

# Seed Setting in Cross Combinations and Identification of Elite Parents for Breeding *Hippeastrum x hybridum* Hort.

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## Summary

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This research aimed to produce new, high-quality hybrids by hybridizing amaryllis cultivars and introducing new cultivars. It was conducted at the Ornamental Plants Research Center, Mahallat, Iran in 2017-2018 to assess seed traits of successful crosses between different cultivars of *Hippeastrum*. Thirty-nine crosses were laid out under a randomized complete block design with three replications. The results showed that the reciprocal crosses of 'Moscow' × 'Barbados', 'Baby Star' × 'Dancing Queen', and 'Moscow' × 'OPRC-201' were successful. The analysis of variance revealed that the difference was significant ( $P \leq 0.01$ ) for all the studied traits, reflecting a diversity of the crosses in all traits. The main traits showed that 'OPRC16' had the highest germination percentage (93.05%) and 'OPRC9' had the lowest (16.19%). The highest and lowest seed numbers per capsule were related to 'OPRC28' (126.67 seeds) and 'OPRC35' (18.00 seeds), respectively. 'OPRC11' had the highest thousand-seed weight of 13.22 g and 'OPRC33' had the lowest one of 6.60 g. The crosses were categorized into four groups. It was found that the diversity in the traits was mostly related to the genetic factors, and the environment was less influential on these traits.

## Key words

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diversity, hybridization, capsule, germination percentage

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## Introduction

Amaryllis (*Hippeastrum x hybridum* Hort.) are used as flowering plants, pot plants, and cut-flowers or limitedly in landscape designing. In Iran, they are mostly grown in the northern regions (the provinces of Mazandaran, Gilan, and Golestan). In Persian, amaryllis is called *Nasrin*. The flowers are trumpet-shaped, zygomorphic, diverse color and striping (Wang et al., 2018) with a wide range of colors including red, white, pink, sandy, orange, and/or with veins of different colors, but purple and yellow do not occur. Its big flower size and easy flowering have turned it into a popular and highly demanded flower throughout the world. Amaryllis is a genus from the family *Amarelidaceae* and the order *Asparagales*. The genus contains 55-75 species, and its endemic species are found in Brazil (Dutilh, 1996; Wang et al., 2018). *Hippeastrum hybridum*, which is a bulbous plant among the top 20 popular flowers in the world, is native to hot regions of South America. The plant is commercially produced in the Netherlands, South Africa, and the USA (Meerow et al., 1991). There are about 300 improved cultivars of amaryllis in the world (Vazquez et al., 2015). The cultivars are tetraploid produced by the crossing of two native Brazilian species, *H. reginae* and *H. vittatum* (Ockenga, 2002). Major markets of Bulbs were produced by the Netherlands, South Africa, Japan, Brazil and the United States. Hippeastrum production in Florida for the 2012 season was valued at seven million dollars (USDA, National Agricultural Statistics Service, 2012). Sales of Amaryllis cut flowers in the major markets of the Netherlands amounted to 72 million flowers in 2012 (Hubner, 2014). and they are exported by the Netherlands, South Africa, Japan, Brazil, and the United States (Vazquez et al., 2015). The Netherlands and South Africa have a 200-year history of amaryllises (*A. herborp*) breeding (Meerow, 2009), including a breeding program with a 15-year perspective to improve heat resistance and produce new florets (Meerow, 2009). Tombolato and Castro (1989) produced tetraploid cultivars of 'Red Lion' and 'Apple Blossom' by controlled pollination.

Several studies have been conducted on morphological attributes and breeding of native bulbous plants of Iran including *Narcissi* (Chehrizi et al., 2007), *Iris* (Azimi et al., 2012; 2018), *Gladiolus* (Azimi 2020a; 2020b; 2020c; Azimi et al., 2020), *Cyclamen* (Naderi et al., 2016), and *Fritillaria* (Momeni et al., 2013). Most breeding programs focus on improving ornamental properties, color, size, flower shape, plant height, growth form, and sensitivity to light quality and quantity (Teixeira da Silva, 2004). The main goal in the management of a germplasm is to collect and identify its diverse forms (Khadari et al., 2003). Genetic diversity is of crucial importance for parent selection in breeding programs. Genetic diversity can be examined by morphological, molecular, and biochemical methods (Krichen et al., 2012). As the first markers, morphological traits can contribute to utilizing genetic diversity in breeding programs (Fu et al., 2008; Shao et al., 2010). Optimal morphological traits are the first criterion for the selection of a suitable germplasm. Breeders usually employ morphological traits to describe and preliminarily categorize a germplasm for the selection of valuable genetic resources for their breeding programs (Krichen et al., 2012). *H. euaniae* is white or cream in color and is used as a parent to change flower color. Recently, yellow flowers have been produced, but their color was not stable, and the flowers were not big enough. Self-

incompatibility may be tackled by embryo culture techniques or other biotechnological methods.

The commercial breeding of tetraploid populations of amaryllis has an intensive history so that the hybrids enjoy optimal traits in terms of flower size and capsule number. Male-sterile triploid generations are the result of crossing between diploid and tetraploid species. Self-incompatibility happens in most diploid species and hybrids, which finally change into tetraploids (Bell, 1973; Bell, 1977). Caroline de Moura et al. (2010) studied bulb traits and yields of amaryllis (*Hippeastrum hybridum* Hort.) in Brazil and reported a good yield. The improvement of high-yielding cultivars requires recognition of the genetic structure of the intersected parents and the combinability of their desirable traits, which is possible using quantitative genetic methods, such as diallel or line-tester intersection (Firuzi et al., 2018). *Hippeastrum hybridum* is propagated by seed offsets and scaling (Sharifi et al., 1994). However, tissue culture is better for the mass production of disease-free bulbs.

So far, four crossing systems have been introduced: open-pollination, poly-cross, top-cross, and diallel cross (Allard, 1960). The diallel cross is rarely used in plant breeding because only a few parents can be examined. In the progenies of open-pollination too, the seeds are taken from the maternal parent whilst the paternal parent is unknown, whereas in the progenies of poly-cross and top-cross, the pollens of the paternal parents are a homogenous pool of pollens of all parental genotypes. In these methods, after crossing, the seeds of the genotypes with a common parent are semi-sib relatives (Kasperbauer, 1990). In the poly-cross method, the clones of a plant are placed among the clones of other plants to provide the genotypes with the chance of random crossing. The constant demand from consumers fuels the constant production (Teixeira da Silva et al., 2014) and introduction of new cultivars for ornamental plants (Tanaka, 2006). The approach towards producing new cultivars with valuable traits will undoubtedly transform the turnover of ornamental plants. On the other hand, the high diversity in color and form in the market will be a motive for introducing new cultivars. Most Iranian producers use imported bulbs to produce amaryllis because of the lack of technical knowledge to produce hybrid cultivars in Iran. So far, no significant effort has been made to produce new cultivars and diversify this plant species in Iran.

The main goals of the present research were to check the hybridization process and assess the seed characteristics and hybridizability of amaryllis cultivars for the sake of breeding and introducing new cultivars.

## Materials and Methods

### Plant Material

The research was carried out in the research greenhouse of the Ornamental Plants Research Center, Mahallat, Iran (Latitude: 33°53' N, Longitude: 50°29' E., and 1732 m above sea level) in 2017-2018. The study used 10 commercial cultivars and five promising genotypes of amaryllis (*Hippeastrum hybridum* Hort.) for hybridization (Table 1), which were selected based on their

superior traits, commercial importance, market position and high diversity available for breeding programs. In terms of flower color, flower size and flowering period traits were important and will increase the potential for diversity.

**Table 1.** Ornamental traits in *Hippeastrum x hybridum* Hort. cultivars

| Code | Cultivar/<br>promising | Flower: color     | Flower type:<br>(Miniature, simple, double) |
|------|------------------------|-------------------|---|
| V1   | Moscow                 | White             | Simple                                      |
| V2   | Baby Star              | Red/ vein white   | Miniature                                   |
| V3   | Hercules               | Hot pink          | Simple                                      |
| V4   | Red Lion               | Light red         | Simple                                      |
| V5   | Pink Rival             | Hot pink          | Simple                                      |
| V6   | Barbados               | Red/ vein white   | Simple                                      |
| V7   | Benfica                | Red               | Simple                                      |
| V8   | Cherry Nymph           | Red               | Double                                      |
| V9   | Dancing Queen          | Red/ white        | Double                                      |
| V10  | OPRC-202               | Indian red        | Simple                                      |
| V11  | OPRC-201               | Dark red          | Simple                                      |
| V12  | OPRC-205               | White/ light pink | Simple                                      |
| V13  | OPRC-206               | Red/ vein white   | Simple                                      |
| V14  | OPRC-203               | Light red/ red    | Simple                                      |
| V15  | Ferrari                | Red               | Simple                                      |

The promising genotypes were produced by imported pollinating cultivars in a five-year period in Iran. All crosses were named based on the name of the institute, i.e. 'OPRCn' where 'n' refers to the numerical identifier for the promising genotypes (Table 2). The growth period for the maternal plants was  $80 \pm 10$  days from February to June 2018. The zone for the parental bulbs was 18-20 cm in size.

### Hybridization Program

After the spike emergence of the varieties, a full diallel crossing was carried out among the varieties to study seed setting in cross combinations and the combining ability of seed characters. In the female parent, flowers at the pre-anthesis stage were selected for emasculation. When flowering started, the hybridization steps including the removal of anthers, bagging, pollination and harvesting for seeds were performed. For the crosses that were assessed to be successful, the seeds were carefully collected in July and then winnowed (Fig. 1). Before the opening of the flowers and the breaking of the anthers, the stamens were removed, and the flowers were isolated (the florets were covered with special bags made of wax paper). In most amaryllis cultivars, the male parts mature earlier and the pollens are released, a condition that is known as protandry.

**Table 2.** Cross combinations for the production of *Hippeastrum x hybridum* Hort.

| Cross codes | Parents (parental code) |                    |
|-------------|-------------------------|--------------------|
|             | ♀                       | ♂                  |
| OPRC7       | Moscow (V1)             | OPRC-206 (V13)     |
| OPRC36      | Moscow (V1)             | OPRC-201 (V11)     |
| OPRC37      | Moscow (V1)             | OPRC-205 (V12)     |
| OPRC1       | OPRC-202 (V10)          | Moscow (V1)        |
| OPRC23      | Ferrari (V15)           | Moscow (V1)        |
| OPRC20      | Hercules (V3)           | Moscow (V1)        |
| OPRC21      | Barbados (V6)           | Moscow (V1)        |
| OPRC13      | Red Lion (V4)           | Moscow (V1)        |
| OPRC29      | OPRC-201 (V11)          | Moscow (V1)        |
| OPRC32      | OPRC-203 (V14)          | Moscow (V1)        |
| OPRC2       | OPRC-201 (V11)          | Baby Star (V2)     |
| OPRC17      | OPRC-201 (V11)          | Pink Rival (V5)    |
| OPRC33      | OPRC-201 (V11)          | Dancing Queen (V9) |
| OPRC31      | Barbados (V6)           | OPRC-201 (V11)     |
| OPRC34      | Benfica (V7)            | OPRC-201 (V11)     |
| OPRC3       | Hercules (V3)           | OPRC-205 (V12)     |
| OPRC19      | Hercules (V3)           | Dancing Queen (V9) |
| OPRC6       | Barbados (V6)           | Hercules (V3)      |
| OPRC4       | Red Lion (V4)           | OPRC-205 (V12)     |
| OPRC15      | Red Lion (V4)           | Baby Star (V2)     |
| OPRC5       | Pink Rival (V5)         | Red Lion (V4)      |
| OPRC8       | OPRC-202 (V10)          | OPRC-206 (V13)     |
| OPRC9       | OPRC-202 (V10)          | Benfica (V7)       |
| OPRC25      | Barbados (V6)           | OPRC-202 (V10)     |
| OPRC10      | Cherry Nymph (V8)       | OPRC-205 (V12)     |
| OPRC22      | Pink Rival (V5)         | Cherry Nymph (V8)  |
| OPRC11      | Pink Rival (V5)         | Dancing Queen (V9) |
| OPRC16      | Pink Rival (V5)         | OPRC-205 (V12)     |
| OPRC28      | Pink Rival (V5)         | Barbados (V6)      |
| OPRC30      | Barbados (V6)           | Pink Rival (V5)    |
| OPRC12      | Baby Star (V2)          | Pink Rival (V5)    |
| OPRC39      | Baby Star (V2)          | Dancing Queen (V9) |
| OPRC24      | Dancing Queen (V9)      | Baby Star (V2)     |
| OPRC14      | Barbados (V6)           | Benfica (V7)       |
| OPRC27      | Barbados (V6)           | Dancing Queen (V9) |
| OPRC38      | Barbados (V6)           | OPRC-205 (V12)     |
| OPRC35      | OPRC-203 (V14)          | Barbados (V6)      |
| OPRC18      | Benfica (V7)            | Dancing Queen (V9) |
| OPRC26      | OPRC-203 (V14)          | Benfica (V7)       |



**Figure 1.** The hybridization process until the formation of initial bulblets in amaryllis *Hippeastrum x hybridum* Hort. (Photo by the researcher). A: Crossing and seed formation inside capsules; B: the seeds harvested with shell papery; C: the seeds germinated in the sowing tray and substrate; D: a view of the initial seedlings as well as the seeds and newly formed bulblets (70-day-old seedlings); E: a view of the bulblets with roots after 70 days; F: the size of six-month-old seedlings taken from the cross.

The stigmas were fully ready for the cross in the greenhouse conditions, i.e., after 3-4 days in winters ( $19 \pm 4$  °C) and 1-2 days in springs ( $27 \pm 5$  °C). It has been established that when the humidity is low and the temperature is high, the pollen sack withers fast and the pollens lose their efficiency. On the other hand, the stigmas are sticky temporarily and they will have a problem in accepting pollens, so the viability rate of the crosses will drop sharply. So, it is recommended to use optimal greenhouse conditions if the goal is to breed and produce amaryllis seeds. In each spike, 3-4 florets were pollinated artificially because similar research on *Gladiolus* (Azimi et al., 2020) produced larger seeds. The seeds of successful crosses were carefully collected in July and were winnowed (Table 2).

### Seedbed Preparation, Sowing and Bulblet Production

The seeds were sown on sowing trays containing 30% perlite, 35% peat-moss, and 35% coco-peat at  $25 \pm 4$  °C and  $65 \pm 5\%$  humidity in the greenhouse in July. They were sown in 1.5-2 cm in trays.

### Data Collection

The recorded traits included capsule length and width (mm), capsule dry weight (g), seed number per capsule, seed length and width (mm), thousand-seed weight (g), and seed germination (%), which were measured by a digital scale, a caliper and a ruler, respectively. The thousand-seed weight was determined with a 0.001-g precision digital scale. Germination percentage was determined by the following equation (Scotte et al., 1984):

$$\text{Seed germination \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

### Data Analysis

The research was based on a randomized complete block design with three replications. Each replication was composed of 100 seeds. Factor analysis was performed to evaluate the contribution of each morphological parameter to the ordination of crosses. The 39 crosses were grouped by using the un-weighted pair group method with arithmetic mean (UPGMA) algorithm and Ward method. The statistical analyses included simple coefficients of correlation, analysis of variance (ANOVA), and means comparison by the LSD test at the 5% level ( $P < 0.05$ ), all performed in the SAS 9.1 software package.

## Results and Discussion

### Combining Ability and Successful Crosses

The highest combining ability (10 crosses) was related to the cross of 'Moscow' (V1) with other cultivars (Table 2). The lowest was observed between 'Ferrari' (V15) and 'Moscow' (V1). Diallel (reciprocal) crosses were between 'Moscow' (V1) × 'Barbados' (V6), 'Baby Star' (V2) × 'Dancing Queen' (V9), and 'Moscow' (V1) × 'OPRC-201' (V11). Other crosses were direct (Table 2). The results showed that the highest pollen reception (maternal parent) was in the crosses of 'Barbados' (V6) (7 crosses) and the lowest was for 'Ferrari' (V15), 'Cherry Nymph' (V8), and

'Dancing Queen' (V9) with one successful cross. The genotypes 'OPRC-205' (V12) and 'OPRC-206' (V13) did not accept any pollen. The pollen donation (tester) was for 'Moscow' (V1) with seven crosses, 'Dancing Queen' (V9) with six crosses, and 'OPRC-205' (V12) with five crosses, and the lowest for 'Hercules' (V3), 'Red Lion' (V4), 'Cherry Nymph' (V8), and 'OPRC-206' (V13) with only one successful cross. 'Ferrari' (V15) failed as a paternal parent (Table 2). The results of Huang et al. (1997) as to the intra- and interspecific hybridization in the genus *Iris* showed that the seedlings of the crosses died after 6-8 weeks due to the low interspecific compatibility.

### Analysis of Variance and Comparison of Means

According to the results of variance analysis (Table 3), the recorded seed traits were significant ( $P \leq 0.01$ ), implying a high diversity in the traits among all crosses. The comparison of the means (Table 4) revealed significant differences in seed germination percentage among the crosses. The highest germination percentage was obtained from 'Pink Rival' × 'OPRC-205' (93.05%), 'OPRC-201' × 'Baby Star' (92.53%), and 'Barbados' × 'OPRC-201' (90.35%), and the lowest from 'OPRC-202' × 'Moscow' (13.36%), 'OPRC-202' × 'OPRC-206' (16.63%), and 'OPRC-202' × 'Benfica' (16.19%). Since the substrate and greenhouse climate were identical for all seeds, the results indicated that when 'OPRC-202' was used as the maternal parent, fewer seeds could germinate. The range of the coefficient of variation in *Iris germanica* varied in 100-seed fresh weight and was the lowest in the number of days from crosses to harvest (Firuzi et al., 2018). The results of progeny tests in *Gladiolus* showed that the seeds of self-pollination had higher germinability in some *Gladiolus* cultivars (Azimi, 2019). The use of more vigorous seeds with higher germination rates, uniformity and percentages can help produce more robust seedlings and attain higher potential yields in different environmental conditions (Sikder et al., 2009). Various factors are involved, such as genetics, environmental conditions during seed development and storage, and seed germination improvement treatments (Di Girolamo et al., 2012). Fast and uniform germination of seeds and simultaneous flowering are the breeding goals of *Cyclamens*, which are seen in modern cultivars (Young-Soon, 2011). Significant differences were observed among the crosses in seed width and length (Table 4). The highest seed width was observed in the crosses 'Barbados' × 'Hercules' (15.76 mm), 'Hercules' × 'OPRC-205' (15.66 mm) and 'Benfica' × 'Dancing Queen' (15.53 mm) and the lowest in 'Moscow' × 'Benfica' (10.82 mm) and 'OPRC-203' × 'Moscow' (10.99 mm). The maximum seed length (Table 4) was related to 'OPRC-201' × 'Pink Rival' (20.57 mm), 'Ferrari' × 'Moscow' (20.43 mm), and 'Moscow' × 'OPRC-201' (20.42 mm) and the minimum to 'Red Lion' × 'OPRC-205' (13.61 mm) and 'OPRC-203' × 'Moscow' (14.59 mm). The results indicated that when 'Hercules' (V3) was used as a parent, the highest seed width was obtained. Similarly, Suresh (2015) reported that the crosses of *Gladiolus* differed significantly in seed width and length.

Based on the comparison of the means (Table 4), the highest 1000-seed weight was related to 'Pink Rival' × 'Dancing Queen' (13.22 g), 'Barbados' × 'Pink Rival' (13.10 g), and 'Baby Star' × 'Pink Rival' (13.04 g) and the lowest to 'OPRC-203' × 'Moscow' (6.70 g) and 'OPRC-201' × 'Dancing Queen' (6.60 g).

**Table 3.** Analysis of variance for the evaluated traits in *Hippeastrum x hybridum* Hort.

| Source of variability | Means of squares |                     |                     |                    |                             |                    |                     |                    |                       |
|-----------------------|------------------|---------------------|---------------------|--------------------|-----------------------------|--------------------|---------------------|--------------------|-----------------------|
|                       | df               | Capsule length      | Capsule width       | Capsule dry weight | Number of seeds per capsule | 1000-seed weight   | Seed length         | Seed width         | Seed germination (%)  |
| Block                 | 2                | 17.4 <sup>*</sup>   | 45.00 <sup>ns</sup> | 0.01 <sup>ns</sup> | 39.00 <sup>ns</sup>         | 0.41 <sup>ns</sup> | 4.01 <sup>ns</sup>  | 0.88 <sup>ns</sup> | 261.37 <sup>**</sup>  |
| Cultivar              | 38               | 74.63 <sup>**</sup> | 85.79 <sup>**</sup> | 0.43 <sup>**</sup> | 1759.02 <sup>**</sup>       | 9.08 <sup>**</sup> | 10.34 <sup>**</sup> | 5.45 <sup>**</sup> | 1632.30 <sup>**</sup> |
| Error                 | 76               | 5.59                | 25.67               | 0.02               | 58.34                       | 0.35               | 1.10                | 2.02               | 10.86                 |
| CV (%)                | -                | 8.88                | 14.92               | 13.21              | 11.44                       | 5.62               | 5.94                | 10.98              | 5.40                  |

It was found that the use of 'Pink Rival' (V5) as a parent increased the 1000-seed weight of the crosses, so it can be considered in breeding programs. In each spike, 3-4 florets were artificially pollinated because they would produce larger seeds. This is consistent with a similar research work on *Gladiolus* (Azimi, 2019). In a study on wheat, Mahfoozi et al. (2004) found that when the plants were exposed to drought stress after pollination and during the grain-filling period, their grain weight decreased. They found that the supply of assimilate was a factor effective in the control of grain weight (Jedel and Hunt, 1990).

The results of the means comparison (Table 4) showed that 'Pink Rival' × 'Barbados' and 'Pink Rival' × 'Dancing Queen' had the highest number of seeds per capsule (126.67 and 123.00 seeds, respectively) and 'OPRC-203' × 'Barbados' had the lowest one (18.00 seeds). Whenever 'Pink Rival' (V5) was the maternal parent, more seeds formed in the capsules. The results in Table 4 show that 'Pink Rival' × 'Barbados' and 'Barbados' × 'OPRC-201' had the highest capsule dry weight (2.12 and 1.92 g, respectively) and 'OPRC-203' × 'Barbados' had the lowest one (0.38 g). The highest capsule width was 53.10 mm obtained from 'Moscow' × 'OPRC-205' and the lowest was 22.73 mm obtained from 'OPRC-203' × 'Moscow'. The crosses 'Baby Star' × 'Dancing Queen', 'Pink Rival' × 'Dancing Queen', and 'Hercules' × 'OPRC-205' produced the longest capsules (35.26, 34.74, and 34.29 mm, respectively), whereas the crosses 'OPRC-203' × 'Barbados' and 'OPRC-201' × 'Dancing Queen' produced the shortest ones (14.70 and 17.68 mm, respectively). In other bulbous plants, there is a high diversity in positive cross parameters in *Gladiolus* genotypes, such as in capsule length, capsule width, seed number per capsule and 1000-seed weight (Azimi et al., 2020). In addition, there is a report as to the significance of studying the process of seed formation, the seed formation percentage of each cross and the compatibility of crossing between optimal parents of *Gladiolus* (Hossain et al., 2012). Suresh (2015) reported that *Gladiolus* crosses differed significantly in the number of seeds per capsule, capsule width and capsule dry weight.

### Correlation of the Quantitative Traits

The coefficients of the correlations were used to decide on the relative importance of the recorded traits and their merit as a selection criterion (Agrama, 1996). The coefficients of the correlation of the traits among the progenies (Table 5) showed that the strongest positive and significant correlations were between capsule dry weight and capsule width ( $r = +0.83$ ), between 1000-

seed weight and seed length ( $r = +0.72$ ), and between 1000-seed weight and seed number per capsule ( $r = +0.68$ ). It was found that the dry weight of the capsules was increased with the increase in their width. In other words, the wider capsules were heavier. Thousand-seed weight was correlated with seed length and seed number per capsule positively and significantly. It is an important characteristic of amaryllis because when seed length and number increase, 1000-seed weight increases too and this can be very effective in germination rate, seed health and germination uniformity and can be used by applying positive selection in the breeding period. The lowest negative and insignificant correlation was for that of germination percentage with 1000-seed weight and seed number per capsule ( $r = -0.09$ ; Table 5). In other words, the germination percentage decreases with the decrease in 1000-seed weight and seed number per capsule. Any factor that can increase germination percentage can be considered by breeders. Germination percentage is of high importance in inter- and intra-variety crosses of amaryllis so that cultivars (parents) with higher germination can be selected in breeding programs. Firuzi et al. (2018) reported that the correlation coefficients in *Iris germanica* showed that the highest positive and significant correlation was observed between 100-seed fresh and dry weight and the lowest was observed between the number of days from crosses to harvest and seed dry weight. The easiest way to find out the relationship between two certain variables is to calculate the coefficient of correlation. A significant correlation signifies that both variables are influenced by the same factors (Montgomery et al., 2006).

### Grouping by Traits

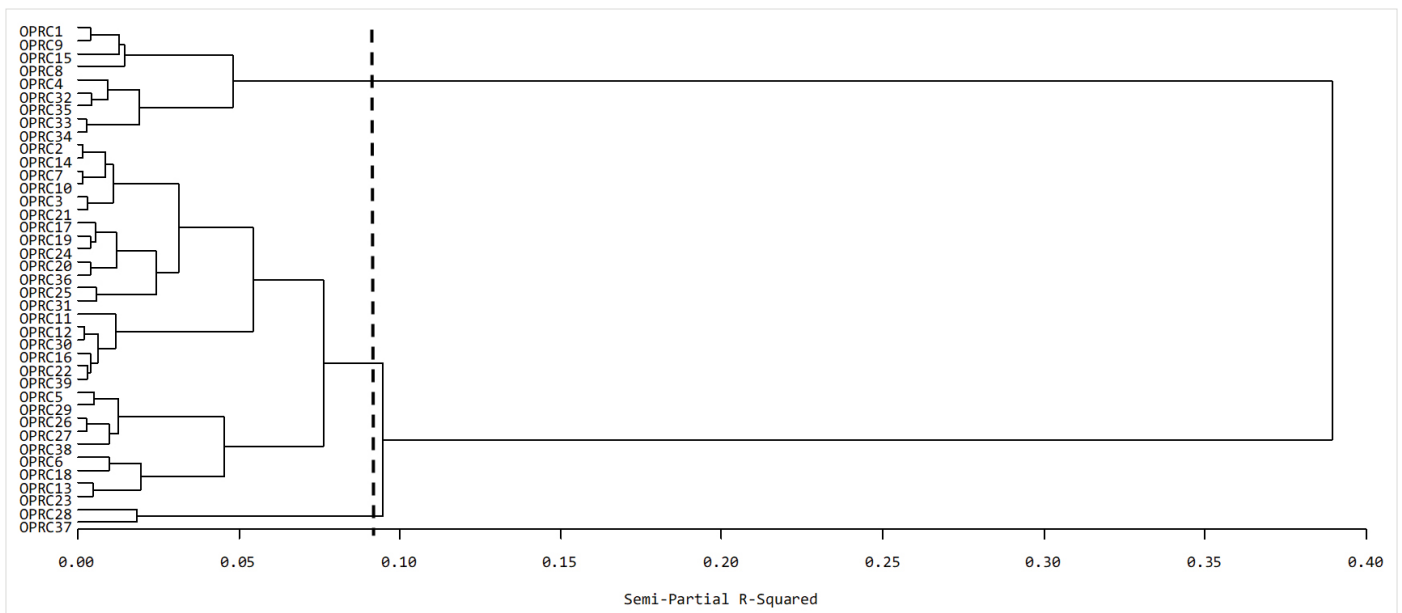
Based on the traits, Ward's method classified the crosses into four main groups (Fig. 2). The first group included two sub-groups and nine crosses. This group had the lowest means of the traits. The second group included two sub-groups and 19 crosses. The results showed that this group had the highest mean seed width of 13.41 mm (Table 6). The third group was divided into two sub-groups. It had nine crosses. These crosses were superior to the other crosses in terms of most traits. As is evident in Table 6, this group showed the highest capsule length (30.99 mm), 1000-seed weight (11.86 g), seed length (13.76 mm), and germination percentage (72.16%). The fourth group included 'OPRC28' (V5 × V6) and 'OPRC37' (V1 × V12) and had the lowest genetic affinity among the crosses. This group was characterized by the highest capsule width (47.25 mm), capsule dry weight (1.83 g), and seed number per capsule (105.33 seeds) (Table 6).

**Table 4.** Mean squares (Mean  $\pm$  Sd) of traits in crosses or hybrids in *Hippeastrum x hybridum* Hort.

| Cod/ cross | Mean $\pm$ Sd        |                  |                  |                       |                             |                        |                    |                     |
|------------|----------------------|------------------|------------------|-----------------------|-----------------------------|------------------------|--------------------|---------------------|
|            | Seed germination (%) | Seed width(mm)   | Seed length(mm)  | 1000-seed weight (gr) | Number of seeds per capsule | Capsule dry weight(gr) | Capsule width (mm) | Capsule length (mm) |
| OPRC1      | 13.36 $\pm$ 3.10     | 12.00 $\pm$ 1.29 | 15.62 $\pm$ 0.94 | 9.45 $\pm$ 0.56       | 53.67 $\pm$ 4.51            | 1.06 $\pm$ 0.14        | 32.99 $\pm$ 0.79   | 30.52 $\pm$ 0.52    |
| OPRC2      | 92.53 $\pm$ 3.08     | 13.47 $\pm$ 0.01 | 17.62 $\pm$ 2.17 | 11.12 $\pm$ 0.02      | 57.00 $\pm$ 4.00            | 1.16 $\pm$ 0.16        | 34.38 $\pm$ 1.30   | 32.70 $\pm$ 1.04    |
| OPRC3      | 68.17 $\pm$ 3.08     | 15.66 $\pm$ 1.13 | 17.90 $\pm$ 2.29 | 12.00 $\pm$ 0.31      | 66.00 $\pm$ 10.58           | 1.26 $\pm$ 0.31        | 37.91 $\pm$ 0.68   | 34.29 $\pm$ 1.90    |
| OPRC4      | 43.59 $\pm$ 4.50     | 11.60 $\pm$ 1.10 | 13.61 $\pm$ 0.29 | 8.37 $\pm$ 0.43       | 31.50 $\pm$ 3.50            | 1.08 $\pm$ 0.04        | 25.31 $\pm$ 0.61   | 20.07 $\pm$ 0.91    |
| OPRC5      | 67.86 $\pm$ 3.08     | 12.00 $\pm$ 0.07 | 16.34 $\pm$ 0.37 | 10.83 $\pm$ 0.60      | 54.00 $\pm$ 9.00            | 1.29 $\pm$ 0.32        | 32.19 $\pm$ 1.27   | 26.59 $\pm$ 0.38    |
| OPRC6      | 61.51 $\pm$ 3.09     | 15.76 $\pm$ 0.47 | 19.47 $\pm$ 0.45 | 11.25 $\pm$ 0.45      | 38.00 $\pm$ 1.00            | 0.97 $\pm$ 0.04        | 30.34 $\pm$ 0.27   | 22.22 $\pm$ 0.74    |
| OPRC7      | 56.72 $\pm$ 4.24     | 14.64 $\pm$ 1.79 | 18.87 $\pm$ 0.63 | 11.36 $\pm$ 0.43      | 68.33 $\pm$ 7.57            | 1.02 $\pm$ 0.07        | 32.00 $\pm$ 0.65   | 28.78 $\pm$ 1.94    |
| OPRC8      | 16.63 $\pm$ 2.87     | 13.35 $\pm$ 5.24 | 15.37 $\pm$ 0.17 | 7.10 $\pm$ 0.30       | 34.00 $\pm$ 3.00            | 0.60 $\pm$ 0.03        | 27.90 $\pm$ 0.05   | 25.15 $\pm$ 0.60    |
| OPRC9      | 16.19 $\pm$ 3.41     | 10.82 $\pm$ 0.29 | 15.48 $\pm$ 0.59 | 9.79 $\pm$ 0.35       | 34.00 $\pm$ 6.56            | 0.75 $\pm$ 0.12        | 31.16 $\pm$ 0.50   | 30.23 $\pm$ 0.47    |
| OPRC10     | 61.73 $\pm$ 4.00     | 14.07 $\pm$ 0.03 | 18.83 $\pm$ 0.39 | 11.87 $\pm$ 0.33      | 55.50 $\pm$ 2.50            | 1.20 $\pm$ 0.02        | 32.45 $\pm$ 3.71   | 28.11 $\pm$ 3.02    |
| OPRC11     | 52.37 $\pm$ 6.20     | 12.56 $\pm$ 0.67 | 18.53 $\pm$ 0.31 | 13.22 $\pm$ 0.99      | 123.00 $\pm$ 7.94           | 1.73 $\pm$ 0.18        | 38.30 $\pm$ 1.54   | 34.74 $\pm$ 3.21    |
| OPRC12     | 84.14 $\pm$ 4.50     | 11.68 $\pm$ 0.41 | 18.46 $\pm$ 0.55 | 13.04 $\pm$ 0.42      | 96.00 $\pm$ 10.54           | 1.55 $\pm$ 0.28        | 29.25 $\pm$ 1.94   | 29.84 $\pm$ 3.10    |
| OPRC13     | 25.26 $\pm$ 4.00     | 13.80 $\pm$ 2.34 | 18.98 $\pm$ 0.37 | 11.46 $\pm$ 0.72      | 62.33 $\pm$ 10.07           | 1.26 $\pm$ 0.13        | 30.96 $\pm$ 1.22   | 26.43 $\pm$ 1.53    |
| OPRC14     | 84.56 $\pm$ 4.50     | 13.77 $\pm$ 1.15 | 17.95 $\pm$ 0.25 | 11.87 $\pm$ 0.28      | 58.67 $\pm$ 12.01           | 1.28 $\pm$ 0.12        | 32.33 $\pm$ 3.29   | 29.56 $\pm$ 4.11    |
| OPRC15     | 25.35 $\pm$ 6.63     | 12.67 $\pm$ 0.89 | 16.35 $\pm$ 0.58 | 8.69 $\pm$ 0.48       | 47.33 $\pm$ 6.03            | 1.56 $\pm$ 0.10        | 30.04 $\pm$ 4.05   | 25.08 $\pm$ 2.91    |
| OPRC16     | 93.05 $\pm$ 3.00     | 11.78 $\pm$ 0.26 | 17.02 $\pm$ 0.61 | 11.71 $\pm$ 0.42      | 74.00 $\pm$ 9.64            | 1.42 $\pm$ 0.04        | 33.70 $\pm$ 4.38   | 29.56 $\pm$ 4.74    |
| OPRC17     | 37.10 $\pm$ 2.53     | 13.43 $\pm$ 1.84 | 20.57 $\pm$ 0.52 | 10.61 $\pm$ 0.43      | 95.00 $\pm$ 9.17            | 1.57 $\pm$ 0.14        | 37.30 $\pm$ 2.66   | 33.01 $\pm$ 2.19    |
| OPRC18     | 54.73 $\pm$ 4.13     | 15.53 $\pm$ 2.96 | 18.73 $\pm$ 0.35 | 10.55 $\pm$ 0.52      | 80.00 $\pm$ 7.00            | 1.40 $\pm$ 0.25        | 35.61 $\pm$ 4.66   | 20.79 $\pm$ 1.40    |
| OPRC19     | 72.01 $\pm$ 5.05     | 14.04 $\pm$ 0.54 | 20.04 $\pm$ 0.67 | 11.36 $\pm$ 0.37      | 85.67 $\pm$ 3.51            | 1.48 $\pm$ 0.14        | 37.64 $\pm$ 1.48   | 33.05 $\pm$ 3.62    |
| OPRC20     | 65.78 $\pm$ 4.00     | 13.18 $\pm$ 0.17 | 19.03 $\pm$ 1.48 | 10.76 $\pm$ 0.33      | 62.67 $\pm$ 11.68           | 1.37 $\pm$ 0.29        | 37.04 $\pm$ 2.43   | 28.10 $\pm$ 1.04    |
| OPRC21     | 87.14 $\pm$ 5.80     | 14.96 $\pm$ 1.63 | 19.44 $\pm$ 1.03 | 12.54 $\pm$ 0.66      | 66.67 $\pm$ 8.02            | 1.22 $\pm$ 0.10        | 36.56 $\pm$ 5.76   | 32.46 $\pm$ 4.30    |
| OPRC22     | 75.07 $\pm$ 4.00     | 12.86 $\pm$ 0.85 | 17.47 $\pm$ 0.34 | 11.46 $\pm$ 0.59      | 92.00 $\pm$ 10.54           | 1.43 $\pm$ 0.07        | 34.55 $\pm$ 2.20   | 31.88 $\pm$ 1.41    |
| OPRC23     | 35.05 $\pm$ 4.13     | 13.08 $\pm$ 1.28 | 20.43 $\pm$ 1.20 | 10.19 $\pm$ 0.33      | 44.67 $\pm$ 8.33            | 0.97 $\pm$ 0.09        | 29.90 $\pm$ 2.74   | 28.53 $\pm$ 4.65    |
| OPRC24     | 56.95 $\pm$ 3.62     | 13.66 $\pm$ 0.75 | 19.80 $\pm$ 0.18 | 11.96 $\pm$ 0.36      | 87.33 $\pm$ 4.51            | 1.47 $\pm$ 0.12        | 30.77 $\pm$ 1.15   | 33.35 $\pm$ 2.05    |
| OPRC25     | 87.33 $\pm$ 3.52     | 14.47 $\pm$ 0.32 | 20.18 $\pm$ 0.38 | 12.77 $\pm$ 0.47      | 80.67 $\pm$ 9.07            | 1.63 $\pm$ 0.20        | 40.81 $\pm$ 1.86   | 25.60 $\pm$ 4.18    |
| OPRC26     | 85.34 $\pm$ 3.52     | 13.38 $\pm$ 0.55 | 18.24 $\pm$ 1.05 | 9.05 $\pm$ 0.91       | 79.67 $\pm$ 6.81            | 1.08 $\pm$ 0.25        | 32.64 $\pm$ 1.71   | 21.65 $\pm$ 1.82    |
| OPRC27     | 84.46 $\pm$ 3.61     | 12.92 $\pm$ 0.30 | 17.75 $\pm$ 0.46 | 9.96 $\pm$ 0.36       | 74.33 $\pm$ 5.69            | 1.13 $\pm$ 0.04        | 38.00 $\pm$ 0.90   | 22.70 $\pm$ 0.42    |
| OPRC28     | 55.99 $\pm$ 3.61     | 11.49 $\pm$ 0.45 | 15.34 $\pm$ 0.95 | 10.05 $\pm$ 0.23      | 126.67 $\pm$ 7.57           | 2.12 $\pm$ 0.12        | 41.40 $\pm$ 1.12   | 27.77 $\pm$ 0.39    |
| OPRC29     | 76.07 $\pm$ 4.67     | 13.16 $\pm$ 0.52 | 16.93 $\pm$ 0.87 | 9.76 $\pm$ 0.56       | 58.67 $\pm$ 6.03            | 0.87 $\pm$ 0.13        | 28.37 $\pm$ 0.72   | 25.71 $\pm$ 0.51    |
| OPRC30     | 75.52 $\pm$ 4.67     | 11.74 $\pm$ 0.36 | 18.27 $\pm$ 0.15 | 13.10 $\pm$ 0.10      | 77.67 $\pm$ 3.06            | 1.69 $\pm$ 0.23        | 32.45 $\pm$ 1.10   | 30.78 $\pm$ 0.59    |
| OPRC31     | 90.35 $\pm$ 5.00     | 14.34 $\pm$ 2.09 | 18.12 $\pm$ 2.29 | 11.08 $\pm$ 0.16      | 94.00 $\pm$ 7.55            | 1.92 $\pm$ 0.17        | 38.21 $\pm$ 2.66   | 28.53 $\pm$ 2.60    |

Continued. Table 4

| Cod/ cross           | Mean $\pm$ Sd        |                  |                  |                       |                             |                        |                    |                     |
|----------------------|----------------------|------------------|------------------|-----------------------|-----------------------------|------------------------|--------------------|---------------------|
|                      | Seed germination (%) | Seed width(mm)   | Seed length(mm)  | 1000-seed weight (gr) | Number of seeds per capsule | Capsule dry weight(gr) | Capsule width (mm) | Capsule length (mm) |
| OPRC32               | 34.41 $\pm$ 4.13     | 10.99 $\pm$ 0.47 | 14.59 $\pm$ 1.06 | 6.70 $\pm$ 0.66       | 26.00 $\pm$ 5.57            | 0.49 $\pm$ 0.11        | 22.73 $\pm$ 2.61   | 19.97 $\pm$ 2.82    |
| OPRC33               | 55.21 $\pm$ 4.61     | 11.42 $\pm$ 1.49 | 14.91 $\pm$ 1.95 | 6.60 $\pm$ 1.40       | 50.33 $\pm$ 15.04           | 0.78 $\pm$ 0.28        | 33.70 $\pm$ 5.35   | 17.68 $\pm$ 2.45    |
| OPRC34               | 50.10 $\pm$ 4.13     | 12.10 $\pm$ 0.39 | 15.95 $\pm$ 2.29 | 7.93 $\pm$ 0.42       | 51.33 $\pm$ 9.50            | 0.79 $\pm$ 0.04        | 33.10 $\pm$ 5.28   | 21.46 $\pm$ 3.40    |
| OPRC35               | 44.72 $\pm$ 3.08     | 11.38 $\pm$ 0.41 | 15.05 $\pm$ 1.49 | 8.00 $\pm$ 1.00       | 18.00 $\pm$ 7.00            | 0.38 $\pm$ 0.10        | 25.63 $\pm$ 1.57   | 14.70 $\pm$ 3.44    |
| OPRC36               | 45.73 $\pm$ 5.22     | 12.31 $\pm$ 0.34 | 20.42 $\pm$ 0.64 | 10.78 $\pm$ 0.92      | 59.67 $\pm$ 4.16            | 1.38 $\pm$ 0.12        | 40.22 $\pm$ 1.55   | 31.17 $\pm$ 1.76    |
| OPRC37               | 74.53 $\pm$ 4.10     | 11.69 $\pm$ 0.67 | 15.19 $\pm$ 2.03 | 9.66 $\pm$ 0.82       | 84.00 $\pm$ 6.00            | 1.54 $\pm$ 0.25        | 53.10 $\pm$ 26.19  | 26.00 $\pm$ 3.63    |
| OPRC38               | 87.02 $\pm$ 3.10     | 11.04 $\pm$ 2.73 | 18.59 $\pm$ 0.90 | 10.85 $\pm$ 0.74      | 72.00 $\pm$ 4.58            | 1.07 $\pm$ 0.06        | 37.75 $\pm$ 9.29   | 27.33 $\pm$ 1.22    |
| OPRC39               | 84.89 $\pm$ 4.67     | 12.06 $\pm$ 0.32 | 18.14 $\pm$ 0.85 | 12.29 $\pm$ 1.00      | 81.67 $\pm$ 3.21            | 1.67 $\pm$ 0.10        | 35.22 $\pm$ 2.44   | 35.26 $\pm$ 1.68    |
| LSD( $P \leq 0.05$ ) | 4.90                 | 5.61             | 0.37             | 24.06                 | 1.65                        | 1.82                   | 1.51               | 22.95               |

Figure 2. Cluster analysis of 39 crosses of *Hippeastrum x hybridum* Hort. using quantitative traits based on the Ward method

The highest genetic affinity was between the crosses 'OPRC39' (V2  $\times$  V9) and 'OPRC22' (V5  $\times$  V8). The results showed that the crosses were placed in different groups. In a breeding program, selection requires genetic diversity. As genetic diversity gets wider within a population, the range of selection increases. The grouping of progenies based on seed characteristics in *Gladiolus* (Azimi, 2019) showed that the progenies of the cross between 'Amsterdam' and 'Advance Red' were the best in most traits and were categorized into different groups.

### Factor Analysis

To differentiate the studied traits in genetic diversity, factor analysis was performed (Table 7). The results of this analysis based on eight important traits showed that two factors accounted for 52.50% of the total variance. In the first factor, which captured 34.50% of the variance, the traits of capsule width and capsule dry weight had the highest positive factor coefficient, so this component was named capsule. The second factor accounted for 18.00% of the variance and the traits of seed length and 1000-seed weight had the highest positive factor coefficient in this factor.

**Table 5.** Correlation of quantitative traits in *Hippeastrum x hybridum* Hort.

| Characters                     | 1      | 2      | 3      | 4      | 5      | 6     | 7      | 8    |
|--------------------------------|--------|--------|--------|--------|--------|-------|--------|------|
| 1. Capsule length              | 1.00   |        |        |        |        |       |        |      |
| 2. Capsule width               | 0.59** | 1.00   |        |        |        |       |        |      |
| 3. Capsule dry weight          | 0.63** | 0.83** | 1.00   |        |        |       |        |      |
| 4. Number of seeds per capsule | 0.27   | 0.58** | 0.64** | 1.00   |        |       |        |      |
| 5. 1000-seed weight            | 0.10   | 0.44*  | 0.51*  | 0.68** | 1.00   |       |        |      |
| 6. Seed length                 | 0.17   | 0.39*  | 0.39*  | 0.61** | 0.72** | 1.00  |        |      |
| 7. Seed width                  | 0.50*  | 0.33*  | 0.45*  | 0.34*  | 0.23   | 0.47* | 1.00   |      |
| 8. Seed germination            | -0.36* | 0.38*- | 0.35*- | 0.09-  | 0.09-  | 0.29- | 0.38*- | 1.00 |

Note: \*\*, \*: significant at  $P < 0.01$  and  $P < 0.05$ , respectively

**Table 6.** Average of traits in different groups according to cluster analysis

| Characters                     | Mean of traits | Cluster4 | Cluster3 | Cluster2 | Cluster1 |
|--------------------------------|----------------|----------|----------|----------|----------|
| Number of hybrids              | -              | 2        | 9        | 19       | 9        |
| 1. Capsule length              | 26.32          | 26.89    | 30.99    | 24.66    | 22.76    |
| 2. Capsule width               | 36.11          | 47.25    | 35.17    | 32.86    | 29.17    |
| 3. Capsule dry weight          | 1.31           | 1.83     | 1.45     | 1.12     | 0.83     |
| 4. Number of seeds per capsule | 70.96          | 105.33   | 77.43    | 62.63    | 38.46    |
| 5. 1000-seed weight            | 10.06          | 9.86     | 11.86    | 10.43    | 8.07     |
| 6. Seed length                 | 16.91          | 15.26    | 18.76    | 18.39    | 15.22    |
| 7. Seed width                  | 12.55          | 11.59    | 13.37    | 13.41    | 11.82    |
| 8. Seed germination            | 58.71          | 65.26    | 72.16    | 64.14    | 33.28    |

**Table 7.** Result of factor analysis for traits in *Hippeastrum x hybridum* Hort.

| Characters                     | Factor1 | Factor2 |
|--------------------------------|---------|---------|
| 1. Capsule length              | 0.70    | 0.04    |
| 2. Capsule width               | 0.88    | 0.10    |
| 3. Capsule dry weight          | 0.88    | 0.09    |
| 4. Number of seeds per capsule | 0.69    | 0.52    |
| 5. 1000-seed weight            | 0.38    | 0.76    |
| 6. Seed length                 | 0.02    | 0.87    |
| 7. Seed width                  | 0.52    | 0.27    |
| 8. Seed germination            | 0.68    | 0.26    |
| Percentage of the variation    | 34.50   | 18.00   |
| Cumulative variation           | 34.50   | 52.50   |

## Conclusion

The diallel crosses 'Moscow' × 'Barbados', 'Baby Star' × 'Dancing Queen' and 'Moscow' × 'OPRC-201' are more important for breeding programs. The highest hybridizability was observed between 'Moscow' and other cultivars, so it can be considered in breeding programs. The traits related to seed number per capsule, 1000-seed weight, and germination percentage are more important in distinguishing the crosses, so the populations of these hybrids can be candidates for the next round of selection.

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## CREDit Authorship Declaration

**Mohammad Hossein Azimi:** designing the experiments, obtaining and analyzing data and writing the scientific article.

## Declaration of Competing Interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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