GENETIC PROPERTIES OF CARBOXYLATING ENZYME CAPACITY IN PLANTS WITH THE C_4-DICARBOXYLIC ACID PATHWAY OF PHOTOSYNTHESIS

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Received on the 2th May 1972

Higher plants can be divided into two groups according to the pathway of photosynthetic CO_2 fixation. The plants producing phosphoglyceric acid as a first stable product of photosynthetic CO_2 fixation, have ribulosediphosphate carboxylase as an effective enzyme in this reaction. Kortschak et al. (1965) reported of a new CO_2 fixation pathway they first found to be operativ in the sugarcane. This pathway is commonly referred to as the C_4 dicarboxylic acid pathway, since oxaloacetate, malate and aspartate are the first stable products of CO_2 fixation, or to β-carboxylation photosynthesis, since it involves β-carboxylation of phosphoenolpyruvate (Maruyama et al. 1966, Johnson and Hatch 1968).

The β-carboxylation pathway seems mainly to be limited to taxonomic groups. Those plants which possess this pathway apparently have different photosynthetic characteristics as the plants which lack this (Downton and Tregunna 1968). In the plants with β-carboxylation photosynthesis there are two consecutive carboxylation reactions. CO_2 is fixed by phosphopyruvate carboxylase and in a Calvin – type carboxylation; it is mediated by ribulosediphosphate carboxylase (Björkman et al. 1970).

The photosynthetic and biochemical characteristics are related to distinctive patterns of leaf-anatomy and to dimorphic chloroplasts (Laetsch 1969).

Plants with β-carboxylation pathway were shown to have a low CO_2 compensation point (Tregunna at el. 1969). The *Sorghum* species are also such a low CO_2 compensation *Gramineae*, in the Panicoid line.

It was interesting to study the activity and distribution of photosynthetic carboxylases in different lines of *Sorghum* sp. and to determine the inheritance of these enzymatic characteristics in their hybrids.

Material and methods

Experiments were made with *Sorghum vulgare* var. *frumentaceum* 106, 509 fertile and cytoplasmic male sterile analogous and with 301, 130 inbred lines and with their first generation hybrid. The seeds were germinated at 20°C and grown in sand, supplemented by twice diluted Knop solution, containing microelements (K n o p 1865, F o x 1963), under continuous illumination of 2500 lux. Leaves 20 – 21 days old were harvested for enzyme extraction.

Mesophyll and parenchyma-sheath cells were separated by using the method of *B j ö r k m a n* and *G a u h l* (1969). Leaf samples were blended with an MSE homogenizer in a medium containing 0.04 M of Tris pH 7.8; 5 mM of GSH (glutathion reduced); 5 mM of ascorbate. The slurry was filtered through a microsieve of about 30 μ pore diameter. The remainder was thoroughly ground in a mortar and the slurry was filtered too. A part of the filtrates was used for pigment determination in ethylether by multiwavelength method (F a l u d i – D á n i e l et al. 1970). The crude enzymes were obtained by centrifuging the filtrates at 100 000 xg for 20 min at 2 – 5°C.

Activities of RuDP-carboxylase (EC 4.1.1.39) and PEP-carboxylase (EC 4.1.1.31) were determined by measuring the incorporation of $H^{14}CO_3^-$ in the presence of D-ribulose-1.5-diphosphate and phosphoenolpyruvate respectively. Reaction mixture contained 30 μmole of Tris, pH 7.8; 3 μ mole of $MgCl_2$; 1.5 μmole of GSH; 0.1 μmole of EDTA and 10 μ mole $NaH^{14}CO_3$ (0.4 – 0.6 μ Ci/μmole^{-1}) in a final volume of 0.6 ml and an aliquot quantity of enzyme extract. For assay of activity of RuDP-carboxylase the reaction was started with D-ribulose-1.5-diphosphate (0.15 μ mole in every sample). For assay of PEP-carboxylase the reaction mixture additionaly contained 1.0 μmole phosphoenolpyruvate and 2.5 μmole sodiumglutamate. Reaction was started with an aliquot quantity of enzyme extract (N a g y et al. 1972).

The reactions were stopped after 2 min by adding 6 N acetic acid. The acid-stable radioactivity was determined by liquid scintillation-counting (Nuclear Chicago 724), using the method of B u s h and H a n s e n (1965).

Results

The chlorophyll content of leaves and PEP-carboxylase capacity were not influenced by the two different cytoplasm *(Table 1)*. The activity of RuDP-carboxylase was relatively low in to 509 lines, independent from the cytoplasm type.

Both in two hybrid leaves the total chlorophyl content was a little higher than or similar to that of the parental plants. Bundle-sheath cells of the hybrid seeds contained a higher percentage of the total chlorophyll content than those of the parental leaves.

Table I.

Total chlorophyll (a+b) content, activities of carboxylating enzymes, in male-sterile and fertile strains of Sorghum vulgare cv. frumentaceum

Material	Chlorophyll (a+b) vmole/g fr. w.	Activities of enzymes RuDP=carboxylase	Nmole CO_2/g fr. w. PEP-carboxylase
106	1529	774	2411
male-sterile			
106	1793	938	2504
male-sterile			
509	1532	334	2758
male-sterile			
509	1894	192	2968
male-sterile			

Table II.

Total chlorophyll (a+b) content and total activity of two carboxylating enzymes in two first generation hybrid strains and in their parental lines

Material	clorophyll $\frac{\text{nmole}}{\text{g fr. w.}}$	% in bundle sheath	CO_2 fixed by enzymes $\frac{\text{nmole}}{\text{g fr. w.}}$	% in bundle sheath
Female				
509	1532	40	3092	37
Hybrid				
509×301	2110	55	4167	55
Male				
301	1990	40	1431	60
Female				
106	1529	48	3185	59
Hybrid				
106×130	1497	64	2464	51
Male				
130	877	44	2413	55

The total CO_2 fixation capacity was affected in different ways by the hybridisation in two hybrid strains. In the leaves of 509×301 hybrid type, the total activity of two carboxylating enzymes was much higher than that of the parental plants. The seed of 106×130 hybrid line showed an intermediate value of enzyme capacity and about half of this value was localized in bundle-sheath.

The tissue activity of RuDP-carboxylase in 509 and 301 inbred lines was very similar, and in their hybrid it was much higher, than the

one in the leaves of parental plants (Fig. 1/1). PEP-carboxylase capacity of 509 female plant and the hybrid plant was significantly higher than that of the other parental seed (Fig. 1/2).

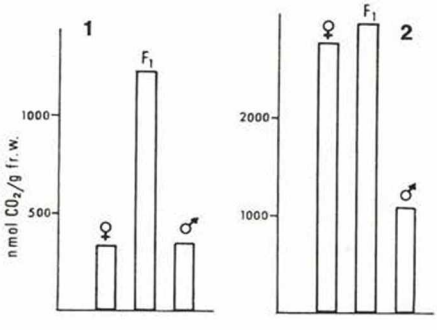

Fig. 1.

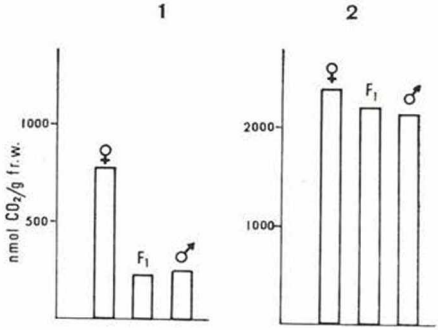

Fig. 2.

Fig. 1. Activities of RuDP-carboxylase and PEP-carboxylase in the leaf extracts of 509 male sterile and 301 inbreed line of *Sorghum vulgare* cv. *frumentaceum* and their first generation hybrid

1 = RuDP-carboxylase,
2 = PEP-carboxylase,

Fig. 2. Activities of RuDP-carboxylase and PEP-carboxylase in the leaf extracts of 106 male-sterile and 130 inbreed line of *Sorghum vulgare* cv. *frumentaceum* and of their first generation hybrid

1 = RuDP-carboxylase,
2 = PEP-carboxylase,

In the other cross type a high difference was experienced between the RuDP-carboxylase activities of the two parental lines (Fig. 2/1). This enzyme capacity of hybrid leaves was very similar to that of the male plant. Concerning PEP-carboxylase activity an intermediate type of inheritance was observed (Fig. 2/2).

Discussion

Many taxonomists classified the cultivated kinds of *Sorghum* into several species and subspecies. A modern cytogenetic concept places them all within one species, *Sorghum vulgare*, because they all possess 10 chromosomes, they intercross freely, and the crosses are fully fertile. Furthermore the various groups have been classified as species or subspecies often differing by only a few genes. One pair of genes controlling sweetness of the stalk-juice distinguishes grain sorghum from sorgo, often regarded as separate subspecies. Even in wide crosses, such as the one between a grain sorghum and Sudan grass, or between grain sorghum and broomcorn, the ratio of F_2 generation-segregates classified with the broad parental groups suggests the possibility that 3 to 6 genes might account for the basic differences between these groups (M a r t i n 1959).

Sterile inducing cytoplasm delayed flowering by 1/2 day and caused a 3 cm increase in plant height. There were considerable differences in grain yield between pairs of hybrids with different cytoplasm, but variation from plot to plot was great enough so that statistically non-significant differences were shown (Quinby 1970). As it was proved in our paper, biochemical differences were not in relation to the cytoplasm types.

Inheritance of biochemical characteristics appear to be predominantly under nuclear control in interspecific hybrids of Atriplex rosea and Atriplex patula (Björkman et al. 1970). In these crosses, the Calvin type CO_2 fixation was of an intermediate inheritance, while the low PEP-carboxylase activity dominanted over the higher one.

The experimental results, presented in this paper, indicated that the hybrids of different inbreed lines of *Sorghum vulgare* cv. *frumentaceum* were not only simple intermediates between the parental types with regard to photosynthetic carboxylases.

We found a great heterotic response for activity of RuDP-carboxylase in 509×301 hybrid line. In the other crossing type (106×130) the hybrid plant had a low RuDP-carboxylase capacity similar to the one of the male parent. The activity of PEP-carboxylase in hybrid leaves showed another type of inheritance. If the enzyme capacity of two parental lines was very different, their first generation hybrid plant had a very similar one to female line (509×301). In the case of similar parental character the activity PEP-carboxylase was practically unchanged in leaves of their hybrid plant.

Heterotic response for vegetative and mature plant characters was studied by Kirby and Atkins (1968). They found significant differences between parental and hybrid plants in grain yield, plant height, stalk diameter etc. According to experimental results of Beil and Atkins (1967) both general and specific combining ability were important in the expression of grain yield and in the other characters of grain sorghum hybrids.

Summary

The activities of RuDP-carboxylase and PEP-carboxylase were studied in different inbred lines of Sorghum vulgare cv. frumentaceum as well as in their hybrid. CO_2 fixation in vitro was similar in male-sterile-inducing cytoplasm plant and in fertile analogous. Heterotic response was observed in one hybrid. In the other case the capacity of carboxylating enzymes was similar to male parent lines. The activity of RuDP-carboxylase and PEP-carboxylase were affected in different ways by the hybridisation.

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