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Genetic Variation within Iranian *Iris* Species Using Morphological Traits

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Abstract

Iris belongs to Iridaceae family and it is monocotyledon. *Iris* is one of the important ornamental and medicinal plants. 34 iris genotypes (14 species) collected from different provinces of Iran were planted at National Institute of Ornamental Plants (NIOP) Iran. All of the species evaluated for 15 quantitative traits and 30 qualitative traits. Results showed that the highest positive correlation was between inner tepal length and width and the lowest of this correlation was between crest length and width. Cluster analysis using Ward similarity coefficient divided *Iris* species into three clusters. Also the highest Nei's genetic distance based on qualitative traits was between *I. iberica* and *I. fosterana* and the lowest was between *I. germanica* and *I. paradoxa*. The maximum genetic diversity was in Khorasan Razavi populations, and the minimum was in Khorasan (North). According to Shannon index, *I. spuria* and *I. germanica* species had the maximum level of genetic diversity. Cluster analysis according to qualitative traits by Jaccard similarity coefficient and UPGMA classified the 34 genotypes into four groups. Classification of populations based on quantitative traits was not similar to classification based on qualitative traits and they were not similar to geographical distribution.

Key words: genetic diversity, iris, Jaccard similarity coefficient.

Introduction

Irises (2n=2x=18-48) are monocotyledonous plants and belong to *Iridaceae* family. The genus of *Iris* is consisted of 4 subgenera: *Hermodactyloides*, *Iris*, *Limniris*, and *Scorpiris* (Khassanov and Rakhimova, 2012). Iris is one of the ornamental and medicinal plants that have been used for a long time and several improvements accomplished to introduce new cultivars. There are more than 300 species of *Iris* in the world that the existence of about 20 species and subspecies are reported in Iran (Wendelbo, 1977). Ghahreman (1979-1998) has reported 26 species landraces of *Iris* in Iran which 21 of them are related to *Iris* and 5 of them belong to *Gladiolus*. Most of Iranian *Iris* species are divided in groups of roots, bulbous, and tuberous types (Azimi *et al*. 2012).

Wendelbo (1977) reported some species which are related to *Iris* genus like *I. iberica*, *I. spuria, I. meda, I. fosterana, I. acutiloba, I. imbricate*, and *I. paradoxa*. Avishai (1977) and Avishai and Zohary (1980) classified *Oncocyclus* subgroup based on morphological traits and they offered seven

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groups for them. Avishai and Zohary (1980) noted that 60 species have been identified from the subgroup. 33 species of the *Oncocyclus* subgroup have been reported by Rix (1997) and also 41 species by Mathew (1989). Sapir *et al.* (2002) studied morphological characteristics of *Oncocyclus* subgroup in order to determine the classification relationships; they measured vegetative and productive characteristics related to 42 populations (9 groups). Rahimi *et al*. (2009) studied about *Iris* landraces of Iran by using morphological traits. Naderi *et al.* (2007), Sapir *et al.* (2002), Feinbrun-Dothan (1986), Arafe *et al.* (2002), and Karam *et al.* (2004) studied different species of *Iris* by morphological traits.

Description and evaluation of plant genetic resources is used for morphological expression of reproductive and vegetative organs, also for classical agronomic evaluation (Low *et al*., 1996). Because of the various environmental conditions in Iran, there are a lot of cultivars in iris genus. Surveying about the diversity of different kinds of iris according to morphological characteristics and identification of valuable commercial characteristics was the goal of this study, which may be useful in breeding programs. Also, clarifying the relationship between patterns of species diversity and Iranian iris geographical distribution was the other goal.

Materials and Methods

Thirty-four *iris* genotypes (14 species) collected from different provinces of Iran (Table 1) were planted in 2008 at the research farm of National Institute of Ornamental Plants (NIOP), Iran, which has 33°, 54' N latitude and 50°, 29' E longitude of geography with height of 1732 m above sea level, 13.4°C the mean annual temperature and 57.1% the mean relative humidity. All of the species were evaluated for 15 quantitative and 30 qualitative traits. This evaluation was based on UPOV (for the Protection of New Varieties of Plants, 2000) guideline.

Qualitative characteristics consisted of: bud color, flower color, the number of flower color, outer shape of blade, recurving of blade compared to claw, outer tepal ground color of upper side of blade, blade width of outer tepal, conspicuousness of veins on upper side of outer tepal, size of spot on blade of outer tepal, color of spot on blade of outer tepal, shape of tip of spot on blade of outer tepal, ground color of upper side of claw, inner tepal shape of blade, inner tepal color, filament color, anthers color, intensity of yellow color of pollen, pistil color of upper side of bridge, depth of incisions of margin of crest, stigma color, flowering time, storage organ status, beard of outer beard, scent flower, the number of flower on branch, and the leaf number.

Quantitative traits analysis consisted of Pierson correlation coefficient, principle component analysis, cluster analysis Ward method basis on quantitative traits, and using UPGMA method and Jaccard similarity coefficient basis on qualitative traits, discriminant function analysis, and descriptive statistics like mean, standard deviation, variation coefficient, Nei's distance coefficient, Nei’s gene diversity (Nei, 1978), Shannon index, and principle component analysis was done. SAS, SPSS 16.0, NTSYS ver 2.01, and GenAlex 6.3 (Peakall and Smouse, 2006) were used for statistical analysis.

Results and Discussion

Quantitative Traits

Pierson correlation coefficient was used to evaluate the relationship among the traits (Table 2). The highest positive correlation was between inner tepal length and width (r= +0.852) and the lowest of this correlation was between crest length and width (r= +0.078). Crest and peduncle length had the highest negative correlation (r= -0.342; Table 2).

Table 1. Irises, their species and geographic origin in Iran

Name	Genotypes code	Location site
Khorasan	*kopetdaghensis*	Khorasane Razavi- Fareman
Nimrouzi	*sisyrinchium*	Khorasan Northern- Esfarayen road
Goldorosht	*iberica*	Azarbayejan (West) – Kajlarat
Limooei	*imbricata*	Tehran- Damavand- Tar Lake
Maad	*meda*	Hamedan
German (Whit flower)	*germanica*	Markazi- Mahallat
Golchamani, Khoogan, Robatmorad	*songarica*	Markazi - Khomein
Rezaiye	*Barnumae*	Azarbayejan (West) -Divankhane
Kamani	*aucheri*	Kordestan- Sanandaj
Paradox	*paradoxa*	Azarbayejan (East)- Tarzom
Parvaneii	*acutiloba*	Khorasane Razavi- Neyshabour
German (Purple flower)	*germanica*	Azarbayejan (East)- Peygham
Namakzar3	*spuria*	Markazi- Poldoab
Namakzar2	*spuria*	Azarbayejan (East)- Ahar
Namakzar1, Namakzar4	*spuria*	Hamedan- Farmen
Dorang	*fosterana*	Khorasan Razavi- Ghuochan
Shaffaf	*pseudacaucasica*	Chaluos road
Bojnord3730, Bojnord3712	*sisyrinchium*	Khorasan (North)- Bojnord
Mashhad3697, Mashhad3752	*sisyrinchium*	Khorasan Razavi- Mashhad
Sarakhs3688	*sisyrinchium*	Khorasan (North)- Sarakhs
Bayanlou	*sisyrinchium*	Kurdestan
Bane1	*sisyrinchium*	Kurdestan- Bane
Boushehr	*sisyrinchium*	Boushehr
Hamedan1332, HamedanA4	*songarica*	Hamedan
Naragh	*songarica*	Markazi- Naragh
Marivan	*songarica*	Kurdestan- Marivan
Bane2	*songarica*	Kurdestan- Bane
Fars2699	*songarica*	Fars

Table 2. Pairwise matrix of Nei's genetic distance in *Iris* spp

Kopetdaghensis	*Acutiloba*	*barnumae*	*Iberica*	*Germanica*	*paradoxa*	*Imbericata*	*aucheri*	*fosterana*	*maad*	*pseudocau*	*casia*	*spuria*	*sisyrinchium*	*songarica*
0.000														*kopetdaghensis*
0.775	0.000													*acutiloba*
0.682	0.446	0.000												*barnumae*
0.775	0.284	0.446	0.000											*iberica*
0.542	0.433	0.361	0.422	0.000										*germanica*
0.682	0.284	0.446	0.314	0.221	0.000									*paradoxa*
0.378	0.597	0.557	0.638	0.295	0.411	0.000								*imbericata*
0.727	0.638	0.481	0.518	0.401	0.597	0.638	0.000							*aucheri*
0.463	0.660	0.537	0.800	0.701	0.704	0.394	0.617	0.000						*fosterana*
0.682	0.446	0.255	0.378	0.351	0.314	0.411	0.446	0.617	0.000					*maad*
0.446	0.775	0.682	0.727	0.485	0.597	0.284	0.727	0.428	0.557	0.000				*pseudocaucasia*
0.633	0.360	0.329	0.429	0.225	0.430	0.450	0.312	0.532	0.341	0.571	0.000			*spuria*
0.727	0.638	0.411	0.597	0.375	0.557	0.597	0.346	0.751	0.314	0.378	0.412	0.000		*sisyrinchium*
0.727	0.411	0.346	0.597	0.488	0.557	0.597	0.446	0.617	0.378	0.682	0.439	0.378	0.000	*songarica*

Therefore, by increasing inner tepal width, the inner tepal length increased. Because flowering branch thickness and crown thickness had a high level of correlation, by increasing one of them, the other one will increase. Additionally, this effect of physiological characteristics causes increasing of transport capability. In principle component analysis (PCA), the four first components had the 81.21% of total variance (Table 3).

Table 3. Principle component analysis (PCA) for 15 traits in *Iris* spp

Component	Eigenvalue	Proportion of variance (%)	Cumulative (%)	Inner tepal width	Pistil width of bridge	Perianth tube length	Crest length	Flowering branch thickness	Crown thickness	Peduncle thickness	Flowering branch length
1	7.11	47.40	47.40	0.334	0.337	0.021	0.194	0.277	0.236	0.128	0.277
2	2.24	14.97	62.37	0.017	-.005	0.536	0.459	-.055	-.196	-.391	-.055
3	1.75	11.69	74.06	-.061	-.004	0.307	0.250	0.344	0.341	-.110	0.341
4	1.07	7.15	81.21	-.365	0.144	0.241	0.221	0.332	-.052	0.358	0.332

The first component had a positive correlation with inner tepal width and pistil width of bridge which was 0.334 and 0.337, respectively. The second principle component had 0.536 correlations with perianth tube length and 0.459 with crest length. Flower branch thickness had 0.344 and crown thickness had 0.341 correlations with the third principle component. The fourth principle component had 0.358 correlations with peduncle thickness and 0.332 correlations with flowering branch length. The first two components separated *Spuria* species from the other species, as for two-dimensional diagram (Fig. 1).

Therefore, width of inner tepal, pistil width of bridge, perianth tube length, and crest length had the maximum effect on the above mentioned species differentiation. Rahimi *et al.* (2009) mentioned that the maximum correlation was between the appearance of the first flower and flower area, also between flower diameter and flower area in Iranian landrace iris. Moradi (2007) found that there is a significant positive correlation between stem thickness and leaf number and a negative correlation between width and length of leaf in *Gladiolus*. In factor analysis among the morphological variation in Iranian landrace iris, three first factors had the 92% of total variance which was reported by Rahimi *et al.* (2009). Arafe *et al*. (2002) reported that the three first components had 72.94% of total variance by studying the genetic variation. Also stem length, leaf size, size, and shape of the flower had a positive correlation with the first component. The maximum variation was related to width of inner tepal and leaf width and the minimum variation was for crown thickness and peduncle length (Table 4).

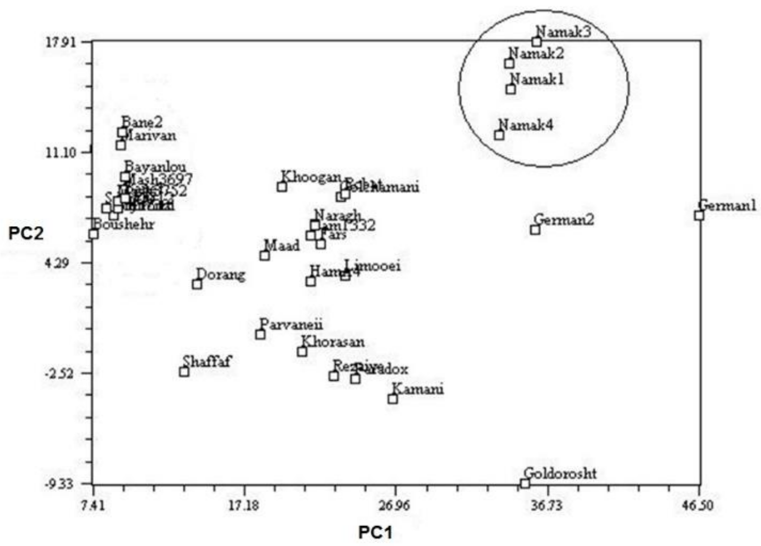

Fig. 1. Plot of the two first principle components in *Iris* spp. according to quantitative traits

Table 4. Descriptive statistics for quantitative characters in *Iris*

Trait	Minimum	Maximum	Mean	Variance	C.V (%)
Leaf width	3.38	38.44	10.59	91.50	90.26
Peduncle length (mm)	3.6	38.4	23.21	74.25	37.11
Peduncle thickness (mm)	3.28	13.48	6.11	6.85	42.80
Flower size (mm)	9.3	110.22	49.67	1087.27	66.37
Perianth tube length (mm)	4.66	51.36	19.61	239.26	78.87
Outer tepal width (mm)	5.34	57.9	13.62	141.93	87.46
Outer tepal width of claw (mm)	3.85	46.5	11.54	78.82	76.89
Inner tepal length (mm)	5.02	76.34	34.41	309.64	51.13
Inner tepal width (mm)	3.29	54.42	12.66	147.79	96.02
Pistil width of bridge (mm)	3.07	20.5	6.39	11.61	53.30
Crest length (mm)	3.62	13.18	7.79	9.47	39.51
Crest width (mm)	2.02	13.9	6.24	9.47	49.27
Plant height (cm)	10.76	106.9	49.03	572.23	48.78
Flowering branch thickness (mm)	3.86	16.8	6.92	11.50	48.95
Crown thickness (mm)	6.3	20.76	9.96	10.95	33.21

Therefore, crown thickness was more stable than the other traits and conversely inner tepal width and leaf width was the most variable than other traits. Also, Karam *et al*. (2004) reported that in morphological traits evaluation in some iris, the maximum amount of diversity was for leaf length. In the survey of quantitative traits which are important economically, about leaf width, those with smooth leaf sections (*I. germanica* and *I. spuria*) had the maximum mean. Often these species with rhizome are higher in length and adapted to wetland. *I. iberica*, *I. germanica*, and *I. paradoxa* species have bigger flower and *I. sisyrinchium* has little flower in comparison to the others. The species with the highest height was *I. spuria*. Also, the highest inner tepal width and outer tepal length were in *I. germanica* and *I. iberica*. Inner tepal length in *I. paradoxa* was the highest.

Cluster analysis using Ward algorithm divided *Iris* species into 3 clusters. The first cluster consisted of these species: *I. kopetdaghensis, I. imberica, I. barnumae, I. aucheri, I. meda, I. paradoxa, I. pseudacaucasica, I. fosterana,* and *I. songarica*. The height of this group is lower than the other species but has higher perianth tube length. The second group consisted of *I. sisyrinchium* species. This group had the minimum leaf width, flower size, perianth tube length, inner tepal length, and inner tepal width. The third group (the last one) consisted of: *I. germanica*, *I. spuria*, and *I. iberica* (Fig. 2). This group had the maximum amount of these traits: height, inner tepal length and width, outer tepal width and length, flower size, and leaf width which were the main distinguishing characteristics of these species.

Identifying different species from each other in discriminant function analysis, all of the quantitative traits were effective, except perianth tube length and crown thickness. Therefore, different species of *Iris* which were studied in this survey are very different in all of the mentioned traits except crown thickness and perianth tube length. Also, this analysis showed that the classification of these iris populations based on the species is correct. Therefore, different studied species had significant distinctness.

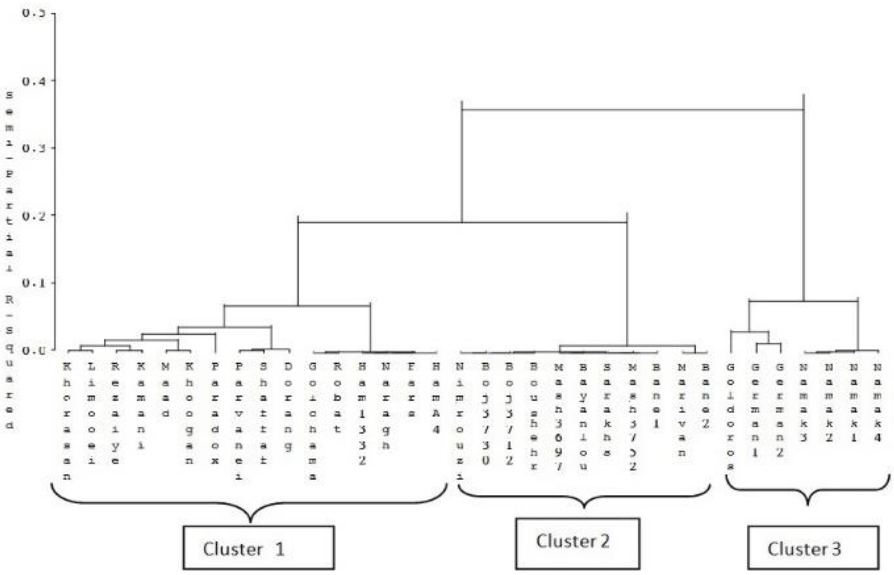

Fig. 2. Dendrogram of 34 genotypes of *Iris* sp. using Ward method basis on quantitative traits

Qualitative Traits

The highest Nei's genetic distance based on qualitative was between *I. iberica* and *I. fosterana* (0.800) and the lowest was between *I. germanica* and *I. paradoxa* (0.221) (Table 2). Geographically, the highest Nei's genetic distance was between Isfahan and Fars populations with Tehran population. The Nei's genetic distance between Fars and Isfahan was zero. Also North Khorasan and Boushehr had not significant genetic distance. Geographically, the maximum genetic diversity was in Razavi Khorasan populations. On the other hand, the minimum was in North Khorasan. By calculating Shannon index, *spuria* and *I. germanica* species had the maximum level of genetic diversity (217, 209) among the species which include several genotypes.

Cluster analysis based on Jaccard similarity coefficient and UPGMA algorithm classified the species into 4 groups (Fig. 3): the first one consisted of *I. kopetdaghensis, I. imbericata*, *I. pseudocaucasia*, and *I. fosterano* species, all of which were bulbous plants except *I. imbericata*. These species based on recently classification of iris are related to bearded iris and are suitable for dry weather conditions. The second group consisted of *I. songarica*, *I. sisyrinchium*, *I. barnumae* and *I.meda species*. *I. songarica* was a beardedless species and are grown in wet areas. *I. germanica*, *I. spuria*, and *I. auchery* classified in the third group which were beardedless and are suitable to wet soils. A unique feature of these species was that their flowers were aromatic which may be useful in the breeding programs. The fourth group consisted of *I. acutiloba*, *I. paradoxa*, *I. iberica*, *I. germanica*, and *I. spuria* (Namakzar1). *I. paradoxa* was more colorful than the other species.

I. germanica species classified into 2 groups based on bud color, flower color, stigma color, pistil color upper side of bride, and color of upper side of crest (Namakzar1) because of bud color, number of flower color, outer tepal ground color of upper side of blade, crest, and pistil and stigma color is different from the others.

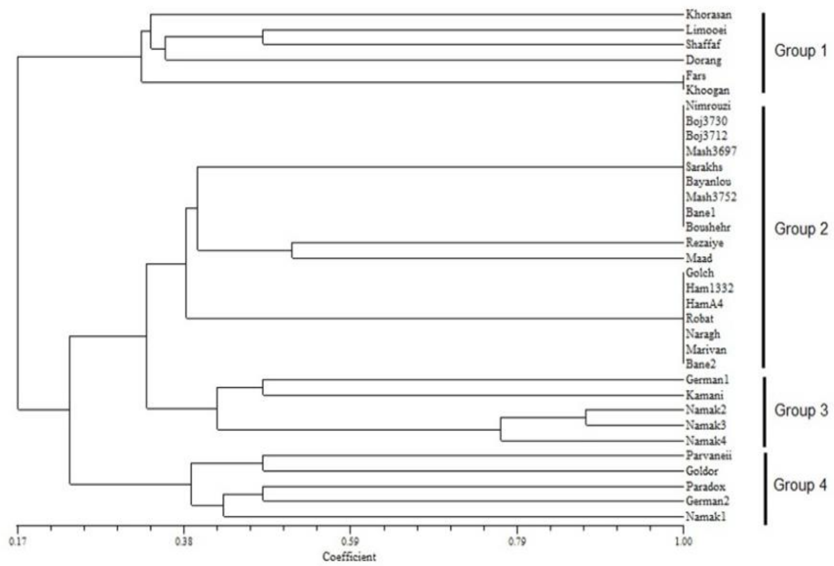

Fig. 3. Dendrogram of 34 genotypes of *Iris* sp. using UPGMA method and Jaccard similarity coefficient basis on qualitative traits

In this study, the results showed that climatic and geographical changes did not affect the vegetative and reproductive traits and the only effective factor in populations' differentiation was on species level. Totally, there was a significant diversity in quantitative traits (33% up to 96%), but vegetative and flower traits did not have significant diversity differences (Fig. 3). Azimi *et al.* (2012) reported that characteristic of correlation coefficients in different *Iris* species, the most correlation (positive and significant) was relating to length and width of inner tepal and length of perianth tube and pistil width of bridge had the lowest correlation (negation and significant).

The variation range was 28% to 83% in group 1 species (*I. kopetdaghensis*, *I. songarica*, etc). In this group, leaf width has the highest diversity; on the other hand, crown thickness and crest length had the lowest diversity. Peduncle length had more diversity in *I. sisyrinchium* species, while flower size, perianth tube length, outer tepal length, and inner tepal width had the lowest diversity (Fig. 3). There was a high diversity in group 3 (8%-95%). In this group, flower size and perianth tube length had the highest and lowest stability, respectively (Fig. 3).

Karam *et al.* (2004) announced that vegetative traits (leaf and stem) were the most variable traits (85%) and flower traits were the most stable traits (15%). Generally, Group 2 which was consisted of *I. sisyrinchium* had more stability in vegetative and reproductive traits in comparison oto the other groups. Group 1 had more species in comparison to group 3, but it had lower diversity, this means group 3 had the most diversity. Therefore, *I. germanica* and *I. spuria* had the highest level of diversity.

These results assimilate with the qualitative results (Fig. 2) because in the qualitative traits analysis, the highest level of Shannon index obtained from *I. germanica* and *I. spuria* while *I. songarica*, and *I. sisyrinchum* did not show diversity (Shannon index =0). In the classification of studied iris populations (Fig. 2), based on quantitative traits of vegetative traits like plant height, leaf width and in reproductive traits like flower size, inner tepal length and width, outer tepal width and length, and perianth tube length had the most effects in differentiation of three groups.

Arafe *et al*. (2002) announced that in the cluster analysis of two iris (*hayneri* and *atrofusca*), the principle differentiation (Table 3) of groups is because of flower and vegetative traits.

Fars populations were similar to Isfahan; also North-Khorasan populations were similar to Boushehr. It is interesting to note that Boushehr is completely different from the North-Khorasan climatically and geographically. Therefore, no clear relationship was found for genetic diversity between populations in relation to geographical distribution. Although there were more genotypes in *I. sisyrinchium* and *I. songarica* species, they had a low level of diversity. Hamrick and Codt (1990) reported that plant species with low expansion or landrace tend to low level of diversity in comparison of the other species. Conversely, some of a little landrace populations showed that they had strong genetic structure despite of ecosystem limitations (Brauner *et al*. 1992), for example, *I. germanica* and *I. spuria*, in spite of their little population, had more diversity.

In Arafe *et al.* (2002) studies on Iris, there was not any relationship between genetic diversity and the size of populations. In this survey, classification of populations based on quantitative traits was not similar to classification based on qualitative traits and they had not similarity to geographical distribution. Therefore, there was not any significant morphological relationship between the populations and geographical areas. Generally, *I. spuria* and *I. germanica* species from the group of which were from wet lands and *I. iberica* and *I. paradoxa* from the dwarf iris group which were from dry lands had the highest amount of economic traits; therefore, the mentioned species were the most valuable *Iris* of Iran (Fig. 4).

Fig. 4. Plant of *Iris* species that have the highest amount of economic traits; A. *I. iberica*, B. *I. paradoxa*, C. I. *germanica*, D. *I. spuria*

Conclusion

In this study, quantitative traits of flower size, outer tepal, and inner tepal width and leaf width were economically important traits that may be considered in breeding programs. Important flower component is consisted of outer tepal and inner tepal width and inner tepal length were also important in breeding. The highest average of traits belonged to *I. germanica*, *I. spuria*, *I. paradoxa*, and *I. iberica* species which may be used in cross breeding programs to improve commercial cultivars.

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