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Investigation of the relationship between SSR markers and agronomic traits in saffron (*Crocus sativus* L.)

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ABSTRACT

Purpose: This experiment aimed find the relationship between morphological, physiological traits, metabolites, with SSR markers. **Research method:** To investigate the relationship between quantitative traits and SSR (Simple sequence repeat) markers, an experiment was conducted under both field and laboratory conditions at Zanjan University. In this experiment, 20 SSR primers were used and agronomic and physiological traits with secondary metabolites were measured during the growing season. **Findings:** Amplified primers generated a total of 38 bands and the average number of bands in all locus was 2.38 bands. The highest number of alleles (5 alleles) and the polymorphic information content (0.9) were for the A2 primer. The stepwise regression analysis results showed that the studied primers (10 primers) had a significant relationship with most traits and the highest R^2 in the first year was harvest index (79%), dry weight of stigma (69%), fresh weight of flower (62%), and yield (62%). In the second year, harvest index (67%), number of leaves (65%), number of flowers (61%), and yield (64%) had the highest coefficient of determination. The C25 and C50 primers were associated with 5 and 4 quantitative traits in 2014 and 4 and 3 quantitative traits in 2015, respectively. The C25 and C50 primers identified 2 and 3 alleles in the study population respectively, and considering the number of identified loci, it can be considered as the important primers concerning quantitative traits. The A8, A10, A48 and A2 primers were associated with at least more than 4 traits during the two years of experiment. According to the observed relationship, these marker loci can be used to select ecotypes with marker-assisted in saffron populations. **Limitations:** No limitations to report. **Originality/Value:** C50, C25, A8, A2, and C36 primers are candidate markers in marker-assisted selection saffron breeding programs.

INTRODUCTION

Iran is known as the largest and most important saffron producing country in the world (Jalali-Heravi et al., 2010), so that 80% of the annual saffron production in the world produced in Iran, which is about 300 tons per year (Kumar et al., 2008). The saffron cultivation area in Iran is about 116 thousand hectares, of which 106 thousand hectares (more than 91%) are in the Razavi and South Khorasan provinces (MAJ, 2020). Since saffron is a triploid and sterile plant, its reproduction is asexual and the diversity in this crop is very low (Grili-Caiola & Canini, 2004). The study of plant diversity is done in different methods. Today, molecular markers are widely used to study genetic diversity. The PCR-based Markers are widely used due to their simplicity and the need for small amounts of DNA. The selection of molecular markers depends on high reproducibility, simplicity of the method, low cost, and high reliability (Han et al., 2007). The microsatellite markers are superior to other markers due to their co-dominance, locus-specificity, and high polymorphism for studying diversity and intraspecific relationships. Therefore, molecular markers such as SSR can be used in breeding programs if they show polymorphism. The reports showed that saffron is genetically considered a monomorphic species and its genotypic diversity is very low (Alavi Kia et al., 2008; Rubio-Moraga et al., 2009). Rubio-Moraga et al. (2009) reported 58 microsatellite markers for saffron, and 9 markers were polymorphic in their studied population. Nemati et al. (2012) reported significant genetic diversity using microsatellite markers among 50 saffron ecotypes collected from 5 geographical areas. Keifi and Beiki (2012) found considerable variation between saffron ecotypes by using RAPD and SRAP markers and placed ecotypes in four different groups by cluster analysis and stated that saffron is not a monomorphic species and its diversity can be used in breeding programs. Other researchers have reported the existence of diversity in saffron using various markers (Babaei et al; 2014; Bayat et al., 2018a; Bayat et al., 2018b). According to this diversity, finding the relationship between markers and morphological traits as a useful tool can help in the indirect selection of traits through markers.

Nowadays, tracking the desired traits and ease of selection with the help of markers has become possible by determining their association (linkage) with important agronomic traits (quantitative and qualitative). This allows for the rapid and accurate selection of desirable genotypes in the early stages of growth and reduces the length of the breeding period. In addition, instead of evaluating the traits, the indirect selection is done with the help of associated markers (Ashraf & Harris, 2005). Genetic linkage between markers and quantitative trait locus (QTL) is the most likely reason for the relationship between molecular markers and quantitative traits (Virk et al., 1996). The study of the relationship between molecular markers and agronomic traits has several applications including the possibility of investigating the genetic potential of specific genotypes before phenotypic evaluation, identification of alleles of desirable traits in germplasm assemblages, facilitation of accurate mapping of QTLs, and confirmation of candidate genes responsible for quantitative traits (Gebhardt et al., 2004). Multiple regression analysis is a suitable method for identifying markers related to morphological traits (Abbasi et al., 2015; Bayat et al., 2018a). In the study of 146 spring barley cultivars using 236 AFLP markers, the relationship between molecular markers and yield traits and yield stability was determined. Multiple stepwise regression showed that 18 to 20 markers explain 40 to 58% of the changes in these two traits (Kraakman et al., 2004). By using SSR markers, Inostroza et al. (2009) showed that 21 chromosomal locations were highly correlated with grain yield, plant height, and yield stability in 8 barley substitution lines. In another study, 33 SSR markers and 135 RAPD markers and 14 fruit

traits were found to be associated with more than one trait (Kgadivi-Khub, 2014). Bayat et al. (2018a) reported the association of 25 informative markers with agronomic traits during two years of experiment, so that SCA382, SCA15, and SCD219 markers showed the highest correlation with traits. The aim of this experiment was to find the relationship between morphological, physiological traits and metabolites with SSR markers.

MATERIALS AND METHODS

Experimental materials and phenotypic traits

To investigate the relationship between phenotypic traits (agronomic, physiological, and secondary metabolites) of saffron and different primers of SSR markers, an experiment was conducted in a randomized complete blocks design (RCBD) with three replications at two years in the research farm and laboratory of Zanjan University. The 18 saffron ecotypes were collected from the regions with the longest history of saffron cultivation from all over the country (Table 1). The total plot area was 4.5 m^2 (3 m×1.5 m). The agronomic traits (including the number of flowers per square meter, fresh weight of stigma, fresh weight of flowers, dry weight of stigma, dry weight of flower, stigma length, stigma yield, number of daughter corms, fresh weight of daughter corms, dry weight of daughter corms, dry weight of leaf, number of leaves, leaf length, leaf width, biomass, and harvest index), physiological traits (transpiration rate, stomatal conductance, and photosynthesis rate) during the growing season. Transpiration rate, stomatal conductance, and photosynthesis rate were measured simultaneously using an Infrared Gas Analyzer (IRGA-Infra Red Gas Analyzer (LCA4)) under light-saturated conditions. Picrocrocin, safranal, and crocin metabolites according to the national standard method of Iran were measured (ISIRI, 2006).

Table 1. Geographic characteristic of regions

Number	Region	Province	Elevation	Longitude	Latitude
1	Bahabad	Yazd	1432	56^{0}02′17″	31^{0}50′55″
2	Bajestan	Razavi-Khorasan	1250	58^{0}19′37″	34^{0}47’38″
3	Bardeskan	Razavi-Khorasan	1000	57^{0}57′22″	35^{0}15′33″
4	Estahbanat	Fars	1730	54^{0}02′56″	29^{0}07′55″
5	Feizabad	Razavi-Khorasan	990	58^{0}42′31″	34^{0}56′53″
6	Ferdows	South-Khorasan	1293	58^{0}10′31″	34^{0}01′43″
7	Ghaen	South-Khorasan	1432	59^{0}10’ 52″	33^{0}43′15″
8	Gonabad	Razavi-Khorasan	1056	58^{0}41′44″	34^{0}21′57″
9	Azadshahr	Golestan	1300	55^{0}33′11″	36^{0}59′44″
10	Kalilabad	Razavi-Khorasan	985	58^{0}17′ 45″	35^{0}15′01″
11	Kashmar	Razavi-Khorasan	1109.7	58^{0}28′ 56″	35^{0}12′07″
12	Natanz	Isfahan	1684.9	51^{0}54′20″	33^{0}31′37″
13	Neishabour	Razavi-Khorasan	1213	58^{0}48′34″	36^{0}16′10″
14	Roshtkhar	Razavi-Khorasan	1141	59^{0}37′02″	34^{0}58′32″
15	Torbat-e-Heidarieh	Razavi-Khorasan	1450.8	59^{0}13′41″	35^{0}16′35″
16	Torbat-e-Jam	Razavi-Khorasan	950.4	60^{0}35′46″	35^{0}15′06″
17	Zarand	Kerman	1650	56^{0}31′54″	30^{0}47′20″
18	Zaveh	Razavi-Khorasan	1300	59^{0}27′48″	35^{0}17′02″

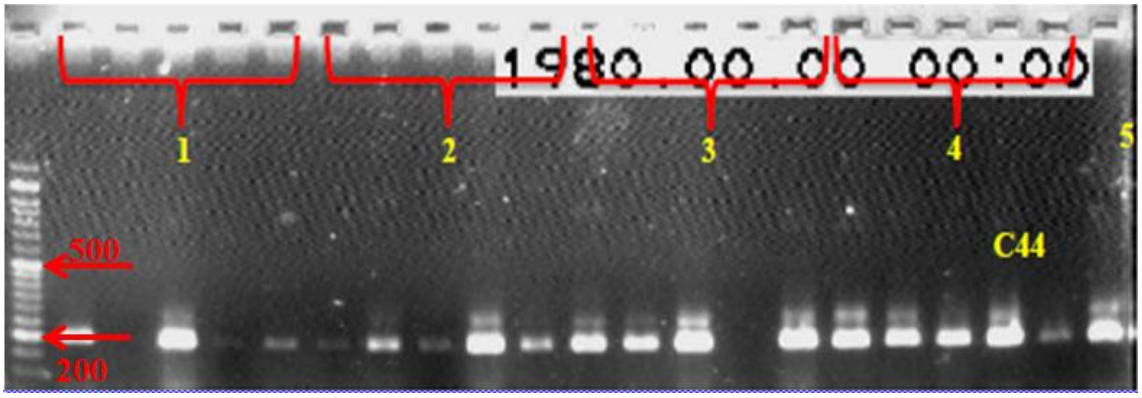

Fig. 1. Amplified fragments by C44 primer (1, 2, 3, 4, and 5 are indicator of Bahabad, Bajestan, Bardeskan, Estahbanat and Feizabad ecotypes respectively)

Genomic DNA extraction and fragment amplification

At the beginning of the growing season, 5 samples were taken separately from each ecotype and stored in the freezer at -80 °C until extraction. DNA extraction from fresh saffron leaves (150 mg) was performed by the CTAB method with a little modification (Saghai-Maroof et al., 1984). After DNA extraction, the quantity and quality of DNA were measured using a nanodrop (Thermo Scientific) spectrophotometry and electrophoresis on 1% agarose gel. In this study, 20 pairs of SSR primers were used to evaluate the genetic diversity of saffron ecotypes (Table 2). These primers had the most polymorphism in the study of Namayandeh et al. (2013) and Nemati et al. (2012).

The polymerase chain reaction was performed with using thermocycler (Bio-Rad) in a volume of 12.5 microliters, including primer and genomic DNA, each with a volume of 1 µL with a concentration of 20 ng, 6.5 µL master mix (2X), and 4 µL ddH_2O. The temperature cycle of the polymerase chain reaction was primary denaturation at 95 ˚C for 5 min, followed by 35 cycles of denaturation at 95 ˚C for 45 s, the recommended temperature for each primer for 40 seconds (for annealing of primers) (Table 3), 1 min at 72 ° C, and final expansion at 72 ° C for 7 min. PCR products were separated using 3% agarose (Bayat et al., 2018a) stained using Robust dye (Arian Gene Gostar co.). A 50 bp marker (Fermentas) was used to determine the band size (Fig. 1).

Scoring and data analysis

All of the variables were tested for normal by Shapiro–Wilk normality test. After normality test, mean data of morphological, physiological traits, and metabolites for each ecotype were calculated. Then, descriptive statistics such as minimum, maximum, range, total mean (The mean of three replications and 18 ecotypes in two years was used as the total mean), variance, standard deviation, and coefficient of variation (CV) were estimated with using SAS software (9.4). After scoring the bands as 0 (absence of band) and 1 (presence of band), the polymorphic information content (PIC) and gene diversity coefficient (H) of the amplified primers were calculated using GeneAlex software version 1.31. The calculation formulas were as follows (1 and 2):

$$H = 1 - \sum_{i=1}^{N} P_i^2 \quad (1)$$

Where P_i is the frequency of the i allele at a given locus, N is the number of loci (Nei, 1973).

$$PIC = 1 - p^2 - q^2 \quad (2)$$

Where, q = 1-p and p is the ratio of individuals with the desired band (Ghislain et al., 1999).

Finally, stepwise multiple regression analysis was used to find the relationship between SSR markers and agronomic traits, physiological and metabolites. In this method, marker locus was considered as independent variables, and phenotypic traits measured were considered as dependent variables. Stepwise regression analysis was performed using SPSS software (21).

RESULTS AND DISCUSSION

The descriptive statistics for different traits are summarized in table 2 based on the data of two years of experiment. Stigma yield (66.78%), harvest index (59.34%), number of flowers (56.19%), and biomass (44.50%) had the highest phenotypic CV among the studied traits, respectively. High CV indicates high diversity among ecotypes. Therefore, the results show the diversity between ecotypes in terms of studied traits, so this diversity can be used in saffron breeding programs. Phenotypic variation in traits has also been reported by other researchers (Bayat et al., 2018a).

Among the 20 pairs of primers, 16 primers amplified scorable bands (Table 3). These primers generated a total of 38 bands, ranging in size from 50 to 1350bp, with an average of 2.38 bands in a total of loci. The highest number of Polymorphic alleles belonged to the A2 primer pair with 5 alleles. The A10, A28, A8, and C50 primer each produced three alleles (Table 3).

The highest polymorphic information content was allocated to A2, C50, A28, and C46 primers with 0.9, 0.66, 0.58, and 0.54, respectively. The lowest PIC values were related to A21, C23, C53, and C59, which identified only one allele and showed no difference between ecotypes in terms of this allele. Nemati et al. (2013), Nemayandeh et al. (2013) and Bayat et al. (2018b) reported an average of 2.6, 2.16 and 2 bands in all loci, respectively. These results indicated that the SSR marker is a powerful tool and a useful and effective method for identifying diversity among different ecotypes that do not have the same origin. It can also be said that saffron is a polymorphic plant and these ecotypes can be used in breeding programs.

Although Alavi-Kia et al. (2008) and Rubio-Moraga et al. (2010) did not report any genetic variation between the studied samples, Nemati et al. (2013), Namayandeh et al. (2013), Bayat et al. (2018a), Bayat et al. (2018b), and Yousefi Javan and Gharari (2018) reported significant genetic diversity among the studied populations by using SSR markers. The diversity of saffron ecotypes, despite the vegetative reproduction of this plant, can be due to the selection of superior genotypes by farmers in each region over many years.

Informative primers related to important agronomic traits including yield and yield components of saffron, especially primers whose chromosomal location is known, can be used in breeding and gene transfer programs or asexual breeding methods. The stepwise regression method was used to determine the relationship between the studied quantitative traits with molecular data and to identify primers related to these traits. For this purpose, each quantitative trait was considered separately as a dependent variable and all SSR marker primers (16 primers) were considered as independent variables, and thus primers that had a significant relationship with the quantitative trait were considered and entered to the regression model in two years (Table 4). It should be noted that in the entered primers to the regression model, all polymorphic bands showed a significant relationship with the traits.

Table 2. Descriptive statistics of studied traits in saffron

Statistic	NF (m^{-2})	F. FW (mg)	S. FW (mg)	F. DW (mg)	S. DW (mg)	Sl (cm)	Yield ($Kg.ha^{-1}$)	N.DC	FW.C (g)	DW.C (g)	DW. L (mg)
Max	51.67	350.37	34.18	51.94	6.06	28.64	2.79	5.60	9.57	3.28	124.43
Min	8.64	266.65	23.18	38.00	3.85	24.71	0.32	2.50	3.71	1.25	71.16
Range	43.03	83.71	11.00	13.94	2.21	3.93	2.47	3.10	5.87	2.03	53.28
Mean	19.61	314.76	27.74	45.27	5.02	26.79	0.95	3.56	5.40	1.85	96.65
variance	121.44	445.12	11.22	11.18	0.33	1.11	0.41	0.42	1.63	0.20	170.79
S.D.	11.02	21.10	3.35	3.34	0.57	1.05	0.64	0.65	1.28	0.44	13.07
Phenotypic CV (%)	56.19	6.70	12.08	7.38	11.36	3.93	66.78	18.19	23.69	24.04	13.52

Table 2. Continued.

Statistic	NL	LL (cm)	LW (mm)	Bio ($g.m^{-2}$)	HI (%)	Trans[1]	SC[2]	P[3]	Pic	Saf	Cro
Max	10.13	31.75	2.27	233.24	0.257	1.12	0.032	6.25	121.74	41.59	207.23
Min	5.83	22.26	1.83	55.30	0.032	0.86	0.022	4.19	97.98	28.41	135.28
Range	4.30	9.49	0.44	177.94	0.225	0.26	0.011	2.06	23.76	13.18	71.95
Mean	7.69	26.65	2.05	120.01	0.088	1.00	0.027	5.18	109.60	35.58	162.54
variance	1.84	5.26	0.02	2851.20	0.003	0.01	0.000	0.41	42.63	11.35	345.92
S.D.	1.36	2.29	0.14	53.40	0.052	0.08	0.003	0.64	6.53	3.37	18.60
Phenotypic CV (%)	17.65	8.61	6.61	44.50	59.34	8.28	10.704	12.37	5.96	9.47	11.44

NF: Flower number, F.FW: Flower fresh weight, S.FW: Stigma fresh weight, F.DW: Flower dry weight, S.DW: Stigma dry weight, SL: Stigma length, Yield: Stigma Yield, N.DC: Number of daughter corm, FW.C:Fresh Weight of Daughter Corm, DW.C: Dry Weight of Daughter Corm, DW.L: Dry Weight of leaf, NL: Number of Leaf, LL: Leaf length, LW:Leaf width, Bio: Biomass, HI: Harvest index, Trans: Transpiration rate, SC: Stomatal conductance, P: Photosynthesis rate, Pic: Picrocrocin, Saf: Safranal, Cro: Crocin [1:] $mmol.m^{-2}.s^{-1}$, [2:] $mmol.m^{-2}.s^{-1}$, [3:] $\mu mol.m^{-2}.s^{-1}$

Table 3. Name, Annealing temperature and diversity indices of used primers.

Number	Primer	Code	T_a[1] (°C)	No of observed allels (Polymorphic bands)	Polymorphic band size (bp)	Gene diversity (h)	PIC[2]
1	ABRII/Cs 10	A10	53	3(3)	a-200, b-250, c-400	0.97	0.31
2	ABRII/Cs 11	A11	52.4	2(2)	a-250, b-300	0.98	0.27
3	ABRII/Cs 2	A2	50	5(5)	a-550, b-600, c-650, d-700, e766	0.53	0.9
4	ABRII/Cs 20	A20	50	3(2)	a-200, b-250	0.94	0.42
5	ABRII/Cs 21	A21	53	1(0)	Monomorphic	0	0
6	ABRII/Cs 28	A28	50	3(3)	a-200, b-600, c-700	0.88	0.58
7	ABRII/Cs 48	A48	50	2(2)	a-200, b-400	0.94	0.44
8	ABRII/Cs 8	A8	50	3(3)	a-150, b-180, c-200	0.1	0.1
9	CSMIC23	C23	50	1(0)	Monomorphic	0	0
10	CSMIC25	C25	53	2(2)	a-50, b-150	0.93	0.45
11	CSMIC36	C36	55.5	3(1)	a-1350	0.1	0.1
12	CSMIC44	C44	61.3	2(1)	a-250	0.93	0.46
13	CSMIC46	C46	56	2(1)	a-200	0.90	0.54
14	CSMIC50	C50	63	4(3)	a-250, b-500, c-575	0.83	0.66
15	CSMIC53	C53	63	1(0)	Monomorphic	0	0
16	CSMIC59	C59	56	1(0)	Monomorphic	0	0
Sum		-	-	38(28)	-	10.82	5.24
Average		-	-	2.38(1.75)	-	0.68	0.33

[1] Anealing temperature

[2] Polymorphism information content

In this study, the number of flowers, picrocrocin, fresh weight of daughter corms, dry weight of daughter corms, number of leaves, leaf width, harvest index, transpiration rate and yield traits are significantly correlated with the SSR primers in two years of experiment. The fresh weight of flower, dry weight of stigma, dry weight of leaf traits only in 2014 and dry weight of flower, safranal, crocin traits only in 2015 are significantly correlated with the SSR primers. However, the stigma fresh weight, stigma length, number of daughter corms, leaf length, biomass, stomatal conductance and photosynthesis rate traits were not related to the SSR primers in two years of experiment. In the first year, harvest index (79%), dry weight of stigma (69%), fresh weight of flower (62%), and yield (62%) had the highest coefficient of determination. In the second year, harvest index (67%), number of leaves (65%), number of flowers (61%), and yield (64%) had the highest coefficient of determination. These traits were associated with A2, A8, A10, A28, A48, C25, C36, C44, C46 and C50 primers (Table 4).

Consideration of Table 4 revealed that C25, C50, A2 and A8 primers are related to stigma yield in two years, but a closer study shows that these primers are also related to the number of flowers (C25, C50, A2 and A8), dry weight of stigma (A8, C25), fresh weight of flower (C25 and C50) and harvest index (A8), which are components of yield. Therefore, this issue shows the importance of these marker positions in determining the stigma yield. The high coefficient of determination also confirms this strong correlation. It was also found that the A10, C44 and A48 primers were associated with the fresh and dry weight of daughter corms in two years, which indicates the importance of these markers in determining traits related to corms. Bayat et al. (2018a) introduced 25 informative primers by examining the relationship between SSR primers and agronomic traits of saffron during two years of experiment and suggested three primers of SCD219, SCA382 and SCA15 have the most correlation with quantitative traits as candidate markers in marker selection. Ivandic et al. (2002) also identified primers associated with flowering time under different growth conditions in barley using 33 SSR markers. Therefore, primers that showed a significant relationship with traits in this experiment can be used to identify superior saffron ecotypes before phenotypic evaluation.

Among the studied primers, 10 primers (62.5%) were associated with quantitative traits, and the other primers were not associated with quantitative traits (Table 4). The C25 and C50 primers were associated with 5 and 4 quantitative traits in 2014 and 4 and 3 quantitative traits in 2015, respectively, which were the most associated with quantitative traits among all primers. These primers were associated with the number of flowers, fresh weight of flower, dry weight of flower, dry weight of stigma, number of leaves, leaf width, dry weight of leaf, transpiration rate, and dry stigma yield. The C25 and C50 primers identified 2 and 3 alleles in the study population respectively, which can be considered as the important primers to quantitative traits. The A8, A10, A48 and A2 primers were associated with at least more than 4 traits during the two years of experiment. The association of a primer with several traits can be due to pleiotropic effects or close association between loci (June et al., 2008; Bayat et al., 2018a). Another feature of association analysis is the lack of need for segregating populations, which saves time. On the other hand, the crossover that occurs in segregating populations is a constraint on accurate mapping (Bayat et al., 2018a). The effectiveness of these methods in identifying and mapping genes controlling Mendelian traits has been determined (Breseghello & Sorrells, 2006). Therefore, informative primers that were identified in association showed a high coefficient of determination in the regression model and can be isolated and cloning, and used in breeding programs. These primers can also be used as optimal sequences for primer design for attractive traits (SCAR) and in marker-assisted selection breeding programs (Ruan, 2010; Bayat et al., 2018a).

Table 4. Informative primer in relation to quantitative traits based on SSR marker

Traits	Informative marker (2014)	R^2 (%)	Informative marker (2015)	R^2 (%)
Flower number	A2, A8	50	C25, C50	61
Flower fresh weight	C25, C50, A48	62	0	0
Stigma fresh weight	0	0	0	0
Flower dry weight	0	0	C25	23
Stigma dry weight	A8, C25, A48	69	0	0
Stigma length	0	0	0	0
Stigma Yield	A8, A2	62	C25, C50	64
Picrocrocin	A8	29	C36	39
Safranal	0	0	C36, C46	47
Crocin	0	0	A28	29
Number of daughter corm	0	0	0	0
Fresh Weight of Daughter Corm	A10, C44	61	A10, A48	50
Dry Weight of Daughter Corm	A10, C44	56	A10, A48	49
Leaf number	C50, C25	52	C50, C25, C44	65
Leaf length	0	0	0	0
Leaf width	C50, C25	48	A48	28
Dry Weight of leaf	C25, A2	42	0	0
Biomass	0	0	0	0
Harvest index	A8	79	A10, A28, C36	67
Transpiration rate	C50	47	A2	23
Stomatal conductance	0	0	0	0
Photosynthesis rate	0	0	0	0

CONCLUSION

The results showed that there was diversity between the studied ecotypes in terms of quantitative traits and SSR markers. Investigation of the relationship between quantitative traits and SSR markers showed that C25, C50, A2 and A8 primers were most associated with quantitative traits and were associated with stigma yield. It was also found that secondary metabolites were associated only with primers A8, A28, C36 and C46, and among physiological traits, only transpiration rate was associated with primers C50, A2. Therefore, these markers can be used as candidate markers in future saffron breeding programs.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Abbasi, Z., Majidi, M. M., Arzani, A., Rajabi, A., Mashayekhi, P., & Bocianowski, J. (2015). Association of SSR markers and morpho-physiological traits associated with salinity tolerance in sugar beet (*Beta vulgaris*). *Euphytica*, *205*(3), 785-797. http://doi.org/10.1007/s10681-015-1408-1

Alavi-Kia, S. S., Mohammadi, S. A., Aharizad, S., & Moghaddam, M. (2008). Analysis of genetic diversity and phylogenetic relationships in crocus genus of Iran using inter-retrotransposon amplified polymorphism. *Biotechnology & Biotechnological Equipment*, *22*(3), 795-800. http://doi.org/10.1080/13102818.2008.10817555.

Ashraf, M., & Harris, P.J. (2005). *Abiotic stresses: Plant resistance through breeding and molecular approaches*. USA, Binghamton: Food Products Press.

Babaei, S., Talebi, M., Bahar, M., & Zeinali, H. (2014). Analysis of genetic diversity among saffron (*Crocus sativus* L.) accessions from different regions of Iran as revealed by SRAP markers. *Sciatica Horticulture*, *171*, 27-31. http://doi.org/10.1016/j.scienta.2014.03.033

Bayat, M., Amirnia, R., Özkan, H., Gedik, A., Ates, D., Rahimi. M., & Tanyulac, B. (2018a). Identification of markers associated with traits for use in marker-assisted selection in saffron. *Genetika*, *50*(3), 971-982. http://doi.org/ 10.2298/GENSR1803971B

Bayat, M., Amirnia, R., Özkan, H., Gedik, A., Ates, D., Tanyulac, B., & Rahimi. M. (2018b). Diversity and phylogeny of saffron (*Crocus sativus* L.) accessions based on iPBS markers. *Genetika*, *50*(1), 33-44. http://doi.org/10.2298/GENSR1801033B

Breseghello, F., & Sorrells. M.E. (2006). Association analysis as a strategy for improvement of quantitative traits in plants. *Crop Science*, *46*(3), 1323-1330. http://doi.org/10.2135/cropsci2005.09-0305

Gebhardt, C., Ballvora, A., Walkemeier, B., Oberhagemann P., & Schüler. K. (2004). Assessing genetic potential in germplasm collections of crop plants by marker-trait association: a case study for potatoes with quantitative variation of resistance to late blight and maturity type. *Molecular Breeding*, *13*, 93-102. http://doi.org/10.1023/B:MOLB.0000012878.89855.df

Ghislain, M., Zhang, D., Fazardo, D., Huamann, Z., & Hismans, R.H. (1999). Marker-assisted sampling of the cultivated Andean potato *Solanum fureja* collection using RAPD markers. *Genetic Resources and Crop Evolution*, *46*, 547-555. http://doi.org/10.1023/A:1008724007888

Grilli-Caiola, M., & Canini, A. (2004). Ultrastructure of chromoplasts and other plastids in *Crocus sativus* L. (Iridaceae). *Plant Biosystems*, *138*, 43-52. http://doi.org/ 10.1080/11263500410001684116

Han, Y. C., Teng, C. Z., & Zhong, S. (2007). Genetic variation and clonal diversity in population of Nelumbo nucifera (Neloumbonaceae) in central China detected by ISSR markers. *Aguatic Botany*, 86, 67-75. http://doi.org/10.1016/j.aquabot.2006.09.007

Inostroza, L., del Pozo, A., Matus, I., Castillo, D., Hayes, P., Machado, S., & Corey, A. (2009). Association mapping of plant height, yield and yield stability in recombinant chromosome substitution lines (RCSLs) using *Hordeum vulgare* sub sp. *spontaneum* as a source of donor alleles in a *Hordeum vulgare* subsp. *vulgare* background. *Molecular Breeding*, *23*, 365-376. http://doi.org/10.1007/s11032-008-9239-6

Iranian Standard & Industrial Research Institute (ISIRI). (2006). 259-2 Saffron (*Crocus sativus* L.).

Ivandic, V., Hackett, C.A., Nevo, E., Keith, R., Thomas, W.T.B., & Forster, B.P. (2002). Analysis of simple sequence repeats (SSRs) in wild barley from the Fertile Crescent: associations with ecology, geography and flowering time. *Plant Molecular Biology*, *48*, 511-527. http://doi.org/ 10.1023/A:1014875800036

Jalali-Heravi, M., Parastar H., & Ebrahimi-Najafabadi, H. (2010). Self-modeling curve resolution techniques applied to comparative analysis of volatile components of Iranian saffron from different regions. *Analytica Chimica Acta*, *662*, 143-154. http://doi.org/10.1016/j.aca.2010.01.013

Jun, T.H., Van, K., Kim, M.Y., Lee, S.H., & Walker, D.R. (2008). Association analysis using SSR markers to find QTL for seed protein content in soybean. *Euphytica*, *62*, 179-191. http://doi.org/10.1007/s10681-007-9491-6

Keifi, F., & Beiki, A. H. (2012). Exploitation of random amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP) markers for genetic diversity of saffron collection. *Journal of Medicinal Plants Research*, *6*(14), 2761-2768. http://doi.org/10.5897/JMPR11.834

Khadivi-Khub, A. (2014). Regression association analysis of fruit traits with molecular markers in cherries. *Plant Systematics and Evolution*, *300*(5), 1163-1173. http://doi.org/10.1007/s00606-013-0953-0

Kraakman, A. T. W., Niks, R. E., Van den berg, P. M. M. M., Stam, P. & Van Eeuwijk, F.A. (2004). Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. *Genetics*, *168*, 435-446. http://doi.org/10.1534/genetics.104.026831

Kumar, R., Singh, V., Devi, K., Sharma, M., Singh, M. K. & Ahuja, P. S. (2008). State of art of saffron (*Crocus sativus* L.) Agronomy: a comprehensive review. *Food Reviews International*, *25*, 44–85. https://doi.org/10.1080/87559120802458503

Ministry of Agriculture Jihad (MAJ). (2020). Communications and information technology center. Available at www://amar.maj. ir/Portal/Home /Default.aspx? Category ID= 117564e0-507c-4565-9659-fbabfb4acb9b.

Namayandeh, A., Nemati, Z., Kamelmanesh, M. M., Mokhtari, M., & Mardi, M. (2013). Genetic relationships among species of Iranian crocus (*Crocus* spp.). *Crop Breeding Journal*, *3*(1), 61-67. http://doi.org/10.22092/CBJ.2013.100451

Nei, M. (1973). Analysis of genetic diversity in subdivided populations. *Proceedings of the National Academy of Sciences*, *70*, 3321-3323.

Nemati, Z., Zeinalabedini, M., Mardi, M., Pirseyedian, S. M., Marashi, S. H. & Khayam Nekoui, S. M. (2012). Isolation and characterization of a first set of polymorphic microsatellite markers in saffron, *Crocus sativus* L. (Iridaceae). *American Journal of Botany*, *116*(3), 359-368. http://doi.org/10.3732/ajb.1100531

RUAN, C. (2010). Germplasm-regression-combined marker-trait association identification in plants. *African Journal of Biotechnology*, *9*(5), 573-580. http://doi.org/10.5897/AJB09.001

Rubio-Moraga, A., Castillo-López, R., Gómez- Gómez, L., & Ahrazem, O. (2009). Saffron is a monomorphic species as revealed by RAPD, ISSR and microsatellite analyses. *BMC Research Notes*, *2*, 189-193. http://doi.org/10.1186/1756-0500-2-189

Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A., & Allard, R. W. (1984). Ribosomal DNAsepacer-length polymorphism in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences*, *81*, 8014-8019. http://doi.org/10.1073/pnas.81.24.8014

Virk, P., Ford-Lloyd, B., Jackson, M., Pooni, H., Clemeno T. & Newburry, H. (1996). Marker-assisted prediction of agronomic traits using diverse rice germplasm. Third International Rice Genetics Symposium. Manila (Philippines). International Rice Research Institute.

Yousefi Javan, I., & Gharari, F. (2018). Genetic diversity in saffron (*Crocus sativus* L.) cultivars grown in Iran using SSR and SNP markers. *Journal of Agricultural Science and Technology*, *20*, 1213-1226.