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Research Article

Effect of Pot Size on Various Characteristics Related to Photosynthetic Matter Production in Soybean Plants

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Despite the wide uses of potted plants, information on how pot size affects plant photosynthetic matter production is still considerably limited. This study investigated with soybean plants how transplantation into larger pots affects various characteristics related to photosynthetic matter production. The transplantation was analyzed to increase leaf photosynthetic rate, transpiration rate, and stomatal conductance without affecting significantly leaf intercellular CO_2 concentration, implicating that the transplantation induced equal increases in the rate of CO_2 diffusion via leaf stomata and the rate of CO_2 fixation in leaf photosynthetic cells. Analyses of Rubisco activity and contents of a substrate (ribulose-1,5-bisphosphate (RuBP)) for Rubisco and total protein in leaf suggested that an increase in leaf Rubisco activity, which is likely to result from an increase in leaf Rubisco content, could contribute to the transplantation-induced increase in leaf photosynthetic rate. Analyses of leaf major photosynthetic carbohydrates and dry weights of source and sink organs revealed that transplantation increased plant sink capacity that uses leaf starch, inducing a decrease in leaf starch content and an increase in whole plant growth, particularly, growth of sink organs. Previously, in the same soybean species, it was demonstrated that negative correlation exists between leaf starch content and photosynthetic rate and that accumulation of starch in leaf decreases the rate of CO_2 diffusion within leaf. Thus, it was suggested that the transplantation-induced increase in plant sink capacity decreasing leaf starch content could cause the transplantation-induced increase in leaf photosynthetic rate by inducing an increase in the rate of CO_2 diffusion within leaf and thereby substantiating an increase in leaf Rubisco activity in vivo. It was therefore concluded that transplantation of soybean plants into larger pots attempted in this study increased the plant photosynthetic matter production by increasing mainly sink capacity that uses leaf starch for whole plant growth, particularly, growth of sink organs.

1. Introduction

Plant photosynthetic matter production is affected by various environments. In studies for understanding how plant photosynthetic matter production responds to various environments and what mechanisms are responsible for the responses, there are cases that potted plants are used. There are also cases that potted plants are dealt with as commercial goods or foods. It is important to accumulate information of how photosynthetic matter production in potted plants is affected by pot size, since even in the future potted plants will be used by many people for various uses. However, the information has been only a few, and only in recent years the importance of pot size for plant photosynthetic matter production was shown with scientific data by Arp [1]. The author collected data of pot size and data related to photosynthetic matter production from a number of studies that had conducted high CO_2 treatment experiments in potted plants, and reported that there were roughly positive correlations between pot size and leaf photosynthetic rate and pot size and increased ratio of root to shoot and pot size and leaf chlorophyll content [1]. The high CO_2 treatment experiments have been conducted to examine responses of plants to high CO_2 environments that will come in the future [2]. Arp pointed out from collected data and his own research data [3] that downregulation of leaf photosynthesis can occur more in potted plants than in field grown plants [1], and with respect to the reason(s), he pointed out the importance of a well-known hypothesis that there is downregulation of leaf photosynthesis through accumulation of photosynthetic carbohydrate in leaf, which occurs from photosynthetic source capacity that is excessive to sink capacity of sink organs such as roots, although the detailed mechanism(s) is still unclear [1]. To our knowledge, since Arp, only one study using cotton seedlings provided information that smaller pots decreased leaf photosynthetic rate and stomatal conductance and increased leaf starch content [4].

In point of photosynthetic source-sink balance, for example, growing plant materials with smaller pots may be similar to removing sink organs (e.g., flowers, fruits, or pods) from plant materials. A number of studies have used the manipulation that removes sink organs from plant materials to examine how reducing plant sink capacity affects photosynthetic matter production [5, 6]. However, removal of sink organs from plant materials is not identical to growing plant materials with smaller pots. Smaller pots should affect in particular sink organs of roots, since roots are mainly present within the pots. In addition, the removal of sink organs gives excisions' damage to plant materials [6]. Thus, to obtain more information of how pot size affects plant photosynthetic matter production, it is important to alter pot size directly. This study investigated the effect of altering pot size on plant photosynthetic matter production using soybean, which is one of the most important crops grown in the world [7, 8]. Actually, with potted soybean plants, it was analyzed how transplantation into larger pots affects various characteristics related to photosynthetic matter production, that is, leaf photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration, initial and total activities of Rubisco in leaf extract, contents of a substrate (RuBP) for Rubisco, major photosynthetic carbohydrates (sucrose and starch), total protein and chlorophyll in leaf, and dry weights of source and sink organs. In similar studies other than this study, the same series of analyses have not been conducted, and transpiration rate and content of total protein in leaf and initial and total activities of Rubisco in leaf extract have not been analyzed.

2. Materials and Methods

Soybean (*Glycine max* L. Merr. cv. Tsurunoko) seeds were sown in plastic pots (11.4 cm in height, 7.5 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 24/17°C, and relative humidity of 60%. After 40 days, half of the plants were transplanted into larger pots (24 cm in height, 20 cm in diameter) and grown with the remaining plants (controls) for 14 or 24 days under the 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 top of the original pot.

Leaf photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO_2 concentration were determined in fully expanded middle trifoliate leaves mainly on day 14 and 24 after transplantation 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, as described previously [5].

The initial and total activities of Rubisco in leaf extract were determined at 25°C as described previously [5]. Content of RuBP in leaf was determined as described below. To a leaf extract obtained by homogenizing a leaf disk with an ice-cold buffer (100 mM HEPES-KOH, pH 7.8, 1 mL), $HClO_4$ (final conc., 0.5 M) was added, and the mixture was centrifuged (10,000 g, 10 min) after leaving on ice for 10 min. The resulting supernatant was centrifuged (10,000 g, 10 min) after neutralizing to pH 5.6 with K_2CO_3, and the supernatant was used for the determination of RuBP content [5]. The content of total protein in leaf was determined by quantifying protein included in leaf extract that had been prepared for determination of Rubisco activity by the method of Bradford [9]. The leaf chlorophyll content was determined according to the method of Mackinney [10]. The contents of sucrose and starch in leaf were determined as described by Sawada et al. [11]. Dry weights of source (leaves) and sink organs (stems, floral organs including pods, and roots) were determined for plants on day 24 after transplantation. Each organ was dried at 75°C for a week.

3. Results

On both days 14 and 24 after transplantation, the analyzed leaf photosynthetic rate and transpiration rate were significantly higher in transplanted soybean plants than in control plants (Figure 1). Leaf stomatal conductance was also higher in transplanted plants than in control plants on both days, while leaf intercellular CO_2 concentration did not differ significantly between control and transplanted plants (Figure 2). Initial and total activities of Rubisco in leaf extract were significantly higher in transplanted plants than in control plants on both days (Figure 3), while the activation ratios (initial activity/total activity) did not differ significantly between control and transplanted plants (not shown). Contents of chlorophyll and total protein in leaf were significantly higher in transplanted plants than in control plants on both days (Figure 4). Leaf RuBP content was significantly (day 14) or on the average lower (day 24) in transplanted plants than in control plants (Figure 5). Leaf sucrose content was significantly higher in transplanted plants than in control plants, while leaf starch content was significantly lower in transplanted plants than in control plants on both days (Figure 6). Dry weights of leaves, floral organs including pods, and roots in transplanted plants on day 24 were significantly heavier than those in control plants, while dry weight of stems did not differ significantly between

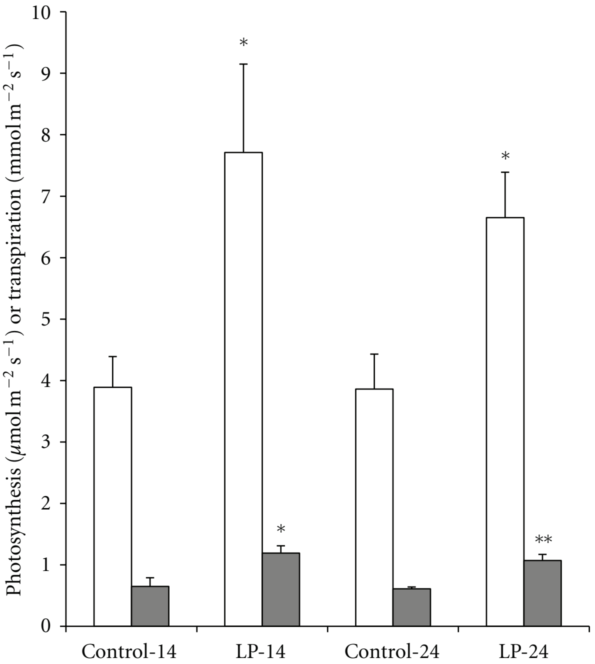

Figure 1: Leaf photosynthetic rate and transpiration rate in soybean plants grown with original pots (Control) and with larger pots (LP). Half of plants with age of 40 days were grown with larger pots for 14 (Control-14 or LP-14) or 24 days (Control-24 or LP-24). Open bar, photosynthetic rate; closed bar, transpiration rate. Vertical bars, S.D. ($n = 3$). $^*P < 0.05$/$^{**}P < 0.01$ (t-test) when compared with control.

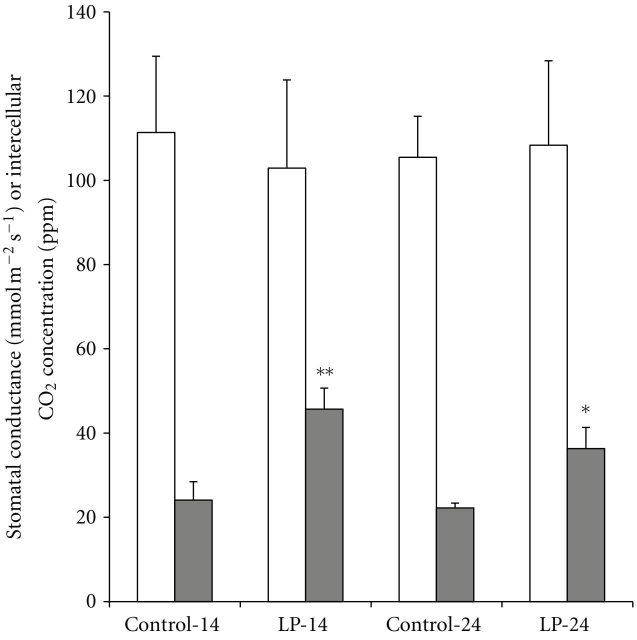

Figure 2: Leaf stomatal conductance and intercellular CO_2 concentration in soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Open bar, intercellular CO_2 concentration; closed bar, stomatal conductance. Vertical bars, S.D. ($n = 3$). $^*P < 0.05$/$^{**}P < 0.01$ (t-test) when compared with control.

control and transplanted plants (Figure 7). When the ratio of sink (stems + floral organs + roots) to source organs (leaves) was calculated, those in control and transplanted plants were on the average 2.26 (100%) and 2.60 (115%), respectively. Transplantation did not affect dry weight of stems. The ratios in control and transplanted plants calculated without stems were on the average 1.66 (100%) and 2.13 (128%), respectively.

4. Discussion

To obtain more information concerning the effect of pot size on photosynthetic matter production in potted plants, this study investigated how transplantation of soybean plants into larger pots affects various characteristics related to photosynthetic matter production. The characteristics were analyzed mainly on day 14 and 24 after transplantation in control and transplanted plants. It was shown that leaf photosynthetic rate, transpiration rate, and stomatal conductance were higher in transplanted plants than in control plants on both days (Figures 1 and 2). As leaf photosynthetic rate, transpiration rate, and stomatal conductance in control plants that were measured just before transplantation did not differ significantly from those measured on day 14 and 24 after transplantation (not shown), these results indicate that the transplantation increased leaf photosynthetic rate, transpiration rate and stomatal conductance and strongly suggest that the transplantation increased the rate of CO_2 diffusion via leaf stomata. It is speculated that transplantation might have increased leaf photosynthetic rate through an increase in the rate of CO_2 diffusion via leaf stomata. However, data of Figure 2 indicate that transplantation did not affect significantly leaf intercellular CO_2 concentration, implicating that transplantation increased equally the rate of CO_2 diffusion via leaf stomata and the rate of CO_2 fixation in leaf photosynthetic cells.

As shown in Figures 3 and 4, initial and total activities of Rubisco in leaf extract and leaf total protein content were higher in transplanted plants than in control plants on both days. When the ratio of Rubisco activity (initial or total activity) and the ratio of leaf total protein content of transplanted plants relative to control plants were calculated from mean values of data, on both days the former and latter ratios were roughly consistent. The former and latter ratios were also roughly consistent with the ratio of leaf photosynthetic rate of transplanted plants relative to control plants calculated from mean values of data on both days. The ratios on days 14 and 24 were 2.6 and 2.6, respectively, for initial activity of Rubisco, and 3.0 and 2.8, respectively, for total activity of Rubisco, and 1.9 and 2.3, respectively, for leaf total protein content, and 2.0 and 1.7, respectively, for leaf photosynthetic rate. These results strongly suggest that transplantation increased leaf Rubisco activity in vivo and suggest that the increase in leaf Rubisco activity, which is likely to result from an increase in leaf Rubisco content, could contribute to the transplantation-induced increase in

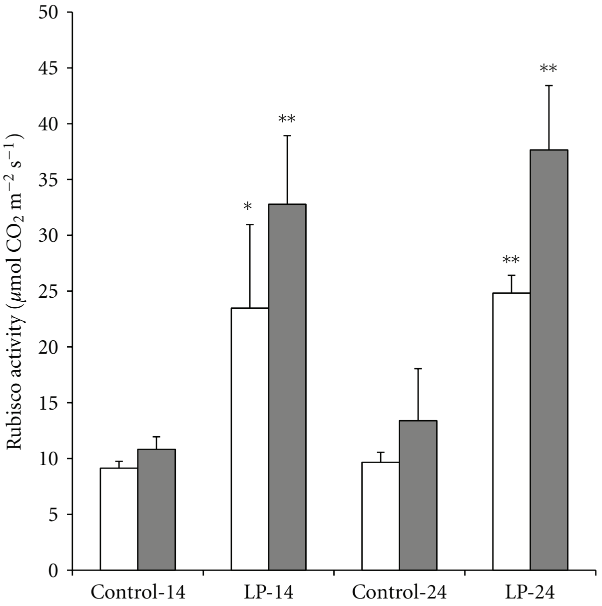

Figure 3: Initial and total activities of Rubisco in leaf extract from soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Open bar, initial activity; closed bar, total activity. Vertical bars, S.D. ($n = 3$). $^{*}P < 0.05$/$^{**}P < 0.01$ (t-test) when compared with control.

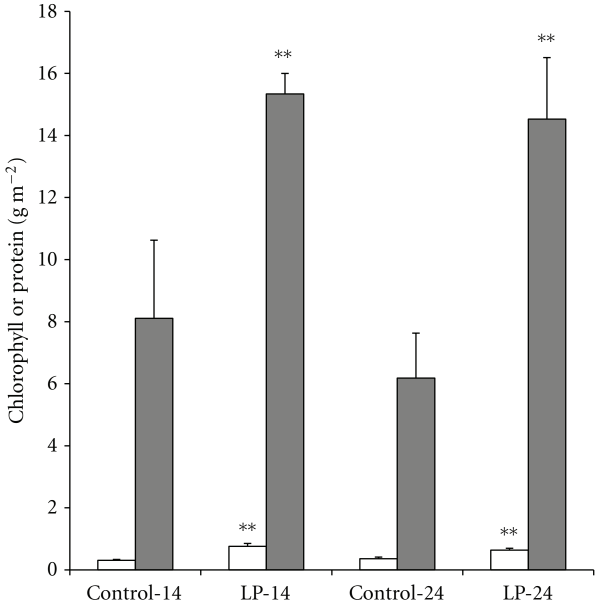

Figure 4: Leaf chlorophyll and total protein contents in soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Open bar, chlorophyll; closed bar, total protein. Vertical bars, S.D. ($n = 3$). $^{**}P < 0.01$ (t-test) when compared with control.

leaf photosynthetic rate. It is well known that in plants, Rubisco is a considerably major protein in leaf [12, 13]. There is evidence from studies altering expressions of Rubisco or its activation enzyme, Rubisco activase that changes in the activity and/or the amount of Rubisco in leaf significantly affect leaf photosynthetic rate [12, 13].

Data of Figure 4 indicate that transplantation increased leaf chlorophyll content. Arp found that a rough and positive correlation exists between pot size and leaf photosynthetic rate or pot size and leaf chlorophyll content [1]. The findings by Arp implicate that a rough and positive correlation may exist between leaf chlorophyll content and photosynthetic rate. Our results support the findings by Arp and the implication. Thus, an increase in leaf chlorophyll content might have also contributed to the transplantation-induced increase in leaf photosynthetic rate. However, there is a report that chlorophyll-less soybean isolines had similar leaf photosynthetic rate as the wild type at full sun photosynthetic photon flux densities [14].

RuBP is a substrate for Rubisco. Thus, it is thought that leaf RuBP content decreases when leaf Rubisco activity increases. Leaf RuBP content in transplanted plants was significantly or on the average lower than that in control plants (Figure 5). This result supports the suggestion that transplantation increased leaf Rubisco activity in vivo. In single-rooted soybean leaves that are the same species as we used in this study, it was demonstrated that continuous exposure to light, which increases photosynthetic source capacity, or treatment of roots with low temperatures, which decreases root sink capacity, decreases the leaf photosynthetic rate and Rubisco activity and increases the leaf RuBP content [15–18].

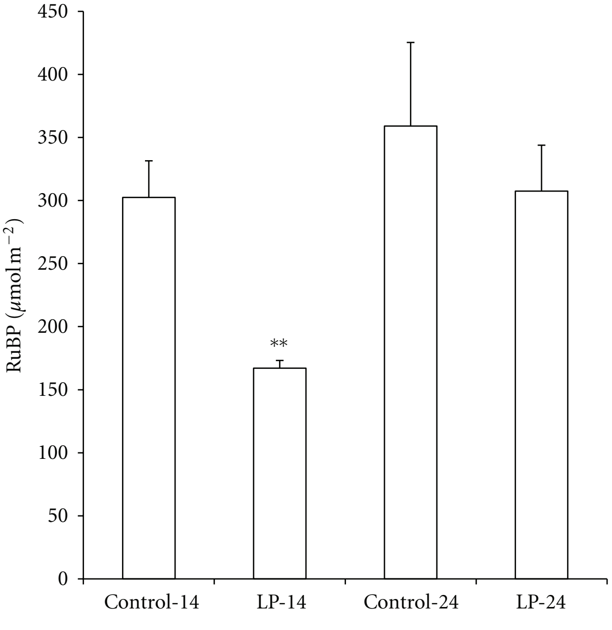

Figure 5: Leaf RuBP content in soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Vertical bars, S.D. ($n = 3$). $^{**}P < 0.01$ (t-test) when compared with control.

Sucrose and starch are the major photosynthetic carbohydrates in plants. In this study, it was shown that whereas leaf sucrose content was higher in transplanted plants than in control plants, leaf starch content was lower in transplanted

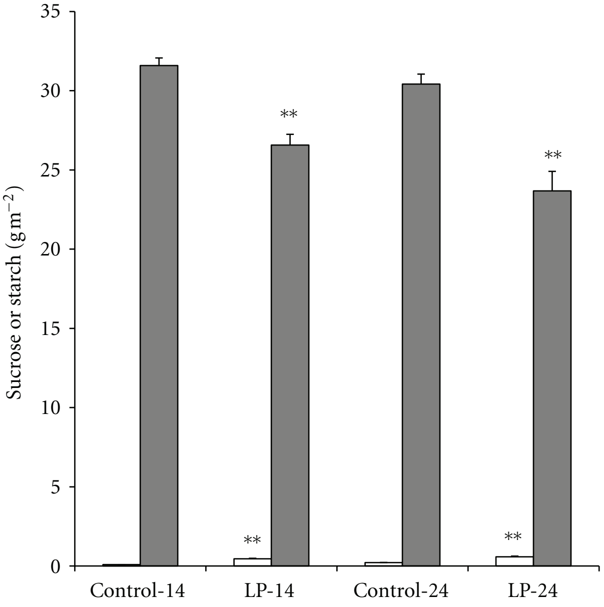

Figure 6: Leaf sucrose and starch contents in soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Open bar, sucrose; closed bar, starch. Vertical bars, S.D. ($n = 3$). $^{**}P < 0.01$ (t-test) when compared with control.

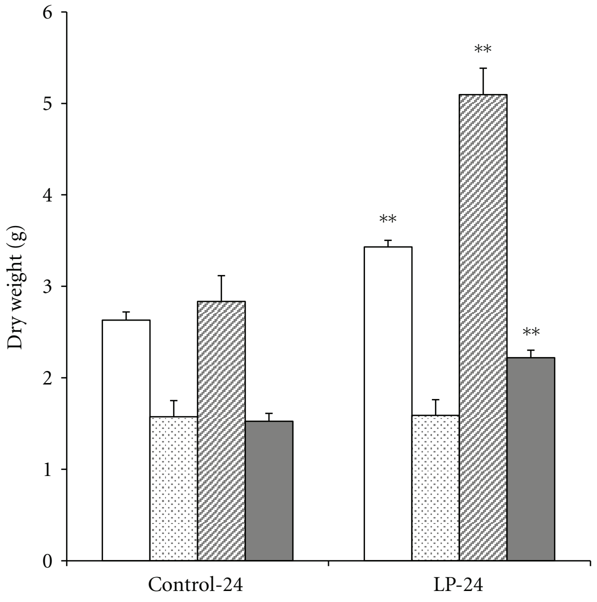

Figure 7: Dry weights of source (leaves) and sink organs (stems, floral organs or roots) in soybean plants grown with original pots (Control) and with larger pots (LP). Half of plants with age of 40 days were grown with larger pots for 24 days (Control-24 or LP-24). Open bar, leaves; dotted bar, stems; shaded bar, floral organs; closed bar, roots. Vertical bars, S.D. ($n = 3$). $^{**}P < 0.01$ (t-test) when compared with control.

plants than in control plants on both days (Figure 6). However, when the total content of sucrose and starch in leaf was calculated, the content was lower in transplanted plants than in control plants on both days (not shown). As data of Figure 7 indicate that transplantation increased dry weights of source and sink organs and the ratio of sink to source organs, these results indicate that transplantation increased the plant sink capacity that uses especially leaf starch for whole plant growth, particularly, growth of sink organs.

There is a hypothesis of inhibition of photosynthesis through accumulation of sucrose in leaf, although the regulatory mechanism(s) is still unclear [5]. For example, in a study conducting continuous exposure to light of single-rooted soybean leaves, it was demonstrated that significant negative correlation exists between leaf sucrose content and photosynthetic rate [15]. In this study, leaf sucrose content was higher in transplanted plants that had higher leaf photosynthetic rate than in control plants on both days (Figures 1 and 6). It is thought that in transplanted plants, sucrose-induced inhibition of leaf photosynthetic rate was, if any, very small. Leaf sucrose content of transplanted plants on day 24, which was the highest content observed in this study, was about one third of a content that led to a small decrease (about 25%) in leaf photosynthetic rate of single-rooted soybean leaves [15]. There is also a hypothesis of inhibition of photosynthesis through accumulation of starch in leaf [5]. In the same study using single-rooted soybean leaves, it was also demonstrated that significant negative correlation exists between leaf starch content and photosynthetic rate [15]. Another study using single-rooted soybean leaves demonstrated that accumulation of starch in leaf decreases the rate of CO_2 diffusion within leaf [19]. Leaf starch content of control plants on day 14, which was the highest content observed in this study, almost corresponded with a content that led to a large decrease (about 45%) in leaf photosynthetic rate of single-rooted soybean leaves [15]. Data of Figure 6 indicate that transplantation decreased leaf starch content. Therefore, it is suggested that the decrease in leaf starch content could cause the transplantation-induced increase in leaf photosynthetic rate by inducing an increase in the rate of CO_2 diffusion within leaf and thereby substantiating an increase in leaf Rubisco activity in vivo. As transplantation increased plant sink capacity that uses leaf starch for whole plant growth, particularly, growth of sink organs, it is concluded from data of this study that transplantation of soybean plants into larger pots attempted in this study increased the plant photosynthetic matter production by increasing mainly sink capacity that uses leaf starch for whole plant growth, particularly, growth of sink organs. Although it is not study investigating the effect of pot size on plant photosynthetic matter production, a number of studies have implicated that there is downregulation of leaf photosynthesis through accumulation of photosynthetic carbohydrate (sucrose and/or starch) in leaf [5].

As described in the Introduction, a study using cotton seedlings showed that smaller pots decreased leaf photosynthetic rate and stomatal conductance and increased leaf starch content [4]. Our results are consistent with the report.

The study, however, did not analyze activity of Rubisco in leaf extract and contents of total protein, chlorophyll and RuBP in leaf. To our knowledge, until now, in similar studies other than this study that have examined the effect of pot size, a series of analyses we carried out have not been conducted, and transpiration rate and content of total protein in leaf and initial and total activities of Rubisco in leaf extract have not been analyzed.

Arp found from collected data that a rough and positive correlation exists between pot size and leaf photosynthetic rate or pot size and increased ratio of root to shoot [1]. The findings by Arp implicate that a rough and positive correlation may exist between leaf photosynthetic rate and increased ratio of root to shoot. Our results essentially support the implication, since transplantation increased leaf photosynthetic rate and the dry weight ratio of sink to source organs (Figure 7). Since the findings by Arp [1] and the study using cotton seedlings [4], substantial information on the effect of pot size on plant photosynthetic matter production has been scarce.

Regarding the phenomena seen in soybean plants in this study, essential similarity has also been seen in plants subjected to other manipulations to alter source or sink capacity. For example, removal of developing pods (soybean plants), which decreases sink capacity, was shown to result in accumulation of major photosynthetic carbohydrate (sucrose) in leaf, decrease in leaf photosynthetic rate, and decrease in Rubisco activity of leaf extract [20]. Data from other studies conducting removal of floral organs or petiole girdling, which decreases sink capacity, or continuous exposure to light, which reduces sink capacity by increasing photosynthetic source capacity, suggest that a decrease in stomatal conductance or Rubisco activity or Rubisco content in leaf, or both decreases in Rubisco activity and Rubisco content in leaf are responsible for the reduced sink capacity-induced decrease in leaf photosynthetic rate [21–28]. In potato and *Arabidopsis*, continuous exposure to light has been shown to accelerate expressions of photosynthetic genes, pigments and proteins, and subsequent declines of the expressions [29, 30]. It is important to elucidate the detailed mechanism(s) of how pot size affects plant photosynthetic matter production. Some studies have described mechanism(s) concerning regulation of plant leaf photosynthesis through photosynthetic source-sink balance, although they are not studies that have investigated the effect of pot size on plant photosynthetic matter production. For example, studies using transgenic plants suggest that hexokinase may be involved in carbohydrate-mediated repression of photosynthetic gene expression [31–33]. Other 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 [34]. 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 still unclear. Data obtained in this study and those from other studies that have investigated the effect of pot size on plant photosynthetic matter production strongly suggest that pot size can largely affect plant leaf photosynthesis and organization of source and sink organs, including the capacities of source and sink. Therefore, further studies are important for elucidation of the mechanism(s) responsible for regulation of plant photosynthetic matter production through photosynthetic source-sink balance.

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