BIOLOGIA PLANTARUM 50 (1): 138-141, 2006

BRIEF COMMUNICATION

Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants

Y. OHASHI, N. NAKAYAMA, H. SANEOKA[1] and K. FUJITA

Graduate School of Biosphere Science, Hiroshima University,
Kagamiyama 1-4-4, Higashi-Hiroshima, 739-8528, Japan

Abstract

Changes in plant growth, photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean [*Glycine max* (L.) Merr.] plants under drought stress were studied. Total plant dry mass was reduced by 30 % compared to well-watered control plants. Leaf water potential was slightly decreased by water stress. Water stress induced daytime shrinkage and reduced night-time expansion of stem. Photosynthetic rate, stomatal conductance and transpiration rate were significantly declined by water stress, while the intercellular CO_2 concentration was changed only slightly at the initiation of stress treatment. The maximum photochemical efficiency of photosystem 2 and apparent photosynthetic electron transport rate were not changed by water stress.

Additional key words: *Glycine max,* growth, net photosynthetic rate, photochemical efficiency of photosystem 2, stomatal conductance, transpiration rate, water stress.

Soybean is one of the major and widespread crops in the world and is rather sensitive to water stress. Plants regulate their diurnal water status at a favourable level by the control of stomatal aperture (Farquhar and Sharkey 1982). Stomatal closure contributes to maintain high leaf water content and high leaf water potential, but it leads to a decrease in leaf photosynthesis. Stomatal closure reduces intercellar CO_2 concentration in leaves which imposes limitations CO_2 assimilation, and it causes an imbalance between photochemical activity at photosystem 2 (PS 2) and electron requirement for photosynthesis, and leads to increased susceptibility to photo-damage (He *et al*. 1995, Flagella *et al*. 1998). Several investigators have shown that the stem diameter of woody plants fluctuate diurnally (*e.g*., Imai *et al*. 1990 in grape, Simonneau *et al*. 1993 in peach, Ito *et al*. 1999 in pear). Simmoneau *et al.* (1993) used the micromorphometric techniques to observe rapid changes in the stem diameter in peach tree, which was closely related to the water status throughout the day. Water status of plants has been estimated directly by measuring the changes in stem diameter, however, the effect of water stress on growth in soybean plants has not been examined by this method.

The present study was undertaken to evaluate the responses of photosynthesis to water stress in soybean plants, both in terms of CO_2 assimilation, as measured by leaf gas exchanges, and of the functionality of the photosynthetic apparatus, as assessed by chlorophyll fluorescence measurements. We also analysed the effect of water stress on stem diameter of intact soybean plants grown under drought.

Seeds of soybean (*Glycine max* (L.) Merr.), late-maturing cultivar New Tanbakuro were sown in mixture of granite regosol soil, *Perlite* and peat moss (2:1:1 (v/v/v)), and were irrigated daily. At 24 d after

---

Received 10 March 2004, *accepted* 1 September 2005.

Abbreviations: c_i - intercellular CO_2 concentration; DAT - days after the stress treatment; E - transpiration rate; ETR - apparent photosynthetic electron transport rate; F_v/F_m - variable to maximum chlorophyll fluorescence ratio (maximum photochemical efficiency of photosystem 2); g_s - stomatal conductance; P_N - net photosynthetic rate; PS - photosystem; ψ_w - leaf water potential.

Acknowledgments: We thank Dr. G.S. Premachandra of Department of Agronomy, Purdue University, USA for the critical review of this manuscript.

[1] Corresponding author; fax:(+81) 824 247917, e-mail: saneoka@hiroshima-u.ac.jp

germination, plants of uniform size were selected and transplanted into 22 cm (diameter) × 24 cm (height) pots (one seedlings per pot), filled with the same soil mixture as mentioned above. The plants were fertilized at each watering with a commercial fertilizer mixture (*Ohotsuka Co.*, Tokyo, Japan) in the irrigation system until the beginning of the water stress treatment. Plants were grown on benches in the glasshouse at Hiroshima University, under approximately 31 °C daytime maximum and 24 °C night-time minimum temperature. Water stress was imposed at 48 d (flowering stage) after germination. The pots were grouped into two, and thereafter, in one group, soil water content was maintained at the field capacity. In the other group, water was withheld until the soil water content decreased to 50 % of field capacity. Thereafter soil water content was maintained at this for 11 d.

Gas exchange and chlorophyll fluorescence were measured simultaneously on attached uppermost fully expanded leaves with a combined open gas-exchange system and a chlorophyll fluorescence system (*LI-6400-40*, *Li-Cor*, Lincoln, NE, USA). The photosynthetic photon flux density was maintained at of 1 500 μmol m^{-2} s^{-1}. During the measurement the air relative humidity was about 75 %, the leaf temperature at 25 °C and the ambient CO_2 concentration at 370 μmol mol^{-1}. Leaf water potential (ψ_w) was measured by using a pressure chamber (*Daiki-Rika Instruments*, Tokyo, Japan) on the same leaves used for gas-exchange and chlorophyll fluorescence measurement.

Plants were harvested at 7 d after the treatment (DAT), and leaf area was measured using a leaf area meter (*AMM-5*, *Hayashi-denko Co.*, Tokyo, Japan). Plant samples then were dried in an air-forced oven at 80 °C for more than 4 d before weighing. Changes in stem diameter were recorded at 5 min intervals after the initiation of the stress treatment with a micro-displacement detector for 10 d. The point of measurement of stem diameter was a part of 10 cm from basal of the main stem. The stems were placed inside of a Hofman pinch-cock and a 10 mm diameter *Tygon* tube. The displacement sensor, and the screw of the pinch-cock was adjusted to hold the stem with the displacement sensor and *Tygon* tube in tandem. The sensor was connected to a computerized data acquisition system (*NEC*, *San-ei Co.*, Ltd., Tokyo, Japan). Based on our control runs with a glass rod (12 mm in diameter), the sensitivity of the measurement of the diameter was within ± 2 μm.

Statistical analysis was performed according to Maxwell and Delaney (1889) using software of *Analyze-it Software, Ltd.* (Leeds, UK) .

In the present study, soil moisture maintained in the water stress treatment was 50 % of the field capacity and therefore, ψ_w in midday was slightly decreased from -0.72 MPa in control plants to -0.98 MPa in water-stressed plants (Table 1). However, plant growth was severely reduced in stress plants (Table 1). The reduction in dry mass of shoot and root was 33 and 25 %, respectively at 7 DAT. Leaf area was also severely reduced by water stress. Soybean plants are sensitive to drought compared to other crop plants as seen in this study and the previous studies (Korte *et al.* 1983, Ohashi *et al.* 1999, De Costa and Shanmugathasan 2002). In the present study, root growth was less affected by water stress compared to shoot growth. Roots seemed to be more resistant to water stress than shoots. The less inhibition of root growth appears to be associated with the maintenance of relatively high water potential in roots (Sharp and Davis 1985).

Table 1. Leaf water potential, gas exchange parameters, parameters derived from chlorophyll fluorescence, dry mass and leaf are in water-stressed and control soybean plants measured 7 d after the initiation of water stress treatment. Means ± SE of 4 replicates and the percentage of control in the last column.

Parameters	Control	Stressed	[%]
ψ_w [-MPa]	0.72 ± 0.04	0.98 ± 0.16	-
P_N [μmol m^{-2} s^{-1}]	19.00 ± 0.23	14.30 ± 2.04	75
g_s [mol m^{-2} s^{-1}]	0.54 ± 0.11	0.18 ± 0.05	34
E [mmol m^{-2} s^{-1}]	12.76 ± 0.24	6.70 ± 1.34	52
c_i [μmol mol^{-1}]	256.30 ± 7.97	187.00 ± 11.89	74
F_v/F_m	0.71 ± 0.03	0.76 ± 0.01	106
ETR	59.80 ± 0.87	90.90 ± 0.83	152
Leaf d.m. [g plant^{-1}]	19.67 ± 1.46	13.59 ± 0.30	69
Stem d.m. [g plant^{-1}]	14.96 ± 1.38	9.46 ± 0.34	63
Root d.m. [g plant^{-1}]	8.32 ± 0.36	6.28 ± 0.18	75
Leaf area [m^2 plant^{-1}]	0.52 ± 0.04	0.39 ± 0.004	76

Photosynthetic rate (P_N) was reduced by water stress and the reduction of P_N by water stress was 21 and 34 % at the 1st and the 2nd DAT, respectively. Stomatal conductance (g_s) and transpiration rate (E) were also reduced similar to the reduction in P_N. The decrease in g_s was 41 and 30 % at the 1st and 2nd DAT, respectively. E was reduced by 30 and 32 % at the 1st and the 2nd DAT, respectively. The intercellular CO_2 concentration (c_i) was also slightly decreased by water stress at the 1st DAT and the reduction was 13 % compared to the control, but c_i in water-stressed plants did not differ from control at the 2nd DAT. P_N, g_s, E and c_i were 25, 66, 48 and 26 % lower in stressed plants than the control at 7 DAT, respectively (Table 1). The inhibition of P_N under water stress may be attributed to stomatal closure, although direct effects on several biochemical and photochemical processes have been also reported (Long *et al.* 1994, Cornic 2000). In the present study, the reduction of P_N and g_s occurred at a similar level suggesting that P_N was mostly reduced due to the reduction in g_s. Our results suggest that the stomatal closure limited leaf photosynthetic capacity in the water-stressed soybean plants. Cornic and Briantais (1991) indicated that g_s declined before leaf water content was affected, and P_N was largely dependent on stomatal aperture in *Phaseolus vulgaris*. Farquhar *et al.* (1989) also reported that stomatal factors are more important than non-stomatal factors under water stress. The E declined correspondingly with the decline in P_N and g_s. The reduction in water loss by stomatal closure is one of the adaptive responses to maintain a high water potential in plants as the drought develops.

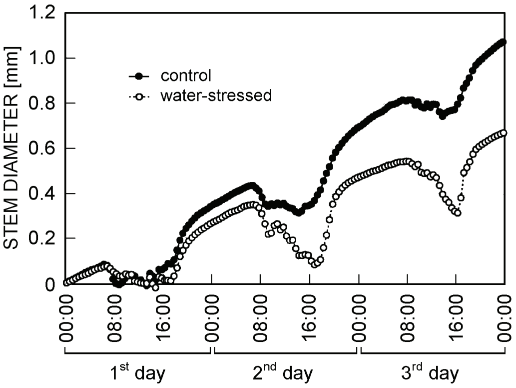

Fig. 1. Diurnal changes of stem diameter in intact soybean plants during the first 3 d of the water stress treatment.

Apparent photosynthetic electron transport rate [ETR = (Fm' - F)/Fm' × PPFD × 0.42, where Fm' is maximum fluorescence yield during a saturation pulse, F is fluorescence yield under natural irradiance, and PPFD is photosynthetically active radiation] and maximum photochemical efficiency of PS 2 (variable to maximum fluorescence ratio, F_v/F_m) were not affected by water stress at 7 DAT (Table 1). These results show that leaf photochemistry in soybean was rather resistant to water stress and the electron transport chain was maintained under water stress conditions, although P_N and g_s were strongly inhibited. Ramanjulu *et al.* (1998) reported that PS 2 activity in mulbery leaves was maintained under mild or moderate water stress condition at which ψ_w was dropped from -0.84 to -2.08 MPa, however, at a severe water stress of ψ_w values less than -3.13 MPa, PS 2 activity was strongly reduced. Genty *et al.* (1987), Lu and Zhang (1998) and Shangguan *et al.* (2000) also suggested that under mild water stress PS 2 photochemistry was not affected and is resistant to water deficits.

The stem diameter was gradually increased after sunset, and it reached the maximum at early morning before sunrise (Fig. 1). The stem diameter decreased after sunrise, and it reached minimum at evening before sunset. The increase in stem diameter in water-stress plant at night was smaller than in the control plants. The decrease of stem diameter, as revealed by shrinkage of stem, was smaller in water stress plants than in control plants. These observations suggested that change of stem diameter was controlled by water status in plants, and begins as plant expose to sun light and water loss by leaves exceed the supply of water by roots. This is consistent with results of Ito *et al.* (1999) on Japanese pear and Fujita *et al.* (2003) on tomato in which fluctuations in circadian rhythm of stem diameter was mainly regulated by the degree of stomatal opening. Stem diameter responded quickly within one or two days after the water stress treatment similarly as P_N, g_s and E, suggesting that stem diameter as an effective parameter characterizing water status of soybean plants under various environmental stresses.

References

Cornic, G.: Drought stress inhibits photosynthesis by decreasing stomatal aperture - not by affecting ATP synthesis. - Trends Plant Sci. **5**: 187-188, 2000.

Cornic, G., Briantais, J.M.: Partitioning of photosynthetic electron flow between CO_2 and O_2 reduction in a C_3 leaf (*Phaseolus vulgaris* L.) at different CO_2 concentrationa and during drought stress. - Planta **185**: 178-184, 1991.

De Costa, W.A.J.M., Shanmugathasan, K.N.: Physiology of yield determination of soybean (*Glycine max* (L.) Merr.) under different irrigation regimes in the sub-humid zone of Sri Lanka. - Field Crops Res. **75**: 23-35, 2002.

Farquhar, G.D., Sharkey, T.D.: Stomatal conductance and photosynthesis. - Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Farquhar, G.D., Wong, S.C., Evans, J.R., Hubick, K.T.: Photosynthesis and gas exchange. - In: Jones, H.G., Flowers, T.J., Jones, M.B. (ed.): Plants under Stress. Pp. 47-69. Cambridge University Press, Cambridge 1989.

Flagella, Z., Campanile, R.G., Stoppelli, M.C., De Caro, A., Di Fonzo, N.: Drought tolerance of photosynthetic electron transport under CO_2-enriched and normal air in cereal species. - Physiol. Plant **104**: 753-759, 1998.

Fujita, K., Okada, M., Lei, K., Ito, J., Ohkura, K., Adu-Gyamfi, J.J., Mohapatra, P.K.: Effect of P-deficiency on photoassimilate portioning and rhythmic changes in fruit and stem diameter of tomata (*Lycopersicon esculentum*) during fruit growth. - J. exp. Bot. **392**: 2519-2528, 2003.

Genty, B., Briantais, J.M., Vieira Da Silva, J.B.: Effects of drought on primary photosynthetic processes of cotton leaves. - Plant Physiol **83**: 360-364, 1987.

He, J.X., Wang, J., Liang, H.G.: Effects of water stress on photochemical function and protein metabolism of photosystem II in wheat leaves. - Physiol. Plant **93**: 771-777, 1995.

Imai, S., Iwao, K., Fujiwara, K.: Measurement of plant physiological information of vine tree and indexation of soil moisture control. (1) Analysis of stem diameter variation affected by environmental factors. - Environ. Control Biol. **28**: 103-108.

Ito, J., Hasegawa, K., Fujita, K., Ogasawara, S., Fujiwara, T.: Effect of CO_2 enrichment on fruit growth and quality in Japanese pear (*Pyrus serotina* Reheder cv. Kousui). - Soil

Sci. Plant Nutr. **45**: 385-393, 1999.

Korte, L.L., Williams, J.H., Specht, J.E., Sorense, R.C.: Irrigation of soybean genotypes during reproductive ontogeny. I. Agronomic responses. - Crop Sci. **27**: 1197-119, 1983.

Long, S.P., Humphries, S., Falkowski, P.G.: Photoinhibition of photosynthesis in nature. - Annu. Rev. Plant Physiol. **85**: 990-995, 1994.

Lu, C., Zhang J.: Effects of water stress on photosynthesis, chlorophyll fluorescence and photoinhibition in wheat plants. - Aust. J. Plant Physiol. **25**: 883-892, 1998.

Maxwell, S. S., Delaney, H.D. (ed.): Designing Experiments and Analyzing Data. - Wadsworth, Belmint 1989.

Ohashi, Y., Saneoka, H., Matsumoto, K., Ogata, S., Premachandra, G.S., Fujita, K.: Comparison of water stress effects on growth, leaf water status, and nitrogen fixation activity in tropical pasture legumes siratro and desmodium with soybean. - Soil Sci. Plant Nutr. **45**: 759-802, 1999.

Ramanjulu, S., Sreenivasalu, N. Giridhara Kumar, S., Sudhakar, C.: Photosynthetic characteristics in mulberry during water stress and rewatering. - Photosynthetica **35**: 259-263, 1998.

Shangguan, Z., Shao, M., Dyckmans J.: Effects of nitrogen nutrition and water deficit on net photosynthetic rate and chlorophyll fluorescence in winter wheat. - J. Plant Physiol. **156**: 46-51, 2000.

Sharp, R.E., Davis, W.J.: Root growth and water uptake by maize plants in drying soil. - J. exp. Bot. **36**: 1441-1456, 1985.

Simmoneau, T., Habib, R., Goutouly, J.P., Huguet, J.G.: Diurnal changes in stem diameter depend upon variations in water content: Direct evidence in peach trees. - J. exp. Bot. **44**: 615-621, 1993.