Image-based approach for postharvest characterization of Red Torch inflorescences

Marina Romano Nogueira¹ (b), Patrícia Duarte de Oliveira Paiva^{1,*} (b), Antonio Rodrigues da Cunha Neto¹ (b), Ângela Maria Pereira Nascimento¹ (b), Caroline de Oliveira Timóteo¹ (b), Michele Valquíria dos Reis¹ (b)

1. Universidade Federal de Lavras 🕅 – Lavras (MG), Brazil.

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*Corresponding author: patriciapaiva@ufla.br

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ABSTRACT: The characterization of cut flowers plays a significant role in detecting senescence signals, which are essential for establishing quality parameters and guiding the development of postharvest technologies. This study aimed to morphologically characterize Red Torch Ginger inflorescences at three harvest points: fully closed, semi-open, and fully open. The inflorescences were standardized at 60 cm, and their width, length, diameter, and fresh weight were measured. Inflorescences were evaluated for visual senescence, wettability, natural wax content, and fluid behavior. Dominant color, gloss, and texture were analyzed using GroundEye. Morphological differences affected the postharvest durability of Red Torch Ginger, with fully open inflorescences being less durable than semi-open and fully closed ones. Hydrophobicity, high-fluid movement, and natural wax content were crucial for decision-making regarding the application of new postharvest technologies and coatings in cut tropical flowers. These findings provide insights into the characterization of cut flowers and their postharvest behavior, which can help improve quality and extend the shelf life of cut flowers, leading to increased economic benefits for the flower industry.

Key words: biospeckle, Etlingera elatior, GroundEye, morphology, tropical flowers.

INTRODUCTION

Native to Southeast Asia, Malaysia, and Indonesia, *Etlingera elatior* (William Jack) Rosemary Margaret Smith, popularly known as Red Torch ginger, is a tropical species of the family Zingiberaceae. The family includes several species with applications in horticulture, mainly for use in cooking, medicine, ornamentation, and landscaping (Cunha Neto et al. 2023, Yunus et al. 2021).

The flowering stems of the Red Torch are composed of floral scapes and inflorescences that consist of differentiated petals and involucral and floral bracts, in addition to true flowers that are found among the floral bracts, in pink (cultivars 'Pink Torch' and 'Porcelain'), red (cultivar 'Red Torch'), and white colors (Juwita et al. 2018, Nogueira et al. 2023). Such characteristics are subjective if only visually analyzed; thus, the morphological characterization of the inflorescences, using quantitative parameters, allows for a better understanding of the species, favoring the application of new postharvest technologies.

Extravagant and striking inflorescences with bright colors make Red Torch suitable for use as a cut flower in floral arrangements, and it can be harvested at different stages of inflorescence opening. The wide variety of uses for Red Torch has led to an increase in its commercialization as cut flowers (Yunus et al. 2021).

The harvest point of a flower stem coincides with an inflorescence opening point, which varies greatly depending on the region, time of year, cultivation conditions (field or greenhouse), variety, and distance from the market (Yunus et al. 2021). Therefore, the harvest should follow regional recommendations, meeting quality standards defined specifically

for each species or cultivar. The stems of Red Torch Ginger are commercialized from the flower bud stage in formation, with closed bracts, to fully open bracts and floral scapes with a minimum size of 60 cm and a diameter greater than 1 cm (Carneiro et al. 2014).

For most tropical species, there is a lack of information about the influence of the stage of bract opening on postharvest durability (Carneiro et al. 2014). Additionally, there is no standardization for marketing and appropriate technology for postharvest preservation for most tropical cut flowers. Compared to other tropical species, torch ginger has low durability. Some studies have been conducted to improve vase life and understand the senescence process, as well as to determine some conservation technologies. The use of carnauba wax applied to the inflorescences did not show an efficient barrier to water and gas exchange and did not affect the physiological or visual quality of the flowering stems (Mattos et al. 2018).

Furthermore, although there are some indications for postharvest conservation, the technological process for torch ginger is not clear. In this sense, inflorescence characterization is an important tool for understanding the specific characteristics inherent to this species and thus allows the indication of effective methodologies to increase vase life.

Considering that studies on the specific characteristics of tropical flowers are scarce, the objectives of this study were to perform the morphological characterization of the inflorescences and bracts of Red Torch Ginger at the three different harvest points and to present a senescence scale of the stems.

MATERIAL AND METHODS

Etlingera elatior inflorescences were collected at different opening points: fully closed (closed bracts), semi-open (first row of involucral bracts open), and fully open (with the first ring of true flowers open) (Carneiro et al. 2014, Mattos et al. 2018) (Fig. 1). All of them were cleaned by immersion in tanks with water and neutral detergent.



Figure 1. Opening points of the Red Torch inflorescences at harvest: (a) closed; (b) semi-open; (c) fully open; (d) distribution of the involucral, floral, and true flower bracts; and upper and lower sides of the bracts.

The senescence scale, morphological characterization, and analysis of natural wax of the torch ginger were performed on inflorescences, floral scapes, involucral bracts outside the true flowers, and floral bracts positioned between the true flowers on their upper and lower sides (Fig. 1).

Evaluation of morphological characteristics

Seven inflorescences were collected at each harvest point (closed, semi-open, and open), totaling 21 flowering stems. The following evaluations were conducted immediately after harvest: inflorescence width, inflorescence length (measured from

the base to the apex), and floral scape diameter (measured 20 cm below the inflorescence) using a digital caliper (Digimess). The floral stems were standardized at 60 cm, and their fresh weight was determined and weighed on a precision scale. The inflorescences and their respective floral scapes were dried in an oven at 60°C until constant weight was reached to obtain the dry weight. The water content (%) was calculated using Eq. 1:

{[fresh weight (g) - dry weight (g)]/fresh weight (g)}
$$\times 100\%$$
 (1)

After harvesting the stems, the involucral and floral bracts of the inflorescences were evaluated for wettability or contact angle. A qualitative analysis was performed through the angle formed by the liquid (water) deposited on the surface of the bract, indicating whether it was hydrophilic or hydrophobic, using an OCA Data Physics absorption tester equipped with a Kruss CCD camera. The behavior of fluids in the bracts was analyzed using Biospeckle, in which they were indirectly illuminated with a red laser diode (632 nm) with 10 mW of power. Images were captured using a Samsung Digital Cam CMOS 6.4 Megapixel full HD camera, characterizing the movement of fluids. Two ImageJ routines were used to obtain the value of the standard parameter spatial temporal speckle. The characteristics of color, gloss, and texture dominance were determined by image analysis using the GroundEye system, version S800.

Senescence scale

After evaluating the morphological parameters of the floral stems, they were kept in water and photographed daily to perform the senescence scale. The inflorescences were analyzed by three evaluators, and visual changes related to senescence were recorded, considering parameters such as turgor, gloss, and the presence of spots.

Characterization of natural wax

For the characterization of the natural wax standard present in the bracts, quantification was performed using the method described by Viana et al. (2010): 1 cm² of bract samples were taken immediately after harvest and introduced into Falcon tubes containing 100 mL of chloroform for 20 seconds, shaking slightly. Then, the samples were filtered using filter paper and transferred to a 25-mL test tube of known weight and evaporated in a water bath to obtain the solid residue (wax). The quantification of the wax was expressed as the amount of wax per unit area (mg/cm²).

Experimental design and statistical analysis

The experimental design was completely randomized, with the three inflorescence opening points characterizing the treatments, each with seven repetitions composed of one inflorescence each. The experiments were replicated twice for all evaluated parameters. The data were analyzed by analysis of variance using the statistical program SISVAR (Ferreira 2019). The comparison of means was performed using Tukey's test with the significance level of 5%.

RESULTS AND DISCUSSION

The lengths of the inflorescences showed small variation between the closed (mean 110.78 mm) and semi-open (mean 108.44 mm) points of development. However, the inflorescence length at these opening points differed from the inflorescence length at the open point, which was 102.98 mm (Fig. 2a).

Unlike the diameter of the floral scape, for which there was no difference between the inflorescence opening points at harvest (mean of 22 mm), inflorescence diameter showed a significant increase with the advancement from semi-open to open inflorescences, with a mean difference of 80 mm from one point to another (Fig. 2b).



Figure 2. Morphological parameters assessed. (a) Length and (b) diameter of the inflorescences (mm) at the three different opening points of Red Torch Ginger. The same letters are not significantly different according to the Tukey's test at the 5% significance level (p < 0.05).

The fresh and dry weights of the inflorescences behaved similarly, increasing as the opening point advanced (Figs. 3a and 3b). This behavior was unlike that of the floral scape weights, which did not differ according to the inflorescence opening point at harvest; in addition, the floral scape fresh weight was on average 213.18 g, and the floral scape dry weight was 20.18 g.



Figure 3. Morphological parameters assessed. (a) Fresh and (b) dry weight of the inflorescences of different opening points of Red Torch Ginger. The same letters are not significantly different according to the Tukey's test at the 5% significance level (p < 0.05).

Physiological changes resulting from the senescence of flowering stems of torch ginger cv. 'Porcelain' were observed, with a 50% increase in fresh weight from fully closed to fully open inflorescences in torch ginger cv. 'Porcelain' (Choon and Ding 2017). In addition, Choon and Ding (2017) concluded that the influx of water for cell expansion is fundamental for the development of a torch ginger inflorescence, as water is the main component of an inflorescence (Choon and Ding 2017).

The water content differed between the inflorescence and floral scape. The water content after harvest was lower in fully closed and semi-open inflorescences compared to fully open ones. For the floral scape, the opposite was observed: water content was lower in fully open inflorescences harvested compared to fully closed and semi-open ones (Figs. 4a and 4b).



Figure 4. Morphological parameters assessed. (a) Water content at the different opening points of the inflorescences and (b) floral scapes of Red Torch Ginger. The same letters are not significantly different according to the Tukey's test at the 5% significance level (p < 0.05).

Water is the main constituent of living cells since it has a series of properties that make it fundamental to all physical, chemical, and biological processes essential for plant development (Sukpitak and Seraypheap 2023).

According to the wettability characteristics, i.e., the property by which the ability of a liquid to adhere to the surface is estimated (Nor and Ding 2020), evaluated by the contact angle (Fig. 5) through the deposition of water on the involucral and floral bracts of the fully closed, semi-open, and open inflorescences, it was observed that, regardless of the inflorescence opening point, they were hydrophobic ($\theta > 90^\circ$).



Figure 5. Droplet of water being deposited on a sample of a Red Torch involucral bract of the fully open inflorescence and its behavior over time (60 seconds) sequentially from left to right.

The characterization of the wettability parameters and contents of the natural wax present in the bracts (Nor and Ding 2020) was conducted to understand and explain factors related to the adhesion of biodegradable coating films used in the postharvest of inflorescences. The biodegradable films are composed of biopolymers in excess water, which, at high temperatures, gelatinize. Thus, the hydrophobic characteristic of bracts (Juwita et al. 2018) is extremely important in the decision on which films or additives to use to achieve satisfactory results related to the adhesion of the films to the coated products, while maintaining the quality and increasing the durability of the inflorescences.

The greater the contact angle between the liquid and the surface, the lower the ability of the liquid to wet it. Many plants have varying degrees of roughness, and Nor and Ding (2020) indicated that increasing the surface roughness increases the resistance of a drop of water to penetrate the grooves of this surface, thereby increasing hydrophobicity.

According to the analysis of biological activity obtained through biospeckle, the involucral and floral bracts of the inflorescences showed no statistically significant difference when comparing different inflorescence opening points. A significant difference was observed when the involucral and floral bracts of an inflorescence were at the same opening point. This means that the involucral bracts had greater biological activity than the floral bracts in the closed, semi-open, and fully open inflorescences (Fig. 6a). This difference can be explained by the sample size since the involucral bracts were larger than the floral bracts.



Figure 6. Numerical values of the biological activity of the involucral and floral bracts at different opening points of Red Torch. (a) Analysis of the spatial-temporal speckle images obtained by biospeckle. (b) The same letters are not significantly different according to the Tukey's test at the 5% significance level (p < 0.05).

Biospeckle is sensitive to levels of roughness that are on the same order of magnitude as the wavelength of the light source, and it is therefore useful in monitoring activities at the microscopic level (Rabelo 2000). It is important to describe the illuminated cellular structures and movement velocity of liquids in cells. The elemental unit of biological tissues is the cell, and cells are mostly composed of water and organic compounds (Rabelo 2000).

The spatial-temporal speckle pattern is a way to evaluate the activity level of a specimen because, if the result presents a completely distorted figure, it is a sign that the movement is intense, as observed in Fig. 6b. Additionally, the movements inside biological material are due to gas exchange, metabolism, and the movement of nutrients in the cells, being more intense in the direction of the fibers than in other directions (Rodrigues et al. 2007).

Color characteristics are expressed through the dominance of the colors present in the bracts. Red was the dominant color at the different inflorescence opening points analyzed, without showing differences. However, there was an interaction between the position of the bracts (involucral and floral) and their side (upper and lower), and a difference in the dominance of red was observed. In comparison to the floral bracts, the involucral bracts had a greater degree of red dominance, both on the upper and lower surfaces. In the case of the floral bracts, a significant difference was observed in that, in comparison to the lower side, the upper side had less red dominance (Fig. 7a).



Figure 7. Color dominance of the involucral and floral bracts of Red Torch Ginger at the fully open point of the inflorescences, obtained through image analysis with GroundEye S800. (a) Red and (b) pink. The same letters are not significantly different according to the Tukey's test at the 5% significance level (p < 0.05).

Pink dominance was also observed (Fig. 7b) in floral bracts, showing higher dominance values than those of the involucral bracts; i.e., the pink dominance values were inversely proportional to red dominance. Additionally, pink dominance was inversely proportional in the floral bracts of the upper side to those on the lower side, in that the upper side had greater levels of pink dominance (Fig. 8).



Figure 8. Images of the bracts captured by GroundEye S800.

The dominance of red and pink colors was observed for the bracts at the three opening points, confirming the observations from visual analysis. It was possible to observe that there was an interaction between the different opening points of the inflorescences and the position of the bracts in relation to the gloss parameter. For the closed and semi-open inflorescences, there was no difference related to gloss for the involucral and floral bracts, and, for the fully open inflorescences, the floral bracts showed more gloss than the involucral bracts. The bracts arranged in the external position showed no difference among the different inflorescence opening points, whereas the floral bracts showed a higher mean gloss in the fully open inflorescence.

There was an interaction between the position of the bracts in the inflorescence and their orientation, where the upper side had the highest mean gloss level in both positions, indicating that the upper side of the bracts has a higher gloss level than the lower side. Moreover, the upper side had a higher gloss level in the involucral bracts than in the floral bracts, while the lower side had a higher gloss level in the floral bracts (Fig. 9).





Compared to the floral bracts, the involucral bracts of fully open inflorescences had a lower gloss level due to a longer period of exposure to the environment, while the floral bracts still have protection against this loss of gloss. This scenario can be explained by the natural production of carotenoids in larger amounts in involucral bracts as a way of protection against light incidence, and this process influences the production of reactive oxygen species, causing lipid peroxidation in these bracts and the loss of their gloss (Nogueira et al. 2023).

The collected data suggest a correlation between inflorescence opening and bract position, in which the involucral bracts had a less rough texture than the floral bracts. Involucral bracts of closed and semi-open inflorescences showed greater roughness compared to bracts of fully open inflorescences. On the other hand, floral bracts showed greater roughness in fully open inflorescences compared to closed and semi-open inflorescences. Additionally, a relationship between the opening points and the side of the bracts was observed, in which the lower sides of the bracts were smoother than the upper sides in all different inflorescence opening phases. This can be explained by the fact that these sides were more exposed to environmental conditions (Fig. 10).



Figure 10. Roughness of the involucral and floral bracts of Red Torch obtained by image analysis with GroundEye S800. Same letters are not significantly different according to the Tukey's test at the 5% significance level (p < 0.05).

GroundEye is a tool that allows for the analysis and extraction of information regarding morphological characteristics, such as size, shape, texture, and color. Widely recognized for its speed, objectivity, and often non-destructive process (Taniguchi et al. 2022), this tool was crucial in characterizing the properties of the Red Torch inflorescence. Additionally, the GroundEye equipment has shown promise in evaluating the physiological potential of various species (Andriazzi et al. 2020).

Based on the data obtained in the quantification of wax in bracts of the torch ginger species, significant differences were observed between the points of inflorescence opening. It was noted that there was a higher amount of wax accumulated in the involucral bracts of fully open inflorescences (1.41 mg/cm^2) compared to those partially open or closed (1.14 mg/cm^2). Similarly, for the floral bracts, a similar trend was observed, with lower values of wax accumulation (with an average of 0.83 mg/cm^{-2}) in semi-open and closed inflorescences.

Wax contents higher than 0.6 mg·cm⁻² are considered very high (Baker 1982). The significant amount of wax observed can be justified by the fact that the involucral bracts of fully open inflorescences were more exposed to the environment and, consequently, required more intense protection against water loss, pathogens, and heat dissipation (Fig. 11).

Through scanning electron microscopy, it was observed that Red Torch inflorescences have epicuticular wax deposited in the form of plates on the lower and upper surfaces of their bracts (Mattos et al. 2018). Therefore, wax is naturally present in the epidermis of the Red Torch. Epicuticular waxes present in plants constitute the first contact surface between the plant and the environment, acting as protection against water loss and a barrier against pathogens, and dissipating excess heat or radiation by forming a bright and reflective layer (Grohar et al. 2023). Although Grohar et al. (2023) observed the presence



of natural wax in the bracts, the amounts of wax in the involucral and floral bracts were not determined, and no difference in this amount was observed in relation to the inflorescence opening point when they were harvested.

Figure 11. Quantification of the wax on the involucral and floral bracts at the three inflorescence opening points of Red Torch. The same letters are not significantly different according to the Tukey's test at the 5% significance level (p < 0.05).

Understanding the specific characteristics of Red Torch, such as hydrophobicity, high fluid movement inside the cells, and high levels of natural wax, assists in decision-making regarding the use of postharvest coatings to increase durability. In addition, the final characteristics of the synthesized biofilms, which in turn depend on the characteristics of both the raw material or polymeric base (polysaccharides and/or proteins) and the lipid base, will be better understood. The association of the compounds and their additives is closely linked to the properties of the biopolymers (Nor and Ding 2020).

Two days after harvest, the plants showed the opening of the bracts. In comparison to the closed and semi-open inflorescences, the fully open inflorescences showed the appearance of new true flowers, as shown in Fig. 12. In the first three days after harvest, all the inflorescences, regardless of the opening point, showed gloss and turgidity; that is, they did not exhibit dryness at the ends of the bracts or have spots on them yet. Starting on the fourth day, the closed and semi-open inflorescences showed a decrease in gloss and a slight drying of the spotted extremities or bracts. The fully open inflorescences already showed marked dryness on the edges and spots on the underside of the involucral bracts, in addition to rows of slightly rotted true flowers.



Figure 12. Red Torch inflorescence senescence scale for closed, semi-open, and open inflorescences over the period of ten days after harvest.

When the true flowers of Red Torch inflorescences begin to open sequentially, the involucral bracts and the upper part of the peduncle begin to darken and dry due to a reduction in soluble sugars and the degradation of the starch grains accumulated by the plant and used during flower formation (Choong and Ding 2017).

On the fifth day, the inflorescences showed no gloss, and the bracts showed an increase in dryness or more pronounced spots, especially on the underside of the involucral bracts. On the sixth day, the inflorescences had no turgidity or gloss, with dryness and darkening of the bracts and very marked spots. These more pronounced characteristics were observed in the closed inflorescences starting on the seventh day.

The postharvest durability of Red Torch inflorescences varies widely. Hoult and Marcsik (2000) observed a postharvest durability of three to ten days for four cultivars. In a study conducted to evaluate the potential of Red Torch as a cut flower, Choon and Ding (2017) observed a postharvest durability of six days and indicated a high density of stomata in the bracts causing severe water loss by transpiration. Carneiro et al. (2014), evaluating the addition of sugar in the preservation of Red Torch, concluded that postharvest durability was correlated with the harvest point: only four days for flowering stems with open inflorescences and 10 days for flowering stems with semi-open inflorescences. This variation in the durability of the inflorescences can be explained by the climatic conditions in which the plants developed and by the different preservation conditions to which they were exposed.

Postharvest durability varied according to the inflorescence opening point at which they were harvested. The harvested inflorescences at a closed stage allowed for a durability of up to six days, while the semi-open and open inflorescences lasted up to four days. The use of senescence scales can reduce the subjectivity of postharvest estimates, allowing better quality stems to be marketed and increasing the useful life of the flowers. That is, the use of these scales allows quantification of the time required for flowers to reach consumers at a high-quality level, allowing a longer ornamental use (Cunha Neto et al. 2023, Nogueira et al. 2023). Thus, to ensure the quality and durability of flowering stems, Red Torch inflorescences should be harvested before the inflorescences fully open and true flowers appear in the closed or semi-open inflorescences.

In light of the findings from this study on Red Torch Ginger (*Etlingera elatior*) inflorescences, future research should delve deeper into the molecular aspects of senescence processes in these plants. This could involve exploring gene expression related to senescence and longevity, as well as investigating the biochemical pathways that influence the postharvest life of the flowers.

CONCLUSION

Based on the results of this study, the postharvest durability of the Red Torch inflorescences is influenced by the stage of inflorescence opening at the time of harvest. Closed and semi-open inflorescences showed greater durability than fully open inflorescences, which showed signs of deterioration more quickly. Additionally, we observed that Red Torch inflorescences have specific characteristics, such as hydrophobicity, high movement of fluids within their cells, a dominance of red staining, and the presence of high levels of wax in the bracts at different inflorescence opening points. The use of senescence scales can reduce the subjectivity of postharvest estimates, allowing better quality floral stems to be marketed and increasing the vase life of flowers. Therefore, it is recommended that Red Torch inflorescences be harvested before they are fully open, and that producers use appropriate postharvest preservation techniques to prolong the durability of the flowers.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Nogueira, M. R., Paiva, P. D. O. and Reis, M. V.; Methodology: Nogueira, M. R., Cunha Neto, A. R. and Timóteo, C. O.; Investigation: Nogueira, M. R., Paiva, P. D. O., Cunha Neto, A. R.; Nascimento, A. M. P. and Reis, M. V.; Writing – Original Draft: Nogueira, M. R.; Writing – Review and Editing: Paiva, P. D. O., Cunha Neto, A. R., Nascimento, A. M. P. and Reis, M. V.; Funding Acquisition: Paiva, P. D. O. and Reis, M. V.; Supervision: Paiva, P. D. O. and Reis, M. V.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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