Journal homepage
www.ajas.uoanbar.edu.iq
Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

PHENOTYPIC CHARACTERISATION OF F1 HYBRIDS DERIVED FROM CROSS-BREEDING DOUBLE AND SINGLE PETUNIAS (PETUNIA HYBRIDA)

A. A. Sharef [1*] **J. M. Faraj [1]** **H. A. Mustafa [1]** **H. S. Othman [2]**
N. H. Abdul [2] **N. H. Samsulrizal [2]**

[1] University of Sulaimani, College of Agricultural Engineering Sciences, Horticulture Department
[2] Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

***Correspondence to:** Ashtekhwaz Ahmad Sharef, Department of Horticulture, College of Agricultural Engineering Sciences, University of Sulaimani, Iraq.
Email: ashtikhwaz.sharif@univsul.edu.iq

Article info
Received: 2024-04-25
Accepted: 2024-05-31
Published: 2024-12-31

DOI-Crossref:
10.32649/ajas.2024.147846.1167

Cite as:
Sharef, A. A., Faraj, J. M., Mustafa, H. A., Othman, H. S., Abdul, N. H., and Samsulrizal, N. H. (2024). Phenotypic characterisation of f1 hybrids derived from cross-breeding double and single petunias (petunia hybrida). Anbar Journal of Agricultural Sciences, 22(2): 962-980.

Abstract
Part of the Solanaceae family, petunias possess a wide range of flower colors and patterns. In this study, we crossed two types of petunias i.e., the single blackberry (as a female parent) and double (male parent). Seeds obtained from this cross were grown and the phenotypic characters of 85 plants were analysed including the number of days of first flower emergence, flower diameter, peduncle length, number of flowers, number of branches, plant height, pollen color, number of petals (single or double flower), and petal color. Of particular interest is that the double flower inherited the F_1 generation and recorded 54.1%, the single flower recorded 45.9%, and a wide range of flower colors were light pink to dark purple. It was noted that 67.1% of the plants had light purple pollens and 32.9% showed yellow colors. Based on the coefficient of variation (CV) and box plot analysis, the flower diameters showed moderate variability than other traits. Principal Component Analysis (PCA) revealed distinct patterns and associations among phenotypic traits. PC1 primarily reflected several traits such as plant height, number of flowers, flower diameter, and single or double

petunias. However, PC2 was predominantly influenced by pollen and petal color, stem length, number of days of first flower emergence and number of branches. Besides, data clustering analysis also identified distinct clusters based on phenotypic traits in the petunia population, which can be exploited for future breeding programs.

Keywords: Petunia hybrida, Cross breeding, Germplasm, Commercial cultivars.

التوصيف المظهري لهجن F1 المشتقة من التهجين بين زهور البتونيا (Petunia hybrida) القطمر والمفردة

اشتخواز أحمد شريف [1]* جمال محمود فرج [1] هيمن عبدالله مصطفى [1]

حليمتون سعدية عثمان [2] نور الهدى عبد [2] نور الهداية سامسولريزال [2]

[1] قسم البستنة، كلية علوم الهندسة الزراعية، جامعة السليمانية

[2] قسم علوم النبات، كلية العلوم، الجامعة الإسلامية العالمية ماليزيا، 25200 كوانتان، باهانج، ماليزيا

***المراسلة الى:** اشتيخواز احمد شريف، قسم البستنة، كلية علوم الهندسة الزراعية، جامعة السليمانية، السليمانية، العراق.

البريد الالكتروني: ashtikhwaz.sharif@univsul.edu.iq

الخلاصة

تنتمي زهور البتونيا (*Petunia hybrida*) إلى العائلة الباذنجانية ولها مجموعة واسعة من الألوان والأنماط الزهرية. في هذه الدراسة قمنا بتهجين نوعين من زهور البتونيا التجارية المفردة (الام) والقطمر (الاب). ثم زرعت البذور التي تم الحصول عليها من هذا التهجين وتم تحليل الصفات المظهرية لـ 85 نبات بما في ذلك عدد أيام ظهور الزهرة الأولى، قطر الزهرة، طول ساق الزهرة، عدد الزهور، عدد الفروع، ارتفاع النبات، لون حبوب اللقاح، عدد البتلات (زهرة مفردة أو قطمرة) ولون البتلات. من اهم النتائج التي تم الحصول عليها هي أن صفة القطمر شهدت أكثر تعبيرا في الجيل الاول بنسبة 54.1% في حين سجلت الزهور المنفردة 45.9% وتم الحصول على مدى واسع من ألوان البتلات من الوردي الفاتح إلى الأرجواني الداكن حيث 67.1% من الأزهار احتوت على حبوب لقاح ذات لون أرجواني فاتح و32.9% منها تميزت باللون الأصفر. استنادا إلى معامل التباين (CV) ونتائج مخطط الصندوق، اظهرت قطر الزهرة تباينا معتدل من الصفات الأخرى. كشف تحليل المكونات الرئيسية (PCA) عن أنماط وارتباطات متميزة بين الصفات المظهرية. حيث ارتبط PC1 في المقام الأول بالعديد من الصفات مثل ارتفاع النبات وعدد الازهار وقطر الزهرة والازهار المفردة أو القطمرة. في حين تأثر PC2 في الغالب بالمتغيرات المتعلقة بلون حبوب اللقاح ولون البتلة وطول الساق وعدد أيام ظهور الزهرة الأولى وعدد الافرع. علاوة على ذلك، افرز تحليل المجموعات مجموعات متميزة بناءً على الصفات المظهرية في المجتمع المدروس. بالإضافة إلى المساهمة في فهمنا لبيولوجيا زهور البتونيا، توفر هذه النتائج أساسًا للبحث المستقبلي حول وراثة التباين

المظهري في زهور البتونيا، والتي يمكن استغلالها في برامج التربية المستقبلية وتطوير أصناف تجارية جديدة في العراق.

كلمات مفتاحية: البيتونيا، تربية النبات، الأصول الوراثية، الأصناف التجارية.

Introduction

Petunias belong to the Solanaceae family and are among the most annual floricultural crops that originated in South America. It is believed that *Petunia hybrida* was generated from an interspecific hybridization between two petunia wild parents, *Petunia axillaris* and *Petunia integrifolia* (or its related species *Petunia inflata*) (29). Petunia species differ significantly in terms of plant size and shape, as well as flower color and morphology. Commercial petunia cultivars do not produce seeds without insect or manual pollination due to heteromorphic self-incompatibility (23). Usually, the female parts of double petunias are sterile, which mean that they have an inbreeding depression. *Petunia axillaris* is native to South America and exhibit small, white, and highly fragrant flowers pollinated by hawkmoths. On the other hand, *Petunia integrifolia*, also originating from South America, display larger, non-scented funnel-shaped flowers with a variety of colors, including shades of purple, red, and pink that are pollinated by bees (14). There is, however, the possibility of manual cross-pollination of natural petunia species. Breeders use interspecific hybridization to create genotypes that combine novel characteristics from other species; it involves crossing species as well as genus barriers. New phenotypes have been developed through heterosis, some cultivated traits improved through cross-breeding between species (7), and also used to overcome the self-incompatibility phenomenon (9 and 31). In the 19th century, through the first interspecific crosses, *Petunia hybrida* or the garden petunia was produced. In addition, European breeders crossed multiple accessions of the wild parent species to produce the first hybrids (26). Commercial petunias have undergone intense breeding since the first interspecific hybridizations resulted in modern varieties of flower traits. Petunia types exhibit enormous variations due to the introgression of mutant alleles from wild species and new mutations occurring during breeding programmes (12).

Renowned for its vibrant and diverse array of flower colors and patterns, the petunia has become a widely cultivated ornamental plant in gardens, parks, and hanging baskets worldwide. Its captivating blooms and versatility in various growing conditions make it a favorite choice among gardeners, horticulturists, and plant enthusiasts. Today, cultivars of petunia are found in an astounding range of colors including whites, yellows, pinks, mauves, reds, magentas, lavenders, and purples with one notable exception of blue. During development, color can be easily quantified and monitored in different tissues. Numerous studies show that flower color is important to pollinators (33) and some have directly linked single genes to pollinator preference (15). The floriculture industry values petunia as an ornamental plant with global commercial value (1). There is a strong demand for this plant in the ornamental plant market, ranking first in auctions and reaching an annual value of US$130 million (3). This is due to seed demand in several countries, such as the Netherlands, Poland, and Germany which import 31.25 kg, 7.3 kg, and 4.16 kg per month, respectively (19). An intriguing aspect of petunias is their floral morphology, specifically the occurrence of single and double flowers. Several studies have identified key genetic and molecular factors associated with single and double-flower formation in petunias. The double-flower trait is often associated with mutations or alterations in floral organ identity genes, such as the class B and class C genes. These genes regulate the development of petals and stamens, and mutations in them can lead to the conversion of stamens into petals, resulting in the formation of extra petals and a double flower appearance (27).

Research shows that mutations in genes like DEFICIENS (DEF) and GLOBOSA (GLO) can lead to double flower formation by disrupting the balance between petal and stamen development (34). The single and double flower traits in petunias have significant implications for horticultural purposes. Double-flowered cultivars are often favored for their ornamental value due to their showy and fuller appearance. They are commonly used in floral displays and landscaping projects to add visual interest and color. Single-flowered cultivars, on the other hand, might be preferred in certain scenarios where a more natural or understated aesthetic is desired.

Currently, there are many commercial petunia cultivars available around the world as a result of vigorous breeding works. Unfortunately, there have only been limited attempts at petunia breeding in Iraq (23). Double petunia seeds are extremely costly and the flowers are very attractive. However, as the female part is sterile it is unable to produce seeds. Therefore, this research is a preliminary attempt at improving petunia breeding, specifically on developing double petunias. The findings of this study can aid in the development of suitable ornamental germplasm for future breeding programs and commercial exploitation either for the local or export markets.

Materials and Methods

Plant material: This research was conducted at the Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Iraq, from October 2022 to May 2023. Earlier, two cultivars of double petunias, one with a purplish pink (male) color and the other a single purplish black (female) color, were grown. As the double petunia was female sterile, it was used as the male parent, and therefore reciprocal crosses were not possible. The two cultivars were compatible together and their seeds were harvested and grown. A total of 85 F1 progenies plants were used in this study and grown in a greenhouse at a temperature of 24/22°C at 16/8 hr day/night cycles. The seeds were placed on the surface of peat moss contained in a small pot with a transparent cover as petunia seeds require light for germination. After two weeks, the seedlings were transferred to the seedling tray and then to the bigger pot after they had reached the appropriate sizes.

Phenotypic traits measurement: Full-bloom flowers were chosen in this study for phenotypic diversity analysis. Nine phenotypic traits were observed i.e., the number of days of first flower emergence, flower diameter, peduncle length, number of flowers, number of branches, plant height, pollen color, single or double flower, and petal color. The phenotypic traits data were recorded after six months of growing the plants. Table 1 shows the standards for determining the respective specific phenotypic features.

Table 1: Morphological diversity indexes and description of petunias.

Traits	Phenotypic Traits Description
Number of days of first flower emergence	Number of days from seed planting until the opening of the first flower
Flower diameter	The two farthest points between the corolla were measured using six flowers from each plant
Flower stem length (peduncle)	Measured from the end of the sepals to the beginning of the stem
Number of flowers	Number of flowers for each plant
Number of branches	Number of branches for each plant
Plant height	Measured from the farthest points of the branch to the plant crown
Pollen color	Light Purple =1, Yellow =2
Single or double flower	Plant classified based on petal fringe
Petal color	Purplish black =1; Purplish pink=2; Dark pink =3 Pink=4; Light orange=5; Dirty pink=6 Light red=7; Rose pink=8; Dark purple=9 Pinkish white=10; Light purple=11 Dark pink and white=12; White and dark purple =13

Statistical analysis: Descriptive statistics analysis was derived using SPSS 22.0. The genetic relationships among different petunia F1 progenies were analyzed through a two-step cluster analysis using SPSS 22.0 which can handle mixed data types including continuous, categorical, and binary variables making it versatile for various types of datasets in phylogenetic studies. The cluster membership data generated by the two-step cluster analysis was later used to create a dendrogram using hierarchical clustering. The XLSTAT software package (version 2021, Addinsoft, Paris, France) was also used for data analysis in this study. This powerful statistical software provides a wide range of analytical tools and functions. In this study, two types of analysis were done using XLSTAT: 1- Box Plot Analysis, and 2-Principal Component Analysis (PCA) (25).

Results and Discussion

Petunia flower variations: The crosses in this F_1 progenies breeding program mainly produced light purple and yellow pollen colors. The pollen color of the maternal parent (blackberry) was purple whereas that of the paternal parent was yellow. Of the 85 progenies, 67.1% showed light purple pollen and the remaining 32.9% were yellow (Figure 1A).

The proportion of single- to double-flowered plants varied slightly from 1:1. The F1 progenies produced after crossing consisted of 45.9% single flowers and 54.1% double flowers (Figure 1B).

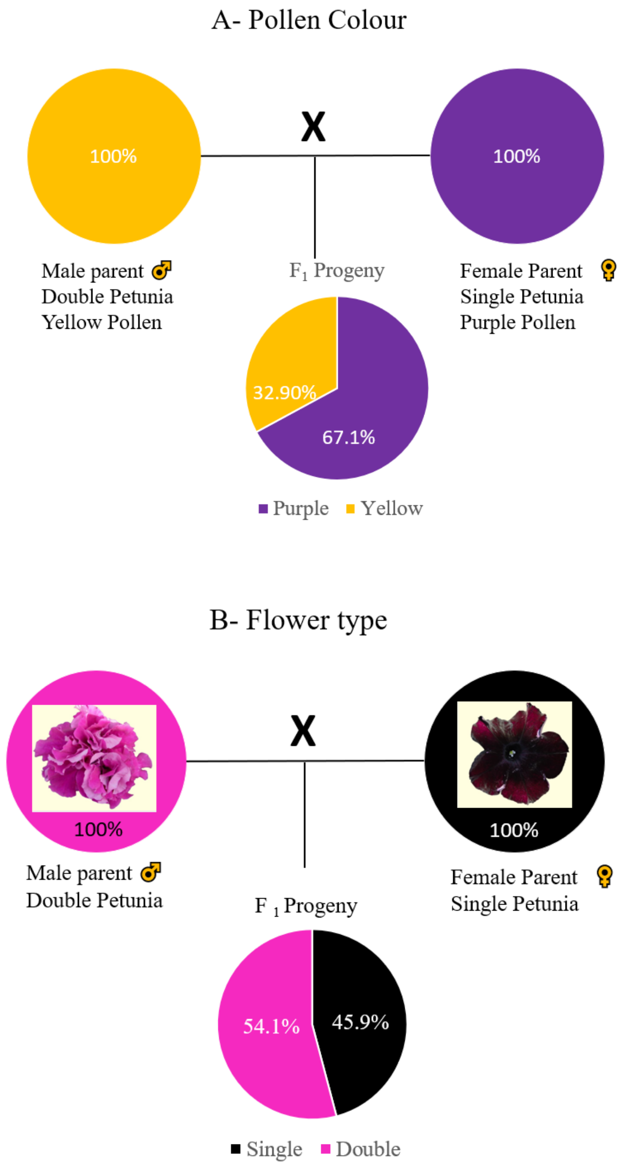

Figure 1: (A) Segregation of pollen color in the F_1 hybrid with 67.1% and 32.9% inheriting purple and yellow colors, respectively. (B) Segregation of flower type in the F_1 hybrid with 54.1% and 45.9% single and double flowers, respectively.

For flower color, the variations were great, with 40% purplish black flowers, 9.4% each of dark pink, dirty pink, light red, and dark purple flowers, 7.1% purplish-pink flowers, 5.9% purplish white flowers, 3.5% pink flowers, 1.2% each of light orange, rose pink, light purple, dark pink and white flowers, and white and dark purple flowers (Figure 2).

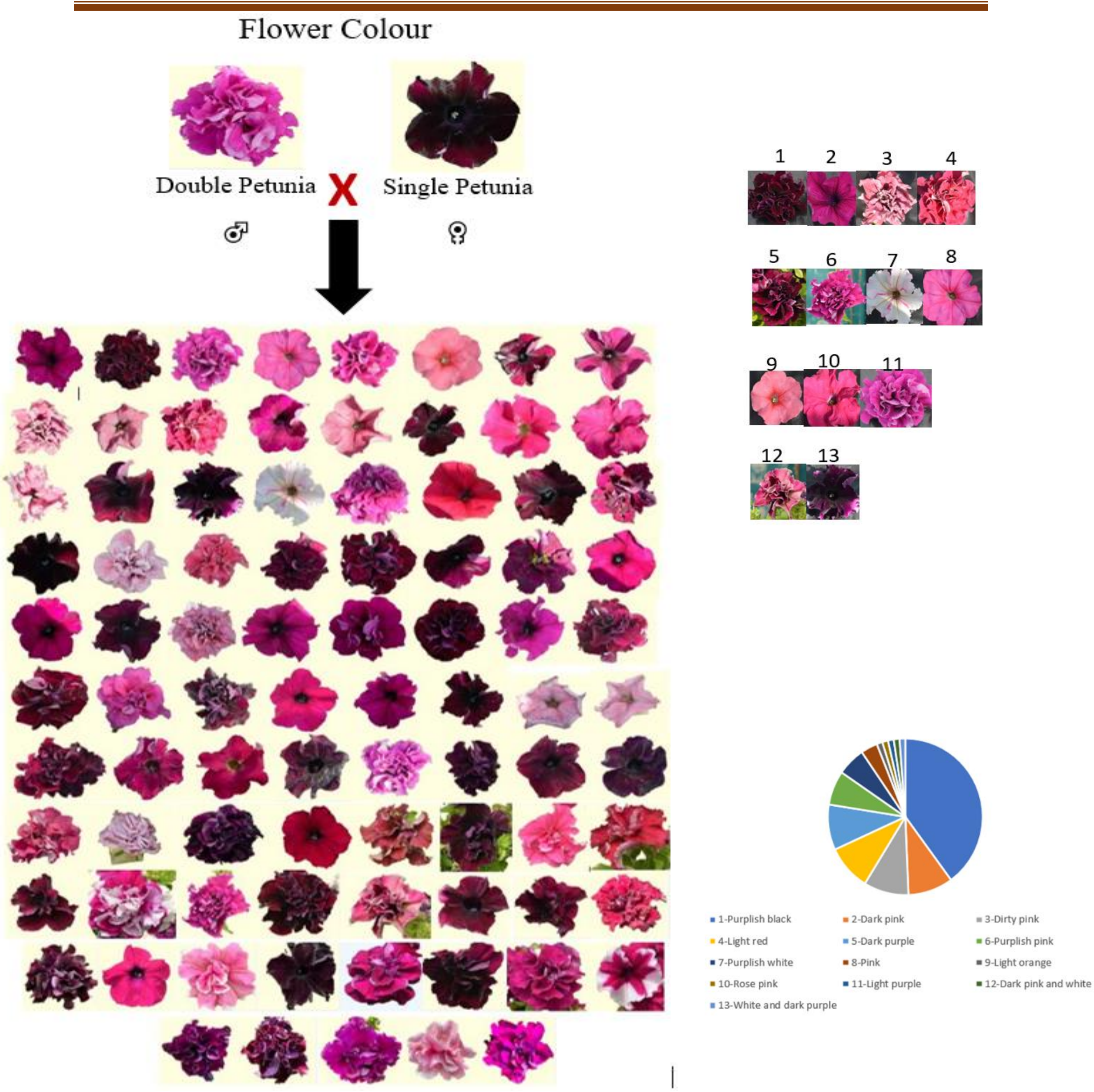

Figure 2: Segregation of flower colors in the F_1 generation: 40% had purplish black flowers 1; 9.4% dark pink flowers 2; 9.4% dirty pink flowers 3; 9.4% light red flowers 4; 9.4% dark purple flowers 5; 7.1% purplish pink flowers 6; 5.9% purplish white flowers 7; 3.5% pink flowers 8; 1.2% light orange flowers 9; 1.2% rose pink flowers 10; 1.2% light purple flowers 11; 1.2% dark pink and white flowers 12; and 1.2% white and dark purple flowers 13. Bar=1cm.

The near-isogenic line (NIL) population used in this study consisted of blackberry for single-flower and purplish pink for double-flower plants. These two cultivars were indistinguishable until upon blooming. Of the two closed buds, the single-flower buds opened earlier, with the absence of sepals to cover the internal organs (Figure 3).

Figure 3: Morphological differences between single and double flowers of petunia (A) Blackberry (♀); (B) Purplish pink (♂); (C) F_1 progeny (single petunia); and (D) F_1 progeny (double petunia). Bar = 1cm

In general, and in terms of ornamental value, there are two different types of petunia i.e., single and double. Doubleness is considered a crucial trait in breeding petunias because of its attractiveness and economic value as it has a bigger flower size and more petals. Flower-doubleness inheritance eluded researchers for a long time. Double-flowered plants were propagated by cuttings, from the time of the mutation in 1855 until the early 1930s. In addition, the double flower was crossed with a single flower to produce single and double flowers. However, reciprocal crosses cannot be done as the double flower is female sterile. For the first time, (22) attempted to understand the genetics of doubleness in petunias. However, similar results as previously were obtained regarding producing seeds due to a lack of pistil development. (8) reported that the single factor (d) is recessive, and the double factor (D) is dominant since crosses between single and double produce both single and double plants, whereas the single self produces only single plants. These results were further confirmed as they were observed in petunias. A single dominant gene, D, is allelic to single flowers, while recessive gene d, controls the inheritance of floral doubleness. Since the females of the double-flowered phenotype are typically sterile, they can only be used as the male parent for seed production. In addition, only the homozygous genotype (DD) of double petunia and female fertile single can be crossed to provide 100% double-flowered hybrid seed for commercial uses (10 and 18).

Petunia colors have long been the subject of research (11). Nine genes were identified in one genetic study, which were involved in the inheritance of flower colour (20). Later, more detailed information about the anthocyanins of the different color classes of petunia hybrids became available, confirming the turning from the inheritance of flower colour to the inheritance of specific anthocyanins (16). The diversity of anthocyanin structures is one of the key factors contributing to the existence of flower color variation in petunias. In addition, the pH inside the vacuole, the vacuolar lumen also largely affects flower color (32). Depending on both the structural transformation and the pH shift of the solvent, anthocyanins exhibit a reversible structural transformation in their chromophores. Flowers grown in different media, have an acidic PH, and exhibit different colors. Very slight changes in pH are known to affect flower color and dramatically affect their absorption spectra (11 and 13). The obtained results indicate that double petunias can be inherited to the next generation, which is a crucial point as we are in the initial stage of petunia breeding in Iraq. In addition, all colors and patterns obtained from crossing these two parents are attractive indicating that they are suitable for use as stock for the continuous hybridization of petunias.

Analysis of the genetic diversity of phenotypic traits in petunias: The genetic diversity of the 85 petunia samples was examined in addition to their parents. The phenotypic data (Table 2) illustrates the coefficient of variation (CV) of the quantitative traits ranging from 0.07% to 78.78%, with the average CV at 30.98%. The CV is a statistical measure for assessing the relative variability of quantitative traits within a population. In this study, the CV was calculated for various quantitative traits in petunia plants to examine the extent of phenotypic variation and determine traits with the highest degree of variability.

The CV analysis revealed notable differences in the variability of quantitative traits among the petunia population. Flower diameter exhibited a CV of 16.23%, indicating a moderate level of variation within this trait. This suggests that genetic and environmental factors contribute to the observed differences in flower diameter among individual petunia plants (2). Other traits, such as number of days of first flower emergence and plant height had CVs of 0.07% and 18.48.7%, respectively, indicating relatively lower levels of variability compared to flower diameter.

Table 2: Genetic diversity analysis of quantitative characters in petunias.

	N	Minimum	Maximum	Mean		SD	CV
Trait	**Statistic**	**Statistic**	**Statistic**	**Statistic**	**Std. Error**	**Statistic**	**Statistic**
Flower Diameter	85	4.90	11.00	8.2928	.14599	1.34592	16.23
Peduncle Length	85	2.00	14.50	3.5968	.18259	1.68340	46.80
Number of Flowers	85	2.00	156.00	33.0824	2.82675	26.06138	78.78
Number of Branches	85	3.00	11.00	6.1529	.17027	1.56985	25.51
Plant Height	85	30.00	66.00	47.2529	.94712	8.73200	18.48
Number of Days for First Flower Emergence	85	116.00	149.00	133.3529	1.01249	9.33466	0.07

Box plot analysis for phenotypic variation in petunias: The box plot analysis (Figure 4) revealed interesting patterns and characteristics of the phenotypic traits in petunia plants. For flower diameter, the box plots showed a relatively symmetric distribution with a median value of 8.5 cm. The interquartile range (IQR) indicated moderate variation, with the lower and upper quartiles spanning 7.4 cm to 9.3 cm, respectively. However, few outliers were identified, displaying unusually large or small flower diameters. In contrast, the box plots for stem length displayed a negative skewed distribution. The median value was 3.1 cm, while the IQR spanned 2.7 cm to 3.6 cm. It also displayed the left-skewed distribution for the number of flowers and branches with median values of 30 and 6.0, respectively. Notably, there were certain outliers observed for this trait, with less variable distribution of stem length, number of flowers and branches within the petunia population.

In comparing the box plots of flower diameter and stem length, and number of flowers and branches, it is evident that the former exhibits a wider range of variation and a greater number of outliers, indicating a higher degree of phenotypic diversity within this trait. This finding suggests that stem length, number of flowers and branches may be influenced by multiple genetic and environmental factors, resulting in a broader range of observed values. In contrast, the relatively narrow distribution of flower diameter indicates a more consistent and genetically controlled trait. The presence of outliers in the box plots warrants further examination. The outliers observed in the number of flower traits may indicate the presence of rare or novel genetic variants that significantly impact this trait (24). Alternatively, these outliers could be attributed to measurement errors or environmental factors that influence flower development in specific cases (28). Further investigations, such as genetic analyses or controlled growth experiments will help elucidate the causes of these outliers (9).

The distribution and variations observed in the phenotypic traits of petunia plants have biological implications. The wide range of flower number values suggests potential adaptations to different pollinators or ecological conditions (4). Petunias with more flowers may attract larger pollinators, compared to those with less flowers. Stem length, on the other hand, may reflect adaptations related to light capture or water regulation (5). It is important to note that this study focused on a specific set of phenotypic traits and a limited sample size. Thus, the observed patterns and variations may not fully represent the entire range of phenotypic diversity within the petunia population. Future research could incorporate additional traits, expand the sample size, or explore different petunia cultivars or species to gain a more comprehensive understanding of phenotypic variations.

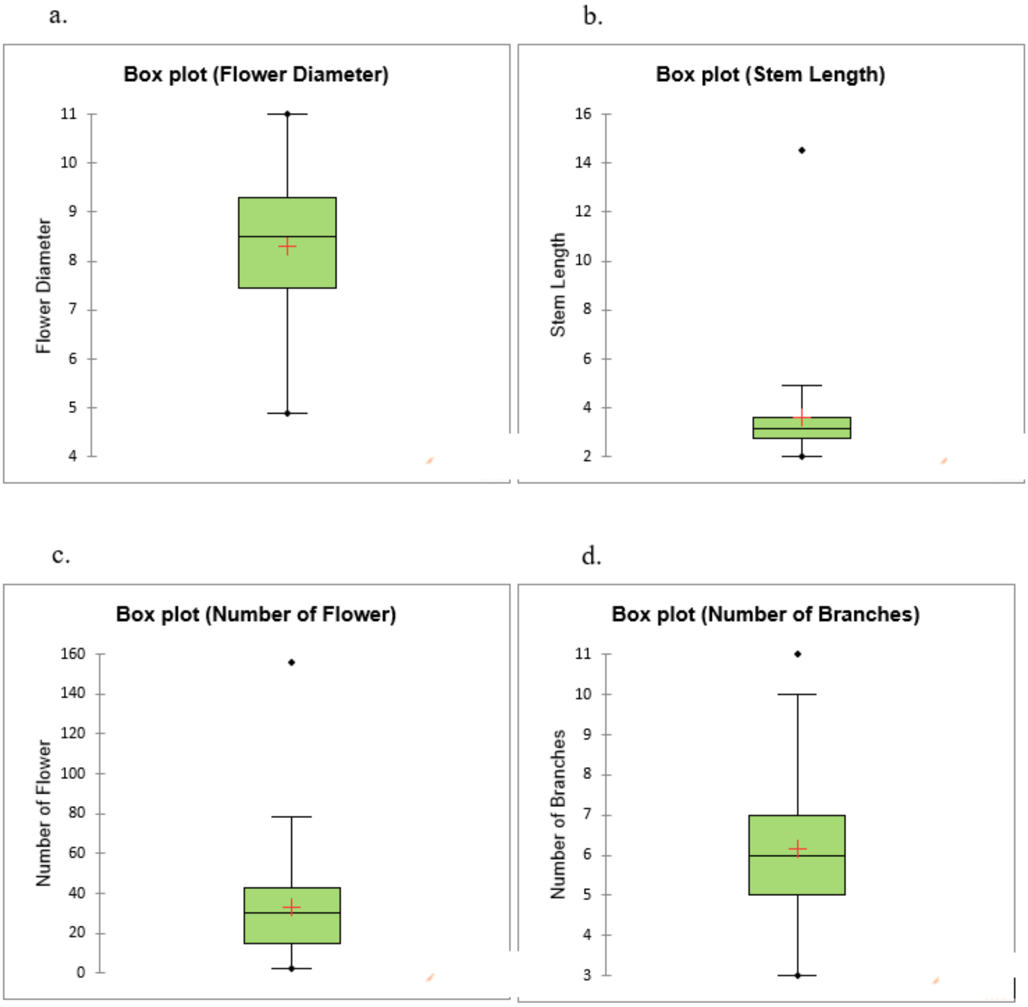

Figure 4: Box plot of phenotypic variation in petunias: (a) flower diameter; (b) stem length; (c) number of flowers; and (d) number of branches.

Principal component analysis (PCA) based on phenotypic traits: One purpose of this study was to investigate the relationship between phenotypic traits in petunia F1 progenies using PCA. By analyzing a comprehensive dataset of various phenotypic characteristics, we aimed to uncover underlying patterns, identify key variables driving phenotypic variations, and gain insights into the biology of petunia plants. The PCA (Figure 5) results revealed that the first two principal components (PCs) accounted for a significant proportion of the total variance, collectively explaining 44.2% of the variation observed in the phenotypic traits. PC1 explained 23.7% of the variance and PC2 explained 20.5% (Table 3). These findings indicate that a substantial portion of the phenotypic diversity in petunia progenies can be attributed to these PCs.

In interpreting the principal components, we observed that PC1 was predominantly influenced by variables related to plant height, number of flowers, flower diameter and single or double petunias. This suggests that these traits are closely interconnected and may be controlled by shared genetic factors or common physiological mechanisms (6). The strong association between types of single or double flowers and the number of flowers aligns with previous studies highlighting the role of specific genetic loci in determining petal number and flower morphology in petunia. These findings confirm the presence of a dominant gene D responsible for the double flowering trait in petunias. Furthermore, the investigation of morphological developments in single and double flower forms of petunias revealed that the shoot apices differed only at the initiation point of petal and sepal primordia (18).

In contrast, PC2 primarily reflected other traits such as pollen and petal colors, stem length, number of days of first flower emergence, and number of branches. These characteristics likely represent adaptations related to the overall reproductive strategy and ecological niche of the petunia plant (35). Pollen and petal colors and number of branches may have evolved as adaptations in petunias to attract specific pollinator species and increase the likelihood of successful cross-pollination. The correlation between certain morphological traits in petunias and their attraction to pollinators highlights the intricate relationship between genetic profiles and flower characteristics (21). The distinct grouping of these traits in PC2 indicates their shared genetic basis or coordinated response to environmental factors.

The patterns observed in the PC scores further support the existence of distinct clusters among the phenotypic traits. For instance, plants with large flowers and thicker stems tended to have fewer flowers and lower plant height. This suggests a coordinated growth and development strategy in these individuals, potentially driven by shared genetic or hormonal mechanisms. The findings of this study align with previous research investigating petunia phenotypic traits, confirming the interrelationships and associations among various morphological characteristics (30). However, it is important to note that this study focused solely on phenotypic traits and did not explore underlying genetic factors or gene expression patterns. Future studies could incorporate genomic approaches, such as quantitative trait loci (QTL) mapping or transcriptomics, to further elucidate the genetic basis of these phenotypic traits.

The knowledge gained from this PCA analysis has implications for plant breeding and horticulture. By understanding the relationships and interdependencies among different phenotypic traits, breeders can strategically select individuals with desired combinations of traits for targeted improvement (17).

Table 3: Principal component analysis of phenotypic traits of petunia.

Trait	PC1	PC2	PC3	PC4	PC5
Flower Diameter	-0.456	-0.300	0.523	0.403	-0.186
Stem Length	-0.078	-0.185	0.826	-0.304	0.054
Number of Flowers	0.836	0.101	-0.195	0.163	-0.071
Number of Branches	-0.158	0.484	0.066	0.624	-0.467
Plant Height	0.781	-0.017	0.337	-0.084	-0.090
Number of Days for First Flower Emergence	0.077	0.384	0.207	0.492	0.748
Pollen Color	-0.079	0.812	0.016	-0.252	-0.073
Single or Double Flower	-0.754	0.135	-0.267	-0.167	0.118
Petal Color	-0.011	0.804	0.307	-0.271	-0.072

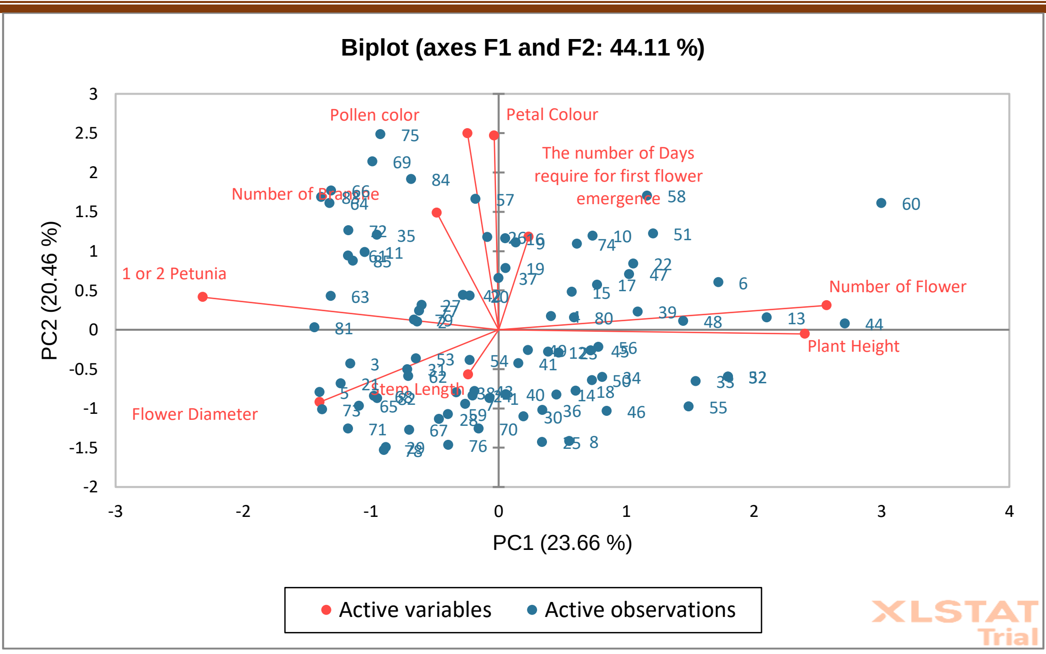

Figure 5: Principal component analysis of phenotypic traits in petunias.

Classification based on data clustering analysis: Data clustering was performed to find separate groups or clusters of petunia F_1 progenies based on phenotypic features. The analysis aimed to identify distinct clusters or groups of cultivars based on their genetic similarities, providing insights into the evolutionary history and classification of petunias. The two-step cluster analysis revealed three distinct clusters, labelled Cluster 1, Cluster 2, and Cluster 3, when nine predictors (inputs) were used. However, the cluster quality was only considered as fair. These three clusters were almost equally distributed with a ratio size of 1:19 between the largest and smallest clusters. Of the nine predictors, pollen color was the most important followed by flower type and flower color (Figure 6), with those having input importance below 0.2 considered less significant.

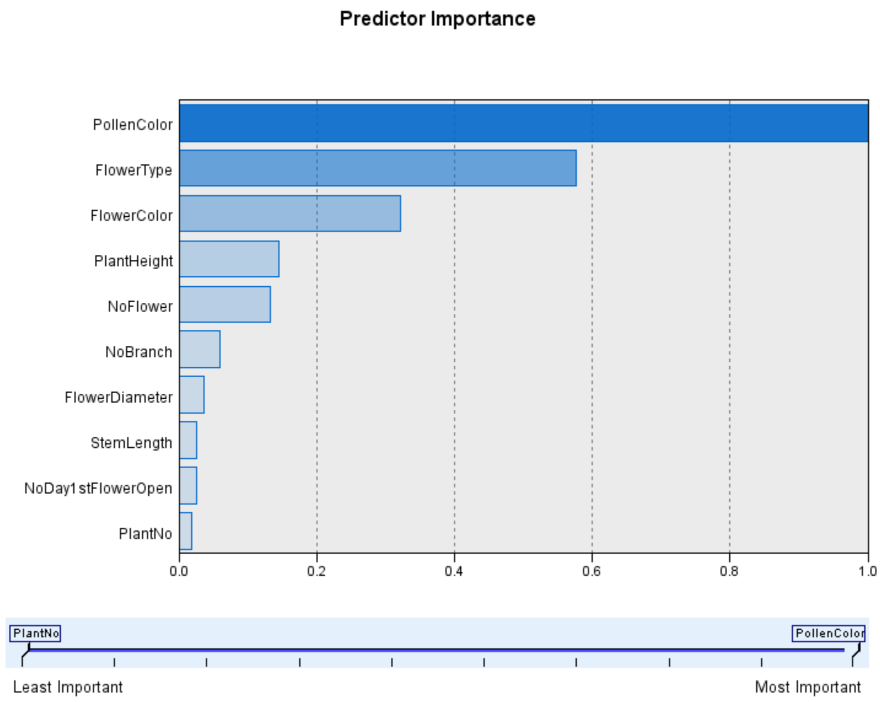

Figure 6: Predictor importance based on the two-step clustering analysis.

Cluster 1 contained a group of closely related petunia progenies with high genetic similarity to the male parent (Figure 7). These progenies share common genetic markers and likely originated from a common ancestor. Cluster 2 consisted of a set of petunia progenies displaying moderate genetic similarity, suggesting a broader range of genetic variations and possibly represent a group of progenies with more complex genetic relationships. Some progenies in this cluster might be the main outcome of hybridization or introgression between male and female parents, resulting in a mix of genetic characteristics. Cluster 3 comprised a group of slightly genetically-distinct petunia progenies. These progenies exhibited genetic dissimilarity from those in Clusters 1 and 2, indicating a separate evolutionary lineage or potentially representing the species origin (wild). In this data, Cluster 3 is a set of petunia progenies that have similarities with the female parent.

These clustering results align with previous studies that have explored petunia phenotypic diversity. However, our findings provide additional insights into the classification and characterization of specific trait profiles within the petunia population. By understanding the distinct clusters and their associated phenotypic traits, breeders and horticulturists can make informed decisions on cultivar selection, targeted breeding programs, or landscape design. It should be noted that this data clustering analysis has some limitations. This study focused solely on phenotypic traits and did not explore genetic or molecular factors that may underlie the observed phenotypic variations.

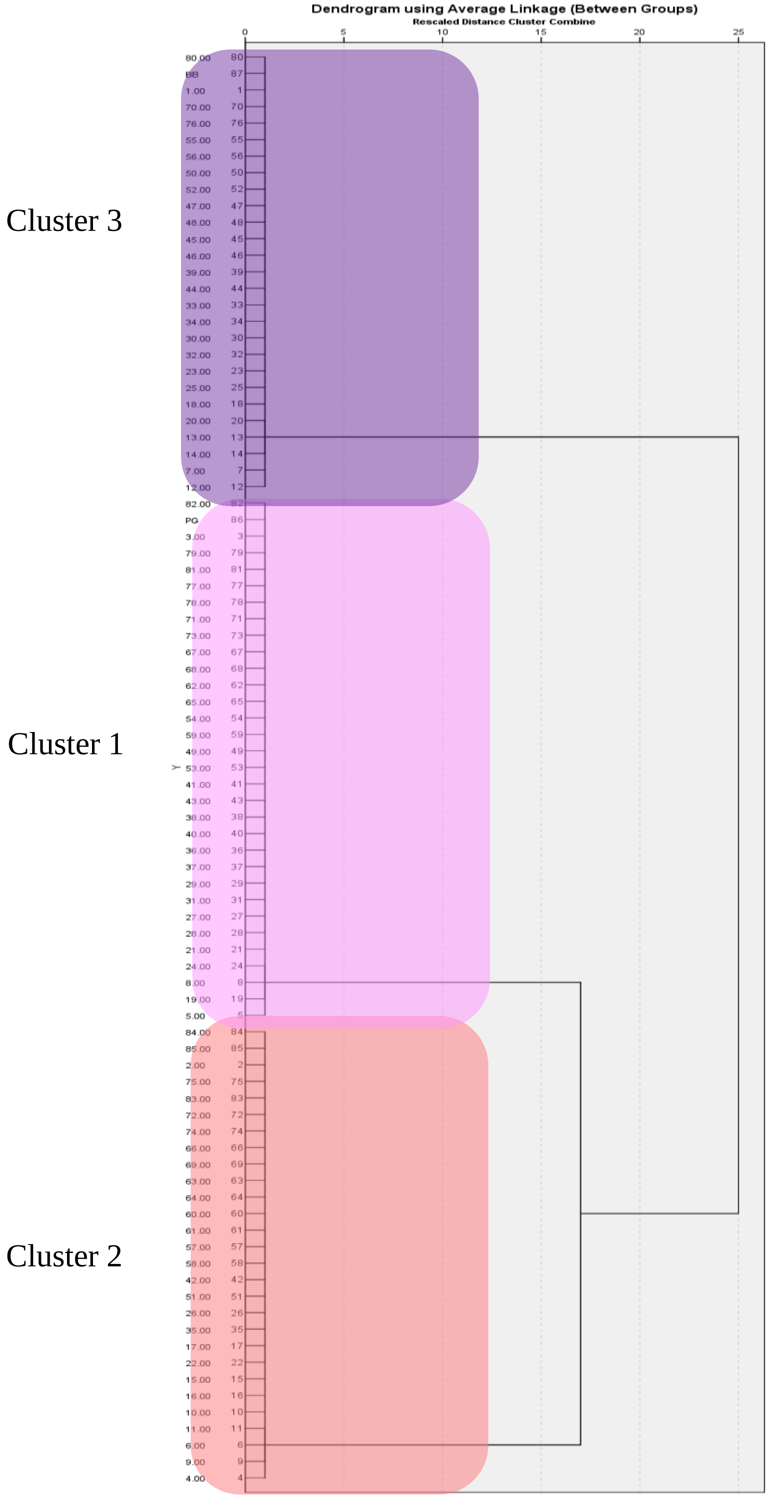

Figure 7: Cluster analysis dendrogram of 85 petunia F1 progenies based on phenotypic characteristics.

While acknowledging the importance of underlying genetics and molecular factors associated with phenotypic variation, this study specifically focuses on the morphological variations of petunias in Iraq. The scope of this research is designed to provide a comprehensive morphological assessment as a foundational step.

Future studies could benefit from integrating genetic and molecular analyses to further elucidate the underlying mechanisms of the observed phenotypic variations.

However, for this paper, we have chosen to concentrate on morphological characteristics to establish a clear baseline for subsequent research in these areas**.**

Conclusions

This study analyzed the phenotypic characteristics of 85 petunia F_1 progenies. We found that the phenotypic traits of the progenies were quite different, and 48 F_1 progenies were considered different types. CV analysis revealed differences in the variability of quantitative traits in the petunia F_1 progenies with a number of flowers exhibiting the highest CV, indicating a high level of variation. The box plot analysis provided insights into the distribution and variation of phenotypic traits in petunia plants. The observed patterns and characteristics of flower diameter and stem length highlight the diversity and genetic control of these traits within the petunia population. The findings contribute to our understanding of petunia phenotypic variations and offer valuable information for further investigations in regard to future petunia breeding.

Further, PCA analysis revealed distinct patterns and associations among phenotypic traits in the petunia plants. Also, data clustering analysis identified distinct clusters based on phenotypic traits in the petunia population. These findings advance our understanding of petunia biology and offer valuable insights for breeding programs and horticultural practices. Additionally, they contribute to our understanding of petunia biology and provide a foundation for future investigations into the genetic mechanisms controlling phenotypic variations. One limitation of this study is the reliance on a specific set of phenotypic traits, which may not capture the entire complexity of petunia morphology and physiology. Future research could consider incorporating additional traits or exploring different growth stages to provide a more comprehensive analysis of petunia phenotypic diversity.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Ashtekhwaz Sharef designed the paper and wrote the parts of the introduction, material and methods. Jamal Faraj and Hemn Mustafa collected and adjusted the data. The data were analyzed and interpreted by of Halimaton Othman, Nurul Abdul and Nurul Samsulrizal, Finally, Ashtekhwaz Sharef critically revised the manuscript for important intellectual content. All authors have read and agreed to the published version of the manuscript.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted by the protocol authorized by the University of Sulaimani, College of Agricultural Engineering Sciences, Horticulture Department and Ethics Committee.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The authors gratefully acknowledge the staff of the Department of Horticulture, College of Agricultural Engineering Sciences, University of Sulaimani, Iraq for their technical and general support. In addition, we appreciate Dr Zainab Sabah Lazim and Dr Haider Anwar Haider for the Arabic abstract evaluation.

Disclaimer/Journal's Note:

The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of AJAS and/or the editor(s). AJAS and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

References

1. Azizah, N. N., Mazieda, M. N., Listyorini, D., and Dahlia, D. (2014). Optimalisasi Isolasi dan Purifikasi DNA *Petunia hybrida* Seri Rose Picotedengan Kit Isolasi Geneaid. In Proceeding Biology Education Conference: Biology, Science, Environmental, and Learning. 11(1): 273-278.
2. Berenschot, A. S., Zucchi, M. I., Tulmann-Neto, A., and Quecini, V. (2008). Mutagenesis in *Petunia x* hybrida Vilm and isolation of a novel morphological mutant. Brazilian Journal of Plant Physiology, 20: 95-103. https://doi.org/10.1590/S1677-04202008000200002.
3. Bombarely, A., Moser, M., Amrad, A., Bao, M., Bapaume, L., Barry, C. S., ... and Kuhlemeier, C. (2016). Insight into the evolution of the Solanaceae from the parental genomes of *Petunia hybrida*. Nature Plants, 2(6): 1-9. https://doi.org/10.1038/nplants.2016.74.
4. Caballero-Villalobos, L., Silva-Arias, G. A., Turchetto, C., Giudicelli, G. C., Petzold, E., Bombarely, A., and Freitas, L. B. (2021). Neutral and adaptive genomic variation in hybrid zones of two ecologically diverged Petunia species (Solanaceae). Botanical Journal of the Linnean Society, 196(1): 100-122. https://doi.org/10.1093/botlinnean/boaa091.
5. Chen, J. J., Sun, Y., Kopp, K., Oki, L., Jones, S. B., and Hipps, L. (2022). Effects of water availability on leaf trichome density and plant growth and development of Shepherdia× utahensis. Frontiers in Plant Science, 13: 855858. https://doi.org/10.3389/fpls.2022.855858.
6. Cooke, J. E., Eriksson, M. E., and Junttila, O. (2012). The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant, Cell and Environment, 35(10): 1707-1728. https://doi.org/10.1111/j.1365-3040.2012.02552.x.
7. DeHaan, L. R., and Van Tassel, D. L. (2014). Useful insights from evolutionary biology for developing perennial grain crops1. American journal of botany, 101(10): 1801-1819. https://doi.org/10.3732/ajb.1400084.
8. Farhan, F., T., & S. Sekhi, Y. (2024). Effect Of Plant Growth Regulators And Explants On The Micropropagation Of Capparis Spinosa L. Anbar Journal Of

Agricultural Sciences, 22(1), 750–762. https://doi.org/10.32649/ajas.2024.183834.

9. Gerats, T., and Strommer, J. (Eds.). (2008). Petunia: evolutionary, developmental and physiological genetics. (2nd ed). Springer-Verlag New York. P: 85-106.
10. Goldsmith, G. A. (1968). Current developments in the breeding of F_1 hybrid annuals. HortScience, 3(4): 269-271. https://doi.org/10.21273/HORTSCI.3.4.269.
11. Griesbach, R. J. (1998). The effect of the Ph 6 gene on the color of Petunia hybrida Vilm. flowers. Journal of the American Society for Horticultural Science, 123(4): 647-650.
12. Griesbach, R. J. (2007). Petunia. In: Anderson, N.O. (eds) Flower Breeding and Genetics. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-4428-1_11.
13. Grlesbach, R. J. (1996). The inheritance of flower color in Petunia hybrida Vilm. Journal of Heredity, 87(3): 241-245. https://doi.org/10.1093/oxfordjournals.jhered.a022993.
14. Hermann, K., Klahre, U., Venail, J., Brandenburg, A., and Kuhlemeier, C. (2015). The genetics of reproductive organ morphology in two Petunia species with contrasting pollination syndromes. Planta, 241: 1241-1254. https://doi.org/10.1007/s00425-015-2251-2.
15. Kellenberger, R. T., Byers, K. J., De Brito Francisco, R. M., Staedler, Y. M., LaFountain, A. M., Schönenberger, J., ... and Schlüter, P. M. (2019). Emergence of a floral colour polymorphism by pollinator-mediated overdominance. Nature communications, 10(1): 63. https://doi.org/10.1038/s41467-018-07936-x.
16. Mol, J., Grotewold, E., and Koes, R. (1998). How genes paint flowers and seeds. Trends in Plant Science, 3(6): 212-217. https://doi.org/10.1016/S1360-1385(98)01242-4.
17. Morris, S. (2011). Plant biotechnology in Ireland: Understanding the factors that shape plant biotechnology of risk management policy in Ireland (Doctoral dissertation, University of Galway).
18. Natarella, N. J., and Sink, K. C. (1971). The morphogenesis of double flowering in *Petunia hybrida* Hort. 1. Journal of the American Society for Horticultural Science: 96(5): 600-602. https://doi.org/10.21273/JASHS.96.5.600.
19. Panji, C., and Herawati, M. M. (2020). Pengaruh suhu dan lama simpan terhadap viabilitas polen petunia (Petunia inflata). Jurnal Penelitian Pertanian Terapan, 20(2): 135-141.
20. Paris, C. D., and Haney, W. J. (1958). Genetic studies in Petunia. I. Nine genes for flower color. Proceedings. American Society for Horticultural Science, 72: 462-472.
21. Rodrigues, D. M., Caballero-Villalobos, L., Turchetto, C., Assis Jacques, R., Kuhlemeier, C., and Freitas, L. B. (2018). Do we truly understand pollination syndromes in Petunia as much as we suppose? AoB Plants, 10(5): ply057. https://doi.org/10.1093/aobpla/ply057.
22. Saunders, E. R. (1910). Studies in the inheritance of doubleness in flowers. Journal of Genetics, 1(1): 57-69.

23. Sharef, A. A. (2023). Phenotypic analysis of *Petunia hybrida* commercial varieties. Passer Journal of Basic and Applied Sciences, 5(2): 335-341. https://doi.org/10.24271/psr.2023.390656.1290.
24. Shim, J. S., and Jang, G. (2020). Environmental signal-dependent regulation of flowering time in rice. International Journal of Molecular Sciences, 21(17): 6155. https://doi.org/10.3390/ijms21176155.
25. Singh, V. K., Avtar, R., Mahavir, N. K., Manjeet, R. K., and Rathore, V. (2020). Assessment of genetic relationship among diverse Indian mustard (Brassica juncea L.) genotypes using XLSTAT. Electronic Journal of Plant Breeding, 11(02): 674-680.
26. Sink, K.C. (1984). Petunia. Monographs on Theoretical and Applied Genetics. Springer, Berlin, Heidelberg. P: 185-202.
27. Soltis, D. E., Soltis, P. S., Albert, V. A., Oppenheimer, D. G., DePamphilis, C. W., Ma, H., ... and Theißen, G. (2002). Missing links: the genetic architecture of flower and floral diversification. Trends in plant science: 7(1): 22-31. https://doi.org/10.1016/S1360-1385(01)02098-2.
28. Tornielli, G., Koes, R., and Quattrocchio, F. (2009). The genetics of flower color. Petunia: Evolutionary, developmental and physiological genetics, 269-299. https://doi.org/10.1007/978-0-387-84796-2_13.
29. Vandenbussche, M., Chambrier, P., Rodrigues Bento, S., and Morel, P. (2016). Petunia, your next supermodel?. Frontiers in plant science, 7: 72. https://doi.org/10.3389/fpls.2016.00072.
30. Venail, J., Dell'Olivo, A., and Kuhlemeier, C. (2010). Speciation genes in the genus petunia. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1539): 461-468. https://doi.org/10.1098/rstb.2009.0242.
31. Wijsman, H. J. W., and De Jong, J. H. (1985). On the interrelationships of certain species of Petunia IV. Hybridization between *P. linearis* and *P. calycina* and nomenclatorial consequences in the Petunia group. Acta botanica neerlandica, 34(3): 337-349. https://doi.org/10.1111/j.1438-8677.1985.tb01925.x.
32. Yoshida, K., Mori, M., and Kondo, T. (2009). Blue flower color development by anthocyanins: from chemical structure to cell physiology. Natural product reports, 26(7): 884-915. https://doi.org/10.1039/B800165K.
33. Yuan, Y. W., Byers, K. J., and Bradshaw Jr, H. D. (2013). The genetic control of flower–pollinator specificity. Current opinion in plant biology, 16(4): 422-428. https://doi.org/10.1016/j.pbi.2013.05.004.
34. Zhang, B., Liu, Z. X., Ma, J., Song, Y., and Chen, F. J. (2015). Alternative splicing of the agamous orthologous gene in double flower of Magnolia stellata (Magnoliaceae). Plant Science, 241: 277-285. https://doi.org/10.1016/j.plantsci.2015.10.017.
35. Zhang, L., Yu, L., and Lou, A. (2017). No evolutionary change in the mating system of *Solanum rostratum* (Solanaceae) during its invasion in China. Scientific Reports, 7(1): 17698. https://doi.org/10.1038/s41598-017-17881-2.