Effects of Source/Sink Ratio and Cytokinin Application on Pod Set in Soybean

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Abstract: A large proportion of soybean flowers abort during development. Pod set is significantly affected by the availability of assimilate and cytokinin in the flowers, but their synergistic effects on pod set remain unclear. The objective of this study was to examine whether pod set at specific nodes of a plant is enhanced by increasing the source-sink ratio and applying cytokinin to the nodes. The source-sink ratio was manipulated by removing floral buds excluding the specific nodes. As the ratio increased, the number of pods per node of control plants increased curvilinearly, reaching a plateau at high source/sink ratios. By contrast, in the cytokinin-applied plants, the number of pods per node increased without the plateau with increasing source/sink ratio, either by increasing the number of flowers or by increasing the pod-set percentage depending on the year. The results indicated that cytokinin plays a promotive role in increasing pod number in the plants with high levels of assimilate availability.

Key words: Cytokinin, Flower abortion, Glycine max, Photoassimilate, Pod set, Source/sink ratio, Soybean.

A large proportion of flowers abort during development in soybean (Kato, 1964; Abernethy et al., 1977). Under normal growing conditions, pod set percentage ranges from 20 to 40% (Wiebold et al., 1981; Jiang and Egli, 1993). By reducing this abortion, we might be able to increase pod and seed numbers, thereby increasing the yield (Kokubun, 2004). Many previous studies have focused on two possible factors that might be involved in regulating the abortion: availability of photoassimilate or nutrients (Brevedan et al., 1978; Antos and Wiebold, 1984; Brun and Betts, 1984; Heitholt, 1986a, 1986b) and quantity of certain hormones (Huff and Dybing, 1980; Heindl et al., 1982; Spollen et al., 1986a, 1986b; Carlson et al., 1987; Yarrow et al., 1988; Kokubun and Honda, 2000).

In soybean, like many other leguminous species, vegetative growth lasts until the middle or late stage of reproductive growth. Therefore, a strong competition for photoassimilate among plant organs can lead to a deficiency of photoassimilate available to reproductive structures (Antos and Wiebold, 1984; Brun and Betts, 1984). Shading treatment during a period from flowering to pod development significantly reduced pod number (Kokubun, 1988; Kurosaki and Yumoto, 2003), but increase in light intensity by supplemental light or plant shape manipulation increased pod number (Schou et al., 1978; Kokubun, 1988). These results suggest that inadequate supply of photoassimilate to flowers is a dominant factor in the abortion.

The formation and abortion of reproductive organs in soybean are influenced by phytohormones. Huff and Dybing (1980) applied extracts from flowers and young pods to growing flowers, and found that the extract from young pods accelerated the abortion of flowers. Then they applied a lanolin paste containing either indoleacetic acid (IAA), gibberellin (GA) or 6-benzylaminopurine (BA) to the raceme, and found that IAA accelerated the abortion like the extract, while GA and BA did not. Their results suggest that IAA plays a critical role in accelerating flower abortion, although there is a conflicting report showing that IAA delays the abortion (Oberholster et al., 1991). Liu et al. (2003) found that the reduced pod-set percentage induced by water deficit was associated with an increase in ABA content of reproductive structures during a period from 3 to 5 days after anthesis. Endogenous cytokinins detected in exudate showed a maximum concentration during a period from 0 to 9 days after anthesis, when most flowers are destined to either initiate or abort pods (Heindl et al., 1982; Carlson et al., 1987). The application of BA to racemes reduced abortion and increased pod number (Crosby et al., 1981; Carlson et al., 1987; Dyer et al., 1987; Peterson et al., 1990; Mosjidis et al., 1993; Reese et al., 1995; Nagel et al., 2001). Within individual racemes, pod set probability at different floral positions was closely associated with the cytokinin concentration (Kokubun...
and Honda, 2000). These results strongly indicate that cytokinin produced in roots and translocated to flowers might be involved in an enhancement of pod initiation.

These previous studies raise a question whether increased supply of photoassimilate and cytokinin application have a synergistic effect on the enhancement of pod set in soybean. The objective of this study was to examine whether pod set of a soybean plant could be enhanced by increasing the ratio of source (leaf) to sink (floral organ) and by applying cytokinin (BA) to the nodes. The ratio of source to sink was manipulated by changing the number of nodes bearing floral structures, and leaf photosynthetic rate and starch accumulation were monitored as criteria for the strength of source activity.

Materials and Methods

Soybean (Glycine max L. Merr.) cultivar Enrei was used in this study. Four seeds per pot (16 cm diameter, 19 cm tall) were sown on May 30 and thinned to two plants per pot after emergence. Prior to seeding, 0.5 g N, 2 g P2O5, 2 g K2O and 5 g of slaked lime per pot were applied by mixing the fertilizers with soil. The soil type was a fine-textured clayey Terrace Yellow Soil (Classification Committee of Cultivated Soils, 1996). The soil was inoculated with Bradyrhizobium japonicum prior to seeding. The plants were grown outdoors with adequate irrigation.

The branches were pruned whenever emerged, and only the main stem was allowed to grow in this study. Source/sink ratio was manipulated by removing floral buds, as shown in Fig. 1. The ratio was adjusted at four levels; N5, N3, N1 and C - with five nodes, three nodes, one node and all nodes allowed to bear floral buds, respectively. The buds were removed by hand when they appeared. The date of flowering of individual flowers was recorded using six plants (three pots) for each source/sink treatment, and thereafter the growth of the flowers (pod set or abortion) was monitored everyday. All the flowers that visibly opened were counted and regarded as the number of flowers. The number of pods was defined as all the pods retained at maturity. Pod set percentage was defined as the number of pods divided by the number of flowers. BA was applied to half of the plants whose source/sink ratio was manipulated. BA solution was prepared as described by Crosby et al. (1981). Briefly, a small quantity of BA was dissolved in 1 N KOH and diluted to 2 mM with 16 mM K-phosphate buffer (pH 6.4) containing 0.05% (v/v) Tween 80. The BA solution was applied, using a syringe, as a drop to individual racemes (0.1 mL per raceme). The applications were made at 3 and 15 DAA (days after anthesis, when the first flower in the plant opened) when the inflorescence had reached developmental stages of R1 and R3 (Fehr et al., 1971), respectively, because these stages were found to be most responsive to BA application (Crosby et al., 1981; Peterson et al., 1990). Control plants received the solution without BA.

The photosynthetic rate in the leaf at the 10th node in three plants of each treatment was measured with an LI 6400 Portable Photosynthesis System (LI-COR, NE, USA). The measurement was carried out during 1000 and 1200 hr on 12, 21 and 32 DAA. The flow rate of air into the leaf chamber was controlled at 500 μmol s\(^{-1}\), and the CO\(_2\) concentration was kept at 350 μmol mol\(^{-1}\). The irradiance on the measured leaves (6 cm\(^2\)) was controlled to 1,500 μmol m\(^{-2}\) s\(^{-1}\) PPFD. The temperature of the chamber was maintained at 25°C. After the measurement of photosynthesis, the leaves were sampled, oven dried at 80°C for three days and weighed. The dried samples were ground in a mill and the starch content was analyzed using the procedure described by Kawabata (1999).

The same experiments were conducted in two years (2002 and 2003).

Results

1. Effects of source/sink ratio in control plants

With increasing source/sink ratio, the numbers of flowers and pods per node increased (Fig. 2, open bars). The number of pods per node was significantly higher in N3 and N1 than in C, but there was no significant difference between N3 and N1 (P<0.05). Pod set percentage ranged from 41 to 78% depending on the source/sink ratio and year, and it was highest in N3 in both years.

The results obtained on the photosynthetic rate and starch content were similar in the two years. Therefore, only the results obtained in 2003 are described here. Generally, the photosynthetic rate in the 10\(^{th}\) leaf tended to become lower at later stages, although it recovered at 32 DAA in C and N5 plots (Fig. 3, open bars).

The starch content of the 10\(^{th}\) leaf was higher at 21 and 32 DAA than that at 12 DAA irrespective of...
source/sink ratio (Fig. 4, open bars). The starch contents at 12 and 21 DAA in N5 and N3 plots were significantly lower than those in the C plot, but those in the N1 plot were not significantly different from those in the C plot at any DAA (P<0.05).

2. **Interactive effects of source/sink ratio and cytokinin application**

The data for the cytokinin-applied plots are indicated as shadowed bars in Fig. 2, 3 and 4. The number of flowers per node increased with increasing source/sink ratio in both the control and cytokinin-applied plots. When the source/sink ratio was raised from N3 to N1, the increase in the number of pods per node was clear in cytokinin-applied plants, whereas the increase was vague in control plants (Fig. 2). The pod set percentage tended to be low in cytokinin-applied plots compared with the control plot. The number of aborted flowers and pods tended to increase with increasing source/sink ratio in cytokinin-applied plants, but this tendency was not obvious in the control plots.

![Fig. 2. Effect of source/sink ratio and cytokinin application on the number of flowers per node, number of pods per node and pod-set percentage in 2002 and 2003. Values represent the mean±SE of six plants.](image-url)
Generally the photosynthetic rate in the 10th leaf did not significantly differ between cytokinin-applied and control plots, although it tended to be higher in cytokinin-applied plants than in the control plants in the N1 plot (Fig. 3). The rates decreased during the period of 12 to 21 DAA in all the plots, and then tended to recover at 32 DAA in C and N5 plots.

The starch content of the 10th leaf at 21 and 32 DAA was higher than that at 12 DAA irrespective of source/sink ratio (Fig. 4). The concentration tended to be lower in N5 and N3 plots of cytokinin-applied plants, although the difference was not significant (P<0.05).

**Discussion**

Even under favorable conditions, a large proportion of soybean flowers are destined to abort without bearing pods. This is probably caused by a deficient supply of photoassimilate to flowers, which might be induced by a competition for photoassimilates among leaves, stems, nodules and reproductive organs (Brun and Betts, 1984; Antos and Wiebold, 1984). In the present study, therefore, we examined the effects of increased supply of photoassimilate to flowers, which was manipulated by increasing the ratio of source (leaves) to sink (the number of nodes bearing floral buds). We expected that the number of pods per node might increase proportionally with increasing source/sink ratio. However, the number of pods per node tended to reach a plateau at a higher source/sink ratio (N3 and N1 plots), although the number of flowers per node increased proportionally to the ratio up to N1 (Fig. 2, control plants). This raised the following question. Can the number of pods be increased
further in the plants with a higher source/sink ratio?

Bruning and Egli (2000) analyzed the correlation of leaf starch accumulation with pod set using the phloem-isolated nodes in which the supply of photoassimilate was manipulated by defoliation and girdling. They found that pod number was directly related to assimilate supplies at low levels of assimilate availability with no accumulation of starch in leaves. At high levels of assimilate availability, however, there was only a slight increase in pod number, and starch accumulated in the leaves. In the present study, there were substantial increases in starch content of leaves during the period of 12 to 21 DAA in all the plots, and the increase was remarkable in the N1 plot than in N5 and N5 plots; for example, the content in the N1 plot increased from about 80 mg g\(^{-1}\) at 12 DAA to 170 mg g\(^{-1}\) at 32 DAA (Fig. 4). It appears that assimilate availability in the N1 plot was such a high level that further enhancement of assimilate supply could not lead to an increase in pod number. Contrary to our expectation, the starch content in the N1 plot was almost the same as that in the C plot, and that in N5 and N3 plots was lower than that in C and N1 plots (Fig. 4). Unknown factors other than source-sink ratio, might affect the leaf starch content.

In the plants with high levels of assimilate availability, a feedback mechanism triggered by starch accumulation in leaves might be working, resulting in a depression of photosynthesis in the leaves (Mondal et al., 1978; Setter et al., 1980; Bruening and Egli, 2000). This phenomenon appeared to have occurred during the period of 12 to 21 DAA when photosynthetic rate declined (Fig. 3) and starch content increased (Fig. 4). During the period of 21 to 32 DAA, the photosynthetic rate in C and N5 plots recovered regardless of less change of starch content, whereas the rate in N3 and N1 plots did not recover. The physiological factor responsible for the contrasting change is not clear.

Several previous studies indicated that cytokinin plays a critical role in pod set (Crosby et al., 1981; Dyer et al., 1987; Peterson et al., 1990; Mosjidis et al., 1993; Kokubun and Honda, 2000; Nagel et al., 2001). Therefore, we examined whether pod number can be increased by applying cytokinin to the plants with a high source/sink ratio. In cytokinin-applied plants, the number of pods per node was markedly higher than that in the control plants in the N1 plot with the highest source/sink ratio, but not in the C plot with a low source/sink ratio (Fig. 2). The increase of pod number was attributed either to the increase of pod-set percentage in 2002, or to the increase of the number of flowers in 2003. It is noteworthy that the pod number in the plants with cytokinin applied increased with increasing source/sink ratio from C to N1, but that in the plots without the cytokinin application reached a plateau at N3.

Previous studies suggested that cytokinin acts to redirect movement of photoassimilate into sinks, increase sink strength and subsequently enhance growth rates of the flowers and pods (Huff and Dybing, 1980; Dybing, 1994; Reese et al., 1995). In the present study, cytokinin application increased the photosynthetic rate and pod number, but not the starch content of leaves (Fig. 2, 3, 4). These results suggest that cytokinin applied to sink organs might enhance translocation of assimilates from leaves to sinks, and hence enhance photosynthesis in the leaves of the plants with a high source/sink ratio, although the exact role of cytokinin in pod development should be examined in further studies.

Nagel et al. (2001) introduced BA into the xylem stream through a cotton wick during anthesis and found that the effect of BA application on pod number and seed yield was consistently significant in the greenhouse, but it was variable in field trials. Their finding suggests that environmental conditions such as temperature and light intensity in the field, which are often suboptimal, might suppress the positive effect of cytokinin on pod number and seed yield. In the present experiment, the plant materials were subjected to these environmental fluctuations. Nevertheless, the positive effect of cytokinin application on the pod number in the plants with the highest source/sink ratio (N1 plot) was consistent in different years.

In this experiment, cytokinin was applied to the racemes on the 10th node of the main stem. Since the magnitude of flower abortion varies with the position within plants and racemes (Hansen and Shibles, 1978; Wiebold et al., 1981; Heindl and Brun, 1984; Gai et al., 1984; Saitoh et al., 1999; Kokubun and Honda, 2000), the effects of cytokinin application might be affected by the position on the plant. Further studies are needed to clarify the environmental and physiological conditions under which the positive effects of cytokinin application on pod number and seed yield are expressed.

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* In Japanese with English abstract.

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