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.

In the present paper, we have examined the possible biochemical basis of these differences. Four key regulatory-enzymes were considered: RubisCo 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.
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 Reys (15). The crude enzyme extract was preactivated for 10 min in 32 mM MgCl₂ 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 cm²) at −196°C in a conical glass homogenizer. The extraction buffer (0.1 M Tris-HCl [pH 8.0], 5 mM dithiothreitol) was added during thawing, and the slurry was centrifuged 3 min at 12,000g, 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 NaHCO₃, 10 mM MgCl₂, 0.2 mM NADH, 3.5 units NAD-malate-dehydogenase (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 MgCl₂, 1 mM Na₂ EDTA, 2.5 mM dithiothreitol, 1% bovine serum albumin, 0.6% insoluble polyvinyl-pyrrolidone). The extract was centrifuged for 1 min in a microcentrifuge at 12,000g. 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 MgCl₂. 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 pelletted by a 30 s centrifugation at 12000g. 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 MgCl₂, 1 mM Na₂ 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 MgCl₂, 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 A₄₅₀. 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 ³²P-pyrophosphate as
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 $10^9$ 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 (Intertechique 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).

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**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.

**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.

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**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
genotypes were grown at 1 month interval, F7 × F2 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
reported a high correlation between Rubisco or PEPcase activities and CO₂ 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 represents a high proportion of soluble protein, ranging from 36% for W64A to 47% for W₁₁₁₁₁₁.

LITERATURE CITED

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