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Regulation of Leaf Photosynthesis Through Photosynthetic Source-Sink Balance in Soybean Plants

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1. Introduction

Plant photosynthesis is the basis for matter production needed for all living organisms. In the future, plant photosynthesis would be more important, since environmental problems such as climatic warming due to increasing environmental CO_2 concentration and problems of food and energy shortages due to increasing populations may be severer (von Caemmerer & Evans, 2010; Raines, 2011). Increasing plant leaf photosynthesis and thereby increasing plant matter production would be expected as a realistic way to resolve the problems. There is, however, a well-known hypothesis that in plants leaf photosynthesis can be down regulated through accumulated photosynthetic carbohydrates in leaf under excessive photosynthetic source capacity, which also means sink limitation, although the detailed mechanism is not clear (see Kasai, 2008). Actually, for example, there is evidence for the excessive photosynthetic source capacity causing down regulation of photosynthesis in crop plants under field conditions (Okita et al., 2001; Smidansky et al., 2002, 2007). Therefore, for the better improvement of leaf photosynthesis in plants, it is important to elucidate the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity and thereby clarify way of the improvement of leaf photosynthesis.

To elucidate the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity, experimental construction of the excessive photosynthetic source capacity is important. Excising sink organs such as pods, fruits or flowers from plant materials is a way to construct excessive photosynthetic source capacity, and it has often been conducted to study the regulatory mechanism of photosynthetic source-sink balance in plants (see Kasai, 2008). However, the way excising sink organs results not directly but indirectly in excessive photosynthetic source capacity by diminishing sink capacity, and can give some damages to plant materials. Recent studies using transgenic plants have shown that overexpression of Calvin cycle enzymes (sedoheptulose-1,7-bisphosphatase and fructose-1,6-bisphosphatase) or leaf plasma membrane CO_2 transport protein increases the leaf photosynthetic rate significantly (Raines, 2003, 2006). Therefore, the use of the transgenic plants with improved higher leaf photosynthetic rate may be useful to study the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity, since the higher photosynthetic rate is likely to result in excessive photosynthetic source capacity. However, it seems difficult to analyze the down regulation of

photosynthesis that may hide in the improved photosynthetic rate. Actually, down regulation of photosynthesis that is associated with excessive photosynthetic source capacity has not been analyzed in the transgenic plants with improved higher photosynthetic rate. Exposure to high CO_2 or continuous exposure to light of plant materials is thought as the other way to construct excessive photosynthetic source capacity. It is well known that leaf photosynthetic rate, especially, in C_3 plants does not reach the saturation at the present atmospheric CO_2 concentration and thus the rate increases initially under high CO_2 conditions (Ward et al., 1999). Therefore, in C_3 plants, exposure to high CO_2 is expected to result in excessive photosynthetic source capacity. However, the way of exposure to high CO_2 may be not suitable to analyze the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity, because of the same reason described for the transgenic plants with improved photosynthetic rate and well-known action of high CO_2 to decrease stomatal aperture (Bredmose & Nielsen, 2009). In contrast, continuous exposure to light of plant materials, which prolongs photosynthetic period, can result in excessive photosynthetic source capacity without affecting directly the sink organs, leaf photosynthetic rate and stomatal aperture and giving direct damage to the plant materials.

Soybean plants, although it is single-rooted soybean leaves, have largely contributed to study the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity through the experimental system using continuous exposure to light. Single-rooted soybean leaves are source-sink model plants with a simple organization of a leaf, a short petiole and roots developed from the petiole in individuals and were developed by Sawada et al. (1986) using the primary leaves of intact soybean plants (*Glycine max* L. Merr. cv. Tsurunoko). Studies using single-rooted soybean leaves have shown that treating the plants with continuous light results in accumulation of photosynthetic carbohydrates (sucrose and starch) in the leaf and decrease in the leaf photosynthetic rate, which correlates with the increase in leaf carbohydrate (sucrose or starch) content (Sawada et al., 1986, 1989, 1990, 1992). Also, it has been shown in the single-rooted soybean leaves that deactivation of Rubisco, a CO_2-fixing enzyme is caused by the treatment of continuous exposure to light (Sawada et al., 1990, 1992). As continuous exposure to light of single-rooted soybean leaves also increased the leaf phosphorylated intermediates' contents (Sawada et al., 1989), and there have been findings that in vitro, inorganic phosphate promotes activation of Rubisco by enhancing the affinity of uncarbamylated inactive Rubisco to CO_2 (Bhagwat, 1981; McCurry et al., 1981; Anwaruzzaman et al., 1995), the studies using single-rooted soybean leaves have suggested that there is a regulatory mechanism of leaf photosynthetic rate through deactivation of Rubisco, which is associated with accumulation of photosynthetic carbohydrates in leaf under excessive photosynthetic source capacity, and that the deactivation of Rubisco may be caused by limitation of inorganic phosphate (Sawada et al., 1990, 1992). Data from a study using single-rooted soybean leaves demonstrate that the plants do not change the leaf area and leaf dry weight other than the weights of major photosynthetic carbohydrates (sucrose and starch) and grow only the roots during experimental period, irrespective of whether light conditions are normal (daily light/dark periods of 10/14 h) or continuous without darkness (Sawada et al., 1986). Although the source-sink model plants with simple source-sink organization have been developed from various plant species, only the single-rooted soybean leaves have been demonstrated to show almost no growth in the source organ (Sawada et al., 2003). No growth of the source organ and the simple organization of source and sink in the single-rooted soybean leaves are

attractive characteristics to analyze comprehensively the regulatory mechanism of photosynthetic source-sink balance in plants, including the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity. Actually, as mentioned above, various analyses have been conducted in the single-rooted soybean leaves, especially in studies for elucidating the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity. Therefore, the single-rooted soybean leaves are important plant materials to elucidate further the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity. However, the plants are made artificially, and do not exist in nature, and in addition, as already mentioned, the plant leaf originates from only the primary leaf in intact soybean plants (Sawada et al., 1986). Therefore, there is the possibility that properties of single-rooted soybean leaves may not reflect those of the original, intact soybean plants or the other intact plants. Thus, it is important to examine the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity using the original, intact soybean plants.

The present study used the original intact soybean plants, and it was analyzed how continuous exposure to light affects the leaf photosynthetic rate and related characteristics, such as leaf stomatal conductance and intercellular CO_2 concentration, contents of water, chlorophyll, major photosynthetic carbohydrates (sucrose and starch), total protein and Rubisco protein in leaf, and activity and activation ratio (ratio of initial to total activity) of Rubisco and amount of protein-bound ribulose-1,5-bisphosphate (RuBP) in leaf extract, which were analyzed to evaluate the amount of uncarbamylated inactive Rubisco (Brooks & Portis, 1988). The same series of analyses have not been conducted together in studies that have performed the experiment of continuous exposure to light using plants.

2. Materials and methods

2.1 Plant materials

Soybean (*Glycine max* L. Merr. cv. Tsurunoko) seeds were sown in plastic pots (13.5 cm in height, 12.5 cm in diameter) containing almost equal volumes of vermiculite and sand that had been mixed, and were grown in growth chambers (Koitotoron, HNL type; Koito Industries Ltd., Tokyo, Japan) under daily light/dark periods of 10/14 h, day/night temperatures of 24/17^{o}C and relative humidity of 60 %. After 8 weeks, plants were divided into two groups, and one group was grown for 3 days with continuous light, and another group was grown for 3 days under daily light/dark periods of 10/14 h as controls. Nutrients were supplied once a week with a 1000-fold diluted solution of Hyponex [6-10-5 type (N:P:K = 6:10:5); Hyponex Co., Osaka, Japan], and tap water was supplied in sufficient amounts. Light was supplied with incandescent lamps at an intensity of 480 μmol photons m^{-2} s^{-1} (400-700 nm) at the middle height of plants grown for 8 weeks.

2.2 Leaf photosynthetic rate, stomatal conductance and intercellular CO_2 concentration

Leaf photosynthetic rate, stomatal conductance and intercellular CO_2 concentration were determined in fully expanded fourth trifoliate leaves at a light intensity of 1000 μmol photons m^{-2} s^{-1}, air flow rate of 200 ml min^{-1}, air temperature of 25 ^{o}C, relative humidity of 60 % and CO_2 concentration of 350 ppm on day 3 after treating plants with continuous light

using a portable photosynthetic analyzer (Cylus-1; Koito Industries Ltd.). After measurements, leaf disks (1.79 cm^2) were cut off from fourth trifoliate leaves, immediately frozen in liquid nitrogen and stored at -80 ^oC until used for the other analyses described below.

2.3 Other analyses

The activity of Rubisco in leaf extract was determined at 25 ^oC as described previously (Kasai, 2008). For the initial activity, 20 µl of a leaf extract obtained by homogenizing a leaf disk with ice-cold buffer (100 mM HEPES-KOH, pH 7.8, 2 ml) was added to a cuvette containing 1.98 ml of assay medium [100 mM Bicine-KOH (pH 8.2), 20 mM $MgCl_2$, 20 mM $NaHCO_3$, 5 mM creatine phosphate, 1 mM ATP, 0.2 mM NADH, 20 units creatine kinase, 20 units 3-phosphoglycerate kinase and 20 units glyceraldehyde-3-phosphate dehydrogenase], immediately followed by the addition of RuBP (final concentration 0.6 mM) and mixed well. For total activity, RuBP was added 5 min later after 20 µl of the leaf disk extract was immediately combined with the assay medium. The change in absorbance at 340 nm was monitored using a spectrophotometer (Model U-2000; Hitachi Co., Tokyo, Japan).

The amount of protein-bound RuBP in leaf extract was determined as described previously (Kasai, 2008). A leaf extract (800 µl) obtained by homogenizing a leaf disk with an ice-cold buffer (100 mM HEPES-KOH, pH 7.8, 1 ml) was centrifuged (100 g, 1 min) after loading onto a column containing Sephadex G-50 (bed volume before centrifugation, 4 ml) that had been equilibrated with the same buffer. The eluent (500 µl) from the column lacking free RuBP was centrifuged (10,000 g, 10 min) after mixing with an acidic solution (5.5 M $HClO_4$, 50 µl) to precipitate protein in the eluent. The resulting supernatant was centrifuged (10,000 g, 10 min) after neutralizing to pH 5.6 with K_2CO_3, and RuBP in the supernatant was determined in the assay medium for determining Rubisco activity using purified spinach Rubisco (0.5 units).

Leaf Rubisco content was determined as described by Makino et al. (1986). Leaf total protein was extracted as described by Makino et al. (1986) and quantified by the method of Bradford (1976). Leaf chlorophyll content was determined according to the method of Mackinney (1941). Leaf sucrose and starch contents were determined as described by Sawada et al. (1999). Leaf water content was analyzed by measuring fresh weight and dry weight of leaf disks. Leaf disks were dried for 2 days at 75 ^oC.

3. Results

Analyzed leaf photosynthetic rate was significantly lower in intact soybean plants grown for 3 days with continuous light than in control plants grown under daily light/dark periods of 10/14h (Fig. 1).

Leaf stomatal conductance was also significantly lower in continuous light-treated plants than in control plants (Fig. 2). Leaf intercellular CO_2 concentration did not differ significantly between control and continuous light-treated plants (Fig. 2).

When activation ratio (percentage of initial activity to total activity) of Rubisco in leaf extract was calculated from analyzed initial and total activities of Rubisco in leaf extract, the ratio was significantly lower in continuous light-treated plants than in control plants (Fig. 3). The ratios in control and continuous light-treated plants were 74.2% and 56.6%, respectively.

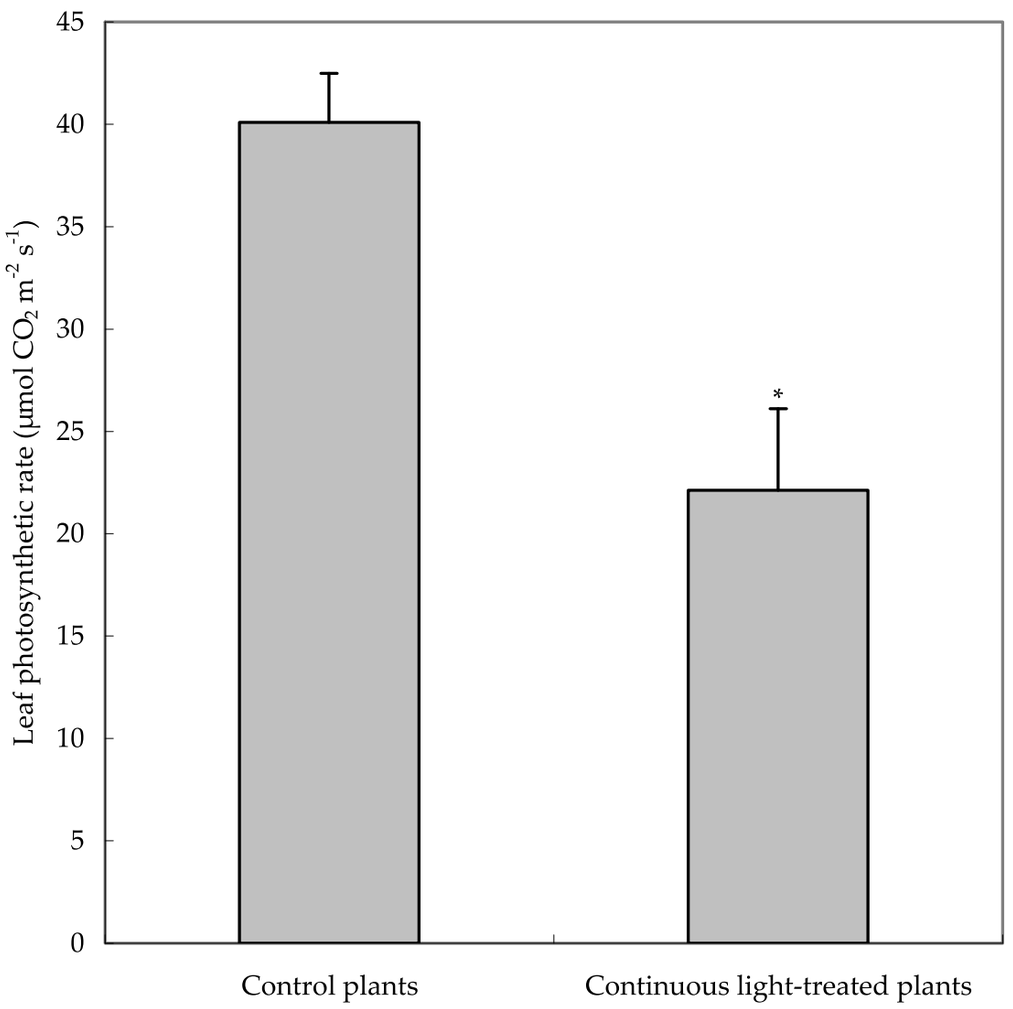

Fig. 1. Leaf photosynthetic rate in soybean plants on day 3 after continuous exposure to light. Control plants were grown under daily light/dark periods of 10/14h for 3 days. Vertical bars indicate S.D. (n=4). $^{*}P<0.01$ (*t*-test) when compared with control plants.

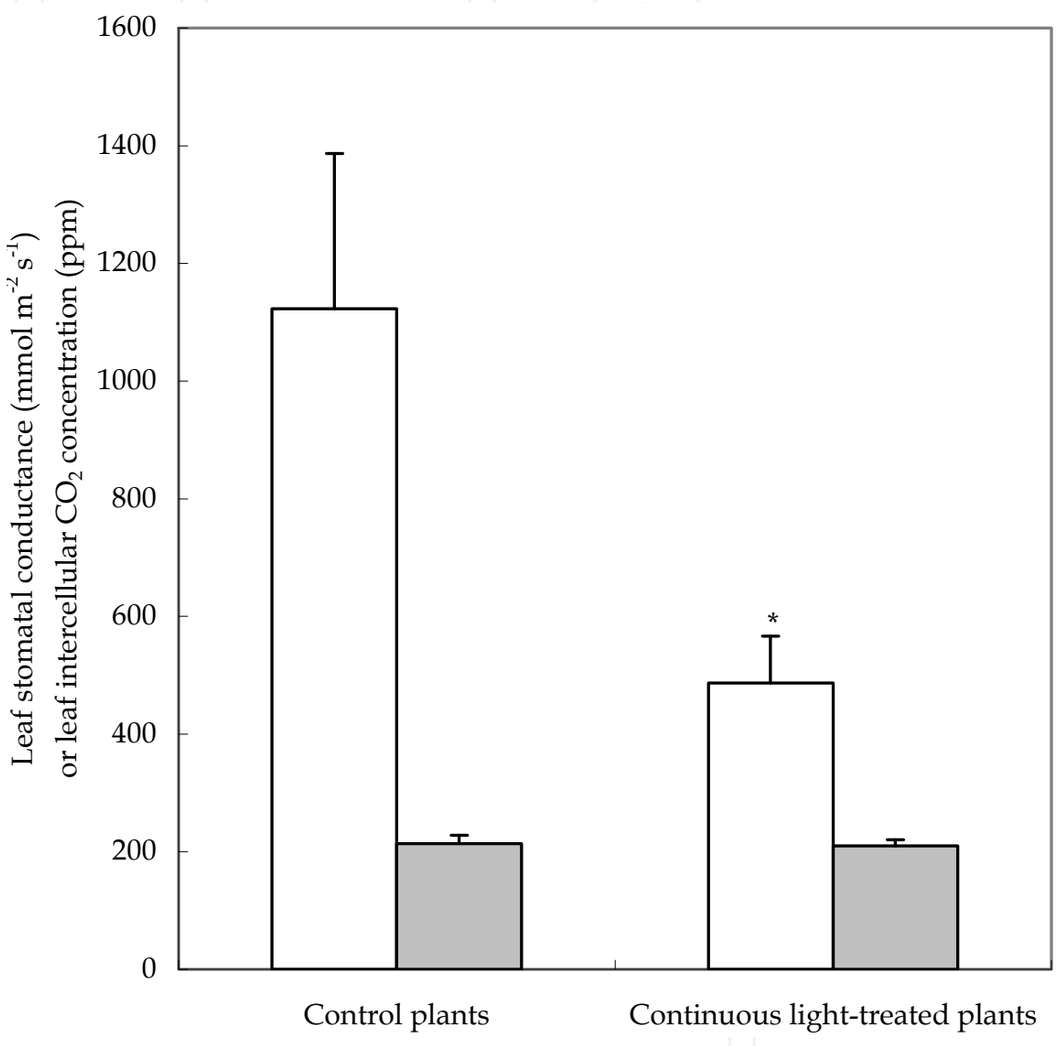

Fig. 2. Leaf stomatal conductance and leaf intercellular CO_2 concentration in soybean plants on day 3 after continuous exposure to light. Control plants were grown as described in Fig. 1. Open bar, leaf stomatal conductance; closed bar, leaf intercellular CO_2 concentration. Vertical bars indicate S.D. (n=4). $^{*}P<0.01$ when compared with control plants. The intercellular CO_2 concentration did not differ significantly ($P>0.05$) between control and continuous light-treated plants.

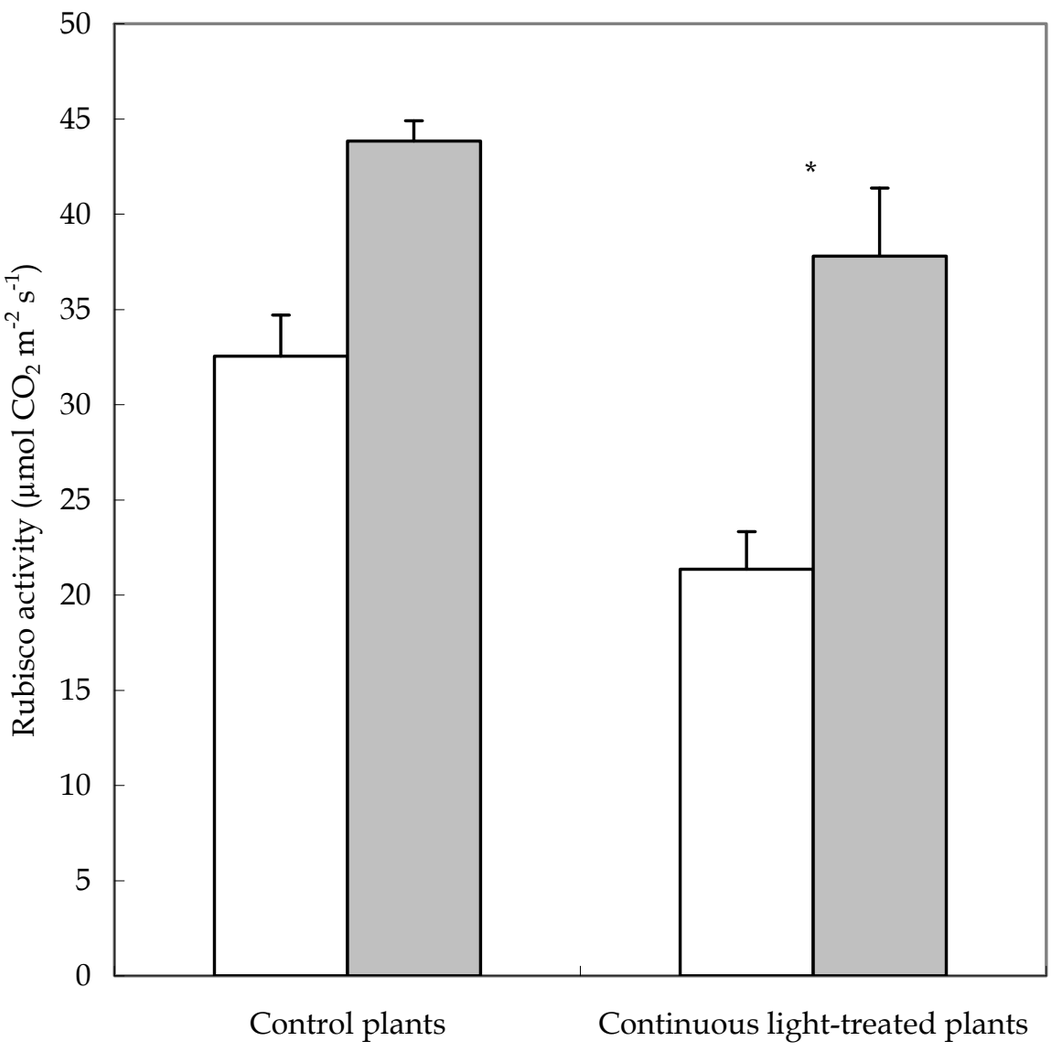

Fig. 3. Initial and total activities of Rubisco in leaf extract from soybean plants on day 3 after continuous exposure to light. Control plants were grown as described in Fig. 1. Open bar, initial activity; closed bar, total activity. Vertical bars indicate S.D. (n=4). In comparison with control plants of the activation ratio of Rubisco calculated as a percentage of the initial activity to total activity, $^{*}P<0.01$.

In a study investigating the light activation of Rubisco using *Arabidopsis thalian*, it was demonstrated that the amount of protein-bound RuBP in leaf extract reflects the amount of uncarbamylated inactive Rubisco (Brooks & Portis, 1988). When the amount of protein-bound RuBP was analyzed, the amount was significantly more in continuous light-treated plants than in control plants (Fig. 4).

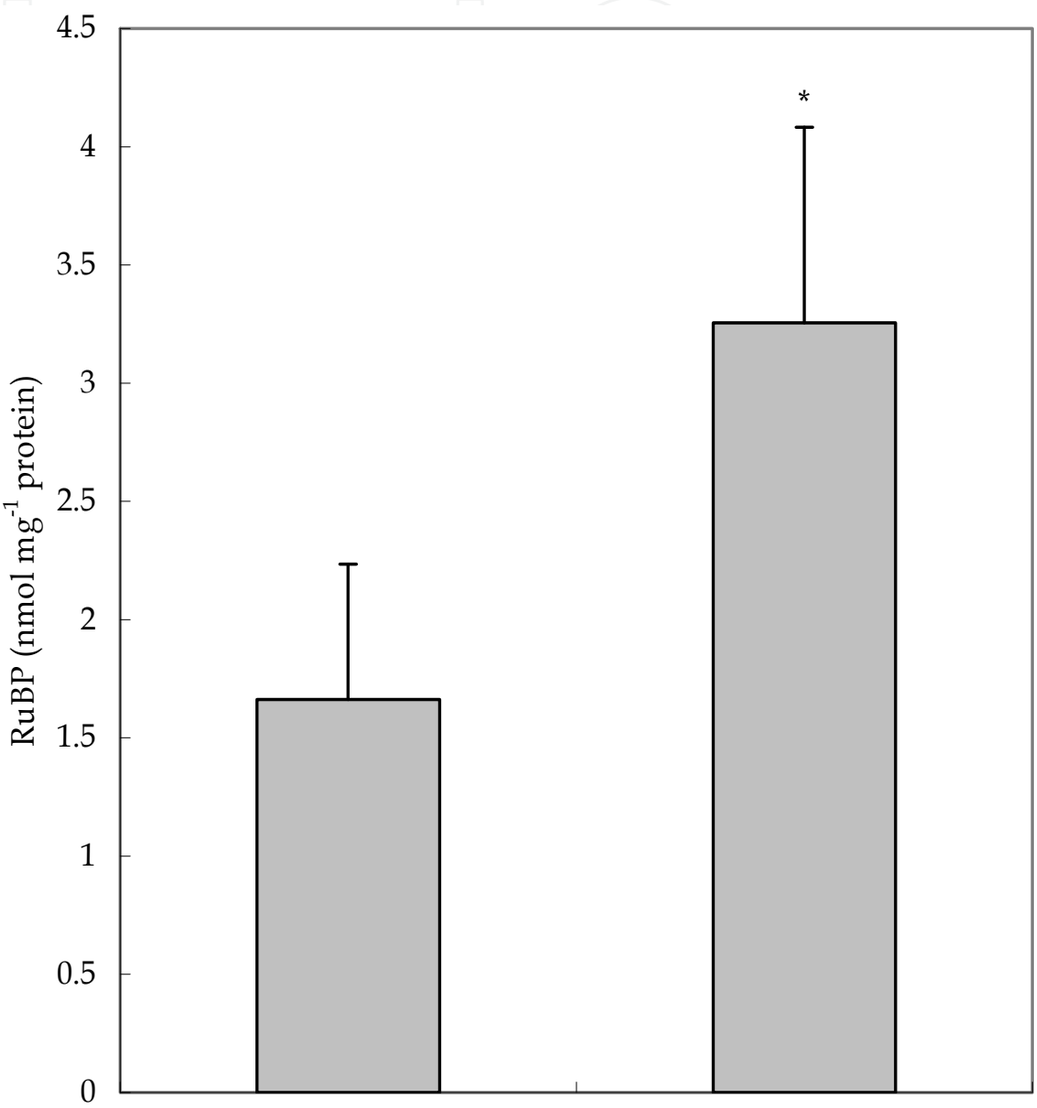

Fig. 4. Amount of protein-bound RuBP in leaf extract from soybean plants on day 3 after continuous exposure to light. Control plants were grown as described in Fig. 1. Vertical bars indicate S.D. (n=4). $^{*}P<0.05$ when compared with control plants.

Contents of sucrose and starch, which are the major photosynthetic carbohydrates, in leaf were both significantly higher in continuous light-treated plants than in control plants (Fig. 5).

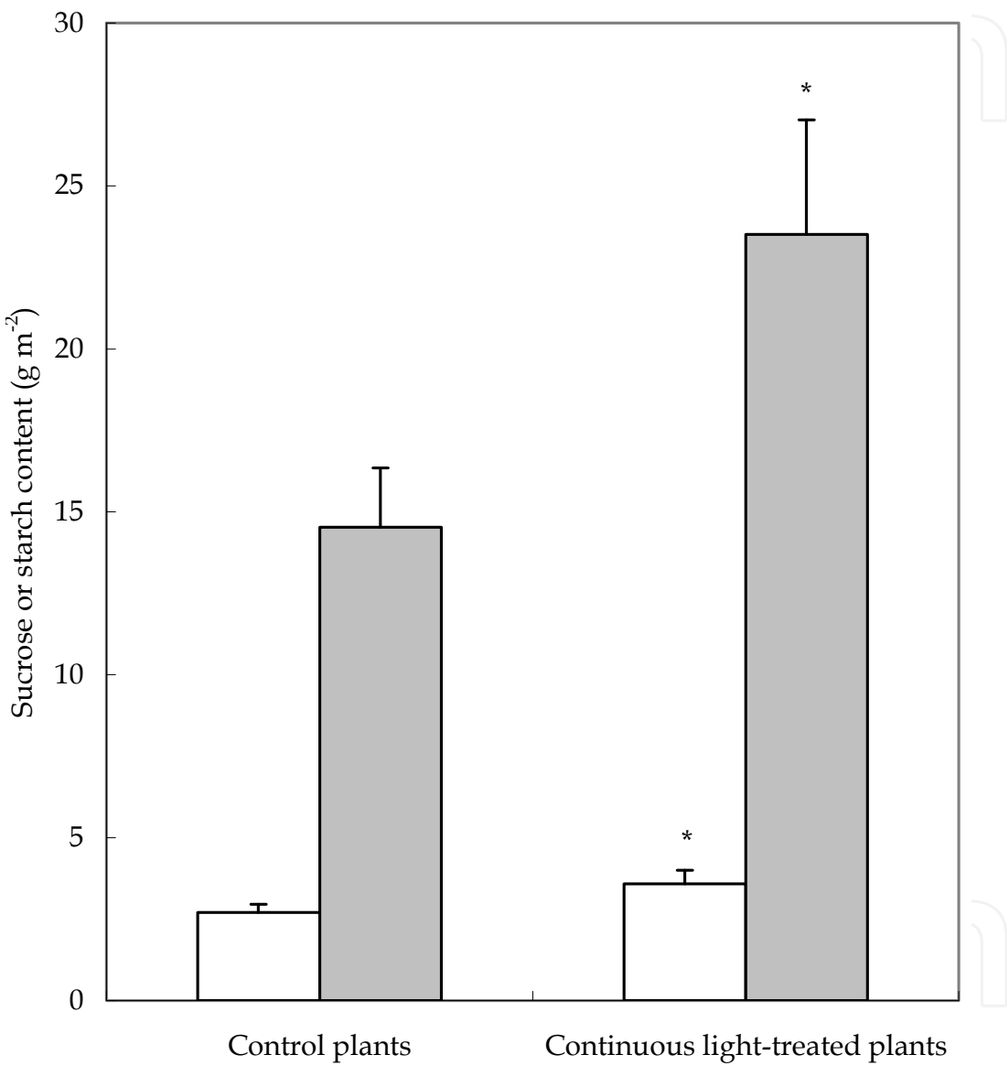

Fig. 5. Leaf sucrose or starch content in soybean plants on day 3after continuous exposure to light. Control plants were grown as described in Fig. 1. Open bar, sucrose content; closed bar, starch content. Vertical bars indicate S.D. (n=4). $^{*}P<0.05$ when compared with control plants.

Analyzed contents of chlorophyll, water, total protein and Rubisco protein in leaf did not differ significantly between control and continuous light-treated plants (Fig. 6 and 7).

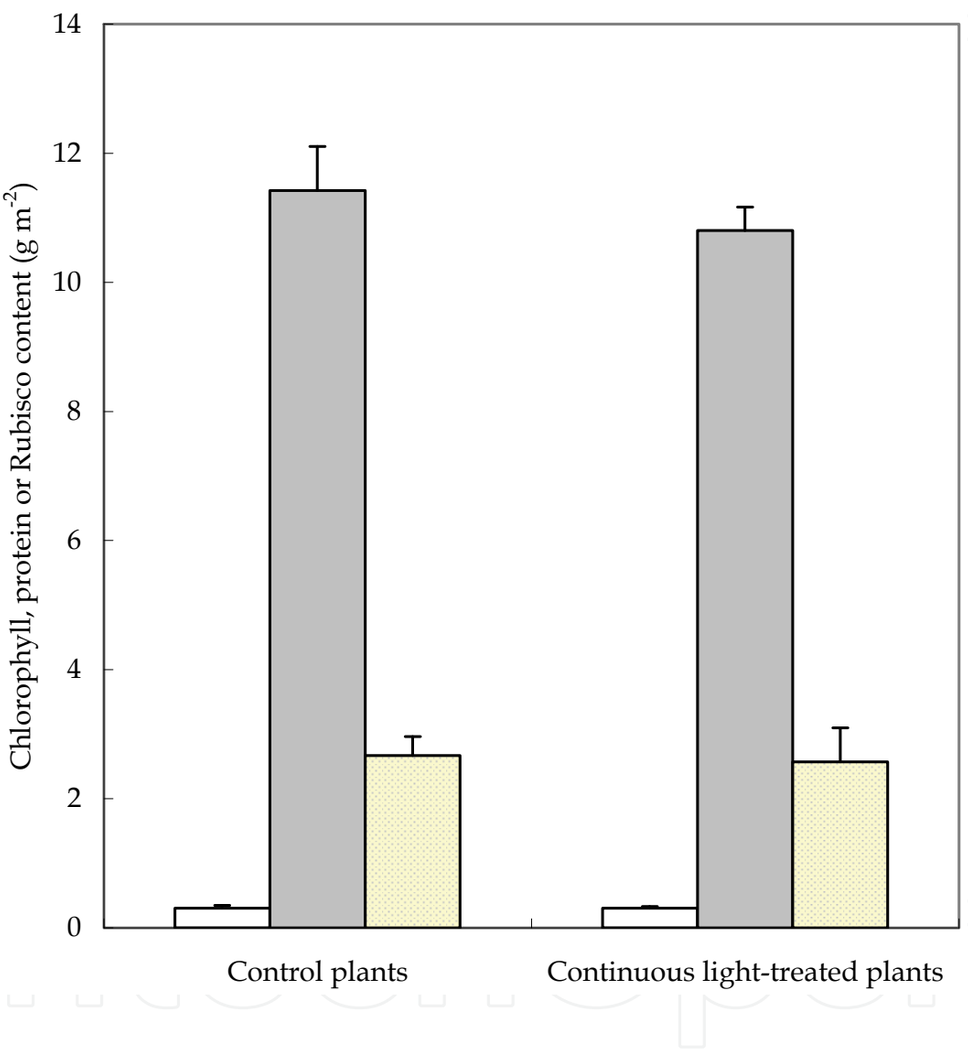

Fig. 6. Leaf chlorophyll, total protein or Rubisco content in soybean plants on day 3 after continuous exposure to light. Control plants were grown as described in Fig. 1. Open bar, chlorophyll content; closed bar, total protein content; dotted bar, Rubisco content. Vertical bars indicate S.D. (n=4). The chlorophyll, total protein and Rubisco contents did not differ significantly ($P>0.05$) between control and continuous light-treated plants.

Analyzed leaf dry weight other than the weights of sucrose and starch was heavier a little in continuous light-treated plants than in control plants (Fig. 5 and 7). The mean dry weights

after subtracting the weights of sucrose and starch in control and continuous light-treated plants were 49.0 g m^{-2} and 57.5 g m^{-2}, respectively.

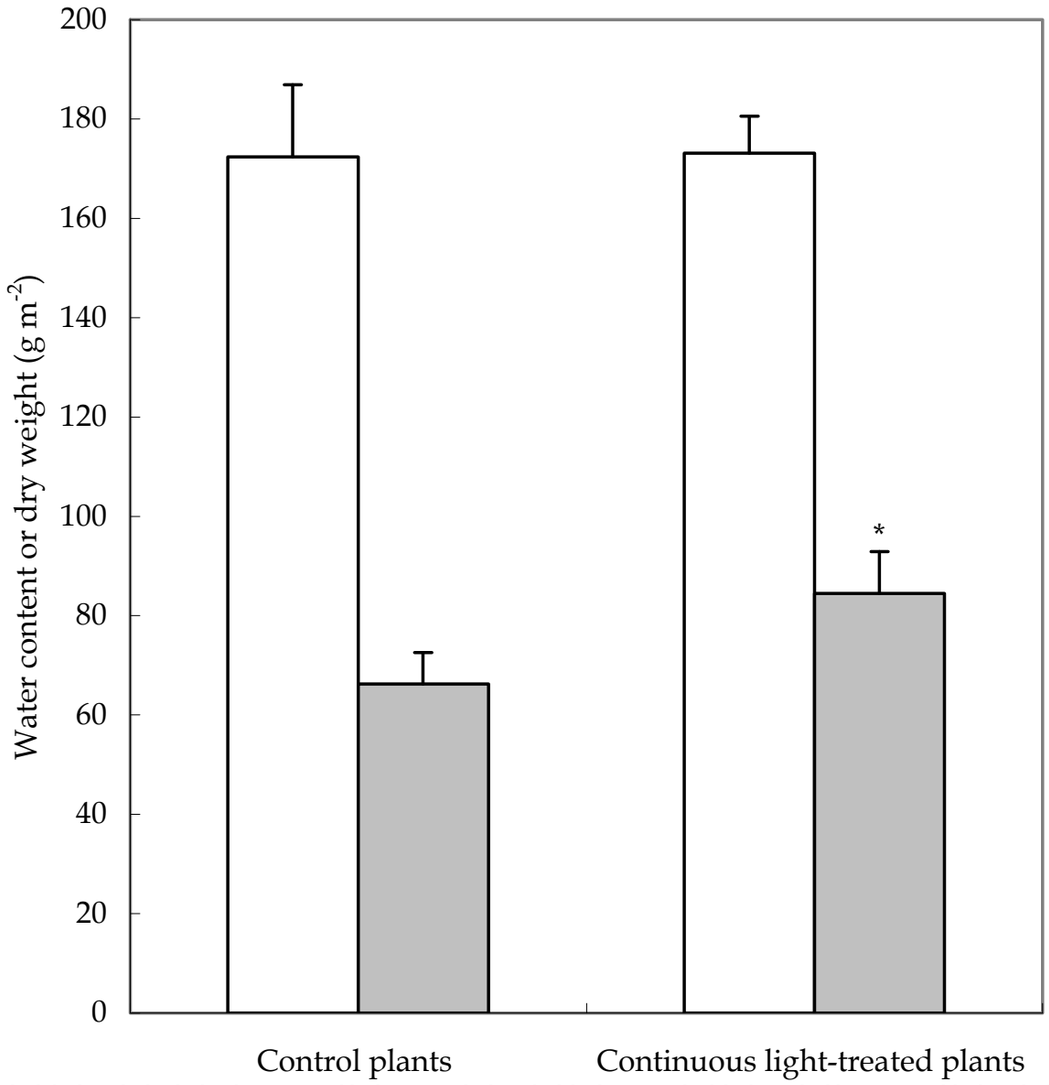

Fig. 7. Leaf water content and leaf dry weight in soybean plants on day 3 after continuous exposure to light. Control plants were grown as described in Fig. 1. Open bar, leaf water content; closed bar, leaf dry weight. Vertical bars indicate S.D. (n=4). *P<0.05 when compared with control plants. The leaf water content did not differ significantly (P>0.05) between control and continuous light-treated plants.

4. Discussion

The present study was conducted to examine the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity in intact soybean plants. The experimental construction of excessive photosynthetic source capacity was conducted by treating the plants with continuous light for 3 days. The data show that the treatment of

continuous exposure to light for intact soybean plants decreased significantly the leaf photosynthetic rate (Fig. 1). Since the light treatment also decreased the leaf stomatal conductance in soybean plants (see Fig. 2), it is thought that the decrease in leaf photosynthetic rate caused by treatment of continuous exposure to light might have resulted from stomatal limitation of CO_2 diffusion. However, the treatment of continuous exposure to light did not affect significantly leaf intercellular CO_2 concentration (see Fig. 2), implicating that the light treatment decreased CO_2 incorporation by leaf photosynthetic cells, as it affected leaf stomatal conductance. In addition, the light treatment decreased activation ratio of Rubisco in leaf extract and did not affect significantly leaf Rubisco content (see Fig. 3 and 6). Furthermore, the light treatment increased the amount of protein-bound RuBP in leaf extract (see Fig. 4). The decrease in activation ratio of Rubisco and increase in the amount of protein-bound RuBP in leaf extract (Brooks & Portis, 1988) strongly suggest an increase in the amount of uncarbamylated inactive Rubisco in leaf. Therefore, it is suggested that the decrease in leaf photosynthetic rate caused by treatment of continuous exposure to light is likely to be due to deactivation of Rubisco in leaf. Treatment of continuous exposure to light for intact soybean plants also increased significantly both the contents of sucrose and starch, which are the major photosynthetic carbohydrates, in leaf (see Fig. 5), indicating that the light treatment could result in an excessive photosynthetic source capacity in the plants. The present study also shows that analyzed leaf chlorophyll, total protein and water contents were not affected significantly by the treatment of continuous exposure to light (see Fig. 6 and 7). Therefore, results obtained in the present study strongly suggest that the decrease in leaf photosynthetic rate in intact soybean plants caused by treatment of continuous exposure to light is unlikely to be due to simple damages such as the breakdown of cellular compartments, but is likely to be due to deactivation of Rubisco, which is associated with accumulation of photosynthetic carbohydrates (sucrose and starch) in leaf under excessive photosynthetic source capacity.

As described in the Introduction, single-rooted soybean leaves have quite been helpful to study the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity, since the plants have simple source-sink organization and have excellent characteristics [growing only the sink organs (roots) without growing source organ (leaf)], which have not been found in other plants (Sawada et al., 1986, 2003). However, as already mentioned, as the plant leaf is constituted from only the primary leaf in intact soybean plants, there is the possibility that properties of single-rooted soybean leaves may not reflect those of the original intact soybean plants or the other intact plants. However, results obtained in the present study of the changes in leaf photosynthetic rate, initial activity and activation ratio of Rubisco in leaf extract, and contents of major photosynthetic carbohydrates (sucrose and starch) and chlorophyll in leaf caused by treatment of continuous exposure to light corresponded with results from studies that have performed similar experiments of continuous exposure to light using single-rooted soybean leaves (Sawada et al., 1986, 1990, 1992). Leaf intercellular CO_2 concentration, amount of protein-bound RuBP in leaf extract and leaf Rubisco content have not been analyzed in the single-rooted soybean leaves. As already mentioned, the present study used the original intact soybean plants from which single-rooted soybean leaves can be made. Therefore, the correspondence of data from original intact soybean plants and those from single-rooted soybean leaves highlights that properties of single-rooted soybean leaves and those of original intact soybean plants are very similar, thus suggesting that properties of single-

rooted soybean leaves and those of original intact soybean plants can reflect each other. As described in the Introduction, studies using single-rooted soybean leaves have implicated that there is a regulatory mechanism of leaf photosynthetic rate through deactivation of Rubisco, which is associated with accumulation of photosynthetic carbohydrates in leaf under excessive photosynthetic source capacity (Sawada et al., 1986, 1989, 1990, 1992, 1999, 2003). Data from the present study using the original intact soybean plants have also suggested the same regulatory mechanism of leaf photosynthetic rate. Therefore, the suggested regulatory mechanism of leaf photosynthetic rate may be a common mechanism in plants. With respect to the excellent characteristic of single-rooted soybean leaves that do not change the leaf dry weight other than the weights of major photosynthetic carbohydrates (sucrose and starch) (Sawada et al., 1986), a little change (increase) of leaf (fourth trifoliate leaves) dry weight other than the weights of major photosynthetic carbohydrates (sucrose and starch) was observed by treatment of continuous exposure to light in the original intact soybean plants (see Fig. 5 and 7). Although the present study conducted various analyses to examine the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity, the same series of analyses have not been conducted together in other studies that have performed the treatment of continuous exposure to light using plants.

Treatment of continuous exposure to light for plants results, in most cases, in accumulation of photosynthetic carbohydrate(s) in leaf and decrease in leaf photosynthetic rate. However, in addition to these effects of the light treatment, there are other effects of the light treatment that are different from those indicated by the present study. In tomato, egg plant, peanut and potato, treatment of continuous exposure to light has been shown to result in leaf decolorization (Bradley & Janes, 1985; Globig et al., 1997; Murage et al., 1996, 1997; Rowell et al., 1999; Wheeler & Tibbitts, 1986; Tibbitts et al., 1990). In young leaves of potato and *Arabidopsis*, the continuous light treatment has been shown to accelerate expressions of photosynthetic genes, pigments and proteins, and subsequent declines of the expressions (Cushman et al., 1995; Stessman et al., 2002). In a study using young apple, a decrease in leaf photosynthetic rate caused by treatment of continuous exposure to light was suggested to be due to stomatal limitation of CO_2 diffusion rather than a reduction of Rubisco activity, although, in the study, leaf water content, which is likely to affect stomatal aperture (Brodribb & McAdam, 2011), was not analyzed (Cheng et al., 2004). Therefore, leaf photosynthetic rate may also be regulated through changes in expressions of photosynthetic genes, pigments and proteins and through a regulation of stomata under excessive photosynthetic source capacity in plants.

Other ways, which indirectly construct excessive photosynthetic source capacity as described in the Introduction, have also been shown to result in accumulation of photosynthetic carbohydrate(s) in leaf and decrease in leaf photosynthetic rate. With respect to the cause(s) of why leaf photosynthetic rate declines under the excessive photosynthetic source capacity, for example, data from photosynthetic carbohydrate-feeding or high CO_2 treatment experiments suggest that decreased expressions of photosynthetic genes, including genes for chlororphyll-related protein and Rubisco protein can be causes (Paul & Foyer, 2001; Martin et al., 2002; Paul & Pellny, 2003). However, there is also evidence from high CO_2 treatment experiments using various C_3 plants that decreased Rubisco activity in leaf rather than changes in leaf Rubisco content is likely to be a main cause (Sage et al., 1989). Data from experiments conducting excisions of sink organs (pods or flower buds and

flowers) or petiole girdling suggest that a decrease of stomatal conductance or Rubisco activity or Rubisco content in leaf, or both decreases of Rubisco activity and Rubisco content in leaf can be responsible for the decrease in leaf photosynthetic rate under excessive photosynthetic source capacity (Mondal et al., 1978; Setter & Brun, 1980; Setter et al., 1980; Wittenbach, 1982, 1983; Xu et al., 1994; Crafts-Brandner & Egli, 1987; Cheng et al., 2008). As described in the Introduction, excising sink organs or high CO_2 treatment can have side effect(s) other than inducing excessive photosynthetic source capacity. In the present study using intact soybean plants in which excessive photosynthetic source capacity was constructed by treatment of continuous exposure to light, visible damages such as leaf decolorization and wilt were not observed. Treatment of continuous exposure to light did not affect significantly leaf chlorophyll, total protein and water contents analyzed. However, as mentioned above, totally, the effects of indirectly constructed excessive photosynthetic source capacity on leaf carbohydrate status, photosynthetic rate, stomatal conductance, Rubisco activity and photosynthetic gene expressions including Rubisco gene expression are similar to those of excessive photosynthetic source capacity that is constructed by treatment of continuous exposure to light.

Regarding the detailed mechanism(s) of why leaf photosynthetic rate declines under excessive photosynthetic source capacity, recent studies using transgenic plants show that hexokinase could be involved in carbohydrate-mediated repression of photosynthetic gene expression (Jang et al., 1997; Dai et al., 1999; Moore et al., 2003). Other recent study shows that protein kinases (KIN10 and KIN11) may be involved in governing the entirety of carbohydrate metabolism, growth and development in response to carbohydrates in plants (Baena-Gonzalez et al., 2007). Data from a study investigating the effect of chilling stress on leaf photosynthetic rate suggest that H_2O_2, a reactive oxygen species can induce deactivation of Rubisco (Zhou et al., 2006). As described in the Introduction, inorganic phosphate has been found to promote activation of Rubisco by enhancing the affinity of uncarbamylated inactive Rubisco to CO_2 (Bhagwat, 1981; McCurry et al., 1981; Anwaruzzaman et al., 1995). Data from a more recent study suggest that pH within the chloroplasts can be an important factor affecting leaf photosynthetic rate, since the study has demonstrated that pH can affect distribution of Rubisco activase within the chloroplasts by affecting binding of the enzyme to the thylakoid membranes (Chen et al., 2010). Distribution of Rubisco activase within the chloroplasts can affect activation state of Rubisco, since Rubisco activase plays a role in promoting the activation of Rubisco by dissociating RuBP from uncarbamylated inactive Rubisco (Crafts-Brandner & Salvucci, 2000), which tightly binds RuBP (Jordan & Chollet, 1983). Since ATP is needed for the catalytic action of Rubisco activase (Crafts-Brandner & Salvucci, 2000) and it is well known that ATP is needed for regeneration of RuBP, a substrate for Rubisco in Calvin cycle (see Kasai, 2008), it is evident that ATP is also an important factor affecting leaf photosynthetic rate. However, the precise mechanism of how hexokinase and protein kinases exercise regulation of photosynthetic carbohydrate metabolism including the carbohydrate-mediated repression of photosynthetic gene expression is not yet clear. In addition, effects of excessive photosynthetic source capacity on the levels of H_2O_2, inorganic phosphate, pH and ATP within the chloroplasts in which central photosynthesis is performed have not been analyzed in intact plants at real times under light. A main reason seems to be the lack of appropriate methods. Therefore, further researches including those following the development of new methods are important to elucidate further the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity.

Recent studies using transgenic plants have shown that overexpression of Calvin cycle enzymes (sedoheptulose-1,7-bisphosphatase and fructose-1,6-bisphosphatase) or leaf plasma membrane CO_2 transport protein increases the leaf photosynthetic rate and the biomass production (Raines, 2003, 2006). Increasing plant leaf photosynthesis and thereby increasing plant matter (biomass) production seems to be an effective way to resolve the serious problems such as climatic warming and food and energy shortages. However, data obtained in the present study and those from other studies strongly suggest that excessive photosynthetic source capacity decreases the efficiency of leaf photosynthetic matter production. This means that under excessive photosynthetic source capacity, efficiency of plant matter (biomass) production decreases. There is also evidence for the excessive photosynthetic source capacity causing down regulation of photosynthesis in plants under field conditions (Okita et al., 2001; Smidansky et al., 2002, 2007). Therefore, it is strongly suggested that for the efficient improvement of plant matter (biomass) production, well-balanced improvement of source and sink would be essential. Further studies are desired for deeper and more comprehensive understanding of the regulatory mechanism of photosynthetic source-sink balance including the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity. Soybean plants (*Glycine max* L. Merr. cv. Tsurunoko) used in the present study from which single-rooted soybean leaves can be made are one of the important experimental materials.

5. Conclusion

Studies using single-rooted soybean leaves, each of which is constituted from a primary leaf, a short petiole and roots developed from the petiole, have implicated that there is a regulation of leaf photosynthesis through deactivation of Rubisco, which is associated with accumulation of photosynthetic carbohydrates in leaf under excessive photosynthetic source capacity. The present study using intact soybean plants from which single-rooted soybean leaves can be made has also suggested the same regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity. It is therefore concluded that for efficient improvement of plant matter (biomass) production, well-balanced improvement of source and sink would be essential. Further studies are desired for more complete understanding of the regulatory mechanism for leaf photosynthesis under excessive photosynthetic source capacity and its application.

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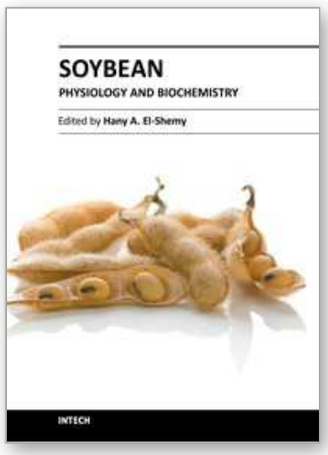

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