Low leaf hydraulic conductance associated with drought tolerance in soybean

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Lack of water is the most serious environmental constraint on agricultural production. More efficient use of water resources is a key solution for increased plant productivity in water-deficit environments. We examined the hydraulic characteristics of a 'slow wilting' phenotype in soybean (*Glycine max* Merr.), PI 416937, which has been shown to have relatively constant transpiration rates above a threshold atmospheric vapor pressure deficit (VPD). The VPD response of PI 416937 was confirmed. Three experiments are reported to examine the hypothesis that the VPD response was a result of low hydraulic conductance in leaves as compared to two other soybean genotypes. Results are reported from experiments to measure transpiration response to VPD when xylem water potential was maintained at zero, leaf rehydration response and leaf carbon assimilation response to petiole cutting. Major interspecific differences in leaf hydraulic properties were observed. The observed low leaf hydraulic conductance in PI 416937 is consistent with an increased water use efficiency, and an increased water conservation by limiting transpiration rates under high evaporative conditions but allowing normal gas exchange rates under more moderate evaporative conditions.

Introduction

Water scarcity is a major constraint on increasing crop yields, particularly in many developing countries (Huang et al. 2002, Rosegrant and Cline 2003). Yield losses in soybean, which is the major source of vegetable protein and oil worldwide (Purcell and Specht 2004), can be especially large because its symbiotic N_2 fixation is sensitive to even modest soil drying (Sinclair and Serraj 1995, Serraj et al. 1999). Slow wilting in genotype PI 416937 was reported in 1990 as a genetic source for drought tolerance (Sloane et al. 1990), and its progeny lines often produce greater yields than commercial cultivars in the field under drought. However, because there is substantial environmental interaction on the yield response, use of this trait for commercial variety development has stalled pending the ability to phenotype breeding material so that the introduction of this complex trait into high-yielding backgrounds can be achieved (Miflin 2000).

Previous studies have shown that slow wilting is not associated with deeper soil water extraction (Hudak and Patterson 1995) or altered plant sensitivity to soil drying (Earl 2002). Plants of genotype PI 416937 were found, however, to have constant transpiration rate when atmospheric vapor pressure deficit (VPD) was greater than 2.0 kPa (Fletcher et al. 2007). This result contrasted with the commercial cultivar A5959, which exhibited a linear increase in transpiration rate with VPD > 2.0 kPa. Here, we explore the hypothesis that low hydraulic conductance in PI 416937 plants results in restricted

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Abbreviations – CER, carbon dioxide exchange rates; VPD, vapor pressure deficit.

transpiration rates under elevated evaporative conditions, allowing soil water conservation that can prolong crop growth during late-season drought, i.e. slow wilting. In the first experiment, the rhizosphere of container-grown plants was pressurized to overcome any hydraulic limitations in the soil or roots that might constrain transpiration rate at high VPD. In the second and third experiments, differences in leaf hydraulic conductance among genotypes were assayed by comparing rehydration rates of detached leaves and by changes in leaf gas exchange when petioles were detached under water.

Materials and methods

The three experiments to investigate the possibility of interspecific variation in leaf hydraulic conductance were each performed using PI 416937 and two conventional soybean genotypes; A5959, a recent commercial cultivar, and Biloxi, a vigorous-growing historical cultivar.

Transpiration vs VPD

Plants were grown in a glasshouse until they had developed five or six fully expanded leaves. Seeds were sown into 1.4-l tubes (7.8-cm diameter, 29 cm tall) in which the bottom end was covered with several layers of cheesecloth. The open bottom of the pots allowed excess water to drain freely drained from the soil, and importantly, allowed pressure treatments to be readily applied to the contents of the pots. The plants were well watered and grown under an extended photoperiod (16 h) to prevent flowering.

The transpiration rate response to VPD was measured in a laboratory by installing the plant shoot in a chamber for regulation of VPD and placing the pot in a chamber to allow pressurization of the rhizosphere. The shoot chamber was not sealed at the base of the plant stem so that air could be readily flowed through the chamber with little back pressure. A box fan was placed in the shoot chamber to rapidly stir the air and minimize boundary layer conductance and its impact on plant water loss rate. Absolute humidity in the shoot chamber was adjusted by changing the airflow rate (15–45 l min^{-1}) through the shoot chamber and the humidity of the air source. The plant shoot was exposed to approximately 1000 μmol m^{-2} s^{-1} photosynthetically active radiation and maintained at a temperature of 30 $\pm$ 1°C. Air temperature and leaf temperature were measured, and rarely differed by more than 0.3°C. Air VPD was calculated based on the measured air temperature and relative humidity of the chamber.

A balance was placed under the rhizosphere chamber to measure changes in plant weight. As the plant stem was not sealed into the shoot chamber, the stem could move freely in a slot of the chamber and not interfere with measurement of plant weight. Weight was recorded every 30 s. After a new humidity was established in the chamber, a new steady transpiration rate was usually reached in about 20 min. Transpiration rate was calculated by linear regression during the steady-state period from the decrease in pot weight over 5 min. Plant transpiration rate was first measured at six to eight VPD levels in the range of approximately 1–3 kPa without pressurizing the rhizosphere chamber. Data were collected from at least five plants for each genotype. Transpiration rate for individual plants and treatments were plotted against the VPD to which the plant shoots were exposed.

The critical comparison in resolving the hydraulic limitation was to measure transpiration rate over the range of VPD while maintaining leaf xylem water potential at zero, so that any hydraulic limitation on water transport between the soil and leaf was eliminated. Therefore, a positive pressure was applied in the rhizosphere chamber in which the plant pots were installed to maintain zero hydrostatic water potential in the leaf xylem (Passioura and Munns 1984, Sinclair et al. 2008). Xylem pressure in the plant was measured every 30 s by coupling the petiolule of the terminal leaflet of one of the uppermost expanded leaves to a pressure transducer using a thick-walled tube filled with water. Pressure deviation from zero at the petiolule was used to adjust by computer monitoring the pressure in the rhizosphere chamber. The pressure applied in the rhizosphere chamber was generally in the range of 0.2–0.4 MPa. Transpiration rate was again measured at 6–8 VPD levels over the 1–3 kPa range for each plant. Following the transpiration measurements, all leaves were removed from the plant and digitally scanned to determine total plant leaf area.

Leaf rehydration

Whole stems were detached from greenhouse-grown plants and allowed to dry briefly on a laboratory bench to a leaf water potential of −0.3 to −0.7 MPa. After brief drying, a stem segment associated with a single leaf was placed in a 'guillotine' apparatus to allow in a single step the severance of the petiole from the stem segment under water and a connection of the cut petiole to a water reservoir (Zwieniecki et al. 2007). The water reservoir was placed on a high-resolution balance ($\pm$10 μg) and the water taken up by the rehydrating leaf was recorded every 1 s. Rehydration was performed while exposing the leaf to approximately 800 μmol m^{-2} s^{-1} photosynthetically active radiation. Water uptake kinetics was analyzed assuming a two-compartment, exponential

model (Zwieniecki et al. 2007). Time constants for rehydration and hydraulic conductance were calculated for the fast-uptake compartment, which was assumed to be indicative of water flow in the epidermal compartment of the leaves.

Short-term gas exchange response to petiole cutting

Carbon dioxide exchange rates (CER) of fully expanded leaves were measured using a photosynthesis chamber (model 6400; Licor, Lincoln, NE) clamped on to attached leaves of soybean plants grown in the greenhouse. After placing the photosynthesis chamber onto a leaf segment and CER had reached a steady-state rate, the leaf petiole was severed underwater and maintained under water for at least 90 s. CER data were recorded at approximately

3-s intervals and normalized with respect to the CER at the time of cutting.

Results

Transpiration vs. VPD

In all three genotypes, transpiration rate increased linearly with VPD increase up to 1.8 kPa (Fig. 1) when the plant rhizosphere was not pressurized. At VPD greater than 1.9 kPa, however, the genotypes differed substantially. PI 416937 exhibited no further increase in transpiration rate above 1.9 kPa, while in Biloxi, transpiration rate continued to increase although the rate of increase was 40% of the rate at lower VPD. Cultivar A5959 was intermediate to the other two genotypes in that transpiration rate above VPD of 1.9 kPa increased
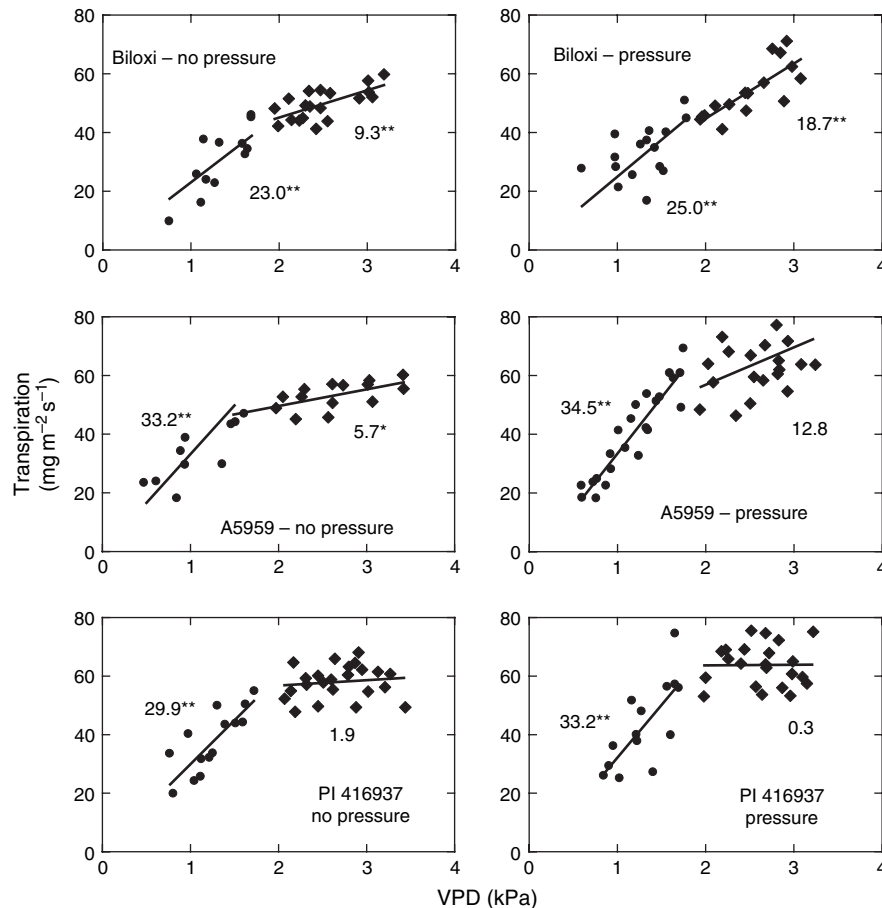

Fig. 1. Transpiration rate plotted against atmospheric VPD for each observation of each of the three genotypes when subjected to either ambient or increased rhizosphere pressure. The transpiration rates were calculated on a leaf area basis of each individual plant (n = 5). The numbers on the graph are the slopes (mg H_2O m^{-2} leaf area s^{-1} kPa^{-1}) obtained by linear regression for the segments where VPD was below (data shown as circles) and above 1.9 kPa (data shown as ♦) (* and **$P <$ 0.05 and <0.01, respectively).

at 19% of the transpiration rate increase rate at VPD < 1.8 kPa.

Differences among the genotypes were even more apparent as a result of the root pressurization treatment. In Biloxi, root pressurization resulted in transpiration rate increasing at a much higher rate than in unpressurized plants when VPD was >1.9 kPa (Fig. 1), indicating much of the hydraulic limitation in the plant was overcome by pressurization. However, transpiration rate of PI 416937 was virtually unchanged by pressurization because transpiration rate was again observed to be constant at VPD > 1.9 kPa. That is, full hydration of the leaf xylem of PI 416937 had no impact on the original hydraulic limitation, indicating that the hydraulic limitation was anatomically beyond the leaf xylem in the water flow pathway. The results with A5959 were intermediate between the two other genotypes in that the transpiration rate increase was 42% of the transpiration rate increase below 1.8 kPa. These results indicate that A5959 might have a hydraulic limitation both in root and shoot xylem as well as in the leaves.

Leaf rehydration

Having established that there was a difference in the hydraulic response among the three genotypes, we undertook experiments to examine specifically leaf hydraulic conductance limitations. In the first of these experiments, rehydration kinetics was studied for leaves collected from plants that were slightly dehydrated (Zwieniecki et al. 2007). The time constant for rapid-phase leaf rehydration of PI 416937 leaves was much slower ($P < 0.05$) than in the other two genotypes (Fig. 2). In fact, the time constant for rehydration of PI 416937 was more than twice as long as that of Biloxi. The longer time constant for PI 416937 indicates a hydraulic limitation in the replacement of water lost from leaves under elevated evaporative conditions, i.e. high VPD.
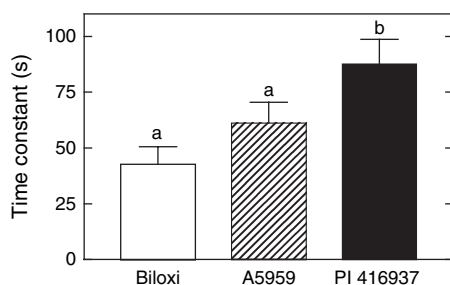
Mean leaf hydraulic conductance was calculated to be 2.4, 6.0 and 7.6 mmol H_2O MPa^{-1} m^{-2} for PI 416937, A5959 and Biloxi, respectively.

Short-term gas exchange response to petiole cutting

The second experiment to examine leaf hydraulic conductance directly was performed by measuring the short-term change in leaf gas exchange when the leaf petiole was severed under water. Absolute CER of the leaves was similar for all three genotypes with initial values of approximately 15 μmol m^{-2} s^{-1}. After cutting the petiole, there was an initial lag of no change in CER followed by a marked decrease (Fig. 3). The decrease in CER was attributed to a surge of water into the epidermis from the cut petiole causing an increase in hydraulic pressure in the epidermal cells. The increase in pressure in the epidermal cells would result in a mechanical advantage with respect to the guard cells causing a decrease in leaf gas exchange (Powles et al. 2006, Sharpe et al. 1987). Indeed, a rapid decrease in CER was observed in all three genotypes, but the response was much slower in PI 416937 than in the other two genotypes (Fig. 3). In PI 416937, initiation of CER decrease was delayed by nearly 10 s and the slope of the decrease was less than observed in the other two genotypes. In addition, minimum CER in PI 416937 was reached 15 s later and was greater than in the other two genotypes.

Discussion

The results from all three experiments indicated that PI 416937 has a low hydraulic conductance in its leaves

Fig. 2. Time constant for rehydration of the fast compartment of soybean leaves (n = 6 for each genotype). Standard error is indicated for each bar. Bars with different letters are significantly different ($P < 0.05$).
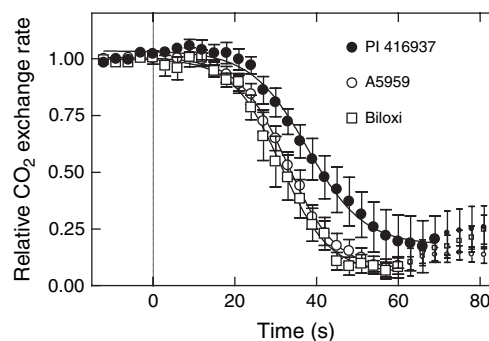
Fig. 3. Relative CO_2 exchange rate measured for leaves prior to and following cutting (time = 0) of the petiole under water (n = 9 for each genotype). The CER of each genotype was normalized using the rate at petiole cutting, which was 16.3, 14.6 and 14.6 μmol m^{-2} s^{-1} for A5959, Biloxi and PI 416937, respectively.

compared with Biloxi and A5959. It is hypothesized that a low hydraulic conductance limits the flow of water to the guard cells under high-evaporative conditions, i.e. high VPD, so that there is a loss of guard cell turgor and a decrease in stomatal conductance. The decrease in stomatal conductance would reestablish the equilibrium between water flow into and evaporation rate from guard cells under the higher VPD environment.

All three genotypes showed a hydraulic restriction on transpiration rate at VPD > 1.9 kPa, and the hydraulic restriction in PI 416937 was sufficiently large to result in a stable, constant transpiration rate at elevated VPD. Such interspecific differences in leaf hydraulic conductance associated with dramatically different responses to atmospheric VPD have not been previously reported.

The restricting hydraulic conductance in PI 416937 was shown to be localized in the leaves by measurement of transpiration rate when imposing a rhizosphere pressurization treatment. In both Biloxi and A5959, the relative transpiration rate at elevated VPD was increased as a result of pressurization indicating that a hydraulic conductance in the water pathway from the soil to the leaf xylem was being overcome. However, the transpiration rate response in PI 416937 was unchanged at high VPD in that transpiration rate was again stable and constant at VPD > 1.9 kPa. These results indicated that in PI 416937 the restricting hydraulic conductance was in the leaves and existed between the leaf xylem and the guard cells.

Two experiments were designed to observe directly possible differences among genotypes in leaf hydraulic conductance. The results of the leaf rehydration experiment showed that the conductance of water into slightly dehydrated leaves was substantially lower in PI 416937 than in the other two genotypes. The time constant for rapid-phase rehydration of PI 416937 leaves was about twice as long as that in Biloxi. The estimate of the hydraulic conductance for rehydration was also greatest in PI 416937.

The results from the measurements of CER following petiole severance further indicated a difference in hydraulic conductance among genotypes. Gas exchange of the leaves dropped substantially shortly after severing petioles as expected as a result of water movement into the leaf epidermis. However, again, a major difference among genotypes was observed. The decrease in CER was delayed in PI 416937 relative to the timing and rate of decrease observed in the other two genotypes. These results indicated a restricted hydraulic flow in the leaves of PI 416937 compared with the other genotypes.

The results from the three experiments all indicated that the water flow from the leaf xylem to the guard cells was obstructed by a lower hydraulic conductance in the leaves of PI 416937 compared with the other two genotypes.

Localizing the hydraulic limitation responsible for the water conservative behavior of PI 416937 is an essential first step in understanding and making use of this trait.

The practical significance of the low leaf hydraulic conductance in PI 416937 is that under VPD > 1.9 kPa, which is common at midday in most crop production areas, there is a limitation on transpiration rate resulting in conservation of soil water. Consequently, these results open a new approach for crop improvement in water-limited environments by allowing increased crop water use efficiency under high-VPD conditions. Conserved soil water will be available to the slow-wilting crop when drought develops later in the season allowing for sustained crop growth and grain formation (Sinclair et al. 2005). This behavior is consistent with the need to increase the efficiency in resource use, i.e. water, in the effort to increase crop productivity (Passioura 2002, Sinclair et al. 2004). The beauty of the complex trait examined here is that it specifically targets water conservation to those periods when the environmental conditions impose the least favorable gas exchange.

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