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Intervarietal hybridization and observation of heterosis in the new hybrids of *Iris germanica*

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Abstract
To produce new, high-quality offsprings, we generated two cross combinations in *Iris germanica*. Three parental plants were selected after preliminary screening of commercial traits in different cultivars. Fourteen quantitative traits mostly related to the flower organ were investigated on 15 progenies and three parental plants in a randomized complete block design with three replications. Significant positive and negative correlations were observed among different studied attributes at 0.01 and 0.05 probability. The highest positive (+0.86) and negative (-0.76) correlations was recorded between diameter of flowering branch with crown diameter as well as peduncle length, respectively. Cluster analysis using Ward's method separated most of the progenies from the parental plants based on evaluated morphological characteristics. However, some of the progenies were grouped with their maternal parent. In PCA analysis, five factors explained 87.84% of the total variations; crown diameters as well as diameter of flowering branch were predominant in the first component (34.19%). Some promising progenies with interesting characteristics were observed and several hybrid plants were superior to their parents and showed positive heterosis over their parents. Progenies NIOP5 and NIOP8 were superior in the most studied attributes, therefore, they suggested for further investigations which can be used in breeding programs. Progenies showed a wider range for flower diameter, peduncle thickness, bush height, as well as inner and outer tepal dimensions. Flower color also showed wider range in the progenies and purple, violet and white colors were the most frequent colors in the hybrid plants, while lilac color was the rarest one. Results of our investigation revealed that intervarietal hybridization is an effective way to contribute to the phenotypic variation in the iris flower for producing new plant materials for breeding purposes as well as releasing new cultivars. The inheritance of some of the traits identified in this study will be important for improving plant architecture in German iris plant.

Keywords: German iris, hybridization, phenotypic variation, progeny, heterosis.

Abbreviation: **PCA**, Principal component analysis.

Introduction

Iris (2n=2x=18-48), an important member of Iridaceae, has been used from ancient time for its medicinal and ornamental properties. More than 300 wild *Iris* species exist in the world, out of which 20 ones are endemic of Iran (Wendelbo, 1977).

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Bearded, aril and beardless irises are three main groups of this genus that are commonly used as garden plants. German iris (*Iris germanica* L.), an important member of bearded irises with primary hybrid origin, is a rhizomatous perennial plant consisted of triplet flower organs, flat leaves, multicolored flowers, and aril-less seeds that is used commonly in landscape settings. This species is moderately resistance to the calcareous soils and other unfavorable environments. Moreover, low water requirement is one of the main important features of German irises. Beside its ornamental values, this plant has medicinal properties due to the aromatic essence of the rhizome (Gozu et al., 1993). Considering these characteristics, German iris is considered as one of the most popular ornamental plants for landscape designers' points of view and is planted in the rocky gardens in the most countries (Azimi et al., 2011; Ghanadi, 1991).

Crossing different plant varieties is one of the main practices that have been carried out by plant breeders with the purposes of generating wider genetic variation and using heterosis of hybrid progenies (Joshi et al., 2001). Interspecific hybridization is a commonly used method in different close related species for producing offsprings with new characteristics. This method has been widely utilized in different *Iris* species (Zhen et al., 1997; Burke et al., 1998; Shimizu et al., 1999; Yuval et al., 2002; Zhen et al., 2003; Arnold et al., 2010). Intervarietal hybridization is another commonly way for transferring desirable attributes among different cultivars and producing progenies with new characteristics which has been used widely in different plant species (Zamaniet al., 2010; Yang et al., 2015). Zhen et al., (1997) performed interspecific and intraspecific crosses in *Iris* genus and reported that F_1 seedlings resulted from two interspecific hybrids grew very weak and died within 6 to 8 weeks due to the low compatibility between species, while F_1 seedlings of three intraspecific hybridizations grew well. These authors reported that some characteristics such as bush height and flower color showed high variation in the F_1 plants and attributed their observations to the hetrozygosity nature of parents for these traits. Based on the wide spectrum of flower color in the offsprings, Zhen et al., (1997) suggested that flower color of the progenies derived from reciprocal crosses of *Iris tectorum* × *I. tectorum f. alba* is being controlled by single gene, whereas that of *I. germanica LP* × *I. germanica PP* being controlled by multigenes. In another study, Zhen et al., (2003) obtained eight dwarf offsprings from five crosses between dwarf and normal sized plants and reported that although bush height had high variation among progenies, the mean values of progenies were lower than theirs parents. These authors showed that it is possible to obtain dwarf German iris through back cross with dwarf parent (Zhen et al., 2003). To elucidate taxonomic relationships of *Oncocyclus* irises, Yuval et al., (2002) studied morphological attributes of this subgroup and suggested that natural selection has an important role in the population differentiations. Shimizu et al., (1999) produced somatic hybrids of *I. ensata* and *I. germanica* through protoplast fusion. Burke et al., (1998) evaluated F_1 progenies derived from interspecific hybridization of *I. fulva* and *I. hexagona* and stated that hybrid plants were significantly superior to their parents. Arnold et al., (2010) studied the hybrid plants of *I. fulva* with other Louisiana irises and reported high compatibility among *I. fulva*, *I. brevicaulis*, and *I. hexagona*species. In the cross between *I. pseudacorus* with *I. laevigata* and *I. revsicolor*, hybrid plants were superior to their parents in most of the characteristics (Austin, 2005).

To keep up flower diversity according to the new consumer demands in the ornamental plant industry, plant breeders need to diversify existing variation and to produce new flowers having new characteristics. Therefore, we hybridized three of the German iris cultivars to produce new progenies with probable potential of commercial importance in the flower industry. German iris was selected in our investigation because of its elegant appearance as well as its tolerance to unfavorable conditions such as water deficiency, which is becoming one of the most important objectives in the plant breeding programs in semi-arid regions of world. This is the first investigation of German iris hybridization in Iran and can open new horizon for designing new breeding programs between commercial and wild irises in future.

Materials and methods

Three cultivars of *Iris germanica* with different colors were used in this investigation. These cultivars were chosen according to the commercial traits including: flower color, number of floret, flowering duration, height plant and resistance to environmental conditions in different cultivars. Two blue (P2) and brown (P5) colors cultivars were used as paternal plants, while yellow color cultivar (P3) was used as maternal plant (Fig. 1). Hybridization of $P_3 \times P_2$ produced 13 offsprings while $P_3 \times P_5$ crosses produced two offsprings. Seeds of successful crosses were collected at the end of summer and stored in the moistened peat moss at 4° C for 45 days. When the dormant period is almost finished and sprouting signs were appeared (middle of December), seeds from each offspring were planted in the clay pots containing rotten manure, sand, clay and leaf mould (1:1:1:1) in the greenhouse condition. At the stage of 4-5 leaves (April), seedlings were planted in 30 × 30 cm^2 spacing in the field conditions.

Totally 18 plants, including 15 progenies and 3 parental plants were planted in the completely randomized block design (CRBD) with three replications in the National Institute of Ornamental Plants in Mahallat city and evaluated during 2011-2014 and the mean values of studied attributes were used for statistical analysis.

At the stage of flowering, 14 quantitative characteristics were recorded from the progenies as well as their parents according to the international union for the protection of new varieties of plants (UPOV, 2000). These attributes included leaf width, peduncle length, flower diameter, outer tepal width, inner tepal length, inner tepal width, bush height, diameter of flowering branch, crown diameter as well as four color related attributes

Color measurements was performed on the digital images and analyzed by using color tester software version 3 (Strecer et al., 2010). Indices of a, b, L was measured and ΔE was calculated according to below formula: $\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2}$

a: tendency of flower color from green to red (-70 for dark green and +70 for dark red).

b: tendency of flower color from blue to yellow (-70 for blue and +70 for yellow).

L: brightness and lucidity of flower (0 for dark and 100 for white).

ΔE: degree of color changing and its differences across flower.

Data analysis

Analysis of variance was performed for all of the morphometric measured traits by SAS software ver. 6 (SAS Inst. 1990). Descriptive statistics including mean, minimum, maximum values as well as coefficient of variation (CV %) were calculated for all traits. The correlation between morphological variables was also evaluated using Pearson correlation coefficient using SPSS statistic software version 16 (SPSS Inc., Chicago, united States, Norusis 1998). Multivariate analysis of variance (principal component analysis; PCA) was performed using SPSS software to characterize the accessions based on their morphological attributes. Tri- and biplot was created based on the first three main components using SPSS software. After normalizing each trait using Z scores, cluster analysis was performed according to the ward's method based on square Euclidean distance coefficient using SPSS software. In addition, percentage of heterosis (percent) was calculated according to Hallauer et al., (1988). Two below formula were used to compute relative heterosis in comparison with average of parents (a) and superior parent (b):

a) MPH= (Mean Hybrid value - Mean parent value / Mean parent value) × 100

b) HPH= (Mean Hybrid value – High parent value / High parent value) × 100

Fig. 1. Photograph of flowers from German iris cultivars that used as parents in this investigation and progenies obtained from their hybridizations. P2 and P5 were used as paternal and P3 as maternal parents. NIOP1-13 obtained from crosses of P3 × P2 and NIOP14 and NIOP15 resulted from crosses of P3 × P5.

Results

Analysis of variance and descriptive statistics

Results of ANOVA revealed that all of the studied attributes were significantly different ($p \leq 0.01$) among offsprings as well as their parents. With Coefficient of variation (CV) varied from 13.45% (Inner tepal width) to 32.23 % (Diameter of flowering branch) without considering of color components (Table 1). While by considering flower color components, a index had the highest CV (75.71%). Results of this investigation revealed that inner tepal width, inner tepal length and outer tepal width, which are considered as the main components of iris flower, had lower coefficient of variation compared with other traits. Traits having higher range of CV are preferred by breeders for selection purposes.

Simple correlation among traits

The correlation between quantitative traits for each pair of iris attributes was calculated in this investigation (Table 2). The highest positive correlation was observed between diameter of flowering branch with crown diameter ($r = 0.85$; $p<0.01$). Our results indicate that size of crown diameter is positively correlated with diameter of flowering branch, which is an important trait in iris and could be used in breeding programs. Flower diameter had significant positive correlation with bush length ($r = 0.68$). Moreover, leaf diameter was significantly correlated with bush length ($r = 0.62$), flower diameter ($r = 0.56$), outer tepal width ($r = 0.50$) as well as peduncle length ($r = 0.42$) and thickness ($r = 0.33$). Inner tepal width also showed significant correlation with peduncle length ($r = 0.65$), flower diameter ($r = 0.50$) and outer tepal width ($r = 0.39$). In addition, diameter of flowering branch was negatively correlated with peduncle length (-0.76) and inner tepal length (-0.73).

Cluster Analysis

Cluster analysis based on Ward's method divided new hybrids and their parents into five distinct groups (Fig. 2). According to the cluster analysis, two main groups were recognizable; one entirely composed of hybrid plants and the other included three parental cultivars and three hybrid plants. Our results showed relatively higher variation among the hybrid plants compared to their parents. The highest phenotypic similarity was observed between NIOP2 and NIOP3, both resulted from crosses of P2 × P3. These two hybrid plants also showed high similarity with their maternal parent (P3) and formed a sub-cluster along with their maternal parent and NIOP15 (a progeny derived from hybridization of P5 × P3). Two paternal parents (P2 and P5) also grouped together and separated from hybrid plants. We observed that three hybrid plants (NIOP2, NIOP3 and NIOP15) had high morphological similarity with their maternal parent and clustered in the same sub-group along with their maternal plant (P3).

Table 1. Descriptive statistics of different studied characteristics among German iris samples.

	unit	Min.	Max.	Mean	SD	CV%
Leaf width	mm	24.01	59.00	40.70	9.45	23.21
Peduncle length	mm	16.52	44.00	32.18	7.69	23.90
Peduncle thickness	mm	6.66	18.00	10.03	3.23	32.18
Flower diameter	mm	88.30	150.00	113.86	20.24	17.77
Outer tepal width	mm	35.20	59.33	46.32	8.38	18.09
Inner tepal length	mm	51.43	90.00	70.83	11.46	16.18
Inner tepal width	mm	35.00	56.00	45.13	6.07	13.45
Bush height	cm	55.00	96.00	74.15	12.14	16.38
Diameter of flowering branch	mm	5.00	12.30	6.99	2.25	32.23
Crown diameter	mm	10.66	18.00	13.12	2.29	17.42
ΔE	-	37.63	90.50	69.99	15.87	22.67
L	-	26.45	75.25	46.70	12.78	27.36
a	-	-14.50	53.90	25.15	19.04	75.71
b	-	-68.75	55.65	-21.82	37.83	173.38

Table 2. Pearson correlation among quantitative traits of iris studied accessions.

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13
Leaf width													
Peduncle length	0.43												
Peduncle thickness	0.33	-0.28											
Flower diameter	0.56*	0.35*	0.47*										
Outer tepal width	0.51*	0.32*	0.57*	0.69**									
Inner tepal length	0.21	0.66**	-0.14	0.51*	0.40								
Inner tepal width	0.02	0.32	0.22	0.37	0.51*	0.39							
Bush height	0.62**	0.23	0.44	0.68**	0.32	0.18	0.07						
Diameter of flowering branch	-0.27	-0.76**	0.25	-0.26	-0.22	-0.73**	-0.18	0.02					
Crown diameter	-0.18	-0.64**	0.48*	-0.04	0.08	-0.57*	0.13	-0.03	0.86**				
ΔE	0.01	-0.02	0.01	0.06	0.04	-0.18	0.34	-0.02	0.23	0.40			
L	-0.12	-0.11	-0.37	-0.39	-0.37	-0.21	-0.25	-0.33	-0.08	-0.17	0.40		
a	0.13	0.06	0.38	0.57*	0.36	0.22	0.47*	0.49*	0.22	0.32	0.22	-0.69**	
b	-0.20	-0.29	-0.25	-0.56*	-0.37	-0.35	-0.48*	-0.52*	0.05	-0.05	-0.53*	0.26	-0.72**

** and *: significant at 1% and 5% probability levels, respectively , Numbers in the first line represent each trait according to Table 4 (according to the chronological appearance in the first column) .

Cluster Analysis

Cluster analysis based on Ward's method divided new hybrids and their parents into five distinct groups (Fig. 2). According to the cluster analysis, two main groups were recognizable; one entirely composed of hybrid plants and the other included three parental cultivars and three hybrid plants. Our results showed relatively higher variation among the hybrid plants compared to their parents. The highest phenotypic similarity was observed between NIOP2 and NIOP3, both resulted from crosses of P2 × P3. These two hybrid plants also showed high similarity with their maternal parent (P3) and formed a sub-cluster along with their maternal parent and NIOP15 (a progeny derived from hybridization of P5 × P3). Two paternal parents (P2 and P5) also grouped together and separated from hybrid plants. We observed that three hybrid plants (NIOP2, NIOP3 and NIOP15) had high morphological similarity with their maternal parent and clustered in the same sub-group along with their maternal plant (P3).

Principal component analysis

According to PCA analysis, three main factors explained more than 81% of total variance (Table 3). A principal component loading of more than 0.55 was considered as being significant for each factor. In the first eigenvector that explained 34.19% of total variance, attributes such as peduncle length (-0.86), inner tepal length (-0.82), diameter of flowering branch (0.93) and crown diameter (0.87) had large loading and were regarded as an important contributor to this principal component. Flower diameter (0.42), bush height (0.41), and flower components (0.90, -0.80, and -0.62, for a, L and b, respectively) were prevalent in the second factor that explained 24.19% of entire variation. Leaf width (0.85) and bush height (0.85) had the highest loadings on the third component that contributed 12.39% of total variance.

Tri- and biplot was depicted based on the three and two main components, respectively (Fig. 3). The first principal component discriminated between three parents and NIOP8, whereas the second and the third components were completely discriminated parental plants from their progenies. As it is obvious from scatterplot, parent cultivars were highly similar and grouped together while hybrid plants had higher diversity and distributed equally across plot.

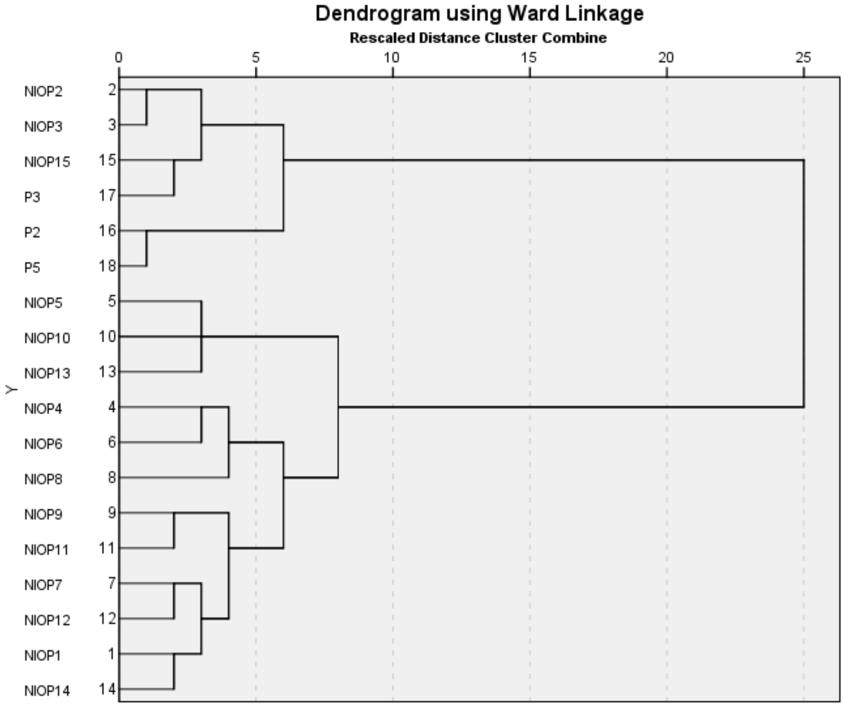
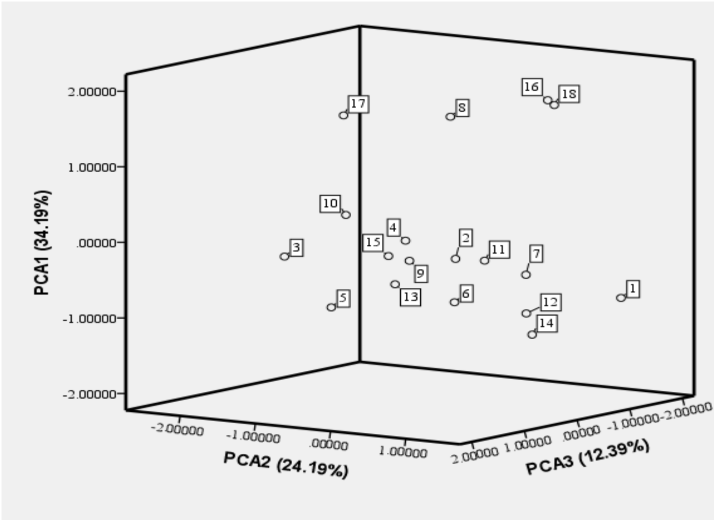


Fig. 2. Dendrogram representing iris samples according to their morphological attributes using Ward's method. Values on the top of figure (0 – 25) are the rescaled differences of studied samples generated by SPSS software.

Table 3. Eigenvalues, proportion of total variation and correlation between *Iris* attributes and five principal components in *Iris germanica* samples.

Row	Traits	Components				
		1	2	3	4	5
1	Leaf width	-0.22	-0.11	0.85	0.24	0.00
2	Peduncle length	-0.86	0.08	0.20	0.09	0.13
3	Peduncle thickness	0.45	0.19	0.44	0.62	-0.11
4	Flower diameter	-0.25	0.42	0.62	0.44	0.10
5	Outer tepal width	-0.17	0.16	0.35	0.84	0.02
6	Inner tepal length	-0.82	0.30	0.04	0.23	-0.02
7	Inner tepal width	-0.24	0.38	-0.21	0.65	0.41
8	Bush height	-0.02	0.41	0.85	-0.02	0.04
9	Diameter of flowering branch	0.93	0.17	-0.06	-0.14	0.09
10	Crown diameter	0.87	0.18	-0.09	0.27	0.21
11	ΔE	0.19	-0.05	0.00	0.07	0.96
12	L	-0.04	-0.80	-0.08	-0.26	0.44
13	a	0.10	0.90	0.19	0.19	0.22
14	b	0.16	-0.62	-0.30	-0.11	-0.61
Eigenvalue	-	4.79	3.39	1.73	1.36	1.03
Variance%	-	34.19	24.19	12.39	9.69	7.39
Cumulative%	-	34.19	58.37	70.76	80.45	87.84



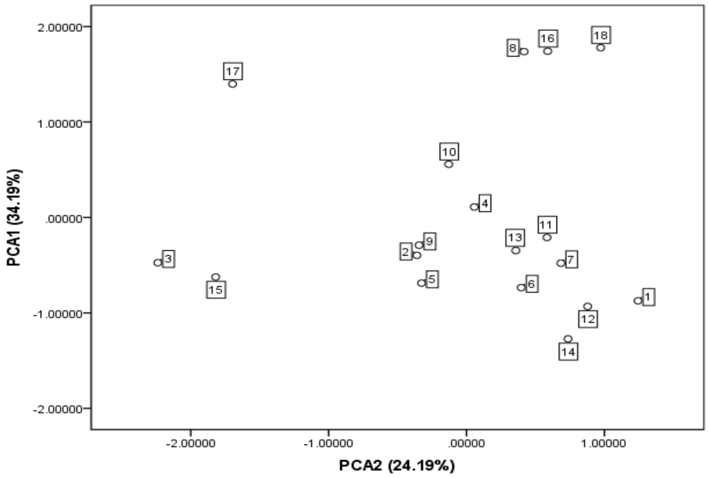


Fig. 3. Three-plot (up) and bi-plot (down) obtained by three and two main components representing three parental German iris cultivars and their progenies.

Evidence of Heterosis in the progenies

Results of heterosis test are presented in Table 4. In comparison with their parents, studied progenies were superior in some traits and inferior in some other. Inner tepal length was the only trait that all of the progenies showed positive heterosis over their parents. The lowest value for this trait (51.43 mm) was recorded from P5 while the highest value (90.00 mm) was recorded from NIOP9, a progeny derived from P3 × P2. This offspring exhibited significant positive heterosis not only compared to the mean parents (Hmp = 64.38%), but also compared to the superior parent (Hsp = 62.16%). Inner tepal width also was among the traits that most of the resulted progenies were superior to their parents. NIOP1 and NIOP12 had the highest value of inner tepal width (56 mm) and were superior to both parents (Hmp = 36.14%) and their superior parent (Hsp = 30.05%). Most of the studied progenies also exhibited positive heterosis for outer tepal width and NIOP5 was the most superior for this trait (Hmp = 51.70% and Hsp = 41.26%). Flower diameter also greatly varied among the progenies and showed positive heterosis in the most progenies. The lowest and the highest flower diameter were recorded in the maternal plant (88.30 mm) and NIOP10 (150.00 mm), respectively. The later also exhibited the highest heterosis for this trait (Hmp = 65.47% and Hsp = 61.29%).

Table 4. Mean of each trait and their heterosis in the parents as well as their progenies of *Iris germanica.*

Code	Crown diameter (mm)	Diameter of flowerin	Bush height (cm)	Inner tepal width	Inner tepal lengt	Outer tepal	Flower diameter (mm)	Peduncle thicknes	Peduncle length (mm)	Leaf width (mm)	ΔE	L	a	b
P2	16.28	11.12	68.30	39.2	55.50	36.2	93.00	9.23	16.52	24.0	53.7	40.0	26.29	24.30
P3	17.60	10.13	62.30	43.0	54.00	42.0	88.30	9.23	27.13	41.2	81.9	60.1	0.71	55.65
P5	15.80	12.30	73.16	42.1	51.43	35.2	94.06	8.75	18.20	25.1	87.9	50.7	40.83	-59.08
NIOP1	13.00	5.43	64.00	56.0	85.00	47.0	125.0	8.66	37.00	30.0	83.9	42.7	53.90	-48.09
Hmp	-23.26	-48.89	-1.99	36.1	55.25	20.1	37.89	-6.18	69.65	-7.99	23.7	-	299.2	-
Hsp	-26.14	-51.17	-6.30	30.0	53.15	11.9	34.41	-6.18	36.53	-	2.38	-	105.0	-
NIOP2	10.66	5.20	58.00	36.0	66.00	40.0	90.00	6.66	31.00	38.0	47.1	36.0	6.53	29.64
Hmp	-37.07	-51.06	-	-	20.55	2.28	-0.72	-27.84	42.14	16.5	-	-	-51.63	-25.85
Hsp	-39.43	-53.24	-	-	18.92	-4.76	-3.23	-27.84	14.39	-7.77	-	-	-75.16	-46.74
NIOP3	11.66	5.20	58.00	36.0	66.00	40.0	90.00	6.66	34.33	41.3	77.4	75.2	-10.72	14.86
Hmp	-31.17	-51.06	-	-	20.55	2.28	-0.72	-27.84	57.40	26.7	14.1	50.2	-	-62.83
Hsp	-33.75	-53.24	-	-	18.92	-4.76	-3.23	-27.84	26.68	0.32	-5.50	25.0	-	-73.30
NIOP4	12.00	6.00f	90.00	44.0	66.00	46.0	108.00	15.00	34.00	59.3	63.9	45.4	26.19	-36.63
Hmp	-29.16	-43.53	37.83	6.96	20.55	17.6	19.14	62.51	55.89	81.9	-5.72	-9.36	94.00	-
Hsp	-31.82	-46.04	31.77	2.18	18.92	9.52	16.13	62.51	25.46	44.0	-	-	-0.38	-
NIOP5	11.33	6.50	91.00	45.0	74.00	59.3	135.00	9.00	41.33	59.0	72.1	49.0	28.05	-45.16
Hmp	-33.12	-38.82	39.36	9.40	35.16	51.7	48.92	-2.49	89.50	80.9	6.34	-2.16	107.7	-
Hsp	-35.63	-41.55	33.24	4.51	33.33	41.2	45.16	-2.49	52.51	43.2	-	-	6.69	-
NIOP6	10.66	5.83	78.00	43.0	81.00	36.0	106.66	7.66	33.00	49.0	65.5	47.5	34.91	-28.41
Hmp	-325.47	-45.13	19.45	4.53	47.95	-7.95	17.66	-17.01	51.31	50.2	-3.44	-5.03	158.5	-
Hsp	-39.43	-47.57	14.20	-0.14	45.95	-	14.69	-17.01	21.77	18.9	-	-	32.79	-
NIOP7	12.73	6.16	75.00	52.0	82.00	54.0	122.00	12.00	35.00	41.0	37.6	26.4	26.45	4.08
Hmp	-24.85	-42.02	14.85	26.4	49.77	38.0	34.58	30.01	60.48	25.7	-	-	95.93	-89.79
Hsp	-27.67	-44.60	9.81	20.7	47.75	28.5	31.18	30.01	29.15	-0.49	-	-	0.61	-92.67
NIOP8	18.00	10.00	75.00	50.0	60.00	58.0	120.00	18.00	25.00	50.0	87.7	33.5	46.80	-66.25
Hmp	6.26	-5.88	14.85	21.5	9.59	48.3	32.38	95.02	14.63	53.3	29.4	-	246.6	-
Hsp	2.27	-10.07	9.81	16.1	8.11	38.1	29.03	95.02	-7.75	21.3	7.10	-	78.01	-
NIOP9	13.00	6.00	77.00	45.0	90.00	56.0	132.00	12.00	26.00	40.0	71.0	55.8	19.67	-39.23
Hmp	-23.26	-43.53	17.92	9.40	64.38	43.1	45.62	30.01	19.21	22.6	4.67	11.4	45.70	-
Hsp	-26.14	-46.04	12.74	4.51	62.16	33.3	41.94	30.01	-4.06	-2.91	-	-7.25	-25.18	-
NIOP1	13.00	7.00	90.00	42.0	65.00	53.0	150.00	15.00	28.00	53.0	52.1	40.0	33.09	5.21
Hmp	-23.26	-34.12	37.83	2.10	18.72	35.5	65.47	62.51	28.38	62.5	-	-	145.1	-86.97
Hsp	-26.14	-37.05	31.77	-2.46	17.12	26.1	61.29	62.51	3.32	28.6	-	-	25.87	-90.64
NIOP1	14.33	6.00	74.00	49.0	75.00	55.0	144.66	11.33	36.00	41.0	90.5	41.1	42.05	-68.75
Hmp	-15.41	-43.53	13.32	19.1	36.99	40.6	59.58	22.75	65.06	25.7	33.4	-	211.4	-
Hsp	-18.58	-46.04	8.35	13.7	35.14	30.9	55.55	22.75	32.84	-0.49	10.4	-	59.95	-
NIOP1	12.66	5.66	80.00	56.0	77.00	52.0	117.00	7.00	43.00	45.0	84.1	38.0	43.27	-61.37
Hmp	-25.27	-46.73	22.51	36.1	40.64	32.9	29.07	-24.16	97.16	38.0	24.1	-	220.5	-
Hsp	-28.07	-49.10	17.13	30.0	38.74	23.8	25.81	-24.16	58.67	9.22	2.71	-	64.59	-
NIOP1	12.66	6.83	96.00	42.0	74.00	36.0	133.00	8.66	38.00	48.0	77.6	50.6	24.23	-53.67
Hmp	-25.27	-35.72	47.01	2.10	35.16	-7.95	46.72	-6.18	74.23	47.2	14.4	1.06	79.48	-
Hsp	-28.07	-38.58	40.56	-2.46	33.33	-	43.01	-6.18	40.22	16.5	-5.25	-	-7.84	-
NIOP1	10.91	5.50	70.00	42.0	86.00	48.0	112.00	7.33	44.00	35.0	51.5	36.1	24.95	-26.98
Hmp	-31.98	-53.03	-1.03	3.31	60.85	34.4	19.75	-18.46	153.46	42.5	-	-	-25.66	55.15
Hsp	-32.99	-55.28	-4.32	-0.24	54.95	32.5	19.07	-20.59	141.76	39.4	-	-	-38.89	-
NIOP1	10.90	5.00	55.00	51.0	67.00	41.0	90.00	7.66	31.33	30.0	73.7	71.9	-14.53	7.11
Hmp	-32.04	-57.30	-	25.4	25.32	14.8	-3.77	-14.79	80.47	22.1	4.12	58.5	-	-
Hsp	-33.05	-59.35	-	21.1	20.72	13.2	-4.32	-17.01	72.14	19.5	-	41.8	-	-70.74

Hmp: Heterosis than mean parents; Hsp: Heterosis than superior parent. Values for heterosis is expressed

All hybrid plants had higher value of peduncle length compared with their parents. The highest value of heterosis among the studied traits was recorded for peduncle length and NIOP14 had the highest values (Hmp = 153.46% and Hsp = 141.76%). NIOP8 had the highest value for peduncle thickness and was superior to its both parents (Hmp and Hsp= 95.02%).

Leaf width varied from 24.01 mm (P2) to 59.33 mm (NIOP4) (Table 2). NIOP4, a hybrid plant obtained from crosses of P3 × P2 had significantly higher leaf width than other offsprings and was highly superior to their parents (Hmp = 81.97% and 44.00%).

Most of the hybrid plants also exhibited negative heterosis for crown diameter, NIOP8 was the only progeny that was superior to their parents (Hmp = 6.26% and Hsp = 2.27%).

Bush height showed a wider range in the offsprings compared to their parents and offspring NIOP13 had the highest value (96.00 mm) for this trait and was superior to its parents (Hmp = 47.01% and Hsp = 40.56%).

We observed a wide range of color in the hybrid plants. From all seedlings resulted from crosses of P3 × P2, blue and purple petal flowers were the most abundant. All of the color related traits showed wider range in the progenies compared to the parents. Hmp for color changing (ΔE) ranged from -44.52 (NIOP7) to 33.42 (NIOP11). The same trend was recorded about color brightness (L) and heterosis for this index ranged from -47.21 (NIOP7) to 58.55 (NIOP15). Color brightness is an important attractive aspect of iris flower, which was improved in some of the progenies in our study. Moreover, a and b indices showed high level of variation in the offsprings. These two indices represent the tendency of flower from green to red and from blue to yellow, respectively. The highest positive heterosis (Hmp = 299.26) was recorded about index a from NIOP1 offspring, which indicates high degree of flower changing. This index showed that most of the progenies were inclined to red color than green. In addition, index b showed a wide range of color changing in the offsprings and Hmp varied from -265.73 (NIOP8) to 55.15 (NIOP14). According to this index, most of the resulted progenies were inclined to blue color than yellow.

Discussion

Analysis of variance and descriptive statistics

German irises possess important and popular ornamental attributes and considerable hardiness to unfavorable environmental conditions. Intervarietal hybridization, in which satisfactory attributes or genes are transferred from one variety into another, is a promising strategy for improving plant characteristics (Yang et al., 2015). Results of our investigation revealed that the main components of iris flower had lower coefficient of variation. In accordance to this investigation, Azimi et al., (2010b) evaluated morphological attributes of Iranian irises and reported that the main components of iris flower had relatively lower variation than other traits and the highest and the lowest CV% were recorded about bush height and inner tepal length, respectively. Another report about Iranian native irises indicated that the highest CV value was recorded for bush height (12.29%) while the lowest value was recorded for inner tepal length (Azimi et al., 2011). We also observed high variation in the leaf width (CV = 23.21%). Similarly, Rahimi et al., (2009) observed that leaf width had the highest coefficient of variation among the morphological attributes of Iranian irises. In comparison with the previous reports, we observed significantly higher variation in the studied traits. These results indicated that intervarietal crossing is an appropriate way that can be used in the breeding programs for expanding existing diversity in the German irises. However, results of present and previous studies indicate that the main components of flower have lower morphological variation than other traits.

Simple correlation among traits

The correlation coefficient can provide information on the characters that are most important in assessing individuals (Norman et al., 2011). According to our results, size of crown diameter was positively correlated with diameter of flowering branch. In addition to its physiological effects, diameter of flowering branch could improve flower tolerance in transferring from field to the market (Azimi et al., 2012; Jozghasemi et al., 2015). Bush length and leaf dimensions also had significant correlation in this investigation. High correlation between leaf dimensions and bush height and flower components were also reported in other ornamental species (Hayakawa et al., 2011). It is well established that increase in the leaf dimension, will provides a better condition for photosynthetic products (Arzani, 1994; Zarei, 2017; Jalilian et al., 2018). Accordingly, higher photosynthetic activity influences the physiological characteristics and led to an improvement in plant architecture (Bell et al., 1996). According to previous study, positive correlation was observed between inner tepal length and inner tepal width, leaf width with claw width, and diameter of flowering branch with crown width among different irises species (Azimi et al., 2011). Characteristics of tepal are very important in the iris breeding program, tepal length increases in parallel with diameter of flowering branch and this factor has great importance in improving shelf life, transporting and quality of iris big flowers. High level of correlation we observed between studied traits can be used to predict each other in the breeding programs of iris.

Cluster Analysis

Cluster analysis revealed that phenotypic similarity was higher among some of the half-sib hybrid plants than the full-sib progenies. On the other hand, some of the progenies were clustered with their half-sib related and separated from their full-sib offsprings. We had two hybrid plants from crosses of P5× P3 (NIOP14 and NIOP15) that separated completely and each placed in one of the two main groups, indicating that hybrid plants, resulted from the same combination of parents, are highly variable in their phenotypic attributes. This could be attributed to the source of parents we used in this study, which were not pure line and may be heterozygous for some of their loci. Although the number of these progenies is not enough, these observations may indicated that cytoplasmic inheritance may be reflected by morphologic traits in *Iris germanica* and have major effects on the crosses of this species. Importance of maternal inheritance have been reported in different plant species (Werlemark et al., 1999; Luo et al., 2002; Kirk et al., 2005; Zamani et al., 2010) and could be of high importance for selection of appropriate parents in the hybridization programs by breeders.

Principal component analysis

PCA was used to estimate the importance degree and relationship of different traits in the total variation, as well as determining the importance of variables in each group. Associations among attributes revealed by PCA analysis may represent the genetic linkage between loci controlling traits or a pleiotropic effect (Iezzoni and Pritts1991).

The relatively low phenotypic differentiation among some offsprings in this study indicated higher genetic similarity between parents mainly due to the intervarietal hybridization.

Evidence of heterosis in the progenies

Heterosis is known to be a multigenic complex trait and can be extrapolated as the sum total of many physiological and phenotypic traits (Kumar Baranwal et al., 2012). According to our results, most of the tepal related characteristics were higher in the offspring compared with their parents. These observations could be attributed to the dominant effect of tepal controlling loci. On the other hand, it is most probable that parental plants used in this study were homozygote for tepal-related loci and hybridization masked the expression of recessive alleles from one parent by dominant alleles from the other. Hybrid vigor occurrence was also reported in other iris breeding programs (Arnold et al., 2010; Burke et al., 1998; Zhen et l., 1997). Length and width of tepal are among the important structural parts of iris flower and have been subjected to many breeding programs (Azimi et al., 2012; Jozghasemi et al., 2015). Therefore, it seems that intervarietal hybridization could improve tepal related characteristics in German irises, therefore, it can be suggested for the breeding programs with the aim of increase in the perianth components.

Peduncle dimensions (length and thickness) were also highly variable in the progenies. It is well documented that peduncle thickness influences the stability of iris flower on the stalk; on the other hand, the higher the peduncle thickness is, the more stable the flower on the stalk will be. Peduncle length and thickness are considered among the valuable characteristics of irises and influences not only the flower physiology but also the stability of cut-flower from field to the market (Azimi et al., 2012). Therefore, offsprings NIOP4 and NIOP7, which have significant heterosis over their superior parent are suggested to be used in the breeding programs for improving cut-flower quality in irises. Interestingly, NIOP14 and NIOP15, two offsprings derived from hybridization of P3 × P5, were inferior to their parents and showed significantly negative heterosis for peduncle thickness. The same trend was observed about the leaf width and these two hybrid plants showed negative heterosis than their parents, while offsprings derived from hybridization of P3 × P2 were superior to their parents. These observations indicate the importance of proper parental selection for crossing programs. Important role of parent selection on the heterosis occurrence was also reported in other hybridization programs (Yang et al., 2015).

NIOP4 showed positive heterosis over their parents for leaf width. Increase in the leaf area positively improves photosynthetic activity and enhances the carbohydrate reservoir of the plant which finally increases the shelf life of the flower. It is obvious that increase in the shelf life of cut-flowers provides the possibility of longer distance transportation of flowers (Jozghasemi et al., 2015). Therefore, offsprings NIOP4 and NIOP5 have potential to be further investigated for these purposes.

Diameter of flowering branch was the only traits that all of the progenies from two cross combinations were inferior to their parents and had negative heterosis. Therefore, crossing the iris varieties in this investigation was not successful for improving the diameter of flowering branch and seedlings had tendency to their weaker parents.

Irises having higher crown diameter are suitable to be used as vase plants. NIOP8 was superior to their parents for this trait. Therefore, this hybrid has potential to be used in the breeding programs with the aim of introducing new long vase life flowers. Previous reports indicated that crown diameter in the hybrid plants showed positive heterosis than their parents (Arnold et al., 2010, Burke et al., 1998; Zhen et al., 2003). However, majority of hybrid plants resulted in this study showed negative heterosis for this trait. These contradictory may be attributed to the differences in the parental plants for loci controlling crown diameter.

Bush height was also among the traits that most of the progenies showed positive heterosis than their parents. In accordance to our results, heterosis and wider range of bush height in the first filial was reported in the offsprings of other irises (Burke et al., 1998; Zhen et al., 2003; Arnold et al., 2010). However, significant negative heterosis for this trait is also reported in other ornamental plants (Yang et al., 2015). Although dominance, over-dominance, epistatic interactions as well as epigenetics factors have been conventionally proposed as the main bearings behind the heterosis manifestation, recent studies suggested that the combination of the parental pair has a major effect on the extent of heterosis and overall performance of the progenies (Kumar Baranwal et al., 2012). Bush height is considered as an important trait in the cut-flower industry and along with flower size are two main factors affecting sorting the iris flowers. Our investigation revealed high positive correlation between these two traits. The taller the bush height is, the higher the number and size of the leaves and reproductive organs are. On the other hand, iris flowers having higher bush height will produce high quality flowers. Therefore, some of the resulted progenies, including NIOP13, that had higher positive heterosis, can be suggested for further breeding programs aiming to improve the quality of iris cut flower.

We observed a wide range of color in the hybrid plants. Flower color is predominantly due to the three types of pigments including flavonoids, carotenoids and betalains (Tanaka et al., 2005). Anthocyanin is considered as the main pigment in the iris flower color (Ishikura, 1980; Mizuno et al., 2015). It is well established that the amount and degree of methylation, glycosylation and acylation will affect the color of different anthocyanidins (pelargonidin, cyanidin and delphinidin). In addition to the anthocyanin structure, pH and co-pigments (typically flavones and flavonols) are also affecting the final color of flower. Therefore, the final visible color of a flower is a combination of a number of factors including the type of anthocyanin accumulating, modifications to the anthocyanidin molecule, co-pigmentation and vacuolar pH (Tanaka et al., 2005). A number of genes regulate each of these factors. Genomic turbulence or genomic shock that can be resulted from combination of two distinct genomes may cause the genome-wide relaxation and subsequent changes such as transposon activation that finally can be resulted in the alteration the gene expressions patterns (Ha et al., 2009). Therefore, wider range of color we observed in this investigation may be attributed to the changes in gene expression of different color related factors.

According to our results, blue, purple, violet and white colors were the most frequent flower color in the hybrid plants, while lilac color was the rarest one. Similar to our observations, reports of previous study from inter- and intraspecific crosses of different iris accessions indicated that purple color was the most frequent color in the progenies (Zhen et al., 1997). High variation in flower color we observed in our investigation will provide a good enterprise for iris producers as well as breeders for breeding programs with the aim of producing iris flowers with unique color and represent high potential of intervarietal hybridization for color improvement in German irises.

Conclusion

Two crosses were carried out using three varieties of German irises and 15 offsprings were obtained. The progenies were investigated using different important morphological characteristics. Cluster analysis showed that although most of the progenies were separated from their parents, however, three of them were grouped with their maternal plant. High correlation was observed between different studied attributes that are important for predicting the traits from each other. Most of the evaluated attributes showed positive and negative heterosis and some progenies were superior to their parents. Regarding flower structure attributes such as length and width of inner tepal as well as width of outer tepal, offsprings NIOP5 and NIOP8 had higher values compared to their parents. This observation could be attributed to the dominance nature of the genomic alleles for these traits. A wider range in the most attributes was observed in the progenies, especially for color related characteristics. Several promising hybrid plants were also observed that have potential to be used in different breeding programs with different objectives or directly used in further complementary studies for multiplication and introduction to the ornamental plant markets. The variability of morphological characteristics among offsprings demonstrated that intervarietal hybridization is an effective breeding method in the genetic improvement of German iris. According to our results, it is possible to expand phenotypic variation in the German irises by crossing different varieties.

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