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Glutamate oxaloacetic transaminase and malate dehydrogenase isozymes of *Zea mays* L. × *Tripsacum dactyloides* L. hybrids and parents

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Abstract

Electrophoretic patterns of glutamate oxaloacetic transaminase (Got) and malate dehydrogenase (Mdh) of *Zea mays* L. × *Tripsacum dactyloides* L. hybrids and their parents have been compared. The results suggested that Got and Mdh isozymes may be used as markers for genic regions on 5 S and 6 L maize chromosomes and for linkage groups D and L on *T. dactyloides* chromosomes, syntenic to genic regions on 5 S and 6 L maize chromosomes. The latter have a regulatory effect on fertility and on the apomictic mode of reproduction.

Additional key words: apomixis, biochemical markers, electrophoresis, isozymes, maize, syntenic regions.

Introduction

The distant relative of maize *Tripsacum dactyloides* may be reproduced by two different modes. The diploid (2n=36) *T. dactyloides* reproduce sexually, whereas polyploids reproduce by apomixis (Le Blanc *et al.* 1995). In apomicts, meiosis is suppressed and the embryo develops from maternal tissue (Koltunow 1993). Thus apomixis makes it possible to fix the genotype of a selected plant variety and the seeds, representing that genotype could be cheaply produced independent of pollination (Koltunow *et al.* 1995). *T. dactyloides* is easily hybridised with maize (Kindiger and Becket 1992) so hybrids with *T. dactyloides* genes determining an apomictic mode of reproduction may be developed (Kindiger *et al.* 1996).

A relationship between the expression of apomixis and fertility as measured by percent seed set has been established (Grimanelly *et al.* 1998, Blakey *et al.* 2001). According to Blakey *et al.* (2001), genes associated with fertility have been preserved in both maize and *T. dactyloides.* Blakey *et al.* (2001) and Sokolov (1998) assumed that the regulation of fertility and hence of apomixis is performed by a multigene system. Blakey *et al.* (2001) identified four major fertility regulatory-associated genic regions on four maize chromosomes (1, 5S, 6L and 7S) which reveal synteny with genic regions in *T. dactyloides.* Isoenzymes may be used for the identification of genetic material of *Z. mays* and *T. dactyloides* which determines an apomictic mode of reproduction. They are much cheaper than DNA markers. The isozymes of 6-Pgd and Mdh from leaves, esterase and peroxidase from coleoptiles have been applied to identify 6 L and 7 maize chromosomes and homologous *T. dactyloides* chromosomes which contain genic regions, associated with fertility (Tsanev *et al.* 2000, 2002). Krulíčková *et al.* (2002) used isozyme markers for identification of fibre flax and linseed cultivars.

We used electrophoretically separated components of the enzymes glutamate oxaloacetic transaminase (E.C. 2.6.1.1) and malate dehydrogenase (E.C. 1.1.1.37) to detect markers for the regions on 5S and 6L chromosomes of *Z. mays* and for syntenic chromosome regions of *T. dactyloides* which contain fertility associated genes.

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Abbreviations: Mdh - malate dehydrogenase; Got - glutamate oxaloacetic transaminase; Rm - relative electrophoretic mobility.
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Materials and methods

The following plants have been used: *1*) tetraploid maize (*Zea mays* L.) line 1259/4n, 2n=40 (female parent of *Z. mays* × *T. dactyloides* F1 hybrids); *2*) four inbred maize lines: A-344, W-401, Mo-17, A-632, 2n=20 (female parents of *Z. mays* × *T. dactyloides* BC2 hybrids); 3) *Tripsacum dactyloides* L. (2n=72), an apomictic form (male parent of F1 and BC2 hybrids); 4) BC2 hybrids with: A-344, W-401, Mo-17 and A-632, respectively. The F1 hybrids developed by crossing of *T. dactyloides* with 1259/4n maize line were backcrossed with the inbred maize lines mentioned above. The BC2 hybrids obtained have been studied.

The leaves were taken from plants, grown in a greenhouse at the Institute of Genetics, Sofia. The hybrids were grown individually in a range of low density (1 plant m^{-2}). About 25 - 35 hybrid plants were obtained from each BC2 combination. *T. dactyloides* was grown in the same conditions. The maternal maize lines were grown in two row plots with one replication for each line. Every plot contained 40 plants with 30 cm plant spacing within rows. Samples of fresh young leaves from the BC2 hybrids and their parents were taken in the morning. *Z. mays* plants were at the 5 - 6 leaf stage. To develop shoots seeds were germinated in the dark at 27 °C on moist filter paper. The experiments were carried out with five to ten individual plants (respectively shoots and seeds) of each combination.

The plant tissues investigated (leaves, seeds and shoots) were homogenised with 0.05 M Tris HCl buffer (1:5; m/v) pH 7.2, containing protective supplements: 6 mM cystein hydrochlorid, 6 mM ascorbic acid and 0.5 M sucrose (Rychter and Levak 1969). The enzymes were analysed using vertical block polyacrylamide gel electrophoresis (Davis 1964) with some modifications - the separating gel and the upper electrode buffer containing 0.03 M Na_2 EDTA (Vladova 1991, Vladova and Petkolicheva 1996). The isozymes of Got and Mdh were visualised in the gels by the methods of Shaw and Prasad (1970): Mdh - 50 mg NAD, 30 mg nitroblue tetrazolium, 2 mg phenazine methosulfate, 10 cm^3 1 M Na L-malate dissolved in 0.05 M Tris-HCl buffer, pH 7.0 to a final volume of 100 cm^3; Got - 532 mg L-aspartic acid, 73 mg γ-ketoglutaric acid, 50 mg pyridoxal phosphate, 200 mg Fast Violet B salt to a final volume of 100 cm^3 0.1 M phosphate buffer, pH 7.0.

The relative electrophoretic mobilities (Rm) of the protein bands were calculated according to Bednář and Provazníková (1994).

Results and discussion

The isozymes of glutamate oxaloacetic transaminase (Got) of the BC2 hybrids and their parents can be divided in two groups according to their electrophoretic mobility - slower moving (Rm from 0.20 to 0.42) and faster moving (Rm from 0.65 to 0.78) (Figs. 1, 2). In most of the maize lines there was one slow moving Got isozyme, in *T. dactyloides* - one or three (depending on the plant tissue analysed), two of which (with Rm 0.22 and 0.42) were specific to it. The staining intensity of the slower moving Got isozymes of the maize lines and *T. dactyloides* was highest in the seeds. In the maize lines and *T. dactyloides* there were two fast moving Got isozymes in the seeds and shoots and one in the leaves; the latter probably consists of 2 fused Got isozymes of high staining intensity. All fast moving Got isozymes of the maize lines were not present in *T. dactyloides* and *vice versa*. The staining intensity of the fast moving Got isozymes of the maize lines and *T. dactyloides* was highest in the leaves. In the BC2 hybrids we found Got isozymes, specific to maize lines and to *T. dactyloides*. Isozymes with intermediate electrophoretic position between those specific to the maize lines and *T. dactyloides* were also present (Figs. 1, 2). Such isozymes seem to be intralocus heterodimers as Got isozymes in maize are all dimers, encoded by three loci (Stuber and Goodman 1979) and no interlocus heterodimer Got isozymes have been found. Their existence indicates the presence of homologous Got loci from the two parents and is in accordance with the synteny observed between genes of *T. dactyloides* and maize (Grimanelli *et al.* 1998, Blakey *et al.* 2001).

The slow moving Got isozymes of the forms studied might be treated as Got-1, the fastest one, and the isozyme next to it - as Got-3 and Got-2 (Stuber and Goodman 1979). They are encoded by loci on: 3 L chromosome - Got-1; 5 L chromosome - Got-2 and 5 S - Got-3 (Scandalios *et al.* 1975, Goodman *et al.* 1980b). Got-1 isozymes are expressed in the glyoxysomes and Got-3 - in the mitochondria (Scandalios *et al.* 1975). Got-2 coded enzymes are plastidial (Coe and Neuffer 1993). The site of the locus, encoding Got-3 isozymes, according to the maize chromosome maps (Sheridan 1982, Coe and Neuffer 1993, Davis *et al.* 1998) is between the UMC 1 marker and the centromere (Fig. 3). In the same region, between the UMC 166 and Bn 14.36 markers is localised the Am 1 gene (ameiotic) (Blakey *et al.* 2001). Stocks homozygous for Am 1 fail to commit to meiosis and result in complete male and female sterility cells (Golubovskaya *et al.* 1997). UMC 166 marker is linked to the genic region which reveals synteny to the *T. dactyloides* linkage group D (Blakey *et al.* 2001). On the basis of the synteny between the genetic regions of

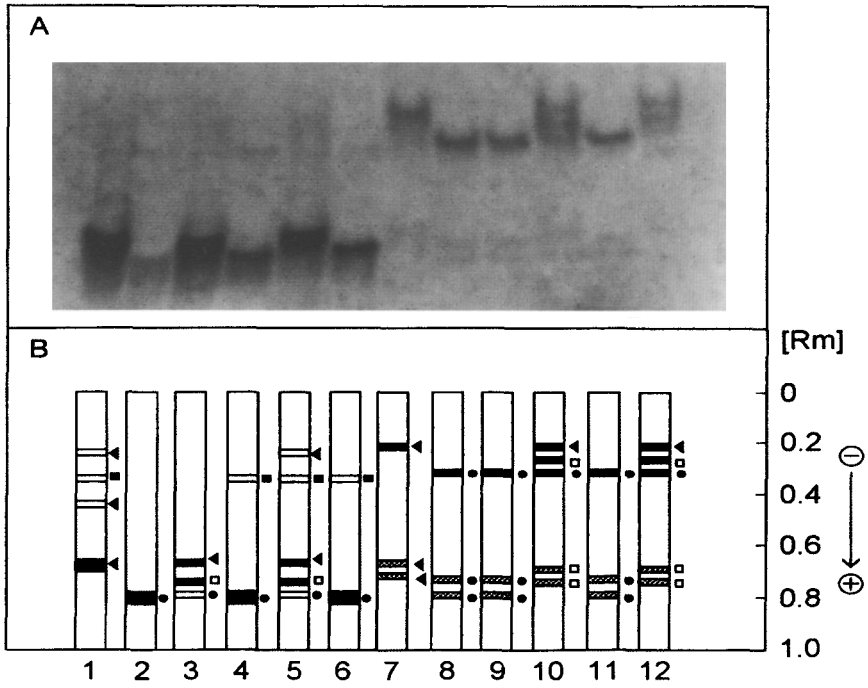

Fig. 1. Electrophoregram (*A*) and diagram (*B*) of the Got isozymes from leaves (1 - 6) and from seeds (7 - 12) of *Z. mays* L. × *T. dactyloides* L. BC2 hybrids and their parents - tetraploid *Z. mays* line 1259/4n, 2n=40; inbred *Z. mays* lines, 2n=20; *T. dactyloides* L., 2n=72. On the diagram of these isozymes, the ones, specific to *T. dactyloides* are indicated with *closed arrows*, specific to *Z. mays* - with *closed circles*, common ones - with *closed squares*, heterodimers - with *empty squares*. 1, 7 - *T. dactyloides;* 2, 4, 9, 11 - inbred *Z. mays* (2 - A-344, 4 - W-401, 9 - A-344, 11 - W-401); 3, 5, 10, 12 - *Z. mays* × *T. dactyloides* BC2 hybrids (3 - BC2 with A-344, 5 - BC2 with W-401, 10 - BC2 with A-344, 12 - BC2 with W-401); 6, 8 - tetraploid *Z. mays*.

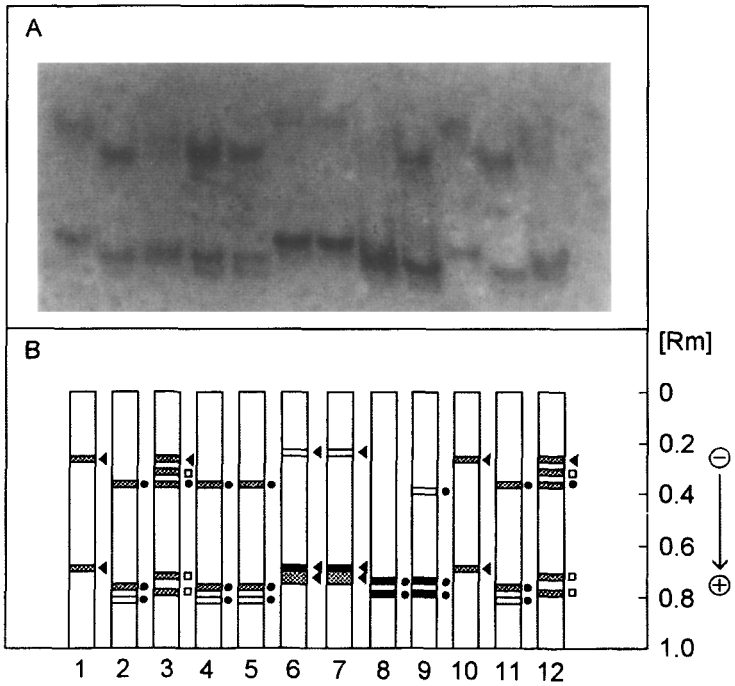

Fig. 2. Electrophoregram (*A*) and diagram (*B*) of the Got isozymes from dark grown (1 - 5, 10 - 12) and light grown (6 - 9) shoots of *Z. mays* L. × *T. dactyloides* L. BC2 hybrids and their parents (as in Fig. 1). The designation of the specific Got isozymes as in Fig. 1. 1, 6, 7, 10 - *T. dactyloides*; 2, 4, 9, 11 - inbred *Z. mays* (2 - A-344, 4 - W-401, 9 - A- 344, 11 - Mo-17); 3, 12 - *Z. mays* × *T. dactyloides* BC2 hybrids (3 - BC2 with A-344, 12 - BC2 with Mo-17); 5, 8 - tetraploid *Z. mays*.

maize and *T. dactyloides* it may be suggested that the isozyme of Got-3 specific to *T. dactyloides* can be used as a marker for the presence of *Tripsacum* linkage group D. The heterodimer Got-3 isozyme in the hybrids indicates the presence of loci from the region of Am 1 gene and from a syntenic *T. dactyloides* genic region.

The number of the malate dehydrogenase (Mdh) isozymes in leaves of A-344 maize line was greater compared to the Mdh isozymes in seeds of the maize lines studied (Fig. 4). In the seeds of the maize lines there were one to three slower moving Mdh isozymes with Rm varying from 0.43 to 0.53 and one or two faster moving Mdh isozymes with Rm 0.60 and 0.65. The slower moving Mdh isozyme of *T. dactyloides* (Rm 0.59) was diffusely stained. The same isozyme was found in all BC2 hybrids. The slower moving Mdh isozymes, specific to the maize lines were also present in most of the BC2 hybrids. The experiments with inhibited leaf cytosolic Mdh isozymes of *Z. mays* × *T. dactyloides* hybrids (Tsanev *et al.* 2002) show that the slow moving Mdh isozymes are from the mitochondrial fraction. They might be treated as Mdh 2 isozymes encoded by loci on the distal end of maize chromosome 6L (Goodman *et al.*

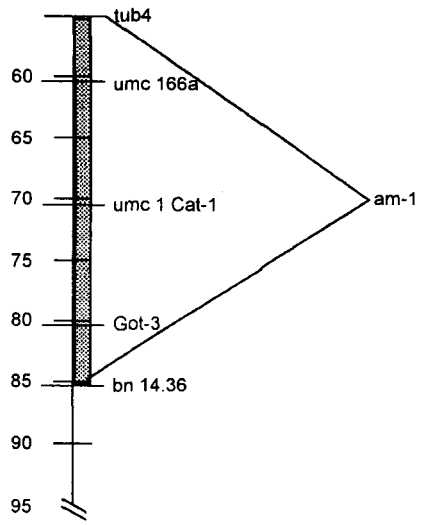

Fig. 3. Genetic segments of 5 S maize chromosome that show syntenic intervals with *T. dactyloides* linkage group D (according to Blakey *et al.* 2001). The site of Got-3 according to maize chromosome maps (Coe and Neuffer 1993, Davis *et al.* 1998, Sheridan 1982, Blakey *et al.* 2001) is between UMC 1 marker and the centromere.

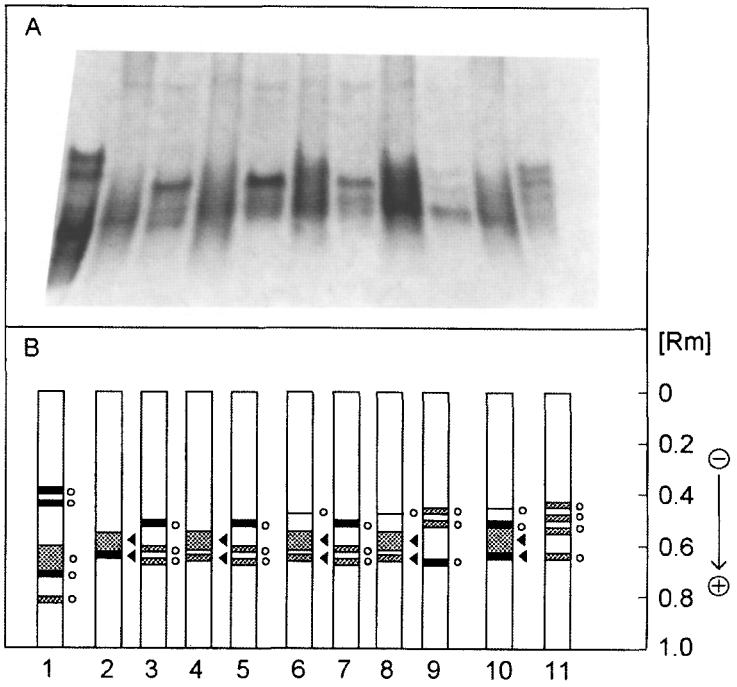

Fig. 4. Electrophoregram (*A*) and diagram (*B*) of the Mdh isozymes from leaves (1) and seeds (2 - 11) of *Z. mays* × *T. dactyloides* BC2 hybrids and their parents (as in Fig. 1). The designation of the specific Mdh isozymes as in Fig. 1. 1, 3, 5, 7, 9 - inbred *Z. mays* (1 - A-344, 3 - A-344, 5 - W-401, 7 - Mo-17, 9 - A-632); 2 - *T. dactyloides*; 4, 6, 8, 10 - *Z. mays* × *T. dactyloides* BC2 hybrids (4 - BC2 with A-344, 6 - BC2 with W-401, 8 - BC2 with Mo-17, 10 - BC2 with A-632); 11 - tetraploid *Z. mays*.

1980a). The site of Mdh 2 locus in maize is between the UMC 238 and UMC 28 markers in maize map (Davis *et al.* 1998) (Fig. 5). According to Blakey *et al.* (2001) in this DNA interval is located a genic region with regulatory effect on fertility. Thus the slow moving Mdh isozymes, specific to the maize lines found in the BC2 hybrids may be used as a marker of the DNA region on 6 L maize chromosome which has a regulatory effect on the fertility. The linkage group L on the proximal end of *T. dactyloides* 16 chromosome is homoelogous to this

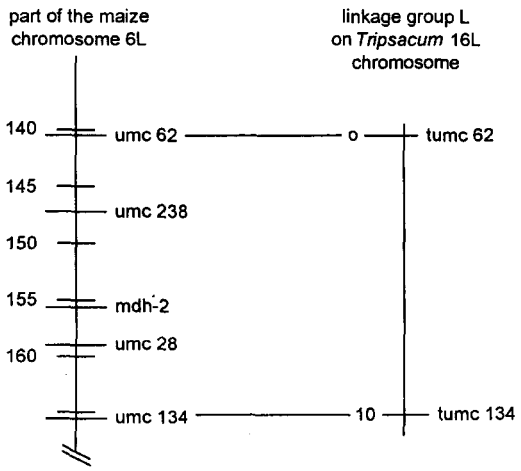

Fig. 5. Genetic segments of 6 L maize chromosome and *T. dactyloides* linkage group L in which the conservation of RFLP marker order is preserved (according to Kindiger *et al.* 1996). The site of Mdh-2, according to the maize chromosome map (Davis 1998) is between 62 and 134 UMC markers.

maize DNA interval (Kindiger *et al.* 1996). It manifests synteny with the genic regions on 6 L maize chromosome (Blakey *et al.* 2001). The slow moving Mdh isozyme specific to *T. dactyloides*, present in the BC2 hybrids may be used as a marker for the linkage group L of *T. dactyloides*.

In conclusion, the Got-3 isozymes in the seeds and shoots of the BC2 hybrids may be used as a marker of the Am-1 gene on 5 S maize chromosome and of the linkage group D of *T. dactyloides*. The same genic regions contain genes, which control the fertility and the apomictic mode of reproduction of the plants The isozymes of Mdh 2 from the seeds of the BC2 hybrids may be used as markers of the genic region on 6 L maize chromosomes and of the linkage group L on *T. dactyloides* 16 chromosome, which are connected as well with the control of fertility and the apomictic mode of reproduction of plants.

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