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Glycine-Glomus-Rhizobium Symbiosis

V. EFFECTS OF MYCORRHIZA ON NODULE ACTIVITY AND TRANSPIRATION IN SOYBEANS UNDER DROUGHT STRESS

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GABOR J. BETHLENFALVAY*, MILFORD S. BROWN, KEIKO L. MIHARA, AND ALAN E. STAFFORD
United States Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Albany, California 94710

ABSTRACT

Soybean (*Glycine max* [L.] Merr.) plants were nodulated (*Bradyrhizobium japonicum*) and either inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe or left uncolonized. All plants were grown unstressed for 21 days initially. After this period, some VAM and non-VAM plants were exposed to four 8-day drought cycles while others were kept well watered. Drought cycles were terminated by rewatering when soil moisture potentials reached −1.2 megapascal. Nodule development and activity, transpiration, leaf conductance, leaf and root parameters including fresh and dry weight, and N and P nutrition of VAM plants and of non-VAM, P-fed plants grown under the same controlled conditions were compared. All parameters, except N content, were greater in VAM plants than in P-fed, non-VAM plants when under stress. The opposite was generally true in the unstressed comparisons. Transpiration and leaf conductance were significantly greater in stressed VAM than in non-VAM plants during the first half of the final stress cycle. Values for both VAM and non-VAM plants decreased linearly with time during the cycle and converged at a high level of stress (−1.2 megapascal). Effects of VAM fungi on the consequences of drought stress relative to P nutrition and leaf gas exchange are discussed in the light of these findings and those reported in the literature.

Many factors affect nodule formation, such as soil moisture, pH, and the availability or toxicity of minerals (16), but the availability of P, carbohydrate serving as a source of electrons for nitrogenase activity, and an adequate plant water status can be singled out as the most limiting to nodule function in legumes (21, 28). While drought has been shown to affect nodulation directly by desiccating sensitive nodule tissues (27), low soil water potential interacts with P availability (7) to produce indirect effects on nodule activity mediated by the host plant. These latter effects may also be adverse, and include a decrease in P supply from host to endophyte (15, 23). The condition is aggravated by the regulatory role played by P on photosynthesis (12, 26): P deficiency lowers the production and subsequent transport of reducing power to the nodule, whose activity is crucially dependent on high levels of ATP and high rates of electron flow (21). A photosynthesis-mediated effect of drought stress on N_2 fixation in field-grown soybeans has recently been suggested (10), probably as a result of impaired gas exchange due to stress- and P-related changes in leaf conductance (1).

The involvement of VAM[1] fungi with plant drought tolerance is generally considered to be related to P nutrition (18) and is therefore of particular interest to N_2 fixation in legumes (9) in view of the direct and indirect impact of P on nodule formation and function. Following our finding that a VAM fungus significantly enhanced the development of soybean plants in comparison to P-fed non-VAM plants of higher P content (5) under drought stress, we were interested in the effects of the fungal symbiont on nodule activity in a similar stress situation. The purpose of the experiment was to determine the capability of VAM fungi to modify the effects of drought or transient rates of nodule activity and leaf conductance in soybean plants in comparison with P-fed plants of comparable size.

MATERIALS AND METHODS

Experimental Design. The experiment was designed as a 2 × 2 factorial of four treatments, with six replications of each for a total of 24 units of single plants. The factors were two levels of drought stress, and two forms of P nutrition, either by P fertilization or mediation by a VAM fungus.

Biological Materials. Soybean (*Glycine max* [L.] Merr. cv Hobbit) seeds were surface sterilized, germinated, selected for uniformity, and inoculated with 10 ml of a suspension containing 10^9 cells/ml of *Bradyrhizobium japonicum* strain USDA 136. The VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe was used as fungal endophyte. It had been collected from a desert site and recultured under controlled conditions on *Sorghum bicolor* L. to serve as a source of inoculum. The inoculum used in this experiment consisted of 50 ml of soil containing approximately 500 sporocarps with 1 to 5 spores per sporocarp. It was mixed uniformly into the growth medium of VAM plants prior to planting. All plants received an inoculum wash, free of VAM-fungal propagules, to equalize the microbiota of the VAM and non-VAM treatments.

Growth Conditions. The plants were grown in a walk-in type growth chamber at day/night regimes of 16/8 h, 27/21°C and 40/60% RH, with photosynthetic photon flux density of 800 $\mu E/m^{-2} \cdot s^{-1}$. Only the center of the chamber platform was utilized, providing uniform light and temperature conditions. The soil (1.25 L/pot) was a Balcom series (Yolo County, California) heavy silt loam (Typic Xerorthent) of pH 7.7 (paste). NH_4HCO_3-extractable P of 3.3 μg (g soil)$^{-1}$, total N of 0.63% and a sand/silt/clay content of 20.5/55.6/23.9%. It was mixed with fine sand (2:1, v:v, soil:sand) to avoid cracking when dry.

Extra plants were checked for colonization by the endophytes at 14 and 21 d after planting. As there was very little nodulation and no fungal colonization at 14 d, 1 mM NH_4NO_3 was added to the basal nutrient solution (1.5 mM $CaCl_2$, 1.0 mM K_2SO_4, 0.25 mM $MgSO_4$, and micronutrients at one-quarter strength Hoagland solution) to encourage nodulation. At 21 d an average

[1] Abbreviations, VAM, vesicular-arbuscular mycorrhizal.

of 48 nodules per plant and numerous VAM infection units were observed. Drought cycling was initiated at this point. Stressed plants were taken through four cycles for a total of 32 to 36 d. The total growth period was 53 to 57 d with a harvest period of 4 d. Plants were harvested before rewatering.

Stressed plants were watered when soil moisture sensors (Soil-moisture Equipment Corp. model 5201), placed in all pots at two-thirds depth, showed soil water potentials in excess of 1.2 MPa. At the beginning of the first cycle, non-VAM plants received nutrient solution 0.3 mM in KH_2PO_4 and, at the start of the remaining three cycles, 0.6 mM in KH_2PO_4. Nonstressed plants were watered as needed with deionized water to keep them at a soil water potential above −0.05 MPa but received nutrient solution timed to coincide with the watering schedule of the stressed plants. Only non-VAM plants received P, and no N was applied except during the week preceding stress cycling. The relationships between sensor readings, soil water potentials, and soil water contents during the final stress cycle are shown in Figure 1 and were derived from pressure-plate apparatus calibration curves (data not shown).

Assays. Transpiration and leaf resistance of stressed VAM and non-VAM plants were measured daily on the center leaflet of the youngest fully expanded leaf during the final stress cycle. Readings were taken during the same time each day. The sampling chamber of the instrument (LI-COR model LI-1600 Steady-State Porometer) was placed on the same area (proximal, abaxial, including midrib) of the leaflet at each measurement. Nodule activity measured as C_2H_2 reduction (6), VAM colonization (4) and soil water parameters (5) were determined as described elsewhere. The data were evaluated by analysis of variance for each parameter, and by *t* test for VAM and non-VAM comparisons at each of the two stress levels.

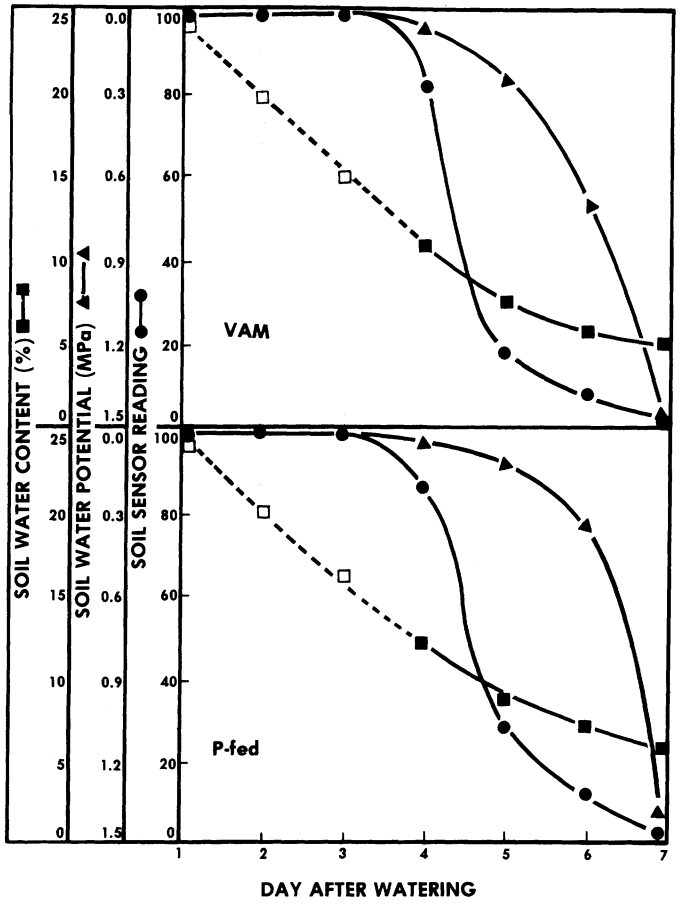

FIG. 1. Soil water content, water potential, and moisture sensor relationships during the final stress cycle of VAM and non-VAM, P-fed soybean plants grown in 1.5 L pots. Soil sensor data points reflect the average of six replications, and are related to soil water data by calibration in a pressure plate apparatus. The dotted portion of the soil water content curve indicates an approximate position of the data points for d 2 and 3 of the cycle. Soil water content values were not measured, but calculated from a calibration curve based on soil sensor readings and the saturation value on d 1 (24%, w:w). Since soil sensors were not sensitive to change during the first 3 d, the curve was interpolated, and the symbols placed at the appropriate position.

RESULTS

Colonization of host roots by VAM fungi was low (Table I) relative to levels achieved by this endophyte in other tests (8). This low colonization is ascribed to differences in the infectivity between different batches of the inoculum. Since host-plant growth closely reflects the extent of colonization in our *Glycine-Glomus*-Balcom soil system, the amounts of P applied to grow non-VAM comparison plants of similar size must be adjusted differently from experiment to experiment. This, in turn, influences comparative P concentrations in VAM and non-VAM tissues (8), and as a major limiting factor, affects the development of all aspects of host and endophyte (2). Both root (Table I) and leaf (Table II) P concentrations were at or below those regarded as critical in soybeans (11), while nodule P was in the range considered to be normal.

In stressed plants, all (nonderived) root (Table I) and host plant (Table II) parameters except N content were greater in VAM plants than in non-VAM plants, a situation which was generally reversed in the unstressed comparisons. The apparent relationship between significantly higher levels of P in stressed VAM-plant tissues and the stimulation of other parameters was particularly prevalent with nodulation and nodule activity (Table I) and leaf area (Table II). The reversal of this phenomenon in nonstressed plants was not as pronounced. Leaf dry matter as percent of fresh weight (Table II) was greater in VAM than in non-VAM plants regardless of stress treatment, indicating a greater degree of hydration in the latter.

Transpiration rates in nonstressed VAM plants tended to be higher than in non-VAM plants but were not significantly different at the 5% level, apparently due to a great deal or variability (results not shown). In stressed plants, transpiration and leaf conductance in VAM plants were significantly greater than in non-VAM P-fed plants during the first part of the stress cycle (Fig. 2). Transpiration rates and leaf conductance declined linearly with time for both VAM and P-fed treatments and converged towards the end of the stress cycle. This decline occurred even during the early part of the drought cycle where moisture sensors were not sensitive enough to detect a change in soil water potentials (Fig. 1). Apparently, the decline in these leaf parameters was in response to soil water depletion within the range between saturation and a soil water potential of −0.05 MPa. The relationship between transpiration and leaf conductance was essentially linear towards the end of the stress cycle (Fig. 3). However, during the first days of the cycle, while soil water potentials were still high (Fig. 1), large changes in leaf conductance were accompanied by relatively small changes in transpiration (Fig. 3).

The data were evaluated by analysis of variance. The main effects, those due to P nutrition or stress are annotated as factors A and B, respectively, in Tables I and II. The apparent lack of significance in the main effect due to P nutrition (factor A) of the analysis of variance (Tables I and II) for most parameters is a result of the stress-related reversal of the effect. This is indicated by the significant interactions, which show that stress affected host and endophyte parameters differently in VAM and non-VAM plants. The evaluation of P-nutrition effects by *t* tests

Table I. *Root Parameters of Stressed or Nonstressed VAM or Non-VAM, P-Fed Soybean Plants*

Stressed plants were exposed to four 8 d drought cycles (watered at −1.2 MPa); nonstressed plants were kept at field capacity, but received the same nutrient supplementation as stressed plants. The four-way comparisons of each parameter were evaluated by analysis of variance (ANOVA), with main effects due to P nutrition (A) or stress (B) and a nutrition × stress interaction (AB). Differences between VAM and P-fed plants were evaluated individually for the two stress conditions by *t* test (NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Parameter	P-Treatment	Condition		ANOVA		
		Stressed	Nonstressed	A	B	AB
VAM colonization (%)	VAM	39.7 NS	38.4			
Root dry weight (g)	VAM	1.57*	2.31 NS	NS	***	NS
	P-fed	1.37	2.38			
Root P content (mg/g)	VAM	1.1*	1.0**	NS	*	***
	P-fed	0.8	1.2			
Root N content (mg/g)	VAM	17.2***	16.6**	***	NS	NS
	P-fed	19.2	18.9			
Nodule dry weight (mg)	VAM	0.14*	0.23**	NS	***	***
	P-fed	0.05	0.32			
C_2H_2 reduction (μmol/h·plant)	VAM	9.4**	6.0*	NS	NS	**
	P-fed	1.7	9.6			
Nodule P content (mg/g)	VAM	2.9*	2.8 NS	NS	NS	**
	P-fed	2.0	3.0			

Table II. *Host Plant Parameters of Stressed or Nonstressed VAM or Non-VAM Soybean Plants*

Growth conditions and data evaluation are as in Table I.

Parameter	P-Treatment	Condition		ANOVA		
		Stressed	Nonstressed	A	B	AB
Plant dry wt (g)	VAM	4.07*	6.98 NS	NS	***	*
	P-fed	3.34	7.58			
Root/shoot ratio	VAM	0.64	0.47 NS	NS	***	*
	P-fed	0.71	0.46			
Leaf fresh wt (g)	VAM	4.30*	7.85*	NS	***	*
	P-fed	3.36	9.53			
Leaf dry wt (g)	VAM	1.17*	2.64*	NS	***	*
	P-fed	0.88	2.91			
Leaf area (cm^2)	VAM	191*	334**	NS	***	**
	P-fed	148	414			
Leaf dry matter (% of fresh wt)	VAM	27.2*	33.6*	***	***	NS
	P-fed	25.0	30.5			
Specific leaf area (cm^2/g dry wt)	VAM	163 NS	127*	***	***	NS
	P-fed	168	142			
Leaf P concentration (mg/g)	VAM	1.2**	0.9*	NS	NS	***
	P-fed	0.9	1.2			
Leaf N concentration (mg/g)	VAM	24.4 NS	23.8*	NS	NS	NS
	P-fed	25.2	24.9			

shows the statistical significance of these comparisons individually for each stress condition.

DISCUSSION

Two aspects of the involvement of VAM fungi in plant drought-stress ecophysiology are at the center of controversy. One concerns the relative importance of water uptake *versus* P nutrition mediated by the endophyte; the other concerns the impact of altered leaf gas-exchange characteristics on water use efficiency (3, 5, 18, 25). We feel that the data base is insufficient for the resolution of these two issues. Both issues also have direct relevance to N_2 fixation, because of the crucial impact of P, reduced C, and H_2O availability on this process (21, 27).

Several studies have shown (as summarized previously, 3, 5, 20) that VAM fungi confer a growth advantage on the host under drought stress, but not necessarily in the absence of stress (19), relative to P-supplemented comparison plants. In some experiments P levels are higher in the symbiotic plants (20, 25; Table II), in others P levels are higher in the nonsymbiotic comparison plants (3, 5, 8). We feel that this depends on the kind, level, and timing of P application, the extent of VAM-fungal colonization, and host-endophyte as well as host-endophyte-soil-climate compatibilities. When P is higher in the stressed VAM plant, we presume that P nutrition is responsible for the growth effect;

when P is lower in the VAM plant than in the P-fed comparison and yet the growth effect persists, we consider other reasons, such as drought alleviation by the fungus to be primary. Unfortunately, truly comparable VAM and non-VAM plants are very difficult to achieve experimentally and even more difficult to replicate from time to time. This may be due to such factors as differences in the infectivity of different batches of inoculum, soil characteristics, harvest timing, and climatic variability even under controlled conditions. However, accumulating evidence (3, 5, 13) suggests that, in addition to plant P levels, a factor influencing growth under stress may be the rate of gas exchange.

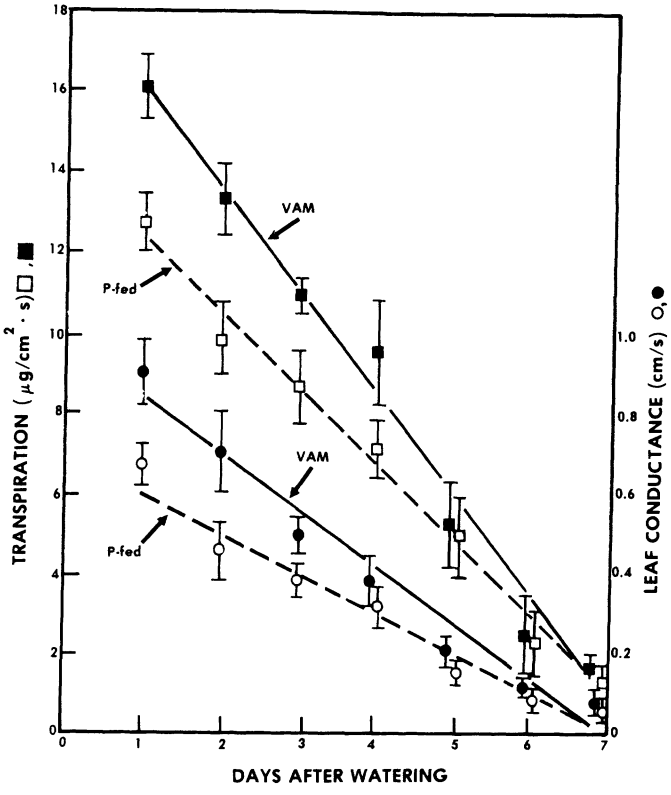

FIG. 2. Changes in transpiration rates and leaf conductance in VAM and non-VAM, P-fed plants during the final stress cycle. Correlation coefficients were $r = -0.99$ (transpiration) and $r = -0.98$ (leaf conductance) for both VAM and non-VAM plants.

Plants are known to have adequate photosynthetic capability not only to supply their own needs, but, in the case of legumes, to respond to the demands of their endophytes (14) as well as to some stresses (4) by increasing their rates of net CO_2 exchange. Thus, the reasonable suggestion was made (9) that the reduction in N_2 fixation usually observed in the field in conjunction with fruit set, and ascribed to competition for reduced C, may be due to drought stress-effected limitations on CO_2 exchange. If the VAM condition, as the present results (Fig. 2) as well as those of others (17) show, enhances transpiration and leaf conductance during an important portion of recurring drought cycles, then the associated process, CO_2 influx, must also necessarily be affected (8). The differences observed in the relationship between transpiration and leaf conductance in VAM and non-VAM plants during the nonstressed portion of a drought cycle (Fig. 3) indicate that the VAM condition may also have an effect on water-use efficiency (18).

The present data relate the highly significant enhancement of nodulation and nodule activity of stressed VAM plants *versus* non-VAM plants (Table I) to the coincidence of higher P levels and better leaf conductance in the former. The reversal of this phenomenon in nonstressed plants is apparently related to the better P nutrition of the non-VAM plants only, in the absence of conclusive conductance data. The effect of P deficiency on reduced leaf development (22) was evident in both stressed non-VAM plants and nonstressed VAM plants (Table II). Percent leaf dry matter (a measure of tissue hydration) was greater in VAM plants regardless of stress, indicating less water retention in VAM plant leaves. This may be related to the higher rates of transpiration observed in stressed VAM plants. However, lacking a simple, direct relationship between leaf water potential and leaf conductance in general (24), this point remains unresolved.

We conclude that the VAM condition in soybean stimulates nodule development and activity under drought stress, and that this effect may result not only from better P nutrition, but also

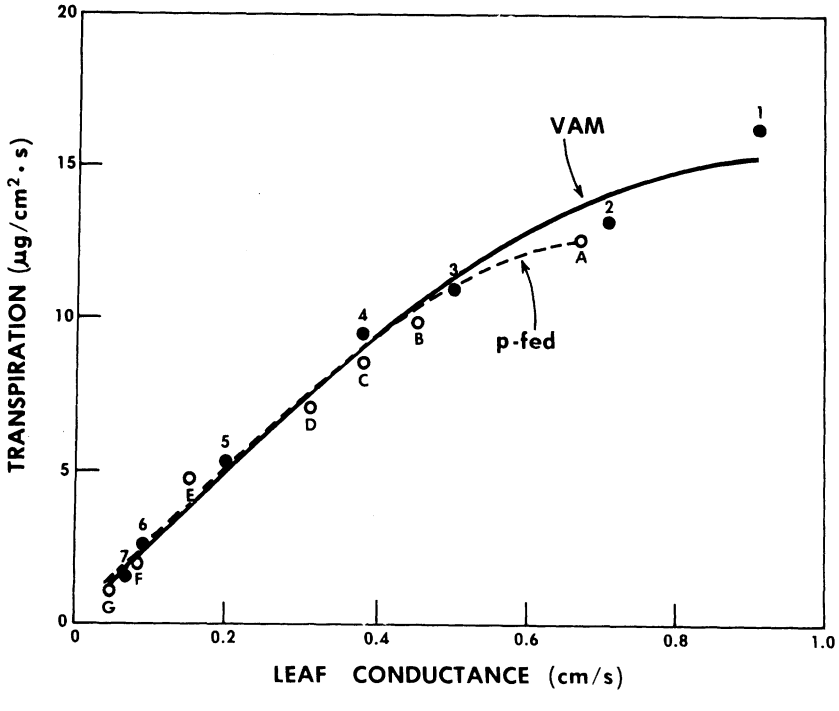

FIG. 3. Relationship between leaf conductance and transpiration for VAM and non-VAM, P-fed plants during the final stress cycle. Numbers (above full circles, VAM) and letters (below open circles, non-VAM) indicate the sequence of days after last watering.

from increased CO_2 uptake due to better leaf conductance. We join others (26) in the opinion that, in the effort to improve such processes of plant productivity as N_2 fixation or photosynthesis, manipulation of the mechanisms themselves at the molecular level must be complemented by an investigation and understanding of existing bioregulatory systems on the symbiotic, nutritional, and general ecophysiological levels.

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