NADH oxidation, H^+ efflux, and nutrient ion uptake of soybean roots[1]

Jim Bidlack,[2] *Gumersindo Ramírez-Oliveras,*[3] *and Charles A. Stutte*[4]

ABSTRACT

Soybean [*Glycine max* (L.) Merr.] yields are influenced by the nutrient content of the soil which might, in turn, be reflected by the ability of roots to efflux hydrogen ions (H^+). Regulation of H^+ efflux by NADH oxidation was investigated to evaluate the physiological response of H^+ efflux and its relation to soybean yield among different cultivars.

Enzyme assays and pH measurements were used to monitor NADH oxidation and H^+ efflux by soybeans grown in a controlled-environmental chamber. Nutrient content, and yields of biomass and seed were measured with field-grown soybeans. Dry matter yields were determined for relative treatment comparisons in all experiments.

Dry weights of different soybean cultivars were negatively correlated to NADH oxidation and positively correlated to H^+ efflux. Comparison of 'Davis' and 'Forrest' soybeans indicated that dry weight, seed yield, and nutrient-ion contents were negatively correlated to NADH oxidation and positively correlated to H^+ efflux.

These fidings suggest that soybean nutrition and yield are closely interrelated with oxidation-reduction reactions in the plant's root tissues. Favorable growth and yield performance appears to require a supply of H^+ ions. The efflux of these ions, in turn, appears to favor nutrient uptake and hence growth and yield potential. The chemical supplier of H^+ ions remains obscure and NADH might or might not be a contributor.

RESUMEN

Oxidación de NADH y la absorción de nutrimentos en raíces de habichuela soya, *Glycine max* (L.) Merr.

Se investigó el efecto regulador de la oxidación de dicotinamida-adenina-dinucleótido reducido (NADH) sobre el flujo de iones de hidrógeno (H^+ para poder evaluar su relación con el rendimiento en diferentes cultivares de habichuela soya *Glycine max* (L.) Merr. El contenido enzimático y pH se correlacionaron con la oxidación de NADH y el flujo de H^+ en plantas de soya cultivadas en cámara de crecimiento con medioambiente regulado. El análisis de rendimiento de materia seca, grano, cantidad de nutrimento iónico y biomasa se determinó para todos los experimentos de campo.

La correlación entre peso seco y oxidación de NADH resultó negativo entre las cultivares de soya mientras que fue positivo para el flujo de H^+.

[1] Manuscript submitted to Editorial Board 18 November 1988.

[2] Ph.D. candidate at Iowa State University.

[3] Professor, Dept. Agron. and Soils.

[4] Distinguished Professor of Agronomy, University of Arkansas, Fayetteville.

Las cultivares Forrest y Davis arrojaron una correlación positiva para el flujo de H^+ en relación al rendimiento de materia seca, grano y contenido de nutrimento iónico pero negativa para los mismos parámetros con relación a la oxidación de NADH.

Los resultados indican una interrelación entre la oxidación de NADH, flujo de H^+, contenido de nutrimento iónico y rendimiento del grano en habichuela soya.

INTRODUCTION

Soybeans, which show increased yield and high nutrient absorption (6) are possibly responding through physiological adjustments that increase nutrient ion uptake. Studies with barley (*Hordeum vulgare* L.) revealed that increased ion uptake parallels an exchange of hydrogen ions from roots for cations in the surrounding solution (4). Soybean nutrient ion uptake might also parallel ion exchange, but it is not clear where the hydrogen ions originate.

Recent research has shown that NADH oxidation may be linked to ion exchange in corn (*Zea mays* L.) root protoplasts (7). The present research focuses on root NADH oxidation and hydrogen ion efflux in relation to nutrient ion uptake, dry matter yield, and seed yield in soybeans.

MATERIALS AND METHODS

Three analyses were performed: NADH oxidation, H^+ efflux, and nutrient ion uptake. Dry matter yield was also measured to provide relative comparisons of growth for selected cultivars.

NADH oxidation experiment

Seeds were obtained from C.E. Caviness at the Arkansas Agricultural Experiment Station at Fayetteville and germinated in vermiculite in a controlled environment chamber. Seedlings were transferred to a nutrient solution (3) at the VC to V1 stage (1) and grown at temperatures ranging from 22 to 27° C diurnally and from 19 to 23° C at night. A simulated 14-h day with constant radiant flux density at 20 to 25 W m^{-2}, and a relative humidity of 65 to 75%, was followed by a 10-h night at the same relative humidity.

At the V2 to V3 stage, 0.10 g of fresh root tissue was removed within a distance of 2.0 cm from root tips of each plant. The tissue was rinsed and ground with 10 ml of deionized water for 30 s in a Ten Broek[5] homogenizer at room temperature. The sample was then vortexed with 5.0 mg of polyvinylpolypyrrolidone for 10 to 20 s to remove phenolic substances. Centrifugation at room temperature for 10 min at 1200 g was

[5]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment of materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

the final step for obtaining a crude extract for the NADH oxidation assay.

Assays for each crude extract were performed within 3 to 5 h of the initial tissue sampling. A 2.0-ml aliquot of each sample extract was mixed with 0.10 mL of 1.0 m*M* NADH buffered at a pH of 7.0. Enzyme activity determined from two-minute, first order decreases in absorbency at 340 nm gave units of micromoles of NADH oxidized per milligram of fresh tissue per minute ($\mu o\lambda$ $\mu\gamma^{-1}$ min $^{-1}$).

Remaining tissue from each plant was used for dry matter determination. The data presented include NADH oxidation and dry matter (DM) yield from three replications of five selected cultivars.

Hydrogen ion efflux experiment

Seeds were obtained from the Arkansas Agricultural Experiment Station at Fayetteville and from commercial companies. Germination and growth in an environmental chamber was essentially the same as the NADH oxidation experiment except that the light provided was 40 W m^{-2}.

At the V3 to V4 stage, plants were transferred from nutrient solution to a solution of 0.5 m*M* $CaSo_4$ and 0.1 m*M* K_2SO_4. The drop in pH from 7.0 over a 24-h period was interpreted as a result of root H^+ efflux (2).

After pH changes were recorded, dry weight of plant tissue was obtained. Data presented include change in pH and dry matter yield from four replications of five soybean cultivars.

Nutrient ion uptake experiment

For this experiment, plants were propagated at the AES-UPR Isabela Substation in 3.6 – 4.5 m field plots. Leaf samples from the lower, middle, and upper leaf canopy were harvested during flowering (R_1) from two of six rows of experimental plots and analyzed for K, Ca, and Mg by flame photometry (10).

Whole plants remaining in the field were harvested for total biomass and yield determination. There were four replications of each treatment in a randomized complete block design.

RESULTS AND DISCUSSION

There was a negative relationship between NADH oxidation and dry weight for five selected soybean cultivars (table 1). This finding suggests that soybean roots may have a lower tendency to oxidize NADH when physiologically mobilized for high growth activity. It has been reported recently that a high-yielding soybean cultivar produced less root DM with lower root respiration than a low-yielding cultivar (5). Whether or not NADH oxidation, as a reflection of respiration, can alter dry matter accumulation remains questionable. Perhaps NADH oxidation influences soybean yield potential through regulated nutrient ion exchange.

TABLE 1.—*Dry matter yield and NADH oxidation of soybean cultivars at the V2-V3 stage compared to dry matter yield and H^+ efflux of soybean cultivars at the V3-V4 stage*

Experiment 1: NADH V2-V3 stage			Experiment 2: H^+ efflux V3-V4 stage		
Cultivar	NADH	Dry wt.	Cultivar	pH change	Dry wt.
	μmol/mg/min	g		(−)ΔpH	g
Pershing	11.0	0.38	TerraVig 708	0.70	0.78
Mack	9.9	0.46	Hood 75	0.80	0.78
Narow	9.3	0.48	Lee 74	1.71	1.26
Forrest	8.9	0.63	Forrest	1.44	1.26
Davis	7.8	0.70	Davis	1.98	1.48
r = −0.95			r = 0.98		

Parallel to the negative relationship between NADH oxidation and dry weight, a positive relationship between H^+ efflux and dry weight was obtained (table 1). Recent studies have shown that H^+ efflux measurement is a useful tool for screening barley cultivars for high yield (2). More research will be needed to determine the validity of H^+ efflux or NADH oxidation measurements as potential indicators of forthcoming yield in soybeans.

Two cultivars presented in table 1 can be found in both experiments. Consistency with overall results is shown in the relationship between dry weight, NADH oxidation, and H^+ efflux of these two cultivars. Yield and nutrient ion measurements show that biomass and seed yield are consistent with dry weight in previous experiments, and that nutrient ion content is higher in the higher-yielding cultivar Davis (table 2).

A scenario from table 2 can be developed to show high and low-yielding situations for different soybean cultivars. In the low yield situation, fewer hydrogen ions are being excreted, apparently resulting in poorer

TABLE 2.—*Relationship among NADH oxidation, H^+ efflux, nutrient ion content, biomass yield, and seed yield of soybean cultivars Forrest and Davis*

	Experiment -1-	Experiment -2-	Experiment -3-				
	Growth stage V2-V3	Growth stage V3-V4	Growth stage R1			Maturity	
Cultivar	NADH μmol/mg/min	pH change ΔpH	K	Ca	Mg	Biomass	Seed
			—meg/100 g DM—			—kg/ha—	
Forrest	8.9	1.4	21.0	106	58	4340	1674
Davis	7.8	2.0	21.5	116	63	6290	1956

FIG. 1—Metabolically-coupled NADH oxidation, and control of H^+ efflux and nutrient ion influx, in low- and high-yielding soybeans.

plant nutrition with lower biomass and seed yield attained by the plant. Alternatively, a high-yielding soybean would have a higher H^+ efflux, and more favorable nutrient uptake, hence better potential for biomass and seed production (fig. 1). What would cause these differences? One can theorize that the oxidation of NADH or another supplier of electrons *in vivo* can materially influence the H^+ efflux ability, and potentiate changes in the plant's metabolism.

The role of NADH in oxidative phosphorylation is a possible mechanism whereby respiratory differences among soybean cultivars can be explained. Previous investigators have demonstrated superior respiratory efficiency among genetic lines in winter wheat (*Triticum aestivum* L.) (9) and ryegrass (*Lolium perenne* L.) (11). In comparing soybean cultivars, "Forrest" and "Davis" (table 2), it could be predicted that Davis has the lower NADH oxidation potential and, consequently, greater potential to utilize its NADH resource in sustaining its growth and seed production activities.

Low yields in cultivars could be an expression of genotype-based, over consumption of H^+ by NADH during oxidative phosphorylation processes. An alternative mechanism to replenish the supply of H^+ in roots might be needed to regenerate ion exchange potential. The existence of an exogenous NADH on the outer surface of corn plasmalemma has already been theorized in the literature (7,8). This could facilitate increased H^+ efflux and ion influx, as depicted on the left side of figure 1. Perhaps this is a contributing mechanism by which respiratory soybeans can maintain a sufficient nutrient supply and balance for growth and development.

CONCLUSIONS

Soybean dry matter yield has been shown to be positively correlated to root H^+ efflux and nutrient ion concentration of leaves, and negatively

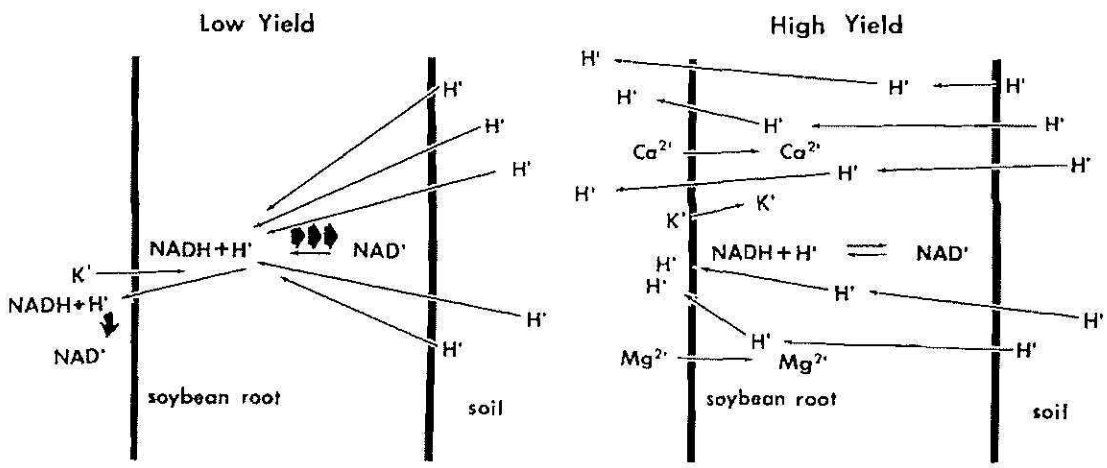

FIG. 1—Metabolically-coupled NADH oxidation, and control of H^+ efflux and nutrient ion influx, in low- and high-yielding soybeans.

correlated to NADH oxidation in roots. Results from NADH metabolism studies suggest that H^+ consumption during coupled NADH oxidation parallels a decrease in H^+ efflux and nutrient-ion exchange. More research will be needed to show how specific NADH oxidases affect nutrient ion exchange and yield in soybeans.

LITERATURE CITED

1. Fehr, W. R. and C. E. Caviness, 1977. Stages of soybean development. Spec. Rep. 80. Iowa State Univ., Ames, Iowa.
2. Glass, A. D. M., M. Y. Siddigi and K. I. Giles, 1981. Correlations between potassium uptake and hydrogen efflux in barley varieties. *Plant Physiol.* 68: 457-59.
3. Hoagland, D. R. and D. I. Arnon (Revised by D. I. Arnon), 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Berkley, CA. Circular 347 (revised).
4. ——— and T. C. Broyer, 1940. Hydrogen ion effect and the accumulation of salt by barley roots as influenced by metabolism. *Am. J. Bot.* 27: 173-85.
5. Kishitani, S. and R. Shibles, 1986. Respiration rates of soybean cultivars. *Crop Sci.* 26: 580-83.
6. Leggett, J. E. and M. H. Frere, 1971. Growth and nutrient uptake by soybean plants in nutrient solutions of graded concentrations. *Plant Physiol.* 48: 457-60.
7. Lin, W., 1984. Further characterization on the transport property of plasmalemma NADH oxidation system in isolated corn root protoplasts. *Plant Physiol.* 74: 219-22.
8. ———, 1982. Responses of corn root protoplasts to exogenous reduced nicotinamide adenine dinucleotide: oxygen consumption, ion uptake, and membrane potential. *Proc. Nat. Acad. Sci.* 79: 3773-776.
9. Pearlman, I., S. M. Thomas and G. N. Thorne, 1981. Dark respiration of several varieties of winter wheat given different amounts of nitrogen fertilizer. *Ann. Bot.* 48: 535-46.
10. Williams, C. H. and J. R. Twine, 1967. Determination of N, S, P, Na, Ca, and Mg in plant material by automatic analysis. Div. Plant Industry Tech. Paper 24, CSIRO Australia.
11. Wilson, D., 1982. Response to selection for dark respiration rate of mature leaves in *Lolium perenne* and its effect on growth of young plants and simulated swards. *Ann. Bot.* 49: 303-12.