Chapter 14

Interaction of Photosynthetic Source-Sink Balance and Activities of Membrane H^+ Pumps in Soybean

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Additional information is available at the end of the chapter

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1. Introduction

There is evidence suggesting that in plants photosynthetic matter production is regulated by photosynthetic source-sink balance, i.e., the ratio of photosynthetic source organs (e.g., leaves) to non-photosynthetic sink organs (e.g., roots) and/or the balance of supply and demand of photosynthetic carbohydrate(s) within the plant (Kasai, 2008, 2011). Plant photosynthetic dry matter production is the source of a variety of metabolic and structural compounds. Because of increasing population, shortages of energy and food may become more severe (von Caemmerer & Evans, 2010; Raines, 2011). Plant photosynthetic dry matter production is also essential for maintaing environmental quality. For example, a well-known environmental problem is climatic warming of the earth, which mainly comes from deforestation (Brovvkin et al., 2004). Improvement of plant dry matter productivity may be an effective way for solving the problems of energy, foods and climatic warming. Thus, it is important to elucidate the mechanism(s) of regulation of plant photosynthetic matter production through photosynthetic source-sink balance.

Data from a number of studies including field investigations implicate that in plants, accumulation of photosynthetic carbohydrate(s) in leaves, which occurs when photosynthetic source capacity exceeds sink capacity, can regulate leaf photosynthetic rate (Sawada et al., 1999; Kasai, 2008, 2011; Kasai et al., 2012). In soybean a significant negative correlation exists between leaf photosynthetic carbohydrate (sucrose or starch) content and photosynthetic rate (Sawada et al., 1986, 2001; Kasai, 2008). There have also been findings of photosynthetic carbohydrate-mediated decrease in the activity or the amount of Rubisco, the CO_2-fixing enzyme in leaves (Sage et al., 1989; Xu et al., 1994; Martin et al., 2002; Paul & Pellny, 2003), although the detailed mechanism(s) is still unclear. To date, many studies have focused on photosynthetic carbohydrate-mediated inhibition of leaf photosynthesis to elucidate the

mechanism(s) of regulation of photosynthetic matter production through photosynthetic source-sink balance. However, in contrast, there is also evidence suggesting that leaf photosynthetic rate is not necessarily affected by accumulated photosynthetic carbohydrate(s) in leaf (Nebauer et al., 2011). Apart from the regulation of leaf photosynthesis through levels of photosynthetic carbohydrate(s), it is important to examine the mechanism(s) of regulation of photosynthetic dry matter production through photosynthetic source-sink balance by focusing on new enzyme(s) thought to be important.

Data from recent studies implicate that in plants, activity(ies) of membrane H^+ pump(s) such as tonoplast H^+ pump(s) can be important in the regulation of photosynthetic dry matter production through photosynthetic source-sink balance (Kasai &Muto, 1990.; Schumacher et al., 1999; Li et al., 2005; Yang et al., 2007; Wang et al., 2011). However, the effect of photosynthetic source-sink balance on the activity(ies) of membrane H^+ pump(s) has not been investigated. We show here experimental data of our recent study relating to this subject. We investigated in soybean plants how removal of pods, which decreases the ratio of sink to source organs, affects various characteristics related to photosynthetic dry matter production. Factors studied were leaf photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration, initial and total activities of Rubisco, chlorophyll, total protein, inorganic phosphate, photosynthetic carbohydrates (sucrose and starch), and dry weights of source and sink organs. We also investigated the effect of pod removal on activities of the H^+ pumps of the leaf plasma membrane (H^+-ATPase) and tonoplast (H^+-ATPase and H^+-PPase). It is now well known that soybean is one of the most important crops grown in the world (Board & Kahlon, 2011; Ainsworth et al., 2012). On the basis of our experimental data and the other relevant information, we also consider how membrane H^+ pump(s) can be important in the regulation of photosynthetic dry matter production through photosynthetic source-sink balance.

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, 8.5 cm in diameter) containing mixed vermiculite and sand (1:1 in volume) and 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 25/17°C and relative humidity of 60 %. After 51 days, pods were all removed together with small floral organs from half of the plants, and the depodded plants were grown with the remaining plants (controls) for 3 days under same growth conditions. Nutrients were supplied twice 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. Intensity of light, which was supplied with incandescent lamps, was 80 μmol photons m^{-2} s^{-1} (400-700 nm) on pots.

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2.2. Leaf photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration

Leaf photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration were determined on day 3 after pod removal in fully expanded middle trifoliate leaves at a light intensity of 800 μmol photons m^{-2} s^{-1}, air flow rate of 200 ml min^{-1}, air temperature of 25 °C, relative humidity of 60 % and CO_2 concentration of 350 ppm using a portable photosynthetic analyzer (Cylus-1; Koito Industries Ltd.). After measurements, leaf disks (1.79 cm^2) were taken from the middle trifoliate leaves for the other analyses (see 2.3), as described previously (Kasai, 2008).

2.3. Rubisco activity, chlorophyll, protein, phosphate, sucrose and starch

Initial and total activities of Rubisco in leaf extract were determined at 25 °C as described previously (Kasai, 2008). Leaf chlorophyll content was determined according to the method of Mackinney (1941). Leaf total protein content was determined by quantifying protein included in the leaf extract that had been prepared for determination of Rubisco activity by the method of Bradford (1976). Leaf inorganic phosphate content was determined according to the method of Saheki et al. (1985). Leaf sucrose and starch contents were determined as described by Sawada et al. (1995).

2.4. Dry weight

For determination of dry weights of source (leaves) and sink organs (stems, floral organs including pods, and roots), organs were separated from plants on day 3 after pod removal and dried at 70°C for a week.

2.5. Plasma membrane, tonoplast and H^+ pump activity

The activities of H^+ pumps of leaf plasma membrane and tonoplast were determined using plasma membrane vesicles and tonoplast vesicles prepared from leaves. Plasma membrane vesicles and tonoplast vesicles were prepared from leaves (25 g in fresh weight) of plants on day 3 after pod removal essentially as described by Nouri & Komatsu (2010) and Maeshima & Yoshida (1989), respectively. For preparation of plasma membrane vesicles, leaf-homogenizing medium consisted of 0.3 M sucrose, 50 mM Tris, 8 mM EDTA (acid form), 2 mM PMSF, 4 mM DTT and 0.2 % (w/v) BSA, and its volume was 200 ml. After homogenization, the medium was filtered through four layers of gauze and the filtrate was centrifuged at 10,000 g for 20 min. After supernatant was centrifuged at 80,000 g for 40 min, the pellets were suspended with a sucrose-containing medium [0.3 M sucrose, 5 mM KH_2PO_4, 5 mM KCl, 0.1 mM EDTA and 0.1 mM DTT (pH 7.8)], of which volume was 10 ml. The plasma membrane vesicles were prepared from the suspension by using aqueous two-phase partitioning method. Dilution of the upper layers that had been obtained was conducted with a sorbitol-containing medium [0.25 M sorbitol, 5 mM HEPES-BTP and 0.1 mM DTT (pH 7.0)]. The final pellets of the plasma membrane vesicles after centrifugation (80,000 g, 40 min) were suspended with another sorbitol-containing medium [1 M sorbitol, 5 mM HEPES-BTP

and 0.1 mM DTT (pH 7.0)] and stored at -80 °C until uses. For preparation of tonoplast vesicles, leaf-homogenizing medium consisted of 0.25 M sorbitol, 50 mM HEPES-KOH, 5 mM EGTA, 1 mM PMSF, 2.5 mM $Na_2S_2O_5$ and 1.5% (w/v) PVP (pH 7.6), and its volume was 200 ml. After homogenization, the medium was filtered through four layers of gauze and the filtrate was centrifuged at 4000 g for 10 min. After supernatant was centrifuged at 80,000 g for 60 min, the pellets were suspended with a sucrose-containing medium [0.3 M sucrose, 10 mM KH_2PO_4, 1 mM EGTA and 2 mM DTT (pH 7.8)], of which volume was 5 ml, and 3 ml of a sorbitol-containing medium [0.25 M sorbitol, 50 mM HEPES-KOH, 1 mM EGTA and 2 mM DTT (pH 7.3)] was put on the suspension and the solution was centrifuged (120,000 g, 40 min). The resulting middle layer was diluted with the same sorbitol-containing medium. The final pellets of the tonoplast vesicles after centrifugation (150,000 g, 20 min) were suspended with another sorbitol-containing medium [0.25 M sorbitol, 5 mM HEPES-BTP, 2 mM DTT (pH 7.5)] and stored at -80°C until uses.

The activity of H^+ pump, i.e., H^+-ATPase of leaf plasma membrane was determined at 30°C as vanadate-sensitive ATP-hydrolytic activity (kasai & Sawada, 1994). The activities of H^+ pumps, i.e., H^+-ATPase and H^+-PPase of leaf tonoplast were determined at 30°C as nitrate-sensitive ATP-hydrolytic activity and Na^+-sensitive PPi-hydrolytic activity (Kasai et al., 1993; Kasai & Sawada, 1994), respectively. Reaction medium (500 μl) for the activity of plasma membrane H^+ pump consisted of 50 mM HEPES-BTP (pH 7.0), 3 mM $MgSO_4$, 3 mM ATP, 1 mM EGTA, 50 mM KCl, ± 0.1 mM Na_3VO_4, 0.02% (w/v) Triton X-100 and membrane vesicles (10 μg). Reaction medium for the activity of tonoplast H^+-ATPase consisted of ±50 mM HEPES-BTP (pH 7.5), 3 mM $MgSO_4$, 3 mM ATP, 1 mM EGTA, ±50 mM KCl, ± 50 mM KNO_3, 0.02% (w/v) Triton X-100 and membrane vesicles (10 μg). Reaction medium for the activity of tonoplast H^+-PPase consisted of 50 mM HEPES-BTP (pH 7.5), 5 mM $MgSO_4$, 0.5 mM PPi, 1 mM EGTA, 50 mM KNO_3, ± 50 mM $NaNO_3$, 0.02% (w/v) Triton X-100 and membrane vesicles (10 μg). Phosphate liberated from substrate ATP or PPi was determined according to the method of Saheki et al. (1985).

3. Results

Analyzed leaf photosynthetic rate and transpiration rate were significantly lower in depodded plants than in control plants (Fig. 1). Leaf stomatal conductance was also lower in depodded plants than in control plants, while leaf intercellular CO_2 concentration did not differ significantly between control and depodded plants (Fig. 2). Initial and total activities of Rubisco in leaf extract did not differ significantly between control and depodded plants (Fig. 3). Contents of chlorophyll, total protein and inorganic phosphate in leaves were all significantly higher in depodded plants than in control plants (Fig. 4). Contents of sucrose and starch in leaves did not differ significantly between control and depodded plants (Fig. 5). Activity of H^+ pump (H^+-ATPase) of leaf plasma membrane and activities of H^+ pumps (H^+-ATPase and H^+-PPase) of leaf tonoplast were all significantly lower in depodded plants than in control plants (Fig. 6). Dry weights of leaves, stems and roots did not differ significantly between control and depodded plants (Fig. 7). When the ratio of sink (stems + floral

organs including pods + roots) to source organs (leaves) was calculated, those in control and depodded plants were on the average 1.25 (100%) and 0.70 (56%), respectively.

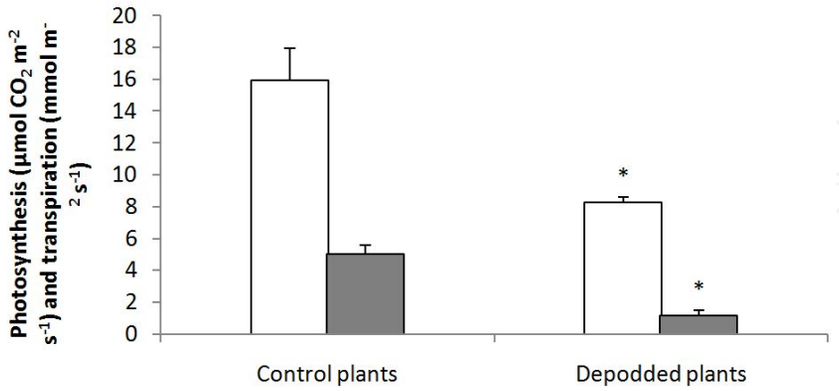

Figure 1. Leaf photosynthetic rate and transpiration rate of soybean plants on day 3 after pod removal. Open bar, leaf photosynthetic rate; gray bar, leaf transpiration rate. Vertical bars indicate S.D. (n=3). *P<0.01 (t-test) when compared with control plants.

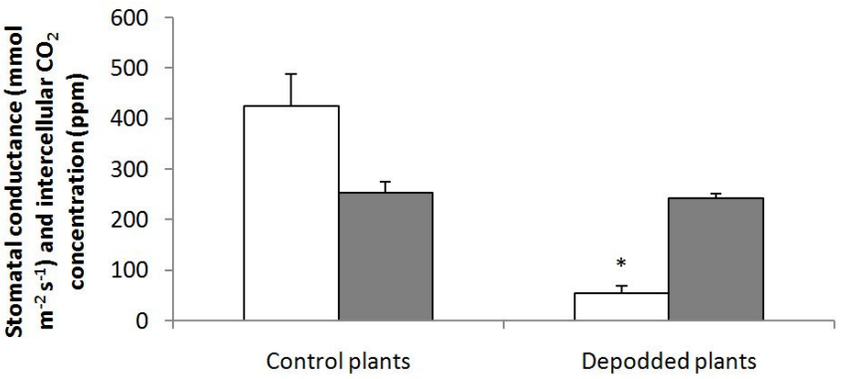

Figure 2. Leaf stomatal conductance and intercellular CO_2 concentration of soybean plants on day 3 after pod removal. Open bar, leaf stomatal conductance; gray bar, leaf intercellular CO_2 concentration. Vertical bars indicate S.D. (n=3). *P<0.01 when compared with control plants. The leaf intercellular CO_2 concentration did not differ significantly (P>0.05) between control and depodded plants.

4. Discussion

As described in the Introduction, it is important to examine the mechanism(s) of regulation of plant photosynthetic dry matter production through photosynthetic source-sink balance by focusing on a new enzyme(s). We focused on H^+ pumping enzymes of leaf plasma membrane (H^+-ATPase) and tonoplast (H^+-ATPase and H^+-PPase), and investigated in soybean plants how removal of pods, which decreases the ratio of sink to source organs, affects vari-

ous characteristics related to photosynthetic dry matter production and the activities of H^+ pumps. Pod removal was shown to decrease largely leaf photosynthetic rate, transpiration rate and stomatal conductance without affecting significantly leaf intracellular CO_2 concentration (Fig. 1 and 2). These results imply that pod removal decreased equally the rate of CO_2 diffusion via leaf stomata and the rate of CO_2 fixation in leaf photosynthetic cells. In plants, Rubisco is a major protein in leaves (Furbank et al., 1996; von Caemmerer et al., 2005), and there is evidence from studies altering the expressions of Rubisco or its activation enzyme, Rubisco activase, that changes in the activity or the amount of Rubisco in leaves significantly affect leaf photosynthetic rate (Furbank et al., 1996; von Caemmerer et al., 2005). There is also a report demonstrating that a rough and positive correlation exists between leaf chlorophyll content and photosynthetic rate (Arp, 1991). Therefore, it is speculated that the pod removal-induced decrease in leaf photosynthetic rate might have resulted from a decrease in the activity or the amount of Rubisco in the leaf or the content of leaf chlorophyll. However, data of Figure 3 and 4 indicate that pod removal did not significantly affect potential activity of Rubisco in the leaf and could not decrease the content of Rubisco or chlorophyll, suggesting that the pod removal-induced decrease in leaf photosynthetic rate did not result from either of these factors.

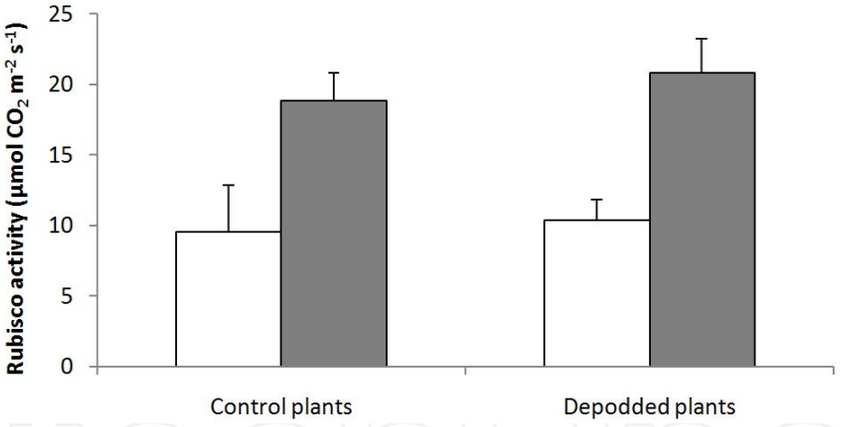

Figure 3. Initial and total activities of Rubisco in leaf extract from soybean plants on day 3 after pod removal. Open bar, initial activity; gray bar, total activity. Vertical bars indicate S.D. (n=3). Both initial and total activities did not differ significantly (P>0.05) between control and depodded plants.

Previously, in single-rooted soybean leaves that are the same species as we used in our pod removal study, it was demonstrated that a decrease in leaf inorganic phosphate content can result in a decrease in leaf Rubisco activity in vivo (Sawada et al., 1990, 1992). In vitro, inorganic phosphate has been found to promote the binding of activator CO_2 to uncarbamylated inactive Rubisco (Bhagwat, 1981; McCurry et al., 1981; Anwaruzzaman et al., 1995). Data of Figure 4 indicate that pod removal did not decrease leaf inorganic phosphate content. This result indicates that the pod removal-induced decrease in leaf photosynthetic rate did not result from a decrease in leaf inorganic phosphate content.

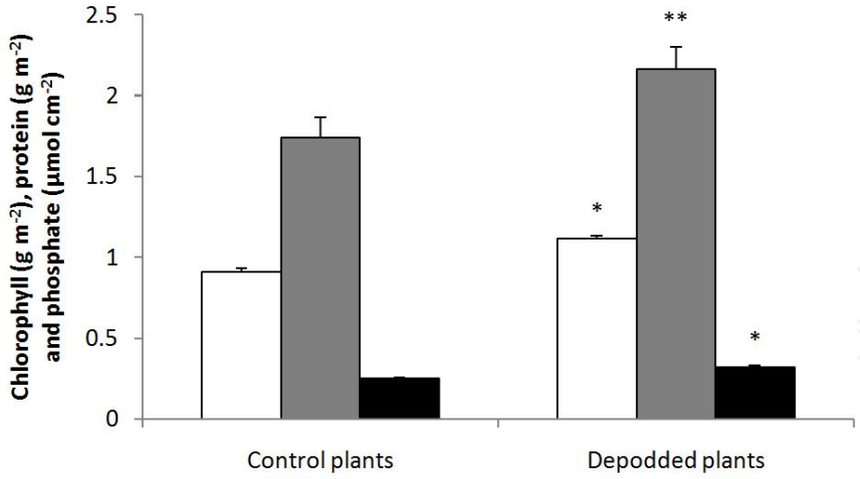

Figure 4. Leaf chlorophyll, total protein and inorganic phosphate contents in soybean plants on day 3 after pod removal. Open bar, chlorophyll; gray bar, total protein; black bar, inorganic phosphate. Vertical bars indicate S.D. (n=3). *P<0.01 (**P<0.05) when compared with control plants.

Figure 5. Leaf sucrose and starch contents in soybean plants on day 3 after pod removal. Open bar, sucrose content; gray bar, starch content. Vertical bars indicate S.D. (n=3). Both sucrose and starch contents did not differ significantly (P>0.05) between control and depodded plants.

There is a hypothesis of inhibition of photosynthesis through accumulation of sucrose in the leaf, although the detailed mechanism(s) is still unclear (Kasai, 2008). For example, in a study having continuous exposure to light of single-rooted soybean leaves, a significant negative correlation was shown between leaf sucrose content and photosynthetic rate (Sawada et al., 1986). It is thought that in both control and depodded plants, sucrose-induced inhibition of leaf photosynthesis was, if any, very small. Leaf sucrose content of control plants, which was higher on the average than that of depodded plants (Fig. 5), corresponded with a content that led to a very small decrease in leaf photosynthetic rate of single-rooted soybean leaves (Sawada et al., 1986). Leaf sucrose content of control plants did not seem to decrease leaf photosynthetic rate in our previous pod removal study us-

ing soybean plants (Kasai et al., 2008). There is also a hypothesis of inhibition of photosynthesis through accumulation of starch in leaves (Kasai, 2008). In the same study, continuous exposure to light of single-rooted soybean leaves resulted in a significant negative correlation between leaf starch content and photosynthetic rate (Sawada et al., 1986). Another study using single-rooted soybean leaves demonstrated that accumulation of starch decreases the rate of CO_2 diffusion (Sawada et al., 2001; Kasai et al., 1996). In our pod removal study, pod removal did not significantly affect leaf starch content (Fig. 5). Therefore, it is suggested that the pod removal-induced decrease in leaf photosynthetic rate did not result from accumulation of sucrose or starch in leaves.

It is believed that in plants, P-type H^+ pump (H^+-ATPase) exists in the plasma membrane, and V-type H^+ pumps (H^+-ATPase and H^+-PPase) exist in the tonoplast (Hall & Williams, 1991; Barkla et al., 2008). In plant leaves, a decrease in H^+ pump activity of the guard cell plasma membrane can induce decreases of stomatal conductance and transpiration rate by inducing a decrease in stomatal pore size (Tominaga et al., 2001). Although we did not analyze the H^+ pump activity of the guard cell plasma membrane, it was shown that pod removal largely decreased the H^+ pump activity of the leaf plasma membrane (Fig. 6). Essentially, almost the same method is used for isolation of the plasma membrane from leaves and guard cells (Becker et al., 1993). Therefore, it is suggested that a large decrease in H^+ pump activity of the guard cell plasma membrane could cause the pod removal-induced decreases of leaf stomatal conductance and transpiration rate. In plant cells, a decrease in H^+ pump activity of the plasma membrane can induce a decrease in the electrochemical potential difference of H^+ across the plasma membrane (Hall & Williams, 1991; Barkla et al., 2008). There is increasing evidence that depolarization-activated Ca^{2+} channel, Ca^{2+}-activated anion (e.g., Cl^-) channel, depolarization-activated anion channel (e.g., HCO_3^-), electrogenic Ca^{2+}/H^+ antiporter (which has a stoichiometry higher than $2H^+/Ca^{2+}$), and CO_2-transportable and Ca^{2+}-inhibitable water channel are present in the plant plasma membrane (Thuleau et al., 1994; Roberts, 2005; Frachisse et al., 1999, Kasai et al., 1990; Song et al., 2011; Chaumont et al., 2005). Therefore, it is speculated that the observed decrease in H^+ pump activity of the leaf plasma membrane could cause the decrease in the rate of CO_2 transport in leaf photosynthetic cells by inducing a depolarization of the plasma membrane, a decrease in the proton motive force across the plasma membrane and a rise of Ca^{2+} concentration inside the plasma membrane in the leaf photosynthetic cells. The suggestion drawn on the basis of data of Figure 1 and 2 that pod removal decreased equally the rate of CO_2 diffusion via leaf stomata and the rate of CO_2 fixation in leaf photosynthetic cells is roughly consistent with the above-mentioned suggestion and speculation proposing the regulation of leaf stomatal conductance, transpiration rate and CO_2 transport in leaf photosynthetic cells by activity of H^+ pump of leaf plasma membrane. As shown in Figure 6, pod removal also greatly decreased activities of the H^+ pumps (H^+-ATPase and H^+-PPase) of the leaf tonoplast. There is increasing evidence that electrogenic Ca^{2+}/H^+ antiporter (which has a stoichiometry higher than $2H^+/Ca^{2+}$) is also present in the plant tonoplast (Blackford et al., 1990; Mei et al., 2007). Therefore, it is speculated that a large decrease in H^+ pump activity of the leaf plasma membrane and a large decrease in activities of H^+ pumps of the leaf tonoplast could cause cooperatively the pod removal-induced decrease in leaf photosynthetic rate by inducing equal

decreases in the rate of CO_2 diffusion via leaf stomata and the rate of CO_2 fixation in leaf photosynthetic cells. To verify our speculations, more evidence is needed. We emphasize, however, that until now, in similar studies other than our study, activities of the H^+ pumps of plasma membrane and tonoplast have not been analyzed.

With respect to the mechanism(s) of why pod removal decreased the H^+ pump activity of the leaf plasma membrane and activities of the H^+ pumps of the leaf tonoplast, it is inferred that plant hormones abscisic acid and cytokinin could be involved in the mechanism(s). In general, cytokinin is known to have positive effect in synthesizing chlorophyll and protein, and its content in plant cells is known to decrease under deficiency of mineral nutrients such as P and N. In contrast, abscisic acid antagonizes the effects of cytokinin, and its content in plant cells increases under conditions of mineral nutrient deficiencies (Pozsar et al., 1967; Kusnetsov et al., 1998; Salama & Wareing, 1979; Mizrahi & Richmond, 1972; Battal et al., 2003). In our pod removal study, it was shown that pod removal, which decreased the ratio of sink to source organs (Fig. 7), increased significantly the contents of chlorophyll, total protein and inorganic phosphate in the leaf (Fig. 4), implicating that pod removal might have increased cytokinin content relative to abscisic acid content in the leaf by influencing the partitioning of mineral nutrients such as P and N within the plant. In barley, it was demonstrated that abscisic acid has stimulatory effects on activities of tonoplast H^+ pumps, whereas cytokinin has opposite effects antagonizing the effects of abscisic acid (Kasai et al., 1993; Fukuda & Tanaka, 2006). In *Phaseolus vulgaris*, excessive levels of cytokinin were demonstrated to decrease leaf stomatal conductance, transpiration rate and photosynthetic rate (Pospisilova, 2003).

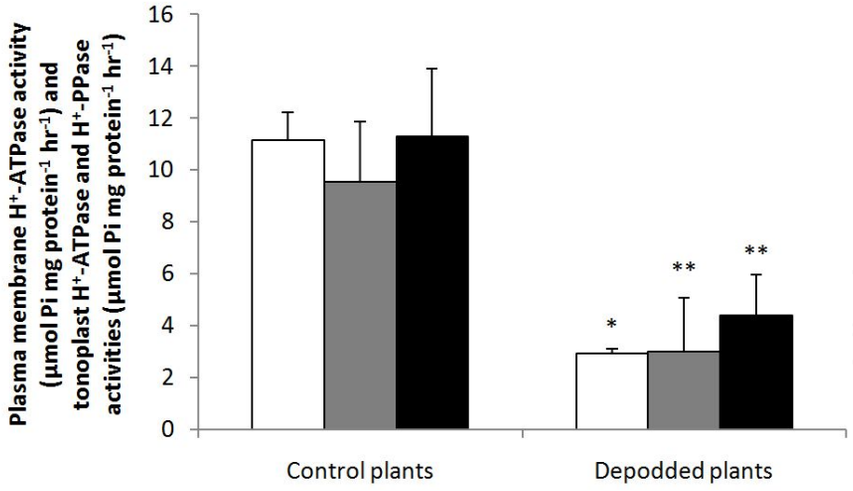

Figure 6. Activities of H^+ pumps of leaf plasma membrane and tonoplast from soybean plants on day 3 after pod removal. Open bar, plasma membrane H^+-ATPase; gray bar, tonoplast H^+-ATPase; black bar, tonoplast H^+-PPase. Vertical bars indicate S.D. (n=3). *P<0.01 (**P<0.05) when compared with control plants.

Data from recent studies using transgenic plants and those from physiological studies implicate that in plants, activity(ies) of membrane H^+ pump(s) can be important in the regulation of photosynthetic dry matter production through photosynthetic source-sink balance. For

example, in a study using *Arabidopsis* plants, overexpression of tonoplast H^+-PPase was shown to result in an increase in whole plant growth, in particular, growth of sink organ roots (Li et al., 2005). In another study using *Arabidopsis* plants, it was shown that overexpression of tonoplast H^+-PPase resulted in a 1.9-fold increase in root dry weight and a 1.5-fold increase in shoot dry weight (Yang et al., 2007). There is also a report that transgenic manipulation increasing tonoplast H^+-ATPase resulted in an increase in root length (Wang et al., 2011). In a study using rye plants, increases of root/shoot ratio and activities of tonoplast H^+ pumps (H^+-PPase and H^+-ATPase) were shown to occur under growth conditions inducing deficiency of mineral nutrients (Kasai et al., 1998). Whereas conditions inducing deficiency of mineral nutrients can increase abscisic acid level in plant cells, it can also decrease cytokinin level (Salama & Wareing, 1979; Mizrahi & Richmond, 1972; Battal et al., 2003). In a recent study using *Arabidopsis* plants, transgenic manipulation decreasing cytokinin level was shown to enhance root/shoot ratio (Werner et al., 2003). In plant cells, levels of abscisic acid and cytokinin can also be affected by levels of photosynthetic carbohydrates, which can interact with the levels of mineral nutrients (Rolland et al., 2002). Interestingly, a more recent study using *Arabidopsis* plants showed that overexpression of tonoplast H^+-PPase resulted in an increase in cell abscisic acid level and a decrease in cell cytokinin (biologically active cytokinin) level (Gonzalez et al., 2010). In the other study using *Arabidopsis* plants, it was shown that a mutant losing about 60% of tonoplast H^+-ATPase activity had a morphology resembling cytokinin-treated plants (Schumacher et al., 1999). As already mentioned, abscisic acid had stimulatory effects on activities of tonoplast H^+ pumps, whereas cytokinin had opposite effects antagonizing the effects of abscisic acid in barley (Kasai et al., 1993; Fukuda & Tanaka, 2006). In our pod removal study using soybean plants, decreasing the ratio of sink to source organs by conducting pod removal was shown to result in a large decrease in activities of H^+ pumps of the leaf plasma membrane and tonoplast. Although characteristics such as leaf photosynthetic rate related to photosynthetic dry matter production were not analyzed in the above studies other than our study, information from these studies and the other information of our data from pod removal study implicate that in plants, changes in activity(ies) of membrane H^+ pump(s) can actually play key roles in the regulation of photosynthetic matter production through photosynthetic source-sink balance. It is suggested from experimental evidence that abscisic acid and cytokinin can be involved at least in the regulation of activities of tonoplast H^+ pumps. Although no direct evidence exists, data from more recent studies let us suppose that now well-known 2C-type protein phosphatase and salt-overly-sensitive 2 protein kinase may be involved (in part) in the regulation of activities of tonoplast H^+ pumps by abscisic acid and cytokinin (Batelli et al., 2007; Huertas et al., 2012). In plants, abscisic acid, cytokinin and membrane H^+ pump(s) are speculated to be symbolic biomolecules that are able to induce or respond to a variety of internal and external environmental changes that cause the regulation of photosynthetic dry matter production through photosynthetic source-sink balance. Many experimental data suggest that interaction actually exists between environmental change and cell abscisic acid and cytokinin levels and environmental change and, for example, activities of tonoplast H^+ pumps and environmental change and photosynthetic matter production and environmental change and photosynthetic source-sink balance (Board & Kahlon, 2011; Peleg & Blumwald,

2011; Kasai, 1999; Rolland et al., 2002). On the basis of a lot of evidence, it seems evident that photosynthetic source-sink balance is an essential factor regulating photosynthetic dry matter production (Kasai, 2008, 2011; Rolland et al., 2002). As described in the Introduction, plant photosynthetic dry matter production is essential for all living organisms and is also essential for creating sound environments. Therefore, further studies are important for elucidation of the detailed mechanism(s) of how membrane H^+ pump(s) are involved in the regulation of photosynthetic dry matter production through photosynthetic source-sink balance. We emphasize that for improvement of plant productivity and for creating sound environments, well-balanced improvement of source and sink would be essential.

Figure 7. Dry weights of leaves, stems, floral organs including pods, and roots in soybean plants on day 3 after pod removal. Open bar, leaves; gray bar, stems; black bar, floral organs including pods; dotted bar, roots. Vertical bars indicate S.D. (n=3). Dry weights of leaves, stems and roots did not differ significantly (P>0.05) between control and depodded plants.

5. Conclusion

Data from recent studies implicate that in plants, activity(ies) of membrane H^+ pump(s) can be important in regulation of photosynthetic dry matter production through photosynthetic source-sink balance. However, the effect of photosynthetic source-sink balance on the activity(ies) of membrane H^+ pump(s) has not been investigated. In our recent study, we investigated in soybean plants how pod removal, which decreases the ratio of sink to source organs, affects various characteristics related to photosynthetic matter production. We also investigated, for the first time, the effect of pod removal on activities of H^+ pumps of leaf plasma membrane and tonoplast. From the data obtained and the other relevant information, it was concluded that in plants, changes in activity(ies) of membrane H^+ pump(s) can actually play key roles in the regulation of photosynthetic dry matter production through photosynthetic source-sink balance, and that hormones abscisic acid and cytokinin may be involved in regulation of activities of tonoplast H^+ pumps. Plant photosynthetic dry matter

production is essential for all living organisms and is also essential for creating sound environments. Therefore, further studies are important to elucidate the detailed mechanism(s) of how membrane H^+ pump(s) are involved in the regulation of photosynthetic dry matter production through photosynthetic source-sink balance.

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