Nitrogen Fixation and Seed Yield in Soybean under Moderate High-Temperature Stress

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Soybean plants (Glycine max. L.) in the central part of Japan occasionally experience high temperatures in the mid- and late summer season, during which they are in their pod setting and seed filling stages, respectively. During this season, the temperature rarely exceed 30°C in daily mean temperature. However, an increase in temperature by three degrees over natural conditions (moderate high-temperature stress) at Kyoto throughout the reproductive period, inhibited seed development and significantly reduced the seed weight per plant (Mochizuki et al., 2005). Although the response of soybean to temperature has been characterized in a number of studies (Raper and Kramer, 1987), neither the substantial effects of moderate high-temperature stress on yield nor the diverse effects of concomitant occurrence of high temperature and water shortage on productivity have been clarified. In soybean, nitrogen (N) availability is a crucial factor limiting both crop photosynthetic capacity and seed growth rate and thus N fixation activity during seed filling can be a key physiological process determining seed productivity (Sinclair and de Wit, 1976). An experiment was conducted using a Temperature Gradient Chamber (TGC) to investigate the effect of high temperatures on N fixation and seed yield in soybean under well-watered and water-limited conditions.

Material and Methods

The cultivar Tachinagaha was grown along with a non-nodulating line, Lee(−) under varied temperature and soil moisture conditions in the TGC at Kyoto University, Japan. The TGC, 2 m in width and 25 m in length, created an almost linear temperature gradient along its longitudinal axis from natural temperature to a several-degree higher temperature. The soil temperature was maintained as uniform as possible by keeping the side cover of TGC fully open and supplying adequate irrigation water. The treatments to vary temperature and soil moisture were started at three days before the beginning of seed filling (R5) and continued until plant maturity (10 October).

The volumetric soil water content (SWC) was monitored in every subplot with the time domain reflectometry (TDR) meter (SONY Tektronix Co. Ltd., Tokyo, Japan) that measured average SWC of a 30 cm-deep soil profile. The W subplots received adequate irrigation almost every evening and the mean SWC during the treatment period was 25.5 % (v/v), while in the D subplots the amount of water applied was reduced to half and the mean SWC was 21.0 %. The physiological status of the plants as affected by soil moisture was checked by measuring the stomatal conductance to water vapor once a week by a steady-state porometer, LI-1600 (LI-COR, NE, USA) on the abaxial side of the upper leaves of Tachinagaha.

Before sowing, 15N labeled ammonium sulfate (4.7 atom %) as N fertilizer was incorporated into the soil in the whole mini-fields at a rate of 4 g m$^{-2}$ together with 10 g m$^{-2}$ of P$_2$O$_5$ and K$_2$O as superphosphate and potassium chloride, respectively. N fixation rate was assessed by the 15N dilution method (Yoneyama, 1985). The aboveground parts of six plants were harvested.
on 51DAS, 84DAS and on 105/110DAS (D/W, at maturity) and dry weights of plant organs were determined. Plant samples from the first two harvests were analyzed for concentrations of total N and $^{15}$N by Tracer MAT (Thermo-electron Co. Ltd., Tokyo, Japan), a tracer mass spectrophotometer combined with the elemental analyzer. The amount of N in the aboveground plant parts ($N_{total}$) was calculated from N concentration and dry weight of plant organs. The fraction of N obtained by symbiotically N fixation in total N in the top of Tachinagaha plant ($F_{Nfix}$) was calculated by the following equation.

$$F_{Nfix} = 1 - \frac{^{15}N \text{atom}\% \text{excess in Tachinagaha}}{^{15}N \text{atom}\% \text{excess in Lee(-)}}$$

Then, the amount of N obtained by symbiotic fixation ($N_{fix}$) and that by absorption from the soil ($N_{abs}$) were obtained by multiplying $F_{Nfix}$ with $N_{total}$ and by subtracting $N_{fix}$ from $N_{total}$, respectively.

**Results and Discussion**

The average air temperature during the treatment period was from 24.2°C in the D subplot in T1 to 28.2°C in the W subplot in T3 and the lowest temperature was almost the same as the normal outdoor temperature in Kyoto for this season. The averaged values of leaf stomata conductance for the treatment period were 0.66 and 0.44 mol m$^{-2}$ s$^{-1}$ in the W and D subplots, respectively.

The seed yield tended to decline with the increase in mean air temperature during seed filling, resulting in

![Fig. 2. Effects of mean air temperature and soil moisture regimes during 32 days after beginning of seed filling on (A) total N accumulation in the top ($N_{total}$), (B) N acquired by N$_2$ fixation ($N_{fix}$) and (C) N absorbed from the soil ($N_{abs}$). Error bars indicate the difference between the two replicates.](image)
30% lower yields in T3 as compared with T1 for both the W and D subplots (Fig. 1A). In the previous studies with growth chambers, a high daily mean temperature above 30°C inhibited pod and/or seed growth and yield (Egli and Wardlaw, 1980; Dornbos and Mullen, 1991; Gibson and Mullen, 1996). In this experiment, the mean temperature in T2 and T3 was around 30°C during the major seed filling period (Fig. 2). Thus this study basically confirmed the previous findings in a near-field condition.

Both the biomass yield and harvest index (HI) tended to decline as the temperature rose, but the response of HI to the high temperature was more consistent than that of biomass yield (Fig. 1B and 1C). Although the detailed processes remain to be elucidated, the growth of reproductive organs appears to be more susceptible to high-temperature stress than biomass production in soybean.

Fig. 2 shows N_{total}, N_{fix} and N_{abs} for a 32-day period after the R5 stage, i.e., the major part of the seed filling period. N_{total} tended to decline as temperature rose under both soil water regimes. In the D subplot, N_{fix} in T2 and T3 was as low as less than half of that in T1, while in the W subplot N_{fix} only slightly responded to the high temperature. On the other hand, N_{abs} did not show a clear response to the higher temperature either in the W or D subplot. These results indicate that the variation in N accumulation among treatments was attributable to varied N fixation activity.

The mean soil temperature at 10 cm depth during the above period in T3 was about 28°C for both the W and D subplots (data not shown). In the previous studies, soybean plants showed high rates of N_{fix} at the temperature up to about 30°C (Munevar and Wollum, 1981; Sinclair and Weisz, 1985). Thus, it appears as if the soil temperature environment in this study was within the optimum range for N fixation. However, its diurnal variation was larger in the D subplot than in the W subplot presumably due to lower water content in the D subplot. The maximum soil temperature exceeded 30°C on 12 days during the 32-day period in the D subplot in T3, while it did only on two days in the W subplot in T3. Although the distribution of nodules was not observed and thus the temperature in the nodulated root zones is unknown, it is likely that plants in the D subplot in T2 and T3 frequently experienced high root temperatures that reduced nodule activity. In any case, N_{fix} was reduced by the high-temperature conditions evidently under the water-limited condition.

The results of this study indicate that a temperature 3 to 4°C higher than normal level for Kyoto during seed filling possibly causes a substantial decline of soybean yield associated with reduced N fixation activity, especially when the soil water is not sufficient. It is suggested that improving adaptability to high temperature environment is an important subject for high-yielding and stable soybean production in the warmer region of Japan.

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