PHYSIOLOGICAL AND ANATOMICAL VARIATION IN

TWO <u>TRITICUM</u> <u>DICOCCOIDES</u> ACCESSIONS

DIFFERING IN PHOTOSYNTHETIC RATE

By

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CHAPTER I

INTRODUCTION

Man has practiced artificial selection on wheat for convenience of cultivation and increased grain yield. He selected for large grain size, ears without brittle rachis, free-threshing and many grains per ear (Feldman, 1976). As a result, plants which have fewer but larger shoots and larger leaves than their progenitors were developed (Austin et al., 1982). Harvest index rather than photosynthetic rate per unit leaf area has been the major physiological factor in the selection of wheat for increased yield (Dunstone, 1973). Once harvest index is optimized, further improvements in yield will likely require increased rates of photosynthesis (Holbrook et al., 1984). Among the Triticum species, average photosynthetic rates are generally highest in diploids, intermediate in tetraploids, and lowest in the cultivated hexaploids (Austin et al., 1982; Khan and Tsunoda, 1970; Dunstone et al., 1973). In several studies, the relatively low photosynthetic rate of hexaploid wheat has been attributed to anatomical and physiological differences in leaf tissue (Parker and Ford, 1982; Austin et al., 1982; Sharkey, 1985). Many researchers, using a variety of species, have attempted to determine the role of specific anatomical and physiological characteristics in the determination of photosynthetic rate. How these and other aspects of cell and tissue composition combine to influence photosynthetic capacity is not yet completely understood.

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Net CO_2 assimilation per unit leaf area is found to be negatively correlated with leaf area across genotypes (Austin et al., 1982; Parker and Ford, 1982). Thus modern cultivated hexaploids generally have larger leaves and lower photosynthetic rates than their wild progenitor species with reduced ploidy (Bhagsari and Brown, 1986; Evans and Dunstone, 1970). An exception to this trend was noted in two accessions of the wild tetraploid species, T. dicoccoides, which differed by about 30% in their photosynthetic rate but did not differ in leaf area. These two accessions were selected for further study because their difference in photosynthesis was not confounded by differences in genomic constitution or leaf area. Since these genotypes belong to the same species, hybrid populations could be developed to determine the genetic basis for increased photosynthesis in T. dicoccoides. Also, the high photosynthesis genotype could be utilized in a back cross program with cultivated wheat to introduce genes for high photosynthesis. But first, research is needed to study the genetic regulation of high photosynthetic efficiency and associated physiological, anatomical and biochemical characteristics in these two T. dicoccoides.

The two parts of this dissertation are separate and complete manuscripts to be submitted for publication in Crop Science.

CHAPTER II

Physiological Variation in Photosynthetic Rate in Two
Triticum dicoccoides Accessions.

ABSTRACT

In the Triticum species, average photosynthetic rates are generally
highest in diploids, intermediate in tetraploids, and lowest in the
cultivated hexaploids. Net CO_2 assimilation per unit leaf area (A) is
also negatively correlated with leaf area. An exception to this trend
was noted in two accessions of the wild tetraploid species, T.
dicoccoides, which differed by ca. 30% in A but did not differ in leaf
area. These accessions were selected for further study because their
difference in A was not confounded by differences in genomic
constitution or leaf area. The objective was to identify the
physiological factor(s) which would explain the difference in
photosynthetic rate between the two T. dicoccoides accessions and
between the T. dicoccoides accessions and a hexaploid wheat used as a
standard for comparison. Photosynthetic response to CO_2 (at 2 and 21%),
light, humidity and temperature, ribulose bisphosphate (RuBP)
carboxylase activity, and sucrose and starch concentration were
determined on newly expanded leaves of growth chamber-grown plants of
two tetraploids, PI 428042 (low A) and PI 428109 (high A), and a
hexaploid cultivated wheat (TAM W-101). PI 428109 showed higher A than
PI 428042 at varying levels of CO_2, light, humidity and temperature.

Response to CO_2 was compared among genotypes using A vs. C_i (internal leaf CO_2 concentration) curve parameters. The slope of the initial linear portion of the curve and the CO_2-saturated A were significantly higher in PI 428109 than in PI 428042 which suggest higher RuBP carboxylase activity and RuBP regeneration capacity, respectively, in PI 428109. A higher RuBP carboxylase activity observed in leaves of PI 428109 supported this result. A higher sucrose concentration was observed in the leaves of PI 428042 than in those of PI 428109, but its relationship with photosynthetic capacity was uncertain. This study indicates that the difference in A between the two T. dicoccoides accessions was mainly due to differences in RuBP carboxylase activity.

Introduction

Flag leaves of the primitive Triticum species have higher rates of photosynthesis per unit leaf area than the cultivated hexaploids (Austin et al., 1982; Khan and Tsunoda, 1970; Dunstone et al., 1973). Diploid species have shown the highest rate of photosynthesis and the cultivated hexaploids have the lowest rate, with the tetraploids being intermediate (Austin et al., 1982). However, differences in photosynthesis have also been found within a ploidy level. Variation in photosynthesis could be caused by several factors. Sharkey (1985) identified three limitations to photosynthesis: (1) supply or utilization of CO_2, (2) supply or utilization of light, and (3) supply or utilization of phosphate. Single measurements of photosynthesis give very little information about the biochemical limitations to photosynthesis in leaves. Models have been developed which allow inferences to be made about biochemistry based on the response of photosynthesis to light or CO_2 (Sharkey, 1985, Farquhar et al., 1980). Sharkey (1985) stated that the first limitation indicated above is most readily measured by determining how CO_2 assimilation rate varies with change in partial pressure of CO_2 inside the leaf. The second limitation can be determined by the quantum requirement of photosynthesis. The third limitation is most readily detected as a loss of O_2 sensitivity of photosynthesis. A significant difference of ca. 30% has been found in photosynthetic rate between two accessions of the wild tetraploid species, T. dicoccoides. Thus these accessions were considered appropriate material for genetic and physiological investigations of the cause(s) of variation in photosynthetic efficiency.

In this study we investigated the photosynthetic response of the two _T. dicoccoides_ accessions (PI 428109 and PI 428042) to CO_2, light, temperature and humidity. Some biochemical tests were made to verify the results. Because the long-term goal is to transfer high photosynthetic potential to hexaploid cultivated wheat TAM W-101 was included as a standard of comparsion with the tetraploid accessions. Our objective was to determine if any of these factors accounted for the difference in photosynthetic rate between the two accessions.

Materials and Methods

Plant material

Unvernalized plants of the $\underline{T.}$ $\underline{dicoccoides}$ accessions, PI 428109 and PI 428042, and the hard red winter wheat ($\underline{T.}$ $\underline{aestivum}$) cv. TAM W-101, were grown in growth chambers at 20/15°C (day/night) and 14 h light (600 umol m^{-2}s^{-1} PPFD) for 4 weeks as described by Johnson et al. (1987). A series of experiments were conducted to determine photosynthetic response of these genotypes to temperature, light and humidity.

Gas exchange measurements

For each plant, gas exchange characteristics were measured on the last fully emerged leaf using a stirred, temperature and humidity controlled reaction chamber (cuvette) (Johnson et al., 1987). Humidity was measured inside the chamber with a condensation dew-point hygrometer (General Eastern 1100DP, Watertown, MA) and CO_2 concentration by passing chamber exhaust through a differential CO_2 gas analyzer (Horiba PIR 2000 R, Irvine, CA). The concentration of CO_2 inside the measurement chamber was varied by mixing gases of known CO_2 concentration. Photosynthetic photon flux density (photons) was measured with a quantum sensor (LI-190SB, LI-COR Inc., Lincoln, NE) at leaf level. Leaf temperature was determined by appressing a thermocouple to the underside of the leaf. Standard measurement conditions were 1800 umol photons m^{-2} s^{-1}, 330 ul l^{-1} air, 20°C, and a leaf-to-air vapor pressure difference of 1.0 KPa.

After gas exchange measurements, leaf area was determined and the leaf sample frozen at -20°C for later determinations of chlorophyll by the method of Inskeep and Bloom (1985). From another tiller on the same

plant, a recently fully emerged leaf was excised, leaf lamina area and dry weight were determined, and specific leaf weight was calculated. Calculations of CO_2 assimilation were made per unit leaf area (A) and per mole of chlorophyll (A/Chl). Transpiration (E), A, stomatal conductance (G_s), internal CO_2 concentration (C_i) and water use efficiency (WUE = A/E) were calculated according to Von Caemmerer and Farquhar (1981).

Photosynthetic <u>response</u> <u>to</u> <u>temperature</u>

CO_2 assimilation per unit leaf area was measured at 8, 14, 20, 26, 32, and 38°C on PI 428109, PI 428042, and TAM W-101. Vapor pressure difference was maintained at all temperatures near 1.0 KPa with a standard deviation of 0.27. Measurements were made by progressing from the lowest to the highest temperature on leaves from six plants of each genotype.

Photosynthetic <u>response</u> <u>to</u> <u>light</u>

Rates of A were determined at 0, 200, 400, 600, 800, 1000, 1400, and 1800 umol photons m^{-2} s^{-1} by progressing from low to high light levels on one group of plants and from high to low light levels on another group of plants. The slope of the initial linear portion of the response curve for each genotype was described as the apparent maximum quantum yield (the efficiency of light utilization by photosynthesis or the number of moles of CO_2 fixed per mole photon absorbed by a leaf) (Long and Hallgren, 1985).

Photosynthetic <u>response</u> <u>to</u> <u>CO_2</u>

Rates of A were determined at ambient CO_2 concentrations of 5, 40, 75, 126, 208, 330, 436, 584, and 622 ull^{-1}. Air with no CO_2 was mixed in the cuvette to obtain a desired ambient CO_2 concentration. Assimilation

vs. internal CO_2 concentration (C_i) response curves were developed for each genotype. The slopes of the initial linear portion of the curves and the CO_2 compensation points were determined. The stomatal limitation to A was calculated as outlined by Farquhar and Sharkey (1982):

$$\%stomatal\ limitation\ =\ (A_{saturated} - A_{ambient})A_{saturated}^{-1}$$

On another set of plants A was determined at the same ambient CO_2 concentrations but at two oxygen levels (2% and 21%). The level of O_2 inhibition on A was calculated as the difference between the A rates at 2% and 21% O_2 (Monson et al., 1982).

Photosynthetic response to humidity

Measurements of gas-exchange were made at five levels of vapor pressure difference (VPD) (0.6, 1.0, 1.4, 1.8, and 2.2 KPa) to determine effect of humidity on A. Leaf water potential (WP) was estimated prior to and after measurements by using leaf cutter psychrometers (J. R. D. Merill, Logan, UT) as described by Johnson et al. (1986) to measure the change in leaf water status.

RuBP carboxylase activity determination

Leaf samples were ground at 4^0C in extraction buffer [50 mM Bicine (pH 8.2), 20 mM MgCl, 1 mM EDTA, 10 mM $NaHCO_3$, 5 mM DTT and 1% Polycar (W/V)]. One ml of the ground tissue was saved for chlorophyll determination using 80% acetone (Inskeep and Bloom, 1985). After grinding, samples were centrifuged in 20 mM $NaHCO_3$ at room temperature for 10 min and then placed in a solution containing assay buffer [50 mM Hepes (pH 8.2), 20 mM $MgCl_2$, 5 mM DTT, 10 mM KCl, 1 mM EDTA] along with enzymes and other factors needed for NADH oxidation (Lilley and Walker, 1974). Then RuBP carboxylase activity was determined as described by

Lilley and Walker (1974) using an NADH linked spectrophotometeric assay. About 0.2 ml of the supernatant from centrifugation was saved for protein determination using the Bio-Rad protein assay method (Bio-Rad, Richmond, CA).

Sucrose and starch determination

Leaf samples were frozen in liquid nitrogen immediately after carbon exchange determinations and were later freeze-dried for 24 h, oven dried at $70^{O}C$ for 24 h to denature enzymes. The dried leaves were ground to pass a 1mm screen using a Udy mill (UDY Corp., Ft. Collins, CO) and stored in brown bottles at $-20^{O}C$. Aliquots of 0.05 g dried leaves were extracted with 2 ml 95% (v/v) ethanol at $80^{O}C$ for 15 min and centrifuged at 10,000 g. The supernatant for four successive ethanol extractions was evaporated in vacuo and resuspended for sucrose, glucose and fructose determination using the enzymatic method of Boehringer Mannheim (Boehringer Mannheim Biochemicals, Indianiapolis, IN, 46250).

The residue remaining after ethanol extraction was suspended in 0.5 ml redistilled water and heated at $90^{O}C$ for 1 hr to gelatinize amylopectin. Samples were cooled and 15 ml 0.2 M acetate buffer (pH 4.5) containing 0.5%(w/v) porcine pancreatic alpha amylase and 2% (w/v) Rhizopus mold amyloglucosidase was added. Samples were incubated at $25^{O}C$ for 1 h and at $55^{O}C$ for 24 h, then centrifuged at 10,000g for 15 min. The residue was then extracted in 5 ml water at $60^{o}C$ for 10 min. The combined supernatants from three water extractions and incubation were utilized for glucose determinations as in the ethanol extractions and multiplied by a factor of 0.9 (to account for the water gained during starch hydrolysis to glucose) to obtain starch determinations.

Statistical methods

Plants in these experiments were grown in a growth chamber in a completely randomized design. Data on photosynthetic response to light, temperature, CO_2, and humidity were analyzed in a split-plot with genotypes as main plot and treatment levels as subplots. Data on RuBP carboxylase activity, soluble protein, sucrose and starch were analyzed in a completely randomized design. In the temperature response study estimates of optimum temperature were made for each genotype using a cubic polynomial regression model (which was found to be the best fit).

Results

At varying light levels PI 428109 had significantly higher A (Fig. 1a) and G_s than PI 428042, but they were not different in C_i (data not shown). TAM W-101 had intermediate A values but had significantly lower G_s than PI 428109 and lower C_i than both accessions averaged over photosynthetic photon flux density (PPFD). The A/chlorophyll values and chlorophyll a/b ratio of PI 428109 were also higher than those of PI 428042 and TAM W-101 (Table 1). There were significant differences in A values between progressions of PPFD, from low to high PPFD and from high to low PPFD. Carbon assimilation (Fig. 1b), G_s and WUE values were consistently higher when progressing from low to high than from high to low PPFD levels, but C_i values showed the reverse (data not shown). On the average, slope was lower on the high-low progression. The initial slope of the curve of the A versus PPFD was determined for each genotype in both progressions. There were no significant differences in slope between genotypes in the low-high progression, but in the high-low progression PI 428109 had a higher slope than the other two genotypes (Table 1).

In the temperature range of 14 to 32°C, PI 428109 had significantly higher A than PI 428042 and TAM W-101 did not differ from PI 428042 (Fig. 2a). Values of C_i were lowest when A rates were highest (Fig. 2b). Even though the higher A of PI 428109 was associated with generally higher G_s (Fig. 2c) than the other genotypes, there was no significant difference among genotypes in C_i. Optimum temperature for photosynthesis for all genotypes was estimated to be about 27°C. There were no significant differences in leaf water potential (WP) between genotypes during the temperature response measurements (data not shown).

Assimilation rate vs. C_i response curves were developed and the slopes of the initial linear portion of the curves were calculated (Fig. 3). A significantly higher slope was observed for PI 428109 than PI 428042 (Table 2). As in the light response study the CO_2 response was also measured at two progressions, and at two O_2 levels, 2 and 21% O_2. No genotype x O_2 interaction was observed in this study. A significant difference was observed in the slopes of the A versus C_i curves between the two progressions at 2% level of O_2 averaged over genotypes (Fig. 3b). The high-low progression showed higher slope than the low-high progression. O_2 had a highly significant effect on the slope. The 2% O_2 brought about 50% increase in the slope (Table 2, Fig. 3c).

The main purpose of the O_2 treatments was to test for differences in sensitivity to O_2, which is also an indicator for triose phosphate utilization limitation between genotypes. The difference between A rates determined with 2 and 21% O_2 represents the amount of inhibition imposed by 21% O_2. This difference is expressed as percentage oxygen inhibition in table 2. These values are similar to the average value reported for C_3 species which is ca. 30% (Brown et al., 1986). The O_2 inhibition, however, was larger at lower CO_2 levels than at saturating CO_2. At low CO_2 levels (less than 80 μl 1^{-1})the O_2 inhibition was as high as 63% but at saturating CO_2 level it was about 27% (data not shown). Stomatal conductance was higher at 2% O_2 than at 21% O_2 and decreased with increased CO_2 level. Stomatal limitation to A was also lower in 2% than in 21% O_2 (Table 2). There was no significant difference in CO_2 compensation point among genotypes at either O_2 levels, but there was a marked reduction of the CO_2 compensation point at the 2% O_2 level. The mean CO_2 compensation value for all genotypes

was 9 and 38 at the 2 and 21% O_2, respectively (Table 2). At the 2% level a significant difference was observed in the CO_2 compensation point between the two progressions of CO_2 (Table 2). The values were higher in the low to high CO_2 progression than in the high to low CO_2 progression. Although stomatal limitation to A was similar in all the genotypes, it was lower at 2% than at 21% O_2, 26 and 32%, respectively (Table 2).

The increase in VPD from 0.6 to 2.2 KPa at constant temperature ($25^{\circ}C$) resulted in significant reduction in A, G_s, and C_i (Fig. 4a, b and c) in all genotypes. PI 428109 still had significantly higher A than PI 428042 at the different VPD levels whereas TAM W-101 showed intermediate values (Fig. 4a). There was no significant decrease in A of PI 428109 until the deficit was greater than 1 KPa whereas there was a significant reduction in PI 428042 and TAM W-101 above 0.6 KPa. Stomatal conductance, C_i and WUE followed the same pattern. Over all genotypes, there was about 44% reduction in G_s with increase in VPD from 0.6 to 2.2 KPa (Fig. 4b). A very high correlation (r = 0.990) was observed between A and G_s (Fig. 5). Leaf (WP) showed a slight decrease with increased VPD (data not shown). There was also a highly significant increase in transpiration with increase in VPD, possibly causing slight reduction in leaf WP. PI 428109 had higher transpiration rate than both PI 428042 and TAM W-101 (data not shown).

A significant difference in RuBP carboxylase activity was detected between the T. dicoccoides accessions. PI 428109 had higher RuBP carboxylase activity than PI 428042 but was not different from TAM W-101 (Table 3). PI 428109 also had higher A/RuBP carboxylase activity and A/chl ratios than PI 428042. However, PI 428109 was not different from

PI 428042 in soluble protein content (Table 3). TAM W-101 had a significantly higher soluble protein content than the T. dicoccoides accessions.

A significantly higher concentration of sucrose was observed in PI 428042 leaves than in PI 428109 (Table 4). TAM W-101 had the highest concentration of sucrose. No difference was detected among genotypes in starch concentration (Table 4).

Discussion

The initial slope of the A vs. PPFD curve measures the photochemical efficiency or light utilization efficiency or, otherwise is called quantum yield (Sharkey, 1985). There were no differences in quantum yield among genotypes in the low-high progression; but a significant difference was detected in the high-low progression where the quantum yield was smaller. Reduced quantum yield suggests that carbohydrate accumulation impairs the production or consumption of ATP/NADPH in photosynthesis which reduces regeneration of RuBP and this results in the reduction of photosynthesis (Azcon-Bieto, 1983).

Other studies devoted to the influence of decreasing irradiance on A have shown a lag in A after the transition from high to low irradiance and this lag is complicated by the presence of a post-lower-illumination CO_2 burst (Stitt et al., 1983; Prinsley et al.,1986). Prinsley et al. (1986) indicated that the explanation for the lag lies in metabolic rather than physical constraints upon CO_2 assimilation. They found that immediately following a reduction in irradiance, the rate of sucrose synthesis considerably exceeds the rate of CO_2 assimilation. They concluded that depletion of Calvin-cycle intermediates by excessive sucrose synthesis, thereby causing metabolite build-up in the Calvin cycle, is partly responsible for the lag phase following a reduction in irradiance.

The higher chlorophyll a/b ratio of PI 428109 could indicate a greater molar ratio of Photosystem II reaction centers to chlorophyll. Thus, for a given chlorophyll content PI 428109 may have a greater density of PS II reaction centers (Edwards and Walker, 1983). At or near light saturation PI 428109 may have a greater capacity for electron

transfer, which is likely to be manifested as a faster rate of regeneration of RuBP and consequently greater A. However, the higher chlorophyll a/b ratio of PI 428109 was not always manifested in experiments on the two T. dicoccoides accessions.

The CO_2 response study showed that 428109 had a higher capacity for mesophyll photosynthesis than PI 428042. Although higher stomatal conductance was associated with A this was manifested as a steeper initial slope of the curve and higher CO_2-saturated A. Stomatal limitation to A was not significantly different, but CO_2-saturated A was significantly higher in PI 428109 than in PI 428042. Interpretation of these results according to the model of Farquhar et al. (1980) suggests a higher RuBP carboxylase activity in association with the steeper initial slope of the curve and higher RuBP regeneration capacity associated with the higher CO_2 saturated A of PI 428109. Verification of this interpretation, however, requires biochemical measurements of those factors. Nevertheless, both carboxylase activity and RuBP regeneration are likely affected by a number of factors involving both the light and dark reactions of photosynthesis.

The high RuBP carboxylase activity of PI 428109 supported the results of the CO_2 response study. Leaves of TAM W-101 had even higher RuBP carboxylase activity, but A was not higher than that of PI 428109. Wittenbach (1979) found that RuBP carboxylase accounts for about 50% of the soluble protein. Ku et al. (1979) indicated that there is a very close relationship between activity and concentration of RuBP carboxylase in leaf extracts. Our results, however, suggest that the difference in enzyme activity between the two accessions was due to actual activity rather than a difference in amount of the enzyme. But

in TAM W-101 the increase in activity might have been accompanied by an increase in the amount of the enzyme itself.

Sharkey (1985) proposed that in addition to the limitation of A by RuBP carboxylase activity and RuBP regeneration, A could also be limited by triose phosphate utilization (TPU). TPU limitation is detected by the lack of stimulation of A by low O_2 at saturating light and high partial pressure of CO_2 (Brown et al., 1986; Sharkey, 1985). When A is TPU-limited, both RuBP carboxylase activity and the rate of RuBP regeneration must be reduced to match the capacity for TPU (Sharkey, 1985). In this study measurement of A at low O_2 pressure with saturating light and high partial pressure of CO_2 (622 ul 1^{-1}) stimulated A by about 32% in all genotypes. Higher A values were also observed at 2% than at 21% O_2 in the A vs. C_i curve due to an increase in carboxylase activity rather than oxygenase activity of RuBP carboxylase. Therefore, the CO_2 response with 2% and 21% O_2 provided evidence that the difference in A between the T. dicoccoides accessions was caused neither by difference in sensitivity to O_2 nor by TPU limitation.

TPU utilization is involved in sucrose and starch synthesis. To verify our finding of no TPU limitation, we measured leaf sucrose and starch concentrations. A higher sucrose concentration was observed in PI 428042, but its relationship with photosynthetic capacity is uncertain. Many investigations have been made in search of a relationship between sucrose content of leaves and A (Stitt et al., 1984; Azco-Bieto, 1983; Stitt et al., 1987). In some studies accumulating sucrose has been correlated with an inhibition of A (Stitt et al., 1984), but this effect may not always be present (Stumpf and

Conn, 1987). Limitation of A by sucrose synthesis could be caused by suboptimal metabolic levels or suboptimal phosphate (Stumpf and Conn, 1987; Stitt et al., 1987). Most of the triose phosphates (TP) are retained in the Calvin cycle to generate RuBP, which is the acceptor for further CO_2 fixation (Sharkey, 1985). During sucrose synthesis, phosphate is released from TP and reenters the chloroplast in exchange for further TP. If this occurs faster than the rate at which TP can be produced, then the stromal metabolite will decrease and A will be inhibited (Stitt et al., 1984; Sharkey, 1985; Azcon-Bieto, 1983). This could be one reason for the higher sucrose content and the lower A in PI 428042 than in PI 428109. But this is not in agreement with the interpretation of the results obtained in the CO_2 response study.

Gas exchange was depressed by about 13% in PI 428109 whereas in the other two genotypes the depression was as high as 27% as VPD increased from 0.6 to 2.2 kPa. This might have a contribution to the lower A of PI 428042. However, it couldn't be the major reason for the difference in A between the two T. dicoccoides, as PI 428019 had consistently higher A than PI 428042 at all levels of humidity. The relatively small reductions in WP and the observed rapid response of G_s to changes in VPD suggest a feed-forward mechanism (Cowan, 1977; Farquhar, 1978) for stomatal aperture regulation based on humidity rather than internal leaf water status. A very high positive correlation was observed between G_s and A ($r = 0.990$) (Fig. 5). Reduced G_s was probably the prime cause of reduction in A. The decrease in C_i (Fig. 4C) is an indication for this relationship.

This study indicates that the difference in A between the two T. dicoccoides accessions was mainly due to differences in RuBP carboxylase

activity. A more thorough examination of the properties of the RuBP carboxylase and determination of metabolites in the accessions will be helpful in explaining the molecular basis of variation in photosynthetic rate. However, this should be supported by anatomical investigations since some anatomical features have been found to influence rate of gas exchange.

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Table 1. Several photosynthetic parameters measured on plants of two
T. <u>dicoccoides</u> accessions (PI 428042 and PI 428109) and a hexaploid
wheat (TAM W-101).

Accession	A	A/Ch	Ch a/b	Slope[+]	Slope[‡]
	$(\mu mol\ m^{-2}s^{-1})$	$(mmol\ mol^{-1})$		Low-high	High-low
PI 428042	16.7	31.9	3.29	0.028	0.020
PI 428109	25.1	39.3	3.78	0.036	0.032
TAM W-101	19.7	30.4	3.39	0.034	0.024
$LSD_{0.05}$	3.4	7.7	0.20	NS	0.004

[+]Slope low-high indicates the slope for the A vs. PPFD curve measured by
 progressing PPFD from low to high level.
[‡]Slope high-low indicated the slope for the A vs. PPFD curve measured by
 progressing PPFD from high to low level.

Table 2. Several parameters of the photosynthetic response to CO_2 at 2 and 21% O_2 for two $\underline{T.\ dicoddoides}$ accessions (PI 428042 and PI 428109) and a hexaploid wheat (TAM W-101).

Accession inhib.	O_2 level	A^*	Initial slope A/C_i	CO_2 comp.	Stomatal lim.	O_2
	%	$\mu mol\ m^{-2}s^{-1}$		$\mu l\ l^{-1}$	%	%
PI 428042	21	24	0.096	39	33	36
	2	37	0.282	10	28	
PI 428109	21	33	0.163	34	30	33
	2	48	0.334	9	23	
TAM W-101	21	25	0.121	40	33	38
	2	41	0.272	7	27	
$LSD_{0.05}$ (genotype)		5	0.089	8	10	9
(O$_2$ level)		4	0.054	6	4	

*measured at ambient CO_2 of 330 $\mu l\ l^{-1}$, 1800 μmol photon $m^{-2}s^{-1}$, 20^0C and 50% RH.

Table 3. Several photosynthetic parameters, RuBP carboxylase activity and soluble leaf protein for two $\underline{T}$. $\underline{dicoccoides}$ accessions (PI 428042 and PI 428109) and a hexaploid wheat (TAM W-101).

Accession	A $\mu mol\ m^{-2}s^{-1}$	RuBP carb. activity $\mu mol\ m^{-2}s^{-1}$	A/RuBP act.	Soluble protein $gm\ m^{-2}$	A/Chl $mmol\ mol^{-1}$
PI 428042	19.8	55.8	0.360	3.44	28.85
PI 428109	26.9	68.1	0.401	3.22	33.7
TAM W-101	25.8	71.7	0.362	5.14	31.1
$LSD_{0.05}$	2.5	6.3	0.037	0.79	2.7

Table 4. Sucrose and starch concentration per unit leaf dry weight (LDW) of two T. dicoccoides accessions (PI 428042 and PI 428109) and a hexaploid wheat (TAM W-101).

Accession	Sucrose $\mu g\ mg^{-1}$ LDW	Starch $\mu g\ mg^{-1}$ LDW
PI 428042	50.6	53.9
PI 428109	34.0	47.1
TAM W-101	66.8	43.8
$LSD_{0.05}$	9.9	NS
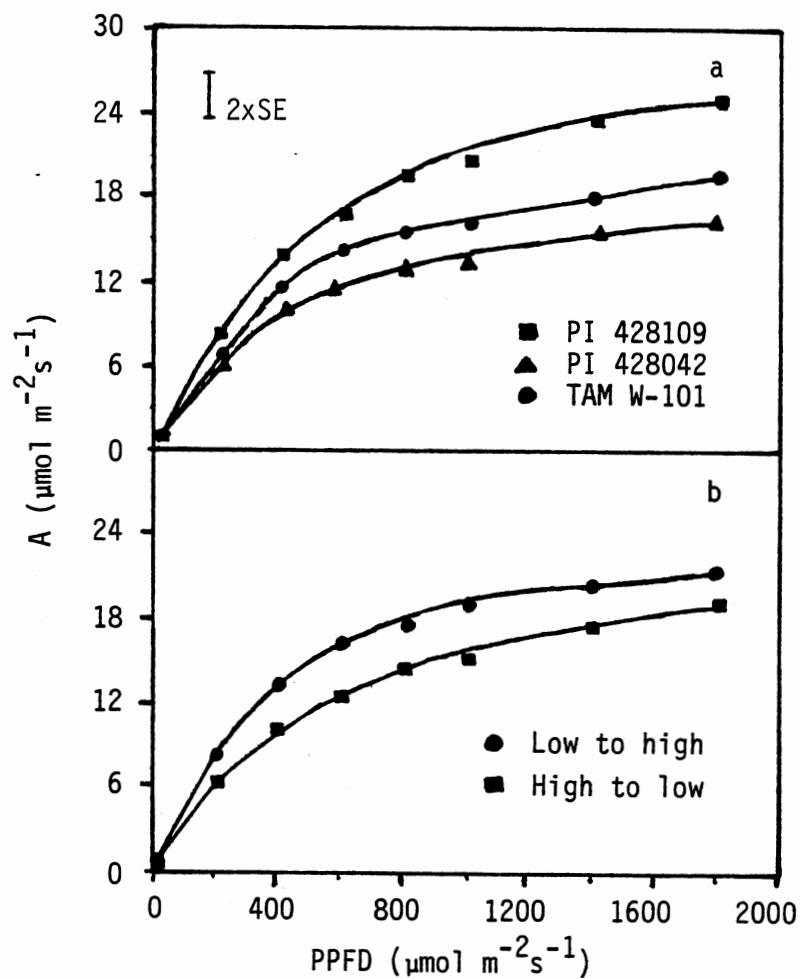
Fig. 1. (a) CO_2 assimilation per unit leaf area (A) of two <u>T</u>. <u>dicoccoides</u> accessions (PI 428042 and PI 428109) and a hexaploid wheat (TAM W-101) at varying photosynthetic photon flux density (PPFD), (b) A averaged over genotypes at two light progressions (low to high and high to low).
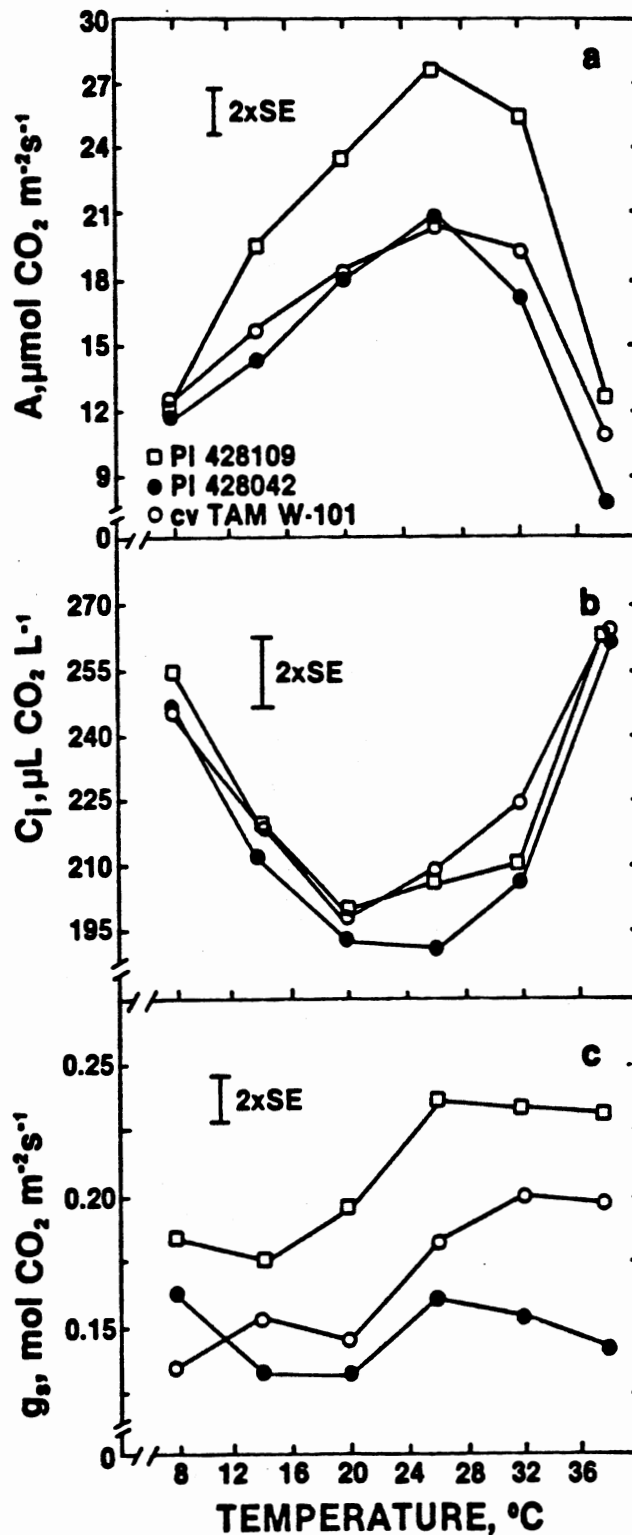
Fig. 2. CO_2 assimilation per unit leaf area
(A), internal CO_2 concentration (C_i), and
leaf conductance to CO_2 (G_s) in leaves of
two T. dicoccoides accessions (PI 428019 and
PI 428042) and a hexaploid wheat (TAM W-101).
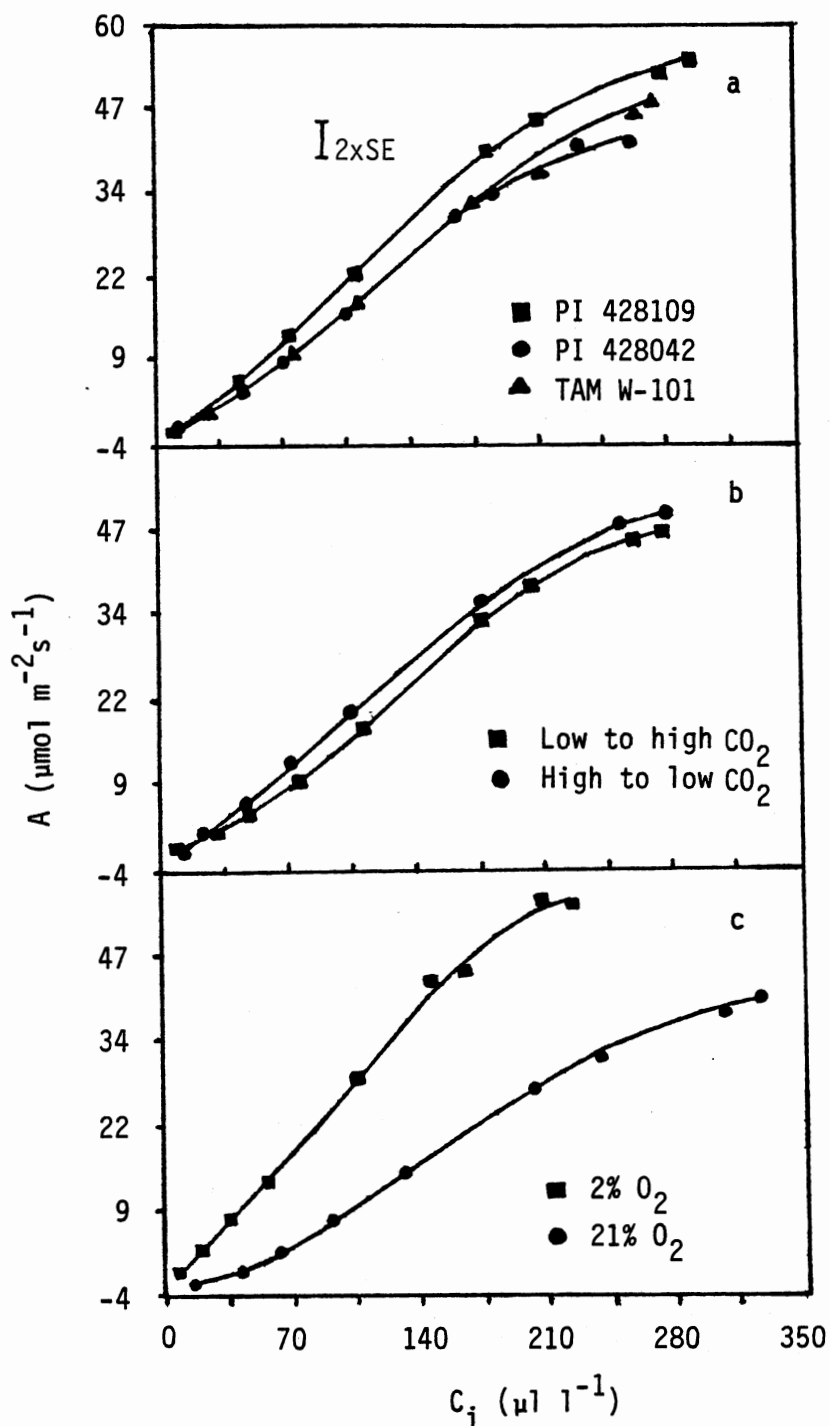
Fig. 3. (a) CO_2 assimilation per unit leaf area (A) vs. internal CO_2 conc. (C_i) in three genotypes, (b) A at two CO_2 progressions (from low to high and from high to low) averaged over genotypes and (c) A at two O_2 levels (2 and 21%) averaged over genotypes.
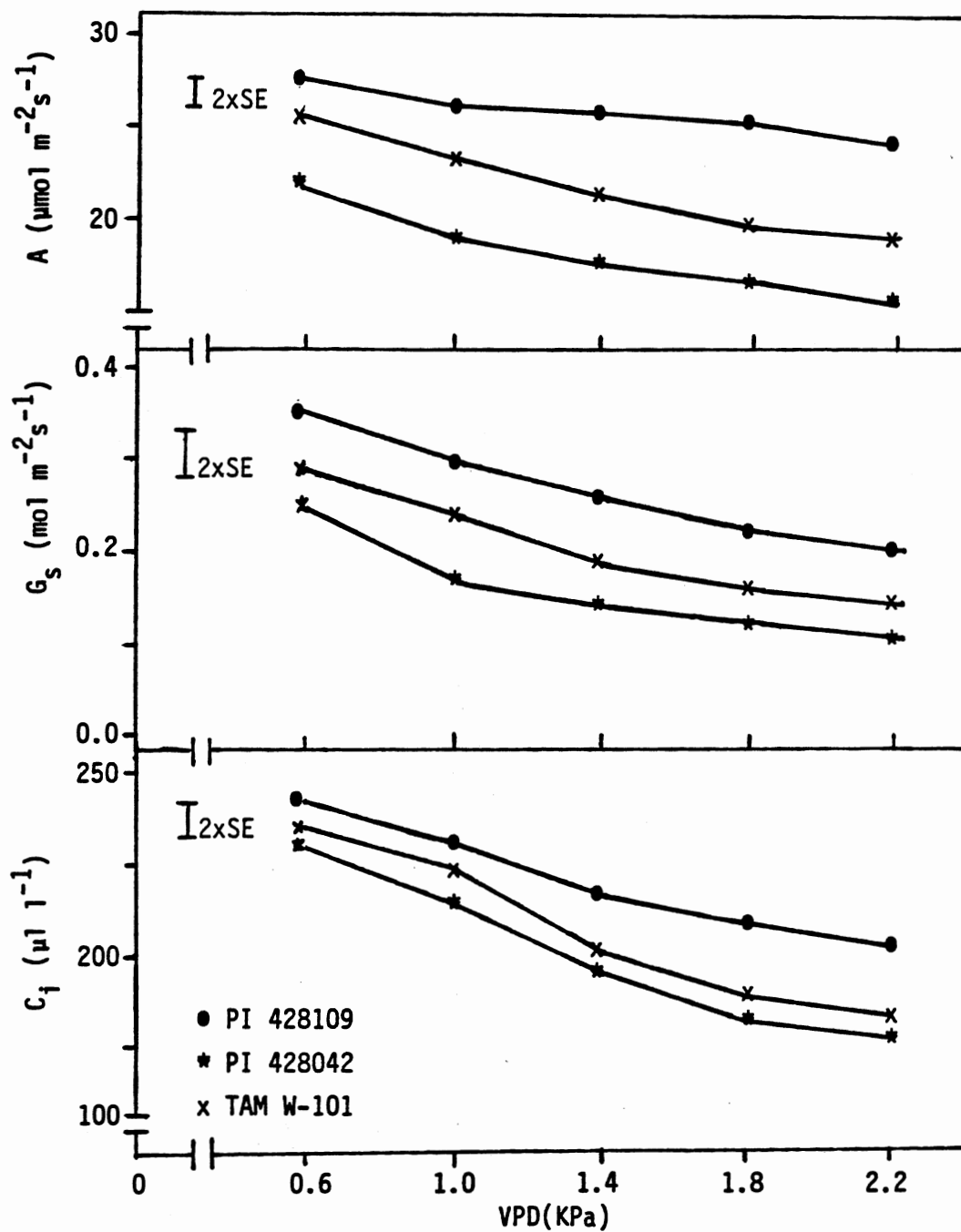
Fig. 4. CO_2 assimilation per unit leaf area (A), leaf conductance to CO_2 (G_s) and internal CO_2 concentration (C_i) in leaves of two T. dicoccoides accessions (PI 428042 and PI 428109) and a hexaploid wheat (TAM W-101) at varying vapor pressure difference (VPD) between leaf and air.
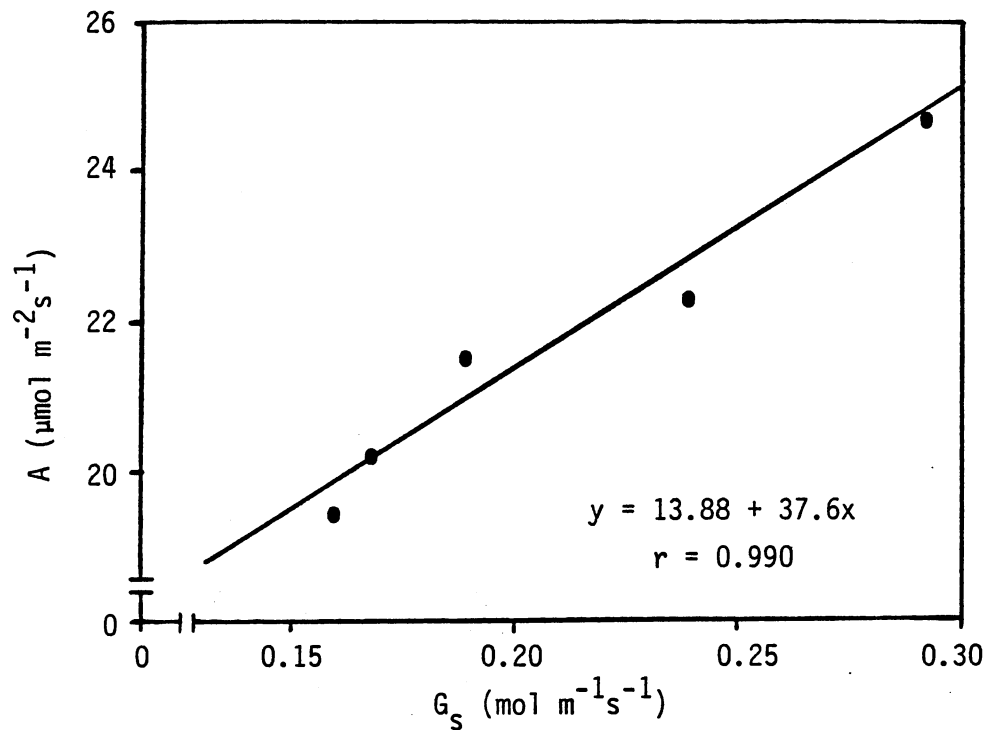
Fig. 5. Relationship between CO_2 assimilation per unit leaf area (A) and leaf stomatal conductance (G_s) in two T. dicoccoides accessions and a hexaploid wheat using means across genotypes at varying levels of vapor pressure difference (VPD) between leaf and air.

CHAPTER III

Comparison of anatomical characters between two
Triticum dicoccoides accessions differing
in photosynthetic rate

ABSTRACT

Variation in photosynthetic rate can be attributed to anatomical,

physiological, and/or biochemical differences in leaf tissue. Leaf

area, leaf width, mesophyll cell size, and interveinal distance are

often associated with photosynthetic rate. The objective of this study

was to determine whether any of these anatomical features were

associated with the reported difference in photosynthetic rate between

two Triticum dicoccoides accessions (PI 428042 and PI 428109). Light

and electron microscopic investigations were made on sections of newly

expanded leaves of the two accessions and a hexaploid wheat, TAM W-101

(included as a standard check). Plants were grown in a growth chamber

for four weeks under 14 h light and 20/15°C (day/night). Volume

fractions of leaf cross-sectional components (mesophyll, intercellular

air space, vascular bundle and bundle sheath, and epidermis), mesophyll

cell volume, surface area. and total mesophyll surface area to leaf

volume ratio, interveinal distance and volume fraction of mesophyll

chloroplasts were determined stereologically. Stomatal density and pore

length, leaf thickness and leaf width were also determined. No leaf

anatomical differences were detected between the two T. dicoccoides

accessions. Therefore, anatomical differences are not contributing toward differences in photosynthetic rate in these accessions. TAM W-101 had larger leaf area, width and mesophyll cell size than the two accessions. This study supports previous results indicating photosynthetic differences between the two T. dicoccoides accessions were mainly due to physiological and/or biochemical factors.

Introduction

Various hypotheses based on leaf anatomy have been developed to explain photosynthetic differences reported among _Triticum_ species. With increasing ploidy level a decrease in photosynthetic rate per unit leaf area is often accompanied by an increase in leaf area (Dunstone et al., 1973; Parker and Ford, 1982). Surface area per unit volume of mesophyll tissue, along with cell size, degree of cell lobing, interveinal distance, and other gross morphometric measurements, have also been used to explain interspecific differences (Austin et al., 1982; Jellings and Leech, 1984). Dunstone and Evans (1974) found that the area of macerated mesophyll cells was larger in cultivated tetraploids and hexaploids than in diploids, and was negatively correlated with photosynthetic rate. The mesophyll surface area per unit leaf area was also larger in diploids than in polyploids (Sasahara, 1982). Jellings and Leech (1984) indicated that cell size rather than cell number is the major component of interveinal distance.

This study was mainly conducted within a single species to reduce the confounding effects of ploidy level and leaf area. We have identified two accessions of the wild tetraploid species _Triticum dicoccoides_, differing in photosynthetic rate but having the same leaf area (Johnson et al., 1987). Our goal was to determine whether any of the anatomical features believed to be associated with photosynthesis were correlated with photosynthetic differences between these two accessions. This anatomical information will be combined with physiological and genetic information to more fully understand the basis for variation in photosynthetic rate in _Triticum dicoccoides_.

Materials and methods

Plants of the two, accessions, PI 428042 and PI 428109, and the hexaploid wheat, TAM W-101 (included for comparison) were grown in pots in a growth chamber for five weeks at 20/15°C (day/night), 14-h photoperiod with photosynthetic photon flux density of 600 mol $m^{-2}s^{-1}$ at pot level. The plants were watered every day with 25% strength Hoaglands's nutrient solution.

Simultaneous measurements of CO_2 assimilation and transpiration rates were made on the most recently fully emerged leaf of the main vegetative tiller using a LI-COR 6200 portable photosynthetic system [LI-COR Inc. Lincoln, NE].

Leaf samples were collected from the mid-lamina section following carbon exchange measurements. The leaf segments were fixed in cold 0.1 M potassium phosphate-buffered 4% glutaraldehyde, post-fixed with 1% osmium tetraoxide (OsO_4) for 4 h in the dark, dehydrated in graded series of ethanol and embedded in a firm-formulation epoxy resin of Spurr (Spurr, 1969).

For light microscopic investigations sections of 0.5 m were made with ultramicrotome using glass knife and stained with 1% toluidine blue in 1% borax. Photomicrographs of the transverse sections were taken at a magnification 200X. Slide pictures of these sections were projected on a viewing screen and stereological measurements were made by randomly superimposing a transparent (1 pseudo-random test grid for volume density determinations by the point-counting method (spacing 1.0 cm) or (2) isotropic Merz grid test system for surface densities by the line intersection method (spacing 1.2 cm) (Weibel, 1979). Measurements were

made on leaf thickness, distance between vascular bundles, and length of mesophyll cell wall exposed to intercellular air space. Volume fractions of mesophyll, epidermis, vascular tissue, and intercellular air space were calculated. Surface area to volume ratio was also determined for mesophyll tissue (Weibel, 1979).

Thin leaf sections (0.01 μm) were cut with ultramicrotome using a diamond knife for analysis by electron microscope. Sections were stained in 5% uranyl acetate and lead citrate. Photomicrographs of the mesophyll cells were taken at magnifications of 3500X, 7000X, and 14000X to determine volume fractions of chloroplasts and other organelles.

Additional leaf samples were taken from the mid-laminal section of the same leaves above and preserved in formalin-propionic acid. Sections of 10 mm^2 were cut and incubated in 6% NaOH at room temperature for 16 h. Cells were teased out in 3 ml of 1% Fast Green FCF in water/glycerate (50:50 mixture). Twenty μl of the cell suspension were withdrawn and placed on a slide. Camera lucida outline drawings were made on a random sample of 25 mesophyll cells from each of six leaves per genotype (recorded on a Zeis MOP 3 Digitizing Tablet) and on these drawings number of lobes on the cells were counted. Surface area, volume and surface area to volume ratio of the mesophyll cells were also calculated.

Leaves at approximately the same developmental stage were excised for stomatal counts and stomatal pore length determination. Impressions of the upper and the lower surface of the leaves were made by applying clear finger-nail polish to the mid-portions of fully emerged leaves. The number of stomata in a 6.5 mm^2 grid were counted and stomatal pore

length of 6 adaxial and 6 abaxial stomata per plant were measured under a microscope.

The plants were grown in a completely randomized design inside a growth chamber. Frequency distribution was made on the number of lobes on the mesophyll cells and Chi-square test was used to see if there were differences in lobe number between genotypes.

Results

No significant differences were detected by the light microscopic
analysis of volume fractions between the two T. dicoccoides accessions
or between the two accessions and TAM W-101 (Table 1). The mesophyll
tissue and the intercellular air space occupied about 38 and 30% of the
leaf volume in all genotypes, respectively. Because photosynthetic
cells are primarily located in mesophyll tissue, we made a closer
investigation of the components of individual mesophyll cells. no
genotypic differences were observed either in the total surface area of
the mesophyll tissue per unit volume of leaf (S_{tot}/V) or the total
surface area exposed to the intercellular air space per unit leaf volume
(S_{air}/V) (Table 2). Surface area (S_A) and volume (V) of individual
mesopyll cells were similar for the T. dicoccoides accessions, but
mesophyll cells of TAM W-101 had larger surface are and volume than T.
dicoccoides. When expressed per unit volume (S_A/V), however, TAM W-101
did not differ from PI 428109 (Table 2). The mesophyll cells of the low
photosynthetic accession, PI 428042, did show significantly larger
surface area per unit volume than those of PI 428109 or TAM W-101
(Table 2).

Camera lucida outline drawings of a random sample of mesophyll
cells from macerated young and fully emerged leaves are illustrated in
Fig. 1. The number of lobes per mesophyll cell were counted in 150
random cells from each of the three genotypes. According to the
frequency distribution in Fig. 2, the majority of mesophyll cells in PI
428109 and PI 428042 had two or three lobes. Although PI 428042 had a
higher percentage of mesophyll cells with four or more lobes (60%) than

PI 428109 (51%) the Chi-square test did not show that the difference was significant. Seventy one percent of the mesophyll cells from TAM W-101 had four or more lobes.

No significant differences were detected among the three genotypes in various cell volume fractions based on electronmicrographs (Table 3). About 36% of the mesophyll cell volume was composed of chloroplasts in these genotypes.

An examination of the external leaf structures showed that the T. dicoccoides accessions had the same leaf size but smaller than that of TAM W-101 (Table 4). However, in leaf thickness and interveinal distance no significant differences were detected among these genotypes.

Stomatal counts on the adaxial and abaxial surface of the leaves showed no differences between the two T. dicoccoides, but TAM W-10 had a higher number of stomata on the adaxial surface of its leaves (Table 5). Stomatal pore lengths were not different among genotypes.

Discussion

In the <u>Triticum</u> species, higher photosynthesis per unit leaf area is associated with small mesophyll cell size (Austin et al., 1982). The diploid species have the smallest mesophyll cells while hexaploids have the largest mesophyll cells (Jellings and Leech, 1984; Austin et al., 1982; Parker and Ford, 1982). Smaller mesophyll cell size in the lower ploidy level has resulted in smaller and narrower leaves with closely spaced veins. In this study also, the hexaploid wheat had larger mesophyll cell size as well as larger and wider leaves than the tetraploid wild wheat accessions. However, in contrast to earlier reports (Austin et al., 1982; Evans and Dunstone, 1970; Parker and Ford, 1982) the two <u>T. dicoccoides</u> accessions having same mesophyll cell size, leaf area and leaf width were significantly different in photosynthetic rate.

Sasahara (1982) reported that the surface area to volume ratio of mesophyll cells increases with increasing number of lobes per cell within a genome. Mesophyll cells of the two <u>T. dicoccoides</u> accessions had significantly different surface area to volume ratio on an individual cell basis. The low photosynthetic rate accession, PI 428042, actually had mesophyll cells with a larger surface area to volume ratio and with greater lobe number than cells of PI 428109, although the difference in lobe number was not statistically significant. Therefore, mesophyll surface area per unit volume was not responsible for the photosynthetic difference between the two <u>T. dicoccoides</u> accessions. This was contrary to what has been reported in

other studies (Sasahara, 1982; Parker and Ford, 1982), i.e., the larger the surface area per unit volume the higher the photosynthetic rate.

High rates of photosynthesis in diploids were also found to be associated with an increase in leaf intercellular air space and mesophyll surface area exposed to the intercellular air space per unit leaf area (De Greef et al., 1979; Parker and Ford, 1982; Jellings and Leech, 1984). These factors may contribute to higher rates of photosynthesis by reducing the residual diffusion resistance of carbon dioxide into the mesophyll (Austin et al., 1982; Parker and Ford, 1982). However, in this study, the difference in photosynthetic rate among genotypes could not be accounted for by differences in the amount of intercellular air space and mesophyll surface area exposed to the intercellular air space since the T. dicoccoides accessions did not differ in these measurements.

Veins of hexaploid wheat were found to be more widely spaced and the cells were less compact than diploid wild species (Parker and Ford, 1982; Austin et al., 1982). Parker and Ford (1982) suggested that greater interveinal distance could impede the movement of photosynthate and water between veins and chloroplasts and thus accounts in part for the lower rate of photosynthesis in hexaploids. This difference was not apparent in this study either between the two T. dicoccoides accessions or between T. dicoccoides and the hexaploid.

Several reports have shown that photosynthetic variation is strongly associated with various anatomical differences among leaves of Triticum species also differing in ploidy (Jellings and Leech, 1984; Nobel et al., 1975). However, our results indicated that the variation in photosynthetic rate between the two T. dicoccoides accessions was not

associated with any of their anatomical features. We have made other physiological and biochemical investigations on these accessions and found differences related to their photosynthesis. Therefore, this study supports that the difference in photosynthetic rate between the two accessions was due to biochemical and/or physiological factors in the leaves rather than anatomical differences.

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Table 1. Photosynthesis and relative volume of leaf cross-sectional components of two T. dicoccoides accessions (PI 428042 and PI 428109) and a hexaploid (TAM W-101).

Accession	Photosynthesis[a] (µmol $m^{-2}s^{-1}$)	Epidermis	Mesophyll	Vascular & bundle sheath %	Intercellular airspace
PI 428042	12.9	23.6	36.1	8.6	29.4
PI 428109	19.8	22.8	39.5	7.6	30.1
TAM W-101	17.0	23.8	38.7	8.0	29.5
LSD.05	2.7	NS	NS	NS	NS

[a] at 600 µmol photon $m^{-2}s^{-1}$, 330 µl 1^{-1} ambient CO_2, 20°C and 50% RH.

Table 2. Surface area, volume, and surface area per unit volume of the mesophyll component in the leaves of two $\underline{T}$. $\underline{dicoccoides}$ accessions (PI 428042 and PI 428109) and a hexaploid (TAM W-101).

Accession	Mesophyll tissue		Mesophyll cell		
	S_{tot}/V [a] $(cm^2\ cm^{-3})$	S_{air}/V [b] $(cm^2\ cm^{-3})$	$S_A\ (\mu m^2)$ [c]	$V\ (\mu m^2)$ [d]	S_A/V [e] $(\mu m^2\ \mu m^{-3})$
PI 428042	954	682	1.17×10^4	6.87×10^4	1.82×10^{-1}
PI 428109	913	661	1.19×10^4	7.52×10^4	1.72×10^{-1}
TAM W-101	946	679	1.31×10^4	8.40×10^4	1.69×10^{-1}
$LSD_{0.05}$	NS	NS	8.02×10^2	7.83×10^3	7.32×10^{-3}

[a] total surface area per unit volume
[b] surface area exposed to intercellular air space per unit volume of leaf tissue
[c] surface area
[d] volume
[e] surface area per unit volume

Table 3. Relative volume of mesophyll cell cross-sectional components of two T. dicoccoides accesions and a hexaploid wheat (TAM W-101).

Accession	Chloroplast	Cell Wall	Mitochondria	Others [a]
			%	
PI 428042	37.0	8.3	3.9	50.8
PI 428109	35.9	7.6	3.1	53.4
TAM W-101	35.8	7.2	3.3	53.7
$LSD_{0.05}$	NS	NS	NS	NS

[a] the rest of the organelles in the mesophyll cell.

Table 4. Some leaf characteristics of $\underline{T}$. $\underline{dicoccoides}$ accessions (PI 428042 and PI 428109) and a hexaploid (TAM W-101).

Accession	Leaf area (cm^2)	Leaf width (mm)	Leaf thickness (mm)	Interveinal distance (mm)
PI 428042	10.5	5.3	0.22	0.42
PI 428109	10.0	5.2	0.23	0.41
TAM W-101	14.4	9.7	0.22	0.43
$LSD_{0.05}$	1.4	0.5	NS	NS

Table 5. Leaf stomatal number and pore length of two
T. dicoccoides accessions and a hexaploid wheat
(TAM W-101).

Accession	No. of stomata (per mm^2)		Stomatal pore length (μm)	
	abaxial	adaxial	abaxial	adaxial
PI 428042	36	42	35.1	39.0
PI 428109	38	41	37.6	41.0
TAM W-101	35	49	34.2	38.0
$LSD_{0.05}$	NS	6	NS	NS

Fig. 1. Camera lucida outline drawings of a random sample of mesophyll cells from macerated young and fully emerged leaves of four weeks old plants.
(a) T. dicoccoides (PI 428042), (b) T. dicoccoides (PI 428109), (c) T. aestivum (TAM W-101).
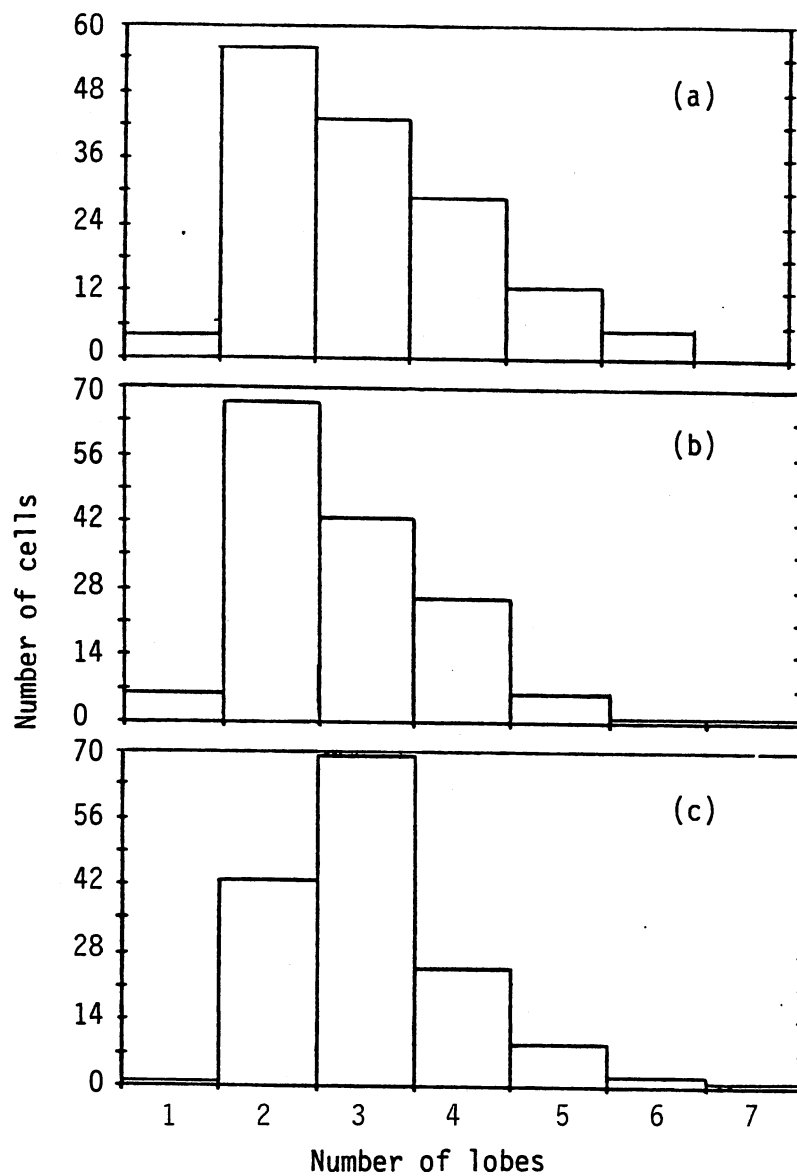
Fig. 2. Frequency distribution of the number of lobes per cell in a random sample of mesophyll cells from young and fully emerged leaves of four weeks old plants of two T. dicoccoides accessions [(a) PI 428042 and (b) PI 428109] and (c) a hexaploid wheat (TAM W-101).

TABLE 1

MEAN VALUES OF ASSIMILATION RATE PER UNIT LEAF AREA FOR LEAVES OF TWO ACCESSIONS OF T. DICOCCOIDES SPECIES (PI 428042 AND PI 428109) AND A CULTIVATED HEXAPLOID (TAM W-101) AT VARYING PHOTOSYNTHETIC PHOTON FLUX DENSITY (PPFD) WITH PPFD PROGRESSING FROM LOW TO HIGH AND FROM HIGH TO LOW LEVELS.

		PPFD							
		0	200	400	600	800	1000	1400	1800
ACCESS-IONS	PROGRE-SSION								
PI428042	Low-High	0.3	7.6	11.5	13.9	14.9	15.9	17.1	17.7
	High-Low	-0.0	5.1	8.1	9.7	10.9	11.4	13.3	15.6
PI428109	Low-High	0.1	8.8	14.5	18.2	21.0	22.4	25.2	26.4
	High-Low	0.2	7.8	13.0	15.4	18.4	19.3	22.1	23.9
T W-101	Low-High	-0.0	8.1	13.6	16.4	17.8	18.3	18.9	20.6
	High-Low	0.0	5.7	9.5	12.6	13.6	14.3	17.0	18.8
Accession									
PI 428042		0.2	6.3	9.8	11.8	12.9	13.7	15.2	16.7
PI 428109		0.1	8.3	13.7	16.8	19.7	20.9	23.6	25.1
TAM W-101		0.0	6.9	11.6	14.5	15.7	16.3	18.0	19.7
ALL		0.1	7.2	11.7	14.4	16.1	17.0	18.9	20.5

TABLE 2

MEAN VALUES OF ASSIMILATION RATE PER UNIT LEAF AREA (a),
INTERNAL CO_2 CONCENTRATIN (b), AND LEAF STOMATAL CONDUC-
TANCE (c) OF TWO ACCESSIONS OF T. DICOCCOIDES SPECIES AND
A CULTIVATED HEXAPLOID (TAM W-101) AT VARYING TEMPERATURE.

(a)

Accession	Temperature (^{o}C)					
	8	14	20	26	32	38
PI 428042	11.7	14.0	18.2	20.8	17.3	7.8
PI 428109	12.6	17.8	23.5	27.7	25.6	12.7
TAM W-101	12.0	15.6	18.1	20.5	19.3	11.0
ALL	12.1	15.8	19.9	23.0	20.7	10.5

(b)

Accession	Temperature (^{o}C)					
	8	14	20	26	32	38
PI 428042	246.33	212.00	194.17	191.00	207.00	260.67
PI 428109	261.43	220.33	200.50	204.67	210.83	266.33
TAM W-101	234.00	219.50	199.00	206.67	224.50	266.17
ALL	248.78	217.28	197.89	200.78	214.11	264.39

(c)

Accession	Temperature (^{o}C)					
	8	14	20	26	32	38
PI 428042	0.163	0.133	0.132	0.162	0.153	0.142
PI 428109	0.181	0.175	0.197	0.237	0.235	0.233
TAM W-101	0.130	0.153	0.145	0.182	0.200	0.198
ALL	0.161	0.154	0.158	0.193	0.196	0.191

TABLE 3

MEAN VALUES OF ASSIMILATION RATE PER UNIT LEAF AREA FOR LEAVES OF TWO T. DICOCCOIDES ACCESSIONS (PI 428042 AND PI 428109) AND A CULTIVATED HEXAPLOID (TAM W-101) AT TWO PROGRESSIONS OF CO_2 (FROM LOW TO HIGH AND FROM HIGH TO LOW CO_2 LEVELS) AND TWO OXYGEN LEVELS (2 AND 21%).

Oxygen	Accession	Progression	Ambient CO_2 concentration ($\mu l\ l^{-2}$)								
			0	40	75	126	208	330	436	584	622
2%	PI428042	Low-high	-1.0	1.9	5.1	10.1	17.6	34.9	40.0	48.2	51.3
		High-low	-0.8	3.4	7.3	13.2	21.7	39.0	42.1	50.4	47.2
	PI428109	Low-high	-0.8	2.5	6.6	12.8	23.5	47.1	52.1	58.9	60.4
		High-low	-0.9	4.4	9.8	17.8	28.1	49.3	52.4	62.6	64.1
	TAM W-101	Low-high	-0.7	2.5	6.2	11.5	20.0	39.2	44.0	53.1	54.7
		High-low	-0.2	4.1	8.4	14.8	23.6	42.7	39.6	55.6	58.0
21%	PI428042	Low-high	-1.2	-0.1	1.7	5.5	10.8	21.7	25.9	31.3	33.2
		High-low	-2.5	-0.7	2.4	6.7	13.3	26.0	27.8	34.3	38.2
	PI428109	Low-high	-2.1	-0.0	3.4	8.8	16.8	29.9	35.5	42.2	42.9
		High-low	-2.0	-0.7	4.0	10.0	18.8	35.3	39.5	47.3	50.1
	TAM W-101	Low-high	-1.5	-0.2	2.2	6.2	13.1	26.1	33.3	38.4	39.3
		HIgh-low	-2.2	-0.5	2.0	6.3	12.9	24.3	30.0	36.8	38.7

TABLE 3 (CONTINUED)

Oxygen	Accession	Ambient CO_2 concentration								
		0	40	75	126	208	330	436	584	622
2%	PI 428042	-0.9	2.7	6.2	11.6	19.7	36.9	41.0	49.3	48.8
	PI 428109	-0.9	3.4	8.2	15.3	25.8	48.2	52.2	60.8	62.3
	TAM W-101	-0.5	3.3	7.3	13.1	21.8	41.0	41.3	54.4	56.3
21%	PI 428042	-1.8	-0.4	2.1	6.1	12.1	23.8	26.9	32.8	34.9
	PI 428109	-2.0	-0.4	3.7	9.4	17.8	32.6	37.5	44.8	46.5
	TAM W-101	-1.9	-0.3	2.1	6.2	13.0	25.2	32.2	37.6	39.0
Oxygen 2%		-0.7	3.1	7.2	13.4	22.4	42.0	44.6	54.8	55.3
21%		-1.9	-0.4	2.6	7.2	14.3	27.2	32.2	38.4	40.6

TABLE 4

MEAN VALUES OF INTERNAL CO_2 CONCENTRATION FOR THE LEAVES OF TWO T. DICOCCOIDES ACCESSIONS (PI 428042 AND PI 428109) AND A CULTIVATED HEXAPLOID (TAM W-101) AT TWO PROGRESSIONS OF CO_2 (FROM LOW TO HIGH AND FROM HIGH TO LOW CO_2 LEVELS) AND TWO OXYGEN LEVELS (2 AND 21%).

Oxygen	Accession	Progression	Ambient CO_2 concentration ($\mu l\ l^{-1}$)								
			0	40	75	126	208	330	436	584	622
2%	PI428042	Low-high	8.50	23.50	37.00	58.00	93.00	142.50	154.50	196.50	206.50
		High-low	5.00	17.00	29.50	49.00	72.50	134.00	143.50	185.00	227.67
	PI428109	Low-high	6.50	23.00	38.00	63.00	97.00	164.00	178.00	245.50	259.00
		High-low	5.00	17.00	29.50	49.50	79.50	149.00	160.00	213.50	231.50
	TAM W-101	Low-high	5.00	22.50	37.50	60.50	91.50	132.50	150.00	200.00	209.00
		High-low	3.00	14.50	29.00	52.00	81.00	151.00	192.67	218.50	228.00
21%	PI428042	Low-high	7.50	40.00	63.00	89.50	127.50	185.50	227.50	290.00	312.00
		High-low	20.50	45.00	63.00	90.00	124.50	197.00	216.00	266.50	291.00
	PI428109	Low-high	12.00	39.00	59.50	89.00	131.00	204.50	249.50	328.00	348.00
		High-low	9.00	42.50	61.00	92.00	133.50	217.00	255.50	329.00	352.00
	TAM W-101	Low-high	11.00	41.00	64.00	95.00	136.00	206.00	247.00	309.50	321.50
		High-low	20.50	42.50	63.50	93.00	134.50	211.00	251.00	324.50	343.00

TABLE 4 (CONTINUED)

Oxygen	Accession	Ambient CO_2 concentration ($\mu l\ l^{-1}$)								
		0	40	75	126	208	330	436	584	622
2%	PI 428042	6.75	20.25	33.25	53.50	82.75	138.25	149.00	190.75	219.20
	PI 428109	5.75	20.00	33.75	56.25	88.25	156.50	169.00	229.50	245.25
	TAM W-101	4.00	18.50	33.25	56.25	86.25	141.75	175.60	209.25	218.50
21%	PI 428042	14.00	42.50	63.00	89.75	126.00	191.25	221.75	278.25	305.00
	PI 428109	10.50	40.75	60.25	90.50	132.25	210.75	252.50	328.50	350.00
	TAM W-101	15.75	41.75	63.75	94.00	135.25	208.50	248.33	317.00	332.25
Oxygen 2%		5.50	19.58	33.42	55.33	85.75	145.50	165.38	209.83	227.00
21%		13.42	41.67	62.33	91.42	131.17	203.50	240.18	307.92	331.27

TABLE 5

MEAN VALUES OF ASSIMILATION RATE PER UNIT LEAF AREA (a),
INTERNAL CO_2 CONCENTRATION (b), AND LEAF STOMATAL CONDUC-
TANCE (c) OF TWO ACCESSIONS OF T. DICOCCOIDES SPECIES AND
A CULTIVATED HEXAPLOID (TAM W-101) AT VARYING VAPOR PRE-
SSURE DIFFERENCE (VPD) FROM LEAF TO AIR.

(a)

	VPD				
	0.6	1	1.4	1.8	2.2
Accession					
PI 428042	21.5	18.8	17.0	16.3	15.7
PI 428109	27.5	26.9	26.4	25.1	24.0
TAM W-101	25.1	23.2	21.2	19.1	18.5
ALL	24.7	23.0	21.6	20.2	19.4

(b)

	VPD				
	0.6	1	1.4	1.8	2.2
Accession					
PI 428042	230.33	214.00	195.17	182.17	178.00
PI 428109	243.00	230.50	216.50	208.50	203.17
TAM W-101	236.83	224.00	200.67	188.50	183.67
ALL	236.72	222.83	204.11	193.06	188.28

(c)

	VPD				
	0.6	1	1.4	1.8	2.2
Accession					
PI 428042	0.388	0.283	0.222	0.195	0.183
PI 428109	0.548	0.480	0.417	0.370	0.342
TAM W-101	0.468	0.392	0.288	0.240	0.225
ALL	0.468	0.385	0.309	0.268	0.250

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