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Determination mechanisms of leaf anatomy and chloroplast characteristics in sun and shade leaves

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Introduction

Plants acclimate to their light environments morphologically and physiologically, and differentiation of sun and shade leaves is one of such examples. Substantial physiological and anatomical information about sun and shade leaves has been accumulated. Sun leaves with thicker palisade tissue have more photosynthetic components, such as RubisCO, cytochromes, and reaction centers than shade leaves on leaf area basis, and show higher photosynthetic rates. On the other hand few developmental studies on differentiation of sun and shade leaves have been conducted, and, thereby, we still do not know their differentiation processes. Thus, we first studied developmental processes of sun and shade leaves at the cellular level.

Plants need to sense their light environments to differentiate sun and shade leaves. We know various photoreceptors such as phytochromes and blue-light receptors. Photosystems, abundance of photosynthates and redox state of Q pool would also sense the light environment (Anderson *et al.* 1995). We have been trying to identify the light-sensing mechanism that regulates leaf differentiation.

In the shoot apex, the developing leaves are covered by elder leaves and not directly exposed to the incident light. With the unfolding of the elder leaves, developing leaves perceive incident light more directly. So the light intensity perceived by leaves would increase with their development. Thus, we hypothesized that the light environment is sensed by mature leaves. Then, the information transmitted to a developing leaf, and the developing leaf differentiates into sun or shade leaf accordingly. To examine this hypothesis, we partially shaded (or exposed) the shoot apex of the plants for six days as follows and analyzed their effects on anatomy of the palisade tissue and chloroplast ultrastructure in developing leaves.

a) LA (low light apex): mature leaves in high light but developing leaves shaded.
b) HA (high light apex): mature leaves shaded but developing leaves in high light.
c) HH (high light to high light): the whole plant under high light condition.
d) HL (high light to low light): the whole plant sifted to low light condition.

Materials and methods

Chenopodium album L. plants were grown in a growth chamber with a 14 h day / 10 h night cycle. Air temperatures during the day and night were 25˚C and 18˚C, respectively. PPFD at the plant level was 360 μmol m^{-2} s^{-1} (high-light) or 60 μmol m^{-2} s^{-1} (low-light). Because of their similar lamina length, leaves above the 10th true leaf were used for analyses. The tissue sample was taken from the basal part of the leaf, near the mid vein, with a razor blade. The specimens were fixed in glutaraldehyde in a cacodylic acid buffer

at pH 7.2 overnight and subsequently in osmium tetroxide for three hours, dehydrated with acetone series, and embedded in Spurr's resin. For light microscopy, cross sections of 1 µm thick were made. Sections were stained with 0.5% toluidine blue. Light micrographs were taken using a digital camera. For chloroplast observation with TEM, 40 nm thick cross sections were made and stained with uranium acetate and lead citrate.

The partial shading

A preliminary study showed that palisade tissue cells of sun leaves started periclinal division when the lamina length was 8 mm. The leaves with laminas less than 8 mm did not show any anatomical differences. So the leaves with lamina length less than 8 mm were subjected to various light treatments. For the low-light apex treatment (LA), the shoot apex with new leaves was covered with a cap made of black shading cloth and the rest of mature leaves were exposed to high-light. In the high-light apex treatment (HA), the shoot apex was exposed and the other mature leaves were covered with black shade cloth. LA and HA treatments lasted for 6 days. After the treatment, tissue samples were taken from the young leaves, whose laminas had been less than 8 mm at the onset of the shading treatment.

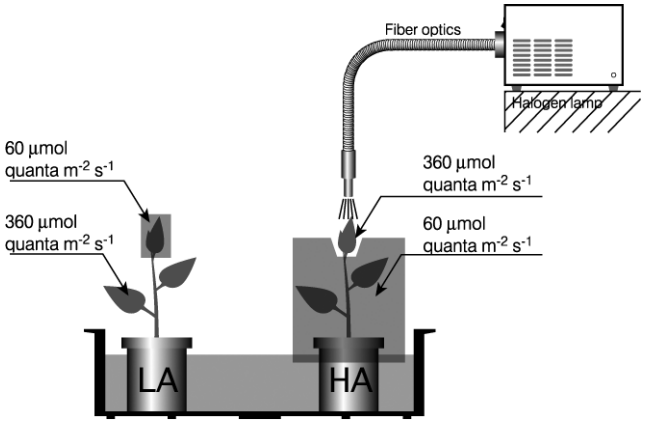

Fig. 1 **LA and HA treatment**

Quantification of leaf anatomy

Cell walls of the palisade tissue cells were traced on the light micrograph on a computer screen by the aid of a photo retouch software. Leaf thickness, cross-sectional area, width, and height of the cellular column, and number of the cells in the palisade tissue were examined with an image analysis software (NIH Image, see Fig. 2 for detailed calculations). A "column" denotes vertical cellular pillar in the palisade tissue whether it consists of one cell or two cells. From these, the average number of cell layers in the palisade tissue (CLP) and the total palisade tissue cell number index (TPN) were calculated.

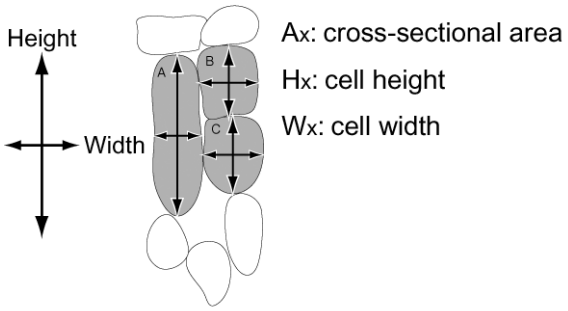

Fig. 2 Analyses of leaf anatomy. When there are two columns consisted of one cell and two cells, respectively, calculations are made as follows.
Cross-sectional column area = {Aa+(Ab+Ac)} ÷ 2
Column height = {Ha+(Hb+Hc)} ÷ 2
Column width = {Wa+(Wb+Wc) ÷ 2} ÷ 2

CLP = Total number of cells in the palisade tissue / Number of cell columns

TNP = (Total number of cells in the palisade tissue on a section / Section width) x Lamina length

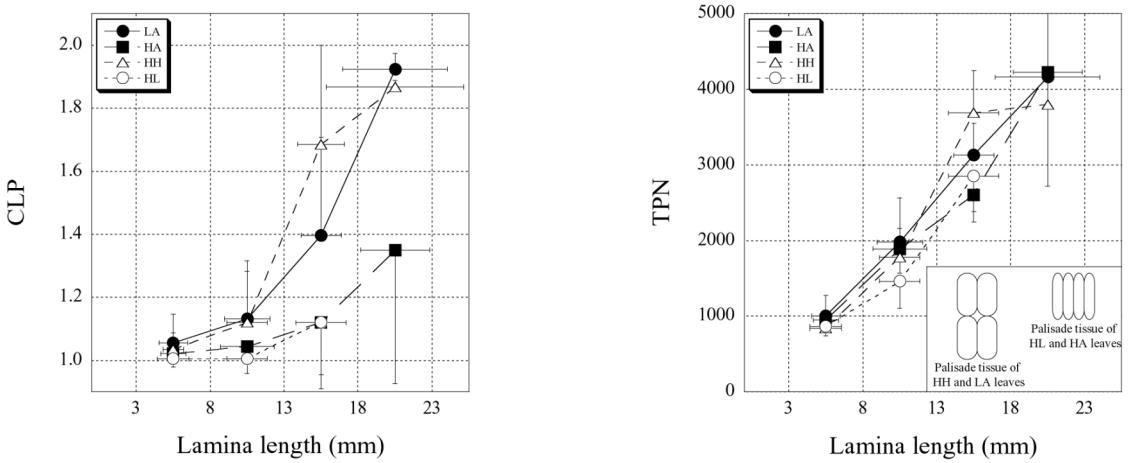

Fig. 3 CLP (a) and TPN (b), both plotted against lamina length at harvest after the treatments for six days. Horizontal and vertical bars indicate standard deviations.

Results & Discussion

3-1. Development of leaves

The periclinal division of the cells in the palisade tissue started when lamina attained 8 mm length in HH/LA leaves. But few periclinal divisions were observed in HL/HA leaves (Fig. 3). TPN increased linearly with the increase in lamina length in HH, HL, LA, and HA leaves (Fig. 3). There was no detectable difference in TPN between leaves of the same lamina length. The width of the column in the palisade tissue of HH/LA leaves started to increase when lamina attained 15 mm length, but it did not increase in the HL/HA leaves (data not shown). So we can conclude that the palisade tissue cells of HH/LA leaves divided both periclinally and anticlinally, but that in HL/HA leaves underwent only anticlinal division. The palisade tissue thickness and the leaf thickness increased markedly with the increase in lamina length in sun leaves, but less in shade leaves (data not shown). These results indicate that the differentiation of sun and shade leaves is caused by the difference in the direction of cell division.

3-2. Site for light sensing

The leaves under LA conditions developed two-layered palisade tissue (Fig. 3), while leaves under HA conditions developed one-layered palisade tissue (Fig. 3). Furthermore, the cross-sectional column area (Fig. 4) in the palisade tissue, the cross-sectional cell width (data not shown), palisade tissue thickness (Fig. 4), leaf thickness (Fig. 4) of the LA leaves were similar to those of the HH leaf, and those of the HA

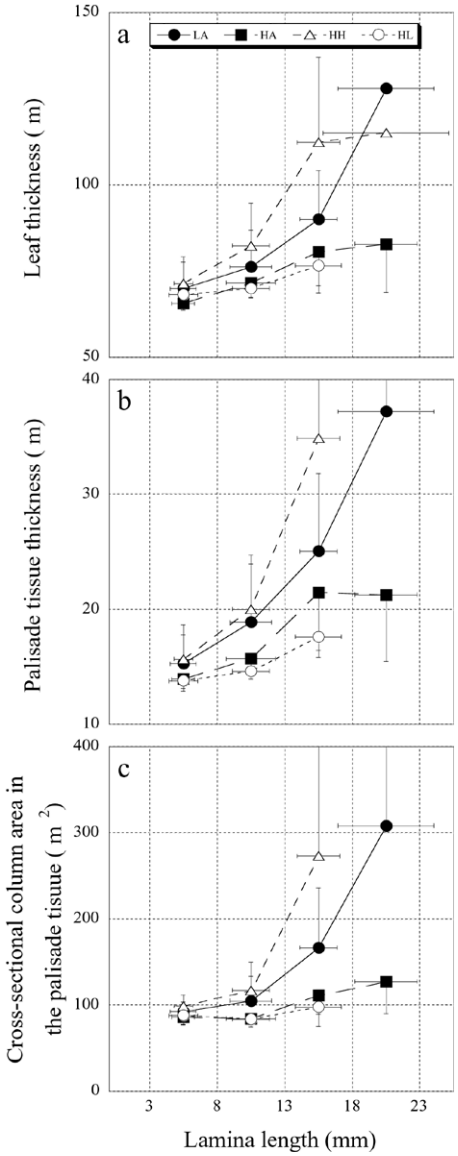

Fig. 4 Changes in leaf thickness (a), palisade tissue thickness (b) and in column area (c). Horizontal and vertical bars indicate standard deviations.

leaf were similar to those of the HL leaf. These indicate that the light sensor that determines anatomy of the developing leaves exists in mature leaves but not in the developing leaves. It is interesting that not only the number of cell layers, but also cell growth (area, width, height) was regulated by light environment of the mature leaves.

There are some candidates regulating leaf development in response to light environment; phytochromes, blue light receptors, photosystems, carbohydrate supply, and redox state of Q pool. So far, four blue light receptors have been identified; CRY1, CRY2, NPH1, and NPL1. Weston *et al.* (2000) tested leaf anatomical response to light using blue-light-perception mutants of *Arabidpsis thaliana* (except for NPL1). But no significant differences in leaf anatomy were observed.

Phytohrome regulations of photomorphogenic processes in plants are well reported. The red/far-red ratio is the major determinant for the phytohrome action in plants (Sims 1994). The red/far-red ratio for daylight ranges from 1.05 to 1.25, and that for canopy shade ranges from 0.05 to 1.15. The red/far-red ratio in this study was 2.5 (fluorescent tube) or 4.4 (halogen light). So the red/far-red light condition is "super sunny" in this study, which excludes the role of phytochrome in the differentiation of sun and shade leaves.

In high atmospheric CO_2 concentration, plants develops thick leaves independently to light environment. Taken all these into account, the role of photosynthates as light signals from mature leaves to the developing leaves is most probable. But, further studies are required to clarify the light-sensing mechanisms regulating leaf anatomy.

Recently, Lake et al. (2001) reported that stomatal density of new leaves was affected by light intensity or atmospheric CO_2 concentration around mature leaves in *A. thaliana*. They proposed long-distance signaling from mature leaves to developing leaves. We do not know the nature of this signal. However, it is likely that the same signal regulates not only stomatal density but also leaf and palisade tissue anatomy.

3-3. Chloroplast ultrastructure

Chloroplasts in LA leaves had many grana and showed characteristics of "the shade type chloroplasts." In HA leaves, chloroplasts had fewer grana than those of LA leaves and had characteristics of "the sun type chloroplasts." These indicate that chloroplasts development was influenced by their local light environment.

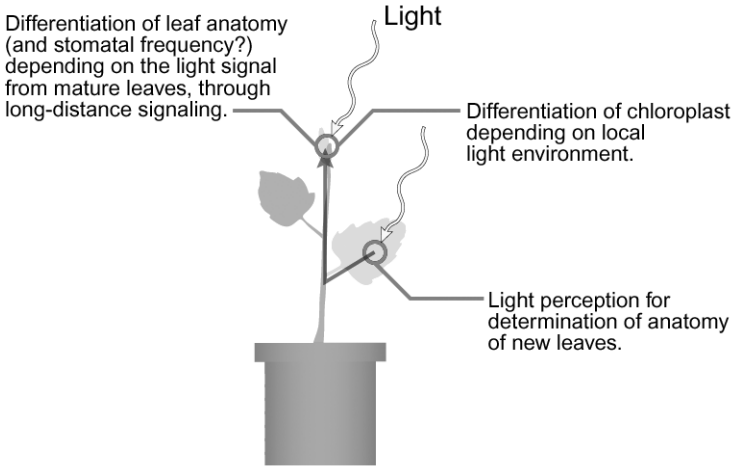

Fig. 5 A model of light sensory mechanisms for developing leaves. Anatomical characteristics of developing leaves are regulated by long distance signal, which bears light environment information sensed in mature leaves (shaded parts indicate the palisade tissue). Chloroplast characteristics of developing leaves are regulated by local light environment.

4. Conclusion

From these results, in sun-shade leaf differentiation, light sensory mechanism(s) for the leaf anatomical development is localized on mature leaves. But that for the chloroplasts is localized on developing leaves. So we can conclude that plants delocalize two light sensory mechanisms, for leaf and chloroplast development, to mature and new leaf, respectively (see Fig. 5).

LA leaves had sun-type leaf anatomy and shade-type chloroplasts, and HA leaves had shade-type leaf anatomy and sun-type chloroplasts (Fig. 6). Thus the present partial shading method enables us to control leaf anatomical and/or chloroplast characteristics separately, and to produce leaves with different anatomical characteristics without changing chloroplast characteristics. Then it would be possible to experimentally examine how much leaf anatomical characteristics contribute to leaf photosynthesis. A study in this line is in progress.

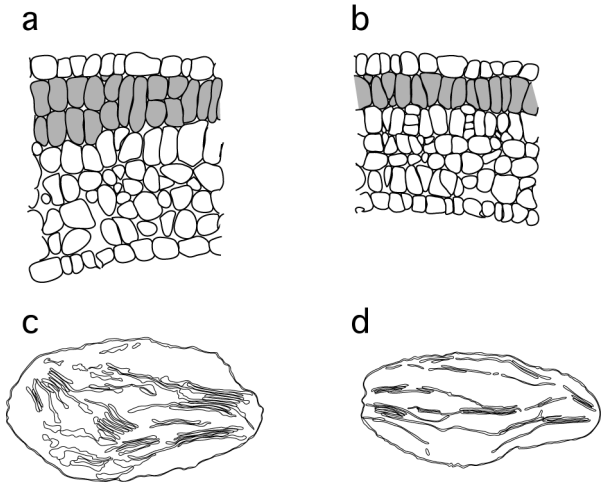

Fig. 6 Traced illustration of LA leaf cross section (a), HA leaf cross section (b), a LA leaf chloroplast (c) and a DA leaf chloroplast (d). Shaded parts in (a) and (b) represent the palisade tissue.

Acknowledgement

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