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Malate dehydrogenase, alcohol dehydrogenase, and 6-phosphogluconate dehydrogenase isozymes of *Zea mays* L. × *Tripsacum dactyloides* L. hybrids and parents

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Abstract

Electrophoretic patterns of malate dehydrogenase (Mdh), alcohol dehydrogenase (Adh), and 6-phosphogluconate dehydrogenase (Pgd) of *Zea mays* L. × *Tripsacum dactyloides* L. hybrids and their parents were compared. The components of enzymes specific to *T. dactyloides* may be used as markers to identify the following *T. dactyloides* chromosomes in the hybrids: Tr 16 (Mdh 2 and Pdg 1), Tr 7, and/or Tr 13 (Adh 2). The isozymes of Mdh 2 are supposed as a possible biochemical marker to evaluate the introgression of genes, determining an apomictic mode of reproduction from *T. dactyloides* (localized on *Tripsacum* 16 chromosome) into *Z. mays*. The isozymes may be used as markers for the identification of maize chromosomes 1 and 6 in the hybrids as well. Chromosome count taken on the examined hybrids showed the addition of 9 to 13 chromosomes of *T. dactyloides* to maize chromosome complement.

Additional key words: biochemical markers, chromosome complement, electrophoresis, isozymes, maize.

Introduction

Tripsacum dactyloides L., a distant relative of maize is a perennial, warm season bunchgrass that is widely distributed in some areas of Northern and Southern America. It is a donor of valuable traits such as drought tolerance (Berthaud and Savidan 1989), resistance to some diseases (Berquist 1981), *etc.* Due to its superior nutritional aspects, numerous efforts have been initiated in the cultivation, dissemination and breeding of this species (Burns *et al.* 1992). *T. dactyloides* is easily hybridized to maize (Kindiger and Beckett 1992). For that reason it can be used as a source of germplasm for the improvement of maize. The ability of *T. dactyloides* individuals with higher ploidy to be reproduced in an apomictic mode is of a particular interest for maize improvement (Burson *et al.* 1990, Leblanc *et al.* 1995). With this way of reproduction the progenies are copies of the female plant. Therefore, it is possible to fix the genotype of a superior female plant and to reproduce it (Koltunow *et al.* 1995). The lack of rapid biochemical or molecular markers for line identification and detection of *T. dactyloides* introgression into maize is a hindrance to breeding *T. dactyloides* and hybridization with *Z. mays*. A set of isozymic markers for this species would be valuable in detecting *T. dactyloides* additions, substitutions, translocations or introgression into maize. They are much cheaper than DNA markers. Kindiger and Vierlig (1994) and Tsanev *et al.* (2000) used electrophoretic spectra of acid phosphatase, malate dehydrogenase, phosphoglucomutase, phosphohexose isomerase, esterase and peroxidase to differentiate between *T. dactyloides* individuals as well as to detect *Tripsacum* introgression into maize. Zlokolica and Milošević (2001) studied the possibilities of using isozymes as genetic markers for quantitative traits in the Yugoslavian maize population. Kato-Noguchi (2000) established that osmotic stress increases alcohol dehydrogenase activity in maize seedling. We used components of several enzymes: malate dehydrogenase (E.C. 1.1.1.37), 6-phospho-

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Abbreviations: Adh - alcohol dehydrogenase; Mdh - malate dehydrogenase; Pgd - 6-phosphogluconate dehydrogenase; Rm - relative electrophoretic mobility.

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gluconate dehydrogenase (E.C. 1.1.1.44), and alcohol dehydrogenase (E.C. 1.1.1.1) separated electrophoretically to assess introgressed *T. dactyloides* genes (chromosomes or chromosome segments) into maize hybrids, especially to detect markers for *T. dactyloides* 16 chromosome, carrying loci responsible for the apomictic mode of reproduction.

Materials and methods

Leaves from 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*) five inbred maize lines: A-632, W-401, Mo-17, M-320/78, A-344, 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-632, W-401, Mo-17, M-320/78 and A-344, 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 obtained BC2 hybrids have been studied.

As the first *Z. mays* × *T. dactyloides* backcrosses are, like *T. dactyloides* perennial and warm season forms, the BC2 hybrids and their parents were grown in greenhouse at the Institute of Genetics, Bulgarian Academy of Sciences, Sofia. Since the fertility of the BC2 hybrids is low, they were grown individually in a range of rather low density (1 plant per 2 m^2). About 25 - 35 hybrid plants were obtained of 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. The experiments were carried out in two replications of 10 plants each. 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. The cytological studies showed 9 - 13 *T. dactyloides* chromosomes in the chromosome complement of the BC2 hybrids analyzed.

The leaves were homogenized with 0.05 M Tris HCl buffer (pH 7.2, 1:5, m/v) containing protective supplements: 6 mM cystein hydrochlorid, 6 mM ascorbic acid and 0.5 M sucrose (Rychter and Levak 1969). The electrophoretic analysis was carried out using vertical block polyacrylamide gel electrophresis after 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). Isozymes of Adh, Mdh and Pgd were visualized in the gels by the methods of Shaw and Prasad (1970): Adh - 50 mg NAD, 30 mg nitroblue tetrazolium, 2 mg phenazine methosulfate, 3 cm^3 ethanol (95 %) dissolved in 0.05 M Tris-HCl buffer, pH 7.1, to a final volume of 100 cm^3; 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; Pgd - 20 mg Na_3 6-phosphogluconate, 10 mg NADP, 10 mg nitroblue tetrazolium, 2 cm^3 1 M $MgCl_2$ dissolved in 0.05 M Tris-HCl buffer, pH 8.0, to a final volume of 100 cm^3. The relative electrophoretic mobilities (Rm) of protein bands were calculated according to Bednář and Provazníková (1994).

Results and discussion

Malate dehydrogenase (Mdh) isozymes of studied hybrids and their parents can be divided into three groups according to their electrophoretic mobility: slow moving, with medium electrophoretic mobility and fast moving Mdh isozymes (Fig. 1). The maternal maize lines are characterized by two or three slow moving Mdh isozymes with Rm varying from 0.50 to 0.71. Slow moving Mdh isozyme in *T. dactyloides* (Rm 0.75) is diffusely stained. Slow moving Mdh isozymes, specific both to maize lines and to *T. dactyloides,* were observed in the electrophoretic spectra of the BC2 hybrids. An intensively stained Mdh isozyme component with medium electrophoretic mobility was present in the electrophoretic spectra of maize lines (Rm 0.80), *T. dactyloides* (Rm 0.82) and BC2 hybrids (Rm 0.80). A fast migrating diffusely stained Mdh isozyme (Rm 0.84), specific to *T. dactyloides* was observed in the electrophoretic spectra of all hybrids studied.

Protein extracts of maize line A-344 and BC2 hybrid with line Mo-17 were treated with urea to distinguish between cytosolic and mitochondrial Mdh isozymes of the studied hybrids and their parents. Their electrophoretic profiles were compared with those of protein extracts untreated with urea (Fig. 2). Urea selectively inhibits the cytosolic Mdh isozymes, but has a little effect on the mitochondrial ones (Vladova 1991). The slow moving Mdh isozymes of the urea treated samples (Fig. 2, *lanes* 2 and 4) were identical to those untreated (Fig. 2, *lanes* 1 and 3). This suggests that they are from the mitochondrial fraction and might be considered as Mdh 1 and/or Mdh 2 isozymes, encoded by loci on maize chromosomes 8 and 6L (Goodman *et al.* 1980a). Since

Mdh 1 isozymes are of low activity, the slow moving ones seem to be Mdh 2 isozymes, encoded by loci on the distal end of 6L maize chromosome (Goodman *et al.* 1980a). Therefore, the slow moving Mdh isozymes in the BC2 hybrids, specific to maize lines may be used as a marker for the distal end of 6L chromosome of maternal maize lines. Similarly, the specific to *T. dactyloides* slow moving Mdh isozyme in the BC 2 hybrids may be used as a marker for 16 chromosome of *T. dactyloides* as the proximal end of *Tripsacum* 16 chromosome is homeologous to the distal end of the 6L maize chromosome (Kindiger *et al.* 1996).

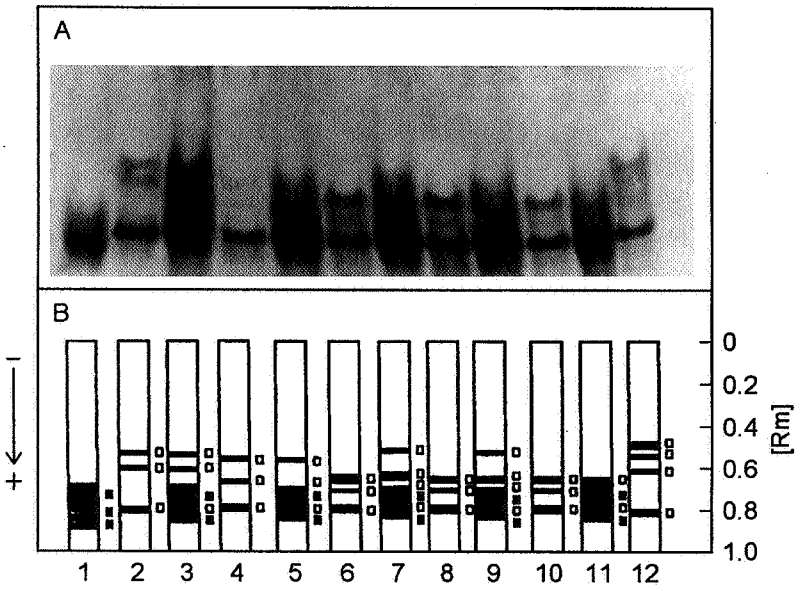

Fig. 1. Electrophoregram (*A*) and diagram (*B*) of the Mdh isozymes from leaves of *Z. mays* L. × *T. dactyloides* L. BC2 hybrids and their parents - tetraploid *Z. mays* line 1259/4n, 2n = 40; inbred *Z. mays* lines, 2n = 20; *T. dactyloides* L, 2n = 72. On the diagram of these isozymes, the ones, specific to *T. dactyloides* are indicated with *closed squares*, specific to *Z. mays* with *empty squares*. 1 - *T. dactyloides;* 2, 4, 6, 8, 10 - inbred *Z. mays* (2 - A-344, 4 - W-401, 6 - M-320/78, 8 - A-632, 10 - Mo-17); 3, 5, 7, 9, 11 - *Z. mays* × *T. dactyloides* BC2 hybrids (3 - BC2 with A-344, 5 - BC2 with W-401, 7 - BC2 with M-320/78, 9 - BC2 with A-632, 11 - BC2 with Mo-17); 12 - tetraploid *Z. mays*.

Genes, conferring apomixis are assigned to the proximal end of the *T. dactyloides* chromosome 16 (Kindiger *et al.* 1996). Therefore, the specific to *T. dactyloides* slow moving Mdh isozyme in the BC2 hybrids may be also used as a marker for introgression of genes, responsible for the apomictic mode of reproduction from *T. dactyloides* into *Z. mays*.

The patterns of protein extracts untreated with urea of maize line A-344 and BC2 hybrid with line Mo-17 exhibited one intensively stained Mdh isozyme with medium electrophoretic mobility (Fig. 2, *lanes* 1 and 3) whereas those of the extracts treated did not contain this isozyme (Fig. 2, *lanes* 2 and 4). So that isozyme is from the cytosolic fraction and might be treated as Mdh 4, encoded by loci on maize chromosome 1 (Goodman *et al.* 1980a). The specific to maternal maize line intensively stained Mdh isozyme with medium electrophoretic mobility, present in the hybrids, may be used as marker for maize chromosome 1.

We observed two phenotypes in the electrophoretic spectra of 6-phosphogluconate dehydrogenase (Pgd)

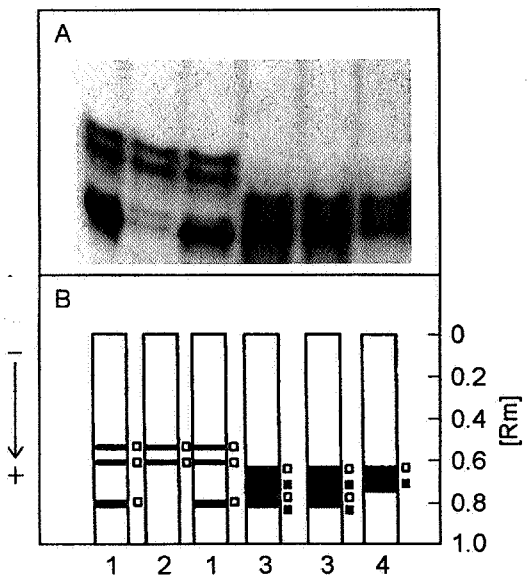

Fig. 2. Electrophoregram (*A*) and diagram (*B*) of Mdh isozymes in untreated (*lanes 1* and *3*) and treated (*lanes 2* and *4*) with urea protein extracts of the maize line A-344 (*lanes 1* and *2*) and BC2 hybrid with line Mo-17 (*lanes 3* and *4*).

of the maternal maize lines (Fig. 3). One of them contained one band (Rm 0.45) (Fig. 3, *lanes* 2, 4, 8, 10 and 12) and the second - two bands (Rm 0.45 and 0.53) (Fig. 3, *lane* 6). Those results agree with the data of Sukhorzhevskaya and Khavkin (1980) who established two phenotypes for maize Pgd isozymes: phenotype A (one band) and phenotype B (two bands). *T. dactyloides* contained two Pgd isozymes, one of them with the electrophoretic mobility of the slow moving maize isozyme (Rm 0.45) and the other - specific to it (Rm 0.58). The electrophoretic spectra of the BC2 hybrids studied have two components: the *T. dactyloides* specific isozyme (Rm 0.58) and the isozyme common to *T. dactyloides* and maize lines (Rm 0.45). The intensity of

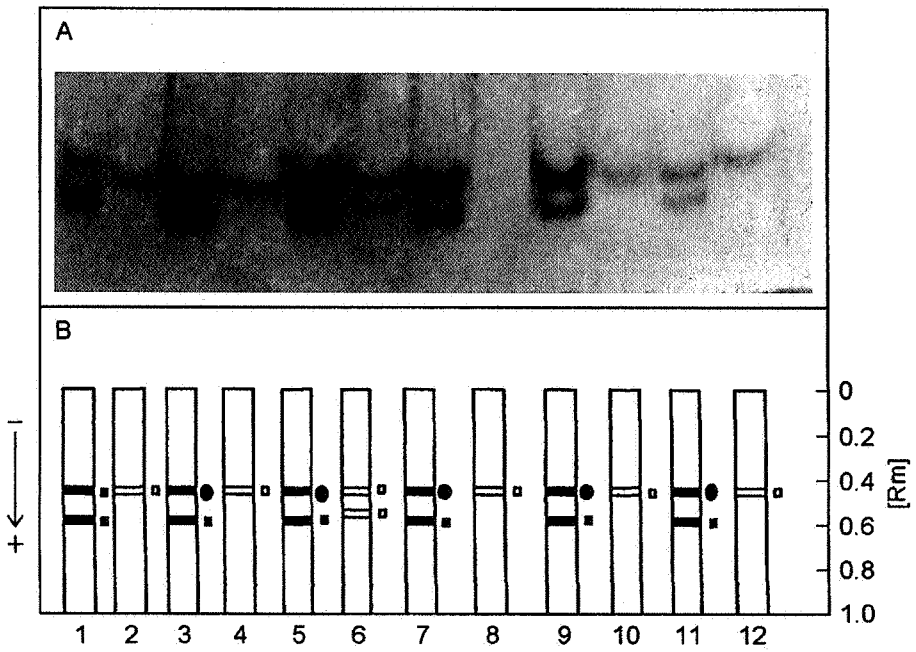

Fig. 3. Electrophoregram (*A*) and diagram (*B*) of the Pgd isozymes from the leaves of the same forms as in Fig. 1. On the diagram the isozymes specific to *T. dactyloides* are indicated with *closed squares*, those specific to *Z. mays* with *empty squares*, and common ones with *closed circles*.

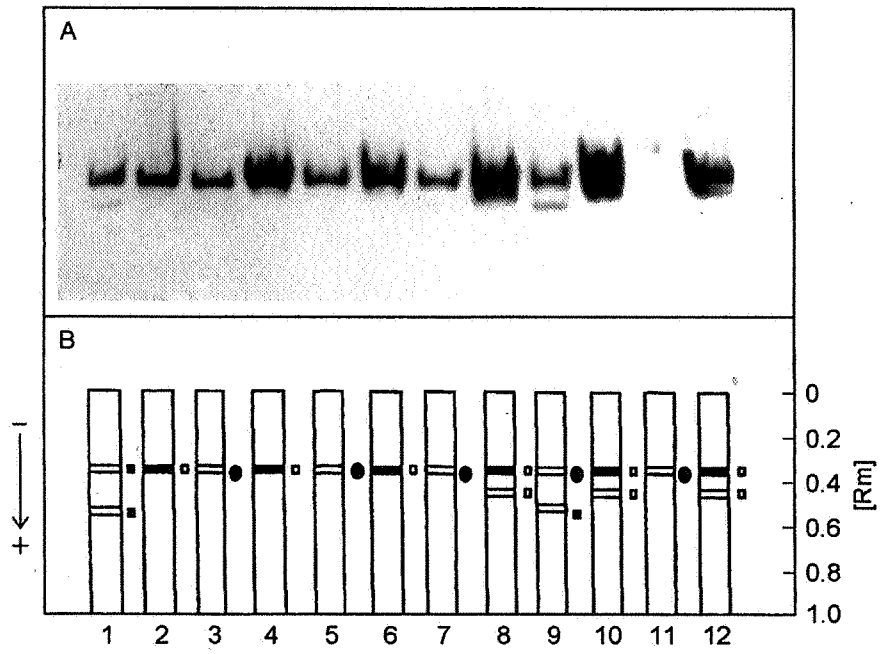

Fig. 4. Electrophoregram (*A*) and diagram (*B*) of the Adh isozymes from the leaves of the same forms as in Fig. 1. On the diagram of these isozymes, the ones, specific to *T. dactyloides* are indicated with *closed squares*, specific to *Z. mays* with *empty squares*, and common isozymes with *closed circles*.

staining of the last one, however, is comparable with that of the *T. dactyloides* component. The fast moving Pgd isozyme specific to *Tripsacum,* observed in the BC2 hybrids may be used as a marker for the 16 chromosome of *T. dactyloides* since chromosome 16 of *T. dactyloides* is homeologous to maize 6 chromosome (Kindiger *et al.* 1996) which carries genes for Pgd 1 isozymes (Goodman *et al.* 1980a, Weissinger *et al.* 1979).

Most of the maize lines contained one alcohol-dehydrogenase isozyme, Adh-1 (Rm 0.35), encoded by loci on maize chromosome 1L (Schwartz 1971) (Fig. 4). In maize lines A-632, Mo-17 and 1259/4n there were two isozymes (Rm 0.35 and Rm 0.45) (Fig. 4, *lanes* 8, 10 and 12). The faster moving Adh band in these lines is Adh-2, encoded by loci on 4S maize chromosome (Goodman *et al.* 1980b). The Adh electrophoretic spectrum of *T. dactyloides* has two isozymes (Fig. 4). Rm of the slower moving one was equal to that of the slow moving maize isozyme (Rm 0.35). Its intensity of staining, however, was lower than that in maize lines. The faster moving one was specific to *T. dactyloides* (Rm 0.52). In most of the BC2 hybrids only one Adh isozyme was present (maize phenotype), but its intensity of staining was similar to that in *T. dactyloides.* In the BC2 hybrid of maize line A-632 there were two Adh isozymes: the faster moving one (Rm 0.52) is inherited from *T. dactyloides* and the other (Rm 0.35) is common for the two species (Fig. 4, *lane* 9). The faster moving Adh isozyme, specific to *T. dactyloides* in the BC2 hybrid with maize line A-632 may be used as a marker for 7 and/or 13 chromosome of *T. dactyloides.* The latter are homologous to maize chromosome 4 (Galinat 1973) which carries Adh 2 loci.

In conclusion the BC2 hybrids studied contain isozymes specific to the parental forms: *T. dactyloides* and the maternal maize lines *i.e.* they contain loci, encoding isozymes from the two species. Isozymes of Mdh, Adh and Pgd might be used as biochemical markers to prove the presence of *T. dactyloides* genes in the BC2 hybrids, including genes determining the apomictic mode of reproduction.

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