Journal of Nature Studies 12 (2): 45-53
ISSN: 2244-5226

Notes and Information

OCCURRENCE OF PIGMENTED FLOWERS AND VARIATION IN OTHER MORPHOLOGICAL TRAITS OF IRRADIATED AND UNIRRADIATED SAMPAGUITA (Jasminum sambac L. Sol.)

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ABSTRACT – The occurrence of pigmented flowers and variation in other morphological traits including the survival of cuttings were investigated in unirradiated and irradiated sampaguita. Cuttings were exposed to varying doses of gamma rays ranging from 0, 3, 5, 10 and 15 Gy. Unirradiated control plants and those irradiated at 15 Gy had the highest percent survival of cuttings which was not significantly different at 70.29% and 67.19% respectively. In terms of the number of branches, plants derived from irradiation with 3 Gy produced the highest at 19.00 which is not significantly different from 15 Gy at 17.75, while the lowest was obtained at 5 Gy at 11.57. The width and length of leaves derived from irradiated and unirradiated cuttings were statistically similar. The highest mean number of petals at 9.33 and sepals length at 9.67 cm was observed in the unirradiated control although this is very similar to irradiated treatments. The mean number of white flowers is highest in the unirradiated control plants at 72.79 followed by those plants derived from 5 Gy at 53.08, while the lowest mean number of white flowers at 18.70 came from plants derived from irradiated cuttings at 15 Gy. Furthermore, the highest mean number of pigmented flowers is not significantly different for the unirradiated control plants which have 6.14, and those plants which were derived from irradiated plants with 3 Gy at 5.0 and with 10 Gy at 5.49. The lowest mean number of pigmented flowers was observed from plants derived from cuttings exposed to 15 Gy. In both the unirradiated control and irradiated treatments regardless of radiation dose, flower fragrance was retained in both pigmented and white flowers. The different doses of radiation used in the present study have no significant effect on the morphological characteristics and mean number of pigmented flowers produced by the sampaguita. While the irradiation treatments have no significant effect on the formation of pigmented flowers, the previously irradiated materials with 3-10 Gy plus the control can be subjected to another round of irradiation to possibly build up the induced mutation.

Keywords: gamma irradiation, Jasminum sambac, mutation, sampaguita

INTRODUCTION

Naturally-occurring and induced mutations are useful for generating genetic variability for use in plant breeding. When genetic variation is limited, mutation induction is the alternative approach to create variation within a crop (Brown and Caligari 2008). This approach offers the possibility of inducing desired traits that cannot be

To cite this paper: Magdalita, P.M., De Leon, M.J.C., Sotto, R.C. and Ponce, K.S. 2013. Occurrence of Pigmented Flowers and Variation in Other Morphological Traits of Irradiated and Unirradiated Sampaguita (Jasminum sambac L. Sol.) *Journal of Nature Studies*. 12 (2): 45-53

found in nature or have been lost during evolution (IAEA 1977). Mutagenic agents such as ionizing radiation like gamma rays and certain chemicals can be utilized to induce mutation and generate genetic variations from which desired mutants may be selected (IAEA 1977). It has been reported that radiation was successfully used in producing variation in crop plants that lead to the development of improved varieties (Lapade et al. 1996). Based on the FAO/IAEA mutant varieties database, there are 2,252 crop varieties that were generated from mutation breeding and have been officially released as varieties, but of these releases, sampaguita has not been included.

The sampaguita (Jasminum sambac L. Sol.) belonging to the family Oleaceae. It is a viny ornamental plant cultivated for its profuse, white and fragrant flowers (Lantin-Rosario 2005). It was claimed that sampaguita is a native to Iran and its neighboring countries and was introduced in the Philippines in pre-historic times (Price 1968). It was also reported that it originated from India (Quisumbing 1977). While the sampaguita is easy to grow and has been popularly utilized for making garlands because of its natural white color symbolizing purity, and its beautiful and unique scent, other flower color/s is desired to enhance diversity. In the past, there was an attempt to develop other colored sampaguita using higher dose of gamma rays but the success was very limited (Casyao 1992).

This study re-explores the possibility of coming up with a sampaguita with other color variation using reduced doses of radiation. For instance, a change in flower color of sampaguita may become a novel trait that could raise the status of this flower and give it a new image. This can make the sampaguita more attractive to consumers which could enhance the income of sampaguita growers. Thus, the objectives of this study were to: i) assess the occurrence of pigmented and white flowers in unirradiated and irradiated sampaguita with lower doses of radiation, and, ii) examine the effect on the survival of cuttings and variation in morphological traits including: number of branches, leaf length, leaf width, number of petals and number of sepals.

MATERIALS AND METHODS

Plant materials

Semi-hard wood branches were obtained from the sampaguita germplasm collection growing in pots at the Crop Science Cluster and Institute of Plant Breeding, College of Agriculture, University of the Philippines Los Baños, College, Laguna. Cuttings measuring 10 cm long were produced, so that they can fit inside the metal canisters that go into the irradiation chamber. The cuttings were bundled by tying them with a rubber band. A total of 15 bundles were prepared with 20 cuttings in each bundle.

Irradiation

The irradiation treatment was done at the Philippine Nuclear Research Institute (PNRI) in Diliman, Quezon City. Bundled cuttings were placed inside the metal canisters which are a part of the irradiation device. The bundled cuttings (three bundles per treatment representing three replications) were then irradiated with different doses of gamma irradiation such as: 0, 3, 5, 10 and 15 Gy from a Cobalt gamma source with a dose rate of 10.01 kr/hr. These doses of radiation used in the present study were based from the recommendations by Casyao (1992). Each treatment was replicated three times with 20 cuttings per replicate. The irradiation experiment was laid out in completely randomized design (CRD). After irradiation, the cuttings both irradiated and unirradiated were brought back to the Plant Breeding Laboratory of the Fruit and Ornamental Crop Section at the Institute of Plant Breeding, and each cutting was dipped quickly in a rooting hormone. A previously prepared sterile sandy soil was used as potting medium that were placed in well-perforated plastic trays measuring 40 cm long, 30 cm wide, 9 cm deep. Five trays representing the five treatments were used. Three subdivisions in each tray were made to represent the three replications. Each cutting was planted into the potting medium in a hole 2.0 cm deep and 0.5 cm wide. All cuttings were incubated inside the misting chamber and allowed to root for two months. The data on percent survival of cuttings was taken two months after incubation in the misting chamber.

Grow-out trial

The grow-out trial was conducted at the sampaguita breeding blocks of the Crop Science Cluster and Institute of Plant Breeding. The rooted cuttings were planted in a mixture of 1 part each of soil, sand, hog manure and slow-release fertilizer (14N:13P:13K) placed in plastic bags (5 x 8 cm). The slow release fertilizer was re-applied at a rate of 25 gm per potted plant every three months. The plants were grown inside the shade house for three months and provided with the standard cultural management practices for sampaguita. After this period, they were re-pottted in earthen pots containing the same growing medium mentioned above and grown for another three months. The plants were then planted in the sampaguita breeding blocks and observed for morphological characters. Data on number of branches, number of petals and sepals, leaf length and leaf width, number of white flowers and number of pigmented flowers were gathered. Leaf length was measured using a ruler from the base to the tip of five fully expanded leaves, while leaf width was measured on the widest portion of the leaf margin. The retention of fragrance in pigmented flowers was also observed. This was done by directly smelling the pigmented flowers while still attached to the plant while comparing with the white flowers.

Statistical analysis

The irradiation experiment was conducted in completely randomized design (CRD) with three replications in each treatment and 20 cuttings in each replicate. The mean and the standard deviation for all parameters measured were taken. All data gathered were subjected to one-way analysis of variance (ANOVA) technique (Gomez and Gomez 1984). Differences between treatment means was detected at 0.05 level of significance using least significant difference (LSD).

RESULTS AND DISCUSSIONS

Survival of unirradiated and irradiated cutting

The cuttings irradiated with the different doses of gamma rays survived at different rates. The highest mean percentage survival of 70.29% of cuttings was obtained in the unirradiated control (0 Gy) followed by 15 Gy with 67.19% survival which is not significantly different from the unirradiated control (Figure 1). The percent survival of irradiated cuttings increased from 3 to 15 Gy. It is commonly believed that irradiation decreases seedling vigor but in the case of sampaguita, semi-harwood to hardwood cuttings were used, hence they could be more resistant to the lethal effects of radiation. In the present study, similar level of survival of irradiated cuttings was obtained using 5-10 Gy (0.5-1.0 Kr) which was also reported by Casyao (1992). Cuttings irradiated with 5 and 10 Gy had similar mean percent survival of 41.38% and 41.82, respectively. However, percent survival was decreased to 67.19% when cuttings were irradiated with higher dose at 15 Gy. It has been reported that radiation causes death of cells and prevention or delay in cell division (Martin and Harbison 1986).

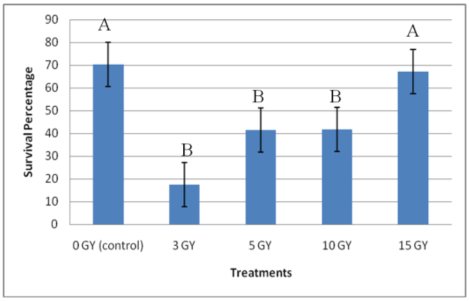


Figure 1. The percent survival of Jasminum sambac L. Sol. Subjected to different doses of gamma rays. Bars with the same letter are not significant different using LSD at 0.05 level.

Assessment of morphological traits in unirradiated and irradiated plants

The effect of irradiation on the plants derived from cuttings exposed to varying doses of radiation was assessed in terms of number of branches, leaf length, leaf width, number of petals, number of sepals, number of white flowers and number of pigmented flowers. In terms of the number of branches, plants derived from cuttings irradiated with 3 Gy produced the highest mean number of branches of 19.00, followed by those that were derived from 15 Gy with 17.75 which are not significantly different, and these were slightly higher than the unirradiated control (Figure 2). This increase in the number of branches could be a stimulatory effect of irradiation on shoot formation which might be due to destruction of inhibitors and release of activators (Barrida and Medina 2003). A similar observation to the present result is that there is enhancement of apical dominance in irradiated crops like the 'Milagrosa' rice variety resulting to the development of greater number of tillers (Santos et al. 1980). In addition, similar result was obtained in irradiated yam where multiple tubers were formed (Lapade et al. 1996). Similarly, it has been found that a radiation dose of 0.5 Kr (5 Gy) stimulated shoot formation in sampaguita (Casyao 1992). The plants obtained from cuttings irradiated with 10 Gy produced an average of 13.50 branches. However, the lowest average mean number of branches of 11.57 was produced by plants derived from cuttings irradiated with 5 Gy. In general, the mean number of branches of plants derived from all irradiated treatments is not statistically different to the unirradiated control (0 Gy).

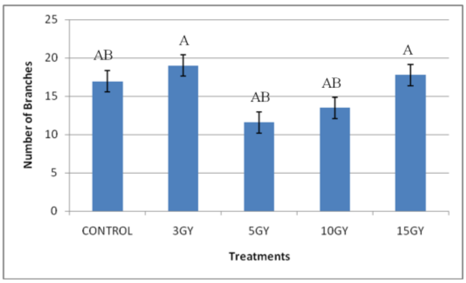


Figure 2. The number of branches of Jasminum sambac L. Sol. subjected to different doses of gamma rays. Bars with the same letter/s are not significantly different using LSD at 0.05 level.

The plants derived from cuttings exposed to 15 Gy had the longest leaf at 7.81 cm followed by plants derived from cuttings irradiated with 10 Gy at 7.52 cm (Figure 3). The leaf length of plants derived from cuttings irradiated with 3 Gy at 7.26 cm is similar to the unirradiated control at 7.18 cm. This result verified previous findings that irradiation either stimulated or inhibited the leaf size in sampaguita (Casyao 1992), which could be associated with increased or decreased in cell division, cell enlargement and a pulling apart of the cells. However, the shortest leaf measuring 7.03 cm was observed from plants derived from cuttings irradiated at 5 Gy. The leaf length of plants derived from cuttings irradiated with 3 Gy at 7.26 cm and 5 Gy at 7.03 is very similar to the leaf length at 7.18 cm of the unirradiated (0 Gy) plants. In general, the leaf length of plants irradiated with 3, 5, 10 and 15 Gy is similar to the unirradiated control (0 Gy) (Figure 3).

In terms of leaf width, the plants derived from cuttings irradiated with 3 Gy and 15 Gy had leaf width of both 4.86 cm and the unirradiated control (0 Gy), at 4.85 cm which are similar. This was followed by plants that came from cuttings irradiated at 10 Gy with a leaf width of 4.79 cm. The leaf width of plants that came from cuttings irradiated at 5 Gy had the narrowest leaves of 4.60 cm. (Figure 3). Nevertheless, the leaf width of plants derived from all the irradiated cuttings is

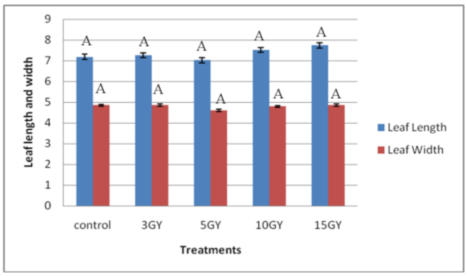


Figure 3. Leaf length and width (cm) of Jasminum sambac L. Sol. subjected to different doses of gamma rays. Bars with the same letter are not significantly different using LSD at 0.05 level

similar to the unirradiated control.

The highest mean number of petals of 9.33 was observed in the plants derived from the unirradiated control (0 Gy) cuttings and those that were obtained from cuttings irradiated with 15 Gy at 9.09 petals (Figure 4). Similar observations were reported by Casyao (1992) that irradiated and unirradiated sampaguita had an average of 8 petals per blossom and that a slight increase in the number of petals of sampaguita irradiated with 10 Gy (1.0 Kr) was observed. The lowest mean number of petals was observed from plants derived from cuttings irradiated with 10 Gy at 7.82 petals. However, the mean number of petals of plants derived from cuttings irradiated with 3, 5, 10 and 15 Gy was similar to the unirradiated control (0 Gy) plants. The result in the present study and those of Casyao (1992) suggest that the dose of radiation used cannot cause changes in petal number of sampaguita.

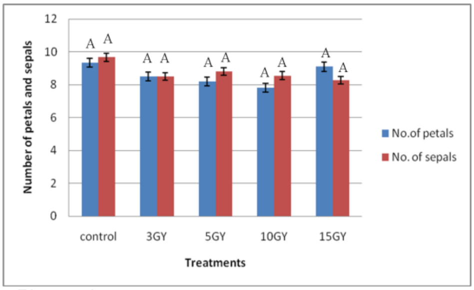


Figure 4. The number of petals and sepals of Jasminum sambac L. Sol. subjected to different doses of gamma rays. Bars with the same letter are not significantly different using LSD at 0.05 level.

In terms of the number of sepals, the highest mean number was observed in the plants derived from the unirradiated (0 Gy) cuttings at 9.67 (Figure 4). This was followed by those plants derived from cuttings irradiated with 5 and 10 Gy, with 8.80 and 8.55 sepals, respectively. The lowest number of sepals was observed from those plants derived from cuttings irradiated with 15 Gy at 8.27. However, the mean number of sepals of all plants derived from irradiated cuttings is similar to the unirradiated control (0 Gy). This result jibed with the result of Casyao (1992) where radiation doses did not affect the number of sepals produced. The present result is also corollary to the results obtained by Scandalios (1964) who reported that the number of flower parts may have a decrease or increase in portions as frequently observed in irradiated plants. Similarly, in Philippine soybean varieties 'BPI-Sy4', 'PSB-Sy4', 'PSB-Sy5' and 'NSIC-Sy8', it was found that gamma irradiation did not significantly enhanced the number of pods that developed (Asencion et al. 2004).

The mean number of white flowers which is 72.79 is highest in the unirradiated (0 Gy) control plants followed by those plants derived from cuttings irradiated with 5 Gy at 53.08 (Figure 5). This was followed by plants derived from cuttings irradiated at 3 Gy with 37.00 and 10 Gy with 36.30 white flowers. The lowest mean number of 18.70 of white flowers came from plants derived from cuttings irradiated with 15 Gy. The result suggests that the irradiated materials produced less number of white flowers compared to the unirradiated control, suggesting that radiation could be inhibitory to white flower formation. However, in general, the number of white flowers of plants derived from the irradiated cuttings and the unirradiated control (0 Gy) are variable. Furthermore, there is no difference in the degree of whiteness of flowers in both the unirradiated control (0 Gy) and among the irradiated plants using the different treatments.

Furthermore, the mean number of pigmented flowers of plants derived from the unirradiated cuttings is 6.14. This is not significantly different to the number of pigmented flowers of the irradiated materials at 10 Gy with 5.49, and those materials that were irradiated at 3 Gy with 5.10 including those that were irradiated at 5 Gy with 4.78 pigmented flowers (Figure 5). An intense pigmentation and almost uniform coloration was observed in the fully opened, partially opened and in the unopened flower buds for both unirradiated control (0 Gy; Figure 6 B) and irradiated treatments (Figure 6 C, D, E & F).

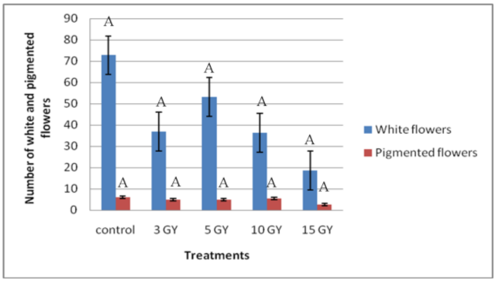
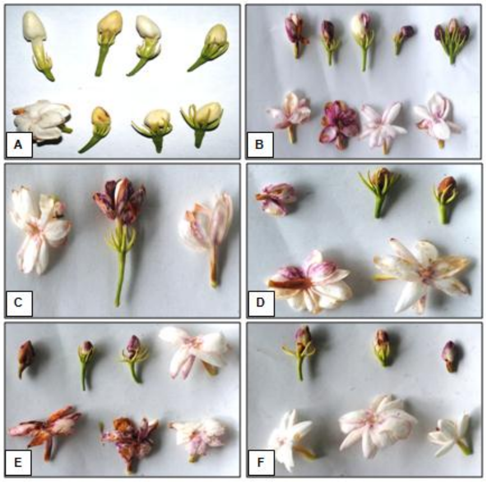


Figure 5. The number of white and pigmented flowers of Jasminum sambac L. Sol. subjected to different doses of gamma rays. Bars with the same letter are not significantly different using LSD at 0.05 level.

The occurrence of pigmented flowers in the unirradiated sampaguita is possible since sampaguita is considered a putative mutant by itself and the occurrence of colored flowers could be due to the effect of a spontaneous mutation. This mutation is controlled by a recessive gene that undergoes reverse mutation (Muthuswamy and Shanmugavelu 1989). Similarly, the sudden occurrence of color variations in crotons commonly known as "San Francisco" is due to the natural propensity of the plant to produce sports mutants or continuous mutation occurring within the plant (Vergara 2007, Brown 1995).

A tinge of pigmentation was also present on the tip of the petals (Figure 6 C), underside of the petals (Figure 6 C & D) and in the unopened flower bud (Figure 6 E). The lowest mean number of pigmented flowers was observed from plants derived from cuttings exposed to 15 Gy with 2.63 pigmented flowers. The pigmented and the white flowers of plants derived from irradiated cuttings had scent similar to the unirradiated (0 Gy) control, suggesting that irradiation has no effect on the scent of sampaguita. This result suggests that the irradiated and the unirradiated plants both produce pigmented flowers at variable numbers. In an earlier study, the same finding was reported by Casyao (1992). While, irradiation in the present study has no significant effect on changes in morphological traits, the variability observed in the number of pigmented flowers in the unirradiated and irradiated materials could be both exploited to select for materials that produces high numbers of flowers with purple pigmentation. The selected materials could be subjected again to a second round of irradiation to possibly build-up mutations that could increase the formation of pigmented flowers. While a definite dose of radiation had no significant effect on occurrence of pigmented flowers in this study, a second round of irradiation using higher dose of irradiation up to 10 Gy of the previously irradiated



materials could be done to further enhance the mutation. In addition, chemical mutagens like sodium azide (NAM_3) and ethyl methane sulphonate (EMS) (Medina et al. nd) can be recommended to be used to induce mutation

Figure 6. White and pigmentedflowers of Jasminum sambac Sol. produced when cuttings were subjected to different doses of rays: 0 Gy (A-white flowers & B-pigmented flowers), 3Gy (C), 5Gy (D), 10Gy (E), and 15Gy (F).

instead of radiation. Alternatively, sampaguita strains that naturally produce pigmented flowers need to be identified and isolated and also be subjected to irradiation.

Pigmented sampaguita genotypes could be the best starting material for irradiation to produce other flower colors in sampaguita like what has been observed in rooted cuttings of the pink-flowered chrysanthemum (Broertjes 1966). Alternatively, sampaguita genotypes that naturally produce high numbers of pigmented flowers should be selected and identified as natural variant/s.

CONCLUSION AND RECOMMENDATIONS

The study was conducted to assess the occurrence of pigmented flowers in unirradiated and irradiated sampaguita cuttings using lower dose of gamma irradiation and, to examine the effects on survival of sampaguita cuttings and variation on the morphological traits of the plants. Five treatments such as 0, 3, 5, 10 and 15 Gy were replicated three times and there were 20 sample cuttings for each replication. Survival of cuttings was assessed two months after incubation of the cuttings in a misting chamber. Percent survival (70.29%) was highest in the unirradiated (0 Gy) cuttings and lowest (17.45%) in the irradiated (3 Gy) cuttings, but not significantly different. Cuttings irradiated with 3 Gy had the highest average number (19) of branches while cuttings irradiated with 5 Gy had the lowest (11.57) but not significantly different. Irradiated cuttings with 15 Gy had the longest (7.74 cm) and widest leaf (4.86 cm) but these are not significantly different with the other treatments. The unirradiated (0 Gy) cuttings produced the highest mean number of petals (9.33) and sepals (9.67) but not significantly different with the rest of the treatments. The unirradiated (0 Gy) cuttings produced the highest mean number (72.79) of white flowers. Furthermore, the unirradiated cuttings produced the highest mean number of pigmented flowers (6.14), but not significantly different with the rest of the treatrments. Pigmented flowers produced by both unirradiated and irradiated cuttings regardless of radiation dose, still retained the typical sampaguita fragrance. On the overall, the irradiated sampaguita regardless of dose had morphological features and number of pigmented flowers not significantly different to the unirradiated cuttings. While irradiation has no significant effect on morphological traits, the existing natural variability with respect to the number of pigmented flowers in the unirradiated cuttings could be exploited to select strains that produce more number of pigmented flowers. Alternatively, the irradiated materials producing pigmented flowers could be re-irradiated using 3 to 10 Gy to enhance mutation frequencies that could lead to increased pigmentation of flowers.

ACKNOWLEDGEMENTS

The Ornamental Crops Breeding Project wherein the sampaguita study is included is supported by the Crop Science Cluster (CSC) and Institute of Plant Breeding (IPB), (UPLB) College of Agriculture (CA), University of the Philippines Los Baños (UPLB). Mr. Fernando B. Aurigue of the Agricultural Research Section, Atomic Research Division, Philippine Nuclear Research Institute (PNRI), Diliman, Quezon City is acknowledged for his technical advice in designing the irradiation treatments. The authors would like to thank Ms. Maria Fe H. Cayaban, Mr. Marcelino T. Gregorio, Mr. Jessie V. Silverio and Mr. Bill Anderson for the various forms of help rendered to this study.

STATEMENT OF AUTHORSHIP

Pablito M. Magdalita is the lead author of this article, he conducted the literature search, formulated recommendations, provided the first draft and did the final revision. Ma. Jenina C. De Leon as co-author help write the first draft, provided the tables and figures, edit and revised the paper. Dr. Rachel Sotto conducted the identification of the varieties and helped in writing the first draft. Ms. Kimberly Ponce helped in gathering the data and provided the figures.

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JOURNAL OF NATURE STUDIES
(formerly Nature's Bulletin)
Print ISSN: 1655-3179
Online ISSN: 2244-5226