



## Intervarietal Hybridization and Observation of High-quality Offspring of Cut Freesia Flowers

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### ABSTRACT

*Freesia* belongs to the family *Iridaceae* and is one of the most popular bulbous species that can be used both as a cut flower and as a pot flower, depending on the situation. This study was carried out to produce new, high-quality offspring by intervarietal hybridization between five commercial cultivars of *freesia hybrids*. Eighteen morphometric traits, which were mostly related to flower organs, were evaluated on twelve progenies and five parental plants in a randomized complete block design (RCBD), with three replications (2014-2018). The assessments were based on instructions of the International Union for the Protection of New Varieties of Plants (UPOV). The highest heritability of broad-sense traits was related to the outer segmental width of the perianth and the inner segmental width and length of the perianth. The lowest heritability was related to the length of stem between the first and second flowers. The number of flowers and buds had the most significant, positive correlation with spike length ( $r=+0.75$ ). Likewise, the strongest positive, significant correlation was observed between the length of the inner and outer segments of the perianth ( $r=+0.79$ ). Superior progenies consisted of the 20A hybrid, with a white color, which originated from the parental cross P1×P5. The 3A hybrid, with a yellow color, was obtained from the parental cross P2×P5, and the 28P hybrid, with a reddish brown color, was obtained from the parental cross P4×P5. These progenies were superior in terms of important traits such as plant height, branch number of the peduncle, spike length, number of buds and florets, compared to the parents. Thus, intervarietal hybridization in this research was proved as an effective way to generate new phenotypic variations of *freesia hybrids*, thereby providing opportunities to produce new plant materials for breeding purposes and release new cultivars.

### Introduction

The history of cultivating freesias dates back to 200 years ago (De Hertogh and Le Nard, 1993; Anderson, 2007), although it is historically less known for its cultivation compared to other economic ornamentals (Bryan, 2002). Freesia is a

bulbous flowering species that is popular in European and American countries due to its aromatic property, long postharvest life, and color variation (Anderson, 2007). Also, owing to its low weight and easy transportation, it is optimally commercialized in the flower markets of European countries (Deyaa, 2012). The low color

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variation in freesias, however; previously limited its marketing development. Following the production of new cultivars with high color diversity, freesias became more important in the world of ornamental plants (Deyaa, 2012).

Freesia is a monocotyledon from the family Iridaceae and the subfamily *Ixioideae* which contains 11 species. The new name of cut freesia is *Freesia hybrids* (Wang, 2007), and its propagation is done by corm, cormlet, seed, and tissue culture (Azimi et al., 2015). The Mediterranean climate areas are appropriate for open-air cultivation of *Freesia hybrid* (Bryan, 2002; Anderson, 2007). The most common freesia varieties are fragrant and they are used to extract essential oils (Wang, 2007). Recently, freesia varieties that can be grown in pots have attracted common interest among consumers (Ehrich et al., 2009). On the other hand, the selection of superior varieties with good cut-flower characteristics is usually considered for commercial production. Factors that should be considered in breeding ornamental plants include flower form, color, aroma, pest and disease resistance, as well as tolerance to abiotic stresses (Benschop et al., 2010). More than 340 varieties of freesia have been registered on the international list of hyacinths and other bulbous plants (Van Scheepen, 1991). Freesia is essentially a self-sterile plant, meaning that the progeny or fertilized parents are partially different from each other. Several cultivars of the F1 hybrids with doubled-flowered forms have been generated and different ploidy levels like diploids, triploids and tetraploids, have developed a floescence of single-flowers. Since these plants are self-sterile and progenies of cross-pollinated parents are partially different (Anderson, 2007), the breeding of freesia has continued dynamically, and new double-flower cultivars have acquired a superficial resemblance to the earlier breeding species. The results of *Freesia refracta* hybridization showed that seed propagation has a faster rate than corm propagation and, as a matter of color heritability, the petals tend to develop a diverse range of colors such as yellow, white, and white with yellow halos (Azimi et al., 2015). In cultivar breeding programs, breeders consider the selection of varieties that bear strong characteristics such as disease resistance, tall cut-flower height, transportation resistance, high growth of lateral branches, strong aroma, and a high number of cormlets (Imanishi et al., 1997). To increase flower diversity according to the new consumer demands in the ornamental plant industry, plant breeders need to diversify the existing variation and produce new flowers with

attractive attributes (Azimi et al., 2018b). Several studies have been conducted on morphological features. In Iran, for instance, the breeding of ornamental bulbous plants, so far, has involved *Hippeastrum hybridum* (Azimi and Karimi Alavijeh, 2020), *Gladiolus* (Azimi 2020a; 2020b; 2020c; Azimi et al., 2020), Daffodils (Chehrazai et al., 2007), Iris (Azimi et al., 2012; 2018a, b; Firouzi et al., 2018), Cyclamen (Naderi et al., 2016) and *Fritillaria* (Momeni et al., 2013).

In recent years, cut freesia has attracted a lot of interest and has faced a growing demand from Iranian consumers because of its aroma. This can be important for the production and introduction of new cultivars. The present study was carried out to explore the production of new hybrids of cut freesia by hybridization. The aim was to create variation and produce new cultivars with superior quantitative, qualitative traits, so as to meet demands in response to the growing markets.

## Materials and Methods

### *Plant materials and crossing*

In order to produce new hybrids, five of the most important and popular cut freesia flowers (Table 1) were crossed in the research greenhouse of Ornamental Plants Research Center (OPRC; N 33°54'30.13" E 50°27'30.34"; H 1747), Mahallat, Iran. This study started in 2014 and continued for four years. All cultivars were grown from 2014. Twelve offspring plants were obtained from the crossing and self-pollination of the five *Freesia hybrid* cultivars (Table 2). Intervarietal hybridization was performed by the removal of the anthers, envelope-making, pollination, and seed collection. With the onset of flowering in March 2015, the artificial pollination was started in the greenhouse. For this purpose, the parental flowers were already prepared for crossing. The flowers were covered with paper envelopes to prevent the inoculation of foreign and unwanted pollens. Emasculation was done 1-3 days before anther maturity and outbreak. The anthers were covered with paper envelopes again. Then, the installed label information was completed on each sample (i.e. the paternity profile). The seeds were harvested and, to accelerate germination, stratification was applied (Azimi et al., 2015). The seed trays contained 25% perlite, 25% peat moss, and 50% cocopeat to enable optimal seed growth (23±4°C; 65±5 RH). The freesia seeds were tiny and, thus, careful attention was paid to the depth of planting (Azimi et al., 2015). The self and hybrid progenies flowered in the spring of the second year and were propagated in the third year. Then, their morphological and genetic traits

were recorded in the fourth year (Table 2).

### **Morphological experiments for hybridity**

For the hybridity test, the hybrids and parents were cultivated based on a completely randomized block design with three replications. At flowering time (February 2017), all traits were

recorded based on the distinctness, uniformity, and stability (DUS) test in accordance with the Instructions of the International Union for the Protection of New Varieties of Plants (UPOV, 2017). Ornamental traits were measured using a digital ruler and a caliper, and the flower color was recorded according to the standard RHS color chart (The Royal Horticulture Society, London).

**Table 1.** Color characteristics of the cut freesia flower varieties used in cross-breeding and combinations

Hybrids (F1) codes	Parents (parental code:p1-p5)	
	♀	♂
11, 12, 13, 14, 29, 30	<i>Freesia hybrid cv. soleil</i> with yellow color (P5) - self	-
2	<i>Freesia hybrid cv. alba river</i> with white color (P1)	<i>Freesia hybrid cv. red river</i> with red and yellow throat color (P4)
20A, 7A	<i>Freesia hybrid cv. soleil</i> with yellow color (P5)	<i>Freesia hybrid cv. alba river</i> with white color (P1)
3A	<i>Freesia hybrid cv. soleil</i> with yellow color (P5)	<i>Freesia hybrid cv. grandeur</i> with milky color (P2)
5A	<i>Freesia hybrid cv. soleil</i> with yellow color (P5)	<i>Freesia hybrid cv. pink passion</i> with pink color (P3)
28P	<i>Freesia hybrid cv. soleil</i> with yellow color (P5)	<i>Freesia hybrid cv. red river</i> with red and yellow throat (P4)

**Table 2.** Successful crosses and self-pollinations between freesia cultivars and plant phenology from cultivation until flowering

Cross date	Seed harvest date	Seed stratification date	Seed cultivation date	Flowering date
March 2014	April 2015	August 2015; for 35 days	October 2015	February 2017

### **Statistical Analysis**

Data analysis was performed using SAS V9.2 software (SAS Institute Inc., Cary, NC, USA). The variances, along with phenotypic, genetic and environmental variability coefficients, were calculated based on the methods of Barton and Turelli (1989) and Santos et al. (2011).

Genetic Coefficient of Variation (GCV):  $(\sqrt{\delta_g} / \mu) \times 100$

Phenotypic Coefficient of Variation (PCV):  $(\sqrt{\delta_{ph}} / \mu) \times 100$

Environmental Coefficient Variation (ECV):  $(\sqrt{\delta_e} / \mu) \times 100$

Genetic Variance ( $\delta^2_g$ ):  $(MS_t - MS_e) / r$

Phenotypic Variance ( $\delta^2_{ph}$ ):  $\delta^2_g + \delta^2_e$

Environmental Variance ( $\delta^2_e$ ):  $\delta^2_{ph} - \delta^2_g$

Estimation of heritability was calculated through a formula by Moll et al. (1964):  $h^2_{BS} = \delta^2_g / \delta^2_{ph}$   
Genetic specifications were calculated according to the following formula:

Broad heritability  $\times K \times$  phenotypic coefficient variation  $\times 100$

Where K equals 2.06 and is the selection rate at the 5% probability level

Cluster analysis was based on the Ward approach

on quantitative traits, using the UPGMA method and Pearson's correlation coefficient, via the SAS software package.

### **Results and Discussion**

#### **Analysis of variance and descriptive statistics of traits**

The analysis of variance showed that progenies and parents differed significantly ( $P \leq 0.01$ ) in all traits, so that the coefficient of variation (CV) differed in terms of the recorded traits and varied from 5.25% to 17.40%. The maximum coefficient of variation was related to stem length between the first and second flowers (17.40%) and the lowest was observed in the length of the inner segment of perianth (5.25%) (Table 3). In general, a higher coefficient of variation for a trait provides a wider opportunity for acting selectively on the trait. In this research, branch number per peduncle and rachis length between the first and second flowers had a high coefficient of variation. The study of morphological traits of native Iranian and *Iris germanica* showed that the highest coefficient of variation was related to plant height and the lowest belonged to the length

of the inner tepal. Furthermore, among the evaluated traits, the main components of the flower had a lower coefficient of variation (Azimi et al., 2011, 2012). Yang et al. (2015) observed a wide scope of the variation coefficient, which

ranged from 15.5 to 79.8% in F1 populations of cut chrysanthemums, thereby suggesting a remarkable potential for the use of hybridization in the improvement of this flower.

**Table 3.** Analysis of variance and estimates of genetic parameters in the evaluated cultivars of freesia

Ms										
Sources of variation	df	Plant height (cm)	Leaf length (cm)	Peduncle length (cm)	Peduncle number of branches	Spike length (cm)	number of flowers and buds	length of stem between first and second flower	Bud length/width ratio	Tube perianth length (mm)
Block	2	0.43ns	6.25ns	28.04*	0.02ns	1.43ns	0.49ns	0.15ns	0.164*	0.51ns
Treat	1	111.96*	90.26*	13.37**	2.38**	25.89*	18.34*	111.56*	0.30**	5.95**
	6	*	*			*	*	*		
Error	3	9.27	18.84	8.34	0.35	1.36	1.25	12.61	0.041	1.22
	2									
CV (%)	-	7.47	13.82	14.85	16.25	11.76	12.34	17.40	8.78	7.30

Sources of variation	df	Perianth throat length (mm)	Perianth throat: width of distal part (mm)	Perianth throat: number of stripes on inner side	Perianth: length of outer segment (mm)	Perianth: width of outer segment (mm)	Perianth: length of inner segment (mm)	Perianth: width of inner segment (mm)	Perianth: ratio length/width of inner segment	Stigma lobes length (mm)
Block	2	0.57ns	0.003ns	0.50ns	0.13ns	3.27ns	0.86ns	0.49ns	0.004ns	0.001ns
Treat	1	18.93**	31.44**	17.96**	42.95**	42.45**	29.88**	79.85**	0.10**	2.10**
	6									
Error	3	2.18	0.85	1.26	2.35	2.55	1.45	3.42	0.016	0.158
	2									
CV (%)	-	6.98	5.60	7.04	5.95	8.40	5.25	7.33	14.02	8.36

### Correlation Coefficients

The correlation coefficients (Table 4) between the traits of hybrids demonstrated that the most significant, positive correlation was observed in the flower count and spike buds count which correlated with spike length ( $r=+0.75$ ), the length of the inner segment of perianth correlated with the length of its outer segment ( $r=+0.79$ ). The width of the outer segment of perianth correlated with the peduncle length ( $r=+0.72$ ). The width of the inner segment of perianth correlated with the length of its inner segment ( $r=+0.72$ ). The length of the inner segment of perianth correlated with the width of distal parts of the perianth ( $r=+0.71$ ), and the length of the inner segment of the perianth correlated with spike length ( $r=+0.71$ ). The positive and significant correlation between these traits is an important factor in the flowering and spike-bearing of freesia because the perianth largely affects marketability and improves floret size. Increasing the length of the inner segment of the perianth and the width of the distal part of the perianth throat resulted in larger florets. Also, an increase in the length of the inner segment of the

perianth which correlated with the length of the spike led to longer spikes and enhanced the quality of cut freesia flowers. In fact, these are major purposes of freesia breeding programs. Moradi (2009) reported a positive and significant correlation between leaf width and leaf length, stem diameter and leaf number, leaf width and flower dry weight in gladiolus. The most significant, positive correlation in various iris species was related to the correlation between petal length and petal width, whereas the most significant, negative correlation was reported between the length of the perianth tube and the width of the pistil, as well as between the length of the perianth tube and the width of the crest (Azimi et al., 2011). It is important to be aware of the relationship between different traits in breeding programs to improve the efficiency of crossing, precisely because trait selection cannot be done by considering each trait separately. Thus, breeding programs should take into account the correlations between traits (Karami et al., 2005).

**Table 4.** Correlation coefficients between traits in hybrids (F1) and parents

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1	1																		
2	0.624**	1																	
3	0.568**	0.379*	1																
4	0.485**	0.476*	0.442**	1															
5	0.296*	0.189	0.642**	0.157	1														
6	0.389**	0.209	0.401**	0.308*	0.638**	1													
7	0.287*	0.044	0.598**	-0.036	0.750**	0.401**	1												
8	-0.120	-0.091	-0.084	-0.163	-0.324*	-0.345*	-0.159	1											
9	0.093	0.255	0.042	-0.098	-0.088	-0.375**	0.083	0.313*	1										
10	-0.100	0.080	-0.060	0.296*	-0.305*	-0.051	-0.225	0.040	0.031	1									
11	0.447**	0.327*	0.654**	0.005	0.630**	0.320*	0.561*	0.009	0.139	-0.223	1								
12	0.027	0.038	0.107	-0.101	0.238	0.181	0.073	0.193	-0.371**	-0.241	0.216	1							
13	0.241	0.283*	0.528**	0.005	0.612**	0.242	0.534*	-0.003	-0.019	-0.075	0.669**	0.610*	1						
14	0.490**	0.530*	0.723**	0.192	-0.482**	0.178	0.442*	0.082	0.192	-0.178	0.653**	0.268	0.671*	1					
15	0.278*	0.320*	0.514**	0.101	0.706**	0.378**	0.530*	-0.164	-0.003	-0.074	0.578**	0.339*	0.791*	0.543**	1				
16	0.646**	0.461*	0.634**	0.134	0.530**	0.268	0.451*	0.051	0.123	-0.323	0.710**	0.364*	0.680*	0.697**	0.721*	1			
17	-0.524**	-0.291	-0.294*	0.083	-0.125	-0.138	-0.161	-0.104	-0.109	0.315*	-0.407**	-0.087	-0.143	-0.365**	-0.118	-0.614**	1		
18	-0.011	0.041	0.032	0.053	0.050	-0.089	-0.067	0.179	-0.229	-0.077	0.057	0.587*	0.477*	0.224	0.282*	0.394**	-	-	1
																			0.220

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

1- Plant height	4- Branches number of peduncle	7- Length of rachis between first and second flower	10- Perianth throat length	13- length of outer segment perianth	16- Width of inner segment perianth
2- Leaf length	5- Spike length	8- Bud length/width ratio	11- Distal part of throat's perianth width	14- Width of outer segment perianth	17- Length/width of inner segment of perianth
3- Peduncle length	6- Bud and flower number of spike	9- Perianth tube length	12- Stripes number on inner side of perianth throat	15- length of inner segment perianth	18- Stigma lobe length

**Table 5.** The mean comparison of progenies or genotypes (1 to 17) and parents to fressia measured traits.

Number	Cross/ self	Hybrid or parent code	Plant height (cm)	Leaf length (cm)	Peduncle length (cm)	Shoot number of peduncle (cm)	Spike length (cm)	Bud and flower number of spike	Length of stem between first and second flower (mm)	Length to width ratio of bud	Perianth tube length (mm)	Length of terminal segment of the perianth throat (mm)	Width of terminal segment of the perianth throat (mm)	Stripes number on inner side of the perianth throat	Length of outer segment of perianth (mm)	Width of outer segment of perianth (mm)	Length of inner segment of perianth (mm)	Width of inner segment of perianth (mm)	Length to width ratio of inner segment of perianth	Stigma lobe length (mm)
1	-	P1	36.11e	25.88efg	17.88d-g	2.83d-f	13.00a-c	13.16a	30.50a	2.38b-f	14.44d-g	19.83cde	14.04e	17.94bc	26.38def	16.66de	24.03ab	22.91fg	1.03bcd	4.64cde
2	-	P2	29.5f	19.16g	10.99h	2.50ef	5.58h	4.50g	16.66cde	2.84a	15.18c-f	22.50abc	13.70e	16.50cd	23.73fgh	14.93ef	19.75c	21.50g	0.91c-f	5.66b
3	-	P3	36.00e	26.33d-g	17.00fg	2.33ef	11.33b-f	10.33bc	23.00bc	1.96g	14.76d-g	21.33bcd	17.50d	15.33df	28.33cd	16.00def	26.00a	23.50efg	1.10abc	3.63fg
4	-	P4	39.33cde	28.50c	19.45c-f	3.58bcd	9.36f-g	10.00bc	18.00b-e	2.01efg	15.25c-f	18.33e	14.93e	13.00fg	21.11hi	17.25de	18.81c	21.75g	0.86c-f	4.54de
5	-	P5	35.83e	31.66a-f	12.75gh	4.50ab	5.66h	6.83f	10.24f	2.73ab	14.99c-g	24.88a	10.05f	14.83def	21.30hi	13.22f	18.89c	15.87h	1.28a	4.52de
6	P5×P4	28P	48.83ab	28.16c-f	22.66b-e	4.66ab	9.16fg	11.50ab	24.33ab	2.09efg	14.32e-g	22.77ab	17.04d	12.00g	19.66i	15.43ef	18.66c	21.58g	0.86c-f	2.98g
7	P5×P2	3A	45.83b	33.00a-e	24.16bc	4.33abc	11.08c-f	9.16c-e	23.16bc	2.22d-g	13.71e-g	19.11de	17.31d	19.50ab	31.50ab	25.46b	26.16a	33.11a	0.77fg	6.75a
8	P5×P1	20A	51.50a	39.00ab	29.66a	4.83a	13.06a-c	13.33a	24.16ab	2.08efg	12.96g	22.95ab	20.58ab	20.50a	32.80a	25.00b	26.16a	33.11a	0.77efg	5.50b
9	Self-P5	11	34.66e	24.33fg	14.00fgh	2.33ef	10.16def	6.66f	20.00bcd	2.24c-g	14.10e-g	17.66e	16.50d	18.00bc	26.93cde	17.83de	23.56b	24.00efg	0.97b-e	4.60cde
10	Self-P5	12	38.33de	30.66b-f	17.66e-g	4.00abc	13.33ab	11.33ab	20.00bcd	2.20d-g	13.16f-g	18.00e	17.33d	18.00bc	25.00efg	15.00ef	24.00ab	24.83d-g	0.96cde	5.33bc
11	Self-P5	29	48.33ab	34.33a-d	22.66b-e	4.33abc	10.00ef	7.33ef	24.33ab	2.56a-d	18.00a	21.66bcd	17.63d	14.66def	27.50cde	18.66d	25.90a	29.66bc	0.87c-f	4.93bcd
12	Self-P5	30	43.33bcd	35.00abc	27.66ab	3.66bcd	13.66a	7.66d-f	30.00a	2.16efg	17.33ab	20.00cde	19.66bc	12.33g	27.43cde	23.33bc	25.16ab	31.00ab	0.80d-g	4.56de
13	Self-P5	14	44.00bc	38.00ab	14.00fgh	3.33cde	7.66gh	9.66b-d	13.66d-f	2.23d-g	15.01c-g	22.30abc	14.28e	14.00efg	23.54gh	18.58d	23.58b	27.54cd	0.58g	5.22bcd
14	P1×P4	2	43.50bcd	33.83a-e	14.00fgh	2.16f	5.71h	6.66f	13.33ef	2.93a	16.51a-d	18.11e	18.20cd	17.80bc	23.60hg	17.46de	18.50c	26.66cdf	0.69fg	4.45de
15	P5×P1	7a	45.67b	39.83a	30.11a	3.83a-d	12.00a-e	9.00c-e	24.33ab	2.61abc	16.95a-c	19.91cde	19.28bc	16.50cd	28.34cd	33.13a	25.36ab	29.58bc	0.86c-f	4.06ef
16	P5×P3	5a	36.66e	34.83abc	23.14bcd	4.16abc	12.25a-d	10.33cc	21.16bc	2.41b-e	15.83b-e	25.00a	21.33a	16.00cde	29.51bc	22.06c	26.16a	25.83def	1.01b-e	4.96bcd
17	Self-P5	13	35.50e	31.83a-f	12.83gh	4.33abc	5.25h	6.83f	10.06f	1.87g	14.93c-g	25.01a	10.15f	14.66def	21.42hi	17.18f	19.12c	16.01h	1.21ab	4.48de

Mean values with similar letters in each column are not significantly different at 5% probability level using Duncan's multiple range test

### ***Heredity of quantitative traits***

According to the results (Table 5), the tallest plants (offspring) were observed in 20A (P5×P1), 3A (P5×P2), and 28P (P5×P4) which were 51.50, 45.83, and 48.83 cm, respectively, and the shortest one was P2 (29.50 cm). Compared to their parents, the progenies were superior in plant height. The flowering stem height, length and diameter are usually considered as valuable structural traits of the cut freesia flower, thereby affecting physiological characteristics and cut-flower resistance to enable a convenient transport from the farm to the market. The height of a cut-flower depends on cultivar, season, and temperature (John-Dole and Wilkens 2004). Since environmental conditions were controlled in this research, the height difference was influenced by genetic variations. In this regard, hybrid vigor was also reported in iris breeding programs (Zhen et al., 1997; Burke et al. 1998; Arnold et al. 2010). Plant height in cut-flowers is an important structural trait that has been the subject of many breeding programs.

The highest leaf length was observed in hybrids 20A (P1×P5) (39.00 cm) and 7a (P1×P5) (39.83 cm), whereas the lowest was observed in P2 (19.16 cm) (Table 5). All offspring had the highest leaf length compared to their parents. The increase in leaf area resulted in a higher photosynthesis rate and a greater level of carbohydrate accumulation, causing enhancements in flower longevity which, thus, would allow the convenient transportation of the flowers to more distant places and destinations (Azimi et al., 2018a; Azimi, 2019). The highest length of the peduncle was obtained in hybrids 7a (P1×P5) (30.11 cm), 20A (P1×P5) (29.66 cm), and 30 (P4×P5) (27.66 cm). The peduncle length of progenies was superior compared to that of the parents. The shortest peduncle was observed in P2 (10.99 cm) (Table 5). The highest and lowest branch numbers per peduncle were observed in the P5 parent (4.5 on average) and in the P parent (2.50 on average), respectively (Table 5). The highest branch number per peduncle in the progeny was obtained in 20A (P1×P5) and 28P (P4×P5). The progenies had more branches per peduncle than their parents. These progenies can become more important as commercial cultivars in cut-flower markets. On the contrary, the progeny that resulted from the hybridization of P5 was superior to the other parents. These observations indicated the importance of proper parental selection for crossing programs (Yang et al., 2015).

The highest spike lengths were measured in progeny 30 (13.66 cm) and 12 (13.33 cm), and

the lowest was observed in progeny 13 (5.25 cm) (Table 5). Most progenies exhibited higher spike lengths than their parents. A higher spike length with more buds and florets meant a bigger commercial value. The length of spike in cut-flowers is an important trait that has been the subject of many breeding programs.

The highest number of buds and flowers per spike was observed in progeny 20A (mid-value: 13.33), P1 (mid-value: 13.16), and 28P (mid-value: 11.50), whereas the lowest was obtained in the P2 parent (mid-value: 4.5). Several progenies had a higher number of buds and flowers per spike, compared to their parents. The flower number per spike is a valuable trait and, as the flower number increases, there are usually enhancements in flower shelf life, beauty, and grading. Since environmental conditions were under control in this study, the differences in flower number per spike were rooted in genetics. Progenies with more flowers than their parents can be successful commercial cultivars in flower markets. Less competition over water, mineral nutrients, and light between the plants usually results in the growth of more flowers on spikes (Mojiri and Arzani, 2003). Since the number of florets is determined by genetic factors, the selection of these traits can be effective. Patra and Mohanty (2014) reported that the highest percentage of genetic inheritance of gladiolus was attributed to floret number.

The highest length of rachis between the first and second flowers was obtained from the P1 parent and in 30 progenies respectively, whereas the lowest one was observed in 13 progenies (Table 5). The results showed that only the P1 parent had a significant difference compared to the progenies. The lower stem length between the first and second flowers increased the marketability and commercial value of cut freesia flowers. This is another important economic trait of cut freesia flowers. One of the physiological disorders of freesia inflorescence is thumbing which refers to the non-uniform spacing between two or three flowers at the bottom of the stem. These stems are very low quality (John-Dole and Wilkens, 2004). In comparison to their parents, the studied progenies were superior in terms of thumbing.

The highest bud length-to-width ratio was observed in progeny 2 and in parent P1, whereas the lowest value was observed in progeny 13 (Table 5). A larger bud length-to-width ratio increases the floret length-to-width ratio. The longest tube of perianth was obtained in progeny 29 and 30, whereas the lowest value was observed in progeny 20A. The average length of a parent's tube perianth was higher than that of the

progeny's tube perianth (Table 5). The results revealed that the shortest distal lengths of the perianth throat were observed in progenies 13 and 12, respectively (Table 5). Also, the maximum and minimum distal part width of the perianth throat was observed in 5A and P5, respectively. In this research, the width of the distal part of the perianth throat in the average progeny was higher than that of the average parent. Higher length and width of the perianth in a floret throat meant higher commercial value and marketability of the cut flower.

The number of strips on the inner side of the perianth throat in 20A (P1×P5) and 3A (P2×P5) was 20.50 and 19.50, respectively. The results indicated that the lowest number of strips on the inner side of the perianth throat belonged to progeny 28P (Table 5).

The highest length of the outer segment of the perianth was measured in 20A (32.80 mm) and 3A (31.50 mm), whereas the lowest was observed in parent P4 (21.11 mm) (Table 7). Also, the highest and lowest width of the outer segment of perianth was obtained in progeny 7A (33.13 mm) and in parent P5 (13.22 mm) (Table 5). In this study, the width of the outer segment of the perianth was higher in genotypes than in the parents. The greater length and width of the outer segment of the perianth in the floret throat enhanced the commercial value of the cut-flowers. The larger length-to-width ratio of the outer and inner segments of the perianth caused the floret size to increase. The results showed that the highest length and width of the inner segment of the perianth belonged to progenies 20A and 3A, whereas the lowest belonged to progeny 2 and parent P5, respectively (Table 5). The highest and lowest length-to-width ratios of the inner segment of the perianth were measured in parent P5 (1.28) and in progeny 14 (0.58), respectively (Table 5). The maximum length of the stigma lobe was related to genotypes 20A (P1×P5) (5.50 mm) and 3A (P2×P5) (6.75 mm) (Table 5). Progeny 28P had the lowest length of stigma lobe. Therefore, it seems that intervarietal hybridization improved perianth-related traits in cut freesia flowers. Thus, breeding programs can aim to increase perianth-related components. In this context, the importance of maternal inheritance has been reported in *Iris germanica* (Azimi et al., 2018a, b).

### ***Hybrids (F1) and parents grouping***

The hybrids and parents were divided into two groups (Fig. 1). The first group consisted of two subgroups. The first subgroup included parents P5 and P2, along with parents P1 and P3 which

were placed in the second subgroup. Also, parent P4 and other progenies were placed in the second group. Since progenies in the same group had closer genetic relationships than progenies in different groups, heterosis can be calculated based on the availability of genotypes in different groups. In breeding programs, selections are usually based on genetic variations and are widened as genetic diversity increases in a population. The evaluation of morphological characteristics is an important step in breeding programs because it allows genetic quality monitoring and the best selected genotype is, thus, used in breeding programs. The relatively low phenotypic differentiation among some progenies in this study indicated higher genetic similarity between parents, which was mainly due to the intervarietal hybridization. Similar results were observed in a previous research on iris flowers (Azimi et al., 2018b).

### ***Estimation of variance components, coefficient of diversity, and inheritance***

According to the results (Table 6), the highest genetic variation coefficient was related to spike length, the width of the outer segment of the perianth, and the length of the perianth throat, whereas the lowest coefficient was observed in the length-to-width ratio of the buds. The coefficient of phenotypic variation was slightly higher than the coefficient of genetic variation, indicating that the environment had smaller effects on these traits. Higher genetic variation coefficients implied the additive effect of controlling genes on these traits. The results of the present study are similar to previous findings on genetic and phenotypic variation coefficients in gladiolus (Moradi, 2009). Langton et al. (1999) reported that the highest genetic diversity of cut chrysanthemums was related to plant length and flowering rate.

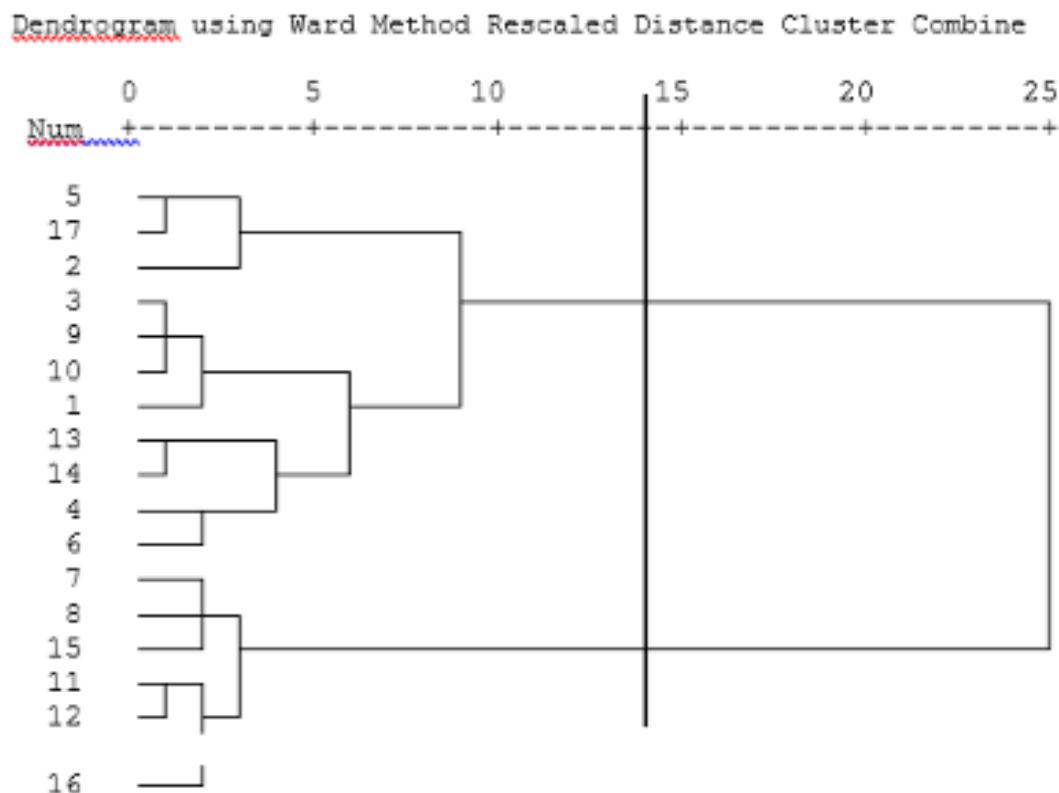
The highest broad heritability was related to the width of the terminal segment of perianth throat (92.31%), the width of the inner segment of perianth (88.16%), and the length of the inner segment (86.73%) of the perianth. The lowest value belonged to the length of the rachis between the first and second flowers (2.7%). The highest narrow heritability was related to the width of the terminal segment of the perianth throat (41.15%) and to the width of the inner segment of the perianth (44.08%), whereas the lowest value was related to the length of the rachis between the first and second flowers (1.35%). In general, most traits had high heritability, indicating a high selection range for these traits in breeding programs. Heritability plays a key role in deciding



on particular trait selection. High heritability shows effective selections based on the phenotypic efficiency.

Heritability, genetic advancement, and high genetic variance were observed in leaf length, the width of the terminal segment of the perianth throat, and the length of the inner and outer segments of the perianth. High heritability coupled with high genetic advancement, which

indicated the control of a trait by additive genes. The highest broad sense heritability was obtained in the corm weight of gladiolus, and the cormlet weight of the variety had the highest genetic advancement (Sarangi et al. 1994). Selvaraj et al. (2011) reported that high genetic variations coupled with high heritability, and genetic advancement provided a clearer insight into genotype selection (Anbanandan et al., 2009).



**Fig. 1.** Cluster analysis of progenies and parents based on the traits studied by the Ward method. The progeny and parent number is based on mid-value comparisons.

## Conclusion

The evaluation of important traits showed a significant difference between progenies and parents, so that progenies 28P, 3A, and 20A (Fig. 2) had a desirable potential for commercialization. The flower structure (plant height, spike length, floret number, and the length and width of the perianth) of these progenies outperformed the qualities of the parents. Most traits exhibited high heritability, indicating a high selection range for these traits in breeding programs. It can be expected that these selected progenies will succeed as a new genotype in flower markets. The results showed that pollen-receptive crosses in Freesia hybrid cv. soleil (P5)

(female parent) are desirable and can be used in breeding programs. Consequently, intervarietal hybridization is an effective way to contribute to the phenotypic variation of the Freesia hybrid, so as to produce new plant materials for breeding purposes and help release new cultivars

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## Conflict of interest:

The authors state that there is no conflict of interest regarding the publication of this article.

**Table 6.** Genotypic and phenotypic variances and broad and narrow sense heritability of the studied traits

Sources of variation		Tube perianth length	Bud length to width ratio	length of rachis between first and second flower	Bud and flower number of spike	Spike length	Branches number of peduncle	Peduncle length	Leaf length	Plant height	Length lobes of stigma	Length to width ratio of inner segment of perianth	Inner segment width of perianth	Inner segment length of perianth	Outer segment width of perianth	Stripes number on inner side of the perianth throat	Width of terminal segment of the perianth throat	Length throat of perianth	Outer segment length of perianth
Variance	Genotype	1.58	0.09	0.35	5.70	8.18	1.99	1.68	23.81	0.90	0.65	0.03	25.48	9.48	13.37	5.57	10.2	5.58	13.53
	Phenotypic	2.80	0.13	12.96	6.95	9.54	10.33	10.02	42.65	10.17	0.81	0.04	28.9	10.93	15.72	6.83	11.05	7.76	15.88
	Environmental	1.22	0.04	12.61	1.25	1.36	8.34	8.34	18.84	9.27	0.16	0.02	3.42	1.45	2.35	1.26	0.85	2.18	2.35
Coefficient of variance (%)	Phenotypic	4.95	0.80	11.28	20.34	28.54	3.89	8.85	13.57	3.93	1.67	1.80	10.45	13.55	29.53	16.11	6.48	27.41	15.46
	Genotype	3.72	0.66	1.85	18.42	26.43	1.70	3.62	10.14	1.17	1.50	1.43	9.81	12.62	27.23	14.55	6.22	23.24	14.27
	Environmental	3.27	0.45	11.13	8.63	10.78	3.49	8.08	9.02	3.75	0.74	1.08	3.59	4.94	11.42	6.92	1.80	14.52	5.95
Broad sense heritability (%)		56.38	67.80	2.70	82.01	85.74	19.24	16.74	55.82	8.82	80.38	63.64	88.16	86.73	85.05	81.54	92.31	71.92	85.20
Genetic advanced (0.05)		1.94	0.50	0.20	4.45	5.45	1.27	1.09	7.51	0.58	1.49	0.27	9.76	5.91	6.95	4.39	6.32	4.13	7.00

**Fig. 2.** Offspring plants of crossing and self-pollination

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