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Correlation between the Maintenance of Photosynthesis and *in Situ* Protoplast Volume at Low Water Potentials in Droughted Wheat[1]

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ABSTRACT

Studies were undertaken to examine the relationship between water deficit effects on photosynthesis and the extent of protoplast volume reduction which occurs in leaves at low water potential (Ψ_w). This relationship was monitored in two cultivars ('Condor' and 'Capelle Desprez') of cultivated wheat (*Triticum aestivum*) that differed in sensitivity to drought, and in a wild relative of cultivated wheat (*Triticum kotschyi*) that has been previously found to be 'drought resistant.' When subjected to periods of water stress, Condor and *T. kotschyi* plants underwent osmotic adjustment; Capelle plants did not. Photosynthetic capacity was maintained to different extents in the three genotypes as leaf Ψ_w declined during stress; Capelle plants were most severely affected. Calculations of internal leaf [CO_2] and stomatal conductance from gas exchange measurements indicated that differences in photosynthetic inhibition at low Ψ_w among the genotypes were primarily due to nonstomatal effects. The extent of protoplast volume reduction that occurred in leaves at low Ψ_w was also found to be different in the three genotypes; maintenance of protoplast volume and photosynthetic capacity in stressed plants of the genotypes appeared to be correlated. When the extent of water stress-induced inhibition of photosynthesis was plotted as a function of declining protoplast volume, this relationship appeared identical for the three genotypes. It was concluded that there is a correlative association between protoplast volume and photosynthetic capacity in leaves of wheat plants subjected to periods of water stress.

Previous investigations focusing on the physiological basis of inhibited capability of chloroplasts to photosynthesize in water-stressed leaves have led to the hypothesis that perturbations in metabolism may be intrinsically linked to the extent of organelle and/or protoplast volume reduction occurring at low Ψ_w[2] (3, 7, 20, 21). Possibly, then, the presence of low cell Ψ_w in the leaves of droughted crop plants may not necessarily be injurious. This research has also led to the speculation that one possible mechanism by which the leaf mesophyll cell can acclimate to an imposed drought (*i.e.* in terms of maintaining relatively greater photosynthesis) is by reducing the extent of protoplast volume reduction which occurs at low leaf Ψ_w.

The line of research which supports these assertions is as follows. Reduction of Ψ_w in isolated chloroplasts only inhibits photosynthesis when a nonpenetrating solute is used to lower the Ψ_s of the external medium (2, 11). Lowering external Ψ_s from isotonicity down to −2 MPa by addition of ethylene glycol does not inhibit photosynthesis of isolated chloroplasts. Presumably, then, the volume reduction that occurs when stromal Ψ_w equilibrates with external Ψ_s by dehydration is the biophysical change which inhibits photosynthesis. This differential effect of nonpenetrating (sorbitol) and penetrating solutes on photosynthesis has also been demonstrated with intact cells (leaf tissue incubated in solutions of high osmotic strength; 9). It should be noted that more recent studies have indicated that isolated chloroplasts exposed to hypertonic Ψ_s may become transiently permeable to sorbitol (19), although not all studies support this contention (21). Studies examining the response of photosynthesis to water stress *in situ* have also illuminated the correlative relationship between volume change and water deficit inhibition of photosynthesis. Photosynthesis *in vitro* of chloroplasts isolated from water-stressed plants was not as inhibited in high osmotic strength media as when chloroplasts were isolated from well-watered plants (1). This acclimation response was also demonstrated in intact cells incubated in high osmotic strength media (3). The acclimation of the chloroplast to low Ψ_s *in vitro* was correlated with the maintenance of both stromal volume and photosynthesis of intact leaves in subsequent studies (21). Other studies have also shown that chloroplast acclimation to low Ψ_w can beneficially impact photosynthesis of water-stressed leaves (13).

Kaiser (10) has used a technique that involves the vacuum infiltration of solutions containing ^{14}C-sorbitol and ^3H_2O to measure the relative protoplast volume of leaf tissue equilibrated in media of varying osmotic strength. In this study, it was demonstrated that the differences between photosynthesis at low Ψ_w *in vitro* of leaf tissue prepared from different species exactly correlated with the extent of protoplast volume reduction occurring in the different species. Using this same dual label infiltration technique, Sen Gupta and Berkowitz (20) indicated that differing ability of wheat cultivars to demonstrate leaf osmotic adjustment was found to result in differential extent of protoplast volume maintenance when tissue

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[2] Abbreviations: Ψ_w, water potential; $[CO_2]_i$, internal leaf CO_2 concentration; Ψ_s, osmotic potential; RWC, relative water content; g_c, stomatal conductance.

was incubated in solutions of high osmotic strength *in vitro*. This differential response of protoplast volume *in vitro* correlated well with differences in photosynthesis at low leaf Ψ_w as measured both *in vitro* and *in situ*. In this study, conventional methods used to generate pressure/volume water isotherms confirmed that differences between the cultivars in osmotic adjustment capability resulted in the contrasting abilities to maintain protoplast volume at low Ψ_w.

Although the above-mentioned studies offer clear evidence that there is a correlation between protoplast and/or chloroplast volume change at low Ψ_w, and the extent of (nonstomatal) water deficit inhibition of photosynthesis, conclusions that can be drawn from this literature are limited. In most of the studies where protoplast volume changes were monitored at varying leaf Ψ_w, leaves were subjected to low Ψ_w *in vitro*, by incubation of leaf tissue in media of varying osmotic strength. The goal of the work described in this report was to extend this line of research, by examining the relationship between the *in situ* protoplast volume, and photosynthesis in water stressed leaves.

This research objective was addressed by adapting the dual label infiltration technique developed by Kaiser (10) so that *in situ* protoplast volume could be estimated. Studies were undertaken by adjusting the infiltration medium osmotic strength such that the Ψ_s matched the declining Ψ_w in leaves of droughted plants. This technique was used to characterize the relationship *in situ* between declining photosynthesis and protoplast volume in leaves of plants subjected to water deficits.

This relationship was studied in wheat genotypes that vary in their response to water stress. The agronomic cultivars 'Condor' and 'Capelle Desprez' of cultivated wheat (*Triticum aestivum*) have been previously characterized as capable and incapable, respectively, of undergoing osmotic adjustment in response to plant water deficits (15). The tetraploid *Triticum* species *T. kotschyi* is a wild relative of cultivated wheat that grows in the Negev desert of Israel. Previous reports indicate that *T. kotschyi* is a 'drought resistant' relative of cultivated wheat, in that photosynthesis is not as inhibited as that of cultivated wheat at low leaf Ψ_w (14). Subsequent work has shown that the basis for the relatively higher leaf photosynthetic rates in *T. kotschyi* at low Ψ_w was less inhibition of chloroplast metabolism under stress (8). However, the specific mechanisms mediating the resistance of chloroplast metabolism to water stress in *T. kotschyi* were not identified. *Triticum kotschyi*, along with the cultivated wheat cultivars 'Condor' and 'Capelle Desprez' was used in the experiments reported here so that the effects of plant water deficits on photosynthesis and protoplast volume could be studied in wheat genotypes that vary in sensitivity to drought.

MATERIALS AND METHODS

Plant Material

Seeds (three per pot) of *Triticum aestivum* L. cvs 'Condor' or 'Capelle Desprez,' or of *Triticum kotschyi* (Boiss) Bowden were planted in pots containing approximately 2000 cm^3 potting mix (1:1 (v/v) peat:vermiculite). Pots were placed in a growth chamber which was maintained at 21°C and 50% RH with an 11 h light (250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) period, and irrigated to runoff twice per week with water, and once per week with commercial (Peter's 'Geranium Special') fertilizer. Seedlings were thinned to one per pot after 2 weeks. Plants were used after 6 weeks of growth. Only fully expanded (*i.e.* auricle exposed on a culm), nonsenescing leaves were used for experiments. Plants were subjected to periods of water stress by withholding irrigation from pots.

Water Relations

Leaf Ψ_w, Ψ_s, and RWC were all measured on the same leaves; three leaves were used as replicates for each of the measurements. Leaf Ψ_w was measured using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA). During the Ψ_w measurement, the pressure chamber walls were lined with wet paper, and the leaf blade was enclosed in plastic wrap to avoid tissue desiccation during the measurement period (typically 30–60 s). Control experiments indicated that the RWC of the wheat leaves was not affected by prior insertion in the pressure chamber. RWC was ascertained by measuring the averaged fresh, rehydrated (overnight at 4°C on distilled water), and dry (80°C for 2 d) weights of 10 leaf sections (1 cm length/section) cut from a leaf. Three discs were also cut from the same leaf, sealed in plastic wrap, immediately frozen in liquid N_2, and stored at −20°C. Leaf Ψ_s was ascertained by measuring the Ψ_w of each of the defrosted discs using a Wescor (Logan, UT) HR33T microvoltmeter (operating in the hygrometric mode) and C-52 leaf chambers. Osmotic adjustment capability of the wheat varieties was evaluated by measuring the leaf Ψ_s at 100% RWC (*i.e.* at full turgor) of plants exposed to drought episodes. Leaves were removed from plants, recut twice under distilled water, covered loosely with plastic wrap, and left to rehydrate overnight at 4°C. Discs (three per leaf) were then cut from each leaf, and used for Ψ_s analysis.

Gas Exchange Analysis of Photosynthesis

An ADC (P. K. Morgan Inst., Andover, MA) infrared gas analysis system was used to measure concomitant H_2O evolution and CO_2 uptake in leaves of wheat plants. Measurements were made under 1700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR provided by a sodium vapor lamp. Air temperature in the leaf cuvette was maintained between 20 and 24°C by immersion of the aluminum heat exchanger of the leaf chamber in a water bath. Calculations of the energy balance of the leaves illuminated in the leaf chamber indicated that leaf temperature never rose above 25°C during any of the photosynthesis assays. Air at 344 to 348 $\mu L/L$ [CO_2] was provided to the leaf from compressed air tanks. Net photosynthesis, transpiration, stomatal conductance, and internal leaf [CO_2] were calculated using equations developed by von Caemmerer and Farquhar (25) as described previously (17). Any current gas exchange studies which purport to evaluate the relative contributions of stomatal and nonstomatal effects of water stress on the inhibition of photosynthesis *in situ* using the analysis presented by Farquhar and Sharkey (6) should acknowledge the important study recently published by Sharkey and Seemann (23). They indicated that under water stress leaves of *Phaseolus vulgaris*

plants demonstrated 'patchy' or heterogenous stomatal closure. This phenomenon would lead to incorrect assumptions regarding the relationship between photosynthetic capacity and internal leaf [CO_2] in water-stressed leaves. However, in a direct examination of this phenomenon using different methodology, Bunce (5) concluded that internal [CO_2] calculations from gas exchange measurements of water stressed leaves are fairly accurate. Also, preliminary evidence from this laboratory (S Mane, D Gunasekera, G Berkowitz, unpublished data) suggests that patchy stomatal closure may not occur in response to water stress in all plant species, and that leaves of water stressed wheat plants do not show patterns of heterogenous stomatal closure in assays similar to those of Sharkey and Seemann (23). Therefore, we accept the analysis of Farquhar and Sharkey (6) as valid for the wheat plants used in our experiments, and present $[CO_2]_i$ calculations in support of contentions regarding nonstomatally mediated effects of water stress on photosynthesis. However, this presentation should be interpreted with caution, and limited by the current controversy.

In Situ Protoplast Volume

The *in situ* protoplast volume in leaves of plants exposed to water deficits was estimated using dual label infiltration methodology as described previously (20) with modifications. The key technical aspect of this report is the way in which the protoplast volume measurement assay was used in our experiments. Previously, in this laboratory (3, 12, 20) and others (10), this technique was used to ascertain changes in the relative protoplast volume which was maintained in leaf tissue as it was instantaneously brought to a range of water potentials *in vitro* by equilibration with solutions of varying osmotic strength. In the present study, leaf tissue was equilibrated with solutions which were made up such that the solution Ψ_s matched the ambient leaf Ψ_w which was measured on a given day in plants exposed to *in situ* drought episodes. The infiltration solution Ψ_s was altered by varying the sorbitol concentration. On a given day, the protoplast volume of tissue prepared in this manner was compared to tissue isolated from the same leaves, but prepared in solutions which lacked the (unlabeled) sorbitol (*i.e.* these solutions were essentially at 0 MPa). The protoplast volume measured at 0 MPa on a given day represented the maximum protoplast volume for the leaf material. A comparison of the volume measured at ambient leaf Ψ_w and at 0 MPa yielded the percentage reduction in protoplast volume which occurred at that leaf Ψ_w.

Three leaves were used for each of the protoplast volume measurements. A total of six discs (two from each of three leaves) were placed in 16 × 100 mm tubes which contained 800 μL infiltration solution. Three tubes were used as replicates for each measurement. In each tube, the infiltration solution contained 25 mM Hepes (pH 7.6), 2.5 μL [^{3}H]H_2O, and varying sorbitol (*i.e.* such that the infiltration solution Ψ_s matched the leaf Ψ_w as measured within 1 h prior to the infiltration experiment). After addition of leaf discs, tubes were placed under a vacuum twice for 15 s (or until vigorous bubbling), covered, and left at room temperature for 30 min. At 30 min, 200 μL of additional infiltration solution was added to the tubes. This aliquot of infiltration solution was at the same Ψ_s, contained the same concentration of ^3H_2O (*i.e.* 0.625 μCi), but also contained 0.5 μCi [^{14}C]sorbitol. The tubes were vortexed, sealed, and left for an additional 30 min at room temperature. Discs were then removed from tubes with the labeled infiltration solution, rinsed twice in 2 mL of unlabeled infiltration solution that was made up at the same Ψ_s, and then frozen by addition of liquid N_2. After evaporation of the N_2, the discs were transferred to 2 mL microfuge tubes, and 1.5 mL of 96% ethanol which contained 10 N formic acid was added to the discs. The microfuge tubes were left on a shaker for 2 d. One mL of the ethanol was then sampled for label using a dual label DPM program on a Beckman 3801 (Beckman Instr., Somerset, NJ) liquid scintillation counter. The label which leached out of the discs was compared with the label in the infiltration solution for each measurement. For each day that leaves were sampled for ambient protoplast volume, a second set of discs were cut from the same leaves for measurement of maximum protoplast volume. In this case, identical procedures were followed except that the infiltration solution contained no unlabeled sorbitol.

This protoplast volume measurement technique was adapted from previous methods used in this laboratory (20) so that maximum protoplast volume could be measured in tissue prepared from well-watered and water-stressed plants using only infiltration solutions made up at 0 MPa. In previous studies (3), it has been noted that incubation of leaf tissue prepared from stressed plants in infiltration solutions made up at 0 MPa Ψ_s did not yield maximum protoplast volume measurements. The maximum volume occurred at lower infiltration solution Ψ_s. It was concluded in these studies that incubation of leaf tissue in strongly hypotonic solutions caused the cell membranes to become transiently permeable to sorbitol. This phenomenon resulted in an incorrectly high apoplast (*i.e.* [^{14}C]sorbitol) volume measurement, thus decreasing the calculated protoplast volume. Control experiments (data not shown) indicated that the modifications in the technique as described here allowed for maximum protoplast volume to be measured in leaf tissue taken from stressed plants using only the 0 MPa infiltration solution, in contrast to the range of solutions that had to be used previously (3, 20).

RESULTS AND DISCUSSION

Osmotic Adjustment in Response to Stress

As pointed out by Morgan (16) and Turner and Jones (24), wheat varieties vary greatly in their ability to undergo leaf osmotic adjustment in response to plant water deficits. As shown in Figure 1, the varieties used in this study demonstrated a broad range of osmotic adjustment during the imposed drought episodes. Leaf Ψ_s at full turgor remained virtually unchanged in Capelle during the stress, dropping by 0.17 MPa as leaf Ψ_w declined from −0.28 down to −1.51 MPa. Osmotic adjustment in response to leaf Ψ_w decline was substantial in both *Triticum kotschyi* and Condor (Fig. 1). Over the course of the imposed drought episodes, leaf Ψ_s at full turgor declined by 1.27 and 1.56 MPa, respectively, in Condor and *T. kotschyi*. Another difference noted between Capelle, and the other wheat varieties, with regard to osmotic

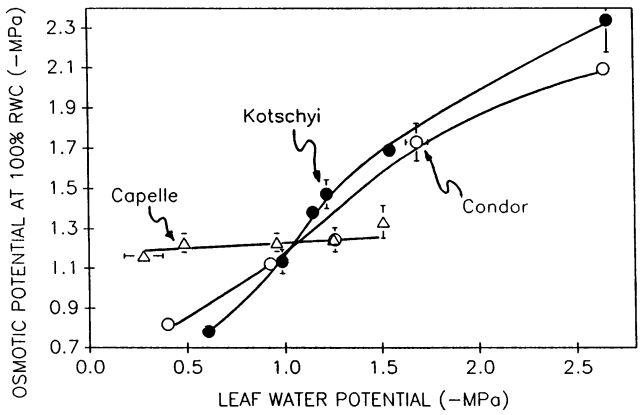

Figure 1. Characterization of osmotic adjustment capability in wheat genotypes exposed to *in situ* water deficits. A decline in Ψ_s at 100% RWC with decreasing Ψ_w denotes osmotic adjustment. Data are presented for the wheat genotypes Condor (○), Capelle (△), and *T. kotschyi* (●). Error bars denote the SE of the means. In many cases, the SE bar is covered by the symbol.

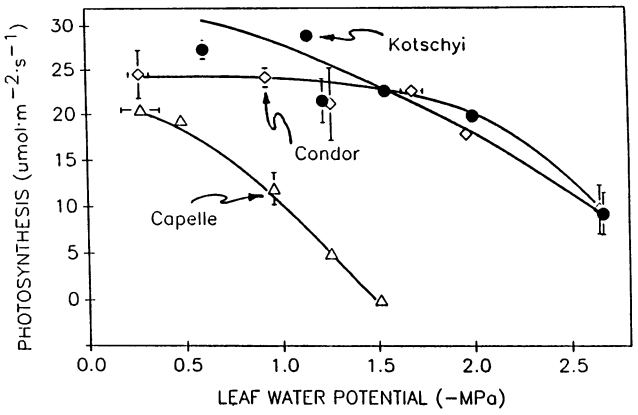

Figure 2. Photosynthetic capacity at declining leaf Ψ_w of Condor (◇), Capelle (△), and *T. kotschyi* (●) plants exposed to water deficits. Error bars denote the SE of the means for photosynthesis and Ψ_w. In many cases, the SE is covered by the symbol.

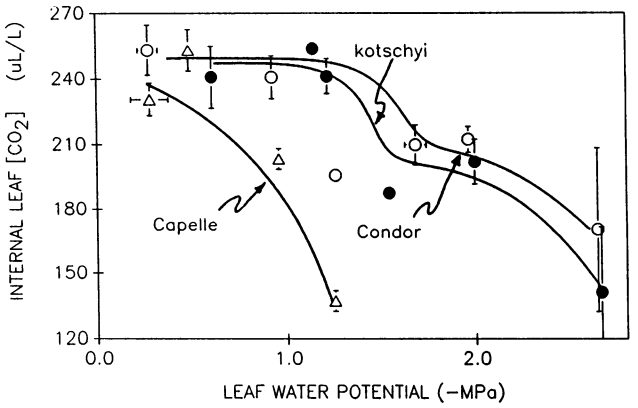

Figure 3. Internal leaf $[CO_2]$ calculated from infrared gas exchange measurements of Condor (○), Capelle (△), and *T. kotschyi* (●) plants exposed to water deficits. Error bars denote the SE of the means for $[CO_2]_i$ and Ψ_w. In many cases, the SE is covered by the symbol.

relations in leaf cells, was that leaves of well-watered Capelle plants had substantially greater solute content (Fig. 1). This effect was reversed in stressed plants due to the lack of osmotic adjustment capability in Capelle.

It should be noted that evaluation of osmotic adjustment using psychrometric analysis of Ψ_s of rehydrated leaves, as described in this study, may suffer from some technical problems such as water injection into the leaf apoplast (20). However, it is an accepted and widely used method of evaluating solute accumulation in leaves (*e.g.* 7). Also, the differences between Condor and Capelle response to stress reported here are qualitatively similar to the differences between these varieties as evaluated using two other techniques of osmotic adjustment analysis (15, 20). Therefore, we conclude that the data presented in Figure 1 indicate that the varieties of wheat used in this study differ in cellular-level response to leaf water deficits.

Water Stress Effects on Photosynthesis

Previous work has indicated that water stress has different effects on photosynthesis *in vitro* of leaf tissue prepared from Capelle and Condor plants (20). In a separate study (8), photosynthesis in *T. kotschyi* was not as inhibited by water stress as photosynthesis in the cultivated wheat variety (TAM W-101) it was tested against. *In situ* photosynthesis of all three wheat genotypes at declining leaf Ψ_w was compared in the experiment shown in Figure 2. The results extend the conclusions of the previously mentioned studies. Photosynthesis in Condor and *T. kotschyi* was relatively 'drought resistant,' while initial Ψ_w decline substantially inhibited photosynthesis in Capelle plants. As compared to the respective maximum rates measured at high Ψ_w, photosynthesis at −1.25 MPa Ψ_w was inhibited by 76, 21, and 13%, respectively, in Capelle, *T. kotschyi*, and Condor plants (Fig. 2).

The results in Figure 2 also indicate that under well-watered conditions (at leaf Ψ_w approaching 0 MPa), *T. kotschyi* has somewhat higher photosynthetic rates than the varieties of cultivated wheat. Also, Condor plants had higher photosynthetic rates at high Ψ_w than Capelle. The photosynthetic rate of the control *T. kotschyi* plants was identical to previously reported values (8), and the differences between control Condor and Capelle plants have been noted in previous studies (20). We conclude, therefore, that the photosynthetic rate under well-watered conditions of the wheat plants used in this study was optimal, and similar to that found in previous studies.

Evaluation of Stomatal and Nonstomatal Effects of Stress on Photosynthesis

Calculated leaf $[CO_2]_i$ of wheat plants exposed to drought are shown in Figure 3. $[CO_2]_i$ remained at the level found in well-watered plants (approximately 240–250 μL/L) as leaf Ψ_w declined to −0.9 MPa in Condor, and −1.2 MPa in *T. kotschyi*. In both of these genotypes, $[CO_2]_i$ then dropped down to approximately 200 μL/L at water potentials ranging down to −2.0 MPa. Further decline in $[CO_2]_i$ occurred at

−2.6 MPa. These data suggest that any differences in water stress inhibition of photosynthesis between Condor and *T. kotschyi* as noted in Figure 2 may not be due to differential response of stomatal conductance to stress. The extent of $[CO_2]_i$ drop in Capelle as leaf Ψ_w declined to −1 MPa was approximately the same as the drop which occurred in Condor and *T. kotschyi* as leaf Ψ_w approached −1.5 MPa (Fig. 3). However, the inhibition in photosynthesis in Capelle at −1.0 MPa was greater than the inhibition which occurred at −1.5 MPa in the other genotypes (Fig. 2). The $[CO_2]_i$ data presented in Figure 3, then, suggest that the extreme sensitivity of photosynthesis to water stress in Capelle was not entirely due to stomatal closure.

As discussed in the "Materials and Methods," $[CO_2]_i$ data should be interpreted with caution with regard to documentation of a nonstomatal effect of water stress on the photosynthetic process. Therefore, stomatal conductance data are also presented (Fig. 4) to support the contention that the difference in stress effects on photosynthesis among the genotypes may not exclusively be due to differences in stomatal response. Decreasing leaf Ψ_w caused a similar degree of reduction in g_c in Condor and *T. kotschyi* plants (Fig. 4). For example, at −9.2 MPa, conductance was reduced from maximal levels by 17% in Condor. At −1.1 MPa, this reduction was 12% in *T. kotschyi*. At −1.97 MPa, conductance was inhibited by 76% in Condor. At −2.0 MPa in *T. kotschyi*, there was a 71% reduction in g_c. These data indicate that stomatal response to declining Ψ_w was similar in Condor and *T. kotschyi* and that any differences in water stress inhibition of photosynthesis between these wheat genotypes as shown in Figure 3 cannot be fully explained by altered stomatal response to stress. In the drought-sensitive genotype Capelle, initial leaf Ψ_w decline from control values resulted in large reductions in g_c (Fig. 4). Therefore, we cannot rule out a stomatal contribution to the relative sensitivity of photosynthesis in Capelle as compared to the other genotypes tested.

Characterization of the Basis for Differential Response of Photosynthesis to Stress

In a recent publication, Sen Gupta *et al.* (22) have demonstrated that altering the osmotic relations of wheat leaf cells by increasing cytoplasmic (and/or vacuolar) $[K^+]$ can substantially alter the relationship between RWC and Ψ_w *in situ*. At a given Ψ_w, RWC was higher under high K^+ fertilization. High K^+ plants were able to maintain photosynthesis to a greater extent at low Ψ_w, primarily due to the maintenance of higher RWC. As concluded previously by Flower and Ludlow (7), examining the relationship between RWC and Ψ_w, then, might be a convenient screening assay to ascertain the ability of a crop plant to maintain photosynthesis at declining Ψ_w. This relationship was investigated in our studies. Results are shown in Figure 5. At water potentials greater than −1 MPa, the relationship between RWC and Ψ_w was similar in all three genotypes. Below −1 MPa, Condor clearly maintained higher RWC than either Capelle or *T. kotschyi*. The relationship between RWC and Ψ_w was similar in Capelle and *T. kotschyi* at all water potentials at which comparisons can be made (*i.e.* down to −1.5 MPa) as shown in Figure 5. Differences among the genotypes with regard to RWC at declining Ψ_w, therefore, were not correlated with the differential effect of declining Ψ_w on photosynthesis in the three genotypes as shown in Figure 2. Although wheat was not included in the studies, Bunce (4) evaluated the relationship between the maintenance of photosynthetic capacity at saturating $[CO_2]$ and RWC at low Ψ_w in a number of species. The data in this study also showed no consistent correlation between the maintenance of high RWC and photosynthesis for the species tested.

The relationship between declining leaf Ψ_w and protoplast volume in Condor, Capelle, and *T. kotschyi* plants is shown in Figure 6. The relationship between protoplast volume and leaf Ψ_w was different in the three genotypes. As shown in Figure 6, declining Ψ_w caused the greatest extent of volume reduction in Capelle; volume in Condor was the least sensitive to low Ψ_w. For example, at approximately −1.5 MPa, protoplast volume reduction was 12.5, 22.7, and 55.5% in Condor, *T. kotschyi*, and Capelle, respectively. The data presented in Figure 6 indicate that the extent of protoplast volume reduction as leaf Ψ_w declines during water deficits in Capelle is much greater than that occurring in Condor or *T. kotschyi*. Preliminary data suggest that changes in the bound water

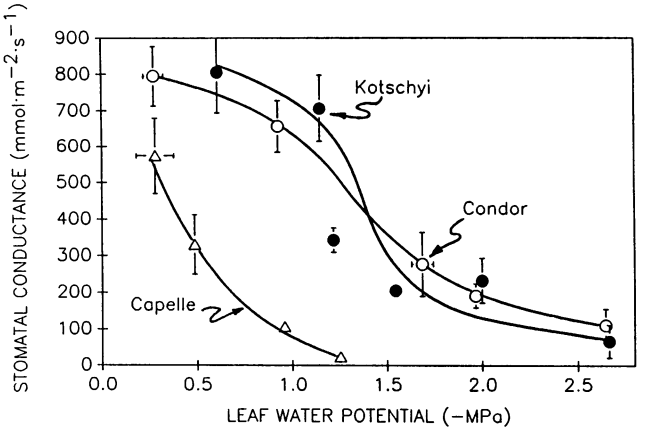

Figure 4. Stomatal conductance, as calculated from infrared gas exchange measurements of Condor (○), Capelle (△), and *T. kotschyi* (●) plants exposed to water deficits. Error bars denote the SE of the means for g_c and Ψ_w. In many cases, the SE is covered by the symbol.

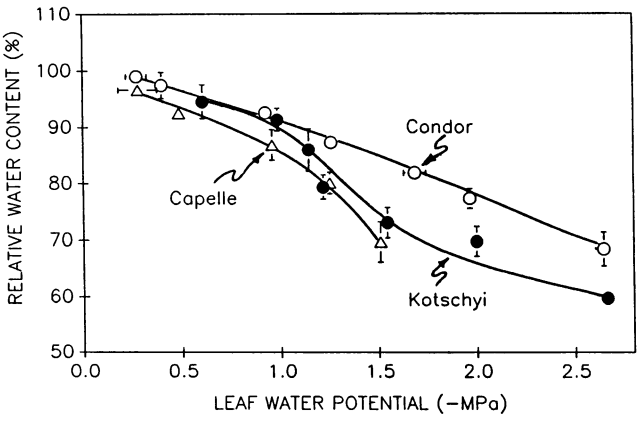

Figure 5. Relationship between relative water content and Ψ_w in Condor (○), Capelle (△), and *T. kotschyi* (●) plants exposed to water deficits. Error bars denote the SE of the means for RWC and Ψ_w. In many cases, the SE is covered by the symbol.

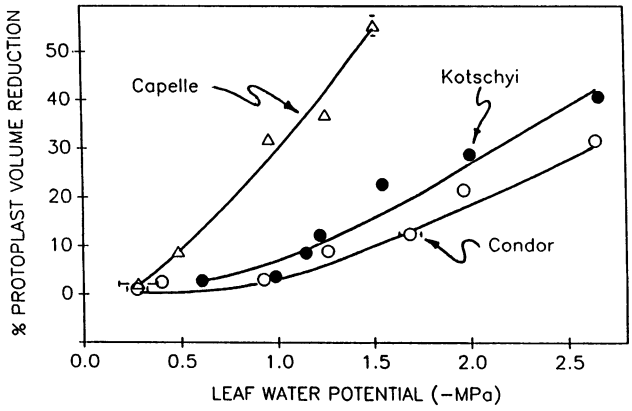

Figure 6. Increasing degree of protoplast volume reduction *in situ* with declining leaf Ψ_w in Condor (○), Capelle (△), and *T. kotschyi* (●) plants exposed to water deficits. Error bars denote the SE of the mean for the extent of protoplast volume reduction, and Ψ_w. In many cases, the SE is covered by the symbol.

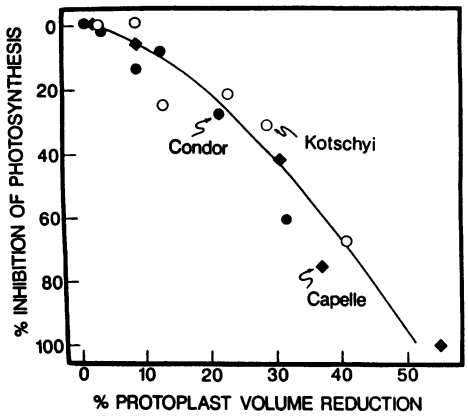

Figure 7. Relationship between degree of protoplast volume reduction, and photosynthetic inhibition in Condor (●), Capelle (◇), and *T. kotschyi* (○) plants during episodes of drought. These data are recalculated from the data presented in Figures 2 and 6.

fraction in stressed Capelle plants may be contributing to this effect (data not shown).

The correlative relationship between water stress-induced protoplast volume reduction and photosynthetic inhibition is portrayed in Figure 7. When the extent of *in situ* photosynthesis inhibition at low Ψ_w is plotted as a function of decreasing protoplast volume, it can be seen that this relationship is virtually identical in all three genotypes. These data suggest, then, that differences between genotypes in terms of relative sensitivity of photosynthesis to low Ψ_w may be explained by the difference in extent of protoplast volume reduction at low Ψ_w. However, the identification of a correlative association between protoplast volume maintenance at low leaf Ψ_w, and the maintenance of photosynthesis in leaves of water stressed wheat plants should not be interpreted as proving that a causal relationship exists between these two physiological parameters.

We believe these data are significant in that the relationship between photosynthesis and protoplast volume at low Ψ_w has been demonstrated for the first time *in situ* with regard to whole plant response to slowly developed leaf water deficits. Whether or not photosynthetic inhibition under water stress is primarily due to stomatal resistance increases, inhibited chloroplast metabolism, or a combination of both factors, it is interesting to note that in wheat, the differences in extent of inhibition at low Ψ_w between genotypes may be a function of differential ability to maintain protoplast volume. If this relationship, as delineated in Figures 2, 6, and 7, is evidenced in a range of agronomic wheat genotypes, then this report points to several interesting areas of further study. The correlation between photosynthetic inhibition and volume reduction suggests that the primary lesion in cell metabolism induced by water stress may be due to the concentrating effect of dehydration on one or several regulatory metabolites.

A second point raised by the data presented in this report is that if the maintenance of protoplast volume at low Ψ_w allows for enhanced chloroplast metabolism over significant periods of time (also see ref. 21), then enhanced carbon gain could be expected at any g_c under water stress. This cellular-level physiological acclimation should allow for some degree of 'drought resistance,' as enhanced transpiration ratio (g carbon uptake/g water loss) would result. The modifications in the *in situ* protoplast volume assay, as described in the "Materials and Methods" section, should allow for screening of a relatively large amount of genetic material for this trait. Of course, to project from the data presented in this report to a full screening program is not warranted. To label a particular physiological trait as conferring drought resistance, repeated long-term studies must be undertaken under field conditions to evaluate if the genotype which displays the potential for the trait actually performs better under water stress on a consistent basis. However, a previous survey by Quarrie (18) supports the possibility raised here of examining protoplast volume maintenance as an important physiological response to low Ψ_w which may allow for enhanced crop performance under drought conditions. In Quarrie's study, 25 agronomic cultivars of wheat were evaluated for ability to increase ABA titer in response to plant water deficits. In this study, it was found that the wheat cultivars which generally performed best under field drought conditions were the cultivars which produced the *least* ABA in response to water stress. Strong stomatal control, therefore, was not identified as conferring drought resistance in the range of wheat cultivars tested. Rather, the ability to maintain photosynthesis under plant water deficit conditions was the physiological trait linked to 'apparent' drought resistance under field conditions.

A third point raised by the data presented here is that differences between genotypes in protoplast volume maintenance at low Ψ_w may be a function of altered gene expression under stress. 'Subtraction profiles' of protein and mRNA in well-watered and stressed plants of the wheat genotypes used in this study may delineate targets for genetic manipulation. These hypotheses will be the focus of our future research in this area.

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