# Source-sink Relationships of Soybean as Influenced by Drought Stress during the Pod and Seed-developing Stage

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**ABSTRACT :** This study was conducted to investigate the influence of drought stress during the pod developing and seed filling stage on source-sink relationships of soybean (*Glycine max*). Drought treatments were imposed by withholding water at the full-pod stage, 19 days after flowering, and then limited watering was relieved at 15 days after the initiation of drought treatment. Soybean seed yield was reduced by 39% mainly due to decreased pod number under drought stress, but the 100-seed weight was relatively less reduced. In spite of the 15-day drought during the full-pod stage, soybean produced good seeds showing similar 100-seed weight, protein, starch and soluble sugar content to those from the well-watered. Although drought during the full-pod stage caused source limitations; i.e. accelerated leaf senescence and reduced leaf soluble sugars, it did not cause limitations of other source characteristics such as SGR and leaf starch level. This is because the reduction in size of sinks, such as pod and seed abortions compensated for source limitations, resulting in balanced source-sink as expressed by LAR and the ratio of leaf area to seed dry weight. Drought stress during the pod developing and seed filling stage did not disrupt the source-sink balance

*Keywords*: soybean (*Glycine max*), drought, source-sink, source limitation

S oybean pod grows rapidly at the full-pod stage ($R_4$) (Fehr & Caviness, 1977) and seed development starts at the beginning of this stage. A certain period of time from the $R_4$ stage is the most critical for soybean seed yield. Dry weight of pods increases greatly during the $R_4$-$R_5$ stages. From late pod formation period at the $R_{4.5}$ to early seed filling period at the $R_{5.5}$ is the most critical for yield. Any stresses during the $R_4$-$R_6$ cause more yield reduction than any other stages, and such reduction is mainly due to the reduced number of pods (Saitoh et al., 1999; Liu, 2004). Right amount of irrigation water needs to be considered during this critical period of time to prevent yield loss from

drought stress because it has been known that drought stress during flowering and early pod-developing stage decreases pod number of soybean (Saitoh et al., 1999). During seed-filling stage, it decreases seed size (Board et al., 1994; Desclaux et al., 2000; Brevedan & Egli, 2003).

Reduced photosynthesis by shading (Egli & Zhenwen, 1991) or defoliation (Board & Tan, 1995) decreases pod and seed number. Depodding to increase assimilate supply to the remaining pods usually increases seed size (100-seed weight), but does not always change individual seed growth rate (SGR) (Egli et al., 1985; Munier-Jolain et al., 1998). Source limitations during seed-filling may cause changes in seed size relatively in common, but SGR is not as responsive to changes in source activity and can be sink limited (Egli & Bruening, 2001). Soybean SGR is generally sink limited if photosynthesis increases during seed filling, but source limited if photosynthesis is reduced (Egli & Bruening, 2001).

Leaf starch level responds quickly to changes in photosynthesis rate and sink activity. Soybean leaf starch levels typically increase during the day and decrease during the night (Upmeyer & Koller, 1973; Huber et al., 1984). Increased source-sink ratio by increasing photosynthesis or decreasing sink size usually results in higher leaf starch levels (Ackerson et al., 1984; Crafts-Brandner et al., 1984; Miceli et al., 1995), while reduction in photosynthesis lowers starch levels (Eglis et al., 1980; Huber et al., 1984). Nonstructural carbohydrates such as sugars and starch accumulated in the pods are significantly reduced under drought stress (Liu, 2004).

Allocation of dry matter, starch, soluble sugar and protein between source and sink may be changed by drought stress, which also affects seed protein and carbohydrate levels as well as seed yield. Recently, it has been reported how the source-sink balance by artificial sink or source modification such as shading and depodding is changed (Dybing et al., 1986; Munier-Jolain et al., 1998; Egli, 1999; Egli & Bruening, 2001).

Therefore, this study was conducted to investigate the influence of drought stress during the pod and seed-develop-

ing stage stages on source-sink relationship and seed quality of soybean. Responses of soybean to drought in source-sink balance and limitation, and assimilate levels such as protein and carbohydrates in soybean were also investigated.

## MATERIALS AND METHODS

### Plant materials and drought treatment

A pot experiment was conducted in the greenhouse at the experimental station of Yeongnam Agricultural Research Institute, NICS, Milyang, Korea in 2005. Two seedlings of soybean (*Glycine max* L. cv. Taekwangkong, a determinate type) were transplanted on 10$^{th}$ June 2005 in a cylindrical PVC pot (dia. 20 cm × 60 cm) containing mixture of sandy loam soil and peat to facilitate drainage. Pots were then placed in a rectangular plastic container filled with water up to 30 cm depth, resulting in50.8 kPa of soil water potential at 10 cm soil depth. The seedlings were thinned to one per pot at 7 days after transplanting. Soybean plants were grown under well-watered condition in the greenhouse. Drought stress was imposed on soybean plants at the $R_4$ stage by withholding water for 15 days. During the drought treatment, the stressed plants at wilting point were watered by watering up to 10 cm level overnight and then withheld water. This transient relief of drought stress was made three times during the treatment. After 15 days of drought treatment, the pots were maintained appropriately with irrigation afterwards. All the treatments were arranged in a completely randomized design with six replicates.

### Assessments of growth and assimilates

Whole plants were harvested at 0, 5, 15, 25, 55 days after initiation of drought treatment (DAT). Dry matters of organs such as leaf, petiole, stem, pod, and seed were individually measured. Leaf area was measured using a leaf area meter (LI-3100, LI-COR, USA). Leaf chlorophyll was extracted with 10 ml of 80% acetone from five leaf discs (21.8 mm$^2$ disc$^{-1}$) of the fully expanded leaves overnight and the content was measured following Lichtentaler and Wellburn (1983)'s method by using a spectrophotometer (S-3100, Scinco, Korea) at 470 and 646 nm.

Leaf and seed soluble sugars were extracted from 20 mg of dry ground tissues twice in 7 mL of 80% ethanol at 80 for 45 min followed by 2 mL for 15 min. After centrifugation at 500×g for 2 min, the supernatants were combined, and brought to 10 mL. An 1.5 mL aliquot was freeze-dried and dissolved with 0.5 mL of deionized water, and then the sample was deproteinized by mixing with 0.5 mL of 0.3 N Ba(OH)$_2$ and 0.5 mL of 0.3 N ZnSO$_4$. After centrifugation

at 23,000×g for 5 min, 0.4 mL of supernatant was reacted with 1.6 mL of freshly prepared anthrone reagent (100 mg anthrone + 50 mL 95% H$_2$SO$_4$) at 100°C for 10 min. After cooling on ice, soluble sugar content was measured using a UV-visible spectrophotometer at 620 nm (S-3100, Scinco, Korea). D-glucose was used as a standard chemical (Egli & Bruenig, 2001; Watanabe *et al.*, 2000).

The extracted pellet was dried at 50°C in an oven, suspended in 5 mL of 0.25% (w/v) benzoic acid and heated in a boiling water bath for 1.5 hr to gelatinize starch. It was then cooled, and the tissue was removed from suspension by centrifugation at 500×g for 2 min. An 80 μ*l* aliquot of starch suspension was incubated in 320 μ*l* of 50 mM potassium acetate (pH 5), containing 2 unit mL$^{-1}$ of amyloglucosidase (A7255, Sigma Chemical Co., USA) for 2.5 hr at 40°C. This starch digest was reacted with 1.6 mL of freshly prepared anthrone reagent (100 mg anthrone + 50 mL 95% H$_2$SO$_4$) at 100°C for 10 min. After cooling on ice, the absorbance at 620 nm was measured using the UV-visible spectrophotometer, and then the starch content was calculated by multiplying the sugar content by 0.9 (Chinnusamy & Khanna-Chopra, 2003). D-glucose was also used as a standard chemical.

Leaf and seed protein content was calculated by multiplying the total N content by 5.71 (FAO/WHO, 2003), which was determined by the Kjeldahl method (Nelson & Sommers, 1973; Herberer *et al.*, 1985).

### Statistical analysis

All measurements were initially subjected to analysis of variance (ANOVA). Means were separated by using Duncan's multiple range test (DMRT) at P≤0.05. All statistical analyses were performed using the SAS EG (SAS Institute Inc., USA).

## RESULTS AND DISCUSSION

### Growth parameters associated with sink

Shoot dry weight was significantly reduced in the drought-stressed soybean by 20 and 31% at 15 and 25 DAT, respectively, as compared with that of the well-watered control (Fig. 1). Drought stress also significantly reduced pod and seed growth (Fig. 2). Pod number was reduced by drought at all sampling dates, with 19, 36, and 31% reductions at 15, 25 and 55 DAT, respectively (Fig. 2A). Seed number also showed similar response to drought stress as pod number, with 20, 37, and 33% reductions at 15, 25 and 55 DAT, respectively (Fig. 2B). Seed and pod numbers increased until 25 DAT in the well-watered but 15 DAT in the drought-stressed, and then remained relatively constant

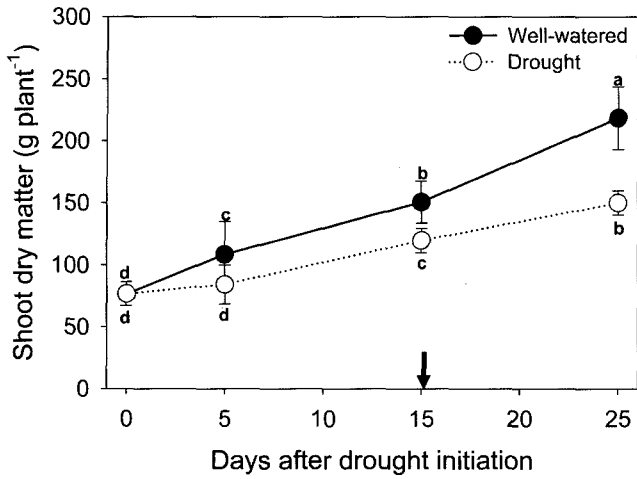afterwards, implying that pod abortion occurred under drought stress in spite of pod formation completed within 5



**Fig. 1.** Changes in shoot dry matter of the well-watered and the drought-stressed soybean. Means followed by the same letter across sampling times are not significantly different at p<0.05 by DMRT. The arrow indicates the initiation time for relieving drought stress.

days after anthesis (Dybing *et al.*, 1986). Interestingly, however, there was no significant difference in 100-seed weight between the drought-stressed and the well-watered at 5, 15, and 25 DAT except at harvest at 55 DAT, when 100-seed weight of the drought stressed was 15% less than that of the well-watered (Fig. 2C). As a consequence of all those influenced by drought stress, seed yield of the drought-stressed soybean was reduced by 39% at harvest (Fig. 2D).

Drought stress did not affect the number of locule per pod at all sampling times, but slightly reduced the ratio of seed to locule number (Table 1). At harvest, the ratio of the well-watered was 96.2%, while that of drought-stressed was reduced to 88.7%. Therefore, it can be concluded that seed yield reduction by drought stress during the full-pod stage is mainly caused by pod abortion in agreement with previous reports (Liu, 2004).

Individual seed growth rate (SGR, mg seed$^{-1}$ d$^{-1}$) during the period of 15-25 DAT was greatest in the both treatments, implying that this period was the most active seed-filling period (Fig. 3). Drought stress significantly reduced the SGR by 11% during this period, but no significant reduction
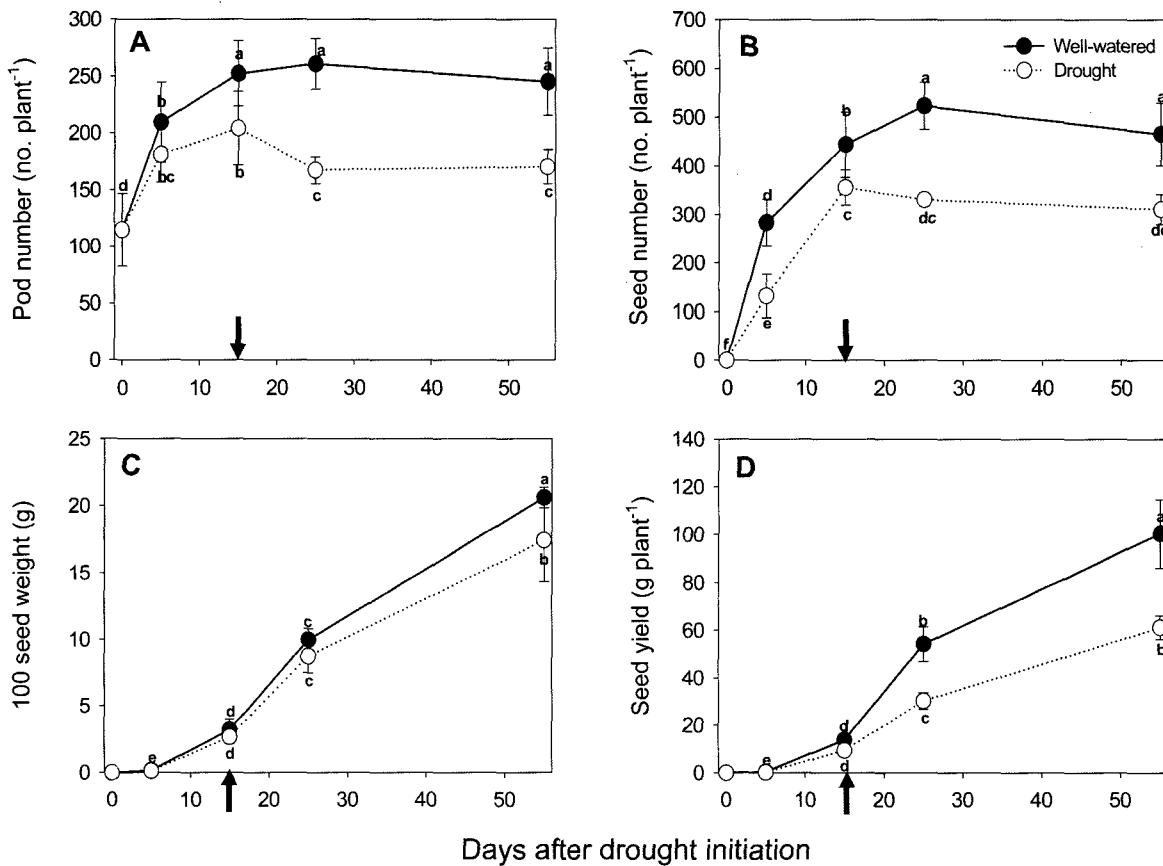


**Fig. 2.** Changes in pod number (A), seed number (B), 100 seed weight (C), and seed yield (D) of the well-watered and the drought-stressed soybean. Means followed by the same letter across sampling times are not significantly different at p<0.05 by DMRT. Arrows indicate the initiation time for relieving drought stress.

**Table 1.** Changes in locule number, and the ratio of normal seed to locule number in the well-watered and the drought-stressed soybean.

| Days after drought initiation | Locule number[†] (no. pod$^{-1}$) | | Ratio of seed to pod locule (seed/pod locule, %) | |
|---|---|---|---|---|
| | Well-watered | Drought | Well-watered | Drought |
| 5 | 2.07 | 2.14 | 96.3[a‡] | 89.4[b] |
| 15 | 2.14 | 2.13 | 98.2[a] | 95.4[a] |
| 25 | 2.06 | 2.09 | 98.3[a] | 96.6[a] |
| 55 | 2.07 | 2.03 | 96.2[a] | 88.7[b] |

[†]There is no significant difference in locule number per pod.
[‡]Means followed by the same letter across sampling times are not significantly different at $p<0.05$ by DMRT.

**Table 2.** Changes in leaf area, specific leaf area, and total chlorophyll content in the well-watered and the drought-stressed soybean.

| Days after drought initiation | Leaf area (m$^2$ plant$^{-1}$) | | Specific leaf area (m$^2$ kg$^{-1}$) | | Total chlorophyll ($\mu$g cm$^{-2}$) | |
|---|---|---|---|---|---|---|
| | Well-watered | Drought | Well-watered | Drought | Well-watered | Drought |
| 0 | 1.26[a†] | 1.26[a] | 32.7[ab] | 32.7[ab] | 32.8[ab] | 32.8[ab] |
| 5 | 1.22[ab] | 1.13[bc] | 31.1[bc] | 34.4[a] | 35.3[a] | 28.8[c] |
| 15 | 1.25[ab] | 1.06[c] | 25.1[d] | 28.7[c] | 31.8[b] | 26.7[c] |
| 25 | 1.30[a] | 1.06[c] | 23.8[d] | 28.6[c] | 27.3[c] | 21.6[d] |

[†]Means followed by the same letter across sampling times are not significantly different at $p<0.05$ by DMRT.
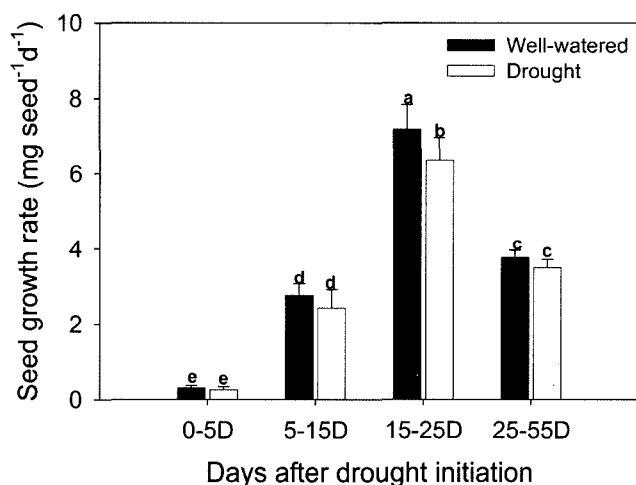


**Fig. 3.** Changes in seed growth rate of the well-watered and the drought-stressed soybean. Means followed by the same letter across sampling times are not significantly different at $p<0.05$ by DMRT.

of the SGR was observed during the other periods. The SGR is limited by reduction of the assimilate supply, influx of sucrose from transient sucrose pools of the fruit (Fader & Koller, 1985), but not so responsive to changes in source activity, implying that the SGR may be sink-limited (Egli & Bruening, 2001). Therefore, sink capacity for assimilates supply may not be significantly limited during the drought stress.

**Growth parameters associated with source**

Drought stress significantly reduced leaf growth and chlo-

rophyll content (Table 2). Leaf area rapidly decreased by 10% during the first 5-days of drought treatment and thereafter did not significantly decrease, particularly with constant leaf area since 15 DAT when drought-stress was relieved by re-watering (Table 2). In comparison with the well-watered, leaf area was significantly much less in the drought-stressed at all sampling times. Specific leaf area (SLA) of the drought-stressed was continuously larger than that of the well-watered and became constant by re-watering at 15 DAT, indicating that the leaves of drought-stressed soybean were thinner than those of the well-watered (Table 2). Total chlorophyll content was also significantly reduced by drought stress, approximately 20% less in the drought-stressed than the well-watered with no recovery until the last sampling at 25 DAT (Table 2). Drought stress during the full-pod stage accelerated leaf senescence, expressed by declines in leaf area and leaf chlorophyll, as previously reported (Sionit & Kramer, 1977; de Souza *et al.*, 1997; Brevedan & Egli, 2003). Our findings suggest that drought stress relief by re-watering can arrest changes in leaf area and SLA but may not stop decline in leaf chlorophyll with time (Brevedan & Egli, 2003).

**Changes in assimilates**

Leaf soluble sugar level was highly dynamic and was reduced during the drought treatment, but showed no significant difference between the well-watered and the drought-stressed at 25 DAT, 10 days after drought-relief by re-watering (Fig. 4A), indicating photosynthesis inhibition by
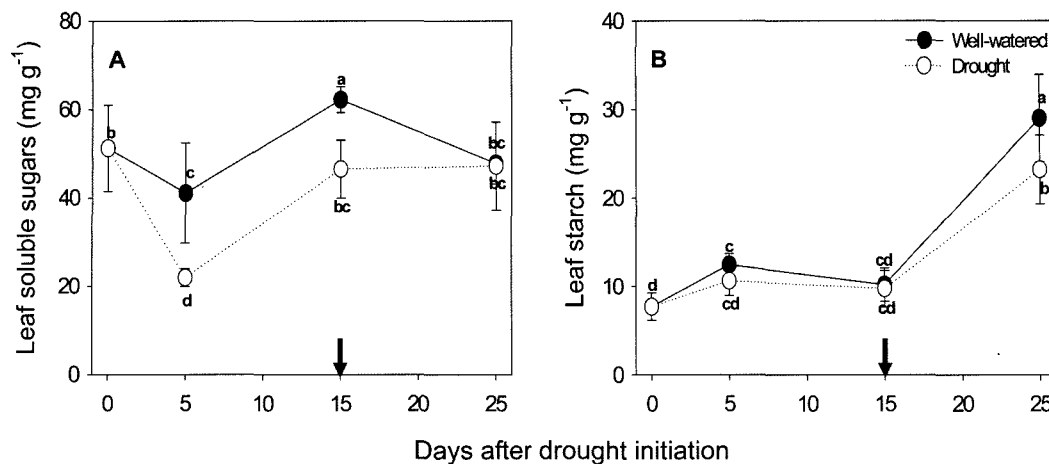
**Fig. 4.** Changes in leaf soluble sugar (A) and starch (B) levels of the well-watered and the drought-stressed soybean. Means across sampling times were separated by DMRT at P0.05. Arrows indicate the initiation time for relieving drought stress.

drought (Westgate et al., 1989; Brevedan & Egli, 2003). Leaf starch level was less dynamic than soluble sugar. No significant difference in leaf starch level was observed between two treatments until 15 DAT, but significant difference was observed at 25 DAT with lower starch level in the drought-stressed (Fig. 4B). In other studies, leaf starch level of the plants exposed to continuous drought stress was significantly lower than that of the well-watered (de Souza et al., 1997; Brevedan & Egli, 2003; Liu, 2004). Leaf total non-structural carbohydrates including both soluble sugar and starch were lower in the drought-stressed than the well-watered, as previously reported (Serraj et al., 1998). Protein content based on leaf weight (%, w/w) was not affected by drought stress, while that based on leaf area (mg cm$^{-2}$) was reduced by drought, with 12% and 15% reductions at 15 and 25 DAT, respectively (Fig. 5). It may be due to the greater SLA in the drought-stressed than in the well-watered (Table 2).

Seed soluble sugar level in the drought-stressed was approximately 33% greater than the well-watered at 15 DAT (Table 2), in agreement with Liu (2004). However, at 25 DAT, 10 days after drought relief by re-watering, the seed soluble sugar level in the drought-stressed became less than that in the well-watered. These results suggest that the capacity of the seeds to utilize the incoming sucrose may be impaired by drought stress and recovered by re-watering. Therefore, no significant difference was observed at harvest (55 DAT). Although seed starch level increased with time, no significant difference between two treatments was observed at all sampling times (Table 2). Seed protein content was relatively constant at about 39% (w/w) and not significantly affected by drought stress, in agreement with Dybing et al. (1986). Our findings thus indicate that drought stress relief for 40 days before harvest after 15-day drought during the full-pod stage cannot recover seed yield but seed
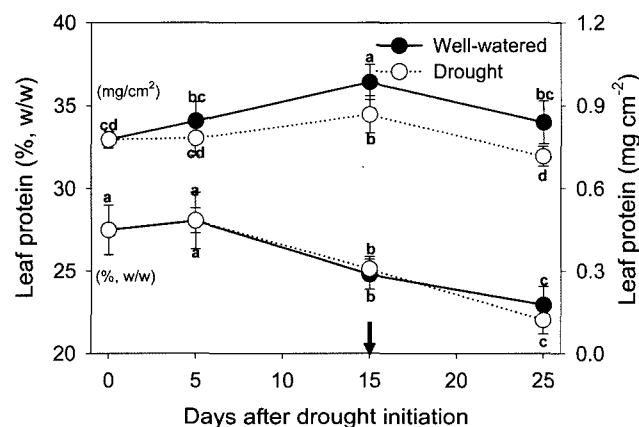


**Fig. 5.** Changes in leaf protein levels of the well-watered and the drought-stressed soybean. Means followed by the same letter across sampling times are not significantly different at p<0.05 by DMRT. The arrow indicates the initiation time for relieving drought stress.

quality-related characters, particularly seed chemical components such as carbohydrates and proteins.

## Source-sink relationship

Leaf area as a main indicator of source was compared with shoot and seed dry weights as indicators of sink to clarify the relationships between sink and source. General decline in the ratio between leaf area and shoot dry weight (LAR, m$^2$ kg$^{-1}$) with time was observed, in agreement with Eagles (1971), but no significant difference in LAR between the two treatments was observed (Fig. 6). The ratio between leaf area and seed dry weight, a similar parameter to LAR, was greater in the drought-stressed than in the well-watered when observed at 5 DAT, but thereafter no significant differ-
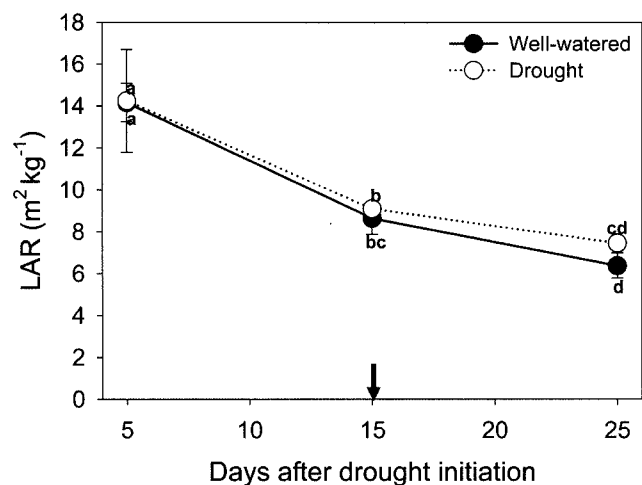
**Fig. 6.** Changes in leaf area ratio (LAR) of the well-watered and the drought-stressed soybean. Means followed by the same letter across sampling times are not significantly different at p<0.05 by DMRT. The arrow indicates the initiation time for relieving drought stress.
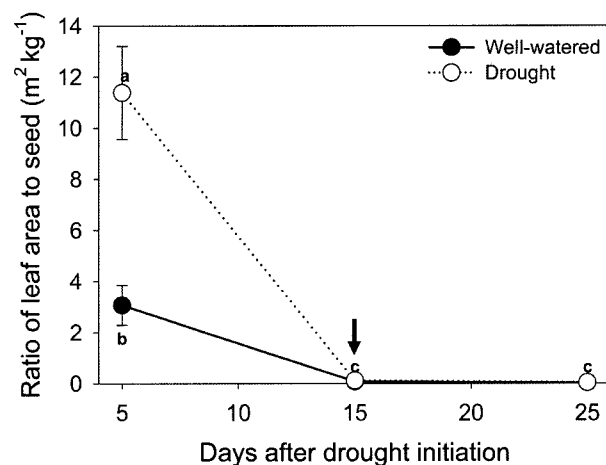
**Fig. 7.** Changes in the ratio of leaf area to seed dry weight of the well-watered and the drought-stressed soybean. Means followed by the same letter across sampling times are not significantly different at p<0.05 by DMRT. The arrow indicates the initiation time for relieving drought stress.

**Table 3.** Changes in soluble sugar, starch, and protein contents of seed in the well-watered and the drought-stressed soybean.

| Days after drought initiation | Soluble sugars (mg g⁻¹) | | Starch (mg g⁻¹) | | Protein (%, w/w) | |
|---|---|---|---|---|---|---|
| | Well-watered | Drought | Well-watered | Drought | Well-watered | Drought |
| 15 | 23.6$^{c\dagger}$ | 31.4$^b$ | 17.4$^c$ | 17.3$^c$ | 39.7$^{ab}$ | 40.3$^a$ |
| 25 | 27.4$^{bc}$ | 16.6$^d$ | 26.9$^b$ | 26.1$^b$ | 36.6$^d$ | 38.1$^c$ |
| 55 | 50.7$^a$ | 52.5$^a$ | 78.7$^a$ | 74.8$^a$ | 38.9$^{bc}$ | 39.2$^{abc}$ |

$\dagger$Means followed by the same letter across sampling times are not significantly different at p<0.05 by DMRT.

ence was observed between the two treatments (Fig. 7). These findings thus indicate that soybean maintained source-sink balance even under drought stress.

Soybean plants try to maintain the source-sink balance under adverse conditions such as shading and depodding (Egli & Bruening, 2001). Our study also showed such source-sink balance by reducing source activities such as leaf growth (Table 2) and production of leaf soluble sugar (Fig. 4) corresponding to reduced sink size such as pod and seed abortions (Table 1 and Figs. 2A and 2B). Several authors demonstrated pod or seed abortion regulations under stresses. Sufficient carbohydrates supply and ABA play a key role for pod or seed setting (Zinselmeier et al., 1999; Setter et al., 2001; Liu, 2004). Recently, Liu (2004) reported that drought-induced reductions of current and reserve carbohydrate supplies contribute to pod abortion during the critical pod development phase. These hormone and carbohydrate supply regulations may adjust the balance between source and sink under drought stress. Our results also suggest that the ratio between source and sink should be balanced in spite of drought stress during the full-pod stage, as

previously reported (Streeter, 2003). Reductions of leaf area, chlorophyll, and leaf soluble sugars and increase of SLA (Brevedan & Egli, 2003) are source-limiting indicators. SGR reduction (Egli, 1999; Egli & Bruening, 2001) and leaf starch level increase (Ackerson et al., 1984; Crafts-Brandner et al., 1984; Miceli et al., 1995; Egli, 1999) are indicators of sink-limitation. As previously discussed, drought-stressed soybean plants adjust to maintain source-sink balance. This source-sink adjustment causes pod abortion that results in increase of assimilate supply to the remaining seed and compensation for reduction in source activity (Munier-Jolain et al., 1998; Egli & Bruening, 2001). In this study, sink size reductions such as pod and seed numbers by drought (Figs. 2 A and B, respectively) also resulted in a similar level of sink activity to that of the well-watered, i.e. no SGR reduction (Fig. 3) and no leaf starch increase (Fig. 4). These findings thus indicate that drought stress did not cause sink limitation.

Our study revealed that soybean maintained its source-sink balance even under drought stress. However, this study had a single drought treatment. Soybean reproductive

organs are developed in order from flowering, followed by podding and seed-filling. There should be different source-sink responses and critical drought duration for maintaining the source-sink balance among the developmental stages and soybean cultivars. Therefore, further work may be required to investigate the effects of various drought stress conditions and soybean cultivars on the source-sink relationships.

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