

Possible roles of sugars on leaf senescence

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Introduction

Availability of nitrogen almost always limits plant growth. Photosynthetic activity and nitrogen content in the leaf start to decrease around at full leaf expansion. In upright plants, especially when they form dense stands, old, lower leaves are shaded with the development of younger upper leaves. Nitrogenous compounds in such shaded leaves are degraded and re-allocated to upper leaves. This process is referred to leaf senescence and important for efficient use of nitrogen in photosynthetic production. Leaf longevity is different among species. It also depends on growth conditions. Leaves senesce faster when plants are grown under high light with low nutrient supply. Carbohydrates accumulate in mature leaves under nitrogen deficiency (Radin and Eidenback, 1986; Paul and Stitt, 1993; Ono et al. 1996). Krapp et al. (1991) fed glucose to detached leaves of *Spinacia oleracea* via petiole to increase carbohydrates in the leaf. This treatment caused decreases in concentrations of photosynthetic components, such as RuBP carboxylase/oxygenase (Rubisco), chlorophyll and in the rate of photosynthesis. Sheen (1990) fed glucose and/or sucrose to the bathing medium of protoplasts of maize leaf cells, and found that expression of photosynthetic genes was suppressed. Therefore, it would be possible that such a mechanism involves in leaf senescence. To test whether the genes for photosynthetic components were suppressed in the plants at low nitrogen availability, sunflower seedlings were grown under nitrogen deficient condition (0.2 mM nitrate). The plants were transferred to high nitrate (8 mM nitrate) condition or DCMU was applied to the leaves to reduce accumulated carbohydrates in the leaves. With the decrease in the carbohydrate contents, the transcript level of the gene encoding Rubisco small subunit (*rbcs*) increased and the decrease in Rubisco was suppressed (Ono and Watanabe 1997). These results strongly suggest that sugar level in the leaf regulates leaf senescence.

If a leaf can sense its light environment or photosynthetic status relative to those of other leaves within the plant and maintain or decrease (senesce) the photosynthetic capacity, the nitrogen distribution within a plant would be maintained near the optimal condition. It is probable that a leaf senses its photosynthetic status by monitoring its sugar concentration and regulates senescence processes. Using *Phaseolus vulgaris* plants, we examined this hypothesis.

Materials and methods

Phaseolus vulgaris (cv. Yamashiro-Kuro-sando) plants were grown in the controlled environment at air temperature of 25°C, day/night cycle of 12h/12h and air humidity of 70%. *P. vulgaris* has a pair of primary leaves. From the second leaves, leaves are trifoliate. After 8 to 10 days of culture in vermiculite, primary leaves began unfolding. Before unfolding, the seedlings were transplanted in vermiculite in pots (11.3 cm in diameter, one plant per pot). In the experiment with nutrient supply, 50 ml of Hoagland's solution containing 6 mM nitrate

was given to each plant everyday. In the experiment without nutrients, only tap water was given. Plants received light at $330 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active photon flux density (PPFD). In one group of plants, younger trifoliate leaves were shaded with primary leaves being exposed. The shading treatment was started when the terminal leaflets of the 1st trifoliate leaf exceeded 2 cm. When the younger leaves were shaded, they received PPFD at $10 \mu\text{mol m}^{-2} \text{s}^{-1}$. This was below the light compensation point. Control plants were unshaded. The lamina length of the primary leaves was measured everyday. The day 0 in the figures denotes when the growth of lamina length stopped.

CO_2 assimilation rates were measured with a portable gas-exchange system (LI-6400; LICOR Inc). Measurements were made on intact primary leaves. Photosynthetic activity (A_n) was measured under the growth condition (the PPFD at $330 \mu\text{mol m}^{-2} \text{s}^{-1}$ and temperature at 25°C).

Leaves were harvested just after the onset of light period. Soluble sugars (glucose, sucrose) and starch were extracted and quantified according the method of Ono et al.(1996).

Results

Fig.1 shows the effects of the shading of the young leaves on the photosynthetic activity (A_n) of the primary leaves. In this experiment with nutrient supply, A_n decreased in the

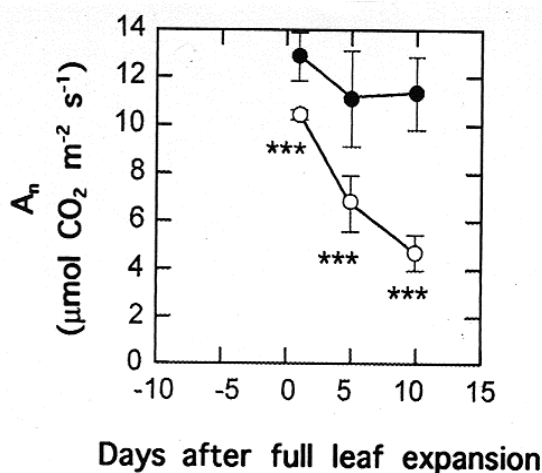
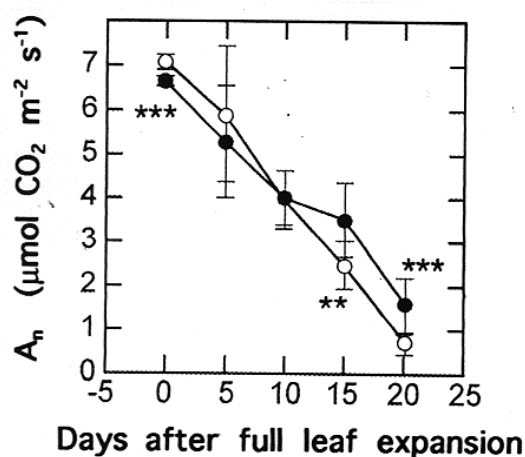


Fig.1 Changes in A_n in the primary leaves of *P. vulgaris* plants grown with nutrients. A_n was measured at $330 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the ambient CO_2 concentration of $360 \mu\text{mol mol}^{-1}$ and at 25°C . Closed circles, plants with the shaded new leaves; open circles, unshaded control plants. Each point shows the mean \pm standard deviation of more than five leaves. *** denotes statistically significant differences between the unshaded control and the shaded plants at $P < 0.001$ (Student's *t*-test).

control plants after full expansion of the primary leaves. Decrease in A_n was decelerated by shading the young leaves.

Changes in carbohydrate contents in the primary leaves with nutrient supply are shown in Fig.2. The contents of carbohydrates in the primary leaves of the unshaded control plants increased after the full leaf expansion. On the other hand, such increases in carbohydrate contents were suppressed in the primary leaves in plants with young leaves shaded.

Fig.2 Changes in the glucose, sucrose and starch contents in the primary leaves of *P. vulgaris* plants with nutrients. Leaves were harvested at the onset of light period. Closed circles, plants with the shaded new leaves; open circles, unshaded control plants. Each point shows the mean \pm standard deviation of more than five leaves. * and ** denote statistically significant differences between the unshaded control and the shaded plants at $P < 0.05$ and $P < 0.01$, respectively



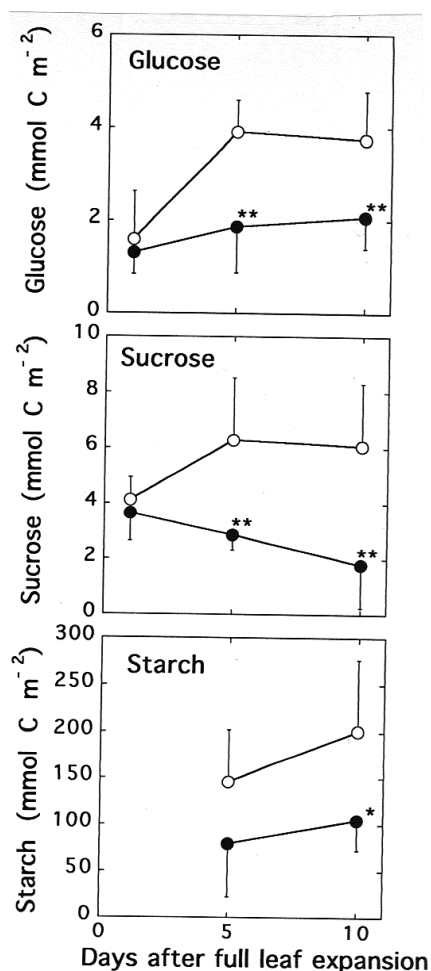


Fig.3 Changes in A_n in the primary leaves of *P. vulgaris* plants grown without nutrients. Closed circles, plants with the shaded new leaves; open circles, unshaded control plants. Each point shows the mean \pm standard deviation of more than five leaves. ** and *** denote statistically significant differences between the unshaded control and the shaded plants at $P < 0.01$ and $P < 0.001$, respectively (Student's *t*-test).

When plants were grown without nutrient supply, A_n decreased rapidly in both unshaded control and shaded plants (Fig.3). Photosynthetic activity in the shaded plant appeared to decline more slowly in later period than that in unshaded control plants. The carbohydrate contents increased markedly in both the control and shaded plants (data not shown).

Discussion

After full leaf expansion, photosynthetic rates under the growth condition started to decrease in the unshaded control plants as shown in Fig.1. Increases in carbohydrates in the unshaded control plants would be attributed to the decrease in the translocation of carbohydrates. It is probable that the marked increase in glucose content in the control plants suppresses the expression of the genes encoding photosynthetic proteins. This was supported by a preliminary northern blot analysis of *rbcS*. In the experiment without nutrient, carbohydrate contents did not decrease by shade treatment of young leaves. Under the condition, nutrient limitation was severe and growth of the younger leaves suppressed (data not shown). We cannot conclude whether sugar-repression involves in senescence process under severe nutrient deficiency. In such a case, other age-dependent mechanisms, such as enhanced protein degradation, would also operate.

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