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Water-deficiency effects on single leaf gas exchange and on C_4 pathway enzymes of maize genotypes with differing abiotic stress tolerance

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Abstract

Responses to drought were studied using two maize inbred lines (B76 and B106) and a commercial maize hybrid (*Zea mays* L. cv. Silver Queen) with differing resistance to abiotic stress. Maize seedlings were grown in pots in controlled environment chambers for 17 days and watering was withheld from one half the plants for an additional 11 days. On the final treatment date, leaf water potentials did not differ among genotypes and were –0.84 and –1.49 MPa in the water sufficient and insufficient treatments, respectively. Greater rates of CO_2 assimilation were retained by the stress tolerant maize inbred line, B76, in comparison to the other two genotypes 11 days after watering was withheld. Rates of CO_2 assimilation for all three genotypes were unaffected by decreasing the measurement O_2 concentration from 21 to 2% (v/v). Activities of phospho*enol*pyruvate carboxylase (PEPC), NADP-malic enzyme (NADP-ME), and NADP malate dehydrogenase were inhibited from 25 to 49% by the water deficiency treatment. Genotypic differences also were detected for the activities of NADP-ME and for PEPC. Changes of transcript abundance for the three C_4 pathway enzymes also varied among watering treatments and genotypes. However, examples where transcripts decreased due to drought were associated with the two stress susceptible genotypes. The above results showed that enzymes in the C_4 photosynthetic pathway were less inhibited by drought in stress tolerant compared to stress susceptible maize genotypes.

Additional key words: C_4 photosynthesis; drought; enzyme activities; gene expression; phospho*enol*pyruvate carboxylase.

Introduction

Maize is the third most important grain crop behind wheat and rice and it is widely grown in many agricultural areas of the world (Turrent and Serratos 2004, Campos *et al.* 2004). Maize is used as a human staple, for animal feed, and for various industrial applications, including the production of biofuels (Sicher and Kim 2011). It is estimated that yields of the global maize crop are diminished about 4% annually due to the effects of excessive temperatures and drought (Lobell *et al.* 2011). There is growing evidence that future climates will be characterized by increased episodes of drought and by abnormally high temperatures (Hatfield *et al.* 2008). Therefore, genotypic improvements of the maize crop will be needed to insure high yields in future environments. Chen *et al.* (2012) examined the responses of several maize inbred lines to drought and demonstrated that relative water content, as well as vegetative and reproductive growth, was enhanced in drought tolerant compared to susceptible lines. Many of the drought tolerant inbred lines also demonstrated resistance to heat stress. It occurred to us that these maize lines could be useful tools for evaluating responses of maize to water stress.

Maize is a C_4 crop that possesses specialized anatomy and a CO_2 concentrating mechanism (Chollet and Ogren 1975). These two factors increase CO_2 concentrations in bundle sheath cells and this saturates rates of net photosynthesis at ambient CO_2 (Sage 2004). High rates of photosynthesis have been also observed in C_4 plants when stomatal conductance was reduced by moderate abiotic stress (Bunce 2004). Consequently, C_4 plants normally maintain greater water use efficiencies than

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Abbreviations: $g_{s(21)}$ – stomatal conductance at 21% O_2; $g_{s(2)}$ – stomatal conductance at 2% O_2; NADP-MDH – NADP-dependent malate dehydrogenase; NADP-ME – NADP-dependent malic enzyme; PEPC – phospho*enol*pyruvate carboxylase; $P_{N(21)}$ – net photosynthetic rates at 21% O_2; $P_{N(2)}$ – net photosynthetic rates at 2% O_2; ψ_w – leaf water potential.
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their C_3 counterparts and this should confer an adaptive advantage to C_4 plants grown in climates with diminished soil moisture. However, C_4 grasses are underrepresented in arid environments (Paruelo and Lauenroth 1996) and it has been suggested that the CO_2 concentrating mechanism is preferentially inhibited by moderate to severe drought (Ripley *et al.* 2007). Evidence for the inhibition of the C_4 photosynthetic pathway in response to water stress is primarily based on gas exchange and on chlorophyll *a* fluorescence analyses. Therefore, a specific enzyme step in the CO_2 concentrating pathway that is impaired by water stress has yet to be identified. An inhibition of the CO_2 concentrating mechanism by water stress would eliminate the apparent advantage of plants having the C_4 compared to C_3 photosynthetic pathway and this could adversely impact the success of maize and related C_4 crop species in future environments.

In the current study, we hypothesized that the inhibition of the CO_2 concentrating mechanism by water stress would be more evident in drought susceptible compared to drought resistant maize genotypes. Our approach was to compare changes of gas exchange and C_4 pathway enzyme activities in response to drought using maize genotypes differing in abiotic stress tolerance. Lastly, we monitored changes of transcript abundance of specific C_4 pathway enzymes in response to water stress.

Materials and methods

Plants: Maize (*Zea mays* L.) genotypes used in this study consisted of two inbred lines, B76 (PI 550483) and B106 (PI 59404), that were developed at Iowa State University to resist European corn borer (Russell and Hallauer 1974). Chen *et al.* (2007) reported that B76 was more heat tolerant than B106, and that B76 was also drought tolerant. Experiments here were also performed using a locally grown maize hybrid, cv. Silver Queen, which was not selected for altered stress tolerance. Seeds of B76 (stress resistant) and B106 (stress susceptible) were obtained from the U.S. Department of Agriculture's Germplasm Resources Information Network (http://www.ars-grin.gov/) and the Silver Queen variety was purchased locally.

Experiments were conducted in indoor controlled environment chambers (*model M-18, Environmental Growth Chambers Corp.,* Chagrin Falls, OH, USA) essentially as described previously (Sicher and Barnaby 2012, Qu *et al.* 2014). Plants were grown from seed in pots filled with vermiculite and were irrigated once daily with a complete mineral nutrient solution (Robinson *et al.* 1984). The growth chambers were programmed to provide a 14 h day/10 h night diurnal cycle, a constant air temperature of 27 ± 1°C, an irradiance of 900 ± 30 μmol(photon) m^{-2} s^{-1} and a chamber air CO_2 concentration of 380 ± 10 μmol mol^{-1}. Irradiance was provided by a mixture of high pressure sodium and metal halide lamps located above an acrylic plastic barrier and chamber air CO_2 concentrations were regulated with an infrared gas analyzer and set-point controller. Relative humidity was uncontrolled but was determined to be 60 ± 10% during the daytime. Three maize genotypes were grown simultaneously in each of two matching growth chambers under well watered conditions. Drought was imposed on all plants in one randomly chosen chamber after the seedlings were 17 d old. Water-stress treatments were obtained by completely withholding watering for 11 d. All measurements reported in this study were performed on the final treatment date.

Single leaf gas-exchange measurements: Steady-state rates of net photosynthesis (P_N) and stomatal conductance (g_s) of individual maize leaves were measured with a portable infrared gas analyzer (*Li-Cor model 6400, Li-Cor, Inc.,* Lincoln Nebraska) essentially as described previously (Sicher and Barnaby 2012, Qu *et al.* 2014). Measurements were performed between 3 and 6 h after the start of the photoperiod. Conditions within the cuvette were controlled by the system and leaf temperature, humidity, light, and CO_2 concentrations were set to match conditions used for plant growth. Gas-exchange measurements were performed on the most recently collared leaf and employed three to four plants in each treatment. Rates of P_N and g_s were determined at both 21% and 2% O_2. These were the $P_{N(21)}$, $P_{N(2)}$, $g_{s(21)}$, and $g_{s(2)}$ measurements, respectively. Gas-exchange values were computed by the instrument using formulas described by Farquhar and von Caemmerer (1982). Values of leaf water potential (ψ_w) were determined on the same leaves used for gas-exchange determinations. Measurements were performed on excised leaf discs (0.33 cm^2) using a model *HR-33T* dewpoint microvoltmeter *(Wescor,* Logan UT, USA) and after a 1-h equilibration period.

Enzyme assays: Single leaf discs from each plant (1.7 cm^2) were extracted with 0.7 cm^3 ice cold extraction buffer consisting of 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA, 1% (w/v) polyvinyl pyrollidone-40 (soluble PVP), 5 mM Na^+-pyruvate, and 10% glycerol. Immediately prior to extraction, the solution was supplemented with 1 mM leupeptin and 5 mM with dithiothreitol. Pyruvate was added to the extraction medium to stabilize enzyme activities. Leaf material was extracted at 0°C using a ground glass tissue homogenizer and homogenates were transferred to 1.5 ml plastic centrifuge tubes on ice. The samples were spun in a microcentrifuge (*Model 5415C, Brinkmann,* Westbury, NY, USA) for 180 s at 14,000 × *g* and 0.23 cm^3 of each supernatant was immediately transferred to a clean 0.5 cm^3 centrifuge tube

and kept on ice. Enzyme activities were determined spectrophotometrically (model *UV2101PC*, *Shimadzu Corp*., Columbia, MD, USA) at 25°C essentially as described by Maroco *et al.* (1999). Briefly, NADP-malate dehydrogenase (NADP-MDH, EC 1.1.1.83) was measured in 1 cm^3 solution containing 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 100 mM oxaloacetic acid (adjusted to pH 6.0), 10 mM NADPH, and 0.025 cm^3 of the leaf extract. PEPC (EC 4.1.1.31) was measured in 1 cm^3 solution containing 50 mM Tris-HCl (pH 8.0), 5 mM $NaHCO_3$, 5 mM $MgCl_2$, 0.2 mM NADH, 2.5 mM phospho*enol*pyruvate (tricyclohexamine salt), 16.7 nkat of MDH, and 0.025 cm^3 of the sample. NADP-ME (EC 1.1.1.40) was measured in 1 cm^3 solution containing 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 5 mM malic acid, 5 mM dithioerythritol, 0.5 mM $NADP^+$, and 0.025 cm^3 sample. Reactions were initiated by adding 22.5 mM $MgCl_2$. All measurements were performed using a spectrophotometer (*Model 2101, Shimadzu Scientific Instruments,* Columbia, MD, USA) operated in the kinetic mode. Enzyme activities were calculated on a leaf area basis from the rate of change in optical density at 340 nm.

Quantitative transcript measurements: Maize leaf sections [approximately 0.5 g of fresh mass (FM)] from either side of the midrib of the most recently expanded leaf were ground to a liquid N powder in a sterile mortar and pestle, and total RNA was extracted using *TRIzol® reagent* according to the manufacturer's instructions (*Invitrogen,* Carlsbad, CA, USA). The amount of total RNA in each sample was quantified with a *NanoDrop* spectrophotometer (*model 2000c, Thermo-Fisher Scientific Inc.,* Waltham, MA, USA). First strand cDNA was synthesized with 2 μg of total RNA (OD_{260}/OD_{280} > 1.95), oligo(dT)20 primers, and SuperScript III RNase H reverse transcriptase from *Invitrogen*. The resultant cDNA was diluted 10-fold and was used as a template for real-time quantitative polymerase chain reaction (QPCR). Amplifications were performed with a model *Mx3005P QPCR System* plus Brilliant SYBR® Green QPCR Master Mix *(Stratagene*, La Jolla, CA). Details of the QPCR procedures were described previously (Bae *et al.* 2009). Primer sequences for maize transcripts encoding PEPC, NADP-MDH, and NADP-ME are listed in Table 1. Assays were performed with four biological samples from each treatment, and measurements were replicated twice. The maize *actin1* gene was used as an expression control and real-time QPCR efficiencies were calculated according to Pfaffl (2001).

Table 1. Primer sequences for *Zea mays* transcripts. PCR efficiencies are shown in parentheses.

Name	Sequence	PCR efficiency [%]	Product length [bp]
NADPME-F	AGGCTCTCTTCAGCCATTCA	106.4 (109.4)	173
NADPME-R	TAGGCCTCTCGTTGAAGGAA		
NADPMDH-F	GGGAAGTCAGCATTGGCATAG	107.4 (88.6)	192
NADPMDH-R	CAACAACTAAGACTTTCGCGT		
PEPC-F	GAGATCCAAGCAGCCTTCAG	90.9 (96.6)	215
PEPC-R	CCACCCATCCAAGAAGAGAA		
Actin1-F	CTATGTTCCCTGGATTGCT	(87.4)	
Actin1-R	GGGCCCAAAGAATTAGAAGC		

Statistical comparisons: Results of two completely replicated experiments were combined and significant differences were determined using a two-way analysis of variance procedure (*ANOVA*, *StatView 5.0*, Mountain View, CA, USA). Leaf measurements were independent variables and genotypes and drought treatments were dependent variables. This study used three genotypes and two CO_2 treatments and had a 3 × 2 design. A *Fisher*'s Protected Least Significant Differences (PLSD) test was used to perform *post hoc ANOVA* comparisons.

Results

Leaf water potential (ψ_w) and single leaf gas exchange: Measurements of ψ_w of three maize genotypes differing in water-stress tolerance are shown in Fig. 1*A*. As shown in Table 2, the ψ_w measurements reported here differed among water stress treatments but were similar among the three genotypes. Mean ψ_w averaged over all three maize genotypes was –0.84 ± 0.01 MPa, when the plants were well watered, and was –1.49 ± 0.05, when plants were water-deficient for 11 d.

The inhibition of P_N by drought was 56, 67, and 95% for the B76, B106, and Silver Queen maize genotypes, respectively, when measurements of P_N were averaged over both O_2 concentrations (Fig. 1*B*,*C*). Measurements of P_N for all three genotypes did not differ among high and low O_2 concentrations, either when measured separately or when combined over the control and drought treatments. Measurements of P_N differed among genotypes and rates of $P_{N(21)}$ and $P_{N(2)}$ of inbred line B76 differed from those of cultivars B106 and Silver Queen. Conversely, measurements of P_N performed at 21% and

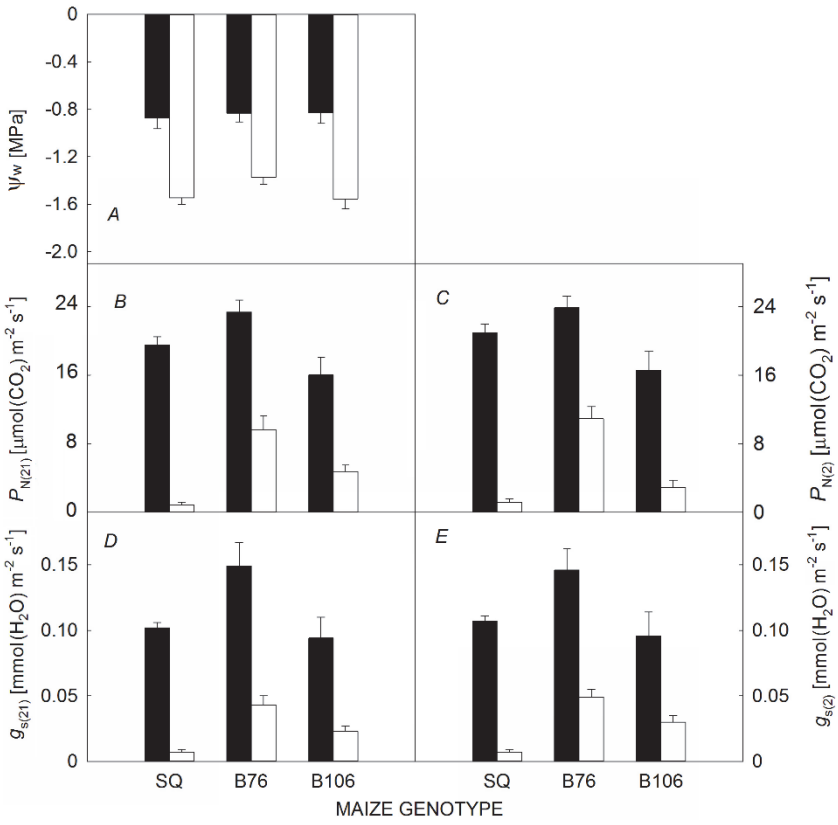

Fig. 1. Effects of water insufficiency on leaf water potential (ψ_w), net photosynthetic rate (P_N), and stomatal conductance (g_s) of three maize genotypes differing in abiotic stress tolerance. Measurements were performed on leaves of water-sufficient (*black columns*) or water-insufficient (*white columns*) maize, *i.e.*, the Silver Queen (SQ) hybrid, and two maize inbred lines, B76 and B106. $P_{N(2)}$ and $P_{N(21)} - P_N$ with 2 and 21% O_2, respectively; $g_{s(2)}$ and $g_{s(21)}$ g_s with 2 and 21% O_2, respectively. Values are means ± SE, $n = 8$.

Table 2. *ANOVA* table showing calculated probabilities (P) for the responses of three maize genotypes exposed to water-sufficient and water-insufficient treatments. P_N – net photosynthetic rate; g_s – stomatal conductance; ψ_w leaf water potential; PEPC – phospho*enol*pyruvate carboxylase; NADP-MDH – NADP-dependent malate dehydrogenase; NADP-ME – NADP-dependent malic enzyme; $P_{N(2)}$ and $P_{N(21)} - P_N$ with 2 and 21% O_2, respectively; $g_{s(2)}$ and $g_{s(21)}$ g_s with 2 and 21% O_2, respectively.

Parameter		Genotype P	Treatment	Interaction
Enzyme activity	PEPC	0.01	0.01	0.17
	NADP-ME	0.98	0.01	0.03
	NADP-MDH	0.01	0.01	0.37
Gene expression	PEPC	0.01	0.66	0.01
	NADP-ME	0.01	0.17	0.01
	NADP-MDH	0.01	0.01	0.47
ψ_w		0.35	0.01	0.46
P_N	$P_{N(21)}$	0.01	0.01	0.03
	$P_{N(2)}$	0.01	0.27	0.88
g_s	$g_{s(21)}$	0.01	0.01	0.27
	$g_{s(2)}$	0.01	0.01	0.24

2% O_2 were similar for the two drought susceptible cultivars (B106 and Silver Queen) based on a PLSD test. Because rates of P_N by inbred line B76 were less susceptible to drought than the other two genotypes, a significant genotype by treatment interaction was detected for these measurements.

Measured values of g_s were between 0.09 and 0.15 mmol m^{-2} for all three genotypes in the well watered treatment (Fig. 1*D*,*E*). Similar to findings for P_N discussed above, measurements of $g_{s(21)}$ and $g_{s(2)}$ differed among genotypes and watering treatments but no interactions were detected. Values of g_s were not altered when the O_2 concentration was lowered from 21 to 2% and g_s decreased by 69, 72, and 93% for the B76, B106, and Silver Queen genotypes, respectively, when averaged across O_2 concentrations. Again, the PLSD test indicated

that g_s of the B76 cultivar differed from that of B106 and Silver Queen, whereas g_s of the latter two genotypes were similar. Effects of water stress on g_s and on evapotranspiration rates were similar in this study (data not shown).

C_4 pathway enzymes: The activities of three C_4 pathway enzymes, NADP-MDH, NADP-ME, and PEPC, decreased in response to water stress (Fig. 2). Measured PEPC activity decreased 44, 46, and 50% in response to drought treatment in the B106, B76, and Silver Queen genotypes, respectively. Moreover, NADP-MDH activity decreased 44, 27, and 42%, and NADP-ME decreased 71, 38, and 38% in the same three genotypes in response to drought. The 71% decrease in NADP-ME activity was the largest change in enzyme activity measured in this study and it was similar to the 67% mean decrease of P_N determined for this genotype. A genotype by treatment interaction

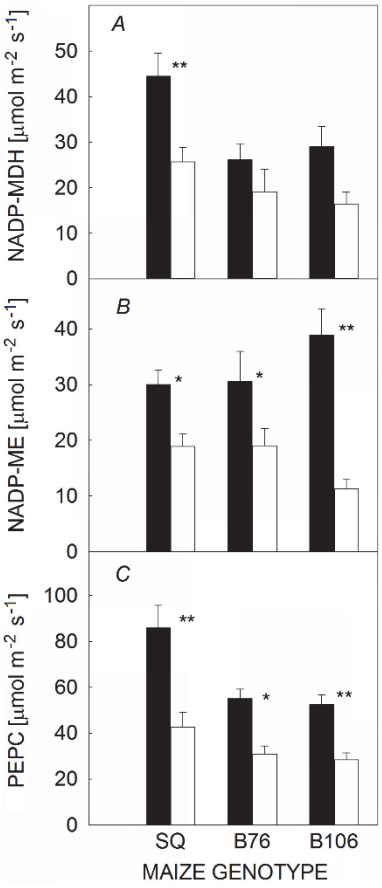

Fig. 2. Effects of water insufficiency on the activities of maize enzymes associated with the C_4 photosynthetic pathway. NADP-MDH – NADP-dependent malate dehydrogenase; NADP-ME – NADP-dependent malic enzyme; PEPC – phospho*enol*pyruvate carboxylase. Measurements were performed on leaves of water-sufficient (*black columns*) or water-insufficient (*white columns*) maize, *i.e.*, the Silver Queen (SQ) hybrid, and two maize inbred lines, B76 and B106. Columns marked with * or ** differ at $P \leq 0.05$ and $P \leq 0.01$, respectively.

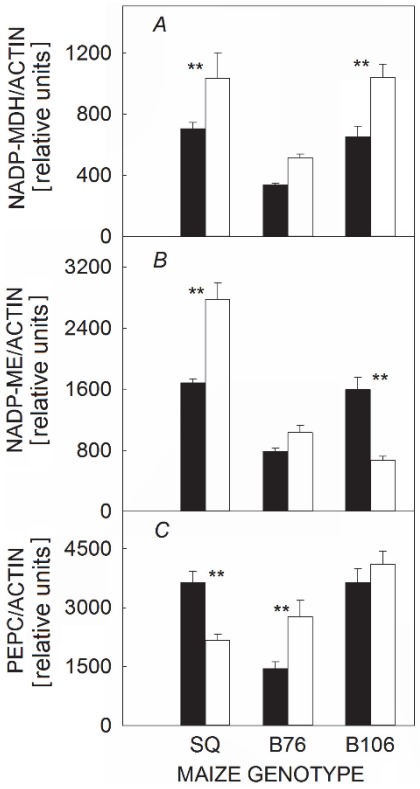

Fig. 3. Effects of water insufficiency on relative transcript abundance of maize enzymes associated with the C_4 photosynthetic pathway. Maize transcript abundance was determined 11 d after water was withheld using quantitative polymerase chain reaction (QPCR). The maize *actin 1* gene served as an expression control. Measurements were performed on leaves of water-sufficient (*black columns*) or water-insufficient (*white columns*) maize, *i.e.*, the Silver Queen (SQ) hybrid, and two maize inbred lines, B76 and B106. Columns marked with ** differ at $P \leq 0.01$.

was detected for the NADP-ME measurements. Unlike measurements of NADP-ME, activities of PEPC and NADP-MDH were greater in Silver Queen than in the other two genotypes. Therefore, genotypic differences were observed for the latter two enzymes in this study.

Maize transcripts encoding C_4 pathway enzymes: Changes of maize leaf transcripts in response to drought are shown in Fig. 3. Data are for the three C_4 pathway enzymes described above and expression ratios were compared with that of the maize *actin1* gene (Fig. 3). Unlike the responses of maize enzyme activity measurements to water stress, the gene expression results in this experiment varied among watering treatments and genotypes. The expression of NADP-MDH increased in response to water stress in both the Silver Queen hybrid and the B106 inbred line. However, the expression of NADP-MDH in the B76 inbred line was unaffected by water stress. The expression of NADP-ME and of PEPC

decreased in the B106 and Silver Queen genotypes, respectively, in the water insufficient compared to the water sufficient treatments. Conversely, the expression of NADP-ME and of PEPC was unchanged by water deficiency in the Silver Queen hybrid and maize inbred line B106, respectively. Therefore, a significant genotype by treatment interaction was detected for the expression of the latter two enzymes in maize leaves.

Discussion

Because water stress affects plant survival and decreases crop yields, the physiological and molecular responses of C_3 and C_4 plant species to drought have received considerable research attention (Lawlor and Cornic 2002, Flexas *et al.* 2004, Ghannoum 2009). One of the earliest responses of plants to drought is stomatal closure, which inhibits P_N by restricting the movement of CO_2 from the atmosphere into the leaf. The stomatal inhibition of P_N can normally be quickly and fully reversed by rehydrating the plant or by exposing leaves to very high CO_2 concentrations (Ludlow and Wilson 1971). Prolonged drought also induces a nonstomatal inhibition of P_N, which is only partially reversed when water stress is alleviated (Lawlor 2002). The nonstomatal inhibition of P_N is likely due to changes of metabolism, including decreased enzyme activities and impaired membrane integrity (Flexas *et al.* 2004). Drought-tolerant maize genotypes utilize adaptive advantages to minimize the nonstomatal inhibition of P_N and they have a greater capacity to recover from drought after rewatering when compared to stress susceptible genotypes (Hayano-Kanashiro *et al.* 2009).

In the current study, we examined drought responses of two maize inbred lines differing in water-stress tolerance with a commercial maize hybrid. After water was withheld for 11 d, rates of P_N were inhibited 56, 67, and 95% for the B76, B106, and Silver Queen genotypes, respectively, in comparison to the well watered, control plants. For these calculations, rates of P_N were averaged over the 21 and 2% O_2 concentrations that were used for gas-exchange measurements. Note that mean ψ_w of all three genotypes was about –1.5 MPa in the water stressed treatment and this did not differ among genotypes. Therefore, differences in rates of P_N among the three genotypes used here were not attributable to differences of ψ_w. Also, the finding that the inhibition of P_N by water stress was not alleviated when the O_2 concentration decreased from 20 to 2% was consistent with results of Lawlor and Fock (1978).

Stomatal conductance was clearly greater in B76 than in B106 or Silver Queen and this was true in both well watered and dry treatments. Conversely, g_s measurements for the two stress susceptible genotypes were similar in both the well watered and water-insufficient treatments. There was a close correlation between P_N and g_s in this study. Therefore, it is likely that stomatal closure and differences of g_s contributed to the variation in the inhibition of P_N among the three genotypes in this study. Note that the least effect of water stress on P_N and g_s was observed for the inbred line previously identified as heat- and drought-tolerant.

Prior investigators reported effects of water stress on various maize enzyme activities. Becker and Fock (1986) observed that Rubisco, PEPC, and NADP-ME decreased from 20 to 33% when the ψ_w was –1.17 MPa and results were expressed on a leaf area basis. Foyer *et al.* (1998) reported that PEPC activity per chlorophyll unit increased slightly in maize leaves when the ψ_w was –1.2 MPa. However, the latter findings may have been affected by drought dependent changes of leaf chlorophyll concentrations. Saccardy *et al.* (1996) observed that PEPC and NADP-ME activity on a leaf area basis were unchanged when the relative water content of the leaf was 50% and Du *et al.* (1996), working with sugarcane, reported that Rubisco, PEPC, NADP-ME, and phosphopyruvate dikinase activities decreased 50 to 89% on a leaf area basis when ψ_w was –1.61 MPa. Results of the current study were in broad agreement with Becker and Fock (1986) and with Du *et al.* (1996) and showed that maize leaf NADP-MDH, NADP-ME, and PEPC activities decreased 38, 49, and 25%, respectively, on a leaf area basis when averaged across genotypes. Taken together with prior findings discussed above, the activities of C_4 pathway enzymes decreased in response to moderate or severe water stress and PEPC was generally less affected by drought than the other enzymes in the cycle.

This study also identified genotypic differences in maize leaf enzyme activities. In both the water sufficient and water insufficient treatments, the Silver Queen hybrid possessed greater activities of PEPC and NADP-MDH than the two inbred lines. In contrast, no genotypic differences were observed for NADP-ME activity in maize leaves. Although the overall *ANOVA* test result was significant, we could not find differences due to water stress of NADP-MDH activity in leaves of the B76 and B106 genotypes when using a PLSD test. Therefore, the effects of drought on enzyme activity measurements varied among specific enzymes and among genotypes.

Unlike maize enzyme activities, seven of nine transcript measurements in this study were unchanged or increased in response to drought treatment. This was clear evidence that the mechanisms controlling gene expression were functioning at the stage of drought used in this study. This also suggested that in seven of nine instances the decreased enzyme activities reported above were not due to insufficient transcript levels and were likely due to changes at the protein level. The two transcripts that decreased in response to water stress were associated

with the two drought susceptible genotypes, *i.e.*, Silver Queen and B106. In our earlier paper (Sicher and Barnaby 2012), we reported that three maize genes, IVR2, HSP82, and Dhn2, showed increased expression during early drought but not during the later stages of drought treatment. The observation that the same gene was increased by drought in one maize genotype and decreased in another is novel and merits further research. It also would be valuable to repeat these experiments and measure changes of gene expression as a function of time of drought treatment.

The three enzymes measured in this study were all partially inhibited by water insufficiency. However, the percent inhibition of P_N and g_s due to drought generally exceeded the inhibition of enzyme activity. The possible exception to this conclusion was that the decreases of NADP-ME activity and of P_N in genotype B106 were similar. Although changes of C_4 pathway enzyme activities in response to drought were capable of contributing to the observed inhibition of P_N, there was insufficient evidence to suggest that the three enzymes measured here were impacted enough by water stress to shut down the entire C_4 pathway. Taken together, the above evidence suggested that the C_4 photosynthetic pathway was more affected by drought in stress susceptible than stress tolerant maize genotypes.

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