Theoretical and Applied Genetics 46, 19-23 (1975)
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# Differences of Cytoplasmic Transaminase Activity in Normal and *Opaque*-2 Maize (*Zea mays* L.) Seedlings*

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Summary. The activity of glutamate-oxalacetate and glutamate-pyruvate transaminases (GOT 2.6.1.1., GPT 2.6.1.2.) was determined in seedlings of normal and *opaque-2* maize. The activity of these transaminases was determined in homozygotes +/+ and $o_2/o_2$, their $F_1$ hybrid, in mixture of +/+ and $+/o_2$ in $S_3F_4$ generation. The GOT and GPT activity in mutants *opaque-2* was always higher than in normal plants. In generation $S_3F_4$ the higher activity of transaminases in *opaque-2* plants was demonstrated without addition of the pyridoxal-5-phosphate (PRP) coenzyme (at GOT 1,36x, at GPT 2,00x) as well as at the optimum PRP concentration (at GOT 1,39x, at GPT 2,48x). The difference in transaminase activity is therefore not due to changes in PRP biosynthesis.

The determination of the GOT and GPT activity in seedlings might be used in breeding experiments as a screening method for selection of mutants.

Jones and Singleton, in the late 20's, described the mutant gene *opaque-2*, which results in the typical opaque structure of the maize kernel endosperm. However, nothing was known of the effect of this mutant gene on the nutritive value of the maize endosperm. Mertz et al. (1964) made a break-through when they found that the recessive gene *opaque-2* nearly doubles the limiting essential amino acids content in the endosperm of maize kernel.

Interconversion of amino acids by transamination is a basic cowise of amino acid metabolism; we were interested in whether the alteration of amino acid synthesis by the *opaque-2* gene is manifest in a modified transaminase activity.

In the present investigation, the enzymatic activity of the glutamate-oxalacetate transaminase (GOT 2.6.1.1.) and of the glutamate-pyruvate transaminase (GPT 2.6. 1.2.) was followed in seven day-old seedlings of normal and *opaque-2* maize, with the aim of finding out whether or not the transaminase activity of the two genotypes already shows differences at an early ontogenic phase.

## Materials and Methods

### Plant material and its cultivation

Transaminase activity was determined on the inbred line SW 437 (normal endosperm) of a collection from the Plant Breeding Station, Stupice, and in the $S_1$ line derived from the synthetic population homozygous for the recessive mutant *opaque-2* gene, kindly given by Prof. A. Bianchi**. Their $S_0F_1$ hybrid, and plants grown from phenotypically normal kernels of segregating ears of the $S_1F_2$ and $S_2F_3$ generations, were also examined. The transaminase activity in the generation $S_3F_4$ was determined in normal homozygous plants (from non-segregating ears - genotype +/+), in mutant homozygous plants (from segregated *opaque-2* kernels - genotype $o_2/o_2$) and in plants from phenotypically normal kernels of a segregating ear - genotypes +/+ and $2/o_2$ (see Fig.1).

For parents +/+ and $o_2/o_2$, and in the subsequent generations up to $S_2F_3$, the activity was determined by means of an average sample taken out of 30 seedlings. In the $S_3F_4$ generation the activity was determined by four replications of 10 seedlings each.

The seeds were sterilized for 2 minutes in 10% formaldehyde, rinsed and left for 6 hours in distilled water before sowing. The seeds were placed on damp cellulose and cultivated at 23° C in the dark.

### Preparation of the enzyme extract

Seven days after sowing the shoots were cut, weighed and cooled down to 4° C. Cool (4° C) phosphate buffer 0.1M at pH 8, containing 0,4M saccharose, 0,01M EDTA and 0,01M 2-mercaptoethanol (Wightman and Cohen 1968) was used for extraction. The plant material together with the buffer (1:3) was ground in the mixer at 4°C. The mixture was left for 30 minutes in solid $CO_2$ cooled bath, then filtered through linen cloth and centrifuged for 20 minutes at 4° C and 12.000 g. Supernatant (enzyme extract, EE) was separated and used for estimating the enzymatic activity.

### Determination of the enzyme activity

The GOT and GPT activity was determined by a modified method of Cabaud et al. (1956), originally used for the determination of transaminase activity in blood serum. The catalyzed enzymatic reactions have this course:

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* Dedicated to Prof. Borriss on the occasion of his 65th anniversary

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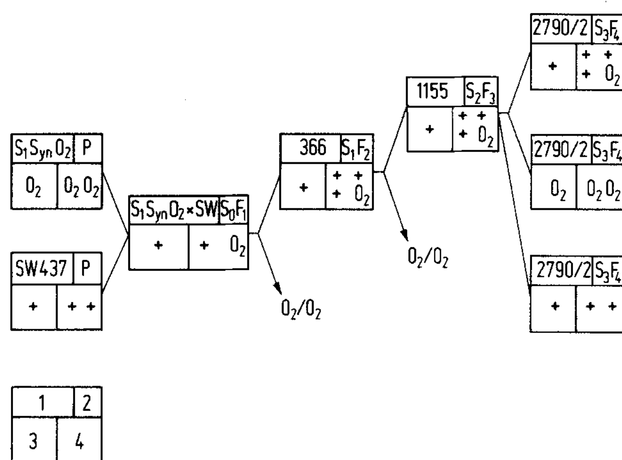
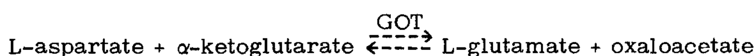
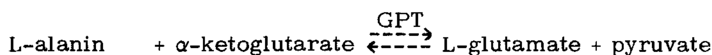



Fig.1. Phenotype and genotype representation of *Zea mays* evolved in Stupice. 1 Specification, 2 Generation, 3 Phenotype of endosperm, 4 Genotype of seedling




We determined the concentrations of the resulting ketoacids, i.e. oxaloacetate in GOT and pyruvate in GPT. By adding anilincitrate reagent, oxaloacetate in the first case was converted to pyruvate. Pyruvate was chemically stabilized in the form of hydrazone by using 2,4-dinitrophenylhydrazine. Hydrazone was shaken into toluene and determined at 490 nm on a Unicam SP 1800 spectrophotometer.

The optimum values for pH, temperature, incubation period and concentration of the coenzyme pyridoxal-5-phosphate (PRP) are given in Fig.2. In our work we used pH = 8, 38° C, 50 µg PRP per ml and 20 minutes incubation period.

Two series of enzyme activity determination were investigated simultaneously in three different genotypes (+/+, +/$o_2$, $o_2/o_2$) of the generation $S_3F_4$, with and without addition of the coenzyme (50 µg/ml).

The resulting activity of GOT and GPT is expressed in international enzymologic units for 1 mg protein (I.U./mg).

Determination of protein in enzyme extracts

The proteins in EE were determined after Lowry et al. (1951). Human serum albumin was used as standard.

## Results

The lines SW 437, $S_1$Syn $o_2$, and their hybrid $F_1$, did not differ in protein content but the GOT and GPT activities were different (Tab.1). In the mutant form $S_1$ Syn $o_2$, the transaminase activities were 1,4 times (GOT) and 1,8 times (GPT) higher, respectively, than in the normal SW 437. The $F_1$ hybrid showed a complete dominance of the low GOT and GPT activities. The same was observed in the $S_1F_2$ and $S_2F_3$ generations. In all cases, the GOT and GPT activities of seedlings grown from the phenotypically normal seeds were lower than in plants from mutant seeds. The absolute values of the GOT and GPT activity tended to decrease in successive generations but the relation between the mutants + and $o_2$ remained. In the $S_3F_4$ without additional PRP the activity of 2790/2 $o_2$ was 1,36 times higher and the GPT activity 2 times higher than in 2790/2 + (mixture of homozygotes +/+, and of heterozygotes +/$o_2$). From the analysis of variance we found that these differences were significant at 0,1 level. The whole transaminase activity is higher in the enzyme system with addition of PRP (50 µg/ml), but the relation between the normal and mutant form is about the same (GOT 1,39 x higher, GPT 2,48x higher) (Tab.2 and 3).

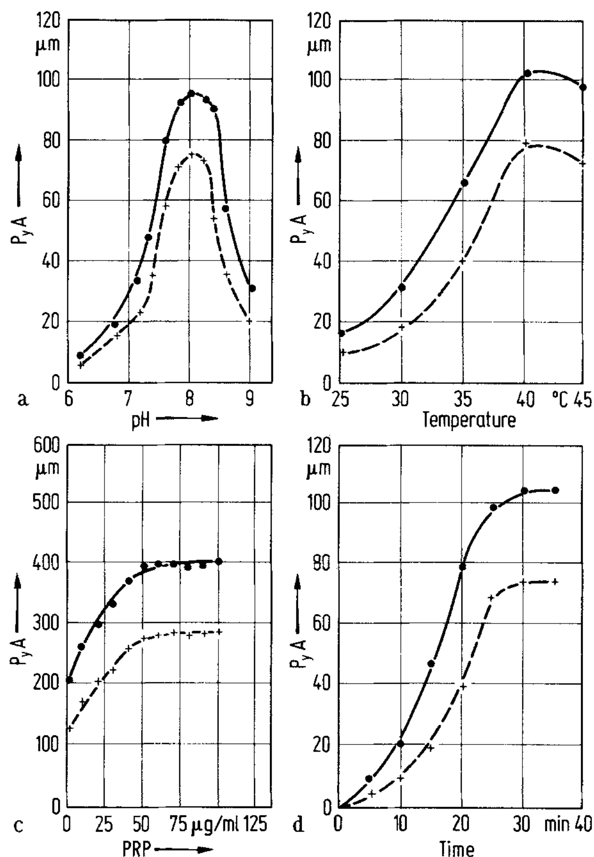



Fig.2. Determination of optimum conditions for measuring GOT (full line) and GPT (dotted line) transaminase activity in maize (µM of pyruvic acid -PyA- evolved) a Optimum pH, b Optimum temperature, c Effect of coenzyme PRP concentration, d Time course of transaminase reaction

## Discussion

The most likely way in which the amino acid composition of the endosperm proteins is shifted by a structural

Table 1. Protein content and GOT and GPT activity in seven days old seedlings (without coenzyme addition)

| Year | Specification | Generation | Embryo genotype | Endosperm phenotype | Proteins mg/ml EE | GOT U.I./mg protein | GPT U.I./mg protein | Proteins % * | GOT % * | GPT % * |
|---|---|---|---|---|---|---|---|---|---|---|
| 1968 | $S_1$Syn $o_2$ | P | $o_2o_2$ | $o_2$ | 5,79 | 1,30 | 0,72 | 103,3 | 144,4 | 180,0 |
| | SW 437 | P | ++ | + | 5,61 | 0,90 | 0,40 | 100,0 | 100,0 | 100,0 |
| 1969 | $S_1$Syn $o_2$xSW 437 | $S_0F_1$ | +$o_2$ | + | 5,88 | 0,95 | 0,36 | 99,5 | 105,4 | 90,0 |
| 1970 | 366 | $S_1F_2$ | ++, +$o_2$ | + | 5,55 | 0,85 | 0,41 | 99,0 | 94,4 | 102,5 |
| 1971 | 1155 | $S_2F_3$ | ++, +$o_2$ | + | 5,52 | 0,87 | 0,38 | 98,4 | 96,6 | 95,0 |
| 1972 | 2790/2 | $S_3F_4$ | ++, +$o_2$ | + | 5,56 | 0,75 | 0,25 | 99,2 | 83,4 | 62,5 |
| | 2790/2 | $S_3F_4$ | $o_2o_2$ | $o_2$ | 5,73 | 1,10 | 0,54 | 102,2 | 122,1 | 135,0 |
| | 2790 | $S_3F_4$ | ++ | + | 5,53 | 0,80 | 0,23 | 98,5 | 88,9 | 57,5 |

* SW 437 = 100%

Table 2. Protein content and GOT and GPT activity in seven days old seedlings of the $S_3F_4$ generation

| Specification | Genotype | Proteins mg/ml EE $\times 10^2$ | | Activity GOT U.I./mg prot. $\times 10^2$ without coenzyme addition | | Activity GOT U.I./mg prot. $\times 10^2$ with coenzyme addition | | Activity GPT U.I./mg prot. $\times 10^2$ without coenzyme addition | | Activity GPT U.I./mg prot. $\times 10^2$ with coenzyme addition | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2790/5 | ++ | 561,4 | a* | 89,4 | a | 277,8 | a | 39,4 | a | 86,8 | a |
| 2790/2 | ++, +$o_2$ | 557,6 | a | 94,8 | a | 292,2 | a | 35,6 | a | 88,2 | a |
| 2790/2 | $o_2o_2$ | 560,0 | a | 129,0 | b | 399,0 | b | 71,0 | b | 205,8 | b |

* Values marked with the same letter do not differ significantly; values marked with different letters differ significantly on the probability level P = 0,01

gene mutation is by a change in the proportions of the single protein fractions. In the case of the mutant gene *opaque-2* synthesis of the nutritionally unsuitable prolamine fraction seems to be partly suppressed, whereas the synthesis of nutritionally valuable proteins in the albumine and globuline fractions is de-repressed (Nelson 1970). The changes, therefore, have to be looked for in the regulating systems of protein synthesis. We suppose that a very important role might additionally be played by the ability to quickly rearrange the amino acid pool under changed conditions of proteosynthesis.

Whereas the effect of the *opaque-2* gene on the composition of the endosperm protein is known in more detail, our knowledge about the effect of this gene on the enzyme system of amino acid metabolism and about proteosynthesis in the vegetative plants is less complete. So far there is no consensus as to when gene $o_2$ starts to influence plant metabolism. Alexander et al. (1969) observed no difference in the amino acid composition of the proteins of the embryo and the leaf of a normal and mutant plant. Ševčenko and Agafonov (1972) were not able to detect differences in RNA content in leaves and stalks caused by the gene $o_2$. They pointed out that the gene *opaque-2* begins to function at the milky-wax stage. Murphy and Dalby (1971) investigating the protein fractions and Mehta et al. (1972) following DNA and RNA fractions and RNA-ase activity came to a similar opinion that gene $o_2$ is acting only during the formation of the kernels. On the other hand, Kadam et al. (1973) reported differences in the isozyme composition of the glutamate dehydrogenase in the embryo and in the germ of *opaque-2* and normal maize. In our recent comparative study of free and bound amino acid content in normal and *opaque-2* ten day-old plants we found significant differences in free amino acid levels of two varieties: the free amino acids content was considerably higher in opaque-2 plants, the bound amino acids content did not differ much (Stránský et al.,1974). The differences in transaminase activity, especially GOT, in *opaque-2* and normal seven day-old seedlings found in our present investigation are in agreement with these results. It is, however, known that the activity of single enzymes may change during ontogenesis (e.g. for maize, Maršálek 1972). The question still remains

Table 3. Variance analysis of protein content and GOT, GPT activity at seven days old seedlings of the $S_3F_4$ generation

| Source of variability | (d.F.) | Proteins mg/ml EE $\times 10^2$ | | Activity GOT U.I./mg prot. $10^2$ without coenzyme addition | | Activity GOT with coenzyme addition | | Activity GPT U.I./mg prot. $10^2$ without coenzyme addition | | Activity GPT with coenzyme addition | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | F | MS | F | MS | F | MS | F | MS | F |
| Genotype | 2 | 18,45 | $4,77^{+}$ | 2.305,8 | $61,9^{+++}$ | 21.404,4 | $355,5^{+++}$ | 1.888,4 | $40,2^{+}$ | 22.515,2 | $595,6^{+++}$ |
| Residue | 12 | 3,87 | | 37,2 | | 62,2 | | 47,0 | | 37,8 | |

$F_{05} = 2,88$ $F_{01} = 6,93$ $F_{001} = 12,97$

to be answered whether the changes in GOT and GPT activity observed in young plants persist during the later phases of ontogenesis.

Jolivet et al. (1970) studied differences between normal and *opaque-2* maize plants in the incorporation of $^{14}CO_2$ into the first metabolites of photosynthesis. They found that in the *opaque-2* mutant the photosynthetic activity of type $C_4$ connected with the formation of amino acids related to the Krebs cycle decreases while the photosynthetic activity of type $C_3$ simultaneously rises, causing an increase in the serine and glycine levels. Compared with our results on transaminases connected with the Krebs cycle, an inverted dependence is apparent. We assume that the decreased accumulation of amino acids connected with the Krebs cycle is again connected with the enhanced activity of their interconversion.

In a separate investigation we found that there was no difference in the activity of tryptophan transaminase in normal and mutant maize. We suppose, therefore, that differences in transaminase activity should be looked for primarily in the metabolism of amino acids related to the Krebs cycle.

The results of pursuing the GOT and GPT transaminases with and without the coenzyme show that the gene $o_2$ does not act through PRP biosynthesis; the differences in GOT and GPT activity in both genotypes are not cancelled by addition of the coenzyme.

From the standpoint of practical breeding it is essential to be able to select the *opaque-2* mutant forms with an improved amino acid composition and without the undesirable side-effects, such as lower yield, soft endosperm, lowered cold resistance, slower ripening etc. From contemporary work (Alexander et al. 1969, 1971; Salamini et al. 1970; Chadžinov 1971) it can be concluded that the final effect of *opaque-2* gene is influenced by a number of modifying genes. Already some results (e.g. Paez et al. 1969, Pollaczek et al. 1971; Pešev et al. 1973) show that phenotypically normal kernels without opaque structure, but with a high proportion of valuable protein fractions, can be obtained. For their selection it is, however, necessary to adopt suitable, simple and rapid screening methods (Kamra 1972). On our results, a screening test based on the determination of GOT and GPT activity in young plants may be suggested. Methods of transaminase activity determination are simple and very well worked out for clinical human biochemistry (see the test sets of the firms Calbiochem, Böhringer and others); according to our experience they can also be applied to plant material after selecting optimum conditions. Taking only a part of the young plant for analysis (e.g. first leaf), it would allow the selected indivudual to grow to maturity. In this way it would be possible to identify among the segregating progeny of normal × *opaque-2* cross seedlings those individuals with changed amino acid metabolism.

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Received October 15, 1974

Communicated by H. Stubbe

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