Fertigation Temperature Adjustment Enhances the Yield and Quality of Saffron Grown in a Soilless Culture System

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Abstract. Saffron is one of the most appreciated, traditional, and expensive spices in the world. The objective of our study was to evaluate the effect of cooling the nutrient solution on the production, and organoleptic and commercial qualities of saffron grown in soilless culture. The nutrient solution was cooled to 4 to 5 °C whereas the control treatment was the fertigation supplied at ambient temperature. Corms were placed in a controlled cultivation chamber. The number of flowers per corms, and the weight and length of stigmas were measured. The amounts of safranal, crocin, and picrocrocin were analyzed spectrophotometrically according to the International Organization for Standardization [ISO/TS 3632-2 (2011) Normative]. Our results show that cooling of the nutritive solution increased flower production, the commercial phytochemical content, and organoleptic properties.

The cultivation of saffron (*Crocus sativus* L.) is one of the oldest in the world and has occurred in the region of the Orient, near the Mediterranean, since the Late Bronze Age (Zohary and Hopf, 1994). It is one of the most expensive spices used in the food industry (García-Rodríguez et al., 2017; Hosseinzadeh and Nassiri-Asl, 2012). It is also highly appreciated in many cuisines around the world, and saffron has acquired increasing interest given the health effects of its chemical components, especially from safranal, crocin, and picrocrocin (Molina et al., 2005). This species has potential medical applications, particularly those based on its antitumor and antichronic stress properties (Amin et al., 2016; Ghadrdoost et al., 2011; Naeimi et al., 2019).

The quality of saffron is evaluated according to its bioactive compound content (Hadi-zadeh et al., 2007). The greater the amount of bioactive compounds, the greater the quality of saffron (Gohari et al., 2013). Since 1980, the International Organization for Standardization provides a standard procedure (ISO/TS 3632) for saffron quality classification:

The quality of saffron. Such quality is determined through the spectrophotometric quantification of picrocrocin, safranal and crocin by direct measuring of the absorbance of 1% standard aqueous solution of dried saffron at 257, 330 and 440 nm, respectively (International Organization for Standardization, 2003).

One of the factors that has proved to be important for the production of high-quality saffron is the environmental temperature at which plants are grown in terms of root and shoot growth, and flower formation (Ahrazem et al., 2015; Molina et al., 2005; Wang et al., 2021). In addition, it is well known that a markedly low temperature ($\approx 17^\circ\text{C}$) is the best for flower emergence or anthesis from

the corm (Molina et al., 2005). This knowledge allows growers to program saffron flowering and increase production in soilless cultivation systems under controlled conditions (Gohari et al., 2013; Wang et al., 2021).

Soilless cultivation systems have been developed through chamber temperature control (Molina et al., 2010; Valero et al., 2004). However, warming the air of the entire greenhouse requires more energy than increasing the root temperature by means of circulating hot water or air through buried tubes (Elwell et al., 1985; Wang et al., 2021). Moreover, warming of the nutrient solution is even cheaper and it is always applied daily as fertigation, given that it is a mandatory action to meet the water and nutrient requirements of the crop (Urrestarazu et al., 2008).

Studies of different species have shown that plant growth is greatly influenced by root temperature (Díaz-Pérez et al., 2007; Nxawe et al., 2009; Solfeld and Johnsen, 2006). For instance, Urrestarazu et al. (2008) reported that heating the nutrient solution in the 12- to 16-°C range was useful for increasing melon yield, and Yan et al. (2012) stated that an increase in root temperature improved nutrient uptake in cucumbers. However, little is known about the effects of increasing the nutrient solution temperature on saffron plants.

The main goal of this work was to determine the effects on yield and bioactive compound content of saffron by modifying the fertigation temperature to a develop low-cost method of obtaining high-quality saffron.

Materials and Methods

Plant growth conditions. The experiment was carried out in controlled growth chambers located at the University of Almería, Almería, Spain, between 2019 and 2020. The corms of *C. sativus* were obtained from Minaya (Albacete, Spain). This is a traditional saffron-producing area in Spain under the Regulatory Council Foundation of the Protected Designation of Origin La Mancha (DOP, 2021). Before planting, corms were incubated at 25 °C (Molina et al., 2004a) for 55 d. On 25 Aug. 2019, corms were transplanted into individual 500-mL containers. The substrate used was commercial coconut fiber, which has defined physicochemical characteristics that have been described by Pozo et al. (2014).

Fertigation treatments. Two nutrient solution temperatures were used: control, 10 to 15 °C; and cooled, 4 to 5 °C (Fig. 1). The plants were fertigated with a nutrient solution in line with the one described by Sonneveld and Straver (1994). The pH of the nutrient solutions was adjusted to 5.8 with dilute nitric acid. The nutrient solution was cooled using a refrigerator. Refrigerator, growth chamber, and substrate temperatures were controlled using an Onset Hobo Data Logger U23 Pro v2 (Onset Computer Corp., Bourne, MA). The temperatures were 5 °C inside the refrigerator, 16 °C in the culture chamber air, 16 to 17 °C in the substrate when applying the control

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Results and Discussion

Effect on growth parameters. Flower stigmas are the main commercial product of the saffron plant (Gresta et al., 2008). When the cooled nutrient solution was applied, an improvement of 20% and 24% was found in the number of flowers formed and the dry weight of the stigmas, respectively (Table 1). Flower formation mean per corm was 50% greater than that reported by Molina et al. (2004b) under similar crop conditions. No significant effects were recorded in the remaining vegetative growth parameters. It is well known that air temperature is one of the most important factors to induce saffron flowering (Gresta et al., 2009; Molina et al., 2004a; Wang et al., 2021). Molina et al. (2004a) reported that flower emergence required the transfer of the corms from the conditions of flower formation to a markedly lower air temperature (17°C). By applying a colder environment, greater flower production was obtained (Gresta et al., 2009). These results were similar to those reported by Urrestarazu et al. (2008) and Yan et al. (2012), who obtained significant benefits in the productivity of horticultural crops under soilless culture by means of modifying the temperature of the nutrient solution and root environment. This is because the root-zone temperature is an important factor in plant growth and water and nutrient uptake (Bode Stoltzfus et al., 1998; Marschner et al., 1996; Mozafar et al., 1993). So, the cooling of the solution is a low-cost and environmentally friendly method of increasing saffron yield.

Effect on chemical components. Under soilless culture, the crocin, safranal, and picrocrocin contents we obtained were significantly greater than those reported by Caballero et al. (2007) (Table 2). After applying cooled fertigation, increases of 16% and 91% in safranal and crocin, respectively, were realized. However, no significant differences were found in the amount of picrocrocin. So, the fertigation temperature affects two of the main bioactive compounds of saffron.

Effect on saffron quality. Saffron quality depends on the concentration of its major metabolites according to ISO 3632/2 (International Organization of Standardization, 2011). Table 3 shows significant differences in the quality of saffron characteristics according to nutrient solution temperature as evaluated by absorption values. It is well known that the secondary metabolites (i.e., crocin, picrocrocin, and safranal) range greatly from country to country based on several factors, including climatic conditions, the harvest and drying processes, and storage (Carmona et al., 2005). The data we obtained were within the ranges reported by Carmona et al. (2005). Under cooled fertigation, absorption values for crocin, safranal, and picrocrocin increased by 16%, 52%, and 13%, respectively, thus leading to an increase

Fig. 1. Schematic experimental unit of saffron crop and point of sample temperature under soilless culture.

nutrient solution, and 15 to 16°C in the substrate when the cooled nutrient solution was supplied. The photoperiod was 16/8 h (day/night) at 16°C, and the relative humidity was 85%.

Fertigation management was carried out according to the methodology proposed by Urrestarazu et al. (2015). The application of new fertigation was effected when the water in the growing unit reached 10% of the easily available water (Cunha-Chiamolera et al., 2017; Rodríguez et al., 2014; Urrestarazu et al., 2017). Each treatment was applied four times, and the drainage from all pots for each treatment was collected. The pH, electrical conductivity, and nitrate and potassium content of the nutrient solution and the drainage were measured using a pH-meter (Crison MM40+; Hach LPV2500.98.0002, Bizkaia, Spain), a conductivity meter (EC-Meter Crison BASIC 30, Hach), and a LAQUATwin B-742 and B-731 (Horiba, Northampton, UK), respectively. The volume was measured using a test tube graduated to one-hundredth of a millimeter.

Growth parameters. After flowering, the number of flowers formed per corm was counted, the stigma length was measured before and after the drying process, and the dry and fresh stigma weights were measured with a resolution of 0.1 mg.

The vegetative growth of the plants (number of corms formed, and roots and leaves in the dry and fresh states) was measured after 90 d of flowering. At the end of the experiment, the corms formed per plant were classified according to diameter, and the fresh and dry weights of the leaves were also measured. Roots were rinsed in distilled water to remove substrate residue, weighed, and transferred to paper bags for drying. This was accomplished by placing the material in a convection oven (Heratherm; ThermoScientific, Waltham, MA) at 75°C until a constant weight was achieved. This was measured using an OHAUS Adventurer Analytical Precision Analytical Balance (model AX 124/E; OHAUS, Parsippany, NJ), with a precision to four-tenths of a gram (Nájera and Urrestarazu, 2019; Urrestarazu et al., 2016).

Saffron quality analysis. The aqueous extracts of saffron were prepared according to ISO 3632 (International Organization of Standardization, 2011). The saffron filaments were crushed and sieved, and 50 mg of the

resulting powder was placed in a 100-mL volumetric flask, to which 90 mL distilled water was then added. The solution was stirred using a magnetic stir bar at 1000 rpm for 1 h while being kept away from light. The flask was filled to 100 mL and the solution was homogenized and filtered. After dilution (1:10, v/v), the samples of each treatment were measured in the 200- to 700-nm range using a quartz cell (path length, 1 cm). Absorbance readings at 257, 310, and 440 nm were related back to the 1% solution and were expressed as E 1% (257 nm) (maximum absorbance of picrocrocin), E 1% (330 nm) (maximum absorbance of safranal), and E 1% (440 nm) (maximum absorbance of crocin), according to ISO/TS 3632-2 (International Organization of Standardization, 2003). A blank system was prepared for each. The results were obtained by direct reading of the absorbance (D) as reported in the following equation:

$$E\ 1\% = (D \times 1000) / m(100 - M),$$

where D represents the specific absorbance, m is the weight of the sample, and M is the moisture of the sample.

Moisture was determined by weighing the sample in a watch glass that had been dried and tared previously. The tare containing the test portion was placed in an oven at 103°C for 16 h, and was then cooled and weighed using an analytical balance. Moisture (M) was calculated according to the following equation:

$$M = (m_i - m_f) \times 100 / m_h,$$

where m_i represents the weight of the tare containing the test sample (measured in grams), m_f is the weight of the tare more the dry test sample (measured in grams), and m_h is the test sample to determine the humidity.

To determine the concentrations of crocin, safranal, and picrocrocin, a standard curve was used according to Khoulati et al. (2019), and the absorbance was recorded using a Helios Gamma UV-Vis Spectrophotometer (Thermo Electron Corporation, Waltham, MA).

Experimental design and statistical analysis. The experiment was performed using a randomized complete block design, with four blocks (Petersen, 1994). The results were subjected to analysis of variance, and the comparison of means was made using Tukey's test ($P \leq 0.05$) with statistical

Table 1. Yield of saffron grown under soilless culture according to nutrient solution temperature.

Yield characteristic	Nutrient solution temperature	
	4 to 5 °C	10 to 15 °C
No. of flowers/corm	3.37 ± 0.45	2.61 ± 0.19**
Stigma (mg/flower)		
Fresh weight	42.60 ± 1.99	40.72 ± 1.89 NS
Dry weight	8.46 ± 0.96	7.02 ± 0.42*
Length of stigma (cm)		
Fresh	3.33 ± 0.07	3.02 ± 0.42 NS
Dry	2.31 ± 0.05	2.28 ± 0.02 NS
No. of corms/plant		
Diameter, >1.5 cm	22.00 ± 2.35	22.00 ± 3.46 NS
Diameter, <1.5 cm	23.80 ± 3.11	22.00 ± 2.45 NS
Leaves (g/corm)		
Fresh weight	81.13 ± 9.72	78.57 ± 11.18 NS
Dry weight	23.80 ± 0.70	23.72 ± 2.47 NS
Roots (g/corm)		
Fresh weight	12.38 ± 3.38	16.85 ± 3.96 NS
Dry weight	1.75 ± 0.39	2.32 ± 0.40 NS

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01 , respectively, using Tukey's test ($n = 4$).

Table 2. Pigment content of saffron according to the nutrient solution temperature (measured in milligrams per gram of stigmas).

Bioactive compound	Nutrient solution temperature	
	4 to 5 °C	10 to 15 °C
Crocin	117.90 ± 0.17	101.12 ± 0.17*
Safranal	3.09 ± 0.01	1.61 ± 0.31**
Picrocrocin	59.74 ± 0.01	59.25 ± 0.01 NS

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01 , respectively, using Tukey's test ($n = 4$).

Table 3. Quality characteristics of saffron according to International Organization for Standardization/ TS 3632-2 (2011) Normative versus nutrient solution temperature.

Nutrient solution temperature ²	Value	Category			
		I	II	III	IV
E 1% (440 nm)					
absorption value of crocin					
4 to 5 °C	163.80*		X		
10 to 15 °C	137.68 NS			X	
E 1% (330 nm)					
absorption value of safranal					
4 to 5 °C	35.71**	X			
10 to 15 °C	17.20 NS		X		
E 1% (257 nm)					
absorption value of picrocrocin					
4 to 5 °C	77.64*	X			
10 to 15 °C	67.69 NS		X		

²E 1% (440 nm) is the maximum absorbance of crocin, E 1% (330 nm) is the maximum absorbance of safranal, and E 1% (257 nm) is the maximum absorbance of picrocrocin.

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01 , respectively, using Tukey's test ($n = 4$).

in one level in the commercial ranking for two of the main bioactive compounds of saffron.

The temperature of fertigation is a behavior of a eustressor according to the work by Vázquez-Hernández et al. (2019).

Conclusion

Fine-tuning the temperature of the nutritive solution increases flower production, and crocin and safranal content without affecting the growth parameters of saffron. Cooling the nutrient solution is an effective, low-cost, and environmentally friendly methodology for

increasing saffron production and commercial quality based on bioactive compounds and organoleptic components.

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