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RESEARCH PAPER

HORTICULTURAL STUDIES

Variety Breeding Studies on *Hesperis isatidea* (Boiss.) D.A. German & Al-Shehbaz

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Abstract

Hesperis isatidea (Boiss.) D.A.German & Al-Shehbaz, a member of the Brassicaceae family, represents a distinctive natural species characterized by its fragrant, conspicuous inflorescences, unique flower coloration, and distinct feather-like foliage. The Turkish name of the species is known as 'Allı Gelin'. This species is native, exhibiting resilience to drought conditions and requiring minimal care. Cultivation studies on this species were carried out at Erzincan Horticultural Research Institute (Türkiye) with project number 106G022 within the scope of TÜBİTAK (Turkish Scientific and Technological Research Council). The research aimed to determine optimal production techniques through generative and vegetative propagation methods. The project, initiated in 2013 and concluded in 2022, focused on developing a cultivar of the 'Allı Gelin' plant using single selection breeding techniques. Thirteen populations of 'Allı Gelin' from institute's gene pool were utilized. Employing the single selection method, four distinct lines were identified: compact, tightly spread compact, pyramid-1 and pyramid-2 shaped. Subsequent baby plant tests revealed stability in the tightly spread compact and pyramid-1 lines, leading to their selection for further breeding. Although breeding efforts continued on the compact line, the pyramid-2 line was discontinued due to an inability to attain the desired stability. The successfully tested variants, namely the pyramid-1 and tightly spread compact forms, have been earmarked as candidates for outdoor ornamental plant varieties. Registration procedures for these candidates are underway. These new varieties promise to enrich diversity within the ornamental plants sector, potentially enhancing competitiveness in foreign markets.

1. Introduction

Türkiye, distinguished for its geographical positioning, geomorphological structure, and diverse ecology, holds significant prominence as one of the world's key genetic centers. The country's flora encompasses approximately 12.000 plant taxa, with around 35% exhibiting endemism (Anonymous, 2021). Among these, the 'Allı Gelin' plant, exclusively found in limited locales, notably along roadside slopes, carries substantial economic prospects. However, the recent intensification of road construction has resulted in the loss or reduction of many of these habitats.

The 'Allı Gelin' plant, scientifically known as *Hesperis isatidea* (Boiss.) D.A. German & Al-Shehbaz belonging to the Brassicaceae family, represents a distinctive biennial species. Its distinct appearance, characterized by fragrant and flamboyant flowers, unique flower colors, and distinctive feather cover, is endemic to various locations in Erzincan (Baytop, 1994; Kandemir et al., 2022). Notably, it exhibits resilience to drought and thrives under limited maintenance conditions.

This species showcases considerable potential in the ornamental plants sector, particularly in landscaping along highways, roadsides, parks, and gardens, where it can serve as seasonal flower.

The 2020 report from the Ornamental Plants Producers Sub-Union underscores the necessity of prioritizing the development of drought-resistant species and varieties, particularly in grass seeds, within the ornamental plants sector. Moreover, it emphasizes establishing a gene pool to facilitate the long-term breeding of crucial species and implementing measures to prevent the introduction of invasive foreign species that could endanger the native flora (SÜSBİR, 2020).

Policy recommendations from the Ornamental Plants Sector Document Workshop for the 2020-2024 period primarily advocate supporting research and development endeavors aimed at creating new varieties adaptable to evolving climate conditions, specifically focusing on drought-resistant species requiring minimal maintenance. The proposed approach entails leveraging high-value plant species from the indigenous flora through collaborative efforts involving governmental bodies, universities, and the private sector. This initiative aims to tackle a key challenge faced by the sector: the developping of new varieties capable of thriving under changing climate conditions and limited maintenance, utilizing species from the local flora (Anonymous, 2021).

Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies (TAGEM) 2021-2025 Agricultural Research Master Plan outlines objectives within its ornamental plants research program, including the development of outdoor ornamental plant varieties resilient to drought and low water consumption, cultivation of economically viable indigenous species for export, and ensuring sustainable utilization. The research program's strategy in the short and medium term aims to develop high-yield, high-performance varieties for both indoor and outdoor ornamental plants adaptable to shifting climate dynamics. This strategic direction aims to curtail water usage in landscaping while meeting market demands for new varieties suited for drought conditions. In the long run, emphasis will be placed on integrating economically valuable species into breeding programs to align with market expectations and develop new drought-resistant varieties utilizing local genetic resources in response to climate change (Anonymous, 2021).

A major hurdle faced by the ornamental plants sector lies in the scarcity of indigenous and nationally recognized varieties (Kaya et al., 2015). The study on the *H. isatidea*, an integral component of our diverse biodiversity and one of the most sought-after outdoor ornamental plants has been specifically designed to address these objectives.

In line with the aforementioned rationale, Erzincan Horticulture Research Institute has concluded cultivation studies on the *H. isatidea* and aims to introduce a selection of candidates derived from a selective breeding (single selection) method into the ornamental plants sector. Initiated in 2013, the project involved 13 ‘Allı Gelin’ populations from our institute's genetic pool. Over the period from 2013 to 2022, purpose-driven self-pollination techniques were employed (compact, pyramid, tightly spread compact), resulting in the selection of three distinct lines. Baby plant trials conducted in the tightly spread compact and pyramid forms in 2022 exhibited a 98% stability rate. These lines in these two forms have been identified as candidates for outdoor ornamental plant varieties, and necessary registration procedures have been set in motion.

2. Material and Method

2.1. Materials

The materials for our study were constituted by individuals from a collection garden, which represented 13 different 'Allı Gelin’ populations. These were gathered as part of the TÜBİTAK-supported project "Cultivation of Some Perennial Plant Species in the Eastern Anatolia Region" (Project No: 106G022) between 2006 and 2009 (Aslay et al., 2009).

2.2. Method

2.2.1. Selection breeding

The most suitable production technique for the species was identified by applying generative and vegetative propagation techniques (Aslay et al., 2013). The "Single-Selection Breeding Method" was utilized in this study aimed at standard variety development (Demir, 1975). The following stages were followed in a single selection:

2.2.2. Population inspection (1-4 Years)

The ‘Allı Gelin’ plant produces seeds biennially, so each generation requires two years. Samples from 13 populations, collected as part of the “Cultivation of ‘Allı Gelin’ Plant in Eastern Anatolia Region” project, were present in the institute's gene pool. Seed sowing of these 13 populations was conducted, and plants possessing desired traits (Figure 1) were marked for self-pollination.

2.2.3. Elite inspection (4-8 Years)

Selected elite plants were numbered and grown in separate rows without repetition. Self-pollination was conducted on plants exhibiting desired forms (compact, tightly spread compact, and pyramid form). This self-pollination process continued until pure lines were obtained.

Figure 1. Preferred plant forms (1: Compact, 2: Pyramid, 3: Tightly spread compact).

Table 1. Morphological measurement and observation criteria for Allı Gelin plant.

No	Morphological measurement and observation criteria	Descriptions
1	Plant height (cm)	Distance from the soil surface to the furthest point of the plant
2	Branching status of stem	Presence or absence of branching on the stem and if present, the number of branches
3	Leaf status on stem	Presence or absence of leaves on the stem
4	Flowering date	Date when the first flowering begins in the plant
5	Flower color	Identification of flower colors of the plant was done using the RHS color catalogue
6	Flower cluster diameter (cm)	Distance between the two farthest points of the flower when evaluated in its full bloom
7	Flower cluster type	Form of the flowers on the plant: compact, pyramid, tightly spread compact
8	Flower longevity (days)	Duration for which flowers remain vibrant on the plant, in days
9	Petal width (mm)	Distance between the two widest points of the petals in the flower
10	Petal length (mm)	Distance between the two furthest points of the petals in the flower
11	Number of petals	Number of petals in the flower
12	Fragrance	Scent of the flowers in the plant was determined sensorially
13	Fuzziness	Presence or absence of fuzziness on the stem and leaves of the plant
14	Number of leaves	Number of leaves on the plant
15	Leaf color	Color of the leaves on the plant was determined using the RHS color catalogue
16	Leaf shape	Shape of the leaves at the base of the plant was determined
17	Leaf width (cm)	Distance between the two widest points of the leaf
18	Leaf length (cm)	Distance between the two furthest points of the leaf
19	Seed maturation time	Date when all the seeds in the plant have matured

2.2.4. *Baby plant testing and selection of suitable variety candidates (9-10 Years)*

Stability checks were performed on plants in the selected lines, and off-target plants were removed. Selection of individuals suitable for outdoor and potted floriculture was done from the baby plants, followed by seed production. Morphological measurements and observations of the obtained pure lines were conducted according to the criteria listed in Table 1.

3. Results and Discussion

3.1. 2013-2014 (Population inspection)

In 2013, seed sowing of the existing 13 populations in the institute's gene pool was carried out, resulting in the production of seedlings. When these seedlings had reached the first true-leaf stage, they were transplanted into 45-cell trays and grown in peat+perlite (3/1) mixture medium (Aslay et al., 2010). The matured seedlings in the trays were then planted in the field using a triangular planting pattern with spacing of 50×50×50 cm between and along the rows. The 'Allı Gelin' plants that matured during this period and went into dormancy in winter, flowered/bloomed in 2014. Among these flowering plants, those with desired traits such as compact, pyramid, and tightly spread compact forms (Figure 1) were numbered, and seeds were obtained by selecting self-pollinated ones and 9 lines.

3.2. 2015-2016 (Elite inspection)

In 2015, the selected elite plants were cultivated in separate rows without repetition. Based on plant observations conducted in 2016, self-pollination was carried out on plants meeting the desired criteria (compact, pyramid, tightly spread compact),

and 5 lines possessing the targeted features were selected. Seed planting for these selected lines was performed in the autumn.

3.3. 2017-2018 (Elite inspection)

Seedlings were obtained from seeds planted in the autumn when they germinated in March. These seedlings were then transplanted into trays. Seedlings that matured in the trays were planted in the field. The following year, in April, among the plants of the five 'Allı Gelin' lines that began forming flower buds, 35 plants possessing the desired traits were isolated in iron cages covered with fly netting (9×6, 30 mesh) for self-pollination (Figure 2). Among these caged plants, 4 lines that formed seeds and met the desired form (compact, pyramid, tightly spread compact) were selected and named.

For the plants of the four selected elite lines (Figure 3), the duration of flower retention varied between approximately 30 to 40 days. The plants transitioned to seed formation by the end of May and matured their seeds in June. The 'Allı Gelin' seeds from these four purposefully selected lines were collected and dried under laboratory conditions. The seeds from these lines were then sown in trays on October 17th for seedling production, with the intention of planting them in the field the following year.

3.4. 2019-2020 (Examination of elite lines)

Our study commenced in March 2019 with the germination of seeds from elite plants, which had been sown in trays in October 2018. During this period, the germinating seeds were transferred to cell packs for seedling development, and necessary care was provided until they reached 6-7 leaves by May. In May, the seedlings were planted in the field.

In April 2020, the plants of the four 'Allı Gelin' lines, which began forming flower buds, were individually isolated within their respective lines using insect mesh (9×6, 30 mesh) covered iron cages (Figure 4). In the 'Allı Gelin' lines, inbreeding depression was observed in the tightly spread compact line, characterized by a reduction in flower diameter and seed size. Gökçora (1969) reported that in cross-pollinating plants, self-pollination of a single plant could lead to a regression in various characteristics in the offspring. To prevent inbreeding depression, each line was subjected to collective isolation within itself. From the plants

Figure 2. Self-pollination study.

Figure 3. Images of the selected four lines (1: Compact, 2: Tightly spread compact, 3: Pyramid, 4: Pyramid 2).

Figure 4. Collective isolation within the lines.

Table 2. Morphological measurements and observations for the tightly spread compact-1 line.

No	Morphological measurement and observation criteria	Descriptions
1	Plant height (cm)	25.6-32.5 (Min.-Max.)
2	Branching condition of stem	20-40 (Min.-Max.)
3	Stem leaf condition	Present
4	Flowering date	09.05.2022-02.06.2022
5	Flower color	RHS 85B-82C-76B-77B-155B-155D
6	Flower cluster diameter (cm)	41-60 (Min.-Max.)
7	Type of flower cluster	Tightly spread compact
8	Flower longevity (days)	35-40 (Min.-Max.)
9	Petal width (mm)	0.3-0.4 (Min.-Max.)
10	Petal length (mm)	0.5-0.7 (Min.-Max.)
11	Number of petals	4
12	Fragrance	Fragrant
13	Hairiness	Present
14	Number of leaves	392-812 (Min.-Max.)
15	Leaf color	RHS 137A-137C
16	Leaf shape	Between reverse lanceolate and spoon-shaped
17	Leaf width (cm)	1.2-3.0(Min.-Max.)
18	Leaf length (cm)	5.4-10.5 (Min.-Max.)
19	Seed maturation time	19.08.2022

caged within the 'Allı Gelin' lines, four lines forming seeds in the desired forms (compact, pyramid, tightly spread compact) were selected and named.

3.5. 2021-2022 (Baby plant testing)

The study in 2021 focused on growing seedlings of elite plants and planting them in the field. In 2022, the project began with the plants emerging from dormancy in March, following the winter of 2021, and the plants were monitored daily. In April, observations were made on the plants of the four 'Allı Gelin' lines as they started forming flower buds. The Pyramid 2 line failed to achieve stability and was therefore eliminated from the project. In the Tightly Spread Compact-1 and Pyramid-1 forms, pure lines were established, and non-target individuals were removed from the field, achieving a 98% rate of stability, and these lines were designated as variety candidates. For the Compact line, selections of individual plants in the desired form were made and isolated in iron cages covered with insect mesh (9×6, 30 mesh). The morphological measurements and observations of the promising variety candidates from the Tightly Spread Compact-1 and Pyramid-1 forms are presented in Table 2 and Table 3.

The plant height of the tightly spread compact variety candidate ranges from 25.6 to 32.5 cm. Among the three forms, only the tightly spread compact form exhibits branching, with 20-40 branches emerging from the stem. The diameter of the flower cluster varies between 41-60 cm, and the flowers remain blooming for 35-40 days. The flowers are fragrant. The number of leaves is quite high, ranging from 392 to 812 leaves (Figure 5).

Among the tightly spread compact, compact, and pyramid forms, individuals of the pyramid form were taller. The height of the plants in the pyramid variety candidate ranged between 38.5-43.5 cm. No branching was observed on the stem in the pyramid form. The diameter of the flower cluster varied between 20-50 cm, and the duration of flower retention was between 49-53 days. The flowers were fragrant. The number of leaves ranged between 50-123 (Figure 6).

Table 3. Morphological measurements and observations for pyramid-1 line.

No	Morphological measurement and observation criteria	Descriptions
1	Plant height (cm)	38.5-43.5 (Min.-Max.)
2	Branching condition of the stem	None
3	Stem leaf condition	Present
4	Flowering date	28.04.2022
5	Flower color	RHS N82C-RHSN81B-RHS76B
6	Flower cluster diameter (cm)	20-50 (Min.-Max.)
7	Flower cluster type	Pyramid
8	Duration of flower retention (days)	49-53 (Min.-Max.)
9	Petal width (mm)	0.3-0.5 (Min.-Max.)
10	Petal length (mm)	0.8-0.9 (Min.-Max.)
11	Number of petals	4
12	Fragrance	Fragrant
13	Hairiness	Present
14	Number of leaves	50-123 (Min.-Max.)
15	Leaf color	RHS137C
16	Leaf shape	Between inverted lanceolate and spoon-shaped
17	Leaf width (cm)	2.2-3.0 (Min.-Max.)
18	Leaf length (cm)	13-15.4 (Min.-Max.)
19	Seed maturation time	19.08.2022

Figure 5. Tightly spread compact variety candidate.

Figure 6. Visuals of the pyramid-1 line.

Both forms of promising variety candidates have wide flower forms, are fragrant, and also possess showy flower clusters. In light of these characteristics, trials for the registration process of these outdoor ornamental plant variety candidates have been set up within 2023, and applications for registration have been made.

Among 2000 Paeonia varieties collected from various countries around the world, a relative evaluation was made considering their general appearances, initially selecting 81 varieties. From these varieties, based on criteria such as leaf type, color and shape, flower cluster type and size, petal size, number and color, stem height, branching number, and flowering date, ultimately 9 varieties were selected for use. These were classified according to their usage possibilities as cut flowers, perennial garden flowers, and pot flowers (Choi, 1994). This study shows parallels with our research.

In the breeding research on the *Anemone coronaria* L. (poppy anemone) species, Kostak and Köse (1998) identified 8 clones of yield and quality suitable for use as cut flowers, based on phenological and morphological observations on samples collected from various regions. However, since the breeding studies were not continued, the variety stage could not be reached. Our study has reached the stage of variety registration by following the appropriate stages for breeding.

In the study conducted by Kaya et al. (2009) all regions of the Turkish Flora were surveyed; 1166 populations encompassing 177 geophyte species, 20 dunes, and 44 outdoor ornamental plant species, totally 241 species, were identified. Plant samples were selected through preliminary selection, transferred to culture conditions, and preserved in responsible institutions. Of these collected plants, 70 species with economic potential were selected and introduced to the ornamental plants sector. The results of this study align with ours.

Similarly, in the study "Cultivation and Breeding of Turkish Peonies" conducted by Kaya (2010) based on selection breeding, 6 varieties suitable for use as outdoor ornamental plants, 2 as pot plants, and 3 as cut flowers were identified, considering criteria like flower color, numerous flower clusters, double or semi-double, flower type, leafy multiple branching, relatively short height, flowering vegetation period, thick flower stem, vase life, etc.

In the project conducted by Kaya et al. (2015) aimed at developing varieties of Turkish *Fritillaria* species, 22 varieties were developed from natural populations using selection and mutation breeding methods. Important selection criteria such as plant propagation speed, plant growth strength, application area, flowering date, number of flowers, flower life, flowering vegetation period, flower shape, flower diameter, tepal size, color, position, leaf shape, number, color, and position were considered in single selection of flowering types, and among the developed varieties, Vuslat (*F. imperialis*), Doğu Güneşi (*F. aurea*), and Aslay (*F. michailovskyi*) were protected for breeder's rights (Aslay, 2015; Yıldız et al., 2022; Aslay et al., 2023).

Our selection study in the 'Allı Gelin' plant differs from other conducted studies in that it develops varieties of a special species capable of tolerating the effects of drought, one of the negative impacts of global climate change, and thriving under limited maintenance conditions, thereby making these developed varieties unique in this aspect.

4. Conclusion

The ornamental plant sector's key objectives involve prioritizing the development of drought-resistant species and varieties that require minimal maintenance. Furthermore, establishing a gene pool for the long-term breeding of crucial species within the sector is crucial. Incorporating native plants into landscaping projects not only boosts success rates and decreases expenses but also bolsters the national economy. To this end, our study focused on the 'Allı Gelin' plant, an endemic species possessing these desired characteristics. We utilized the single selection breeding method, resulting in stable compact and pyramid-1 forms. We have also initiated registration procedures for the newly developed variety.

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