

Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration

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Abstract

Nitrogen fixation activity by soybean (*Glycine max* (L.) Merr.) nodules has been shown to be especially sensitive to soil dehydration. Specifically, nitrogen fixation rates have been found to decrease in response to soil dehydration preceding alterations in plant gas exchange rates. The objective of this research was to investigate possible genetic variation in the sensitivity of soybean cultivars for nitrogen fixation rates in response to soil drying. Field tests showed substantial variation among cultivars with Jackson and CNS showing the least sensitivity in nitrogen accumulation to soil drying. Glasshouse experiments confirmed a large divergence among cultivars in the nitrogen fixation response to drought. Nitrogen fixation in Jackson was again found to be tolerant of soil drying, but the other five cultivars tested, including CNS, were found to be intolerant. Experiments with CNS which induced localized soil drying around the nodules did not result in decreases in nitrogen fixation rates, but rather nitrogen fixation responded to drying of the entire rooting volume. The osmotic potential of nodules was found to decrease markedly upon soil drying. However, the decrease in nodule osmotic potential occurred after significant decreases in nitrogen fixation rates had already been observed. Overall, the results of this study indicate that important genetic variations for sensitivity of nitrogen fixation to soil drying exist in soybean, and that the variation may be useful in physiology and breeding studies.

Introduction

Symbiotic fixation of atmospheric nitrogen is an important activity for obtaining high yields in legumes. However, several studies with soybean have shown that even moderate soil dehydration can result in decreased rates of nitrogen fixation, and these decreases in nitrogen fixation rates precede decreases in leaf gas exchange. Using 3-week-old plants of cultivar Chippewa 64 exposed to various concentrations of polyethylene

glycol, Kuo and Boersma (1971) found a much greater decline in nitrogen fixation than CO₂ assimilated during a 10-day exposure. Sinclair (1986) in a study with cultivar CPI 26671 grown in pots, found that symbiotic fixation rates decreased before transpiration rates in plants subjected to a drying cycle. A pot experiment performed by Durand *et al.* (1987) with the cultivar Clark showed a decrease in nitrogen fixation preceded a decrease in plant photosynthesis as the soil dried.

Field studies have also shown that nitrogen fixation in soybean is adversely influenced upon soil dehydration (*e.g.* Weisz *et al.*, 1985). Hunt *et al.* (1981) found with the cultivar Bragg that the

imposition of a non-irrigated treatment resulted in decreased leaf nitrogen concentration, indicating a decrease in nitrogen accumulation relative to carbon accumulation. A direct comparison of the relative sensitivity of nitrogen and carbon accumulation to soil dehydration in the cultivar Biloxi was reported by Sinclair *et al.* (1987). They found that nitrogen accumulation was more adversely affected by soil drying than carbon accumulation for all the water-limiting treatments.

Important questions remain about the possible genetic variation among soybean genotypes in sensitivity of nitrogen fixation to soil dehydration. Ray (1987) examined the performance of 28 soybean genotypes in response to soil drying during early reproductive development. He found a substantial variation in the shortening of the duration of the R5 to R7 stage among cultivars due to drought stress. If it is assumed that at least part of the shortening of this period results from decreased nitrogen availability in the plant as suggested by Sinclair and de Wit (1976), then genotypic differences in sensitivity of nitrogen fixation to soil drying may be indicated. The first objective of this study was to determine directly if genotypic differences among soybean cultivars exist for sensitivity of nitrogen fixation to soil dehydration. A second objective was to examine changes in nodule osmotic potential as a possible basis for the differences among cultivars.

Materials and methods

Field study

Based on the comparison of 28 cultivars reported by Ray (1987) for divergence in the duration of the R5 to R7 stage in response to drought treatment, eight cultivars (Table 1) were selected for a subsequent, direct comparison of nitrogen accumulation. The comparison was performed in 1987 on an Arredondo fine sand (loamy, siliceous, hyperthermic Grossarenic Paleudult) at Gainesville, FL. Before sowing, 83 g m⁻² of 0-10-20 (N-P₂O₅-K₂O) fertilizer was applied to the soil, and on 21 July 42 g m⁻² of muriate of potash was applied. The cultivars were sown on

17 June into four randomized replications of the main plots for the well-watered and water-limited treatments. Subplots for each cultivar consisted of 11 rows with 0.3 m between rows and a row length of 7.3 m. The sowing rate was 20 seeds per m of row and final plant populations of 44 to 50 plants m⁻² were achieved. The water-limited treatment was imposed on 8 August at the R2 stage after fully canopy closure. The water-limited treatment was irrigated only when leaves had become wilted, and then the plots were watered with only 10 mm of water at each irrigation.

Due to the lack of nitrogen fertilizer and the low organic matter concentration (5.5 g kg⁻¹ organic C) of this fine sand soil, the plants quickly became dependent on symbiotic N fixation as their major source of nitrogen. An estimate of the rate of nitrogen fixation by each cultivar was obtained from the difference in plant nitrogen accumulation measured between 4 August and 1 September. On each of these dates plants were harvested from a 0.56 m² area in each replication by cutting the stems at soil level. The entire sample was oven dried at 70°C to determine dry weight. After grinding of the entire sample, a 1-g subsample was collected for measurement of plant nitrogen content by the Kjeldahl technique.

Pot study

Based on the 1987 field study, six cultivars (Jackson, CNS, Coker 368, SCE 82-222, Coker 156, SCE 82-303) were selected for further study under glasshouse conditions. The first test was to compare the decrease in nitrogen fixation activity against transpiration rate as the soil was allowed to dehydrate. These experiments were done by growing plants in pots constructed from 10-cm diameter × 30-cm long sections of PVC pipe. The bottom of the pipe was sealed with an end cap in which a 3-mm Swagelok elbow fitting (Crawford Fitting Co., Solon, OH) had been mounted. With a 20-mm thick layer of gravel inside the end cap, the elbow fitting provided both a drainage port and an entry port for the acetylene-air gas mixture during measurements of nodule activity. The top of the pot was fitted with a PVC toilet flange which had been mach-

Table 1. Screens of nitrogen accumulation tolerance to drought among soybean cultivars grown under field conditions

Cultivar	Maturity group	Decrease in R5 to R7 ^a (days)	1987 N accumulation	
			Drought treatment	Drought/well-watered
			(g m ⁻² d ⁻¹ (SE))	
Jackson	VII	1	0.23 (0.03)	0.86
CNS	VII	2	0.19 (0.04)	0.72
Coker 368	VIII	1	0.18 (0.05)	0.72
Roanoke	VII	3	0.14 (0.02)	0.67
SCE 82-222	VIII	3	0.11 (0.04)	0.55
Coker 156	VI	7	0.14 (0.08)	0.53
Asgrow 7372	VII	6	0.10 (0.05)	0.46
SCE 82-303	VIII	7	0.08 (0.02)	0.19

^a Ray, 1987.

ined so that a two-piece lid could be attached for sealing the pot during the dehydration treatment (Denison *et al.*, 1983). The pots were filled with a mixture of 2/3 potting soil (Vitagreen, Inc., Clermont, FL) and 1/3 vegetable plug mix (W.R. Grace and Co., Cambridge, MA). A single plant was allowed to develop in each pot.

In each dehydration experiment of a cultivar nine pots were used; the soil in six pots was allowed to dehydrate and three pots were well-watered. The experiments were initiated 5 to 6 weeks after sowing by sealing the top of the pots with the two-piece lids. Sealing the pots not only facilitated measurement of nodule activity, but prevented evaporation from the soil so that daily weighing of the pots yielded a measure of transpiration rate per pot. The transpiration rates of the dehydrated plants usually decreased to less than 10% of the well-watered plants in 6 to 9 days.

The nodule activity was measured daily during the dehydration cycle using a continuous-flow acetylene reduction technique. It was found that between 5 and 15 min after initiating a 1000 cm³ min⁻¹ flow of a 10% acetylene-90% air mixture through the pot, a stable rate of ethylene production was measured in the efflux from the pot. All pots were exposed simultaneously to the acetylene-air mixture and three gas samples were collected in 1-mL syringes from the efflux of each pot during the 5 to 14 minute period after initiating the flow of the acetylene-air mixture. After the gas samples were collected, the pots were flushed with only air for another 15 min.

The gas samples in the syringes were injected into a gas chromatograph having a flame ionization detector (Model 5710A, Hewlett-Packard Corp., Palo Alto, CA) to determine ethylene concentration. On each day the individual measurements of transpiration rate and nodule activity were expressed as a fraction of the values observed for the well-watered pots.

To investigate further the influence of soil dehydration on nodule activity, experiments were performed with cultivars CNS and Jackson where the soil layer around the nodules was allowed to dehydrate while the lower soil layer which contained few, if any, nodules was watered. An arrangement of soil in pots similar to that suggested by Khanna-Chopra *et al.* (1984) was used. The top 8-cm layer of soil was separated from the lower layer by a 5-cm layer of gravel. The gravel layer minimized upward conduction of water, yet provided a uniform layer into which water could be added to irrigate the lower soil layer only. Using the gravel layer to divide the soil into a top and bottom layer, three treatments of three pots each were imposed: fully watered by surface irrigation, only bottom soil layer irrigated, and no irrigation of either soil layer. Nodule activity was measured daily by the same techniques as described previously.

Osmotic potential

Nodule osmotic potential was measured for all six cultivars as a possible physiological variable which responded directly to soil dehydration. At

the termination of the previously described pot experiments, nodules were removed from the soil, immediately placed in liquid nitrogen, and the frozen nodules were stored until osmotic measurements were made. Nodules greater than 2-mm diameter were used for measurement of osmotic potential by squeezing the liquid from thawed nodules onto paper sampling discs. The discs were placed in an osmometer (Model 5500, Wescor, Inc., Logan, UT) for determination of osmotic potential. The fractional water content ($((\text{fresh wt} - \text{dry wt})/\text{fresh wt})$) was determined using a combined sample of all the other nodules harvested from each treatment.

To examine the course of change in nodule osmotic potential during soil dehydration, two cultivars which were intermediate in their nitrogen fixation response to drought were studied. Plants of Coker 368 and SCE 82–303 were grown in 1 L pots and the soil drying experiment was begun 6 weeks after sowing. During the drying cycle all pots were sealed in plastic bags to prevent soil evaporation. The pots were weighed daily in order to calculate transpiration rate. Nodules were harvested daily from three treatment pots and a control pot by removing the soil from the pot and gently detaching nodules. The nodule osmotic potential and transpiration rate relative to the control were plotted as a volumetric fraction of transpirable soil water, FTSW (transpirable soil water is the quantity of water lost from the pots through transpiration).

Results

Field study

Ray (1987) found that drought during early reproductive growth shortened the length of the R5 to R7 stage by 1 to 7 days for the 28 cultivars studied. Our subsequent field experiment with eight selected cultivars also showed a wide diversity among these cultivars in their ability to accumulate nitrogen when the soil was allowed to dry (Table 1). As a further comparison, the ratio was calculated for each cultivar of the mean rate of nitrogen accumulated in the dry plots to the mean nitrogen accumulation rate in the well-watered plots. The values of this ratio ranged

from 0.19 for SCE 82–303 to 0.86 for Jackson (Table 1). These results provide direct evidence that genetic diversity exists in the tolerance of nitrogen fixation to dry soil. Also, a correlation is evident in the ability to accumulate nitrogen under dry conditions and the shortening of the R5 to R7 stage. Cultivars which proved to have low nitrogen accumulation rates under dry conditions in our experiment also were found by Ray (1987) to have the greatest shortening of the interval between the R5 and R7 stages.

Pot studies

Observations of relative nitrogen fixation rate and transpiration rate measured in the pot studies in the glasshouse were plotted against each other. If these two processes were equally sensitive to soil dehydration, their rates would decrease in parallel and the plot of data would be clustered around the equality line. Cultivars Jackson and CNS represented the extremes in response to soil drying (Fig. 1). The data for Jackson were unique in that they indicated an equality of sensitivity to soil drying between nitrogen fixation and transpiration. Such a response is consistent with the superiority of Jackson observed in the field for nitrogen accumulation under drought conditions. On the other hand, the data for the other five cultivars showed nitrogen fixation activity was depressed relative to transpiration. CNS showed the greatest sensitivity in nitrogen fixation to soil dehydration with most of the data falling below the equality line (Fig. 1), which is in contrast to the response observed in the field.

Due to the differences in CNS between the field and pot experiments in the nitrogen fixation response to soil drying, an additional pot experiment was done to examine the consequences of allowing only the soil around the nodules to dry. Dividing the soil in the pot between a top and bottom zone with a gravel layer allowed the top soil layer around the nodules to dehydrate while the bottom soil layer was irrigated. The nitrogen fixation rates relative to the well-watered treatment were not affected for the plants subjected to only top soil layer drying (Fig. 2). Seemingly, soil dehydration immediately around the nodules did not induce decreases in nodule activity. In

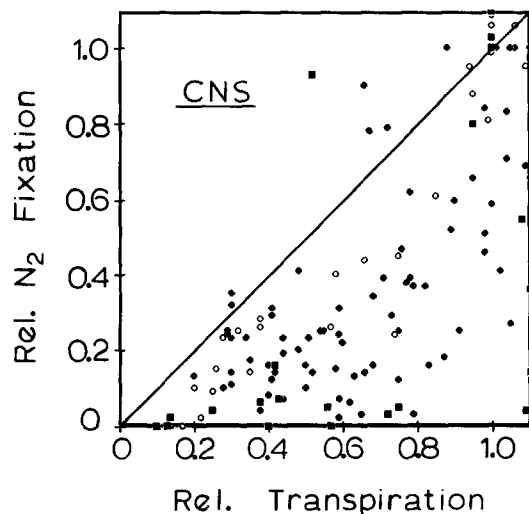
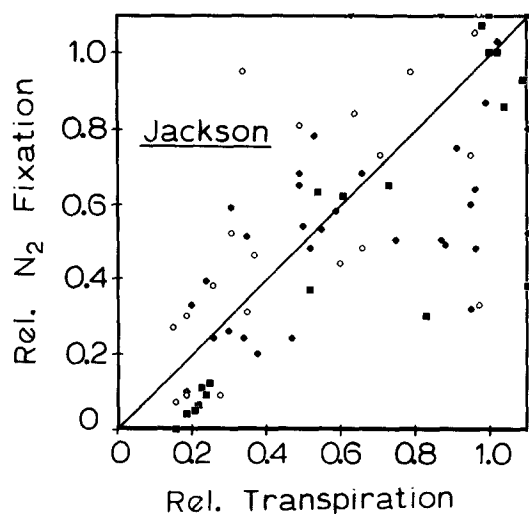


Fig. 1. Daily observations from soil dehydration pot experiments where acetylene reduction rates are plotted against transpiration rates. The data from the dry pots has been normalized relative to the daily rates of the well-watered pots. Different symbols in each plot represent data obtained in three separate soil-drying experiments for each cultivar.

contrast, the treatment in which all the soil was allowed to dry resulted in a marked decline in relative nitrogen fixation rates on the fourth day of the experiment (Fig. 2).

Osmotic potential

A possible explanation for the observed decreases in nitrogen fixation and nodule gas permeability in response to soil dehydration (Weisz

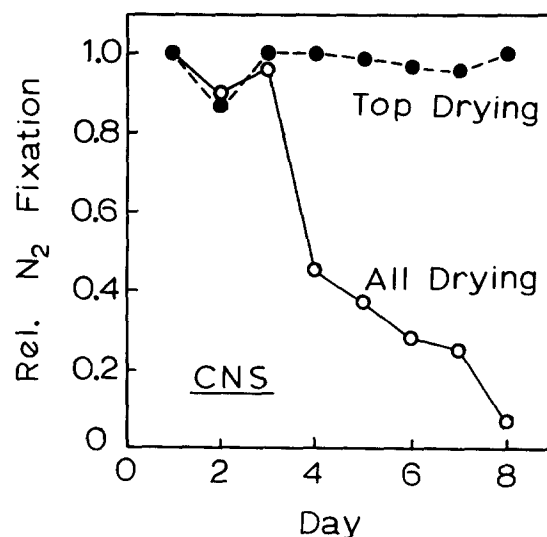


Fig. 2. Acetylene reduction rates normalized relative to well-watered pots for treatments where all the soil was allowed to dry or where only the top layer was allowed to dry.

et al., 1985) could be related to decreases in cell turgor within nodules. An important feature of the nodule turgor loss may be changes in osmotic potential of the nodules when the soil dries. Nodules were harvested after a soil dehydration cycle for each of the six cultivars and osmotic potential was measured. These data (Table 2) showed large differences in nodule osmotic potential between the irrigated and soil-drying treatments in all cultivars. At least a 1.2 MPa decrease in nodule osmotic potential of the dehydrated treatment relative to the well-watered was observed. While Jackson was found to have the largest difference between the soil-drying and control treatments, the osmotic potential of nodules from the dehydrated treatment of Jackson were not significantly different from those of CNS. The decreases in nodule water volume were too small to account for the observed decreases in osmotic potential, therefore, these results indicate an accumulation of solutes in the nodules during the soil dehydration.

To determine if the changes in nodule osmotic potential during the soil dehydration cycle paralleled the changes in nitrogen fixation activity, daily harvests of nodules for osmotic potential measurement were made throughout a soil dehydration cycle with cultivars Coker 368 and SCE 82-303. The results of this experiment showed

Table 2. Osmotic potential (standard error) and fractional water content of nodules at termination of soil-drying experiments

Cultivar	Osmotic potential (MPa)		Fraction H ₂ O content (g g ⁻¹)	
	Control	Dry	Control	Dry
Jackson	-0.74 (0.02)	-2.70 (0.13)	0.71	0.58
CNS	-0.83 (0.03)	-2.73 (0.15)	0.67	0.58
Coker 368	-0.80 (0.07)	-2.02 (0.30)	0.67	0.54
SCE 82-222	-0.93 (0.10)	-2.31 (0.20)	-	-
Coker 156	-0.88 (0.03)	-2.14 (0.09)	-	-
SCE 82-303	-0.95 (0.05)	-2.21 (0.23)	0.69	0.54

that for both cultivars the dramatic decrease in osmotic potential did not occur until FTSW had reached about 0.25 (Fig. 3A). In both cultivars the decrease in osmotic potential at an FTSW of about 0.25 corresponded well with the decline in plant transpiration rates, but occurred at a lower FTSW than the decline in nitrogen fixation rates (e.g. Coker 368 in Fig. 3b). Therefore, the large

changes in nodule bulk osmotic potential appear not to coincide with the decrease in nitrogen fixation rate.

Discussion

Both the field and pot experiments indicated substantial differences exist among genotypes in the response of nitrogen fixation to soil dehydration. Cultivars Jackson and CNS were found to be tolerant to soil drying in two years of field tests (Table 1). Surprisingly, the response of these two cultivars in pot-drying experiments was different. While nitrogen fixation activity in Jackson also proved to be drought tolerant in pots, the nitrogen fixation rates of CNS dropped rapidly relative to decreases in plant transpiration rates (Fig. 1). The four other cultivars tested in pot experiments in the glasshouse also were found to have greater sensitivity in nitrogen fixation rates than transpiration rates to soil drying. CNS showed the greatest sensitivity of nitrogen fixation to soil dehydration.

The decrease in nitrogen fixation activity in CNS at a relatively high soil water content upon soil dehydration was not due to the soil water content immediately surrounding the nodules, but rather to the drying of the entire soil volume (Fig. 2). The lack of response of nitrogen fixation by CNS to drying of the top soil layer is in contrast to the results for cowpea reported by Khanna-Chopra *et al.* (1984), who found in a similar experiment that soil dehydration only in the top layer resulted in a severe decrease in nitrogen fixation activity. However, they measured acetylene reduction on excised nodules after a 60-min incubation. It seems possible that

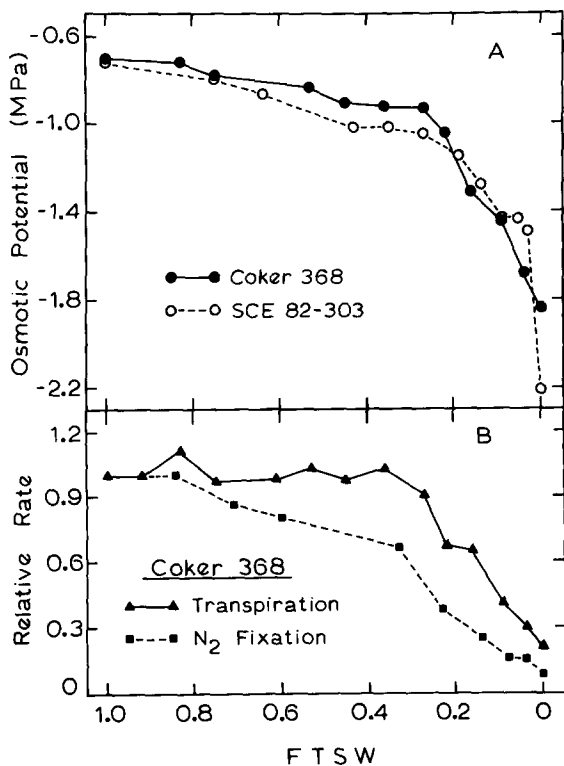


Fig. 3. (A) Osmotic potential measured daily for nodules harvested from Coker 368 and SCE 82-303 during a soil-drying cycle plotted against fraction transpirable soil water (FTSW). (B) Relative transpiration rate and nitrogen fixation rate for Coker 368 during a soil-drying cycle plotted against FTSW.

the disturbance and removal of nodulated roots in their experiments may have resulted in decreased acetylene reduction rates, especially for stressed nodules (Minchin and Witty, 1989), in contrast to our intact system.

Measurement of nodule osmotic potential at the end of the drying cycle showed decreases in osmotic potential as compared to control nodules in all six cultivars (Table 2). Although dehydration of nodules was found, the change in nodule water content was insufficient to account for the change in osmotic potential. Observations of the changes in osmotic potential with soil dehydration showed that large decreases in osmotic potential occurred after the decreases in nitrogen fixation rates had begun (Figure 3). These results are consistent with those of Albrecht *et al.* (1984), who found that large decreases in soybean nodule activity occurred when the nodule water content decreased from 0.60 to 0.55.

The results of this study indicate two approaches may exist for use in developing cultivars which avoid, at least in part, the decreases in nitrogen fixation rate resulting from modest decreases in soil water content. One approach would be to develop cultivars in which the nitrogen fixation appears to be insensitive to soil drying such as observed in Jackson. Particularly important for this alternative will be the identification of specific traits in the plant and/or nodule which confer this drought tolerance. The second approach would be to develop cultivars which do not readily transmit a drought 'signal' to the nodules. The performance of CNS in pots and in the field is reconciled if a drought 'signal' from its deeper roots was inhibited in the field experiments relative to the pot experiments. Of course, one possibility for the 'signal' may be a hydraulic one and the transport of water to the nodules from deep roots may ameliorate the effects of soil drying in the upper layers. In any event, the results presented here indicate that important genotypic variation exists in the soy-

bean germplasm for ameliorating the effects of soil dehydration on nitrogen fixation rates.

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