

Progeny Evaluation from the Crossing of Novelty-Type *Phalaenopsis* I Hsin Bee x *Phalaenopsis pulcherrima* var. *champorensis*

(Penilaian Progeni daripada Kacukan Jenis Kebaharuan *Phalaenopsis* I Hsin Bee x *Phalaenopsis pulcherrima* var. *champorensis*)

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ABSTRACT

The floriculture industry has rapidly developed in Indonesia. One of the potential genera of orchids is *Phalaenopsis*. New varieties must be developed to compete with imported ones. Genetic improvement of *Phalaenopsis* has been carried out through interspecific and intergeneric crossing - the crossing between *P. I Hsin Bee* x *P. pulcherrima* var. *champorensis* is dedicated to introduce harlequin type to *P. pulcherrima*. Forty progenies of the first generation (F_1) and their parents are used for the characterization and evaluation. The results show that *P. pulcherrima* is more dominant (50%) in flower shape and colour based on the progeny composition. The introduction of the harlequin type into *P. pulcherrima* is indicated by 35% of progenies have dark color at the flower and matched with the spotted pattern of the female parent, indicating that the female contributed partially to the respected traits. The progenies are divided into five distinct groups based on nine qualitative characteristics. Three progenies, namely D829-4, D829-6, and D829-8, are considered to have prominent features, such as more stable and symmetric coalescence of red-brownish blotches on the flowers, representing harlequin type than other progenies. These progenies will potentially be developed to be commercial varieties.

Keywords: Harlequin; hybridization; moon orchid; qualitative characters; quantitative characters

ABSTRAK

Industri florikultur telah berkembang pesat di Indonesia. Salah satu genus orkid yang berpotensi ialah *Phalaenopsis*. Varieti baharu daripada genus tersebut harus dikembangkan untuk menandingi varieti import. Penambahbaikan genetik *Phalaenopsis* telah dilakukan melalui kacukan antara spesies dan antara genus. Kacukan antara *P. I Hsin* x *P. pulcherrima* var. *champorensis* dilakukan untuk mengintegrasikan ciri harlequin pada *P. pulcherrima*. Sebanyak 40 progeni generasi pertama (F_1) dan induk mereka telah digunakan untuk penilaian dan pencirian. Hasil menunjukkan bahwa *P. pulcherrima* lebih dominan (50%) dalam ciri bentuk dan warna bunga berdasarkan komposisi progeni. Integrasi ciri harlequin ke dalam *P. pulcherrima* ditunjukkan dengan banyaknya progeni (35%) yang mempunyai bunga berwarna hitam yang sama dengan pola bercak pada induk betina. Sembilan ciri kualitatif yang membahagikan kesemua progeni menjadi lima kumpulan, berdasarkan variasi warna dan bentuk daripada petal, sepal dorsal dan lateral, serta lobus-tengah. Tiga progeni, iaitu D829-4, D829-6 dan D829-8, menunjukkan ciri yang menonjol, seperti lebih stabil, gabungan simetri tompok merah kecoklatan pada bunga yang memperlihatkan ciri harlequin berbanding dengan progeni lain. Ketiga-tiga progeni tersebut memiliki potensi untuk dikembangkan lebih lanjut menjadi varieti komersial.

Kata kunci: Ciri kualitatif; ciri kuantitatif; harlequin; kacukan; orkid bulan

INTRODUCTION

Phalaenopsis is an economically important horticultural crop, which accounts for at least 75% of the world orchids market (Yuan, Chin & Chen 2015). The orchid is cultivated

as a potted ornamental plant or as a cut flower. As ornamentals, *Phalaenopsis* are recognized for their exotic flower color and shape, elegant appearance, and extended longevity (Chen 2020; Liang et al. 2020; van Tongerlo et

al. 2021) and can be scheduled to flower quickly throughout the year (Cai et al. 2015). This orchid has also raised issues regarding plant varieties and intellectual property rights protection (varieties, cultivars, or hybrids) because these plants are easily propagated clonally (Vo et al. 2019). Among the members of Orchidaceae, the genus *Phalaenopsis* consists of more than 70 species from subtropical and tropical Asia to northern Australia. Genome and chromosome sizes vary considerably among *Phalaenopsis* species (from 1C = 1.39–8.74 pg) (Lee & Chung 2020). The chromosome number is $2n = 38$, except for species in the section Aphyllae ($2n = 34$ and 36).

The orchid can be divided into three major groups, i.e., standard type with large flowers, multi-flora, and novelty types (Lee & Chung 2020). The group can be produced through interspecific and intergeneric hybridization using species and/or varieties that inherit the targeted characteristic (Tang & Chen 2007). The interspecific and/or intergeneric hybridizations among subgenera/sections have successfully extended qualitative and quantitative traits, such as flower color and size, to current cultivars (Tsai et al. 2010). The first successful artificial intergeneric crossing was reported on *Doritis pulcherrima* with *P. equestris* producing *Doritaenopsis* hybrids with more flowers and more intense pink than those of the *Phalaenopsis* parent (Hsu et al. 2010; Li et al. 2021). 1923 Baron Toshita Iwasaki crossed the species with *P. lindenii* Loher and produced *Doritaenopsis* hybrids such as *Doritaenopsis* Asahi (Lin et al. 2001). All *Doritis* species are now included in the genus *Phalaenopsis* (Yukawa et al. 2005).

Other reported interspecific or intergeneric hybridizations involving *Phalaenopsis* between *Ascocenda* John De Biase Blue and *Phalaenopsis* Chi Shang's Stripes through embryo rescue technique. The scheme of unique blue color was maternally inherited through cpDNA from *Ascocenda* to *Phalaenopsis* (Liu et al. 2016; Tsai et al. 2010). Dwiati, Susanto and Prayoga (2020) and Hartati (2010) also reported the successful intergeneric crossing between female *Vanda tricolor* and three male *Phalaenopsis* cultivars, i.e., *Phalaenopsis* Joane Kileup June, *Phalaenopsis* Pinlong Cinderella, and *Phalaenopsis* Fortune Buddha x *Phalaenopsis* Princess Kaiulani. They found that the highest fruit formation was observed when the crossing was conducted not more than two weeks after the flower was blooming.

Other reported intersection hybrids on novelty-type *Phalaenopsis*. These plants are produced primarily for the hobbyist and orchid breeder markets. One of the novelty types of *Phalaenopsis* is Harlequin. Breeding of the type began in Taiwan when the hybrid *P. Golden Poeker* 'Brother' originated from the crossing of *P. Misty Green* and *P. Liu Tuen Shen* and was mericlone in the 1990s (Chen, Lin & Chen 2020). All harlequin cultivars were

derived from a micropropagated mutation of *P. Golden Poeker* 'Brother'. The most distinguished characteristic of this group is the appearance of large blotches of coalesced spots with intense color against the light creamy white or other colors (Lee & Chung 2020). Another type of Harlequin is *P. I Hsin Bee* from the cross between *P. Golden Poeker* and *Sogo Lisa* and registered by Chien in 2003 (Figure 1). *Phalaenopsis* Golden Poeker originated from the cross between hybrids *P. Misty Green* x *P. Liu Tuen Shen*. Parent hybrids were developed from 12 species through 11 generations. Notably *P. gigantea* contributed about 25% of the morphological features (Hsu et al. 2019). *Phalaenopsis* Sogo Lisa was derived from nine species, with *P. venosa* as the major contributor (25%) (Hsiao et al. 2011).

The formation of *P. I Hsin Bee* involve approximately 14 species, and the largest contribution is consecutively detected on *P. amabilis* subsp. *amabilis*, *P. gigantea*, *P. lueddemanniana*, *P. amboinensis*, and *P. venosa* (16.91%, 15.63%, 14.07%, 12.5%, and 12.5%), respectively. No species from the original *Phalaenopsis* genus were directly involved in forming the harlequin flower (Hsu, Chen & Chen 2018).

Phalaenopsis pulcherrima is an orchid species originating from Hainan Island to western Malaysia. It is a terrestrial and sometimes lithops orchid indicated by the erect upright inflorescence with high lignification content on the inflorescence (Pramanik et al. 2022). *Phalaenopsis pulcherrima* var. *champorensis* white is a cross hybrid grown in Zynah Orchid farms in Kochi, Kerala, India. The common ones are purple and yellow color form on the petals. The peloric form is believed to be due to mutation, which caused the two top petals to look like the bottom petals (lip). The species is endemic to Southeast Asia (Hu et al. 2021).

Many studies have reported interspecific and/or intersection crossings among *Phalaenopsis* species, hybrids, and types. However, evaluating the characteristic derivation from the parents into their progenies still becomes an interesting case to investigate. The paper described the qualitative and quantitative evaluation of progenies from crossing the harlequin type of *P. I Hsin Bee* and *P. pulcherrima* var. *champorensis*.

MATERIALS AND METHODS

PREPARATION OF PLANT MATERIALS

The crossing between *P. I Hsin Bee* with *P. pulcherrima* var. *champorensis* was carried out in 2008 under the screen house and the tissue culture laboratory of the Indonesian Instrument Assessment Institute for Ornamental Crops (formerly the Indonesian Ornamental Crops Research

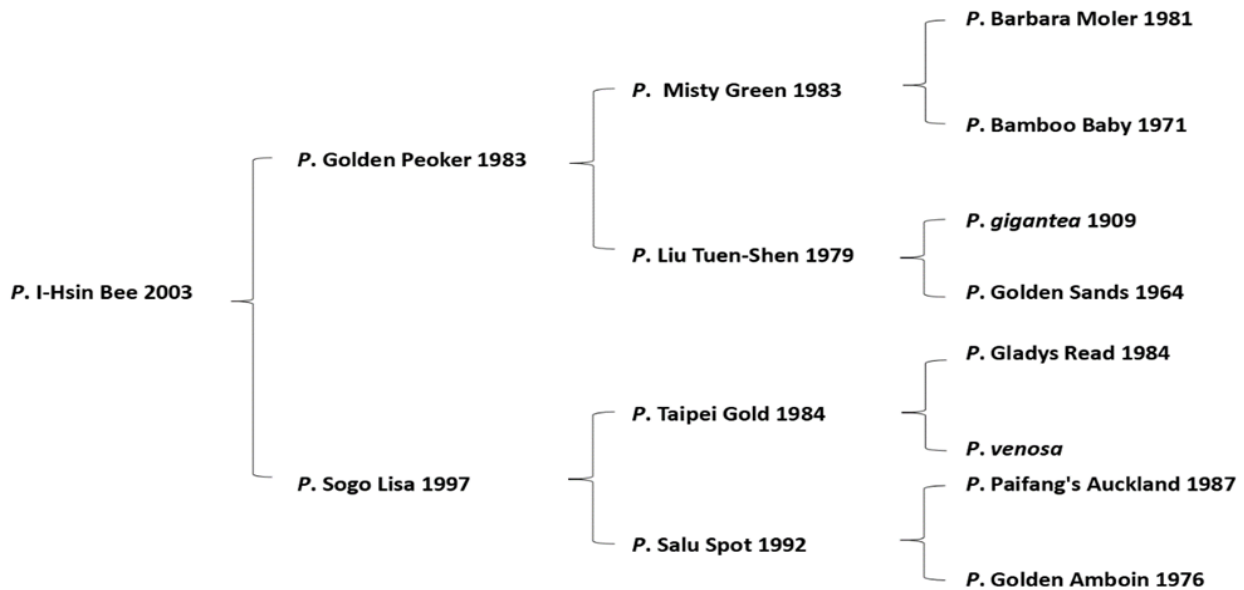


FIGURE 1. Family tree of the female parent, *Phalaenopsis* I Hsin Bee

Institute). The site was 1100 m above sea level. The crossing was conducted in the morning, and the pod was harvested six months later. The pods were disinfected and sown in MS culture media. The germination of seedlings was carried out inside the laboratory. After two years, the plantlets were acclimatized and then transferred into community pots. After six months of maintenance, the young plants were selected for fast-growing and healthy performances instead of those with poor performances, such as leaf deformity and abnormal growth. The selected young plants were planted into single pots. From this population, 40 progenies were obtained and further maintained under greenhouse conditions until flowering. The progeny's first flowering period varied, ranging from 3 to 5 years after acclimatization.

The Progeny genotypes and their two parents were used for the characterization and evaluation. The evaluation of the progenies was carried out from 2010 up to 2022. The observation of qualitative characters was carried out on flower colors and the shape of flower parts. The assessment of flower color encompassed the evaluation of petal, sepal, lip, column, and side-lobe colors using the RHS color chart. Simultaneously, observations on the shape of flower parts were conducted for petals, lateral sepals, dorsal sepals, and lips. The quantitative parameters assessment was focused on four plant characters, i.e., the length and width of the leaf and flower.

DATA ANALYSIS

This study used a one-way analysis of variance (ANOVA) to examine the variability in quantitative data. In the presence of significant differences among the treatments, mean values for each treatment underwent additional testing through the Tukey (HSD) method at a significance level of 0.05. The ANOVA, together with the HSD test, was conducted using SPSS statistic program version 29 (SPSS, IBM) (Table S1).

To understand the relationship between genotypes (parental lines and progenies) and four quantitative characters, this study employed PCA analyses using R and the factoextra package developed by Kassambara and Mundt (2020). For assessing the similarity or dissimilarity between different genotypes, data matrix coefficients were computed utilizing the Dice similarity coefficient via the NTSYS program. In this analysis, genotypes can be represented as binary vectors, where the presence or absence of specific genetic markers is indicated by 1s and 0s, respectively. The coefficient ranges from 0 (indicating no similarity or no common elements) to 1 (indicating perfect similarity, where the sets are identical). In practical terms, a higher Dice coefficient suggests a greater degree of similarity between the sets being compared.

Correlation analyses were used to explore the relationship between quantitative and qualitative characters, such as the shape of flower parts, flower length and width,

and leaf length and width. For assessing the shape of flower parts, a scoring system was applied to the six recognized flower shapes: a score of 1 for a semi-circular shape, a score of 2 for an oval shape, a score of 3 for a mod-lobe shape, a score of 4 for elliptic shape, a score of 5 for elliptic with wing shape, and a score of 6 for rhomboid shape (Figure 2). Pearson correlation coefficients were calculated using R version 3.6.2 (R Team 2016) in R Studio version 1.2.5033 (R Team 2019), employing the cormat package (Meyer 2010). The resulting Pearson correlation coefficients were classified as high ($\geq \pm 0.50$ to ± 1), medium (≥ 0.30 to $< \pm 0.50$), and low ($< \pm 0.30$) based on predefined criteria (Pramanik et al. 2022).

RESULTS AND DISCUSSION

This wide range of values across leaf and flower dimensions among parental lines and progenies indicates substantial genetic diversity within the population, as also reported in other breeding programs of *Phalaenopsis* (Hsu, Chen & Chen 2018). This diversity highlights the existing variability and presents a valuable opportunity for trait improvement through targeted selective breeding (Watanabe & Watanabe 2000). Understanding the genetic basis of these variations is essential for understanding how

desirable traits are inherited within this population (Zhang et al. 2022). This knowledge is foundational for guiding future breeding efforts to enhance specific characteristics of *Phalaenopsis*. Further, the selection of superior candidates based on quantitative was addressed in the last part of the discussion section.

QUANTITATIVE CHARACTERS OF THE F₁ PROGENIES

Through the analysis of variance, this research identifies significant differences in all quantitative characters among parental lines and the 40 tested progenies (Table S1). To better understand the relationships among the quantitative characters and genotypes under investigation, this study performed a Principal Component Analysis (PCA) on the four quantitative characters (Table 1).

The outcomes showed two PCA factors, where longer lines corresponded to more significant loadings for these characters. The first PCA factor positively associated with flower length and flower width characters (0.97 and 0.96, respectively). In contrast, the second PCA factor is positively correlated with leaf length and width (0.75 and 0.82, respectively) (Table 1 & Figure 3).



FIGURE 2. The shape of the petal, sepal, and mid-lobe of the parents (*P. I Hsin Bee* and *P. pulcherrima* var. *champorensis*) and their progenies. Petal shape semi-circular (A), elliptical (B), oval (C), and rhomboid (D). Dorsal and lateral sepal shape: oval (E), elliptic (F), mid-lobe shape (G), rhomboid (H), elliptic with wing (I)

The biplot examines the relationships between variables and genotypes, where genotypes positioned close to variables indicate stronger correlations. Genotypes on the right side of the first PCA axis are associated with leaf length and width, suggesting a correlation with longer and wider leaves. For example, genotype number 40 (D829-38) exhibited a mean leaf length of 26.33 cm, contrasting with genotypes on the lower-left side of the PCA axis, such as genotype number 9 (D829-7), which has a mean leaf length of 11.9 cm (Table 1 & Figure 3). This pattern also held for flower characters. For instance, genotype number 2 (*Phalaenopsis pulcherrima* var. *Champorensis*), with an average flower length of 6.4 cm, is positioned in the upper right of the second PCA axis, contrasting with genotype number 19 (D829-17) which has an average flower length of 5.1 cm (Table 1 & Figure 3).

Identifying two PCA factors suggests the potential grouping of quantitative characters into distinct sets based on their relationships. The first factor is linked to flower length and width, while the second is associated with leaf length and width. Additionally, the scattering of parental lines and progenies across the plot indicates substantial genetic diversity within the population, consistent with findings in other *Phalaenopsis* breeding programs (Hsu, Chen & Chen 2018). This diversity underscores the existing variability and provides a valuable opportunity for targeted selective breeding to improve traits (Watanabe & Watanabe 2000). Understanding the genetic basis of these variations is crucial for comprehending how desirable traits are inherited within this population (Zhang et al. 2022). This foundational knowledge is essential for guiding future breeding efforts to enhance specific characteristics in *Phalaenopsis*.

QUALITATIVE CHARACTERS OF THE F₁ PROGENIES

The crossing between *P. I Hsin Bee* x *P. pulcherrima* var. *champorensis* resulted in progenies with different flower patterns and sizes from both parents, like both parents and

a mixture of the parents on sepal and petal features. Regarding flower type, 14 of 40 progenies have a harlequin type, 23 accessions resembled *P. pulcherrima*, the remaining three accessions have diverse types from both parents. Based on the composition of progenies, the number of progenies with *P. pulcherrima* type is more than 50%. These indicate the contribution of *P. pulcherrima* was more dominant than harlequin. The more dominant derived characters are predicted due to the origin of the *P. pulcherrima* var. *champorensis*. This male parent is a primary hybrid since it was produced from the crossing between species (Hu et al. 2021). Thus, it tended to have less heterogeneous segregated gametes during meiosis (Marwoto et al. 2012).

Regarding flower size and color patterns, four progenies have the same or larger flower than the female parent but similar color and patterns to the male parent (Figure 4(C)- C). Nine progeny accessions show small flowers like the male parent, but the color pattern is dominated by the harlequin type with a symmetrical blotches pattern (Figure 4(D)). From the 40 progenies, 14 progenies also have a dark color at the base of the mid lobe that resembled the female parent (D829-8, D829-33, D829-12, D829-28, D829-19, D829-20, D829-15, D829-6, D829-39, D829-3, D829-4, D829-17, D829-32, and D829-23), and 23 genotypes show a light color resembling the male parent (D829-13, D829-37, D829-40, D829-30, D829-2, D829-24, D829-10, D829-7, D829-11, D829-21, D829-16, D829-34, D829-36, D829-29, D829-31, D829-5, D829-9, D829-14, D829-27, D829-25, D829-35, D829-26, and D829-38) (Figure 4). The color of the base of the mid-lobe matches with the spotted pattern on the sepals and petals, except the accessions D829-21 (Figure 4-(F21)), where the sepals and petals resembled the male parents, but the lips resembled the female parents. On the other hand, D829-17 (Figure 4-(F17)) has a harlequin pattern. It has a dark base of the mid-lobe, like its female parent.

The progenies from the cross between *P. I Hsin Bee* and *P. pulcherrima* var. *champorensis* also show petal,

TABLE 1. Loadings derived from the Principal Component Analysis (PCA) of the quantitative characters and analyzed *Phalaenopsis* genotypes

Variable	PCA1	PCA2
Leaf length	0.75	0.342
Leaf width	0.822	0.174
Flower length	-0.19	0.969
Flower width	-0.225	0.963

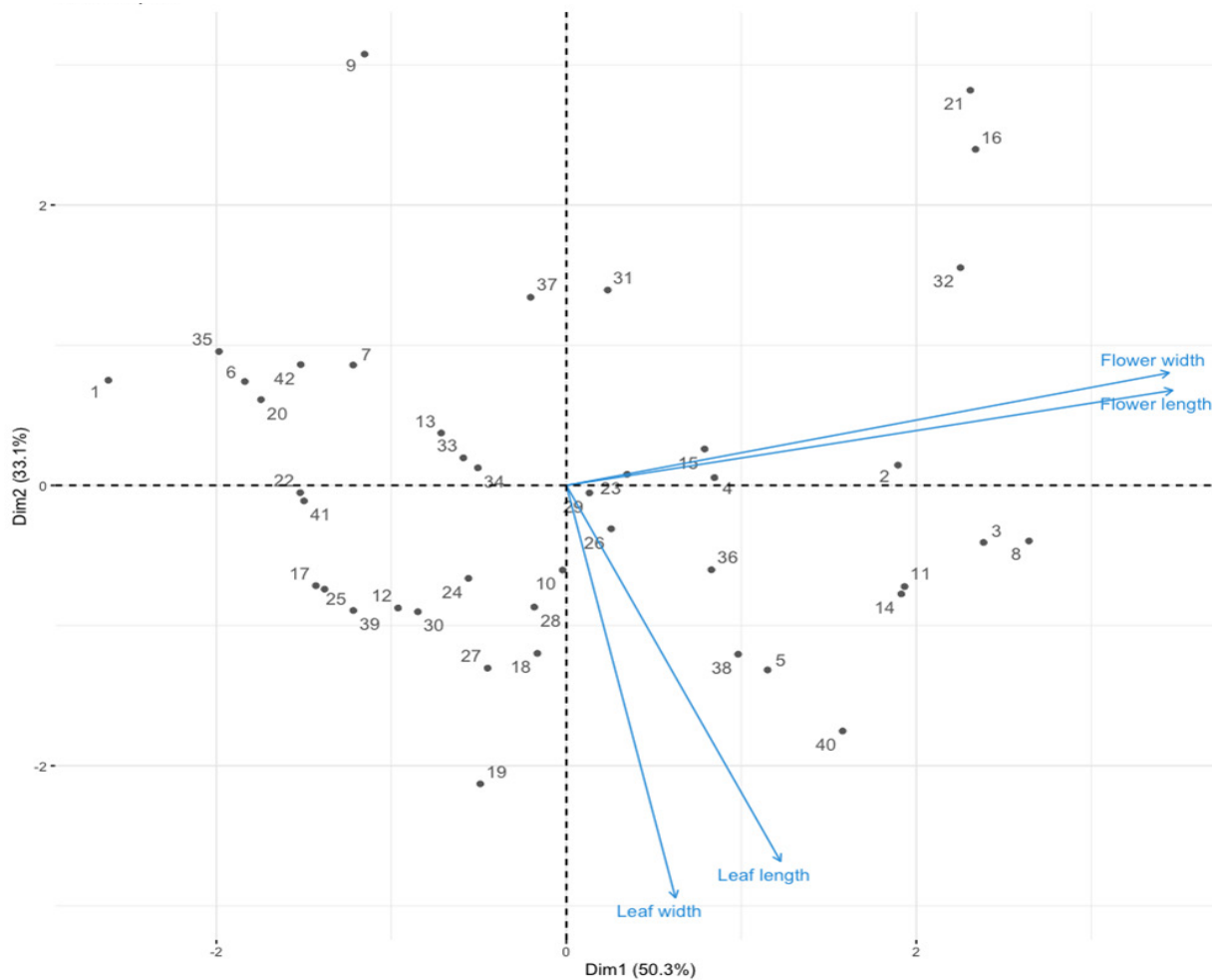


FIGURE 3. Principal component analysis of quantitative traits in both parental lines and 40 *Phalaenopsis* progenies. In this figure, the numbers 1 and 2 correspond to *P. I Hsin Bee* and *Phalaenopsis pulcherrima* var. *champorensis*, respectively, while numbers 3 to 42 represent F1 plants labeled with the codes D827-1 to D829-40

mid-lobe, dorsal, and lateral sepal shapes variations. Among the progenies, at least four petal shapes have been observed, i.e., semi-circular, elliptic, oval, and rhomboid, two dorsal and lateral sepal shapes (oval and elliptic), and two mid-lobe shapes (rhomboid and elliptical). The visual description of all these characteristics is presented in Figure 3. *Phalaenopsis pulcherrima* has a distinctive mid-lobe shape that looks like wings (Hu et al. 2021; Zhang et al. 2019), whereas, in *Phalaenopsis I Hsin Bee*, the wing is absent in the mid-lobe (De 2020; Hsu et al. 2010). Like the female parent, all the progenies have midlobes with

narrowed and widened sizes and no wing-like features (Figure 5). However, the contribution of the male parent (*P. Pulcherrima* var. *champorensis*) is considered high (>50%). Another important finding in evaluating progenies is the presence of spotted marks in the petals and sepals. These blotch marks varied from few to many, regular and symmetrical. These features indicate the contribution of the harlequin type of *P. I Hsin Bee* into progenies population. Several studies also reported these phenomena on interspecific crossings involving harlequin parents (Tang & Chen 2007).

CLUSTER ANALYSIS BASED ON QUALITATIVE CHARACTERS

Cluster analysis was carried out based on nine qualitative characters, i.e., petal color, sepal color, lip color, column color, side lobe color, petal shape, dorsal sepal shape, lateral sepal shape, and lip shape on the 40 progenies and the parents, *Phalaenopsis* I Hsin Bee and *P. pulcherrima* var. *champorensis*. The 42 accessions are clustered into two major groups, the parents and their offspring, with a similarity coefficient of 0.147 - 0.264 (Figure 6).

The first group was only made up of two parents. In contrast, the second group consisted of the progeny, which was divided into five subgroups. The first subgroup consisted of eight progenies that resembled female parents, namely D829-1; D829-3; D829-4; D829-8; D829-19; D829-32; D829-33; and D829-39. The typical features of these first groups are petal color (Red Purple 59A), lip color (Orange 26A), column color (White NN155D), and elliptic lips. The members of subgroup 2 consisted of 11 progenies (D829-14; D829-16; D829-20; D829-24; D829-25; D829-26; D829-27; D829-29; D829-31; D829-36; and D829-38). The 11 progenies have similar characteristics in petal color (Yellow, 6C), lip color (Orange 26A and Orange Red 35A), and column color (White NN155D). Subgroup three has ten progenies divided into two sub-subgroups, 3A and 3B. Sub-subgroup 3A consist of progeny D829-2; D829-11; and D829-35) which have similarities in six characters, petal color (Yellow 4C), lip color (Orange Red 35A), side lobe color (Yellow 14C), elliptic petal shape, oval dorsal and lateral sepal shapes. The members of sub-subgroup 3B were D829-6; D829-12; D829-15; D829-17; D829-18; D829-23; and D829-28, which have the common features in petal color (Yellow 4C) and column color (White NN155D). Group four comprise of two progenies, namely D829-9 and D829-37, which have six similar characters: petal color (Yellow 1C), sepal color (Purple 75A and Yellow 1C), lip color (Yellow 1C, Orange 26A, Orange Red 35A, and Purple 75A), column color (White NN155D), side lobe lip color (Purple 75A, Red Purple 59A, Orange Red 35A) and rhombic lip shape. Group five has eight progenies, i.e., D829-5; D829-7; D829-10; D829-13; D829-21; D829-30; D829-34; and D829-40 and have four similar features of petal color (Yellow 1D), sepal color (Yellow 1D), lip color (Yellow 14C and Purple 75C) and column color (White NN155D). Meanwhile, D829-22 are the only progeny that not included in any group (Figure 6).

Each group has distinct features representing the typical common characteristics from its members. Petal color becomes a distinct feature of group 1 from the others. The petal color of other groups fell into a yellow group. In other characters like sepal, lip and side colors, petal, dorsal and lateral sepal, and lip shapes, each group has

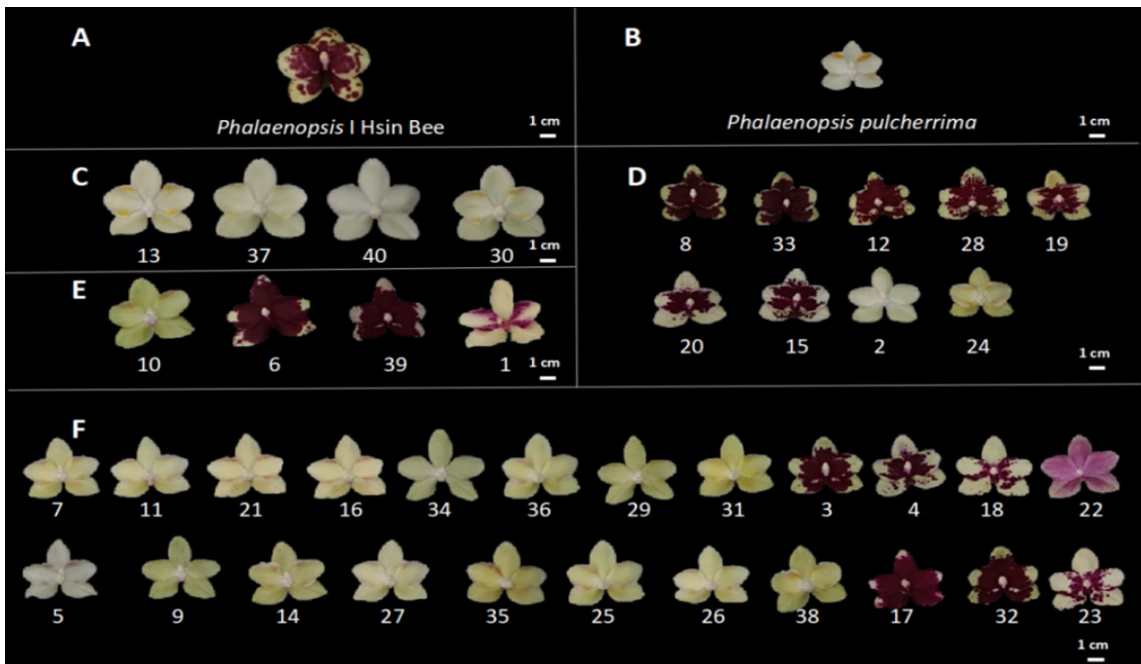
common difference. All groups seem to have a similar feature only in the column color characteristic. The grouping of progenies based on the nine qualitative characteristics indicate that the derivation of these characteristics differed from the parents. However, the color type on a petal, sepal, lip, column, and side lobe is less varied, except on D829-22. Further studies are needed to elucidate the phenomena behind the mixture color on D829-22. However, the typical blotch colors represent the prominent harlequin pattern (Chen, Lin & Chen 2020).

RELATIONSHIP BETWEEN QUANTITATIVE AND QUALITATIVE CHARACTERS

The correlations among the four qualitative (shape of petal, shape of dorsal sepals, shape of lateral sepals, and shape of lips) and four quantitative characters (flower length, flower width, leaves length, and leaf width) are illustrated in Figure 6. Notably, within the qualitative characters, the shape of dorsal sepals exhibit a highly positive correlation with the shape of lateral sepals ($r = 1$; $P < 0.01$). Conversely, the shape of lips show a medium negative correlation with the shapes of both dorsal and lateral sepals (both with $r = -0.39$; $P = 0.01$). Among the quantitative characters, flower width demonstrates a high positive correlation with flower length ($r = 0.96$; $P < 0.01$), while leaf width exhibits a positive medium correlation with leaf length ($r = 0.37$; $P = 0.016$). Additionally, this study explores the correlation between qualitative and quantitative characteristics. First, there is a negative medium correlation between leaf length and the shapes of dorsal and lateral sepals ($r = -0.37$; $P = 0.016$). Second, there is a medium, negative correlation between leaf width and the shapes of dorsal and lateral sepals (both with $r = -0.41$; $P = 0.007$) (Figure 7).

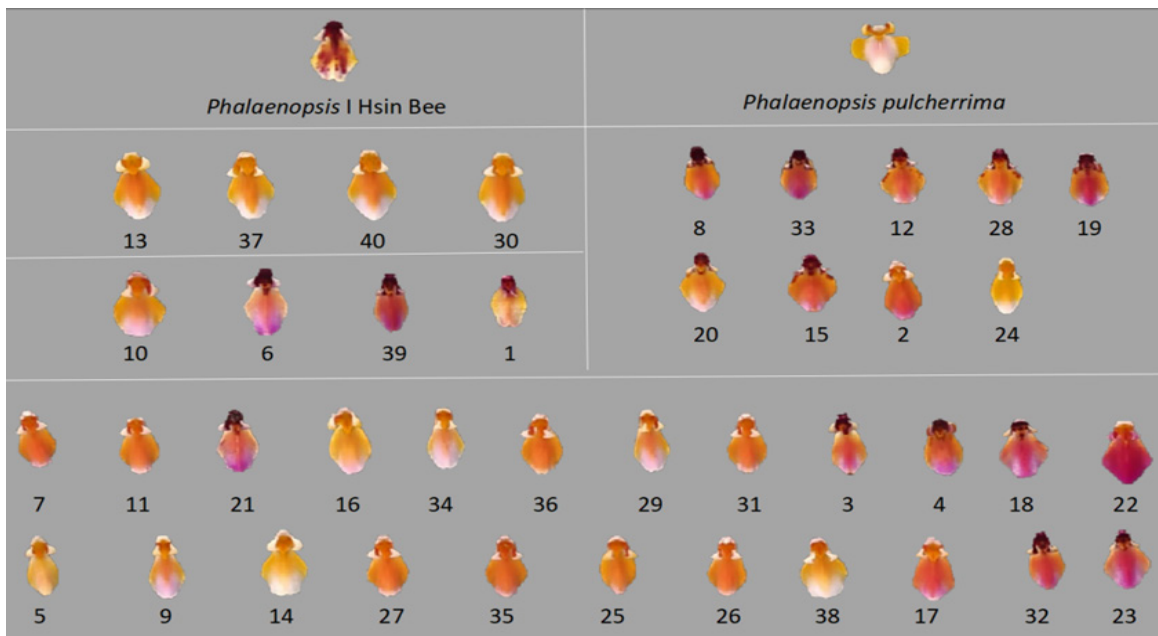
The shape of dorsal and lateral sepals in orchids is significant because it reflects characteristics inherited from parental lines, indicating similar shapes in both parent and their progenies. In this study, the feature, dominant in both parent lines, plays a role in orchid appearance, with potential connections to molecular processes influencing floral development and symmetry (Huang et al. 2015). The arrangement and shape of these sepals impact the flower's visual appeal, especially in the commercial market. Furthermore, there is a negative medium correlation between lip shape and the shape of lateral and dorsal sepals, suggesting an inverse relationship or proportionality in their growth patterns.

A strong positive correlation between the width and length of a flower implies that the flower is likely to exhibit a nearly round shape. This correlation signifies a uniform and harmonized growth pattern in flower dimensions, leading to a more rounded or symmetric appearance. The



The numbers in the figure range from 1 to 40 and correspond to the codes assigned to the progeny, namely D829-1 to D829-40

FIGURE 4. Size and color pattern of sepals and petals of *P.* I Hsin Bee (female parent) (A), *P. pulcherrima* var. *champorensis* (male parent) (B), and their offspring (C-E)



The numbers in the figure range from 1 to 40 and correspond to the codes assigned to the progeny, namely D829-1 to D829-40

FIGURE 5. The shape and color of the lip of *P.* I Hsin Bee (female parent), *P. pulcherrima* var. *champorensis* (male parent), and their offspring

round flower is identified as the ideal type for the commercially favored *Phalaenopsis* by Indonesian consumers, as highlighted in the study by Nuralinda et al. (2011). Meanwhile, the leaf width has a positive medium correlation with leaf length, which implies that these two characters are not independent. The moderate correlation means that as one-dimension increases, the other is likely to increase as well, demonstrating a coordinated growth pattern between leaf width and length (Schrader et al. 2021).

The medium negative correlation between qualitative and quantitative characteristics is found between leaf length and width with the shape of dorsal and lateral sepals. In practical terms, this indicates that as leaf length and width increase, there is a tendency for the shape of lateral and dorsal sepals to become rounder. The scoring system used, with half-round receiving the lowest score (1) and rhomboid the highest score (6), suggests that the correlation is based on the relationship between the quantitative measurements of leaf dimensions and the qualitative scoring of sepal shapes.

These findings highlight the interconnected nature of floral traits and dimensions, offering insights into the significance of correlations within both qualitative and quantitative characters of parental lines and the F_1 generation of *Phalaenopsis*. These correlations may contribute to understand the hereditary aspects of these traits and hold importance for commercial preferences.

SELECTION OF SUPERIOR CANDIDATES BASED ON QUANTITATIVE AND QUALITATIVE CHARACTERS

Based on the evaluation of the qualitative and quantitative characters of progenies, three progenies namely D829-4, D829-6, and D829-8, are considered to have prominent and preferred performance among the other progenies. The three progenies have longer leaves, yet their leaf width, flower length, and width resembled their parents (Table 2). Compared to their parents, two progenies, namely D829-4 and D829-8, have oval dorsal and lateral sepal shapes and resemble their male parents. Likewise, D89-6 has elliptic dorsal and lateral sepal shapes, like its female parents

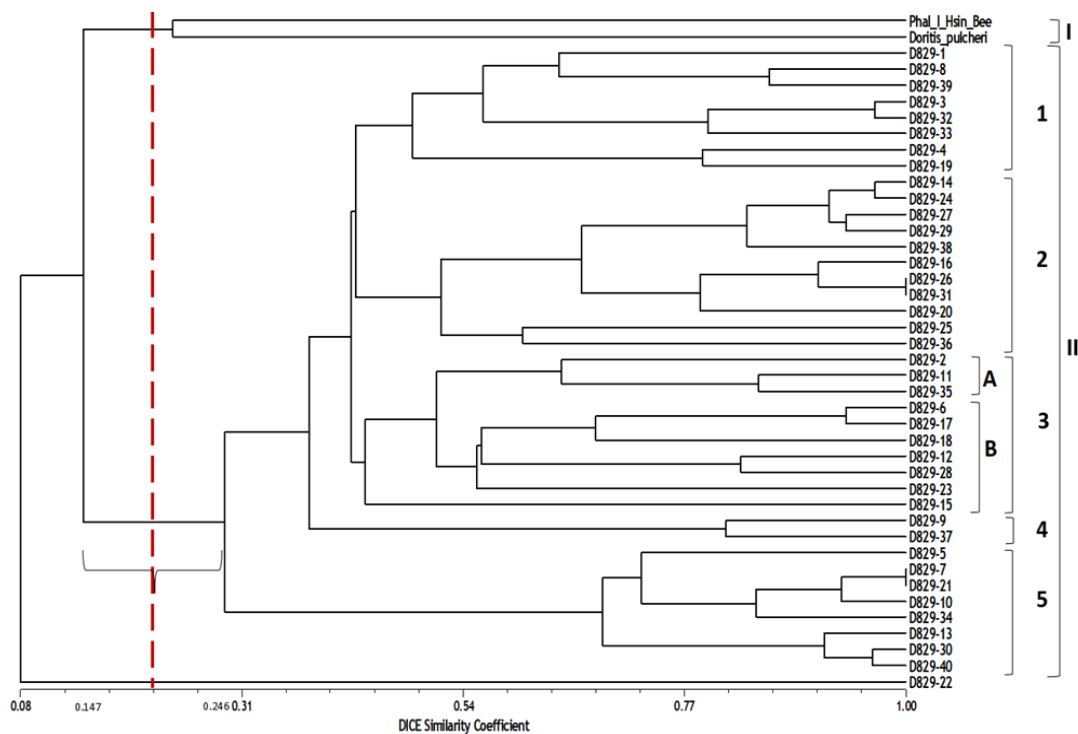


FIGURE 6. The constructed dendrogram of *Phalaenopsis* I Hsin Bee (female parent), *P. pulcherrima* var. *champorensis* (male parent), and their 40 progenies using the Dice similarity coefficient from nine qualitative characters and Unweighted Pair Group Method with Arithmetic mean (UPGMA)

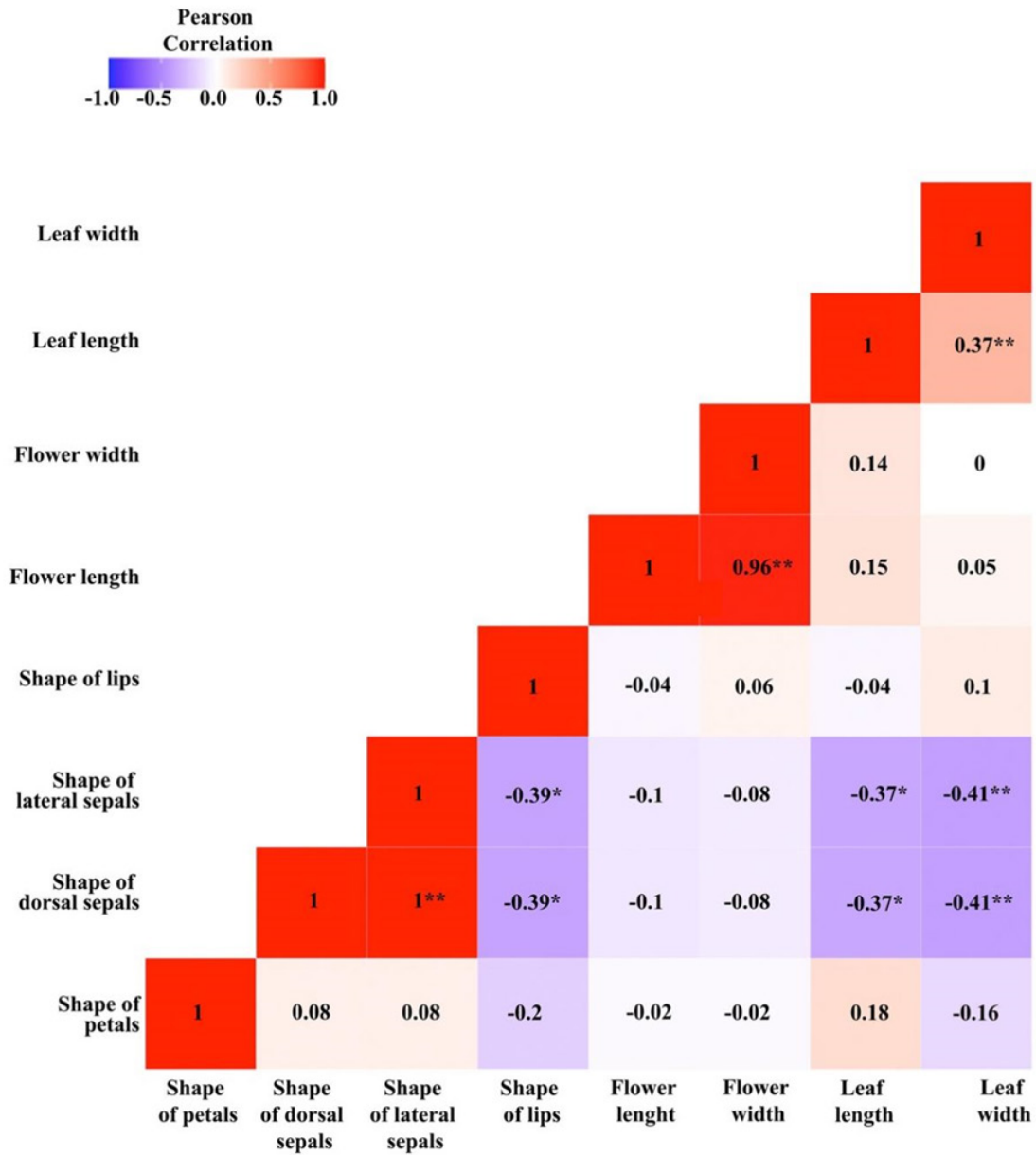


FIGURE 7. A Pearson correlation plot depicting the relationships among the dependent variables of *Phalaenopsis* I Hsin Bee (female parent), *P. pulcherrima* var. *champorensis* (male parent), and their 40 progenies analyzed in this study is presented. **indicates that the correlation is significant at the 0.01 level (2-tailed), while *indicates significance at the 0.05 level (2-tailed). The color key for Pearson correlation is as follows: blue represents negative correlation (below 0), white indicates no correlation (0), and red signifies positive correlation (above 0). The correlation coefficients exhibit varying degrees: high (≥ 0.50 to ± 1), medium (≥ 0.30 to $< \pm 0.50$), and low ($< \pm 0.30$)

TABLE 2. Comparative values on leaf and flower dimensions of progeny candidates and their parents

Genotype	Leaf length (cm)	Leaf width (cm)	Flower length (cm)	Flower width (cm)
<i>P. I Hsin Bee</i>	16.50 ± 0.35	7.37 ± 0.09	6.40 ± 0.00	6.70 ± 0.00
<i>P. pulcherrima</i> var. <i>champorensis</i>	14.73 ± 1.02	5.67 ± 0.19	4.47 ± 0.13	4.80 ± 0.10
D829-4	17.57 ± 0.30	5.27 ± 0.15	4.90 ± 0.00	4.97 ± 0.34
D829-6	24.40 ± 1.23	6.43 ± 0.37	6.60 ± 0.10	6.83 ± 0.07
D829-8	23.83 ± 0.75	6.00 ± 0.06	5.10 ± 0.06	5.97 ± 0.03

Means followed by number showed the standard error



FIGURE 8. Plant and flower performances of the female parent, *Phalaenopsis* I Hsin Bee (A, F, K); male parent *P. pulcherrima* (B, G, L); and the candidates: D829-4 (C, H, M); D829-8; and D829-6

(Table 3). All candidates have oval shapes on flower petals and lips and are different from their parents.

The selection of candidates also considered those progenies with *P. pulcherrima* var and *champorensis* characteristics but also have a harlequin type with spotted characters (Figure 8). These three candidates have been observed to have stable and symmetric coalescence of red-brownish blotches on the flowers, representing the

harlequin type (Bolaños-Villegas & Chen 2022; Hsu et al. 2010). D829-4, for instance, the combination of *P. I Hsin Bee* and *P. pulcherrima* var. *champorensis* are between the two parents. On the flower, a stable symmetric pattern of yellow color is present on the edge of the petal, with its lip resembling *P. Hsin Bee* (Figure 9).

TABLE 3. Comparative values of three candidates and their parents on qualitative characters

Qualitative characters	<i>P. IHsin Bee</i>	<i>P. pulcherrima</i> var. <i>champornensis</i>	D829-4	D829-6	D829-8
Petal color	Yellow 12C, Red Purple 59A	White NN155A, Yellow – Orange 14A, Purple – Violet N80A	Yellow Group 1D, Red Purple 59A	Yellow 4C, Red Purple 54A	Yellow 4C, Red Purple 59A, Yellow Orang 14 A
Sepal color	Yellow 12C, Red Purple 59A	White NN155A	Yellow 1D, Red Purple 59A, Yellow Orange 14C	Yellow 4C, Red Purple 54A	Yellow 4C, Red Purple 59A
Lip color	White NN155D, Greyed– Orange 172C, Greyed Purple 183A, Yellow 12A, Purple NN78D	White NN155AD, Purple 75B, Yellow – Orange 14A, 23A	Yellow 14A, Orange 26A, Purple N75A	Orange 26A-35A, Purple 74B, Red Purple 59A	Orange 26A-35A, Purple B74B-75A, Red Purple 59A
Column color	White NN155A	White NN155A	White NN155D	White NN155D	White NN155D
Side-lobe color	White NN155A, Greyed Purple 183A, Yellow 12A,	Yellow – Orange 14A, 23A, Greyed Purple 183A	Yellow Orange 14 A, Greyed Purple 187A, Red Purple 59A	White NN155A, Red Purple 59A. Greyed Purple 187A	Yellow 1C, Greyed Purple 187A
Petal shape	Semi circular	Semi circular	Oval	Oval	Oval
Dorsal sepal shape	Elliptic	Oval	Oval	Elliptic	Oval
Lateral sepal shape	Elliptic	Oval	Oval	Elliptic	Oval
Lip shape	Rhombic	Winged Elliptic	Oval	Oval	Oval



FIGURE 9. The performance of selected genotype D829-4

CONCLUSIONS

The evaluation of progenies derived from *P. I Hsin Bee* x *P. pulcherrima* var. *champorensis* shows that *P. pulcherrima* is more dominant in flower shape and color than harlequin. About 35% of progenies have a dark color at the base of the mid-lobe and matched with the blotched pattern on the sepals and petals, resembling the female parent, indicating the harlequin type from *P. I Hsin Bee* can be introduced into *P. pulcherrima* var. *champorensis*. The cluster analysis based on nine qualitative characters divide the progenies into five groups with petal, sepal dorsal, and lateral and mid-lobe shape variations. These separated the progenies from their parents' group. Based on the evaluation of qualitative and quantitative characteristic of progenies and the parents, three progeny accessions, namely D829-4, D829-6, and D829-8, are considered to have prominent features compared to the rest. These candidates show stable and symmetric coalescence of red-brownish blotches on the flowers, representing the harlequin type.

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SUPPLEMENTARY TABLE 1. Mean of quantitative characters measured from two parental lines and 40 progenies

Genotype	Leaf length (cm)	Leaf width (cm)	Flower length (cm)	Flower width (cm)
<i>P. I Hsin Bee</i>	16.50 efghij	7.37 abcd	6.40 cde	6.70 b
<i>P. pulcherrima</i> var. <i>champorensis</i>	14.73 efghij	5.67 efgh	4.47 p	4.80 p
D829-1	18.07 defghi	7.80 ab	6.67 abc	6.73 b
D829-2	19.60 cdefg	6.43 abcdefgh	6.03 ef	6.03 def
D829-3	20.47 abcdef	7.93 a	6.07 ef	6.03 def
D829-4	17.57 efghij	5.27 ghi	4.90 o	4.97 nop
D829-5	14.2y ghij	6.07 defgh	5.30 jklmn	5.23 jklmn
D829-6	24.40 abc	6.43 abcdefgh	6.60 abc	6.83 ab
D829-7	11.90 j	4.03 i	5.50 ghijkl	5.50 hij
D829-8	23.83 abcd	6.00 defgh	5.10 nmo	5.97 ef
D829-9	24.17 abc	6.43 abcdefgh	6.50 bcd	6.27 cd
D829-10	23.83 abcd	6.00 defgh	5.10 nmo	5.10 lmno
D829-11	17.60 efghij	6.03 defgh	5.40 ijklm	5.40 ijk
D829-12	24.43 abc	6.43 abcdefgh	6.40 cde	6.33 c
D829-13	19.03 cdefghi	6.30 bcdefgh	6.13 def	5.93 efg
D829-14	13.27 ij	5.60 fgh	6.90 a	7.03 a
D829-15	14.47 ghij	7.80 ab	5.00 no	5.10 lmno
D829-16	19.10 cdefghi	7.70 abc	5.50 ghijkl	5.50 hij
D829-17	25.67 ab	7.20 abcde	5.10 nmo	5.27 jklm
D829-18	15.27 efghi	5.97 defgh	5.00 no	5.00 mnop
D829-19	13.4o hij	5.07 hi	6.93 a	7.03 a
D829-20	18.40 cdefghi	6.10 defgh	5.00 no	5.00 mnop
D829-21	20.57 abcde	6.03 defgh	5.77 ghi	5.83 fg
D829-22	20.20 abcdefg	6.70 abcdefg	5.40 ijklm	5.30 jkl
D829-23	14.67 efghij	7.80 ab	5.00 no	5.13 klmno
D829-24	19.33 cdefgh	6.73 abcdefg	5.83 fgh	5.67 ghi
D829-25	19.10 cdefghi	7.73 abc	5.40 ijklm	5.33 jkl
D829-26	19.13 cdefghi	7.30 abcd	5.53 ghijk	5.50 hij

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D829-27	20.23 abcdefg	6.20 defgh	5.63 ghij	5.77 fgh
D829-28	20.47 abcdef	6.83 abcdef	5.17 klmn0	5.23 jklmn
D829-29	16.30 efghij	5.43 ghi	5.87 fg	5.97 ef
D829-30	17.47 efgihj	5.60 fgh	6.83 ab	6.77 ab
D829-31	19.27 cdefghi	5.90 defgh	5.43 ijklm	5.40 ijk
D829-32	16.97 efghij	6.43 abcdefgh	5.47 hijklm	5.50 hij
D829-33	13.33 hij	5.93 defgh	4.90 o	5.00 mnop
D829-34	19.13 cdefghi	7.30 abcd	6.03 ef	5.93 efg
D829-35	14.23 ghij	5.83 defgh	5.60 ghij	5.90 efg
D829-36	20.53 abcde	7.73 abc	6.03 ef	5.93 efg
D829-37	19.73 bcdefg	6.87 abcdef	5.17 klmn1	4.93 op
D829-38	26.23 a	7.27 abcd	6.10 ef	6.13 cde
D829-39	18.73 cdefghi	6.10 defgh	5.00 no	5.00 mnop
D829-40	13.27 ij	6.20 defgh	5.13 lmno	5.17 klmno

Means followed by the same letter in the same column are not significantly different based on Tukey test, $p < 0.05$