salicylic acid	0.5 Mm	2.57 c	2.64 ab	67.0 a	0.097 abc	4.6 bc
	1 Mm	3.73 b	2.06 abc	70.0 a	0.111 ab	5.2 b
	2 Mm	4.39 a	3.28 a	71.2 a	0.114 a	6.7 a
SE		0.2396	0.2429	3.06	0.00486	0.000336

Note: Mean values in each column followed by the same letter are not significantly different (p = 0.05) by the LSD

Corolla dry weight was significantly affected by treatments; however, there was no difference among control, KNO_3, and 0.5 mM SA. Although the highest value of this variable was observed in plants treated with 1 and 2 mM of SA, there was no difference among them. Stigma is the most economically important part of saffron flower. Application of both KNO_3 and SA treatments significantly influenced this trait (Table 2). The data showed that KNO_3 at 1000 ppm and also all SA concentrations significantly increased stigma dry weight and the highest value was observed at 2 mM of SA (Table 2). More stigma yield in plants treated with SA may be due to the fact that SA acts as an endogenous signaling molecule that promotes flowering (Klessig & Malamy 1994). KNO_3 also provides potassium and nitrogen for plant growth and is considered as a flowering stimulator in plants such as saffron (Jabbari et al. 2017).

Color characteristics

Analysis of variance (Table 2) indicated that all treatments significantly influenced *a*, *b*, and chroma values at 1% and *L* at 5%; however, differences in hue value were not significant. The highest *a* (redness), *L*, and chroma and the lowest *b* values were observed at 2 mM SA (Table 4). Redness in saffron may be resulted from accumulated carotenoids (Rubio-Moraga et al. 2009). Moharekar et al. (2003) suggested that total carotenoid content, size of xanthophyll pool, and de-epoxidation rate increased significantly with an increase in SA concentration in both wheat and moong plants. It is suggested that SA may influence carotenoids biosynthesis pathway, which led to the highest *a* value. There was no significant difference among control and KNO_3 levels on *a*, *b*, *L*, and chroma. Increment of SA level from 0.5 to 2 mM led to a significant reduction in b value (Table 4).

Vegetative characteristics

The highest leaf appearance rate was observed in plants treated with 500 ppm KNO_3 and 2 mM SA and the lowest value was observed in control. Increment of SA concentration from 0.5 to 2 mM significantly increased this variable (Table 5). Khayyat et al. (2010) stated that spraying with potassium nitrate significantly increases petiole length of strawberry plants. Shakirova et al. (2003) indicated that SA treatments reduced the damaging action of salinity on wheat seedling growth, raising indole acetic acid content and enhancing cell division and extension of root cells. Khan et al. (2003) reported that SA stimulated the root formation of some crops. The number of leaves from corms and cormels was not affected by the applied treatments. The lowest EL value was obtained at 1000 ppm KNO_3 and 2 mM SA, in comparison with control and other treatments. Specifically, increment of SA concentration decreased this variable that is in agreement with Yildirim et al. (2008) results obtained under salt stress. On the other hand, the highest membrane stability index was shown in 1000 ppm KNO_3 and 2 mM SA and a trend similar to EL was observed (Table 5). Barkosky and Einhellig (1993) stated that exogenous application of SA influences membrane permeability.

Table 4. Effect of different levels of SA and KNO_3 on stigma color characteristics of saffron

Treatment		*a*	*b*	*L*	Hue	Chroma
Control (distilled water)	0	9.22 b	−15.44 b	38.50 b	58.24 a	18.24 b
KNO_3	250 ppm	8.83 b	−15.67 b	42.70 b	61.39 a	19.44 b
	500 ppm	10.71 b	−15.69 b	44.2 b	55.35 a	18.80 b
	1000 ppm	10.71 b	−17.26 b	38.60 b	55.91 a	18.89 b
SA	0.5 Mm	10.59 b	−19.00 b	35.3 b	60.78 a	21.75 b
	1 Mm	10.51 b	−19.23 b	39.70 b	61.30 a	21.94 b
	2 Mm	17.34 a	−25.34 a	56.3 a	55.14 a	30.92 a
SE		0.830	1.908	3.72	2.303	1.878

Note: see Table 3

Table 5. Effect of different SA and KNO_3 levels on vegetative characteristics of saffron

Treatment		Leaf appearing rate (day^{-1})	Corm leaf number	Cormel leaf number	Electrolyte leakage (%)	Membrane stability index
Control(distilled water)	0	1.25 e	6.75 a	4.75 a	27.30 a	72.7 c
KNO_3	250 ppm	1.86 d	4.00 b	4.00 a	21.10 ab	78.9 bc
	500 ppm	3.79 ab	6.50 a	4.75 a	19.60 ab	80.4 bc
	1000 ppm	3.54 b	6.50 a	5.25 a	14.60 abc	85.1 ab
SA	0.5 Mm	2.46 c	6.75 a	4.25 a	21.70 ab	78.3 bc
	1 Mm	3.35 b	6.50 a	4.25 a	21.50 ab	78.5 bc
	2 Mm	4.05 a	7.75 a	5.25 a	8.40 c	91.6 a
SE		0.1717	0.591	0.493	3.75	3.75

Note: see Table 3

Table 6. Effect of different levels of SA and KNO_3 on chlorophyll content and fluorescence efficiency of saffron

Treatment		Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Chlorophyll a /chlorophyll b	Fv/Fm
Control	0	0.34 b	0.077 ab	4.47 bc	0.054 b
KNO_3	250 ppm	0.35 b	0.096 a	3.66 c	0.093 ab
	500 ppm	0.34 b	0.086 ab	3.93 c	0.094 ab
	1000 ppm	0.31 bc	0.072 abc	4.33 c	0.069 ab
SA	0.5 Mm	0.27 c	0.045 c	6.56 ab	0.049 b
	1 Mm	0.27 c	0.060 bc	5.46 abc	0.100 ab
	2 Mm	0.44 a	0.066 bc	6.71 a	0.273 a
SE		0.01516	0.00927	0.711	0.0715

Note: see Table 3

Chlorophyll measurement and chlorophyll fluorescence

Chlorophyll content and related variable Fv/Fm were significantly influenced by the treatments (Table 2). Contents of Chl a decreased below control when SA was applied at 0.5 and 1 Mm but at 2 mM was significantly higher than control (Table 6). KNO_3 application did not affect this variable compared with control. Klessig and Malamy (1994) and Yildirim et al. (2008) reported that the application of SA significantly increased chlorophyll concentration. There was no significant difference among control, 500 and 1000 ppm of KNO_3 and 1 and 2 mM of SA on Chl b. Only at 0.5 mM SA content of Chl b was lower than control. Application of KNO_3 and 0.5 and 1 mM of SA did not affect Chl a/Chl b ratio, compared with control; however, the highest level was observed at 2 mM SA (Table 6). FV/Fm ratio was significantly higher at 2 mM SA, compared with control. Lower SA concentration and KNO_3 application did not affect Fv/Fm, which shows that probably natural chilling stress during vegetative growth of saffron has exerted a negative effect on photosynthetic apparatus (Jabbari et al. 2017).

Chlorophyll fluorescence yield can inform about stress or damage to the photosynthetic apparatus (Jabbari et al. 2017). Björkman and Demmig (1987) reported that Fv/Fm ratio is almost constant for many C3 plant species under optimal conditions and it ranges between 0.80 and 0.86. Thus, our control plants were likely under stress conditions and SA application at 1 and 2 mM was effective. It is suggested that endogenous levels of SA in our plants is not effective to face stressful conditions, so any endogenous or exogenous increment of SA can be useful for improvement of assimilation and flowering. It has been reported that SA application increases carbon dioxide (CO_2) assimilation and rate of photosynthesis (Khan et al. 2003; Fariduddin et

al. 2003; Szepesi et al. 2005), which may be related to Fv/Fm. Bongi and Loreto (1989) showed a positive correlation between photosynthetic inhibition and reduction of Fv/Fm. Under stress condition, radical oxygen species (ROS) are generated and carotenoids protect photosynthetic apparatus from damages (Young 1991). Tajik et al. (2015); Jabbari et al. (2017) reported that SA has a main role in a plant's growth, development, and defense system. It is also involved in some signal transduction systems to stimulate the enzymes related to biosynthesis of secondary metabolites in plants (Tajik et al. 2015). Thus, the increment of Fv/Fm in plants treated with 2 mM SA may be related to this process. On the other hand, because of the presence of nitrate-N in potassium nitrate, efficiency of photosynthesis and net CO_2 rates increases. Thus, these lead to increases in cell division and elongation in the growing areas. Moreover, this chemical has nutritional effects that interfere with growth and developing processes.

CONCLUSION

The important results of this experiment were the discovery of effectiveness of saffron corm dipping in solutions of salicylic acid, especially at 2 mM. This treatment improved vegetative and/or reproductive characteristics of saffron. SA treatment increased stigma dry weight and their redness that is important for market value. Consequently, it is advised to apply dipping of corms in SA for the improvement of saffron yield and its quality.

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