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Phenotypic analysis of *Petunia hybrida* commercial varieties

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ABSTRACT

Petunia hybrida belongs to the Solanaceae family. It has a significant commercial value worldwide. Petunias grow very well in Kurdistan, with their long flowering period becoming an attractive addition to gardens. In Kurdistan, petunias seeds are often imported, and commonly face problems such as low germination rate; sometimes, the plants' colour is not as described. In addition, it seems that commercial cultivars usually do not produce seeds. *Petunia* as a model plant, plays a crucial role in most research carried out at molecular, biological, and physiological levels, whereas, the inbred line should be used in such types of research. So far, here in Kurdistan, there has been no research for developing inbred lines or developing petunias for ornamental purposes. This study mainly evaluated four commercial, attractively coloured cultivars: Red Frost, Blackberry, White, and Blue Star. All cultivars showed the same colour as was described. The chosen sample has pin flowers, meaning that self-incompatibility in all cultivars is heteromorphic, but after manual self and cross-pollination, they all produced seeds**.** In addition, the focus was on several essential phenotypic characteristics that led to exciting results. Present results revealed that the largest diameter belongs to Blackberry (8.319 cm). In contrast, the Red Frost cultivar recorded the most extended peduncle length (4.994cm). In comparison, White cultivars recorded the highest plant height (32.933cm). These characteristics can be exploited to improve the breeding ornamental sector in Kurdistan.

Keywords: Petunia, Flower Colour, Commercial Cultivars, Compatibility.

1. Introduction

Petunia belongs to the Solanaceae family, and it has the same scientific, local, and common name. Despite being a winter annual plant, it blooms from spring to autumn in Kurdistan. Petunias have a wide range of colours. However, white, purple, pink, red, and yellow are the most popular colours, and there are also some cultivars with multi-colored flowers. The best soil for growing petunias should consist of well-rotted compost and silt. Petunias need 16 hours of light and 25°C temperature to grow appropriately.

Several plants within the Solanaceae family have ornamental value, but the petunia is the one that has the most significant economic value worldwide[1]. Consequently, petunia seeds have become an economically valuable ornamental resource for several countries[2,3]. It is believed that the origin of *Petunia* is South America and the name *Petunia* is derived from the word "petun" in the Tupi-Guarani language[1]. Species in the *Petunia* genus have played a crucial role as an experimental model for horticultural, biological, and biomolecular research[3].

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Interestingly, the whole Petunia genome was recently sequenced, which helps carry out more complicated research in plant breeding and genetics[4]. Researchers have tried to improve petunia using different techniques varying from conventional methods to sophisticated techniques for breeding plants[5,6]. In floriculture, criteria such as flowering season, flower colour, and flower diameter are crucial, however, the ability of plants to produce seeds for the next season and provide attractive plants is by far the most essential trait. In Kurdistan, petunia grows very well, and the long flowering season (which lasts from April until September) makes it a valuable addition to gardens. The petunia seeds, however, are imported from other countries, which is very expensive, especially the rare varieties. Understanding the phenotypic characteristics of petunia commercial varieties is important for several reasons. First, introduce and evaluate commercial cultivars as a starting point for breeding programs. Secondly, understanding inbreeding depression (such as self-incompatibility and male sterility) is the first step toward developing an inbred line[7,8]. Seeds collected from commercial cultivars obtained from self or cross-pollination usually gave different kinds of plants compared to parents. However, petunia has a short life cycle lasting about three to five months which helps to develop several generations in one year and produce inbred lines from self-pollination in a few years. Inbred lines of petunia have an essential role in the research carried out at the molecular level, particularly after sequencing the whole petunia genome. In addition, crossing two inbred lines to produce an F1 hybrid, which can be exploited for ornamental purposes for use locally or for export to other countries. Self-incompatibility as a helpful tool can be used for producing an F1 hybrid[8]. Nowadays, petunia has a crucial role in the floricultural trade thanks to F1 hybrid cultivars. According to the National Agricultural Statistics Service for Floriculture Crops in 2014, the annual wholesale value of garden petunia in the United States exceeded US$130 million[9].

This study aims to analyse several phenotypic traits for four commercial cultivars to develop an inbred line and F1 hybrid cultivars in the future.

2. Methods and Materials

2.1 Sowing Seeds and Growth Condition

This research was carried out in the Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, from September 2020 to May 2021. Petunia commercial cultivars {Red Frost (RF), Blackberry (BB), White (WH), and Blue Star (BS)} were grown in Greenhouse in (24/22°C, 16/8 hr day/night cycle). The seeds were germinated in small pots using compost. After three weeks, they were transferred to a seedling tray. After six weeks, the plants were transferred to 5-liter pots containing a standard petunia potting mix comprising compost, silt, and perlite in a ratio of 3:2:1. Pesticides were used as appropriate and one osmocote exact tablet {14 (N), 8 (P2O5), 11(K2O), TE}, and more information available at https://multisite-assets.icl-growingsolutions.com/wp-content/uploads/2023/07/07070826/9078-1-3.pdf} was used for each pot. A completely Randomized Design (CRD) was used for designing this research. Three plants were used for each replication and three replications for each cultivar were used. In total nine plants were used for each cultivar.

2.2 Observations and Parameters

This research focused on several critical phenotypic traits. For example, to see to what extent the described flower colour is present in reality, anther colour , and the position of anthers and stigma, pollen viability were observed by the eye. Flower diameters, however, were obtained by measuring the two farthest points between the corolla, and six flowers were measured for each plant. Peduncle length was measured from the end of the sepals until the beginning of the stem. Testing for self-compatibility was carried out using ten flowers/plant. The pollen of an individual flower was used to pollinate the stigma of the same flower. If the plant produced capsules and set seed it was considered as self-compatible. To test cross compatibility, five flowers/plants were emasculated three days before anthesis by removing all the stamens to prevent self-pollination but leaving the stigma intact. Two days later, the pollen was applied to the stigma using sterilized forceps. All pollinations were labelled with a string tag placed around the pedicel recording the date and type of pollination. Days to set seed and the number of pods observed when the pod colour changed to light brown. The pod weight was measured a few days later after it dried out. The plants were considered self or cross-compatible if they set seed and produced capsules after self or cross-pollination. The seed set was calculated using the number of pods produced after self and cross-pollination. Plants' heights were measured after six months of planting. Moreover, germination percentages were measured. To start with, all seeds were surface sterilized with household bleach available in the local market called (buzhna).

One hundred seeds were put in folded filter paper, and labels, then closed from all corners using paperclips. All folded filter papers were put in %2 (v/v) household bleach for 20 min. After discarding the bleach, the seeds were washed three times using distilled water, the paperclips were opened carefully using sterilized forceps and then the samples were left overnight to dry out. The seeds were then spread in a petri dish on wet filter paper. All Petri dish lids were closed using parafilm and left at room temperature and exposed to 16 hours of light. Every day the Petri dishes were observed for germinated seeds. The germination percentages were calculated using the following formula:

Germination Percentage (GP) = All germinated seeds/total seeds x 100.

Statistical Analysis

XLSTAT | Statistical Software for Excel was used for data analysis.

3. Results and discussion

In this research, four cultivars with four different flower colours were used. Sometimes, flower colours in reality are slightly, or in some cases, totally different, from the description. Seed source, reliability, and environmental factors, including light, temperature, and type of soil, all play an essential role in influencing the phenotype of commercial cultivars. In this study, however, we were satisfied that all cultivars produced flowers of the exact colour described, as illustrated in Figure (1). In addition, all cultivars had a yellowish anther except for BB, which has light purple anthers.

A- Red Frost (RF) B-Blackberry (BB) C-Blue Star (BS) D-White (WH)

Figure 1: *Illustration of four Petunia cultivars used in this Research.* A- RF has red petals with a white border. B-BB has a dark purple colour. C-BS has purple petals with a white star in the middle. D- WH has white to yellowish petals. Bar= 2cm.

All cultivars did however present pin flowers as all stamens were in the lower position to the style. This indicated that these cultivars show heteromorphic self-incompatibility. Regarding male sterility, in this study, all cultivars had viable pollen, and there was no male sterile cultivar. There was variation in the flower diameter between the different cultivars. As described in Table 1, the most extended flower diameter belonged to BB (8.319 cm), which varied significantly from the other cultivars. There was no significant difference between the flower diameter of RF and WH which were 7.365 cm and 7.210 cm respectively.

The most minor flower diameter was recorded in the BS cultivar (6.139 cm).

As illustrated in Table 1, the most extended peduncle length was recorded in the RF cultivar (4.994cm), and this was only significantly different from the peduncle length of the WH cultivar (3.936 cm). Analysis of plant height revealed that WH cultivars were the highest (32.933cm), however, this was only significantly different from BB (27.33 cm).

Table 1: Summary of flower diameter, Peduncle length, and plant height for all cultivars.

Cultivars	Flower Diameter (cm)	Peduncle Length (cm)	Plant Height (cm)
RF	7.365 b	4.994 a	29.300 ab
BB	8.319 a	4.842 a	27.333 b
WH	7.210 b	3.936 b	32.933 a
BS	6.139 c	4.557 ab	32.800 a

* According to Duncan's multiple range test (P < 0.05), there are no significant differences between the mean value with the same letter.

Ten flowers from each plant were self-pollinated, and five were cross-pollinated with all other cultivars (reciprocal cross). It was observed that all cultivars are self and cross-compatible. On the other hand, all flowers in all plants and cultivars without manual pollination remain incompatible. The level of compatibility, however, was different between cultivars. Analysis of self-pollination (summarized in Table 2), showed that both RF and BS produced the most significant number of pods 29.333 and 29 pods out of 30 pollinated flowers, respectively). The quantity of pods varies markedly from BB (22) and WH (4.667 pods). WH cultivars produced a minimal number of pods compared to others.

Table 2: Seed set after self-pollination and cross-pollination in all cultivars.

Cultivars	Seed Set (number of pods) out of 30 self - pollinations	Reciprocal Cross Between Different Cultivars	Seed Set (number of pods) out of 15 pollinations
RF	29.333 a	RF X BB	14.000 a
		RF X WH	13.000 ab
		RF X BS	11.667 abc
BB	22.000 b	BB X RF	9.000 bcd
		BB X WH	7.667 cd
		BB X BS	9.000 bcd
WH	4.667 c	WH X RF	6.667de
		WH X BB	6.000 de
		WH X BS	3.667 e
BS	29.000 a	BS X RF	10.000 abcd
		BS X BB	12.333 ab
		BS X WH	12.333 ab

* According to Duncan's multiple range test (P < 0.05), there are no significant differences between the mean value with the same letter.

Examination of cross-pollination showed that crossing RF with BB gave 14 pods out of 15 pollinations. This number, does not vary significantly from the number of pods obtained from crossing RF X WH (13.000), RF X BS (11.667), BS X RF (10.000), and both BS X BB and BS X WH (12.33) pods. The same number of pods were obtained from crossing BB with RF and BS (9), but fewer pods were produced by crossing BB and WH (7.667). The lowest pod numbers (6.667, 6, and 3.66) were recorded using the WH cultivar as the maternal parent. The number of days to set seed, or produce pods, varied between all cultivars (Table 3). The length of time for setting the seed was calculated from the pollination date until the capsule or pods became brown.

Table 3: Number of days to set seed after self and cross-pollination.

Cultivars	Days to Set Seed (Self-pollination)	Reciprocal Cross Between Different Cultivars	Days to Set Seed (Cross-Pollination)
RF	35.000 abc	RF X BB	35.333 abc
		RF X WH	32.000 cd
		RF X BS	32.000 cd
BB	33.333 bcd	BB X RF	36.000 abc
		BB X WH	35.000 abc
		BB X BS	33.000 bcd
WH	36.000 abc	WH X RF	37.333 abc
		WH X BB	38.333 ab
		WH X BS	39.667 a
BS	28.333 d	BS X RF	37.333 abc
		BS X BB	33.667 bc
		BS X WH	33.667 bc

* According to Duncan's multiple range test (P < 0.05), there are no significant differences between mean values with the same letter.

As shown in Table 3, after self-pollination the WH cultivar needed the most prolonged period for setting seed (36 days). However, this number is not significantly different from the days required for seed setting RF and BB. BS required the minimum number of days to set seed (28.333) which was significantly different from all the other cultivars except BB (33.333 days).

Evaluation of cross-pollination showed that pods obtained from crossing WH X BS required the maximum period for seed set, (39.667days), which varied significantly from crossing RF X WH (32days), RF X BS (32days), BB X BS (33 days), BS X BB (33,667days) and BS X WH (33.667 days). When comparing self and cross-pollination for this trait, we observed that pods obtained from BS took the minimum number of days for the seed set (28.333) and pods produced from crossing WH X BS took the maximum number of days (39.667).

Another phenotypic trait that was investigated in this study was pod weight, and the results are described in Table 4. When self-pollinated, BS produced the heaviest pods (53.913 mg), however, this was not statistically different from the weight of the pods produced by the other cultivars.

Table 4: Pod weight obtained from self and cross-pollination between all cultivars.

Cultivars	Pod Weight (mg)	Reciprocal Cross Between Different Cultivars	Pod Weight (mg)
RF	46.997 abc	RF X BB	54.101 ab
		RF X WH	58.329 a
		RF X BS	55.058 ab
BB	45.363 abc	BB X RF	47.110 abc
		BB X WH	48.438 abc
		BB X BS	46.454 abc
WH	37.940 bcd	WH X RF	44.667 abc
		WH X BB	25.387 d
		WH X BS	32.909 cd
BS	53.913 ab	BS X RF	55.058 ab
		BS X BB	46.454 abc
		BS X WH	47.110 abc

* According to Duncan's multiple range test ($P < 0.05$), there are no significant differences between mean values with the same letter.

Cross-pollination between RF and WH produced the largest pods (58.329 mg), which were significantly heavier than the pods produced by crossing WH with BB and BS (25.387 mg and 32.909 mg, respectively). The weight also varied significantly when compared to pods obtained from self-pollination of the WH cultivar (37.940 mg).

As illustrated in Table 5, the highest number of seeds per gram was obtained from self-pollinating BB (14261.667 seeds/gm), which is significantly different from all other data for this trait except crossing BB X RF (12633.055 seeds/gm), BB X BS (12676.000 seeds/gm), WH X BB (12685.056 seeds/gm) and BS X WH (12632.000 seeds/gm).

Table 5: Illustration of seeds per gram harvested from self and cross-pollination.

Cultivars	Number of Seed/gm	Reciprocal Cross Between Different Cultivars	Number of Seed/gm
RF	9666.667 cd	RF X BB	11574.667 bc
		RF X WH	9393.333 cd
		RF X BS	9398.333 cd
BB	14261.667 a	BB X RF	12633.055 ab
		BB X WH	11666.667 bc
		BB X BS	12676.000 ab
WH	10437.667 bcd	WH X RF	8620.667 d
		WH X BB	12685.056 ab
		WH X BS	9444.333 cd
BS	11798.667 bc	BS X RF	11111.000 bc
		BS X BB	10438.000 bcd
		BS X WH	12632.000 ab

* According to Duncan's multiple range test ($P < 0.05$), there are no significant differences between mean values with the same letter.

The fewest seeds per gram were obtained from crossing WH X RF (8620.667 seeds/gm). This result is significantly different from the number of seeds per gram harvested from all other pollinations except the self-pollination of RF and WH and the cross-pollination of RF X WH, RF X BS, WH X BS, and BS X BB (9666.667, 10437.667, 9393.333, 9398.333, 9444.333 and10438.000seeds/gm respectively). Moreover, germination percentage is considered for seeds obtained from self and cross-pollinations between all cultivars, as illustrated in Table 6.

Table 6: Summary of germination percentage.

Cultivars	Self-Pollination	Reciprocal Cross Between Different Cultivars	Cross-Pollination
	*GP (%)		*GP (%)
RF	95.000 a	RF X BB	98.000 a
		RF X WH	95.333 a
		RF X BS	80.000 b
BB	42.000 c	BB X RF	97.333 a
		BB X WH	92.333 a
		BB X BS	92.000 a
WH	95.333 a	WH X RF	98.667 a
		WH X BB	32.667 d
		WH X BS	20.000 e
BS	96.667 a	BS X RF	97.333 a
		BS X BB	96.333 a
		BS X WH	97.667 a

*GP = Germination Percentage (GP).

* According to Duncan's multiple range test ($P < 0.05$), there are no significant differences between mean values with the same letter.

Analysis of germination percentage showed that seeds harvested from crossing WH X RF had the highest germination percentage (98.667%). This result significantly differed from the germination percentage of harvested seeds from the self-pollination of BB, and the cross-pollinations of RF X BS, WH X BB, and WH X BS (42, 80,32.667, and 20%, respectively).

4. Discussion

Petunia is an important floricultural crop and an attractive addition to gardens due to its wide range of cultivars, colours, and long flowering season. Consequently, the petunia seed has a high commercial value, and rare cultivars are usually expensive. Here in Kurdistan, we have depended on other countries for most ornamental plant seeds, including petunia due to a lack of relevant research. In this study, we compared four different cultivars imported from different countries. Four different cultivars were used in this research. In total, only nine plants were grown for each cultivar. We observed a low germination rate, possibly caused by transportation problems or unsuitable seed storage conditions. This highlights the need to develop our cultivars to obtain fresh seeds rather than depending on those imported from other countries.

Flower colour is very crucial, both for attracting pollinators and making plants desirable to the customer. Furthermore, it can be exploited in garden design and decoration. Unfortunately, flower colour is not always as described in commercial varieties of petunia. However, observation of flower colour in this study revealed that all four cultivars have the exact flower colour as described. This trait can be easily observed in various tissues during development[10]. The RF cultivar had solid red flowers with white edges while BB presented very dark purple flowers. BS had distinctive purple flowers with a white star in the middle, while plants obtained from the WH cultivar had white to yellowish flowers with wavy edges.

Flavonoids are responsible for flower colour in petunia, of which there are two groups: anthocyanins and co-pigments. Five different anthocyanidins (cyanidin, peonidin, delphinidin, petunidin, and malvidin) are found in petunia, as well as three types of acyl moieties (coumaric, ferulic, and caffeic acid), and two different sugars (glucose and rhamnose)[11,12]. The major anthocyanins responsible for visible colours such as orange, red purple, magenta, violet, and light pink are Pelargonidin, Cyanidin, Peonidin, Delphinidin, Malvidin, and Petunidin respectively. Flavanols, however, are responsible for ultraviolet [UV] colour. Nine genes with three alleles are responsible for flower colour in petunia, and 768 possible phenotypes were identified[13]. Moreover, another two genes were identified[14]. Flower size is another important morphological trait. *Petunia Grandiflora* with large flowers and *Petunia multiflora* with small flowers were reported[15], and the genes for both species were designated G and g, respectively. Quantitative trait loci (QTL) responsible for the various phenotypes of *Petunia axillaris* and *Petunia integrifolia* were mapped[16]. It was observed that flower size and nectar volume are highly polygenic. The largest flower diameter in this study belongs to BB (8.319 cm), which was significantly different from the other cultivars. As described in Table 1, RF had the most extended peduncle length (4.994 cm), however, this was only significantly different from the WH cultivar, which had an average peduncle length of 3.936 cm.

Self-incompatibility is an obvious barrier that can prevent inbreeding and encourage cross-pollination. Most cultivated Solanaceae exhibit self-compatibility (SC), which greatly facilitates the production of seeds for annual crops. *Petunia*, however, is a member of the Solanaceae family that exhibits the S-Ribonuclease mechanism of self-incompatibility. Heteromorphic self-incompatibility occurs because of differences in the flower morphology, particularly, the lengths of the style and stamens, and this system is called heterostylous self-incompatibility. Style and anther positioning in these types are genetically controlled by dominance between alleles at a single S-locus[17]. Types of heterozygous self-incompatibility include Distylous (short style and long anthers) Thrum, (long style with short anthers) Pin, and Tristylous which has three flower forms (short style with middle and high stamens, middle style with low and high stamens and long style with low and middle stamens). Heteromorphic incompatibility as a mechanism to encourage outcrossing is revealed in several species[18,19] revealed two alleles responsible for pin and thrum flowers at the Primula S locus. In this study, we observed that all cultivars had pin flowers. Consequently, all plants produced no seeds except those pollinated manually in both self and cross-pollinations (as illustrated in Table 2). When self-pollinated the WH cultivar only produced 4.667 pods out of 30. This result was significantly different from the number of pods obtained from the remaining cultivars. These results could be because of pseudo-self-compatibility in addition to heteromorphic self-incompatibility in the WH cultivar. Examination of cross-pollination via reciprocal crosses produced different results. Crossing WH and BS produced the lowest number of pods (only 3.667 out of 15 pollinations). The variation observed could be attributed to varying physiological factors between the cultivars.

The number of days required for the seed set varied after self and cross-pollination. As illustrated in Table 3 WH took the maximum number of days to set seed after self-pollination compared to all the other cultivars (36.000) days. On the other hand, pods obtained from crossing WH and BS took the maximum number of days to set seed (39.667) compared to pods obtained from all the other reciprocal crosses. The reason for that could be due to the effect of light[20]. Pods are often exposed to different levels of light as they are surrounded and covered by plant branches, and pods in shade places need more time to reach maturity[21]. In addition, different cultivars have different genetic backgrounds and need a varying number of days for setting seeds and producing pods.

Measuring pod weight showed no significant difference between the pods obtained from the self-pollination of all the varieties (Table 4). There was, however, a significant difference in pod weight between pods obtained from reciprocal crosses. The reason for that could be genetic background, as different cultivars produce different pods.

Moreover, seeds harvested from crossing WH X RF had the highest germination percentage (98.667%). Our results revealed that these commercial cultivars are all heteromorphic self-incompatible, but they can produce seeds after manual self and cross-pollination. Seeds obtained from the self and cross-pollinations will need to be grown to analyze the F1 and F2 hybrids. This evaluation will show whether they can be used locally instead of depending on imported seeds from other countries, which are usually expensive and have a low germination rate. In addition, several inbreed lines can develop from harvested seeds. Even if they do not have commercial value, they can be exploited and used for research purposes.

Conclusions

Through this study, we can conclude several significant points. All cultivars gave the same flower colour as described confirming the reliability of the source of production. Evaluation of these cultivars in terms of flower colour, flower diameter, and plant height is acceptable. However, they had a low germination rate. Our results overturn previous beliefs, as people used to believe that petunia commercial cultivars do not produce seeds without understanding the reasons behind this phenomenon. Here we observed that all cultivars exhibit heteromorphic self-incompatibility, which prevents seed production. However, after self-pollination and reciprocal crosses, they show self and cross-compatibility and the collected seed gave a nearly high germination rate. The obtained seed can be used in different breeding programs to improve the ornamental sector in Kurdistan.

From the results obtained in this research, we can recommend several points. First, seeds collected from self-pollination will give different plants from parents but they can be grown, and after self-pollination of several generations inbreed line can be developed and used either for research purposes or for crossing together and producing an F1 hybrid. Second, although plant growers believe that seeds collected from commercial cultivars, in general, do not have economic value, this idea might be overrated as regarding petunia there is no strong evidence to confirm this thought in practice. Consequently, seeds collected from reciprocal crosses can be grown and see if they have ornamental value, if so they can be exploited to improve the ornamental sector and produce our petunia seed instead of depending on other countries even if we can export to other countries. We expect to obtain different plants in F2 hybrid if it is an adverse point for other crops in ornamental crops is a positive point. In this case, as the petunia is an ornamental plant, more variation between the plants gives more attractiveness.

Conflict of interests

None.

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