PN-AAP-194
ISN:33196

Reprinted from *Crop Science*
Vol. 23, Sept.-Oct. 1983, p. 815-818

DEC 19 1983

Effect of Salinity on the Functional Components of the Soybean-*Rhizobium japonicum* Symbiosis[1]

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ABSTRACT

A split-root solution culture system was used to partition the effects of salinity on the functional components of the soybean [*Glycine max* (L.) Merr] - *Rhizobium japonicum* symbiosis. Nodules were confined to half of the root system. After nodules were well developed (40 days from planting), 120 m*M* NaCl was applied to the half-root systems in the following combinations: 1) unstressed, no salt to either half-root system; 2) partially stressed shoot and unstressed nodules, salt to only the nonnodulated half-root system; 3) partially stressed shoot and stressed nodules, salt to only the nodulated half-root system; and 4) stressed shoot and nodules, salt to both half-root systems. Leaf osmotic potentials (ψ_s) for the four treatments were: 1) −1.02; 2) −1.12; 3) −1.23; and 4) −1.83 MPa. Nodule ψ_s for the treatments were: 1) −0.70; 2) −0.72; 3) −1.12; and 4) −1.21 MPa. While total and specific nitrogenase activity as well as shoot N content were suppressed in treatment 3, the greatest decrease in activity and shoot N was observed when the plant was stressed by having both half-root systems in salt (treatment 4). The rate of leaf expansion in treatment 4 was half the rate observed in the other treatments. We conclude that reduced N_2 fixation by nodulated soybeans growing in saline environments was more a result of the effect of salt on leaf ψ_s and expansion, than the direct action of salt on the functional processes of the nitrogenase system.

Additional index words: Nitrogenase activity, Split-root, Osmotic potential, *Glycine max*.

THE legume-*Rhizobium* symbiosis is a highly integrated process involving a complex interaction between the host and microorganism. A constant exchange of metabolites takes place between plant photosynthetic organs and root-nodules. Photosynthate enters the nodule by phloem transport where it is either respired for nodule growth, maintenance, and reduction of N_2 or reexported to the shoot combined with fixed N_2 (Pate, 1976). The production and flow of amino compounds, as with other assimilates, is regulated and ultimately partitioned to growth points and reproductive sinks in relation to growth potential (Lewis and Pate, 1973). Therefore, any stress that decreases photosynthesis and plant growth will undoubtedly affect N_2 fixation.

Soil stress may decrease symbiotic efficiency to levels below the genetic potential of a host-*Rhizobium* strain combination. The stress could decrease the accumulation of symbiotically derived N by either affecting shoot photosynthesis and yield potential or by directly affecting processes within the nodule. Due to the integrated nature of the functional processes of the symbiosis it becomes difficult to assess the relative sensitivity of host and microorganism to stress. By identifying the components or processes of the symbiosis that are most sensitive to the soil stress, efforts to increase N_2 fixation in these environments may then focus on the proper component. Singleton et al. (1982) have indicated that the ability of *Rhizobium* to grow and survive probably does not limit N_2 fixation in saline environments.

The same soil stress that decreases plant growth,

[1] This research was supported in part by Grants AID/DSAN-G-0100 (211-d) and AID/ta-c-1207 (NifTAL Project) from the United States Agency for International Development. Received 13 Dec. 1982.

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may also directly affect the processes within the root-nodule. Sprent (1972) observed a 50% decline in nitrogenase activity within 19 min of exposing the entire nodulated root system of soybean plants to 120 m*M* NaCl. Exposure of detached soybean nodules to 1 *M* NaCl also depressed nitrogenase activity.

Lauter et al. (1981) showed that salinity adversely affected nodule weight and total nitrogenase activity of chickpea (*Cicer arietinum* L.). Similar results were obtained for soybean and alfalfa (*Medicago sativa* L.) by Bernstein and Ogata (1966) and for *Glycine wightii* by Wilson (1970). Since salinity is known to decrease photosynthesis and growth of most plants (Gale, 1975), the above studies do not determine how salinity limited the symbiosis.

This study was conducted to assess the effects of salinity on the components of the soybean—*Rhizobium japonicum* symbiosis. We partitioned the effects of salinity on N_2 fixation into effects that directly relate to nodule function vs. indirect effects that are mediated through the host photosynthetic apparatus.

MATERIALS AND METHODS

Plant Culture. The split-root solution culture growth system that confined the nodules to half the root system of soybean [*Glycine max* (L.) Merr cv. Davis] and the planting procedure have been described earlier (Singleton, 1983). At planting NH_4NO_3 (7.1 m*M* N) was added to the basal nutrient solution: (m*M*) 0.5 P; 0.96 K; 1.56 S; 0.82 Mg; and 0.75 Ca. Micronutrients were added according to Broughton and Dilworth (1971). Solutions were changed 22 days from planting and half of the split-root system was inoculated with *Rhizobium japonicum* strain USDA 110 (USDA, Beltsville Md.) at a density of 9.7×10^5 cells/mL of culture solution. The opposite half of the root system remained uninoculated. NH_4NO_3 (0.75 m*M* N) was added to the basal nutrient solution of the uninoculated half to supply the plant requirement for N during nodule development. Significant nodule development was observed 40 days from planting. At this time solutions were changed, nodulated sides reinoculated (7.1×10^4 cells/mL) and NaCl was introduced in two equal increments over a 2-day period to designated containers to a final concentration of 120 m*M* NaCl. No N was given to plants after treatment initiation. Solutions were again changed at day 46, reinoculated (1.7×10^5 cells/mL) and designated containers resalinized. Plants were harvested at 50 days from planting.

Salinity Treatments to Half-Root Systems. Four treatment combinations between the source of symbiotic N and salinity were instituted and are displayed in Table 1. Treatments were arranged in a completely randomized design and replicated three times.

Measurement of Leaf Expansion. The most recently emerged leaflets of three plants were selected at random and tagged and their initial leaf area determined with a Licor area meter. The measurement was performed 3 days after the initiation of salinity treatments. The same leaflets were measured 5 days later. Leaf expansion was expressed as cm^2/day.

Measurement of Osmotic Potential (ψs). Eight days after treatment initiation, leaf and nodule ψs were determined. The most recently expanded trifoliolate leaf of three plants not used for measurement of leaf expansion were removed and placed in 9 cm lengths of Tygon R-3603 tubing (12 mm i.d., 2 mm wall). The tubes were sealed with rubber stoppers and frozen on dry ice. Nodule and root samples were blotted dry prior to placing them in the tubes for freezing. Tubes were thawed (20 min), pressed at 10.52×10^6 kg/m^2, and the expressed sap sampled with filter paper discs that were placed in a Wescor 5130 vapor pressure osmometer.

Harvest. Shoots were cut and oven dried at 65°C. Root halves were removed, placed in 2 L plastic bottles, and incubated for 30 min in 50 mL/L acetylene. Ethylene production was determined by gas chromatography.

Roots and nodules were separated and oven dried. Potassium and Na contents of nodules were determined by flame photometry from water extracts of ground tissue. Chloride in nodules was determined by potentiometry using the titrating method in conjunction with a Ag - AgCl electrode and a glass reference electrode (Chapman and Pratt, 1961).

Table 1. The effect of NaCl (120 m*M*) applied to half-root systems of soybean on shoot dry weight and shoot N.

	Treatment to half-root system†		Dry weight	Shoot N	
	Nod	Nonnod			
			g pot^{-1}	g pot^{-1}	g kg^{-1}
1.	−	−	150.6	4.1	26.9
2.	−	+	118.3	3.2	27.0
3.	+	−	130.9	2.6	20.1
4.	+	+	80.5	1.8	22.9
	LSD 0.05		34.9	1.2	5.3

† Half-root systems nodulated (Nod) or nonnodulated (Nonnod) in either normal nutrient solution (−) or nutrient solution plus 120 m*M* NaCl (+). Salt treatments instituted at day 40 from planting until harvest at 50 d.

Table 2. The effect of shoot and nodule osmotic potential (ψs) on nitrogenase and leaf expansion of soybean.

	Treatment to half-root system†		ψs	ψs	Nitrogenase activity		Expansion of new trifoliolate
	Nod	Nodnod	Leaf	Nodule	Total‡	Specific§	
			MPa		nmole C_2H_4		cm^2 d^{-1}
1.	−	−	−1.02	−0.70	88.6	18.6	34
2.	−	+	−1.12	−0.72	60.3	15.8	31
3.	+	−	−1.23	−1.12	46.7	11.9	31
4.	+	+	−1.83	−1.21	13.0	6.4	16
	LSD 0.05		0.39	0.15	41.4	7.5	12

† Half-root systems nodulated (Nod) or nonnodulated (Nonnod) in either normal nutrient solution (−) or nutrient solution plus 120 m*M* NaCl (+). Salt treatments instituted at day 40 from planting until harvest at day 50.

‡ Total nitrogenase activity of nodulated half-root systems: nmole C_2H_4 pot^{-1} s^{-1}.

§ Specific nitrogenase activity: nmole C_2H_4 (g nodule dry weight)$^{-1}$ s^{-1}.

RESULTS

Shoot dry weight and shoot N accumulation decreased when half and whole root systems were exposed to NaCl (Table 1). The concentration of N in shoots was decreased when nodules were exposed to NaCl.

Leaf osmotic potential (ψs) of plants with only half the root system salinized was between 0.1 and 0.2 MPa lower than the nonsalinized control. With the whole root system salinized leaf ψs was 0.8 MPa lower than the control (Table 2). Nodules exposed to salt had a ψs 0.4 MPa lower than nonsalinized nodules (Table 2).

Nitrogenase activity was reduced when the nodulated half of the root system was exposed to salt. However, there was a much greater decline in nitrogenase

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Table 3. The effect of NaCl (120 m*M*) applied to half-root systems of soybean on the concentration of shoot and nodule Na^+, Cl^-, and K^+.

Treatment to half-root system†		Na^+		Cl^-		K^+	
Nod	Nodnod	Shoot	Nodule	Shoot	Nodule	Shoot	Nodule
		g kg^{-1}					
1. −	−	0.5	2.0	2.1	0.3	23.0	21.3
2. −	+	1.8	4.0	8.8	0.5	22.0	17.2
3. +	−	2.3	37.0	10.5	4.4	19.0	8.8
4. +	+	9.8	37.0	33.5	4.9	25.0	8.2
LSD 0.05		3.6	1.5	5.9	1.6	2.2	4.3

† Half-root systems nodulated (Nod) or nonnodulated (Nonnod) in either normal nutrient solution (−) or nutrient solution plus 120 m*M* NaCl (+). Salt treatments instituted from day 40 from planting until harvest at day 50.

activity when the whole root system was salinized (Table 3). Specific nitrogenase activity was reduced when nodules were exposed to salt. Specific activity of nodules on plants with both half-root systems salinized was approximately half of that of nodules on plants with only the nodulated half salinized (Table 2).

Leaf expansion, while affected slightly by salinizing either half the root system, was reduced by 50% when both half-root systems were salinized (Table 2).

Data for the concentrations of Na^+, Cl^-, and K^+ in shoots and nodules are given in Table 3.

DISCUSSION

Studies on the effect of salinity on the legume-*Rhizobium* symbiosis have always subjected the entire symbiotic system to the stress. Increasing salinity resulted in a reduction of nodule number, nodule weight and total N_2 fixed by *Glycine wightii* (Wilson, 1970). Bernstein and Ogata (1966) and Lauter et al. (1981) observed similar effects of salinity on nodulation and N_2 fixation by *Glycine max* and *Cicer arietinum*, respectively. In both studies, NO_3^--fed plants subjected to salinity had higher relative yields than similarly treated symbiotic plants. This suggests that the processes of the symbiosis are relatively more sensitive to salinity than the mechanisms of uptake and metabolism of mineral N. The sensitivity of particular symbiotic processes cannot be determined from these studies since both the shoot and nodule were stressed simultaneously. To identify the symbiotic process most susceptible to salinity, it is necessary to subject shoot and nodules to salinity stress independently.

Huang et al. (1975b) partitioned the effects of soil moisture tension to photosynthesis and nodule function of soybean. Photosynthesis of drought stressed plants was enhanced by CO_2 enrichment that resulted in an increase in nitrogenase activity. Their methodology, however, requires a great deal of preliminary investigation and more complex procedures than the split-root technique used in this study.

Since symbiotic N_2 fixation is dependent on recently-fixed photosynthate (Minchin and Pate, 1974; Huang et al., 1975a) root environment stress that reduces photosynthesis (or the translocation of photosynthate) to the nodules will have a rapid effect on nodule function. Despite osmotic adjustment of roots and shoots to reduced water potentials in the root environment (Bernstein, 1961), photosynthetic assimilation of carbon by glycophytes such as soybean is adversely affected (Sung and Kreig, 1979; Jensen, 1975; Gale, 1975; Turner et al. 1978). Sung and Kreig (1979) concluded that photosynthesis was reduced with water stress in sorghum and cotton prior to any reduction in translocation rates. Reduced photosynthesis by plants in saline environments is characterized by reduced leaf expansion (Jensen, 1975; Hawker and Walker, 1978) and increased leaf resistance to CO_2 assimilation (Jensen, 1975).

The osmotic potential of shoots and nodules (Table 2) indicates that leaves and nodules of the same plant could be subjected to differential salinity stress. Nodules of treatments 2 and 3 are functioning at a 0.4 MPa ψs differential while leaves of both treatments have approximately the same ψs. Treatments 3 and 4 have nodules at the same ψs but leaves carrying on growth and photosynthesis at a 0.5 MPa difference. Turner et al. (1978) and Huang et al. (1975a) found that photosynthetic rates in soybean were reduced substantially when leaf water potentials were below −1.40 MPa.

Exposing either the nodulated or nonnodulated half of the root system to salt resulted in a reduction in both shoot dry weight and total shoot N (Table 1). When only the nodulated half was salt stressed (treatment 3) shoot N concentration was reduced. Apparently, the stressed nodules ($\psi s = -1.12$ MPa) could not supply adequate N to meet the growth requirements of the relatively unstressed shoot. The greater N concentration in the shoot when both half-root systems were stressed (treatment 4) indicates that it was not N availability that was limiting shoot growth in this treatment.

The direct effects of salinity on nodule function can be determined by comparing nitrogenase activities of treatments 2 and 3. Total and specific nitrogenase activity was reduced when salinization of nodulated half-root systems resulted in a −0.4 MPa change in nodule ψs. When the whole symbiotic system was salt stressed (treatment 4), however, a large reduction in nitrogenase activity was observed. This reduction can best be explained by the large reduction in leaf ψs and leaf expansion rather than nodule ψs. Sprent (1972) demonstrated that nitrogenase activity by detached soybean nodules decreased by 50% when exposed to 100 m*M* NaCl. Since nodules in that experiment were detached during exposure to salinity, little or no osmoregulation could occur. By exposing nodules to salinity in the split-root assembly in this experiment a more realistic test of the effects of salinity on nodule function was possible.

Low water potentials in the root environment affect both photosynthetic rates (Boyer, 1970) and leaf expansion (Hawker and Walker, 1978). Since shoots are both the source of energy for t nitrogenase system and the sink for nodule products, leaf expansion can be considered as a measure of change in photosynthetic capacity and strength of the sink for fixed N_2. The effect of reducing leaf ψs on leaf expansion is illustrated in Table 2. Although leaf expansion rates were slightly affected when one-half the root system was exposed to salt and leaf ψs declined by 0.1 or 0.2 MPa, a 50% reduction in expansion rate was observed when leaf ψs declined to −1.83 MPa. Nitrogenase activity in the soybean *R. japonicum* symbiosis is, therefore, limited by salinity more through

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a reduction in leaf ψs and leaf expansion. The nitrogenase system can withstand substantial reductions in ψs from exposure to extremely saline environments provided the shoot remains relatively unstressed.

Tissue analysis of shoots and nodules help to explain these results (Table 3). Nodules, like roots (data not shown), accumulated more Na^+ and excluded more Cl^- relative to the shoot. Uptake of Na^+ by nodules was negatively correlated with nodule K^+. Shoot K^+ was not greatly affected by the salinity treatments. Our results show that nodule activity can proceed at near normal rates despite the presence of high concentrations of Na^+, and an apparent reduction in K^+.

In conclusion, differentially salinized soybeans with nodules confined to one-half the root system provide a simple system in which the processes of the symbiosis may be subjected to stress independently. Plants with half their roots exposed to salt had leaf ψs and shoot yield potentials approximately equivalent to nonsalinized controls. Nodule ψs was a function of the ψs of the solutions to which they were exposed regardless of leaf ψs. Nitrogenase activity of salinized nodules was more a function of the degree of stress in the shoot rather than the direct effect of salt on nodule processes. With the high concentration of salt used in this experiment (120 m*M* NaCl), there appears to be some direct effect of salt on nodule function. However, reduced nitrogenase activities observed in plants with the whole root system salt stressed was due more to salt limiting leaf expansion, shoot growth and demand for N. The relative tolerance of established nodules to low water potentials may have evolutionary and ecological relevance. Annual legumes such as soybean may mature using water deep in the soil profile while, as the surface soil dries, nodules must adjust and function in an environment of declining soil matric and osmotic potentials.

ACKNOWLEDGMENTS

We would like to thank D.P. Bartholomew and S.A. El-Swaify for review, E. Okazaki for assistance in tissue analysis, and J. Tagami, D.L. Ota and P. Cainglit for manuscript preparation. We would also like to thank the Dep. of Botany at the Univ. of Hawaii for providing greenhouse facilities.

REFERENCES

1. Bernstein, L. 1961. Osmotic adjustment of plants to saline media: I: steady state. Am. J. Bot. 48:909–918.
2. ----, and G. Ogata. 1966. Effects of salinity on nodulation, nitrogen fixation, and growth of soybean and alfalfa. Agron. J. 58:201–203.
3. Broughton, W.J., and M.J. Dilworth. 1971. Control of leghemoglobin synthesis in snakebeans. Biochem. J. 125:1075–1080.
4. Boyer, J.S. 1975. Differencial sensitivity of photosynthesis to low leaf water potentials in corn and soybean. Plant Physiol. 46:236–239.
5. Chapman, H.D., and P.F. Pratt. 1961. Methods of analysis for soils, plants, and waters. University of California.
6. Gale, J. 1975. Water balance and gas exchange of plants under saline conditions. p. 168–187. *In* A. Poljakoff-Mayber and J. Gale (ed.) Plants in saline environments. Springer-Verlag, New York.
7. Hawker, J.S., and R.R. Walker, 1978. Effect of sodium chloride on expansion rates and invertase activity of leaves. Aust. J. Plant Physiol. 5:73:80.
8. Huang, C.Y., J.S. Boyer, and L.N. Vanderhoff. 1975a. Acetylene reduction and metabolic activities of soybean having various leaf and nodule water potentials. Plant Physiol. 56:222–227.
9. ----, ----, and ----. 1975b. Limitation of acetylene reduction by photosynthesis in soybean having low water potentials. Plant Physiol. 56:228–232.
10. Jensen, C.R. 1975. Effects of salinity in the root medium: I: Yield, photosynthesis and water relationships at moderate evaporative demands and various light intensities. Acta Agric. Sc. 25:3–10.
11. Lauter, D.J., D.N. Munns, and K.L. Clarkin. 1981. Salt response of chickpea as influenced by N supply. Agron. J. 73:961–966.
12. Lewis, O.A., and J.S. Pate. 1973. The significance of transpirationally derived nitrogen in protein synthesis in fruiting plants of pea (*Pisum sativum* L.). J. Exp. Bot. 24:596–606.
13. Minchin, F.R., and J.S. Pate. 1974. Diurnal functioning of the legume root nodule. J. Exp. Bot. 25:295–308.
14. Pate, J.S. 1976. Physiology of the reaction of nodulated legumes to the environment. p. 335–360. *In* P.S. Nutman (ed.) Symbiotic nitrogen fixation in plants. Cambridge University Press, Cambridge.
15. Singleton, P.W. 1983. A split-root growth system for evaluating the effect of salinity on the components of the soybean *Rhizobium japonicum* symbiosis. Crop Sci. 23:259–262.
16. ----, S.A. El Swaify, and B.B. Bohlool. 1982. The effect of salinity on *Rhizobium* growth and survival. Appl. Environ. Microbiol. 44 (4): 884–890.
17. Sprent, J.I. 1972. The effects of water stress in nitrogen fixing root nodules. New Phytol. 71:451–460.
18. Sung, F.J., and D.R. Krieg. 1979. Relative sensitivity of photosynthetic assimilation and translocation of ^{14}Carbon to water stress. Plant Physiol. 64:852–856.
19. Turner, N.C., J.E. Begg, H.M. Rawson, S.D. English, and A.B. Hern. 1978. Agronomic and physiological responses of soybean and sorghum crops to water deficits. III. Components of leaf water potential, leaf conductance $^{14}CO_2$ photosynthesis, and adaptation to water deficits. Aust. J. Plant Physiol. 5:179–194.
20. Wilson, J.R. 1970. Response to salinity in Glycine:VI. Some effects of a range of short term salt stresses on the growth, nodulation and nitrogen fixation of *Glycine wightii*. Aust. J. Agric. Res. 21:571–582.

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