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DIFFERENCES IN TOMATO SEED PROTEIN PROFILES OBTAINED BY SDS-PAGE ANALYSIS

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Abstract: The protein profiles of tomato seeds from sub-species (*subsp. cultum* Brezh., *subsp. subspontaneum* Brezh. and *subsp. spontaneum* Brezh.) were analyzed using SDS-PAGE technique. Electrophoreograms and denzitograms of total, soluble and non-soluble proteins of 31 different samples have showed quantitative and qualitative differences. Qualitative differences in electrophoregrams of total seed proteins refer to protein fragments in zone A (114 kDa, 83 kDa and 65 kDa) and protein fragment in zone C (17 kDa). Qualitative differences in electrophoregrams of soluble seed proteins refer to protein fragment in zone A (94 kDa). Qualitative differences in electrophoregrams of non-soluble seed proteins refer to protein fragments with molecular weights of: 210 kDa, 85 kDa, 67 kDa and 26 kDa.

Key words: tomato; SDS-PAGE; protein profile; total proteins; soluble proteins; non-soluble proteins

Introduction

Endosperm seed protein analysis constitutes a valid and/or improved approach to cultivar identification which is commonly based on morphological traits recorded in the field. Seed storage proteins analyses results are very useful for differentiating and characterizing the studied ecotypes (*Mennella et al.,* 2001).

Specific proteins and enzymes as markers have application in selection of parental material for hybridization, cultivar purity testing, explanation of phylog-

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eny and taxonomy relations among different genus and species, F_1 hybrid purity testing etc. (*Markova et al.,* 2003). Analyses of seed storage proteins in many horticultural species allowed the differentiation among species, cultivars and androgenetic lines (*Kononkov et al*., 1987; *Smith and Smith,* 1992; *Dinelli and Bonetti,* 1992; *Cooke,* 1995; *Mennella et al.,* 1995, 1996, 1998, 1999).

For variety identification and genetic purity control of self-pollinated and open-pollinated species, storage and functional proteins as direct gene products could be used. These genetic markers are widely used 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 (*Zlokolica et al.,* 1997).

The electrophoretic protein profiles and their high stability and independence of the ecological conditions could be used as genotype markers (*Sullivan and Freytag*, 1986; *Lioi,* 1991; *Stoyanova et al.,* 1992). The information that protein electrophoreograms gives us, can assist to the process of valid selection of starting hybridization material (*Konarev,* 1983).

The purpose of this study was to detect differences in seed protein profiles of assayed tomatoes and possibility for their use in identification of different tomato lines, hybrids, populations and varieties.

Material and Methods

Plant material: The plant material from *Lycopersicon esculentum* Mill species was obtained from the Genebank of the Republic of Macedonia. Eleven lines from the sub-species (*subsp. cultum* Brezh.) were analyzed from the Agricultural Institute - Skopje (*L-120/86, L-10 mercedes, L-100/92, L-55/91, L-12 mercedes, L-63/97, L-5/91, L-26/97, L-6/97, L-223/92* and *L-136/98*), one population (*Volovsko srce*), one variety (*var. grandifolium*) and 12 of their F_1 reciprocal hybrids (*hybrid-447, hybrid-306, hybrid-412, hybrid-285, hybrid-677, hybrid-312, hybrid-127, hybrid-261, hybrid-14, hybrid-158, hybrid-230* and *hybrid-670*) (Table 1). Four varieties (*var. cerasiforme-red, var. cerasiforme-yellow, var. pruniforme and var. pyriforme*), and one line (line obtained by Dascalov by crossing of *subsp. spontaneum* Brezh. *var. racemigerum* and *subsp. cultum* Brezh.) were analyzed from the sub-species (*subsp. subspontaneum* Brezh.). One variety (*var. racemigerum*) was analyzed from the sub-species (*subsp. spontaneum* Brezh.).

Protein extraction: Tomato seed proteins were extracted using the method described by *S. Doonan* (1996 and modified by *G.D. Efremov* (1998). Exact quantity of each seed was homogenized in electric mixer and proteins were extracted using two procedures.

Soluble and non-soluble proteins were extracted in one fraction using procedure A. Powdered seed was immersed in buffer A (0,0625 M TRIS-HCl (pH 6.8), 2% (W/V) SDS, 5% (V/V) -mercaptoethanol, 10% (W/V) glycerol and 0,002% (W/V) bromphenol blue) in proportion 1:2,5. The seed sample was homogenized

by vigorous vortexing, kept at room temperature for 2 hours, and denaturized at 95^{o}C in water bath for 2 minutes. After this treatment the sample was centrifuged at 10 000 - 20 000 rpm, and the supernatant was used for electrophoretic analysis.

Procedure B was performed in order to get the soluble and non-soluble proteins in two separate fractions. For that purpose powdered seed was immersed with buffer B which contained 0,1M TRIS-HCl (pH 8.0), 0,01 M $MgCl_2$, 18% (W/V) sucrose and 40 mM -mercaptoethanol in proportion 1:3. In the first step after the homogenization, procedure A was performed and the soluble proteins were extracted in supernatant. In the second step non-soluble proteins were extracted by rinsing of the sediment with the solution which contained 150l 2% (W/V) SDS, 50l 6% (W/V) sucrose and 40 mM -mercaptoethanol. After the homogenization by vortexing the procedure A was repeated.

Sodium – dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE): Separation of the tomato seed proteins was done by using 10% SDS-PAGE (*Laemmli,* 1970). Calibration kits of Amersham Pharmacia Biotech, Uppsala, Sweden were used for analyzing of obtained electrophoreograms. The high molecular weight-SDS (HMW-SDS) calibration kit is mixture of five characterized proteins (212kDa, 170kDa, 116kDa, 76kDa and 53kDa). The low molecular weight (LMW) calibration kit is mixture of six characterized proteins (94kDa, 67kDa, 43kDa, 30kDa, 20.1kDa and 14.4kDa).

SDS-polyacrilamide gel electrophoresis was carried out in a vertical gel. Stacking gel contained: 30% acryl amide, 4X Buffer A composed by 0,5M TRIS (pH 6.8) and 0,4% SDS, 0,03% TEMED and 10% APS. Running gel contained: 30% acrylamide, 4X Buffer B composed by 1,5 m TRIS (pH 8.8) and 0,4% SDS), 0,1% TEMED and 10% APS. Samples were prepared of four parts of a dissolved extracted proteins and one part of Loading buffer, which contained: 0,25 M TRIS (pH 6.8), 36% glycerol, 2% SDS, 4% -mercaptoethanol, and 0,1% bromphenol blue. The mixture of buffer and sample was denatured in Thermal cycler on 99°C / 3minutes. The electrophoreses was performed at 136 V/60mA for 50 minutes in running buffer, which contained: 14,4% glycine, 3% Tris and 1% SDS.

The gel was stained using 1% Commassie Blue G-250 in buffer that consisted: 30% methanol and 7% acetic acid. The gel was unstained by the same buffer. Obtained electrophoreograms were analyzed using PHOTO-CAPT SOFTWARE program and for each protein profile was obtained denzitogram.

Results and Discussion

Tomato seed proteins separated by SDS-PAGE were divided in three zones: A, B and C from cathode to the anode. Zone C contains protein fragments with smaller molecular weight, zone B contains proteins fragments with medium molecular weight and zone A contains protein fragments with the biggest molecular weight (**Fig.1**) (Stoilova et al., 2001).

In the zone A commonly appeared protein fragments with following molecular weights: 114 kDa, 83 kDa, 65 kDa, 54 kDa and 38 kDa. Namely, the protein fragment with the molecular weight of 114 kDa was identified in *Lycopersicon esculentum* Mill. *subsp. cultum* Brezh. (*L-10 mercedes*, *L-100/92*, *L-55/91*, *L-12 mercedes*, *hybrid-677*, *hybrid-312*, *L-63/97*, *L-6/97*, *hybrid-14*, *hybrid-158*, *L-223/92*, *hybrid-230, hybrid-670* and *var.grandifolium*) (Fig 2). The protein fragment with the molecular weight of 83kDa was not present in *Lycopersicon esculentum* Mill. *subsp. subspontaneum* Brezh. (*var. pyriforme* and line obtained by Dascalov by crossing of (*subsp. spontaneum* Brezh. *var. racemigerum*) and (*subsp. cultum* Brezh.). 65kDa protein fragment was absent in *Lycopersicon esculentum* Mill. *subsp. cultum* Brezh. *population Volovsko srce* and *Lycopersicon esculentum* Mill. *subsp. subspontaneum* Brezh. *var. racemigerum* (**Fig. 2**). The protein fragment with molecular weight of 17 kDa was found in zone C in every analyzed tomato, except *Lycopersicon esculentum* Mill. *subsp. cultum* Brezh. (*L-63/97*) *and Lycopersicon esculentum* Mill. *subsp. spontaneum* Brezh. (*var. cerasiforme*-red, *var. cerasiforme*-yellow and *var. pruniforme)*.

Analyses of SDS-PAGE electrophoregrams and denzitograms showed quantitative and qualitative differences between soluble seed protein profiles in the three zones. Qualitative differences refer to protein fragment with molecular weight of 94 kDa in zone A. The protein fragment was not identified only in *Lycopersicon esculentum* Mill. *subsp. cultum* Brezh. *hybrid 447* (**Fig 3**). In the zone C appeared protein fragments with following molecular weights: 28 kDa, 25 kDa, 20 kDa, 18 kDa, 16 kDa and 14 kDa. Protein differences also were found in number of fragments in zone C. In *Lycopersicon esculentum* Mill. *subsp. cultum* Brezh.(*L-63-97, L-5/91, hybrid 127, hybrid 261, L-26/97, L-6/97, hybrid 14, hybrid 158, L-223/92, L-136/98, hybrid 230* and *hybrid 670*) 4, 5 or 6 protein fragments were noticed, that are more compared to other analyzed tomatoes.

SDS-PAGE electrophoreograms and denzitograms of non-soluble proteins showed quantitative differences in intensity of some protein fragments in the three zones of protein profiles. The protein fragment with the molecular weight of 210 kDa was found in *Lycopersicon esculentum* Mill. *subsp. cultum* Brezh. (*hybrid 677*, *hybrid 127*, *hybrid 261*, *L-26/97*, *L-6/97*, *hybrid 14*, *hybrid 158*, *L-223/92*, *L-136/98* and *hybrid 230*) (**Fig. 4**). 85 kDa protein fragment was identified in *Lycopersicon esculentum* Mill. *subsp. cultum* Brezh. (*L-120/86*, *L-10 mercedes*, *hybrid 447*, *hybrid 306*, *population Volovsko srce*, *L-12 mercedes, hybrid 677*, *hybrid 312*, *hybrid 158*, *L-223/92*, *var. grandifolium*), *Lycopersicon esculentum* Mill. *subsp. subspontaneum* Brezh. (*var. cerasiforme*-yellow*, var. pyriforme*, line obtain by Daskalov by crossing of (*subsp. spontaneum* Brezh., *var. racemigerum* and *subsp. cultum* Brezh.) and *Lycopersicon esculentum* Mill. *subsp. spontaneum* Brezh. *var. racemigerum* (**Fig. 5**). The protein fragment with the molecular weight of 67 kDa was found in *Lycopersicon esculentum* Mill. *subsp. cultum* Brezh. (*L-120/86*, *hybrid 447*, *hybrid 306*, *L-100/92*, *L-55/91*, *hybrid 412*, *L-12 mercedes*, *hybrid 312, hybrid 158* and *L-223/92*) and *Lycopersicon esculentum* Mill. *subsp. spontaneum* Brezh.*var. racemigerum* (**Fig. 4-6**). The protein

fragment with the molecular weight of 26 kDa was identified in *Lycopersicon esculentum* Mill. *subsp*. *cultum* Brezh. (*L-120/86*, *hybrid 306*, *population Volovsko srce*, *L-12 mercedes, hybrid 677*, *hybrid 312*, *L-223/92*, *L-136/98*, *hybrid 230* and *hybrid 670*) (**Fig 6**).

Differences between protein profiles in different tomatoes mainly were quantitative. Qualitative differences in SDS-PAGE electrophoregrams of total seed proteins refer to protein fragments with molecular weights of 114 kDa, 83 kDa, 65 kDa and 17 kDa. Qualitative differences in SDS-PAGE electrophoregrams of soluble seed proteins refer to protein fragment in zone A with molecular weight of 94 kDa. Qualitative differences in SDS-PAGE electrophoregrams of non-soluble seed proteins refer to protein fragments with molecular weights of: 210 kDa, 85 kDa, 67 kDa and 26 kDa. Protein fragments that were detected as qualitative differences in SDS-PAGE electrophoreograms of assayed tomatoes are presented in Table 2. A weak polymorphism in SDS-PAGE banding patterns of *L. esculentum* Mill. ecotypes was detected and also by Mennella et al. (2001).

Conclusion

In this study, detected differences in seed protein profiles of analyzed tomatoes were not enough to use them in identification of different tomato lines, hybrids, populations and varieties. It is obvious that morphological and biochemical methods are not alternative methods, but they are complementary between each other.

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Table 1. Figure of hybridization and obtained hybrid combinations

	Parents (mother)	**Parents (father)**	**F_1 hybrids**
1.	*L-120/86*	*L-10 mercedes*	*hybrid 447*
2.	*L-10 mercedes*	*L-120/86*	*hybrid 306*
3.	*L-100/92*	*L-55/91*	*hybrid 412*
4.	*L-55/91*	*L-100/92*	*hybrid 285*
5.	*population Volovsko srce*	*L-12 mercedes*	*hybrid 677*
6.	*L-12 mercedes*	*population Volovsko srce*	*hybrid 312*
7.	*L-63/97*	*L-5/91*	*hybrid 127*
8.	*L-5/91*	*L-63/97*	*hybrid 261*
9.	*L-26/97*	*L-6/97*	*hybrid 14*
10.	*L-6/97*	*L-26/97*	*hybrid 158*
11.	*L-223/92*	*L-136/98*	*hybrid 230*
12.	*L-136/98*	*L-223/92*	*hybrid 670*

Table 2. Detected qualitative differences in assayed tomatoes

	Tomato	**Proteins** present/ absent (+ / -)								
		Total (kDa)				Soluble (kDa)	Non-soluble (kDa)			
		17	65	83	114	94	26	67	85	210
1.	***L-120/86***	+	+	+	-	+	+	+	+	-
2.	***L-10 mercedes***	+	+	+	+	+	-	-	+	-
3.	***hybrid 447***	+	+	+	-	-	-	+	+	-
4.	***hybrid 306***	+	+	+	-	+	+	+	+	-
5.	***L-100/92***	+	+	+	+	+	-	+	-	-
6.	***L-55/91***	+	+	+	+	+	-	+	-	-
7.	***hybrid 412***	+	+	+	-	+	-	+	-	-
8.	***hybrid 285***	+	+	+	-	+	-	-	-	-
9.	***population Volovsko srce***	+	-	+	-	+	+	-	+	-
10.	***L-12 mercedes***	+	+	+	+	+	+	+	+	-
11.	***hybrid 677***	+	+	+	+	+	+	-	+	+
12.	***hybrid 312***	+	+	+	+	+	+	+	+	-
13.	***L-63/97***	-	+	+	+	+	-	-	-	-
14.	***L-5/91***	+	+	+	-	+	-	-	-	-
15.	***hybrid 127***	+	+	+	-	+	-	-	-	+
16.	***hybrid 261***	+	+	+	-	+	-	-	-	+
17.	***L-26/97***	+	+	+	-	+	-	-	-	+
18.	***L-6/97***	+	+	+	+	+	-	-	-	+
19.	***hybrid 14***	+	+	+	+	+	-	-	-	+
20.	***hybrid 158***	+	+	+	+	+	-	+	+	+
21.	***L-223/92***	+	+	+	+	+	+	+	+	+
22.	***L-136/98***	+	+	+	-	+	+	-	-	+
23.	***hybrid 230***	+	+	+	+	+	+	-	-	+
24.	***hybrid 670***	+	+	+	+	+	+	-	-	-
25.	***var. grandifolium***	+	+	+	+	+	-	-	+	-
26.	***var. cerasiforme (red)***	-	+	+	-	+	-	-	-	-

27.	***var. cerasiforme (yellow)***	-	+	+	-	+	-	-	+	-
28.	***var. pruniforme***	-	+	+	-	+	-	-	-	-
29.	***var. pyriforme***	+	+	-	-	+	-	-	+	-
30.	***line obtained by Dascalov***	+	+	-	-	+	-	-	+	-
31.	***var. racemigerum***	+	-	+	-	+	-	+	+	-

Figures

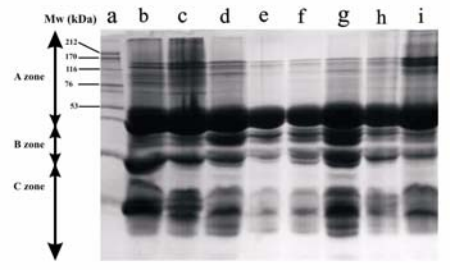

Fig.1 *SDS-PAGE electrophoreograms of tomato seed proteins: b- hybrid 230; c- hybrid 670; d-var. cerasiforme; e- var. pruniformee; f- var. pyriforme; g- line obtained by Dascalov by crossing of (subsp. spontaneum Brezh. var. racemigerum) and (subsp. cultum Brezh.); h- var. racemigerum; i- hybrid 306;*

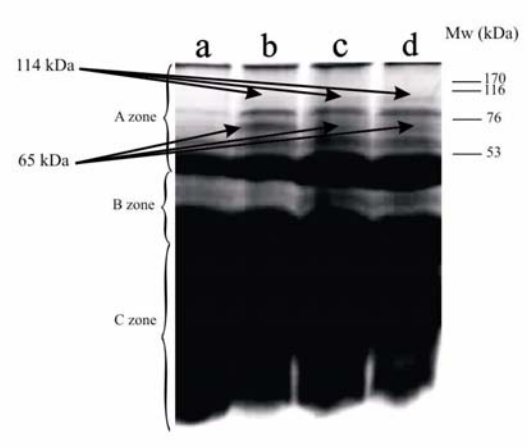

Fig.2 *SDS-PAGE electrophoreogram of total tomato seed proteins a-population Volovsko srce; b- L-12 mercedes; c-hybrid 677; d-hybrid 312*

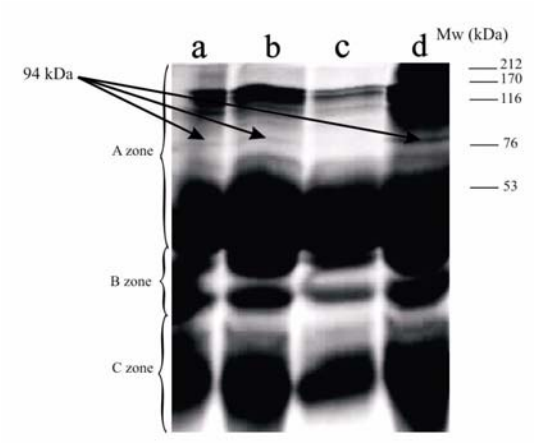

Fig.3 *SDS-PAGE electrophoreogram of soluble tomato seed proteins:*
a-L-120/86 ; b- L-10 mercedes; c-hybrid 447; d-hybrid 306

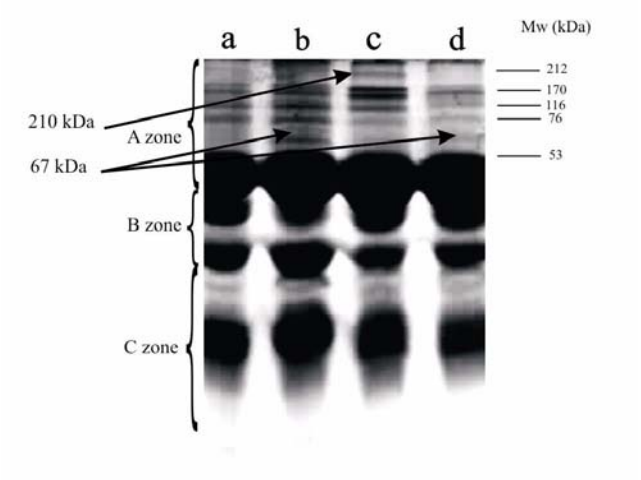

Fig. 4 *SDS-PAGE electrophoreogram of non-soluble tomato seed proteins:*
a- population Volovsko srce ; b- L-12 mercedes; c-hybrid 677; d-hybrid 312

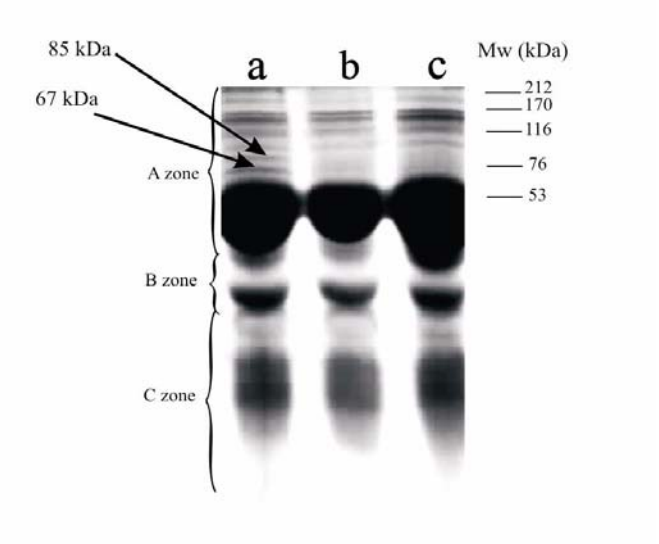

Fig.5 *SDS-PAGE electrophoreogram of non-soluble tomato seed proteins:*
a-L-223/92 ; b- L-136/98; c-hybrid 230

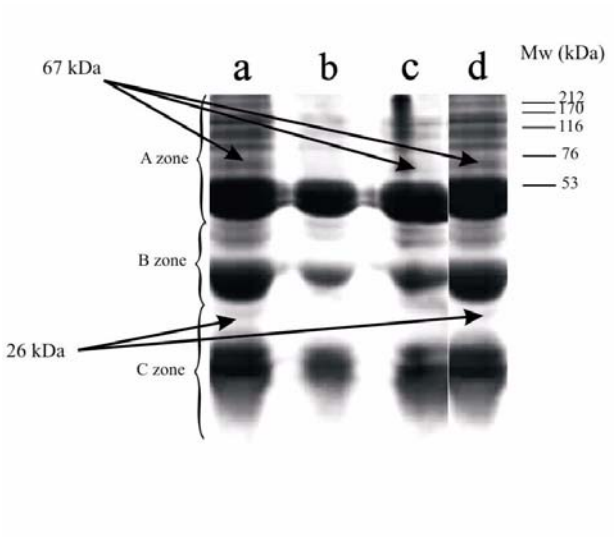

Fig.6 *SDS-PAGE electrophoreogram of non-soluble tomato seed proteins:*
a-L--120/86 ; b- L-10 mercedes; c-hybrid 447; d-hybrid 306;

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RAZLIKE U SEMENSKIM PROTEINSKIM PROFILIMA PARADAJZA DOBIJENIM SDS-PAGE ANALIZAMA

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Rezime

Semensko proteinske profile paradajza iz podvidova (*subsp*. *cultum* Brezh., *subsp*. *subspontaneum* Brezh. i *subsp*. *spontaneum* Brezh.) bili su analizirani SDS-PAGE tehnikom. Elektroforeograme i denzitograme ukupnih, rastvorljivih i nerastvorjivih semenskih proteina kod 31 različitog uzorka pokazale su kvantitativne i kvalitativne razlike. Kvalitativne razlike u elektroforeogramima ukupnih semenskih proteina odnose se na proteinske fragmente u zoni A (114 kDa, 83 kDa and 65 kDa) i proteinski fragmenat u zoni C (17 kDa). Kvaltativne razlike u elektroforeogramima rastvorljivih semenskih proteina odnose se na proteinski fragmenat u zoni A (94 kDa). Kvalitativne razlike u elektroforeogramima nerastvorljivih semenskih protina odnose se na proteinske fragmente sa molekularnom masom: 210 kDa, 85 kDa, 67 kDa i 26 kDa.

Ključne reči: paradajz, SDS-PAGE, proteinski profil, ukupni proteini, rastvorljivi proteini, nerastvorljivi proteini

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