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BRIEF COMMUNICATION

Application of seed esterase isoenzymes in testing hybridity of tomato

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Abstract

Expression of esterase isoenzymes in 1272 seeds was used to estimate hybridity of *Lycopersicon esculentum* Mill. Individual seeds (440) of the parental cultivars taken from different experimental stations in Bulgaria were also analysed. The banding patterns were obtained by means of vertical block electrophoresis in polyacrylamide gels. It was established that quantitative variation of locus Est-1 can be applied to prove hybridity of F_1 tomato seeds. This marker is related to the genetic nature of tomatoes and is not the result of the environmental influence. The reason for this conclusion is the fact that the isoenzymes of the Est-1 locus are indicative for hybridity determination of all examined seeds taken from different regions in Bulgaria. Use of this locus is to be recommended for both its universality and the fact that the reagents for esterase isoenzymes staining are not expensive.

Additional key words: isoenzyme pattern, *Lycopersicon esculentum*, quantitative variation, F_1 hybrids.

The proving of F_1 tomato hybridity is of great significance for commercial seed production. It is known that the isozymes are reliable markers for seed hybridity testing, because of the rapidity and low economic cost of electrophoretic methods (Qi *et al.* 1994). The isozyme expression is almost independent of the environmental factors in contrast to morphological markers and thus far more efficient than morphological markers in tomato breeding (Tanksley and Rick 1980b).

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Esterase isoenzymes in red fruit tomato species have been investigated by Rick and Zobel (1972). The authors found that the heterozygotes at some loci are distinguished by the formation of "hybrid" or double (co-dominant) bands. Detailed electrophoretic investigations of esterases in different *Lycopersicon* species have been carried out by Tanksley and Rick (1980a). The authors established seven loci that code esterase isoenzymes in tomato roots.

The quality variations in the electrophoretic patterns of the enzyme alcohol dehydrogenase (ADH) are proposed as an isozyme test for proving F_1 tomato seeds genetic purity and hybridity (Tanksley and Jones 1981, Van den Berg 1991, Vodenicharova *et al.* 1993). There are no investigations for application of quantitative variations in esterase isozyme patterns for tomato hybridity proving. Quantitative variation of peroxidase and esterase phenotypes was, however, used successfully in cultivar identification (Endo 1971, Payne and Kossikowsky 1978). In the present investigation we proposed the quantitative variation of the isozyme patterns of Est-1 locus as genetic hybridity marker in tomato seeds.

Individual F_1 hybrid seeds (1272) from ripe tomatoes (*Lycopersicon esculentum* Mill.) and 440 seeds from the respective parental lines (cultivars) were investigated. The seeds for analysis were received from different lots and experimental stations in Bulgaria (Table 1). The seeds were imbibed in water for 36 h. The isoenzyme extracts

Table 1. Parental lines and F_1 tomato hybrid seeds obtained from different experimental stations in Bulgaria and a number of investigated seeds using Est-1 locus.

Maternal phenotype	Number of seeds	Paternal phenotype	Number of seeds	F_1 hybrids phenotype	Number of seeds	Experimental station	Total seeds
Line 6944	36	line 2413	36	Kristi	152	Sadovo, Sekirovo, Chalakovo	224
Line 6944	38	Standard	36	Standard 69	242	Proslav, TS. SDK Proslav, Sekirovo	316
Line 8	18	line 46	14	Hybrid	30	Sofia	62
Line 15	58	line 16	43	Maritsa 15	300	Krichim, ISK Maritsa, Strjama, Haskovo	402
Line 15	22	line 17	20	Maritsa 25	313	Gen. Nikolaevo, Parvenets, Strjama	355
Line 50	20	line 19	15	Hybrid	42	Sofia	77
Line 15	25	Bolivar	19	Hybrid 80	133	Gorna Orjahovitsa	177
Line BM	20	line BB	20	Hybrid 82B	60	Gorna Orjahovitsa	100
Total	237		203		1272		1712

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 the apparatus *Biotech Hoefer SE 600* (*Pharmacia*, Uppsala, Sweden) and electrophoretic power source *EPS 600*. The electrophoretic resolution was carried out at 90 V/50 mA

for 20 min and 300 V/50 mA for 8 h. The chemicals used were from *Chemapol* (Prague, Czech Republic) and *Serva* (Heidelberg, Germany). The amount of the enzyme extract laid was 0.03 cm^3. The staining of the α-naphthyl esterases (EST), (EC 3.1.1.1) was done according to the method described by Scandalios (1964).

According to electrophoretical separation of the esterase isozyme of Est-1 locus of the hybrid Standard 69 and the parental lines (line 6944 and cv. Standard) the difference between the maternal and the paternal phenotypes is only with regard to the quantitative expression of isozyme No1 (Fig. 1*a,b*). In the paternal line the staining intensity of the same isozyme, respectively its activity, is higher. In F_1 hybrid pattern there is an esterase phenotype (Fig. 1*c*) with the same staining intensity as in the paternal line. In other cases the staining intensity can be higher than in the paternal line as shown in most of Maritsa 25 F_1 hybrid seeds (Fig. 2, *tracks* 6, 8-10).

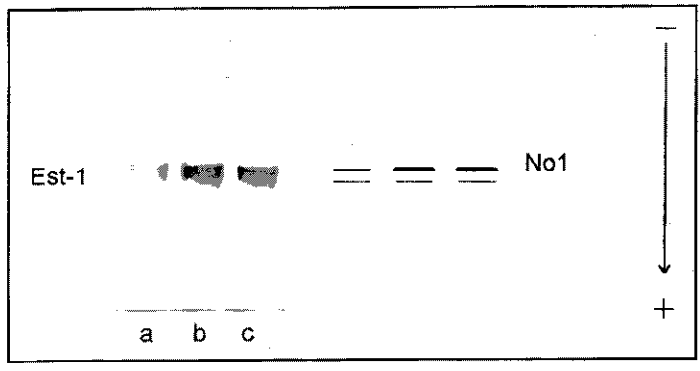

Fig. 1. PAAG electrophoretic patterns and scheme of EST (Est-1) locus in tomato seeds (*a* - maternal parent line 6944, *b* - paternal cv. Standard, *c* - F_1 hybrid Standard 69).

It is evident that isozyme No1 has equal or higher activity in the 7 hybrids shown with respect to the paternal form. This conclusion is confirmed in all 1272 hybrid seeds investigated.

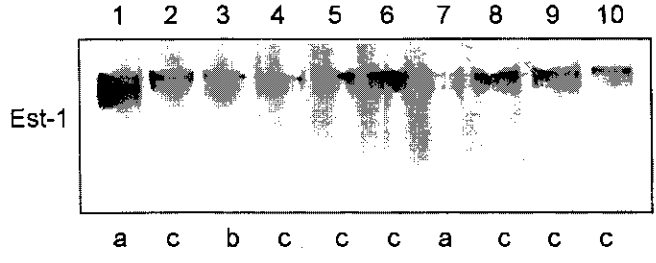

Fig. 2. PAAG electrophoretic patterns of EST (Est-1) locus in tomato seeds (*a* - maternal parent line 15, *b* - paternal line 17, *c* - F_1 hybrids Maritsa 25, 1 - 10 - number of tracks).

According to some authors the quantitative variation of the isoenzyme activity is not as significant as the qualitative one in proving hybridity (Gatos and Boulter 1979) and the environment conditions are considered to be a reason for this (Kawase and

Sakamoto 1984, Levy *et al.* 1985, Castle and Randall 1987). Regardless of that, the quantitative variation of the peroxidase and esterase phenotypes was successfully used in cultivar identification as well as in determining purity of rice and soybean (Endo 1971, Payne and Kossikowsky 1978).

Some authors suggested that the relative quantity of the peroxidase enzymes in the *Oryza perennis* heterozygote is a result of factors influencing the different stages of the chain of reactions from alleles activation to enzyme synthesis (Endo 1971). It is presumed that two kinds of inductors are available that activate the two alleles of the parental lines of *Oryza perennis* between which specific or concurrent activation is taking place (Endo 1971). As a result of that the quantity of the allelic isoenzyme in the heterozygote is different. Possible alternative of the latter explanation is that different quantities of allelic function isoenzymes are produced under the influence of the protein modifiers. Our investigations show that isoenzyme No1 of Est-1 locus in the hybrid seeds has intensity equal to that of the paternal parents or higher than that. A possible explanation may be the interaction between the quantitative variants of isoenzyme No1 of the parents which differ phenotypically in the expression of this enzyme. Independently of the causes, the isoesterase pattern established by us is indicative equally for tomato hybridity in all 1272 hybrid seeds obtained from different lots and experimental stations in Bulgaria. Hence, the tomato hybridity marker is related to their genetic nature and does not result from environmental influence. This fact gives us ground to consider that isozyme No1 of locus Est-1 can be a reliable marker for hybridity identification.

The locus Est-1 proposed by us for tomato hybridity is more universal compared to Adh-1 locus, for example. The application of the latter is possible, according to our investigations, only in hybrids Kristi and Standard 69 (Vodenicharova *et al.* 1993). The fact that isozyme staining is fast and the reagents for histochemical reaction are significantly cheaper can not be neglected.

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