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IDENTIFICATION OF NOVEL VARIANTS OF THREE *ZEPHYRANTHES* SPECIES THROUGH MULTILOCATION ASSESSMENT

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Abstract

Zephyranthes spp. (Rain lily) are priced ornamentals known for their brightly coloured flowers that gently naturalize the surroundings. They contain rich sources of alkaloids that are well known for their pharmacological activities. Breeding work in rain lily is extensively difficult; it is performed to add value as ornamentals. The present study was undertaken to identify and develop three novel variants of *Zephyranthes*- *Z. rosea, Z. citrina, Z. candida* through multilocation assessment. Initial field trial was conducted with fifteen germplasm for each species during spring 2018, 2019 and identified one lines for each species- RRL-PRL-05 (*Z. rosea*), RRL-YRL-02 (*Z. candida*) and RRL-WRL-27 (*Z. citrina*). RRL-PRL-05 with bright pink flowers and flat, broad leaves; RRL-YRL-02 showed bright yellow flowers with flat narrow leaves while RRL-WRL-27 showed white flowers with fine hairlike leaves. Multilocation study conducted for two consecutive years showed the identified lines to be stable for quantitative characters and colour combinations.

Introduction

Zephyranthes is a deciduous or evergreen bulbous perennial wildflower belonging to the family Amaryllidaceae. It is also commonly called rain lily, rain flower, zephyr lily because these plants have the tendency to burst into bloom after a heavy rain (Katoch and Singh 2015). The genus *Zephyranthes* is one amongst 75 genera under Amaryllidaceae and consists of about 90 species (Tapia-Campos *et al.* 2012). It is native to warm temperate regions of western hemisphere and also to Southern North America, Central America and South America (Meerow 1999). The wild *Zephyranthes* plants has been naturalised and cultivated by farmers as ornamental plant and are widespread in areas like Indonesia, India, Thailand, Hawaii etc. The genus *Zephyranthes* vary in its morphological characteristics, most notably in flower colour, such as- white, pink and yellow (Fellers 1996, Fernandez-Alonso and Groenendijik 2004). *Z. citrina* (yellow rain lily) is native to Mexico, *Z. rosea* (pink rain lily) is native to Peru and Colombia and *Z. candida* (white rain lily) is native to South America including Paraguay, Brazil, Argentina and Uruguay. The plants of this genus contain rich sources of Amaryllidaceae alkaloids that are well known for their pharmacological activities and used in the treatment of various health problems (Luo et al. 2012, Xiang-Yang and Shu-Feng 2022). Many toxic alkaloids, including lycorine are found in this genus which possess potent biological and pharmacological properties, like antibacterial, antiviral, anticancer as well as anti-inflammatory activity (Jahn *et al.* 2012, Katoch and Singh 2015). Lycorine hydrochloride (LH), a derivative of lycorine is an anti-ovarian cancer agent which can be used as a novel drug in future (Cao et al. 2013, Wang *et al.* 2014). *Z. rosea* flower and bulb extracts are used for a number of medical conditions, which are used as immunomodulators in modern days (Ghosal et al. 1985). In China it is used for treatment of breast cancer (Tapia-Campos *et al.* 2012). In India Z. *rosea* and *Z. flava* were used in various traditional ailments such as treatment of chest and ear ailments, diabetes and against viral infections (Wu *et al.* 2009).

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Z. candida is used to treat tetanus, infantile convulsions and epilepsy (Luo *et al.* 2012, Xiang-Yang and Shu-Feng 2022). In Africa, indigenous people use the leaves of *Z. candida* for treatment of diabetes mellitus. *Z. citrina*, which is known to contain one of the richest sources of phytochemicals, possesses potent pharmacological applications like antimicrobial, antioxidant, analgesic, antiprotozoal, anti-inflammatory, cytotoxic and anti-alzheimer properties (Biswas and Paul 2022).

Breeding work in rain lily is extensively difficult due to their differences in chromosome number, varying flowering time etc. (Raina and Khoshoo 1972). However, in spite of these difficulties breeding work is performed to add value as ornamental. The method to introduce new variants with desired floral characteristics and pharmacological properties is through hybridization between elite cultivars. In the present study some unique lines were identified having distinct characteristics, which were further evaluated through multilocation trials for testing their stability. Moreover, these plants flourish well in warm and humid environments having slightly acidic soil. So, the studied region is the best suited for the experiment to conduct.

Materials and Methods

Fifteen germplasm for each of the three *Zephyranthes* species, *viz- Z. rosea* (pink rain lily), *Z. candida* (white rain lily), and *Z. citrina* (yellow rain lily) were collected from different Northeast regions during 2018. These germplasms were identified by the breeder of the institute. The germplasm was planted using Randomized Block design at CSIR-NEIST experiment field for two consecutive years, *i.e.* spring 2018 and 2019. Required agropractices for maintaining good healthy plants were followed, such as a well-drained soil with good amount of sunlight and regular watering. Fertilizer dose (NPK) of different concentrations (40:30:30) were provided, where nitrogen was applied in two split doses and phosphorous, potassium was applied during plantation time.

Morphological and agronomic traits were recorded for two consecutive seasons. Characters recorded include plant height (cm), tillers/plant, flower colour, flower length (cm), leaf shape, bulb shape and fresh 100 bulb weight (kg). During two years evaluations, three lines were identified and named as RRL-PRL-05, RRL-YRL-02, and RRL-WRL-27 for each of three species, *i.e., Z. rosea*, *Z. citrina* and *Z. candida* respectively which were evaluated for two seasons (spring 2020, 2021) at different locations, *viz-* Tinsukia (Assam), Runne (Pasighat, Arunachal Pradesh) and Nongpoh (Meghalaya) along with a check variety for each of the species. Multilocation evaluation was done for all the characters taken during initial field trial.

Results and Discussion

Development of elite lines with attractive floral characteristics is the most desired criteria in *Zephyranthes* breeding. However, producing such unique phenotypes through seed is very complex because some species reproduce via pseudogamy or apomixis process (Gupta *et al.* 1998). Also, the differences in chromosome number, flower time, pollen production and self-incompatibility etc. also greatly reduce the chance of crossing among them (Raina and Khoshoo 1971, Raina and Khoshoo 1972, Roy-Chowdhury and Hubstenberger 2006). However, breeding work is done to add value as ornamental despite these challenges. Through hybridization between elite cultivars, new variations with desired floral traits and pharmacological effects can be introduced. The germplasm in the present study possesses many variations in the studied traits and these variations can be further applied in their improvement programme and the current study is an attempt to the same. In this study, the initial field experiment carried out with fifteen germplasm during spring 2018 and 2019 for each species which showed variations in terms of plant height,

tillers/plant and flower length (Table 1). Plant height in *Z. candida* ranged from 49-57 cm, 4-7 tillers/plant, 4.1-4.8 cm flower length and 4.1-4.9 kg fresh 100 bulb weight. In *Z. citrina* and *Z. rosea* plant height ranged from 39-56, 36-42 cm, 6-12, 9-26 tillers/plant, 4.1-4.7, 3-3.8 cm flower length and 5-5.5, 4.8-5.3 kg fresh 100 bulb weight respectively. Variations was also observed for qualitative characters, such as the leaf shape in *Z. candida* was somewhat fine, hair-like; in *Z. citrina* it was flat and narrow, while in *Z. rosea* it was comparatively flat and broad. With regards to bulb shape it was elongated and oblong in *Z. candida*, flattened globe in *Z. citrina* and broad elliptical in *Z. rosea*. Following the two years assessment three unique lines for each species were recognized and named as RRL-WRL-27 for *Z. candida*, RRL-YRL-02 for *Z. citrina*, RRL-PRL-05 for *Z. rosea*. For RRL-WRL-27, plant height of 55.6 cm, flower length of 4.8 cm, fresh 100 bulb weight of 4 kg and 6 tillers/plant was obtained. Similarly, for RRL-YRL-02 plant height of 54 cm, flower length of 4.5 cm, fresh 100 bulb weight of 5.3 kg and 10 tillers/plant was

Table 1. Range of characters of three *Zephyranthes* species during spring 2018 and 2019.

Characters	*Z. candida*	*Z. citrina*	*Z. rosea*
Plant height (cm)	49-57	39-56	36-42
Tillers/plant	4-7	6-12	9-26
Flower colour	white	yellow	pink
Flower length (cm)	4.1-4.8	4.1-4.7	3-3.8
Leaf shape	fine, hairlike	flat and narrow	flat and broad
Bulb shape	oblong, elongated	flattened globe	broad elliptic
Fresh 100 bulb weight (kg)	4.1-4.9	5-5.5	4.8-5.3

observed. With respect to RRL-PRL-05, 41.7 cm plant height, 3.6 cm flower length, 5.1 kg fresh 100 bulb weight and 23 numbers of tillers/plant were observed (Fig. 1). The number of tillers/plants was comparatively more in RRL-PRL-05, thereby making the plant somewhat bushy in appearance than RRL-YRL-02 and RRL-WRL-27. Fresh 100 bulb weight was found to be higher in RRL-YRL-02, which may be related to their rounder, globose nature compared to the other two species. Previous literature revealed that detailed investigation on identification of superior Zephyranthes species is very scarce. Till date no detailed investigations has been found on selection of superior genotypes of Zephyranthes species and thus present study can be considered a novel report. A study by Vu *et al.* (2023) reported morphological diversity of Zephyranthes species in Vietnam where it was found great diversion in growth performance in terms of leaf length (8.3 - 22 cm), leaf thickness (1.0 to 1.7 mm), number of leaves (4.4 to 12.7), leaf width (2.4 - 9.7 mm), leaf angle (23-50o), tillering ability in six months (0 to 5.8 branches/bulb), number of petals (6 -12 petals) with diverse flower colours such as white, light pink, dark pink, orange. Whereas in this study tillers per plant was found to be between a range of 4 - 7. Alves-Araujo (2012) studied morphoanatomical characteristics of Amaryllidaceae family where *Zephyranthes candida* possess to have subcircular and unifacial leaves whereas in this study *Z. candida* was found to have fine, hairlike shape. Again, a breeding work was performed by Roy-Chowdhury and Hubstenberger (2006) on cross pollination evaluation of Zephyranthes and Habranthus species where variations in petals shape; foliage colour; flower scent, colour, picotees, stripes and bicolour was observed.

Earlier Lal (2012) in his study suggested that multilocation study is necessary to check the stability of the crop with regards to development of a variety. So, keeping this criterion in mind

multilocation evaluation was performed for these lines for two consecutive years *i.e.*, spring 2019 and 2020. A check variety for each species was taken at three different locations, i.e., Tinsukia (Assam), Runne (Pasighat, Arunachal Pradesh) and Nongpoh (Meghalaya) (Table 3). The line RRL-WRL-27 showed superior performance than its check for all the characters except for bulb weight, which was found to be of similar weight. However, in case of RRL-YRL-02 and RRL-PRL-05 all the characters showed superior performance than their respective check counterpart for two seasons. In case of check-3, a slight variation was observed for flower colour i.e., light pink. It may be due to unstable performance of the check at different locations, a common phenomenon with change in environment condition, as also reported by Sevik et al. (2014). In this regard, our identified lines were found stable for the characters studied and can be considered for cultivation at different places.

Fig. 1. Morphological variations observed in (A) flower, (B) bulb and (C) Leaf characteristics.

Table 2. Morphological characteristics of selected lines of three *Zephyranthes* species.

Characters	*Z. candida*	*Z. citrina*	*Z. rosea*
Plant height (cm)	55.6	54	41.7
Tillers/plant	6	10	23
Flower colour	white	yellow	pink
Flower length (cm)	4.8	4.5	3.6
Leaf shape	fine, hairlike	flat and narrow	flat and broad
Bulb shape	oblong, elongated	flattened globe	broad elliptic
Fresh 100 bulb weight (kg)	4	5.3	5.1

Table 3. Average two seasons data of selected lines at three sites along with check varieties.

Variety	Plant height	Tillers/ plant	Flower colour	Flower length	Leaf shape	Bulb shape	Fresh 100 bulb weight (kg)
RRL-WRL-27 (*Z. candida)*	53.7	5	white	4.6	fine, hairlike	elongated, oblong	3.9
Check 1	51.4	4	white	4.4	fine, hairlike	elongated, oblong	3.9
RRL-YRL-02 (*Z. citrina)*	53	8	yellow	4.4	flat and narrow	flattened globe	5.1
Check 2	51.5	6	yellow	4	flat and narrow	flattened globe	4.7
RRL-PRL-05 (*Z. rosea)*	40.3	20	pink	3.4	flat and broad	broad elliptic	4.7
Check 3	37	15	Light pink	2.8	flat and broad	broad elliptic	4.5
SD	7.23	6.41	-	0.71	-	-	0.39
SE (Mean)	2.95	2.62	-	0.29	-	-	0.16
CV	15.12	66.29	-	17.81	-	-	9.08

The aim of the current study is to uncover three new lines of *Zephyranthes* species, *i.e*., RRL-PRL-05 for *Z. rosea*, RRL-YRL-02 for *Z. citrina* and RRL-WRL-27 for *Z. candida*. These plants are mostly priced not only for their attractive flowers but also for their pharmacological properties, which make them ideal from the rest of the ornamental plants. Besides flower colour improvements programme can be done with respect to flower longevity, development of scented variety etc. So, a planned breeding experiment was conducted to identify lines with unique traits and floral characteristics. Also, the lines were found stable in performances, as verified through multilocation trial studies, which is a necessary criterion during varietal development study. As far our conscience, this study on *Zephyranthes* species is the first of its report till date and no such studies were previously been reported. The identified lines can be taken for large scale evaluation as confirmed through multilocation studies.

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