The Differential Response of Two Chrysanthemum Cultivars to Shading: Photosynthesis, Chloroplast, and Sieve Element-companion Cell Ultrastructure

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Abstract. The response to reduced light intensity of two contrasting cultivars Puma Sunny (shade intolerant) and Gongzi (shade tolerant) was characterized in terms of plant height, the root/shoot ratio, photosynthetic capacity, and the morphology and ultrastructure of their chloroplasts and phloem companion cells. The initial response to shading of cultivar Puma Sunny plants was to extend their stems, and while the equivalent response of cultivar Gongzi was less marked. Shading depressed the maximum relative electron transport rate (rETR) in both cultivars, and while the efficiency of light capture in cultivar Puma Sunny was compromised by shading, this was not the case for cultivar Gongzi. Low levels of incident light inhibited the formation of starch grains in the chloroplast and increased the volume of interspace between the grana lamellae. In cultivar Puma Sunny, but less so in cultivar Gongzi, the chloroplasts became more slender and the stroma lamellae more swollen. Adjusting chloroplast morphology by developing extra layers of grana lamellae and maintaining the integrity of the phloem companion cells are both adaptations which help make ‘Gongzi’ a more shade-tolerant cultivar.

Light intensity significantly affects plant’s morphology, so reduced levels of illumination are likely to have an impact on both the plant’s morphology, anatomy (Craven et al., 2010; Dai et al., 2009; Deng et al., 2012), physiology, cellular biochemistry, phenology, and economic yield (Craven et al., 2010; Favaretto et al., 2011; Raveh et al., 1998). The relationship between the level of photosynthetically active radiation (PAR) and the plant’s relative rETR is frequently assessed by means of the “rapid light curve” (RLC) technique, produced by plotting ETR against actinic irradiance (Belshe et al., 2007; Ralph and Gademan, 2005; Seródio et al., 2006), and thereby providing a measure of photosynthetic enzyme activation, the plant’s circadian rhythm, and activity in the lutein epoxide cycle (Ralph et al., 2002).

Although the effect of suboptimal illumination on photosynthetic activity, chlorophyll content, and chlorophyll fluorescence is well established (Craven et al., 2010; Deng et al., 2012), the relationship—if any exists—between photosynthesis and chloroplast ultrastructure has not been widely characterized. Phloem sieve elements are nonnucleated cells, largely controlled by their nucleolated companion cells, and their function is concerned with the loading and transport of assimilate from the leaf into the phloem (Wang and Huang, 2003). In maize, companion cells in the main vein have been shown to be responsible for the loading and transport of sucrose synthase into the phloem (Nolte and Koch, 1993). The rice gene OsSUT1 is expressed in companion cells and is known to be regulated both by light and the presence of sugar (Matsukura et al., 2000). Whether there is any influence of incident light on the phloem’s ultrastructure has yet to be determined.

Recently, chrysanthemum has been widely cultivated, especially in shaded greenhouse in China. The low light growth conditions affect the ornamental value. Thus, it is of commercial importance to understand the physiological causes of this constraint. Here, we describe the contrasting behavior of the shading intolerant cultivar Puma Sunny and the more tolerant cultivar Gongzi. Our aim was to contrast their responses to suboptimal illumination at the level of plant morphogenesis, photosynthetic activity, and chloroplast and phloem companion cell ultrastructure.

Materials and Methods

Plant growth and shading treatment. Samples of the chrysanthemum cultivars Puma Sunny and Gongzi were obtained from the Chrysanthemum Germplasm Resource Preserving Center, Nanjing Agricultural University. Rooted cuttings at the seven-leaf stage were planted in 4-L pots containing a 2:1:1 mixture of garden soil, vermiculite, and perlite (Han et al., 2013). The two cultivars are obviously different in morphology, growth, florescence, especially flower quality: ‘Puma sunny’ with anemone petal owns the higher ornamental value, while ‘Gongzi’ exhibits short duration tubular flower. Shading was conducted in net-house (2.5-m high, 4-m long and 4-m wide) covered with various thicknesses of commercial black shading nets, one restricted the incident light to 55% of full sunlight (referred to as “CK,” the preliminary experimental result showed the maximum PAR of 55% full sunlight was close to the light saturation point) and the other to 15% of full sunlight (referred to as “Shade”). Diurnal variations of photosynthetic photon flux density (wavelength 400–700 nm) under two light conditions were frequently monitored with external quantum sensor connected to photosynthesis system (LI-6400XT, LI-COR, Lincoln, NE), and displayed in Fig. 1. For this experiment, the rooted cuttings were subjected to two irradiance levels for 20 d. Average canopy temperatures under two contrasting light treatments were always within <1.0 °C of each other, and in no case were consistent differences in temperature detected between treatments. The average minimum/maximum temperature, photoperiod (day/night), and air humidity were 30/21 °C, 14/10 h and 70%, respectively. Rapid light curves were measured on the day 20. Each treatment involved 60 pots, at each conducted time point, three or above plants of each treatment was selected randomly and determined.

Plant growth measurement. The height of each plant was measured at 4-d intervals between days 4 and 20 following the establishment of the cuttings, at which time both cultivars expressed symptoms of shade avoidance in the “Shade” treatment, with flimsy and bigger leaf, elongated petiole and stem. At day 20, the material was harvested and divided into leaf, stem, and root, dried at 80 °C for 48 h and weighed, 10 plants per treatment.

RLC analysis. A portable pulse-amplitude modulation fluorescence system (MINI-PAM, Walz, Germany) was used to measure
fluence characteristics associated with photosynthesis. Rapid light curves were obtained using a preinstalled software routine, which required the actinic illumination to be increased in eight incremental steps starting from effective darkness. The RLC was based on the relationship $P = P_m \exp(-\alpha \times \text{PAR}/P_m) \times \exp(-\beta \times \text{PAR}/P_m)$ (Platt et al., 1981), where $P_m$ (equivalent to $R_{\text{ETR}}$ max) expresses the photosynthetic capacity at saturation, $\alpha$ the initial slope (representing the maximum light-use efficiency) and $\beta$ is a photoinhibition parameter. Parameter values were derived using a nonlinear regression method. There are three replicates per treatment.

**Chloroplast and phloem companion cell ultrastructure.** Chloroplast ultrastructure was investigated in the fourth leaf from the apex of each plant after fixation in 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for at least 48 h. Phloem companion cells were sourced from the main veins in these leaf samples. After fixation, the material was immersed in 1% (v/v) osmium acid, embedded in resin and ultrasectioned for inspection by transmission electron microscopy (H7650, HITACHI, Tokyo, Japan).

**Statistical analysis.** Statistical analysis was conducted by two-way analysis of variance, means were compared by Duncan’s test at 5% level. The light level and cultivar were treated as the two fixed factors (SPSS v17.0, SPSS Inc., Chicago, IL).

**Results**

**Plant height and root/shoot ratio.** Plant height of shaded ‘Puma Sunny’ significantly increased from day 8 to 16 in comparison with the control but reached plateau day 20. The height of cultivar Gongzi plants was rather less affected by low light intensity, but the shaded plants were nevertheless statistically taller than the nonshaded ones on day 20 (Fig. 2). The effect of the low level of incident light was to reduce the root/shoot ratio in both cultivars, although the magnitude of the effect was smaller for cultivar Gongzi (Fig. 3).

**RLC analysis.** RLC describe the response of plants to a range of light level. Figure 4 shows clearly that average $r_{\text{ETR}}$ is significantly influenced by light irradiance. Under the “Shade” regime, the $P_m$ and $E_k$ values of both cultivars were substantially lower than under the “CK” regime; however, although the $\alpha$ value of cultivar Puma Sunny was substantially affected by the light intensity, that of cultivar Gongzi was not (Table 1).

**Chloroplast ultrastructure.** Both chloroplast size and shape were materially influenced by the level of incident light (Fig. 5). The abundance of chloroplasts and the number of starch grains within each chloroplast were both inhibited by low light conditions. In cultivar Puma Sunny exposed to the “Shade” treatment, the chloroplasts became elongated (Fig. 5E), their stroma appeared irregular and swollen and their grana lamella interspaces were wider (Fig. 5F). In contrast, the appearance of the majority of stroma lamellae in cultivar Gongzi, whether grown under the “CK” or the “Shade” regime, was regular; however, the grana lamellae of plants of this cultivar subjected to low light intensity were thickened compared with those of plants grown under the “CK” regime (Fig. 5H and K).

**Phloem companion cell ultrastructure.** Companion cells in plants of both cultivars grown under “CK” conditions tended to contain more cytoplasm and a greater number of inclusions than those in plants grown under “Shade” conditions (Fig. 6). The effect of low incident light was to depress the amount of cytoplasm and the number of mitochondria present in cultivar Puma Sunny companion cells, to distort the shape of the companion cell nucleus and to promote vacuolization (Fig. 6B). In cultivar Gongzi, the same effect was observed with respect to the companion cell cytoplasm and mitochondria, but there was no change in the shape of the nucleus (Fig. 6D). Overall, therefore, the companion cell ultrastructure of this cultivar was less affected by low light intensity than that of cultivar Puma Sunny.

**Discussion**

Exposure to low intensity light has profound effects on plant morphology, as shown by its influence on height, internode length, root growth, and phenology (Cavagnaro and Trione, 2007; Craven et al., 2010; Deng et al.,
2012; Quero et al., 2006). Some of these characters are similarly affected in chrysanthemum, in particular, plant height (Fig. 2) and the root/shoot ratio (Fig. 3). The reallocation of biomass induced by low levels of incident light is consistent with the “balanced growth hypothesis,” which suggests that the effect of shading is to induce plants to divert energy supply from root to shoot growth to promote height as an escape from shade (Shipley and Meziane, 2002).

The ability to adjust to different light intensities can vary from cultivar to cultivar within a given species (White and Critchley, 1999). One such adaptation involves resetting the photosynthetic machinery to cope with a changed light regime. Medium- and short-term adjustments in photosynthetic efficiency can be captured by RLC analysis (Walhoff et al., 2002), since this technique delivers information regarding the saturation characteristics of electron transport, as well as being able to quantify overall photosynthetic performance (Ralph and Gademann, 2005). A clear effect of low light intensity was to reduce both the rETRmax and the minimum saturating irradiance (Table 1), consistent with the behavior of seagrass (Ralph and Gademann, 2005). When exposed to low light intensity, the α value of cultivar Puma Sunny was lower than that of plants of the same cultivar exposed to more intense light (Table 1), suggesting that exposure to 15% natural light reduced photosynthetic activity. The analysis demonstrated that cultivar Gongzi was more adaptable than cultivar Puma Sunny, and so was better able to maintain its level of photosynthetic activity under low-intensity light conditions.

The core reaction of photosynthesis takes place within the thylakoid, so the structure and quantity of thylakoid membrane present is a critical determinant of photosynthetic activity (Allen and Forsberg, 2010). The chloroplasts of plants exposed to the “CK” light regime were well developed, forming well-organized grana and stroma lamellae (Fig. 5A–C). However, in the leaves of cultivar Puma Sunny plants exposed to 20 d of the “Shade” regime, the chloroplasts appeared slender, the stroma swollen, and the thylakoid membrane disorganized (Fig. 5D–F), reminiscent of the reaction of jasmine plants when stressed by shading (Deng et al., 2012). The development of a higher number of grana lamellae is the one of adaptive mechanisms used by cultivar Gongzi to tolerate shading stress. The stacking of the thylakoids improves the plant’s ability to capture light and to increase its efficiency of energy transfer (Staehelin and Arntzen, 1986).

The amyloplast is the site of starch storage, so a relevant indicator of photosynthetic efficiency is the number of amyloplasts present (Zhang et al., 2010). In jasmine plants subjected to shading, the number of starch grains formed within each chloroplast is lower (Deng et al., 2012). The same behavior was shown by chrysanthemum, as the chloroplasts present in the “CK” treated leaves contained more starch grains than did those in the “Shade” treated leaves (Fig. 5A, D, G, and J). The effect was more evident in cultivar Puma Sunny than in cultivar Gongzi, a further expression of the superior shade tolerance of the latter cultivar.

Companion cells in plants exposed to full sunlight form a dense cytoplasm that contains abundant mitochondria, endoplasmic reticulum, multivesicular bodies, vesicles, and plastids (Wang and Huang, 2003). The vacuolization of the cytoplasm was an indication that weak light triggers the breakdown of the cytoskeleton (Hoffmann-Thoma et al., 2001). Vacuolization and irregular nuclei were commonly observed in cultivar Puma Sunny plants subjected to the “Shade” treatment (Fig. 6B), symptomatic of a weakened cytoskeleton. The efficiency with which assimilate is loaded into the phloem depends on the status of the companion cell. When this has been compromised by shading stress, the flow of assimilate through the phloem will also be reduced (Wang and Huang, 2003). In pea and spinach, transferring plants from low- to high-light intensity increases the

Table 1. The rapid light curve fitting parameters used for plants grown under 55% (CK) and 15% (Shade) full sunlight.

| Light irradiance | Cultivars | Pm     | α     | E50 |
|------------------|----------|--------|-------|------|
| 55% (CK)         | Puma Sunny | 45.59 ± 2.3 a | 0.41 ± 0.01 a | 110.10 ± 6.7 a |
| 15% (Shade)      | Gongzi   | 39.51 ± 1.6 ab | 0.43 ± 0.03 a | 92.83 ± 7.4 a |
|                  | Puma Sunny | 21.18 ± 0.8 c | 0.24 ± 0.01 b | 90.56 ± 6.0 a |
|                  | Gongzi   | 27.56 ± 0.4 bc | 0.43 ± 0.01 a | 68.36 ± 2.7 b |

Significance

| C     | L     | C × L |
|-------|-------|-------|
| NS    | *     | *     |
| **    | NS    | NS    |
| **    | **    | NS    |

A two-way analysis of variance based on cultivar (C) and light treatment (L) as the fixed factors, and the interaction (C × L) is shown for each parameter.

*P < 0.05; **P < 0.01.

Not significant.

Data shown in the form of mean value ±SE (n = 3, one leaf per replicate).

Fig. 5. Chloroplast ultrastructure in the leaves of cultivar Puma Sunny (A–F) and cultivar Gongzi (G–L) at day 20 grown under “CK” (A–C, G–I) and “Shade” (D–F, J–L) light treatments. A, D, G and J: 5 μm, in B, E, H and K: 1 μm and in C, F, I and L: 250 nm.

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rate of apoplastic transport without the development of additional veins (Amiard et al., 2005). When the growing point is compromised, the transport of assimilate from source to sink tends to be reduced (Li et al., 2002). The implication of the changed morphology of the companion cells in shaded cultivar ‘Puma Sunny’ plants is that assimilate export to sink tends to be reduced (Li et al., 2002).

Fig. 6. Phloem companion cell ultrastructure in the leaves of cultivar Puma Sunny (A, B) and cultivar Gongzi (C, D) plants grown under “CK” (A, C) and “Shade” (B, D) light treatments. SE = sieve element; CC = companion cell; PP = phloem parenchyma cell; C = cytoplasm; M = mitochondrion; N = nucleus; NP = nucleoplasm; K = karyotheca; V = vesicle. Scale bar: 1 μm.

Conclusion

The more tolerant cultivar Gongzi appeared able to maintain a higher level of photosynthetic activity by expanding its grana lamellae, while cultivar Puma Sunny was unable to avoid damage to its photosynthetic machinery. Exposure to low-intensity light transformed the ultrastructure of the companion cells in plants subjected to the “Shade” treatment.

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