A Vertical Gradient of the Chloroplast Abundance among Leaves of Chenopodium album

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The abundances of chloroplasts in leaves on the main stems of Chenopodium album at different height levels were investigated in relation to the photosynthetic capacity and light environment of the leaves. (1) The number of chloroplasts per mesophyll cell decreased with descending position of leaves, except for young developing leaves at the top of plants that had smaller chloroplast numbers per cell than matured leaves beneath them. Contents of chlorophyll and ribulose-1,5-bisphosphate carboxylase/oxygenase per leaf area were highest in the topmost young leaves and decreased with decreasing height level indicate that there is a vertical gradient of chloroplast abundance per leaf area decreasing from the top of the leaf canopy with depth. (2) Light-saturating rate of photosynthetic oxygen evolution per leaf area of matured leaves decreased more steeply with decreasing leaf position than the chloroplast number per cell. (3) The chloroplast number per cell in newly expanded second leaves was comparable to those in leaves that have developed at later stages of the plant growth but decreased gradually during leaf senescence both in the dark and light. The formation of the vertical gradient of chloroplast abundance is, therefore, ascribed to loss of whole chloroplasts during senescence of leaves. (4) Irradiance that a leaf receives is highest at the top of the plant and decreases with decreasing position of the leaf or increasing number of leaves developed at upper positions (Monsi and Saeki 1953). The light saturating rate of photosynthesis is realized in leaves at the top of the canopy but not necessarily in lower leaves in shade. Individual leaves located at different height levels, therefore, contribute differently to the photosynthetic production of the whole plant.

The entire process of photosynthesis takes place in chloroplasts. The photosynthetic capacity of a leaf is, therefore, determined by the quantity and activity of chloroplasts present per unit area of the leaf. For understanding of the photosynthetic organization of a whole plant, it is essential to investigate distribution of chloroplasts among leaves developed at different height levels in relation to their light environments. It would be beneficial for plants to have a higher concentration of chloroplasts in the top leaves which receive full sun light than in lower leaves in shade. Because chloroplasts carry a major proportion of proteins (Morita 1980, Makino and Osmond 1991) and all the chlorophyll molecules present in leaf cells, and because nitrogen, an essential constituent of proteins and Chl, is very often a limiting nutrient for naturally growing plants, the photosynthetic productivity of a plant would be improved if chloroplasts in lower leaves serve as a source of nitrogen for synthesis of Chl and proteins in upper developing leaves. There are several lines of evidence supporting the occurrence of such a recycling of nitrogen in plants. The number of chloroplasts per mesophyll tissue or cell decreases gradually during senescence of leaves (Kura-Hotta et al. 1990, Ono et al. 1995) and the loss of chloroplasts is accelerated in the absence of nitrogen nutrient (Ono et al. 1995). Mae and Ohira (1981) showed that more than 60% of nitrogen in the youngest leaf blade of rice derives from older leaves. The nitrogen content of a leaf is highest at the top of canopies of various plants and decreased with depth (Field 1983, DeJong and Doyle 1985, Hirose and Werger 1987a, b, Werger and Hirose 1991). A recent study with a vine which had been grown horizontally to avoid mutual shading of leaves showed that the gradient of nitrogen contents in leaves is generated by advance of the leaf age at low nitrogen availability but also strongly regulated by light environment of leaves (Hikosaka et al. 1994).

In the present study, the vertical distribution of chloro-

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plasts among leaves of *Chenopodium album* L. was investigated in relation to light environment of leaves. The numbers of chloroplasts per mesophyll cell in leaves attached to the main stems of the plants at different height levels were determined. Light-saturating rates of photosynthesis and contents of Chl and RuBP carboxylase of the leaves were also measured. An experiment was carried out with newly developed second leaves to examine effects of the age and light environment of leaves on the vertical distribution of chloroplasts.

**Materials and Methods**

The plant used was a broad-leaved summer annual, *Chenopodium album* L. The seeds were stratified at 4°C for 2 weeks and sown on Jun 11, 1993, in a plot in the campus of Toho University (35°42’N). Plants were fertilized once a week with a nutrient solution (4 ml Hyponex 5-10-5, Hyponex Japan, per m²) and watered every two days. Experiments were performed from Aug. 2 through Sept. 30 with plants of about 1 m in height that were grown at a relatively high population density (about 20 plants per m²).

Seedlings with newly expanded second leaves were also used. Plants were grown on vermiculite in stainless pots (20 cm x 24 cm) which were placed in a growth chamber. Temperature and relative humidity were kept at 25°C and 75%, respectively. Irradiance was 250 μmol photons m⁻² s⁻¹ at the top of seedlings and light and dark periods were 14 and 10 h, respectively. Irradiance was determined with a Li-Cor quantum sensor. Plants were watered everyday but no nutrients were added.

Photosynthetic evolution of oxygen was determined with a Hansatech leaf disc oxygen electrode at 25°C and in air containing 4% CO₂. Leaf discs were illuminated with white light of 2,000 μmol photons m⁻² s⁻¹ from a 150 W halogen lamp (Luminar Ace LA-150SE, Hayashi) through a Hoya HA heat-absorbing filter.

Materials and Methods

The leaves used for the assay of photosynthetic oxygen evolution were analyzed for the numbers of chloroplasts per mesophyll cell. At least 100 cells that contained approximately equal numbers of palisade tissue cells and spongy tissue cells were examined for each leaf sample. As illustrated in Fig. 2, the number of chloroplasts considerably varied even among cells derived from a single leaf segment. There was, however, a trend that the number of chloroplasts per cell gradually decreases with decreasing position of leaves, except for the top two leaves. Thus, the mean number of chloroplasts per cell showed a maximum at 70

**Results**

Plants that had been grown at a relatively high population density had thin and erect main stems with many leaves attached, although leaves below 10 cm above-ground height had been mostly withered at the time of experiments. From each main stem, leaves were harvested at 10 cm intervals and analyzed for the photosynthetic activity and abundance of chloroplasts. Fig. 1 shows rates of photosynthetic oxygen evolution in leaves at different above-ground heights. The activity determined on the basis of leaf area was highest in the topmost leaf and decreased with descending position of leaves. The rate of oxygen evolution in the lowest leaves was about 24% of that of the top leaf.

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**Fig. 1** Rates of photosynthetic oxygen evolution in leaves at different above-ground heights. Each data point represents mean of oxygen evolution rates determined with 4 leaf discs. Bars indicate standard deviations.
A vertical gradient of the chloroplast abundance

Fig. 2 Frequency distribution of the chloroplast number per mesophyll cell in leaves at different above-ground heights. Number in each figure shows above-ground height in cm.

cm above-ground height (Fig. 3). Cells from the spongy tissue, round in shape, contained larger numbers of chloroplasts than did elongate cells from the palisade tissue, but similar vertical distributions of the chloroplast number with a maximum at 70 cm above-ground height were found in both types of cells. The activity of leaf photosynthesis is, therefore, related to the number of chloroplasts per cell up
to 70 cm above-ground height, but not above. When leaves at 10 cm and 70 cm above-ground height are compared, loss of the photosynthetic capacity was significantly larger than that of the chloroplast number per cell.

A C. album leaf that expanded at an early stage of plant growth had a smaller area than a leaf that developed later. The areas of the leaves employed in the above experiments are shown in Fig. 4. Here again, a maximum was

Fig. 3 Mean numbers of chloroplasts per mesophyll cell in leaves at different above-ground heights. •, total cells; ○, cells from spongy tissues; △, cells from palisade tissues.

Fig. 4 Areas of leaves at different above-ground heights. Each data point represents mean of areas of 6 leaves. Bars indicate standard deviations.

Fig. 5 Chlorophyll contents and chlorophyll a/b ratios of leaves at different above-ground heights. •, Chl contents; ○, Chl a/b ratio. Each data point represents mean of Chl contents or Chl a/b ratios of 6 leaves. Bars indicate standard deviations.
found at 70 cm above-ground height and the leaf area sharply decreased at higher positions. This indicates that the top two leaves were still developing. The small numbers of chloroplasts per cell found in these leaves are, therefore, related to immature states of the leaves.

The following experiments, however, provided evidence that the developing leaves are most abundant in chloroplast on the basis of leaf area. In contrast to the chloroplast number per cell, the content of Chl per leaf area was the highest in the top two leaves and gradually decreased with decreasing leaf position, accompanied by a monotonous decline in Chl a/b ratio (Fig. 5). The top leaves were also most abundant in RuBP carboxylase on the basis of leaf area (Fig. 6). The content of the enzyme protein decreased more steeply than that of Chl in lower leaves. Because Chl-proteins and RuBP carboxylase are major membrane and soluble proteins of chloroplasts, respectively, the results indicate that the abundance of chloroplasts per unit leaf area (but not per cell) is highest in the youngest leaves at the top of plants and decreases with decrease in the leaf position. This is the reason why the developing leaves showed the highest photosynthetic capacity per leaf area. No good correlation was found between the photosynthetic capacity and the content of RuBP carboxylase. This may be ascribed to measurement of photosynthesis in the presence of a saturating concentration of CO₂.

Light environment inside the canopy was monitored under different light conditions and typical data determined on a uniformly overcast day are shown in Fig. 7. Irradiances near the upper surfaces of leaves that attached to the main stems were measured with 10 cm intervals from the top to the bottom of plants using the sensor with the head plane held parallel to ground. Note that irradiance was attenuated by more than 90% by the top 20 cm leaf layer of the canopy. Thus, a major population of lower leaves were present in dark environments where irradiance levels were only a few percent of that above the canopy.

Finally, experiments were performed to investigate how the vertical gradient of the chloroplast number per cell during senescence of the second leaves of Chenopodium album under different light conditions. O, 250 μmol photons m⁻² s⁻¹; □, 5 μmol photons m⁻² s⁻¹; •, dark. Bars indicate standard deviations. P < 0.05 versus the next value except for between day 2 and day 4 where change in the chloroplast number of leaves kept in the light was insignificant.
is formed in the canopy of *C. album*. Effects of light were examined because, as indicated above, leaves were present in different light environments and light is known to have long-term regulatory effects on the redistribution of nitrogen among leaves (Hikosaka et al. 1994). Seedlings with newly expanded second leaves were divided into three groups; the first and second groups were kept under the growth irradiance (250 μmol photons m⁻² s⁻¹) and an attenuated irradiance (5 μmol photons m⁻² s⁻¹), respectively, and the third group was placed in the total darkness. Fig. 8 shows a typical set of the data obtained. The newly expanded second leaves originally had a chloroplast number of about 90 chloroplasts per cell which is in the range of chloroplast numbers per cell found in the leaves attached to the main stems of the plants. The chloroplast number gradually but statistically significantly decreased over 13 days of dark incubation but effect of light on the loss of chloroplasts was not clear. It is concluded, therefore, that the vertical gradient of the chloroplast number is mainly generated as a consequence of loss of whole chloroplasts during senescence of leaves.

**Discussion**

The present study indicates that there is a distinct vertical gradient of the abundance of chloroplasts per leaf area among leaves attached to the main stems of *Chenopodium album*. The number of chloroplasts per mesophyll cell decreased with decreasing leaf position among matured leaves. Exceptionally, the chloroplast number per cell decreased as the leaf position increased above 70 cm above-ground height but developing leaves at the top of plants were most abundant in chloroplasts per leaf area as judged from their contents of Chl and RuBP carboxylase per leaf area (Fig. 5, 6). Thus, the light-saturating rate of photosynthesis, which also decreases from the top of plants downward (Fig. 1), is related to the vertical distribution of chloroplasts among leaves.

Loss of the photosynthetic capacity in lower leaves is, however, larger than that of chloroplasts. Decline in the leaf position from 70 cm to 10 cm above-ground height resulted in 74% loss of the photosynthetic activity (Fig. 1) compared with 50% reduction in the chloroplast number per cell (Fig. 3). Thus, the loss of whole chloroplasts is a major but not the sole cause of the activity loss. Contents of RuBP carboxylase per leaf area sharply decreased below 70 cm above-ground height. The gradient of the Chl content per leaf area was also steeper than that of the chloroplast number per cell. Those results show that there are significant losses of these functional components from chloroplasts remaining in lower leaves. The observed declines in the light-saturating rate of photosynthesis is, therefore, ascribed partly to the loss of whole chloroplasts and partly to the reduced photosynthetic capacity of the remaining chloroplasts.

It has been shown for various herbaceous plants that the nitrogen content of a leaf decreases from the top of a canopy with depth and there is a correlation between the photosynthetic capacity and the nitrogen content of leaves (Field 1983, Hirose and Werger 1987a, b). These important features of the leaf canopies are now related to the vertical distribution of chloroplasts and chloroplast components described here because chloroplasts carry a large proportion of proteins or nitrogen present in leaf cells (Morita 1980, Makino and Osmond 1991).

A simple explanation for the formation of the vertical gradient of the chloroplast abundance in the leaf canopy of *C. album* would be that a leaf always has a smaller number of chloroplasts per cell than a leaf that develops next. Then, because a younger leaf develops at an upper position of the main stem, a vertical gradient of chloroplast number will be generated during growth of the plants. This possibility is, however, ruled out by our observation that newly expanded second leaves have a large number of chloroplasts per cell that is comparable to those of leaves developed at much later stages of the plant growth.

The recent studies demonstrated that the chloroplast number in mesophyll tissue or cells gradually decreases during senescence of leaves (Kura-Hotta et al. 1990, Ono et al. 1995). The present study also shows a gradual loss of whole chloroplasts during senescence of the second leaves of *C. album*. Effects of light on the loss of chloroplasts were not clear during the experiment period of 13 days. We conclude, therefore, that the vertical gradient of the chloroplast abundance is mainly generated as a consequence of loss of chloroplasts during senescence of leaves. Although the present study dealt with only the leaves attached to the main stems of plants, we expect that a similar gradient of the chloroplast abundance occurs among leaves developed on branches because the age of a leaf increases from the tip to the base of each branch.

The vertical distribution of chloroplasts found in the present study is of an important physiological or ecophysiological significance when light environment inside a canopy is taken into consideration. Young leaves at the top of the canopy are able to greatly contribute to the photosynthetic production of the whole canopy because they are abundant in highly active chloroplasts and receive full sun light. Contribution of leaves that are located in lower levels of the canopy is small because their photosynthesis is limited by light (see Fig. 7), unless strong irradiance reaches them in the form of sun flecks. A decline in the chloroplast number should, therefore, have only a minor influence on the photosynthetic performance of the lower leaves. Ono et al. (1995) showed recently that the number of chloroplasts per cell decreases during senescence of the primary leaves of wheat more slowly in plants that had been grown with a sufficient supply of nutrients than in plants that had been grown
without nutrients. They suggested that loss of chloroplasts is influenced by the availability of nutrients because whole chloroplasts in aged leaves serve as a source of nitrogen for synthesis of proteins in upper young leaves. Degradation of chloroplasts in senescing leaves would be, therefore, an important process to remobilize nitrogen in less or non productive leaves in shade for construction of the photosynthetic apparatus in young leaves exposed to strong sun light. Thus, the formation of the vertical gradient of the chloroplast abundance among leaves is an acclimational response of erect herbaceous plants to maintain a high photosynthetic productivity during their growth that is inevitably associated with the mutual shading of leaves.

References


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