



# Genetic diversity of Iranian *Chrysanthemum morifolium* cultivars using morphological traits and sequence-related amplified polymorphism (SRAP) markers

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## Abstract

Knowledge of genetic diversity and relatedness of cultivars; is essential for a successful breeding scheme. The present study was conducted to assess the genetic diversity of 30 Iranian *Chrysanthemum morifolium* cultivars using sequence-related amplified polymorphism (SRAP) molecular markers and morphological traits. For this purpose, 30 cultivars were planted in a completely randomized block design with three replications at the Research Farm of Isfahan University of Technology in 2015. The evaluation of morphological traits during the two consecutive years showed a wide range of variations among different cultivars. The results obtained by the analysis of variance also revealed the significant effects of cultivar, environment and cultivar × environment interaction on all studied traits. Correlation analysis of variables further showed that flowering durability was affected by plant morphology. Results of cluster analysis based on the phenotypic data divided the cultivars into six groups. Molecular analysis using SRAP markers produced a total of 213 identifiable fragments with an average of 21.3 polymorphic bands per combination. The polymorphic information content (PIC) value was 0.46. Along with the Bayesian structure analysis which showed that the highest value of the maximum delta log likelihood ( $\Delta K$ ) was  $K = 3$ , cluster analysis also grouped the cultivars into three main clusters. The high value of admixture revealed ancestral relationships between the studied cultivars. Overall, our findings revealed the potential of SRAP markers and morphological traits for the analysis of genetic diversity in *Chrysanthemum*. This can be, in turn, used in selecting the desired parents in the hybrid breeding programs as well as developing superior cultivars for ornamental applications.

**Keywords** Ornamental · Molecular marker · Phenotypic traits · Genetic structure · Interaction

## 1 Introduction

Genetic diversity is crucial for the successful exploitation of novel traits in commercial ornamental plants. In traditional breeding, the recognition of ornamental plant cultivars has been based on morphological parameters as a common index (Fu et al. 2008). DNA markers have widely been employed in characterizing genetic resources; generally considered as fingerprinting, these markers contribute to assessing the genetic diversity and supplying the knowledge necessary

for ecology, evolution, and population genetics (Arzani and Ashraf 2016). The use of molecular markers along with morphological traits can provide new insights useful for further breeding programs in ornamental plants (Martí et al. 2002; Da Silva 2004; Hassanein and Al-Soqeer 2017). Among molecular markers, sequence-related amplified polymorphism (SRAP) is a novel genetic marker system recognized as an effective marker in cultivars identification and analysis. This marker, which serves as a PCR-based technique, amplifies open reading frames (ORF) (Mokhtari et al. 2013).

*Chrysanthemum* (*Chrysanthemum morifolium* L.), as a short-day and perennial herbaceous member from the Asteraceae family (Roein et al. 2015), is one of the most popular and economically important ornamental and medicinal species in Asian and European countries (Zhang et al. 2011a). The breeding of perennial and heterozygous crops depends mostly on individual selection and hybridization (Yao et al. 2008). Today, different cultivars of *Chrysanthemum* have

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been obtained in different countries through different methods, especially by hybridization. These cultivars exhibit a wide range of diversity in their morphological traits. Hence, evaluation and identification of valuable cultivars and their cultivation can be of great importance for the ornamental purposes. Interestingly, most *Chrysanthemum* cultivars look like each other in terms of the morphological traits. So, more accurate evaluation of the cultivars based on DNA molecular markers seems to be necessary. There are some reports on the genetic variation of *C. morifolium* based on different molecular tools and different countries; these include ISSR, SRAP, RAPD and AFLP markers from India, China, Pakistan and Iran, respectively (Palai and Rout 2011; Shao et al. 2010; Naz et al. 2015; Roein et al. 2014).

In Iran, many *C. morifolium* cultivars with different flower colors have been introduced; however, not much information is available on their genetic diversity. SRAP markers have been used to characterize Chinese medicinal *Chrysanthemum* (Shao et al. 2010), but no similar studies have been reported on the genetic variation of the Iranian *Chrysanthemum* cultivars. In addition, there is still comparatively little work on using molecular markers to discriminate desirable plant types to improve plants. Furthermore, most of the previous studies on the variations of *C. morifolium* cultivars have been carried out regardless of the environmental effects (E) and their interactions with the genotype (G), G×E (Shao et al. 2010; Banerji et al. 2012; Roein et al. 2014). Therefore, the present study was aimed to investigate the morphological variation in *C. morifolium* during two constitutive years and its relationship with the molecular markers data.

In the light of the mentioned information, the present study was carried out (1) to evaluate the morphological characteristics of different *Chrysanthemum* cultivars

including vegetative and reproductive characters in 2 years, (2) to assess the extent of genetic diversity and the population structure of *Chrysanthemum* cultivars by using SRAP markers, and (3) to explore the suitable parents for targeted hybridization in *Chrysanthemum*.

## 2 Materials and methods

### 2.1 Plant materials

Thirty cultivars of *Chrysanthemum* (*Chrysanthemum morifolium* Ramat.), which had been provided by the Iranian Research Center for Ornamental Plants, Mahallat, Iran, were used in this study (Table 1). Stock plants were grown at the research farm of Isfahan University of Technology during two constitutive years, including 2015 and 2016, using a completely randomized block design with three replications.

### 2.2 Phenotypic evaluation

The agro-phenological and morphological characteristics were recorded for the germplasm. The following phenotypic traits were evaluated: plant height, flower head diameter, flower dry weight, flower fresh weight, number of flowers per plant, yield of flowers per plant, diameter of the plant canopy, days for the color appearance of the flower bud, and flowering durability (days to 15% flower senescence).

### 2.3 DNA extraction and PCR amplification

Genomic DNA was extracted using the young leaves of *Chrysanthemum* cultivars according to the modified Murray and

**Table 1** *Chrysanthemum* cultivars used in this study

Code	Name	Breeder's reference	Code	Name	Breeder's reference
Chr1	Shokoh	BR504	Chr16	Marmar	BR312
Chr2	Atashgoon	BR657	Chr17	Aydin	BR641
Chr3	Bolor	BR117	Chr18	Poya3	BR59
Chr4	Romina	BR658	Chr19	Sahand2	BR261
Chr5	Armita2	BR425	Chr20	Mitra	BR18
Chr6	Javaneh	BRB1	Chr21	Farhood	BR477
Chr7	Forough	BR246	Chr22	Khalijfars2	BR24
Chr8	Azin2	BR159	Chr23	Taraneh	BR336
Chr9	Atash2	BR326	Chr24	Dorna2	BR292
Chr10	Fariba2	BR373	Chr25	Sahel	BR168
Chr11	Ziba2	BR202	Chr26	Afsaneh	BR499
Chr12	Erica	BR421	Chr28	Ashna	BR462
Chr13	Azita	BR551	Chr29	Hour	BRA6
Chr14	Roshanak	BR480	Chr30	Mehrnoosh2	BR120
Chr15	Sahar	BR563	Chr31	Tarannom2	BR501

Thompson's (1980) method. The quality of the extracted DNA was estimated using agarose gel electrophoresis and staining with ethidium bromide.

A set of ten different SRAP primer (Table 2) combinations were employed for the analysis of *Chrysanthemum* samples (Ghafoori and Rahimmalek 2018; Talebi et al. 2015). DNA amplification was performed in a 10 µl reaction mixture. PCR mixture consisted of 1 µl genomic DNA, 5 µl of the Master Mix Red (Ampliqon, Finland), 1 µl of each primer (0.5 µM), and distilled water to the final volume. PCR amplification program included 5 min of the initial denaturation at 94 °C, 5 cycles during 1 min at 94 °C, 1 min annealing at 35 °C, and 1 min elongation at 72 °C; these were followed by 35 cycles during 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C, and a final stage of 5 min at 72 °C. Separation of DNA amplified fragments was performed on 12% acrylamide gels [acrylamide: bisacrylamide (29:1), 1×TBE] at 230 V for 2.5 h. Then, the gels were stained with silver nitrate.

## 2.4 Data analysis

The morphological data obtained during 2 years were subjected to the analysis of variance (ANOVA) using a split-plot completely randomized block design with the SAS v9 software (SAS institute, 2002). Mean comparisons were performed using the Fisher's (protected) least significant difference (LSD,  $p \leq 0.05$ ) test. The genotypic and phenotypic variances as well as heritability were calculated for the phenotypic traits by the following formula:

$$\sigma_g^2 = (\text{MSG} - \text{MSE})/r \quad \% \text{GCV} = \sqrt{\frac{\sigma_g^2}{\bar{X}}}$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 \quad \% \text{PCV} = \sqrt{\frac{\sigma_p^2}{\bar{X}}}$$

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

**Table 2** Characteristics of SRAP primer sequences used in this study

Name	Sequence (5'–3')	Annealing temp (°C)
Forward		
Me1	5'-TGAGTCCAAACCGGATA-3'	50
Me2	5'-TGAGTCCAAACCGGAGC-3'	50
Me3	5'-TGAGTCCAAACCGGAAT-3'	50
Me4	5'-TGAGTCCAAACCGGACC-3'	50
Me5	5'-TGAGTCCAAACCGGAAG-3'	50
Reverse		
Em1	5'-GACTGCGTACGAATTAAT-3'	50
Em2	5'-GACTGCGTACGAATTTGC-3'	50
Em3	5'-GACTGCGTACGAATTGAC-3'	50
Em4	5'-GACTGCGTACGAATTTGA-3'	50
Em5	5'-GACTGCGTACGAATTAAC-3'	50

MSG refers to genetic mean squares, MES is the environmental mean squares,  $\sigma_g^2$  is the genetic variance,  $\sigma_p^2$  is the phenotypic variance, GCV refers to the genotypic coefficients of variation, and PCV represents the phenotypic coefficients of variation.

The cultivars were clustered for all morphological traits by the STATGRAPHICS, ver.6, statistical package using the Ward method. Pearson's correlation coefficient was calculated using SPSS (version 16; SPSS Inc. Chicago, IL, USA).

For each SRAP marker, the presence (1) or absence (0) of the bands was scored. To estimate the number of genetically distinct clusters (K), the STRUCTURE 2.3.4. program was used. Analysis was conducted using Markov chain Monte Carlo from 5000 to 50,000 repeats (MCMC), which were correlated with the allele frequencies ( $K = 2-10$ ). Cluster analysis was conducted based on the Neighbor-Joining method using MEGA, version 4.0. Polymorphic information content (PIC) was calculated according to the formula:  $\text{PIC} = 1 - \sum P_{ij}^2$ , where  $P_{ij}$  is the frequency of the  $j$ th allele for the  $i$ th marker. The correlation between the morphological traits and the molecular markers was estimated using the Mantel's test. The cophenetic correlation coefficient was also obtained using the COPH routine to check the goodness of fit between the clustering and the similarity coefficient matrix.

## 3 Results

### 3.1 Morphological evaluation

The results of the 2-year analysis of variance (ANOVA) revealed the significant effect of cultivar, year and cultivar × year interactions on all studied morphological traits (Table 3). The highest phenotypic (PCV) and genotypic (GCV) coefficients of variation were attributed to the number of flowers per plant in both years (i.e., > 57%). Among the studied traits, the highest heritabilities were obtained for the flower fresh weight and the days to color appearance of the flower bud, while flower yield recorded the lowest heritability (Table 4).

During the first year, cultivars Mehrnoosh2 (100 cm), Afsaneh (28 g), Romina (184 days) and Taraneh (38.66 days) exhibited the highest values for the canopy diameter, flower dry weight, days to color appearance of the flower bud and flowering durability, respectively; on the other hand, in the second year, the highest values of these traits were recorded for the cultivars Tarannom2 (83.33 cm), Armita2 (24 g), Poya3 (189.33 days), and Afsaneh (37.66 days). In 2015, the plant height varied from 22 cm (Poya3) to 89.66 cm (Tarannom2) with an average value of 46.82, while in 2016, it ranged from 17.66 cm (Taraneh) to 112.66 cm (Tarannom2), with the average value being 49.57 cm. Flower head

**Table 3** Results of analysis of variance for the morphological traits in *Chrysanthemum* cultivars grown in 2 years in the field

Means of square		df	Plant height	Head diameter	Dry weight	Fresh weight	Flower number	Flower yield	Canopy diameter	Days to flowering	Flowering durability
Block	2	94.85 <sup>ns</sup>	0.22 <sup>ns</sup>	27.92 <sup>**</sup>	24.55 <sup>**</sup>	491.46 <sup>ns</sup>	74.59 <sup>ns</sup>	16.37 <sup>ns</sup>	10.03 <sup>ns</sup>	4.35 <sup>ns</sup>	
Cultivar	29	1532.29 <sup>**</sup>	2.46 <sup>**</sup>	135.72 <sup>**</sup>	5261.19 <sup>**</sup>	69584.14 <sup>**</sup>	13862.71 <sup>**</sup>	844.23 <sup>**</sup>	579.3 <sup>**</sup>	97.68 <sup>**</sup>	
Cultivar*block	58	11.37 <sup>ns</sup>	0.02 <sup>ns</sup>	0.49 <sup>ns</sup>	4.60 <sup>ns</sup>	384.03 <sup>ns</sup>	111.71 <sup>ns</sup>	8.12 <sup>ns</sup>	4.79 <sup>ns</sup>	4.65 <sup>ns</sup>	
Year	1	341.68 <sup>**</sup>	7.56 <sup>**</sup>	12.01 <sup>**</sup>	490.77 <sup>**</sup>	148092.05 <sup>**</sup>	27135.35 <sup>**</sup>	11360.55 <sup>**</sup>	888.88 <sup>**</sup>	322.67 <sup>**</sup>	
Year*cultivar	29	164.61 <sup>**</sup>	0.36 <sup>**</sup>	16.33 <sup>**</sup>	345.11 <sup>**</sup>	11928.8 <sup>**</sup>	5358.93 <sup>**</sup>	219.64 <sup>**</sup>	30.75 <sup>**</sup>	26.29 <sup>**</sup>	
Error	2	3.07	0.02	5.62	13.65	304.8	47.79	11.50	0.87	5.08	

*ns* non-significant, *df* degree of freedom

\*, \*\*Significant at 5% and 1% level of probability, respectively

diameter varied from 2.9 in the cultivar Romina to 5.3 in the cultivar Ashna. The highest flower yield (245.19 g) and number of flowers (521.67) in both years belonged to the cultivar Shokoh. Also, the highest fresh weight was recorded for the cultivar Taraneh with an average value of 148.90 g (Table 5).

### 3.2 Correlation analysis of the morphological traits

Correlation analysis was conducted for all morphological traits obtained in 2 years (Table 6). The highest positive and significant correlation was observed between the fresh and dry weight of flower ( $r = +0.924$ ), as well as between flower head diameter and flower dry weight ( $r = +0.753$ ); these were followed by canopy diameter and plant height ( $r = +0.665$ ). Moreover, a negative correlation was found between the duration of flowering and days to flowering ( $r = -0.753$ ). In addition, flowering durability established significant correlations with flower dry weight ( $r = +0.52^{**}$ ), flower head diameter ( $r = +0.38^*$ ), days to color appearance of the flower bud ( $r = -0.75^{**}$ ), and plant height ( $r = -0.43^*$ ).

### 3.3 Hierarchical cluster analysis of morphological traits

Cluster analysis was performed based on the morphological traits obtained in 2 years; all the 30 studied cultivars were divided into six groups using the Ward method (Fig. 1). Cluster 1 was characterized mostly by small flowers, the least vase life and flower weight, but plant height, flower yield, and flower number were high. Cluster 2 consisted of seven cultivars with the lowest plant height, canopy diameter, and flower yield. The cultivars in the third group with the medium flower durability, late flowering time, and low flower yield were classified in two separate subgroups based on their flower diameter. Having the large flowers was characteristic of the first subgroup of the third main group. Cluster 4 was characterized by the lower number of flowers, as well as the large flower head diameter and long flowering durability. The cultivar Taraneh in this group with the lowest plant height was placed in a separate subgroup. Cluster 5 comprised the cultivars Hour and Fariba2 with the earliest flowering times. Finally, Cluster 6 was characterized by the early flowering time and appropriate flowering durability. Whenever the purpose of breeding is the production of cultivars for cut flower applications, the cultivars with the higher height, attractive colors, large flowers and high vase life are suitable for selection. Therefore, the crossing of the first group with the fourth and fifth groups could be effective. If the purpose is to produce pot or garden cultivars, having features such as lower height and the high number of flowers per plant can be effective. The probability of

**Table 4** Range, mean, genetic and phenotypic coefficient of variation (CV) and broad-sense heritability for the morphological traits of *Chrysanthemum* cultivars in 2 years

Traits	Range		Average		Genotypic CV (%)		Phenotypic CV (%)		Broad-sense heritability (%) Two year
	2015	2016	2015	2016	2015	2016	2015	2016	
Flower diameter (cm)	3–6	2.8–5.3	4.28	5.30	17.94	14.91	18.14	14.99	85.71
Plant height (cm)	20–93	17–115	46.82	115	31.04	38.08	31.34	38.20	89.04
Canopy diameter (cm)	35–100	26–90	61.52	90	23	27.16	23.13	27.33	73.57
Flower number	47–516	24–600	196.6	600	57.75	86.16	58.31	86.25	82.96
Flower yield (g)	44.4–225.8	20.2–292.2	113.07	292.20	39.91	74.82	40.59	74.88	61.40
Flower fresh weight (g)	31–149.9	29.77–149.2	68.67	149.21	44.51	42.19	44.59	42.19	93.44
Flower dry weight (g)	6–29	6–24	12.68	24	42.14	35.37	42.36	35.38	87.93
Days to flowering	144–185	146–190	172.02	190	5.11	6.31	5.15	6.34	94.35
Flowering durability (days)	20–40	17–45	28.71	45	12.32	19.96	12.85	19.96	72.05

reaching this goal with the crossing of the cultivar Taraneh, which had small shrubs and large flowers, was like that with the cultivars for the first group, such as Shokoh, which had many flowers.

### 3.4 SRAP amplification and levels of polymorphism

Ten primer combinations were selected based on clarity, sharpness, and number of bands in a prescreening assay performed by 20 primer combinations on 30 cultivars. A total of 222 identifiable fragments were obtained; out of these, 213 bands (95.94%) were polymorphic, ranging from 11 to 33 with an average of 21.3 polymorphic bands per combination. The Shannon's information index (*I*) and the Nei's gene diversity index (*H*) were calculated from this analysis to be 0.53 and 0.35, respectively. Shao et al. (2010) reported lower values (*I*: 0.34, *H*: 0.21) for the Chinese *Chrysanthemum* cultivars in comparison with the Iranian ones. The average polymorphism information content (PIC) value was 0.46 (Table 7). The highest (0.496) and lowest values (0.433) of the PIC were attributed to Me2-Em3 and Me5-Em2 primer combinations. Figure 2 shows the pattern of Me4-Em3 SRAP primer combination in different cultivars.

### 3.5 Phylogenetic relationships and principal coordinate analysis (PCoA) of the molecular data

Cluster analysis of the molecular data based on the NJ method divided the cultivars into three major groups (Fig. 3). The first group included 11 individuals, the cluster II had eight cultivars, and eleven individuals were assigned to the cluster III.

Principal coordinate analysis (PCoA) was conducted to ensure a better view of the genetic relationships in *Chrysanthemum* cultivars. According to the PCoA analysis, the first

three principal coordinates accounted for 17.78% of the total variation, such that the contribution of the first component was 8.89% and those of the second and third components were 4.63% and 4.24% of the total variation respectively (Fig. 4). According to Mohammadi and Prasanna (2003), cluster analysis could be more sensitive for the detection of germplasm relationships than PCoA, as the first two or three principles explained less than 25% of the total variation.

The Bayesian approach for clustering information from SRAP primer combinations revealed the highest value of  $\Delta K$  as  $K=3$ . Genetic admixture was observed among the three clusters, which could be related to the amount of cross pollination and the level of heterozygosity of *Chrysanthemum*. According to a membership value of 0.75, the STRUCTURE analysis divided 15 cultivars into three clusters (Fig. 5). Cultivars with membership probabilities less than 0.75 were attributed to an admixture group. Cluster a included four cultivars (20, 21, 18, 11), cluster b consisted of six cultivars (26, 31, 14, 30, 13, 25), and cluster c comprised five cultivars (5, 10, 1, 6, 7). Clustering according to structure analysis was in line with the clustering-based NJ method. The diversity parameters in each cluster were calculated (Table 8). Among the three groups, the admixed cluster showed the highest diversity parameters (*H* and PIC), and the cluster a exhibited the lowest ones. Also, based on the genetic similarity index, genetic distances ranged from 0.202 in Ashna and Hour to 0.545 in Fariba2 and Sahel with a mean of 0.370.

## 4 Discussion

Given that biodiversity is fundamental to the breeders' programs and there is a wealth of empirical evidence regarding the benefits of plant genetic resources in developing improved plant cultivars, biotechnological approaches could substantively enrich this use (Arzani and Ashraf 2016). The

**Table 5** Means of the morphological traits of *Chrysanthemum* cultivars in 2 years

Cultivars	Flower diameter (cm)		Plant height (cm)		Canopy diameter (cm)		Flower number/plant (g)		Flower yield/plant (g)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Shokoh	3.30	2.90	52.66	76.33	78.33	48.33	493.33	550.00	222.54	267.85
Atashgoon	4.80	4.13	35.66	46.66	70.00	46.00	148.00	91.00	127.33	73.79
Bolor	4.06	4.66	29.66	41.33	58.00	50.00	97.00	66.00	114.10	68.79
Romina	3.00	2.93	31.66	31.33	50.00	36.00	209.00	103.00	70.37	38.14
Armita2	5.26	4.63	45.33	48.66	80.00	53.00	80.33	96.00	119.90	125.94
Javaneh	3.93	3.16	40.33	34.66	38.66	40.00	116.33	47.00	50.96	20.24
Forogh	5.23	3.90	50.66	40.66	48.33	34.00	122.00	40.00	131.67	51.08
Azin2	5.16	4.50	43.33	62.66	73.33	51.33	158.00	240.00	70.27	114.43
Atash2	3.63	3.56	30.33	32.33	51.66	32.00	200.00	91.00	96.70	49.14
Fariba2	3.73	3.50	35.66	37.33	40.33	39.33	327.00	305.00	171.88	192.91
Ziba2	5.40	3.83	39.66	39.00	60.00	40.00	175.00	95.00	70.86	56.04
Erica	3.50	3.50	46.33	41.00	56.33	42.00	206.00	164.00	101.10	92.11
Azita	4.30	3.83	49.33	46.66	57.33	44.66	164.00	54.00	86.47	31.81
Roshanak	4.90	4.73	49.00	60.00	55.66	34.33	199.00	42.00	172.01	41.86
Sahar	3.56	3.70	53.33	68.66	80.00	69.33	278.33	83.00	96.98	35.56
Marmar	3.46	3.83	73.66	68.33	81.66	45.00	132.67	157.00	51.12	100.04
Aydin	5.16	4.66	38.66	56.00	60.00	49.33	155.00	147.00	119.75	156.87
Poya3	4.00	3.80	22.00	35.33	44.33	31.66	88.33	70.00	55.64	50.37
Sahand2	4.50	3.76	50.33	57.66	63.00	53.66	200.33	322.00	163.81	254.15
Mitra	3.50	3.70	43.33	37.00	58.66	35.33	223.00	97.00	131.37	67.16
Farhood	3.83	3.53	55.66	62.33	70.00	59.33	129.33	63.00	114.50	57.94
Khalijfars2	4.90	3.73	77.66	69.00	63.66	59.66	165.33	193.00	135.55	197.97
Taraneh	5.50	4.80	26.66	17.66	61.00	35.66	48.00	24.00	72.00	35.81
Dorna2	4.30	3.86	41.66	28.33	50.00	26.66	257.00	71.00	164.47	50.12
Sahel	3.26	3.50	49.00	46.00	73.66	51.66	478.67	166.00	157.65	49.41
Afsaneh	4.60	4.30	44.33	34.66	55.66	32.33	183.67	46.00	187.72	24.79
Ashna	5.50	5.10	62.66	67.66	51.00	55.00	89.00	100.00	60.19	90.28
Hour	4.80	4.00	48.33	44.66	41.66	37.00	169.33	135.00	83.43	68.75
Mehrnoosh2	3.93	3.00	48.00	42.66	100.00	53.00	123.00	119.00	52.69	63.35
Tarannom2	3.33	3.23	89.66	112.66	73.33	83.33	482.00	400.00	147.14	128.68
Mean	4.28	3.87	46.82	49.57	61.52	45.63	196.60	139.23	113.07	88.51
LSD	0.35	0.22	5.80	4.25	4.38	3.87	45.06	14.92	23.81	7.26

Cultivars	Flower dry weight (g)		Flower fresh weight (g)		Days to flowering		Flowering durability (days)	
	2015	2016	2015	2016	2015	2016	2015	2016
Shokoh	9.00	10.00	45.13	48.23	176.00	183.00	27.33	22.66
Atashgoon	12.66	13.00	85.53	81.03	168.00	171.33	33.66	30.66
Bolor	18.00	17.00	118.32	104.08	169.66	177.66	28.33	26.66
Romina	6.00	7.00	31.31	37.01	184.00	188.00	22.00	22.33
Armita2	24.50	24.00	136.16	131.06	166.33	173.32	30.00	27.66
Javaneh	8.00	9.00	40.32	43.02	176.66	182.00	29.66	22.00
Forogh	18.50	22.00	103.12	127.23	162.33	160.00	31.00	30.66
Azin2	10.00	9.00	48.23	47.22	180.00	189.33	27.66	28.33
Atash2	8.33	9.00	46.45	54.00	170.00	174.33	26.00	24.33
Fariba2	12.33	12.00	61.87	63.08	148.66	147.66	34.00	35.33
Ziba2	12.50	11.00	58.37	58.33	173.66	178.66	25.66	21.00
Erica	8.50	10.00	44.37	56.05	172.66	164.33	23.33	33.66

**Table 5** (continued)

Cultivars	Flower dry weight (g)		Flower fresh weight (g)		Days to flowering		Flowering durability (days)	
	2015	2016	2015	2016	2015	2016	2015	2016
Azita	9.50	11.00	47.01	58.30	179.00	186.33	23.00	21.00
Roshanak	12.00	18.00	72.64	99.22	175.00	180.00	25.66	22.33
Sahar	7.00	11.00	36.33	42.28	177.00	187.33	28.33	20.00
Marmar	10.00	13.00	48.05	63.24	175.00	181.66	29.00	19.66
Aydin	16.33	18.00	78.24	106.24	180.00	184.66	29.33	26.00
Poya3	14.00	16.00	75.68	71.32	182.00	189.33	29.33	25.00
Sahand2	14.66	14.00	80.61	78.31	173.33	180.33	32.66	32.33
Mitra	10.00	10.00	66.90	69.08	169.00	172.33	29.66	26.00
Farhood	11.00	15.00	67.10	91.32	180.33	186.66	28.33	23.66
Khalijfars2	15.00	14.00	90.77	102.19	173.00	176.00	31.66	25.00
Taraneh	22.00	22.00	148.74	149.07	161.66	163.33	38.66	34.33
Dorna2	14.66	14.00	85.01	70.20	158.66	170.00	30.00	25.33
Sahel	6.00	6.00	32.39	29.25	178.66	180.33	18.66	25.66
Afsaneh	28.00	15.00	105.40	53.30	167.33	159.66	29.66	37.66
Ashna	14.33	19.00	75.70	90.09	175.00	183.00	27.33	19.66
Hour	12.66	9.00	55.35	50.31	149.00	152.00	33.00	32.33
Mehrnoosh2	8.00	11.00	42.26	53.08	178.66	184.00	26.33	22.33
Tarannom2	7.00	7.00	32.74	32.05	180.00	187.33	22.00	17.33
Mean	12.68	13.20	68.67	71.97	172.02	176.46	28.71	26.03
LSD	4.98	0.29	1.55	0.32	3.11	3.26	2.97	3.64

Means with difference less than the LSD provided are not significantly different ( $p < 0.05$ )

**Table 6** Correlation coefficients between the morphological characteristics on *Chrysanthemum* cultivars

Traits	1	2	3	4	5	6	7	8	9
Flower diameter	1								
Plant height	-0.13	1							
Canopy diameter	-0.13	0.66**	1						
Flower number	-0.52**	0.46**	0.35	1					
Flower yield	-0.97	0.33	0.22	0.72**	1				
Flower dry weight	0.75**	-0.26	-0.16	-0.49**	0.06	1			
Days to flowering	-0.23	0.31	0.48**	0.07	-0.15	-0.36*	1		
Flowering durability	0.38*	-0.43*	-0.35	-0.17	0.22	0.52**	-0.75**	1	
Flower fresh weight	0.72**	-0.26	-0.14	-0.50	0.06	0.924**	-0.35	0.53**	1

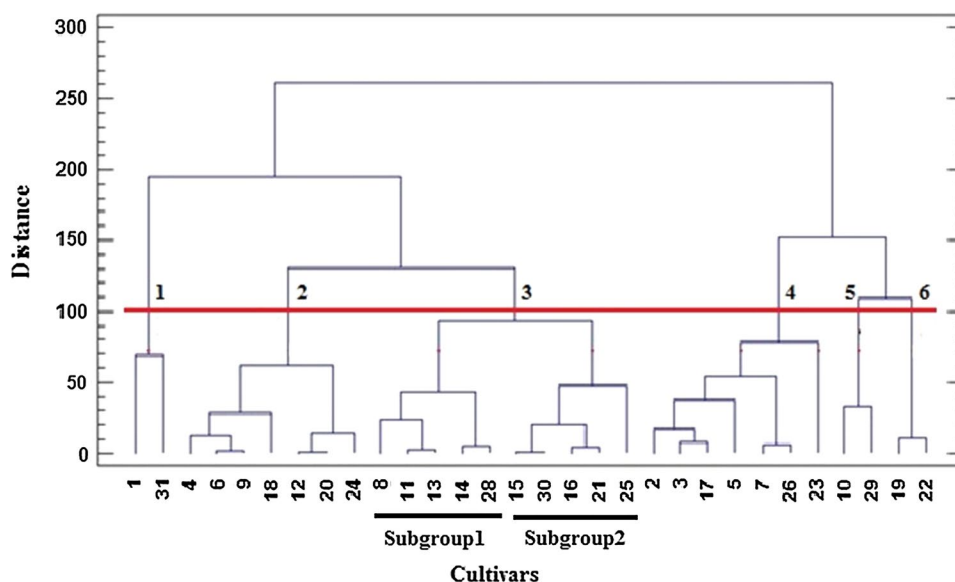
\*, \*\*Significant at  $p < 0.05$ ,  $p < 0.01$ , respectively

results obtained from the phenotypic data revealed a wide range of variation among all studied cultivars. In agreement with our findings, previous studies reported a high phenotypic variation among the Indian and Chinese *Chrysanthemum* cultivars (Kulkarni and Reddy 2004; Shao et al. 2010).

According to the analysis of variance in two constitutive years, the significant interaction effect between the genotypic and environmental factors as well as higher PCV values against the GCV values reflected the important effect of environmental conditions on the morphological traits among the *Chrysanthemum* plants. Different patterns of temperature

and rainfall during the 2 years of field experiments led to a high level of phenotypic variation for all traits. The lower amount of rainfall in the second year rather than the first one (the monthly rainfall average in 2016 ~ 29.1 < 49.1 ~ in 2015 cm) might have reduced the average of flower head and canopy diameter, flower number and yield, and flower durability in the second year. Flowering time and vegetative period are important factors in selecting flowers and introducing new cultivars. The cultivars Fariba2 and Hour were early flowering, while Romina and Poya3 were recorded as the latest flowering cultivars.

**Fig. 1** Dendrogram of 30 cultivars of *Chrysanthemum* based on the morphological traits



**Table 7** The number of total and polymorphic fragments along with polymorphic information content (PIC) per SRAP primer combination

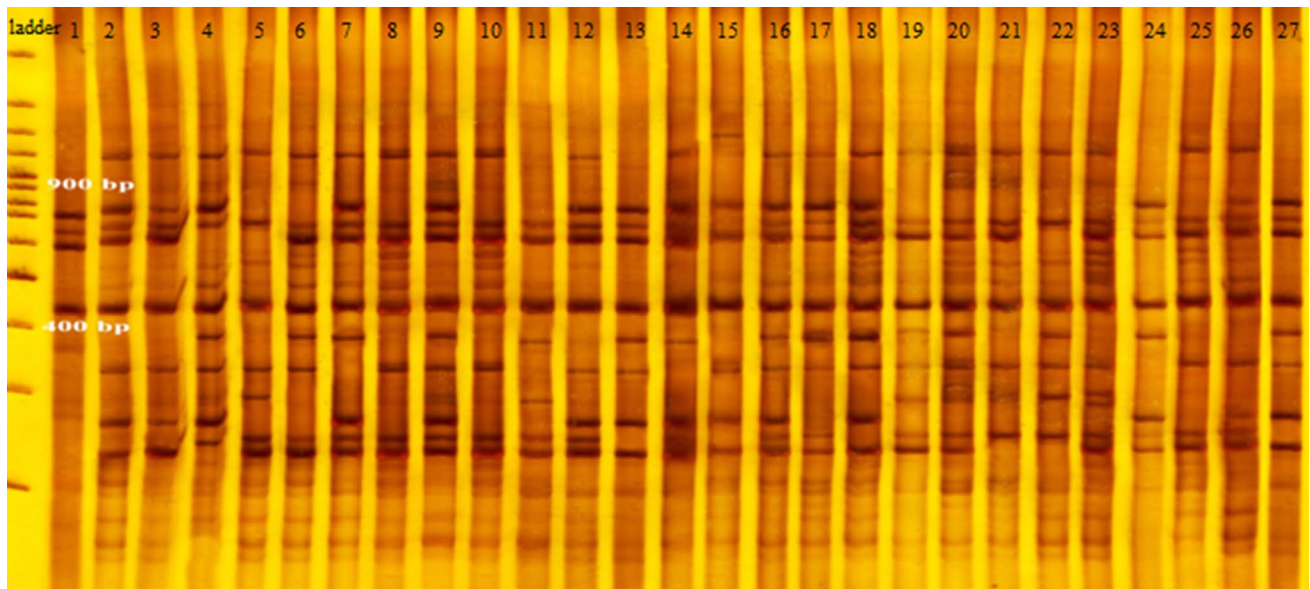
Primer combination	No. total amplified bands	No. polymorphic bands	% of polymorphism	PIC
Me5-Em2	13	13	100	0.43
Me5-Em5	18	17	94.44	0.47
Me2-Em2	17	17	100	0.45
Me2-Em3	30	27	90	0.49
Me3-Em2	28	28	100	0.46
Me4-Em2	18	18	100	0.47
Me4-Em3	20	18	90	0.48
Me4-Em5	32	31	96.87	0.47
Me4-Em4	34	33	97.05	0.43
Me5-Em4	12	11	91.66	0.46

Furthermore, in the ornamental plants, freshness and flower durability are critical traits for the selection of the superior cultivars. In the present study, the cultivars Taraneh, Atashgoon and Fariba2 had the highest duration of flowering (> 33d). The tallest cultivars were Tarannome2 and khalijfars2, while the shortest one was Taraneh during both years. The effect of genotypic variation on the plant height has also been reported by Ahmad et al. (2012). The dwarf and compact *Chrysanthemum* cultivars are suitable for pot cultures, while the erect and tall growing ones are suitable for use as cut flowers. Therefore, among the studied cultivars, “Taraneh” was found to be the best one for pot culture due to its maximum vase life and minimum plant height; also, the cultivars “Tarannome2”, “Khalijfars2”, “Ashna”, “Shokoh”, “Azin2”, “Sahar” and “Marmar” could be ideal for applying as cut flowers. Gaikwad et al. (2002) also recommended nine

elite Indian *Chrysanthemum* cultivars as cut flowers based on some morphological traits including flowering time, yield and vase life.

Correlation analyses also showed that by increasing the vegetative stage, the flowering period of the plant, which was responsible for its ornamental value, was decreased. The results of the correlation analysis revealed that the cultivars with larger flower diameter had a higher fresh and dry weight, less flower numbers, and a higher flowering durability. Also, it could be deduced that the cultivars with the higher plant height, in addition to canopy diameter, had more flowers and less flowering durability. Heritability, as an index estimating the reliability of phenotypic values, is necessary for effective breeding programs (Falconer et al. 1996). The results showed the high percentage of heritability for the days to color appearance of the flower bud, flower fresh weight, flower dry weight and plant height, which revealed the potential of these traits for the genotypic selection of *Chrysanthemum* germplasm. Similar observations have been made in the case of blooming time in *Chrysanthemum* (Roein et al. 2014; Zhang et al. 2011b). The low heritability was recorded for flowering durability, thereby suggesting the indirect selection based on the flower dry weight and days to color appearance of the flower bud, which had high heritabilities and high correlation with flowering durability, would be more effective. Selection of parents could be useful in wide crossing for developing different hybrid combinations and new *Chrysanthemum* cultivars. According to the morphological data, the highest Euclidean distance was observed between the cultivars “Taraneh” and “Shokoh” (9.15), as well as between the cultivars Tarannome2 and Forogh (7.95), while the lowest one was observed between the cultivars Mitra and Ziba2 (0.76). So, the more diverse cultivars could be introduced as the parental materials.





**Fig. 2** Bands patterns of some *Chrysanthemum* cultivars revealed by the Me4-Em3 SRAP primer combination

Previous studies have reported the potential of SRAP markers in cultivar identification (Shao et al. 2010). In the present research, a large number of polymorphic bands assessed by applying SRAP markers confirmed that SRAP analysis could be useful for identifying various *Chrysanthemum* cultivars. In the present research, the average percentage of polymorphic bands (96%) was higher than that reported in the Chinese *Chrysanthemum* cultivars using SRAP (81.87%) and AFLP (93%) markers (Shao et al. 2010; Han et al. 2007). Accordingly, 213 polymorphic bands were produced as it was in range of many previously published reports for the SRAP markers (Talebi et al. 2015; Aneja et al. 2013; Shao et al. 2010). The PIC value is often used to determine the degree of gene diversity among different cultivars (Feng et al. 2016). According to Xie et al. (2010), the applied SRAP primers revealed moderate polymorphism among the studied cultivars ( $0.25 < \text{PIC} < 0.5$ ), which was suitable for the analysis of genetic variation in the studied cultivars.

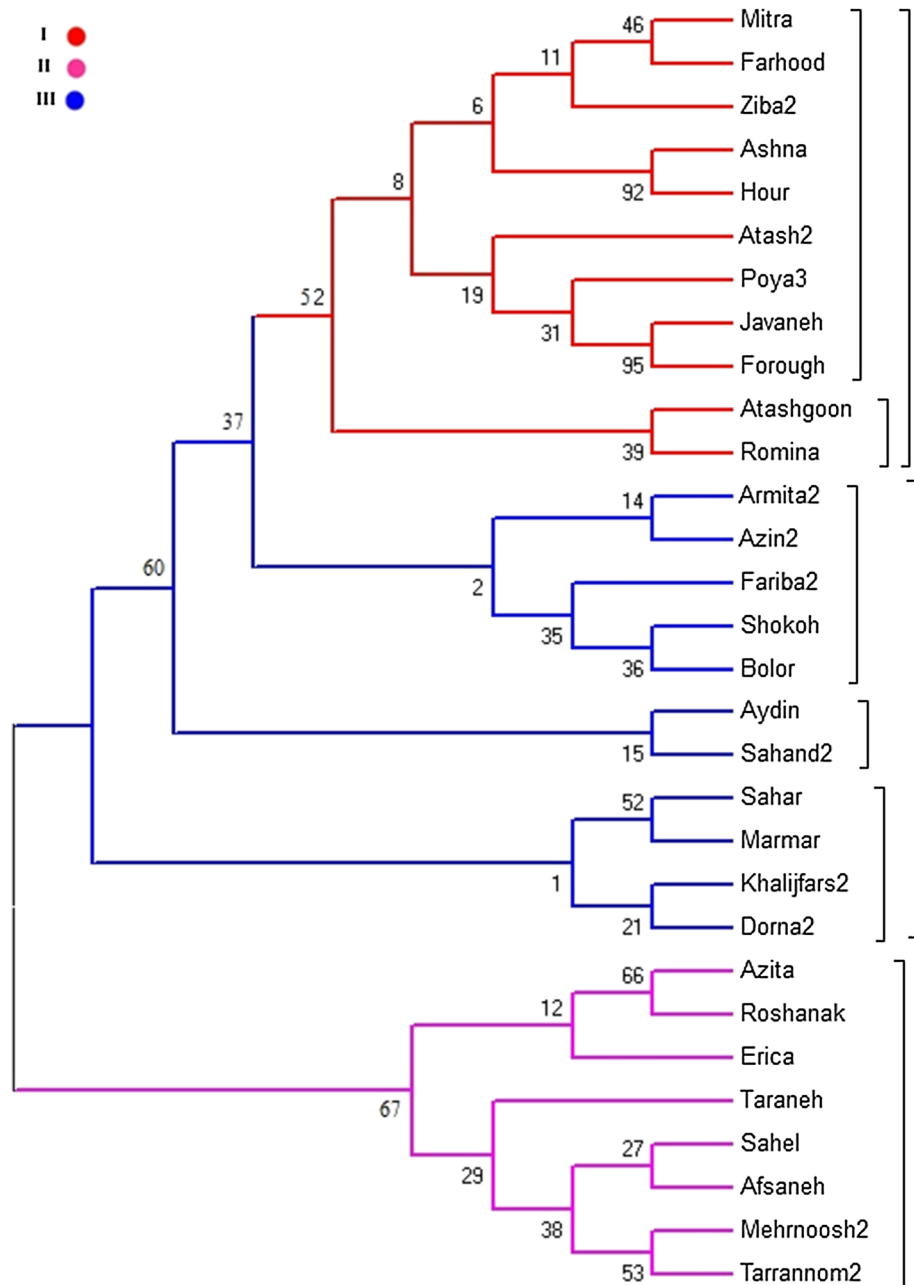
In the present study, less than 25% of the total variation was explained with the first three principal components, which showed that there was a low correlation between the primer combinations and markers distribution across the genome. So, the results of PCoA did not confirm the clustering analysis.

Subgroups showed a membership value of the cultivars that was higher than 0.75, suggesting the presence of admixture. According to STRUCTURE software analysis, the mixed population ancestry was observed among the separate groups. It could be the result of the incorporation of more than a single gene pool as well as gene flow between different cultivars during hybridization programs (Soriano

et al. 2016). The admixture models for the individual cultivars could also provide useful information regarding the hybrid origins (Chen et al. 2008). SRAP phylogenies showed agreement with the previous molecular phylogenetic data explaining the hybrid lineage among *Vitis* varieties and the related species (Robarts and Wolfe 2014). Admixtures have also been reported in the Iranian and Indian *Chrysanthemum* cultivars based on AFLP and RAPD markers, respectively (Roein et al. 2014). In the present study, twenty-three out of 30 cultivars showed some degree of admixture. Fifteen cultivars with membership probabilities  $> 0.75$  were assigned to three groups (Fig. 5a–c). The remaining cultivars, viz. Atashgoon, Azin2, Sahar, Marmar, Sahand2, Khalijfars2, Hour, Erica, Ashna, Bolor, Atash2, Romina, Dorna2, Aydin and Taraneh, were highly variable in respect to the molecular level (membership value  $< 0.75$ ), consequently forming the admixed group. These cultivars could be suggested for the improvement of mapping population and tagging QTLs of important ornamental characteristics (Kumar et al. 2017). Also, evaluation of genetic diversity values ( $H$ ) for each group showed that the admixed group had a higher genetic diversity index (0.33), revealing that this group had more allelic richness and genetic diversity. The highest genetic distance was obtained between the clusters a and b (0.287); this was followed by the distance between the clusters b and c (0.280). These differences might be attributed to the phenotypic variation of the cultivars in the groups. It indicated that the cultivars in these clusters were more diverse and could be introduced as the parental candidates to improve the new *Chrysanthemum* cultivars.

The clusters inferred by structure analysis could also be used for distinguishing the flower colors. White flowers

**Fig. 3** Dendrogram of studied *Chrysanthemum* cultivars using the SRAP data



were only found in the admixed group, while purple and red ones were the most abundant in the group b. The yellow color was also dominant in the clusters a and c. Similarly, recent studies have reported the application of SRAP markers to differentiate among flower color, which can lead to association with the behavior of pollinators or seed-dispersing organisms (Robarts and Wolfe 2014). Also, according to Li et al. (2013), the *Chrysanthemum* cultivars were separated in terms of their flower size by using the UPGMA clustering results, which had been obtained from conserved DNA-derived polymorphism (CDDP). Furthermore, based on the genetic similarity index, the

greatest genetic distances were observed between the cultivars Fariba2 (which belonged to the cluster c) and Sahel (which belonged to the cluster b), while the most similar cultivars were Ashna and Hour (both belonged to the admixed group). Ashna and Hour possessed a semi-double flower head type. The cultivar Sahel had purple flowers, needed medium days for the flowering stage, with single flower head type and the medium plant height, while the cultivar Fariba2 had a yellow flower color, early flowering time, and a semi-double flower head type with the low plant height. The high genetic distance between some cultivars including Sahel and Fariba2, as well as Sahel and

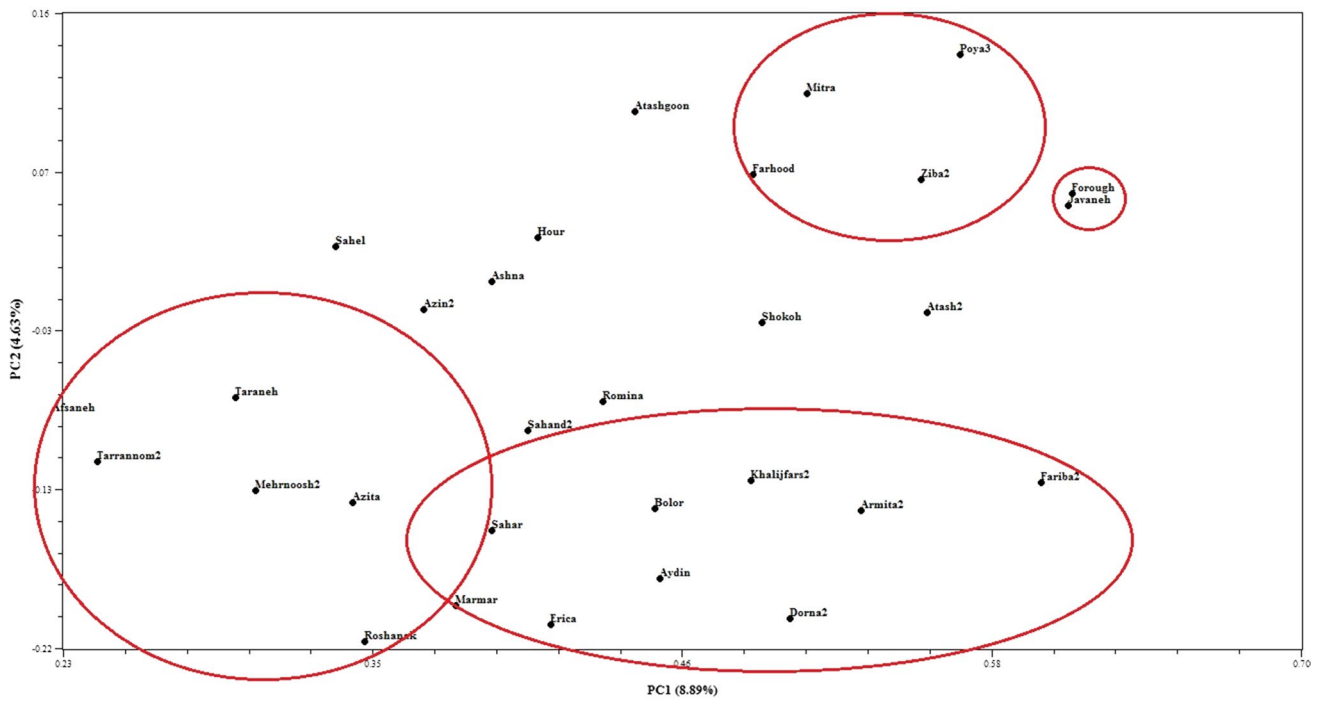


Fig. 4 Principle coordinate analysis for the SRAP markers based on the first two components of *Chrysanthemum* cultivars

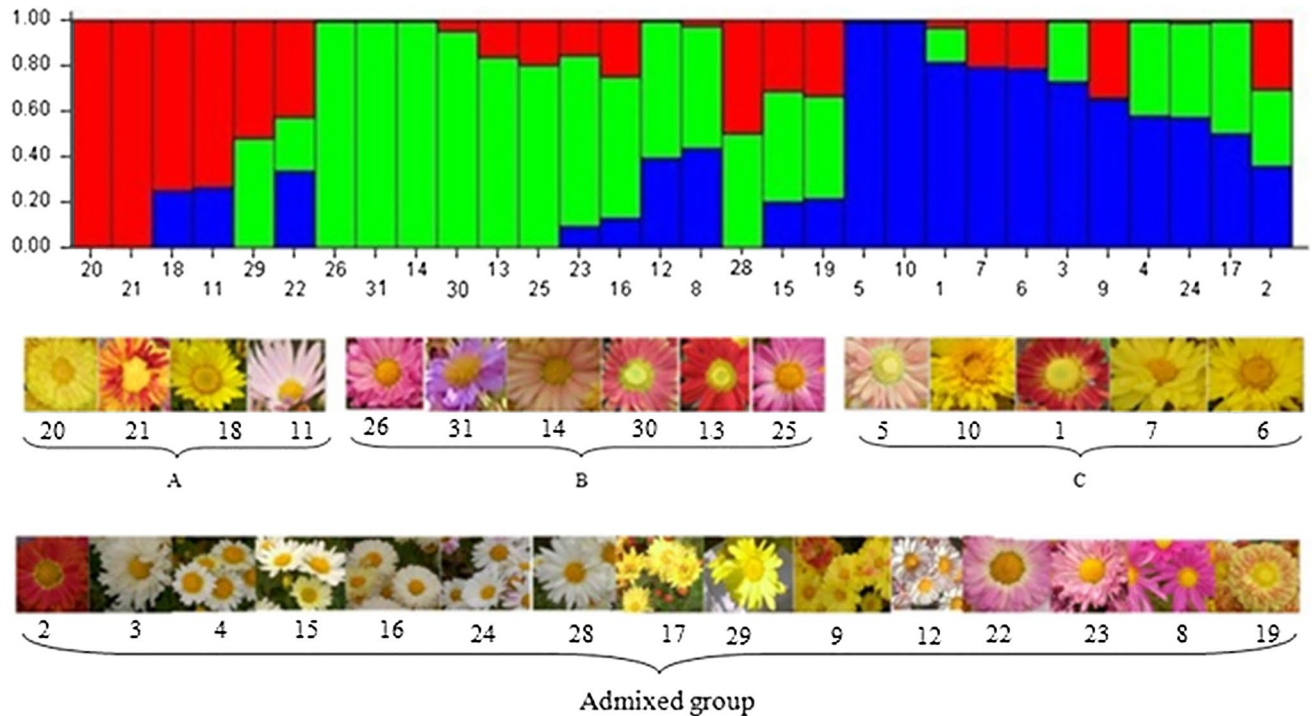


Fig. 5 Genetic structure of 30 *Chrysanthemum* cultivars as inferred by using structure software with ten SRAP marker data sets and K=3 clusters. **a** Red cluster; **b** green cluster; and **c** blue cluster. The X and

Y axis shows the cultivars and the proportion membership of each individual into various clusters, respectively

**Table 8** Diversity parameters in three main clusters differentiated based on structure analysis

Groups	A	B	C	Admixed	H	PIC (%)
A	***	0.75	0.81	0.87	0.23	5.87
B	0.28	***	0.75	0.91	0.24	67.14
C	0.20	0.28	***	0.88	0.26	65.73
Admixed	0.13	0.09	0.11	***	0.33	93.90

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Forough, could provide valuable genetic information to introduce the parental materials for the purpose of breeding programs.

The Mantel test did not show any significant correlation between the results of morphological and molecular data ( $r = -0.16$ ,  $p > 0.05$ ). The negative correlation showed that the two methods (morphological and molecular approach) were distinct and highly variable (Kumar et al. 2013). Choudhary et al. (2017) also suggested that molecular markers should not be seen as a substitute of morphological traits as each marker system evaluates different aspects of genetic variation. Moreover, Solouki et al. (2008) suggested the role of environmental factors in determining the low correlation between phenotypic and molecular data, which could be due to the differentiation of traits-related genes from those associated with the adaptive response of the cultivars (Soriano et al. 2016).

## 5 Conclusion

*Chrysanthemum morifolium* is commercially an important plant with various applications. For the first time, the genetic diversity and population structure of Iranian *Chrysanthemum* cultivars were analyzed using SRAP markers and morphological traits in two constitutive years. Remarkable phenotypic and molecular variations were observed among the studied cultivars. Overall, the results of the present study showed that the morphological traits as well as SRAP molecular markers could be valuable resources to facilitate germplasm identification. Selection of divergent parents could be useful in wide crossing for developing different hybrid combinations and new *Chrysanthemum* cultivars. Finally, according to the results of the two morphological and molecular parts for Sahel and Forough cultivars as well as those for Taraneh and Shokoh, a relatively high genetic distance was observed; therefore, these cultivars could be recommended for use as the parental crosses.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interests.

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