⁶⁰Co Irradiation Influences Germination and Phenotype of Three *Pavonia* Species

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Abstract. The genus *Pavonia* is one of the largest genera in the Malvaceae species; it is mainly distributed in South America. Three species of *Pavonia* were identified based on different flower colors and potential for landscape use in the southeastern United States. These species produce a large amount of seed at the end of the blooming season, which is not ideal for ornamental use. To reduce the seed set, gamma irradiation was used for mutation induction and propensity to induce compactness and sterility. A preliminary study indicated that the seed of *Pavonia hastata* would germinate at irradiation rates up to 2000 Gy. Seeds of three species were treated with six different dose rates ranging from 0 Gy to 1000 Gy to determine the ideal rate for *Pavonia* breeding and how gamma irradiation affected seed germination. M₁ (the first mutant generation) *P. lasiopetala* and *P. missionum* were sown in 2018 and planted in the field at the University of Georgia Durham Horticulture Farm on 1 May 2019, as were M₂ (the second mutant generation) seeds of *P. hastata*. Seed germination in 2019 showed no significance due to treatment but significance due to species and species by treatment interaction. Field evaluation performed in 2019 indicated that height was not influenced by irradiation for any of the three species but that the width index was. Flower diameter and leaf area of *P. missionum* became smaller as the irradiation rate increased, but the other two species showed no trends. Chlorophyll mutations were observed on *P. hastata* at the 1500 Gy level, which has attractive traits for ornamental use.

The genus *Pavonia* Cavanilles is usually found in tropical and subtropical areas and may be the largest genus in the Malvaceae species (Fryxell, 1999). The genus is mostly distributed in South America, Central America, the West Indies, and Mexico. The flowers of *Pavonia* are usually monadelphous, with a central staminal column and numerous anthers (Fryxell, 1999). Five petals are inserted at the base of the staminal column and form a gamosepalous calyx (Fryxell, 1999). The unique feature that distinguishes *Pavonia* from the other seven genera of the tribe of Malvaceae is that it has two-times more styles and stigmas (10) in the flower than carpels (5) in the fruit (van Heel, 1978). The very young ovary has 10 carpels, but alternate

carpels abort during their development. The fruits of *Pavonia* are composed of five mericarps, with each containing a single seed. In *Pavonia* sect. *Pavonia* and *P.* subsect. *Lebretonia*, the mericarps are indehiscent and variously ornamented (Fryxell, 1999). The different kinds of ornamentation involve a hard tissue that is protective of the enclosed seed, presumably creating dormant conditions for seeds to survive longer within the mericarp. Only a few are used as ornamental plants, such as *P. hastata* in Australia (Mitchell, 1982) and *P. lasiopetala* in central Texas (Miller, 1990; Nokes, 1986; USDA, 2010).

All three species of *Pavonia* are perennial shrubs with a similar size of ≈1.5 m tall and 1.5 m wide (Fryxell, 1999). Both *P. hastata* and *P. missionum* are native to South American countries such as Brazil, and *P. hastata* has been introduced in Georgia and Florida (Carter et al., 2009; Duncan, 1981; Fryxell, 1999; Wunderlin et al., 2008). *P. lasiopetala* is native to Texas and northern Mexico and inhabits dry and rocky soils (Hatch et al., 1990). All three species have different flower colors that are attractive to different people. *P. hastata* has a white flower with a deep red center, *P. lasiopetala* has a pink-rose flower, and *P. missionum* has an orange-red flower.

Flowers of all three species only last 1 to 2 d, like most species in the Malvaceae species. Blooming periods for the three species are long, from summer to the first frost, which is ideal for ornamental use. Leaf shapes are different; they are hastate in *P. hastata* and cordate in *P. lasiopetala* and *P. missionum*. Pubescence is observed on all three species, with stellate hairs on *P. hastata* and *P. lasiopetala* and stellate and simple glandular pubescence on *P. missionum* (Fryxell, 1999). Unlike species in other families, *Pavonia* has an epicalyx external to the calyx (Esteves, 2000). All three species produce a hard mericarp with a reticulate-costate shape for *P. hastata*, smoothly rounded shape for *P. lasiopetala*, and reticulate-rugose shape for *P. missionum* (Fryxell, 1999).

Germination is one of the critical steps for plant studies and breeding. Seed dormancy is a mechanism that allows plants to regulate when and where to grow. One crucial function of delayed germination is allowing time for dispersal. Physical dormancy caused by the impermeability of seedcoats to water is common (Baskin and Baskin, 2001). This impermeable layer prevents the seed from taking up water or gases to prevent seeds from germinating until dormancy is broken. In nature, there are several ways to break physical dormancy, including high temperatures, widely fluctuating temperatures, fire, freezing/thawing, drying, and passage through the digestive tracts of animals (Baskin et al., 2000). Fifteen families of angiosperms show physical dormancy, including Anacardiaceae, Bombacaceae, Cannaceae, Cistaceae, Convolvulaceae, Cucurbitaceae, Fabaceae, Geraniaceae, Malvaceae, Musaceae, Nelumbonaceae, Rhamnaceae, Sapindaceae, Sterculiaceae, and Tiliaceae (Baskin and Baskin, 2001).

The fruit of *Pavonia* is a dry schizocarp that splits into one-seeded mericarps. The hard mericarp of *Pavonia* protects the seed and is also the cause of dormancy. Some species of *Pavonia* subgen. *Malache* have woody mericarp walls as well as indehiscent mericarps (Fryxell, 1999). The seeds of *P. candida* can be extracted from the mericarp only with the help of a hammer because the mericarp walls are 1 to 2 mm thick and bony (Fryxell, 1999). The physical or bacteriological degradation of such mericarp walls requires a significant amount of time.

Torres et al. (2008) conducted a germination study of *Pavonia cymbalaria* (*Pavonia* subgen. *Pavonia* sect. *Lebretonia* subsect. *Hastifoliae*, the same as *P. hastata* and *P. missionum*). Their four treatments including removal of mericarp walls, mechanical scarification, nude seeds, and a control. The results indicated that germination only occurred on naked seeds, which implies the existence of a natural dormancy because of the thick mericarp walls. Mechanical scarification via sandpaper was not enough to induce germination. Nokes (2001) mentioned that the germination of *P. lasiopetala* seeds is delayed or staggered for several months. More uniform germination may be achieved by an overnight hot water soak or acid

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scarification. All the information indicates that species of *Pavonia* have physical dormancy, and pretreatments are needed for uniform germination. Few germination studies of *Pavonia* have been conducted; therefore, there is no standard protocol. However, there are some germination studies involving plants in the Malvaceae species, which would be good references to follow. Sulfuric acid scarification is routinely used to break seed physical dormancy and promote rapid and uniform germination (Sakhanokho, 2009). Sakhanokho (2009) conducted germination studies of *Hibiscus* (Malvaceae), and both *H. acetosella* and *H. dasycalyx* seed germination rates increased significantly after sulfuric acid scarification. Wang et al. (2012) also found that a 15-min sulfuric acid scarification treatment increased the seed germination rate and germination energy of *Hibiscus hamabo*.

Most dormant seeds have a palisade layer of lignified cells in the seedcoat (Baskin and Baskin, 2001). To understand how sulfuric acid aids seed germination, one approach is the use of scanning electron microscopy (SEM). SEM can achieve resolution of less than 1 nm; therefore, even small changes on seedcoat structures can be observed. Ruter and Ingram (1991) conducted a germination study of the hard-seeded legume *Sophora secundiflora*. The SEM results showed that acid scarification treatment removed the seed cuticle. By using SEM, researchers can achieve a better understanding of the seed structure and morphology of *Pavonia* species.

Pavonia hastata was previously known to only be naturalized in Charlton County, GA, and in Citrus and Levy counties in Florida (Jones and Coile, 1988); however, it has now spread to Camden County, GA (Carter et al., 2009), and Harris County, TX (Brown et al., 2007). Small and Rydberg (1913) in their *Flora of the Southeastern United States*, noted the presence of *P. hastata* on sandy soils in Georgia. Dr. John Ruter observed both *P. hastata* and *P. missionum* reseeding in Tifton, GA (zone 8b) (personal communication, 9 Feb. 2018). Flowers of all three species only last 1 d, but numerous flowers are produced during the growing season. Because each flower can provide five seeds, a high number of seeds can be produced in one season. Plants with reduced fertility often bloom longer during the flowering season (Acquaah, 2009). Breeding for sterile plants can reduce maintenance and labor costs of gardeners, landscapers, and nurseries.

Mutagenesis is a way to obtain new cultivars for sterility and compactness. Mutation breeding generates random variation, resulting in mutant plants with unique morphological traits (Ibrahim et al., 2018; Loewe and Hill, 2010; Schum, 2003). Plant breeding requires genetic variations for segregation and recombination. There are two main types of mutation: physical and chemical. Among the physical mutagens, X-rays and gamma rays are the two most commonly used for plant breeding. During the past 40 years, gamma irradiation was predominant compared with X-rays for mutation induction

because of its wide availability and versatility of use. According to the Joint FAO/IAEA Division of the Nuclear Techniques in Agriculture, there are more than 1700 cultivars propagated by seed in 154 plant species developed from direct or indirect mutants (Maluszynski et al., 1995). Mutation breeding can improve quality traits for crops such as plant height, maturity, seed shattering, and disease resistance. According to the FAO/IAEA database, there have been 465 mutants released that are propagated vegetatively, most of which are ornamental plants with a few fruit trees. Among the ornamental plants, *Chrysanthemum* (187), *Alstroemeria* (35), *Dahlia* (34), *Streptocarpus* (30), *Begonia* (25), *Dianthus* (18), and *Rhododendron* (15) have been studied the most in mutation breeding (Maluszynski et al., 1992). Mutation breeding can be a faster method that is suitable because ornamental plant traits are often visible. Because many mutations are recessive, it is essential to keep the treated material for testing until at least the M₂ generation to see if irradiation could enhance the segregation of novel traits.

Research by Li et al. (2010) indicated that irradiation could promote *P. hastata* seed germination at doses of less than 200 Gy. The putative radiation dosage suitable for mutation breeding was more than 250 Gy for *P. hastata*. High-dose irradiation can be used to develop desirable morphological traits or induce sterility. Preliminary research by Dr. John Ruter showed a curvilinear response of seed germination to radiation dosages between 0 and 800 Gy (Fig. 1) (unpublished data). Seed germination peaked at ≈350 to 400 Gy, increasing from 36% germination for the control seed to up to 55% germination. Radiation treatments have been shown to stimulate plant growth and development at low dosages (Sax, 1963).

Because seeds germinated at 800 Gy, additional seeds of *P. hastata* were treated with 1000 Gy, 1500 Gy, and 2000 Gy. Germinated seedlings from all three treatments were transplanted to the University of Georgia

Durham Horticulture Farm (UGA Hort Farm) at Watkinsville, GA, and M₂ seeds were collected for further evaluation. To determine the influence caused by gamma irradiation and the ideal rate for breeding, germination studies of three species of *Pavonia* were conducted in 2018 and 2019.

Materials and Methods

An SEM study was conducted at the Georgia Electron Microscopy Laboratory at the University of Georgia to determine the mericarp thickness of three *Pavonia* species: *P. hastata*, *P. lasiopetala*, and *P. missionum*. Mericarps were first treated with 95% to 98% sulfuric acid (Avantor Performance Materials, LLC, Center Valley, PA) for 10 min, 20 min, and 30 min and then rinsed for 10 min under running tap water to remove any remaining acid. Liquid nitrogen was applied to completely dry the mericarp after the mericarp surfaces had been air-dried. Mericarps were separated in half for gold sputter coating, which deposits a thin layer of gold to prevent the charging of a specimen with the electron beam used for SEM. An FEI Teneo FE-SEM (Thermo Fisher Scientific, Hillsboro, OR) was used to obtain high-resolution pictures of the mericarps.

Seeds of the three *Pavonia* species (*P. hastata*, *P. lasiopetala*, and *P. missionum*) were obtained from the University of Georgia Durham Horticulture Farm (Hort Farm) in Watkinsville, GA, in 2017. Seed germination studies were conducted in both 2018 and 2019. Because sulfuric acid scarification for 15 min was applied to the seeds before each study, the germination response was quicker than expected; early data in 2018 was not collected. To record the rapid response of germination, the seed germination study was repeated in Mar. 2019.

In 2018, a completely randomized design was used for each species in the seed germination study. Germination data were collected every Monday, Wednesday, and Friday, beginning at 7 d after treatment. Seeds were first

Fig. 1. Germination percentage of nonacid-scarified *P. hastata* seeds with 50 seeds per treatment. M₁ seedlings were grown and flowered, and M₂ seed was collected and grown in 2018.

Fig. 2. *Pavonia* mericarp before and after acid scarification under scanning electron microscopy (SEM). (A) Interior side of mericarp from control *P. hastata*. (B) Exterior side of mericarp from control *P. hastata*. (C) Interior side of mericarp from *P. hastata* with 20-min acid scarification. (D) Exterior side of mericarp from *P. hastata* with 20-min acid scarification.

Table 1. Germination percentages among three *Pavonia* species in a 2018 study using six different treatment levels of ^{60}Co irradiation. Data were analyzed using R with the Bonferroni least significant difference test. Means followed by the same letter are not significantly different.

Treatment	Germination percentage in 2018		
	<i>P. hastata</i>	<i>P. lasiopetalata</i>	<i>P. missionum</i>
Control	60.5%	76.0% a	96.0% a
200 Gy	51.0%	24.5% b	96.0% a
400 Gy	55.5%	64.5% a	98.0% a
600 Gy	49.0%	16.5% b	64.5% b
800 Gy	30.5%	85.5% a	97.0% a
1000 Gy	34.5%	63.5% a	68.5% b
Significance	NS	***	***

NS, ***Nonsignificant or significant at $P \leq 0.001$, respectively.

treated with ^{60}Co gamma rays at rates of 0, 200 Gy, 400 Gy, 600 Gy, 800 Gy, and 1000 Gy at the University of Georgia Center for Applied Isotope Studies Laboratory Athens, GA. Each treatment contained four replicates of 50 seeds that were treated with sulfuric acid (Avantor Performance Materials, LLC) for 15 min. Seeds were then rinsed with tap water for ≈ 10 min to remove all residue. An overnight soak in boiled water was applied to the seeds after the acid treatments to improve germination. The next morning, seeds were sown at the University of Georgia Trial Gardens greenhouses in 15.2-cm $\times$ 14.0-cm (diameter $\times$ depth) pots (Growers Solution, LLC., Cookeville, TN) with Jolly Gardener Pro-Line C/L Growing Mix (Oldcastle, Shady Dale, GA). All pots were placed under mist (8 s every 5 min from 7:00 AM to 7:00 PM) for 31 d.

In 2019, a completely randomized design was used for all three species in the seed germination study. Germination data were col-

lected every day for 30 d, beginning on 2 March. Radiation and seed treatments were the same as those used in 2018. Four replicates for each treatment and 50 seeds for each replicate were sown in a greenhouse at the UGA Hort Farm. Seeds were planted in 200-well plug trays (Hummert International, Earth City, MO) with potting media (Pro-Mix BX Mycorrhizae; Premier Tech Horticulture, Rivière-du-Loup, QC, Canada) amended with micronutrients (Micromax; Everris NA Inc., Dublin, OH) at 594 g m $^{-3}$. The greenhouse temperature was set at 23.9 °C during the daytime and 18.3 °C at night. Hand-watering was provided as needed.

To avoid the inaccuracy caused by seed vigor and early response, each treatment was assigned a single numerical value that considers both speed and totality of germination, with higher values indicating faster germination (Czabator, 1962). Because the product of the peak value (PV) and mean daily germination (MDG) are so closely correlated with both speed and totality of germination, the germination value (GV) is written as $\text{GV} = \text{PV} \times \text{MDG}$ (Czabator, 1962). The peak value is the maximum value of [(germination percent)/days] for 1 d (the peak day). The mean daily germination is the average number of seeds germinating per day during the test period. Germination value, peak value, and mean daily germination were calculated for the 2019 germination study.

M_2 seeds from Dr. Ruter's preliminary *P. hastata* study using 400 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 1000 Gy, and 1500 Gy were collected in Dec. 2017 and sowed in Feb. 2018 at the UGA Hort Farm with Pro-Mix BX Mycorrhizae substrate (Premier Tech Horticulture, Rivière-du-Loup, QC, Canada). In June 2018, M_2 *P. hastata* were trans-

planted to 2.8-L pots and placed on the container pad under overhead irrigation at the UGA Hort Farm. In Fall 2018, 50 plants from each dose rate with notable phenotypes were cut back to 15 cm and overwintered on the container pad at the Hort Farm for the evaluation of cold hardiness. The average low temperatures in December, January, and February were 3.9 °C, 1.4 °C, and 4.0 °C, respectively (University of Georgia Weather Network, 1997–2019). In May 2019, M_2 plants that survived the winter were transplanted to a field at the UGA Hort Farm to be evaluated for compactness and sterility. Plants were spaced ≈ 1.22 m apart in rows, and each row was 1.83 m apart. Drip irrigation was prepared before planting, with emitters 30.5 cm apart. Approximately 28.3 g of All Season's Lawn & Garden 5N-4.3P-12.5K fertilizer (The Earl Ricketts Co., Tampa, FL) was top-dressed around each seedling 2 weeks after transplanting to the field. The M_1 seedlings of *P. lasiopetalata* and *P. missionum* germinated from the 2018 germination study were transplanted to 2.8-L pots and kept in an overwinter house at the UGA Hort Farm with the temperature set to 7 °C. Seeds from irradiated plants were collected and grown in 2019 because mutations are usually seen in the M_2 generation. In May 2019, M_1 plants of *P. lasiopetalata* and *P. missionum* were transplanted to a field at the UGA Hort Farm for evaluation. Plant morphological traits including plant height, plant width index [(east to west width + north to south width)/2], flower diameter, leaf chlorophyll content, and leaf area were collected from M_2 *P. hastata* and M_1 *P. lasiopetalata* and *P. missionum* after 3 months of growth in the field. Plant height, plant width index, and flower diameter were measured using a ruler. The leaf chlorophyll index was measured by a chlorophyll meter (SPAD-502; Minolta Camera Comp., Osaka, Japan). The leaf area per leaf sample was measured on fully expanded leaves for five leaves per plant by using a LI-3100C leaf area meter (LI-COR Biosciences, Lincoln, NE). The germination percentage and germination value were analyzed using linear regression, and morphological traits comparisons were performed and analyzed using an analysis of variance and least significant difference with the Bonferroni correction. All statistical analyses were performed using R computing software (R Development Core Team, 2018).

Results

Scanning electron microscopy. Seeds of *P. hastata* without acid scarification have a thick mericarp of ≈ 0.33 mm that prevents germination. Acid scarification for 20 min or more helped to degrade the mericarp, thus breaking the physical barrier and allowing seeds to imbibe water and germinate (Fig. 2).

Effect of gamma-ray dosage on seed germination. The germination study in 2018 indicated that seed germination of *P. hastata* decreased as radiation dosages increased from 0 to 1000 Gy (Table 1). The control (0

Fig. 3. Germination value interaction among three species, *P. hastata*, *P. lasiopetala*, and *P. missionum*, and treatment ranging from 0 Gy to 1000 Gy during the 2019 germination study. GV = PV × MDG. The germination value expresses the completeness and rate of germination. The peak value is the cumulative germination on the day of the greatest germination divided by the number of days to reach that level. Mean daily germination is the germination percentage divided by the number of days for germination. Data points are the mean ± SE.

Fig. 4. Germination percentage interaction among three species, *P. hastata*, *P. lasiopetala*, and *P. missionum*, and treatment ranging from 0 Gy to 1000 Gy during the 2019 germination study. Data points are the mean ± SE.

Gy) appeared to have the highest germination percentage (60.5%) after 31 d, whereas 800 Gy had the lowest germination percentage (30.5%). However, there were no differences among treatments ($P = 0.23$). Germination percentages showed no trend for *P. lasiopetala*, with the highest germination percentage (85%) occurring with the 800-Gy treatment and the lowest germination percentage (17%) occurring with the 600-Gy treatment (Table 1). *P. missionum* was the most vigorous among the three species, with more than 90% germination ($P < 0.001$) for four levels of irradiation (0 Gy, 200 Gy, 400 Gy, and 800 Gy) and more than 60% germination for two irradiation levels (600 Gy and 1000 Gy) (Table 1). Interestingly, 64% germination was observed for *P. missionum* with the 1000-Gy treatment, but the seedlings remained at the cotyledon stage until the end of the study.

In the 2019 germination study, the responses of the germination percentage and germination value were quite different

(Figs. 3 and 4). The germination percentage increased slightly with increasing irradiation rate in *P. lasiopetala* and *P. missionum*. However, the germination value of *P. hastata* and *P. missionum* decreased, but the germination value of *P. lasiopetala* increased with the increasing irradiation rate. High significance was observed ($P < 0.001$), with *P. missionum* having the highest germination percentage (92.0%) and *P. lasiopetala* having the lowest germination percentage (17.2%) (Fig. 5). However, the treatment did not affect the germination percentage ($P = 0.26$). The species × treatment interaction was significant ($P = 0.022$) due to the species effect. The germination value was influenced by species, treatment, and the species × treatment interaction ($P < 0.001$, $P < 0.001$, and $P = 0.006$, respectively). The peak value responded similarly to germination value with regard to species, treatment, and species × treatment interaction ($P < 0.001$, $P < 0.001$, and $P = 0.0014$, respectively). The mean daily germination was only significant for species

($P < 0.001$). Interestingly, LD₅₀ was not observed during either the 2018 or the 2019 germination studies. Even at the rate of 1000 Gy, seeds of all three species germinated at more than 50% compared with controls.

Effect of gamma ray dosage on plant morphological traits. After 3 months of growth in the field, plant heights of M₂ *P. hastata* ranged from 60.5 cm to 77.3 cm, but there were no differences in height among the rates of irradiation (Table 2) ($P = 0.099$). The height of M₁ *P. lasiopetala* ranged from 65.2 cm (600 Gy) to 82.7 cm (400 Gy), but there were no differences among the rates of irradiation (Table 3) ($P = 0.125$). Similar results were observed for M₁ *P. missionum*; the plant height ranged from 62.1 cm (1000 Gy) to 76.9 cm (400 Gy), but there were no differences among irradiation rates (Table 4) ($P = 0.268$). In contrast, the width indices of M₂ *P. hastata* were different ($P = 0.013$) among irradiation rates, with the widest (158.0 cm) occurring with the 500-Gy treatment and the most compact plants (125.3 cm) occurring with the 1500-Gy treatment. There was a treatment difference ($P < 0.001$) in the width index for M₁ *P. lasiopetala*, with the widest (146.8 cm) occurring with the 400-Gy treatment and the most compact (116.2 cm) occurring with the 800-Gy treatment, on average. The width index was significant ($P < 0.001$) among treatments for *P. missionum*, with the longest (135.5 cm) occurring with the control treatment and the shortest (110.2 cm) occurring with the 1000-Gy treatment. Height was not influenced by the dosage of irradiation for any of the three species, but the width index was.

The flower size was different ($P = 0.004$) among treatments, with the largest flowers (5.35 cm) occurring with 1500 Gy and the smallest flowers (4.91 cm) occurring with 600 Gy for M₂ *P. hastata* (Table 2). Flower size differed ($P < 0.001$) with different treatment, with the largest (4.21 cm) occurring with 1000 Gy and the smallest (3.86 cm) occurring with 800 Gy for M₁ *P. lasiopetala* (Table 3). Flowers of *P. missionum* were largest on the control plants (4.1 cm) and smallest on plants treated with 1000 Gy (2.6 cm) ($P < 0.001$) (Table 4).

The leaf area was significantly different ($P < 0.001$) for M₂ *P. hastata*, with the smallest (7.20 cm²) occurring with 800 Gy and the largest (9.37 cm²) occurring with 700 Gy (Table 2). With the increasing radiation dosage, the leaf area of *P. lasiopetala* was reduced ($P < 0.001$) (Table 3). Control plants had the largest leaf area, with an average of 15.35 cm², and 1000-Gy plants were the smallest, with an average of 9.53 cm². *P. missionum* responded in a manner similar to that of *P. lasiopetala*, with the largest (14.98 cm²) leaf area occurring with the control treatment and the smallest (5.45 cm²) leaf area occurring with 800 Gy ($P < 0.001$) (Table 4).

The leaf chlorophyll index was different ($P < 0.001$) among all treatments, and there was no trend among treatment, with the highest (69.5) occurring with the control treatment and the lowest (56.7) occurring

Fig. 5. The germination percentage among three *Pavonia* species across all rates of irradiation during the 2019 study. Data points are the mean $\pm$ SE.

Table 2. Morphological traits, including flower size, leaf area, leaf chlorophyll index, plant height, and plant width index, of M_2 *P. hastata* were measured. Data were analyzed using R with the Bonferroni least significant difference test.

Treatment	<i>P. hastata</i>				
	Flower diam (cm)	Leaf area (cm ²)	Leaf chlorophyll index	Ht (cm)	Width index (cm)
Control	5.3 ab	7.9 ab	69.5 a	62.2	133.2 ab
400 Gy	5.1 ab	7.8 b	56.7 b	63.9	145.9 ab
500 Gy	5.2 ab	8.2 ab	57.9 b	77.3	158.0 a
600 Gy	4.9 b	7.6 b	56.9 b	72.2	152.4 ab
700 Gy	5.2 ab	9.4 a	57.9 b	67.8	145.3 ab
800 Gy	5.2 ab	7.2 b	59.7 ab	60.5	130.4 ab
1000 Gy	5.0 ab	7.6 b	66.3 ab	69.6	140.1 ab
1500 Gy	5.3 a	7.6 b	68.8 a	77.3	125.3 b
Significance	*	***	***	NS	*

NS, *, ***Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

Table 3. Morphological traits, including flower size, leaf area, leaf chlorophyll index, plant height, and plant width index, of M_1 *P. lasiopetala* were measured. Data were analyzed using R with the Bonferroni least significant difference test.

Treatment	<i>P. lasiopetala</i>				
	Flower diam (cm)	Leaf area (cm ²)	Leaf chlorophyll index	Ht (cm)	Width index (cm)
Control	4.0 b	15.3 a	59.4 ab	79.0	127.2 ab
200 Gy	4.2 a	12.3 b	65.5 a	70.7	143.0 ab
400 Gy	4.1 b	11.1 bc	58.6 ab	82.7	146.8 a
600 Gy	4.1 ab	11.0 bc	54.6 b	65.2	134.7 ab
800 Gy	3.9 c	10.0 c	55.2 b	66.5	116.2 b
1000 Gy	4.2 a	9.5 c	57.1 b	75.1	141.2 ab
Significance	***	***	***	NS	***

NS, ***Nonsignificant or significant at $P \leq 0.001$, respectively.

with the 400-Gy treatment for M_2 *P. hastata* (Table 2). There was a difference ($P < 0.001$) in treatments for M_1 *P. lasiopetala*, with the highest leaf chlorophyll index (65.5) occurring with 200 Gy and the lowest leaf chlorophyll index (54.6) occurring with 600 Gy (Table 3). However, there were no differences in M_1 *P. missionum* treatments (Table 4), with the leaf chlorophyll index ranging from 49.7 (600 Gy) to 58.1 (200 Gy).

Discussion

In ornamental plant breeding, most plant material has a variable dosage of radiation that is effective. The ideal rates for bougainvillea stem cuttings, chrysanthemum root cuttings, gladiolus bulb, and hibiscus stem cuttings are 2.5 Gy, 10 to 35 Gy, 2.5 to 50 Gy, and 10 to 40 Gy, respectively (Datta, 2009). The ideal dosage to breed *Hibiscus syriacus*

(Song et al., 1997) was determined to be 100 to 120 Gy. The rates for seeds are often higher, up to 500 Gy, for fennel (*Foeniculum vulgare* Mill.) (Zeid et al., 2001), 300 to 600 Gy for long bean (*Vigna sesquipedalis*) (Kon et al., 2007), 300 to 800 Gy for tomato (*Lycopersicon esculentum* cv) (Norfadzrin et al., 2007), and 300 to 600 Gy for chili (*Capsicum annuum*) (Omar et al., 2008). However, all three species of *Pavonia* can survive higher doses of irradiation. During the 2018 germination study, it was interesting to see the significance of treatments used for *P. lasiopetala* and *P. missionum*, but not for *P. hastata*. At the end of the 30-d studies, LD₅₀ was not observed for all three species. However, *P. missionum* plants at 1000 Gy remained at the cotyledon stage until the end of the study; eventually, only two seedlings survived after 3 months, indicating an LD₅₀ between 800 and 1000 Gy.

During the 2019 germination study, a significant difference was observed for species. *P. missionum* had the highest germination percentage among all treatments, but *P. lasiopetala* had the lowest germination percentage. However, when the germination value was added to the germination study, treatment, species, and the treatment $\times$ species interaction were different. The peak value counted in the early response of seed germination and *P. missionum* showed a more rapid response than the other two species. Research of bald cypress and pond cypress seed germination (Murphy and Stanley, 1975) showed that even though the final germination rate at day 60 did not show differences between the control and the other three treatments, the germination percentages were different at day 30. The germination value also indicated that the control treatment had lower germination values than the other three treatments, which explained that the three treatments worked to improve the uniformity and resulted in faster germination. The 2018 results also indicated that *P. missionum* at all treatment levels except 1000 Gy finished germination in less than 10 d. Differences in seed germination between years can be explained by location and timing. The 2018 germination study was conducted at the UGA Trial Garden greenhouse in Athens, GA, whereas the 2019 germination study was conducted at the Hort Farm greenhouse at Watkinsville, GA, at different times of the year and under different environmental conditions.

Temperature and irrigation were the two factors that may have caused a different response in seed germination in two different years. Temperatures in 2018 were regularly higher than 38 °C in June and July; however, in 2019, daily high temperatures rarely exceeded 31 °C in March and April. The seeds of the 2018 study were placed under mist (8 s every 5 min from 7:00 AM to 7:00 PM), and the seeds of the 2019 study were hand-watered every day in small plug trays. Seeds of *Sida spinosa* tended to have a higher germination percentage when moved from 20/10 and 25/15 °C to 35/20 and 40/25 °C (Baskin and Baskin, 1984). In Malvaceae,

Table 4. Morphological traits, including flower size, leaf area, leaf chlorophyll index, plant height, and plant width index, of M₁ *P. missionum* were measured. Data were analyzed using R with the Bonferroni least significant difference test.

Treatment	<i>P. missionum</i>				
	Flower diam (cm)	Leaf area (cm ²)	Leaf chlorophyll index	Ht (cm)	Width index (cm)
Control	4.1 a	15.0 a	56.4	72.4	135.5 a
200 Gy	3.9 b	9.4 b	58.1	74.7	124.6 ab
400 Gy	3.6 cd	8.3 bc	56.0	77.0	135.3 a
600 Gy	3.7 c	8.2 bc	49.7	74.9	120.3 ab
800 Gy	3.5 d	5.4 d	51.5	71.8	113.2 b
1000 Gy	2.7 e	7.4 c	57.1	62.1	110.2 b
Significance	***	***	NS	NS	***

NS, ***Nonsignificant or significant at $P \leq 0.001$, respectively.

Fig. 6. Flower mutation of M₂ *P. hastata* treated with control and 1500 Gy. (A) Control *P. hastata* with normal pollen and five petals. (B) *P. hastata* treated with 1500 Gy with reduced pollen and four petals.

Fig. 7. (A) Compact M₂ *P. hastata* treated with 1500 Gy. (B) Standard size M₂ *P. hastata* treated with 1500 Gy.

Fig. 8. Newly formed leaves of some *P. hastata* treated with 1500 Gy showed different colors. This plant has a *chlorina* mutation (yellowish-green color), which is stable.

seed dormancy was broken after removal of the chalazal cap, thus allowing for imbibition of water by the seed (Baskin and Baskin, 2001); therefore, the moisture of substrate is essential after physical dormancy is broken. The results of both studies in 2018 and 2019 indicated that all three species of *Pavonia* could germinate and be grown under high light intensities in a greenhouse.

Irradiation did not influence the height of M₂ *P. hastata* seedlings growing in the field, but the width index was generally variable. No significance in both parameters among treatments was seen because the SE was too large. Generally, dwarf or compact plants were observed in M₂ generation under higher

rates of irradiation. Irradiation treatments appeared to increase the vigor of *P. hastata* at 500 Gy and 600 Gy. Even though the mean plant sizes at 1500 Gy and 1000 Gy were not the smallest plants, there was more variation; for example, several dwarf plants were observed with reduced pollen formation and abnormal petal numbers (Fig. 6). These dwarf plants will be kept for further evaluation (Fig. 7). Another interesting finding was that after 1500 Gy, *P. hastata* plants without desired traits were cut back to the ground and sprouted, and the newly formed leaves showed a new color (Fig. 8). The variegated leaf color could benefit the ornamental use of *P. hastata*, but further evaluation of its stability is necessary. There are different types of chlorophyll mutations, including *albina*, with which no carotenoids or chlorophylls are formed, *xantha*, with which carotenoids prevail or chlorophylls are absent, *alboviridis*, with which different colors occurs at the base and the top of the leaf, and *viridis*, with which light green or yellowish-green color shows at the early seedling stage Gustafsson (1940). The mutation in Fig. 8 belongs to *viridis* (*virescens* or *chlorina*). Before the plant was cut back, the leaf color was yellowish-green and eventually became a normal color, which fit *virescens*. However, after the plant was cut back, the newly formed leaves showed a yellowish-green color again, which fit *chlorina*. All three parameters of the M₁ *P. lasiopetala* plant size were significant. Like *P. hastata*, the shortest height did not occur with the highest irradiation treatment. One of the possible explanations for this is that many traits are usually recessive, and dwarf plants may not be seen until the M₂ generation. However, the most compact plants occurred with the 1000-Gy treatment for *P. missionum*. The growth responses were similar to those of *Hibiscus sabdariffa* L. (El Sherif et al., 2011). The height of both *P. missionum* and *H. sabdariffa* L. increased at lower irradiation rates and decreased at higher rates.

The flower diameter of *P. hastata* was significantly different among treatments. Interestingly, plants treated with 1500 Gy had the largest mean flower diameter. The studies of *Chrysanthemum morifolium* (Kumari et al., 2013) and *Rosa hybrida* L. (Bala and Pal Singh, 2013) indicated that the flower diameter became smaller with an increase in the irradiation rate. However, a smaller flower diameter was observed on dwarf plants. Smaller flowers might be the side effect of dwarf plants. Sometimes the flower diameter does not decrease with the increased irradiation rate. The flower size of the interspecific hybrid between *Torenia fournieri* and *Torenia baillonii* remained the same with the increased irradiation rate (Sawangmee et al., 2011). With the increased gamma irradiation, the flower diameter of *P. missionum* decreased, which would be expected for some ornamental plants.

The leaf area of *P. lasiopetala* and *P. missionum* tended to be smaller as the rate of gamma irradiation increased. In contrast, the

leaf area of *P. hastata* first increased and then decreased as the irradiation rate increased. Research involving *Jatropha curcas* L. (Nayak et al., 2015) and *Hibiscus sabdariffa* (Shukla and Dube, 2017) indicated that with increased gamma irradiation, the leaf area had small increases and then decreased. In many cases, gamma irradiation could cause abnormal leaf shapes (Hanafiah et al., 2017; Minisi et al., 2013). The leaf chlorophyll index of *P. hastata* and of *P. missionum* first decreased and then increased. On the contrary, the leaf chlorophyll index of *P. lasiopetala* first increased before decreasing. The changes in the leaf chlorophyll index were mainly caused by chlorophyll mutations. Some of the M₁ *P. missionum* showed that the *chlorina* mutation causing a yellowish-green color was prevailing throughout life according to the definition reported by Gustafsson (1940). The M₂ *P. hastata* treated with 1500 Gy showed a *virescens* mutation causing the younger leaves to be light green before gradually changing to dark green and eventually becoming the standard leaf color.

Gamma irradiation has routinely been used to develop novel plant traits in many species. The results of this research indicated that variations induced by gamma ray irradiation in three *Pavonia* species varied among plants and species. The *P. hastata* at 2000 Gy produced a limited amount of pollen, there was no seed produced over 2 years, and growth was weak. Based on observations from the fieldwork during this study, the LD₅₀ for *P. hastata* appears to be between 1500 Gy and 2000 Gy. The highest LD₅₀ found in the literature for the seed of a plant in the Malvaceae species (*Althaea rosea*) is 1200 Gy (Yamaguchi, 1988). More variations were observed with the 1500-Gy treatment for M₂ *P. hastata* than with the other treatments. More variations with higher irradiation treatments were also observed for M₁ *P. lasiopetala*. The increased irradiation decreased the height of *P. missionum*, but the flower diameter also decreased. Due to the limited space and labor, only 600 mutant plants from the three species were grown in 2.8-L pots, and the surviving plants from 2018 were transplanted in the field at the UGA Hort Farm in 2019. Mutation breeding requires large numbers of seeds because of the random nature of mutations and mortality due to the treatments. With enough M₁, M₂, and even M₃ plants, there is a higher chance of finding desired traits. There were only three blast-resistant rice mutants selected from 51,530 M₂ plants treated with gamma radiation (Yamasaki and Kawai, 1968). Jorgensen (1975) screened 951,000 plants of barley to find five M₂ seedlings that were resistant to powdery mildew. More than 2.5 million M₂ barley seedlings were tested to find 95 mildew-resistant mutants. Burton et al. (1980) used ⁶⁰Co to irradiate 500,000 Coastcross 1 Bermuda grass stems, and only one of four plants had improved winterhardness and the same yield as Coastcross 1 Bermuda grass. The information in this study is useful for further breeding work or research of these and other *Pavonia* species.

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