Evaluation of Plant and Flower Characteristics of selected 15-Gy irradiated *Phalaenopsis aphrodite*

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Abstract

Phalaenopsis or butterfly orchid is found in tropical countries and can be classified as terrestrial, epiphytic, or lithophytic. Because Phalaenopsis aphrodite's flowers are purely white, attempts were made to modify them through gamma irradiation. A 15-Gy dose of gamma irradiation effectively induced mutation in P. aphrodite. In this study, three mutant plant selections, previously irradiated with 15 Gy, were characterized based on the leaf, root and flower traits and compared these traits with that of the untreated P. aphrodite. The plant selections have purple to light purple flowers in contrast to the control plants with pure white flowers. Flower, leaf and root characters of irradiated plants were observed to be significantly different from untreated plants. Irradiated plants are smaller, compact and dwarf at the flowering stage. In addition, more primary roots and increased chlorophyll content were observed in irradiated plants. They also have shorter spikes with lesser flowers. Furthermore, smaller flowers and thinner leaves were also detected in the irradiated plants. In addition, after self-pollination, irradiated plants obtained shorter pods. Currently, the three 15-Gy irradiated P. aphrodite plants are being self-pollinated and grown in vitro. They will then be further grown to assess the stability of phenotypic traits observed in the first generation.

***Keywords**:* breeding, butterfly orchids, gamma irradiation, mutation, *Phalaenopsis*

1. Introduction

Phalaenopsis, also known as moth orchids or butterfly orchids, are one of the most common commercially available potted orchids worldwide (Hinsley *et* *al*., 2017; Magdalita *et al*., 2019). These species have high commercial value with cultivars due to their floral quality (Hsieh *et al*., 2020). The traits important to *Phalaenopsis* species include varying sizes of flowers with long inflorescences/flower spikes and varying flower color (Magdalita *et al.*, 2019). The *Phalaenopsis* genus comprises more than 63 epiphytic species found in Southeast Asia and the Pacific (Padolina, 2006). Because of its long shelf life and graceful flowering habit, it is a valuable potted flowering plant commercially produced worldwide (Blanchard and Runkle, 2006). In the Philippines, it is believed that 14 species and 17 varieties of *Phalaenopsis* are endemic (Valmayor, 1984; Magdalita *et al*., 2019).

Due to the country's rich diversity, the utilization of these species can give a competitive edge, especially in this time of ornamental boom this pandemic. Important species with novel traits can be utilized for variety development via mutation breeding. These include *P. aphrodite* for the prolific flowering habit, *P. lueddemanniana* for unique flower markings such as bars and blotches in its flowers, *P. pallens* for fragrance, *P. schilleriana* for pale purple flower color and prolific flowering habit and *P. stuartiana* for red coloration of the lip. These orchid species have contributed greatly to the improvement and diversification of *Phalaenopsis* hybrids (Vergara, 1997). Griesbach (2002) also claimed that *P. equistris* is heavily used in *Phalaenopsis* breeding programs due to its unique flower with white petals and red lip. However, incompatibility among parents in *Phalaenopsis* was a barrier to the production of intergeneric hybrids (Aziz and Sukma, 2015). To overcome this limitation, the utilization of a highly heterozygous population complemented with mutation breeding via irradiation among *Phalaenopsis* can be exploited to increase variability to produce useful mutants in a short period (Li *et al*., 2014).

In the past, gamma radiation was used by Lapade *et al*. (2001) to induce variation to breed new varieties of *P. schilleriana*, *Vanda sanderiana*, and *Dendrobium* sp. conducted at the Philippine Nuclear Research Institute (PNRI) in Quezon City, Philippines. In their study, irradiation of immature embryos with gamma rays at doses ranging from 5 to 10 Gy increased the germination rate of *P. schilleriana* and *Dendrobium sp*.. Furthermore, protocorms of *V. sanderiana* were subjected to a radiation dose of 10 Gy and developed bigger and more vigorous plantlets (Lapade *et al*., 2001). Mutation breeding done to increase variability was reported in *Spathoglottis plicata* (Suyitno *et al*., 2017) and *D. odoardi* (Fathin *et al*., 2021). Induction of insect resistance was carried out through mutation breeding in *Dendrobium* (Ahmad *et al*., 2015) and D. 'Sonia Kai' (Arifin and Basiran, n.d.).

Since *P. aphrodite* has large, white flowers, attempts to improve certain flower characters such as color, form and size have been done through mutation via ionizing gamma radiation. Previous reports have shown that exposure to 15 Gy of *P. aphrodite* effectively induced distinct early flowering by one year and eight months when compared with other doses (Magdalita *et al*., 2019). The mutants irradiated with 15 Gy were purposely chosen for this study because they consistently exhibited mutated traits. Also, they can be potential selections for variety release or breed with others and become a source of segregating populations. The study aimed to describe the leaf, floral and root morphological characters of the selected 15-Gy irradiated *P. aphrodite* plants and compare them with the unirradiated ones.

2. Methodology

2.1 Plant Material

Four-year old-flowering plants of *P. aphrodite*, previously irradiated with a 15-Gy dose of gamma irradiation and unirradiated plants, were used in this study. They were utilized based on the previous result (Magdalita *et al*., 2019). Among the 30 first-generation mutants exposed to 15 Gy, three plants flowered and were used in the study. The plants were grown in the screenhouse of the Fruit and Ornamental Crops Section of the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB), Laguna, Philippines.

2.2 Characterization of Flowers of 15-Gy irradiated P. aphrodite Plants

Selected flowering plants (three plants) of 15-Gy irradiated *P. aphrodite* from previous studies of Magdalita *et al*. (2019) and unirradiated control plants (10 plants) were characterized. The parameters measured were flower length and width; and petal and sepal length, thickness and width. Spike length, number of flowers, the distance between flowers and length of two-month-old self-pollinated pods were also measured. Distance between flowers was determined by measuring the distance of one flowering node with another in a spike. Other morphological characters such as leaf length, width, thickness and chlorophyll content, and root number and length were also measured. The morphological characters were compared with the untreated *P. aphrodite* plants. Measurement of the spike length, leaf length and width, pod length and root length were measured using a ruler. Petal and sepal length and width were measured using a Vernier caliper (531-128, Mitutoyo, Japan), while the petal, sepal and leaf thickness were measured using a micrometer caliper (Series 103, Mitutoyo, Japan). The chlorophyll content of the leaves was measured using an automatic chlorophyll meter (TYS-B, Hangzhou Mindfull Technology Co., Ltd, China). Mature primary roots were measured from the matured flowering plants from the endpoint of the shoot to the root tips. Qualitative traits such as gross plant appearance were also determined. The flower and sepal colors were identified based on the color chart of the Royal Horticultural Society (RHS) Color Chart (RHCC) (RHS, 1966).

2.3 Data Analysis

Random sampling was used for the characterization of flowers, leaf and root traits of the three selected 15-Gy irradiated *P. aphrodite* plants. The completely randomized design (CRD) was employed since all experiments were conducted in the screenhouse. Three replications with five flowers per replicate were measured for the flower characteristics giving a total of 15 flowers per plant selection. Four fully expanded leaves per selection were measured for the leaf characteristics while four roots were measured for root length. The Statistical Tool for Agricultural Research (STAR) program (International Rice Research Institute [IRRI], 2014) was utilized in the analysis of all gathered data. They were subjected to one-way analysis of variance (ANOVA) using the F-test (α = 5%). The significant difference between treatment means was detected using the least significant differences (α = 5%).

3. Results and Discussion

3.1 Morphological Characterization of 15-Gy irradiated P. aphrodite Plant Selections

The changes observed in 15-Gy irradiated *P. aphrodite* plant selections are presented in this paper. Individual flowering plants from 30 15-Gy irradiated *P. aphrodite* plants with novel traits were selected. They can be further bred with other genotypes and commercial hybrids or self-pollinated to generate an improved population where selection can be made. In addition, individual *P.*

aphrodite mutant selections can also be used as sources of new genes as parents to produce hybrids.

In the present study, three irradiated plants produced purplish flowers (Figures 1a to 1c). Specifically, a purplish tinge on the petals was also observed in the 15-Gy irradiated plant. In contrast, the unirradiated or control plants have pure white petals and sepals (Figure 1d).

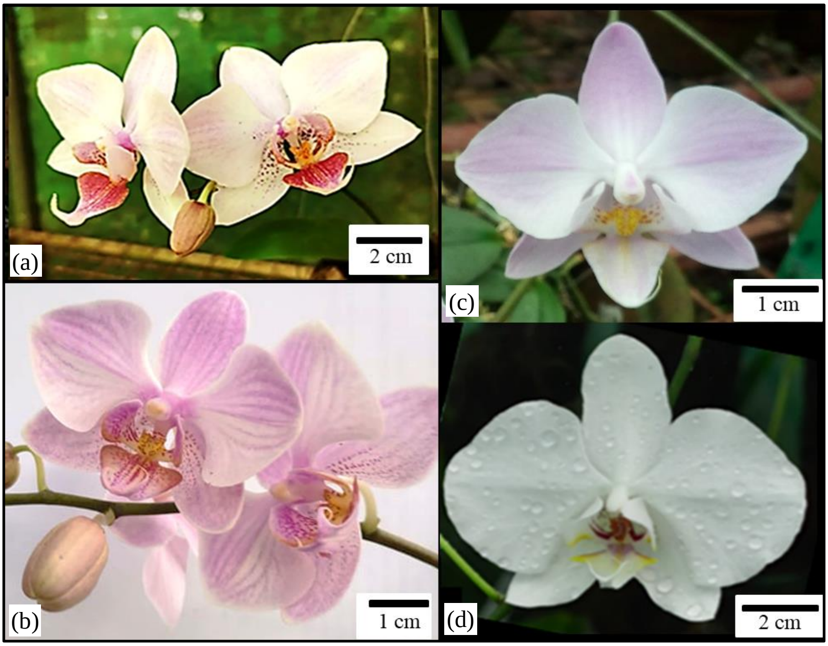


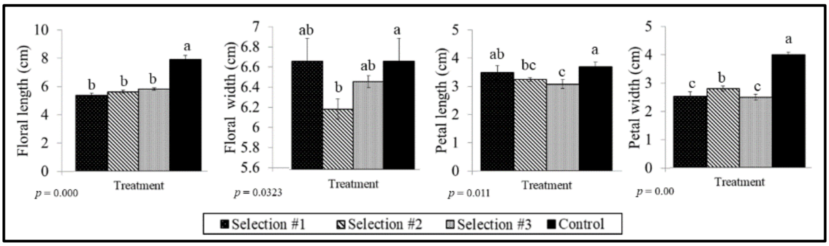
Figure 1. Blooms of 15-Gy irradiated *P. aphrodite* selections #1 (a), #2 (b) and #3 (c) and untreated one (d)

The 15-Gy irradiated *P. aphrodite* plants have a more compact and dwarf growth habit and are smaller than the unirradiated control plants (Figure 4). The spike is shorter and leaves are smaller than the control plants, suggesting that 15-Gy gamma irradiation is responsible for this induced variation. This result corroborated with those of Hameed *et al*. (2008), who reported that gamma irradiation could stress the plants' biochemical and physiological processes that could affect plant processes like flowering. Mutations caused by irradiation can be observed on morphological, structural and functional features of the plants. Dehgahi and Joniyasa (2017) claimed that one determinant of irradiation stress to the plant is the changes observed in its morphological structure.

Multicoloration of *P. aphrodite* petals was also induced by gamma irradiation as observed in the three plant selections (Figure 1). Pink to purple blotches were observed in the petals in plant selection #1 (Figure 1a), while the other *P. aphrodite* exposed to 15 Gy was observed to have pinkish venation in the white petals and sepals in both plant selections #2 (Figure 1b) and #3 (Figure 1c). This observation corroborated with the previous report of Pallavi *et al*. (2017), who also found variation among gamma rays-treated *Zinnia elegans*. The same authors observed color variants on the plants exposed to 75, 100 and 125 Gy. Compared with the control, which is pink, they were able to obtain yellow, orange-yellow and other colored flowers. Color mutations were also achieved, such as pink variants in 40-Gy irradiated *Torenia hybrida* (Suwanseree *et al*., 2011), yellow mutants in 30-Gy *Exacum affine* (Limtiyayotin *et al*., 2018). Earlier studies by Mukherjee and Khoshoo (1970) indicated interesting variegations such as spots, flecks and streaks in irradiated *Canna* sp.

The *P. aphrodite* selections having flower color mutations can be isolated and multiplied to produce genetic stocks and new varieties. The pinkish mutants isolated from *P. aphrodite* were observed to produce stable mutation because, after three cycles of flowering, similar purplish flower characters were seen. The three selected 15-Gy irradiated *P. aphrodite* plants mutants showed differential phenotype in terms of flower colors, while the untreated *P. aphrodite* control plants produce uniform white petal flowers. In contrast, selection #1 had red-purple (RHCC 60B) spots in the sepals and lilac purple (RHCC 70A) tinge in the petals and Indian yellow (RHCC 14A) based on the lip (Figure 1a), while plant selection #2 had magenta (RHCC 64B) small spots on the base of the sepals with *Spiraea* red (RHCC 63B) lining on the petals and magenta rose (RHCC 64B) base of the lip (Figure 1B) and plant selection #3 had light amethyst violet (RHCC 84B) linings on both petals and sepals with Indian yellow (RHCC 14A) tinges on the base and side of the lip (Figure 1c). Aside from color mutation, a variation in flower quantitative characters of the 15-Gy irradiated plants was also observed. In *P. aphrodite*, smaller flowers in terms of flower length were perceived in the three selected 15-Gy irradiated plants.

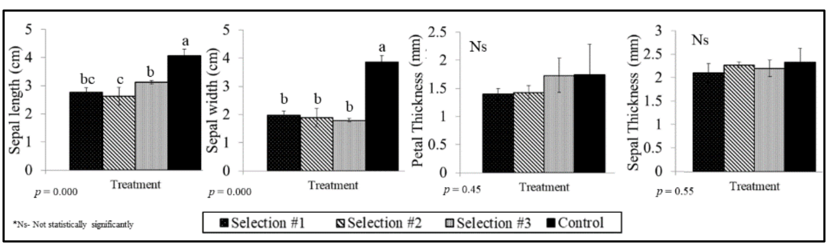
In terms of flower parameters, significant differences were noticed in the mean length and width of the flower in general, petals and sepals of blooms of 15-Gy irradiated plants compared with the untreated plants (Figures 2 and 3). For example, when compared with control plants, the three selected 15-Gy irradiated *P. aphrodite* plants had flowers that were 5.57-, 5.6- and 5.8-cm long, respectively (Figure 2). Significant differences were also discovered in floral width and petal length and width of the three selected 15-Gy irradiated *P. aphrodite* plants. A similar finding was reported by Srivastava and Mishra (2005), who spotted smaller flowers in *Hibiscus rosa-sinensis* plants exposed to gamma irradiation. Other reports indicated that varied flower sizes were observed across gamma-irradiated plants, such as in chrysanthemum (Lamseejan *et al*., 2000), *Calisthepus chinensis* (Anwar *et al*., 2020) and Buddleia (Dai and Magnusson, 2012). Additionally, fewer flowers ranging from three to four were produced in contrast to the control plants with five to eight flowers (Table 1). The same was observed in *Exacum affine* irradiated plants (Limtiyayotin *et al*., 2018).



Means with different letters are significantly different at α = 5%.

Figure 2. Mean flower and petal quantitative characters of 15-Gy irradiated and untreated (control) *P. aphrodite*

On the other hand, significant differences were identified in sepal length and width. However, no significant differences were observed in petal and sepal thickness. Sepal length and width of selected flower diameter were smaller in the flower of 15-Gy irradiated plant compared with control. Petal widths of the three plant selections were shorter compared with the control. Furthermore, shorter and stouter sepals were observed in blooms of the three 15-Gy irradiated plant selections compared with the control (Figure 3).



Means with different letters are significantly different at α=5%.

Figure 3. Mean sepal characters and petal thickness of 15-Gy irradiated and untreated (control) *P. aphrodite*

This result suggests that ionizing radiation could be damaging or have imposed a deleterious effect on the chromosomal composition of *P. aphrodite* while inducing mutation to the plant. In the future, cytogenetic analysis can be done on the *P. aphrodite* mutants to establish chromosomal aberrations as one of the causes of reduced flower size and dwarfism. For instance, it has been reported that ionizing radiation causes mutagenesis via chromosomal aberration in plants. These aberrations may induce reduction or damage of chromosomes correlated to a corresponding decrease in flower diameter as observed in chrysanthemum (Ichikawa *et al.*, 1970).

Interestingly, spike length, the distance between flowers, number of flowers and pod length of two-month-old self-pollinated pods showed significant differences when all the three plant selections were compared with the control (Table 1). It was noted that shorter spikes with a shorter distance between flowers were observed in the three plant selections than in the control (Table 1).

Table 1. Flower-associated plant characteristics of 15-Gy irradiated and untreated (control) *P. aphrodite*

Treatment	Spike length (cm)	Distance between flowers (cm)	No. of flowers	Pod length (cm)
15-Gy plant selection #1	19.47±2.34^b	1.37±0.12^b	3.00±0.02^b	4.37±0.59^b
15-Gy plant selection #2	18.73±2.42^b	1.43±0.32^b	2.67±0.57^b	4.13±0.45^b
15-Gy plant selection #3	18.97±1.31^b	1.57±0.15^b	3.33±0.31^b	5.09±0.64^b
Untreated (Control)	31.6±2.55^a	2.05±0.13^a	8.33±0.58^a	7.3±0.87^a

Means with different letters are significantly different at $\alpha = 5\%$.

In terms of the number of flowers in a spike, in comparison to control plants, lesser flowers were produced by the 15-Gy irradiated plant selections. Self-pollinated flowers developed pods after two months. The pods of the 15-Gy irradiated plant selections were shorter compared with the unirradiated P. *aphrodite* plants. The aforementioned findings suggest that there is dwarfing effect brought about by irradiation which is a common phenomenon in mutation breeding (Billore *et al*., 2019). This was evident not only in the leaf characteristics of *P. aphrodite* but also in the other plant traits, such as spike length and distance between flowers on the spike. Since the spike was shorter, it follows that fewer flowers were also produced. Hence, treated plants were more compact, as evidenced by the shorter distance of nodes (Figure 4).

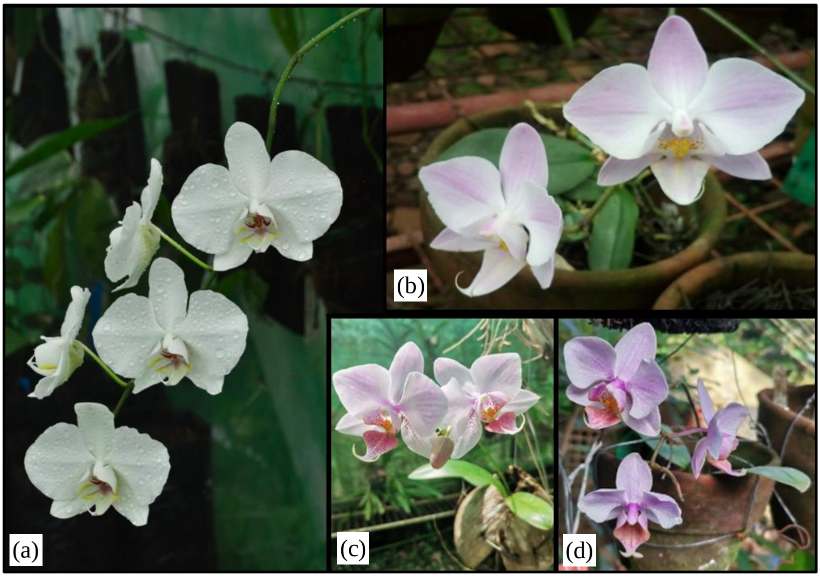


Figure 4. Flowering habit of untreated *P. aphrodite* plant (control) (a) and 15-Gy irradiated *P. aphrodite* plant selections #3 (b), #1 (c), #2 (d)

Significant differences were observed in vegetative parameters such as leaf length, width, thickness and chlorophyll content, and primary root length in 15- Gy irradiated plants compared with the control. Leaves of the three 15-Gy irradiated *P. aphrodite* plant selection were smaller than control plants. These effects can be linked to the destruction of meristematic cells that cause changes in the size of plant organs. According to Taheri *et al*. (2014), these changes are caused by free radical production due to the interaction of radiation with cellular substances such as water. They also added that free radicals lead to the destruction of cells.

In the present study, significant differences were discerned in the leaf length and thickness of 15-Gy irradiated *P. aphrodite* plants (Table 2). Shorter leaves were recognized in the three 15-Gy irradiated plant selections when compared with the control. Further, stouter leaves were observed in plant selections #1 and #2 but comparable leaf widths were noticed in the control and plant selection #3. Additionally, thicker leaves were spotted in the control and plant selection #2. These results agree with the previous report of Taheri *et al*. (2014) indicating significant differences in leaf characters and plant height among gamma-induced mutants of *Curcuma alismatofolia* and those of 15-Gy irradiated *Graptophyllum pictum* (Rosmala *et al*., 2016). Generally, Billore *et al*. (2019) claimed that reduced plant stature or dwarfism is a common expression of induced mutation among organisms. Similarly, Abdullah *et al.* (2018) also sighted dwarfing effect on mutants of *Zingiber officinale* Roscoe mutants irradiated with 9-13 Gy, while Billore *et al*. (2019) reported reduced shoot length and leaf area in *Dendrobium* 'Sonia Kai' irradiated with 15 to 45 Gy. In general, gamma radiation has either a promotive or destructive effect on the enzyme activity of the plant that is related to metabolic processes associated with the growth of these anatomical structures of the leaves.

Table 2. Leaf characteristics of 15-Gy irradiated and untreated (control) *P. aphrodite*

Treatment	Leaf length (cm)	Leaf width (cm)	Leaf thickness (mm)	Chlorophyll reading (SPAD)
15-Gy plant selection #1	12.67±0.12^{b}	4.90±0.4^{b}	2.42±0.44^{b}	97.20±0.75^{a}
15-Gy plant selection #2	13.67±0.45^{b}	4.40±0.36^{b}	3.41±0.32^{a}	97.25±1.33^{a}
15-Gy plant selection #3	12.93±0.4^{b}	5.65±0.40^{a}	2.47±0.27^{b}	94.82±1.68^{a}
Untreated (Control)	21.54±0.38^{a}	5.99±0.46^{a}	3.61±0.31^{a}	86.55±1.45^{b}

Means with different letters are significantly different at α = 5%.

A significant difference in the chlorophyll content of the leaves was observed on 15-Gy irradiated *P. aphrodite* plant selections compared with the control plants (Table 2). Higher chlorophyll content was detected in all three plant selections than in control plants. This finding corroborated with the recent report on *G. pictum* and *Dendrobium* 'Sonia Kai' orchids where there was an elevated level of chlorophyll content of mutants exposed to lower doses of gamma irradiation including those on *Cullen cotylifolium* exposed to 10 Gy (Jan *et al*., 2013; Rosmala *et al*., 2016; Billore *et al*., 2019). In addition, some chlorophyll mutants develop variegation, albinism and lighter coloration; their expression was observed when higher doses of irradiation were used. The chloroplast of the leaves where the plastids are located is a sensitive organelle to a high dose of gamma irradiation exposure. In contrast to the above findings, lower doses of gamma irradiation cannot induce severe alterations in the chloroplast structure (Rosmala *et al*., 2016).

Plant selections #1 and #3 had five roots, while the control and plant selection #2 had six primary roots. In terms of mean root length, longest roots were observed in plant selection #3 followed by plant selection #2 (Table 3). The shortest roots were noticed in the control plants. Similarly, Marcu *et al*. (2013)

observed variation in root length among maize plants exposed to varying doses of gamma irradiation.

Table 3. Number of roots and root length of 15-Gy irradiated and untreated (control) *P. aphrodite*

Treatment	No. of roots	Root length (cm)
15-Gy plant selection #1	5	4.90±0.2^{b}
15-Gy plant selection #2	6	5.20±0.62^{ab}
15-Gy plant selection #3	5	5.93±0.26^{a}
Untreated (Control)	6	4.81±0.35^{b}

Means with different letters are significantly different at α = 5%.

4. Conclusion and Recommendation

Phalaenopsis or butterfly orchid is an economically important orchid species in the country. Non-conventional breeding techniques such as mutation through physical mutagens were explored in *P. aphrodite*, a purely white *Phalaenopsis* species. Mutants were generated and the 15-Gy dose of gamma irradiation-induced novel variability wherein three plant selections produced unique flower colors. In this study, it was observed that the three 15-Gy irradiated *P. aphrodite* plants produced purplish flowers compared with untreated control plants having white flowers. To the knowledge of the authors, this is the first report of mutated flower color on the native *P. aphrodite* existing in the Philippines via mutation breeding. The irradiated *P. aphrodite* plants showed significant differences in the mean flower and sepal length and in sepal and petal width. Shorter flowers were observed in irradiated plant selections. In addition, smaller and thinner sepals were also detected in the irradiated plant selections. In terms of leaf characters, shorter and thinner leaves but higher chlorophyll content and longer roots were noticed in irradiated mutants.

As of this writing, further breeding is being done on the three irradiated *P. aphrodite* plant selections by self-pollination and in vitro culture of the embryos. They will then be grown in vitro, potted out and further characterized to assess the stability of phenotypic traits observed in the first generation.

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