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Full Length Research Paper

Comparisons of stomatal parameters between normal and abnormal leaf of *Bougainvillea spectabilis* Willd

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In this study, stomatal density (SD), stomatal index (SI), epidermal cell density (ED), guard cell length (GCL) and guard cell width (GCW) of normal and abnormal leaf of *Bougainvillea spectabilis* Willd were studied. This can be useful for further research of physical mechanism of abnormal leaf. Epidermal cells were polygonal with straight anticlinal walls in the adaxial epidermis of normal leaf; while in the abaxial epidermis of normal leaf, the epidermal cells were irregular with repand anticlinal walls. The stomata were anomocytic on normal leaf. No stomata were found in the adaxial epidermis of abnormal leaf. In abaxial epidermis of abnormal leaf, the epidermal cells were repand or sinuous. The stomata were anomocytic on abnormal leaf. The guard cells were kidney-shaped on normal and abnormal leaf. SD and SI of normal leaf were significantly higher than that of abnormal leaf. ED and GCL of abnormal leaf were higher than that of normal leaf in relative leaves length 37.5 and 50%. At other four stages, ED and GCL of abnormal leaf were smaller than that of normal leaf. SD, SI and ED had a negative correlation with relative leaf length; a significant positive correlation was observed between GCL and relative leaf length.

Key words: *Bougainvillea spectabilis* Willd, normal leaf, abnormal leaf, stomatal density, epidermal cell density, stomatal index.

INTRODUCTION

Stomata are small pores on the surfaces of leaves and generally comprised of two guard cells. Stomata control the exchange of gasses between the interior of the leaf and the atmosphere (Hetherington and Woodward, 2003). They also make a significant contribution to global water and carbon cycles (Ciais et al., 1997). At present, stomata were observed in the seeds, primary root, leaf and other parts of plants (Paiva et al., 2006; Christodoulakis et al., 2002). Stomata play an important role in plant innate immunity (Melotto et al., 2006). Some researchers seek the possible track of plant genetic variation according to studying leaf epidermal stomatal morphology (Li et al., 2005; Lu et al., 2005). In addition, leaf epidermis stomatal morphology has significance in taxonomy (Wang et al., 2007). In recent years, the research about stomatal distribution and function of the plant's different parts attract more people's attention.

Bougainvillea, a member of the Nyctaginaceae family, comprises of spiny tress, shrubs and vines. *Bougainvillea spectabilis* Willd is one of the major ornamental plants in the south of China. The abnormal leaf is the ornamental part, that is, bract. As the flowers are small and often clustered in three large bracts, while the outer bracts are large and beautiful, so people often mistake the bracts for petals. Current researches about *B. spectabilis* Willd focus on the cutting, medicinal values of extracts and special characteristics of reproductive organs (Lopez and Galetto, 2002; Xu et al., 2009). However, stomatal distribution on the normal and abnormal leaf of *B. spectabilis* Willd has not been reported. The objective of this study was to know the differences of stomatal distribution and dynamic changes by comparing stomatal distribution on the normal and abnormal leaf during the development of *B. spectabilis* Willd and lay foundation for further research of physical mechanism of abnormal leaf.

MATERIALS AND METHODS

Plant materials

Plants of *B. spectabilis* Willd were grown in the Jinan University.

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Plants grew under natural conditions. The ages of plants were 2 years. The place where the plants grow has plenty of sunlight. The experiment was carried out in April, in which the average day/night temperature was 25/17°C. Normal and abnormal leaf were collected during the bracts opening. Ten plants were selected. When this plant flowered, leaf length was divided into six stages. Normal leaves which length was 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 cm were selected to observe and used the length of 8.0 cm as 100%. Abnormal leaves which length was 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 cm were selected to observe and used the length of 4.0 cm as 100%. So, the relative leaf lengths were 37.5, 50, 62.5, 75, 87.5 and 100%, respectively. Ten temporary installation slides were made from normal and abnormal leaf of each length.

Methods

Epidermis of abnormal leaf was directly torn off from the leaves. Epidermis of normal leaf was too fragile to be separated, the epidermis was painted with clear fingernail polish and a piece of transparency sheet was pressed on it by fingers, later, the imprint of stomata was obtained by striping the transparency sheet from the leaves. Observations were made by using a light microscope with a 40 × 10 magnification in 0.159 mm^2 vision field. Each length of normal and abnormal leaf was calculated 50 vision fields and stomatal density, stomatal index and size of guard cells were calculated (Pandey, 2007). Photos were taken using Sony W5 digital camera. All data were subjected to analysis using SPSS 13.0. Stomatal density (SD) was measured as the number of stomata per square millimeter. Epidermal cell density(ED) was measured as the number of epidermal cells per square millimeter. The stomatal index (SI) was calculated as the number of stomata (S) divided by number of stomatal and number of epidermal cells (E), that is, SI = (S / (E + S))× 100%. Guard cell length (GCL) and Guard cell width (GCW) were measured in micrometres.

RESULTS

Morphological comparisons on adaxial epidermis of normal and abnormal leaf

Stomatal existed on the adaxial of normal leaf, but no stomatal were found on the adaxial of abnormal leaf. The shape of the adaxial epidermal cells of abnormal leaf was irregular and the patterns of anticlinal walls were undulate (Figure 1b, f). The shape of the adaxial epidermal cells of normal leaf was polygonal and the patterns of anticlinal walls of epidermal cells were straight (Figure 1c, g). The stomata on the adaxial epidermis of normal leaf were anisocytic and the guard cells were kidney-shaped (Figure 1c, g).

Morphological comparisons on abaxial epidermis of normal and abnormal leaf

Stomata were observed on the abaxial epidermis of normal and abnormal leaf. With the development of abnormal leaf, the ripple of epidermal cells became more and more significant. The form of epidermal cells was polygonal and the patterns of anticlinal walls were straight in the relative leaf 37.5%, while the form of epidermal cells was irregular and the patterns of anticlinal walls were sinuous in the relative leaf length 100% (Figure 1d, h); the stomata were anisocytic. The form of epidermal cells was polygonal and the stomata were anisocytic of normal leaf, the pattern of anticlinal was repand and no significant changes with the development of normal leaf was observed (Figure 1e, i). The guard cell was kidney-shaped both on the normal and abnormal leaf.

Comparisons of SD, SI on the abaxial epidermis between normal and abnormal leaf

SD and SI of normal leaf were significantly higher than that of abnormal leaf (Figure 2a, b). With the development of leaf, SD of normal leaf decreased but it slightly increased in the relative length to 62.5 and 87.5%; SD of abnormal leaf decreased from 27.2 ± 8.57 to 4.2 ± 3.73 stomata / mm^2 and had significant difference ($P < 0.01$). SI of abnormal leaf increased first and then decreased. SI reached the maximum of 0.81 ± 0.50% in the relative length of 62.5% and the minimum was 0.58 ± 0.51%. SI of normal leaf had no significant differences between each others; it decreased at first and then, increased slightly.

Comparisons of ED on the abaxial epidermis between normal and abnormal leaf

ED of abnormal leaf was higher than that of normal leaf in the relative leaf length 37.5 and 50% (Figure 2c). At the other four stages, ED of abnormal leaf was smaller than that of normal leaf. On the other hand, it has significant difference of ED between normal and abnormal leaf ($P < 0.01$) except in the relative leaf length 62.5% ($P > 0.05$).

Comparisons of GCL and GCW on the abaxial epidermis between normal and abnormal leaf

The GCL of normal and abnormal leaf increased (Figure 2d). GCL of normal leaf was significantly larger than that of abnormal leaf, when the relative leaf length was 37.5 and 50%. In the relative leaf length, it was 62.5, 75, 87.5 and 100%, the GCL of normal leaf were smaller than that of abnormal leaf and there was no significant difference ($P > 0.05$) between normal and abnormal leaf. The GCW of normal leaf increased, but the GCW of abnormal leaf decreased (Figure 2e). The GCW of abnormal leaf was larger than that of normal leaf and the difference was very significant ($P < 0.01$), except for the stage that the relative leaf length was 100%.

Correlation analysis for stomatal characters of normal and abnormal leaf

From the correlation studies among the stomatal para-

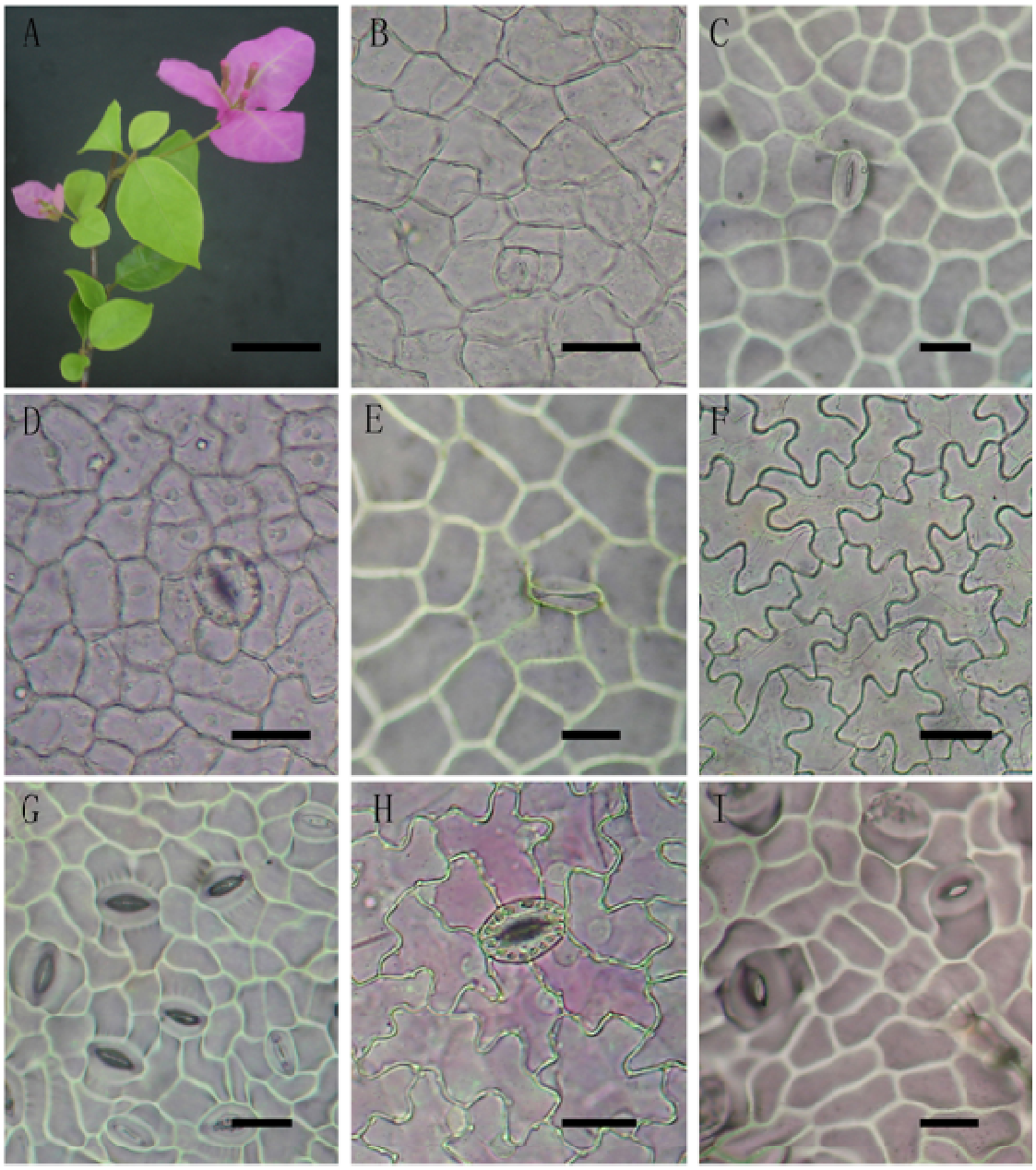

Figure 1. Epidermal cells and stomata in the normal and abnormal leaf of *B. spectabilis* Willd. (A) Normal leaf (green) and abnormal leaf (red); (B) the epidermal cells in the adaxial epidermis of abnormal leaf in the relative leaf length 37.5%; (C) the epidermal cells and stomata in the adaxial epidermis of normal leaf in the relative leaf length 37.5%; (D) the epidermal cells in the abaxial epidermis of abnormal leaf in the relative leaf length was 37.5%; (E) the epidermal cells and stomata in the abaxial epidermis of normal leaf in the relative leaf length was 37.5%; (F) the epidermal cells and stomata in the adaxial epidermis of normal leaf in the relative leaf length was 100%; (G) the epidermal cells and stomata on the adaxial epidermis of normal leaf in the relative leaf length was 100%; (H) the epidermal cells and stomata in the abaxial epidermis of normal leaf in the relative leaf length was 100%; (I) the epidermal cells and stomata in the abaxial epidermis of normal leaf in the relative leaf length was 100%. Bar = 20 µm.

meters (Table 1), it was observed that stomatal density of normal leaf was weakly correlated with stomatal index (R^2 = 0.85*) and epidermal cell density (R^2= 0.89*), while a negative correlation was observed with guard cell width (R^2 = -0.90*). Guard cell length had significant (P = 5 and 1%) positive correlation with guard cell width (R^2 = 0.95**) and relative leaf length (R^2=0.95**).

Guard cell length of abnormal leaf (Table 2) had signifi-

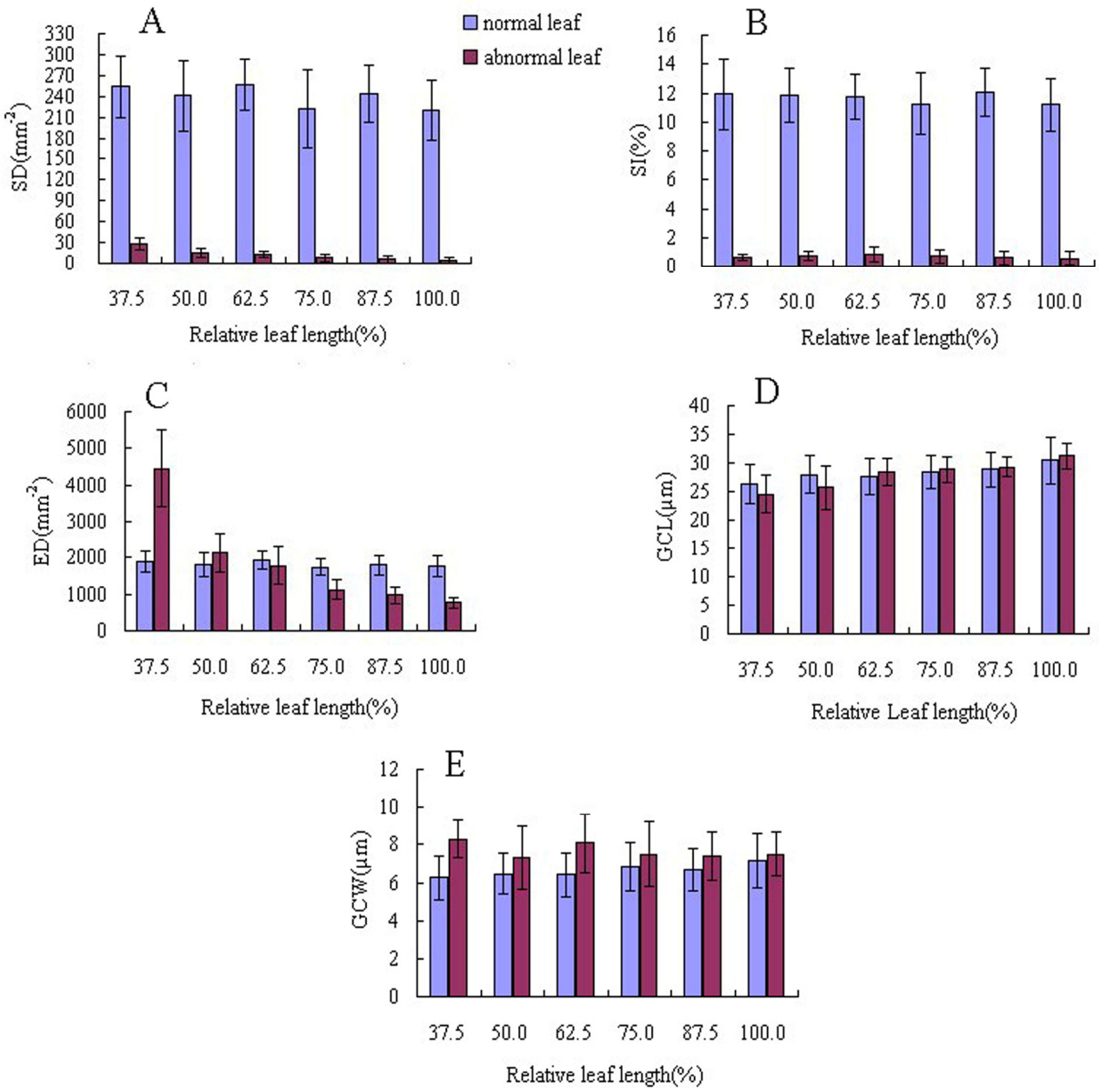

Figure 2. (A) Stomatal density (SD); (B) stomatal index (SI); (C) epidermal cell density(ED); (D) guard cell lengths (GCL); (E) guard cell width (GCW).

cant (P = 5 and 1%) positive correlation with relative leaf length (R^2 = 0.95**). However, a negative relationship was noted between epidermal cell density and guard cell length (R^2 = -0.89*), epidermal cell density and relative leaf length (R^2 = -0.89*).

DISCUSSION

Leaf variation is very common phenomenon in the plant kingdom, which is the result of biological long-term adaptation to the environment. The main function of normal leaf is photosynthesis. Abnormal leaf of *B. spectabilis* Willd, that is, bracts, much like the petals. So, its function may protect inflorescences and attract pollinators, rather than photosynthesis. Because of the stated reasons, the SD of normal leaf was significantly higher than that of abnormal leaf.

The significant positive correlation between GCL and relative leaf length suggests that relative leaf length is strongly associated with the systemic control of stomatal development. SD, SI and ED had a negative correlation with relative leaf length, that is, with the development of leaf, SD, SI and ED decreased.

Table 1. Correlation matrix for stomatal characters of normal leaf.

Character	SD	SI	ED	GCL	GCL
SD	1.00				
SI	0.85*	1.00			
ED	0.89*	0.53	1.00		
GCL	-0.76	-0.59	-0.69	1.00	
GCW	-0.90*	-0.77	-0.76	0.95**	1.00
RLL	-0.66	-0.51	-0.62	0.94**	0.90*

SD, Stomatal density; SI, stomatal index; ED, epidermal cell density; GCL, guard cell length; GCW, guard cell width; RLL, relative leaf length.
*Significant at 0.05 level; **significant at 0.01 level.

Table 2. Correlation matrix for stomatal characters of abnormal leaf.

Character	SD	SI	ED	GCL	GCW
SD	1.00				
SI	0.56	1.00			
ED	-0.12	0.70	1.00		
GCL	-2.02	-0.57	-0.89*	1.00	
GCW	-0.42	0.38	0.74	-0.45	1.00
RLL	-0.14	-0.52	-0.89*	0.97**	-0.57

SD, Stomatal density; SI, stomatal index; ED, epidermal cell density; GCL, guard cell length; GCW, guard cell width; RLL, relative leaf length.
*Significant at 0.05 level; **significant at 0.01 level.

In dicot leaves, the processes of cell division and expansion may overlap spatially, but cell expansion usually occurs after cell division (Croxdale, 2000; Volkenburgh, 1999). In several studies on dicot leaf growth, it is noted that the majority of cells are already initiated when the leaf is 20 to 50% of its final area, after which SD decreases due to expanding leaf area (Brouwer, 1963; Gay and Hurd, 1975). SD reached a peak before the leaf expanded to 10% of its final area and SD decreased from then, till leaf expansion ceased (Gay et al., 1975). SD in other species already starts to decrease at different rates at about 10% of final leaf area. However, ontogenetic changes in SD vary considerably between different species. In this study, SD decreased with the development of leaf. At the early stages, cell divided and the stomatal numbers of normal leaf were larger than that of abnormal leaf; in the later stages of development, epidermal cells expanded without the formation of new stomata and the degree of cell expansion of abnormal leaf maybe larger than that of abnormal leaf.

Pandey (2007) reported that under high temperature and CO_2 enriched environment, increase in ED must have been owing to the increased epidermal cell divisions. In this study, the ED of normal and abnormal leaf has the trend of decline. At the early stages of development, differences were significant and then, ED was relatively constant. This suggests that at the early stages of development, epidermal cell division occurs and then the degree of cell expansion became larger than that of cell division.

Holland (2009) showed that guard cell length generally increased with elevation. Pandey (2007) reported that the changes in the length and width of guard cell did not show a clear pattern in response to higher temperature under CO_2 enrichment, which suggests that it is a genotypic character. Franks (2001) showed that guard cell length does not change appreciably as the pore opens. In this study, the GCL of normal and abnormal leaf increased, which is in agreement with previous study. The GCW of normal leaf increased, but the GCW of abnormal leaf decreased. This maybe related with the development of stomata. The changes of guard cell size may relate with CO_2 concentration and water content. This also shows that plant needs a certain amount of pore area for photosynthesis and other physiological activities.

In this study, our analyses showed the differences between normal and abnormal leaf at the aspects of morphology and stomata distribution. But the reasons leading to the difference and the mechanism causing abnormal leaf required further study.

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