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Exemplifying the breeding potential of ornamental banana hybrids through palynological studies

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ABSTRACT

Ornamental bananas (OBs) are known for their bright colour, bract, and attractive foliage with significant ornamental value. The OBs are tolerant to abiotic (cold and drought) and biotic stresses (pests and diseases), having immense scope for studying their breeding potential and the development of two hybrids from the cross of Karpooravalli (ABB) × *M. acuminata* subsp. *zebrina* (ZB) has sparked curiosity to explore the Ornamental Banana Hybrids (OBHs). Therefore, the present study explored the breeding potential of OBHs and wild species (*M. acuminata* subsp. *zebrina* [ZB]). Among these, maximum pollen viability (PV), pollen germination (PG), pollen output/anther (PO/A) and pollen size (PS) were recorded in 'ZB' (99.60%), 'OZ2' (64.70%), 'OR14' (2859.80) and 'OV1' (115.50 µ), respectively. Boxplot was performed to study the distribution pattern of all three cross combinations, which provided a good result with larger variability in 'OZ' followed by 'RZ' for PV, PG and PS. In contrast, higher variability for PO/A was obtained in 'OR'. A positive correlation was recorded between PV, PG and PS, whereas PO/A was negatively correlated with all the attributes studied. Hierarchical cluster analysis categorized the OBHs and wild species into three clusters, with clusters I, II and III exhibiting the highest PG (%), PV (%) and PO/A, respectively. No significant difference was observed in the case of PS. Hence, these OBHs can be utilized as a pollen source for breeding and developing a novel hybrids.

Key words: *M. acuminata* ssp. *zebrina*, Boxplot, Pollen, Novel hybrids.

INTRODUCTION

In recent times, there has been a growing pursuit of novelty, originality, uniqueness, and aesthetic appeal in the realm of plant species. This surge is primarily driven by the ever-evolving ornamental market, which consistently craves fresh and innovative additions. One striking example of this phenomenon is banana, which is currently the most widely produced, consumed, and traded fruit worldwide. Surprisingly, despite its immense popularity as a fruit crop, the ornamental potential of the banana plant still needs to be explored. Consequently, researchers and breeders have been actively exploring ways to unlock the ornamental beauty concealed within the *Musa* species.

There are three main genera in the Musaceae family, *viz., Musa*, *Musella*, and *Ensete*. Cheesman (3) divided *Musa* into four sections: AustraliMusa, CalliMusa, EuMusa, and Rhodochlamys. Among these sections, Rhodochlamys stands out with its erect inflorescence and negatively geotropic fruits. *Musa* and Rhodochlamys share common features, including the same chromosome number (n = x = 11) that facilitate hybridization, resulting in vigorous offspring. Notably, *M. acuminata* (sect. Musa) readily crosses with *M. laterita*, *M. ornata*, and *M. velutina* (Simmonds, 12) and enables the development of promising hybrids with desirable traits.

Exotic ornamental banana species, *viz.*, *M. acuminata* subsp. *zebrina*, possesses significant ornamental value and is used in banana breeding programmes. Recent hybridization efforts have led to developing two hybrids by crossing Karpooravalli (ABB) with *M. acuminata* subsp. *zebrina* (AA). These hybrids are showing promising growth to date. This achievement has sparked our curiosity and enthusiasm for exploring the breeding potential of ornamental banana hybrids. Unravelling the secrets hidden within their genetic makeup can unlock the possibilities for creating novel ornamental varieties and potential hybrids when combined with intended varieties of *Musa* spp. However, exploring palynology is crucial to fully harness the breeding potential. Palynological studies analyse pollen biology, shedding light on the mechanisms behind successful pollination, fertilization, and seed production. Understanding pollen behaviour is vital for effective hybridization, as it directly influences the desired traits in the offspring.

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In this context, the present study was undertaken for the first time to unravel the breeding potential of ornamental banana hybrids through comprehensive palynological investigations to gain a deeper understanding of the reproductive mechanisms. Hence, the findings of this study will be invaluable for breeders and horticulturists striving to create new and visually captivating varieties.

MATERIALS AND METHODS

Ornamental Banana Hybrids (OBHs) undertaken for the study were developed at ICAR-National Research Centre for Banana, Tiruchirappalli, Tamil Nadu with four inter-specific hybridization of *Musa* species: *M. rubra* × *M. acuminata* subsp. *zebrina* (RZ), *M. ornata* × *M. rubra* (OR), *M. ornata* × *M. acuminata* subsp. *zebrina* (OZ), and *M. ornata* × *M. velutina* ssp. *markkuana* (OV) (Ramajayam *et al*., 9). These OBHs are in the multi-location trial (MLT) for their potential in ornamental use at Tamil Nadu Agricultural University, Coimbatore, and the present study was undertaken at the Department of Fruit Science, Horticultural College and Research Institute, TNAU, Coimbatore. A total of 29 OBHs and one *M. acuminata* subsp. *zebrina* were explored to understand their breeding potential (Fig. 1). Coimbatore is located at an elevation of 426.72 meters above sea level with 11°N latitude and 77°E longitude. During the study period, the temperature ranged from 21.90 to 31.11°C, with morning RH at 85.17% and noon RH at 49.30%. The annual rainfall in the area was 697.96 mm.

For pollen studies, the functional male florets were collected early morning (7.00 to 9.00 AM). The anthers were delicately twisted to release the pollen grains, which were then transferred to glass slides. A drop of 2% acetocarmine solution was added and covered with a cover slip, ensuring no bubble formation. The stained pollen grains were counted among 100 pollen grains, and the percentage of viable pollen grains was calculated using the formula below.

$$\text{Pollen viability (\%)} = \frac{\text{No. of } \frac{\text{viable}}{\text{stained}} \text{ pollen grains}}{\text{Total No. of pollen grains}} \times 100$$

In case of pollen germination study, the pollen grains were carefully transferred onto a cavity slide and a solution containing 10% sucrose and 10 ppm boric acid was added and covered with a cover slip. The prepared slides were then positioned inside petriplates, elevated by glass rods, and surrounded by moist filter paper with water at the base of the dishes. Additionally, a piece of moist filter paper was affixed to the underside of the petriplates cover. The entire setup was subjected to a 24 h incubation period at room temperature. After incubation, the following formula was used to calculate the germination percentage.

$$\text{Pollen germination (\%)} = \frac{\text{No. of germinated pollen grains}}{\text{Total No. of pollen grains}} \times 100$$

The pollen diameter was assessed as the standard size under 100X magnification, utilizing a Binocular Microscope with Image Analyser (HOVERLABSTM). Before its measurement, the microscope scale was calibrated using a stage micrometre with a 1mm size (0.01 mm division) to ensure accurate size determination. From each of the five samples, 100 pollen grains were measured, and the mean diameter was calculated and expressed in micrometres (μ). The haemocytometer method was used to study pollen output per anther (Sathiamoorthy, 11).

The data obtained were subjected to one-way ANOVA, followed by Tukey's test at a significance level of 5%. The Pearson correlation coefficient was used to determine the correlations between the observations. A box plot was generated to better understand the distribution pattern of the OBHs based on their cross combinations. The statistical analyses mentioned above were conducted using the web

Fig. 1. Variations in Ornamental Banana Hybrids (OBHs) (a. male flower with bract; b-c. male flower consisting stamen, pistil, fused & free tepal, nectar and reduced ovary, c. male flower colour flushed with pink; d. anther with dehisce pollen).

application KAU-GRAPES1.0.0. Additionally, for the Hierarchical Cluster Analysis (HCA), the variables were subjected to the libraries "dendextend," "circlize," "factoextra," and "cluster" in RStudio (v 4.1.1) to generate robust hierarchical clustering.

RESULTS AND DISCUSSION

One of the significant challenges faced in conventional banana breeding is the occurrence of male or female sterility, hindering fertilization and hybrid development. To overcome this, identifying strong male parents becomes crucial for breeding programs to succeed. This emphasizes the need for a diverse and robust male breeding pool in banana breeding programs. The acetocarmine staining method was employed in the present study to assess pollen viability. Viable pollen grains exhibited red upon staining, while non-viable or sterile pollen grains remained transparent or unstained. Due to the lack of exine in *Musa* pollen, the stained pollen grains did not display the typical attractive red colour. Instead, the cytoplasm appeared deep red, and the intine appeared light-coloured, allowing for clear differentiation between viable and non-viable pollen (Fig. 2).

Among all the OBHs and ZB, varying results for pollen viability were observed, which ranged from 55.30% ('OZ1') to 99.60% ('ZB') with an overall mean of 78.28%. While stainability can estimate pollen viability, *in vitro* pollen germination is considered a reliable palynological attribute for assessing male fertility status. The *in vitro* pollen tube growth reflects its ability to transport male gametes to the ovules and facilitate fertilization. The findings depicted the highest pollen germination in 'OZ2' (64.70%), followed by 'OZ3' (62.85%) and 'OV1' (62.25%), while it was lowest in 'OZ1' (22.30%) with an overall mean of 45%. Significant difference among the hybrids, with a wide range of variations, was observed concerning the pollen output. This variance in pollen output per anther spanned from 957.00 ('OZ7') to 2859.80 ('OR14') with an overall mean of 1679.75. Pollen size was determined in terms of pollen diameter, as these were circular, and the size among the OBHs ranged from 83.88 mm ('OZ8') to 115.50 mm ('OV1') (Table 1). The present findings conform with the findings of Adeleke *et al*. (1), who observed a notably high level of pollen viability in diploids. Similarly, the investigations by Panda *et al*. (7) revealed a wide range of pollen viability percentages, ranging from 59.90 to 92.84%. Furthermore, their research revealed varying pollen germination rates (22.17 to 85.88%), diverse pollen output, and different pollen sizes (87.20 to 122.74 μm) in diploid genotypes. Krishnamoorthy (6) also found a mean pollen germination percentage of 43.78%.

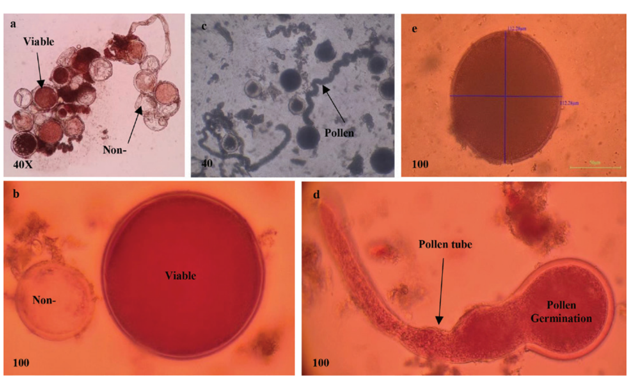

Fig. 2. Microscopic view of pollen of Ornamental Banana Hybrids (OBHs) at 40X and 100X magnification (a-b. Viable and non-viable pollen at 40X and 100X; c-d. pollen tube growth at 40X and 100X; e. Pollen diameter at 100X – bar: 50 μ).

Boxplot was performed to understand the overall variation and distribution pattern in the dataset of three different cross combinations namely *M. ornata* × *M. rubra* (OR), *M. ornata* × *M. acuminata* subsp. *zebrina* (OZ) and *M. rubra* × *M. acuminata* subsp. *zebrina* (RZ) (Fig. 3). The *M. ornata* × *M. velutina* (OV) hybrid was excluded due to its singular presence. The pollen viability percentages of the hybrids developed from 'OR' exhibited a range between 69.87% ('OR1') to 85.64% ('OR13'). For the hybrids developed from 'OZ', the pollen viability ranged from 55.30% ('OZ1') to 86.95% ('OZ7'). As for the OBHs obtained from RZ, the pollen viability varied from 64.71% ('RZ3') to 89.37% ('RZ4'). Pollen germination of the OBHs obtained from OR, OZ and RZ ranged from 33.25% ('OR1') to 52.10% ('OR13'), 22.30% ('OZ1') to 64.70% ('OZ2') and 29.21% ('RZ2') to 51.58% ('RZ4'), respectively. The number of pollens per anther of the OBHs obtained from OR, OZ and RZ ranged from 1083.20 ('OR5') to 2859.80 ('OR14'), 957 ('OZ7') to 2673 ('OZ6') and 1059.20 ('RZ4') to 2434.40 ('RZ1'), respectively. Pollen size of the OBHs obtained from OR, OZ and RZ ranged from 91.43 mm ('OR1') to 114.41 mm ('OR13'), 83.88 mm ('OZ8') to 108.81 mm ('OZ2') and 103.49 mm ('RZ1') to 113.31 mm ('RZ2'), respectively. The finding showed good results for three cross combinations, with larger variability in OZ and RZ for pollen viability, germination, and size. In contrast, higher variability for pollen output/ anther was observed in OR.

One possible reason for the observed pollen-viability variations among the hybrids could be using *zebrina* as one of the parents. The study noted that *zebrina* had the highest pollen viability of 99.60% and pollen germination of 54.09%. Thus, including *zebrina* in the hybridization process may have influenced and contributed to the higher pollen viability in certain hybrids. This favourable characteristic of *zebrina* has consequently led to the development of two

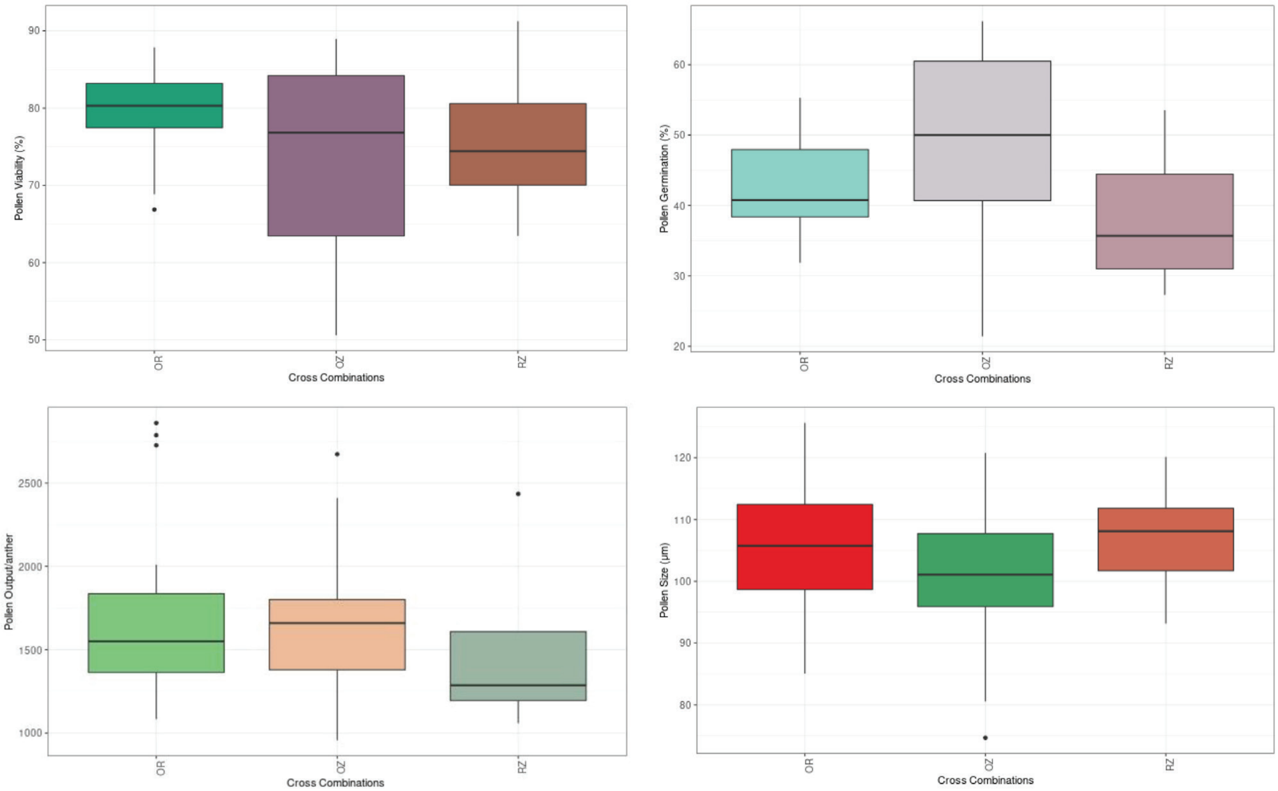

Fig. 3. Boxplot of palynological attributes depicting the distribution between three different cross combinations (OR – *M. ornata* × *M. rubra*; OZ – *M. ornata* × *M. acuminata* subsp. *zebrina* and RZ – *M. rubra* × *M. acuminata* subsp. *zebrina*).

Fig. 4. Two hybrids of cross – Karpooravalli (ABB) × *M. acuminata* subsp. *zebrina* under evaluation at TNAU, Coimbatore, Tamil Nadu, India.

Karpooravalli (ABB) hybrids using *zebrina* as the male parent (Fig. 4). In contrast, the findings by Rachman *et al*. (8) indicated much lower germination in *zebrina* (6.97%). These divergent results between studies may be attributed to various factors, such as experimental conditions, genetic differences, or environmental influences.

Nonetheless, using *zebrina* as a male parent in the current study has successfully contributed to developing high-pollen-viability ornamental banana hybrids. As per Fortescue and Turner (5), *M. acuminata* and *M. ornata* exhibited a remarkable three-fold increase in viable pollen production as compared to the edible tetraploids (AAAB). Furthermore, Damayanti (4) reported a notably high pollen viability of 98.60% in *M. ornata*. Hence, considering the impressive pollen viability of both *M. ornata* and *M. acuminata* subsp. *zebrina*, utilizing them as parents in hybridization processes hold great potential for generating novel hybrids harbouring desired traits. These attributes establish them as promising parents for further crop improvement.

A Pearson's correlation analysis was carried out to assess the relationship among palynological attributes. The results revealed a positive correlation coefficient between pollen viability, germination, and size. In contrast, pollen output per anther displayed a negative correlation with all attributes (Fig. 5). These findings align with that of Panda *et al*. (7), who also reported a negative correlation between pollen output and size and a positive correlation between viability and germination. Hence, smaller pollen size is linked to higher production, and a greater number of viable

Table 1. Palynological attributes of ornamental banana hybrids (OBHs).

Interspecific hybrid	Pollen viability (%)	Pollen germination (%)	Pollen output/ anther	Pollen size (μ)
OR1	69.87	35.20	1225.20	91.43
OR2	82.00	43.57	1662.40	103.78
OR3	71.18	39.57	2787.00	101.39
OR4	78.05	52.10	2726.40	114.20
OR5	83.64	40.54	1083.20	95.12
OR6	78.14	35.82	1378.60	107.60
OR7	83.09	41.77	2009.80	105.29
OR8	78.57	33.25	1659.40	107.30
OR9	80.03	49.68	1347.80	102.66
OR10	83.76	37.99	1516.00	109.19
OR11	73.83	38.76	1384.20	110.39
OR12	81.45	44.51	1108.20	108.65
OR13	85.64	51.27	1550.60	114.41
OR14	85.04	45.46	2859.80	106.55
OR15	80.24	48.32	1563.40	105.61
OV1	81.25	62.25	1303.00	115.50
OZ1	55.30	22.30	1801.20	103.82
OZ2	85.92	64.70	999.20	108.81
OZ3	63.38	62.85	1772.60	104.42
OZ4	72.96	49.33	2410.40	101.68
OZ5	78.88	60.39	1378.80	103.11
OZ6	84.98	41.65	2673.00	96.47
OZ7	86.95	48.23	957.00	105.22
OZ8	78.19	38.25	1659.80	83.88
OZ9	62.88	54.64	1393.60	99.94
RZ1	77.09	32.18	2434.40	103.49
RZ2	72.29	29.21	1239.80	113.31
RZ3	64.71	40.40	1333.20	106.82
RZ4	89.37	51.58	1059.20	104.38
ZB	99.60	54.09	2115.20	114.06
Mean	78.28	45.00	1679.75	104.95
SE(d)	1.13	1.10	52.40	4.497
SE(m)	0.80	0.78	37.05	3.18
CV (%)	2.28	3.86	4.93	6.78

OR: *M. ornata* × *M. rubra*; OV: *M. ornata* × *M. velutina*; OZ: *M. ornata* × *M. acuminata* subsp. *zebrina*; RZ: *M. rubra* × *M. acuminata* subsp. *zebrina*; ZB: *M. acuminata* subsp. *zebrina*.

pollens corresponds to a higher germination rate. However, Rodriguez-Riano and Dafni (10) reported a negative correlation between viability and *in vitro* germination rate, indicating that not all viable pollen can germinate. Consequently, evaluating pollen germination potential could aid in selecting improved parent plants based on the assumption that germinable pollen would indicate fertility (Barrow, 2). These conflicting findings underscore the need for further research to understand factors influencing pollen fertility and germination.

Hierarchical clustering was employed to categorise OBHs and *zebrina* based on pollen traits. The analysis revealed three clusters, clusters I, II, and III, exhibiting the highest pollen germination, viability, and output/anther, respectively. In contrast, no significant difference was observed in the case of pollen size (Fig. 6). This suggests the potential of these hybrids as male parents. Further studies are essential to screen the hybrids for resistance traits against biotic and abiotic stresses. Identifying hybrids with natural resistance to pests, diseases, and environmental challenges will be invaluable in developing resilient and sustainable banana varieties.

The study highlights the significance of using OBHs as a pollen source for breeding, aiming to create new hybrids with exceptional ornamental qualities. OBHs being cross compatible with choice varieties of banana, present a significant opportunity to establish a robust male breeding pool for banana breeding programmes, thereby providing a diverse selection of potential parent plants. The findings conclude that OBHs and *zebrina* can be used to produce hybrids

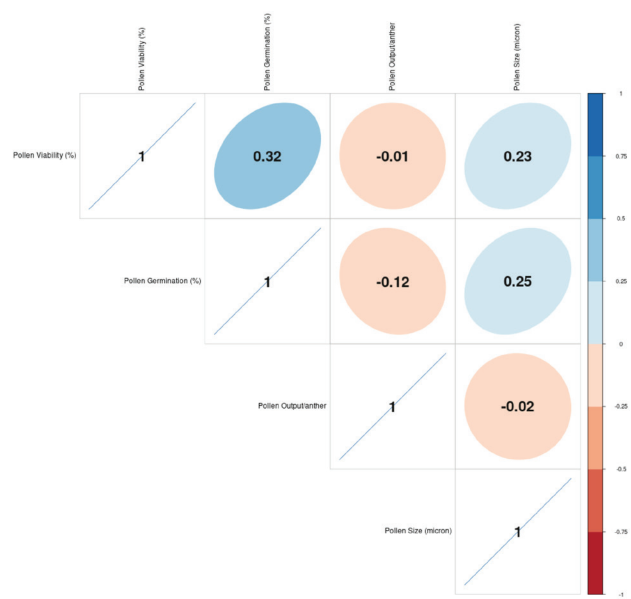

Fig. 5. Correlation coefficient of the relationship between different palynological attributes.

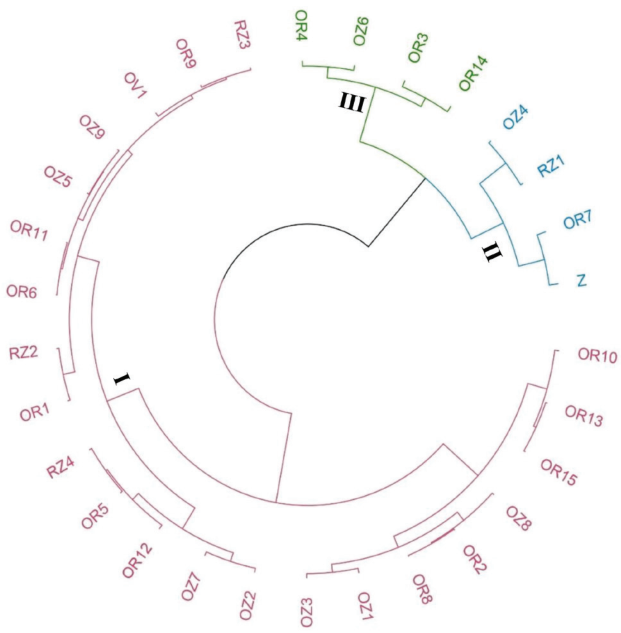

Fig. 6. Circular HCA based on the palynological attributes of 29 OBHs and one wild species (*M. acuminata* subsp. *zebrina*).

with multiple benefits, addressing a critical gap in the literature on pollen characteristics of OBHs. This paper serves as a vital reference for future researchers and breeders interested in leveraging ornamental bananas for crop improvement programmes, offering guidance and inspiration for future research in this field. The comprehensive assessment of pollen viability, germination, output per anther, and size will lead to more targeted and informed breeding efforts, contributing to the development of more resilient and visually captivating ornamental varieties and potential banana hybrids in the future.

AUTHORS' CONTRIBUTION

Conceptualization, statistical analysis, original draft, review and editing (SC). Conceptualization, validation, review and editing (JA). Review of the manuscript and statistical analysis (RD and CPS). Review, comments and corrections on previous versions of the manuscript (IM, MR, GK and AS).

DECLARATION

The authors declare that they do not have any conflict of interest.

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