

Response of Growth, Photosynthetic Gas Exchange, Translocation of ^{13}C -labelled Photosynthate and N Accumulation in two Soybean (*Glycine max* L. Merrill) Cultivars to Drought Stress

NOBUHIRO NAKAYAMA, HIROFUMI SANEOKA¹, REDA E.A. MOGHAIEB[†], GNASIRI S. PREMACHANDRA[‡] AND KOUNOSUKE FUJITA

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

[†]Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt

[‡]Department of Agronomy, Purdue University, West Lafayette, IN, USA

¹Corresponding author's e-mail: saneoka@hiroshima-u.ac.jp

ABSTRACT

Two soybean (*Glycine max* L. Merrill) cultivars, Shin-Tanbakuro and Midori, were grown under mild water stress conditions for 11 days at the vegetative growth stage in order to examine the effect of drought stress on plant biomass production, photosynthetic gas exchange, chlorophyll fluorescence and photoassimilate translocation along with changes in carbohydrates and nitrogen. Relative growth rate (RGR), net assimilation rate (NAR), plant growth rate (GR) and relative leaf area expansion rate (RLER) were decreased by drought and this reduction was greater in cultivar Shin-Tanbakuro than in Modori. Photosynthetic gas exchange (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) in both cultivars were severely decreased by drought; however, the maximum photochemical efficiency of photosystem II (F_v/F_m) and apparent photosynthetic electron transport rate (ETR) were not affected by drought. Drought stress did not affect the export of ^{13}C -labelled photosynthate from leaves to stem and roots. Drought decreased the starch concentration in leaves of both cultivars, but the soluble sugar concentration increased more in Midori than in Shin-Tanbakuro. The nitrogen accumulation in plants was decreased by drought in both cultivars, but the reduction in N accumulation in leaves and roots was higher in Shin-Tanbakuro. Results suggested that Midori is more tolerant to drought stress than Shin-Tanbakuro by virtue of greater nitrogen uptake and supply to developing leaves in improving growth.

Key Words: $^{13}\text{CO}_2$ carbohydrate; Chlorophyll fluorescence; Nitrogen; Photosynthesis; Soybean; Drought stress

INTRODUCTION

Drought stress is the primary factor that limits yield of soybean around the world. The detrimental effects of drought stress on plants are a consequence of osmotic strain on the cytoplasm. In many plants, drought stress decreases stomatal conductance and transpiration (Earl, 2002; Ribas-Carbo *et al.*, 2005). Under drought conditions, stomatal closure helps to maintain higher leaf water potential and thereby leaf water content; however this leads to a decrease in leaf photosynthesis (Lawlor, 1995). The limitation of CO_2 assimilation imposed by stomatal closure reduces the intercellular CO_2 concentration in leaves and causes an imbalance in photosystem II (PSII) photochemical activity, electron requirements for photosynthesis and leads to an increased susceptibility to photo-damage (Epron *et al.*, 1992; He *et al.*, 1995; Flagella *et al.*, 1998). Fluorescence induction patterns and derived indices have been used as empirical diagnostic tools in stress physiology (Schreiber *et al.*, 1994). The F_v/F_m ratio, which represents the maximum quantum yield of the primary photochemical reaction of PSII, is widely used to estimate the degree of

photoinhibition (Osmond & Grace, 1995). This parameter has been shown to respond to drought in various plants (Cornic & Massacci, 1996; Flagella *et al.*, 1998; Tezara *et al.*, 1999). For example, Cornic and Briantais (1991) demonstrated that photosynthetic gas exchange rate and F_v/F_m ratio in *Phaseolus vulgaris* were concomitantly decreased by drought stress. However He *et al.* (1995) reported that drought stress did not affect electron donation to PS II in wheat plants.

Many plants accumulate soluble sugars in leaves, stems and roots in response to drought stress (Castrillo, 1992; Pelleschi *et al.*, 1997). Accumulation of soluble sugars leads to lowering of the osmotic potential in plant tissues, which maintains the driving force for extracting soil water under drought conditions (Wright *et al.*, 1983). Accumulation of soluble sugars also helps plants to perform better under drought in terms of growth and productivity through maintaining a higher turgor and thereby water supply to the plant and maintain a comparatively higher photosynthesis rate and growth (Morgan, 1984; Massacci *et al.*, 1996; Subbarao *et al.*, 2000).

In addition to drought, nitrogen nutrition is another

limiting factor for plant growth and yield (Radin & Boyer, 1982; Purcell & King, 1996). A large part of nitrogen in the plant is allocated to leaves throughout the life of the plant and a large part of leaf nitrogen is invested in the photosynthetic apparatus. Photosynthetic activity is closely related to leaf nitrogen and the photosynthetic rate increases with higher levels of leaf nitrogen (Evans, 1989; Sugiharto *et al.*, 1990). Nitrogen deficiency also has a substantial effect on leaf growth. An increase in N supply stimulates leaf growth under drought stress, resulting in an increase both in the rate of leaf expansion and the number of emerging leaves (Radin & Paker, 1979; Tóth *et al.*, 2002).

The objective of the present study were to investigate the differences in drought tolerance between two soybean cultivars and examine the physiological characteristics causing the differences in drought tolerance in terms of growth, photosynthetic gas exchange, photosystem II activity, translocation of ^{13}C -labelled photosynthate and nitrogen accumulation.

MATERIALS AND METHODS

Plant growth and drought stress treatment. Two soybean (*Glycine max* (L.) Merrill) cultivars Shin-Tanbakuro and Midori were planted in seed beds containing a mixture of soil, perlite and peat moss in a volume ratio of 4:2:1 and irrigated daily to keep the moisture at field capacity. Twenty-four days old plants of uniform size were transplanted to 3.8 L plastic pots filled with the same soil mixture at 1 seedling per pot. Each pot was fertilized at a rate of 40 kg N ha⁻¹, 12 kg P₂O₅ ha⁻¹ and 10 kg K₂O₅ ha⁻¹ using fertilizer mixture and soil pH was adjusted to 6.0 with dolomitic calcium carbonate (300 kg ha⁻¹). The seeds were not inoculated with *Bradyrhizobium japonicum* and therefore the roots were not nodulated. The plants were grown on benches in a greenhouse at Hiroshima University at a maximum temperature of 31°C during the day and minimum temperature of 24°C during the night. Drought stress was imposed at 16 days after transplanting by withholding watering until soil moisture content reached 50% of field capacity. Thereafter soil water content was maintained at 50% field capacity using an ECH₂O-10 soil moisture monitor (Decagon Devices Inc., Pullman WA USA). Well-watered control plants were irrigated to field capacity.

Plants were harvested after 11 days of water stress imposition. Total leaf area was determined using an AAM-9 leaf area meter (Hayashidenko Co. LTD. Tokyo, Japan). Stems, leaves and roots were separated and dried in a ventilated oven at 80°C until constant weight was reached in order to measure relative growth rate (RGR), net assimilation rate (NAR), growth rate (GR), leaf area ratio (LAR), relative leaf area expansion rate (RLER) and growth rate (GR) of leaf, stem and root during 11 days of water stress treatment using the procedure of Poorter (1989).

Measurement of gas exchange, chlorophyll fluorescence

and leaf water potential. Gas exchange and chlorophyll fluorescence were measured simultaneously on attached upper most fully expanded leaves with a portable open gas-exchange system (Li-6400, Li-Cor, Lincoln, NE, USA) and chlorophyll fluorescence system (Li-6400-40, Li-Cor, Lincoln, NE, USA). Fully expanded leaves were at least two tri-foliated further down the main stem and were judged visually to be the most recently produced, but fully expanded. While taking measurements, the photosynthetic photon flux density was at 1500 mol m⁻² s⁻¹, the relative humidity 75% the leaf temperature 25°C and ambient CO₂ concentration 370 mol mol⁻¹. Leaf water potential was measured using a pressure chamber (Daiki-Rika Instruments, Tokyo, Japan). The leaves used for measurements of photosynthesis and chlorophyll fluorescence were used for the water potential measurement.

^{13}C CO₂ feeding and ^{13}C analysis. Plants were fed with ^{13}C CO₂ at the beginning of drought stress by supplying stable carbon isotope. Whole plants that were individually enclosed in transparent vinyl bags in which ^{13}C CO₂ was evolved from Ba ^{13}C CO₂ (99.5 atom % ^{13}C) by the addition of 30% lactic acid solution, while being treated for a period of 1 h under natural light. Plants were harvested after 11 days of water stress. Plant parts were separated, frozen in liquid nitrogen and freeze-dried. Dry weights of each plant parts were recorded before grinding into fine powder using a vibrating sample mill (Model T1-100, Heiko Co. Ltd., Japan). The ^{13}C abundance in the plant material was determined using a mass spectrometer (Delta plus, Finnigan MAT, Bremen, Germany) and excess (%) of ^{13}C atom was calculated as difference in ^{13}C atom percentage between plant samples and glycine.

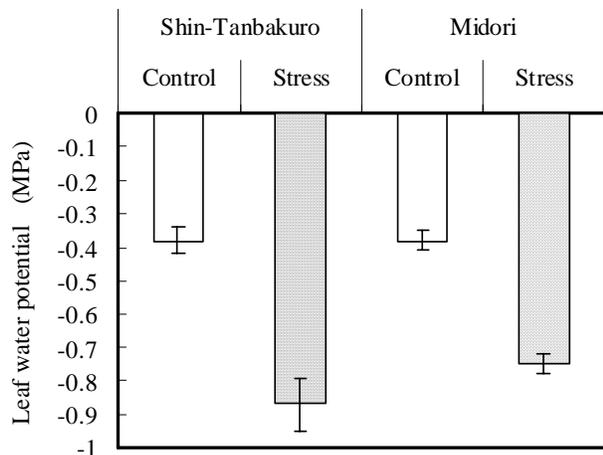
Measurement of carbohydrate and nitrogen concentrations. Powdered plant materials were extracted with 80% ethanol (v/v). After the homogenates were centrifuged at 12000 x g, the ethanol extract was evaporated and the quantification of total soluble sugar content was carried out using the Anthrone method (Spiro, 1966). Starch content was measured according the method of Yoshida *et al.* (1976) after perchloric acid extraction. The total N content was determined using a Kjeldahl nitrogen digester and distillator (Kjeldatherm Type TT100 & Vapodset Type 20, Gerhardt, Germany).

Statistics. Data has been presented as the mean ± S.E. for each treatment and cultivar (n = 3). Significant differences between the treatments were analysed by ANOVA. LSD values were calculated at the P < 0.05 probability level (JMP v. 6.0, SAS Institute Inc., Cary, NC, USA).

RESULTS

Leaf water potential (LWP) was greatly reduced in drought stressed than control plants (Fig. 1). Control plants maintained higher levels of LWP at -0.38 MPa, while the drought stress plants of Shin-Tanbakuro indicated a more reduction in LWP (-0.87 MPa) than cultivar Midori

Fig. 1. Leaf water potential of soybean cultivars Shin-Tanbakuro and Midori under water stress (after 11 days water ofstress treatment) and control. Result are the means \pm standard errors (n = 3)



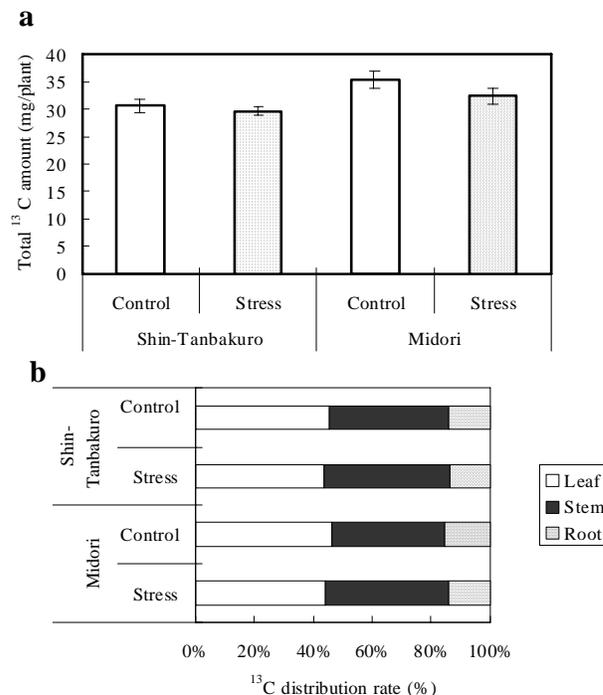
(-0.75 MPa).

Relative growth rate (RGR) and net assimilation rate (NAR) in both cultivars was decreased by drought stress after 11 days of treatment (Table I). The percentage reduction in RGR and NAR compared to the control of cultivar Midori was 15% and 8%, while these values for cultivar Shin-Tanbakuro were 44% and 41%, respectively. Leaf area ratio (LAR) in cultivar Midori was slightly decreased by drought stress; however a significant difference was not observed in the reduction of LAR in Midori. The relative leaf area expansion rate (RLER) in both cultivars was more affected by drought during 11 days of treatment and the reduction of RLER by drought stress was 61% in Shin-Tanbakuro compared to 44% in Midori. Growth rate (GR) was also decreased by water stress and the decrease in GR was larger in Shin-Tanbakuro than in Midori. The GR of leaves was severely decreased compared to that of stems and roots.

Water stress did not affect the ^{13}C fixation in plants of either cultivar (Fig. 2a). The distribution percentage of ^{13}C to leaves in both cultivars was slightly decreased and that to stems slightly increased by drought stress (Fig. 2b). However, there was no cultivar difference in the distribution percentage of ^{13}C to each plant part. The soluble sugar concentration in all plant parts of Midori was slightly higher under drought stress compared to Shin-Tanbakuro (Table II). The starch concentration was decreased in the leaves and stems of both cultivars, while increased in the roots. The concentration of total non-structural carbohydrates was decreased by drought stress in the leaves but increased in the stems.

Total N content in leaves was decreased severely in Shin-Tanbakuro and slightly in Midori under drought stress. This parameter in the root of Shin-Tanbakuro was not changed but increased significantly in Midori due to drought stress (Table III). Total N accumulation during the 11 days

Fig. 2. Total ^{13}C amount in plant (a) and ^{13}C distribution rate to leaf, stem and root (b) in soybean cultivars Shin-Tanbakuro and Midori at 11 days after water stress treatment. Result are the means \pm standard errors (n = 3)



of drought period was significantly reduced in the leaves and stems of drought stressed plants than the control plants in both cultivars. The N content of root of stressed plants was greater than the control plants in cultivar Midori. Total nitrogen accumulation rate was decreased in both cultivars by drought stress during 11 days of water stress treatment.

Photosynthetic gas exchange (P_n), the maximum photochemical efficiency of photosystem II (F_v/F_m) and apparent photosynthetic electron transport rate (ETR) were not affected by drought at 3 days after treatment; however stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) were decreased slightly by drought stress in both cultivars (Table IV). The P_n , g_s , C_i and E were severely decreased by drought at 7 days after treatment in both cultivars; however a significant difference was not observed in the cultivars for reduction in g_s , C_i , or E under drought stress. The F_v/F_m and ETR were not affected by drought stress at 7 days after the treatment.

DISCUSSION

In this experiment, drought was imposed at a soil moisture level of 50% of field capacity; therefore, leaf water potential in midday was slightly decreased by drought stress from -0.38 in the control to -0.87 MPa in cultivar Shin-Tanbakuro and from -0.38 MPa in the control to -0.75 MPa in cultivar Midori (Fig. 1). These findings showed that drought stress caused large effects on the growth of the

Table I. Relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), relative leaf area expansion rate (RLER), and growth rate (GR) of leaf, stem and root in soybean cultivars Shin-Tanbakuro and Midori after 0 to 11 days of water stress treatment. Results are the means \pm standard errors (n=3). Means followed by the same letter within each line are not significantly different (P<0.05)

	Shin-Tanbakuro		Midori	
	Control	Stress	Control	Stress
RGR (mg g ⁻¹ day ⁻¹)	62.7 ^a \pm 6.6	35.1 ^c \pm 4.5	69.6 ^a \pm 1.8	59.2 ^{ab} \pm 3.8
NAR(g cm ⁻² day ⁻¹)	0.509 ^a \pm 0.033	0.299 ^b \pm 0.051	0.584 ^a \pm 0.025	0.542 ^a \pm 0.038
LAR(cm ² g ⁻¹)	122.8 ^a \pm 7.8	121.3 ^a \pm 17.9	119.5 ^a \pm 3.8	109.3 ^a \pm 1.6
RLER (cm ² cm ⁻² day ⁻¹)	0.0593 ^a \pm 0.008	0.0225 ^c \pm 0.014	0.0552 ^a \pm 0.009	0.0312 ^b \pm 0.003
GR (g/day)				
Total plant	1.74 ^a \pm 0.23	0.71 ^c \pm 0.11	1.51 ^a \pm 0.2	1.06 ^b \pm 0.09
Leaf	0.55 ^a \pm 0.1	0.11 ^c \pm 0.04	0.56 ^a \pm 0.11	25.3 ^b \pm 0.03
Stem	0.95 ^a \pm 0.12	0.486 ^d \pm 0.05	0.75 ^b \pm 0.08	0.641 ^c \pm 0.03
Root	0.24 ^a \pm 0.06	0.111 ^b \pm 0.03	0.201 ^a \pm 0.02	0.17 ^a \pm 0.03

Table II. Concentration of sugar and starch in leaf, stem, and root of soybean cultivars Shin-Tanbakuro and Midori after 11 days after of water stress treatment. Results are the means \pm standard errors (n=3). Means followed by the same letter within each column are not significantly different (P<0.05)

Cultivar	Treatment	Sugar concentration (mg g ⁻¹ dry weight)			Starch concentration (mg g ⁻¹ dry weight)		
		Leaf	Stem	Root	Leaf	Stem	Root
Shin-Tanbakuro	Control	37.57 ^c \pm 0.03	57.64 ^c \pm 4.15	7.51 ^c \pm 0.03	242.22 ^a \pm 0.10	179.92 ^a \pm 15.35	14.36 ^b \pm 0.04
	Stress	44.77 ^b \pm 0.03	70.19 ^b \pm 1.92	11.44 ^b \pm 0.04	116.49 ^c \pm 0.07	80.44 ^b \pm 2.10	17.81 ^a \pm 0.04
Midori	Control	37.87 ^c \pm 0.01	44.06 ^d \pm 2.29	2.87 ^d \pm 0.07	189.87 ^b \pm 0.03	189.87 ^a \pm 4.05	14.23 ^b \pm 0.05
	Stress	50.93 ^a \pm 0.03	86.44 ^a \pm 0.45	16.12 ^a \pm 0.06	85.92 ^d \pm 0.05	85.92 ^b \pm 0.23	19.56 ^a \pm 0.06

Table III. N content in plants after 11 days of water stress treatment and N accumulation rate in leaf, stem and root in soybean cultivars Shin-Tanbakuro and Midori at the beginning and after 11 days of water stress treatment. Results are the means \pm standard errors (n=3). Means followed by the same letter within each column are not significantly different (P<0.05)

Cultivar	Treatment	N content		N accumulation rate (mg day ⁻¹ plant ⁻¹)		
		(mg/plant)		Leaf	Stem	Root
Shin-Tanbakuro	Control	609.8 ^a \pm 38.5		6.96 ^b \pm 2.33	4.53 ^a \pm 0.93	3.30 ^a \pm 1.27
	Stress	478.6 ^c \pm 19.5		0.39 ^d \pm 1.07	2.49 ^b \pm 0.61	0.76 ^c \pm 0.58
Midori	Control	591.6 ^a \pm 34.1		13.09 ^a \pm 2.34	2.60 ^b \pm 0.31	1.30 ^b \pm 0.28
	Stress	502.4 ^b \pm 25.5		3.98 ^c \pm 1.39	2.30 ^b \pm 0.21	3.08 ^a \pm 0.6

tested soybean cultivars. Relative growth rate (RGR) is one of the most appropriate parameters to compare plant growth between different water stress treatments. The RGR gives a relative basis on which to compare growth rate of plants since it takes into account both initial and final plant weights over a specified time period (Poorter, 1989). RGR is a function of the net assimilation rate (NAR), which is an index of the photosynthetic capacity of the plant per unit leaf area and the leaf area ratio (LAR), which is index of the leafiness of the plant (Poorter, 1989). During 11 days of water stress treatment the reduction in RGR was 44% in cultivar Shin-Tanbakuro and 15% in Midori (Table I). In this study, LAR was not affected in either cultivar; however NAR was significantly reduced under mild water stress. Decrease in NAR by water stress was greater in Shin-Tanbakuro compared to Midori, suggesting that the decrease in RGR due to water stress was mainly due a decrease in NAR.

In this study water stress produced significant effect on the relative leaf area expansion rate (RLER) of plants during 11 days of stress treatment. Drought sensitive cultivar

Shin-Tanbakuro showed a greater reduction in RLER and the growth rate of leaves compared to Midori (Table I). Hsiao (1973) and Sadras *et al.* (1993) reported that leaf area development is the most sensitive to soil water deficits.

Drought stress did not affect the partitioning of ¹³C from leaves to stems and roots (Fig. 2b). However, carbohydrate concentration was significantly changed. The starch concentration of all organs in both cultivars was significantly decreased; however soluble sugar concentrations were increased by water stress (Table II). Increase in the soluble sugar concentration by drought was larger in the drought tolerant cultivar compared to sensitive one. Several previous studies report an increase in sugar concentration under drought stress (Heitholt & Schmidt, 1994; Premachandra *et al.*, 1995). Premachandra *et al.* (1995) reported that drought tolerant sorghum lines accumulated more soluble sugar than drought sensitive lines. Al Hakimi *et al.* (1995) also reported that a wheat line capable of accumulating greater soluble sugar maintained higher relative water content in leaves. Accumulation of soluble sugars leads to lowering of the osmotic potential of

Table IV. Photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration rate (E), maximum photochemical efficiency of Photosystem II (F_v/F_m), and apparent photosynthetic electron transport rate (ETR) in water stress treatment and controls of soybean cultivars Shin-Tanbakuro and Midori after 3 and 7 days of water stress treatment. Results are the means \pm standard errors ($n=3$). Means followed by the same letter within each line are not significantly different ($P<0.05$)

	Shin-Tanbakuro		Midori	
	Control	Stress	Control	Stress
After 3 days of treatment				
P_n	20.8 ^a \pm 0.5	19.5 ^a \pm 0.6	21.8 ^a \pm 0.7	20.4 ^a \pm 0.8
g_s	0.501 ^a \pm 0.056	0.398 ^b \pm 0.039	0.524 ^a \pm 0.039	0.35 ^b \pm 0.055
C_i	280 ^a \pm 6	269 ^a \pm 5	281 ^a \pm 7	249 ^b \pm 11
E	6.34 ^a \pm 0.38	5.63 ^b \pm 0.39	6.76 ^a \pm 0.31	5.14 ^b \pm 0.56
F_v/F_m	0.815 ^a \pm 0.004	0.818 ^a \pm 0.004	0.777 ^a \pm 0.002	0.795 ^a \pm 0.005
ETR	135 ^a \pm 2	134 ^a \pm 1	139 ^a \pm 6	139 ^a \pm 5
After 7 days of treatment				
P_n	20.6 ^a \pm 1.5	9.8 ^b \pm 1.5	21.2 ^a \pm 1.9	7.8 ^b \pm 1.9
g_s	0.617 ^a \pm 0.089	0.105 ^c \pm 0.089	0.37 ^b \pm 0.077	0.089 ^c \pm 0.077
C_i	289 ^a \pm 6	247 ^b \pm 6	240 ^b \pm 17	218 ^c \pm 17
E	11.4 ^a \pm 0.81	2.02 ^c \pm 0.81	6.02 ^b \pm 0.86	1.6 ^d \pm 0.86
F_v/F_m	0.823 ^a \pm 0.002	0.823 ^a \pm 0.006	0.771 ^a \pm 0.005	0.786 ^a \pm 0.002
ETR	121 ^a \pm 9	125 ^a \pm 23	128 ^a \pm 1	108 ^a \pm 25

the plant, which maintains the driving force for extracting soil water under water deficit conditions (Morgan, 1984; Sharp *et al.*, 1990, Premachandra *et al.*, 1992).

Drought decreased the N accumulation in both cultivars and although this reduction was larger in Shin-Tanbakuro (Table III). During 11 days of drought treatment, drought sensitive cultivar Shin-Tanbakuro did not accumulate N in leaves. It is well known that drought impairs the uptake of N in the plants (Purcell & King, 1996; Xia, 1997). Chipman *et al.* (2001) found that drought sensitive soybean accumulated less N than drought tolerant one. The deficit in N nutrition affects leaf growth, leaf expansion and other physiological factors (Radin & Boyer, 1982; Purcell & King, 1996; Ciompi *et al.*, 1996). Radin and Boyer (1982) have shown that N deficiency in sunflower decreased the leaf hydraulic conductivity and leaf growth by changes in turgor pressure. Tóth *et al.* (2002) also reported that leaf growth was highly affected by nitrogen deprivation in the elongation zone of the basal section of the leaf in maize plants. In this study, water stress reduced N uptake by plants and restricted the translocation of N to the leaves; therefore we suggest that mild water stress might limit photosynthetic activity and growth through altering biochemical metabolism in relation to N levels and N availability in plants.

Applied drought stress inhibits leaf photosynthesis, which may be due to stomatal closure, although direct effects on several biochemical and photochemical processes have been also reported (Long *et al.*, 1994; Cornic, 2000). In this study, P_n of both cultivars did not change at 3 days of stress treatment; however g_s and C_i decreased slightly (Table IV). P_n was decreased severely along with a decrease in g_s and C_i at 7 days of treatment. Results from this study suggest that stomatal closure limited leaf photosynthetic capacity in the drought-stressed soybean. Likewise, Cornic and Briantais (1991) indicated that g_s in *Phaseolus vulgaris* declined before leaf water content was affected and P_n was

largely dependent on stomatal aperture. The E value declined correspondingly with a decline in P_n and g_s . Reduced water loss by stomatal closure is one of the adaptive responses for maintaining a high water potential as the drought develops. Farquhar *et al.* (1989) reported that stomatal factors were more important than non-stomatal ones under drought stress.

Drought also affects photochemical processes (He *et al.*, 1995). However we did not observe any difference in the maximum photochemical efficiency (F_v/F_m) or apparent photosynthetic electron transport rate (ETR) between well-watered and stressed plants in this study (Table IV). These results show that the photochemical activity of soybean leaves was resistant to drought stress and electron transport chain is capable of maintaining well under drought even where P_n and g_s are strongly inhibited. Ramanjulu *et al.* (1998) reported PSII activity in mulberry leaves was maintained under mild or moderate drought stress at which leaf water potential values dropped from -0.84 to -2.08 MPa. However, at severe drought stress, leaf water potential values were lesser than -3.13 MPa and PSII activity was strongly reduced. Our results agree with the findings of Genty *et al.* (1987), Lu and Zhang (1998), Shangguan *et al.* (2000) and Xu and Zhou (2005) in which PSII photochemistry was not affected under mild water stress.

CONCLUSION

Mild water stress had no significant effect on Photosystem II or the translocation of ^{13}C -labelled photosynthate; however photosynthetic gas exchange, growth and biomass production were severely affected. Since the reduction in growth rate of the cultivar Midori was lower than of cultivar Shin-Tanbakuro, it can be concluded that drought tolerance in cultivar Midori is associated with its ability to maintain higher leaf water potential, leaf area expansion and N uptake under water stress.

REFERENCES

- Al Hakimi, A., P. Monneveux and G. Galiba, 1995. Soluble sugars, proline and relative water content (RCW) as traits for improving drought tolerance and divergent selection for RCW from *T. polonicum* into *T. durum*. *J. Genet. Breed.*, 49: 237–44
- Castrillo, M., 1992. Sucrose metabolism in bean plants under water deficit. *J. Exp. Bot.*, 43: 1557–61
- Ciampi, S., E. Gentili, L. Guidid and G.F. Soldatini, 1996. The effect of nitrogen deficiency on gas exchanges and chlorophyll fluorescence parameter in sunflower. *Pl. Sci.*, 118: 177–84
- Chipman, R.B., C.D. Raper and R.P. Patterson, 2001. Allocation of nitrogen and dry matter for two soybean genotypes in response to water stress during reproductive growth. *J. Pl. Nutri.*, 24: 873–84
- Comic, G. and J.M. Briantais, 1991. Partitioning of photosynthetic electron flow between CO₂ and O₂ reduction in a C₃ leaf (*Phaseolus vulgaris* L.) at different CO₂ concentration and during drought stress. *Planta*, 185: 178–84
- Comic, G. and A. Massacci, 1996. Leaf photosynthesis under drought stress. In: Baker, N.R. (ed.), *Photosynthesis and the Environment*, pp: 347–66. Kluwer Academic Publication, The Netherlands
- Comic, G., 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture-not by affecting ATP synthesis. *Trends Pl. Sci.*, 5: 187–8
- Earl, H.J., 2002. Stomatal and non-stomatal restrictions to carbon assimilation in soybean (*Glycine max*) lines differing in drought use efficiency. *Environ. Exp. Bot.*, 48: 237–46
- Epron, D., E. Dreyer and N. Breda, 1992. Photosynthesis of oak trees (*Quercus petraea* (Matt.) Liebel.) during drought under field conditions: diurnal course of net CO₂ assimilation and photochemical efficiency of photosystem II. *Pl. Cell Environ.*, 15: 809–20
- Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia*, 78: 9–19
- Farquhar, G.D., S.C. Wong, J.R. Evans and K.T. Hubick, 1989. Photosynthesis and gas exchange. In: Jones, H.G., T.J. Flowers and M.B. Jones (eds.), *Plants Under Stress*, pp: 47–69. Cambridge University Press, Cambridge
- Flagella, Z., R.G. Campanile, M.C. Stoppelli, A. De Caro Di and N. Fonzo, 1998. Drought tolerance of photosynthetic electron transport under CO₂-enriched and normal air in cereal species. *Physiol. Pl.*, 104: 753–9
- Genty, B., J.M. Briantais and J.B. Vieira Da Silva, 1987. Effects of drought on primary photosynthetic processes of cotton leaves. *Pl. Physiol.*, 83: 360–4
- He, J.X., J. Wang and H.G. Liang, 1995. Effects of drought stress on photochemical function and protein metabolism of photosystem II in wheat leaves. *Physiol. Pl.*, 93: 771–7
- Heitholt, J.J. and J.H. Schmidt, 1994. Receptacle and ovary assimilate concentrations and subsequent boll retention in cotton. *Crop Sci.*, 34: 125–31
- Hsiao, T.C., 1973. Plant response to drought stress. *Annu. Rev. Pl. Physiol.*, 86: 908–13
- Lawlor, D.W., 1995. Limitation of water deficit on photosynthesis. In: Smirnoff, N. (ed.), *Environmental and Plant Metabolism Flexibility and Acclimation*, pp: 129–61. Bios Scientific Publishers, Oxford
- Long, S.P., S. Humphries and P.G. Falkowski, 1994. Photoinhibition of photosynthesis in nature. *Annu. Rev. Pl. Physiol.*, 85: 990–5
- Lu, C. and J. Zhang, 1998. Effects of drought stress on photosynthesis, chlorophyll fluorescence and photoinhibition in wheat plants. *Australian J. Pl. Physiol.*, 25: 883–92
- Massacci, A., A. Battistelli and F. Loreto, 1996. Effect of drought stress on photosynthetic characteristics, growth and sugar accumulation of field-grown sweet sorghum. *Australian J. Pl. Physiol.*, 23: 331–40
- Morgan, J.M., 1984. Osmoregulation and water stress in higher plants. *Ann. Rev. Pl. Physiol.*, 35: 299–319
- Osmond, C.B. and S.C. Grace, 1995. Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reaction of photosynthesis? *J. Exp. Bot.*, 46: 1351–62
- Pelleschi, S., J.P. Rocher and J.L. Prioul, 1997. Effect of water restriction on carbohydrate metabolism and photosynthesis in maize mature leaves. *Pl. Cell Environ.*, 20: 493–503
- Poorter, H., 1989. Growth analysis: towards a synthesis of the classical and functional approach. *Physiol. Pl.*, 75: 237–44
- Premachandra, G.S., H. Saneoka, K. Fujita and S. Ogata, 1992. Leaf drought relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing drought deficits in sorghum. *J. Exp. Bot.*, 43: 1569–76
- Premachandra, G.S., D.T. Hahn, D. Rhodes and R.J. Joly, 1995. Leaf water relations and solute accumulation in two grain sorghum lines exhibiting contrasting drought tolerance. *J. Exp. Bot.*, 46: 1833–41
- Purcell, L.C. and C.A. King, 1996. Drought and nitrogen source effects on nitrogen nutrition, seed growth and yield in soybean. *J. Pl. Nutri.*, 19: 969–93
- Radin, J.W. and J.S. Boyer, 1982. Control of leaf expansion by nitrogen nutrition in sunflower plants. Role of hydraulic conductivity and turgor. *Pl. Physiol.*, 69: 771–5
- Radin, J.W. and L.L. Paker, 1979. Water relations of cotton plants under nitrogen deficiency. I. Dependence upon leaf structure. *Pl. Physiol.*, 64: 495–8
- Ramanjulu, S., N. Sreenivasalu, S. Giridhara Kumar and C. Sudhakar, 1998. Photosynthetic characteristics in mulberry during drought stress and redroughting. *Photosynthetica*, 35: 259–63
- Ribas-Carbo, M., N.L. Taylor, L. Giles, S. Busquets, P.M. Finnegan, D.A. Day, H. Lambers, H. Medrano, J.A. Berry and J. Flexas, 2005. Effects of water stress on respiration in soybean leaves. *Pl. Physiol.*, 139: 466–73
- Sadras, V.O., F.J. Villalobos, E. Fereres and D.W. Wolfe, 1993. Leaf responses to soil drought deficits: comparative sensitivity of leaf expansion rate and leaf conductance in field-grown sunflower (*Helianthus annuus* L.). *Pl. Soil*, 153: 189–94
- Schreiber, K., W. Bilger, H. Hormann and C. Neubauer, 1994. Chlorophyll fluorescence as a noninvasive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E.D. and M.M. Caldwell (eds.), *Ecophysiology of Photosynthesis*, pp: 49–70. Springer Verlag, Berlin
- Shangguan, Z., M. Shao and J. Dyckmans, 2000. Effects of nitrogen nutrition and drought deficit on net photosynthetic rate and chlorophyll fluorescence in winter wheat. *J. Pl. Physiol.*, 156: 46–51
- Sharp, E.S., T.C. Hsiao and W.K. Silk, 1990. Growth of the maize primary root at low drought potentials. II. Role of growth and deposition of hexose and potassium in osmotic adjustment. *Pl. Physiol.*, 93: 1337–46
- Spiro, R.G., 1966. Analysis of sugars found in glycoprotein. In: Neufeld, E.S. and V. Ginsburg (eds.), *Methods in Enzymology, Vol. VIII. Complex Carbohydrates*, pp: 3–26. Academic Press, New York
- Sugiharto, B., K. Miyama, H. Nakamoto, H. Sasakawa and T. Sugiyama, 1990. Regulation of expression of carbon-assimilating enzymes by nitrogen in maize leaf. *Pl. Physiol.*, 92: 963–9
- Subbarao, G.V., N.H. Nam, Y.S. Chauhan and C. Johansen, 2000. Osmotic adjustment, water relations and carbohydrate remobilization in pigeonpea under water deficits. *J. Pl. Physiol.*, 157: 651–9
- Tezara, W., V. Mitchell, S.P. Driscoll and D.W. Lawlor, 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature*, 401: 914–7
- Tóth, V.R., I. Mészáros, S.J. Palmer, S.Z. Veres and I. Précsényi, 2002. Nitrogen deprivation induces changes in the leaf elongation zone of maize seedlings. *Biol. Pl.*, 45: 241–7
- Wright, G.C., R.C.G. Smith and J.M. Morgan, 1983. Differences between two grain sorghum genotypes in adaptation to drought stress: III. Physiological responses. *Australian J. Agric. Res.*, 34: 637–51
- Xia, M.Z., 1997. Effects of soil drought during the generative development phase on seed yield and nutrient uptake of faba bean (*Vicia faba*). *Australian J. Agric. Res.*, 48: 447–51
- Xu, Z.Z. and G.S. Zhou, 2005. Effects of water stress on photosynthesis and nitrogen metabolism in vegetative and reproductive shoots of *Leymus chinensis*. *Photosynthetica*, 43: 29–35
- Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez, 1976. *Laboratory Manual for Physiological Studies of Rice*, p: 83. The International Rice Research Institute, Los Banos, Philippines

(Received 27 February 2007; Accepted 04 April 2007)