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BRIEF COMMUNICATION

Variation in seed protein and isoenzyme patterns in *Cucurbita* cultivars

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Abstract

The genetic variability in the seed proteins and the enzyme alcohol dehydrogenase (ADH) in representative species of the genus *Cucurbita* was studied. The banding patterns were obtained by means of vertical block electrophoresis in polyacrylamide gel. A specific protein components and ADH isoenzymes were established in the polymorphic banding patterns which can be applied individually or in combination as potential biochemical markers for breeding purposes.

Additional key words: alcohol dehydrogenase, electrophoretic spectra, polymorphism.

The genus *Cucurbita* includes 25 species and each of them has 20 couples of relatively short chromosomes. Five of the species are known as cultural plants. According to Brown and Myers (2002) the genetic improvement of this genus is complicated by the numerous types belonging to different species. However, the interest to the genus *Cucurbita* increases in the last years because of its dietetic qualities.

The studying of polymorphism of different enzyme systems in *Cucurbita* is conducted with different purpose during the last 30 years. Wall and Whitaker (1971) investigated the genetic bases of hybrid breakdown of *Cucurbita* by esterase (EST) and leucine aminopeptidase (LAP) phenotypes and demonstrated that the variation was produced by codominant alleles at the unlinked loci Est-1 and Lap-1. The polymorphic isoenzyme loci of the peroxidase (PER), β-galactosidase and EST of *Cucurbita* are used for clarification of haemotaxonomic questions (Puchalski and Robinson 1978). On the base of segregation data of the isoenzyme spectra glutamate oxaloacetate transaminase, isocitrate dehydrogenase, malate dehydragenase, peroxidase, phosphoglucoisomerase, and phosphoglucomutase in F_2 progeny between wild and domesticated species of *Cucurbita*, a genetic interpretation for 6 gene loci and 12 alleles was made (Kirkpatrick *et al*. 1985). The other identified polymorphic isoenzyme loci in *Cucurbita* were summarised by Dane (1983).

The investigations cited above were conducted with embryos, 7-10 day-old cotyledon tissue, seedlings or young leaf tissue. There are a few reports on the genetic variability in *Cucurbita* cultivars on the base of their seed protein and isoenzyme polymorphism. Our studies were conducted with seeds, which is economically more reasonable because the time and costs for the growing of the plants are spared.

In this study we report the genetic polymorphism of the most frequently grown *Cucurbita* cultivars using seed protein and ADH isoenzyme electrophoretic patterns. Besides, we investigate the effectivity of the protein components and isozymes as biochemical markers for cultivar identification.

Electrophoretic data were obtained for *Cucurbita* cultivars, belonging to the three species: *Cucurbita pepo* L. var. *geromontia* cvs. Black beauty, Izobilna and Gornooriahovska-1, *C. maxima* Duch. cvs. Gigant, Kiodzinska morska and Plovdivska and *C. moschata* Poir. cv. Tsigulka. The seeds were received by the Executive Agency For Variety Testing, Field Inspection & Seed Control, the commersial seed company M&M Trading or by private collection. *C. pepo* cvs. Izobilna, Gornooriahovska-1 and *C. maxima* cv. Plovdivska were created in Bulgaria. The other *Cucurbita* cultivars were of foreign origin.

The seed proteins and the enzyme alcohol dehydrogenase (ADH) (E.C. 1.1.1.1) were investigated. The enzyme was extracted with 0.05 M TRIS-HCL buffer, pH 7.2, containing 6 mM ascorbic acid, 6 mM cystein hydrochloride and 0.5 M sucrose as protective supplements (Rychter and Lewak 1969). For protein extraction at reducing conditions a media with pH 2.7 included 0.05 % pyronine G diluted in 2-chloroethanol (20 %) containing urea (18 %) and 2-mercaptoethanol (1 %) according to Cooper (1987) was used.

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Abbreviations: ADH - alcohol dehydrogenase; EST - esterase; LAP - leucine aminopeptidase.
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For protein and enzyme electrophoresis samples of the *Cucurbita* cultivars were crushed and homogenized with the respective buffer at 4 °C and the residues were removed by centrifugation at 10 000 *g* for 20 min on the centrifuge *Eppendorf 5402* (Hamburg, Germany). An extract corresponding to 6.6 mg flour was applied for the purpose of the protein analysis, and the same, corresponding to 8.0 mg for enzyme analysis.

For the enzyme electrophoresis 10 % acrylamide and 0.8 % bisacrylamide according to Davis (1964) and electrode buffer containing TRIS-EDTA-boric acid, pH 8.3 was used. For the reduced proteins the gel system of 10 % acrylamide, 0.4 % bisacrylamide, 6 % urea, 0.1 % ascorbic acid, 0.005 % ferrous sulphate and glucine-glacial acetic acid, pH 3.2, as electrode buffer were applied (Cooper 1987). The chemicals were from *Chemapol* (Prague, Czech Republic) and *Janssen Chimica* (Beerse, Belgium).

The electrophoretic division was done by the method of vertical block PAGE on apparatus SE 600 (*Pharmacia*, Upsalla, Sweden) and power source EPS 600. The gels were stained for protein components with 0.03 % solution of Coomassie blue R 250. For ADH isoenzymes, a standard histochemical method described by Shaw and Prassad (1970) was applied. The data presented are the results of 5 different protein and enzyme extractions at *Cucurbita* cultivars.

The number of the protein components in the pattern of *C. pepo* var. *geromontia* cvs. varied from 9 to 12 (Fig. 1). In zone C at the cv. Black beauty a specific anode component was observed and in zone A at the cv. Gornooriahovska 1 a specific cathode component (Fig. 1, *arrow*). For the cv. Izobilna the lack of the slow moving component in zone C was characteristic. The patterns specificity of *C. maxima* cvs. Kiodzhinska morska and Plovdivska in comparison with the var. *geromontia* cultivars were defined by the absence of the two slowest moving components of zone C, while at the cv. Gigant lacked one component of zone A and zone B. Kiodzinska morska and Plovdivska cultivars had a reduced component composition. A considerable polymorphism was found in *C. moschata* cv. Tsigulka where the three slowest moving components of zone C were specific.

The electrophoretic patterns of the ADH of *C. pepo* var. *geromontia* demonstrated one band in the cv. Black beauty and six bands in cvs. Izobilna and Gornooriahovska 1 (Fig. 2). In the *C. maxima* cultivars four isozymes were present, but two anodic components typical for var. *geromontia* were not expressed. Three isozymes were visualized in the spectrum of *C. mocshata* cv. Tsigulka, whose specificity was determined by the absence of one isozyme homologous for the other two species.

On the base of ADH isoenzyme patterns of *C. pepo* var. *geromontia* cultivars a suggestion for expression of one polymorphic locus with two alleles could be made. The patterns of *C. maxima* cultivars were probably a result of the expression of one polymorphic locus with one allele.

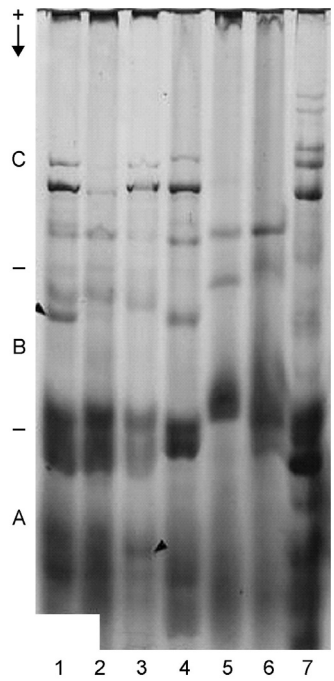

Fig. 1. PAGE patterns of reduced seed proteins of *Cucurbita*: *C. pepo* var. *geromontia* cvs.: Black beauty (1); Izobilna (2); Gornooriahovska-1 (3); *C. maxima* cvs.: Gigant (4); Kiodzinska morska (5); Plovdivska (6); *C. moschata* cv. Tsigulka (7).

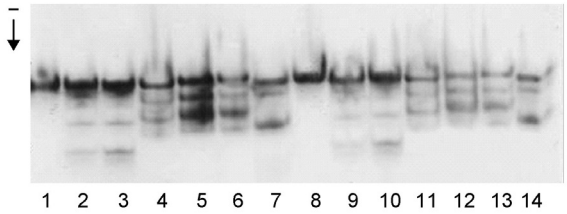

Fig. 2. Alcohol dehydrogenase isoenzyme patterns of *Cucurbita* cultivars: *C. pepo* var. *geromontia* cvs.: Black beauty (1, 8); Izobilna (2, 9); Gornooriahovska-1 (3, 10); *C. maxima* cvs. Gigant (4, 11); Kiodzinska morska (5, 12); Plovdivska (6, 13); *C. moschata* cv. Tsigulka (7, 14).

The interspecific variability in EST and LAP in crude seed extracts of several *Cucurbita* species was characterized by Wall (1969). Weeden and Robinson (1986) established polymorphism in the patterns of the same and other enzyme systems in young leaf extracts and built the first map of *Cucurbita*. Eleven isoenzyme loci were identified in five linkage groups. EST and ADH isoenzyme patterns were substantiated enough for application as markers for genetic purity and F_1 hybridity of tomato lines (Markova *et al*. 1998, 2003) and for identification of winter and summer garlic ecotypes (Cholakova 2000).

The efficiency of the studied proteins and ADH isoenzymes in *Cucurbita* species is determined by the possibility to apply each of them, individually or in combination for cultivar identification and differentiation. The spectrum of the reduced proteins can be used individually as a marker for differentiating the

Cucurbita cultivars. As for the species *C. pepo* cv. Black beauty and *C. moschata* cv. Tsigulka, the isoenzyme patterns of ADH is quite reliable.

In summary, this study provides useful information on seed protein and ADH isoenzyme polymorphism of the most frequent used *Cucurbita* cultivars. The results may be applied as a potential biochemical markers for solving several genetic-breeding purposes.

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