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The Effect of Plant and Environmental Factors on Photosynthetic Efficiency

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Abstract

The photosynthetic mechanism of three wheat and tobacco cultivars was studied. Among the wheat cultivars the two unicum 380 and 492 had higher photosynthetic rates than the commercial cultivar Lakhish. Lakhish had the highest stomatal and mesophyll resistance and the lowest CO_2 compensation point. Increased oxygen concentrations and temperature reduced the photosynthetic rate of Cv 380 and 492 more than of Cv Lakhish. The post illumination burst of Lakhish was lower than that of 380 and 492. It was concluded that Lakhish had the lowest photorespiration rate and its low photosynthetic rate was due mainly to high mesophyll and stomatal resistance. Oxygen increased the internal CO_2 and stomatal resistance in all three cultivars. The photosynthetic rate of isolated protoplasts was high in Cv 380 medium in 492 and low in Lakhish. The advantage of Cv 380 over the other two cultivars was greater than in whole leaves. It was concluded that the transport of CO_2 in the liquid phase was relatively slow in Cv 380 and limited the rate of photosynthesis of whole leaves. Isolated chloroplasts of Cv 380 and 492 had a higher photosynthetic rate than Lakhish. This is a further proof that chloroplasts and protoplasts of Cv 380 could utilize CO_2 at a faster rate than Lakhish and leaves of 380 could photosynthesize faster if CO_2 transport was not limiting. Electron transport and cyclic and non cyclic photophosphorylation rates were relatively higher in Cv 380 and so was the quantum yield.

Among the Tobacco cultivars glutinosa had a higher net photosynthesis rates than samsun and oriental. All three cultivars responded similarly to oxygen, temperature and photosynthetic photon flux density. Oriental had the highest stomatal resistance among the cultivars. Glutinosa had the lowest mesophyll resistance and oriental the highest. The CO_2 compensation point did not vary among the cultivars. Glutinosa was relatively less responsive to CO_2 and oxygen indicating that this cultivar had somewhat lower photorespiration rate. Isolated protoplasts of Cv Oriental had the highest photosynthetic rate among the cultivars. It was concluded that the CO_2 transport in the liquid phase was limiting the photosynthetic rate of whole leaves. The carboxylase activity of Rubisco was found to be relatively higher in Cv Oriental when calculated on a leaf area basis. It was concluded that photorespiration was not the major cause for the differences in the photosynthetic efficiency of the various cultivars. CO_2 transport and mesophyll resistance were probably more important in these respects.

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Objectives of the original research proposal.

1. To improve the method for the measurement of photorespiration based on $^{14}\text{CO}_2$ uptake by the leaf.
2. To study the relationships between mesophyll resistance RuBp carboxylase oxygenase activity and net photosynthesis.
3. To study the effect of environmental conditions on various photosynthetic parameters.

Abbreviations

A - net assimilation rate

Dr - Dark respiration

T - CO_2 compensation point

r_s - Stomatal resistance

r_m - mesophyll resistance

RuBp - Ribulose biphosphate

pR - Photorespiration

P.I.B. - Post Illumination Burst

PPFD - Photosynthetic photon flux density.

Introduction

The mechanism responsible for the differences in the photosynthetic efficiency between various cultivars is not yet fully clear. The photosynthetic efficiency of single leaves is the basic unit which influences the whole plant or whole field photosynthesis and consequently the productivity.

It is commonly accepted that quantum yield of most plants is similar (Zelitch 1970) and the differences in photosynthetic efficiency between plant cultivars are due mainly to the carboxylation efficiency. The carboxylation efficiency is represented by the slope of the net photosynthesis vs the intercellular CO_2 concentration (Ku and Edwards 1977, Farquhar & Sharkey 1982). However this method of calculation does not allow to differentiate between the biochemical resistance of the enzyme Rubisco and physical resistance to the CO_2 transfer from the atmosphere to the fixation site inside the chloroplasts. Raven and Glidwell (1981) as well as Nobel (1983) who used calculations based on pathway dimensions came up with transport resistance values which are higher than many of the measured values found in the literature. On the other hand Farquhar and Sharkey (1982) concluded on the basis of carbon isotope discrimination that the transfer resistance is very small as compared to the values obtained by Gaastra (1959) who was mistaken by considering the intracellular CO_2 concentration in the chloroplasts to be zero. Nevertheless Evans (1983) who used Farquhar's model and consultation found that the resistance is not necessarily insignificant.

The rate of photorespiration was also found to have an important

impact on the net photosynthetic rate (Zelitch 1979). However due to difficulties in measuring the rate of photorespiration the values in the literature vary greatly and it is not clear whether and to what extent do the differences in photosynthetic efficiencies between plant species and cultivars could be attributed to photorespiratory rates. Recent techniques in measuring photosynthesis of isolated cells, protoplasts and chloroplasts (Rathnam and Edwards 1976, Rathnam and Chollet 1980) were used in this study to compare the photosynthetic efficiency of isolated cells, protoplasts and chloroplasts as compared to whole leaves of various peanuts tobacco and wheat cultivars differing in their photosynthetic efficiency.

An attempt was made to study the mechanisms responsible for the differences in photosynthetic efficiency of various cultivars of the species. Peanuts (**Arachis hypogaea*) wheat (*tritium aestivum*) and Tobacco (*Nicotiana Tabaccum*) were used for this study.

Materials and Methods

Three wheat and tobacco cultivars served as model plants in this project. The cultivars were selected after preliminary experiments revealed that they vary in their photosynthetic rate. The following cultivars were selected.

1. Wheat.

- (a) *Triticum aestivum* L. Cv 492
- (b) *Triticum aestivum* L. Cv 380
- (c) *Triticum aestivum* L. Cv Lakhish

The first two cultivars are unicum bred by Prof. Dan Atsmon of the Weizman Institute. The third one is a commercial cultivar grown in Israel.

2. Tobacco.

- (a) *Nicotiana tabaccum* Var Samsun
- (b) *Nicotiana tabaccum* Var Oriental
- (c) *Nicotiana Glutinosa*.

All plants were grown in one l pots containing peat, sand and sandy loam at equal proportions. The plants were grown in a greenhouse at 25C and natural sunlight. The measurements on wheat were made at flowering time and photosynthetic rate was measured on their flag leaves. Protoplasts and chloroplasts were isolated from 7 days old seedlings grown at 24°C 15 hours light per day by fluorescent lamps and 70% relative humidity.

Tobacco plants were measured at an age of 50 to 60 days when plants reached 5-6 leaves stage.

reached 5-6 leaves stage.

The gas exchange system

The system consisted of an IRGA (Unor2 Maihak W. Germany) for CO_2 measurements, Dew point hygrometer (EG&G 880 USA) for water vapor measurements. Air with different CO_2 concentrations was provided by a series of gas mixture pumps (Wosthoff West Germany).

Light was provided by 400 w HPLR (Phillips Holland). Leaf and air temperature were measured by copper constantan thermocouples.

The leaf chamber was a double wall transparent plexiglass, its temperature was controlled by circulating water from a temperature controlled bath. Air flow was measured by flow meters (rotameters). The system operated as an open system and the results of net photosynthesis, dark respiration and transpiration were calculated from the rate of air flow, and the difference in CO_2 and H_2O concentration between the inlet and the outlet of the leaf chamber.

Leaf resistance to water vapor were calculated from transpiration and leaf temperature as measured by fine gauge copper constantan thermocouples. These calculation is based on the assumption that the mesophyll cell wall is always saturated with water (Kuiper 1964, Von Cammerer and Farquhar, 1981). Intercellular CO_2 concentration was calculated according to Moss and Kawlins (1963). Mesophyll resistance was calculated as the reciprocal of slope of the net photosynthesis response curve to CO_2 , (Bravdo 1986, 1969).

CO_2 compensation point was calculated as the intercept of net photosynthesis response curve to CO_2 on the concentrations axis.

Protoplasts preparation

Protoplasts were isolated from 50-60 days old tobacco leaves and from 7-8 days old wheat seedlings. The isolation procedure was according to Rathnam and Edwards (1976) with few modifications. After rinsing in distilled water, the leaves were dipped in 70% alcohol for two minutes and then to a 5% sodium hydroxide solution for 5 minutes. The rest of the procedure was conducted under sterile conditions. The leaves were striped cut with a razor blade and transferred to the incubation solution composed of CaCl_2 5 mM, Sorbitol 0.5 M and an enzyme solution containing 1% cellulase (Onozuka R-10-Japan) 0.1% pectinase (Mecerozyme R-10-Japan) at pH 5.5. The enzyme solution was filtered through a 0.45 M filter and the leaf strips were immersed in the enzyme solution for 17-18 hours at 25°C in the dark in petri dishes. After a gentle shaking the solution was filtered through nitex 150 U and were centrifuged at 500 RPM for 5 minutes. The supernatant was discarded and precipitate was dissolved in 21% sucrose in a tricine buffer at pH=7.3. The solution was then centrifuged again at 1700 RPM for 5 minutes. The protoplasts were then separated by collecting the upper layer with a pipette.

Protoplasts integrity tests were made by phase microscop examinations. Intact protoplasts with a dark and a wide wall were counted. In most cases 80-90% intact protoplasts were obtained. The number of intact protoplasts varied between 8×10^5 to 1.25×10^6 per ml.

Net photosynthetic rate of isolated protoplasts

The measurements were made according to Rathnam and Edwards (1976) with a few modifications. The medium contained 50 mM Hepes buffer, 0.4 M sorbitol, 10 mM NaHCO_3 , 50 mM NaEDTA adjusted to pH 7.8 with KOH. An aliquote of 250 ml of the medium solution was transferred to small test

tubes and the chloroplasts suspension was added. Each test tube contained about 50 ug chlorophyll. After 3 minutes equilibration in dark at 30°C the protoplasts were exposed to light for 2 minutes. The reaction was initiated by adding 50 ml of the reaction mixture containing 0.15 ml 1M NaHCO₃, 2.35 ml distilled water, 0.1 ml NaH¹⁴CO₃ with a specific activity of 0.47 mCi/mg. The reaction was terminated by adding 250 ml 5% T.C.A. Light was provided by a mercury HPLR 400 watt lamp (Phillips Holland). Oxygen concentration in the medium solution was controlled by bubbling air with different oxygen concentrations from the wosthoff pumps to the medium solution. The test tubes were evacuated and the plugged with a rubber septum and another gas mixture at the desired concentration was injected in order to keep the suspension in equilibrium with the appropriate oxygen concentration. The reaction was initiated and terminated by injecting all the ingredients through the rubber septum. The septum was then removed and nitrogen gas was bubbled through the solution to remove the excess of ¹⁴C. The test tubes were then left overnight at 60°C until all the solution was evaporated.

Four ml of scintillation solution was then added and the samples were read in a scintillation counter. The scintillation solution contained 5 g p.p.o. (2,5-Diphenyloxazol), 200 g p.o.p.o.p (1,4-Phenyloxaxolyl benzene-2,2 Phenylenebis 5-Phenyloxazole), 650 ml toluene and 350 ml triton x 100. Two controls were used; one with TCA added before the reaction mixture (black) and the other was a standard containing 10 ul of the reaction mixture in a basic scintillation solution consisting of toluene, p.p.o and bis MSB (packard). The CO₂ uptake was calculated as follows:

$$\text{CO}_2 \text{ uptake (umol, mg chl}^{-1} \times \text{h}^{-1} = \text{dpm} - \text{dpm blank} \cdot \text{umol}$$

CO₂x20x20. Chlorophyll was determined according to Arnon (1948).

Chloroplasts preparation

Chloroplasts were manufactured from protoplasts according to the method of Rathnam and Edwards, 1976. A nitex filter with 20 μ m holes was used to break 25 to 40 μ m protoplasts into 2-4 μ m chloroplasts.

CO uptake determination of chloroplasts

The measurements were made according to Rudolf and Hind (1977). An oxygen electrode (yellow springs 4004) was used to measure the oxygen evolution of chloroplasts containing about 50 μ g chlorophyll brought to 3 ml with the medium solution. Catalase (1600 units) were added to prevent accumulation of H₂O₂. Light was provided by a 6V microscope lamp (Olympus, Japan). The measurements were conducted in a double wall glass chamber, temperature of the reaction was controlled by circulating water from a controlled temperature bath. The chloroplasts were added to the medium solution, illuminated for 5 minutes after which a constant rate of oxygen evolution was observed. The integrity of the chloroplasts was tested according to the method of Heber and Santorius (1970). The method consists of comparing oxygen evolution of normal chloroplasts with chloroplasts which received an osmotic shock.

Rubisco activity

The carboxylase activity was assayed according to Lorimer (1977) with a few modifications. About 1.5 g leaves were freezed with liquid nitrogen and crushed by the use of pistil and mortar in 10 ml solution of 100 mM tris HCl buffer at pH 7.8, 5 mM DTT, 20 mM MgCl₂, 0.5 g p.v.p. After 5 minutes centrifugation at 4°C, 30000 g; the supernatant was used

for the assay.

A sample of 0.4 ml made of 100 mM tris buffer 20 mM MgCl at pH 7.8 was transferred to a test tube. Fifty μ l of the reaction mixture made of 1ml Tris buffer HCl at pH 7.8, 1.475 ml 0.2 M NaHCO₃ and 50 μ l NaH¹⁴CO₃ (S.A. 40 mci/mmol. After 3 minutes equilibration the substrate RuBp (20 μ l of 20 mM) was added. The reaction was initiated by adding 50 μ l of the enzyme solution. The reaction was stopped after 2 minutes with 500 μ l 5% TCA. The rest of the procedure and the calculations were made as with the protoplasts assay (see above).

The oxygenase activity was made with an oxygen electrode using a similar system as for the protoplasts.

Five hundred μ l solution containing tris buffer 100 mM pH 8.6 and 20 mM MgCl₂ were introduced into the glass chamber for 3 minutes temperature equilibration. The reaction was initiated by adding 50 μ l RuBp 20 mM and 50 μ l enzyme extract. Soluble protein was assayed according to the method of Bradford ().

Electron transport and photophosphorylation were assayed according to the methods of Avron (1960) and Gromet-Elhanan (1968).

Results and Discussion

1. Whole plants measurements

1.1. Photosynthesis of wheat

1.1.1. Effect of PPFD

Figures 1,2,3 show the effect of PPFD on the means of A , T and r_s for all three cultivars. It is apparent that A was saturated at PPFD of about $800 \text{ MEm}^{-2}\text{s}^{-1}$ and so were transpiration and stomatal resistance. The effect of C_i on stomatal resistance was not consistent (Fig. 1A). The intercellular CO_2 was almost constant at all light intensities (Fig. 1B). This coincides with the results of many other authors (Farquhar and Sharkey 1982). Comparison of Figures 1, 1A and 1B show that stomatal resistance is not solely dependent on the intercellular and external CO_2 concentration and that the interaction between photosynthesis and light intensity probably affects the stomatal resistance. Mesophyll resistance however was already saturated at $400 \text{ MEm}^{-2}\text{s}^{-1}$ (Fig. 4). Thus, the increase in A between 400 and $800 \text{ MEm}^{-2}\text{s}^{-1}$ was due mainly to stomatal opening. No significant differences between the cultivars were apparent in this particular experiment. However, the average figures from all the experiments conducted under standard conditions; PPFD $800 \text{ MEm}^{-2}\text{s}^{-1}$, 25°C , 21% O_2 and 0.03% CO_2 show that the cultivar Lakhish had the lowest A and the highest leaf resistance whereas its PR and T were the lowest (Table 1). This table also shows that gr $\ddot{o}$ ss photosynthesis of Cv Lakhish was considerably lower than that of Cv 380 and 492.

Table 1 Photosynthesis and related parameters of three wheat cultivars. Data pooled from all experiments performed under 21% O_2 , 25°C and 800 $\mu\text{Em}^{-2}\text{s}^{-1}$ PPFD ($P=0.05$). T-Transpiration $\text{mg dm}^{-2}\text{h}^{-1}$, P-net photosynthesis $\text{mg dm}^{-2}\text{h}^{-1}$, R_s -leaf resistance (H_2O) s cm^{-1} , Γ -CO₂ compensation concentration $\mu\text{l/l}$, PR-photorespiration $\text{mg dm}^{-2}\text{h}^{-1}$, R_m -mesophyll resistance s cm^{-1} .

Cultivar	T	P	R_s	Γ	PR	R_m
Lakhish	1445 a	16.11 a	3.90 a	38.8	3.62	6.94
380	1542 a	20.00 b	2.95 b	50.47	6.32	5.17
492	1520 a	18.64 b	3.28 ab	53.64	6.90	4.96

Fig. 1 Effect of PPFD on photosynthetic rate of wheat flag leaves. (Averages for the three cultivars).

Fig. 2 Effect of PPFD on transpiration rate of wheat flag leaves. (Averages for the three cultivars).

Figure 1 A
Effect of intercellular CO₂
on stomatal resistance. Average for 3
wheat cultivars

Figure 1B
Effect of intercellular CO₂ on
stomatal resistance. Average
for three Tobacco cultivars.

Fig. 3 : Effect of PPFD on leaf resistance to water vapor (Averages for the three cultivars).

Fig. 4 Effect of PPFD on mesophyll resistance of wheat flag leaves (Averages for the three cultivars).

1.1.2. Effect of Temperature

The photosynthetic response of all three cultivars to temperature was similar in spite of the wide range of temperatures measured. Photosynthesis rate was the highest and leaf resistance the lowest at 25°C. Leaf resistance was higher at 40°C than at 10°C (Figs. 5,7). The lowest photosynthetic rate was measured in Cv Lakhish at all three temperatures (Fig. 5). This was due mainly to non-stomatal factors as apparent from the relatively higher intercellular CO₂ concentrations of this cultivar (Fig. 5A). Further support to the fact that the major reason for the lower photosynthetic rate in Cv Lakhish was due to non-stomatal factors is seen in Fig. 5B which shows that the lower photosynthetic rate of Cv Lakhish was associated with a relatively higher internal CO₂ concentration. Transpiration was mainly affected by leaf temperature and by the VPG and to a lesser extent by stomatal resistance (Figs. 5, 6,7). CO₂ compensation of Cv 380 was lowest at 10°C and highest at 40°C. This may mean that photorespiration was the highest in this cultivar (Fig. 9). Mesophyll resistance was the highest in Cv Lakhish at all three temperatures (Fig. 10). The results of the temperature experiment indicate that the lowest photosynthetic rates of Cv Lakhish were due to higher mesophyll resistance and not due to stomatal resistance or photorespiration. The indirect evidences for a higher photorespiration in Cv 380 together with the higher mesophyll resistance of Cv Lakhish may indicate that the CO₂ transfer resistance may play an important role in the differences obtained between the photosynthetic efficiencies of the three cultivars.

Fig. 5 Effect of leaf temperature on photosynthetic rate of flag leaves of three wheat cultivars. 380 O-O, 942 Δ-Δ, □-□ Lakhish. Letter separation by Duncan's MRT. P = 0.05.

Fig. 6 Effect of leaf temperature on flag leaf transpiration rate of three wheat cultivars. Symbols and statistics as in Fig. 5.

Fig. 7 Effect of leaf temperature on flag leaf resistance to water vapor of three wheat cultivars. Symbols and statistics as in Fig. 5.

Figure 5A.
Effect of leaf temperature on
intercellular CO₂ in three
wheat cultivars

Figure 5B.
Effect of intercellular CO₂ on stomatal
resistance of three
wheat cultivars at three leaf
temperatures.

Fig. 8 Effect of leaf temperature on flag leaf vapor pressure gradient in flag leaves of three wheat cultivars. Symbols and statistics as in Fig. 5.

Fig. 9 Effect of leaf temperature on CO_2 compensation point in flag leaves of three wheat cultivars. Symbols and statistics as in Fig. 5.

Fig. 10 Effect of leaf temperature on mesophyll resistance of flag leaves in three wheat cultivars. Symbols and statistics as in Fig. 5.

1.1.3 Effect of Oxygen Concentrations

The photosynthetic rates of Cv Lakhish were the lowest at all three oxygen concentrations measured (Fig. 11). The photosynthetic rates of Cv Lakhish at 200 $\mu\text{l/l}$ external CO_2 were the lowest only at 2% oxygen (Fig. 12) whereas no significant differences between cultivars in photosynthesis were apparent at external CO_2 concentrations of 100 $\mu\text{l/l}$ (Fig. 13). No significant differences in leaf resistance and transpiration were apparent between the cultivars at all three oxygen concentrations. It is apparent however that leaf resistance was increased and transpiration decreased with increasing O_2 concentrations in all the three cultivars (Fig. 14,15). This increase in intercellular CO_2 concentration may be responsible for the increase in stomatal resistance as already found by others (i.e. Raschke 1979). The increase in the intercellular CO_2 (Fig. 16) may be attributed to increased photorespiration due to increasing oxygen concentrations. No differences between cultivars in intercellular CO_2 and in stomatal resistance was found (Fig. 16, 16A). Mesophyll resistance was also increased with oxygen, however the increase in all three cultivars was similar between 2 and 21% O_2 (Fig. 18). Between 21 and 100%, the increase in 392 and Lakhish was greater than in 380. It seems therefore that the mesophyll resistance of Cv 380 is less sensitive to O_2 concentrations. The fact that oxygen concentration was found to affect differently the mesophyll resistance and the CO_2 compensation concentration among the three cultivars may reflect differences between CO_2 transfer resistance and photorespiration (Fig. 17, 18).

Fig. 11 Effect of oxygen on photosynthetic rate of flag leaves of three wheat cultivars at 300 μ l/l CO₂.

Fig. 12 Effect of oxygen on photosynthetic rate of flag leaves of three wheat cultivars at 200 μ l/l symbols and statistics as in Fig. 5.

Fig. 13 Effect of oxygen on photosynthetic rate of flag leaves of three wheat cultivars at 100 $\mu\text{l/l}$ CO_2 .

Fig. 14 Effect of oxygen on transpiration of flag leaves of three wheat cultivars at 300 μ l/l CO₂. Symbols and statistics as in Fig. 5.

Fig. 15 Effect of oxygen on leaf resistance to water vapor of flag leaves of three wheat cultivars at 300 $\mu\text{l/l}$ CO_2 . Symbols and statistics as in Fig. 5.

Fig. 16 Effect of oxygen on the intercellular CO₂ concentration of flag leaves of three wheat cultivars.² Symbols and statistics as in Fig. 5.

Figure 16A.
Effect of intercellular CO₂
on stomatal resistance.

Fig. 17 Effect of oxygen on the CO₂ compensation concentration of flag leaves of three wheat cultivars. Symbols and statistics as in Fig. 5.

Fig. 18 Effect of oxygen on mesophyll resistance of flag leaves of three wheat cultivars. Symbols and statistics as in Fig. 5.

Fig. 19 Effect of CO₂ on photosynthetic rate of flag leaves of three wheat cultivars. Symbols and statistics as in Fig. 5.

Figure 19A.
Effect of intercellular CO₂
on net photosynthetic rate. Of three
wheat cultivars.

Figure 19B.
Effect of intercellular CO₂
on stomatal resistance. Of three wheat
cultivars.

1.1.4. Effect of CO₂ Concentration

At normal CO₂ concentration photosynthesis of 380 was the highest. At 500 and 1000 ul/l CO₂ 492 and 380 had higher photosynthetic rates than Lakhish (Fig. 19). The lower photosynthetic rate of Cv Lakhish at higher CO₂ concentration was due to both increased stomatal as well as non stomatal resistance (Fig. 19A, 19B, 21). At external CO₂ concentration of 500 ul/l all three cultivars had almost the same intercellular CO₂ concentrations. Nevertheless, 380 has the highest net photosynthetic rate. The higher transpiration of Cv 492 corresponded with the lower stomatal resistance of this cultivar at 600 and 1000 ul/l external CO₂ concentration (Fig. 20). The non-stomatal photosynthetic limitation of Lakhish at 1000 ul/l external and 750 ul/l internal CO₂ (Fig. 19A).

1.1.5. Effect of photorespiration

Photorespiration as measured by the post illumination burst method did not vary significantly between the cultivars when the slope of the burst was measured (Fig. 22). The CO₂ photorespiratory pool as estimated from the integration of the area under the post illumination burst curve was the highest at 21% O₂ in all cultivars and that of Cv Lakhish the lowest at both 100 and 21% O₂ and 100 ul/l CO₂ (Fig.22). No difference between the CO₂ pools was apparent at 21 and 100% O₂ and external CO₂ free air(Fig. 22).

1.1.6. Effect of dark respiration

Dark respiration of Cv Lakhish was also lower than that of the other two cultivars at 100 and 21% O₂ and 100 ul/l external CO₂

Fig. 21 Effect of CO₂ on leaf resistance to water vapor of three wheat cultivars. Symbols and statistics as in Fig. 5.

Fig. 20 Effect of CO₂ on transpiration of flag leaves of three wheat cultivars. Symbols and statistics as in Fig. 5.

Fig. 22 Post illumination CO₂ outburst (PIB), dark respiration, and CO₂ pool size in flag leaves of three wheat cultivars. Symbols and statistics as in Fig. 5.

concentration and at 100% O_2 and zero external CO_2 concentration (Fig. 22). It seems therefore that the lower net CO_2 uptake of Cv Lakhish is associated with low photorespiration and dark respiration rates and should be attributed to non photorespiratory factors included in the calculated mesophyll resistance.

By the same token, the higher net CO_2 uptake of Cv 380 was associated with higher rates of photorespiration and relatively lower mesophyll and stomatal resistance. Although it is not possible to separate the CO_2 transfer resistance from the measured mesophyll resistance, these results stress its importance. In addition to the direct evidences for this conclusion which are derived from Post Illumination Burst, CO_2 compensation measurements and mesophyll and stomatal resistance calculations (Table 1, Fig. 22), there are also some indirect indications. Increased sensitivity to high temperature of its CO_2 uptake, stomatal resistance and of CO_2 compensation point of Cv 380 as compared to Cv Lakhish (Fig. 5,5A,5B,7,9). It is important to note that Cv Lakhish had the highest mesophyll resistance at all temperatures particularly at high temperature (Fig. 10). It is not clear why there were differences in CO_2 uptake between the cultivars greater at lower oxygen concentrations particularly at low external CO_2 concentrations (Fig. 11,12,13). In spite of the higher photorespiration in Cv 380 the internal CO_2 concentration of this cultivar was even lower than that of Cv Lakhish which had the lowest photorespiration rate. This may be attributed to the almost 2.5 times faster turnover of the CO_2 in this cultivar (Table 1). This faster turnover is a consequence of high gross photosynthesis and photorespiration. However, the sensitivity of stomatal resistance to

intercellular CO_2 seems to be greater in Cv Lakhish as compared to Cv 380 (Fig. 15,16,16A). The smaller photosynthetic response of Cv Lakhish to elevated internal CO_2 concentrations also shows that this cultivar has a relatively lower photosynthetic rate which could only be attributed to non-stomatal factors.

2.1. Photosynthesis of isolated wheat protoplasts

The photosynthetic efficiency of isolated protoplasts of Cv 380 was found to be by far higher than that of Cv 492 and that of Cv Lakhish was the lowest. Similar differences were also found in their quantum yield as can be seen from the initial slopes of the light response curve (Fig. 23).

The advantage of the photosynthetic CO_2 uptake of Cv 380 protoplasts was also apparent in the second experiment at 30 and 40°C whereas no significant differences were found at 10°C (Fig. 24).

This decrease in difference of CO_2 uptake between the cultivars is probably not due to limitations in the photochemical processes which are known to be insensitive to temperature. It seems therefore that the CO_2 transport or its utilization by the carboxylation process was responsible for the different rates of CO_2 uptake by the isolated protoplasts.

The advantage of Cv 380 protoplasts in CO_2 fixation was apparent also in the third experiment at all three external oxygen concentrations measured. The highest CO_2 fixation rate was found at 2% external oxygen concentration in all three cultivars and the reduction in CO_2 uptake was greater between 2 and 21% as compared to the reduction between 21 and 100% oxygen (Fig. 25). The reduction in CO_2 uptake by the protoplasts

Fig. 23 Effect of PPFD on photosynthetic rate of isolated protoplasts of three wheat cultivars leaves seedlings. Symbols and statistics as in Fig. 5

Fig. 24 Effect of medium temperature on photosynthetic rate of isolated protoplasts of three wheat cultivars seedlings. Symbols and statistics as in Fig. 5.

between 2 and 21% O_2 was 220, 80 and 30 $\mu\text{mole CO}_2/\text{mg chl h}$ respectively for 380, 492 and Lakhish respectively, O_2 in relative terms 52%, 44% and 30% respectively. This is an additional indication that 380 had the highest and Lakhish the lowest rate of photorespiration. Since stomatal resistance of Cv 380 was the lowest among the cultivars it seems that a non-stomatal resistance other than that observed in whole leaf measurement was involved.

3.1. Photosynthesis of Isolated Chloroplasts

Measurements of CO_2 uptake by isolated chloroplasts showed also that isolated chloroplasts of Cv 380 fixed CO_2 at a significantly higher photosynthetic rate than those of Cv Lakhish. The chloroplasts of Cv 492 also fixed CO_2 at a higher rate than those of Cv Lakhish (Fig. 26). Since the differences in CO_2 uptake between the cultivars were higher in protoplasts than in chloroplasts it seems that either transport resistance to CO_2 in chloroplasts had an additive effect on top of the resistance encountered in the protoplasts. The possible explanation that shortage in metabolites in isolated protoplasts and chloroplasts was the reason for the differences in CO_2 uptake between the cultivars is not consistent with the greater difference in isolated protoplasts as compared to whole leaves and isolated chloroplasts.

4.1. Rubisco Measurements

No significant differences between cultivars were found in both carboxylative and oxygenative activities when calculated per unit protein (Fig. 27). The fact that the Rubisco activity could not explain the differences in CO_2 uptake between the cultivars is a further indication

Fig. 25 Effect of oxygen on photosynthetic rate of isolated protoplasts of three wheat cultivars leaves seedlings. Symbols and statistics as in Fig. 5.

Fig. 26 Photosynthetic rates of isolated chloroplasts and protoplasts of wheat leaves seedlings. Columns from left to right are 380, 492 and Lakhish respectively.

that the major part of the measured mesophyll resistance of the various cultivars which was responsible for the differences in CO_2 uptake was the CO_2 transfer resistance rather than the Rubisco resistance. It may well be however that RuBp substrate supply was the limiting factor for the Rubisco activity in vivo (Farquhar and Sharkey 1982). Species variation in the specificity of ribulose biphosphate carboxylase/oxygenase were also reported in the literature (Jordan and Ogren 1981). Since the differences in chlorophyll content per unit leaf area and weight between cultivars were small (Fig. 28) it seems that calculations based on fresh weight or chlorophyll basis would give similar results as on leaf area basis.

Since wheat chloroplasts and protoplasts were isolated from 7 day old seedlings we have also measured gas exchange of such seedlings.

Only when calculated on a unit chlorophyll basis was the photosynthetic CO_2 uptake lowest in Cv Lakhish and highest in Cv 380 (Fig. 32). These results confirm the conclusion based on measurements conducted on old leaves that chloroplasts and protoplasts CO_2 fixation of Cv 380 are relatively more efficient than in whole leaves. Whether or not this efficiency is due to CO_2 transfer or due to availability of various metabolites in the protoplasts or chloroplasts is still an open question.

5.1. Photophosphorylation and Electron Transport Measurements

The rate of electron transport in broken chloroplasts was significantly lower in Cv Lakhish as compared to 492 and 380 (Fig. 29). Similar results were obtained with the cyclic and non cyclic

Fig. 27 Carboxylative and oxygenative activities of Rubisco enzyme from three wheat leaves cultivars. Columns from left to right are Lakhish, 492 and 380 respectively.

Fig. 28 Specific leaf weight, chlorophyll content per unit leaf weight and leaf area in flag leaves of three wheat cultivars. Columns from left to right are Lakhish, 380 and 492 respectively.

Fig. 29 Rate of electron transfer in broken chloroplasts of three wheat cultivars. Columns from left to right are Lakhish, 380 and 492 respectively.

Fig. 30 Cyclic photophosphorylation in broken chloroplasts from leaves of three wheat cultivars. Columns from left to right are Lakhish, 380 and 492 respectively.

Fig. 31 Non cyclic photophosphorylation rate in broken chloroplasts from leaves of three wheat cultivars columns from left to right are Lakhish, 380 and 492 respectively.

Fig. 32 Photosynthetic and transpiration rates and stomatal resistance to water vapor of broken chloroplasts from leaves of three wheat cultivars. Columns from left to right are 380, Lakhish and 492 respectively.

Fig. 33 Effect of PPFD on net photosynthetic rate of three tobacco cultivars. Glutinosa O - O, Samsun Δ - Δ and Oriental $\square$ - $\square$. Letter separation according to Duncan's MRT $P = 0.05$.

Figure 33A.
Effect of PPFD on intercellular CO₂ of
three
Tobacco cultivars.

photophosphorylation rate (Fig. 30, 31). These findings show that the lowest photosynthetic rate of Cv Lakhish as compared to the other two cultivars is a result of a combined effect of higher stomatal and mesophyll resistance, and lower electron transfer rate, and cyclic as well as non cyclic photophosphorylation rates.

The lower gross photosynthesis of Lakhish also explains why net photosynthesis was also lower in spite of the low photorespiratory rate.

1.2. Photosynthesis of Tobacco

1. Whole plants measurements

1.2.1. Effect of PPFD

The effect of PPFD on the net photosynthetic rate of the three cultivars was distinctive. Although all three cultivars were saturated around $300 \text{ MEm}^{-2} \text{ s}^{-1}$ the saturated rate of Glutinosa was 50% higher than that of Samsun and 100% higher than that of Oriental. The quantum yield of Oriental was lower than that of the other two cultivars (Fig. 33). The internal CO_2 concentration was lower in Glutinosa than in the other two cultivars in spite of its higher photosynthetic rate (Fig. 33A). This indicates that the photosynthetic efficiency of Glutinosa compared to the other cultivars was even higher than what is apparent in Fig. 33. the almost constant intercellular CO_2 during the light response curve experiment in each of the cultivars is consistent with the findings of others (Farquhar and Sharkey 1982, Berry and Downton 1982). Transpiration was increased and stomatal resistance decreased with increasing PPFD. Stomatal resistance decreased sharply between 40 and $75 \text{ MEm}^{-2} \text{ s}^{-1}$

and at a slower rate between 75 and 240 $\text{ME}_m^{-2}\text{s}^{-1}$ where it was saturated similarly to photosynthesis (Fig. 34,35).

No significant differences between cultivars was found in the transpiration and stomatal resistance.

1.2.2. Effect of temperature

Colutinosa had the highest photosynthetic rate at the entire range of 10 to 40°C. The photosynthetic rate of Samsun and Oriental was similar at this range (Fig. 36). The higher photosynthetic rate of Glutinosa at all three temperatures was associated with the lowest intercellular CO_2 concentration. However, the advantage of this cultivar in CO_2 uptake over Samsun was by far greater than the relatively small difference in the intercellular CO_2 concentration (Fig. 36A). From this figure it is also apparent that the efficiency of the non stomatal photosynthetic apparatus of Oriental was by far lower than that of the other two cultivars. The inverse relationships between the intercellular CO_2 and the net photosynthetic rate indicate that the non stomatal part of the photosynthetic apparatus differs more than does the net photosynthetic rate of the whole leaves. It is also apparent that the interaction between the internal, external CO_2 concentrations, and the net photosynthetic rates and the stomatal resistance varies greatly among the various cultivars. The transpiration of all three cultivars increased steadily with leaf temperature (Fig. 37). Leaf resistance of Glutinosa and Oriental was steady between 10 and 30°C and decreased sharply between 30 and 40°C. Leaf resistance of Samsun was higher at 10 and 20°C but did not differ from the other cultivars at 30 and 40°C (Fig. 38).

Fig. 34 Effect of PPFD on transpiration rate of three tobacco cultivars. Symbols and statistics as in Fig. 34.

Fig. 35 Effect of PPFD on leaf resistance to water vapor in three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 36 Effect of leaf temperature on net photosynthetic rate of three tobacco cultivars. Symbols and statistics as in Fig. 33.

Figure 36A.
Effect of leaf temperature
on intercellular CO₂ Of three
Tobacco cultivars.

Fig. 37 Effect of leaf temperature on transpiration of three tobacco cultivars. Symbols and statistics as in Fig 33.

Fig. 38 Effect of leaf temperature on leaf resistance to water vapor of three tobacco cultivars. Symbols and statistics as in Fig 33.

1.2.4. Effect of CO₂ Concentration

The photosynthetic rate of Glutinosa was higher than that of the other cultivars at all external CO₂ concentration between 300 to 1000 ul/l (Fig. 39). The photosynthetic rate was saturated at 500 ul/l external CO₂ or 450 ul/l internal CO₂ concentration in all cultivars (Fig. 39,39A). Transpiration was decreased and stomatal resistance increased with the increase in external and internal CO₂ concentration. The response of leaf resistance to CO₂ concentrations was similar for all cultivars except for Samsun who showed a very high sensitivity at 1000 ul/l external CO₂ or 700 ul/l internal CO₂ concentration (Fig. 39B,40,41,41A). The saturation of the photosynthetic apparatus at 450 ul/l intercellular CO₂ concentration and the almost similar differences in the photosynthetic rate between the cultivars in the RuBp regeneration limited region (Farquhar and Sharkey 1982) may indicate that RuBp regeneration rate is probably not the major cause for the differences between the cultivars photosynthetic rate. It may also indicate that the rate of photorespiration did not vary greatly among the cultivars.

1.2.3.1. The effect of the interaction between oxygen concentration and PPFD

Glutinosa had the highest photosynthetic rate at 240 and 800 ME m⁻² s⁻¹ and at external oxygen concentrations of 1 and 21%. No differences were found between Samsun and Oriental at all oxygen concentrations. No significant differences were found between the cultivars at 100% oxygen at all PPFD (Fig. 42). The photosynthetic rate of all the cultivars increased with PPFD at 1 and 2% oxygen. No increase in photosynthetic

Fig. 39 Effect of CO_2 on net photosynthetic rate of three tobacco cultivars. Symbols and statistics as in Fig. 33.

Figure 39A.
Effect of intercellular CO₂ On
net photosynthetic rate of three
Tobacco cultivars.

Figure 39B.
Effect of intercellular CO₂ On
stomatal resistance of three Tobacco
cultivars.

Fig. 40 Effect of CO₂ on the transpiration of three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 41 Effect of CO₂ on leaf resistance to O₂ in three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 42 Effect of PPFD and oxygen concentration on photosynthesis of tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 43 Effect of PPFD and oxygen on the intercellular CO₂ in three tobacco cultivars. Symbols and statistics as in Fig. 33.

rate was found as PPFD was increased from 240 to 800 $\text{ME m}^{-2} \text{s}^{-1}$.

The photosynthetic rate of all cultivars decreased with the increase in oxygen concentrations at all PPFD levels measured (Fig. 42). The intercellular CO_2 concentration has increased in all three cultivars at all PPFD measured with the increase in the external oxygen concentration.

The intercellular CO_2 concentration has decreased in all three cultivars with the increase in the PPFD at 1% external O_2 concentration. No distinctive change in the intercellular CO_2 concentration was apparent as PPFD was increased from 75 to 800 PPFD (Fig. 43). Transpiration was decreased and stomatal resistance increased with increasing external oxygen concentrations. Leaf resistance was increased with the increase in external oxygen concentration in all three cultivars at all PPFD levels measured. However, the increase in leaf resistance was relatively more pronounced in Glutinosa between 21 and 100% oxygen at PPFD of 75 $\text{ME m}^{-2} \text{s}^{-1}$. This may indicate a slightly higher sensitivity of Glutinosa's stomata to intercellular CO_2 since the intercellular CO_2 did not vary among the cultivars (Fig. 43).

1.2.2.3. The effect of interaction between temperature and oxygen

The rate of net photosynthesis decreased with the increase in the external oxygen concentrations in all three cultivars at the three temperatures measured. Glutinosa had the highest photosynthetic rate at 30 and 40°C and 1% oxygen and at 40°C and 21% oxygen (Fig. 46). Oriental had the lowest photosynthetic rate at 1% oxygen and 10°C (Fig. 46). These results show that at 1% oxygen when photorespiration becomes

Fig. 44 Effect of PPFD and oxygen on the transpiration in three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 45 Effect of PPFD and oxygen on leaf resistance to water in three tobacco cultivars.

Fig. 46 Effect of leaf temperature and oxygen on net photosynthetic rate in three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 47 Effect of leaf temperature and oxygen on the intercellular CO_2 in three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 49 Effect of leaf temperature and oxygen on leaf resistance to water vapor in three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 48 Effect of leaf temperature and oxygen on the transpiration of three tobacco cultivars symbols and statistics as in Fig. 33.

negligible, the advantage of Glutinosa becomes obvious (Fig. 46). This may again indicate that Glutinosa had the highest photorespiration rate. The low photosynthetic rate of Oriental and the relatively higher rate of Glutinosa at low oxygen concentration may be more related to differences in mesophyll resistance which were greater than the differences in photorespiration. At 21% oxygen and 40°C Glutinosa has still the highest net photosynthetic rate probably because its low mesophyll resistance is more dominant than photorespiration in its effect on net photosynthesis rate (Fig. 46). Net photosynthetic rate of all cultivars increased with temperature from 10 to 25°C and then decreased again at 40°C at 21 and 1% oxygen. At 100% oxygen photosynthetic rate was the highest at 10°C and decreased continuously with the increase in leaf temperature to 30 and 40°C (Fig. 46). Oxygen concentrations have increased the intercellular CO_2 concentration in all cultivars and at all temperatures. No differences in that respect were found between the cultivars, however the highest increase was at 40°C and the lowest at 10°C. Increasing temperatures decreased the intercellular CO_2 concentrations in all cultivars at 1 and 21% oxygen, however the internal concentrations increased with leaf temperatures at 100% oxygen (Fig. 47). The decrease at 1% oxygen is explained by the increase in gross photosynthesis under conditions of minimal photorespiration whereas at 21% oxygen it is probably due to the fact that the effect of the increase in stomatal resistance and in gross photosynthesis was greater than the increase in photorespiration. Transpiration was decreased and leaf resistance increased with oxygen concentration at 40 and 30°C. The effect of oxygen at 10°C was minimal. Differences between cultivars in leaf resistance

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were only observed at 40°C were 21% oxygen; Samsun had the highest leaf resistance (Fig. 49) and the lowest net photosynthetic rate (Fig. 46) whereas Oriental had the lowest leaf resistance but its photosynthetic rate was still lower than that of Glutinosa. This indicates that the mesophyll resistance of Oriental was higher and played a major role in determining the photosynthetic rate. At 100% oxygen Oriental had the highest leaf resistance, whereas Glutinosa had the lowest (Fig. 49). In spite of these big differences in leaf resistance no difference was observed in the photosynthetic rate between the three cultivars at 100% O₂ (Fig. 46). This again may indicate that the dominant factor determining the photosynthetic rate in this case was the mesophyll resistance.

1.2.4.3. Effect of the interaction between CO₂ and oxygen on the net photosynthetic rate

The photosynthetic rate of Glutinosa at 1% oxygen was the highest at all three external CO₂ concentrations 300, 500 and 1000 ul/l. Photosynthesis of Glutinosa was saturated at 500 ul/l. Saturation in Samsun and Oriental was probably at higher concentrations. At 100% oxygen and 1000 ul/l CO₂ Glutinosa had the highest photosynthetic rate and Oriental the lowest (Fig. 51). The difference in net photosynthetic rate and Oriental at 1% oxygen may be attributed to mesophyll resistance whereas the differences at 100% oxygen indicate that gross photosynthesis, stomatal and mesophyll resistance were more important than photorespiration in determining the net photosynthetic rate. Stomatal resistance was involved in the differences between the cultivars at 500 and 1000 ul/l CO₂ and 100% oxygen (Fig. 52-54). In the case of 1000 ul/l CO₂ the stomatal resistance of Oriental and Samsun increased at 100% O₂ while that

Fig. 50 Protein content per unit fresh weight and leaf area in three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 51 Effect of oxygen and CO₂ on net photosynthetic rate of three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 52 Effect of CO₂ and oxygen on the intercellular CO₂ in leaves of three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 53 Effect of CO₂ and oxygen on the transpiration of three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 54 Effect of CO₂ and oxygen of leaf resistance to water vapor in three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 55 Effect of oxygen on mesophyll resistance and CO₂ compensation point of three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 56 Post illumination outburst, dark respiration and CO₂ pool size of three tobacco cultivars. Columns from left to right, Samsun, Oriental and Glutiousa.

of Glutinosa was stable in spite of the fact that the internal CO_2 of Glutinosa and Samsun increased and that of Oriental decreased. It seems therefore that in this case the interaction between high oxygen and CO_2 rather than the internal CO_2 affected the stomatal resistance. A good correlation between internal CO_2 and stomatal resistance was only found at 300 $\mu\text{l/l}$ CO_2 when oxygen concentration was varied. In the case of 500 $\mu\text{l/l}$ CO_2 stomatal resistance reacted similarly to 1000 $\mu\text{l/l}$ CO_2 , however, here there were no differences in internal CO_2 among the three cultivars. Thus, no correlation between internal CO_2 and stomatal resistance was observed here as well. It may well be that internal CO_2 does not affect stomatal resistance at saturating CO_2 concentrations. Further support to the interpretation which do not attribute a major role to photorespiration in determining the differences in photosynthetic rate among the cultivars comes from the effect of oxygen on CO_2 compensation point and on mesophyll resistance (Fig. 55). Although the sensitivity the CO_2 compensation point of all cultivars to oxygen was similar mesophyll resistance of Glutinosa was less sensitive to oxygen than that of Oriental. Since CO_2 compensation point is a product of mesophyll resistance and photorespiration (Bravdo 1968, 1982) Glutinosa must have the highest photorespiration rate.

1.2.5. Dark and photorespiration

Oriental had the lowest post illumination burst at 200 $\mu\text{l/l}$ CO_2 (external concentration) whereas no significant differences between cultivars were apparent at 100 and 0 $\mu\text{l/l}$ CO_2 (Fig. 56). Samsun had the lowest dark respiration rates at 0 and 100 $\mu\text{l/l}$ while no significant

differences were apparent at 200 $\mu\text{l/l}$ CO_2 . Samsun had the highest CO_2 pool for photorespiration at 100% oxygen (Fig. 56). These results show again that photorespiration was not probably a major factor in the differences in the photosynthetic rates among the cultivars. Moreover, it seems that the differences between cultivars in net photosynthesis were in spite of the rates of photorespiration which seemed to be correlated to the photosynthetic rate. When the results of all individual experiments were pooled together, significant differences in net photosynthetic rate, transpiration and stomatal resistance between Glutinosa and between Samsun and Oriental (Fig. 57).

2. Protoplasts measurements of tobacco

2.2.1. Effect of PPFD on protoplast photosynthesis

Quantum yield and rate of CO_2 uptake by isolated chloroplasts at all PPFD levels was the highest in Oriental and the lowest in Glutinosa (Fig. 58). This is opposite to the findings in whole leaves (Fig. 33). This shows that the best protoplasts capability for CO_2 fixation is in Oriental and the lower rate obtained in whole leaves is due to factors located between the protoplasts membrane and the atmosphere. Although it is obvious that stomatal resistance was the highest in Oriental it was only partially responsible for the low photosynthetic rate of its whole leaves. This is evident from the fact that Oriental had the highest mesophyll resistance (Fig. 55) and also from evidences showing that Glutinosa had a higher photosynthetic rate even with lower internal CO_2 concentrations (Fig. 33A). Furthermore, the fact that Glutinosa could maintain the lowest internal CO_2 concentrations with lower stomatal

Fig. 57

Analysis of gas exchange of the pooled data from all experiments conducted at 300 $\mu\text{l/l}$ CO_2 , 21% O_2 and 30°C in three tobacco cultivars. Symbols as in the columns of photosynthesis and transpiration, from left to right. Glutinoso, Samsun and Oriental.

Fig. 58 Effect of PPFD on the photosynthetic rate of isolated protoplasts of three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 59 Effect of medium temperature on the photosynthetic rate of isolated protoplasts of three tobacco cultivars. Symbols and statistics as in Fig. 33.

resistance and still have the highest net CO_2 fixation rates indicates that the limitations were probably located in the cell wall.

2.2.2. Effect of temperature on protoplasts photosynthesis

The rate of CO_2 uptake by isolated protoplasts of Oriental was higher than that of Glutinosa and Samsun at 28 and 40°C whereas no differences were apparent at 10°C (Fig. 59). Since the biochemical process of photosynthesis is known to be temperature sensitive it is very likely that the advantage of Oriental protoplasts lies in the carboxylation reactions.

2.2.3. Effect of oxygen on protoplasts photosynthesis of Tobacco

CO_2 uptake was higher in protoplasts of Oriental than that of Glutinosa at all oxygen concentrations measured between 1 and 100% oxygen. The rates of Samsun did not vary significantly from Oriental (Fig. 60). The almost constant difference between the photosynthetic rates of the three cultivars throughout the entire range of oxygen, supports the interpretation that photorespiration was not the major cause for the difference between cultivars. The reduction in CO_2 uptake with oxygen shows, however, that photorespiration was considerable in all three cultivars.

4.2. Activity of Rubisco

No significant differences in the carboxylative and the oxygenative activities were apparent when calculated on a protein basis (Fig. 62). When the activities were calculated on a leaf area basis, both activities, the carboxylative as well as the oxygenative were significantly

higher in Oriental. The ability of the Oriental protoplasts to fix CO_2 faster than the other cultivars may be attributed to its higher protein content per unit leaf area and fresh weight. Oriental had also the highest specific leaf weight (Fig. 61) and its average area per leaf was the greatest among all three cultivars (not shown). This is a further support to the conclusion that the greater efficiency of the protoplasts of Cv Oriental to fix CO_2 lies in its carboxylation reactions and this advantage is not being expressed in whole leaves due to some other non stomatal as well as stomatal factors.

Fig. 60 Effect of oxygen on photosynthetic rate of isolated protoplasts of three tobacco cultivars. Symbols and statistics as in Fig. 33.

Fig. 61 Chlorophyll concentration per unit leaf area and weight.
 Symbols of columns from left to right, Glutinoso, Samsun and Oriental.

Fig. 62 Carboxylative and oxygenative activity of the enzyme Rubisco calculated per mg protein and unit leaf area. Columns from left to right Samsun, Glutinosa and Oriental.

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e. Evaluation of the research achievements with respect to the original research proposal.

The mechanisms responsible for differences in photosynthetic efficiency among various cultivars were studied as proposed. The relative contribution of various components of the photosynthetic system to the overall photosynthetic rate in a series of peanuts, tobacco and wheat cultivars were quantified. The potential of the chloroplasts and the protoplasts of each of the cultivars was compared with the whole leaf photosynthetic rate. The contribution of the photorespiratory rate to the overall photosynthetic rate could only be determined in relative terms. Our results indicate that although photorespiration limits the photosynthetic rate in all cultivars it is not necessarily correlated with the differences in net photosynthetic rate but rather with the net photosynthetic rate namely the cultivars with the higher photosynthetic rate may have had the highest photorespiration rate and vice versa.

Unfortunately the method for assaying photorespiration was not further improved as expected due to the discontinuation of the Vibrating Reed Electrometer by "Varian". Attempts to construct such an instrument in our electronic shop are still in progress and so far the noise level was too high to allow precise measurements. For this reason preliminary results brought in the first and second reports were not repeated in this final report. However some results were obtained with an old type of vibrating reed electrometer are presented in the papers on peanut photosynthesis and photorespiration.

The differences found between the wheat cultivars in the rate of electron transport, cyclic and non cyclic photophosphorylation rather

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electron transport, cyclic and non cyclic photophosphorylation rather than in Rubisco activity are interesting and contradict some commonly accepted views.

In conclusion, this study contributed to the understanding of some mechanisms and plant factors involved in photosynthetic efficiency. Breeders can already utilize some of our findings in their breeding programs. A more precise knowledge of the gene mapping may open new possibilities in breeding towards more efficient plants.

f. Description of Cooperation

Methods for measuring the rate of photorespiration were developed in Georgia using an old model "varian" vibrating reed electrometer. Unfortunately the company have discontinued the production of this instrument and therefore only one joint paper entitled "photosynthesis photorespiration and RuBp carboxylase oxygenase activity in selected peanut genotypes" was published (Photosynthetica 16:36-42, 1982). Bravdo visited Georgia once in 1982 to complete experiments on peanuts photosynthesis. Pallas visited Israel once in 1983 to study cell isolation and photosynthesis of isolated cells, protoplasts and chloroplasts. Dr. N. Paz spent a postdoctoral year with Dr. Pallas in Georgia and applied cell isolating techniques which were developed for tobacco and wheat in Israel to peanuts.

g. Benefit to agriculture including a concise description of the nature of the problem and the contribution of the project towards its solution .

The relative contribution of various components of the photosynthetic apparatus to the photosynthesis of whole leaves and plants was found to

vary among cultivars of the same species and among species. This study showed that the ability of the chloroplasts, protoplasts or isolated cells to fix CO_2 varies among cultivars of the same species under similar experimental conditions. The results show that barriers to CO_2 transport as well as photochemical related processes like the rate of electron transport and photophosphorylation may also vary even within the same species. The rate of photorespiration played an important roll in determining the photosynthetic rate however not always was the photorespiratory rate correlated with the net photosynthetic rate. The ability to asses the relative contribution of the various photosynthetic components opens up new possibilities for breeding and selection of better productive cultivars. For example, a cultivar combining the protoplasts capabilities of Cv Oriental with the CO_2 transport of Glutinosa could result in a more efficient plant. A cultivar with reduced photorespiration and/or reduced sensitivity to intercellular CO_2 concentration may have an advantage . Reduced photorespiration is no doubt an advantage provided that its rate will not be positively correlated with gross photosynthesis as seems to be the case with a few peanut, tobacco and wheat cultvrs. The possible linkage between gross photosynthesis and photorespration is interesting from the genetic point of view and further work is required in order to determine whether these two parameters are always linked together.

List of Publications

- 1) Bravdo, B. and J.E. Pallas, Jr. 1982. Photosynthesis photorespiration and RuBp carboxylase/oxygenase activity in selected peanut genotypes.
- 2) Paz, N. and J.E. Pallas. 1985. Photosynthetic capacity of isolated leaf cells from *Arachis hypogaea* L. *Photosynthetica* (in press).
- 3) Paz, N. and J.E. Pallas, Jr. 1986. Photosynthetic capacity of whole leaf compared with isolated leaf cells of peanut and soybean.