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Original Article

Effects of Different Water Supply and Corm Planting Density on Crocin, Picrocrocin and Safranal, Nitrogen Uptake and Water Use Efficiency of Saffron Grown in Semi-Arid Region

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Abstract

Saffron's color, taste and odor result from the chemicals crocin, picrocrocin and safranal, respectively. Hence, in addition to quantitative yield, secondary metabolites content are known as crucial factors for a successful saffron production. Moreover, enhancing resources efficiency, especially water and nitrogen, is becoming increasingly important for agricultural improvement in arid and semi-arid regions. Thus, the effects of irrigation levels and corm planting on crocin, picrocrocin and safranal content, water use efficiency (WUE) as well as nitrogen use efficiency (NUE) of saffron were investigated as a two-year field experiment based on a randomized complete block design arranged in split-plot with three replicates. The irrigation levels (100, 75 and 50% of saffron water requirement) and corm planting pattern (50, 100, 200 and 300 corms m^{-2}) were allocated to main and sub-plots, respectively. Based on the results, crocin and picrocrocin content increased with decreasing irrigation levels. The highest WUE_S (WUE based on dry stigma yield) was obtained when 50% of saffron water requirement was supplied. However, the lowest WUE_C (WUE based on daughter corms yield) and NUE_C (NUE based on daughter corms yield) were obtained when 50% of saffron water requirement was applied. Irrespective of irrigation levels, WUE_S, WUE_C and NUE_C increased with increasing the planting density. The results demonstrated that although relatively severe water stress increases WUE_S and secondary metabolites in saffron stigmas, it could decrease WUE_C and NUE_C through affecting daughter corm growth.

Keywords: crocin, daughter corm, dry stigma yield, picrocrocin, safranal, water requirement

Introduction

Saffron (*Crocus sativus* L.) is a sterile geophyte plant growing in arid and semi-arid regions of the world (Sepaskhah and Kamgar-Haghighi, 2009). The medical aspects of saffron are primarily related to its secondary metabolites, such as crocin, picrocrocin and safranal, found in stigmas (Escribano *et al*., 1996; Tamaddonfard *et al*., 2014; Talaei *et al*., 2015). Crocin, picrocrocin and safranal are responsible for color, taste and odor of saffron, respectively (Lage and Cantrell, 2009; Srivastava *et al*., 2010). Hence, in addition to quantitative yield (flower or corms weight) (Koocheki *et al*., 2014; Khorramdel *et al*., 2015), qualitative yield (secondary metabolites content) is known as a crucial factor in successful saffron production (Omidi *et al*., 2009; Koocheki *et al*., 2016).

Water use efficiency (WUE) is defined as yield of marketable crop produced per unit of water used in evapotranspiration (Dong *et al*., 2011). It is generally believed that in the future, water availability will become increasingly scarce, particularly in arid and semi-arid regions, due to rapid urbanization, higher population growth and expanding areas of irrigation (Abbaspour and Sabetraftar, 2005; Chiew *et al*., 2011; de Souza and Costa da Silva, 2014). Hence, enhancing WUE is becoming increasingly important for agricultural improvement in these regions (El-Hendawy *et al*., 2008; Barati *et al*., 2015).

According to saffron's irrigation schedule, an optimal irrigation schedule consists of five to six irrigation rounds (Koocheki *et al*., 2014; 2016). These irrigation rounds are usually performed in mid-summer (for flowering induction), in early October (for flowering acceleration), in November (after flower picking and leaves appearance), in December (after winter weeding), in March and finally in April (supplementary irrigation for optimum daughter corm growth). However, in some arid and semi-arid regions, saffron fields are irrigated only once (in October), mainly due to water shortage, causing a significant reduction in flower and corm yields (Kafi *et al*., 2002; Koocheki *et al*., 2014). Hence, in spite of being a crop compatible with arid and semi-arid regions, with low water requirements (Alizadeh *et al*., 2009; Sepaskhah and Kamgar-Haghighi, 2009; Yarami *et al*., 2011), water shortage is the most important challenge in sustainable saffron production (Yarami and Sepaskhah, 2015). Therefore, it is critical to determine the amount of water by which saffron can produce maximum yield.

In addition to WUE, more attention should be paid to nitrogen use efficiency (NUE) as an important index in saffron sustainable production (Koocheki and Seyyedi, 2015).

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Nitrogen use efficiency, which is defined as the ratio of the crop yield to the total input of N applied, is split into acquisition and physiological efficiency (Lea and Azevedo, 2006; Salvagiotti *et al.*, 2009). Due to being a perennial species, at least in field conditions (Kumar *et al.*, 2009; Babaei *et al.*, 2014), as well as having dynamic N allocation among leaves or underground organs (Ourry *et al.*, 1988; Dordas, 2009), it appears that NUE in saffron is more complicated than within other annual plants (Koocheki and Seyyedi, 2015).

Optimum planting pattern based on mother corms density is one of the most factors affecting daughter corms behavior, resulting in more flower yield and of better quality (Kumar *et al.*, 2009; Koocheki *et al.*, 2011; 2014). Dense corm planting pattern can increase saffron production, especially during early years (Koocheki *et al.*, 2011, 2012 and 2014). Accordingly, it was hypothesized that dense corm planting pattern would increase WUE and NUE. Therefore, this experiment was aimed to study the crocin, picrocrocin and safranal content, WUE and NUE in response to different levels of saffron water requirement (SWR) and corm planting patterns.

Materials and Methods

Site description

Two-year field experiment was carried out during 2012-2013 and 2013-2014 growing seasons, at experimental station of Faculty of Agriculture, Ferdowsi University of Mashhad (latitude: 36°15′ N; longitude: 59°28′E; elevation: 985 m). The study site was classified in semi-arid region located in Northeast of Iran. Monthly rainfall and average temperature during both growing seasons are given in Fig. 1. The soil was clay (US system) and alkaline in reaction (pH 8.16). The soil (0-30 cm) has bulk density 1.29 g cm^{-3}, EC 1.13 dS m^{-1}; organic carbon 0.54%; available N 18 mg kg^{-1}; available P mg kg^{-1}; available K 165.19 mg kg^{-1}; clay, sand and silt, 49.80, 18.23 and 31.79%, respectively.

Experimental design and field management

The experimental design was a randomized complete block arranged in split-plot with three replicates. The irrigation levels (100, 75 and 50% of SWR equal to no water stress, mild water stress and relatively severe water stress) and corm planting pattern (50, 100, 200 and 300 corms m^{-2}) were allocated to the main and sub-plots, respectively.

SWR was calculated according to total potential evapotranspiration values in the first (523 mm) and second (640 mm) year of the experiment, respectively (Yarami *et al.*, 2011). More information about determination of SWR and irrigation schedule is given in Table 1.

Before mother corms (4-6 g) planting, composted cattle manure (25 ton ha^{-1}) was mixed into the soil and then plots were established. The plots were 2.5 × 1.5 m in size and 0.5 m apart. Composted cattle manure (N 1.65%; P 0.41%; K 0.87%; organic carbon 28.36%) was applied just in the first year of the experiment as the level of soil organic carbon was low (0.54%).

Mother corms planting was done on 17th of June 2012. Inter-row distance for each density was 20 cm. Irrigation (Table 1) was performed using polyethylene irrigation network equipped with counter. During both years of the experiment, weeds were controlled manually when required.

Flower and corm measurements

In the first year, flowers were manually picked up from mid to late of November 2012 and dried stigma yield was measured. Stigmas were dried (Fig. 2) in an oven at 30°C for 48 h. At the end of first growing season, daughter corm yield was determined using a 0.5 × 0.5 m quadrate per plot. The rest of the daughter corms were devoted for the second year. In the second year, flowers were manually picked up from early to late of November in 2013. Other measurements were performed same as in the first year of the experiment.

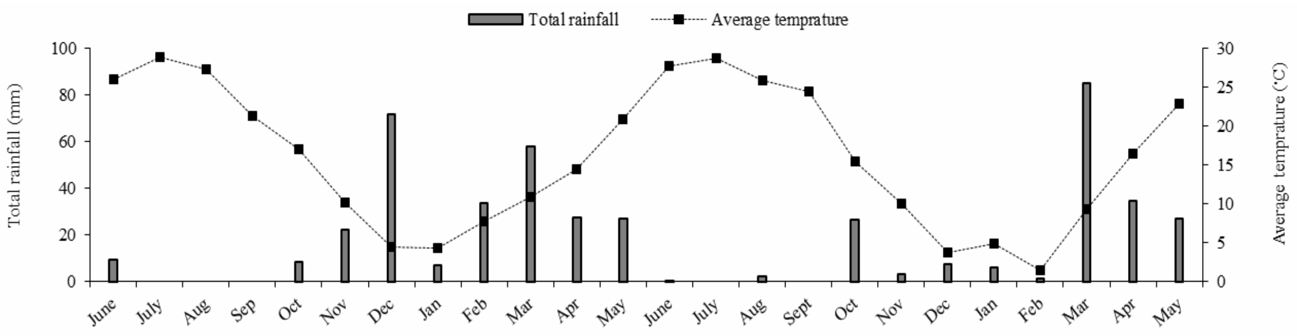

Fig. 1. Monthly rainfall and average temperature during both growing seasons (from June 2012 to May 2014)

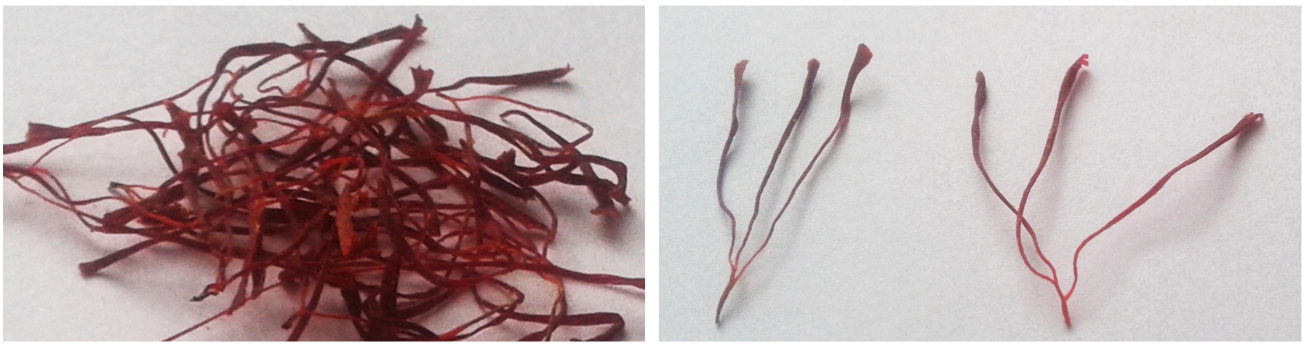

Fig. 2. Saffron stigmas after drying. Stigmas were dried in an oven at 30 °C for 48 h

Table 1. Determination of saffron water requirement (SWR) and total amount of applied water (TAAW) in the first and second years of the experiment

Irrigation frequency	30-year average precipitation (mm)	Irrigation times	Growth stages	Crop coefficien t (Kc)	Irrigation rate (percentage of total)	SWR (mm)	TAAW (mm) to plots		
							100% of SWR	75% of SWR	50% of SWR
First	4.41 (from first to second irrigation)	8th of August 2012	Initial	0.41	1.051	54.98	50.57	37.93	25.29
Second	13.94 (from second to third irrigation)	8th of October 2012	Initial	0.41	10.51	54.98	41.04	30.78	20.52
Third	26.05 (from third to fourth irrigation)	16th of November 2012	Middle	0.93	23.85	124.72	98.67	74.00	49.34
Fourth	96.11 (from fourth to fifth irrigation)	17th of December 2012	Middle	0.93	23.85	124.72	28.61	21.46	14.31
Fifth	57.55 (fifth first to sixth irrigation)	3th of March 2013	Middle	0.93	23.85	124.72	67.17	50.38	33.59
Sixth	71.70 (from sixth irrigation to harvest)	4th of April 2013	Final	0.29	7.44	38.89	-	-	-
Total (first year)	269.76				100	523	286.06	214.55	143.03
First	4.41 (from first to second irrigation)	8th of August 2013	Initial	0.45	10.32	66.06	61.65	46.24	30.83
Second	13.94 (from second to third irrigation)	8th of October 2013	Initial	0.45	10.32	66.06	52.12	39.09	26.06
Third	26.05 (from third to fourth irrigation)	16th of November 2013	Middle	1.05	24.08	154.13	128.08	96.06	64.04
Fourth	96.11 (from fourth to fifth irrigation)	20th of December 2013	Middle	1.05	24.08	154.13	58.02	43.52	29.01
Fifth	57.55 (fifth first to sixth irrigation)	16th of March 2014	Middle	1.05	24.08	154.13	96.58	72.44	48.29
Sixth	71.70 (from sixth irrigation to harvest)	4th of April 2014	Final	0.31	7.11	45.50	-	-	-
Total (second year)	269.76				100	640	396.45	297.34	198.23

SWR was calculated according to Kc coefficients at initial, middle and final growth stages in the first and second years of the experiment, respectively.

Determination of crocin, picrocrocin and safranal

In the first and second year, crocin, picrocrocin and safranal were measured based on ISO 3632 trade standard (ISO/TS 3632, 2003), using UV–vis spectrometric method. Crocin (440 nm), picrocrocin (257 nm) and safranal content (330 nm) were expressed as direct readings of the absorbance of 1% aqueous solution of dried stigma saffron (Lage and Cantrell, 2009).

Determination of WUE

WUE (water use efficiency) was calculated as follows:

WUE_S= Dry stigma yield (g ha^{-1}) / Total water use (mm)

WUE_C= Daughter corm yield (kg ha^{-1}) / Total water use (mm)

The total water used (TWU) was measured using the following equation (Dong *et al.*, 2011):

$TWU = P + I + \Delta W$

Where: P is the precipitation (mm), I is the irrigation (mm), ΔW is the soil moisture change (mm).

Due to plots design, there was no surface water runoff under the conditions of this experiment. The soil water drainage below the crop root zone (mm) and capillary water rise to the root zone (mm) were considered to be negligible.

Determination of NAE and NUE

Nitrogen concentration (g kg^{-1}) in daughter corms (plus corm tunics) and aerial part was measured based on Kjeldal method (AOAC, 2000). On the basis of dry stigma and daughter corms yields, nitrogen acquisition efficiency (NAE) and nitrogen use efficiency (NUE) were calculated using the following equations (Brennan *et al.*, 2014; Koocheki and Seyyedi, 2015):

$NAE\ (\%) = (Nt / Na) \times 100$

$NUE_S\ (g\,g^{-1}) = SY / Na$

$NUE_C\ (g\,g^{-1}) = CY / Na$

Where: Nt is g N in the total plant m^{-2}, Na is g N applied m^{-2}, SY is g dry stigma yield m^{-2}, and CY is g daughter corms yield m^{-2}.

Applied N was determined by the sum of following resources: 1- initial N content into soil before establishment of the trial (based on soil bulk density at the depth of 30 cm), 2- N added by composted cattle manure, and 3- N content in mother corms (Table 2).

Statistical analysis

Analysis of variance (ANOVA) and Duncan's multiple range tests were performed using SAS 9.3 software (SAS, 2011). Saffron traits were analyzed as split-split plot arrangement in time. The irrigation levels, corm planting density and harvesting (in the first and second year) were considered as first, second and third factors, respectively.

Results and Discussion

Flower quality

Although the effect of irrigation levels on crocin and picrocrocin content was significant, safranal content was not affected by irrigation levels (Table 3). In addition, the effect of planting density and harvesting year were not significant on above mentioned compounds (Table 3).

Interestingly, crocin and picrocrocin content increased with decreasing SWR levels. In other words, the highest crocin and picrocrocin content was observed under relatively severe water stress (50% of SWR) condition (Table 4).

The mechanism of saffron organic compound synthesis in response to water stress is not fully understood. As mentioned earlier, crocin and picrocrocin are the most important compounds found in saffron stigmas (Tarantilis *et al.*, 1995; Escribano *et al.*, 1996), so that higher amounts of these compounds leads to an increase in the quality of saffron (D'Auria *et al.*, 2004; Srivastava *et al.*, 2010; Koocheki *et al.*, 2016). On the other hand, it has been reported that the quantity of essential oils and secondary metabolites in plants would increase in response to drought stress (Singh-Sangwan *et al.*, 1994; Reddy *et al.*, 2004). In addition, an increase in

Table 2. N content (g m^{-2}) in saffron mother corms (4-6 g)

Average weight of mother corm (g)	N concentration in mother corms (g kg^{-1})	Planting density (corm m^{-2})	N content in mother corms (g m^{-2})
5	12.70	50	3.18
5	12.70	100	6.35
5	12.70	200	12.70
5	12.70	300	19.05

Table 3. Analysis of variance for the studied traits of saffron

S.O.V	df	Crocin	Picrocrocin	Safranal	WUE_S	WUE_C	NAE	NUE_S	NUE_C
Block	2	69.67 ns	7.39 ns	0.49 ns	3.16 *	85.83 **	64.54 **	0.0000014 *	38.88 **
Irrigation (*I*)	2	13789.51 **	1418.15 **	4.61 ns	44.55 **	2213.76 **	4114.38 **	0.0000030 **	2426.94 **
Error I	4	66.48	16.50	6.36	0.615	31.01	30.95	0.0000002	15.45
Planting density (*D*)	3	61.41 ns	7.58 ns	20.79 ns	159.76 **	4024.60 **	942.64 **	0.0000518 **	1135.66 **
I × *D*	6	95.45 ns	29.63 ns	4.38 ns	3.85 **	191.32 **	103.84 **	0.0000001 *	95.16 **
Error II	18	85.72	9.30	6.75	0.92	21.26	12.71	0.0000004	11.68
Harvesting or year (*H*)	1	120.07 ns	5.01 ns	6.70 ns	0.18 ns	227.66 **	2725.56 **	0.0000103 **	725.36 **
I × *H*	2	14.71 ns	0.02 ns	15.33 ns	5.93 **	183.69 **	165.40 **	0.0000014 *	85.40 **
D × *H*	3	108.72 ns	8.85 ns	6.83 ns	16.88 **	97.77 **	29.25 *	0.0000056 **	36.03 **
I × *D* × *H*	6	179.45 ns	10.42 ns	7.26 ns	1.17 ns	1.93 ns	4.39 ns	0.0000002 ns	2.86 ns
Error III	24	104.52	27.20	11.02	0.86	4.79	7.38	0.0000003	2.26
CV (%)	-	4.46	6.55	8.97	17.27	16.50	6.70	14.24	16.07

The asterisks *, **, or ns indicate statistical differences at p ≤0.05, p ≤0.01, or non-significant, respectively. WUES: water use efficiency (WUE) based on dry stigma yield; WUEC: WUE based on daughter corms yield; NAE: nitrogen acquisition efficiency; NUES: nitrogen use efficiency (NUE) based on dry stigma yield; NUEC: NUE based on daughter corms yield.

Table 4. Effects of irrigation, planting density and harvesting (year) on studied traits of saffron

Experimental treatments	UV–visible			WUE_S (g ha^{-1} mm^{-1})	WUE_C (kg ha^{-1} mm^{-1})	NAE (%)	NUE_S (g g^{-1})	NUE_C (g g^{-1})
	Crocin ($E^{1\%}_{440}$)	Picrocrocin ($E^{1\%}_{257}$)	Safranal ($E^{1\%}_{330}$)					
Irrigation (percentage of water requirement)								
50	251.05 a	87.67 a	37.29 a	6.66 a	26.66 c	25.48 c	0.0032 b	13.19 c
75	233.68 b	78.71 b	37.24 a	5.50 b	44.63 a	49.12 a	0.0038 a	31.43 a
100	203.67 c	72.37 c	36.51 a	3.94 c	29.77 b	47.05 b	0.0039 a	29.64 b
Planting density (corm m^{-2})								
50	232.00 a	79.08 a	37.80 a	2.00 d	19.33 d	32.50 d	0.0016 c	16.64 d
100	229.49 a	80.22 a	37.76 a	3.75 c	24.04 c	36.67 c	0.0029 b	19.91 c
200	228.72 a	80.06 a	35.50 a	7.44 b	39.23 b	44.80 b	0.0051 a	28.38 b
300	227.66 a	78.97 a	36.99 a	8.26 a	52.14 a	48.24 a	0.0050 a	34.07 a
Harvesting (year)								
First harvest (first year)	230.76 a	79.85 a	37.32 a	5.31 a	31.91 b	34.40 b	0.0033 b	21.58 b
Second harvest (second year)	228.18 a	79.32 a	36.71 a	5.41 a	35.46 a	46.70 a	0.0040 a	27.93 a

Values followed by the same letter were not significantly different at p ≤ 0.05 (DMRT). WUES: water use efficiency (WUE) based on dry stigma yield; WUEC: WUE based on daughter corms yield; NAE: nitrogen acquisition efficiency; NUES: nitrogen use efficiency (NUE) based on dry stigma yield; NUEC: NUE based on daughter corms yield.

protein content, peroxidase and superoxide dismutase activity, in response to drought stress, has been documented by Maleki *et al*. (2011). Consequently, it seems that increase in crocin and picrocrocin synthesis in response to drought stress is a compatibility mechanism in saffron.

Interaction between irrigation and planting pattern

Irrespective of irrigation levels, WUE_S, WUE_C, NAE, NUE_S and NUE_C increased with increasing planting density (Table 5). For instance, in full irrigated plants (100% of SWR), an increase in planting density from 50 to 300 corms m^{-2} increased WUE_S and NUE_C by 4 and 2 times, respectively.

As mentioned above, dense corm planting pattern not only improved flower yield during early years, but also promoted sustainable production of saffron (Koocheki *et al*., 2011; 2016). According to the literature, an increase in planting density can be a good approach to deal with water loss in arid regions (Stroosnijder *et al*., 2012). Hence, it seemed that dense planting pattern can increase NUE in saffron through more N uptake and help to reduce water loss.

In each level of planting density, the highest WUE_S was obtained when 50% of SWR was applied (Table 5). This might be due to flowering adaptation mechanisms to drought stress (Sepaskhah, 2009). In other words, drought stress is an incentive factor for flowering which in turns resulted in maximum WUE_S.

Regardless of the planting density, the lowest and highest WUE_C were obtained when saffron plants were irrigated with 50 and 75% of SWR, respectively. Under same conditions, in terms of planting density, the lowest NAE, NUE_S and NUE_C were recorded when 50% of SWR was applied (Table 5). For example, when 50 corms m^{-2} were cultivated, a reduction in irrigation water from 100 to 50% of SWR decreased NAE by 41.98%.

It appeared that mild water stress (supplying 75% of SWR), stimulated daughter corms growth through increasing root growth and better nutrients uptake, especially N. In other words, a slight reduction in water availability would increase corm yield per unit of available water. Nevertheless, considering the sensitivity of saffron to water shortage (Sepaskhah and Yarami, 2009; Renau-Morata *et al*., 2012; Yarami and Sepaskhah, 2015), sever stress would negatively affect daughter corm growth, NAE, NUE_S and NUE_C. In this regard, Renau-Morata *et al*. (2012) observed a decrease in the photosynthetic rate of saffron under water stress. Motalebifard *et al*. (2013) showed that water deficit stress caused a significant reduction in tuber numbers, yield and WUE of potato (*Solanum tuberosum* L. cv. 'Agria').

Interaction between irrigation and harvesting (year)

The results revealed that when 75 and 100% of SWR was applied, more WUE_S, WUE_C, NAE, NUE_S and NUE_C were registered in the second year rather than the first year of the experiment (Table 6). For instance, in full irrigated plots, NAE in the second year increased by 32.6% compared with the first year (Table 6).

Saffron is a plant that propagates by mean of corms, which are renewing each year. Above and underground organs grow more from year to year (Kumar *et al*., 2009; Koocheki *et al*., 2014), thus more nutrients are absorbed from the soil over the time (Koocheki *et al*., 2016). Moreover, higher growth of aerial part and roots cause lower water loss. According to the previous figures, developed above ground organs stimulate root growth and in turns improve plant capability to uptake water and nutrients. Therefore increasing plant density would increase WUE in crops (Sani *et al*., 2008; Stroosnijder *et al*., 2012).

Interaction between planting density and harvesting (year)

From the results obtained, when 50, 100 and 200 corms m^{-2} were cultivated, more WUE_S, WUE_C, NAE, NUE_S and NUE_S were recorded in the second year compared with the first year (Table 7). However, when 300 corms m^{-2} were cultivated, more WUE_S and WUE_C were recorded in the first year compared with the second year (Table 7). This might be due to more small corms, formed at the end of the first year, in 300 corms m^{-2} treatment. In this regard, Koocheki *et al*. (2012) found that an increase in planting density up to 400 corm m^{-2} increased the ratio of small daughter corms to total daughter corm. It has been reported that there is a positive relationship between corm size and flowering ability (Gresta *et al*., 2008; Douglas *et al*., 2014). Therefore, small corms formation in the first year and less flower production in the second year may be considered as the main reasons for reduction of WUE_S and WUE_C in saffron.

Correlation between water consumption and N uptake

There was a positive and significant correlation between WUE_C and NAE (Fig. 3A), WUE_C and NUE_S (Fig. 3B) and WUE_C and NUE_C (Fig. 3C). These results suggested that effective approaches for increasing WUE_C can be practiced through stimulating daughter corms growth and more N uptake from the soil, which improve flower yield per each unit of absorbed N. From the other point of view, considering the key role of N in stimulating vegetative growth, daughter corms formation and flower production (Chaji *et al*., 2013), it seems that higher NUE causes less soil evaporation and more saffron yield per unit of consumed water.

Table 5. Interaction effects of irrigation and planting density on studied traits of saffron

Irrigation (percentage of water requirement)	Planting density (corm m^{-2})	UV–visible			WUE_S ($g\,ha^{-1}\,mm^{-1}$)	WUE_C ($kg\,ha^{-1}\,mm^{-1}$)	NAE (%)	NUE_S ($g\,g^{-1}$)	NUE_C ($g\,g^{-1}$)
		Crocin ($E^{1\%}_{440}$)	Picrocrocin ($E^{1\%}_{257}$)	Safranal ($E^{1\%}_{330}$)					
50	50	252.27 (4.86)	88.23 (4.31)	37.82 (3.20)	2.38 (0.60) hi	15.99 (2.13) h	22.03 (2.81) g	0.0014 (0.0005) d	9.24 (1.45) f
	100	246.08 (4.20)	89.38 (6.53)	37.37 (2.56)	4.69 (0.79) ef	18.61 (2.93) gh	22.48 (6.12) g	0.0026 (0.0005) bcd	10.26 (2.50) ef
	200	254.04 (5.76)	88.27 (6.55)	35.66 (4.58)	9.20 (1.59) ab	28.83 (1.12) def	26.93 (3.91) f	0.0045 (0.0010) a	14.15 (1.70) e
	300	251.82 (9.81)	84.81 (4.25)	38.33 (3.09)	10.35 (3.19) a	43.21 (5.92) c	30.48 (3.77) f	0.0045 (0.0011) a	19.09 (2.14) d
75	50	239.02 (5.11)	79.50 (1.96)	37.94 (3.86)	2.18 (0.92) hi	24.42 (5.69) efg	37.49 (9.71) e	0.0018 (0.0008) bcd	20.18 (5.45) d
	100	237.48 (4.02)	76.98 (0.86)	37.74 (3.09)	3.72 (1.18) fg	29.80 (8.90) de	42.25 (11.71) cd	0.0029 (0.0010) bc	23.30 (7.84) cd
	200	229.63 (3.63)	77.99 (2.86)	35.87 (1.63)	7.67 (1.84) c	57.01 (9.74) b	58.77 (9.40) a	0.0054 (0.0015) a	39.74 (7.82) a
	300	228.58 (5.98)	80.37 (2.24)	37.42 (2.42)	8.41 (1.19) bc	67.29 (5.12) a	57.96 (9.40) a	0.0053 (0.0006) a	42.49 (4.29) a
100	50	204.71 (15.80)	69.51 (5.19)	37.65 (2.72)	1.46 (0.39) i	17.58 (2.05) h	37.97 (9.02) de	0.0017 (0.0006) cd	20.51 (4.27) d
	100	204.90 (18.58)	74.31 (1.96)	38.18 (3.30)	2.82 (0.76) gh	23.70 (4.06) fg	45.27 (10.17) bc	0.0031 (0.0011) b	26.17 (6.71) c
	200	202.48 (16.30)	73.93 (1.69)	34.98 (0.64)	5.46 (0.64) de	31.85 (3.62) d	48.70 (6.72) b	0.0054 (0.0009) a	31.25 (4.87) b
	300	202.58 (8.93)	71.75 (5.03)	35.22 (1.93)	6.03 (1.11) d	45.93 (4.76) c	56.28 (7.27) a	0.0053 (0.0005) a	40.63 (3.40) a

Values followed by the same letter were not significantly different at $p \le 0.05$ (DMRT). Values in the parenthesis indicate standard deviation (±) of means. WUE_S: water use efficiency (WUE) based on dry stigma yield; WUE_C: WUE based on daughter corms yield; NAE: nitrogen acquisition efficiency; NUE_S: nitrogen use efficiency (NUE) based on dry stigma yield; NUE_C: NUE based on daughter corms yield.

Table 6. Interaction effects of irrigation and harvesting (year) on studied traits of saffron

Irrigation (percentage of water requirement)	Harvesting (year)	UV–visible			WUE_S ($g\,ha^{-1}\,mm^{-1}$)	WUE_C ($kg\,ha^{-1}\,mm^{-1}$)	NAE (%)	NUE_S ($g\,g^{-1}$)	NUE_C ($g\,g^{-1}$)
		Crocin ($E^{1\%}_{440}$)	Picrocrocin ($E^{1\%}_{257}$)	Safranal ($E^{1\%}_{330}$)					
50	First harvest (first year)	252.80 (7.10)	87.97 (5.55)	37.04 (4.16)	7.09 (4.43) a	26.85 (13.14) d	22.14 (4.40) e	0.0031 (0.0017) b	12.03 (4.62) e
	Second harvest (second year)	249.30 (6.31)	87.38 (5.57)	37.54 (2.51)	6.22 (3.05) b	26.47 (9.95) d	28.81 (4.07) d	0.0034 (0.0015) b	14.34 (3.96) d
75	First harvest	234.07 (6.18)	78.94 (2.77)	38.46 (3.27)	4.94 (3.25) c	39.69 (20.93) b	40.59 (11.14) c	0.0033 (0.0019) b	26.53 (11.69) c
	Second harvest	233.29 (7.03)	78.47 (2.01)	36.02 (1.57)	6.05 (2.63) b	49.57 (17.92) a	57.65 (9.78) a	0.0044 (0.0017) a	36.32 (9.91) a
100	First harvest	205.41 (12.65)	72.64 (4.90)	36.45 (3.36)	3.91 (2.47) d	29.18 (13.56) c	40.46 (8.61) c	0.0034 (0.0019) b	26.17 (9.48) c
	Second harvest	201.93 (16.23)	72.11 (3.27)	36.57 (1.82)	3.97 (1.62) d	30.35 (9.34) c	53.65 (7.36) b	0.0043 (0.0015) a	33.11 (6.94) b

Values followed by the same letter were not significantly different at $p \le 0.05$ (DMRT). Values in the parenthesis indicate standard deviation (±) of means. WUE_S: water use efficiency (WUE) based on dry stigma yield; WUE_C: WUE based on daughter corms yield; NAE: nitrogen acquisition efficiency; NUE_S: nitrogen use efficiency (NUE) based on dry stigma yield; NUE_C: NUE based on daughter corms yield.

Table 7. Interaction effects of planting density and harvesting (year) on studied traits of saffron

Planting density (corm m^{-2})	Harvesting (year)	UV–visible Crocin ($E^{1\%}_{440}$)	Picrocrocin ($E^{1\%}_{257}$)	Safranal ($E^{1\%}_{330}$)	WUE_S (g ha^{-1} mm^{-1})	WUE_C (kg ha^{-1} mm^{-1})	NAE (%)	NUE_S (g g^{-1})	NUE_C (g g^{-1})
50	First harvest (first year)	233.55 (25.52)	79.91 (9.56)	38.99 (3.47)	1.50 (0.49) f	17.21 (2.59) g	26.09 (5.04) e	0.0011 (0.0002) f	13.55 (4.05) e
	Second harvest (second year)	230.44 (20.83)	78.25 (8.34)	36.61 (2.28)	2.51 (0.63) e	21.45 (6.25) f	38.90 (10.98) c	0.0022 (0.0004) e	19.73 (7.43) d
100	First harvest	234.12 (12.98)	79.74 (6.93)	37.89 (3.50)	3.30 (1.18) de	19.62 (2.90) f	28.92 (9.44) d	0.0023 (0.0003) e	14.94 (5.59) e
	Second harvest	224.86 (26.98)	80.70 (8.83)	37.64 (2.20)	4.19 (1.04) d	28.46 (7.74) e	44.42 (13.40) b	0.0035 (0.0009) d	24.87 (9.57) c
200	First harvest	228.65 (23.38)	79.87 (7.44)	35.26 (3.42)	6.80 (2.08) c	37.41 (12.14) d	39.04 (12.10) c	0.0043 (0.0008) cd	25.24 (10.44) c
	Second harvest	228.79 (25.42)	80.25 (7.76)	35.74 (1.88)	8.09 (2.00) b	41.06 (16.51) c	50.56 (16.41) a	0.0059 (0.0008) a	31.52 (13.36) b
300	First harvest	226.71 (25.30)	79.87 (8.31)	37.14 (3.60)	9.66 (2.67) a	53.40 (10.18) a	43.54 (12.11) b	0.0055 (0.0005) ab	32.57 (10.79) b
	Second harvest	228.61 (20.00)	78.08 (5.05)	36.84 (1.66)	6.86 (1.88) c	50.89 (14.36) b	52.94 (16.02) a	0.0046 (0.0009) bc	35.58 (12.40) a

Values followed by the same letter were not significantly different at $p \leq 0.05$ (DMRT). Values in the parenthesis indicate standard deviation (±) of means. WUES: water use efficiency (WUE) based on dry stigma yield; WUEC: WUE based on daughter corms yield; NAE: nitrogen acquisition efficiency; NUES: nitrogen use efficiency (NUE) based on dry stigma yield; NUEC: NUE based on daughter corms yield.

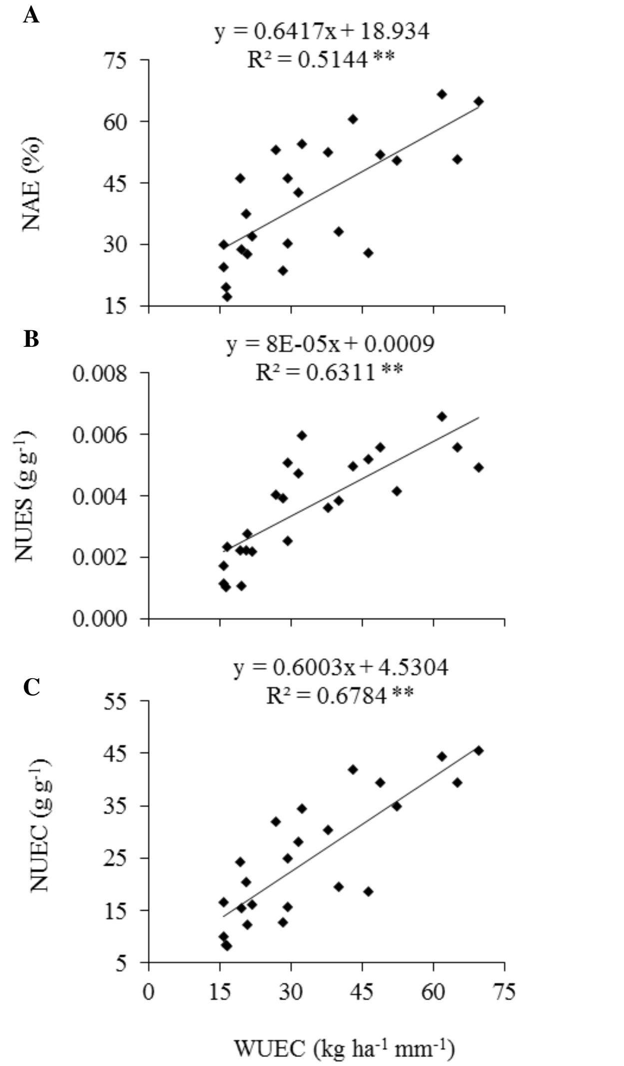

Fig. 3. Relationship between WUE_C and NAE (A), WUE_C and NUE_S, (B) and WUE_C and NUE_C (C) WUE_C: water use efficiency based on daughter corms yield; NAE: nitrogen acquisition efficiency; NUE_S: nitrogen use efficiency (NUE) based on dry stigma yield; NUE_C: NUE based on daughter corms yield.

Conclusions

Generally, the results demonstrated that dense planting pattern can be an effective approach for increasing WUE and NUE in saffron production. Furthermore, the results indicated that although relatively severe water stress increases WUE_S and secondary metabolites in saffron stigmas, it could decrease WUE_C, NAE, NUE_S and NUE_C through affecting daughter corm growth. Considering the positive relationship found between WUE_C and NAE, WUE_C and NUE_S, as well as between WUE_C and NUE_C, it can be stated that the crucial factors affecting daughter corms growth can lead to more efficient use of water and nitrogen in saffron crop.

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