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Genetic Variability in Carbon Fixation, Sucrose-P-Synthase and ADP Glucose Pyrophosphorylase in Maize Plants of Differing Growth Rate

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ABSTRACT

The net photosynthetic rate and the activities of ribulose 1,5 bisphosphate carboxylase (RubisCo), phosphoenolpyruvate carboxylase, sucrose-P-synthase, and ADP glucose-pyrophosphorylase, key enzymes of the leaf carbohydrate metabolism were compared in eight maize (*Zea mays* L.) genotypes presenting large differences in growth rate. The sucrose-P-synthase activity varied in the ratio 1 to 3 from the less active to the more active genotype and this variation was highly correlated with those in growth rate. ADPglucose pyrophosphorylase activity was not significantly different from one genotype to another whatever the basis for expression, leaf area, or soluble protein. The photosynthetic rate varied with similar amplitude (1:1) to the RubisCo activity or RubisCo quantity but the correlation with growth rate was highly significant for photosynthesis and nonsignificant for RubisCo or phosphoenolpyruvate carboxylase. So, in our series of genotypes the sucrose synthesis capacities as expressed by sucrose phosphate synthase activity seem to have a good predicting value for mean growth rate at a young stage.

Plant growth is not only regulated by carbon assimilation but also by carbon partitioning parameters (6). The regulation of these processes is environmentally and genotypically controlled. Comparisons of genotypes of the same species differing in ability for growth provide a useful tool for evaluating the relative contributions of selected or unselected parameters in breeding improvement. Studies performed in wheat (1, 4) have shown that modern hexaploid varieties are superior to ancestral diploid varieties in their harvest index (grain:total dry matter) but that the photosynthetic rate per unit leaf area in hexaploid varieties has nearly decreased by half compared to early ancestors; the greater leaf expansion of modern wheat compensated for decrease of photosynthetic rate. A different situation apparently occurs in maize where photosynthetic rate tends to have increased slightly from pre-Colombian to modern races (3). A significant variability in carbon exchange rate is also reported among presently cultivated genotypes (7).

More recently, a comparison of maize genotypes of contrasting earliness established a high correlation between photosynthetic rate and growth rate but further showed that explanation of intergenotype variability in growth should encompass carbon partitioning in source leaves. The carbon flux to starch was negatively correlated with growth rate whereas the opposite was true for sucrose pool sizes or sucrose export (16).

In the present paper, we have examined the possible biochemical basis of these differences. Four key regulatory-enzymes were considered: RubisCo[1] and PEPcase for carbon fixation, SPS and ADPG-PPase for sucrose and starch synthesis, respectively. RubisCo is often thought to be limiting photosynthesis *in vivo* because of its low specific activity (12). PEPcase in maize was recently reported to be better correlated with photosynthetic rate than RubisCo (2). ADPG-PPase is the main regulatory point on the starch synthesis pathway (14). SPS activity is apparently just sufficient to account for the *in vivo* rate of sucrose synthesis. Interspecific variation in SPS has been observed and activity was shown to be inversely related to starch content (8). We report here a very high intraspecific correlation between early growth rate and SPS activity in maize whereas variations in RubisCo activity or quantity and ADPG-PPase activity were not significant.

MATERIALS AND METHODS

Plants

Eight maize (*Zea mays* L.) genotypes were grown in a glasshouse in April (for photosynthesis measurements), May–June (for SPS), in August–September (for ADPG-PPase and RubisCo) and November (PEPcase); minimal day/night temperatures were 25°C/15°C, RH was 50 to 60%, natural light was supplemented 16 h a day with fluorescent lamps (Phytoclaude 300 μmol quanta m^{-2} s^{-1}). Genotypes were chosen for large differences in growth rate and earliness index (16). In some experiments one genotype was omitted (HS222) due to unavailability of seeds and another one (DEA) was added. The seeds were provided by the same breeding station (INRA, Mons-en-Chaussée) in order to reduce intragenotype effect due to seed origin. Germination conditions and nutrient solution were as described elsewhere (17). The plants were used when the fourth leaf from the base was fully expanded. The criterion used was 50% of these leaves having a visible ligule in a batch of 10 plants taken at random. Discs (0.5 cm^2 $\simeq$ 7 mg), were punched in the medial part of the leaf, frozen in liquid N_2 and stored at −80°C, until used. The rest of plants (shoot + root) was harvested for total dry matter. The mean growth rate was calculated from the increase in dry matter between germination and fourth leaf stage divided by the corresponding time in days. For each batch of culture the

[1] Abbreviations: RubisCo, ribulose 1,5 bisphosphate carboxylase; SPS, sucrose phosphate synthase; ADPG-PPase, ADPglucose pyrophosphorylase; PEPcase, phosphosoenolpyruvate carboxylase.

growth rate was determined and correlated with the enzyme activity measured on the same plants. From one experiment to another the ranking of the genotypes was rather similar; however, some interchanges occurred in the more rapidly growing genotypes.

Enzyme Measurements

All the samplings for enzyme determination were made at 10:00 AM, *i.e.* 6 h after the beginning of the photoperiod. So, the light activable enzymes were activated. This point was checked for RubisCo and SPS. The discs were sampled in the medial part of the leaf. Preliminary experiments with discs punched in different parts of the fourth leaf showed that the medial region was representative of mean leaf activity. The area of the fourth leaf is higher than 50% of total leaf area at sampling stage.

The RubisCo activity and quantity were measured on two discs ground in 100 μL extraction buffer as in Prioul and Reyss (15). The crude enzyme extract was preactivated for 10 min in 32 mM $MgCl_2$ and 12 mM bicarbonate before adding 0.4 mM RuBP. Rabbit antiserum was raised against purified tobacco RubisCo. The calibration curves relating height of the immunorocket and enzyme quantity were prepared with purified enzyme from spinach and then the equivalence between spinach and maize purified enzyme was established to obtain absolute RubisCo content.

PEPcase was extracted by grinding three leaf discs (0.5 cm^2) at −196°C in a conical glass homogenizer. The extraction buffer (0.1 M Tris-HCl [pH 8.0], 5 mM dithiotreitol) was added during thawing, and the slurry was centrifuged 3 min at 12,000*g*, 4°C in a microcentrifuge. Activity was measured spectrophotometrically at 340 nm: 20 μL extract was added to a reaction mixture (final volume 1 mL) containing 0.1 M Tris-HCl (pH 8.0), bovine serum albumin 6 mg mL^{-1}, 5 mM $NaHCO_3$, 10 mM $MgCl_2$, 0.2 mM NADH, 3.5 units NAD-malate-dehydrogenase (Sigma). The reaction was started by addition of 5 mM phosphoenolpyruvate(tri-cyclohexyl-ammonium salt). The activity was calculated from the linear part of the time dependent kinetic.

SPS activities were determined by a method derived from Huber (9). Extracts were obtained by grinding two leaf discs at −196°C in an Eppendorf tube with a glass rod. The leaf powder was warmed to 4°C with 250 μL extraction buffer (50 mM Hepes-NaOH [pH 7.5], 5 mM $MgCl_2$, 1 mM Na_2 EDTA, 2.5 mM dithiotreitol, 1% bovine serum albumin, 0.6% insoluble polyvinyl-pyrrolidone). The extract was centrifuged for 1 min in a microcentrifuge at 12,000*g*. An aliquot of the supernatant (50 μL) was added to a reaction mixture (final volume 80 μL) containing 13 mM UDP glucose, 10 mM fructose-6-P, 14 mM $MgCl_2$. The reaction was run for 15 min at 30°C and terminated by adding 100 μL 1 M NaOH. The tubes were immersed in boiling water for 10 min in order to destroy free hexoses. Insoluble material was pelleted by a 30 s centrifugation at 12000*g*. The fructose moiety of sucrose-P was determined by 0.12% resorcinol reagent in 3.2 N HCl incubated 12 min at 100°C. The reaction product concentration was measured spectrophotometrically at 520 nm by comparison with controls assayed without fructose-6-P and with sucrose standard.

ADPG-PPase was extracted as described for SPS with two discs for 150 μL buffer (50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM Na_2 EDTA, 0.5% bovine serum albumin). Reactions were assayed at 25°C (11) in reaction mixture (500 μL) containing: 40 mM Hepes NaOH (pH 7.5), 4 mM $MgCl_2$, 0.1 mg bovine serum albumin, 1 mM ADP glucose, 1 unit of phosphoglucomutase and of glucose-6-P dehydrogenase, 0.3 mM NAD, 20 μL of extract. Sodium pyrophosphate (1 mM) was added when a plateau was reached at 340 nm (<5 min). The activity was calculated on the initial linear part of the time dependent increase in A_{340}. Concentration of sodium pyrophosphate and volume of extract were adjusted to get the highest activity. In order to confirm results of the enzymatic method, a more specific method using ^{32}P-pyrophosphate as

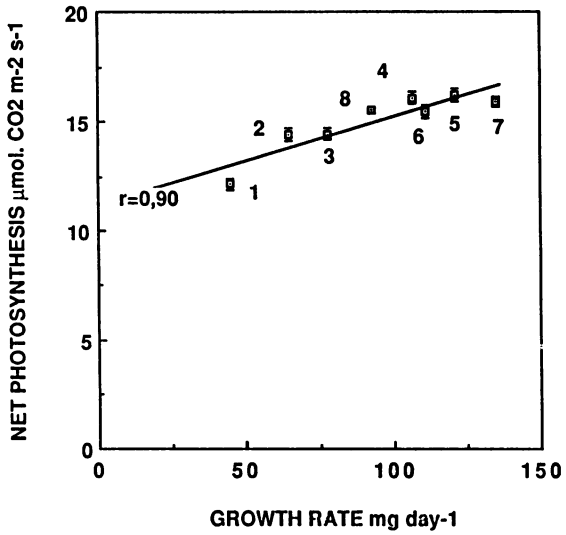

Figure 1. Net photosynthetic rate of the medial part of mature fourth leaf in different maize genotypes in relation to mean growth rate from germination to fourth leaf stage. Genotype identification: 1, W64A; 2, F546; 3, INRA 508; 4, BIP; 5, INRA 250; 6, W_HW_J; 7, F_7F_2; 8, HS222. Mean ± SE, 10 repetitions. The size of error bars is about the same as the symbols.

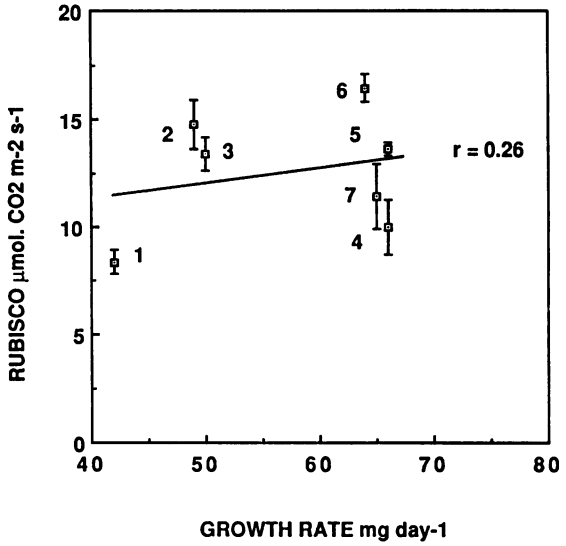

Figure 2. RubisCo activity on a leaf area basis as a function of mean growth rate in a series of maize genotypes (numbered as in Fig. 1). Mean ± SE, 6 repetitions.

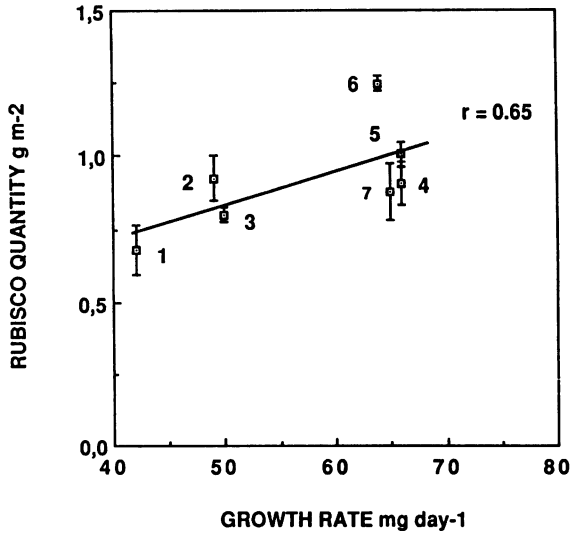

Figure 3. Quantity of RubisCo per leaf area as a function of mean growth rate in a series of maize genotypes (same genotype as Fig. 2). Mean ± SE, 6 repetitions.

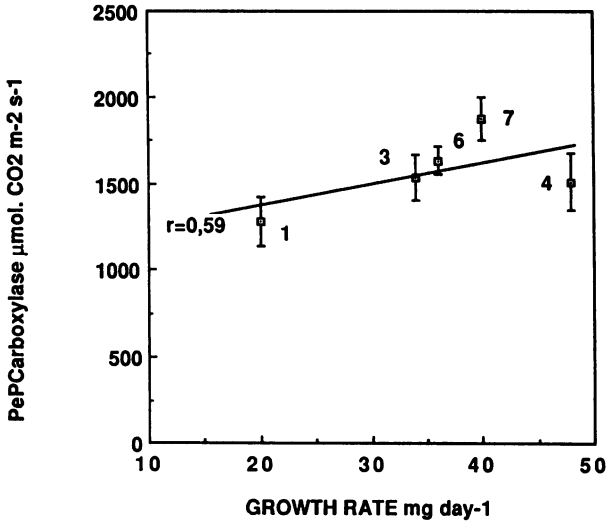

Figure 4. PEPcase per leaf area as a function of mean growth rate in a series of maize genotype (numbered as in Fig. 1) Mean ± SE, 6 repetitions.

substrate and measuring ATP^{32} formation was tried (5, 20). One leaf disc was ground in the same extraction buffer as in the other method. The reaction mixture (100 µL) contained 40 mM Hepes-NaOH (pH 7.5), 4 mM $MgCl_2$, 1 mM ADP glucose, 0.5 mM P-glycerate, 2 mM ^{32}P-pyrophosphate (2–3 10^6 cpm µmol^{-1}). The reaction performed in an Eppendorf tube at 25°C for 10 min was initiated by adding 5 µL of the enzyme extract and was stopped with 1 mL of 5% TCA containing 6 mg activated Charcoal (Baker-acid-washed), 40 µL 10 mM Na_4 pyrophosphate. The pellet was washed twice with 1 mL 5% TCA and boiled in 800 µL 1 N HCl for 10 min. An aliquot (400 µL) of the supernatant was counted in 2.5 mL of scintillation liquid in a counter (Intertechnique SL 30). The yield of ATP adsorption by the activated charcoal was checked with ^{32}P-ATP and was higher than 90%.

Soluble protein content was determined by the Sedmak method (18).

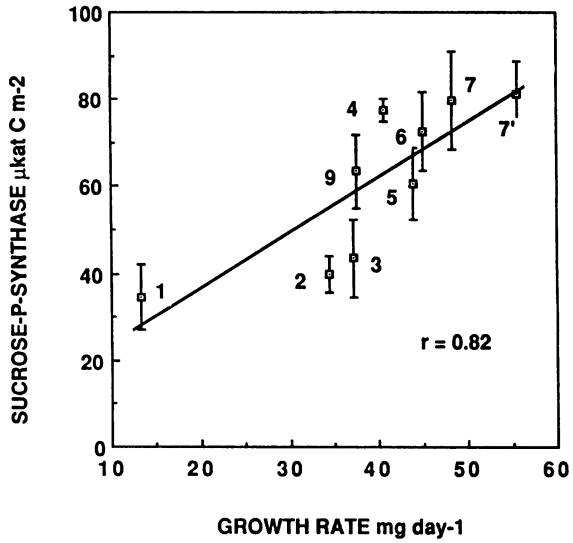

Figure 5. SPS activity per leaf area as a function of mean growth rate in a series of maize genotype (numbered as in Fig. 1) (8 = HS222 was omitted and replaced by 9 = DEA) Mean ± SE, 6 repetitions.

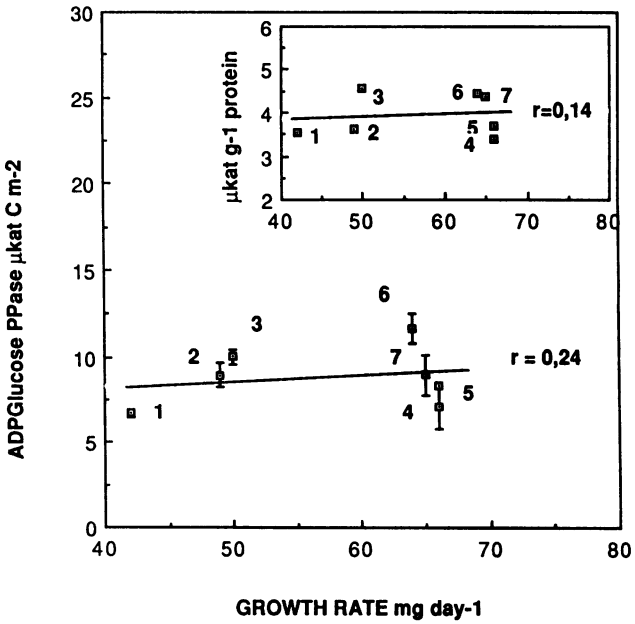

Figure 6. ADPG-PPase activity per leaf area as a function of mean growth rate in a series of maize genotypes (same genotype as Fig. 2). Mean ± SE, 6 repetitions. Inset, activity expressed per soluble proteins.

Net Photosynthetic Rate

A 10 cm^2-zone in the medial part of a fully expanded fourth leaf was enclosed in a small perspex chamber. Net CO_2 fixation was measured with an IRGA in an open circuit under the same irradiance as for growth (300 µmol quanta m^{-2} s^{-1}) as in Rocher (16).

RESULTS

Net Photosynthetic Rate

Net photosynthetic rate on leaf area basis was related with growth rate (Fig. 1). The correlation coefficient was highly

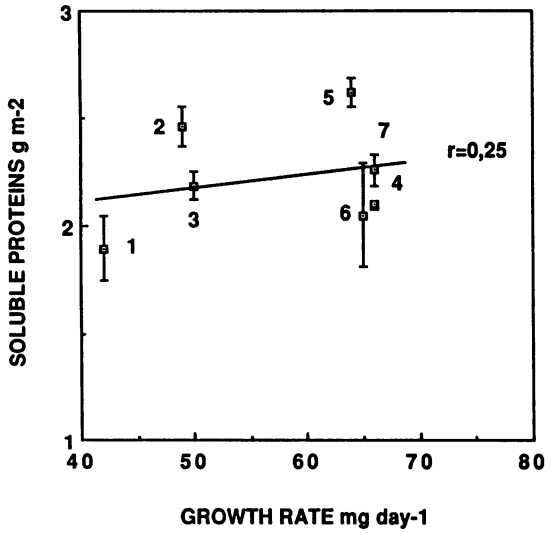

Figure 7. Soluble protein content per leaf area as a function of mean growth rate in a series of maize genotypes (same genotype as Fig. 2). Mean ± SE, 6 repetitions.

Table I. *Comparison of ADPG-PPase Activity in Seven Maize Genotypes by Two Different Methods*

1) with coupling enzymes (= enzymatic method) 2) with ^{32}P pyrophosphate and fixation of synthesized $AT^{32}P$ on activated charcoal (radiochemical method). Mean ± SE for 6 plants per genotype

	1 Enzymatic Method	2 Radiochemical Method
	μKat m^{-2}	*μKat m*$^{-2}$
W64A	1.12 ± 0.04	0.70 ± 0.01
F546	1.49 ± 0.12	1.37 ± 0.15
W_HW_J	1.95 ± 0.14	1.11 ± 0.19
F_7F_2	1.50 ± 0.20	1.30 ± 0.17
BIP	1.19 ± 0.22	0.73 ± 0.20
INRA 250	1.39 ± 0.12	0.78 ± 0.08

significant ($r = 0.90$) but it should be noted that the range of variation in P_N was much narrower (1:1.34) than in growth rate (1:3).

Carboxylases Activity

RubisCo activity was rather variable from one genotype to another and correlation with growth rate was rather poor and nonsignificant (Fig. 2). RubisCo-protein content varied very closely with total soluble protein content ($r = 0.86$) but was loosely correlated with growth rate (Fig. 3). RubisCo specific activity on a RubisCo protein basis was nearly constant. The PEPcase activity was much higher than that of RubisCo but the relative variation was similar (Fig. 4). The correlation with growth rate was better than for RubisCo but the correlation coefficient was not significant.

SPS

The SPS activity of the fourth leaf expressed on a leaf area basis was highly correlated with the mean growth rate from germination to fourth leaf stage (Fig. 5). Two sets of five genotypes were grown at 1 month interval, $F_7 \times F_2$ being in common so that it could be used as internal standard. Good repeatability was noted for this genotype (*cf.* points 7 and 7′ Fig. 5). The magnitude of variations in SPS and growth rate were similar (1:3) which is different from what was noted for net photosynthetic rate.

ADPG-PPase

The activity of ADPG-PPase on a leaf area basis increased a little with growth rate but the correlation ($r = 0.24$) was not significant (Fig. 6) when measured by the enzymic method. The soluble protein content per leaf area varied in the same way (Fig. 7) so that the ADPG-PPase on a soluble leaf protein basis was nearly constant over all the genotypes (inset, Fig. 6). The results from the radiochemical method lead to the same conclusion, *i.e.* an absence of correlation with growth rate. Except for some genotypes (F546) the agreement between the two methods was not excellent (Table I). In general, the radiochemical method gave lower values which can be partly explained by the fact that they represent an averaged rate over 10 min and the fixation of ATP on activated charcoal was not total (approximately 90%).

DISCUSSION

In most plants, including maize, the assimilate exported out of the leaf is sucrose. So sucrose metabolism is of importance in understanding the regulation of photosynthate utilization for growth. Our previous studies on the same maize genotypes showed that the size of the two main sucrose pools (storage and export), the exchange rates between these pools and the export rate (16) were correlated with growth rate ($0.68 < r < 0.8$). The presently observed correlation between growth rate and SPS activity (0.82) is higher than with the sucrose pools and equivalent to that with sucrose flux, which tends to indicate that this enzyme could be rate limiting for growth. The measured *in vitro* rate (Fig. 5) is only 3 times the actual photosynthetic rate (Fig. 1) when expressing activity in moles carbon. These results are in agreement with those of Pollock (13) and Huber (8) who observed that SPS activity was just sufficient to account for sucrose synthesis *in vivo* in several C_3 species. Huber *et al.* (10) further established a very high correlation between export rate and SPS activities in fully expanded leaves from the same species grown in different conditions or from different genotypes.

SPS activity was also correlated to growth rate in two soybean cultivars grown under N_2-dependent or NO_3-supplied conditions (11). In most situations, starch accumulation was inversely related to SPS (8, 11) but ADPG-PPase activity, when measured, was not significantly correlated with *in vivo* starch synthesis (11, 19). The same situation is presently observed since ADPG-PPase remained constant on a leaf area or a protein basis when the proportion of photosynthetic carbon input which is stored into starch varied from 17% in the slower to 12% in the faster growing genotype (16).

Carbon fixation in C_4 plants operates through the cooperative action of PEPcase and RubisCo. It is well established that the activity of the first carboxylase exceeds largely the *in vivo* rate, whereas the RubisCo activity is just equivalent to the CO_2 fixation rate (2 and references therein). Usuda (22)

reported a high correlation between RubisCo or PEPcase activities and CO_2 assimilation in maize leaves of different ages. A correlation was also observed with dry matter accumulation but PEPcase paralleled more tightly biomass than RubisCo in maize seedling grown with different nitrate levels (21) or in senescing source-leaves during kernel growth (2). The presently observed variation in RubisCo is of the same magnitude as that in net photosynthesis but the higher intragenotype variability tends to obscure the correlation with growth rate. PEPcase activity varied similarly but the correlation coefficient, although higher, was not significant.

The expression of the activities on a leaf basis in the place of leaf area basis lead to high correlation with growth but this simply expresses the great importance of leaf area differences as discussed earlier (16). RubisCo protein content or activity was highly correlated with soluble protein content. This is consistent with the fact that RubisCo-protein represent a high proportion of soluble protein, ranging from 36% for W64A to 47% for W_HW_J.

LITERATURE CITED

1. **Austin RB, Morgan CL, Ford MA, Bhagwat SG** (1982) Flag leaf photosynthesis of *Tricitum aestivum* and related diploid, and tetraploid species. Ann Bot **49:** 177–189
2. **Crafts-Brandner SJ, Poneleit CG** (1987) Carbon dioxide exchange rates, ribulose bisphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase activities and kernel growth characteristics of maize. Plant Physiol **84:** 255–260
3. **Duncan WG, Hesketh JD** (1968) Net photosynthetic rates, relative growth rates and leaf numbers of 22 races of maize grown at eight temperatures. Crop Sci **8:** 670–674
4. **Evans LT** (1975) Crop physiology. Cambridge University Press, New York, pp 327–355
5. **Ghosh HP, Preiss J** (1966) Adenosine diphosphate glucose pyrophosphorylase. A regulatory enzyme in the biosynthesis of starch in spinach leaf chloroplasts. J Biol Chem **241:** 4491–4504
6. **Gifford RM, Evans LT** (1983) Photosynthesis, carbon partitioning and yield. Annu Rev Plant Physiol **32:** 485–509
7. **Heichel GH, Musgrave RB** (1969) Varietal differences in net photosynthesis of *Zea mays* L. Crop Sci **9:** 483–486
8. **Huber SC** (1981) Interspecific variation in activity and regulation of leaf sucrose phosphate synthase. Z Pflanzenphysiol **102:** 443–450
9. **Huber SC** (1983) Role of sucrose-phosphate synthase in partitioning of carbon in leaves. Plant Physiol **71:** 818–821
10. **Huber SC, Rufty TW, Kerr PS** (1984) Effect of photoperiod on photosynthate partitioning and diurnal rhythms in sucrose phosphate synthase activity in leaves of soybean (*Glycine max* L. Merr) and tobacco (*Nicotiana tabacum* L). Plant Physiol **75:** 1080–1084
11. **Kerr PS, Huber SC, Israel DW** (1984) Effect of *N*-source on soybean leaf sucrose phosphate synthase starch formation and whole plant growth. Plant Physiol **75:** 483–488
12. **Miziorko HM, Lorimer GH** (1983) Ribulose-1,5-bisphosphate carboxylase-oxygenase. Annu Rev Plant Physiol **52:** 507–535
13. **Pollock CJ** (1976) Changes in the activity of the sucrose synthetizing enzymes in developing leaves of *Lolium temulentum*. Plant Sci Lett **7:** 27–31
14. **Preiss J** (1982) Regulation of the biosynthesis and degradation of starch. Annu Rev Plant Physiol **33:** 431–454
15. **Prioul JL, Reyss A** (1987) Acclimation of ribulose bisphosphate carboxylase and mRNAs to changing irradiance in adult tobacco leaves. Differential expression in LSU and SSU mRNA. Plant Physiol **84:** 1238–1243
16. **Rocher JP** (1988) Comparison of carbohydrate compartmentation in relation to photosynthesis, assimilate export and growth in a range of maize genotypes. Aust J Plant Physiol **15:**(5) (in press)
17. **Rocher JP, Prioul JL** (1987) Compartmental analysis of assimilate export in a mature maize leaf. Plant Physiol Biochem **25:** 531–540
18. **Sedmak J, Grossberg SE** (1977) A rapid, sensitive and versatile assay for protein using Coomassie brillant blue G 250. Anal Biochem **79:** 544–552
19. **Silvius JE, Chatterton NJ, Kremer DF** (1979) Photosynthate partitioning in soybean leaves at two irradiance levels. Comparative responses of acclimated and unacclimated leaves. Plant Physiol **64:** 872–875
20. **Spilatro SR, Preiss J** (1987) Regulation of starch synthesis in the bundle sheath and mesophyll of *Zea mays* L. Intercellular compartmentalization of enzymes of starch metabolism and the properties of the ADP glucose pyrophosphorylase. Plant Physiol **83:** 621–627
21. **Sugiyama T, Mizuno M, Hayashi M** (1984) Partitioning of nitrogen among ribulose-1,5-bisphosphate carboxylase/oxygenase, phosphoenolpyruvate carboxylase and pyruvate orthophosphate dikinase as related to biomass productivity in maize seedlings. Plant Physiol **75:** 665–669
22. **Usuda H** (1984) Variations in the photosynthesis rate and activity of photosynthetic enzymes in maize leaf tissue of different ages. Plant Cell Physiol **25:** 1297–1301