Response of Saffron Ecotypes to Growing Season: Growth Analysis, Plant Nutrition, and Dry Matter Production

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ABSTRACT

Development of saffron corm resources with higher ability to acquire nutrients and produce more dry matter may offer one solution to mitigate the yield loss problem in growing areas. In the present study, variability in growth, nutrition, and biomass production among saffron ecotypes grown for a two-year field experiment was investigated at Kerman, a semi-arid region of Iran, during the 2015-2016 and 2016-2017 growing seasons. The results indicated that the studied ecotypes significantly differed in the mentioned parameters and responded differently to growing seasons. High-agronomic performance (yield) and nutrient-efficient ecotypes, e.g. Ferdows, Sarayan, and Bajestan, accumulated more nutrients as a result of increased Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) before the critical stage, resulting in higher dry matter production. In contrast, ecotypes with lower potential to acquire nutrients, e.g. Zarand and Torbat, had lower growth and dry matter. Further, the results showed that variation in nitrogen (N) concentration in corms and leaves was not significant, although significant variation existed in N uptake, N uptake efficiency, and N use efficiency. This can be due to variation observed in the ability of corms to utilize nutrients for dry matter production. Cluster analysis revealed the presence of highly efficient, moderately efficient, and inefficient ecotypes. Generally, the results indicated that ecotypes with higher growth rate before critical stage showed more potential to uptake and utilize nutrients to produce more dry matter, and exhibited more nutrients use efficiencies. Overall, this study suggested that the nutrient acquisition capacity of ecotypes, a desired feature associated with higher biomass production, can be an important factor in selection programs.

Keywords: Biomass production, Corm provenance, *Crocus sativus*, Growth indices, Nutrient-efficient corms.

INTRODUCTION

Saffron (*Crocus sativus* L.) is one of the most precious medicinal and aromatic products globally (Cardone *et al.*, 2020). This plant is used not only for food purposes (food colorant and flavoring agent) but also for cosmetics and pharmacological industries due to the latest health-promoting properties owing to its metabolites and bioactive compounds (Cardone *et al.*, 2020; Ghanbari *et al.*, 2019b, 2019a).

Of the total world production, about 250 tons (more than 90%) is produced by Iran (Agayev *et al.*, 2007; Kafi *et al.*, 2018). Nevertheless, saffron yield has dramatically decreased per unit area despite the expansion of cultivated lands (Agayev *et al.*, 2007; Cardone *et al.*, 2020). Production of saffron is adversely affected by limiting factors such as low soil fertility, inappropriate corms selection, and low corm quality (Baghalian *et al.*, 2010; Ghanbari *et al.*, 2019b; Ghanbari and Khajoei-Nejad, 2021).

Saffron is a triploid plant ($2n= 3x= 24$) that makes genetic improvement difficult through molecular plant breeding

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approaches (Agayev *et al.*, 2007). Since corm cultivation is the only possible way for saffron propagation, clonal selection is a viable way to create a new high- agronomic-performance and high-quality cultivars of saffron plant (Agayev *et al.*, 2007; Ben El Caid *et al.*, 2020; Ghanbari *et al.*, 2019b). Although saffron ecotypes with different geographic origins indicated extremely low genetic diversity (Busconi *et al.*, 2018), high phenotypic variability has frequently been observed in the field (Agayev *et al.*, 2007; 2009; Amirnia *et al.*, 2013; Ben El Caid *et al.*, 2020; Cardone *et al.*, 2019; Ehsanzadeh *et al.*, 2003; Ghanbari *et al.*, 2019b; Siracusa *et al.*, 2013). Agayev *et al.* (2009) studied five Iranian saffron ecotypes for flowering and corms production. They found that saffron ecotypes behaved inhomogeneously, despite similar clonal origin. However, recent works based on flowering, bioactive compounds, and quality indices of saffron ecotypes indicated significant effects of corm origin and corm origin-by-growing season interaction (Baghalian *et al.*, 2010; Ghanbari *et al.*, 2019b). Cardone *et al.* (2021) evaluated four ecotypes from different European contries in order to detect the variation of traits related to saffron flowering, yield and daughter corms production, leaf traits, and spice quality. They found phenotypic variation among ecotypes during two consecutive years.

Enhancement in vegetative growth can guarantee the growth and development of daughter corms by supplying the photosynthesis reserves (Rezvani-Moghaddam, 2020). The most important factor for improving growth and dry matter accumulation is Nutrient Uptake (NU) and nutrient utilization for producing the highest yield (Xu *et al.*, 2012). The information from research suggests that NU and Nutrient Utilization Efficiency (NUtE) are important for adaptation to different levels of soil fertility (Fukai *et al.*, 1999). Ideal corm would be those that perform well concerning the acquisition and use efficiency of the nutrients (Koocheki and Seyyedi, 2015). Hence, they may perform better in

producing vegetative biomass, corm production, and stigma yield of saffron (Koocheki and Seyyedi, 2015). Thus, enhancing the saffron's capacity for the acquisition of nutrients, improving vegetative growth, and increasing yield and Nutrient Use Efficiency (NUE) by the selection of the most suitable corms could be a very promising approach to guarantee productivity of the saffron farms.

Previous studies evaluated NUE in saffron under different fertilization (Bicharanloo *et al.,* 2021; Ghanbari and Khajoei-Nejad, 2021; 2022; Koocheki and Seyyedi, 2015) and irrigation conditions (Bicharanloo *et al.*, 2020). On the other hand, although considerable variation for flowering and corm yield, quality, and aroma profile were reported among corms with different origins when cultivated in new environmental conditions (Baghalian *et al.*, 2010; Ghanbari *et al.*, 2019b, 2019a), growth analysis, nutrition, and NUE components have not been studied among different saffron ecotypes. Furthermore, the relationship between growth parameters at different vegetative growth stages with nutrition and dry matter production in saffron has not been well studied.

For all the mentioned reasons, this study was undertaken to evaluate growth, nutrition, and biomass production and final NUE of saffron as a result of the growing location of the mother corms (or ecotype effect).

MATERIALS AND METHODS

Site Description and Experimental Design

A field experiment was conducted during two growing seasons of 2015-2017 at the experimental field of Shahid Bahonar University of Kerman, Kerman, Iran. The experimental site is located at 30.1440° N; 57.0715° E and 1,774 m above sea level, characterized by a semi-arid climate with an annual average temperature of 16°C and

rainfall of 206 mm during the experiment. Corms were collected from nine climatically dispersed provenances of saffron in Iran with a long tradition in saffron cultivation, namely, Bajestan, Estahban, Ferdows, Gonabad, Natanz, Qaen, Sarayan, Torbate-Heydarieh, and Zarand. The experiment was carried out in experimental plots of 12.8 m^2 during two consecutive growing seasons of 2015-2016 and 2016-2017. A split-plot in time design was arranged based on a completely randomized block design with three replications. The ecotypes constituted the main factor and growing season constituted the sub factor (harvest time).

Corms of 4–8 g weight were sown manually on 18 October 2015 in raised beds at 10-15 cm depth, 20 cm spacing and in row and 10 cm within rows; density of 50 corms m^{-2} and 640 corms (about 3.8 kg, on average) plot^{-1}. The planting bed was amended with 20 t ha^{-1} cattle manure compost, containing 160 kg N and 40 kg P$_2$O$_5$ .ha^{-1}. Irrigation was done based on Iranian indigenous knowledge of producers (Kafi *et al.*, 2018). Weeds were controlled manually over the experimental period. The other agronomic practices were uniformly applied to all the plots throughout the experiment.

Plant Samplings and Growth Analysis

Leaves were harvested in each growing season at three stages: once after the flowering period, once at the critical stage (almost 139 days after sowing and first irrigation in the first and second growing seasons, respectively; (Behdani *et al.*, 2016; Rezvani-Moghaddam, 2020), and the last one at the end of growing seasons. Leaf Area (LA) was determined using a leaf area meter (WinArea_UT_11, Iran) in cm^2 for harvested leaves in 10 plants randomly selected from 0.2 m^2, and the data were then converted back to Leaf Area Index (LAI) using Equation (1). Leaf Numbers (LN) per m^2 and Leaf Length (LL) were also recorded at the last sampling time. Subsequently,

leaves were washed, and leaf dry weight was recorded after drying at 70°C to constant weight. Different growth indices were then calculated by Equations (2), (3), and (4):

$$LAI = \left(\frac{(LA_2 + LA_1)}{2}\right) \div GA \quad (1)$$

$$CGR \ (g \ m^{-2} \ d^{-1}) = \left(\frac{(W_2 - W_1)}{(t_2 - t_1)}\right) \div GA \quad (2)$$

$$RGR \ (mg \ g^{-1} \ d^{-1}) = \frac{(\ln W_2 - \ln W_1)}{(t_2 - t_1)} \quad (3)$$

$$NAR \ (mg \ cm^{-2} \ LA \ d^{-1}) = \left(\frac{(W_2 - W_1)}{(t_2 - t_1)}\right) \times$$

$$\left(\frac{(\ln LA_2 - \ln LA_1)}{(LA_2 - LA_1)}\right) \quad (4)$$

Where, GA: The soil surface covered by the plant; W$_1$ and W$_2$: Leaf dry Weight at t$_1$ and t$_2$, respectively (g); LA$_1$ and LA$_2$: Leaf Area at t$_1$ and t$_2$, respectively (cm^2); t$_2$ – t$_1$: The period of time between two consecutive samplings (day); CGR: Crop Growth Rate; RGR: Relative Growth Rate, and NAR: Net Assimilation Rate.

Corm and leaves were sampled to measure Leaf Dry Matter (LDM), Corm Yield (CY), and Corm Dry Matter (CDM), as well as plant nutrient analysis, in a 1.6 m^{-2} from each subplot at the end of the first (4 May 2016) and second growing seasons (20 April 2017). Corm yield was determined after drying the corms at room temperature for 10 days (corms are commonly used for planting). Leaves and corms were washed two times with distilled water, dried in a forced-air oven at 70°C for 48 hours, and then LDM and CDM were measured.

Nutrient Analyses and Calculations

Nitrogen (N) concentrations in corms and leaves (%) were directly determined using an Elemental Analyzer (vario Macro, Elementar, Germany). Phosphorus (P) concentrations (%) in corms and leaves were photometrically determined according to the ammonium molybdate blue method (Mills and Jones, 1996).

Nutrient (N and P) Uptake (NU; g m^{-2}) in corms and leaves were calculated by Equation (5). Nutrient Utilization Efficiency

(NUtE; g g^{-1}) were determined based on the data of both growing seasons (Equation 7). Nutrient Uptake Efficiency (NUpE) and Nutrient Use Efficiency (NUE; g g^{-1} for corm yield or mg g^{-1} for stigma yield) were calculated based on corm or stigma yield data obtained at the end of the experiment (Equations 6 and 8; Koocheki and Seyyedi, 2015; Ghanbari and Khajoei-Nejad, 2021).

$$NU = \frac{Dry\ matter \times Nutrient\ concentration}{100} \quad (5)$$

$$NUpE = \frac{NU\ by\ corms}{Nutrient\ applied + Soil\ nutrient\ content} \quad (6)$$

$$NUtE = \frac{Corm\ yield}{NU\ by\ corms} \quad (7)$$

$$NUE = \frac{Corm/stigma\ yield}{Nutrient\ applied + Soil\ nutrient\ content} \quad (8)$$

Statistical Analyses

The data were subjected to Analysis Of Variance (ANOVA) using the SAS version 9.1 (SAS, Cary, NC, USA) to evaluate significant differences. Fisher's Least Significant Difference test (LSD; Probability values< 0.05) was employed to compare means. To investigate the variation of ecotypes and to identify the most effective traits contributing to final yield and NUE, Principal Component Analysis (PCA) and agglomerative hierarchical clustering based on Ward's method were performed by XLSTAT 2016 (Addinsoft, New York, NY, USA). Pearson's correlation analysis was performed to identify the relationships between parameters using the IBM SPSS Statistics ver. 22.0 program package (IBM SPSS statistics, Armonk, NY, USA).

RESULTS

Growth Indices and Dry Matter Production

As can be seen from the results, dry matter production and different growth indices (RGR, NAR, CGR, and LAI before and after the critical stages (BCS and ACS, respectively)) were significantly (P< 0.01) affected by ecotype by growing season interaction effect. Growth analysis showed that the highest growth was in Ferdows, Gonabad, and Bajestan in the second season. On the other hand, the lowest RGR$_{ACS}$ was recorded for the same ecotypes. In contrast, Zarand, Estahban, and Natanz, which had the lowest growth before critical stage, showed the highest RGR$_{ACS}$ and NAR$_{ACS}$ (Table 1). Pearson's correlation analysis showed a negative correlation between RGR$_{BCS}$ and NAR$_{BCS}$ with RGR$_{ACS}$ and NAR$_{ACS}$ (Figure 2a).

The highest LAI values in both stages were recorded by Bajestan and Ferdows in the first season and Ferdows in the second season (Table 1). Relationships between the leaves-related parameters demonstrated that LN contributed more to increasing LAI and LDM production compared with LL (Figure 2a). The highest LAI and LN was recorded in Ferdows at the end of the second season. Such changes contributed to its highest LDM production. As expected, Zarand and Torbat, with the lowest LN, LAI, NAR$_{BCS}$, and RGR$_{BCS,}$ produced the lowest LDM at the second season (Table 2). RGR and NAR before the critical stage showed positive correlation coefficients with growth, LAI, LN, dry matter production, and stigma yield of saffron, while RGR and NAR after the critical stage were negatively correlated with them. It was also found that CGR before the critical stage showed stronger correlations with growth, nutrition, dry matter production, and stigma yield than CGR after the critical stage, confirming that promoting saffron vegetative growth before the critical stage can lead to production of corms with higher flowering capacity. As expected, the corms of Ferdows and Sarayan produced considerably higher corm and total dry matter, while Zarand and Torbat had the lowest dry matter at the end of the second season (Table 2).

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Table 1. Growth indices, Relative Growth Rate (RGR), Net Assimilation Rate (NAR), Crop Growth Rate (CGR), and Leaf Area Index (LAI) before and after critical stage (BCS and ACS, respectively), for saffron ecotypes measured in the first (2015-2016) and second (2016–2017) growing seasons.[a]

Growing season	Ecotype	RGR (mg g⁻¹ d⁻¹) BCS	ACS	NAR (mg cm² LA d⁻¹) BCS	ACS	CGR (g m⁻² d⁻¹)[b] BCS	ACS	LAI[b] BCS	ACS
2015-2016	Bajestan	7.74^f	4.62^{cd}	7.90^{gh}	5.35^{fgh}	0.15^{cd}	0.14^{cd}	0.100^a	0.139^a
	Estahban	14.6^c	10.3^a	14.5^a	12.4^a	0.18^{bc}	0.27^a	0.064^e	0.113^{cd}
	Ferdows	12.9^{cd}	4.37^d	13.8^{abc}	5.12^{gh}	0.25^a	0.14^{cd}	0.094^{ab}	0.141^a
	Gonabad	13.6^c	1.30^e	14.1^{ab}	1.22^j	0.22^{ab}	0.03^e	0.082^c	0.133^{ab}
	Natanz	8.68^{ef}	9.13^{ab}	10.1^{efg}	11.4^a	0.11^{de}	0.22^{ab}	0.060^e	0.100^{cd}
	Qaen	9.01^{ef}	4.47^{cd}	9.4^{fgh}	4.14^{hi}	0.16^{cd}	0.11^d	0.086^{bc}	0.135^a
	Sarayan	13.2^{cd}	5.36^{cd}	14.2^{ab}	5.95^{e-h}	0.24^a	0.16^{bcd}	0.087^{bc}	0.139^a
	Torbat	11.1^{de}	6.89^{bc}	12.1^{b-e}	7.82^{cde}	0.17^c	0.17^{bcd}	0.072^d	0.117^{bc}
	Zarand	6.91^f	9.04^{ab}	7.50^h	10.5^{ab}	0.09^e	0.18^{bc}	0.058^e	0.098^d
2016-2017	Bajestan	17.0^{ab}	6.22^f	14.3^{ab}	5.96^{e-h}	1.23^b	0.82^{bc}	0.470^c	0.713^{cd}
	Estahban	14.5^c	7.29^{de}	11.4^{c-f}	5.88^{e-h}	0.83^c	0.76^{cd}	0.420^d	0.657^d
	Ferdows	18.7^a	6.32^f	15.2^a	6.18^{d-h}	1.67^a	1.09^a	0.610^a	0.945^a
	Gonabad	17.7^a	3.18^g	13.7^{abc}	2.78^{ij}	1.34^b	0.40^f	0.519^b	0.751^{bc}
	Natanz	15.2^{bc}	8.25^{bc}	13.4^{abc}	8.36^{bcd}	0.86^c	0.89^b	0.350^e	0.534^e
	Qaen	14.6^c	8.60^b	12.0^{b-e}	8.43^{bc}	0.82^c	0.84^{bc}	0.361^e	0.556^e
	Sarayan	15.2^{bc}	6.85^{ef}	13.1^{a-d}	6.67^{c-g}	1.28^b	1.04^a	0.530^b	0.787^b
	Torbat	14.0^c	7.91^{cd}	10.5^{ef}	6.74^{c-g}	0.67^d	0.66^e	0.348^e	0.522^e
	Zarand	14.0^c	9.38^a	10.7^{def}	7.37^{c-f}	0.58^d	0.73^{de}	0.280^f	0.487^e
P-value		0.0008	0.0022	0.0005	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a] Mean±standard error (n= 3) for each trait; different superscript letters indicate significant differences (LSD test, P< 0.05).[b] Means for these traits were compared separately for each growing season due to the multiplier changes in these traits during consecutive growing seasons.

Table 2. Leaf Length (LL), Leaf Number (LN), Leaf Dry Matter (LDM), Corm Dry Matter (CDM), and Total Dry Matter (TDM) for saffron ecotypes measured at the end of the first (2015-2016) and second (2016–2017) growing seasons.[a]

Growing season	Ecotype	LL (cm)	LN per m⁻²	LDM (g m⁻²)	CDM (g m⁻²)	TDM (g m⁻²)
2015-2016	Bajestan	31.5^{de}	338^d	34^{abc}	164^a	198^a
	Estahban	26.0^i	579^a	34^{ab}	120^{cd}	154^{cd}
	Ferdows	31.2^{def}	432^{bc}	36^a	157^{ab}	193^{ab}
	Gonabad	31.1^{def}	264^f	25^f	113^{cde}	138de
	Natanz	27.0^{hi}	410^c	30^{cde}	91^e	121^e
	Qaen	29.4^{fg}	272^{ef}	29^{def}	120^{cd}	149^{cd}
	Sarayan	31.5^e	351^d	36^a	134^{bc}	170^{bc}
	Torbat	28.2^{gh}	461^b	31^{bcd}	108de	139de
	Zarand	27.6^{ghi}	311de	26^{ef}	64^f	91^f
2016-2017	Bajestan	32.9^{cd}	1369^{bc}	153^c	961^b	1114^b
	Estahban	35.9^b	1194^{cd}	123^e	675^d	797^d
	Ferdows	35.2^b	1777^a	199^a	1080^a	1279^a
	Gonabad	30.8^{ef}	1440^b	140^d	838^c	978^c
	Natanz	38.5^a	1056de	127^e	659^d	786^d
	Qaen	34.0^{bc}	1060de	121^e	807^c	929^c
	Sarayan	34.6^{bc}	1467^b	176^b	1062^a	1238^a
	Torbat	34.4^{bc}	1011^e	101^f	515^e	616^e
	Zarand	35.0^b	923^e	93^f	550^e	643^e
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a] Mean±standard error (*n* = 3) for each trait; different letters indicate significant differences (LSD test, P< 0.05).
Means for these traits (except for LL) were compared separately for each growing season due to the multiplier changes in these traits during consecutive growing seasons.

Nutrient Concentration and Nutrient Uptake

Nitrogen concentration in corm and leaf and Phosphorus (P) concentration in leaves of saffron were not significantly affected by the studied factors. However, significant variation in corm P concentration (P< 0.05) was observed among different ecotypes. The P concentration of Zarand and Torbat ecotypes were significantly higher than Sarayan, Ferdows, and Bajestan (Figure 1). Negative, and in most cases significant, correlations were observed between growth indices before the critical stage, leaf parameters, and dry matter production with corms and leaves N and P concentrations, while significantly positive correlation coefficients were found between the mentioned parameters with the N and P uptake by leaves and corms (Figure 2a and Table S2). The results further demonstrated that N and P uptake by leaf, corm, and whole saffron plant differed significantly under the interactive effect of ecotype and growing season (P< 0.05). The highest nitrogen uptake (NiU) and P Uptake (PU) by leaves, corms (NiU_C and PU_C) and whole plant (NiU_T and PU_T) were found in Sarayan, Ferdows and Bajestan ecotypes at the end of the second season. On the other

hand, the lowest values were recorded for the corms originating from Torbat and Zarand in the second season (Table 3).

Nutrient Use Efficiency Components

Statistically significant differences existed (P< 0.05) among the ecotypes for N and P Uptake Efficiency (NiUpE and PUpE), P Utilization Efficiency (PUtE), and N and P Use Efficiency examined for both corms ($NiUE_C$ and PUE_C) and stigmas ($NiUE_S$ and PUE_S) of saffron. No significant difference was observed in the NiUtE between the different ecotypes (Table 4).

With a similar variation trend between NiU_C in the second season, the highest NiUpE was recorded by Sarayan and Ferdows. Zarand and Torbat ecotypes showed the lowest values. Similarly, Sarayan recorded the highest PUpE, which did not significantly differ with Ferdows, Gonabad, and Bajestan. The lowest values were observed for Zarand and Torbat (Table 4).

The Pearson correlation analysis showed that NiUpE and PUpE correlated positively with NiUtE and PUtE (Table S2). Also, Ferdows and Sarayan with higher PUpE utilize P more efficiently than others, resulting in considerable PUE_C. The highest
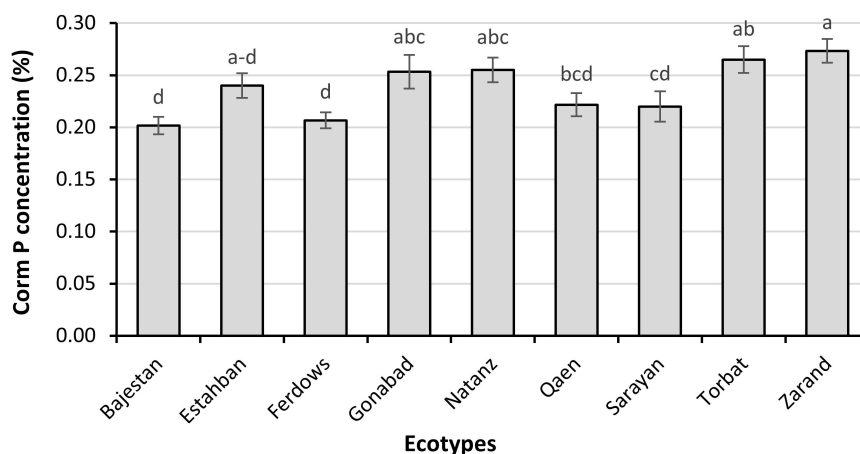

Figure 1. Phosphorous concentrations in corm of different saffron ecotypes average of two studied growing seasons.

Table 3. Nitrogen (N) and Phosphorous (P) uptake by corm, leaf, and whole plant of saffron ecotypes measured at the end of the first (2015-16) and second (2016–2017) growing seasons. [a]

Growing season	Ecotype	N uptake (g m^{-2})			P uptake (g m^{-2})		
		Leaf	Corm	Whole plant	Leaf	Corm	Whole plant
2015-2016	Bajestan	1.15^{ab}	4.77^a	5.92^a	0.065^{abc}	0.33^a	0.40^a
	Estahban	1.21^a	3.88^{abc}	5.09^{a-d}	0.068^{ab}	0.28^{ab}	0.35^{ab}
	Ferdows	1.23^a	4.57^{ab}	5.80^{ab}	0.069^{ab}	0.31^a	0.38^a
	Gonabad	0.87^c	3.44^{bcd}	4.32^{cde}	0.052^d	0.29^{ab}	0.34^{ab}
	Natanz	1.05^{abc}	3.11^{cd}	4.16^{de}	0.062^{a-d}	0.24^{bc}	0.30^{bc}
	Qaen	1.02^{abc}	3.78^{abc}	4.79^{a-d}	0.059^{bcd}	0.27^{ab}	0.33^{ab}
	Sarayan	1.19^a	4.17^{abc}	5.37^{abc}	0.070^a	0.28^{ab}	0.35^{ab}
	Torbat	1.13^{abc}	3.55^{abc}	4.67^{bcd}	0.069^a	0.28^{ab}	0.35^{ab}
	Zarand	0.93^{bc}	2.29^d	3.22^e	0.057^{cd}	0.18^c	0.24^c
2016-2017	Bajestan	5.13^{bc}	25.2^{ab}	30.3^{ab}	0.290^{bc}	1.89^{a-d}	2.18^{bcd}
	Estahban	4.25^{cd}	19.9^{bc}	24.2^{cd}	0.258^{cd}	1.64^{cd}	1.89^{cde}
	Ferdows	6.84^a	28.2^a	35.1^a	0.383^a	2.30^{ab}	2.69^{ab}
	Gonabad	5.00^{bc}	23.9^{ab}	28.9^{bc}	0.324^{ab}	2.13^{abc}	2.45^{abc}
	Natanz	4.43^{cd}	19.7^{bc}	24.2^{cd}	0.283^{bc}	1.65^{cd}	1.93^{cde}
	Qaen	4.29^{cd}	23.8^{ab}	28.1^{bc}	0.246^{cd}	1.78^{bcd}	2.02^{cde}
	Sarayan	5.93^{ab}	28.6^a	34.5^a	0.347^{ab}	2.41^a	2.76^a
	Torbat	3.62^{de}	15.2^c	18.9^d	0.229^{cd}	1.37^d	1.60^e
	Zarand	3.29^e	16.6^c	19.9^d	0.211^d	1.49^d	1.70^{de}
P-value		<.0001	0.0017	0.0002	0.0007	0.0119	0.0044

[a] Mean ± standard error (*n* = 3) for each trait; different letters indicate significant differences (LSD test, P< 0.05). Means for these traits were compared separately for each growing season due to the multiplier changes in these traits during consecutive growing seasons.

Table 4. Nitrogen (N) and Phosphorous (P) Uptake Efficiencies (NUpE and PUpE), corm and stigma N and P Use Efficiencies (NUE and PUE) of saffron ecotypes calculated at the end of the experiment (n= 3), as well as N and P Utilization Efficiencies (NUtE and PUtE) measured for the data obtained from two growing seasons (n= 6).[a]

Ecotype	NUpE	PUpE	NUtE (g g^{-1})	PUtE (g g^{-1})	NUE		PUE	
					Corm (g g^{-1})	Stigma (mg g^{-1})	Corm (g g^{-1})	Stigma (mg g^{-1})
Bajestan	0.148^{ab}	0.00105^{a-d}	147	2017^{abc}	23.6^b	2.21^c	2.23^b	0.209^c
Estahban	0.117^{bc}	0.00091^{cd}	136	1746^{bcd}	16.0^d	1.10^{ef}	1.51^d	0.104^{ef}
Ferdows	0.166^a	0.00128^{ab}	164	2141^a	26.6^a	3.02^a	2.51^a	0.285^a
Gonabad	0.141^{ab}	0.00118^{abc}	147	1697^{bcd}	20.6^c	2.12^c	1.95^c	0.200^c
Natanz	0.116^{bc}	0.00092^{cd}	131	1643^{cd}	16.3^d	1.34^{de}	1.54^d	0.127^{de}
Qaen	0.140^{ab}	0.00099^{bcd}	144	1982^{a-d}	20.1^c	1.58^{de}	1.89^c	0.149^{de}
Sarayan	0.168^a	0.00134^a	152	2040^{ab}	26.8^a	2.53^b	2.53^a	0.239^b
Torbat	0.090^c	0.00076^d	136	1630^d	12.8^e	0.76^g	1.21^e	0.072^g
Zarand	0.098^c	0.00083^d	134	1603^d	13.6^e	0.85^{fg}	1.28^e	0.080^{fg}
P-value	0.0006	0.0087	0.343	0.043	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a] Mean±standard error for each trait; different letters indicate significant differences (LSD test, P< 0.05).

NiUE$_C$ was also calculated for Ferdows and Sarayan, which can be due to their higher NiU and NiUpE. All NUE components correlated positively with NiUE and PUE and, ultimately, dry matter production and stigma yield of saffron (Table S2; Figure 2-a). The most heightened NUE$_S$ and PUE$_S$ were also recorded for Ferdows, followed by Sarayan ecotypes at the end of the experiment. Zarand and Torbat, with the lowest PUpE, utilized P less efficiently, leading to the lower PUE$_C$ and PUE$_S$ (Table 4).

Variation among Ecotypes Studied by Multivariate Statistical Analyses

PCA was conducted to identify the most influential variables explaining the variance among different ecotypes and explore their interrelationships. The main differentiation was achieved by the PC1 (80% of total

variation) that successfully classified different ecotypes based on their performances. Further, PC1 showed a positive correlation with vegetative growth parameters before the critical stage, NU, dry matter production, final yield, and NUE components of saffron, but negatively correlated with RGR$_{ACS}$ and NAR$_{ACS}$, and nutrient concentrations in corms and leaves. PC1 scores successfully distinguished Ferdows, Sarayan, and Bajestan from the other saffron ecotypes, pointing out their higher performance. Zarand, Torbat, Natanz, Estahban, and Qaen positioned the negative ranges of the first component and characterized by higher N and P concentrations and lower agronomic performance. The second PC (PC2) provided about 10% contribution to the differentiation. Gonabad was differentiated from the others based on PC2 scores. This was greatly due to the considerable RGR$_{BCS}$ and NAR$_{BCS}$ and the lowest RGR$_{ACS}$ and
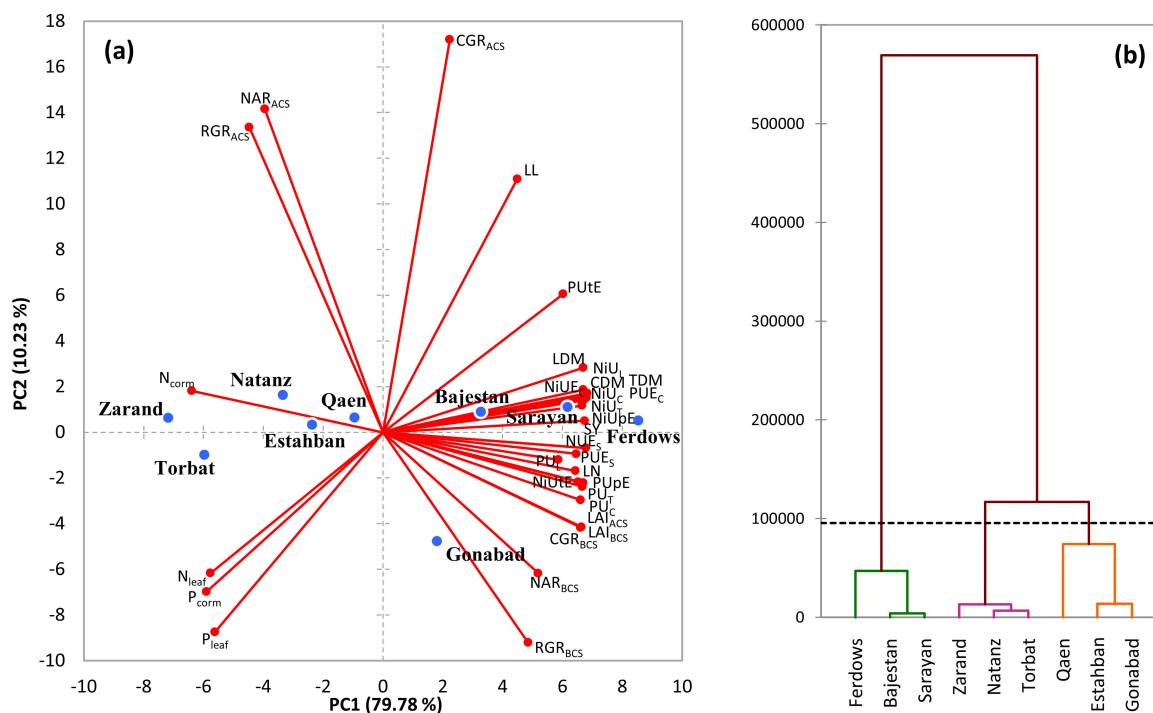

Figure 2. (a) PCA scatter plot and **(b)** Agglomerative hierarchical clustering to the classification of the studied saffron ecotypes based on growth, nutrition-related parameters, dry matter production, and stigma yield.

NAR$_{ACS}$ (Figure 2-a).

The cluster analysis results (Figure 2-b) partially confirmed PCA results since Ferdows, Sarayan, and Bajestan were classified as highly-efficient ecotypes. Gonabad, Estahban, and Qaen were classified in the second group as moderately-efficient ecotypes. The third consisted of three ecotypes as inefficient ecotypes (Zarand, Torbat, and Natanz), which were characterized by the weakest performance as well as higher N and P concentrations in corm and leaf (Figure 2-b).

DISCUSSION

Variation among Ecotypes

The ANOVA results revealed that most of the total variance (more than 80 percent) in growth (CGR and LAI), LN, LDM, CDM, and TDM, NiU, PU, NiUE, and PUE was explained by the growing season, reflecting a much wider range of year than ecotype effect and ecotype by year interaction effect. Such observations on the year effect in saffron can be due to the unique growth pattern of saffron as a perennial plant. Increasing the vegetative growth, dry matter production, and improving nutrition-related parameters in the second season can be due to new daughter corms production during the corms-formation in the first growing season (Gresta *et al*., 2009; Ghanbari *et al*., 2019b).

In the PCA analysis (Figure 1-a), PC1 efficiently discriminated different ecotypes. The most influential variables, nutrient uptake, nutrient uptake efficiency, dry matter production, and nutrient use efficiency contributed to the ecotype variation. Also, the current work results indicated significant differences among tested traits of saffron ecotypes in response to the studied growing seasons. These responses resulted from differences in growth parameters examined, which, in turn, caused differences in nutrition and dry matter production. Variation observed among saffron ecotypes resulted from differences in growth parameters examined, which, in turn, caused differences in nutrition and dry matter production. According to the response of growth, nutrition, and agronomic performance, ecotypes examined in this study were divided into highly efficient, moderately efficient, and inefficient ecotypes (Figure 2-b). High-performing ecotypes, however, exhibited a larger dry matter production that could be attributed to increased RGR and NAR before the critical stage, which resulted in the higher CGR, LN, LL, LAI, and finally LDM (Tables 1 and 2). High agronomic performing and high nutrients-efficient ecotypes accumulated more nutrients because of increased density in root system and size of daughter corms as new sinks for photosynthetic reserves, which resulted in higher CDM and TDM. In contrast, corms with a lower potential to acquire nutrients had lower growth and produced considerably lower dry matter in the present experiment (Tables 1, 2, and 3). For many attributes, large ecotype variations have been reported, e.g. for growth and yield (Baghalian *et al*., 2010), quality (Baghalian *et al*., 2010; Ghanbari *et al*., 2019b), bioactive compounds (Ghanbari *et al*., 2019b), and aroma profile (Ghanbari *et al*., 2019a).

Several studies evaluated genetic diversity among *C. sativus* species using different molecular markers (Babaei *et al*., 2014; Bayat *et al*., 2016; Busconi *et al*., 2018). For instance, Bayat *et al.* (2016), detected narrow and restricted genetic diversity among different saffron accessions from different countries (Iran, Spain, and Turkey) by SSR marker. Mir *et al.* (2021) reported limited genetic differences among Indian saffron samples. They concluded that different accessions of saffron were genetically differentiated minimally. Close relationships among 28 Iranian saffron accessions was detected in the study conducted by Babaei *et al.* (2014). They reported that this could be due to vegetative propagation, superior genotypes selection by

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farmers, and narrow genetic base of saffron. Variation among 17 Saffron accessions during four consecutive years was studied by Busconi *et al.* (2018), and it was reported that, despite the low genetic variability, MS-AFLP analysis revealed a very high epigenetic difference among the accessions. Although extremely low genetic diversity was indicated, saffron ecotypes showed high phenotypic variability in the field (Busconi *et al.*, 2018; Ehsanzadeh *et al.*, 2003; Ghanbari *et al.*, 2019b). Phenotypic variation among saffron ecotypes was observed in vegetative growth (Ehsanzadeh *et al.*, 2003; Cardone *et al.*, 2021), flowering and stigma yield (Baghalian *et al.*, 2010; Ghanbari *et al.*, 2019b; Cardone *et al.*, 2021), and quality related traits (Siracusa *et al.*, 2013; Ghanbari *et al.*, 2019a) is consistent with the results of the present study.

Vegetative Growth Analysis

The higher rates of dry matter accumulation in leaves before the critical stage were due to higher amounts of RGR and NAR at this stage. PCA analysis also confirmed the positive relationships between these indices before the critical stage with nutrition-related parameters, dry matter production, and stigma yield of saffron (Figure 2-a). These findings suggest that growth rate enhancement in this stage mainly contributes to increased LAI and dry matter production, which could lead to the increased final yield of saffron. As stated previously, while CGR follows an additive trend before the critical stage, a decreasing trend occurs afterward. Regarding the RGR and NAR, similar trends were also recorded in this stage (Rezvani-Moghaddam, 2020). Increased RGR and NAR before the critical stage may be associated with greater participation of young leaves in photosynthesis, resulting in more efficient photosynthesis and faster growth of the leaves (Rezvani-Moghaddam, 2020). The reduced RGR and NAR indices after the

critical stage can be due to the enhanced leaf age and changes in the destination of assimilates during the post-critical stage in saffron (Behdani *et al.*, 2016; Rezvani-Moghaddam, 2020). After the critical stage, developing daughter corms become the leading sinks for photosynthetic reserves and accumulation of nutrients in saffron plants (Behdani *et al.*, 2016).

The variations in response to the studied seasons have been linked to the corm's adaptability to environmental conditions where they originated. These adapted corms reacted differently to the cultivation in new environmental conditions (Baghalian *et al.*, 2010). Similar to these findings, Busconi *et al.* (2018) believe that epigenetic variations, which could be influenced by environmental conditions, caused the phenotypic variation among corms of different cultivation areas. Bud initiation, bud emergence, flowering, and vegetative growth of saffron are affected by many environmental factors (Gresta *et al.*, 2009; Molina *et al.*, 2005). For instance, it is reported that a combination of different environmental factors, including temperature and soil water content, regulated the flowering of saffron (Gresta *et al.*, 2009). Therefore, differences in buds emergence have been made during the summer when bud initiation occurs (Behdani *et al.*, 2016; Molina *et al.*, 2005). These differences resulted in differential responses in the initial growth of saffron (Ghanbari and Khajoei-Nejad, 2018). Baghalian *et al.* (2010) also reported the significant interaction of saffron ecotypes by year for flowering traits.

Nutrition, Dry Matter Production, and Nutrient Use Efficiency

High agronomic performance ecotypes had high NU, NUpE but lower nutrients concentrations. A possible reason could be increased utilization of up taken nutrients in the production of leaf and corm dry matter. In contrast, nutrients concentrations in inefficient ecotypes were higher, but

produced less dry matter, suggesting that more growth may dilute the nutrient concentrations. The negative correlation between growth and biomass production with N and P concentrations confirmed these findings (Figure 2a). The results obtained are consistent with those of the previous studies, in which nutrient concentrations were negatively related to higher yield (Inthapanya *et al.*, 2000) and NUE (Fukai *et al.*, 1999).

Nutrient acquisition ability of different plant species could result in maintaining superior growth under different soil fertility conditions (Fukai *et al.*, 1999). One efficient strategy to increase the yield potential of plants like saffron is to use corms that are capable of growing faster and uptake and utilize nutrients more efficiently. Such attributes accumulate more dry matter, particularly in daughter corms, to achieve the maximum yield (Agayev *et al.*, 2007; Ghanbari *et al.*, 2019b; Koocheki and Seyyedi, 2015). Likewise, variation in NU and NUpE can be attributed to variation in the root system's intensity (Xu *et al.*, 2012). Therefore, selecting suitable corms is crucial for improving saffron productivity (Baghalian *et al.*, 2010) as stronger corms are directly connected to the extended root system for exploitation and acquisition of soil nutrients (Koocheki and Seyyedi, 2015). For instance, Ferdows, Sarayan, and Bajestan acquired nutrients from nutrient sources in the soil more efficiently. They also showed a tendency to utilize high amounts of nutrients in the production of dry matter. Low NU of inefficient ecotypes could essentially be attributed to their poor growth before the critical stage. Generally, a greater capacity of efficient corms in NU account for the higher dry matter production by leaf and daughter corms.

However, the ability of ecotypes to take up nutrients appears to be affected by growing seasons. In response to growing seasons, differences among the ecotypes in nutrient-related parameters could be associated with differences in growth response to the studied seasons. As revealed by the results (Figure 2-b), the lower potentials of nutrients-inefficient ecotypes, e.g. Zarand and Torbat, might result from poor growth before the critical stage, leading to low NU and less activity for efficient nutrient utilization (Tables 1 and 3).

Change in NUE mainly depends on plant genotype and nutrient supply conditions (Fukai *et al.*, 1999; Xu *et al.*, 2012). Increasing the NUE is essential to maintain a high productivity level, particularly in a comparatively low nutrient supply condition (Xu *et al.*, 2012). In the current work, in response to growing seasons, variation in dry matter production and yield among ecotypes were closely related to the variation observed in NU (Figure 2-a). Depending on the nutrient supply systems, NUpE or NUtE mainly contributed to major variations in dry matter production, yield, and NUE (Xu *et al.*, 2012). Notably, some ecotypes with high NU, NUpE, and NUtE (e.g. Ferdows and Sarayan) exhibited high NUE, demonstrating that the high agronomic performance corms could also use the nutrients more efficiently. The effects of different agronomic management systems on NUE in saffron were studied (Bicharanloo *et al.,* 2021; Ghanbari and Khajoei-Nejad, 2021; 2022; Koocheki and Seyyedi, 2015). To the best of our knowledge, this is the first report on the NUE of saffron as a result of ecotype effect.

CONCLUSIONS

The obtained results suggested that growth indices of saffron were strongly affected by ecotype and ecotype response to the growing season. The current study further indicated that the diversity existed in Iranian saffron ecotypes, denoting great potential for improving saffron yield based on growth and nutrient-related parameters. According to the results, the ideal saffron corm would be the one that grows more rapidly prior to critical stage and is efficient in the acquisition of nutrients and utilizes them to produce dry matter more efficiently. Highly

efficient ecotypes in nutrient acquisition and acquisition efficiency had faster growth prior to critical stage and showed a higher capability to produce more dry matter. However, further and detailed studies are required to select the best-suited ecotypes for cultivation in specific soil and climatic conditions. Nonetheless, the field-scale periodic selection of superior corms by producers can lead to the gradual increase in yield.

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پاسخ اکوتیپ‌های زعفران به فصل رشد: تحلیل رشد، تغذیه گیاه و تولید ماده خشک

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چکیده

بهبود ذخایر بنه زعفران با قابلیت بالاتر برای کسب عناصر غذایی و تولید ماده خشک بیشتر، ممکن است یک راه حل برای تخفیف مشکل افت عملکرد در مناطق در حال کشت باشد. در مطالعه حاضر، تنوع در رشد، تغذیه و تولید زیست‌توده بین اکوتیپ‌های زعفران کشت شده در آزمایش مزرعه‌ای دو ساله در کرمان، منطقه‌ای نیمه‌خشک در ایران، طی فصول زراعی ۹۵-۱۳۹۴ و ۱۳۹۶-۱۳۹۵ مورد بررسی قرار گرفت. نتایج نشان داد که اکوتیپ‌های مورد مطالعه از نظر پارامترهای ذکر شده تفاوت معنی‌داری داشته و به فصول رشد واکنش متفاوتی نشان دادند. اکوتیپ‌های دارای عملکرد زراعی بالا و کارآمد در جذب عناصر غذایی، به عنوان مثال فردوس، سرایان و بجستان با افزایش سرعت رشد نسبی (RGR) و سرعت جذب خالص (NAR) قبل از مرحله بحرانی، عناصر غذایی بیشتری جذب کردند که منجر به تولید ماده خشک بیشتر شد. در مقابل، اکوتیپ‌های با پتانسیل کمتر برای کسب عناصر غذایی، به‌عنوان مثال زرند و تربت، از رشد و تولید ماده خشک کمتری برخوردار بودند. علاوه بر این، نتایج نشان داد که غلظت نیتروژن در بنه‌ها و برگ‌ها معنی‌دار نبود، اگرچه در جذب نیتروژن، کارایی جذب نیتروژن و کارایی مصرف نیتروژن تفاوت معنی‌داری مشاهده شد. این می‌تواند به دلیل تنوع مشاهده شده در قابلیت بنه‌ها برای استفاده از عناصر غذایی برای تولید ماده خشک باشد. نتایج تجزیه خوشه‌ای وجود اکوتیپ‌های با کارایی بالا، کارایی متوسط و ناکارآمد را نشان داد. به طور کلی، نتایج نشان داد که اکوتیپ‌های با سرعت رشد بالاتر قبل از مرحله بحرانی، پتانسیل بیشتری برای جذب عناصر غذایی، استفاده از عناصر برای تولید ماده خشک و کارایی بالاتر در مصرف عناصر غذایی نشان دادند. در مجموع، این مطالعه نشان داد که ظرفیت کسب عناصر غذایی اکوتیپ‌ها، به عنوان یک ویژگی مطلوب مرتبط با تولید زیست‌توده بالاتر، می‌تواند عامل مهمی در برنامه‌های انتخابی بنه زعفران باشد.

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