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.

In the present paper, we have examined the possible biochemical basis of these differences. Four key regulatory-enzymes were considered: RubisCo1 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). ADP-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 (Phyto-clade 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 ligue in a batch of 10 plants taken at random. Discs (0.5 cm2 ≈ 7 mg), were punched in the medial part of the leaf, frozen in liquid N2 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

1Abbreviations: RubisCo, ribulose 1,5 bisphosphate carboxylase; SPS, sucrose phosphate synthase; ADPG-PPase, ADPglucose pyrophosphorylase; PEPcase, phosphoenolpyruvate 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 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-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 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 pelleted 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 mM 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 A₅₄₀. 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 $\text{ATP}^{32}$ formation was tried (5, 20). One leaf disc was ground in the same extraction buffer as in the other method. The reaction mixture (100 $\mu$L) contained
Table 1. Comparison of ADPG-PPase Activity in Seven Maize Genotypes by Two Different Methods

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Enzymatic Method (μKat m⁻²)</th>
<th>Radiochemical Method (μKat m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W64A</td>
<td>1.12 ± 0.04</td>
<td>0.70 ± 0.01</td>
</tr>
<tr>
<td>F546</td>
<td>1.49 ± 0.12</td>
<td>1.37 ± 0.15</td>
</tr>
<tr>
<td>W74H</td>
<td>1.95 ± 0.14</td>
<td>1.07 ± 0.13</td>
</tr>
</tbody>
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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 photosynthetic 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
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 represent a high proportion of soluble protein, ranging from 36% for W64A to 47% for W₁W₁.

**LITERATURE CITED**

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