High Carbon Requirements for Seed Production in Soybeans

[Glycine max (L.) Merr.]

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Abstract: In soybeans, increase in the dry weight of seed (IWS) during the seed-filling period (SFP) is nearly half that of the whole plant (IWP). Hence, the contributions of assimilate during SFP to seed growth in soybeans is apparently lower than that in other cereal crops. However, analysis of the assimilate budget for seed production based on dry matter could be misleading in soybeans because the carbon (C) contents of seed and that of other organs are different due to the differences in lipid and protein content and respiration loss in each organ results in underestimation of the C contribution to seed production. In field soybeans, irrespective of shading and thinning, IWS/IWP and harvest index (HIW) for dry weight were slightly lower than ICS/ICP and HIC for C, respectively. Of the ¹³C fed at the vegetative stage and early SFP, 3.4% and 16.4%, respectively were accumulated in the seed, 0.9% and 28.2%, respectively, in the pod shell and 26.8% and 37.8%, respectively, were respired before maturity. Ninety-six percent of seed C was assimilated during the SFP. Respiratory loss of ¹³C fed at the early SFP was larger than that from ¹³C at the vegetative stage, showing that seed production requires much more C than leaf or stem growth. These results suggest that the difference in C contents between plant organs has little effect on the estimation of assimilate partition into soybean seeds based on dry matter, and high respiration loss for seed growth reduces the partition of dry matter into seeds.

Key words: Harvest index in the carbon (HIC), Harvest index in the dry weight (HIW), Increase in the carbon of seed (ICS), Increase in the carbon of whole plant (ICP), Increase in the dry weight of seed (IWS), Increase in the dry weight of whole plant (IWP), Respiration, Soybean.

Soybean production in the world has increased during the past several decades, but the yield is lower than that of other cereals (United States Department of Agriculture, 2006). The dry weight of vegetative organs in soybeans increases for 4−5 weeks after flowering, and the seed dry weight markedly increases when assimilate is translocated from vegetative organs (Ojima and Fukui, 1966b). Soybean seeds grow continuously accompanied with the growth of leaves and stems, and a higher percentage of carbon (C) fixed during the seed-filling period (SFP) is partitioned to the seeds (Hume and Criswell, 1973; Akao et al., 1987). In soybeans, regardless of the growth habitat, there was a linear relationship between increase in plant dry-matter increase and seed dry-matter; half of the dry matter produced during the SFP was consumed for vegetative organs and the remaining half for seed production in diverse soybean cultivars, and the reserves stored before flowering apparently did not contribute to seed production (Kakiuchi and Kobata, 2004, 2006).

Soysbeans are crops with a low seed production efficiency compared with other cereal crops; in soybean, 2 g of photosynthate is needed to produce 1 g of seed while in cereal crops only 1.3−1.4 g is needed according to the calculations based on conversion of chemical components (Sinclair and de Wit, 1975). Soybean seeds contain a large amount of protein and lipids (Taira and Taira, 1971), and the depositing of protein and lipids in the seeds requires a large amount of energy (Ojima and Fukui, 1966a; Osaki et al., 1988). Therefore, the contribution of assimilate to seed production based on the analysis of C content would result in an underestimation. Furthermore, the greater respiration loss for synthesis of the seed materials might result in underestimation of the contribution of assimilate to the seed production. However, there is little evidence directly indicating the C loss for seed production compared with that for the whole plant in soybeans.

The use of carbon isotopes can allow one to directly estimate C translocation and respiration loss (Palta et al., 1994). The rate of distribution to seed of ¹⁴C fed at the start of the flowering, pod elongation or seed growing stage increased in this order, i.e., with the progress of the growing stage (Hume and Criswell, 1973), although in their study C loss by leaf abscission was ignored. Most of the ¹³C fed during the reproductive period in soybean was translocated to seeds, while the contribution of C assimilated...
during the vegetative stage to seed production was not estimated (Akao et al., 1987). Furthermore, C loss due to the fall of leaves and respiration, and the role of C stored in seed production should be incorporated to evaluate accurately the contribution of assimilate to seeds.

The objectives of the present study were to clarify the effect of differences in C contents between the seed and other organs and that of respiration loss on the dry-matter increase during SFP of the whole plant (IWP) and that of seed (IWS) and also on harvest index (HI). Contents of C in plant organs and C translocation among organs and respiration loss were estimated by $^{13}$C analysis before and after flowering.

**Materials and Methods**

1. **Effect of difference in C content among plant organs on IWS/IWP and HI**

   **(1) Plant materials**

   Field experiments were conducted in 1999 at a farmer’s field in Matsue, Shimane, Japan. The experimental site was a 20×15 m plot of silty clay loam in a well-drained paddy field. Seeds of the soybean cv. Tamahomare were sown on 16 June 1999 in rows 0.60 m apart, with 0.15 m hill distance (11.1 hills m$^{-2}$) (standard density). Two seeds were sown in 0.03 m-deep holes of each hill and covered with adjacent soil by hand. After establishment (two weeks after germination), one seedling per hill. Magnesium lime at 66.7 g m$^{-2}$, N at 1.05 g m$^{-2}$ [as (NH$_4$)$_2$SO$_4$], P at 7.0 g m$^{-2}$ (as P$_2$O$_5$) and K at 6.9 g m$^{-2}$ (as KCl) were applied following conventional procedure and mixed with the soil before sowing. Soil water potential monitored with a tensiometer fell below −40 kPa only three times. When soil water potential decreased below −40 kPa, the field was surface-irrigated to raise it above −18 kPa.

   **(2) Thinning and shading treatment**

   The plants were thinned or shaded from R2 (flowering) to R8 (maturing) stages (Fehr and Caviness, 1977) in both years to change IWP. On 7 August 1999 at R2 the plant density was reduced to half (5.6 plants m$^{-2}$, 1/2 thinning treatment) or a quarter (2.8 plants m$^{-2}$, 1/4 thinning treatment). Shading treatment was applied to 1.8×4.2 m areas in the plots at standard density on 11 August at R2 using wooden frames 1.1 m high covered with a single white cheesecloth (light shading treatment), a single black cheesecloth (moderate shading treatment) or a double layered black fabric (heavy shading treatment). On a fine day on 29 August short-wave radiation inside and outside the shading frame was alternately measured with a solar meter (SOLAR130, HAEJNI, Jegenstorf, Switzerland) at 1100, 1330, and 1700. It showed that the heavy shading, moderate shading and light shading treatment reduced full-sun radiation by 71%, 53% and 22%, respectively. The control plots were neither thinned nor shaded, and the plants in these plots were grown under full sun conditions throughout the growing season. The experimental plots were laid out in a randomized block design with three replications of the treatment (control, thinning, and shading treatments).

   **(3) Measurements**

   Five plants were harvested from each treatment plot on August 5 at the R2 stage and on November 6 at the R8 stage. The sampled plants were dried in an oven at 80°C for 48 h and then weighed. The numbers of filled pods, total pods and seeds on each plant were measured. Abscised organs were carefully gathered two or three times each week, dried at 80°C for 48 h and then weighed. These weights were added to the weights measured above. Carbon content of each organ sampled at R2 and R8 was measured using an infrared carbon analyzer equipped with a combustion device (CID-301, Nippon Bunko Co. Tokyo).

2. **$^{13}$C Feeding for estimations of C partition and respiration loss**

   **(1) Plant material**

   Pot experiments were conducted in 2004 at Matsue, Shimane, Japan. Three seeds of the soybean cv. Tamahomare were sown on 23 June in pots with a surface area of 0.2 m$^2$, which were filled with 5 kg pot$^{-1}$ of soil for planting rice seedlings (Green-soil, Izumo Green Epoch Co., Izumo, Japan). Seeds were inoculated with leguminous bacteria. Magnesium lime at 15 g pot$^{-2}$, N at 0.1 g pot$^{-2}$ [as (NH$_4$)$_2$SO$_4$], P at 0.7 g pot$^{-2}$ (as P$_2$O$_5$) and K at 0.3 g pot$^{-2}$ (as KCl) were applied and mixed with the soil before sowing. Two seedlings were removed on 7 July, and one seedling was left to grow. Pots were laid outside, and sprinkler irrigation was applied every morning.

   **(2) $^{13}$C feeding**

   The plants fed $^{13}$CO$_2$ on 1 August, immediately before flowering (V10) (Fehr and Caviness, 1977), and on 3 September, at the start of seed filling (R5). Eight pots were placed in the airtight feeding chamber (0.69×0.69×0.90 m), covered with high-transparency polyester sheets designed for greenhouse rooftop. CO$_2$ and water (H$_2$O) in the chamber were deleted as much as possible by adding soda lime and silica gel before $^{13}$C feeding. The $^{13}$CO$_2$ level in the chamber was monitored with a $^{13}$CO$_2$ analyzer (CI-302, CID, INC, WA, USA) using the known relationship between $^{13}$CO$_2$ and $^{12}$CO$_2$ (Svejcar et al., 1990), and the $^{13}$CO$_2$ concentration was maintained around 400 μ moles mol$^{-1}$. $^{13}$CO$_2$ in plants without $^{13}$CO$_2$ feeding was used as natural abundance (NA). Experimental design was complete random of...
two treatments $^{13}$CO$_2$ feeding at V10 ($^{13}$C-V10) and at R5 ($^{13}$C-R5) with four replications.

3 Measurements

The plants fed $^{13}$C at V10 and without $^{13}$CO$_2$ feeding (NA plots) were sampled at R2 (7 August) and R8 (29 October), and the plants fed $^{13}$C at R5 and those in the NA plots were sampled immediately after feeding (R5) (4 September) and at maturity (R8) (29 October). Four plants were harvested from each plot, and after counting the numbers of total nodes, floral organs, total pods and seeds in each plant. They were separated into the stem, leaves, pod shells, seed and roots, and dried at 80°C for 48 h and then weighed. Abscised organs were carefully gathered two or three times each week, dried at 80°C for 48 h and then weighed. These weights were added to the dried sample weights.

The $^{13}$C content of each organ was measured using an infrared carbon analyzer equipped with a combustion device (EX-130S, Nippon Bunko Co. Tokyo). Total C content and $^{13}$C content were calculated using the following equations (Palta et al., 1994):

\[
\text{total C content} = \frac{\text{TC}}{\text{dw} \times (1 - \text{WC})} \times \text{DW}
\]

\[
^{13}\text{C content} = \left(^{13}\text{Catom}\% - ^{13}\text{CNA atom}\%\right) \times \text{total C}
\]

Where, TC is the carbon content of analyzed material, dw is the dry weight of analyzed material, WC is the water content of analyzed material, DW is the total dry weight of a plant, $^{13}$Catom% is the ratio of $^{13}$C to total carbon in $^{13}$CO$_2$ fed plant and $^{13}$CNA atom% is the ratio of $^{13}$C to total carbon in each NA plant. The amount of carbon reserved in vegetative organs before flowering and translocated to seed ($Cv$), the amount of carbon assimilated during the SFP and contributed to seed production ($C_{SFP}$) and the respired ($Cr$) were
estimated by the following equations:

\[ C_v = \left( \frac{\text{total } C}{\text{C}_{\text{start}}} \right) \times \text{C}_s \]

\[ C_{\text{app}} = \text{total } C_s - C_v \]

\[ C_r = \left( \frac{\text{total } C_{\text{start}}}{\text{C}_s} \right) \times \left( \frac{\text{C}_{\text{start}} - \text{C}_{\text{res}}}{\text{C}_s} \right) \]

where \( \text{C}_{\text{start}} \) is the \( ^{13} \)C content of the plant immediately after \( ^{13} \)C feeding at V10, \( \text{C}_s \) is the \( ^{13} \)C content of the seed at R8, total \( C_s \) is the C content of the seed at R8, total \( C_{\text{start}} \) is the C content of the plant immediately after \( ^{13} \)C feeding, \( C_{\text{start}} \) is the \( ^{13} \)C content of plant immediately after \( ^{13} \)C feeding at V10, and \( \text{C}_{\text{res}} \) is the \( ^{13} \)C content of the plant at R8.

### Results and Discussion

1. **Effect of carbon content on IWS/IWP and HI**

   Seed yield of controls in the field experiment was 248 g m\(^{-2}\) at 14% water content. When plants were grown under shading or thinning treatments during the SFP, IWP was changed, but there was a linear relationship between IWP and IWS (Fig. 1-a). The ratio of IWS/IWP was 0.54 and that of ICS/ICP (ICP: Increase in C content of whole plant during SFP and ICS: Increase in C content of seed) was 0.57 (Fig. 1-b). The average HIC (harvest index for C base) (0.42) was higher than the average HIW (0.38) (Fig. 2).

   These results showed that the difference between the dry-matter increase and C increase was small, but IWS/IWP and HIW were underestimated by 6% and 11% compared with ICS/ICP and HIC, respectively.

2. **Allocation of C fed at the vegetative stage and the effects of respiration on IWS/IWP and HI**

   IWS/IWP and HIW in pot-grown plants were 0.52 and 0.36, respectively, although these values were slightly lower than that under field conditions (Fig. 1 and 2). Most of the \( ^{13} \)C fed at the vegetative stage (V10) accumulated in the leaves at R2 (6 days after feeding) (Table 1). \( ^{13} \)C in the leaves, stem and root at flowering (R2) was 77.9, 35.6, and 28.3 mg plant\(^{-1}\), respectively. The amount of \( ^{13} \)C in the leaves, stem, pod shell and seed at maturity (R8) was 46.5, 25.9, 25.2, 0.9% and 3.4% of the

|  |  |  |  |  |  |
|---|---|---|---|---|---|
|  | Stem (mg) | Leaf (mg) | Root (mg) | Pod (mg) | Seed (mg) |
| 13C-V10 R2 | 35.6 ± 0.24 | 77.9 ± 7.95 | 28.3 ± 2.19 |  |
|  | R8 | 25.9 ± 2.30 | 46.5 ± 4.29 | 25.2 ± 1.66 | 1.2 ± 0.09 |
|  | 4.9 ± 0.30 |  |  |  |  |
| 13C-R5 R5 | 4.0 ± 0.41 | 17.4 ± 2.59 | 7.3 ± 0.71 | 16.7 ± 1.28 |
|  | R8 | 1.2 ± 0.29 | 4.8 ± 0.88 | 2.0 ± 0.32 | 12.8 ± 2.29 |
|  | 7.5 ± 0.69 |  |  |  |  |

*Data show average of four replications ± standard error.

Fig. 3. Distribution of \( ^{13} \)C fed at the vegetative stage (V10) to each organ at R2 and R8, and to respiration before R8.

Fig. 4. Distribution of \( ^{13} \)C fed at R5 to each organ at R5 and R8, and to respiration before R8.
C was distributed in the stem, leaves, root, pod and seed at R8, respectively (Fig. 3). These results indicate that the stored C in vegetative organs before flowering slightly contributes to pod shell and seed production. Our result supports a result that reserved carbohydrate is scarcely needed for seed filling under suitable conditions without stress such as water deficit (Rawson et al., 1978).

3. Allocation of C fed at early SFP and the effects of respiration on IWS/IWP and HI

Total amount of $^{13}$C fed and fixed at the early SFP (R5) was 45.5 mg plant$^{-1}$, which was distributed as follows: 4.0 mg to the stem, 17.4 mg to the leaves, 7.3 mg to the root and 16.7 mg to the pod (Table 1). The amount of $^{13}$C in the leaves, stem, root, pod shell and seed at R8 was 4.8, 1.2, 2.0, 12.8 and 7.5 mg plant$^{-1}$, respectively. As a result, 17.2 mg plant$^{-1}$ of $^{13}$C, that is, 37.8% of fixed C, was consumed by respiration between R5 and R8 (Fig. 4). The fed C was stored as follows: 8.8% in the stem, 38.3% in the leaves, 16.1% in the root and 36.8% in the pod at R5, and 2.7% in the stem, 10.6% in the leaves, 4.3% in the root, 28.2% in the pod and 16.4% in the seed at R8 (Fig. 4). Thus, most of the storage carbon in vegetative organs was used for seed production and respiration during the SFP. The respiratory loss of C fixed at the early SFP was greater than that of C fixed at the vegetative stage (Figs. 3 and 4).

These results suggested that respiration loss during seed filling is a significant reason for lower IWS/IWP and HI in soybeans.

Conclusion

Differences in C content between seeds and other organs have little effect on IWS/IWP and HI in soybeans. If respiration loss for seed production is incorporated, nearly 80% of C fixed at the early SFP could be used for seed and pod production, although only less than half of it remains in the seeds. The lower IWS/IWP or HI in soybeans than other cereals seems to come from high respiration loss more than from a high concentration of C in seeds. The greater requirement of C in the pod-shell production might be due to feeding at the early SFP. Our result showed that lower seed production in soybeans than in other starch grain crops results from greater C requirements for growth of seed and reproductive organs.

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