BIOLOGIA PLANTARUM 43 (2): 211-215, 2000

Composition of esterase and peroxidase isoenzyme complex, zein-2 protein fraction, and SDS-protein complex of *Zea mays* L. × *Tripsacum dactyloides* L. hybrids and parents

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Abstract

The patterns of esterase and peroxidase isoenzymes, subunits of zein-2 fraction and protomers of SDS-protein complex of *Zea mays* L. × *Tripsacum dactyloides* L. hybrids and their parents were compared. The study has been made to detect specific to *Tripsacum* isoesterases and isoperoxidases, zein subunits and SDS-protein protomers which could be used as markers for introgression of gene loci encoding these proteins from *Tripsacum* into hybrids of *Tripsacum* with *Zea mays*. Isoesterases and isoperoxidases as well protomers of SDS-protein complex specific to *Tripsacum* were detected in all hybrids analyzed. Zein subunits, specific to *Tripsacum* were detected in some of the analyzed hybrids which indicates that introgression frequency of the loci encoding proteins studied was different. Chromosome counts taken on the examined hybrids showed the addition of 9 - 13 *Tripsacum* chromosomes to maize chromosome complement.

Additional key words: chromosome complement, electrophoresis, introgression frequency, maize.

Introduction

Tripsacum dactyloides L., a wild relative of maize, is a source for its improvement being a donor of valuable characters such as drought tolerance (Berthaud and Savidan 1989), resistance to some diseases (Berquist 1981) and ability for apomictic mode of reproduction (Kindiger *et al.* 1996, Leblanc *et al.* 1995). An important prerequisite for transfer of genetic material from *Tripsacum* into *Z. mays* and retaining *Tripsacum* characters in *Z. mays* × *T. dactyloides* hybrids during the genetic manipulations with them, such as backcrosses with *Z. mays* and breeding programs is the detection of markers for identifying introgressed genetic material from *T. dactyloides* into *Z. mays* (Kindiger and Vierlig 1994).

In the last years a lot of investigations have been carried out for detection of molecular markers suitable to identify *T. dactyloides* genetic material introgressed into *Z. mays* since they have considerable advantage over the cytological and morphological markers. Kindiger and Vierlig (1994) demonstrated that acid phosphatase, malate dehydrogenase, phosphoglucomutase and phosphohexose isomerase are satisfactory markers for detecting *T. dactyloides* introgression in maize. Wet and Prunty (1987) showed that zein proteins are also suitable for identification of gene introgression from *T. dactyloides*. Leblank *et al.* (1995) and Kindiger *et al.* (1996) demonstrated the possibilities for detection of apomictic mode of reproduction of maize - *T. dactyloides* hybrids using maize - RFLP markers.

The aim of the present paper was to detect specific to *T. dactyloides* esterase (E.C. 3.1.1.1) and peroxidase (E.C. 1.11.1.7) isoenzymes, zein-2 subunits and SDS-protein protomers, which could be used to identify introgression of gene loci encoding these proteins from *T. dactyloides* into hybrids of *T. dactyloides* with different *Z. mays* lines.

Received 21 April 1997, *accepted* 5 October 1999.

Abbreviations: BC - back crosses; ME - 2-mercaptoethanol; PAGE - polyacrylamide gel electrophoresis; SDS - sodium dodecyl sulphate; SDS-PAGE - electrophoresis in SDS denaturating conditions; TCA - trichloroacetic acid.

Acknowledgements: The financial support of the National Scientific Fund is much appreciated (Project B-620/1996).

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Materials and methods

Plants used in this study were: a tetraploid maize (*Zea mays* L.) line (2n = 40) - 1259/4n (female parent of *Z. mays* × *T. dactyloides* F1 hybrids); five inbred maize lines (2n=20) - A-619, Mo-17, W-401, M-320/78, A-344 (female parents of *Z. mays* × *T. dactyloides* BC hybrids); *Tripsacum dactyloides* L. (2n=72), an apomictic form (male parent of F1 and BC hybrids); nine *Z. mays* × *T. dactyloides* BC hybrids. F1 hybrids were developed, which originated from the crosses of *T. dactyloides* with 1259/4n tetraploid maize line. For overcoming the sterility of F1 hybrids, they were backcrossed with the above mentioned inbred maize lines, and BC hybrids were developed. The following BC hybrids were analyzed: BC1(Mo-17) = F1(1259/4n × *T. dactyloides*) × Mo-17; BC2(Mo-17) = BC1 (Mo-17) × Mo-17; BC3(Mo-17) = BC2(Mo-17) × Mo-17; BC1(A-619) = F1(1259/4n × *T. dactyloides*) × A-619; BC2(A-619) = BC1(A-619) × A-619; BC3(A-619) = BC2(A-619) × A-619; BC2(W-401) = {F1(1259/4n × *T. dactyloides*) × W-401} × W-401; BC2(M-320/78) = {F1(1259/4n × *T. dactyloides*) × M-320/78} × M-320/78; BC2(A-344) = {F1(1259/4n × *T. dactyloides*) × A-344} × A-344.

Cytological studies showed 9 - 13 *T. dactyloides* chromosomes in the chromosome complement of the analyzed BC hybrids.

Coleoptiles were used for study of esterase and peroxidase isoenzymes. Seeds were germinated in the dark at 27 °C on moist filter paper. Following emergence, the coleoptiles were removed from the seedlings and homogenized with Tris (200 mM) - HCl (0.02 M) buffer, pH 6.8, containing 6 mM ascorbic acid, 1 mM cystein hydrochloride, 2.1 mM Na_2EDTA and 20 % (m/v) sucrose (Bednář and Provazníková 1994). Electrophoretic separation of esterase and peroxidase isoenzymes was performed after the method of Davis (1964) on 7.5 % polyacrylamide gel, pH 8.3, Tris (5 mM) - glycine (38 mM) buffer, pH 8.3, is used as electrode buffer system. The isoesterases and isoperoxidases were localized on gels by colour reaction: 0.04 % (m/v) 1-naphthylacetate, 0.07 % (m/v) Fast Blue RR for detection of isoesterases (Vallejos 1983); 0.005 M benzidine in acetate buffer pH 4.7, 0.02 % H_2O_2 for detection of isoperoxidases (Vallejos 1983).

Seeds were used for analysis of zein-2 fraction and of SDS-protomers. The extraction of zein-2 fraction was made according to Peruanskiĭ and Savin (1985) with the following modification: the extraction medium contained 70 % (v/v) ethanol, 2 M urea and 1 % (v/v) ME. Electrophoretic separation was performed after the method of Peruanskiĭ and Savin (1985) on 10 % polyacrylamide gel containing 6 M urea, pH 3.0. The electrode buffer contained 0.4 % (v/v) acetic acid and 5.3 mM glycine, pH 8.3. The extraction of proteins for SDS-PAGE was performed with 0.05 M Tris - HCl buffer pH 7.5, containing 1 mM phenylmethyl-sulphonyl fluoride (Frova *et al.* 1989). Samples were denaturated with 2 % (m/v) SDS and 1 % (v/v) ME prior to electrophoresis. The method described by Shah and Stagemann (1986) was used for SDS-PAGE on 15 % polyacrylamide gel, pH 8.9. Tris (25 mM) - glycine (0.2 M) buffer, pH 8.3, containing 0.1 % (m/v) SDS was used as electrode buffer.

For determination of molecular masses of the SDS-protein protomers the following protein markers were used: lysozyme (14.3 kDa) and bovine albumin (66 kDa).

The prolamin proteins and protein protomers separated by SDS-PAGE were fixed in 10 % (m/v) TCA and stained by 0.05 % (m/v) Coomasie Brilliant Blue R-250 in 12 % (v/v) methanol, and 6.8 % (v/v) acetic acid. Relative electrophoretic mobilities (Rm) of protein bands were calculated according to Bednář and Provazníková (1994).

Results

Electrophoretic profiles of esterase enzyme complex of maize lines and *T. dactyloides* are significantly different (Fig 1*a*). *T. dactyloides* is characterized by a higher number of isoesterases with middle and high electrophoretic mobility compared to maize lines. For *T. dactyloides* there are two intensively stained fast migrating isoesterases, Rm 0.81 and Rm 0.84 and one less intensively stained isoesterase with middle electrophoretic mobility, Rm 0.50 which are not presented in maize lines. The isoesterases, specific to *T. dactyloides* (Rm 0.50, 0.81, and 0.84) and one intensively stained fast moving isoesterase, Rm 0.78, specific to maize lines are observed in all BC2 hybrids analyzed.

T. dactyloides is characterized by a higher number of isoperoxidases compared to maize lines (Fig. 1*b*). For *T. dactyloides* there are two slow migrating isoperoxidases, Rm 0.19 and 0.23, one intensively stained isoperoxidase with middle electrophoretic mobility, Rm 0.32 and one fast migrating isoperoxidase, Rm 0.64 which are not presented in maize lines. One isoperoxidase with middle (Rm 0.32) and one - with high (Rm 0.64) electrophoretic mobility, which are specific to *T. dactyloides* as well one isoperoxidase with low

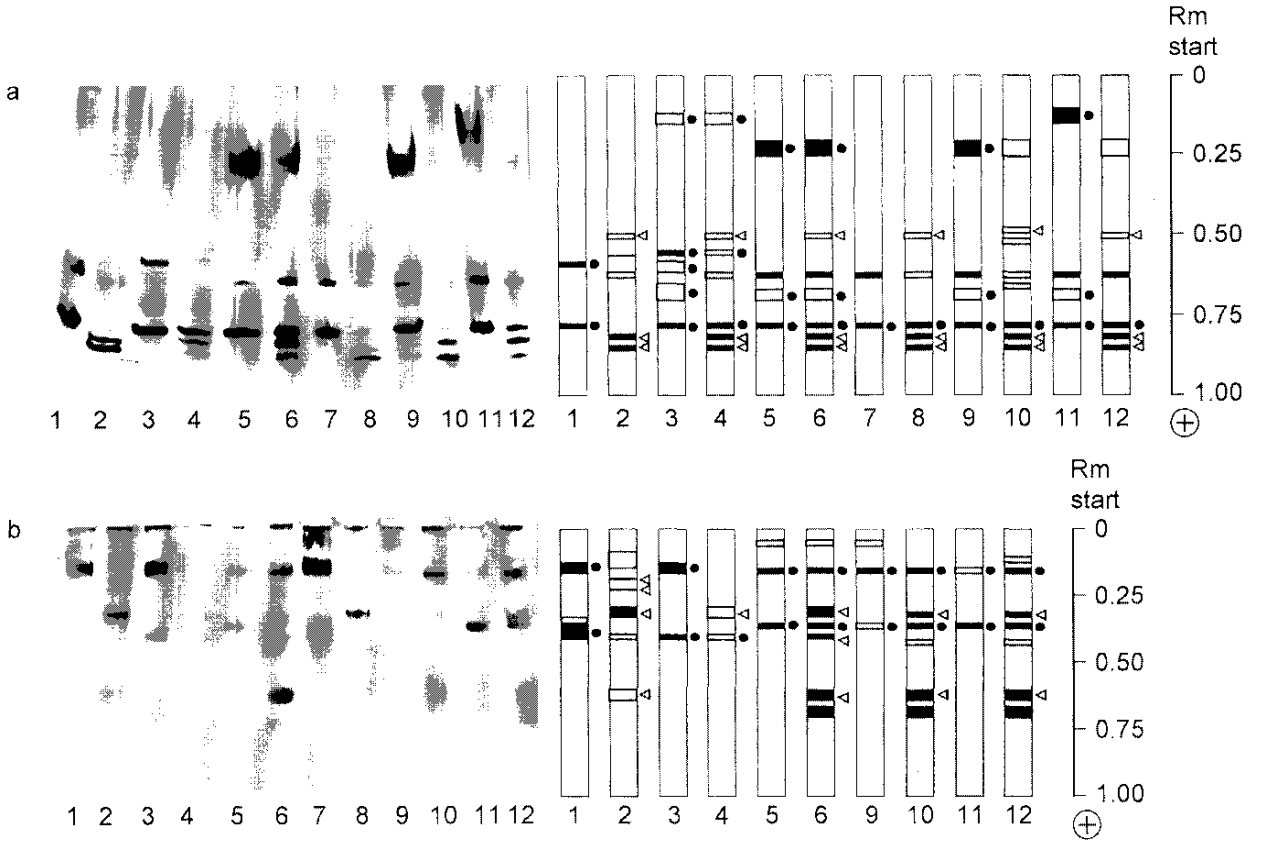

Fig. 1. Electrophoregrams of esterase and peroxidase isoenzymes from coleoptiles of *Z. mays* × *T. dactyloides* BC hybrids and their parental forms - tetraploid *Z. mays* line 1259/4n, 2n=40; inbred *Z. mays* lines, 2n=20; *T. dactyloides* L., 2n=72. On the diagrammatic representation of esterase and peroxidase isoenzymes, the ones, specific to *T. dactyloides* are indicated with the *arrows*, specific to *Z. mays* with *closed circles*.

a) esterase isoenzymes: 1 - tetraploid *Z. mays*; 2 - *T. dactyloides*; 3, 5, 7, 9, 11 - inbred *Z. mays* (3 - Mo-17, 5 - A-344, 7 - M-320/78, 9 - A-619, 11 - W-401); 4, 6, 8, 10, 12 - *Z. mays* × *T. dactyloides* BC2 hybrids (4 - BC2 with Mo-17, 6 - BC2 with A-344, 8 - BC2 with M-320/78, 10 - BC2 with A-619, 12 - BC2 with W-401).

b) peroxidase isoenzymes: 1, 7 - tetraploid *Z. mays*; 2, 8 - *T. dactyloides*; 3, 5, 9, 11 - inbred *Z. mays* (3 - Mo-17, 5 - A-344, 9 - M-320/78, 11 - A-619); 4, 6, 10, 12 - *Z. mays* × *T. dactyloides* BC2 hybrids: (4 - BC2 with Mo-17, 6 - BC2 with A-344, 10 - BC2 with M-320/78, 12 - BC2 with A-619).

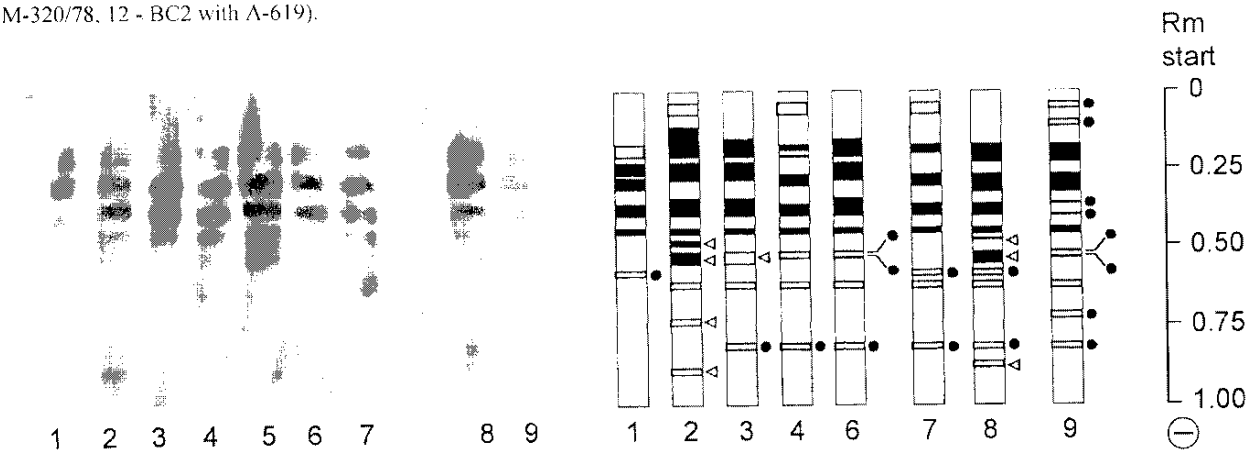

Fig. 2. Electrophoregrams of zein-2 fraction from seeds of *Z. mays* × *T. dactyloides* BC hybrids and their parental forms - tetraploid *Z. mays* line 1259/4n, 2n = 40; inbred *Z. mays* lines, 2n = 20; *T. dactyloides* L., 2n = 72. On the diagrammatic representation of zein subunits, the ones, specific to *T. dactyloides* are indicated with the *arrows*, specific to *Z. mays* with *closed circles*. 1, 4, 7 - inbred *Z. mays* (1 - Mo-17, 4 - M-320/78, 7 - W-401); 2, 5 - *T. dactyloides*; 9 - tetraploid *Z. mays*; 3, 6, 8 - *Z. mays* × *T. dactyloides* BC2 hybrids (3 - BC2 with Mo-17, 6 - BC2 with M-320/78, 8 - BC2 with W-401).

(Rm 0.15) and two - with middle (Rm 0.35 and Rm 0.38) electrophoretic mobility, specific to maize lines are observed in the BC2 hybrids. In the BC2 hybrids one isoperoxidase with middle and one - with high electrophoretic mobility not presented in the parental forms are also observed (Rm 0.47 and Rm 0.74).

Most of the slow migrating zein subunits of *T. dactyloides* and *Z. mays* lines possess similar electrophoretic mobility (Fig. 2). The main differences in zein profiles between the analyzed *Z. mays* lines and *T. dactyloides* are related to the subunits with middle and high electrophoretic mobility, which is in accordance with data of other authors (Paulis and Wall 1977, Magoja and Nivio 1981). For *T. dactyloides*, there are two intensively stained zein subunits with middle electrophoretic mobility, Rm 0.48 and 0.53 and two fast migrating zein subunit, Rm 0.74 and 0.89 which are not observed in maize lines. In the BC2 hybrid of maize line W-401 two zein subunits with middle electrophoretic mobility (Rm 0.48 and 0.53) and one with high mobility (Rm 0.89), specific to *T. dactyloides* are presented; in the BC2 hybrid of line Mo-17 one of the zein subunits with middle electrophoretic mobility, Rm 0.53, specific to *T. dactyloides* is observed. In zein profile of the BC2 hybrid of line W-401 a presence of zein subunits, specific to this line (Rm 0.81) and to *T. dactyloides* is observed.

The differences between electrophoretic profiles of SDS- protein complex of *Z. mays* and *T. dactyloides* are related to the protein protomers of low molecular mass - up to 19 kDa (Fig. 3). For *T. dactyloides* there is one specific protein protomer of molecular mass about 16 kDa. The same protomer is observed in the electrophoretic profiles of all analyzed BC hybrids.

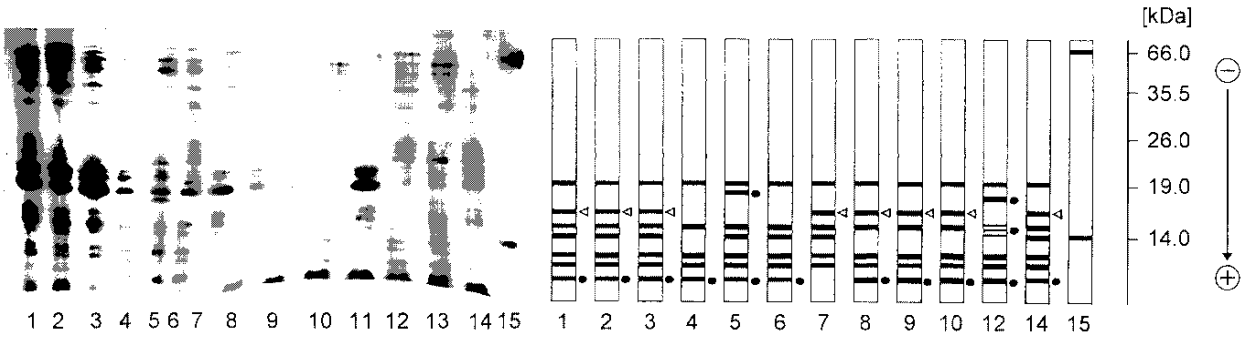

Fig. 3. Electrophoregrams of SDS protein protomers of *Z. mays* × *T. dactyloides* BC hybrids and their parental forms - tetraploid *Z. mays* line 1259/4n, 2n = 40; inbred *Z. mays* lines, 2n = 20; *T. dactyloides*, 2n = 72. On the diagrammatic representation of SDS-protomers of molecular masses up to 19 kDa specific to *T. dactyloides* are indicated with the *arrows*, specific to *Z. mays* with *closed circles*. 6, 13 - tetraploid *Z. mays*; 7, 11 - *T. dactyloides*; 4, 5, 12 - inbred *Z. mays* (4 - A-619, 5 - Mo-17, 12 - M-320/78); 1, 2, 3, 8, 9, 10, 14 - *Z. mays* × *T. dactyloides* BC hybrids (1, 2, 3 - BC1, BC2, BC3 with A-619; 8, 9, 10 - BC1, BC2, BC3 with Mo-17; 14 - BC2 with M-320/78); 15 - protein markers.

Discussion

The present studies showed major differences in esterase and peroxidase electrophoretic profiles between analyzed maize lines and *T. dactyloides*, and lesser differences in zein profiles between the two species. Similar electrophoretic mobility of slow moving zein subunits of *T. dactyloides* to those of maize lines may indicate identical alleles and corresponds to the observed complementary gene action between alleles of maize and *T. dactyloides* (Galinat 1973).

In the examined hybrids isoesterases and isoperoxidases as well zein subunits, specific to *T. dactyloides* and to the analyzed maize lines were observed, which shows the presence of loci, encoding these proteins from the two species. The detected isoesterases and isoperoxidases, SDS-protein protomers and zein subunits, specific to *T. dactyloides* in the examined hybrids may be used to identify introgression of loci, encoding these proteins from *T. dactyloides* into *Z. mays*.

The introgression of *T. dactyloides* loci, encoding the investigated isoenzyme systems and seed proteins into the hybrids may be due to the addition of *T. dactyloides* chromosomes to maize complements in the hybrids. It may be also a result of recombinations between chromosomes of maize and *T. dactyloides*. The introgression of loci, encoding isoesterases and isoperoxidases from *T. dactyloides* into the hybrids may be consequence of recombination between maize

chromosome 7 and homeologous chromosome of *T. dactyloides*; the introgression of loci, encoding zein subunits from *T. dactyloides* into the hybrids may be result of recombination between maize chromosomes 4 and 7 and homeologous *T. dactyloides* chromosomes. The assumptions are based on the following: *1*) Genetical analyses showed that esterase loci E1 and E16 are located on maize chromosome 7 (Goodman and Stuber 1983); peroxidase locus PH 3 is located on maize chromosome 7 as well (Weber 1982); most of zein genes in maize are located on chromosomes 4 and 7 (Valentini et al. 1979, Viotti *et al.* 1980). *2*) Cytological analyses of maize - *T. dactyloides* hybrids carried out by Galinat (1973) indicated homology between maize chromosomes 4 and *T. dactyloides* chromosomes 7 and 13; maize chromosome 7 and unidentified chromosome of *T. dactyloides*.

The isoesterases and isoperoxidases specific to *T. dactyloides* were observed in all analyzed BC hybrids, and zein subunits specific to *T. dactyloides* were detected in BC2 hybrids of *T. dactyloides* with part of the maize lines. It may be proposed that the frequency of introgression of loci from *T. dactyloides*, encoding esterase and peroxidase isoenzymes to the analyzed hybrids was higher as compared to introgression frequency of loci, encoding zein subunits.

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