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BRIEF COMMUNICATION

Esterase isoenzymes as markers for the *VA* 1 gene of *Zea mays* and for the B linkage group of *Tripsacum dactyloides*

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Abstract

It is suggested that cathodal esterase isoenzyme (E_1) might be used as a marker for the *VA* 1 gene on 7 S maize chromosome and for the "B" linkage group of *Tripsacum dactyloides* in *Zea mays* L. × *T. dactyloides* L. hybrids. The latter genic zones have a regulatory effect on fertility and on the apomictic mode of reproduction.

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

The use of clonal seeds is of great advantage to the modern agriculture (Spillane *et al.* 2001). Clonal seeds of maize may be obtained by apomictic reproduction of *Z. mays* × *T. dactyloides* hybrids since *T. dactyloides* is a donor of genes, determining that way of propagation (Sokolov *et al.* 1998). Blakey *et al.* (2001) identified four genic regions on maize chromosomes 1, 5 S, 6 L and 7 S and syntenic to them genic regions in *Tripsacum* which have a regulatory effect on fertility and apomixis. Isoenzymes may be used as markers for these genic regions. They are much cheaper than DNA probes. In our previous studies we showed that the isoenzymes of malate dehydrogenase-2, glutamate oxaloacetic transaminase-3 and 6-phosphogluconate dehydrogenase may be used as markers for the *Am* 1 and *Afd* 1 genes on 6 L and 5 S maize chromosomes and for the "L" and "D" linkage groups in syntenic genic regions of *T. dactyloides* (Tsanev *et al.* 2002, 2003/4). Kruličková *et al.* (2002) used isoenzyme markers for identification of fibre flax and linseed cultivars.

In the present studies we have used the isoenzymes of cathodal esterase (E.C.3.1.1.1.) to detect markers for the *VA* 1 gene on 7 S maize chromosome and for the "B" linkage group of *T. dactyloides* which have a regulatory effect on fertility and apomictic mode of reproduction.

Zea mays × *Tripsacum dactyloides* hybrids and their parents were used. The parental forms were: 1) tetraploid maize (*Zea mays* L.), line 1259/4n, 2n=40; 2) two inbred maize lines: A-632 and Mo-17, 2n = 20; 3) *Tripsacum dactyloides* L. (2n = 72), an apomictic form. The F_1 hybrids developed by crossing *T. dactyloides* with 1259/4n maize line were backcrossed with the inbred maize lines mentioned above. The developed BC hybrids have been studied.

Seeds were germinated in the dark at 27 °C on moist filter paper. We used 3 cm shoots cut off from 4 cm long seedlings for protein extraction. To obtain 4 cm long seedlings maize seeds were germinated four days, *T. dactyloides* seeds - 7 d and *Z. mays* × *T. dactyloides* hybrid seeds - 5 d. Ten seedlings of each hybrid and of the parental forms were analyzed. The protein extracts were prepared by grinding shoots of the seedlings with 0.05 M Tris-HCl buffer pH 7.2, containing 6 mM ascorbic acid, 6 mM cysteine hydrochloride and 0.5 M sucrose (Rychter and Lewak 1969). The electrophoretic separation of cathodal esterases was conducted on 7.5 % polyacrylamide gel (Davis 1964). The electrode buffer (pH 4.5) contained 35 mM β-alanine and 7.5 mM acetic acid (Reisfeld *et al.* 1962). The origin was at the anode and the cathode was at the bottom. Esterases in Fig. 1 were stained with α-naphtylacetate according to Shaw and Prasad (1970) and those in Fig. 2 - with β-naphthyl-acetate according to Brown and Allard (1969). The relative electrophoretic mobilities (Rm) of protein bands

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Abbreviations: E - esterase; Rm - relative electrophoretic mobility.

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were calculated according to Bednář and Provazníková (1994).

The maize esterase isoenzymes are encoded by 12 loci: E_1 - E_{10} (McDonald and Brewbaker 1975), E_{12} and E_{16} (Brown and Allard 1969). The isoenzymes, associated with E_1 and E_3 migrate to cathod. E_1 and E_3 esterase isoenzymes can be distinguished on the basis of different staining with α- and β-naphthylacetate. E_3 isoenzymes develop black colour with α-naphthylacetate whereas the E_1 esterases develop reddish colour with β-naphthylacetate (Brown and Allard 1969). Therefore, it may be assumed that the esterases in Fig. 1 are E_3 and those in Fig. 2 are E_1. In *T. dactyloides* E_1 and E_3 esterase isoenzymes had similar Rm (Figs. 1 and 2); in *Z. mays* lines Rm of E_1 esterases was greater than those of the

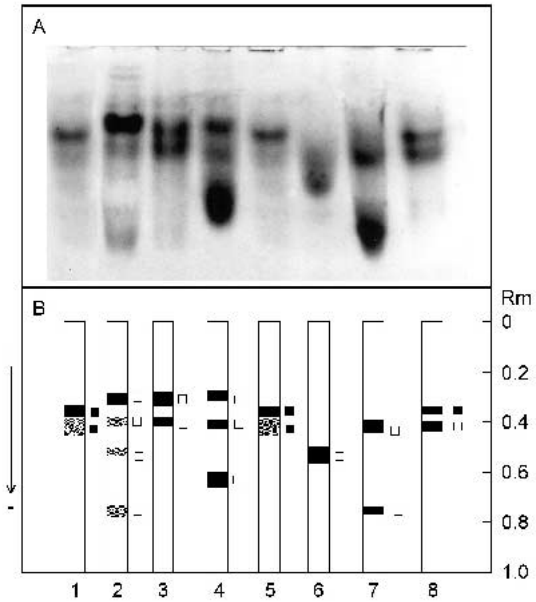

Fig. 1. Electrophoregram (*A*) and diagram (*B*) of E_3 esterase isozymes of *Z. mays* L. × *T. dactyloides* L. BC hybrids and their parents - tetraploid *Z. mays* line 1259/4n, 2n = 40; inbred *Z. mays* lines, 2n = 20; *T. dactyloides* L., 2n = 72. The isozymes, specific to *T. dactyloides* are indicated with closed squares and those specific to *Z. mays* - with empty squares. 1, 5 - *T. dactyloides*; 2, 7 - inbred *Z. mays* (2 - Mo-17, 7 - A-632); 3, 4, 8 - *Z. mays* × *T. dactyloides* BC hybrids (3 - BC3 with Mo-17, 4 - BC2 with Mo-17, 8 - BC2 with A-632); 6 - tetraploid *Z. mays.*

most of E_3 esterases. The number of E_3 esterases in maize lines (1-4) was greater than that of the E_1 esterases. The intensively stained slow moving E_3 esterase isoenzymes specific to Mo-17 maize line (Rm 0.30) and to A-632 maize line (Rm 0.43) were found in the BC2 hybrids of those lines. An E_3 esterase isoenzyme, specific to *T. dactyloides* (Rm 0.37) was detected in the BC2 hybrid of maize line A-632 (Fig. 1). The E_1 esterase isoenzyme, specific to *Z. mays* (Rm 0.67) and two of the E_1 isozymes, specific to *T. dactyloides* (Rm 0.38, 0.40) were found in the BC2 hybrids studied (Fig. 2).

Linkage analyses have shown that E_1 loci are on chromosome 7 (Schwartz 1967). The site of E_1 on maize chromosome 7 according to the UMC maize chromosome map (Davis *et al.* 1998) is localized close to the *VA* 1 gene (variable sterility) (Davis *et al.* 1998; Blakey *et al.* 2001) (Fig. 3). Plants homozygous for *VA*1/*VA1* gene

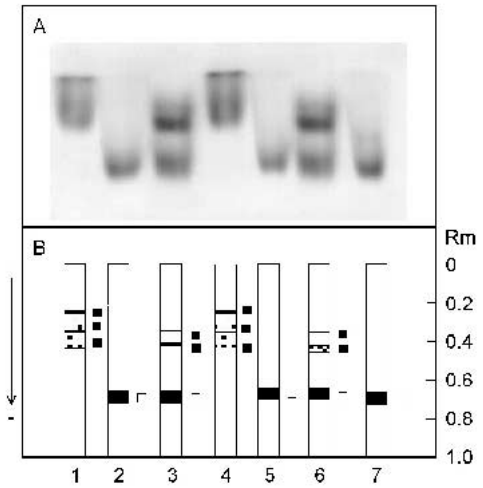

Fig. 2. Electrophoregram (*A*) and diagram (*B*) of E_1 esterase isozymes of *Z. mays* L. × *T. dactyloides* L. BC hybrids and their parents - tetraploid *Z. mays* line 1259/4n, 2n = 40; inbred *Z. mays* lines, 2n = 20; *T. dactyloides* L, 2n = 72. The designation of the specific E_1 esterase isozymes as in Fig. 1. 1, 4 - *T. dactyloides*; 2, 5 - inbred *Z. mays* (2 - Mo-17, 5 - A-632); 3, 6 - *Z. mays* × *T. dactyloides* BC2 hybrids (3 - BC2 with Mo-17, 6 - BC2 with A-632); 7 - tetraploid *Z. mays.*

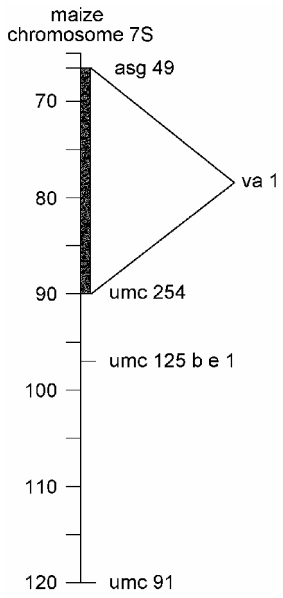

Fig. 3. Genetic segments of 7 S maize chromosome after Davis *et al.* (1998) and Blakey *et al.* (2001). The site of E_1 is between the *umc* markers 254 and 91, close to the *VA* 1 gene. The *umc* 91 marker belongs to the *tda* 30 - *umc* 91 genic region, syntenic to the *T. dactyloides* linkage group "B".

result in variable male and female fertility (Blakey *et al.* 2001). On that ground it may be presumed that the E_1 esterase isoenzyme detected in the BC2 hybrids, specific to the maize lines (Rm 0.67) may be used as a marker for the *VA* 1 gene on maize chromosome 7. The site of E_1 is close to the *umc* 91 marker as well; the latter belongs to the *tda* 30 - *umc* 91 genic regions (Blakey *et al.* 2001). The same region is syntenic to the *T. dactyloides* "B" linkage group which is also associated with the fertility (Blakey *et al.* 2001). Therefore it might be assumed that the gene order in *tda* 30 - *umc* 91 zone of *Z. mays* and in the "B" linkage group of *T. dactyloides* is the same and, hence, E_1 esterase loci are present in the two gene zones mentioned. On that account the specific to *T. dactyloides* E_1 esterase isoenzymes found in the BC2 hybrids (Rm 0.38, 0.40) may be used as markers for the presence of genetic material from the "B" linkage group of *T. dactyloides.*

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