Investigation of Stomata in Cut ‘Master’ Carnations: Organographic Distribution, Morphology, and Contribution to Water Loss

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Abstract. Leaf stomata are the main channels for water loss of plants including cut flowers. In this study, we investigated the organographic distribution, morphological characteristics, light–dark response, and water loss contribution of stomata in cut carnations (*Dianthus caryophyllus* L. ‘Master’), which are prone to typical water deficits despite a few and small leaves. Stomata were observed in the upper and lower leaf epidermis, stem surface, abaxial bract epidermis, and abaxial sepal epidermis. Stomatal density (SD) on the stem surface was the highest and significantly greater than that on the upper and lower leaf and abaxial bract epidermis. The sepal epidermis had the lowest SD and the smallest stomata whereas the upper leaf epidermis had the largest stomata. Changes in the water loss rate increased in the light and decreased in the dark in both intact and leaves-removed cut carnations. The water loss rate of the former was greater than that of the latter. However, the water loss rate for the stem-only cut carnations had weak change rhythms and was much lower than that for the intact and leaves-removed cut carnations. These findings demonstrate the differential contributions of stomata in leaves, stems, and floral organs to water loss, and help to elucidate further the mechanism underlying postharvest water deficit in cut carnations.

Carnation (*Dianthus caryophyllus* L.) is an important ornamental plant worldwide. It is popular because of its abundant flowers, which are available in various colors, sizes, and shapes. It is cultivated mainly as a cut-flower crop (Boxriker et al., 2017; Onozaki et al., 2001). However, cut carnations are prone to petal wilting and stem bending or breakage during postharvest storage and display, which adversely affect their ornamental performance and commercial value (Kim et al., 1998; Lin et al., 2019). These posthar-

vest disorders of cut carnations are generally attributed to ethylene damage. Nonetheless, they are at least partially related to water deficit (Liu et al., 2018; van Doorn, 2012). Water deficit symptoms in many cut flowers, including carnations, are the result of stomatal water loss that gradually exceeds the rate of water uptake through the xylem vessels in the cut-stem ends (Mattos et al., 2017; van Doorn, 2012).

The stomata of higher plants occur mainly on the leaves and regulate transpirational water loss (Wolz et al., 2017). Cut flowers lose water primarily via transpiration (Aliniaiefard and van Meeteren, 2016; Carpenter and Rasmussen, 1974; In et al., 2016). Excessive transpiration from open stomata in cut flowers causes typical water deficit symptoms such as tissue wilting and rapid senescence (Fanourakis et al., 2012; Farrell et al., 2012). Excessive foliar stomatal water loss causes petal and leaf wilting and the bent-neck phenomenon in cut roses (Fanourakis et al., 2012). Stomata may be induced to close by treating them with abscisic acid or acetylsalicylic acid. In this way, the transpiration rate and water deficit are reduced, and cut flower vase life is extended (Fanourakis et al., 2016; Kitamura and Ueno, 2015).

Previous studies on stomatal function in cut-flower transpiration focused mainly on the leaves (Aliniaiefard and van Meeteren, 2016; Schroeder and Stimart, 2005). How-

ever, stomata may also be distributed on other parts, such as stems and floral organs, including petals, sepals, and spathes. Some of them participate in transpiration whereas others have no apparent function (Carpenter and Rasmussen, 1974; Elibox and Umaharan, 2010; Huang et al., 2018; Zhang et al., 2018; Zieliński et al., 2010). Our recent work indicated that stomata are widely distributed on the petals, sepals, and stem surface of cut gerberas, and the stomata in the lower epidermis of the sepals play a critical role in postharvest water loss (Huang et al., 2018). In the case of cut carnations (‘White Sim’), it was reported that actively transpiring stomata in their stems which was associated with the water uptake rate during their vase period (Carpenter and Rasmussen, 1974).

To our knowledge, there is much less known about the distribution, characteristics, or postharvest water loss contribution of the stomata in the nonleaf parts of cut flowers, including carnations. Consequently, the mechanism underlying cut flower water deficit is poorly understood. The objectives of our study, then, were as follows: a) to investigate the stomatal distributions on the leaves, stems, and flowers of cut ‘Master’ carnations; b) to characterize SD, morphology, and light–dark responses; and c) to assess the potential role of these stomata in postharvest water loss.

Materials and Methods

Plant materials

Freshly harvested standard flowering carnation (*D. caryophyllus* ‘Master’) stems at the commercial (“paintbrush”) stage were purchased from a local cut flower market in Guangzhou City, China (lat. 23°06′49″ N, long. 113°12′18″ E). ‘Master’ is one of the most popular varieties of cut carnations in most cut-flower markets in China. They were harvested in the early morning in May 2017 (the temperature was about 20 °C and the relative humidity was 70% to 80%). They were placed immediately upright in plastic buckets filled partially with tap water, covered with low-density plastic film to minimize mechanical injury and water loss, and were transported by van at ≈27 °C within 1 h to the postharvest laboratory at Zhongkai University of Agriculture and Engineering. Upon arrival, the flowering stems were visually inspected and selected for uniformity of size, color, and freedom from defects. They were recut under deionized water (DIW) to ≈25 cm in length. Each stem bore two pairs of leaves (Liu et al., 2018).

Experimental design

Expt. 1: Observation of stomatal distribution, density, and morphology. Three carnations were sampled after being maintained in darkness for 3 h. Three samples (≈2 × 5 mm) were excised with a razor blade from the upper and lower leaf epidermis; adaxial and abaxial epidermis of the petals, sepals, and bracts; and stem surface. All sections were observed using a scanning electron microscope (SEM).

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Expt. 2: Assessment of the roles of different organs in cut carnation water loss. The experiments were conducted in a phytotron at $20 \pm 2^\circ\text{C}$, $60\% \pm 10\%$ relative humidity, and white light [light-emitting diode (LED) light source; Guangzhou Bluesatec Co. Ltd., Guangdong, China] as described by Huang et al. (2018). Each carnation was placed in its own 150-mL glass vase containing 100 mL DIW. Cut carnations were subjected to the following treatments: a) removal of all leaves (leaves removed), b) removal of both the flower and leaves (stem only), or c) intact cut flowers (intact). The cut flowers were maintained in DIW and incubated in the phytotron under an alternating 12-h light (LED white light, $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and 12-h dark cycle.

SEM observations

Samples were prepared and observed using an SEM as described by Huang et al. (2018), with a slight modification. All tissues excised from the various parts of cut carnations were immediately fixed in 4% (v/v) glutaraldehyde in $0.1 \text{ mol}\cdot\text{L}^{-1}$ phosphate buffer (pH 6.8) for 36 h at 4°C , then dehydrated in a graded ethanol series of 30%, 50%, 70%, 85%, 95%, and 100%. The samples were dried with supercritical carbon dioxide (critical-point drying), coated with gold, observed at a 10-kV accelerating voltage with a JSM-6360LV SEM (JEOL Ltd., Tokyo, Japan), and photographed.

Measurements

Stomatal density and morphological parameters. The stomatal density (SD; measured in stomata per square millimeter) of the samples of the upper and lower leaf epidermis; adaxial and abaxial epidermis of the petals, sepals, and bracts; and stem surface were determined using a SEM as described by Balasooriya et al. (2009). The morphological parameters included stomatal area (SA; measured in square micrometers), relative stomatal area (RSA; measured as a percentage), and stomatal shape coefficient (SSC). They were calculated according to the following equations (Huang et al., 2018):

$$\text{SA} = \pi \times \frac{W_g}{2} \times \frac{L_g}{2} \quad [1]$$

$$\text{RSA} = \text{SA} \times \text{SD} \times 100 \quad [2]$$

$$\text{SSC} = \frac{W_g}{L_g} \quad [3]$$

where L_g and W_g are the length and width (both measured in micrometers) of the stomatal guard cell, L_g is the length of the longest axis, and W_g is the width of the widest point perpendicular to the longest axis.

Water loss from cut carnations. Water loss from intact-, leaves-removed-, and stem-only cut carnations was monitored continuously with an automatic apparatus as described by Lü et al. (2011). Data were collected for three individual carnation stems at 2-h intervals over a 72-h period.

Statistical analysis

The data were analyzed with SPSS (version 13.0; IBM Corp., Armonk, NY). The data are presented as mean $\pm$ SE. Means were compared by Duncan's new multiple range test at the $P \leq 0.05$ level.

Results

Stomatal distribution. Stomata were observed on the abaxial epidermis of sepals (Fig. 1A) and bracts (Fig. 1B), upper leaf epidermis (Fig. 1C), lower leaf epidermis (Fig. 1D), and stem surface (Fig. 1E) of the cut 'Master' carnations. However, no stomata were found on the adaxial or abaxial petal epidermis (Fig. 1F and G), adaxial epidermis of the sepals (Fig. 1H), or bracts (Fig. 1I). Most of the stomata were embedded in the epidermal cells. On the bracts, however, they were nearly flush with the epidermal cells (Fig. 1B).

Stomatal density and morphological parameters. There were significant differences among the various parts of cut carnations in terms of SD (Table 1). The stem surface had the greatest stomatal density ($90.6 \text{ stomata}/\text{mm}^2$), whereas the SD on the upper and lower leaf epidermis was significantly less (69.4 and $37.7 \text{ stomata}/\text{mm}^2$, respectively). An even lower SD was determined for the abaxial bract epidermis ($31.7 \text{ stomata}/\text{mm}^2$). The abaxial sepal epidermis had the lowest SD ($3.3 \text{ stomata}/\text{mm}^2$).

Stomatal morphology differed among the cut carnation parts (Table 1). The upper leaf epidermis had the largest stomata, followed by those on the lower leaf and abaxial bract epidermis. The stomata in the stem surface had very small L_g , SA, and RSA, and the smallest W_g . The stomata in the abaxial sepal epidermis had the smallest L_g , SA, and RSA. The cut carnation parts differed substantially in terms of stomatal shape coefficient

Fig. 1. Scanning electron micrographs of stomata distributed on cut 'Master' carnations. (A) Stomata in abaxial sepal epidermis. (B) Stomata in abaxial bract epidermis. (C) Stomata in upper leaf epidermis. (D) Stomata in lower leaf epidermis. (E) Stomata in stem surface. (F) Absence of stomata in adaxial petal epidermis. (G) Absence of stomata in abaxial petal epidermis. (H) Absence of stomata in adaxial sepal epidermis. (I) Absence of stomata in adaxial bract epidermis. Scale bar = $100 \mu\text{m}$; scale bar in inset = $10 \mu\text{m}$.

($SSC = W_g/L_g$) (Table 1). The stomata in the lower leaf epidermis and abaxial sepal and bract epidermis were elliptical ($SSC = 0.66–0.79$). However, the stomata in the upper leaf epidermis were mostly suborbicular ($SSC = 0.89$). Those on the stem surface were long-oval ($SSC = 0.31$).

Roles of different organs in cut carnation water loss. Synchronous water loss measurement with a continuous automatic apparatus showed significant differences in water loss rate among the cut carnation organs. For both intact and leaves-removed cut carnations, the changes in water loss rate exhibited distinct rhythms that increased in the light and decreased in the dark over a 72-h period under an alternating 12-h light/dark cycle (Fig. 2A). During the light periods, the leaves-removed cut carnations showed significantly less water loss rates than the intact cut carnations. However, there was no significant difference between the intact and leaves-removed cut carnations in terms of water loss rate during the dark periods. The stem-only cut carnations showed only very weak rhythms for the change in water loss rate under the same conditions. The water loss rate in stem-only cut carnations was much less than that of the intact or leaves-removed cut carnations in both the light and dark periods (Fig. 2A).

The accumulated daily water loss of the intact cut carnations significantly increased on measurement days 1–3. The accumulated daily water loss of the leaves-removed carnations also increased, but there were no significant differences between days 1 and 2 or between days 2 and 3. However, the accumulated daily water loss of the stem-only cut carnations significantly decreased on days 1–3. The accumulated daily water loss of intact cut carnations was significantly greater than that of leaves-removed cut carnations, which in turn was significantly greater than that of the stem-only cut carnations (Fig. 2B). The relative differences in accumulated daily water loss among intact, leaves-removed, and stem-only cut carnations increased with time (Fig. 2B).

Discussion

The stomata of certain cut flowers are distributed mainly on the leaves but may also occur on other vegetative and floral organs (Huang et al., 2018; Schroeder and Stimart, 2005). A previous study reported stomata in the leaves and stems of cut ‘White Sim’ carnations (Carpenter and Rasmussen, 1974). In our study, SEM imaging of stomatal distribution demonstrated that stomata are widely distributed on various parts of cut ‘Master’ carnations (Fig. 1). Stomata also occur on the abaxial bract and sepal epidermis. However, they are absent on the lower and upper petal, and the upper sepal and bract epidermis. These results indicate that the leaves of cut carnations are amphistomatous; their stomata are distributed on both the upper and lower epidermis. In contrast, the sepals, bracts, and stems of cut carnations are hypostomatous (Table 1). In addition, SEM

Table 1. Stomatal density and morphology in various parts of cut ‘Master’ carnations.^z

Parts	SD (stomata/mm ²)	Stomatal morphological parameters				
		L _g (μm)	W _g (μm)	SA (μm ²)	RSA (%)	SSC
ULE	69.4 ± 1.70 b ^y	31.2 ± 0.70 b	27.6 ± 0.60 a	679.7 ± 26.30 a	4.72 ± 0.18 a	0.89
LLE	37.7 ± 2.30 c	34.4 ± 0.70 a	24.2 ± 0.60 b	655.1 ± 24.10 a	2.47 ± 0.09 b	0.71
ASE	3.3 ± 0.80 e	16.2 ± 0.60 d	10.2 ± 0.40 c	131.5 ± 7.93 c	0.04 ± 0.003 d	0.66
ABE	31.7 ± 1.30 d	29.9 ± 0.50 b	23.7 ± 0.40 b	556.5 ± 14.59 b	1.76 ± 0.05 c	0.79
SS	90.6 ± 1.60 a	27.2 ± 0.50 c	8.4 ± 0.30 d	178.8 ± 7.89 c	1.62 ± 0.07 c	0.31

^zStomatal density data are presented as mean ± SE based on five randomly selected observation areas. Stomatal morphological parameter data are presented as mean ± SE of ≈30 stomata in five randomly selected observation areas.

^yDifferent letters within the same column indicate significant differences in stomatal morphological parameters among the various parts of cut carnations according to Duncan’s new multiple range test at the $P \leq 0.05$ level.

SD = stomatal density; ULE = upper leaf epidermis; LLE = lower leaf epidermis; ASE = abaxial sepal epidermis; ABE = abaxial bract epidermis; SS = stem surface; L_g = guard cell length; W_g = guard cell width; SA = stomatal area; RSA = relative stomatal area; and SSC = stomatal shape coefficient.

imaging revealed that the stomata in the leaves (Fig. 1C and D), stems (Fig. 1E), and sepals (Fig. 1A) are embedded in epidermal cells whereas those on the bracts (Fig. 1B) are almost flush with the epidermal cells.

SD and size are closely associated with transpiration (Franks et al., 2009, 2015; Lawson and Blatt, 2014) and may contribute significantly to water loss in cut flowers (Carvalho et al., 2015; Huang et al., 2018; In and Lim, 2018). There was a negative correlation between the longevity of certain cut snapdragon genotypes and their foliar SD (Schroeder and Stimart, 2005). Roses grown at high relative humidity had significantly greater SD and larger stomata than those raised under low humidity. The former also had comparatively greater water loss and wilting rates (Carvalho et al., 2016; Fanourakis et al., 2012; Torre and Fjeld, 2001). In our study, there were considerable differences in SD and morphology among the leaves, stems, and floral organs of cut carnations (Table 1). The stem had the greatest SD, but its stomata were significantly smaller than those on the leaves and bracts. Therefore, the stomata in the various parts of cut carnations may play different roles in postharvest water loss. The stomata in the stems and bracts may also participate in cut carnation water loss. In contrast, the stomata in the sepals of cut carnations may have little or no function, and their density and area are extremely small. It was reported that the stomata in the sepals of cut hydrangea (*Hydrangea* spp. ‘Endless Summer’) were not involved in postharvest transpiration (Kitamura and Ueno, 2015). However, the stomata in the sepals of cut gerberas may, in fact, play an important role in floral water loss (Huang et al., 2018).

Water loss in cut flowers is attributed mainly to leaf stomata (Fanourakis et al., 2016; In et al., 2016; Schroeder and Stimart, 2005). However, stomata in the nonleaf organs of certain cut flowers may also contribute to postharvest water loss (Azad et al., 2007; Huang et al., 2018). In our study, we evaluated the contributions of the stomata in leaves, stems, and floral organs to postharvest water loss by continuously measuring the changes in the rate of water loss from intact, leaves-removed, and stem-only cut carna-

Fig. 2. Water loss measurement in cut ‘Master’ carnations in deionized water under an alternating 12-h light (light-emitting diode white light; 100 μmol·m⁻²·s⁻¹) and 12-h dark cycle. (A) Water loss rate of intact, leaves-removed, or stem-only cut carnations. Open and solid blocks indicate light and dark periods, respectively. Dynamic synchronous data were collected from three individual carnation stems at 2-h intervals over 72 h and are mean ± SE (n = 3). (B) Accumulated daily water loss of intact (control), leaves-removed, or stem-only cut carnations. Data were obtained from three individual carnation stems at 2-h intervals over 72 h and are mean ± SE (n = 3). Different letters indicate significant differences among intact, leaves-removed, and stem-only cut carnations according to Duncan’s new multiple range test at the $P \leq 0.05$ level.

tions over a 72-h vase period under an alternating 12-h light/12-h dark cycle (Fig. 2A). The changes in water loss rate in leaves-removed and stem-only cut carnations exhibited rhythms—namely, increased in the light and decreased in the dark. This finding was consistent with that observed for carnations (Fig. 2A) and other cut flowers

(Doi et al., 1999; Lü et al., 2011) with intact leaves. Water loss in cut gerberas, which typically presented without leaves, showed a distinct rhythm over a 96-h vase period, possibly because of light-induced stomatal opening on the nonleaf organs (Huang et al., 2018). Relative to intact cut carnations, however, leaves-removed cut carnations showed a significantly lower water loss rate during the same light period. The stem-only cut carnations had the lowest water loss rates of all (Fig. 2A). The accumulated daily water loss of intact cut carnations was significantly greater than that of leaves-removed cut carnations. In turn, the latter had a significantly greater accumulated daily water loss rate than stem-only cut carnations (Fig. 2B). These results suggest that the stomata in the leaves, stems, and floral organs of cut carnations differ in terms of their relative contribution to water loss. Nevertheless, leaf stomatal transpiration might have the strongest impact on water loss in cut carnations.

Conclusions

Current results demonstrated that stomata are widely distributed on the leaves, stems, bracts, and sepals of cut carnation flowers. Of these, the stem had the greatest SD; nevertheless, its stomata were significantly smaller than those on the leaves and bracts. Moreover, although leaf stomata were mainly responsible for postharvest water loss in cut carnations, the stomata in the stem and floral organs also had a potentially significant contribution. These findings provide insight into the function of the stomata in nonleaf organs (such as stems and flowers) to water loss and help to elucidate further the mechanism underlying postharvest water deficit in cut carnations and other cut flowers. Future research objectives may include physicochemical inhibition of stomatal opening on the nonleaf parts of cut carnations (especially the stems and bracts) and optimization of postharvest cut carnation storage conditions.

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