N$_2$ Fixation Response to Drought in Common Bean (*Phaseolus vulgaris* L.)

RACHID SERRAJ* and THOMAS R. SINCLAIR†

USDA-ARS, Agronomy Department, Agronomy Physiology Laboratory, IFAS Building #350, University of Florida, P.O. Box 110965, Gainesville, FL 32611-0965 USA

Nitrogen fixation activity in common bean is generally thought to be low and sensitive to soil drying and, consequently, drought can have important negative effects on N accumulation and yield potential. The objectives of this research were to examine the response of N$_2$ fixation to drought stress in common bean, and to test the hypothesis that drought sensitivity of N$_2$ fixation in common bean is linked to ureide levels in the plants. Two glasshouse experiments were conducted to compare the responses of leaf transpiration and acetylene reduction activity (ARA) to soil water contents. ARA decrease during soil dehydration was found to lag behind the decline in transpiration. This indicates that ARA is relatively less sensitive to soil dehydration compared to leaf gas exchange. Further, in comparing two cultivars there was no consistent difference in the relative response of ARA and transpiration to soil drying. The ureide concentrations measured in common bean plants were low, ranging from 0·1 to 1·0 mmol l^{-1} in xylem sap exudates. Ureide concentrations in the sap exudate varied significantly among the two genotypes even though there was no difference in ARA response to drought. It was concluded that in common bean, the lower sensitivity of N$_2$ fixation to drought compared to leaf gas exchange could be related to low ureide concentrations in petioles and xylem sap. © 1998 Annals of Botany Company

Key words: *Phaseolus vulgaris*, nitrogen fixation, drought stress, nodules, ureides.

INTRODUCTION

About two-thirds of common bean (*Phaseolus vulgaris* L.) production in the developing world occurs under conditions of significant drought stress (Graham and Ranalli, 1997). These regions include large areas in Africa and in Central and South America where the growing season is short and marginal soil conditions are very common. Common bean is important in many cropping systems in these regions because of its ability to fix atmospheric N$_2$; this importance is anticipated to increase with the need to develop sustainable agricultural practices.

Establishment and activity of the legume-rhizobium symbiosis have long been recognized as being sensitive to drought stress (Wilson, 1931; Sprent, 1972). For a number of grain legumes, N$_2$ fixation has been shown to decline early during the soil dehydration cycle, preceding leaf gas exchange and all other measures of drought stress (Sinclair *et al.*, 1987; Sinclair and Serraj, 1995). Because common bean is frequently grown on drought-prone soils, a high sensitivity to soil dehydration may constitute an important constraint on N accumulation and yield potential.

Common bean has usually been considered a poor N$_2$-fixing grain legume, although there is evidence of large genotypic variability for N$_2$ fixation potential (Bliss, 1993). Because little benefit from N$_2$ fixation has been proven so far, even monoculture beans are often highly fertilized with

N, which sometimes results in inhibition of nodulation and N$_2$ fixation (Graham and Ranalli, 1997). However, several authors suggested that dramatic enhancement of biological N$_2$ fixation could be achieved in common bean by exploitation of the genetic diversity and implementation of adequate selection techniques (Bliss, 1993; Hardarson *et al.*, 1993; Giller and Cadisch, 1995).

If improved N$_2$ fixation potential is to be realized in the dry regions where common bean is grown, improved drought tolerance of N$_2$ fixation should be considered an important objective. Surprisingly few investigations have studied the response of N$_2$-fixing common bean to water deficits (Peña-Cabriales and Castellanos, 1993; Sinclair and Serraj, 1995), but both these studies indicated that N$_2$ fixation in common bean was tolerant of soil drying. However, the interaction between N$_2$ fixation and the water economy of the plants was not resolved.

An approach that is especially useful in comparing plant responses to water deficits is to express responses as a function of volumetric soil water content (Ritchie, 1981). This allows plant behaviour, when subjected to the same reservoir of water, to be compared. This approach has proved effective in making comparisons among plant species (Sinclair and Ludlow, 1986) and cultivars (Ray and Sinclair, 1997). By expressing the response in terms of soil water content it is possible to resolve whether there is any inherent difference in plant response to the same level of soil-water deficit. Therefore, the first objective of this study was to determine the response of common bean plants to soil drying. In these comparisons, plant response was characterized by transpiration and N$_2$ fixation rate as a function of relative soil water content.

* Permanent address: Laboratoire de Physiologie Végétale, Département de Biologie, Faculté des Sciences-Semlalia, BP S 15 Marrakech, Morocco.
† For correspondence. Fax 352-392-6139, e-mail trsincl@nervm.nerdc.ufl.edu

bo980670

Recently, there have been indications that N_2 fixation drought tolerance may be associated with ureide levels in the plant: de Silva, Purcell and King (1996) found a correlation between ureide level and the degree of drought stress and the drought-tolerant soybean cultivar 'Jackson' was found to have lower ureide levels than drought-susceptible cultivars (Serraj and Sinclair, 1996). Importantly, the association of ureide levels and N_2 fixation drought response in soybean has been confirmed in both field and glasshouse studies (Serraj and Sinclair, 1996; Serraj et al., 1997). Sinclair and Serraj (1995) reported major differences in N_2 fixation response to drought among grain legume species. Legume species producing ureides were more sensitive to drought than amide producers, which may mean that ureides are involved in the N_2 fixation sensitivity to water deficits. Surprisingly, among the ureide producers tested, common bean showed relatively lower levels of ureides and a higher degree of N_2 fixation drought tolerance compared to soybean, black gram or cowpea (Sinclair and Serraj, 1995). Therefore, a second objective of this study was to test the hypothesis that N_2 fixation sensitivity in common bean is linked to ureide levels in the plants.

MATERIALS AND METHODS

Plant culture

Two soil dehydration experiments were conducted in the glasshouse. Experiment 1 was conducted in February 1995 with the common bean 'Roma'. Experiment 2 was conducted in June 1995 to compare cultivars 'Roma' and 'Kentucky Wonder'. Plants were grown in 10 cm diameter × 30 cm tall PVC pots. Fittings were inserted into the bottom of the pots to allow water drainage and to flow air into the pots during acetylene reduction activity (ARA) measurements. The pots were filled with a 2:1 mixture of potting soil (Vitagreen, Inc., Clermont, FL, USA) and vegetable plug mix (W. R. Grace and Co., Cambridge, MA, USA). Each pot contained about 2 kg of soil mixture inoculated with a commercial preparation of *Rhizobium tropici* (Nitragin, Milwaukee, WI, USA). The pots were overseeded and thinned to one plant per pot after emergence.

Plants were grown in a glasshouse under well-watered conditions for about 6 weeks before the drought treatment was initiated. Throughout the experiment natural light was supplemented with incandescent lamps to extend the daylength to a 16 h photoperiod so that the plants remained in vegetative development. Day/night temperatures were approx. 28/20 °C.

Drought treatment

At the initiation of the soil dehydration treatment, pots were sealed around the plant stem using a lid with a gas exit port, which allowed non-destructive flow-through measures of acetylene reduction activity. The pots were fully watered, allowed to drain overnight, and weighed to determine the hydrated weight of the soil.

For each cultivar, three plants were maintained as well-watered controls, and the soil of five plants was allowed to

dehydrate over 13 d as a result of plant transpiration. Pots were weighed each afternoon to compute soil dehydration and daily transpiration rates. After weighing, the well-watered pots were watered, if needed, to a weight 200 g less than the fully hydrated weight. The water-deficit pots were allowed to dehydrate as a result of transpiratory water losses. The fraction of transpirable soil water (FTSW), as defined by Sinclair and Ludlow (1986), was calculated daily for each dehydrating pot. This was done by first determining the difference between the hydrated pot weight and the final pot weight when the transpiration rate had decreased to less than 10 % of the well-watered pots. After the experiment, the FTSW on each day was calculated from the daily weight measurements. Therefore FTSW on any given day was the amount of transpirable water in the pot expressed as a fraction of the transpirable soil water available in the fully hydrated pot.

A normalized transpiration (NTR) for each drought-stressed plant was calculated daily by dividing the transpiration rate of individual treatment plants by the mean transpiration rate of the well-watered plants of that cultivar. To facilitate comparisons among plants, the normalized transpiration rates were adjusted so that the mean value of each stressed plant during the early, well-watered phase was equal to 1.0. Cumulative transpiration (CTR) for each plant was calculated as the sum of the total water transpired during the experimental period.

In situ *acetylene reduction*

Acetylene reduction activity was measured each afternoon during the dehydration cycle by flowing a mixture of 0·1 acetylene:0·9 air (v/v) at 1 l min⁻¹ through the pots. Ten min were allowed for establishment of an equilibrium rate of ethylene production. Ethylene concentrations in the three 1-cm³ samples collected at the exit port of each pot were analysed in a gas chromatograph equipped with a flame ionization detector. Collection of the gas samples from the pots took approx. 5 min which limited the total time of exposure to the acetylene:air mixture to 15 min. Following collection of the gas sample, pots were flushed with air for at least 45 min to remove all residual acetylene and ethylene. ARA of each stressed plant was calculated daily relative to the mean rate of the well-watered plants of that cultivar. For ARA, the values were further normalized (NARA) so that the mean value of each plant during the early, well-watered phase was equal to 1·0. Cumulative ARA (CARA) was calculated as the sum of daily ARA measurements during the experiment.

Ureide measurements

For both experiments, measurements were made at the end of the experimental period to determine ureide concentrations of sap exudate from each plant as described previously (Sinclair and Serraj, 1995). Shoots were cut between ground level and the cotyledons at approx. 1200 h and then placed in a pressure chamber (Scholander et al., 1965) and subjected to a 1 MPa overpressure. The sap that exuded from the shoots was collected in microcentrifuge

tubes and stored immediately at −20 °C until the ureide concentrations could be measured. Colorimetric procedures were used to determine ureide concentrations (Young and Conway, 1942).

For expt 2, ureide concentrations, as a possible indicator of nitrogen transport, were also measured in individual petioles harvested from well-watered and drought-stressed plants at the end of the drying cycle (Serraj and Sinclair, 1996). The petioles were oven-dried at 75 °C for 2 d and ground. Ureide in the petiole samples was extracted in 5 ml boiling water and measured by colorimetric assay (Young and Conway, 1942). Whole plants were harvested at the end of the drying cycle of expt 2. Shoots and nodules, which had been separated from the roots, were dried for 2 d at 75 °C and weighed.

The experimental design was a randomized complete block design with a factorial of water treatment for expt 1 and a factorial genotype and water treatment for expt 2. The biomass and ureide data were analysed by analysis of variance. The regressions relating NTR and NARA to FTSW in expt 2 were generated by SigmaPlot (Jandel Scientific Software, San Rafael, CA, USA). For each parameter, a single curve was generated for both cultivars by combining data from individual plants. An r^2 value was computed for each curve and statistical analysis was based on comparison of 95% confidence intervals of coefficients (Ray and Sinclair, 1997).

RESULTS

Soil dehydration expt 1 was conducted with the common bean cultivar 'Roma'. Drought stress induced a significant decrease in both leaf gas exchange and ARA as illustrated in Fig. 1 in which NTR and NARA are plotted against FTSW. When FTSW values were greater than 0·4, there was no significant change in NTR and NARA. As soil dried to FTSW values less than 0·4, NTR decreased almost linearly
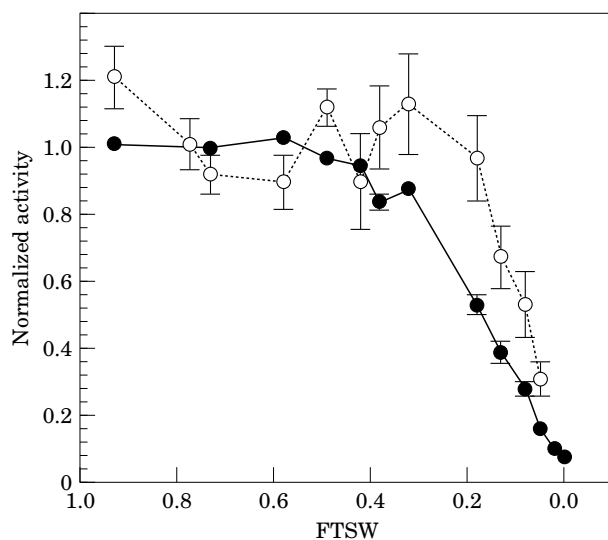

FIG. 1. Normalized acetylene reduction activity (ARA) (--○--) and transpiration (—●—) as a function of fraction of transpirable soil water (FTSW) for common bean 'Roma' during a dehydration cycle. Each data point is the mean of five plants. Error bars are ±s.e.
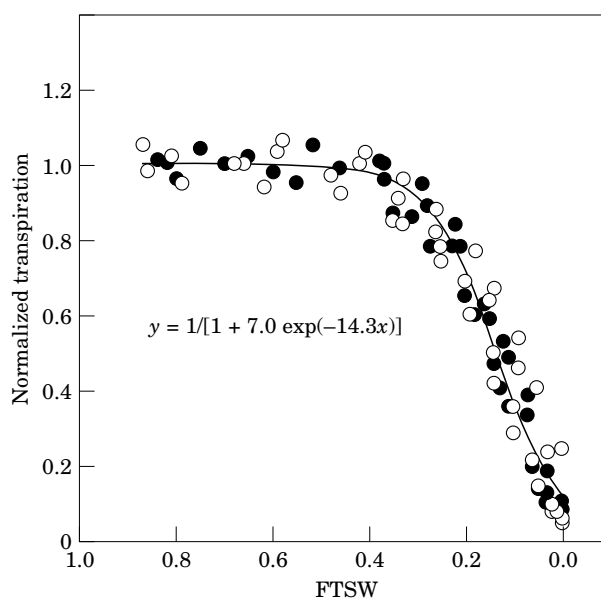

FIG. 2. Plot of normalized transpiration as a function of the fraction of transpirable soil water (FTSW) for common bean plants during a dehydration cycle. Kentucky Wonder (●); Roma (○).
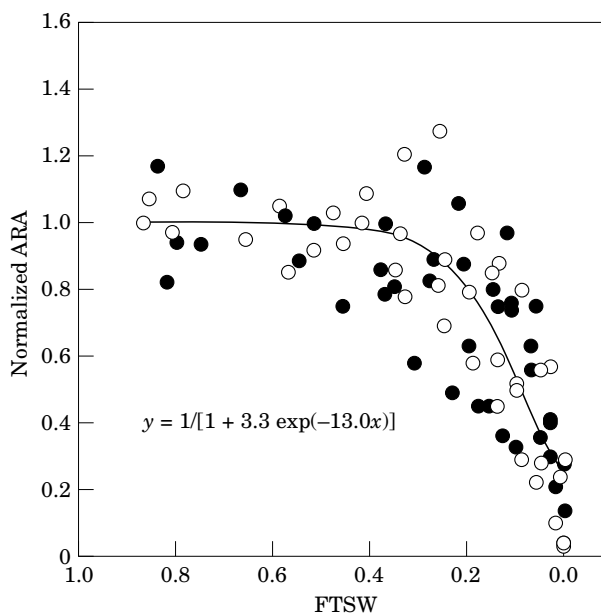

FIG. 3. Plot of normalized acetylene reduction activity (ARA) as a function of the fraction of transpirable soil water (FTSW) for common bean plants during a dehydration cycle. Kentucky Wonder (●); Roma (○).

whereas NARA lagged behind, starting to decrease only at FTSW less than 0·2. This indicates a lower sensitivity of N₂ fixation to drought, compared to transpiration.

The response to drought stress in expt 2 was similar to that in expt 1. Further, no difference between the two cultivars was found for the relative response of NTR or NARA to FTSW (Figs 2 and 3). The plots of NTR and NARA against FTSW were represented for both cultivars by the following logistic equations:

TABLE 1. *Shoot and nodule biomass, cumulative transpiration (CTR) and cumulative acetylene reduction activity (CARA) in two common bean cultivars following 13 d exposure to drought or control treatments*

		g per plant			CARA μmol C_2H_4 per plant
Genotype	Treatment	Shoot d.wt	Nodule d.wt	CTR	
Kentucky Wonder	Well-watered	7.76 ± 2.59	0.63 ± 0.12	2139 ± 65	1192 ± 78
	Drought-stressed	4.02 ± 1.53	0.42 ± 0.18	1311 ± 41	694 ± 72
	Ratio	0.52	0.67	0.61	0.58
Roma	Well-watered	5.30 ± 1.83	0.51 ± 0.18	1756 ± 94	927 ± 41
	Drought-stressed	4.61 ± 0.73	0.40 ± 0.07	1211 ± 30	600 ± 61
	Ratio	0.87	0.78	0.69	0.65

Data are means ($\pm$s.d.) of three (well-watered) or seven (drought stress) replicates.

TABLE 2. *Ureide concentration in petioles and sap exudates of two common bean cultivars following 13 d exposure to drought (DS) or well-watered (WW) treatments*

	Petiole ureide (μmol g^{-1} d.wt)			Sap ureide (μmol l^{-1})		
Genotype	WW	DS	Ratio	WW	DS	Ratio
Kentucky Wonder	4.64 ± 0.99	9.98 ± 2.18	2.15	195 ± 60	692 ± 228	3.55
Roma	2.11 ± 0.71	4.11 ± 1.53	1.95	126 ± 60	210 ± 125	1.67

Data are means ($\pm$s.d.) of three (WW) or seven (DS) replicates.

$$NTR = 1/[1 + 7.0 \exp(-14.3 FTSW)] \quad r^2 = 0.96 \quad (1)$$

$$NARA = 1/[1 + 3.3 \exp(-13.0 FTSW)] \quad r^2 = 0.73 \quad (2)$$

These equations reflect the fact that, for both cultivars, little change in NTR and NARA occurred at FTSW values greater than 0·4. As soil dried to FTSW values less than 0·4, NTR decreased almost linearly (Fig. 2) whereas NARA started to decrease at FTSW less than 0.3 (Fig. 3). The difference in response of NTR and NARA to FTSW was confirmed by the fact that there were statistical differences ($P < 0.05$) in the coefficient of eqns (1) and (2).

For both cultivars used in expt 2, the cumulative transpiration data for well-watered plants showed that the average water loss was significantly higher for 'Kentucky Wonder' compared to 'Roma' (Table 1). The total amount of water lost by transpiration under drought over the 12 d experimental period averaged 69 and 61 % of the control for 'Roma' and 'Kentucky Wonder', respectively. The cumulative ARA data for well-watered plants showed that 'Kentucky Wonder' had an average ARA 30 % higher than 'Roma'. The total cumulative ARA under drought over the experimental period averaged 65 and 58 % of the control for 'Roma' and 'Kentucky Wonder', respectively (Table 1).

The effect of drought on shoot and nodule dry weight measured at the end of expt 2 is given in Table 1. 'Kentucky Wonder' had a higher shoot dry weight than 'Roma' under well-watered conditions, whereas there was no significant difference in dry weight accumulation under drought. Comparing the ratios of shoot and nodule dry weight in droughted relative to control plants shows drought has a more inhibitory effect in 'Kentucky Wonder' than in 'Roma'.
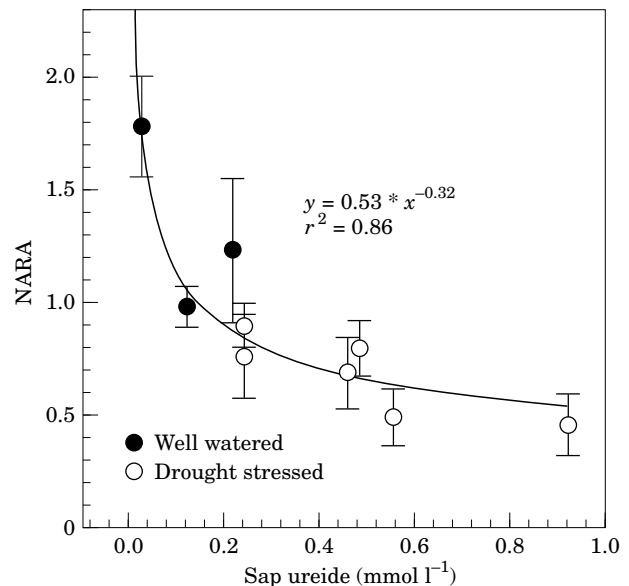

FIG. 4. Plot of normalized acetylene reduction activity (NARA) as a function of ureide concentration in sap exudates for common bean 'Roma' plants during a dehydration cycle. Each data point is the result for an individual plant based on the sap ureide concentration at harvest and the mean NARA on the 3 d prior to harvest. Error bars are $\pm$s.e.

Drought stress in expt 2 induced a significant increase in ureide concentration in both petioles and sap exudate in both cultivars (Table 2). 'Kentucky Wonder' had significantly higher petiole and sap ureide contents than 'Roma' under both well-watered and drought conditions. The drought-induced accumulation of ureides was also higher in

'Kentucky Wonder' than in 'Roma', although this difference was more pronounced in sap ureide.

Sap ureide concentration was determined at the end of the drying cycle in expt 1. The plot of normalized ARA against the concentration of ureide in xylem sap exudate is represented by a logistic curve that indicates a negative correlation between these two parameters (Fig. 4).

DISCUSSION

The drought sensitivity of N$_2$ fixation in common bean was assessed in two experiments by comparing the responses of normalized leaf transpiration and ARA to FTSW. The response of transpiration rates to dehydrating soil for the two common bean cultivars tested in this work was consistent with results obtained with other crop species and experimental conditions (Meyer and Green, 1981; Ritchie, 1981; Sinclair and Ludlow, 1986; Rosenthal et al., 1987). In particular, little change in response to soil drying occurred until there was a substantial decrease in FTSW. In common bean, NTR fitted a logistic curve for both cultivars and it started to decrease only for FTSW less than 0·4 (Fig. 2). Because of the large variability in instantaneous ARA measurements, NARA values were more scattered. NARA fitted a logistic curve showing a decrease starting for FTSW less than 0·3 (Fig. 3). These results show that ARA is less sensitive than transpiration to soil dehydration, indicating that N$_2$ fixation in these two cultivars of common bean is relatively tolerant to drought stress. This conclusion is in agreement with the results of Peña-Carbiales and Castellanos (1993) who found that even though drought affected nodulation in several common bean genotypes, it did not result in a significant decrease in percent N fixed.

The response of N$_2$ fixation to drought has been examined in different grain legumes and was found to be associated with ureide metabolism and transport (Sinclair and Serraj, 1995). It was discovered that N$_2$ fixation in legume species producing ureides was more sensitive to drought than in amide producing species. Exceptionally among the ureide producers, common bean was found to have relatively lower levels of ureides coupled with a higher degree of N$_2$ fixation drought tolerance compared to soybean or cowpea (Sinclair and Serraj, 1995).

The ureide concentrations measured in this study varied between 2 and 10 μmol g^{-1} in the petioles and between 0·1 and 1·0 mmol l^{-1} in sap exudates. These levels are much lower than the concentrations measured in soybean grown in the same conditions, where ureide levels reached 100 μmol g^{-1} in petioles under drought and 5 mmol l^{-1} in sap exudates (Serraj and Sinclair, 1997). These differences in ureide concentrations between species are consistent with the fact that N$_2$ fixation in common bean is less sensitive to drought than in soybean (Sinclair and Serraj, 1995). Furthermore, a negative correlation was found between sap ureide and ARA when individual well-watered and drought-stressed plants of the cultivar 'Roma' were compared (Fig. 4). These results are consistent with the hypothesized involvement of ureide metabolism in drought stress effects on N$_2$ fixation (Sinclair and Serraj, 1995; de Silva et al., 1996; Serraj and Sinclair, 1997).

The two cultivars of common bean were compared to examine possible variations in response to water deficits. Although no differences between genotypes in the response to NTR and NARA response to soil drying (FTSW) were observed (Fig. 3), there was a difference in ureide levels at the end of the drying experiment. Ureide levels increased substantially in both genotypes during soil dehydration (Table 2), but the ureide concentration of petioles was higher in 'Kentucky Wonder' than in 'Roma'. Ureide concentration in the sap exudate was also higher in 'Kentucky Wonder' than in 'Roma' and showed a significant increase under soil dehydration (Table 2). The fact that differences in ureide levels of these common bean genotypes were not associated with differences in the NARA response to drought may indicate that there is a threshold response between ureide levels and N$_2$ fixation activity. That is, the ureide levels in both common bean cultivars may have been sufficiently low so as not to induce the drought sensitivity seen in N$_2$ fixation by soybean, which is associated with much higher ureide levels.

In summary, the results of these experiments confirm the somewhat lower sensitivity of N$_2$ fixation to drought, compared to transpiration, in common bean. This was related to a relatively low ureide concentration in petioles and xylem sap. Our results also indicate a strong consistency in the relationship of NTR and NARA to FTSW independent of cultivar. The logistic functions obtained provide an effective method for describing the response of leaf gas exchange and nodule activity to soil water deficits.

ACKNOWLEDGEMENTS

LITERATURE CITED

Bliss FA. 1993. Breeding common bean for improved biological nitrogen fixation. *Plant and Soil* **152**: 71–79.

de Silva M, Purcell LC, King CA. 1996. Soybean petiole ureide response to water deficits and decreased transpiration. *Crop Science* **36**: 611–616.

Giller KE, Cadisch G. 1995. Future benefits from biological nitrogen fixation—an ecological approach to agriculture. *Plant and Soil* **174**: 255–277.

Graham PH, Ranalli P. 1997. Common bean (*Phaseolus vulgaris* L.). *Field Crops Research* **53**: 131–146.

Hardarson G, Bliss FA, Cigales-Rivero MR, Henson RA, Kipe-Nolt JA, Longeri L, Manrique A, Peña-Cabriales JJ, Pereira PAA, Sanabria CA, Tsai SM. 1993. Genotypic variation in biological nitrogen fixation by common bean. *Plant and Soil* **152**: 59–70.

Meyer WS, Green GC. 1981. Plant indicators of wheat and soybean crop water stress. *Irrigation Science* **2**: 167–176.

Peña-Cabriales JJ, Castellanos JZ. 1993. Effects of water stress on N$_2$ fixation and grain yield of *Phaseolus vulgaris* L. *Plant and Soil* **152**: 151–155.

Ray, JD, Sinclair TR. 1997. Stomatal closure of maize hybrids in response to drying soil. *Crop Science* **37**: 803–807.

Ritchie JT. 1981. Water dynamics in the soil-plant-atmosphere system. *Plant and Soil* **58**: 81–96.

Rosenthal WD, Arkin GF, Shouse PJ, Jordan WR. 1987. Water deficit effects on transpiration and leaf growth. *Agronomy Journal* **79**: 1019–1026.

Scholander PF, Hammel HT, Bradstreet ED, Hemingsen EA. 1965. Sap pressure in vascular plants. *Science* **148**: 339–345.

Serraj R, Sinclair TR. 1996. Processes contributing to N$_2$-fixation insensitivity to drought in the soybean cultivar 'Jackson'. *Crop Science* **36**: 961–968.

Serraj R, Sinclair TR. 1997. Variation among soybean cultivars in dinitrogen fixation response to drought. *Agronomy Journal* **89**: 963–969.

Serraj R, Bona S, Purcell LC, Sinclair TR. 1997. Nitrogen fixation response to water-deficits in field-grown 'Jackson' soybean. *Field Crops Research* **52**: 109–116.

Sinclair TR, Ludlow MM. 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. *Australian Journal of Plant Physiology* **13**: 329–341.

Sinclair TR, Serraj R. 1995. Dinitrogen fixation sensitivity to drought among grain legume species. *Nature* **378**: 344.

Sinclair TR, Muchow RC, Bennett JM, Hammond LC. 1987. Relative sensitivity of nitrogen and biomass accumulation to drought in field-grown soybean. *Agronomy Journal* **79**: 986–991.

Sprent JI. 1972. The effects of water stress on nitrogen fixing root nodules. *New Phytologist* **71**: 451–460.

Wilson JK. 1931. The shedding of nodules by beans. *Agronomy Journal* **23**: 670–674.

Young EG, Conway CF. 1942. On the estimation of allantoin by the Rimini-Schryver reaction. *Journal of Biological Chemistry* **142**: 839–853.