Cultivar Differences in the Stomatal Characteristics of Cut Roses Grown at High Relative Humidity

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Abstract

High relative air humidity (RH>85%) during cultivation is known to reduce the vase life of cut roses, but the magnitude of such effect is cultivar dependent. The reasons behind this genotypic variation are not yet known. In this study, the stomatal density and stomatal responses to two closing stimuli (i.e. desiccation and abscisic acid (ABA) application) were evaluated using detached fully expanded leaves of two contrasting rose cultivars in their sensitivity to high RH ('Frisco' and 'Prophyta') which were grown at moderate (60%) and high (90%) RH. High RH significantly increased the stomatal density in both cultivars, but the effect was stronger in the tolerant cultivar (14% increase for 'Frisco', 8% increase for 'Prophyta'). 'Frisco' also showed a higher stomatal density at moderate RH (53 stomata/mm^2) as compared to the sensitive cultivar (43 stomata/mm^2). Moreover, high RH decreased the speed and the degree to which stomata responded to different closing stimuli in both cultivars, resulting in higher transpiration rates. This effect was more pronounced in the sensitive cultivar. It was concluded that the tolerance to high RH during cultivation is related to more responsive stomata, while the stomatal density is apparently an irrelevant character. Furthermore, this study showed that the rose guard cell dimensions are not representative for the pore dimensions.

INTRODUCTION

High relative air humidity (RH>85%) during the cultivation period (pre-harvest) is a critical environmental factor decreasing the keeping quality of several cut flowers (e.g. rose and *Bouvardia*) and pot plants (e.g. rose, begonia and *Kalanchoe*). Plants grown under such high RH levels do not undergo a natural senescence process, but end their vase or shelf life because of: premature leaf and flower wilting, brittle leaves, bent neck, earlier and more flower and bud drop (Torre and Fjeld, 2001). This phenomenon is related to a water imbalance (water loss>water uptake) that occurs during the postharvest phase due to the high evaporative demand conditions (i.e. high leaf-air vapour pressure deficit (VPD) within the normal temperature range). Stomatal malfunctioning is a major cause for such negative water balance (Mortensen and Fjeld, 1998; Torre et al., 2003). Plants grown under high RH have shown less responsive stomata, compared to plants grown under low RH, when subjected to a number of closing stimuli such as desiccation, ABA and darkness (Fordham et al., 2001; Torre and Fjeld, 2001; Rezaei Nejad and van Meeteren, 2005). The role of ABA in the control of stomatal functioning under drought stress is well described for several species (e.g. Zhang and Davies, 1990; Zhang and Outlaw, 2001). However, the reasons why stomata from leaves developed at high humidity seem to be malfunctioning are still not clear (Torre and Fjeld, 2001). Furthermore, Mortensen and Gislerød (1999, 2005) have shown that the impact of high RH on the vase life of cut roses is strongly cultivar dependent, but these striking cultivar differences are still poorly understood. Most studies were focused on one cultivar, while

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an in-depth systematic analysis of the underlying morphological and physiological mechanisms that explain cultivar differences (tolerant vs. sensitive) is not available.

This study aims at understanding the tolerance mechanisms to high RH. The stomatal density and stomatal responses to two closing stimuli will be evaluated in two contrasting cut rose cultivars in their tolerance to high RH. Moreover, since the effect of growth conditions on stomatal anatomy is usually assessed based on the guard cell dimensions (Rezaei Nejad and van Meeteren, 2005; Torre et al., 2003), whereas the transpiration rate depends on the pore dimensions (Parlange and Waggoner, 1970), we also aim to analyze the relationship between these parameters.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Rooted cuttings of two cut rose cultivars (*Rosa hybrida* L. cv. 'Frisco' and 'Prophyta') were obtained from a commercial propagator (Kordes Roses, De Kwakel, the Netherlands), and planted in 3.6 L pots containing a mixture of cocopeat (Jongkind Grond B.V., Aalsmeer, the Netherlands) and perlite (Agraperlite, Pull Rhenen B.V., Rhenen, the Netherlands) (3:1, v/v). These cultivars were selected based on their contrasting behaviour, i.e. a small (13%, 'Frisco') and a large (53%, 'Prophyta') vase life reduction when grown under high RH as compared to moderate RH (Mortensen and Gislerød, 2005). Twenty four plants per cultivar were randomly distributed over four growth chambers (l×w×h=1.3×0.8×1.3 m). Plants were grown as single stem at a density of 40 plants m^{-2}. In two chambers the RH was set at 60% and in the other two at 90%, resulting in an average of 60±3% for moderate RH and 95±1% for high RH during the complete cultivation period. The four chambers were submitted to a constant day/night temperature of 19±1°C leading to a VPD of 0.90±0.07 kPa for moderate RH and 0.21±0.07 kPa for high RH. The difference in the temperature and RH between the borders and the centre of the chambers was below 1%. These climate parameters were recorded automatically every 5 min. by data loggers (Fourier Microlog EC650, MicroDAQ.com, Ltd.; Contoocook NH, USA). The CO_2 concentration during the light period was 370±50 μmol mol^{-1} (Indoor Air Quality Meter Model 8760, TSI Incorporated; Shoreview MN, USA). CO_2 fertilization was not employed, to avoid effects on stomatal density and responsiveness. Fluorescence tubes (TLD 58W/84, Philips; Eindhoven, the Netherlands) were on for 18 h/day (from 07:00 to 01:00) providing 300±20 μmol m^{-2} s^{-1} photo-synthetically active radiation (LI-COR Model LI-250; Lincoln NB, USA). The light intensity was measured at 70 cm from the pot base, which was just above the canopy of the full grown plants.

Plants were watered regularly with the following nutrient solution: major nutrients: K 4.0, Ca 3.5, Mg 1.38, NO_3 9.49, SO_4 1.5, H_2PO_4 1.25 (mM), and minor elements: Fe 25, Mn 5, Zn 3.5, B 20, Cu 0.75, Mo 0.5 (μM). Two different concentrations were used to water the plants (1.5 and 2 dS/m) with a pH of 5.5. Their use was dependent on the EC of the drain which was monitored daily (Inolab pH/Cond level 1 WTC, Sensor EC WTW TetraCon 325 and Sensor pH Electrode SenTix 61; Weilheim, Germany) and kept in the range of 1.5-2.5 dS/m.

Four treatments were studied resulting from the combination of two RH levels (60 and 90%) and two contrasting cut rose cultivars ['Frisco' (tolerant) and 'Prophyta' (sensitive)]. Four weeks after planting the measurements started on fully developed leaves.

Stomatal Responses to Closing Stimuli

To study the effect of desiccation and ABA application on leaf transpiration rate the terminal leaflets (Fig. 1B) of, respectively, the 1st and 2nd penta-foliate leaves from the top of the plant (Fig. 1A) were detached, their petioles were re-cut under degassed water, and were placed in small flasks filled with water. For the desiccation stimuli the leaflets were further incubated in a saturated humidity environment (100% RH, 21ºC; VPD close

to 0) for 1 h to establish their maximum fresh weight. The re-hydration process took place under light (15 μmol m^{-2} s^{-1}), since following a darkness treatment the light-induced stomatal opening requires up to 1h in roses (Blom-Zandstra et al., 1995). Subsequently, the petioles were removed from water and the leaflets were placed in the test room (50±3%, 21ºC, 1.47 kPa VPD and 50 μmol m^{-2} s^{-1}), where their transpiration rate was measured gravimetrically for 4 h.

For the ABA stimuli the leaflets, with their petioles into water, were left to stabilize in the test room for 1 h. The transpiration rate was further measured gravimetrically for 20 min after which the leaf petioles were moved from flasks containing water into vials containing 100 μM (±) ABA solution (Sigma, St. Louis, MO). The time course changes in transpiration rate were measured gravimetrically for 2 h 50 m. At the end of the application of both closing stimuli the leaf area was determined using a leaf area meter (LI-COR, model 3100 Area Meter; Lincoln NB, USA), and the transpiration rate was calculated per unit leaf area for 12 leaves (1 leaf/plant)/treatment.

Leaf and Guard Cell Anatomical Measurements

Stomatal anatomical characters were determined using the silicon rubber impression technique (Smith et al., 1989). Fully expanded leaflets were sampled (left leaflet of the 1st penta-foliate leaf from the apex; Fig. 1B), and the part of the leaf midway between the tip and the base of the leaf was used. Areas in the vicinity of first and second order veins as well as the leaflet edges were avoided. For the stomatal density, magnification of 100× was used, and five circular 0.55 mm diameter fields (corresponding to total field area of 4.73 mm^2) were counted on the lower epidermis of twelve plants from each treatment (n=60). For the stomatal size evaluation, a magnification of 400× was used, and 20 randomly selected stomata per replicate were measured (n=240). The stomata length, stomata width, pore length and pore aperture under the absence of closing stimuli were determined for both cultivars grown under low RH.

Digitized video images were taken using a microscope (Leica Aristoplan; Bensheim, Germany) connected to a digital imaging camera (Nikon DXM-1200; Tokyo, Japan). Image processing was done using the software UTHSCSA IMAGETOOL (University of Texas Health Science Centre, San Antonio TX, USA).

RESULTS

Stomatal Responses to Desiccation

A general pattern in the leaf transpiration response to desiccation was observed in both cultivars independently of the RH level during the cultivation period (Fig. 2). Leaf transpiration rate increased for a period of 10 min, when the humidity around the leaf was reduced and the petiole was removed from water, and decreased strongly thereafter in all the treatments (Fig. 2). However, the maximum transpiration rate was significantly higher (13.1%) in plants grown at high RH. Moreover, the speed and degree to which the stomata close upon desiccation was largely influenced by the cultivar and RH level. For instance, leaf transpiration rate of 'Prophyta' after 4 h of desiccation when grown at high RH did not stabilize (whereas in the tolerant cultivar 'Frisco' it reached a stable value after 90 min) and reached a final value six times higher than in leaves from plants grown under moderate RH (the respective increase in 'Frisco' was only a factor 3.5).

Stomatal Responses to ABA Feeding

After shifting the leaflets from water into an ABA solution (100 μM; >20 min), stomata from both cultivars grown at moderate RH showed an immediate and pronounced decrease in the transpiration rate (Fig. 3). Nevertheless, high RH decreased the degree and speed of stomatal response to ABA feeding, especially in the sensitive cultivar. 'Prophyta' grown under high RH presented a final transpiration rate 5.8 times higher compared to plants of moderate RH, while for the cultivar 'Frisco' it increased by factor four.

Leaf and Guard Cell Anatomical Measurements

Stomatal density was significantly higher (11.3%) in leaves grown at high RH compared to leaves from moderate RH in the studied cultivars (Table 1). 'Frisco' had higher number of stomata in both RH levels (22 and 29% for moderate and high RH respectively).

In general, poor relationships were found between both pore length and stomata length, and between pore width (aperture) and stomata width in the two cultivars (Fig. 4). However, in 'Prophyta' (cultivar with a broader range of stomata length) the correlation between pore length and stomata length was improved, fact that did not take place in the pore width versus stomata width relation.

DISCUSSION

Fully rehydrated rose leaves underwent the Iwanoff effect when subjected to desiccation, a phenomenon reported for cut rose stems as well (Spinarova et al., 2007). The desiccation treatment mimics the water stress, leaves are subjected during the postharvest life when no root (hydraulic or hormonal) signals are present, and is a powerful signal for stomatal closure. The more hydrosensitive the stomata are, the faster the transpiration rate will decline, and the better the conservation of the leaf water balance will be. Therefore, the tolerant cultivar is advantageous in preserving for longer time positive water balance, since it acquires more hydrosensitive stomata, after growth in high RH, compared to the sensitive one. In contrast with desiccation, ABA application consists of a theoretical situation, which does not happen normally during vase life. The ABA feeding through the transpiration stream showed that short term lack of ABA is not responsible for the weaker stomatal responses of the high RH leaves, as shown in *Tradescantia virginiana* (Rezaei Nejad and van Meeteren, 2005), and revealed similar cultivar behaviour differences in stomatal responses as the desiccation treatment.

The degree and the speed to which stomata respond to different closing stimuli were impaired by high RH, resulting in higher transpiration rates and higher cumulative water losses over time, which is in agreement with previous work conducted in roses (Torre and Fjeld, 2001) and in other species (Fordham et al., 2001; Rezaei Nejad and van Meeteren, 2005). However, we show here that there is a strong genotypic variation in this response, which seems to be closely related to the tolerance mechanisms.

The effect of high RH on the stomatal density is species dependent (Torre et al., 2003; Rezaei Nejad and van Meeteren, 2005), although the majority of the species tends to increase the stomatal density, as an attempt to increase the transpiration rate in a low VPD environment. In the current study it was found that high RH during growth increased the stomatal density in the abaxial leaf surface, which is in line with previous work on roses (Torre et al., 2003). Unlike what was found in other species, namely a significant correlation between pore width (aperture) and stomata width (*Commelina communis* L.: R^2=0.92, *Phaseolus vulgaris* L.: R^2=0.71; Lawson et al., 1998), in roses neither pore length was correlated with stomata length nor pore width with stomata width. The current results impose a cautious examination of changes in stomatal anatomy, as these do not necessarily represent respective trends in the pore dimensions, which are decisive for the transpiration rate.

CONCLUSIONS

From this study we can conclude that the analysed cut rose cultivars differ significantly in the way that they respond to long term high RH conditions, and that stomatal physiology is a key process in these contrasting responses, while stomatal density is apparently a less relevant trait. An understanding of such mechanisms is highly relevant to establish selection criteria and procedures for breeding tolerant cultivars to high RH and to reduce the variation in the keeping quality of ornamental plants accounted across diverse cultivation regimes.

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Tables

Table 1. Stomatal density in the abaxial leaf surface of cut rose cv. 'Frisco' and 'Prophyta' grown at high (90%) and moderate (60%) RH. Different letters indicate significant differences according to LSD-test at 5% level (comparison in columns).

	Stomatal density (stomata mm^{-2})		
	90%	60%	Mean
'Frisco'	60.3	52.7	56.5
'Prophyta'	46.6	43.3	45.0
Mean	53.5 [a]	48.0 [b]	
F prob.			
Cultivar (C)	0.101		
RH	<0.001		
C×RH	0.072		

Figures

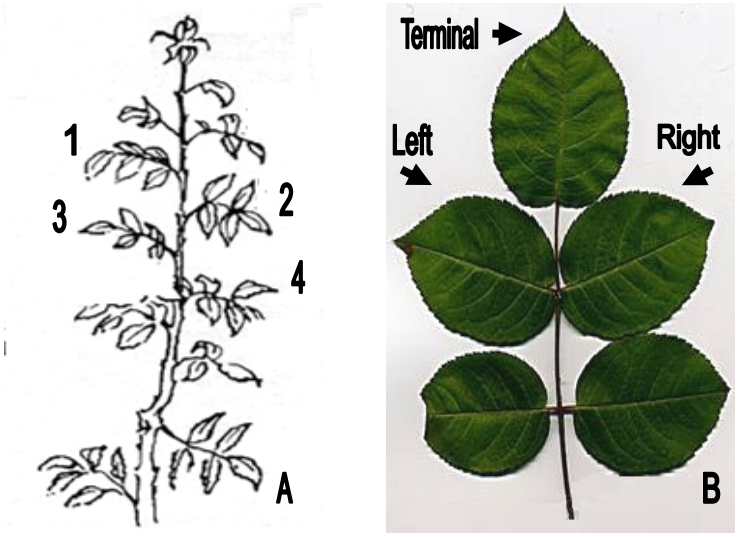

Fig. 1. Illustration of the leaf (A) and leaflet position (B) used in the measurements. Leaf one corresponds to the first five leaflet leaf counting from the apex.

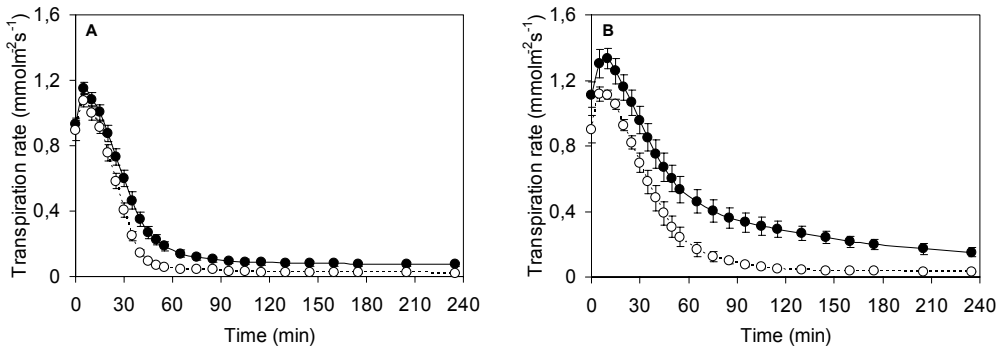

Fig. 2. Transpiration rate during 4 h of desiccation of cut rose cv. 'Frisco' (A), and 'Prophyta' (B), grown at high RH (●, 90%) and moderate RH (○, 60%). Vertical bars indicate S.E.M. (*n*=12).

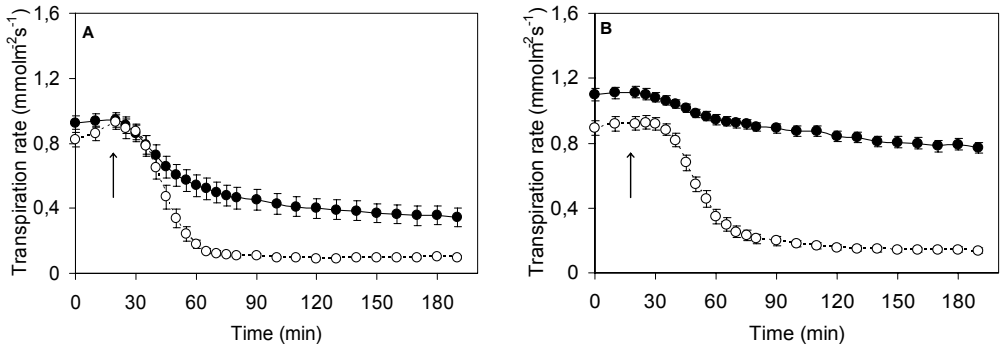

Fig. 3. Transpiration rate of cut rose cv. 'Frisco' (A), and 'Prophyta' (B), grown at high RH (●, 90%) and moderate RH (○, 60%) when kept in water for 30 min and subsequently transferred to 100 µM ABA (black arrows). Vertical bars indicate S.E.M. (*n*=12).

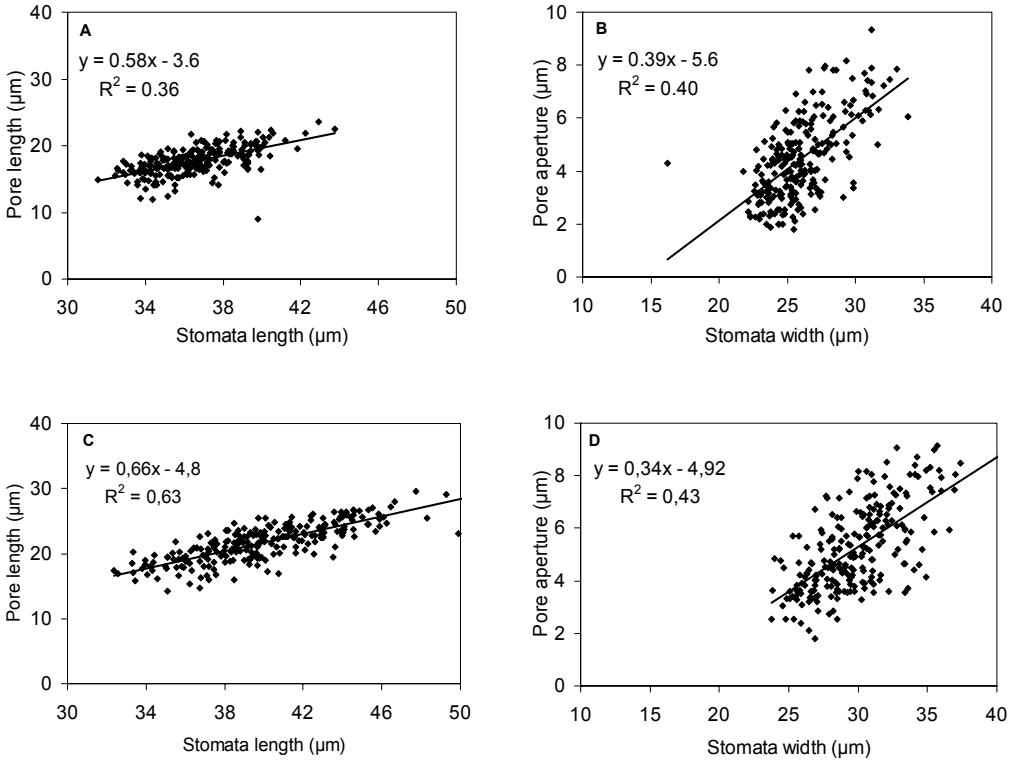

Fig. 4. Relationship between stomata length and pore length (A, C), and between stomata width and pore width (B, D) of cut rose cv. 'Frisco' (A, B) and cv. 'Prophyta' (C, D), grown at moderate RH. Line represents linear regression. The differences in scale on the axes should be noted.