

Diversity in flower characteristics and chromosome numbers of Rain Lily (*Zephyranthes* spp.)

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Abstract: The study examined the flower characteristics and chromosome numbers of twenty rain lily genotypes with different origins. The bulbs of investigated genotypes were grown sequentially in an experimental garden under net-house conditions at the Vietnam National University of Agriculture. The data was collected for flower parameters including flower size and color, flower longevity, lengths of stamen, pistil, and anther, and the number of chromosomes. The results showed that the rain lily genotypes exhibited diversity in flower color and flower diameter, making advances for bedding flowers and landscaping. Furthermore, a wide range of chromosome numbers (from $2n = 18$ to $2n = 54$) was found among the genotypes from $2n = 18$ in genotype D21 to $2n = 54$ in genotype D6. The new chromosome numbers of *Z. atamasco* ($2n = 22$), *Z. ajax* ($2n = 28$), *Z. candida* ($2n = 30$), *Z. rosea* ($2n = 28$), and *Habaranthus robustus* ($2n = 18$) from Vietnamese genotypes and new hybrids were updated. The information will be valuable for the breeding programs of this species.

Keywords: Rain lily, *Zephyranthes*, chromosome, growth, development

Introduction

Rain lily (*Zephyranthes* spp.) belongs to the *Zephyranthes* genus in the subfamily of Amaryllidoideae (Jin, 2013). Rain lilies are classified into four main types: *Zephyranthes candida*, *Z. carinata*, *Z. tubispatha*, and *Z. atamasco* with differences in color, bloom size, and leaf form (Afroz et al., 2018). They are not only cultivated as ornamental plants, along garden walkways or in flower beds, but they are also used as traditional medicine in many countries such as China and India due to their high levels of antioxidants, antiviral, antimicrobial, antimitotic, anticholinesterase, and cytotoxic activities (Zhan et al., 2016; Singh et al., 2019; Syeed et al., 2021).

The genus *Zephyranthes* is known for its diversity in morphological characteristics and chromosome numbers (Flix et al., 2011). While many species have similar features, the number of chromosomes in the genus varies greatly, with counts ranging from $2n = 10$ in *Z. seubertii* to $2n = 200$ in certain hybrids (Daviña et al., 2019; Flory and Smith, 1980). To date, karyotypes of around 50 species of *Zephyranthes* have been studied, revealing a wide range of chromosome numbers. For example, *Z. candida* has been found to have $2n = 38$, 40, and 41 (Raina and Khoshoo, 1971), while *Z. selberti* from Argentina has $2n = 10$, 20, 30, and 40 (Daviña and Fernández 1989). These differences in chromosome count can impact the growth and development of the plant and its morphological characteristics. Further

studies are needed to fully understand the impact of chromosome count on the genus *Zephyranthes*.

Despite its potential, its low species diversity has limited the use of the rain lily. For the rain lily to be widely adopted as a major summer decoration, further research and development are required. Currently, the rain lily species found in Vietnam are relatively uniform, with only a few distinguishable by petal color, including pink, white, and light yellow. Future research should consider hybridization to increase diversity in this species.

Results and discussion

Flower color and petal pattern of rain lily genotypes

The rain lily (*Zephyranthes* spp.) has been identified as a promising species for landscape decoration due to its diverse and attractive flower colors and shapes. In our study, there is a diversity in the petal and pedicel color of investigated genotypes (Table 1, Figure 1). Based on the petal color they could be divided into four main groups: white, yellow, pink, and orange. The white group includes genotypes D4, D7, D8, D9, D19, D22, and D23. The yellow group includes genotypes D5, D13, and D14. The pink group could be divided into three smaller groups including dark pink (D10 and D11), pink (D6, D12, and D17), and pinkish white (D15, D18, and D21). The orange group includes genotypes D16 (orange-yellow)

Table 1. Flower characteristics of rain lily genotypes.

Genotype	Petal color	Pedice color	Flower height (cm)	Flower diameter (cm)	Blooming duration (days)
D4	White	Green	3.48 ± 0.15	4.47 ± 0.55	2 - 3
D5	Yellow	Yellow	5.74 ± 0.33	4.30 ± 0.51	2 - 3
D6	Pink	White	5.75 ± 0.65	8.73 ± 0.50	2 - 3
D7	White	Green	3.83 ± 0.12	5.88 ± 0.23	2 - 3
D8	White	Green	3.22 ± 0.75	4.56 ± 0.63	2 - 3
D9	White	Green	5.13 ± 0.34	5.80 ± 0.43	2 - 3
D10	Dark pink	White	2.94 ± 0.14	3.68 ± 0.22	2 - 3
D11	Dark pink	White	3.06 ± 0.13	3.87 ± 0.22	2 - 3
D12	Pink	White	4.92 ± 0.29	8.02 ± 0.55	2 - 3
D13	Yellow	Yellow	4.52 ± 0.38	6.33 ± 0.50	2 - 3
D14	Yellow	Yellow	3.65 ± 0.21	2.97 ± 0.46	2 - 3
D15	Pinkish white	Yellow	4.50 ± 0.06	5.03 ± 0.12	2 - 3
D16	Orange yellow	Yellow	5.12 ± 0.95	7.30 ± 0.54	2 - 3
D17	Pink	White	7.25 ± 0.48	7.05 ± 0.68	2 - 3
D18	Pinkish white	White	5.14 ± 0.15	4.74 ± 0.25	2 - 3
D19	White	Yellow	6.52 ± 0.32	7.56 ± 0.23	2 - 3
D20	Bold brick	Yellow	4.20 ± 0.13	3.65 ± 0.45	2 - 3
D21	Pinkish white	Olive	7.15 ± 0.35	5.25 ± 0.33	2 - 3
D22	White	Blue	3.22 ± 0.25	5.50 ± 0.70	2 - 3
D23	White	Blue	3.23 ± 0.52	6.10 ± 0.53	2 - 3

and D20 (bold brick). The pedicel color of genotypes are yellow (D5, D13, D14, D15, D16, D19, and D20), white (D6, D11, D12, D17, and D18), green (D4, D7, D8, and D9), blue (D22 and D23), and olive (D21). Afroz et al. (2018) found a variation in flower color among *Zephyranthes* species with white in *Z. atamasco* and *Z. candida*, pink in *Z. carinata*, and yellow in *Z. tubispatha*. *Z. rosea* Lindley had pink color, whereas *Z. citrina* Baker's flower was yellow (Flagg, 2014). *Z. drummondii* is known as the white flower rain lily (Pacific Bulb Society, 2021). In this study, genotypes including D4, D8, D9, and D22 belonging to *Z. candida*, and D7 belonging to *Z. atamasco* presented white flowers. Genotypes D6 and D12 in the *Z. carinata* family, and D10 and D11 in the *Z. rosea* family produced pink flowers. *Z. citrina* genotype D14 showed yellow flowers. *Z. labuffarosea* flowers vary from white to pink, and also bi-colors (Pacific Bulb Society, 2021) such as genotypes D17, D18, and D23. The genotype D21 is a *Habranthus robustus* bloomed pinkish-white flower (Pacific Bulb Society, 2023). D5, a hybrid genotype of *Z. candida* x *Z. citrina* had yellow flowers. The genotype D15, a *Z. batik* made by Fadjara Marta of Indonesia had pinkish flowers and a faint yellow stripe along the middle of each petal (Pacific Bulb Society, 2016). *Z. krakatau* as genotype D20 presented reddish orange petals similar to the report by the Pacific Bulb Society (2019). By observation, the majority of the rain lily genotypes exhibited a petal arrangement of six petals with three inner and three outer petals. However, genotypes D10 and D13 showed a difference from the remaining genotypes with the number of petals being more than six (Figure 1). In addition, several genotypes (including D4, D7, D8, D9, D10, and D11) had petals joined at the sepal to form a small floral tube before separating. In contrast, the petals of the remaining genotypes separated from each other. Vu et al. (2023) also found the same result with the difference in the petal number of IM5 genotypes (12 petals) with other genotypes (6 petals) in their investigation.

Flower and sexual organ size of rain lily genotypes

Flagg and Smith (2008) found that rain lilies are distinct from one another based on the lengths of floral parts such as the

perianth and stamen. The flower height and flower diameter of genotypes ranged from 2.94 to 7.25 cm and from 2.97 to 8.3 cm, respectively (Table 1). D17 (7.25 cm) and D21 (7.15 cm) were the genotypes that had the highest flower height which was approximately higher 2.5 times than that of the lowest ones D10 (2.94 cm) and D11 (3.06 cm). D6 (8.73 cm) was the largest flower diameter genotype followed by genotype D12 (8.02 cm). D14 showed the smallest flower diameter at 2.97 cm. The longevity of the blooms of all investigated genotypes was from 2 to 3 days (Table 1). Vu et al. (2023) found similar results the flower diameter ranging from 16.1 to 27.1 cm, with the durability of one flower from 1 to 2 days. The variations in lengths of the stamen, pistil, and anther of rain lily genotypes are presented in Table 2. The genotypes D17 and D18 had the shortest stamens with values of only 0.12 and 0.13 cm, while D6 (4.35 cm) and D19 (4.69 cm) had the longest stamens. D6 also had the longest pistil (6.40 cm) and D8 (1.26 cm) and D14 (1.38 cm) were the shortest ones. The anther length of genotypes ranged from 0.50 to 1.64 cm, D6 also had the longest anther followed by D12 (1.15 cm). The genotypes D8, D7, and D18 showed the shortest anthers with values of 0.50, 0.54, and 0.56 cm, respectively. Afroz et al. (2018), Dash et al. (2020) found similar results in three *Zephyranthes* species *Z. candida*, *Z. carinata*, and *Z. tubispatha* with the style and anthers ranging from 1.7 to 3.0 cm and 0.7 to 0.9 cm, respectively. Based on the characteristics of stamen, pistil, and anther, the lily genotypes could be classified into three groups with the difference in the relative positioning of the stigma and anther: group 1, included genotypes D5, D17 and D18 with stigma being in position lower than the anther; group 2, included genotypes D4, D9, D14, D15, D16 and D20 which stigma and anther being at the same level; group 3, included the remaining genotypes which stigma positioned higher than anther.

Chromosome numbers of rain lily genotypes

Previous research has shown the wide range of chromosome numbers of the *Zephyranthes* genus. Raina and Khoshoo (1971) reported that it varies from $2n = 18$ to $2n = 96$. The

Table 2. Size of reproductive organs of rain lily genotypes

Genotype	Stamen length (cm)	Pistil length (cm)	Anther length (cm)
D4	1.63 ± 0.15	1.70 ± 0.18	0.68 ± 0.04
D5	3.92 ± 0.11	3.20 ± 0.14	0.99 ± 0.20
D6	4.35 ± 0.25	6.40 ± 0.46	1.64 ± 0.17
D7	1.75 ± 0.21	2.87 ± 0.32	0.56 ± 0.05
D8	0.59 ± 0.10	1.26 ± 0.12	0.50 ± 0.05
D9	1.25 ± 0.20	2.26 ± 0.19	0.58 ± 0.06
D10	1.32 ± 0.13	2.93 ± 0.17	0.86 ± 0.05
D11	1.23 ± 0.09	2.80 ± 0.07	0.71 ± 0.07
D12	2.53 ± 0.29	4.84 ± 0.42	1.15 ± 0.20
D13	1.86 ± 0.15	3.95 ± 0.32	0.80 ± 0.04
D14	2.15 ± 0.09	1.38 ± 0.07	0.75 ± 0.05
D15	2.58 ± 0.17	3.24 ± 0.21	0.92 ± 0.11
D16	2.85 ± 0.28	3.72 ± 0.22	0.98 ± 0.09
D17	0.12 ± 0.02	2.73 ± 0.07	0.68 ± 0.05
D18	0.13 ± 0.01	2.48 ± 0.10	0.54 ± 0.05
D19	4.69 ± 0.21	3.05 ± 0.17	0.78 ± 0.06
D20	1.98 ± 0.12	2.45 ± 0.11	0.88 ± 0.05
D21	2.25 ± 0.23	3.74 ± 0.28	0.68 ± 0.18
D22	1.76 ± 0.12	1.89 ± 0.15	0.72 ± 0.07
D23	1.57 ± 0.10	1.87 ± 0.15	0.94 ± 0.10

**Figure 1.** Flower of the experimental rain lily genotypes.

wider range of chromosome numbers was reviewed by Bhattacharyya (1972) from $2n = 12$ to $2n = 120$. The smallest chromosome number was revealed by Daviña et al. (2019) with $2n = 10$ in *Z. seubertii*. Meanwhile, the largest chromosome number was found in a horticultural hybrid $2n = 200$ (Flory and Smith, 1980). In our study, the chromosome numbers of rain lily genotypes are presented in Table 3 and Figure 2. The genotype D18 ($2n = 18$) had the lowest chromosome number followed by D7 ($2n = 22$). D6 is the genotype that had the highest number of chromosomes

with $2n = 54$. The genotypes having the same chromosome number were D10, D17, and D18 ($2n = 24$); D5, D11, and D15 ($2n = 28$); D4 and D8 ($2n = 30$); D12 and D14 ($2n = 42$); D16, D19, and D23 ($2n = 44$). The remaining genotypes D20, D9, D13, and D22 had 32, 36, 38, and 40 chromosomes, respectively. Interestingly, genotypes that had larger chromosome numbers also had larger flower sizes. The information was supported by a positive significant correlation between chromosome number and flower diameter, stamen length, and anther length in Table 4.

Table 3. Number of chromosomes of rain lily genotypes

Genotype	Number of chromosomes	Genotype	Number of chromosomes
D4	30	D14	42
D5	28	D15	28
D6	54	D16	44
D7	22	D17	24
D8	30	D18	24
D9	36	D19	44
D10	24	D20	32
D11	28	D21	18
D12	42	D22	40
D13	38	D23	44

Table 4. Correlation of chromosome number and flower characteristics

Flower characteristics	Flower height	Flower diameter	Pistil length	Stamen length	Anther length
Chromosome number	0.05 ^{ns}	0.54*	0.33 ^{ns}	0.54*	0.64**

ns, * and ** mean non-significant, significant at $P < 0.05$ and $P < 0.01$, respectively.

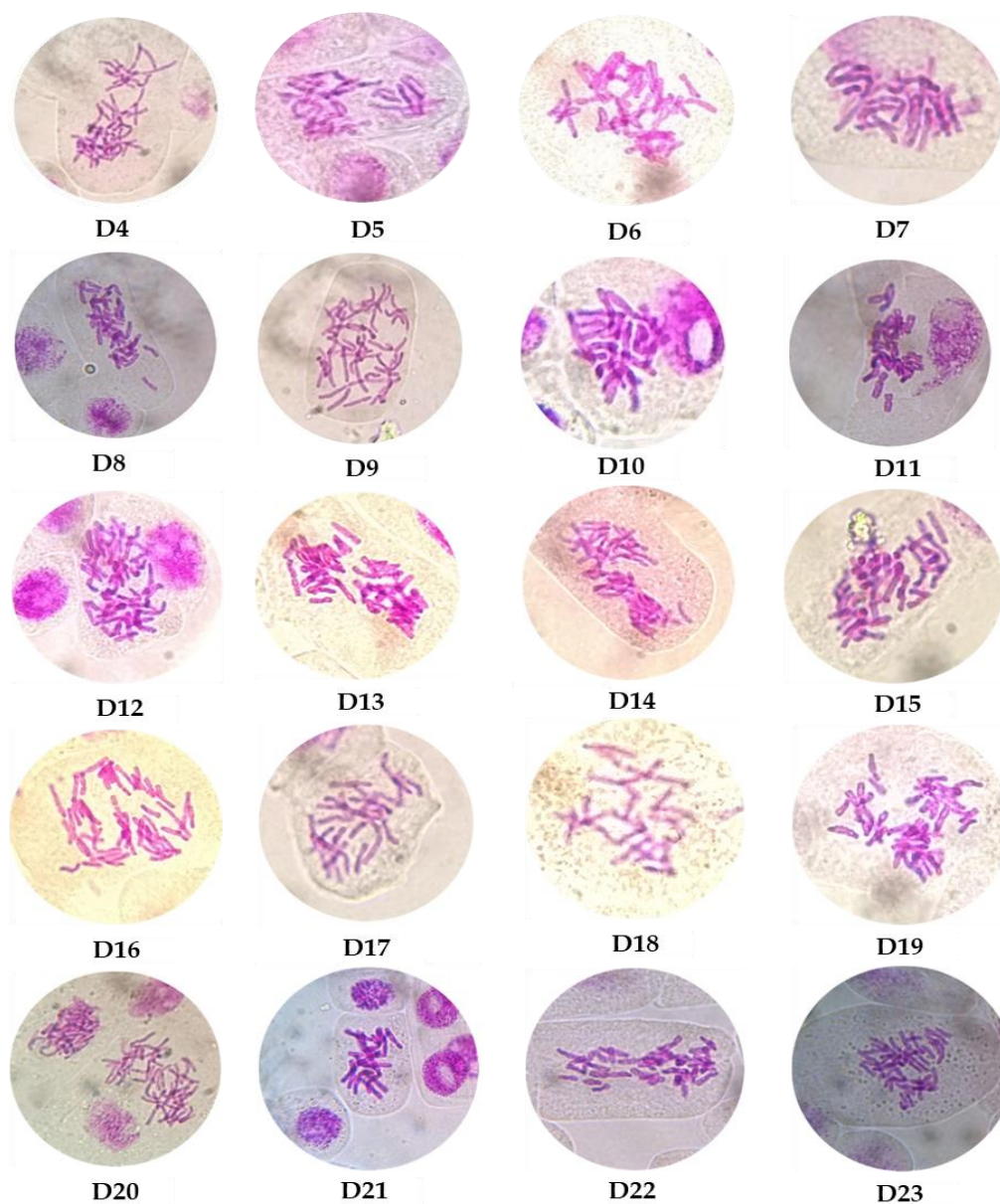
**Figure 2.** Chromosome numbers of experimental rain lily genotypes.

Table 5. Identification code and origins of rain lily genotypes.

ID code	Origin	Scientific name
D4	Ha Noi city, Vietnam	<i>Z. candida</i>
D5	Ha Noi city, Vietnam	<i>Z. ajax</i>
D6	Hung Yen province, Vietnam	<i>Z. carinata</i>
D7	Ha Noi province, Vietnam	<i>Z. atamasco</i>
D8	Thanh Hoa province, Vietnam	<i>Z. candida</i>
D9	Hung Yen province, Vietnam	<i>Z. candida</i>
D10	Ha Noi province, Vietnam	<i>Z. rosea</i>
D11	Vinh Phuc province, Vietnam	<i>Z. rosea</i>
D12	Lao Cai province, Vietnam	<i>Z. carinata</i>
D13	Japan	<i>Z. san antonio</i>
D14	Da Nang province, Vietnam	<i>Z. citrina</i>
D15	America	<i>Z. batik</i>
D16	America	<i>Z. rose perfection</i>
D17	America	<i>Z. pink panther</i> (laffurosea selection)
D18	America	<i>Z. lily pies</i>
D19	America	<i>Z. drummondii fedora</i>
D20	America	<i>Z. krakatau</i>
D21	Lao Cai province, Vietnam	<i>Z. robusta</i> (<i>Habranthus robustus</i>)
D22	Nam Dinh province, Vietnam	<i>Z. candida</i>
D23	Hung Yen province, Vietnam	<i>Z. lily pies</i> (laffurosea selection)

Zephyranthes is one of the evolutionarily dynamic genera of Amaryllidaceae which displays and retains an array of chromosome numbers (Dash et al., 2020). Polyploidy is a common feature in the genus (Daviña and Fernández, 1989) with the basic chromosome numbers $x = 5$ and $x = 6$. Aquino et al. (2023) claimed that *Zephyranthes* had different basic chromosome numbers ($x = 5, 6, 7$, and 9) with $x = 6$ being the most common. Felix et al. (2011) reported *Z. robusta* and *Z. sylvatica* as diploid ($2n = 2x = 12$), *Z. rosea*, *Z. brachyandra*, and *Z. grandiflora* (synonym of *Z. carinata*) as tetraploid ($2n = 4x = 24$). Dash et al (2020) reported In this study, $2n=4x=24$ in *Z. carinata* as tetraploid ($2n = 4x = 24$) and *Z. tubispatha* as octoploid ($2n = 8x = 48$). On the other hand, aneuploidy arises during meiosis due to imbalanced gametes and is perpetuated through asexual reproduction (Flix et al., 2011). The significant variation in the number of chromosomes among rain lily genotypes is a result of their existence in both polyploid and aneuploid forms, a characteristic that sets rain lily flowers apart from others.

The chromosome number of *Zephyranthes* is very varied even in the same species. Raina and Khoshoo (1971), Bhattacharyya et al. (1972), Kumar and Subramaniam (1986), Felix et al. (2011), Tapia-Campos et al. (2012), and Dash (2020) summarized in chromosome number of species in the *Zephyranthes* and *Habranthus* genus related to genotypes in our study as follows:

Habranthus robustus: $2n = 12$;

Z. atamasco: $2n = 12$ and 24 ;

Z. ajax: $2n = 42, 43$ and 48 ;

Z. candida: $2n = 19, 20, 24, 28, 32, 36, 38, 40-42$, and $48-50$;

Z. carinata and its synonyms *Z. grandiflora* and *Z. minuta*: $2n = 24, 36, 38, 42$, and $46-54$;

Z. citrina and its synonym *Z. sulphurea*: $2n = 24, 28, 42-48, 50, 54, 51, 66, 73$ and 96 ;

Z. drummondii and its synonym *Z. pedunculata*: $2n = 12, 14, 24, 28, 44-46, 48-50, 56, 58-60, 68-72$;

Z. rosea: $2n = 24$ and 25 ;

In our study, several genotypes belonging to *Z. candida* (D9, D22), *Z. carinata* (D6, D12, and D14), *Z. rosea* (D10), and *Z. drummondii* (D19) had the same chromosome number as previous reports. Moreover, this study provides new information about chromosome numbers of rain lilies

originating in Vietnam with different chromosome numbers with previous research such as *Z. atamasco* genotype D7 ($2n = 22$), *Z. ajax* D5 ($2n = 28$), *Z. candida* D4 and D8 ($2n = 30$), *Z. rosea* D11 ($2n = 28$) and *Habranthus robustus* D21 ($2n = 18$). Our study also supported the information of *Z. laffurosea* (D17 and D18, $2n = 24$ and D23, $2n = 44$) and hybrids including *Z. krakatau* (D20, $2n = 32$), *Z. rose perfection* (D16, $2n = 44$), *Z. batik* (D15, $2n = 28$) and *Z. san antonio* (D13, $2n = 38$) which has been not mentioned in previous studies.

Materials and Methods

Plant materials

The experimental material was twenty rain lily genotypes with different origins including twelve *Zephyranthes* and one *Habranthus* genotypes collected in Vietnam, and seven *Zephyranthes* genotypes imported from America and Japan. Details of genotype name and origin are shown in Table 5.

Evaluation of flower characteristics of rain lily genotypes

The 15 to 30 bulbs of each genotype were planted sequentially on the seedling bed in the experimental garden of the Department of Horticultural Science, Faculty of Agronomy, Vietnam National University of Agriculture. At blooming time, flower indicators were recorded including petal color, pedicel color, flower height, flower diameter, blooming duration, stamen length, pistil length, and anther length. The collected data was then analyzed using Microsoft Excel software to perform statistical analysis.

Determining the number of chromosomes of rain lily genotypes

The root caps of healthy roots from five sample plants of each genotype were sampled to identify chromosomal numbers using the squash method. The procedure involved cutting 1 cm length of the juvenile roots and treating them in 0.05% colchicine solution for 4 hours at 4°C . The roots were then fixed in Carnoy solution (3 ethanol : 1 acetic acid, v/v) for 24 hours or more at 4°C . To visualize the chromosomes, the roots were hydrolyzed in 1N HCl solution for 15 minutes at 60°C and then dyed in Fuchsin solution.

The stained roots were examined under an electron microscope at magnifications of 10-100x to determine the number of chromosomes.

Conclusions

In conclusion, our study revealed the genetic diversity in flower characteristics and chromosome numbers among the different rain lily genotypes. The chromosome counts ranged from $2n = 18$ in genotype D21 to $2n = 54$ in genotype D6. This range of chromosomes highlights the genetic richness of the rain lily populations and their ability to adapt to varying environmental conditions. The new chromosome numbers of *Z. atamasco* ($2n = 22$), *Z. ajax* ($2n = 28$), *Z. candida* ($2n = 30$), *Z. rosea* ($2n = 28$), and *Habaranthus robutus* ($2n = 18$) from Vietnamese genotypes and new hybrids were updated. Our findings offer important insights into the diversity of rain lilies and their potential for use in future breeding programs to develop or improve new cultivars.

Conflict of Interest: The authors declare no conflict of interest.

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