CARBON ISOTOPE DISCRIMINATION, WATER USE EFFICIENCY, AND RELATED PHOTOSYNTHETIC PROPERTIES IN WINTER WHEAT

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INTRODUCTION

Water limitation is one of the main concerns to agricultural productivity, particularly now that marginal land is being cultivated in order to satisfy the world's population demand for food. Irrigation of dry lands has been used to alleviate problems caused by droughts, but in many cropping systems this practice is not economically viable. A different approach used to solve the problem of water availability is to select and breed cultivars requiring less water for growth. This is to improve their water use efficiency (WUE), but, so far little progress has been made. Breeding for increased WUE has been limited by the lack of screening methods that can be used to select desirable genotypes from large populations under field conditions. Traditional methods of measuring season-long water use efficiency (WUE_{sl}) require a careful accounting of the amount of water used, which can not be done in experiments conducted in the field. Carbon isotope discrimination (Δ) has recently been used to evaluate the variation of WUE_{sl} in pot grown plants. Season-long water use efficiency is related to Δ in C_3 plant species via independent links to C_i/C_a (ratio of intercellular CO_2 concentration to ambient CO_2 concentration). The Δ approach to improving WUE was presented in pot-grown wheat by Farquhar and Richards (1984). Pot studies, where both water use and total biomass can be measured accurately, have almost invariablily shown a negative relationship between WUE_{sl} and Δ. Crops in

which the negative correlation between Δ and WUE_{si} has been found in pot-grown plants include wheat, cotton, peanut, barley, tomato, potato, cowpea, bean, wildrye, sunflower, and wheatgrass. In field experiments this correlation has been confirmed only for several peanut varieties. Before Δ is used as tool to select for WUE_{si} in wheat breeding programs, it is necessary to determine weather Δ is also related to WUE_{si} in plants growing directly in the field, as it is in pots.

The objectives of this study were to 1) identify genetic variation in Δ in winter wheat cultivars, 2) define the relationship between Δ and WUE_{si} in field-grown wheat under normal conditions (closed canopy) and compare with isolated individual plants, 3) determine the association among mean values of gas exchange characteristics, dry matter production, and plant-water relations for wheat cultivars grown under different canopy conditions, 4) determine the control and variability of gas exchange efficiency and limitations of photosynthesis during progressive moisture stress, 5) determine genotypic variation in rubisco activity among three winter wheat cultivars selected for Δ, 6) analyze the effect of severe water stress on rubisco initial and total activities, and 7) assay electron transport in thylakoids isolated from wheat leaves.

CHAPTER II

CARBON ISOTOPE DISCRIMINATION, WATER USE EFFICIENCY, AND GAS EXCHANGE

ABSTRACT

Before carbon isotope discrimination (Δ) is used as a tool to select for season-long water use efficiency (WUE_{sl}) in wheat breeding programs, it is necessary to determine if these two traits are negatively related in field-grown plants as they usually are in pot-grown plants. Studies were conducted for two years to i) examine variation in Δ in winter wheat, ii) investigate the relationship between crop WUE_{sl}, and Δ taking into account the contribution of root to total dry matter, iii) study the relationship between gas exchange characteristics and different agronomic parameters. Twelve winter wheat genotypes spanning the entire range in Δ were grown under field conditions. The experiment was conducted according to a split-plot design in which the whole-plot treatments were comprised of three combinations of canopy density and containerization (open, closed and field). The split-plot treatments were 12 genotypes. Significant canopy effects were observed for Δ and shoot biomass. Genotype effects were significant for Δ, shoot biomass, WUE_{sl}, and total biomass. No genotype x environment interaction was detected. A positive correlation between total biomass and WUE_{sl} was observed in both closed- and open-canopies. Genetic variation in Δ was not associated with genetic variation in WUE_{sl}, indicating that these two parameters are influenced by environmental factors not present in pot-grown plants.

INTRODUCTION

Carbon isotope discrimination (Δ) has been proposed as an indirect selection criterion in selection of C_3 genotypes with superior season-long water use efficiency (WUE_{sl}) (Farquhar and Richards, 1984; Farquhar et al., 1989). Plants discriminate against the naturally occurring and heavier stable isotope of carbon (^{13}C) during CO_2 assimilation, thus accumulating more of the lighter carbon (^{12}C) in their tissue. The ratio of leaf intercellular to atmospheric concentration of CO_2 (C_i/C_a) largely determines the extent of the discrimination (Farquhar et al., 1982a). The C_i/C_a value differs among plants because of changes in stomatal opening and in chloroplast demand for CO_2. The C_i/C_a ratio and Δ will increase if the stomatal conductance (g_s) increases while the mesophyll photosynthetic capacity remains constant. On the other hand, low Δ values result from lower C_i/C_a ratios which occur if g_s remains constant and the mesophyll photosynthetic capacity increases or if decreasing g_s coincides with constant mesophyll capacity.

Farquhar et al. (1982) developed an expression for discrimination in leaves of C_3 plants which defines the theoretical link between Δ and leaf gas exchange characteristics

$$\Delta = a\,\frac{C_a - C_i}{C_a} + b\,\frac{C_i}{C_a} = a + (b-a)\,\frac{C_i}{C_a} \qquad (1)$$

In equation (1) **a** is the fractionation due to diffusion of $^{13}CO_2$ in air (4.4 ‰) and **b** is the net fractionation caused by carboxylation (27 ‰). C_a and C_i are the concentrations of CO_2 in the external atmosphere and the intercellular spaces, respectively. Farquhar et al. (1982a, 1989) pointed out that if stomatal conductance is low in relation to the capacity for CO_2 fixation, then the predicted isotope fractionation approaches 4.4‰. Conversely, when the conductance is large so C_i approaches C_a, then the discrimination approaches 27 ‰. Since CO_2 is being continually fixed by plants, Δ provides a long term estimate of C_i/C_a.

To explain the correlation between Δ and WUE_{si}, an expression for instantaneous water use efficiency (WUE_i) has to be established:

$$\frac{A}{E} = \frac{g_c\,(C_a - C_i)}{g_w\,(e_a - e_i)} = \frac{C_a(1 - C_i/C_a)}{1.6(e_i - e_a)} \qquad (2)$$

where g_c and g_w are the conductances to diffusion of CO_2 and water vapor in air, respectively, and e_i and e_a are the intercellular and atmospheric vapor concentrations. The factor 1.6 is the ratio of gaseous diffusivities of CO_2 and water vapor in air (Farquhar and Richards, 1984; Farquhar et al., 1989). According to equation (2), a smaller C_i/C_a value results in an increase in A/E for a constant vapor pressure difference.

Some carbon and water losses are unique to whole-plant growth and are, therefore, not included in the short-term relationship at the leaf level described by equation (2). By allowing for the proportion, Φ_c, of carbon that is fixed during the day, but respired by the plant at night, and the water that is lost from the plant at night, or

by non-photosynthetic tissue, Φ_w, a modified equation can be used to describe the long-term molar ratio of carbon gain to water loss. Therefore, at the whole-plant level, WUE_{sl} is defined as

$$WUE_{sl} = \frac{C_a\,(1-C_i/C_a)\,(1-\Phi_c)}{1.6(e_i-e_a)(1+\Phi_w)}, \qquad (3)$$

$$\text{where } \Phi_c = \frac{\textit{Total plant respiration - leaf respiration}}{\textit{Gross photosynthesis - leaf respiration}} \qquad (4)$$

From equation (3) it can be concluded that WUE_{sl} is affected by environmental factors (drought, humidity, light intensity and quality, CO_2 concentration, and temperature) as well as by physiological responses of the plant.

Because Δ and WUE_{sl} depend on the C_i/C_a ratio (equation (1) and equation (3)), discrimination may be used as a tool for selecting genotypes with high WUE, as mentioned above.

The relationship between WUE_{sl} and Δ is not causal but occurs because of independent links with C_i/C_a. To analyze the relationship between WUE_{sl} and Δ, two independent equations are used. Hubick et al. (1986), replaced equation (1) by

$$\Delta = a - d + (b-a)C_i/C_a \qquad (5)$$

where the new term **d** is fractionation due to dissolution of CO_2, liquid phase diffusion

and respiratory fractionation. By combining equation (3) and equation (5), WUE_{sl} and Δ are related (Evans and Farquhar, 1991) as follows:

$$WUE_{sl} = \frac{(1-\Phi_o)\ C_a(b-d-\Delta)}{1.6(e_i-e_a)\ (b-a)\ (1+\Phi_w)} \qquad (6)$$

Due to independent links of Δ and WUE_{sl} to C_i/C_a, Δ measured in plant dry matter should be positively correlated with the ratio of C_i/C_a, and negatively associated with WUE_{sl} (Farquhar et al., 1982b; Farquhar et al., 1989). The nondimensional units of Δ are "per mil," or ‰. The Δ values of C_3 plants range from 14‰ to 27‰.

Measurement of Δ has been attempted in different C_3 plant species to determine its usefulness in predicting WUE_{sl}. Farquhar and Richards (1984) reported a negative correlation between WUE_{sl} and Δ for different bread wheat genotypes grown in pots in a glasshouse experiment. The correlation coefficient for the spring-summer experiment was -0.44 and for the winter experiment it was -0.75 ($P < 0.01$). Discrimination was greater in the winter plants, probably due to greater g_s and increased C_i/C_a. Hubick et al. (1986) found a strong correlation ($r = -0.81$) between WUE_{sl} and Δ in plants of pot-grown peanut (*Arachis villosa* and *Arachis hypogaea*) genotypes in conditions of unlimited water availability or very restricted water supply. They measured Δ in various parts of plants and found that Δ values of all parts were highly correlated. The authors reported a strong correlation ($r = 0.91$) between total dry matter production and total water use. Dry matter production was well correlated with leaf discrimination in stressed plants ($r = -0.75$) but less so in well-watered plants ($r = -0.44$). A negative correlation between Δ and WUE_{sl} was also reported for pot-

grown tomato by Martin and Thorstenson (1988). Hubick and Farquhar (1989) tested the relationship between Δ and WUE_{sl} in pot-grown barley cultivars (*Hordeum vulgare* L.). It was shown that those two traits were highly correlated (r= -0.86) in the whole plant and that Δ measured in different parts was also highly correlated with WUE_{sl}, the coefficients being -0.81, -0.84, -0.82, and -0.75 in leaves, roots, stems and heads, respectively. Condon et al. (1990) studied the relationship between WUE_{sl} and Δ in pot-grown wheat cultivars. A negative correlation under well watered conditions and under gradually increasing terminal water stress was found. They observed that genotypic variation in Δ was similar in both experiments. The authors also noticed significant variation in C_i/C_a. Similar results were obtained by Ismail and Hall (1993) in cowpea. Subjecting pots to wet or dry treatments, they found a highly significant negative correlation between WUE_{sl} and Δ. Genotypic rankings for both Δ and WUE were preserved over both watering regimes and years. Analogous observations were also reported for other crops (Farquhar and Richards, 1984; Hubick and Farquhar, 1989).

The results mentioned above showed that pot studies, where both water use and biomass can be evaluated accurately, have generally shown a negative relationship between Δ and WUE_{sl}. It is evident from most experiments carried out in pots that Δ can be used as a predictor of WUE_{sl}. But, before Δ is used as a tool to select for WUE_{sl} in breeding programs in different plant species, it is necessary to determine whether Δ is also related to WUE_{sl} in field grown plants, as it is in isolated pots and in greenhouses. There are several studies in which both Δ and shoot biomass have been determined in field grown plants. Condon et al. (1987) studied the genotypic variation and relationship between yield of field-grown plants and Δ in a

group of wheat genotypes. Results showed substantial genotypic variation in Δ. Genotypic ranking for Δ was consistent across field sites. Under field conditions of nonlimiting water, shoot biomass and grain yield were both positively correlated with Δ.

Experiments conducted in small canopies in the field showed a strong negative correlation between Δ and WUE_{si} of peanut (Wright et al., 1988). Total above-ground dry matter yield was negatively correlated with Δ. The ranking of Δ among cultivars was not affected by the different environments (open- and closed-canopies). This study demonstrated that there is genetic variability of total dry matter yield and WUE_{si} in peanut grown in the field under non-limiting water conditions. White et al. (1990) evaluated the relations between root growth, productivity, and Δ for genotypes of common bean (*Phaseolus vulgaris*) grown under rainfed and irrigated conditions in different sites. In one of the sites under rainfed conditions, Δ was positively correlated with all growth parameters, while at the other rainfed site the linear correlations of Δ with seed yield, biomass, and leaf area index were not significant. Under irrigation there was no association between growth parameters or yield and Δ. A nonsignificant correlation was found between Δ and leaf nitrogen content. The authors inferred that given the positive correlation between root density and Δ at the rainfed sites it would seem unproductive to use Δ as a tool for studying variation in C_i related to genetic differences in stomatal function or photosynthetic capacity under water deficit unless root effects are controlled.

Ehdaie et al. (1991) studied the differences in total dry matter, WUE_{si}, and Δ of flag leaves of diverse wheat genotypes grown in pot experiments and in field conditions under well-watered and drought-stressed conditions for 2 years. The Δ was

negatively and significantly correlated with total dry matter, shoot dry matter and root dry matter in the wet and dry pot experiments. Correlation between Δ and WUE was negative in the well-watered experiment. In the dry field experiments, Δ was positively correlated with above ground dry matter only 1 year. In the wet field experiments, there was a positive correlation between Δ and grain yield only 1 year. In this investigation the patterns of WUE_{si} in wet and dry pot experiments were not consistent over the two years, probably due to an inadequate system in the greenhouse. In general in the field experiments, Δ was not consistently correlated with either above ground dry matter or grain yield.

Johnson and Bassett (1991) noted a negative relationship between dry matter yield and Δ in field-grown cool-season grasses. The tendency toward negative correlations suggested that for the species studied, high WUE and dry matter productivity were not incompatible. They found a negative correlation between pot WUE_{si} and field Δ in a way consistent with Farquhar's theory. Craufurd et al. (1991) measured grain yield and grain Δ in yield trials with barley over two seasons at three sites (seasonal rainfall, irrigation and drought conditions). Considerable variation in trial mean Δ was found. Discrimination was negatively correlated with grain yield in the irrigated trial. Where water was more limiting, the relationship between Δ and grain yield was strongly positive; this correlation contrasts with the one reported by Condon et al. (1987). Craufurd et al. (1991) suggested that the negative correlation between grain yield and grain Δ observed in the irrigated trials may have occurred because the late flowering varieties yielded more grain than the early ones, and the late ones would haven assimilated more of their grain carbon when atmospheric drought was greater. They concluded that high grain Δ may be a helpful indicator of good yield in

barley in water-limited Mediterranean environments. Condon et al. (1993) studied the relationship between Δ and WUE for wheat (*Triticum durum* Desf. and *Triticum aestivum* L.) grown in a dryland environment characterized by winter/spring-dominant rainfall and late-season drought. No relationship was observed between final dry matter production and Δ. For the period to anthesis, when water supply was non-limiting the relationship between dry matter production and Δ was positive and significant. There was no correlation between post-anthesis dry matter production and Δ. Contrary to other studies by the same researchers on pot grown-wheat, no relationship was observed between genotypic variation in WUE_{si} and genotypic variation in Δ. It seems that the relationship between WUE_{si} and Δ was complicated by differences in genotype development and by soil water depletion after anthesis.

Condon and Richards (1992) identified the most effective growth stage or plant part to characterize genotypic variation in Δ in field-grown wheat. They determined that either dry matter formed early in crop development or the grain would be better plant material for characterizing genotypic differences in Δ than other plant materials. Early-formed dry matter may be preferred because in general there is little water stress during the early phase of growth. Estimates of heritability were greatest when green leaves and stems sampled during early stem elongation were used for Δ analysis. Grain Δ values may yield equivocal results. Grain carbon is made up of both current photosynthate and retranslocated carbon. Studies by Hall et al. (1990) in cowpeas demonstrated that broad sense heritability (entry mean basis) for Δ was substantially greater for samples from leaves (0.76) than from grain (0.35). Therefore, leaves seem to be more appropriate than grain for detecting genotypic differences in Δ.

The quite consistent negative correlation between Δ and biomass observed in

pot studies is not always found in field studies, indicating that results in the field include larger environmental effects. As mentioned earlier, Condon et al. (1987) reported a positive correlation between Δ and shoot biomass production for wheat genotypes (*Triticum* spp.) grown in a field environment, suggesting that the positive relationship may be due to genotypic variation in leaf conductance. On the other hand, Hubick et al. (1988) observed a negative correlation between Δ and total dry matter production for peanut (*Arachis hypogaea*) growing under field and well-watered glasshouse conditions, and suggested that these correlations may be due to genotypic variation in photosynthetic capacity. Morgan et al. (1993) reported a positive correlation between Δ and grain yield ($r = 0.71^{**}$) under irrigated conditions in winter wheat. Therefore, results indicate that in some environments, cultivars with high Δ may yield more. The expectation of a negative relationship between biomass and Δ is based on several conjectures which may not be fully valid, especially in field experiments. Among these conjectures the most important are: i) genotypes have the same growth characteristics, ii) soil surface evaporation is the same for all genotypes, and iii) genotypes possess the same relative root biomass. If any of these assumptions fail the negative relationship is weakened and may even be invalidated.

The objectives of this study were to 1) examine variation in Δ among a set of wheat cultivars, 2) investigate the relationship between crop WUE_{sl} and Δ taking into account the contribution of roots to total dry matter, 3) determine the association among mean values of leaf gas exchange characteristics, dry matter production, plant-water relations, and Δ for wheat genotypes grown under different canopy conditions. Genotypes were selected on the basis of a preliminary survey of Δ of whole-plant material (Cai, 1992).

MATERIALS AND METHODS

To determine whether genotypic differences in WUE_{si} exist under field conditions and whether the Δ technology can predict them, we evaluated the variation in WUE_{si} and its correlation with Δ in various canopy types in the field. Based on the information obtained in a preliminary experiment (Cai, 1992), twelve winter wheat genotypes spanning the range in Δ were selected and grown under field conditions at the Agronomy Research Station at Stillwater, OK in two consecutive years (1992 and 1993). Genotypes were separated into two groups with respect to time of anthesis. The soil type was Kirkland silt loam (Fine, mixed, thermic Udertic Paleustoll).

The experiment was conducted according to a split-plot design in a randomized complete block arrangement with three replications. The whole-plot treatments were comprised of three combinations of canopy density and containerization (open, closed, and field). For the closed-canopy whole plot treatment, the space between containers was filled in with the cultivar Chisholm at normal planting density. In another series the inter-plot space remained open (open-canopy whole plot). In the non-containerized closed canopy the twelve genotypes were grown directly in the ground. The split-plot treatments were 12 genotypes. Split-plot experimental units for containerized whole-plot treatments consisted of 12.7 cm diameter x 84 cm deep holes spaced 1.2 m apart within rows drilled in the field. The whole plot consisted of two rows (6 containers per row). Each hole was fitted with a

plastic cylinder with the same dimensions. A second cylinder (container) of 10.2 cm diameter x 81.3 cm length, containing about 2 Kg of Redi-Earth peat-lite mix (Grace Sierra Co., CA) was inserted into the ground-fitted plastic cylinder. The base of these containers was sealed with plastic caps to prevent exchange of water between containers and soil, and also to prevent roots from growing into the ground. One plant was randomly assigned to each cylinder. Four plantless containers were used to correct for soil precipitation and evaporation. The soil surface in the containers was covered by a layer of small rocks to avoid excessive water evaporation.

The experiment was completely enclosed within a rainout shelter, and covered with one layer of plastic film before the time of heavy spring rains. The main purpose of this plastic was to protect containerized plants from heavy preharvest rain, not to impose drought stress beyond natural field conditions. The soil in the cylinders was watered to 50% soil capacity and weighed at the start of the experiment. Throughout the experiment plants were watered at first sign of wilting, and the amount of water added was recorded. The first year (1992), 0.4 g of water-soluble 20-9-17 N-P-K [20-20-20 oxide form] fertilizer was applied, giving 0.1 g N $plant^{-1}$, which corresponded to 36 kg N ha^{-1} on an area basis. Based on laboratory results obtained from plant tissue analyses, the amount of fertilizer was doubled the second year (1993). Dead leaves were collected and weighed during the season and the weight included in the final measurement of total dry matter.

Gas exchange measurements were carried out on the topmost fully-expanded leaf of each plant from jointing to grain-filling (four times). Measurements were made between 1100 and 1600 h with a LI-COR 6200 portable photosynthesis system (LI-COR, Inc., Lincoln, NE). Data were collected when photosynthetically active

radiation (PAR) incident upon the leaf was greater than 1800 μmol m^{-2} s^{-1}. Calculations of gas exchange characteristics were made on a per unit leaf-area basis. Gas exchange WUE was calculated as the ratio of photosynthesis rate (A) to g_s.

When plants reached maturity, the cylinders were lifted out of the ground and weighed. The difference in weight between the start and end minus the same difference of the containers without plants (to correct for soil evaporation and precipitation) represented the season-long water use by each plant. Plants were cut at the soil surface and the roots washed free of soil. Roots and shoots were separately dried to constant weight at 70^{0}C. Shoot and root weights were added to obtain total plant dry weight. Season-long water use efficiency (WUE_{sl}) was calculated by dividing dry weight (dw) by water use (g dw/kg H_2O).

Dry shoots were ground to a fine powder using a Thomas-Wiley laboratory mill (Model 4, Arthur U. Thomas Co., PA). A subsample was used to measure the stable carbon isotope composition ($\delta^{13}C$) by ratio mass spectrometry. With this technique the powder is combusted at high temperature to generate CO_2, and the formed CO_2 is purified and released into a ratio mass spectrometer for determination of the $^{13}C/^{12}C$ ratio. Determinations of the $\delta^{13}C$ were performed at the University of Utah Stable Isotope Ratio Facility for Environmental Research as described by Ehleringer (1990). Determinations of total nitrogen were made on the same tissue samples used for the Δ analysis. In 1993, leaf samples were also collected for Δ determinations. Data for all traits were analyzed using SAS (SAS/STAT, 1989).

RESULTS AND DISCUSSION

Genotypic differences in Δ were observed in 1992 and 1993 (Tables 1, 2 and 3). In 1992, the range in Δ of 1.008‰ in the open-canopy was less than 1.114‰ in the closed-canopy and 1.454‰ in the direct planting (Table 1). These ranges were less than the range of 1.8‰ observed among wheat genotypes by Condon et al. (1990), and the range of 2.2‰ observed among leaves of cowpea cultivars by Hall et al. (1990) and in wheat cultivars by Ehdaie et al. (1991). Genotype ranking across canopies was relatively consistent both years. The correlation in Δ values across canopies was significant (r = 0.64*) indicating that the rankings of genotypes were relatively stable. Rankings of Siouxland, Lamar and Chisholm were the same in the closed- and open-canopies (4th, 8th, and 12th, respectively) in 1992. The ranking of Abilene, Mesa, TAM W-200, and Lamar was consistent in the open-canopy and in the direct planting in 1992 (Table 1).

Carbon isotope discrimination values were greater in plants grown directly in the field than in plants grown in containers (closed or open canopies). The highest and the lowest mean Δ values were 18.55 ‰ (Mesa) and 17.68 ‰ (Quantum 554), respectively. These values correspond to C_i/C_a ratios (ratio of internal CO_2 concentration in the leaf to ambient CO_2 concentration) of 0.63 in Mesa and 0.59 in Quantum 554 based on the expression $\Delta = 4.4 + 22.6\ C_i/C_a$ reported by Farquhar et al. (1982a) and Farquhar and Richards (1984).

In the 1993 study, the same twelve cultivars were grown under the same conditions as in 1992. The range in Δ of 1.837‰ in the open-canopy was greater than the range of 1.008‰ in the closed-canopy and the range of 1.052‰ in the direct planting in 1993 (Table 2). The highest and the lowest mean Δ values were 19.13‰ in TAM W-200 and 18.21‰ in Chisholm which correspond to C_i/C_a ratios of 0.65 and 0.61. Differences in C_i/C_a can arise from changes in the balance between leaf g_s and photosynthetic capacity. Carbon isotope discrimination values were larger in the direct planting than in the other two canopies.

Carbon isotope discrimination values were also determined for leaf tissue in 1993 (Table 3). Genotype Δ values varied across canopies. In the closed-canopy, Δ had a range of 1.506‰, in the open-canopy 2.045‰, and direct planting 1.048‰. In general Δ was higher in leaf tissue than in the whole plant. The lower ‰ values in the whole plant might have resulted from using tissue which contained carbon assimilated during different time periods. Hubick and Farquhar (1989) reported differences in Δ among plant parts, and these differences varied significantly among cultivars. In general stems had greater Δ values than the other plant parts, while spikes showed smaller values than leaves, stems and roots. Hubick and Farquhar (1989) noted that Δ based on total plant carbon eliminates variation originated by any mechanisms which cause spatial fractionation, because it results from combusting the whole plant or a representative sample for analysis.

Analysis of variance of gas exchange data revealed significant differences between environments (years) for g_s, A/g_s, and C_i (Table 4). No significant genotype effects were detected for any of the gas exchange parameters. In contrast, Ismail and Hall (1993) found significant differences for A and g_s among cowpea genotypes and

Kirchhoff et al. (1989) reported genotypic effects for A in cowpea. Environment x canopy interaction was significant for all four parameters indicating that the canopy effect was different in each environment (year). Genotype x canopy interaction was not significant, indicating that genetic differences were relatively consistent across the three canopies. No genotype x environment interaction was observed, suggesting that these characters may be under strong genetic control (Table 4).

The analysis of variance for Δ, shoot biomass, WUE_{si}, root biomass and total biomass (Table 5) showed significant differences between environments for all five characters. Significant canopy effects were detected for Δ and shoot biomass. Genotype effects were observed for Δ, shoot biomass, WUE_{si}, and total biomass. Environment x canopy interaction for Δ and shoot biomass was shown to be large. Genotype x canopy interaction was significant for shoot biomass and total biomass indicating that genetic differences were inconsistent across the three canopies. No genotype x environment interaction was observed for either trait (Table 5). Lack of environment x genotype interaction was also detected by Condon et al. (1987) in wheat, by White et al. (1990) in common bean, and by Johnson et al. (1990) in wheatgrass. These results indicate that selection for either of the three traits could take place in a single environment. Results presented by Hall et al. 1990 for cowpea, suggested that there may be some environment x genotype interaction for Δ, at least when very contrasting locations are compared. However, in wheat (Condon et al., 1987) and in peanuts (Wright et al., 1980) no environment x genotype interaction was found.

Genotypic differences in A were highly associated with genotypic differences in g_s in all three canopies (Tables 6, 7 and 8). Similar results were reported for wheat by

Johnson et al. (1987) Condon et al. (1990), and Morgan et at. (1990). The high correlation between A and g_s tends to conserve the C_i/C_a ratio, which agreed with the nonsignificant genotypic variation for A/g_s found in this study (Table 4).

Genetic variation in intrinsic gas exchange efficiency (A/g_s) was negatively related to g_s (r = -0.84**) in the open canopy in 1992 (Table 7). Martin and Ruiz-Torres (1992) observed that A/g_s increased with reduced g_s in pot-grown plants. This is similar to a previous study in winter wheat in which a negative correlation was found between A/g_s and g_s under irrigated conditions (Morgan et al., 1993). Our data indicated that genotypic changes in A/g_s were mainly due to changes in g_s. Johnson et al. (1987) and Morgan and LeCain (1991) reported analogous findings in wheat suggesting that genotypic variation in A/g_s of wheat is more closely related to variation in g_s than in A.

Genotypic differences in total biomass correlated positively with genotypic differences in WUE_{si} in both closed- and open-canopies, both years (Tables 6, 7, 9, and 10). Ismail and Hall (1993) also found a positive correlation between biomass production and WUE_{si} in cowpea. The positive correlation between total biomass and WUE_{si} may indicate that, for this group of winter wheat cultivars, total biomass was more influenced by variation in photosynthetic capacity than by variation in g_s. Season-long water use efficiency was also positively related to shoot biomass (both years) in the closed- and in the open-canopies (Tables 6, 7, 9, and 10). A positive correlation was also reported between WUE_{si} and shoot biomass in cowpeas by Ismail and Hall (1993). There was a strong positive correlation between total biomass and shoot biomass in the closed and the open canopies (Tables 6, 7, 9, and 10), showing that this correlation was consistent across canopies. Root biomass was positively

related to WUE_{sl} (r = 0.61*) and Δ (r = 0.58*) in the open-canopy in 1992 (Table 7). In this particular year, WUE_{sl} and Δ increased with increasing root biomass. In 1992, A and g_s were positively correlated with shoot biomass (r = 0.72** and r = 0.65*, respectively), in cultivars grown directly in the field (direct planting) (Table 8). A positive correlation between shoot biomass and Δ (r = 0.71**) suggested that wheat genotypes acquiring a greater shoot biomass had higher C_i values (Table 8). The same year (1992), the correlation between shoot nitrogen content and shoot biomass was negative (r = -0.60*) (Table 8). This correlation is consistent with a previous study carried out in *Phaseolus vulgaris* by White et al. (1990), in which leaf nitrogen content and crop biomass were negatively correlated. In 1992, laboratory results showed very low nitrogen content in shoot tissue, suggesting that plants were nitrogen starved. The negative correlation may be the result of lower rates of CO_2 assimilation due to nitrogen starvation. Von Caemmerer and Farquhar (1981) showed that *Phaseolus vulgaris* plants grown at low nitrogen levels had lower rates of CO_2 assimilation. The negative correlation between shoot nitrogen content and shoot biomass (r = - 0.63*) observed in the closed-canopy in 1993 cannot be completely attributed to poor fertilization. Therefore, this negative relationship may also be due to reduced stomatal conductance, reduced photosynthetic capacity, or both.

Leaf Δ was also determined in 1993 (Table 3). Correlations between leaf Δ and other agronomic characters are shown in Table 12. In the open-canopy the relationship between Δ and measured C_i was negative (r = -0.68*). Based on theory (Farquhar, 1982a), this is an unexpected correlation. The physiological basis for variation in Δ in C_3 plant species is related to variation in the C_i/C_a ratio. Low Δ arise from low C_i/C_a ratio and leads to a higher ratio of net photosynthesis rate to

transpiration. Since C_a is relatively constant, average C_i has to be positively related to Δ. Direct planting mean values of Δ were positively correlated with measured C_i supporting Farquhar's theory (Table 12). Contrary to theory, variation in measured A/g_s was positively correlated with Δ ($r = 0.69^*$) in the open canopy. Similar results were reported by Morgan et al. (1993) in wheat. An explanation for this positive correlation is that instantaneous gas exchange measurements do not always reflect long-term genotypic variation in WUE_{sl}. Previous studies demonstrated that a negative association can occur between A/g_s and Δ that are consistent with theory (Farquhar et al., 1989; Condon et al., 1990). It is important to point out that Δ represents a time integrated, and assimilation-weighted estimate of C_i/C_a, whereas gas exchange data represent point measurements.

Mean values (Table 13) showed environment (years) and canopy effects for several traits. C_i values were much higher in 1992 than in 1993. Mean values ranged from 196 μmol mol^{-1} (direct planting) to 214 μmol mol^{-1} (closed-canopy) in 1992, and from 173 μmol mol^{-1} (open canopy) to 177 μmol mol^{-1} (direct planting) in 1993. Stomatal conductance was much lower in 1993, resulting in higher A/g_s values than in 1992. Total biomass was similar in both closed- and open-canopies within each environment. Notable differences in total biomass were observed between environments. Greater shoot biomass values were observed in 1993 than in 1992. The low fertilizer rate and the low winter precipitation in 1992, might have resulted in lower biomass production. The low genotypic mean for shoot biomass under field conditions in 1992 may be evidence of the nitrogen and water limitation that year. In general higher genotypic mean Δ values were observed in all three canopies in 1993 than in 1992. This is contrary to expectation considering the observed C_i values in the

two years. Genotypic means for Δ were lower in the open-canopy than in the closed-canopy or direct planting (Table 13). Reduced stomatal aperture due to greater soil evaporation in the open-canopy resulted in decreased Δ values. Season-long water use efficiency was higher in the open-canopy than in the closed-canopy in 1992. Drier soil conditions in the open-canopy reduced g_s and increase WUE by reducing A proportionally less than the transpiration rate.

In this study, a weak negative correlation (r = -0.51, P = 0.11) between WUE_{sl} and whole plant Δ was observed in the closed-canopy in 1992 (Table 6). A similar correlation (r = -0.57, P = 0.06) was detected between leaf Δ and WUE_{sl} in 1993 (Table 12). The strong negative relationship between Δ and WUE_{sl} observed in pot-grown wheat studies (Farquhar and Richards, 1984; Condon et al., 1990; Ehdaie et al.,1991) was not observed in this study. In order to understand why the strong relationship observed in pot studies diminishes or disappears when plants are grown in the field, one must examine the factors that contribute to variation in Δ and WUE_{sl}. Photosynthetic capacity and stomatal conductance are the two mayor factors that cause variation in Δ. In common bean, it was noted that genotypic variation in Δ mainly results from variation in stomatal conductance (Ehleringer, 1990) while, in peanut, much of the genotypic variation in Δ seemed to be due to variation in mesophyll photosynthetic capacity (Hubick et al., 1988). Condon et al. (1990) reported that genetic variation in Δ in wheat was attributed approximately equally to variation in leaf conductance and in mesophyll capacity. The lack of a strong relationship between Δ and WUE_{sl} may arise from scaling up from a leaf or a single plant in a container to a crop canopy in the field. When plants are grown in the field, the canopy boundary layer resistance is the dominant component of the total diffusive resistance to gases.

Significantly different, but much smaller variation in stomatal resistance, may become insignificant when it is superimposed on the much larger canopy boundary layer resistance. Hence, significant variation in WUE among genotypes noted at the leaf or isolated plant level, may disappear in a closed canopy.

Results suggested that genetic variation in Δ was mainly due to variation in photosynthetic capacity. The manner in which this experiment was carried out minimized stomatal conductance as a source of variation in Δ. All plants received the same amount of water and used approximately 10kg water. Therefore, little variation in water use was observed.

SUMMARY

Genotypic differences in Δ were observed in both years (1992 and 1993). Genotypic ranking across canopies was relatively consistent. The Δ values were greater in plants grown directly in the field in both years. Analyses of variance showed significant genotype effects for shoot biomass, total biomass, and WUE_{sl}. Significant canopy effects were detected for Δ and shoot biomass. No genotype x environment interaction was observed, suggesting that these characters may be under strong genetic control.

Total biomass was positively correlated with WUE_{sl} in both, closed and open canopies in both years. This positive correlation indicated that for this group of wheat cultivars, total biomass was more influenced by variation in mesophyll photosynthetic capacity than by variation in g_s. Under field conditions a positive correlation between shoot biomass and Δ was observed, indicating that wheat genotypes with greater shoot biomass had higher C_i values.

The correlation between Δ and WUE_{sl} was negative but weak, therefore the usually strong negative relationship found in pot studies was not verified in the field. Factors unique to a field environment must have complicated the relationship between Δ and WUE_{sl} when plants were grown in the field. Those factors include relative humidity, CO_2 concentration, light intensity and quality, temperature, and periods of drought.

Table 1. Mean carbon isotope discrimination of whole plant tissue of winter wheat genotypes grown under three different canopy densities in 1992.

Genotypes	Earliness†	Closed-canopy		Open-canopy		Direct planting		Δ	
		Rank	Δ	Rank	Δ	Rank	Δ	Rank	Overall
			‰		‰		‰		‰
Abilene	L	1	18.780	2	17.617	2	19.217	2	18.538
Cody	L	2	18.641	6	17.335	10	18.070	5	18.015
Mesa	E	3	18.519	1	17.780	1	19.353	1	18.551
Siouxland	L	4	18.470	4	17.485	12	17.899	7	17.951
TAM W-101	L	5	18.460	10	17.001	4	18.941	4	18.134
TAM W-200	E	6	18.244	3	17.530	3	19.018	3	18.264
Pony	E	7	18.244	5	17.419	9	18.195	6	17.952
Lamar	L	8	18.066	8	17.259	8	18.216	8	17.847
Pioneer 2157	E	9	17.986	11	16.786	7	18.428	10	17.732
Cimarron	E	10	17.770	9	17.005	6	18.498	9	17.758
Quantum 554	L	11	17.718	7	17.321	11	18.003	12	17.681
Chisholm	E	12	17.666	12	16.772	5	18.690	11	17.709
LSD (0.05) Genotypes			0.5501		0.5156		0.7426		

† E indicates early first flowering, and L is late.

Table 2. Mean carbon isotope discrimination of whole plant tissue of winter wheat genotypes grown under three different canopy densities in 1993.

Genotypes	Earliness†	Closed-canopy		Open-canopy		Direct planting		Δ	
		Rank	Δ	Rank	Δ	Rank	Δ	Rank	Overall
			‰		‰		‰		‰
TAM W-200	E	1	19.178	2	18.307	2	19.905	1	19.130
Mesa	E	2	19.084	6	17.485	3	18.782	4	18.784
Lamar	L	3	18.983	3	18.017	8	19.381	3	18.749
Abilene	L	4	18.780	1	18.582	11	19.064	2	18.854
Pioneer 2157	E	5	18.794	7	17.436	1	19.982	5	18.737
Cimarron	E	6	18.666	9	17.290	7	19.461	8	18.472
Chisholm	E	7	18.662	12	16.745	9	19.234	12	18.214
Cody	L	8	18.568	4	17.965	10	19.220	6	18.584
Quantum 554	L	9	18.505	10	17.186	5	19.493	9	18.395
Siouxland	L	10	18.446	8	17.325	4	19.726	7	18.499
Pony	E	11	18.442	5	17.704	12	18.930	10	18.359
TAM W-101	L	12	18.170	11	17.019	6	19.479	11	18.223
LSD (0.05) Genotypes			0.9105		0.9869		1.0483		

† E indicates early first flowering, and L is late.

Table 3. Mean carbon isotope discrimination of leaf tissue of winter wheat genotypes grown under three different canopy densities in 1993.

Genotypes	Earliness†	Closed-canopy		Open-canopy		Direct planting		Δ	
		Rank	Δ	Rank	Δ	Rank	Δ	Rank	Overall
			‰		‰		‰		‰
Abilene	L	1	19.360	1	19.377	7	19.989	2	19.575
Cody	L	2	19.182	7	18.174	3	20.122	3	19.327
Mesa	E	3	19.147	4	18.286	2	20.163	1	19.999
TAM W-200	E	4	19.144	2	18.582	1	20.254	4	19.327
Pioneer 2157	E	5	19.091	6	18.209	10	19.772	7	19.024
Siouxland	L	6	18.955	8	18.028	4	20.122	6	19.035
Pony	E	7	18.641	9	17.913	11	19.269	10	18.608
Quantum 554	L	8	18.620	3	18.397	5	20.104	5	19.040
Cimarron	E	9	18.519	12	17.332	6	20.051	9	18.634
Lamar	L	10	18.303	5	18.237	12	19.206	11	18.582
Chisholm	E	11	18.184	11	17.815	8	19.926	8	18.642
TAM W-101	L	12	17.854	10	17.815	9	19.894	12	18.521
LSD (0.05) Genotypes			0.8850		0.6444		0.8699		

† E indicates early first flowering, and L is late.

Table 4. Summary of analyses of variance for rate of net photosynthesis (A), stomatal conductance for H_2O (g_s), intrinsic gas exchange efficiency (A/g_s), and intercellular CO_2 concentration (C_i).

Source	df	A	g_s	A/g_s	C_i
Environment (Env.)†	1	NS	*	*	*
Canopy	2	NS	NS	NS	NS
Genotype (Gen.)	11	NS	NS	NS	NS
Canopy x Gen.	22	NS	NS	NS	NS
Env. x Canopy	2	*	**	**	*
Env. x Genotype	11	NS	NS	NS	NS
CV(%)		19	22	9	6

*, ** Significant at P = 0.05 and 0.01, respectively; NS = nonsignificant.
† Years, 1992 and 1993.

Table 5. Mean squares from analyses of variance for carbon isotope discrimination (Δ), shoot biomass, season-long water use efficiency (WUE_{sl}), root biomass, and total biomass.

Source	df	Δ	Shoot biomass	df	WUE_{sl}	Root biomass	Total biomass
Environment (Env.)†	1	17.9**	36212**	1	52.8**	27.5*	5483**
Rep/Env.	4	0.2	361	4	0.4	1.8	56
Canopy	2	46.0*	2512**	1	1.1	80.9	55
Genotype (Gen.)	11	1.2*	531*	11	1.7**	12.0	231**
Canopy x Gen.	22	0.4	570**	11	0.4	4.1	76*
Env. x Canopy	2	1.8*	11344**	1	1.1	28.2	82
Env. x Genotype	11	0.4	240	11	0.3	5.5	34
CV(%)		2.6	24		8	28	10

*, ** Significant at P = 0.05 and 0.01, respectively.
† Years, 1992 and 1993.

Table 6. Correlation coefficients for the genotypic association between different parameters measured under closed-canopy conditions in 1992.

	g_s	C_i	A/g_s	Total biomass	WUE_{si}	Shoot nitrogen	Δ†	Shoot biomass	Root biomass
A	0.87**	0.26	-0.51	0.27	0.13	-0.04	0.34	0.29	-0.07
g_s		0.37	-0.54	0.11	-0.06	0.13	0.53	0.12	-0.01
C_i				-0.29	-0.22	0.39	0.09	-0.29	-0.08
A/g_s				0.08	0.07	-0.30	-0.11	0.08	-0.02
Total biomass					0.95**	-0.43	-0.37	0.98**	0.27
WUE_{si}						-0.40	-0.51‡	0.95**	0.18
Shoot nitrogen							-0.07	-0.51	0.39
Δ								-0.33	-0.34
Shoot biomass									0.13

*, **, ‡ Significant at P = 0.05 and 0.01, and 0.11, respectively.
† Determined in whole plant tissue.

Table 7. Correlation coefficients for the genotypic association between different parameters measured under open-canopy conditions in 1992.

	g_s	C_i	A/g_s	Total biomass	WUE_{si}	Shoot nitrogen	Δ†	Shoot biomass	Root biomass
A	0.73**	0.27	-0.51	-0.24	-0.32	-0.05	0.29	-0.35	0.22
g_s		0.70*	-0.84**	-0.15	-0.21	0.20	0.23	-0.28	0.37
C_i				-0.06	-0.09	0.09	0.32	-0.16	0.26
A/g_s				0.20	0.26	-0.22	-0.30	0.31	-0.22
Total biomass					0.98**	-0.30	0.07	0.98**	0.71**
WUE_{si}						-0.35	-0.03	0.99**	0.61*
Shoot nitrogen							0.32	-0.37	0.05
Δ								-0.09	0.58*
Shoot biomass									0.55

*, **, Significant at P = 0.05 and 0.01, respectively.
† Determined in whole plant tissue.

Table 8. Correlation coefficients for the genotypic association between different parameters measured under field conditions in 1992.

	g_s	C_i	A/g_s	Shoot nitrogen	Δ†	Shoot biomass
A	0.93**	0.06	-0.44	-0.39	-0.30	0.72**
g_s		0.26	-0.51	-0.44	0.26	0.65*
C_i				-0.43	-0.19	-0.16
A/g_s				0.48	0.13	-0.14
Shoot nitrogen					-0.27	-0.60*
Δ						-0.71**

*, **, Significant at P = 0.05 and 0.01, respectively.
† Determined in whole plant tissue.

Table 9. Correlation coefficients for the genotypic association between different parameters measured under closed-canopy conditions in 1993.

	g_s	C_i	A/g_s	Total biomass	WUE_{si}	Shoot nitrogen	Δ†	Shoot biomass	Root biomass
A	0.76**	-0.29	0.12	-0.15	0.05	-0.08	-0.21	-0.18	0.12
g_s		0.38	-0.54	-0.23	-0.15	0.09	-0.23	-0.21	-0.01
C_i				-0.10	-0.24	0.18	-0.04	-0.03	-0.20
A/g_s				0.11	0.24	-0.29	0.22	0.09	0.04
Total biomass					0.87**	-0.60*	0.24	0.95**	-0.09
WUE_{si}						-0.63*	-0.16	0.86**	-0.17
Shoot nitrogen							-0.52	-0.63*	0.23
Δ								0.29	-0.21
Shoot biomass									-0.40

*, **, Significant at P = 0.05 and 0.01, respectively.
† Determined in whole plant tissue.

Table 10. Correlation coefficients for the genotypic association between different parameters measured under open-canopy conditions in 1993.

	g_s	C_i	A/g_s	Total biomass	WUE_{sl}	Shoot nitrogen	Δ†	Shoot biomass	Root biomass
A	0.97**	-0.58*	0.37	-0.11	-0.22	-0.22	0.26	-0.11	-0.01
g_s		-0.44	0.20	-0.22	-0.32	-0.15	0.28	-0.21	-0.09
C_i				-0.33	-0.28	0.22	-0.25	-0.35	-0.02
A/g_s				0.45	0.41	-0.26	0.42	0.46	0.05
Total biomass					0.97**	-0.34	-0.05	0.97**	0.33
WUE_{sl}						-0.30	-0.09	0.96**	0.24
Shoot nitrogen							-0.07	-0.51	0.56
Δ								-0.01	-0.17
Shoot biomass									0.08

*, **, Significant at P = 0.05 and 0.01, respectively.
† Determined in whole plant tissue.

Table 11. Correlation coefficients for the genotypic association between different parameters measured under field conditions in 1993.

	g_s	C_i	A/g_s	Shoot nitrogen	Δ†	Shoot biomass
A	0.89**	0.37	-0.67*	0.41	-0.28	0.13
g_s		0.68*	-0.93**	0.53	-0.14	0.05
C_i				0.32	0.30	0.08
A/g_s				-0.55	-0.05	-0.03
Shoot nitrogen					-0.26	0.34
Δ						-0.10

*, **, Significant at P = 0.05 and 0.01, respectively.
† Determined in whole plant tissue.

Table 12. Correlation coefficients for the genotypic associations between mean carbon isotope discrimination (Δ) determined in leaf tissue and different parameters measured under field conditions in 1993.

Canopy	A	g_s	C_i	A/g_s	Total biomass	Shoot biomass	Root biomass	WUE_{si}
				1993				
Closed	-0.02	0.09	0.20	-0.09	-0.46	-0.46	0.12	-0.57†
Open	0.53	0.45	-0.68*	0.69*	-0.04	-0.07	0.10	-0.14
Field	0.09	0.36	0.65*	-0.56†	n.a.	0.29	n.a.	n.a.

*, **, † Significant at P = 0.05, 0.01, and 0.06, respectively.
n.a. = Not available.

Table 13. Mean net CO_2 assimilation (A), stomatal conductance to H_2O (g_s), intercellular CO_2 concentration (C_i), water-use efficiency (A/g_s), total biomass, shoot biomass, root biomass, season-long water-use efficiency (WUE_{sl}), and Δ for wheat cultivars grown under three different canopy densities.

Canopy	A	g_s	C_i	A/g_s	Total biomass	Shoot biomass	Root biomass	WUE_{sl}	Δ
	$\mu mol\ m^{-2}s^{-1}$	$mol\ m^{-2}s^{-1}$	$\mu L\ L^{-1}$	$\mu mol\ mol^{-1}$	$g\ plant^{-1}$	$g\ plant^{-1}$	$g\ plant^{-1}$	$g\ kg^{-1}$	‰
					1992				
Closed	14.8	0.29	214	67.9	57.0	48.9	8.1	5.3	18.21
Open	17.4	0.30	204	76.2	59.8	51.1	8.8	5.7	17.27
Field	15.5	0.23	196	76.5	n.a.	38.6	n.a.	n.a.	18.54
LSD (0.05)	2.0	0.05	11	7.1	2.7	5.5	0.6	0.3	0.23
					1993				
Closed	15.4	0.18	175	89.1	70.9	62.8	8.1	6.7	18.70
Open	11.4	0.12	173	95.7	70.6	60.1	10.5	6.6	17.59
Field	19.7	0.26	177	79.7	n.a.	93.3	n.a.	n.a.	19.47
LSD (0.05)	1.6	0.02	9	5.0	4.3	9.3	1.6	0.3	0.29

n.a. = Not available.

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CHAPTER III

EFFECT OF DROUGHT STRESS ON GAS EXCHANGE PROPERTIES

ABSTRACT

Leaf exchange of CO_2 and H_2O was determined in six growth-chamber grown winter wheat cultivars (*Triticum aestivum* L. cvs Chisholm, Cimarron, Abilene, Pioneer 2157, Quantum 554, and Cody) previously selected based on their stable carbon isotope discrimination (Δ). The photosynthetic capacity was determined and broken down into light- and CO_2-saturated rate of photosynthesis (A_{max}), carboxylation efficiency (c.e.), and stomatal conductance (g_s) components. Leaf water potential (ψ) declined with increasing water stress. Significant genotypic differences among cultivars were observed when A_{350}, A_{max}, g_s, c.e. and intrinsic gas exchange efficiency (A/g_s) were regressed on leaf ψ. Seven days after withholding water, drought stress lowered A_{350} (net photosynthesis rate measured at 350 μL CO_2 per L air), A_{max}, gs, and c.e by approximately 70, 60, 70, and 60%, respectively. Chisholm had lower stomatal limitation (L_{gs}) than the other cultivars suggesting that the latter cultivars were relatively more limited by mesophyll capacity. Abilene had consistently higher A/g_s than the other cultivars, due mainly to the lower mean g_s and the greater c.e. shown through the experiment. Based on the Δ values from field grown plants, Abilene was expected to have the lowest gas exchange efficiency and Chisholm the highest. Abilene unexpectedly maintained a high WUE under water stress in the growth chamber.

INTRODUCTION

Rate of photosynthesis (**A**) under drought conditions is reduced to a significant extent by stomatal closure (Brix, 1962; Farquhar and Sharkey, 1982) and, possibly, by decreased mesophyll capacity (Boyer, 1971; Hutmacher and Krieg, 1983; Matthews and Boyer, 1984; Gunasekera and Berkowitz, 1993). Stomatal closure may be caused by a decrease in plant water potential (ψ) or by an increase in the water vapor difference between the leaf and the air. Decreased stomatal conductance (g_s) produces a reduction in CO_2 diffusion rates into the leaf resulting in a lowering of the intercellular carbon dioxide concentration (C_i) relative to the ambient CO_2 concentration (C_a). Under ambient conditions low intercellular CO_2 concentration limits A. However it can be overcome by increasing the partial pressure of CO_2 in the air surrounding the leaf.

Impaired mesophyll capacity at low leaf ψ reduces the ability to fix available CO_2 resulting in elevated C_i. Chloroplast activity inhibition cannot be fully overcome by increasing the partial pressure of CO_2 in the air surrounding the leaf as can stomatal constriction and any other diffusion restriction.

Matthews and Boyer (1984) conducted an study in sunflower plants (*Helianthus annuus* L.) to evaluate the contribution of stomata and chloroplasts to the acclimation of A to low leaf ψ. The plants were subjected to water deficit pretreatments for two weeks. Gas exchange measurements showed similar A for rewatered acclimated

plants and control (non-acclimated) plants. When leaf ψ was lower (-1.3 to -1.4 MPa), A was inhibited at all photosynthetic photon flux densities (PPFD), but more in control plants than in acclimated plants. The differences in inhibition of A at low PPFD were not overcome at high PPFD. These differences, where photochemical conversion was limiting, suggested that changes occurred at the chloroplast level. Quantum yields for CO_2 fixation measured at low PPFD showed that at leaf ψ between -1.8 and -2.0 MPa values ranged from 0.005 to 0.009 mol CO_2 mol^{-1} quanta for controls and from 0.024 to 0.034 mol CO_2 mol^{-1} quanta for acclimated plants. Their results showed that nonstomatal acclimation was the major contributor to acclimation of A in intact leaves. This study suggested that there were losses of chloroplast activity at low leaf ψ.

Leaf gas exchange characteristics of a desert annual wild wheat (*Triticum kotschyi* [Boiss.] Bowden) and the wheat cultivar TAM W-101 (*Triticum aestivum* L.) were studied over a range of leaf ψ from -0.5 to -2.9 MPa (Johnson et al., 1987). The A *versus* C_i response curves under well watered conditions were similar in shape between species. Under well watered conditions *Triticum kotschyi* had higher g_s, C_i and A, and lower water use efficiency (WUE) than *Triticum aestivum* at an ambient CO_2 concentration of 330 $\mu L\ L^{-1}$. Hence, g_s had greater control on A than had the mesophyll capacity. At lower leaf ψ (-2.2 MPa) little difference in C_i at an ambient CO_2 concentration of 330 $\mu L\ L^{-1}$ between species was observed. *T. kotschyi* had higher A under water stress than *T. aestivum* basically because its A vs C_i response curves had higher A at C_i values above about 150 $\mu L\ L^{-1}$. They concluded that g_s was very important in maintaining the high A of *T. kotschyi* under well watered conditions, but under water deficits the high A of *T. kotschyi* was related more to the maintenance of a higher mesophyll capacity. In a previous study (Wong et al., 1979), *T. kotschyi* had

higher photosynthesis under well watered conditions and at reduced ψ compared to *T. aestivum*.

Sobrado (1990) studied the gas exchange characteristics of six tropical cultivars of corn (*Zea Mays* L.). Glasshouse-grown plants were subjected to water stress or to irrigated conditions over the entire experiment. The g_s decreased from 0.8 mol m^{-2} s^{-1} at a leaf ψ of -0.8 MPa (irrigated) to about 0.02 mol m^{-2} s^{-1} at a leaf ψ of approximately -1.6 MPa. Photosynthetic rate was approximately 38 μmol m^{-2} s^{-1} in irrigated plants, and declined gradually at lower leaf ψ until it reached about 1 μmol m^{-2} s^{-1} at ψ of -1.6 MPa. Little variation in gas exchange characteristics among cultivars was observed indicating that the genetic potential for improving net photosynthetic rate of tropical corn under drought conditions may be limited.

Ritchie et al. (1990) compared the leaf relative water content (RWC) and gas exchange parameters between a drought-resistant winter wheat cultivar (TAM W-101) and a drought-susceptible cultivar (Sturdy) subjected to water stress. Measurements were made at anthesis or after four weeks of vegetative growth. Relative water content remained relatively stable in both cultivars through the first 5 days after watering was stopped. In the vegetative experiment the RWC of Sturdy plants began to decline sharply after the fifth day and in TAM W-101 after the seventh day. In both growth stages, TAM W-101 maintained a higher RWC and A than Sturdy under moderate to severe drought stress. In both experiments stomatal conductance declined before RWC declined. These results support findings reported by Schonfeld et al. (1988), who showed that RWC was maintained significantly higher in TAM W-101 than in Sturdy plants exposed to water stress. TAM W-101 tended to have higher WUE (A/g_s) than Sturdy under moderate to severe stress conditions on a RWC basis

(Ritchie et al., 1990). The authors concluded that high leaf RWC and A are traits that may contribute to drought resistance in TAM W-101. Earlier, Johnson et al. (1984) reported that TAM W-101 plants undergo greater osmotic adjustment under stress than Sturdy plants.

Soldatini and Guidi (1992) investigated the photosynthetic activity of sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* L.) plants subjected to water stress, and the process of recovery from desiccation when the plants were rewatered. In sunflower, A at saturating light intensities became progressively lower as the water stress treatment continued. After seven days of treatment A was 22% of the control. Stomatal conductance decreased parallel to A. Plants were rewatered and allowed to recover for three days. The recovery of A was about 70% compared to the control. Stomatal conductance recovered only to 50% of the control levels. Soybean plants responded very rapidly to water stress. Photosynthetic rate decreased by 61% after only 24h of water stress and 83% after 48h. Plants were rewatered and allowed to recover for 7 days after which A reached 50% and g_s reached 65% of the control.

Earlier Berkowitz and Gibbs (1983) and Kaiser et al. (1981) reported that reduced stromal volume may be the reason for the inhibition of A. Gupta and Berkowitz (1988) studied the effects of continuous plant water deficit on spinach plants (*Spinacia oleracea* L.). During the first six days of the stress cycle, leaf ψ declined by 0.63 MPa, RWC by 12.5%, and photosynthetic rate by 4.0%. Eleven days after the stress cycle was initiated, A was inhibited by 56.5%. Gas exchange results suggested that chloroplast photosynthetic capacity declined mainly during the later part of the stress cycle. Photosynthetic capacity of chloroplasts isolated from plants subjected to stress was also studied. During the first six days of water deficit, the CO_2-saturated

photosynthetic rates decreased by 3.9%. At the end of the stress cycle the photosynthetic rate of chloroplasts declined in parallel with leaf photosynthesis. Chloroplast stromal volume appeared to be maintained when leaf ψ dropped from -0.29 MPa to -0.83 MPa (RWC declined by 22%). As RWC dropped below 65% (and leaf ψ approached -1.2 MPa), stromal volume started to decline. The apparent maintenance of stromal volume over the initial phase of the stress cycle suggested that chloroplasts were capable of osmotic adjustment in response to leaf water deficits. Stromal volume maintenance could be attributed to solute accumulation above that occurring in the cell as a whole.

Santakumari and Berkowitz (1991) studied the association between nonstomatally-mediated acclimation of A to low leaf ψ and the maintenance of chloroplast volume during water stress in spinach (*Spinacia oleracea* L.). Photosynthesis was inhibited as leaf ψ declined in both acclimated and non-acclimated spinach plants. At ψ below -1.0 MPa photosynthesis rate was greater in acclimated plants. At ψ of -1.2 MPa, photosynthesis in non-acclimated and acclimated plants was 10.2 and 18.3 μmol m^{-2} s^{-1}, respectively. Calculations of C_i at high and low ψ indicated that non-stomatal factors contributed to the acclimation of photosynthesis to low leaf ψ. At high ψ, C_i was similar in acclimated and non-acclimated plants. At low ψ, C_i was significantly higher in non-acclimated plants. Since patchy stomatal closure does not seem to occur in spinach, the authors concluded that the derived relationship between photosynthesis and C_i is valid. Chloroplast volume was maintained constant in both acclimated and non-acclimated plants at ψ greater than -0.8 MPa. As leaf ψ declined below -0.8 MPa, chloroplast volume was reduced in the leaves of non-acclimated plants. These results support the hypothesis that cellular

level acclimation to low ψ is associated with maintenance of chloroplast volume and with differences in photosynthesis evident during the latter part of the drought treatments.

Gimenez et al. (1992) studied the effect of short-term water stress on A of two sunflower hybrids (*Helianthus annuus* L.) differing in productivity under field conditions. Under well-watered conditions (ψ = -0.6 MPa) and at low C_a, photosynthetic rate was not statistically different between cultivars. However, at CO_2 saturation there was a 30% difference. At lower leaf ψ (-2.7 MPa), photosynthesis rate decreased to 12 and 10% of the control values. In both hybrids the rate of light- and CO_2-saturated photosynthesis (A_{max}) decreased linearly with decreasing leaf ψ. With water stress the g_s of both hybrids decreased linearly to the same value (0.05 mol m^{-2} s^{-1}) at about -1.2 MPa with a very similar decrease of cell volume (11 and 13%). The decrease in A at low leaf ψ was not caused by low g_s because maintaining C_i at saturation did not increase A, suggesting that metabolic damage was limiting A.

Castonguay (1992) studied the leaf gas exchange characteristics of common bean (*Phaseolus vulgaris* L.) and tepary bean (*Phaseolus acutifolius* A.) which is adapted to hot arid conditions. Both species were subjected to controlled levels of water stress. *Phaseolus acutifolius* had higher A than *P. vulgaris* at high to moderately low leaf ψ. Photosynthetic rates of *P. acutifolius* declined faster with more negative leaf ψ than those of *P. vulgaris* due to reduced stomatal conductance. *Phaseolus acutifolius* showed higher carboxylation efficiency (c.e.) than *P. vulgaris.* Similar mesophyll palisade surface area in both genotypes suggested that differences in A at low C_i may be related to biochemical rather than anatomical leaf characteristics. A

higher WUE combined with stomata that are more sensitive to a decline in ψ could contribute to make *P. acutifolius* a species with better adaptation to arid environments.

Martin and Ruiz-Torres (1992) investigated the effects of water-deficit stress on photosynthesis in two winter wheat cultivars (TAM W-101 and Sturdy). No differences between cultivars were observed when A_{350} (rate of net photosynthesis at an ambient CO_2 concentration of 350 $\mu L\ L^{-1}$) was compared at identical leaf ψ. A_{350}, A_{max}, g_s, and c.e. decreased with decreasing leaf ψ. A_{350} was reduced from 24.1 to 4.7 $\mu mol\ m^{-2}\ s^{-1}$ and A_{max} from 32.2 to 9.5 $\mu mol\ m^{-2}\ s^{-1}$ when leaf ψ drop from -0.84 MPa to -2.0 MPa. Drought-inhibition of chloroplast biochemistry was suggested by the association between A_{max} and leaf ψ, and by decreasing photosynthetic O_2 evolution rate measured at 5% C_a with a leaf disc electrode. Ephrath et al. (1993) also observed a decrease in g_s and A in water stressed cotton plants (*Gossypium hirsutum L.*) grown under field conditions.

This research was conducted to determine the effects of water stress on A_{350}, A_{max}, c.e., A/g_s, and other photosynthetic parameters in six pot-grown winter wheat cultivars selected to span a wide range of stable carbon isotope discrimination values.

MATERIALS AND METHODS

Plant material. Six winter wheat genotypes (*Triticum aestivum* L. cvs. Chisholm, Cimarron, Abilene, Pioneer 2157, Quantum 554 and Cody) were selected from among 24 cultivars grown in the field (Cai, 1992). Selection was based on their different stable carbon isotope discrimination value (Δ): Chisholm 17.58, Pioneer 2157 17.70, Quantum 554 17.96, Cimarron 18.05, Cody 18.57, and Abilene 18.96.

Plants from the six cultivars were grown in a Sherer Controlled Environment Chamber, model CEL 25-7 HL (Sherer-Gillett Co., Marshall, MI). Three seeds were planted per pot holding 1.3 L of Soil Conditioner (Green Country Soil, Inc., Miami, OK). After a week the seedlings were thinned to one per pot. The growth chamber was maintained at 25°C/18°C day/night temperature, 14 h/10 h day/night length (600 μmol m^{-2} s^{-1} PAR at pot level) and 50% RH. The pots were watered every other day with Peters 20-20-20 (W.R. Grace & Co., Allentown, PA). Unvernalized plants of the six genotypes were grown for 5 weeks under well-watered conditions before water deficits were imposed by withholding water from the pots. Plants were brought from the growth chamber to the laboratory and an intact young fully expanded leaf was selected for measurement. Gas exchange measurements were made over a period of 7 days with a greater stress level (lower leaf ψ) reached every day.

Gas-exchange measurements. Measurements of photosynthesis rate (A), stomatal conductance (g_s) and other parameters were made on intact leaves using a

temperature controlled infrared gas analysis system described by Bingham et al. (1980), Coyne et al. (1982), and Johnson et al. (1987). Leaves were measured at 1800 μmol m^{-2} s^{-1} PAR, 25°C and 50% RH. Humidity in the assimilation chamber was measured with a condensation dew-point hygrometer (General Eastern 1100DP, Watertown, MA) and CO_2 concentration was regulated by passing through the cuvette CO_2-free air from a compressed air cylinder mixed at different ratios with air from another cylinder containing 1700 μL L^{-1} CO_2. Carbon dioxide concentration in the air (C_a) was monitored by passing the chamber exhaust through a differential infrared gas analyzer (PIR 2000 R, Horiba Instrument, Inc., Irvine, CA) followed by adjustment to the desired value. The A versus intercellular CO_2 concentration (C_i) curves were generated by measuring A at about 14 C_a values ranging from 0 to 1700 μL L^{-1}. A concentration of 350 μL CO_2 per L air was used as normal ambient CO_2 concentration.

The A/C_i curve represents the observed photosynthetic demand function, which is the dependence of rate of CO_2 assimilation on the CO_2 concentration in the intercellular air spaces. Conductance to CO_2 (g_s) and C_i were calculated according to von Caemmerer and Farquhar (1981). A_{max} was measured as the greatest A (light- and CO_2-saturated). Carboxylation efficiency was determined from the initial slope at low C_i of the A/C_i curve. The stomatal limitation (L_{gs}) was obtained as described by Farquhar and Sharkey (1982), and the intrinsic gas exchange efficiency A_{350}/g_s was used as a measure of WUE.

Water potential (ψ). Leaf water status was followed by monitoring Ψ on leaves subsequent to their use for gas exchange measurements. Two 0.31 cm^2 leaf disks were collected from each leaf by using leaf cutter psychrometers (J.R.D. Morril

Specialty, Logan, UT). The psychrometers were connected to a microvolt meter (Wescor HP-115, Logan, UT) and Ψ was determined after 4 h equilibration of the psychrometers in a 30°C water bath.

Relative water content (RWC). The remaining leaf section was used for the RWC analysis. The leaf samples were sliced into 2 or 3 sections (depending on the length of the leaf), weighed to obtain fresh weight (FW), then rehydrated in distilled water for 24 h at 4°C to obtain turgid weight. Finally, leaves were oven dried for 72 h at 70°C to obtain dry weight. Relative water content was determined as

$$RWC = \frac{fresh\ weight - dry\ weight}{turgid\ weight - dry\ weight}$$

RESULTS AND DISCUSSION

Leaf ψ declined with increasing water stress (Table 1). Cimarron plants showed higher leaf ψ (-0.46 MPa) than the other cultivars under unstressed conditions. Seven days after withholding water from pots, Chisholm exhibited significantly higher leaf ψ (-1.11 MPa) than the rest of the cultivars. The largest decrease in leaf ψ was observed in Abilene, in which leaf ψ dropped from -0.59 MPa (control) to -3.10 MPa seven days after withholding water. Relative water content declined approximately 15% relative to the unstressed plants over the entire drought period. Cimarron, Cody, and Chisholm showed higher RWC at the final phase of the stress period than the other three cultivars.

Net photosynthesis rate at normal ambient CO_2 concentration of well watered plants ranged from 21.64 μmol m^{-2} s^{-1} in Abilene to 17.07 μmol m^{-2} s^{-1} in Cimarron (Table 1). Drought stress lowered A_{350} by approximately 70% by day 7 when A_{350} ranged from 6.45 μmol m^{-2} s^{-1} in Cody to 3.06 μmol m^{-2} s^{-1} in Quantum 554. Significant differences among cultivars were detected when A_{350} was related to leaf ψ (Table 3). Figures 1a and 1b show the relationship between leaf ψ and A_{350}. The A_{350} showed a polynomial decrease with drought-induced reduction in leaf ψ. Johnson et al. (1987) reported that reduction in leaf ψ significantly decreased A in wheat. Similar findings were reported by Gupta and Berkowitz (1988) in spinach, Graan and Boyer (1990) in sunflower, and Martin and Ruiz-Torres (1992) in winter wheat. The light- and

CO_2-saturated rate of photosynthesis was measured in unstressed and stressed plants (Table 1). A_{max} is a measure of steady-state chloroplast capacity to generate NADPH and/or ATP. Unstressed plants of Abilene and Pioneer 2157 showed higher A_{max} than the other cultivars. Seven days after withholding water, A_{max} was decreased approximately 60% relative to the control. Martin and Ruiz-Torres (1992) reported that water stress reduced A_{max} 3.8 fold when leaf ψ dropped from -0.84 to -2.0 MPa in two winter wheat cultivars (TAM W-101 and Sturdy).

Although no differences were observed among cultivars when C_i was regressed on leaf ψ (Table 3), C_i generally decreased as leaf ψ declined (Table 1). By relating C_i to leaf ψ, one is able to distinguish between stomatal and mesophyll limitation of photosynthesis (Johnson et al., 1987). Intercellular CO_2 concentration increases when the mesophyll is damaged by decreasing leaf ψ. On the other hand, if the C_i is reduced with decreasing leaf ψ, then photosynthesis has become more limited by stomata relative to chloroplast biochemistry. In this study, C_i decreased as leaf ψ decreased in five of the six cultivars (Abilene, Cody, Pioneer 2157, Cimarron, and Chisholm), suggesting that the stomatal limitation was greater than the mesophyll limitation. For Quantum 554, photosynthesis was more prominently limited by the mesophyll. Castonguay and Markhart (1992) showed that limitation to photosynthesis in beans is more affected by reductions in g_s than by reductions in mesophyll capacity. Johnson et al. (1987) working with two different wheat species (*Triticum kotschyi* and *Triticum aestivum*), reported that with water deficits the average C_i of both species first declined as leaf ψ declined, but then increased when ψ fell past -1.9 MPa. This response is similar to the one observed in Quantum 554.

Carboxylation efficiency (a measure of Calvin-cycle activity) decreased with

declining leaf ψ (Table 2). Seven days after withholding water, a reduction in c.e. of about 60% was observed. Abilene maintained a higher c.e. than the other cultivars through the entire experiment. Regression analysis showed a polynomial relationship between leaf ψ and c.e. There were significant differences at the 1% level among cultivars (Table 3). According to the von Caemmerer and Farquhar (1981) model of photosynthesis in C_3 plants, the initial slope of a A/C_i curve is a function of the activity of the Calvin cycle, of which rubisco is a key component. In a related study (Chapter III), it was shown that Abilene had higher initial and total rubisco activities than Chisholm and Cody (Table 1, Chapter III) in plants subjected to water deficits. In the same experiment Chisholm had higher rubisco initial activity than Cody. These enzyme data support the outcome of our gas exchange measurements. However, it is very important to note that rubisco activity was not affected to the same magnitude as A and c.e. in this experiment. These results suggest that rubisco is not the main site affected by severe water stress in wheat. The main biochemical limitation must reside in some other part of the Calvin cycle as previously suggested by Sharkey and Seeman (1989).

Regression analysis showed genotypic differences in g_s among cultivars as leaf ψ declined (Table 3). As expected, reduction in leaf ψ caused a reduction in g_s. Stomatal conductance in Chisholm was more sensitive to decreased leaf ψ than the other five cultivars (Figures 2a and 2b). On the other hand, Abilene initially had low g_s and it proved to be proportionally less sensitive to decreasing leaf ψ.

Figures 3a and 3b show L_{gs} as a function of g_s for all six cultivars. The logarithmic relationship showed significant differences among cultivars (Table 3). L_{gs} increased as conductance decreased (Figures 3a and 3b, Table 2). On the average,

L_{gs} increased about 50% in plants subjected to water deficit for seven days (Table 2).

As expected from theory (Cowan and Troughton, 1971), intrinsic WUE increased as g_s declined (Figures 4a and 4b). This negative relationship results from the linear increase in the rate of transpiration with increasing g_s, coinciding with a relatively smaller increase in A when A approaches CO_2 saturation at high g_s. In this experiment, significant genotypic differences in WUE were found with decreasing g_s (Table 3). Abilene had consistently higher WUE than the other five cultivars. The greater WUE observed in Abilene was due to the lower mean g_s and the greater c.e. shown through the complete experiment. Cimarron and Chisholm showed an increase in WUE of 2.2 and 2.4 fold, respectively, whereas WUE in Abilene increased just 1.9 fold due to the high WUE of the unstressed plants. Ritchie et al. (1990) reported that WUE generally increased with water stress until very severe stress levels were attained. TAM W-101 had higher WUE than Sturdy under water deficits, but not under well watered conditions. Comparisons were made on RWC basis to avoid the confounding effect of stress severity on WUE. In this experiment the pattern observed by Ritchie et al. (1990) in WUE with declining g_s was not detected. In a similar study, Martin and Ruiz-Torres (1992) observed that WUE initially increased in both TAM W-101 and Sturdy, as g_s decreased. But based on the predicted curve fit, below about 0.25 mol H_2O m^{-2} s^{-1} WUE decreased. Therefore, at extremely low g_s inhibition of the mesophyll photosynthetic capacity may be responsible for the drop in WUE.

In relation to the Δ values previously observed in field-grown plants, Chisholm was supposed to have the highest WUE value and Abilene the lowest. Opposite to the anticipated, Abilene had the ability to maintain high WUE in pot grown-plants. An explanation for this is that gas exchange measurements in pot-grown plants do not

reflect genetic variation in Δ determined in field-grown plants. In this experiment, wide differences in growth stage for different measurements in the field and in the growth chamber played an important role.

SUMMARY

The continuous decline with lower leaf ψ of A_{350}, g_s, C_i and c.e. (Figures 1a and 1b, and Tables 1 and 2) implies that both reduced g_s and impairment of the Calvin cycle accompanied the negative effect on A_{350}. Abilene had higher A_{350}, A_{max}, c.e. and WUE than the other cultivars under nonstressed conditions. It also showed low g_s and C_i. Abilene appears to have a uniquely high mesophyll capacity from the other cultivars. This was supported by the large L_{gs} observed during the entire experiment. Based on the Δ values used as a selection criterion prior to the initiation of the experiment, Abilene was expected to have the lowest intrinsic gas exchange efficiency value and Chisholm the highest. Contrary to the expected, when different traits were evaluated under water stress in the growth chamber, Abilene was able to maintain a high intrinsic gas exchange efficiency.

In general, CO_2 uptake in wheat plants exposed to water deficit stress is limited by both mesophyll capacity and stomatal conductance. The degree at which each one of them affects CO_2 fixation rate, depends mainly on the genetic makeup of the cultivars.

Table 1. Photosynthetic parameters measured on intact leaves of winter wheat genotypes at different leaf ψ.

Days of withholding water	Genotypes	ψ	RWC	A_{350}§	A_{max}	C_i§
d		MPa	%	μmol m^{-2} s^{-1}	μmol m^{-2} s^{-1}	μL L^{-1}
0	Abilene	-0.59b*	89.9ab	21.64a	32.20a	263c
	Cody	-0.78c	90.2ab	17.99bc	22.84bc	304a
	Pioneer 2157	-0.61b	93.0a	21.37ab	33.45a	283b
	Quantum 554	-0.85c	91.5a	18.45abc	26.80b	310a
	Cimarron	-0.46a	88.3ab	17.07bc	21.25c	318a
	Chisholm	-0.61b	90.7ab	18.10bc	25.10bc	313a
3	Abilene	-0.95c	87.9c	17.28ab	26.17a	246c
	Cody	-0.88bc	84.5d	13.62bc	18.38bc	308ab
	Pioneer 2157	-0.84ab	91.0ab	14.37bc	24.05ab	278bc
	Quantum 554	-0.92bc	89.9bc	15.27abc	22.43abc	310ab
	Cimarron	-0.78a	82.6d	12.53c	17.66c	314a
	Chisholm	-0.78a	92.5a	19.11a	26.54a	312ab
5	Abilene	-1.61c	82.6c	10.02c	16.32a	190b
	Cody	-0.96a	86.8b	12.35ab	18.05a	280a
	Pioneer 2157	-1.37b	87.3b	10.53ab	17.75a	249a
	Quantum 554	-0.99a	88.5b	9.41c	13.73a	288a
	Cimarron	-1.11a	91.1a	9.67c	17.41a	267a
	Chisholm	-0.96a	88.6b	13.64a	20.10a	270a
7	Abilene	-3.10e	76.5ab	5.15ab	9.87ab	198c
	Cody	-1.63b	82.1a	6.45a	12.94a	279ab
	Pioneer 2157	-2.20d	77.8ab	5.04ab	1.16ab	255b
	Quantum 554	-1.76c	72.8b	3.06c	4.27c	301a
	Cimarron	-1.61b	80.9a	4.35ab	7.58bc	260b
	Chisholm	-1.11a	79.0a	5.55ab	9.84ab	260b

§ Measured at an ambient CO_2 concentration of 350 μL L^{-1}; C_i, intercellular CO_2 concentration.

* Within columns and days after water withholding, means followed by the same letter are not significally different at the 0.05 probability level.

Table 2. Other photosynthetic parameters measured on intact leaves of winter wheat genotypes at different leaf ψ.

Days of withholding water	Genotypes	ψ	c.e	g_s	L_{gs}	A_{350}/g_s
d		MPa	μmol m^{-2} s^{-1}	mol m^{-2} s^{-1}	%	μmol mol^{-1}
0	Abilene	-0.59b	0.103a	0.270c	18.02a	80.80a
	Cody	-0.78c	0.068cd	0.434ab	7.52cd	42.25c
	Pioneer 2157	-0.61b	0.094a	0.376bc	14.44ab	61.27b
	Quantum 554	-0.85c	0.077b	0.480ab	11.38bc	39.43c
	Cimarron	-0.46a	0.060d	0.478ab	5.55d	36.25c
	Chisholm	-0.61b	0.077b	0.531a	7.12cd	34.01c
3	Abilene	-0.95c	0.089a	0.194c	24.24a	92.76a
	Cody	-0.88bc	0.054cd	0.361abc	8.32b	39.00bc
	Pioneer 2157	-0.84ab	0.072b	0.318bc	17.40a	66.41ab
	Quantum 554	-0.92bc	0.059c	0.408ab	8.60b	38.29bc
	Cimarron	-0.78a	0.048d	0.413ab	6.50b	30.52c
	Chisholm	-0.78a	0.075b	0.513a	7.94b	38.66bc
5	Abilene	-1.61c	0.067a	0.065b	34.76a	155.33a
	Cody	-0.96a	0.042b	0.188ab	17.13b	72.96bc
	Pioneer 2157	-1.37b	0.052ab	0.164ab	22.78ab	120.49ab
	Quantum 554	-0.99a	0.048b	0.177ab	11.85b	57.73c
	Cimarron	-1.11a	0.048b	0.131ab	18.00b	74.11bc
	Chisholm	-0.96a	0.051b	0.204a	18.62b	74.72bc
7	Abilene	-3.10e	0.042a	0.035c	38.14a	155.80a
	Cody	-1.63b	0.040ab	0.104a	11.33c	62.01c
	Pioneer 2157	-2.20d	0.029cd	0.050bc	27.54ab	100.40b
	Quantum 554	-1.76c	0.026d	0.077ab	6.05c	51.23c
	Cimarron	-1.61b	0.018e	0.056bc	26.62ab	77.94bc
	Chisholm	-1.11a	0.034bc	0.069abc	15.15bc	80.23bc

* Within columns and days after water withholding, means followed by the same letter are not significally different at the 0.05 probability level.

Table 3. Summary of best fit regressions among photosynthetic parameters measured in six winter wheat genotypes.

Trait	ψ	g_s	c.e.	L_{gs}	A_{350}/g_s
	MPa	mol m^{-2} s^{-1}	mol m^{-2} s^{-1}	%	µmol mol^{-1}
ψ	-	Pol*	Pol**	Lr*	Lr*
A_{350}	Pol*	Ln	Lr	Lr*	Lr
A_{max}	Pol*	Ln	Lr	Lr*	Lr
C_i	Pol	Ln	Lr	Pol**	Ln**
c.e.	-	Pol*	-	Pol	Lr
g_s	-	-	-	Ln**	Ln**
L_{gs}	-	-	-	-	Ln*

Pol = polynomial fit (quadratic); Lr = linear fit; Ln = logarithmic fit.
*,** Significant differences among genotypes at P = 0.05 and P = 0.01, respectively.

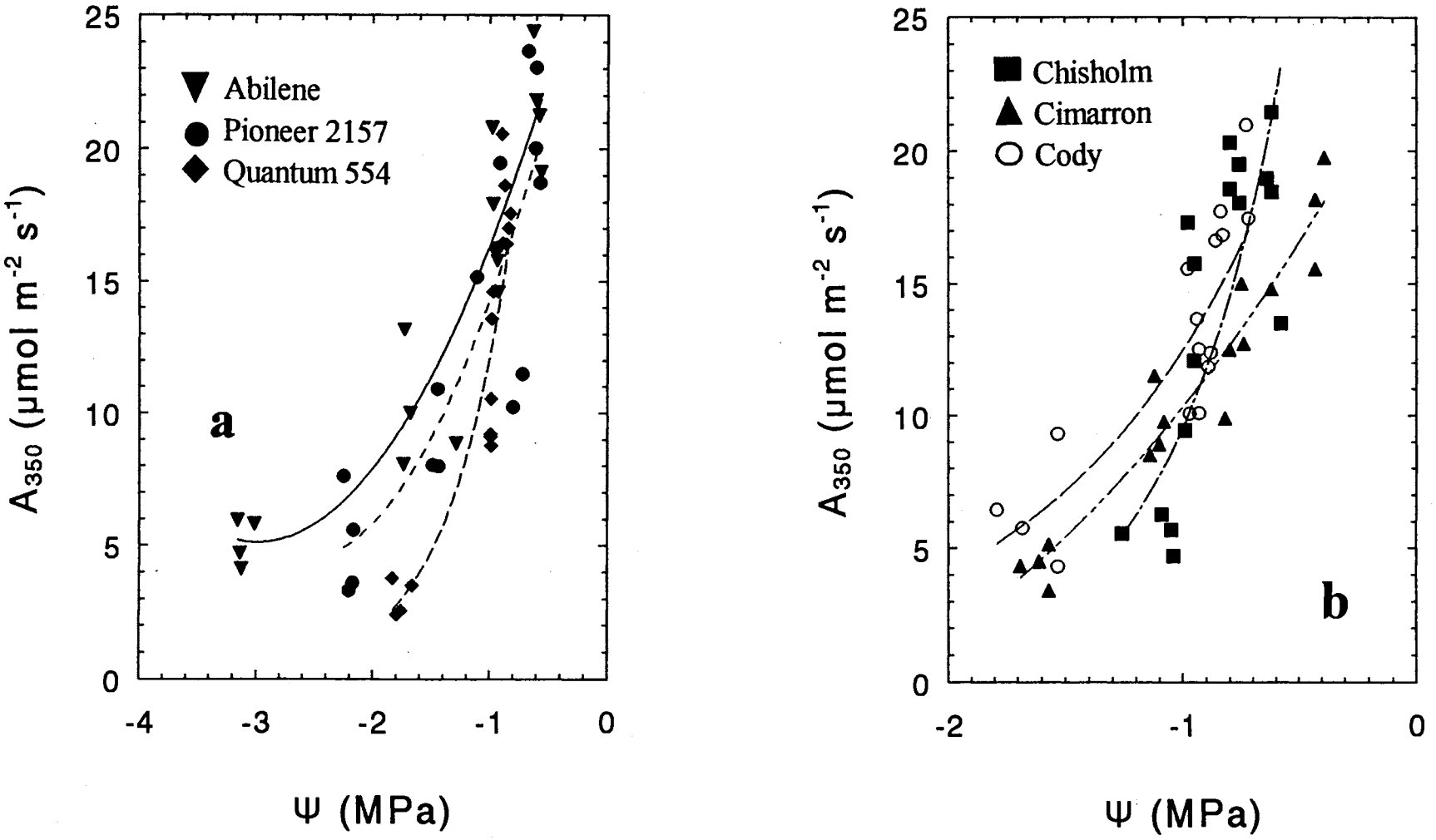

Figure 1. (a) Dependence of A_{350} on leaf ψ. ▼, cv Abilene; ●, cv Pioneer 2157; ♦, cv Quantum 554. (b) ■, cv Chisholm; ▲, cv Cimarron; ○, cv Cody. Abilene, A_{350} = 30.52 + 17.01 x ψ + 2.85 x ψ^2, r = 0.94; Pioneer 2157, A_{350} = 30.49 + 20.10 x ψ + 3.88 x ψ^2, r = 0.87; Quantum, 554 A_{350} = 85.61 + 110.37 x ψ + 35.93 x ψ^2, r = 0.95; Chisholm, A_{350} = 8.92 - 37.50 x ψ - 34.60 x ψ^2, r = 0.82; Cody, A_{350} = 52.23 + 60.02 x ψ + 19.43 x ψ^2, r = 0.97; Cimarron, A_{350} = 24.16 + 16.52 x ψ + 2.68 x ψ^2, r = 0.87.

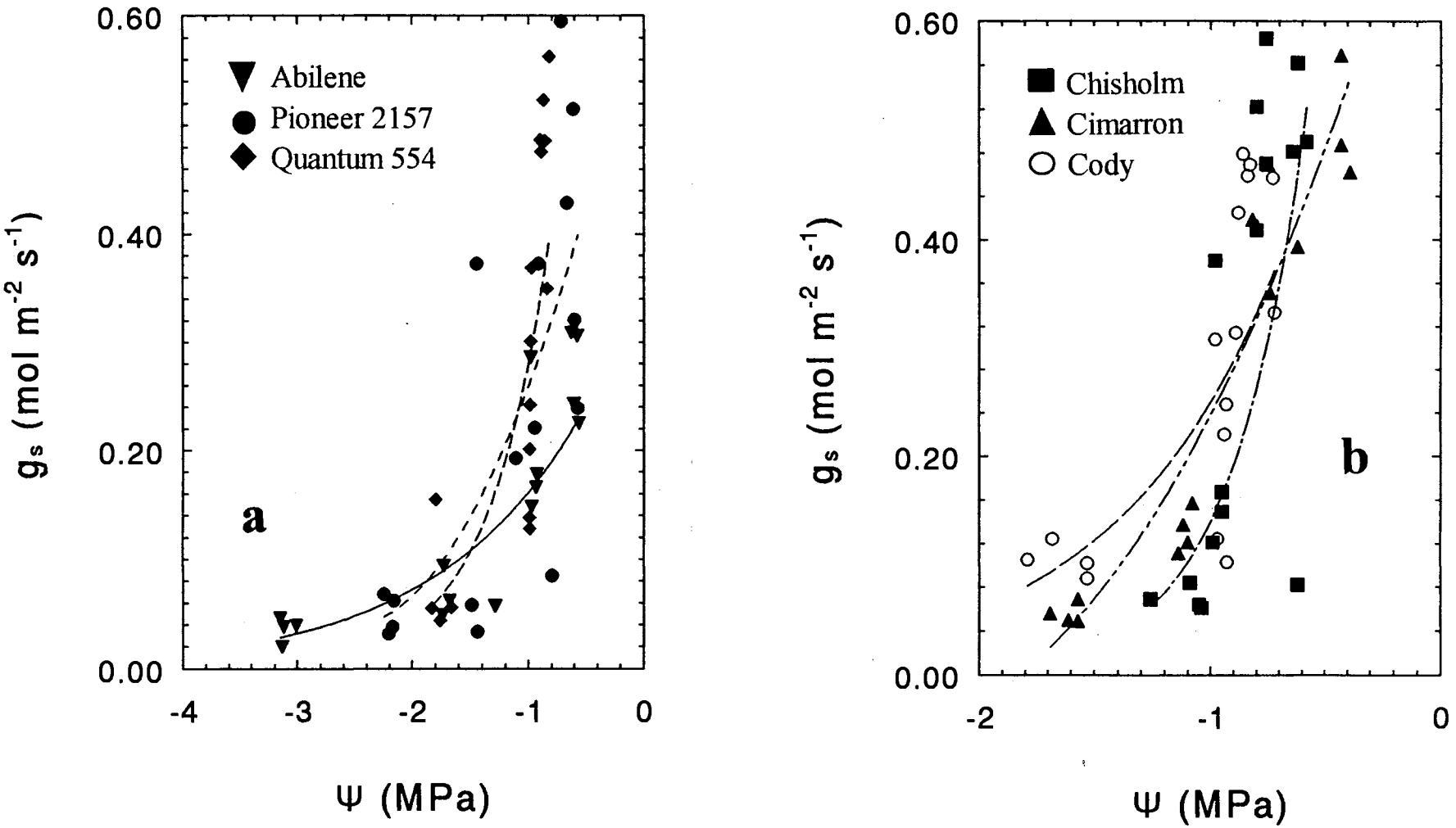

Figure 2. (a) Dependence of g_s on leaf ψ. ▼, cv Abilene; ●, cv Pioneer 2157; ♦, cv Quantum 554. (b) ■, cv Chisholm; ▲, cv Cimarron; o, cv Cody. Abilene, $g_s = 0.449 + 0.340 \times \psi + 0.066 \times \psi^2$, r = 0.90; Pioneer 2157, $g_s = 0.639 + 0.470 \times \psi + 0.093 \times \psi^2$, r = 0.72 ; Quantum 554, $g_s = 3.23 + 4.57 \times \psi + 1.57 \times \psi^2$, r = 0.85; Chisholm, $g_s = 0.498 - 0.292 \times \psi - 0.580 \times \psi^2$, r = 0.72; Cody, $g_s = 0.815 + 0.721 \times \psi + 0.153 \times \psi^2$, r = 0.76; Cimarron, $g_s = 1.504 + 1.901 \times \psi + 0.634 \times \psi^2$, r = 0.95.

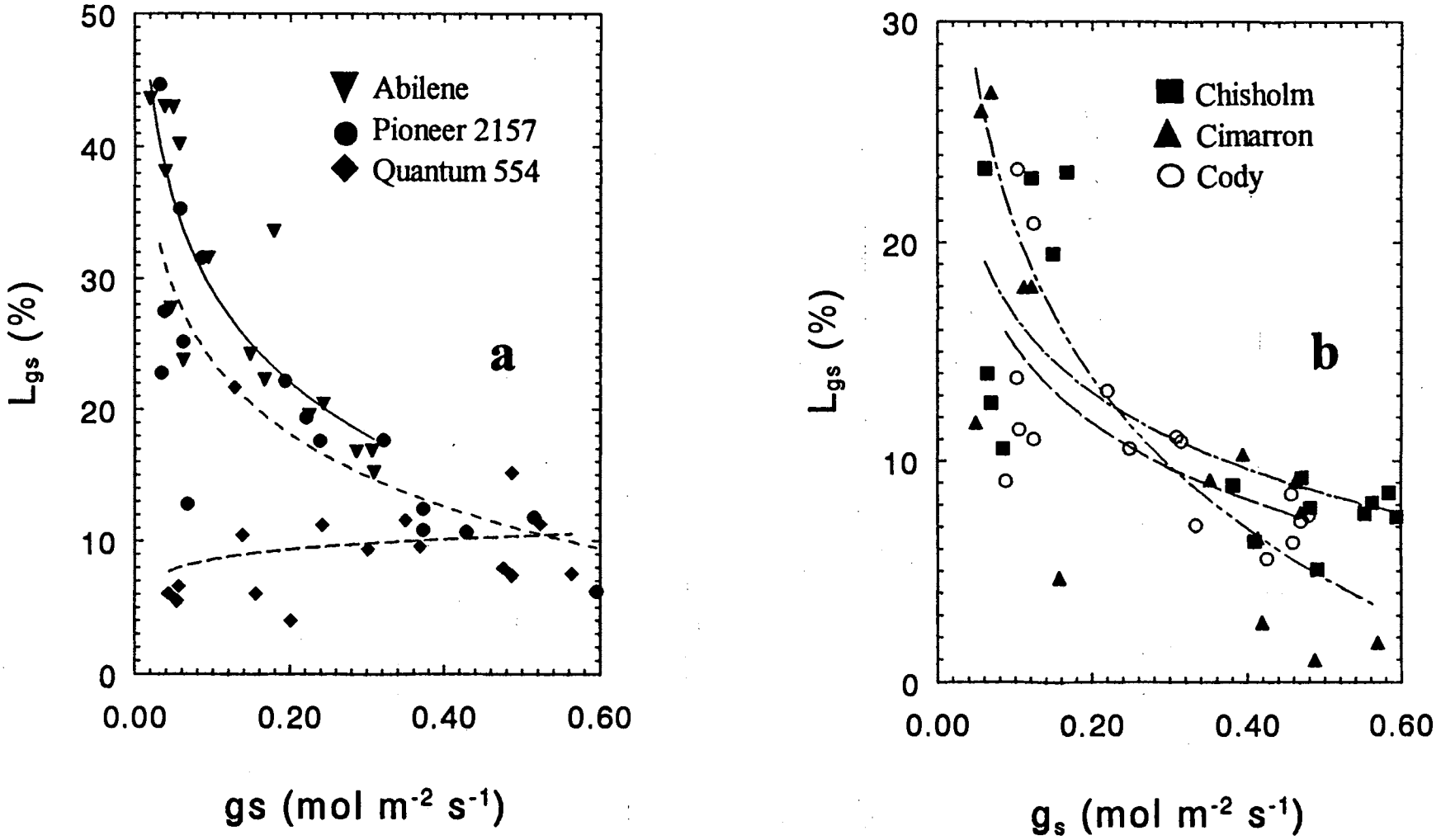

Figure 3. (a) Dependency of L_{gs} on g_s. ▼, cv Abilene; ●, cv Pioneer 2157; ◆, cv Quantum 554. (b) ■, cv Chisholm; ▲, cv Cimarron; ○, cv Cody. Abilene, ln L_{gs} = ln 6.0 - 9.9 x g_s, r = 0.86; Pioneer 2157, ln L_{gs} = ln 5.3 - 7.9 x g_s, r = 0.80; Quantum 554, ln L_{gs} = ln 11.0 + 1.1 x g_s, r = 0.61; Chisholm, ln L_{gs} = ln 5.0 - 5.0 x g_s, r = 0.71; Cody, ln L_{gs} = ln 3.6 - 5.0 x g_s, r = 0.66; Cimarron, ln L_{gs} = ln 2.2 - 9.9 x g_s, r = 0.78.

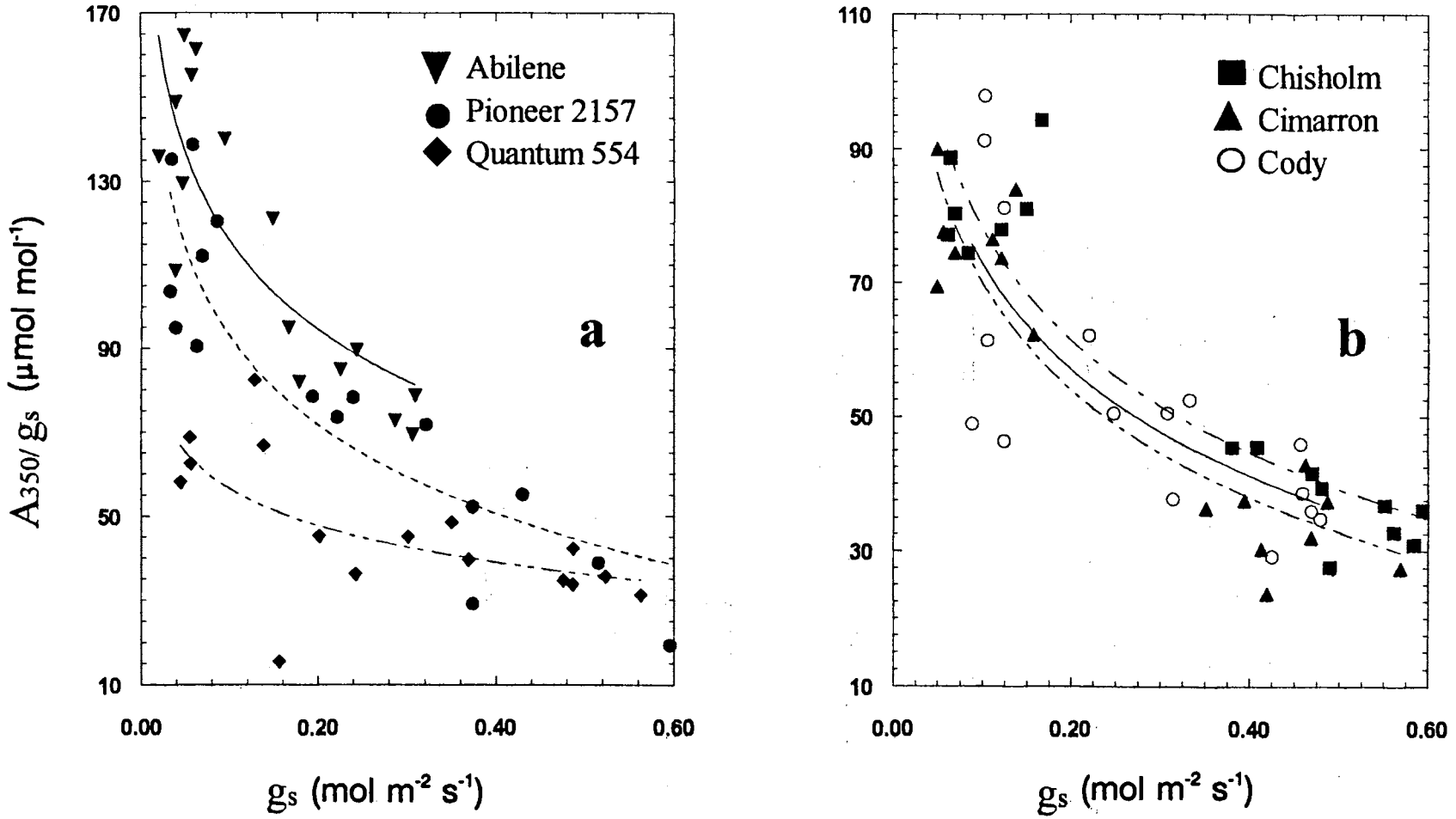

Figure 4. (a) Dependency of A_{350}/g_s on g_s. ▼, cv Abilene; ●, cv Pioneer 2157; ♦, cv Quantum 554. (b) ■, cv Chisholm; ▲, cv Cimarron; ○, cv Cody. Abilene, ln A_{350}/g_s = ln 20.37 - 44.21 x g_s, r = 0.81; Pioneer 2157, ln A_{350}/g_s = ln 11.81 - 39.36 x g_s, r = 0.88; Quantum 554, ln A_{350}/g_s = ln 27.39 - 12.60 x g_s, r = 0.64; Chisholm, ln A_{350}/g_s = ln 22.56 - 24.11 x g_s, r = 0.91; Cody, ln A_{350}/g_s = ln 20.17 - 22.90 x g_s, r = 0.74; Cimarron, ln A_{350}/g_s = ln 16.72 - 23.14 x g_s, r = 0.92.

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CHAPTER IV

CULTIVAR DIFFERENCES IN ELECTRON TRANSPORT AND DROUGHT STRESS EFFECTS ON RUBISCO ACTIVITY

ABSTRACT

The effect of severe drought stress on rubisco activity and activation and electron transport in thylakoids from well-watered leaves were investigated in three winter wheat cultivars (*Triticum aestivum* L. cvs. Abilene, Chisholm, and Cody) . The decrease in initial rubisco activity relative to the nonstressed plants ranged from 18% in Cody to 41% in Chisholm when leaf ψ dropped to -2.5 MPa. Rubisco activity following incubation with $NaHCO_3$ and $MgCl_2$ in the assay medium showed that this enzyme was not differently activated in the three cultivars at similar leaf ψ.

Results suggest that severe drought stress causes a small reduction in rubisco activity. Reductions in photosynthesis rate originated by severe drought stress are not caused by reduced rubisco activity.

The results reported in this chapter show that Abilene had higher rates of rubisco activity and electron transport through PS I than Chisholm and Cody.

INTRODUCTION

Photosynthetic rate in higher plants is negatively affected by water stress (Boyer and Bowen, 1970; Hanson and Hitz, 1982; Gui-Ying et al., 1987; Martin and Ruiz-Torres, 1992). The photosynthetic process is limited by stomatal and nonstomatal factors hence, a reduction in the photosynthetic capacity could be caused by a decline in stomatal conductance (Vaadia et al., 1961; Brix, 1962; Farquhar and Sharkey, 1982) or by lowered chloroplast activity (Boyer, 1971; Matthews and Boyer, 1984; Graan and Boyer, 1990). Changes in the chloroplast activity result from either an alteration in the efficiency with which the photosynthetic machinery operates and/or a change in the leaf content of photosynthetic machinery (Sharkey, 1985; Seemann and Critchley, 1985).

It is likely that stomatal closure is largely accountable for the reduction in net photosynthesis rate of plants subjected to mild water deficit (Lange et al., 1971; Bunce, 1981; Soldatini and Guidi, 1992; Cornic et al., 1992). Impaired chloroplast metabolism may be the main reason for photosynthetic inhibition in plants stressed to lower water potential (ψ) (Kaiser, 1987).

The effect of water deficit on electron transport is not completely clear. For years it was thought that reduced ψ mainly affects electron flow through PSII. In 1976, Fellows and Boyer established that PSII activity in sunflower chloroplasts decrease to

50% of the control activity at a ψ of -2.6 MPa. Matthews and Boyer (1984) showed that, in sunflower, inhibition of chloroplast activity accounts for most of the inhibition of photosynthesis at low ψ. They acclimated plants to low ψ through water deficit pretreatments and observed that at high ψ rates of PSII electron transport were sufficient to support 80 to 90% of maximum rates of CO_2 fixation. However, PSII activity was sensitive to low ψ, although to a lesser extent in chloroplasts from pretreated plants. Meyer and Kouchkovsky (1993) subjected two cultivars of *Lupinus albus* L. to water deficits for up to two weeks, lowering ψ to -4.3 MPa. They reported that drought progressively inhibited the uncoupled electron transport supported by the whole chain. The decrease of uncoupled electron flow dependent on the whole chain was exclusively due to reduced capacity of PSII. According to the above information, it is likely that PSII is less tolerant to drought than PSI.

Declining initial slope of photosynthetic CO_2 response curves in several drought studies suggests a direct effect of dehydration on the Calvin cycle (Matthews and Boyer, 1984; Heitholt et al., 1991; Martin and Ruiz-Torres, 1992). O'Toole et al. (1976) studied the response of *Phaseolus vulgaris* L. to decreasing ψ. They observed that ribulose-1,5-bisphosphate carboxylase (rubisco) activity decreased as internal water stress increased. Their results supported reports by Jones (1973) and Plaut (1971) in cotton and spinach, respectively. Earlier Huffaker (1970) had studied the effects of water stress on nitrate reductase and rubisco in barley. He noted that nitrate reductase activity tended to decrease, while rubisco was little affected by water stress. He concluded that the rapid decrease in photosynthetic fixation of CO_2 at the onset of water stress may be due to reduced stomatal aperture.

Vu et al. (1987) reported that when 50 days-old soybean plants were exposed

to drought stress there was a reduction in the total ($NaHCO_3/MgCl_2$-activated) extractable rubisco activity. When ψ values were lowest (-2.0 MPa), a 50% reduction in the initial (nonactivated) activity was observed. They also noticed that the ribulose-1,5-bisphosphate (RuBP) levels were 10 to 30% lower in drought stressed plants. They concluded that at least in soybean leaves drought stress causes mainly a reduction of the *in vivo* activity of rubisco, and that the decline in canopy CO_2 exchange rates during drought stress cannot be solely attributed to limitations in the RuBP regeneration capability.

Sharkey and Seemann (1989) explored the effect of mild water stress on photosynthetic chloroplast reactions of *Phaseolus vulgaris* leaves by measuring rubisco activity and the pool sizes of RuBP, 3-phosphoglycerate (PGA), hexose monophosphates, and ATP. They found that mildly stressed leaves had photosynthetic rates which were approximately 50% of control rates. The pool size of PGA was markedly reduced by both mild and severe water stress, and the level of fructose-6-P was unchanged by mild stress but reduced by severe stress. The level of ATP was not affected by mild water stress. The percent activation of rubisco (ratio of activity before and after incubation with $NaHCO_3$ and $MgCl_2$) was moderately reduced by severe stress but not by mild stress. Mild stress had no effect on the carboxylation capacity of rubisco. They concluded that the major effect of mild water stress on photosynthetic CO_2 assimilation is stomatal closure, not a reduction in the capacity of the chloroplasts for photosynthesis.

In a recent study Gunasekera and Berkowitz (1993) used tobacco (*Nicotiana tabacum* L.) transformed with antisense rubisco DNA sequences to evaluate whether rubisco or other enzymes of the Calvin cycle limits the rate of photosynthesis at low

leaf ψ. They concluded that the photosynthetic decline under water stress was not due to reduction in leaf conductance but, rather, was caused by impaired chloroplast metabolism. They observed that low ψ reducing rubisco activity to 68% did not enhance photosynthetic inhibition, suggesting that rubisco is not the main limitation in tobacco chloroplast metabolism under water stress. The RuBP levels were observed to decline as the stress increased. The results obtained in this study suggest that an enzyme involved in RuBP regeneration rather than rubisco activity limits photosynthesis in water-stressed tobacco plants. Declining steady state levels of RuBP as ψ dropped in spinach were also reported by Santakumari and Berkowitz (1991). Gimenez et al. (1992) had reported similar results when studying the effect of short-term water stress on photosynthesis of two sunflower hybrids (*Helianthus annuus* L.). They found that water stress decreased CO_2 assimilation of the hybrids to the same extent, by decreasing the RuBP content rather than via stomatal or rubisco regulation.

Based on previous research water stress can reduce the rate of photosynthesis by impairing the carboxylation reaction catalyzed by rubisco, or by reducing the availability of CO_2 and/or RuBP. The objectives of this study were to analyze the effect of severe water stress on rubisco initial and final activities of three winter wheat cultivars selected from previous gas exchange studies (Chapter III), and to assay electron transport in thylakoids from well-watered plants in the same wheat cultivars. Another objective was to examine the relationship between CO_2 assimilation rate at high CO_2 concentration (Chapter III) and photosynthetic electron transport capacity.

MATERIALS AND METHODS

Plant material. This experiment was conducted using 3 winter wheat cultivars (*Triticum aestivum* L. cvs. Abilene, Cody and Chisholm). These cultivars were selected from among six cultivars previously used in gas exchange studies. Plants were grown from seed in 1.3 L pots containing soil conditioner (Green Country Soil, Inc., Miami, OK) in a Sherer Controlled Environment Chamber, model CEL 25-7 HL (Sherer-Gillet Co., Marshall, MI) with a light intensity of approximately 600 μmol m^{-2} s^{-1} PAR at the top the plants. The chamber was maintained at 25^0C/18^0C day/night temperature, 14h/10h day/night length and 50% RH. Plants were watered as necessary with Peters 20-20-20 (W.R. Grace & Co., Allentown, PA). Plants were grown for 5 weeks under well watered conditions (unstressed control) before water deficits were imposed by withholding water from pots. Water was withheld until the leaf ψ was reduced to approximately -2.0 MPa to -2.5 MPa (about 5 and 8 days, respectively). The unstressed control had a ψ around -1.3 MPa. The ψ of each plant used in each experiment was determined using a pressure bomb (PMS Instrument Co., Corvalis, OR.) by detaching a leaf and determining the balance pressure. About 80% of the leaves of each plant was used to assay rubisco activity. From two of the remaining leaves 0.31 cm^2 leaf samples ware taken with a hole puncher for chlorophyll analysis. Each sample was extracted with 2 ml 80% (v/v) acetone. Values were found to be very constant among leaves. Therefore, average values of the two subsamples per

plant were used.

Rubisco. Determination of rubisco activation and activity was made following the procedure described by Salvucci et al. (1986), and Kobza and Seemann (1989). Fully expanded leaves were plunged into liquid nitrogen after being light activated for 30 min and then ground into a fine powder with a mortar and a pestle. The powder was rapidly mixed into 3.0 ml of extraction mixture kept on ice and filtered through a kimwipe. The extraction mixture consisted of 100 mM Tricine (pH 8.1), 10 mM $MgCl_2$, 1 mM EDTA and 15 mM β-mercaptoethanol. For the initial activity, which is dependent upon the concentration and activation state of rubisco, one aliquot of the leaf homogenate was immediately assayed in 0.5 ml assay mixture. The assay mixture consisted of 100 mM Tricine (pH 8.1), 10mM $MgCl_2$, 1mM EDTA, 0.4 mM RuBP, and 10 mM $NaHCO_3$ contained in a 7 ml scintillation vial. The assay mixture was maintained at 25^{0}C in a shaking water bath and the assay started by the addition of 5 µl [^{14}C]$NaHCO_3$ (1 Ci mol^{-1} final specific activity). The assay was stopped after 1 min by injection of 0.1 ml 4 M HCOOH plus 1 M HCl.

To determine the total activity the enzyme was first fully activated *in vitro* by incubation of an aliquot of the crude leaf extract with 10 mM $MgCl_2$ and 10 mM $NaHCO_3$ for 4 min at 25^{0}C. The fully activated enzyme was assayed as described above.

Samples were oven dried for 5 h at 100^{0}C, redissolved in 0.5 ml 0.1 N HCl and mixed with 5 ml ScintiVerse II (Fisher Scientific, Houston, TX). Scintillation vials were immediately vortexed and the acid-stable radioactivity measured. An aliquot of the enzyme extract was used for chlorophyll determination following the procedure of Arnon (1949).

The percent (%) activation of the enzyme was calculated as: (initial activity/total activity) x 100.

Electron transport. The same wheat cultivars (*Triticum aestivum* L. cvs. Abilene, Chisholm and Cody) were grown in a growth chamber (as for rubisco activity). Five plants per pot were grown for 5 weeks under well watered and fertilized conditions.

Determinations of linear electron transport over photosystem II (PSII), photosystem I (PSI) and whole chain electron transport including PSII and PSI were made as described by Martin and Ort (1982) and Kee (1984). Twenty grams of fully expanded leaves from five plants were rinsed in distilled H_2O, dried with a paper towel and cut into small pieces. Pieces were homogenized in 200 ml ice-cold grinding medium in a chilled blender (Waring Commercial Blendor, New Hartford, CT.). Homogenization was made in three 1 sec grinding bursts at high speed. The grinding medium consisted of 30 mM Tricine (pH 6.5), 300 mM NaCl, 3 mM $MgCl_2$, and 0.5 mM EDTA. The homogenate was filtered through 16 layers of cheese cloth, decanted into centrifuge tubes and centrifuged at 1500 x g for 2 min in a table top centrifuge (Adams electric centrifuge, Parsippanny N.J.). The supernatant was decanted gently and the chloroplast pellet resuspended in 60 ml of ice-cold resuspension medium. The resuspension medium contained 200 mM sorbitol, 2 mM $MgCl_2$, 5 mM HEPES-KOH (pH 6.5), and 0.05% (w/v) freshly dissolved BSA. The resuspended chloroplasts were cleared from debris by terminating centrifugation when the centrifugation force reached 1500 x g, followed by filtration of the supernatant into two centrifuge tubes using a small funnel and 1 layer of kimwipe. After centrifugation at 1500 x g for 2 min the supernatant was removed and the pelleted chloroplasts

gently resuspended with a cotton tip in a centrifuge tube containing 6 ml chilled resuspension medium. About 20 min were required to prepare the chloroplasts which were immediately used.

Steady-state electron transport measurements were performed using a combined oxygen electrode and reaction chamber (Hansatech, Ltd., Kings Lynn, U.K.). Temperature was kept constant (25^{0}C) by circulating water from a thermostatically-controlled water bath through a water jacket surrounding the chamber. Briefly, the oxygen electrode disc consists of an anode and a cathode which are maintained at a constant polarization voltage. The electrical current passing between the anode and cathode varies linearly with the oxygen concentration. Consequently, the current is proportional to the oxygen concentration in the reaction mixture (Walker, 1987).

The electron transport rates were calibrated by the complete reduction of a known amount of $K_3Fe(CN)_6$. All reactions were initiated by turning on the high intensity light source that generated 2500 μmol m^{-2} s^{-1} PAR at the surface of the sample (LS2, Hansatech, Ltd., Kings Lynn, U.K.) for 60 seconds.

Whole-chain electron transport. To determine the whole-chain electron transport rate the autoxidation of methyl viologen (MV) was measured. This was achieved by monitoring the net O_2 consumption with MV as an exogenous electron acceptor. Reduction of MV by the whole chain is represented by the following formula:

$$H_2O + 2MV^{2+} \xrightarrow[Chloroplasts]{Light} 2MV^{+} + 1/2O_2 + 2H^{+} \qquad (1)$$

showing oxygen evolution in the usual stoichiometry of the Hill reaction ($O_2/2e^- = 1/2$). However, oxygen is simultaneously consumed at twice this stoichiometry by a combination of the autoxidation reaction

$$2MV^+ + 2O_2 \rightarrow 2MV^{2+} + 2{\cdot}O_2^- \quad (2)$$

with dismutation of superoxide

$$2{\cdot}O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (3)$$

The sum of these three equations (Mehler reaction) is

$$H_2O + 1/2O_2 \xrightarrow[chloroplasts]{2e^-,light,MV} H_2O_2 \quad (4)$$

which gives a stoichiometry of oxygen uptake of

$$O_2 / 2e^- = 1/2 \quad (5)$$

This is, using MV as an artificial electron acceptor of whole chain electron transport

oxygen is consumed at the same overall rate as that at which it would be evolved in a classical Hill reaction. The $\cdot O_2^-$ formed in equation 2 is highly reactive in the presence of reductants in the medium. Therefore the dismutation rate of $\cdot O_2^-$ was maximized by addition of the enzyme superoxide dismutase (SOD). It was also necessary to inhibit the endogenous catalase activity of the chloroplast preparation by sodium azide to avoid uncontrolled release of oxygen from H_2O_2 formed in equation 3.

The 1.5 ml reaction mixture contained 50 mM HEPES-KOH (pH 7.5), 100 mM sorbitol, 5 mM KCl, 2.5 mM $MgCl_2$, 5 mM Na_2HPO_4, 0.5 mM ADP, 100 μM MV, 10 mM NH_4Cl, 0.03 μM valinomycin (Sigma Chemical Company, St. Louis, MO.), 300 units SOD from horseradish (Sigma Chemical Company, St. Louis, MO), 5 mM sodium azide and chloroplasts containing 30 nmol chlorophyll. NH_4Cl and valinomycin were used to uncouple the electron transfer from photophosphorylation.

Photosystem II (PSII) electron transport. Rates of PSII electron transport were measured by monitoring the O_2 evolution resulting from photosynthetic water oxidation. Diaminodurene (DAD) in the oxidized state was used as electron acceptor. The DAD was maintained in the oxidized state by ferricyanide ($K_3Fe(CN)_6$). Photosystem I was inhibited by DBMIB (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone). The general equation for this reaction is

$$2H_2O + 2DAD_{OX} \xrightarrow[chloroplasts]{4e^-,light} O_2 + 2DAD_{RED} \qquad (6)$$

The 1.5 ml reaction mixture for determination of the electron flow rate from H_2O

to $DAD_{(ox)}$ contained 50 mM HEPES-KOH (pH 7.5), 100 mM sorbitol, 5mM KCl, 2.5 mM $MgCl_2$, 5 mM Na_2HPO_4, 0.5 mM ADP, 1.5 mM $K_3Fe(CN)_6$, 1.0 μM DBMIB, 0.5 mM DAD, and 15 nmole chlorophyll.

Photosystem I (PSI) electron transport. PSI activity was measured as electron flow from Durohydroquinone (DHQ) to methylviologen. PSII electron transport was eliminated by DCMU (Diuron) and the reaction uncoupled with nigericin. In the overall reaction (equation 4) water is substituted by DHQ

$$DHQ_2 + O_2 \xrightarrow[chloroplasts]{2e^-,light,MV} DQ + H_2O_2 \qquad (7)$$

The stoichiometry of O_2 uptake to electron flow is $O_2/2e^- = 1$.

The reaction mixture was similar to the one used for chain whole electron transport except for the substitution of valinomycin and NH_4Cl for 0.5 μM nigericin and the addition of 5 μM DCMU and 0.5 mM DHQ. Chloroplasts containing 15 nmoles of chlorophyll were used in a reaction volume of 1.5 ml.

Determination of chlorophyll. Chlorophyll content was determined by adding 250 μl of chloroplast suspension to 9.75 ml of 80% acetone in a centrifuge tube. The centrifuge tube was vigorously agitated and non-soluble components sedimented by centrifugation at 275 x g for 3 min. The absorbance at 645 and 663 nm of the green, clear supernatant was determined using a spectrophotometer (Spectronic 1201, Milton Roy Co. NY). Total chlorophyll concentration was determined following the procedure of Arnon (1949).

RESULTS AND DISCUSSION

Photosynthesis rate per unit leaf area is affected by water deficits. The effects of water deficit stress on plants depend on the magnitude and duration of the stress and the genetic constitution of the plant species. These effects involve both stomatal closure and reduction in biochemical processes. It has been suggested that the rate of CO_2 assimilation initially decreases as a result of stomatal closure (Vu et al., 1987; Sharkey and Seemann, 1989; Heitholt et al., 1991). When ψ becomes even lower net photosynthesis rate decreases due to a variety of factors, one of them being decreased RuBP concentration in the chloroplasts (Santakumari and Berkowitz, 1991; Gimenez et al., 1992; Gunasekera and Berkowitz, 1993). Our experiments showed that rubisco activity decreased in wheat plants exposed to severe water stress. Decrease in the reaction catalyzed by rubisco may be caused by reduced amount or specific activity of rubisco.

Chlorophyll content, initial and total rubisco activities and percent (%) activation were studied in leaves of three winter wheat cultivars. Results are shown in Table 1. Leaves of well-watered Abilene contained slightly but significantly more chlorophyll per unit leaf area than leaves from water-stressed plants. Chisholm leaves stressed to about -2.5 MPa had higher chlorophyll content per unit leaf area than leaves from plants stressed to approximately -2.0 MPa and nonstressed leaves. The case was different in Cody, in which the nonstressed leaves (control) and the most water

stressed leaves (-2.5 MPa) showed higher chlorophyll content per unit leaf area than did the leaves from plants stressed to approximately -2.0 MPa. These differences in chlorophyll content were small but statistically significant.

Nonstressed plants of all three cultivars showed higher initial and total rubisco activities than stressed plants (Table 1). The decrease in initial rubisco activity relative to the nonstressed plants ranged from 18% (Cody) to 41% (Chisholm) when leaf ψ dropped from -1.3 to -2.5 MPa. Initial rubisco activity in Abilene plants stressed to approximately -2.0 MPa decreased 32% relative to the control. Initial enzyme activity was 39% lower, relative to the control when leaf ψ reached -2.5 MPa. Chisholm plants stressed to about -2.0 and -2.5 MPa showed 30 and 41% lower initial rubisco activity, respectively relative to the control. In Cody the difference between nonstressed and stressed plants was smaller. Stressed plants (-2.0 MPa and -2.5 MPa) showed initial enzyme activity decreases of 7 and 18%, respectively. In general, reduction in initial rubisco activity was more drastic at lower ψ. These results agree with data previously by Johnson et al. (1974), who studied the effect of water stress on the initial activity of rubisco from flag leaves and awns of wheat and barley. They reported a decrease in carboxylase activity with decreasing leaf ψ. Rubisco activity decreased approximately 30% in the awns and approximately 50% in flag leaves, when leaf ψ decline to -3.3 MPa. The authors also observed that at a leaf ψ of -3.3 MPa, photosynthetic rate was zero in all tissues assayed, yet considerable initial rubisco activity was still measured in the crude extracts. They concluded that "if *in vivo* rubisco activities are comparable to the *in vitro* rates measured, this enzyme did not likely limit photosynthesis at lower water potentials".

The increase in activity of rubisco extracted from leaves of the three cultivars

caused by incubation with $NaHCO_3$ and $MgCl_2$ in the assay medium ranged from 21 to 35%. Higher percent activation was observed in rubisco extracted from non-stressed leaves than in rubisco extracted from stressed leaves. Rubisco activity following activation showed that this enzyme was not differently activated in the three cultivars at similar ψ. Incubation with $NaHCO_3$ and $MgCl_2$ of leaf extract from stressed plants (-2.5 MPa) increased rubisco activity by about 30% in all cultivars, relative to the control.

The percentage activation decreased with lower leaf ψ. Changes in the percentage activation of rubisco were more substantial in Chisholm than in Abilene and Cody. Percent activation in stressed plants ranged from 74% (Chisholm) to 79% (Abilene) while, % activation in nonstressed plants ranged from 80 (Cody) to 82.5% (Abilene) (Table 1).

There were no differences among cultivars in the relation between initial and total activities of rubisco. Figure 1 shows that the activity of rubisco incubated with $NaHCO_3$ and $MgCl_2$ was proportional to the initial activity in all three cultivars. In a different but related experiment (Chapter II), net photosynthesis rates (A) of all three cultivars were measured. Five-weeks old plants were stressed for several days and the steady-state gas exchange rates evaluated. Intercellular CO_2 concentration (C_i) was plotted against measured A to construct photosynthetic C_i response curves. Carboxylation efficiency was calculated as the slope of the linear part of the A/C_i curve at low C_i. Carboxylation efficiency was significantly higher in Abilene than in Chisholm and Cody (Fig. 2). According to the model of Farquhar et al. (1980) a higher Calvin cycle activity is associated with a steeper initial slope of the curve. The high rubisco activity observed in Abilene is consistent with the results obtained in the

steady-state gas exchange studies. Carboxylation efficiency decreased substantially with decreasing ψ in leaves of all three cultivars. The decrease was more obvious in Chisholm and Cody than in Abilene.

Rates of electron flow were very similar in the three cultivars, yet, some significant differences were found (Table 2). To measure electron transport through PSII, PSI was inhibited by DBMIB. This inhibitor is a plastoquinone antagonist that blocks electron flow through the Cyt b_6/f complex (Trebst, 1980). An artificial electron acceptor (DAD) was required to intercept electrons from the PSII reaction center. We found that Chisholm had higher PSII electron transport rates than Abilene and Cody. Electron flow through PSI was measured by using an artificial electron donor (DHQ), an artificial electron acceptor (MV), and DCMU as PSII inhibitor. DHQ substitutes for plastoquinone as the donor to the Cyt b_6/f complex (Izawa, 1980) and electrons flow from DHQ to MV. Abilene showed significantly higher rates of electron flow through PSI than the other two cultivars. When electron transport through both PSII and PSI (whole chain electron transport) was studied water was used as electron donor and MV as electron acceptor. Cody showed significantly higher electron flow rates than Chisholm and Abilene.

von Caemerer and Farquhar (1981) reported a correlation between *in vitro* measurements of whole chain electron transport and CO_2 assimilation rate at high CO_2 concentration. In this study such a relationship was not observed. Contrary to electron transport data, gas exchange data of Abilene (Chapter III, Table 1) showed higher CO_2 assimilation rate at high CO_2 concentration than the other two cultivars. A reason may be that chloroplast extraction procedure disrupts normal thylakoid performance, or alternatively, whole chain electron transport may *in vivo* be limited by

phosphorylation.

Campbell and Ogren (1990a) demonstrated a requirement for light and photosynthetic electron transport for full activation of rubisco by rubisco activase in lysed chloroplasts at physiological concentrations of CO_2. These results indicated a direct involvement of the thylakoid membrane in the activation of rubisco *in vivo*. In a related study by the same researches (Campbell and Ogren, 1990b), it was found that stimulation of light activation of rubisco by rubisco activase requires electron transport through PSI but not PSII. In agreement with Campbell and Ogren (1990a, b), in this study it was observed that Abilene had higher rates of rubisco activity and electron transport through PSI than the other two cultivars.

The observations reported here and in Chapter III indicate that Abilene has higher mesophyll capacity than Chisholm and Cody.

SUMMARY

The reported results suggest that in winter wheat severe drought stress causes a small reduction in rubisco activity, which is proportionally much smaller than the decrease in photosynthesis rate observed at lower leaf ψ (Chapter III). Photosynthesis was practically eliminated at a leaf ψ of -2.0 MPa, while rubisco activity showed just a reduction of 31, 30 and 7% relative to the control in Abilene, Chisholm, and Cody, respectively. The findings of this study suggest that reduced rubisco activity is not the main cause of reduced photosynthesis rate in water-stress wheat.

The higher electron transport rate through PSI of Abilene may enable the high rubisco activity observed in this cultivar.

Table 1. Mean water potential, chlorophyll content, initial and total activities, and percent activation of rubisco.

Genotype	Days of withholding water	ψ†	Chl‡	Initial activity	Total activity	Activation§
	d	MPa	g m^{-2}	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	%
Abilene	0	-1.3	0.69a*	62.6a	75.8a	82.5a
	5	-2.0	0.61b	42.7b	54.2b	78.9ab
	8	-2.5	0.62b	38.2c	49.9b	76.4b
Chisholm	0	-1.3	0.58a	55.8a	67.9a	82.1a
	5	-2.0	0.59a	39.2b	53.3b	73.6ab
	8	-2.5	0.63b	33.1c	44.2c	74.9b
Cody	0	-1.3	0.64a	48.0a	59.7a	80.4a
	5	-2.0	0.58b	44.7b	56.7b	78.7ab
	8	-2.5	0.62a	39.6c	52.7b	75.2b

† Measured before each experiment.
‡ Chl, chlorophyll a plus chlorophyll b.
§ Initial (*in vivo*)/total ($NaHCO_3$ - $MgCl_2$ activated) activity x 100.
* Within a column and cultivar, means followed by the same letter are not significantly different at the 0.05 probability level.

Table 2. Rates of PSII, PSI, and whole chain electron flow of three winter wheat genotypes (n = 28).

Genotypes†	PSII	PSI	Whole Chain
		μmol e^- mg Chl^{-1} hr^{-1}	
Abilene	134.9b*	72.2a	128.5b
Chisholm	149.7a	64.4b	127.2b
Cody	128.6c	65.7c	136.4a

* Different letters within a column indicate significant differences at $p < 0.05$.
† Well watered-plants.
Chl = chlorophyll a plus chlorophyll b.

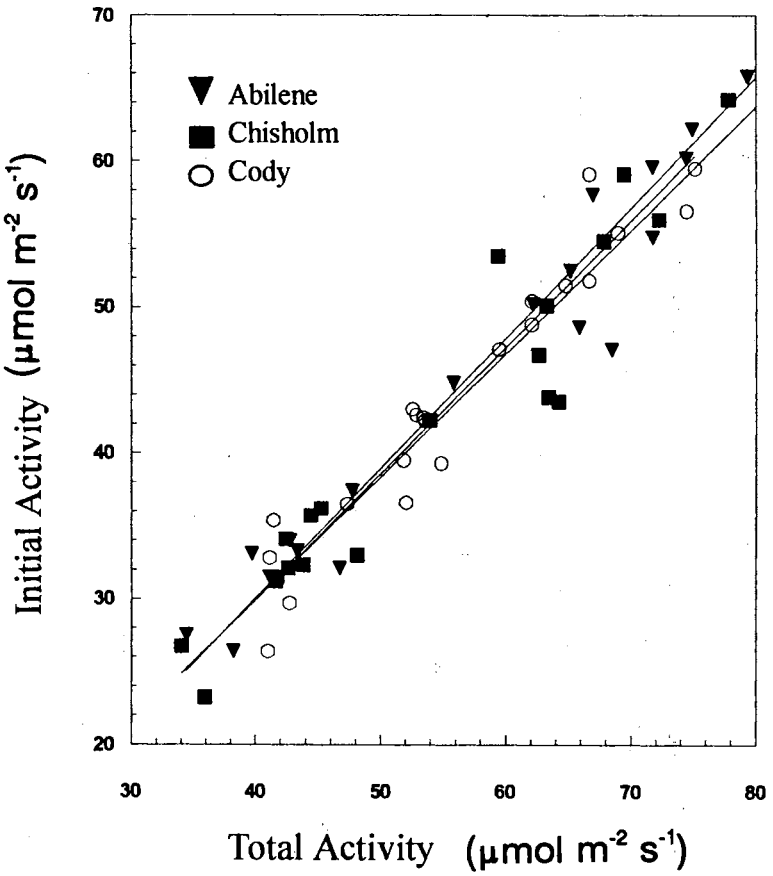

Figure 1. Relationship between initial activity and final activity. ▼, cv Abilene; ■, cv Chisholm; o, cv Cody.
Abilene, initial activity = - 5.63 + 0.89 x Total activity, r = 0.98;
Chisholm, initial activity = - 3.85 + 0.84 x Total activity, r = 0.97;
Cody, initial activity = - 4.81 + 0.86 x Total activity, r = 0.96.

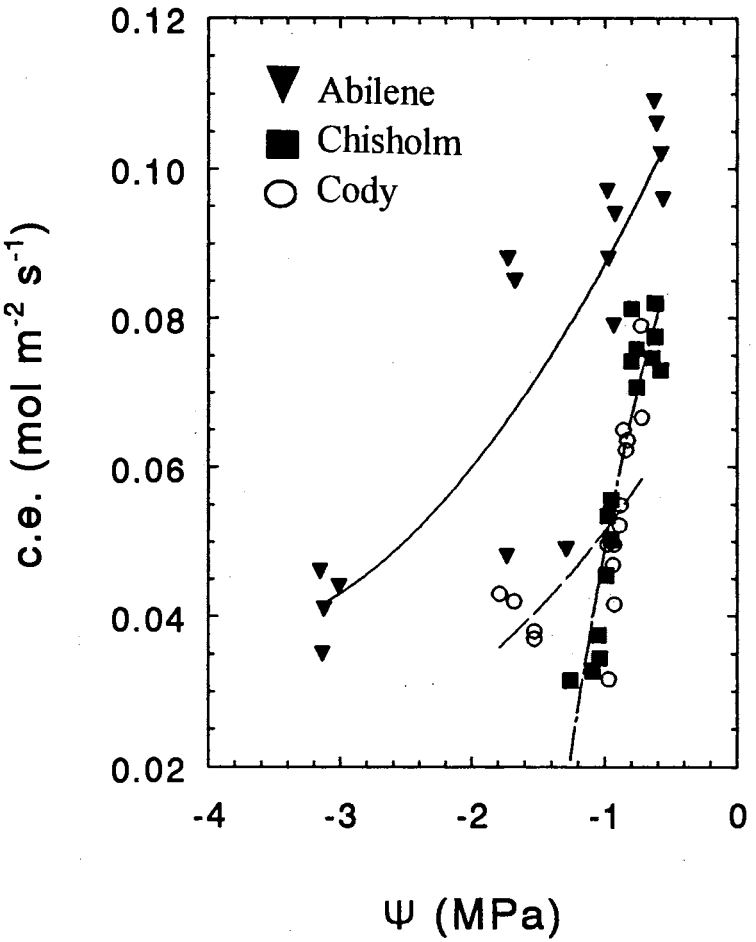

Figure 2. Dependence of c.e on ψ. ▼, cv Abilene; ▪, cv Chisholm; o, cv Cody. Abilene c.e. = 0.125 + 0.043 x ψ + 0.005 x ψ^2, r = 0.88; Chisholm c.e. = 0.101 + 0.008 x ψ - 0.044 ψ^2, r = 0.93; Cody c.e. = 0.223 + 0.282 x ψ + 0.103 x ψ^2, r = 0.73.

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