BIOLOGIA PLANTARUM 46 (2): 309-311, 2003

BRIEF COMMUNICATION

Application of alcohol dehydrogenase loci in testing F_1 hybridity of tomato

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Abstract

Expression of alcohol dehydrogenase loci was used to estimate hybridity of *Lycopersicon esculentum* Mill. in 1012 seeds. The banding patterns were obtained by means of vertical block electrophoresis in polyacrylamide gels. It was established that qualitative variation of locus Adh-2 can be applied to prove hybridity of F_1 tomato seeds. This genetic marker is indicative for hybrids with fertile maternal line or with position male sterility line and not only for maternal line with pollen male sterility.

Additional key words: isoenzyme patterns, *Lycopersicon esculentum*, qualitative variation, male sterility.

The proving of genetic purity of F_1 hybrid seeds in tomato is of great significance for their seed production. It is known that isoenzyme expression is almost independent of the environmental factors in contrast to morphological markers and thus far more efficient (Tanksley and Rick 1980). Besides, the electrophoretic methods of isozyme analysis are rapid and cheaper (Qi *et al.* 1994). By the isoelectric focusing nine alcohol dehydrogenase patterns were distinguished which, with three acid phosphatase patterns, identified twelve of the seventeen tomato cultivars (Henn *et al.* 1992). Differences in alcohol dehydrogenase (ADH) patterns have been suggested for hybridity determination by using different methods (Tanksley and Jones 1981, Van den Berg 1991). Our studies have shown that ADH loci are indicative for tomato hybridity only in two of the six hybrid combinations studied in a large number (2113) of individual seeds (Vodenicharova *et al.* 1996). The maternal line of these two hybrids was the same and had a gene for pollen male sterility (ms).

The aim of our study is to establish whether pollen male sterility is connected with the expression of ADH loci indicative for testing F_1 hybridity. For this purpose hybrids whose maternal parent has a gene for functional (position) male sterility (*ps-2*) and a hybrid with fertile maternal line were also compared.

Individual seeds (1012) from ripe tomato (*Lycopersicon esculentum* Mill.) of three F_1 hybrids and their respective parental lines were studied. The maternal line of hybrid 6944 × 2413 has a gene for pollen male sterility (*ms10*^{35}), of hybrid P-1a × BV has a gene for position male sterility (*ps-2*), and the maternal parent of hybrid 2197 × 2263 is a fertile line (f).

The seeds were imbibed in water for 36 h. The isoenzyme extracts were prepared by grinding the individual seeds with 0.15 cm^3 0.05 M Tris-HCl buffer, pH 7.2 containing 6 mM ascorbic acid, 6 mM cystein hydrochloride and 0.5 M sucrose (Rychter and Lewak 1969). Homogenates were centrifuged at 11 600 *g* for 15 min. All operations were carried at 4 °C. The supernatant was analyzed by vertical block electrophoresis in 7.5 % polyacrylamide gel and discontinuous buffer system of Tris-EDTA-boric acid, pH 8.3 (Peacock *et al.* 1965). The electrophoresis was done on *Biotech Hoefer SE 600 (Pharmacia,* Uppsala, Sweden) and electrophoretic power source *EPS 600*. The gel size was 180 × 160 × 1.2 mm. The electrophoretic resolution was carried out at 90 V/50 mA for 20 min and 300 V/50mA

Received 2 November 2001, *accepted* 4 March 2002.

Abbreviations: ADH - alcohol dehydrogenase; f - fertile; ms - male sterility.

Acknowledgements: The research has been partially supported by a grant from the National Foundation for Scientific Investigations NEC (Project 609/96).

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for 8 h. The chemicals used were from *Chemapol* (Prague, Czech Republic) and *Janssen Chimica* (Beerse, Belgium). The amount of the enzyme extract laid was 0.09 cm^3. The staining of ADH (EC 1.1.1.1) isoenzymes was done according to the method described by Shaw and Prassad (1970).

The ADH patterns in F_1 hybrid seeds and their parental lines are presented by two loci (Fig. 1*A,B,C*). *Adh-1* is localized on IV chromosome (Tanksley 1979), while *Adh-2* is on VI chromosome (Tanksley and Jones 1981). Locus *Adh-1* shows two homologous isoenzymes each in all parental and hybrid phenotypes; therefore, it is invariant and is thus of no interest as a molecular marker of genetic variations. Contrary to this, the isoenzymes encoded by the *Adh-2* locus have differences both between the parental pair, as well as between it and the hybrid. The isoenzymes of the parental lines (a and b) in all three hybrid combinations have differences in the position of their allele expression, irrespective of their equal number - 4 isoenzymes. In all three F_1 hybrid genotypes (c) the *Adh-2* locus expresses six isoenzymes each, *i.e.* it includes both isozyme differences between the parental lines. For that reason, the isoenzymes of the *Adh-2* locus in tomato seeds of F_1 hybrids can be used as a genetic molecular marker to establish hybridity by polyacrylamide gel (PAAG) electrophoresis.

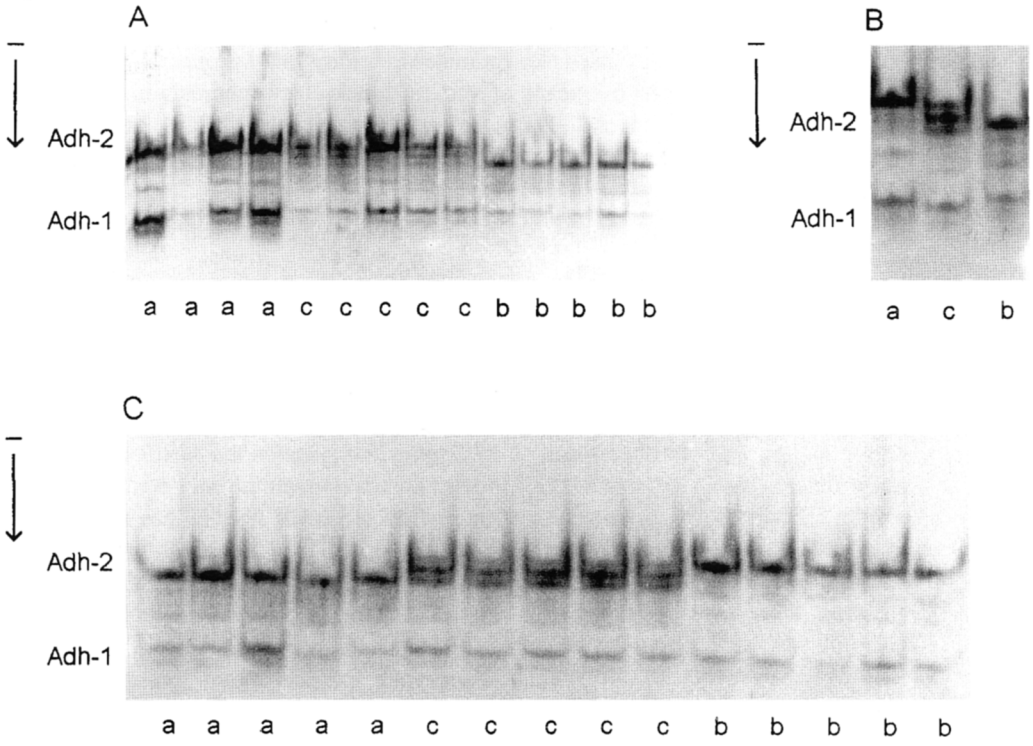

Fig. 1. PAAG electrophoretic patterns of ADH (Adh-2, Adh-1) loci in tomato seeds:
A: a - maternal parent line 6944 *ms10*^{35}, b - paternal parent line 2413, c - F_1 hybrid 6944 × 2413.
B: a - maternal parent line P-1a (*ps-2*), b - paternal parent line BV, c - F_1 hybrid P-1a × BV.
C: a - maternal parent line 2197 f, b - paternal parent line 2263, c - F_1 hybrid 2197 × 2263.

The application of genetic markers in recent years is so wide and varied that the term "marker-assisted selection" was introduced. The suggested ADH pattern is based on qualitative differences between the paternal and the hybrid pattern. This marker is indicative both for the combination with the fertile maternal line (Fig. 1*C*) and in the line with position male sterility (*ps-2*), (Fig. 1*B*), and not only in the 6944 *ms10*^{35} line with pollen male sterility (Fig. 1*A*) (Vodenicharova *et al.* 1996). According to our other studies of *Adh-2* locus in tomato cotyledons of the same line 6944 *ms10*^{35} aa, compared to the fertile line aa$^+$, we established that this locus can be applied as a quantitative molecular marker to identify fertile from sterile plants (Markova *et al.* 2000). The assumption that the expression of the *Adh-2* locus depends to a certain extent on pollen male sterility (ms) was not proven by the results of this study. The application of *Adh-2* locus as a genetic marker in testing F_1 hybridity in tomato by PAAG is based only on the qualitative differences between the parental and the hybrid patterns and is in no way connected to pollen male sterility. This conclusion agrees with the established localization of gene *ms10*^{35} on II chromosome (Philouze 1974), and of *Adh-2* locus on VI chromosome (Tanksley and Jones 1981), explaining the lack of connection between pollen sterility and expression of the *Adh-2* locus.

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