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Use of X-ray Mutagenesis to Increase Genetic Diversity of *Zantedeschia aethiopica* for Early Flowering, Improved Tolerance to Bacterial Soft Rot, and Higher Yield

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Abstract: The development of new cultivars is important for the profitability of the floriculture industry. There is a limited number of cultivars of *Zantedeschia aethiopica*, an iconic ornamental cut flower, garden plant, and potted plant, because of the incompatibility of interspecific crossings within the genus. Most present-day varieties are the result of spontaneous mutations or classical breeding within the species, followed by a long selection process. Here, *Z. aethiopica* mutants were generated by treating seeds with 100 Gy of X-ray radiation. The resulting putative mutants were selected based on particular flowering parameters and compared to nonirradiated, control plants. Over two growing seasons, characteristics such as early flowering, flower size and shape, yield, and response to soft-rot disease were monitored, and considerable variation was observed among the mutated lines. Out of 319 mutants, 20 lines were selected based on their phenotypes and then propagated and further analyzed. Within this group, only two phenotypes displayed at least five improved flowering properties under natural Mediterranean conditions. The rest displayed two to four desired combinations of flowering traits, some with great commercial potential.

Keywords: *Zantedeschia aethiopica*; X-ray mutagenesis; cultivation; *Pectobacterium*; mutation breeding

1. Introduction

Zantedeschia aethiopica (L.) Spreng., also known as arum lily or calla lily, is an iconic ornamental, herbaceous perennial with high yields that grows well in a variety of soils [1]. The genus *Zantedeschia* belongs to the family Aracea and is native to southern Africa. *Zantedeschia* species are cultivated commercially worldwide for cut flowers, garden plants, and potted plants [2,3]. Letty [4,5] grouped the species into two informal sections: *Zantedeschia*, containing two species of the evergreen *Z. aethiopica* and the summer dormant *Z. odorata*, both with white spathes and rhizomatous storage organs; and *Aestivae*, containing six winter-dormant species with colored spathes and tuberous storage organs [4,5]. One of the major threats to *Z. aethiopica* crops around the world is the soft-rot disease caused by *Pectobacterium* spp., although *Z. aethiopica* is more resilient than the colorful *Zantedeschia* hybrid (*Aestivae*) group [6–8]. A major factor enabling commercial development of high-value cultivars is the species' breeding potential, but unlike members of the *Aestivae* group, which can

be easily crossed through conventional interspecific hybridization, members of the *Zantedeschia* group can only be crossed with other members of their own species [9]. Moreover, the two groups cannot intercross because of plastome–genome incompatibility [9–11].

The market demand for classical arum flowers, with their bright white, funnel-like spathes surrounding yellow, upright spadices, is relatively steady, mainly because of their traditional role as wedding and funeral flowers in many cultures. These flowers are exported to the European market from several Mediterranean countries, including Israel. Israeli growers use a single variety, namely *Z. aethiopica* var. *Israeli* (*ZAI*), which naturally blooms during the late winter (weeks 6–18). This variety has never been registered as a commercial cultivar and is derived from several genetic resources of South African origin that have been vegetatively propagated by flower growers over the last decades [12]. The *ZAI* variety has solid, dark green, ovate to cordiform leaves; its spathe is white, and its spadix is yellow to orange and odorless. Growing a crop that lacks distinctive characteristics and is not uniform is a significant limitation for calla-lily growers in Israel. *Z. aethiopica* has been cultivated mainly for export to EU markets, taking advantage of the subtropical Mediterranean climate that allows production during the winter season. Although there is a stable demand for this flower, the prices of the cut stems vary significantly depending on their current availability on the market [12].

New flower phenotypes may increase the flower's commercial value by virtue of their uniqueness. Several *Z. aethiopica* cultivars developed using classical breeding and selection are recognized worldwide. These include the dwarf selection 'Childsiana'; a pink spathe selection, 'Pink Mist'; and the very popular "Green Goddess", which has a green spathe. 'Colombe de la Paix' was selected for its abundant flowering, and the cultivar 'Crowborough' was selected for its cold tolerance. A more recent example of a classically bred and selected cultivar is 'Deja Vu', the first calla lily variety registered in Mexico, which has three colors in its spathe: white, pink, and green [13]. Another example comes from a Korean breeding program that has focused on resistance to soft rot as the most important breeding trait [14]. That program is based on the evaluation of resistance levels and conventional crossings of resistant accessions. Using this approach, two soft rot-resistant lines were selected, 'Silky White' and 'Mont Blanc', which also exhibit good flowering characteristics, homogeneity, and stability [14].

Classical breeding requires a great deal of time and labor, and the presence of a typical phenotype in the parental accession lines is a prerequisite for success. Mutation induction can introduce new phenotypes that are not present in the parental line and accelerate the selection procedure. This approach holds good potential for increasing genetic variation in *Z. aethiopica*, overcoming the inability to cross it with other *Zantedeschia* species. Mutation breeding has been proven successful throughout the development of hundreds of cultivars in the ornamental-plant industry, especially among vegetatively propagated species. Ionizing radiation involving X-rays, gamma rays, or neutrons and chemical mutagens have both been used to improve ornamental crops [15]. The key factor in irradiation of plant material is the amount of radiation energy (dose) absorbed by the tissue. The dose is measured in units of Gray (Gy). Doses of radiation above 10 kGy are considered to be high, medium doses are between 1 and 10 kGy, and lower doses are less than 1 kGy. Irradiation of seed material usually requires doses between 60 and 700 Gy [15]. Successful mutagenesis is also dependent on the plant material being used (e.g., stem cuttings, cell suspensions, callus cultures, or seeds) and varies among different plant species. Thus, each system needs to be calibrated in order to determine the optimal irradiation dose before application [15–17]. The resulting mutant plants with altered phenotypes make the selection process straightforward and easy. Hence, mutation techniques have become an important tool for breeding ornamental plants with hundreds of varieties, including *Chrysanthemum*, *Alstroemeria*, *Dahlia*, *Rosa*, *Begonia*, *Dianthus*, *Azalea*, and others [15].

The main objective of the present study was to select an early flowering *Z. aethiopica* cultivar with high yield and improved tolerance to soft rot. The traditional use of vegetative

propagation in this ornamental crop has made its genetic variability relatively low. To improve the genetic variation, seeds of *Z. aethiopica* were irradiated using X-ray technology. This was followed by a phenotype-selection procedure and the identification of improved cultivars under natural Mediterranean conditions.

2. Materials and Methods

2.1. Plant Materials, Bacterial Strains, and Growth Media

Three seed varieties of *Z. aethiopica* were imported: a local variety from "Seeds and All", Port Elizabeth, South Africa; "Pink Mist" from "Seeds for Africa", Cape Town, South Africa; and unregistered variety, "Thompson and Morgan", from Ipswich, United Kingdom. In addition, a local variety grown in Israel (*ZAI*) was acquired from a local grower. *Pectobacterium brasiliense* strain *Pcb1692* (originally isolated from potato) was used for infection assays. *Pcb1692* was cultivated in lysogenic broth (LB; Difco Laboratories, Detroit, MI, USA) at 28 °C and inoculated in minimal medium (MM) as described [18]. Murashige and Skoog (MS), agar medium and vitamins were purchased from Duchefa (Duchefa Biochemie, The Netherlands).

2.2. Germination Rates of Z. Aethiopica Varieties

Z. aethiopica seeds of all varieties (30–175 seeds, depending on the seed source) were soaked in distilled water overnight, sown in 30 mL Styrofoam containers filled with RAM8 planting mix (Section 2.4), and placed in a greenhouse that was kept at 25 °C with natural daylight. In addition, disinfected seeds were sown on MS agar plates as described in [19]. Seedlings grown from seeds that exhibited early germination were counted, and their survival rates were calculated. The germination rates of the imported seeds and *ZAI* were examined. The seeds from the company "Seeds and All" (Port Elizabeth, South Africa) germinated with the highest efficiency (88%) in both the planting mix and the Petri dishes and exhibited highly uniform germination. According to the manufacturer, these seeds were produced from recognized accessions and maintained high uniformity over the years. Therefore, we decided to continue with this seed source. The *ZAI* seeds exhibited the least uniform germination.

2.3. Mutagenesis

Z. aethiopica seeds were irradiated in the Life Sciences Core Facilities of the Weizmann Institute of Science (Rehovot, Israel), using the X-RAD 320 (North Branford, CT, USA) system that provides a highly homogenous beam and a precise dose of radiation.

2.4. Growth Conditions and Field Experiment

During September 2016, 3000 *Z. aethiopica* seeds (irradiated and control) were soaked in water overnight and then sown in 30 mL Styrofoam containers filled with RAM8 planting mix (Tuff Marom Golan, Golan Heights, Israel), which consisted of 20% peat moss, 70% coconut fiber, and 10% compost (by volume). The containers were placed in a greenhouse with natural daylight, which was kept at 25 °C. After germination (in December 2016), 319 radiated and 72 untreated control seedlings grown from early germinating seeds were planted in 500 mL plastic pots filled with the same planting mix for further growth. The pots were placed in a net house with 10% shade, where they were kept under natural light/temperature conditions. The plants were irrigated and fertilized according to local commercial recommendations. During July 2017, the plants were transplanted into 10 L plastic pots filled with the same planting mix. Each plant represented an individual line originating from a single seed, labeled *Ca* for "control" or *CaX* for "irradiated", and each plant was also assigned a serial number. From August 2017 through June 2018 (the first growing season), growth and flowering parameters were monitored for each of these plants, after which selected lines were isolated. During August 2018, rhizomes from each selected line were split into 10 comparable units, which were planted and grown under the same

conditions. From August 2018 through June 2019 (the second growing season), growth and flowering parameters were again monitored for each line.

2.5. Collection of Data about Plant Traits

All flowering data (i.e., early flowering, flower yield, and flower size and shape) were collected separately for each individual plant and for each line over two consecutive growing seasons. The qualitative parameter of flower scent was treated as a binary parameter (i.e., present or not present). Data calculations and graphs were generated using Excel16 (Microsoft, Redmond, WA, USA). Venn diagrams were generated using VennDIS Ver.1.2 [20].

2.6. Tolerance to Pectobacterium

The infection assays were conducted as described previously [21], with minor modifications. All assays were performed during the second growing season, starting from rhizome division. The second fully open young leaves were harvested, soaked in 0.7% sodium hypochlorite (20 min) for external disinfection, and then washed twice in sterile distilled water. Leaf discs (20 mm in diam.) were then excised, pierced at the center with a sterile 10 µL tip, and placed onto Petri dishes containing 20 mL of half-concentrated MS supplemented with 6 g of agar per mL. Inoculation was performed using 10 µL of mid-log-phase cultures of *Pcb1692* at 10^9 colony-forming units (CFU) mL^{-1} (OD_{600} ~ 0.1). Prior to inoculation, the culture was cultivated in LB at 28 °C, washed twice with minimal media (MM), and then resuspended in MM. For the untreated control, the leaf discs were inoculated with MM alone. The plates were incubated at 28 °C for 21 h. After that incubation, they were scanned, and the necrotic area was measured using ImageJ software [22] and divided by the total area of the disc to calculate the percentage of decayed tissue. Leaf discs in which no infection was observed were omitted from the final calculations for lines in which high levels of infection (>75%) were observed among the tested leaf discs. The assays were conducted on 30–40 leaf discs from three different plants for each line. All virulence assays were repeated twice.

2.7. Statistical Analysis

Multiple comparison tests using Dunnett's method were conducted as subsequent tests. JMP-Pro version 13.0 (SAS Institute Inc., Cary, NC, USA) was used for statistical processing, and the significance probability was set at $p \leq 0.001$.

3. Results

3.1. Calibration of the Dose of X-ray Irradiation

Calibration of the dose of X-ray irradiation was performed on the chosen seeds. To determine the optimal dose of radiation that would allow a survival rate of 20–50% (LD_{50}) of the treated seeds, 50 seeds in five Petri dishes (250 seeds per dose) were irradiated in an X-RAD 320 system. As expected, the results revealed a negative correlation between the radiation dose and the seed survival rate (R^2 = 0.967). A dose of 50 Gy with an exposure time of 10 min was the best fit for an LD_{50} (46%) survival rate. To increase phenotypic variation, we chose 20 min of exposure (100 Gy) as the standard dose for the experiment. The radiation dose and the duration of exposure along with the survival rates of the treated seeds are presented in Table 1 and Figure S1.

3.2. X-ray Radiation of Z. Aethiopica Seeds

X-ray mutagenesis with 100 Gy of radiation was performed on 1800 new, dry *Z. aethiopica* seeds imported from South Africa (*CaX*). Then, the seeds were sown in planting mix. Some 180 seeds from the same origin (*Ca*) were used as a control and were sown under the same conditions without treatment. The survival rate of the irradiated seeds was 17.72% (319 plants), and the survival rate of the control seeds was 40% (72 plants).

All surviving seeds from both groups were transferred to 10 L pots filled with planting medium for continued monitoring of growth and development.

Table 1. Calibration of the radiation dose for *Zantedeschia aethiopica* seeds, duration of exposure and survival rates of treated seeds. The chosen conditions are marked in bold; Gy = Gray.

Duration (Min)	0	2	4	10	20	100	150	200
Radiation dose (Gy)	0	10	20	50	**100**	500	750	1000
Survival rate (%)	70	75	59	46	**16**	0	0	0

3.3. First-Season (2017–2018) Mutant Phenotypes

The season in Israel for outdoor growing of *Z. aethiopica* (*ZAI*) is autumn to spring (September to May), while the flowering season is from February through the beginning of May (a 12-week period). During the first growing stage (before flowering), a few individual mutant lines (*CaX*) showed morphological changes that differentiated them from the nonirradiated control lines (*Ca*). The differences were mainly in leaf shape, number of stems, and mosaic stains, as presented in Figure 1. However, most differences were detected during the flowering season. These included differences in flowering time, number of flowers per plant (yield), flower shape and color, flower size, and floral scent (Figure S2). Some of the mutants exhibited combinations of desirable traits (Figure 2a). A summary of the phenotypes is provided, based on pairwise intersections (Table 2, Figure 2). Out of 319 *CaX* lines, about 6% (20 lines) exhibited valuable traits and were thus chosen for further analysis. Most of those selected lines presented several desired phenotypes, some with a combination of five or six desirable traits (*CaX215* and *CaX100*, respectively; Figure 2).

Figure 1. Morphological changes in the mutated lines during the first growing season. (**a**) Leaves from the *Z. aethiopica* control line (*Ca*) that were not irradiated. (**b–d**) *Z. aethiopica* mutant lines (*CaX*) that were irradiated with X-rays; each image represents an individual line. Changes included leaves with (**b,c**) mosaic-like appearance, (**b**) distortions and (**d**) rounded leaves. These morphological changes indicate that mutations have occurred at different positions.

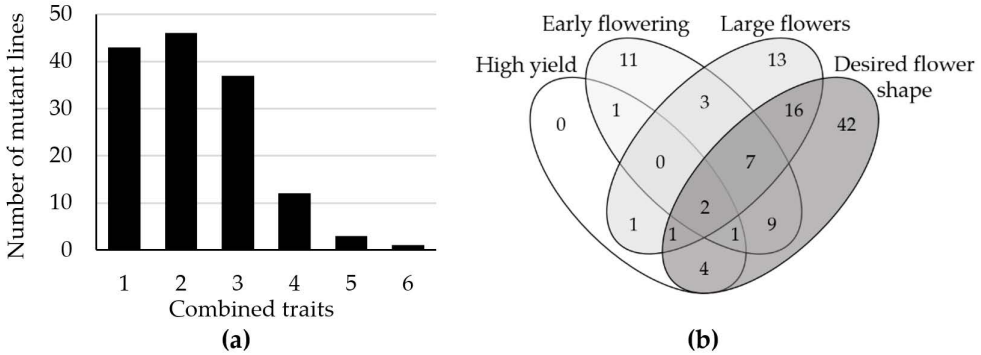

Figure 2. Summary of mutated *Z. aethiopica* (*CaX*) phenotypes from the first season. (**a**) The number of common traits observed among 142 different mutant lines (*CaX*). (**b**) Venn diagram calculated based on the four most desired properties of the mutated lines from the first season.

Table 2. Pairwise intersection of nine traits observed in 143 mutant lines (*CaX*) during the first season (2017–2018). The intersection is displayed as a minimal triangular matrix, in which the different numbers represent mutant lines that share a specific trait. The total number of mutant lines that exhibited the trait is written in parentheses next to each trait. High yield means high number of flowers produced; fl., flowering; and F., flowers. "Scent" is related to the presence of scent in flowers, and "Mosaic" to a mosaic appearance on the leaves.

	Early fl. (34)	**Large F. (43)**	**Small F. (39)**	**Desired F. Shape (82)**	**Flower Scent (53)**	**Shades on Spathe (23)**	**Dark Spadix (29)**	**Mosaic (3)**
High yield (10)	4	4	2	8	6	2	3	0
Early fl. (34)		12	8	19	14	7	1	0
Large F. (43)			1	26	22	7	6	0
Small F. (39)				17	8	7	8	0
Desired F. shape (82)					32	11	18	1
Scent (53)						5	14	0
Shades on spathe (23)							1	0
Dark spadix (29)								0

3.4. Phenotypes of Mutated Plants during the Second Growing Season (2018–2019)

To ensure that the traits of each of the mutated selected lines (*CaX*) were stable, bulbs from each of the lines were split into 10 similar units, and each unit was grown separately for the next season (i.e., the second season), as described previously. The control consisted of two different nonirradiated *Z. aethiopica* lines (*Ca*), as well as two different *ZAI* lines in which the bulbs were split similarly to those in the *CaX* lines. The two *Ca* control lines exhibited a stable phenotype in the first growing season, consistently with the phenotype described by the supplier ("Seeds and All"). During the second growing season (2018–2019), the phenotypic combinations of the selected mutant lines (*CaX*) were compared to those of the control lines (*Ca* and *ZAI*).

During the second growing season, all of the chosen lines (10 clones each) were inspected for the following traits: flowering time, number of flowers per mutant line (yield), flower size, flower shape, presence of floral scent, color of flowers, leaf patterns, and tolerance to *Pectobacterium* infection. All of those traits were compared to those observed in the control lines. Examples of the variability in the flowers of the selected mutant lines from the second season are presented in Figure 3 and Figure S2.

In the second season, 80% of the phenotypic characteristics that were observed in the first year appeared once again in the same lines, 10% retained some of the characteristics observed in the first year, and the remaining 10% were completely different in the two years.

Figure 3. Flower diversity of mutated lines during the second growing season (2018). (**a**) Control line *Ca*. (**b**) The Israeli ecotype *ZAI*. (**c–f**) Flower variations observed in the X-ray-irradiated mutant lines, *CaX*. Numerous flower formations were observed among the mutants. Some of them had (**c**) small and rounded-shape flowers, (**d**) very large flowers, (**e**) flowers with curved borders, and/or (**f**) a colored spathe. The vertical, white, dashed bar on the right of each image represents a length of 6 cm.

3.4.1. Early and Late Flowering

To monitor early flowering phenotypes, we established two definitions: onset of flowering (OOF)—the flowering time of the first 10% of the total flowers for each mutant line—and early flowering (EF)—mutant lines in which OOF was 8 weeks earlier than that of the control lines. We used an 8-week period to overcome the natural variation in OOF. As illustrated in Figure 4, the OOF of the control lines took place during March 2019, while the timing of the OOF of the mutant lines ranged from November 2018 to March 2019. Therefore, in the second season, the EF was calculated based on OOF obtained earlier than mid-January 2019. Among the mutant lines, 50% had an OOF from February 2018, and 40% were EF; one of the mutants' OOF was as early as 3 months before that of the control line (Figure 4).

3.4.2. Flower Yield

Flower yield was measured as the average amount of flowers produced by all of the plants of each line during the second growing season (2018–2019). As shown in Figure 5a, the average yield of the control lines (*Ca*) was less than two flowers per plant, similar to that of 25% of the mutated lines. Only 15% of the mutated lines had lower yields (about one flower per plant). However, 60% of the selected mutated lines had higher yields (i.e., two to four flowers per plant), and four of the mutated lines had significantly higher yields ($p < 0.001$) of more than three flowers per plant (Figure 5a). The local Israeli line (*ZAI*) had significantly lower yields than the South African lines (*Ca*, *CaX*).

3.4.3. Flower Size

The flower-size trait was based on the full length of the spathe before opening and was divided into four groups: (1) small—flower length of 5 cm or less; (2) medium—flower length of 6–10 cm (this included the *Ca* and *ZAI* groups); (3) large—flower length of 11–14 cm and (4) extra-large—flowers longer than 14 cm. As shown in Figure 5b, one mutated line had significantly smaller flowers than the control, 11 lines had significantly larger flowers than the control, and four lines had extra-large flowers that were more than 14 cm long.

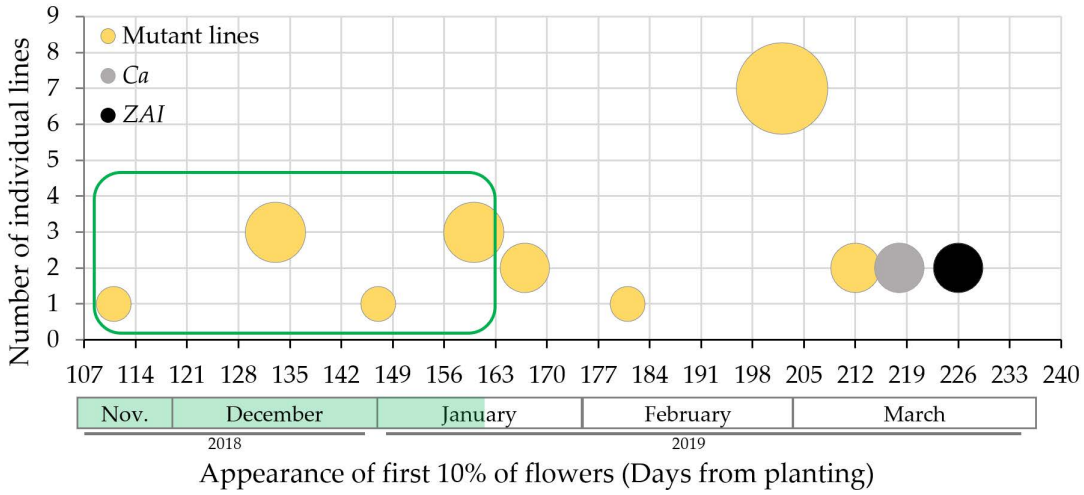

Figure 4. Onset of flowering (OOF) of 20 mutated *Z. aethiopica* lines compared to the control line during the 2018–2019 growing season. Each bubble represents a different group of mutant lines in which more than 10% of the total flowers from those lines appeared, and the bubble size represents the number of lines (y-axis) that flowered at that time (x-axis). Mutant lines (*CaX*) are presented in yellow, the control (*Ca*) is shown in gray, and the local Israeli line *ZAI* is shown in black. Time was measured as days from planting, and the months (and years) are listed below the x-axis, with the green background and the green box representing the period defined as early flowering.

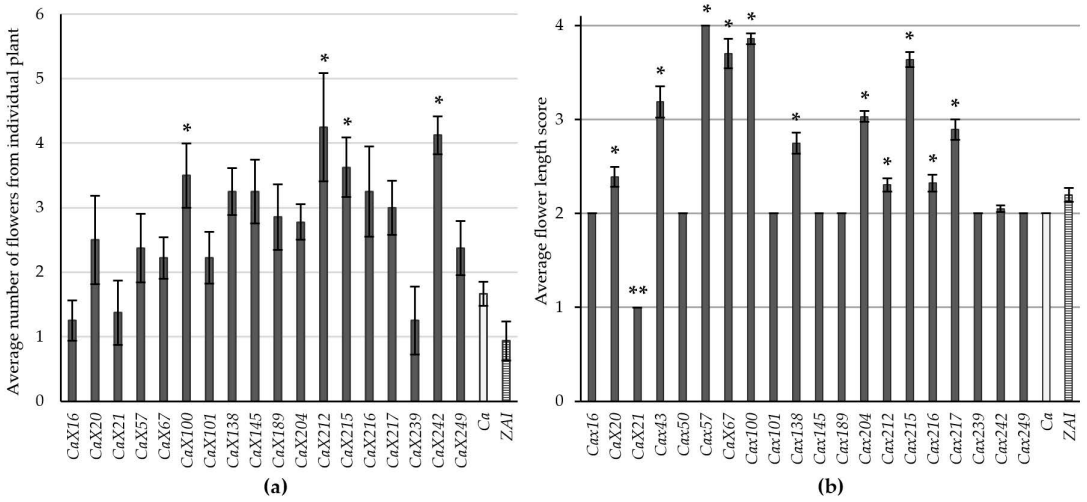

Figure 5. (**a**) Flower yield and (**b**) flower size obtained from the selected *Z. aethiopica* mutated lines compared to the control lines during the second growing season (2018–2019). (**a**) The average numbers of flowers per plant from each line are presented as bars, with standard errors indicated for each column. (**b**) The average flower sizes of the selected lines are represented in length groups with the following scores: small (<5 cm), group 1; medium (6–10 cm), group 2; large (11–14 cm), group 3; and extra-large (>14 cm), group 4. The mutated lines are labeled "*CaX*", and control lines are labeled "*Ca*". The Israeli local line is labeled "*ZAI*". Values significantly greater than the corresponding control values are marked with a single asterisk (*) ($p \leq 0.001$). Double asterisks (**) indicate significantly smaller size ($p \leq 0.001$).

3.4.4. Desired Flower Shape

The mutated lines had a variety of flower shapes. To define the preferred flower shape, we focused on the side and upper projections. With this method, we defined four projections, two side views and two top views. The side view included the open or closed spathe, and the top view included the drop or rounded shape of the spathe, as presented in Figure 6. The desired flower shape consisted of a combination of closed and rounded shape (Figure 6b,d), with the spathe appearing as a smooth cup. About 75% of all of the selected mutated lines had cup-shaped flowers.

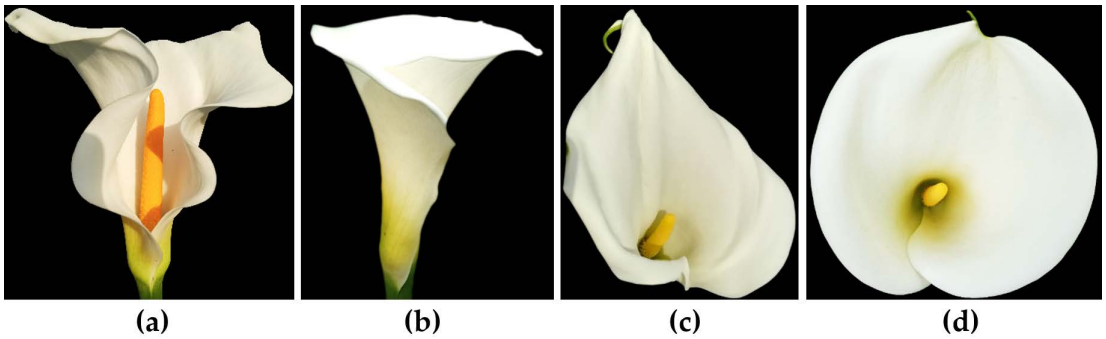

Figure 6. Examples of flower shapes. Side view: (**a**) open or (**b**) closed. Top view: (**c**) drop shape or (**d**) rounded shape. The most desired flower shape was a combination of closed and rounded shapes (**b**,**d**).

3.4.5. Tolerance to *Pectobacterium* Infection

Another desired property for *Zantedeschia* plants is tolerance to soft-rot disease caused by bacterial pathogens of the genus *Pectobacterium*. Since it would have been both time- and labor-intensive to scan large numbers of individual mutated lines during the first growing season, we decided to focus on the 20 best mutated lines (*CaX*) from the second growing season. As *CaX* plants were generated from irradiated seeds, it was important to assess the natural variation of disease tolerance among the control lines (*Ca*). To do so, we analyzed 19 individual *Ca* lines by inoculating leaf discs of each line with 10^8 CFU of *Pectobacterium* strain *Pcb1692*. The relative size of the decayed area was recorded at 21 h postinoculation (Figure 7a). The decay area (% of total area) was calculated using 19 individual *Ca* lines and compared to that observed on the local Israeli line *ZAI* (Figure 7b). The average extent of decay of the *Ca* lines was significantly lower (18%) than that of the local Israeli line *ZAI* (28%).

Next, the infection assays were performed for all of the selected *CaX* lines; two *Ca* lines and the local Israeli line *ZAI* were used as controls (Figure 7d). Compared to the *Ca* lines, the mutated lines exhibited greater variability; eight *CaX* lines were significantly more tolerant to *Pcb1692* infection, and two lines were significantly more susceptible, being similar to *ZAI* (Figure 7d). A comparison of the degrees of variation in each of the two groups revealed that the mean and median values for the two groups were very similar, but that there was greater variability among the irradiated lines (Figure 7e).

3.5. Summary of the Mutated Phenotypes (2018–2019)

At the end of the second growing season, we could detect mutant lines with different desired traits, most of which (75%) had more than two of the desired traits. Two mutated lines (10%) had five or six of the desired traits (*CaX215* and *CaX100*, respectively), 30% had four of the desired traits, 15% had three of the desired traits, and 25% had two of the desired traits. A single line (*CaX101*) did not exhibit any of the desired traits. A summary of all of the examined traits in the 20 selected mutated lines is presented in Table 3.

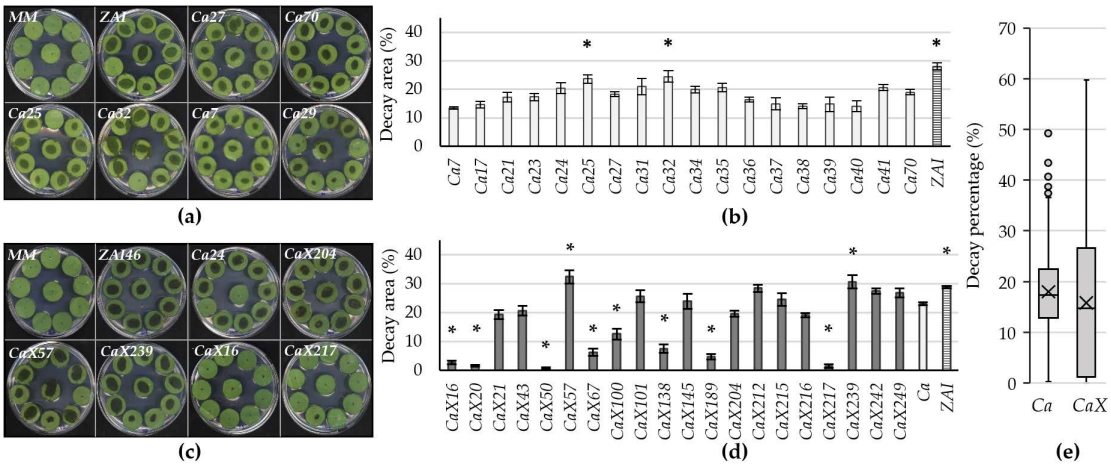

Figure 7. Assessment of *Pectobacterium brasiliense Pcb1692* infection of leaf discs of the control (*Ca*) and selected mutated lines (*CaX*) of *Zantedeschia aethiopica*. Leaf discs were pierced in the center and inoculated with 10 µL of *Pcb1692* (10^9 CFU mL^{-1}). Infection was measured as area of decayed tissue (%) 21 h after inoculation and incubation at 28 °C. (**a**) A representative photo of the infected leaf discs of the *Ca* lines. (**b**) Percentage of tissue area covered by decay among the *Ca* lines. (**c**) A representative photo of the infected leaf discs of the mutated lines (*CaX*). (**d**) Percentage of tissue area covered by decay among 20 *CaX* lines, compared to the average of the control lines (*Ca24* and *Ca31*) and *ZAI*. In (**a**) and (**c**), the line labels are marked in the upper-left corner of each plate. MM stands for noninoculated control (minimal medium). The local Israeli line is marked as *ZAI*. The bars represent average values with standard errors. Lines that are significantly different from the control are marked with asterisks ($p \leq 0.001$). (**e**) Comparison of variation between the *Ca* lines and the *CaX* lines, in terms of the percentage of tissue area covered by decay. "x" is the average and "-" is the median.

Table 3. Summary of traits observed during the 2018–2019 season among the selected X-ray-mutated lines (*CaX*). + indicates presence of the trait.

Mutant Line	High Yield	Early Flowering	*Pectobacterium* Tolerance	Large Flowers	Desired Flower Shape	Scent in Flowers	Total Intersection Occurrences
CaX100	+	+	+	+	+	+	6
CaX215	+	+		+	+	+	5
CaX189		+	+		+	+	4
CaX242	+	+			+	+	4
CaX138			+	+	+	+	4
CaX20			+	+	+	+	4
CaX67			+	+	+	+	4
CaX217		+	+	+	+		4
CaX21		+			+	+	3
CaX57				+	+	+	3
CaX43		+		+		+	3
CaX212	+	+					2
CaX16			+		+		2
CaX50			+			+	2
CaX239					+	+	2
CaX249					+	+	2
CaX204				+			1
CaX216					+		1
CaX145					+		1
CaX101							0

4. Discussion

The main goal of this study was to increase the genetic variation in *Z. aethiopica* in order to select a *Z. aethiopica* variety that was better suited for Mediterranean growers. We hypothesized that the best way to promote this aim would be to select a better-performing

cultivar under the local winter conditions, as winter is the preferred growing season for Israeli growers. Although the use of ionized radiation is an established method for inducing mutations in cultivated plants [15], as far as we know, this was the first time that X-rays were used to induce mutation in *Z. aethiopica* seeds. As *Z. aethiopica* is propagated vegetatively, this method of cultivation is especially feasible [15]. In the present study, the radiation dose was set at 100 Gy to efficiently select for potentially valuable *Z. aethiopica* mutated lines. In previous studies, similar doses of radiation were found to be effective for mutagenesis of other crops, including ornamental crops [15].

Here, we created 319 individual putative mutant lines (*CaX*), from which we selected and further propagated 20 lines that exhibited some of the desired traits that we defined: high flower yield, early flowering time, large flowers with the desired shape, and tolerance to soft rot caused by *Pectobacterium* [8,23–25]. Most of the selected varieties had more than one of these traits (i.e., two to six of these traits), resulting in greater potential for commercialization. The mutated lines we produced and examined had a variety of desirable traits, which were found to be stable even after two consecutive growing seasons. Some of the traits that we examined (e.g., tolerance to soft rot) have also been examined in other *Z. aethiopica* breeding programs that employed a classical breeding approach [14].

Higher yields can easily improve growers' incomes. Here, four mutated lines had flower yields that were significantly higher than those of the controls. None of the selected mutated lines yielded significantly less than the controls.

Early flowering may contribute to yield, as it extends the flowering season. Moreover, it allows marketing during the period that includes Christmas, Valentine's Day, and Easter. The winter season in the northern hemisphere is the time of year when competition from other producers in the market is relatively low and prices per flower stem are relatively high. Early flowering may also minimize the amount that growers spend on chemical treatments, which are required when temperatures rise, as well as the amount spent on growth regulators, which are currently applied at least twice during the growing season. Our study identified eight varieties that flowered significantly earlier than the local variety (*ZAI*) and the nonirradiated *Z. aethiopica* (*Ca*). Five of these early-flowering phenotypes flowered during the period of peak flower prices (i.e., marketing weeks 46–52, Figure S3).

Greater tolerance to *Pectobacterium* can help growers in the battle against soft rot, especially in warmer climate zones. This trait is highly ranked among *Z. aethiopica* growers who are coping with climate change and unexpected heat waves that occur even during the coldest time of the year in the Middle East. The warm conditions that already prevail in Israel present a challenge for soft-rot control [26]. We used *Pcb1692*, a type strain of *P. brasiliense*, which is a rising soft-rot bacterium under warm conditions, to examine tolerance to soft rot. Soft rot is a major problem for the production of economically important crops around the world [27]. Here, we demonstrated that controlled exposure to X-ray radiation could introduce a range of tolerance levels to *Pectobacterium*. About 40% of the selected mutated lines were significantly more tolerant to *Pectobacterium* than the control.

Another important trait is flower appearance, which we divided into flower shape, size, and differences in color. We included this quality as part of our preferred selection for new cultivars by focusing on large, relatively closed, cup-shaped flowers with round projections from the top side. Although some of the mutants in this study were not suitable for commercial cultivation, they could still be used for conventional crossings to increase genetic variation and pass some of their traits along to hybrids with better combined phenotypes.

Overall, X-ray mutagenesis of *Z. aethiopica* seeds effectively expanded the range of traits we observed in *Z. aethiopica* plants and appears to have potential as an effective, time-saving method for enhancing genetic variability in this species. This is of particular interest for breeders working with the genus *Zantedeschia*, for which conventional breeding and interspecific crossings are limited by breeding barriers within the genus. The mutated lines are good candidates for commercialization and cultivation under warm climate conditions.

5. Conclusions

X-ray irradiation of *Z. aethiopica* seeds is an effective, feasible tool for increasing the phenotypic diversity of this ornamental plant. This approach allowed the improvement of traits such as yield, early flowering under warm climate, appearance, and resistance to devastating pathogens such as *Pectobacterium*. The results presented here demonstrate the potential of X-ray mutagenesis for future development of *Z. aethiopica* varieties.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11122537/s1, Figure S1: Calibration of the radiation dose for *Zantedeschia aethiopica* seeds, Figure S2: Flower diversity of mutated lines (*CaX*) during two growing seasons (2018–2020), Figure S3: *Zantedeschia aethiopica* sales in Royal FloraHolland over time (weeks) during the 2019, 2020, and 2021 marketing seasons.

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