

SCIENTIFIC ARTICLE

Heterosis and genetic diversity in the crossings of gladiolus cultivars Amsterdam and White Prosperity

Mohammad Hossein Azimi^{1*}**Abstract**

Gladiolus is one of the most important and popular cut-flower bulbous in Iran. The aim of this research was to produce new, high-quality hybrids through hybridization. We generated two promising hybrids (OPRC16 and OPRC57) combination from the varieties “Amsterdam” and “White Prosperity”. The Research was conducted in Ornamental Plants Research Center in Mahallat from 2014 to 2018. Values of Hm for traits were defined as the difference between the mid-F1 value and the mid-parent value, Mean Mid-parent Heterosis (MPH) or (Hmp) and High Parent Heterosis (HPH) or (Hsp). The results showed that the morphological analysis revealed the heredity and variation in the promising hybrids. The results for the hybrid OPRC16 showed that the Hm and Hmp values were negative for peduncle length but positive for other traits. The Hsp value was observed to be negative for the traits of peduncle length, leaf width, stem diameter, and cormels diameter, but it was positive for other traits. According to the results for the hybrid OPRC57, the Hm and Hmp values were negative for plant height, floret number, peduncle length, floret diameter, stem diameter, spike length, cormles weight, and cormlet diameter and negative for other traits. Peduncle length was positive in other traits. The Hmp value was negative for peduncle length, but positive for other traits. The Hsp value was recorded to be positive for leaf length, bud diameter, vase life, cormlet number, and cormles yield but negative for other traits. All in all, OPRC57 showed a negative heterosis in most traits. Based on the results, when ‘Amsterdam’ is used as the maternal plant in crosses, it has more positive heterosis effects than when it is used as the paternal parent. These results indicate that major of traits in the phenotypic and genetic diversity coefficient was very low, indicating that they had less environmental effects, since the genotypes were cultivated under similar and controlled conditions.

Keywords: morphological, heterosis, hybridization, diversity.

Resumo**Heterose e diversidade genética nos cruzamentos das cultivares de gladiolo Amsterdam e White Prosperity**

O gladiolo é um dos bulbos de flores de corte mais importantes e populares do Irã. O objetivo desta pesquisa foi produzir novos híbridos de alta qualidade por meio de hibridação. Duas combinações de híbridos promissores (OPRC16 e OPRC57) foram produzidas a partir das variedades “Amsterdam” e “White Prosperity”. A pesquisa foi realizada no Centro de Pesquisa de Plantas Ornamentais em Mahallat de 2014 a 2018. Os valores de Hm para as características foram definidos como a diferença entre o valor médio da F1 e o valor do meio dos pais, Heterose Média dos Pais Média (MPH) ou (Hmp) e Heterose Alta dos Pais (HPH) ou (Hsp). Os resultados mostraram que a análise morfológica revelou a hereditariedade e a variação nos promissores híbridos. Os resultados para o híbrido OPRC16 mostraram que os valores de Hm e Hmp foram negativos para o comprimento do pedúnculo, mas positivos para outras características. Observou-se que o valor de Hsp foi negativo para as características de comprimento do pedúnculo, largura das folhas, diâmetro do caule e diâmetro dos cormos, mas foi positivo para outras características. De acordo com os resultados para o híbrido OPRC57, os valores de Hm e Hmp foram negativos para altura da planta, número de flores, comprimento do pedúnculo, diâmetro da flor, diâmetro do caule, comprimento da haste, peso dos cormos e diâmetro dos cormos, e negativos para outras características. O comprimento do pedúnculo foi positivo em outras características. O valor de Hmp foi negativo para o comprimento do pedúnculo, mas positivo para outras características. O valor de Hsp foi positivo para o comprimento da folha, diâmetro da brotação, vida útil do vaso, número de cormos e rendimento de cormos, mas negativo para outras características. Ao todo, o OPRC57 mostrou heterose negativa para a maioria das características. Com base nos resultados, quando ‘Amsterdam’ foi utilizada como planta materna em cruzamentos, houve mais efeitos positivos de heterose do que quando foi utilizada como matriz paterna. Esses resultados indicam que a maioria das características do coeficiente de diversidade fenotípica e genética foi muito baixa, indicando que tiveram menos efeitos ambientais, uma vez que os genótipos foram cultivados em condições semelhantes e controladas.

Palavras-chave: morfologia, heterose, hibridação, diversidade.

¹Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran. *Corresponding author: m.h.azimi58@gmail.com, mh.azimi@areeo.ac.ir

Received Sep 29, 2019 | Accepted Apr 20, 2020 | Available online May 6, 2020

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<https://doi.org/10.1590/2447-536X.v26i2.2095>

Area Editor: Gilmar Schafer

Introduction

Gladiolus is the next most popular cut-flower after rose and chrysanthemum in Iran. It is being grown over an area of 350 ha and centers of production are in pakdasht, varamin, karaj, damavand, mahallat, dezful and jiroft (Ministry of Agriculture Jihad, 2015). Gladiolus (*Gladiolus grandiflora* Hort.) belongs to *Iridaceae* family (Ranjan et al., 2010), and is usually called as the queen of bulbous flowers (Randhawa and Mukhopadhyay, 2000). So, *Gladiolus* popularly known as Sword lily or Corn Lily and originated from South Africa (Poon et al., 2012).

The genus of *Gladiolus* contains over 276 species throughout the world, but they are mostly native to the western, southern, and eastern parts of Africa although 12 species have been originated from the Mediterranean regions (Rina & Hiroshi, 2016). In Iran it is one of the main bulbous cut flowers and also in the world which has an important role in exporting as cut flowers (Azimi, 2020b).

Evaluations on genetic association are useful to ascertain the important component trait on which choice can be made (Choudhary et al., 2011). Several studies have been conducted on gladiolus: phenology (Schwab et al., 2015); estimation of genetic variability (Azimi, 2020b, Rashmi and Kumar, 2014); heritability and genetic advance (Patra and Mohanty, 2014); genotypic and phenotypic variability (Pattanaik et al., 2015, Bhujbal et al., 2013); and hybridization (Ohri and Khoshoo, 1983a; 1983b; Hossain et al., 2012; Azimi, 2019). In gladiolus, new varieties are evolved through hybridization which is recognized as the most important sources of evolution and crop improvement. Crossing different plant varieties aims to generate wider genetic variation and to use heterosis of hybrid progenies (Joshi et al., 2001). Interspecific hybridization is a commonly used method in different close related species for producing off-springs with new characteristics. Among the three different methods of pollination of gladiolus cultivars via natural self-pollination, artificial self-pollination and open pollination, artificial self-pollination generally gave the best results (Dhaduk et al., 1987). Inter-varietal hybridization is another common way for transferring desirable attributes between different cultivars and producing progenies with new characteristics (Azimi et al., 2018; Yang et al., 2015; Zamani et al., 2010). Azimi (2020a, b) and Azimi and Banijamali (2019) indicated significant differences between gladiolus genotypes for morphological traits. The hybrid plants had the highest variability, making them the most indicated for future improvement programs. Diallel analysis provide the greatly influences genetic information for breeding programs (Fan et al., 2014). Reduced natural cross-pollination may have resulted from a lack of pollinating insects (Ohri and Khoshoo, 1981).

Moreover, participation of inhibitory specificities of pollen cholinesterase in the pollen-stigma interaction failed to obtain matured seed from the crosses (Semenova and Roshchina, 1993). Presence of dry type of gladiolus stigma may be another reason for their incompatibility (Clarke et al., 1977). Gladiolus varieties are good general combiners

for many traits and additive type of gene action has been noticed for many traits (Kumar et al., 2008). Corms and cormels are the propagating materials for gladiolus. Cormles grow in between mother and daughter corms (Larson, 1992). Gladiolus is largely propagated by corms and cormles whereas seeds propagation is followed to evolve new cultivars and for the recovery and maintenance of the threatened germplasm. Moreover, seed production is higher than cormles production in gladiolus (Gonzalez et al., 2003). Although, plants raised from seeds require four seasons to come to bloom under ordinary conditions but under best cultural treatments, it may be reduced to two seasons (Bose et al., 2003) and plants produced from seeds can flower in the second year (Cohat, 1993). Environmental factor in combination with genetic and physiological factors play an important role in determination of plant potential for propagating material. These characters appear to be under strong genetic control (Sukarin et al., 1987; Roy et al., 2004). The decision on the tip-top improvement breeding, on the appropriate selection intensity, and on the characteristics to be considered in the selection is based on the knowledge of the genetic structure of the population (Singh et al. 2018).

The key for any success of any genetic breeding program lies in the availability genetic variability for desired traits (Heller, 1996). Wide hybridization enables the interspecific gene transfer, which may lead to the additional source of variation for the desirable characters (Anandhi et al., 2013) and Takatsu et al., (2001) made interspecific hybridization between a modern cultivar of *Gladiolus grandiflora* Hort. ($2n=60$) and the wild species *G. tristis* L. ($2n=30$) to introduce characteristics of the wild species in to the cultivated one and reported best pollen tube growth, fertility, and fruit set in their cross at low air temperatures for F1 hybrid plants production (Takatsu et al., 2001). Hossain et al. (2012) indicate the existence of wide variability among the gladiolus genotypes considering crossing parameters and variations were observed for seed. Significant differences in all traits and a wide variation in progenies observed for all traits among the gladiolus genotypes in F1 seed (Azimi, 2019).

To keep up flower diversity according to the new consumer demands in the ornamental plant industry, plant breeders need to create a diversification and to produce new flowers having new characteristics. Therefore, two popular cultivars of *Gladiolus* were hybridized in order to produce new hybrids with probable potential of commercial importance in the ornamental industry. This is the first investigation of *Gladiolus* hybridization in Iran and can open new horizon for designing new breeding programs between commercial and wild *Gladiolus* in future.

Material and Methods

Plant material

The two varieties of gladiolus include 'Amsterdam' (P1) and 'White prosperity' (P2) were used for hybridization. It was done in the ornamental plants research center (OPRC), Mahallat, Iran (2014-2018).

Hybridization program

After spike emergence of gladiolus varieties, a full diallele crossing was carried out among the two varieties to study seed setting in cross combinations and the combining ability of seed characters. In the female parent, flowers at pre-anthesis stage were selected for emasculation. Emasculation was carried out between early evenings and bagged with butter paper cover. Similarly, in the male parents, a few selected flower buds at pre-anthesis stage were bagged without emasculation to avoid contamination by foreign pollen for collection of pollen grains. Pollen from bagged flowers of pollen parents were collected between morning and Sundown and dusted on the stigma of emasculated flowers of the respective female parents. The flowers were bagged with butter paper and then labelled. The covers were removed after ensuring proper pod set. The F1 pods were harvested at full physiological maturity, when the capsules start to burst. Each individual cross was harvest by hand.

Seedbed preparation, sowing and produce cormles

All seeds of parental crosses (300 seeds from each hybrid) were planted on well prepared and raised seedbeds to produce seedlings. Before sowing in the trays, the seeds are rubbed between two layers of cloth to remove the waxy covering and finally shake for 6 hours. It has been reported that the waxy covering contains some substances that retard the germination process. Seeds are sown in 1.5-2.0 cm deep in trays. The seeds planted in January in the tray with contents 30% perlite and 70% coco peat in the greenhouse at temperature of 23 ± 4 °C and humidity $65\% \pm 5\%$, the moisture content of the bed must be maintained. Hundred seeds were planted for each replication. The seedlings matured in 125 days in greenhouse condition and were harvested cormles. Characteristic and pictures (Fig 1) of all steps are in the Table 1. Among the populations created, two hybrids were selected and propagated. The total entries were therefore four genotypes (parents and superior crosses: OPRC16 and OPRC57) chosen for evaluation.

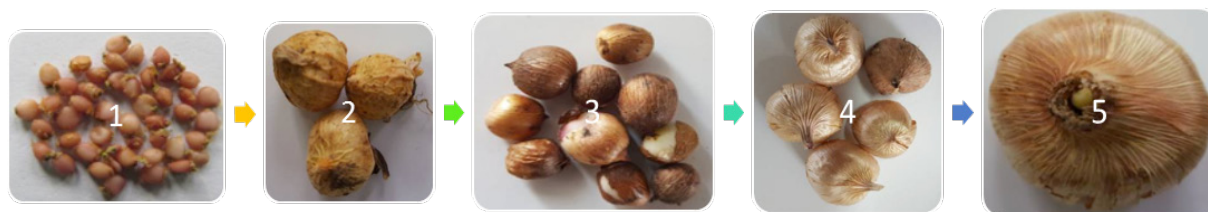


Figure 1. Seed alteration to corm in during growth.

Table 1. Seed alteration to Corm with applied information (2014-2018)

Steps	Description	Yield (number)	Mid-Rest period (day)	Growth period (day)	Mid-Weight (g)	Diameter (mm)
1- seed	125 days after planting becomes mini-corm	10 to 15 seeds per capsule are formed	-	35 days after the cross can be harvest	0.08 g (10 seed)	-
2- Cormlet-1	no flowering ability	3 mini-corm	30.00	110.00 ±10	0.22	7.00 ± 4.36
3- Cormlet-2	no flowering ability	3 mini-corm and 5 small cormlets	35.00	120.00± 20.00	1.33	0.74± 10
4- Corm-1 (Commercial corm)	flowering ability	7 mini-corm, 3 small, and 2 medium cormlets	35.00	90.00± 10.00	7.46	27.00 ±18.00
5- Corm-2 (Commercial corm)	flowering ability	10 mini-corm, 7 small, and 5 medium cormlets	35.00	85.00 ± 15.00	28.44	46.54 ± 32.00

Data collection

Hybrids and parents were cultivated based on completely randomized block design with three repetitions. At flowering period in greenhouse condition (February, 2017), 28 morphometric traits were recorded based to the distinctness, uniformity and stability (DUS) test, according on International Union for the Protection of New Varieties of Plants (UPOV, 2013) instructions. Ornamental traits were measured using digital ruler and caliper, and flower color was recorded according to the standard RHS color chart (the royal horticulture society, London).

Color measurements were performed on digital images and analyzed by using color tester software version 3 (Strecker et al., 2010). Indices of 'a', 'b' and 'L' indexes were measured and ΔE was calculated as follows:

$$\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2}$$

Where: *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. Descriptive statistics including mean, minimum, maximum values as well as coefficient of variation (CV%) were calculated for all traits. Heterosis is compute as mid-parent heterosis or (Hm) (Li and Wu, 1997). Values of Hm for traits was defined as the difference between the mid-F1 value (mean value of that trait in F1 populations) or (Fm) and the mid-parent value (MPV), that is, $Hm = Fm - MPV$; MPV is the mean value of that trait in the two parents (Yang et al., 2015). Percentage of heterosis (percent) was calculated

according to Hallauer et al. (1988). Below formulas were used to compute relative heterosis in comparison with average of parents (a) and superior parent (b):

a) Mean Mid-parent Heterosis (MPH) or (Hmp) = (Mean Hybrid value - Mean parent value / Mean parent value) $\times 100$

b) High Parent Heterosis (HPH) or (Hsp) = (Mean Hybrid value - High parent value / High parent value) $\times 100$

Statistical analysis

This study randomized complete block design with three replications was conducted. Statistical analysis included descriptive statistics, analysis of variance, and mean comparison with Duncan's multiple range tests. All statistical analysis was performed with SAS 9.1 software.

Results and Discussion

Analysis of variance and descriptive statistics of quantitative traits

The comparison of the traits between the promising hybrid OPRC16 and the parents showed that they differed significantly for all traits except for leaf width, spike length, vase life, and cormles diameter. The coefficient of variations (CV) between the traits ranged from 0.00 to 12.20 percent. The highest CV was related to cormlet weight and the lowest to vase life (Table 2).

Concerning the traits of the promising hybrid OPRC57 and the parents, significant differences were observed in all traits unless vase life. The CV was in the range of 0.00-30.69 percent. The highest and lowest CVs were obtained for spike length and vase life, respectively (Table 3). Traits with higher CV exhibit a wider range of quantity, which is regarded as a wider range of selection for that trait.

Table 2. Genetic characteristics of ornamental traits in *Gladiolus grandiflorus* ‘Amsterdam’ (p1: ♀), *Gladiolus grandiflorus* ‘White Prosperity’ (p2: ♂) and their F1 hybrid

Ornamental traits	Parent plants			Hybrid F1 (oprc16)							Parent and F1
	♂	♀	MPV	mid-F1 value	SD	CV (%)	Extreme value	Hm	Hmp	Hsp	Mean Square
Plant height (cm)	128.00	143.20	135.61	193.0	9.65	5.00	183.40-202.70	57.40	42.32	34.78	35.05*
Num. of florets per spike	12.10	16.50	14.31	19.0	0.95	4.99	18.10-20.00	4.70	32.77	15.15	0.36*
Peduncle length (cm)	19.94	20.53	20.24	17.00	0.85	4.99	16.20-17.90	-3.20	-16.01	-17.19	0.94*
Floret width (cm)	8.39	9.43	8.91	12.00	0.60	5.00	11.40-12.60	3.10	34.68	27.21	0.10*
Floret length (cm)	6.59	7.82	7.20	9.00	0.45	5.00	8.55-9.45	1.80	25.00	15.16	0.17**
Leaf width (cm)	2.85	4.71	3.78	4.20	0.21	5.00	3.99-4.41	0.40	11.11	-10.76	0.01 ^{ns}
Leaf length (cm)	51.30	59.30	55.30	79.00	3.95	5.00	75.10-83.00	23.70	42.86	33.21	23.66**
Stem diameter (mm)	10.67	18.08	14.38	11.80	0.60	5.08	11.20-12.40	-2.60	-17.94	-34.74	0.69**
Spike length (cm)	42.47	52.25	47.36	71.00	3.55	5.00	67.45-74.55	23.60	49.92	35.89	0.16 ^{ns}
Bud diameter (mm)	8.07	7.67	7.87	10.44	0.52	4.98	9.92-10.96	2.60	32.66	36.17	0.37*
Vase life (day)	7.00	9.00	8.00	11.00	0.00	0.00	11.00-11.00	3.00	37.50	22.22	0.00
Corm weight (g)	20.11	46.50	33.30	88.21	0.52	0.59	87.61-88.51	54.90	164.89	89.71	11.13**
Corm diameter (mm)	37.26	58.15	47.71	84.76	1.10	1.30	83.66-85.86	37.10	77.66	45.75	56.44**
Num. of cormlets per plant	19.67	50.00	34.83	59.00	1.50	2.54	57.50-60.50	24.20	69.39	18.00	76.36*
Cormlet weight (g)	0.23	0.27	0.25	0.41	0.05	12.20	0.36-0.46	0.20	65.33	51.22	0.00
Cormlet diameter (mm)	5.76	11.02	8.39	8.46	0.69	8.12	7.98-9.25	0.10	0.87	-23.22	0.62 ^{ns}
Cormlet yield per plant (g)	7.10	8.47	7.79	24.39	0.20	0.82	24.19-24.59	16.60	213.05	187.80	0.05**
Corm and cormlet yield per plant (g)	27.20	55.00	41.09	112.6	0.36	0.32	112.20-112.90	71.50	174.02	104.83	11.80**

* and ** indicate a significant difference at the 0.05 and 0.01 level, respectively, Hm= The difference between mean values in F1 and mid-parents value (MPV), Hmp or MPH = (Mean Hybrid value - Mean parent value / Mean parent value) × 100, Hsp or HPH = (Mean Hybrid value – High parent value / High parent value) × 100

Table 3. Genetic characteristics of ornamental traits in *Gladiolus grandiflorus* 'White Prosperity' (p2: ♀), *Gladiolus grandiflorus* 'Amsterdam' (p1: ♂) and their F1 hybrid

Ornamental traits	Parent plants			Hybrid F1 (oprc57)							Parent and F1
	♀	♂	MPV	mid-F1 value	SD	CV (%)	Extreme value	Hm	Hmp	Hsp	Mean Square
Plant height (cm)	128.0	143.2	135.61	135.0	6.75	5.00	128.30-141.80	-0.61	-0.45	-5.72	19.68**
Num. of florets per spike	12.10	16.5	14.31	12.7	2.91	22.92	10.51-16.00	-1.61	-11.48	-23.23	4.77**
Peduncle length (cm)	19.94	20.53	20.24	15.67	4.64	29.53	12.4-21.00	-4.57	-22.60	-23.68	3.25*
Floret width (cm)	8.39	9.43	8.91	8.33	1.49	17.85	7.13-10.00	-0.58	-6.47	-11.66	0.70**
Floret length (cm)	6.59	7.82	7.20	7.33	0.68	9.21	6.65-8.00	0.13	1.85	-6.16	0.28**
Leaf width (cm)	2.85	4.71	3.78	3.87	0.83	21.36	3.25-4.80	0.09	2.29	-17.85	0.32**
Leaf length (cm)	51.30	59.30	55.30	62.33	16.35	26.22	50.40-81.00	7.03	12.72	5.11	116.23**
Stem diameter (mm)	10.67	18.08	14.38	8.26	2.99	36.22	6.20-11.70	-6.12	-42.56	-54.32	1.43**
Spike length (cm)	42.47	52.25	47.36	43.67	13.40	30.69	34.20-59.00	-3.69	-7.80	-16.43	64.09**
Bud diameter (mm)	8.07	7.67	7.87	8.54	1.09	12.79	7.55-9.71	0.67	8.47	11.35	1.07**
Vase life (day)	7.00	9.00	8.00	9.00	0.00	00.00	9.00-9.00	1.00	12.50	0.00	0.00
Corm weight (g)	20.11	46.50	33.30	40.57	0.51	1.25	39.99-40.89	7.27	21.84	-12.74	11.02**
Corm diameter (mm)	37.26	58.15	47.71	53.43	1.10	2.06	52.33-54.53	5.72	11.99	-8.12	56.44**
Num. of cormlets per plant	19.67	50.00	34.83	57.00	1.50	2.63	55.50-58.50	22.17	63.65	14.00	76.36*
Cormlet weight (g)	0.23	0.27	0.25	0.17	0.05	29.41	0.12-0.22	-0.08	-32.00	-56.55	0.007**
Cormlet diameter (mm)	5.76	11.02	8.39	4.79	0.66	13.74	4.07-5.36	-3.60	-42.91	14.36	0.97*
Cormlet yield per plant (g)	7.10	8.47	7.79	9.69	0.20	2.06	9.49-9.89	1.90	24.39	-8.56	0.05**
Corm and cormlet yield per plant (g)	27.20	55.0	41.09	50.30	0.34	0.68	49.88-50.53	9.21	22.32		11.69**

* and ** indicate a significant difference at the 0.05 and 0.01 level, respectively, Hm= The difference between mean values in F1 and mid-parents value (MPV), Hmp or MPH = (Mean Hybrid value - Mean parent value / Mean parent value) × 100, Hsp or HPH = (Mean Hybrid value – High parent value / High parent value) × 100

Assessment of heritability and heterosis in traits

The results revealed that the promising hybrid OPRC16 produced taller plants (193.00 cm) than its parents (135.61 cm). The difference between OPRC16 and the mid-parent value in this trait was positive ($H_m = 57.40\%$) and it exhibited a positive heterosis ($H_{sp} = 34.78\%$) over parents and superior parent ('Amsterdam') (Table 2). OPRC57 had lower plant height than the parents. The difference in this trait between the hybrid and the mid-parent value was negative ($H_m = -0.61$). A negative heterosis ($H_{sp} = -5.72\%$) was observed over the parents ($H_{mp} = -0.45$) and the superior parent ('Amsterdam') (Table 3). The hybrid OPRC16, which showed a high positive heterosis over the superior parent and parents (maternal parent 'Amsterdam' and paternal parent 'White Prosperity'), can be used in breeding and quality improvement programs of gladiolus. A negative heterosis was observed for plant height in OPRC57 over the parents and the superior parent. These results imply that the diversity of plant height is mostly rooted in genetic factors, but it is less influenced by the environment.

The height of the flowering stem is another important trait for the marketability of cut flowers. Thus, this finding may imply that the higher the plant height is, the larger the dimension and number of leaf and other reproductive parts will be, and this will contribute to producing flowers with higher quality. Stem length and flowering branch length are invaluable features of the visual structure of gladiolus that improve the resistance of plants to the transport from farms to markets in addition to influencing their physiological traits. In the present study, the cormlets used were in the same size, so the difference observed in plant height of the gladiolus cultivars may arise from genetic structure and environmental factors, which is consistent with the findings of Hossain et al. (2012). Thus, selection for this trait can be effective. The difference in plant height may be related to their competition for light, moisture, space, nutrients, and aeration (Karavadi and Dhaduk, 2002). The increase in corm size of gladiolus resulted in higher plant height and flowering spike length. Similar results have been reported by Bijimol and Singh (2001) and Moradi (2013) for tuberoses.

The promising hybrid OPRC16 had more florets than the parents. The difference in this trait between the hybrid and the parents was positive ($H_m = 4.70$). This hybrid had a positive heterosis over the parents and the superior parent ('Amsterdam') (Table 2). The number of florets per spike was fewer in OPRC57 than in the parents. The results revealed a negative difference ($H_m = -1.61$) in this trait between OPRC57 and the parents (Table 3). The heterosis was negative over both the parents ($H_{mp} = -11.48\%$) and the superior parent 'Amsterdam' ($H_{sp} = -23.23\%$). OPRC16 showed a high positive heterosis over the superior parent and the parents in terms of floret number whereas the heterosis of OPRC57 over the parents and the superior parent was negative in this trait. These results imply that the diversity of floret number is mainly related to genetic factors, and environmental factors are involved in this trait

to a less extent. Floret number is a key economic trait, so it is important to introduce commercial cultivars with higher number of florets (Azimi, 2020a).

For the trait of peduncle length, the parents outperformed the promising hybrids OPRC16 and OPRC57 (Table 2 and 3). Based on the results, the difference between the mean of the hybrids and the mid-parent value in this trait was negative. Heterosis was negative over the parent and the superior parent 'Amsterdam' (Tables 2 and 3).

The promising hybrid OPRC16 had higher floret width and length than the parents with the difference between the hybrid and the mid-parent value being positive for these traits. This hybrid exhibited a positive heterosis over the parents and the superior parent 'Amsterdam' (Table 2). The promising hybrid OPRC57 showed lower floret width and length than the parents. The results revealed that the difference between hybrid and the mid-parent value in these traits were ($H_m = -0.58$ and 0.13), respectively. For the trait of floret width, the heterosis over the parents and the superior parent ('Amsterdam') was negative (Table 3).

For the trait of floret length, heterosis was 1.85% over the mid-parent value and -6.16% over the superior parent ('Amsterdam') (Table 3). The heterosis points to the relative superiority of F1 hybrids over the mid-parent value or the superior parent for this trait. Mousavi Bazzaz et al. (2007) reported the moderate superiority of hybrids for the traits of double flowers and flower size in stocks. According to the study of Arnold et al. (2010) on iris, the hybrids were superior to the parents in traits and the study of Azimi et al. (2018) on German iris revealed the superiority of some hybrids to the parents. OPRC16 outperformed its parents in flower size. Flower size (floret width and length) is a major economic trait. Larger flowers increase flower sale in domestic and international markets and enhance the economic income as they give the plants more beauty and attractiveness (Azimi et al., 2012). This hybrid showed a positive heterosis in terms of flower size, so it can be included in breeding programs. Obviously, the introduction of commercial cultivars can contribute to the prosperity of flower industries of Iran by increasing the diversity in flower markets.

The results indicated that the hybrids OPRC16 and OPRC57 outperformed their parents in leaf width and length. The difference in these traits between the average of the promising hybrids and the mid-parent value was positive (Tables 2 and 3). The heterosis of leaf width in OPRC16 and OPRC57 was positive over the parents but it was -10.76 and -17.85 over the superior parent ('Amsterdam'), respectively (Tables 2 and 3) whilst it was positive over both parents and superior parent for the trait of leaf length (Tables 2 and 3). The promising hybrids exhibited a significant difference from their parents. Higher leaf area means higher photosynthesis, which increases carbohydrate accumulation. Furthermore, it influences the physiological traits of the flower and affects flower and cormlet yield of gladiolus. Cultivars that have higher leaf area show better quantitative and qualitative traits (Azimi and Banijamali, 2019).

Based on the results, the differences of the average of OPRC16 and OPRC57 with the mid-parent value were -2.6 and -6.12 for this trait, respectively (Tables 2 and 3). The heterosis of stem diameter was negative for both hybrids as compared to their parents and the superior parent (Tables 2 and 3). Stem diameter (thickness) is a decisive factor dictating cut flower quality because not only does it determine stem strength, but it is also important for the vase life of cut flowers (Turkoglu et al., 2008). Flowering stem diameter is an invaluable feature of cut flowers so that it augments the resistance of plants during their transport from farms to markets (Azimi et al., 2012). In this respect, Moradi and Azimi (2017) evaluated the yield of different gladiolus cultivars and did not find any statistically significant differences in this trait among the cultivars. Likewise, Daneshvar and Heidary (2009) reported that planting type and inter-cormlet spacing had no significant effect on stem diameter of gladiolus.

The results revealed that the promising hybrid OPRC16 had higher spike length (71.00 cm) than its parents (47.36 cm). The difference in this trait between OPRC16 and the mid-parent value was positive (Hm= 23.60). So, it showed a positive heterosis over its parents and the superior parent (Table 2). However, OPRC57 produced shorter spikes than its parents. Indeed, the difference in this trait between this hybrid and the mid-parent value was negative (Hm= -3.69), indicating a negative heterosis over its parents and the superior parent (Table 3). The promising hybrid OPRC16 was superior to its parents in terms of spike length. Spike length is an economic trait, so it is important to introduce commercial cultivars with taller spikes (Chanda et al., 2000). As well, spike length is significantly increased by nutrient reserves of corms (Uddin et al., 2002; Bhat et al., 2009; Memon et al., 2009; Kareem et al., 2013) and the increase in planting spacing (Sharma and Goupa, 2003) due to the lower competition of the plants on water, minerals, nutrients, and radiation (Mojiri and Arzani, 2003). These results imply that the diversity in spike length is mainly related to genetic factors, and the environment (greenhouse conditions) is less involved in this trait.

The promising hybrids OPRC16 and OPRC57 had higher bud diameter than their parents, showing a positive difference from the mid-parent value. They had a positive heterosis over their parents and the superior parent in this trait (Tables 2 and 3). The hybrids differed from their parents significantly.

Based on the results, the promising hybrid OPRC16 had a significantly longer vase life (11.00 days) than their parents (8.00 days) and the difference between OPRC16 and the mid-parent value was positive (3.00 days) in this trait. It had a positive heterosis over its parents and the superior parent (Table 2). The vase life of the promising hybrid OPRC57 was one day longer than that of its parents. The difference between this hybrid and the mid-parent value was positive, but it did not differ from the superior parent (Table 3). Since a major goal in cut flower production is to produce larger flowers with higher stem strength, it has been suggested that the strength and

thickness of flowers under the natural conditions plays a crucial role in their vase life (Daneshkhah et al., 2007). Longer vase life allows their transport to more remote export destinations. Thus, these hybrids can be used as candidate commercial cultivars in flower markets. Given the commercial and economic importance of cut flowers, it is critical to introduce cultivars with longer vase life. Vase life is influenced by preharvest and postharvest factors. The quality and vase life of cut flowers depend on growing conditions and their postharvest handling (Azimi and Banijamali, 2019).

OPRC16 and OPRC57 outperformed their parents in corm weight and diameter. A positive difference was observed in these traits between the hybrids and the mid-parent values (Tables 2 and 3). The heterosis of corm weight and diameter was 164.89 and 77.66% for the hybrid OPRC16 over its parents and 89.71 and 45.75% over the superior parent, respectively (Table 2). The heterosis of these traits in OPRC57 was positive over the parents but negative over the superior parent (Tables 2 and 3). OPRC16 and OPRC57 differed from their parents significantly.

In cormlet number, OPRC16 and OPRC57 performed better than their parents did so that their difference from the mid-parent value was positive in this trait. They showed a positive heterosis over their parents and the superior parent (Tables 2 and 3). The range of cormlet variations was 57.50-60.50 and 55.50-58.50 for OPRC16 and OPRC57, respectively. Sanjai and Singh (2000) reported that cormlet number varied from 18.7 to 55.5 in gladiolus cultivars and that 'White Prosperity' had the highest number of cormlets (50.2 cormlets). Propagation rate (cormlet number) in commercial cultivars of gladiolus is an important parameter to be considered.

The hybrid OPRC16 outperformed its parents in cormlet weight and diameter. It showed a positive heterosis over its parents and a negative heterosis (Hsp= -23.22%) over the superior parent ('Amsterdam') in terms of these two traits (Table 2). OPRC57 was not superior to its parents in cormlet weight and diameter. Its heterosis over its parents and the superior parent was negative (Table 3).

Corm and cormlet yield per plant were higher in OPRC16 and OPRC57 than in their parents. The difference between OPRC16 and the mid-parent value was strongly positive in these two traits. The heterosis of this hybrid for corm and cormlet yield per plant was 213.05 and 174.02% over the parents and 187.80 and 104.83% over the superior parent, respectively (Table 2). The heterosis of this hybrid in corm weight and diameter was positive over its parents but negative (Hsp= -23.22%) over the superior parent ('Amsterdam') (Table 2). OPRC57 exhibited higher corm and cormlet yield per plant than its parents. The heterosis values for these traits were 24.39 and 22.32% over the parents and 14.36 and -8.56% over the superior parent, respectively (Table 3).

Analysis of qualitative traits

The flower color was white in parents but it was light coral and light pink in OPRC16 and OPRC57 (Table 4).

Table 4. Ornamental traits in *Gladiolus grandiflorus* cv. ‘Amsterdam’ (p1), *Gladiolus grandiflorus* ‘White Prosperity’ (p2) and their F1 hybrids

Ornamental traits	Parents		Hybrid F1	
	Amsterdam	White prosperity	oprc16	Oprc57
Flower color	White	White	Light coral	Light pink
Perianth tube: distribution of color spots in the bottleneck	-	Interrupted band	Continuous band	-
Spike: arrangement of flowers (one row, zig-zag, two rows, irregular)	One row	Irregular	One row	Irregular
Flower: shape in front view (triangular, star-shaped, round)	Star-shaped	Star-shaped	Star-shaped	Star-shaped
Flower: attitude (upright, semi-upright, horizontal)	Upright	Upright	Upright	Upright
Outer tepal: shape of blade (ovate, Elliptic, obovate)	Elliptic	Obovate	Obovate	Obovate
Inner tepal: shape of macule (type 1, type 2, type 3, type 4)	Type 3 (the base)	Type 3 (the base)	Type 3 (the base)	Type 3 (the base)
Inner tepal: border of marginal zone (slightly, irregular, moderately irregular, very irregular)	Moderately irregular	Moderately irregular	Slightly	Slightly
Median inner tepal: attitude (semi-erect, semi-erect to horizontal, horizontal)	Semi-erect	Semi-erect	Semi-erect to horizontal	Semi-erect
Median inner tepal: attitude of apex (moderately recurved, Straight, moderately reflexed, strongly reflexed)	Straight	Straight	Moderately reflexed	Straight

The distribution of floret color in the bottleneck was in the form of red veins in ‘White Prosperity’ and the hybrids. The floret arrangement on spike was one-row in ‘Amsterdam’ and OPRC16 but irregular in ‘White Prosperity’ and OPRC57. The florets in the parents and hybrids were star-shaped. The petals were semi-erect in the parents and OPRC57 and semi-erect to horizontal in OPRC16. The form of floret standing on the spike was semi-upright in OPRC16 but upright in others. The shape of blade in the outer petal was elliptic in ‘Amsterdam’ and obovate in others. The form of color spots in inner petals was at the base or bottleneck in all parents and hybrids. The amount of wrinkles at the margins of inner petals was moderately irregular in the parents and lowly

irregular in the hybrids. The form of the apex of the inner petal was straight in the parents and OPRC57 and moderately reflexed in OPRC16.

Flower color analysis

Flower color is one of the most appealing features of ornamental plants from the customers’ and economic perspectives. A major goal of ornamental flowers and plants breeding programs is to manipulate flower color. In this work, flower color was measured by the digital imaging method using the color measurement software. The highest and lowest color variations were observed in OPRC16 and OPRC57 (76.36-87.16), respectively (Table 5). The lowest color variation level (82.68-86.16) was observed in the parents (Table 5).

Table 5. Color measurements of parents and their F1 hybrid performed on digital images and analyzed by using color tester software

Color parameters	Parent		Hybrid F1	
	Amsterdam	White Prosperity	oprc16	Oprc57
Average Red	213.20	203.48	211.18	223.11
Average Green	214.27	202.37	102.26	215.89
Average Blue	201.49	180.85	80.95	207.65
Average Luminosity	195.81	181.53	137.47	202.83
Average L Value	85.24	80.94	56.56	86.57
Average a Value	-6.91	-7.38	37.15	-3.40
Average b Value	10.69	15.20	35.38	9.60
Average Hue	122.87	115.89	43.59	109.52
Average Chroma	12.73	16.89	51.31	10.18
ΔE	86.16	82.68	76.36	87.16

where: *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.

The assessment of color tendency of flower from green to red (*a*) showed that the hybrid OPRC16 had the highest color tendency to red and 'White Prosperity' had the highest color tendency index to dark green. Based on the results for the tendency index of flower colors to blue-yellow (*b*), the hybrid OPRC16 had the highest color tendency to yellow and the promising hybrid OPRC57 had the highest tendency to blue (average blue = 207.65) (Table 5). The highest color luminosity (average L of 202.83) was obtained from OPRC57. In a study on German iris, Azimi et al. (2017) found the highest heritability for the colors purple, violet, and white. Huang Su *et al.* (1998)'s study on iris revealed that the flower color inheritability was for a combination of purple.

Conclusions

Based on the results, when 'Amsterdam' is used as the maternal plant in crosses, it has more positive heterosis effects than when it is used as the paternal parent. These results indicate that major of traits in the phenotypic and genetic diversity coefficient was very low, indicating that they had less environmental effects, since the genotypes were cultivated under similar and controlled conditions. Most traits that were found to have high heritability are important in improving plant quality and quantity of Gladiolus. Promising hybrid OPRC57 showed a negative heterosis in most traits. Consequently, inter-varietal hybridization is an effective way to contribute to the phenotypic variations of Gladiolus, produce new plant materials for breeding purposes, and release new cultivars.

Acknowledgements

The financial support of Ornamental Plants Research Center (OPRC) is gratefully acknowledged.

Author contribution

M.H.A.⁰⁰⁰⁰⁻⁰⁰⁰³⁻¹⁵³³⁻¹⁸⁷⁶: designing the experiments, obtaining and analyzing data and writing the scientific article.

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