

Assessment of genetic diversity in a collection of local tomatoes by SDS-PAGE method

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Abstract The work presented in this paper resides in electrophoretic analysis of proteins extracted from seeds of several Romanian tomato varieties. The protein profiles of tomato seeds were obtained by Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) method using 2 variants of extraction solution in order to obtain a wider viewing of tomato seed protein subunits. Analysis of protein patterns of 12 different samples showed no significant qualitative differences between genotypes. Four varieties showed a higher number of bands (Pontica, Carisma, Siriana and Coralina). Both extraction methods have led to the development of several bands located between 3 kDa and 142 kDa in most genotypes. Small quantitative differences were observed in the level of 90 kDa and 41 kDa using the first version of solution extraction compared to the second one. Anyway, a high level of homogeneity was detected with both methods used. Therefore these varieties should be studied also at the level of DNA.

Key words

tomato varieties, protein profiles, SDS-PAGE, extraction solution, homogeneity, protein subunits

Seed protein analysis are very useful for differentiating and characterizing the studied ecotypes (1), in selection of parental material for hybridization, cultivar purity testing, explanation of phylogeny and taxonomy relations among different genus and species, F1 hybrid purity testing (2), differentiation among species, cultivars and androgenetic lines (3, 4, 5, 6, 7, 8, 9, 10), variety identification and genetic purity control of species.

The electrophoretic protein profiles and their high stability and independence of the ecological conditions could be used as genotype markers (11, 12, 13). These genetic markers are widely used also as tools for the identification and estimation of the quantitative traits in plant resources, resistant to disease and environmental stress conditions, and other desirable agronomic traits (14).

The aim of this work was assessment of genetic variability in some tomato varieties of native collection, based on seed protein patterns obtained by testing two protocols of work.

The proposed theme and overall results will allow a deeper view of local germplasm of tomato, especially as a matter of great theoretical and practical importance, namely the genetic diversity of tomato.

Material and Method

The biological material from *Lycopersicon esculentum* Mill species (seeds) was obtained from Romanian research institutes (ICDLF Vidra, SCDL

Buzau). 12 varieties of tomatoes were analyzed in the University of Agricultural Sciences and Veterinary Medicine of Banat from Timisoara regarding their SDS-PAGE profiles. Varieties are shown below (table no.1).

Protein extraction:

- exact quantity of each seed was homogenized in electric mixer and proteins were extracted in one fraction using procedure A described by S. Doonan (1996), modified by G.D. Efremov (1998). Powdered seed was immersed in buffer (0,0625 M TRIS-HCl (pH 6,8), 2% (W/V) SDS, 5% (V/V) β -mercaptoethanol, 10% (W/V) glycerol and 0,002% (W/V) bromphenol blue) in proportion 1:2,5. The seed samples were homogenized by vigorous vortexing, kept at room temperature for 2 hours, and denaturated at 95°C for 2 minutes. After this treatment the samples were centrifuged at 13.000 rpm for 10 minutes, and the supernatant was used for electrophoretic analysis.

another exact quantity of each seed was homogenized in electric mixer and proteins were extracted using the extraction procedure B, where extraction solution was prepared immediately before use from 4,25 ml stock solution for extraction, 0,75 ml 2-mercaptoethanol and distilled water to 10 ml. The extraction stock solution contains: 6,25 ml 1 M Tris HCl buffer, pH 6,8, 12 ml of distilled water, 2 g SDS, 10 mg Bromphenol Blue, 10 ml glycerol. The seed samples were homogenized by vigorous vortexing, kept at room temperature for 2 hours, and denaturated at 100°C for 10 minutes. After this treatment the

samples were cooled at 30°C-24°C and centrifuged at 13.000 rpm for 10 minutes, and the supernatant was

used for electrophoretic analysis.

Table 1

Tomato varieties studied

No.	Variety	Provenance
1.	Pontica	ICDLF Vidra
2.	Vidra 533	ICDLF Vidra
3.	Vipon	ICDLF Vidra
4.	Viorica	ICDLF Vidra
5.	Carisma	SCDL Buzau
6.	Siriana	SCDL Buzau
7.	Coralina	SCDL Buzau
8.	Darsirius	SCDL Buzau
9.	Buzau 47	SCDL Buzau
10.	Buzau 4	SCDL Buzau
11.	Buzau 22	SCDL Buzau
12.	Buzau 1600	SCDL Buzau

The Polyacrylamide Gel was composed of a resolving gel with an acrylamide concentration of 10% and pH 8,8 and a stacking gel with an acrylamide concentration of 3% and pH 6,8. The running buffer (pH 8,3) contained Glycine, TRIS-base, SDS and distilled water.

For the electrophoretic separation, 7µL of the samples were applied in different lanes of the gel. The same volume of protein marker was applied in a separate lane of the gel, in order to allow the estimation of the molecular masses of the separated proteins.

After protein migration through the gel, the gel was fixed in 15% trichloroacetic acid solution and was stained in staining solution containing trichloroacetic acid solution, ethanol, glacial acetic acid and Coomassie Brilliant Blue (F. FURDI, Use of molecular markers in selection for protein content and technological quality of wheat, PhD thesis, USAMVB Timisoara, 2009). The excess of migration dye was removed rinsing the gel with distilled water. The gel was visualized and photographed in white light.

Results obtained

Two methods of protein extraction from seeds were tried in order to obtain a wider viewing of tomato seed protein subunits.

After image processing and data analysis using Photo-CaptMw program, was observed that both

extraction methods have led to the development of several bands located between 3 kDa and 142 kDa in most genotypes. When the first variant of protein extraction (A) was used, the tomato patterns showed some protein subunits in the area of 45 kDa and 57-90 kDa that were not visible when the second procedure for extraction (B) was used. At the same time, subunits that didn't appear after extraction by procedure A were present in the area of 94-96 kDa, 46-55 kDa and 3-10 kDa, after extraction by procedure B (fig. 1).

Accuracy of the second protocol of work appear to be better than the first version of the extraction. Protein subunits appear more clearly in the area of 94-150 kDa, 3-55 kDa. Using the first method, protein subunits appeared clearer at the molecular level of 90 kDa.

Analysis of SDS-PAGE electropherograms showed no significant qualitative differences between seed protein profiles. A weak polymorphism in SDS-PAGE banding patterns of *L. esculentum* Mill. varieties was detected with both methods used. Four varieties showed a higher number of bands (Pontica, Carisma, Siriana and Coralina). Additional bands were scored with an arrow in figure 1 and in figure 2.

Small quantitative differences were observed in the level of 90 kDa and 41 kDa using the first version of solution extraction compared to the second one.

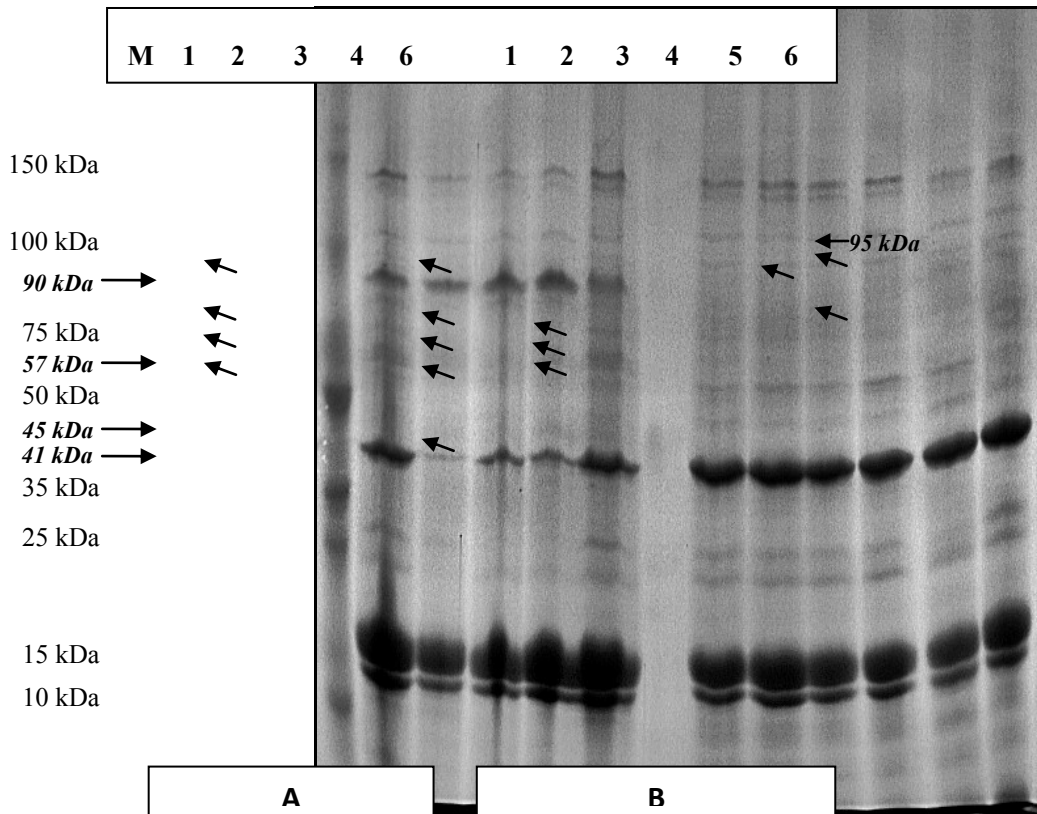


Fig. 1. SDS-PAGE protein patterns of some Romanian tomato varieties using two procedures (A&B) (M=Protein Marker, 1=Pontica, 2=Vidra 533, 3=Vipon, 4=Viorica, 5= Carisma, 6= Siriana)

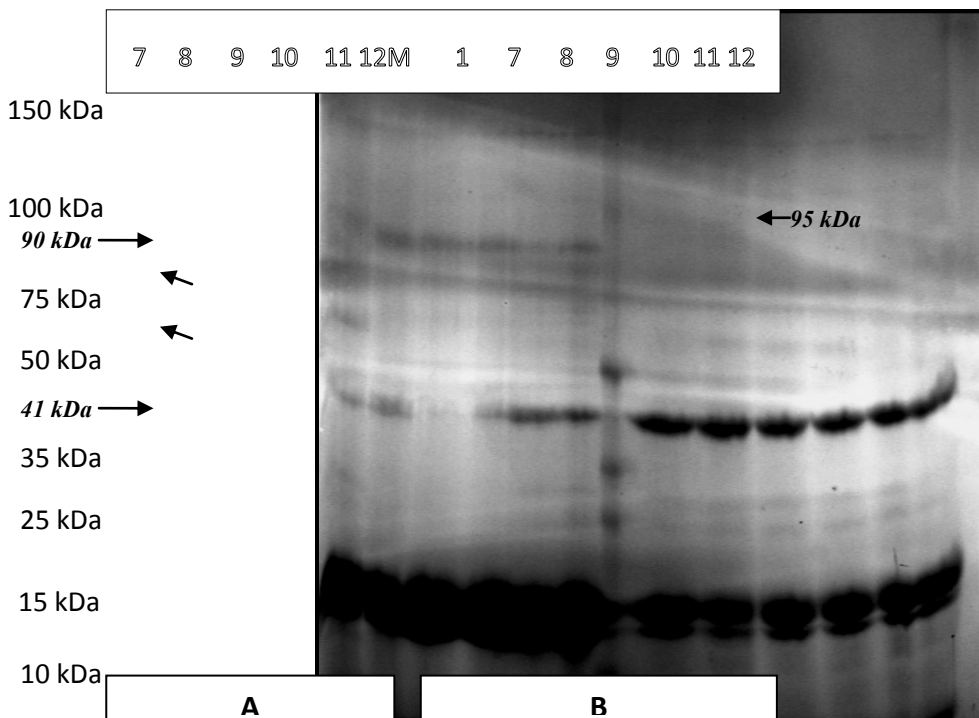


Fig. 2 SDS-PAGE protein patterns of some Romanian tomato varieties using two procedures (A & B) (M=Protein Marker, 7=Coralina, 8=Darsirius, 9=Buzau 47, 10= Buzau 4, 11= Buzau 22, 12= Buzau 1600)

Conclusions

In this study can be observed an extensive homogeneity of Romanian varieties of tomatoes analyzed. The conclusion is that the seed protein polymorphism in the best-known varieties of this species in our country has been shown to be very low at this level. Therefore these varieties should be studied also at the level of DNA by PCR technique, using different DNA markers.

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