Pak. J. Bot., 46(3): 827-832, 2014.

CHARACTERIZATION OF TOMATO GERMPLASM THROUGH SEED STORAGE PROTEIN PROFILING BY SDS-PAGE

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Abstract

The 24 tomato genotypes, including 5 hybrids (Avinash-II, CKD-1092, CKD-1093, CKD-1695, CKD-1088), seven germplasm lines (07039, 09091, 27-07, 42-07, 07011, 09076, 09078) and twelve cultivars (Excellence, Nagina, Naqeeb, Advanta-1202, PTM-1431, Pakit, Rio Grande, Lyp#1, Roma, Continental, VCT-I, Peto-86), were analyzed by SDS-PAGE for total soluble seed storage proteins on 10% gels. A low level of variability was observed in protein profiles of tomato genotypes. Dendrogram based on electrophoretic data clustered the 24 genotypes in four major groups. All germplasm lines illustrated identical profiles, therefore could not be differentiated on the basis of seed storage protein profiles. However, among tomato cultivars, VCT-l found to be the most divergent and could be distinguished from others on the basis of two peptides i.e. 58 kDa and 15 kDa. Similarly, in case of hybrids, a peptide of 58 kDa was absent in Avinash while present in all other hybrids. Another peptide of 64 kDa was unique to Avinash and absent in all other hybrids. Therefore, among hybrids, Avinash can be distinguished from others based on these peptide differences. Uniprot and NCBI protein databases were searched for already reported and characterized seed storage proteins in tomato. Among 42 resolved peptides, eleven could be identified from databases. On the basis of molecular weight similarity, identified peptides were SSP-83 as Alkaline alpha-galactosidase, SSP-78 as BiP, SSP-66 as vicilin, SSP-64 as DELLA Protein, SSP-58 as SNF1, SSP-41 as SNF4, SSP-36 as putative galactinol synthase 1, SSP-33 as Xyloglucan endotransg-lycosylases, SSP-26 as expansin family, SSP-10 as Putative vicilin and SSP-8 as albumin protein. In conclusion, seed storage profiling by SDS-PAGE can economically be used to asses the genetic variation in different tomato genotypes.

Introduction

Tomato (*Lycopersicon esculentum*) is widely grown around the world and constitutes a major agricultural industry. Worldwide, it is the second most consumed vegetable after potato and unquestionably the most popular garden crop. Tomato belongs to the nightshade family *Solanaceae.* Originally, *Solanum lycopersicum* was the name given to cultivated tomato. Latter, cultivated tomatoes were further separated and designated as the genus *Lycopersicon* and the species *esculentum*.

Seed is not only an organ of propagation and dispersal but also the major plant tissue harvested by humankind. The amount of protein present in seeds varies from 10% (in cereals) to 40% (in certain legumes and oilseeds) of the dry weight, forming a major source of dietary protein (Shewry *et al.,* 1995). Seed proteins can be broadly classified into two categories, viz. storage, structural and biologically active proteins. Storage proteins are encoded by families of polymorphic genes and gene copy number can be very high. Multigene families of seed proteins indicate that probably they are evolved by a complicated series of gene duplication (Mandal & Mandal, 2000).

Tomato seed storage proteins include albumin, globulin, gliadin and glutenin. The most abundant proteins in endosperm are seed storage proteins i.e., legumins, vicilins, albumin. Seed storage proteins have been used as genetic markers in four major areas: (1) analysis of genetic diversity within and between accessions, (2) plant domestication in relation to genetic resource conservation and breeding, (3) establishing genome relationships, and (4) as a tool in crop improvement (Iqbal *et al.,* 2005; Hameed *et al.,* 2009; Hameed *et al*., 2012a, b).

SDS-PAGE is considered to be a practical and reliable method because seed storage proteins are largely independent of environmental fluctuations (Javaid *et al.,* 2004; Iqbal *et al.,* 2005; Hameed *et al.,* 2009). Polyacrylamide gel electrophoresis technique allows to identify variation among the taxa of each species, screen the purity of the ever expanding number of cultivars, verify whether or not two or more morphologically identical accessions in the collection are identical. This method can also be used as a promising tool for distinguishing cultivars of particular crop species (Jha & Ohri, 1996). Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary relationships among crops and their wild relatives (Das & Mukarjee, 1995). Cytological characters and protein profile of family Solanaceae suggest that genus *Lycopersicon* and *Solanum* are closer at molecular level as compared to other species (Bhat & Kudesia, 2011).

Bioinformatics analysis can be applied to understand the nature of proteins which are resolved on SDS-PAGE. The National Center for Biotechnology Information (NCBI) provides a comprehensive website for biologists that includes biology-related databases, and tools for viewing and analyzing the data inherent in the databases. UniProtKB/Swiss-Prot is a high quality manually annotated (reviewed) and non-redundant protein sequence database, which brings together experimental results and computed features. UniProtKB/TrEMBL is a computer-annotated (unreviewed) supplement to Swiss-Prot, which strives to gather all protein sequences that are not yet represented in Swiss-Prot. The resolved proteins by SDS-PAGE can be identified by searching NCBI, SWISSPORT. Protein name, length, functions, family and also protein homologus sequences reported in databases can be identified by searching Swiss-Prot/TrEMBL database using UniProtKB (http://www.uniprot.org) and NCBI (www.ncbi.nlm.nih.gov).

Present study was conducted with the following objectives 1) seed storage protein profiling of selected tomato cultivars/hybrids/lines using SDS-PAGE, 2) to assess the genetic diversity in tested tomato germplasm based on electrophoretic data and 3) possible identification of resolved tomato seed storage proteins using Uniprot and NCBI protein databases.

Materials and Methods

A total of 24 tomato genotypes including 12 released cultivars (Excellence, Nagina, Naqeeb, Advanta-1202, PTM-1431, Pakit, Rio Grande, Lyp#1, Roma, Continental, VCT-I, Peto-86), 5 commercial hybrids (Avinash-II, CKD-1092, CKD-1093, CKD-1695, CKD-1088) and 7 germplasm lines (07039, 09091, 27-07, 42-07, 07011, 09076, 09078) were used for seed protein analysis using SDS-PAGE (Table 1). Seeds of germplasm were kindly provided by NARC, Islamabad, CKD & Company Gujranwala, and AARI, Faisalabad.

Seed protein extraction: For extraction of soluble proteins, seeds of individual tomato genotypes were grounded in 50 mM phosphate buffer (pH 7.8) and centrifuged in micro-centrifuge machine (Sigma 1-14) for 10 min at 14,000 rpm. The supernatant was separated and used for protein profiling. Protein concentration of extracts was measured by dye binding assay as described by Bradford (1976). Supernatant was mixed with cracking solution (10 ml containing 1 g SDS, 0.01 g bromphenol blue, 2 ml Mercaptoethanol, 1.5 ml 0.5M tris, pH 6.8, 5 g sucrose and 6.5 ml water) with (4:1) ratio on vortex mixer and then heated in a boiling water bath for five minutes to denature the proteins.

Seed protein profiling: Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by Laemmli (1970). Equal quantities (5 µl) of samples along with protein molecular weight marker (Fermentas SM0431) were loaded on 10% gels. Electrophoresis was performed at constant voltage (100 volts). At the end of electrophoresis, gels were fixed in solution containing 10% Acetic acid and 40% Ethanol for 15 minutes with constant agitation on a shaker. After fixing gel was washed with distilled water for 50 minutes with changing the water after every 10 min. Gels were then stained with coomassie blue G-250 dye for 30 min. Stained gels could be documented immediately or for more clear background, stained gels can be placed in distilled water for overnight.

Gel documentation and analysis: Gels were photographed using UVI proplatinum gel documentation system (UVItec, UK). Computerized gel analysis was performed using UVI pro Platinum 1.1 Version 12.9 for windows (copyright ® 2004-2006). Cluster analysis was performed using software UVI BANDMAP version 11.3 by UVItec.

Peptide search in databases: Uniprot database (www.uniprot.org) and NCBI protein database (www.ncbi.nlm.nih.gov) were searched for the tomato seed storage protein by using different queries, with their molecular weights that have been reported earlier in order to identify the peptides resolved in this study.

Table 1. Germplasm used for seed storage protein profiling.

Sr. No.	Accessions	Source
		Hybrids
1.	Avinash-II	National Agricultural Research Centre (NARC), Islamabad, Pakistan
2.	CKD-1088	CKD & Company, Gujranwala, Pakistan
3.	CKD-1093	CKD & Company, Gujranwala, Pakistan
4.	CKD-1695	CKD & Company, Gujranwala, Pakistan
5.	CKD-1092	CKD & Company, Gujranwala, Pakistan
		Germplasm lines
1.	09078	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
2.	09076	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
3.	07011	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
4.	42-07	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
5.	27-07	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
6.	09091	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
7.	07039	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
		Cultivars
1.	VCT-I	National Agricultural Research Centre (NARC), Islamabad, Pakistan
2.	Peto86	National Agricultural Research Centre (NARC), Islamabad, Pakistan
3.	Continental	National Agricultural Research Centre (NARC), Islamabad, Pakistan
4.	Roma	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
5.	Lyp#1	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
6.	Rio Grande	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
7.	Pakit	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
8.	PTM-1431	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
9.	Advanta-1202	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
10.	Nagina	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
11.	Naqeeb	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan
12.	Excellence	Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan

Results

Seed storage protein profiling of tomato germplasm was done and almost forty-two peptides were resolved on SDS-PAGE. Peptides of different molecular weights ranged from 8 kDa to 114 kDa were identified. A representative diagram showing the seed storage protein profiles of sixteen genotypes is presented in Fig. 1.

Using electrophoretic data, dendrogram was constructed that grouped 24 genotypes in 4 clusters at 80% homology (Fig. 2). The first cluster has one genotype i.e. VCT-l and it was most distinct among all other genotypes. The 2nd cluster included following genotypes viz., Peto-86, Avinash-ll, Continental, CDK-1088, CDK-1093, CDK-1695 and CDK-1095. The VCT-l has 90% homology with 2nd cluster. Within 2nd cluster, the CDK-1088, CDK-1093, CDK-1695 and CDK-1095 were grouped together by having 100% homology. Actually, the CDK-1088, CDK-1093, CDK-1695 and CDK-1095 were hybrids of the same origin. Peptides of molecular weights 58 kDa and 15 kDa were found in all hybrids except Avinash-ll. Avinash-ll has 97% homology with above hybrids, and therefore distinguished from other hybrids. Cultivars Peto-86 and Continental were having almost 99% homology with each other and 97% homology with other members of 2nd cluster.

The 3rd cluster consisted of the seven genotypes. In this cluster, the genotypes viz., 09078 and 07011, and 47-07 and 27-07 showed 100% homology with each other. However, these two groups have 99% homology with each other. The two lines 09078 and 07039 have 99% homology, while these two members of 3rd cluster showed 98% homology with above members of 3rd cluster. Another member of 3rd cluster 09091 showed 90% homology and showed more divergence as compared to other members.

The nine genotypes were grouped together by forming the 4th cluster, and in this cluster the genotypes Rio Grande, Pakit, Naqeeb and Excellence showed 100% homology. Another group in 4th cluster having LYP#1, PTM 1431, Advanta-1202 and Nagina which also showed 100% homology. However, the above two groups showed 98% homology with each other. Roma was placed alone in 4th cluster and showed 99% homology with Rio Grande, Pakit, Naqeeb and Excellence.

In first cluster, the protein profile of VCT-I showed that this genotype was distinct from all other genotypes. Peptide having molecular weight of 58 kDa was found in VCT-I but absent in Peto86, Continental and Avinash-II. Among above genotypes, the Peto86 and Continental were the cultivars and Avinash-II was hybrid. Peptide having molecular weight of 15 kDa was absent in all cultivars except VCT-I, Peto86, lines and hybrids. The VCT-l and Peto-86 can be distinguished on the basis of single peptide having molecular weight of 58 kDa. In VCT-I, Peto-86, Avinash-ll and Continental, the 64 kDa peptide were present but absent in all other hybrids. Therefore, this peptide was unique in Avinash-ll when compared the hybrids. Rio Grande, Pakit, Roma, Naqeeb and Excellence have unique peptide with molecular weight of 114 kDa. On the basis of this peptide, the Rio Grande, Pakit, Naqeeb and Excellence were grouped together with 100% homology, however, Roma has 99% homology with these genotypes.

The first and 2nd clusters lack 95 kDa, 78 kDa, 66 kDa and 38 kDa peptides and it was present among all other genotypes. Peptide having molecular weight of 85 kDa was present in 27-07, 09091, 07039, LYP#1, PTM-1431, Advanta-1202, Nagina, Rio Grande, Pakit, Roma, Naqeeb and Excellence but absent in remaining genotypes. Peptides with molecular weight of 26 kDa, 25.5 kDa and 25 kDa were present in LYP#1, PTM-1431, Advanta-1202, Nagina, Rio Grande, Pakit, Roma, Naqeeb and Excellence but absent in other genotypes. Peptides with molecular weight of 36 kDa 35 kDa have same banding pattern i.e., absent in 09078, 07011, 42-07 and 27-07 and present in remaining genotypes while all other peptides were found in all the genotypes.

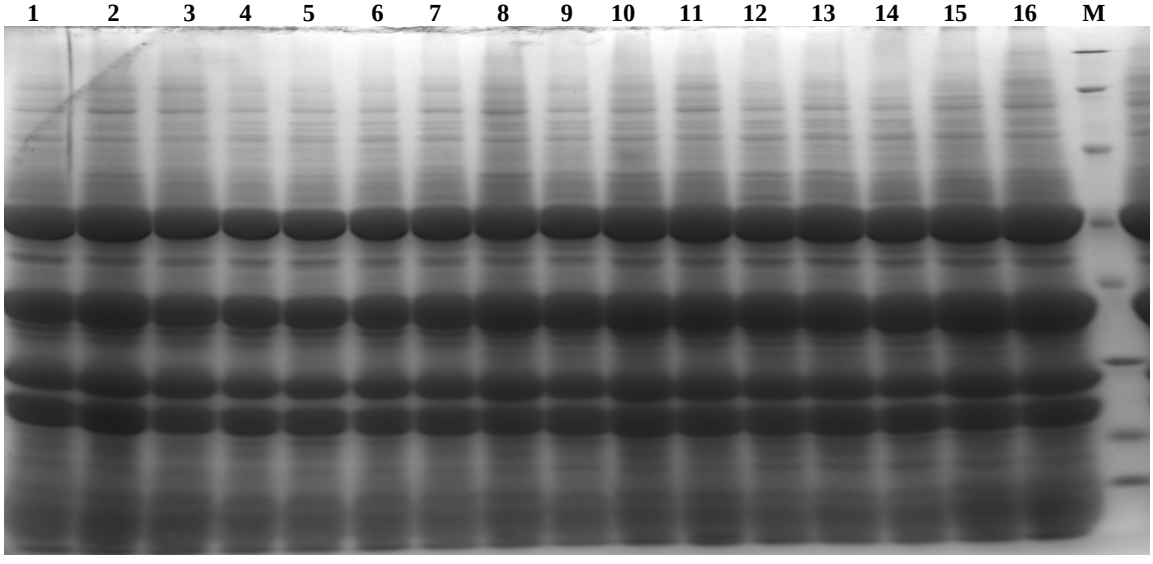

Fig. 1. A Representative diagram showing the seed storage protein profiles of sixteen genotypes. Lane 1: 09078, Lane 2: 09076, Lane 3: 07011, Lane 4: 47-07, Lane 5: 27-07, Lane 6: 09091, Lane 7: 07039, Lane 8: Roma, Lane 9: LYP#1, Lane 10: Rio Grande, Lane 11: Pakit, Lane 12: PTM-1431, Lane 13: Advanta-1202, Lane 14: Nagina, Lane 15: Naqeeb, Lane 16: Excellence, M: Protein Marker (SM0431).

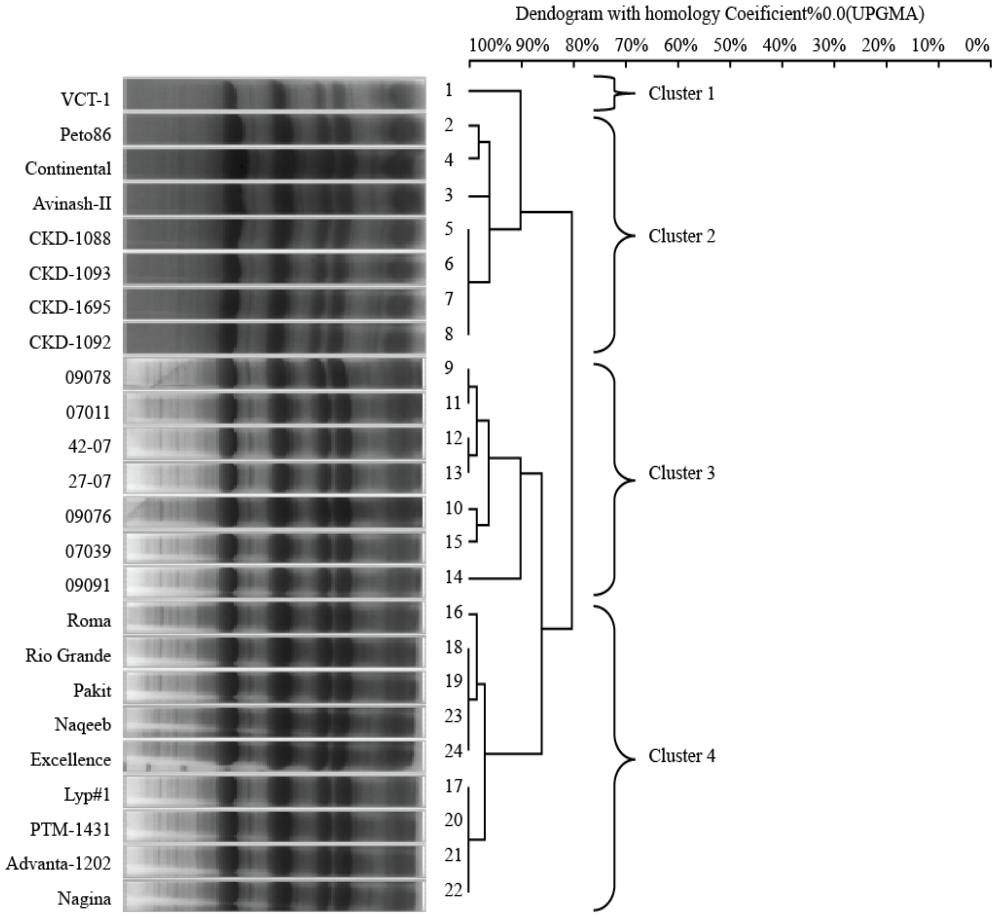

Fig. 2. Dendrogram based on the electrophoretic data of 24 tomato genotypes.

Identification of the peptides using database search and available literature: The banding pattern obtained by resolving peptides on SDS-PAGE can differentiate cultivars, hybrids and lines of tomato germplasm. Along with assessment of genetic variation in germplasm and characterization of cultivars, hybrids and lines, resolved seed storage proteins were searched in protein databases (Uniprot & NCBI) and in previously reported literature for their possible similarity based on their approximate molecular weights. Different queries were used to search seed storage proteins in databases. Information about these proteins available in the databases is given in Fig. 3. According to available literature accessed by different search engines (Google scholar, Pubmed, Pubmed central), tomato seed proteins mainly consists of expansin family, GRAS family, cupin super family, 2S albumin protein and SNF1/AMP-activated protein kinase subfamily. Based on available information in the literature we were able to identify several seed storage proteins those we resolved by SDS-PAGE.

Discussion

Protein electrophoresis is a powerful tool for studying the population genetics (Parker *et al.,* 1998). As the seed storage proteins are not affected by environmental fluctuations, their profiling using SDS-PAGE is particularly considered as a reliable and economic tool for characterization of germplasm (Javaid *et al.,* 2004; Iqbal *et al.,* 2005; Hameed *et al.,* 2009; Hameed *et al*., 2012a; b). Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species (Jha & Ohri, 1996; Mennella *et al.,* 1999). The SDS-PAGE has been reported to be a practical and reliable method for species identification of family *Solanaceae* (Bhat & Kudesia, 2011). According to present findings, we were able to differentiate the tomato hybrids, germplasm lines and approved cultivars from one another using seed storage protein profiles. All the approved tomato cultivars i.e. Lyl#1, PTM-1431, Advanta-1202, Nagina, Rio Grande, Pakit, Roma, Naqeeb and Excellence displayed similar banding pattern but have three distinct peptides (25 kDa to 27 kDa) that differentiate them from hybrids and germplasm lines. Similarly, germplasm lines of tomato i.e., 09091, 07039, 09078, 09076, 07011, 42-07 and 27-07 exhibited two peptide markers (61 kDa and 15 kDa) that were absent in all approved cultivars. While, the 58 kDa peptide was absent in all germplasm lines while present in all approved cultivars and hybrids. Previously, a weak polymorphism in SDS-PAGE banding patterns of *L. esculentum Mill.* ecotypes has been reported (Mennella *et al.,* 2001). According to another report differences in seed protein profiles of tomatoes were not enough to use them in identification of different tomato lines, hybrids, and cultivars (Miskoska-Milevska *et al.,* 2008).

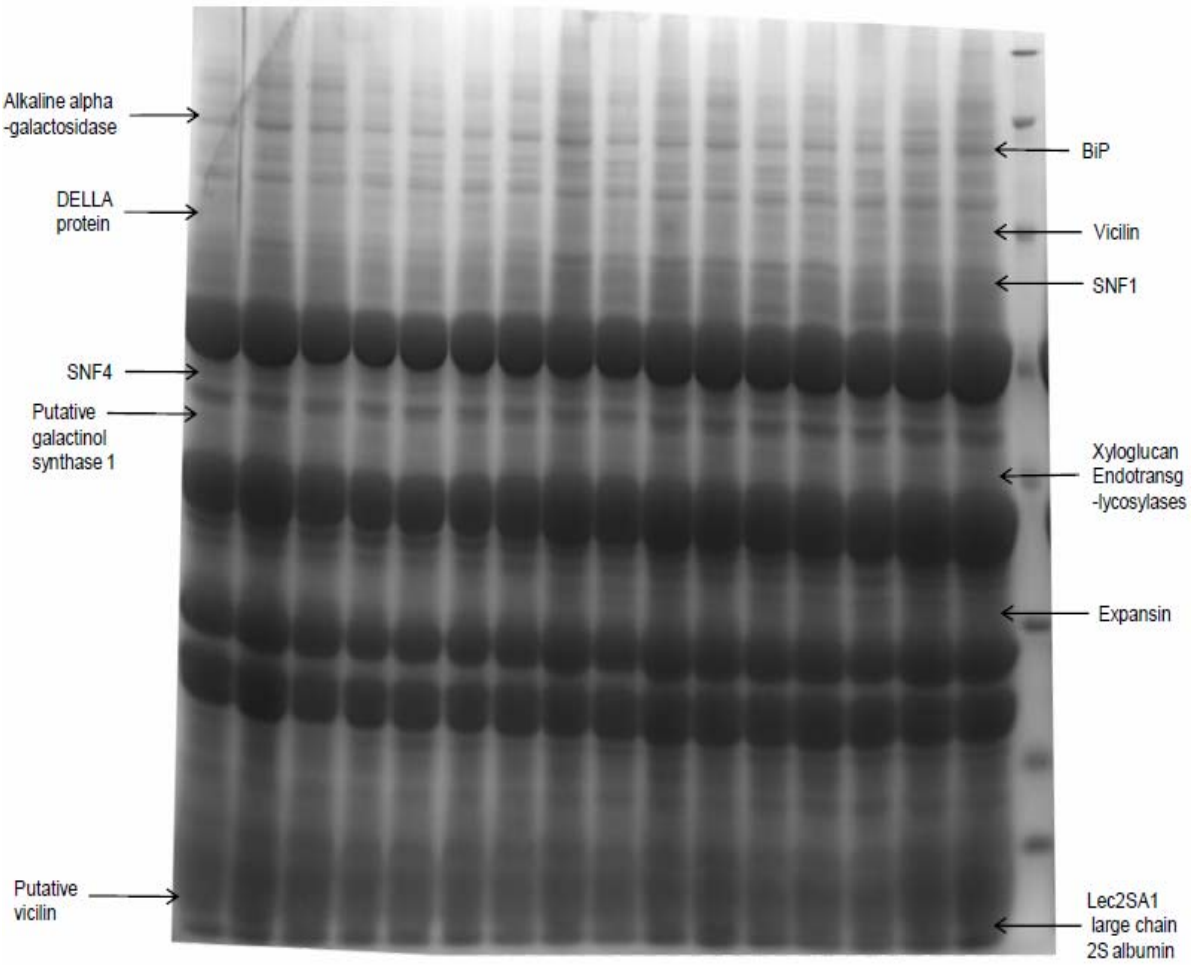

Fig. 3. Representative electrophorogram of tomato genotypes with possible identification Lane 1: 09078, Lane 2: 09076, Lane 3: 07011, Lane 4: 47-07, Lane 5: 27-07, Lane 6: 09091, Lane 7: 07039, Lane 8: Roma, Lane 9: LYP#1, Lane 10: Rio Grande, Lane 11: Pakit, Lane 12: PTM-1431, Lane 13: Advanta-1202, Lane 14: Nagina, Lane 15: Naqeeb, Lane 16: Excellence, M: Protein Molecular weight Marker.

Even going beyond from differentiation among hybrids, approved cultivars and germplasm lines revealed some differences among tomato hybrids and were able to separate one tomato hybrid from other included in the study. Actually a peptide of 58 kDa was absent in Avinash while present in all other hybrids and a peptide of 64 kDa was unique to Avinash and absent in all other hybrids. Based on these peptide differences, it was possible to distinguish Avinash from others tomato hybrids. Additionally, among approved tomato cultivars, VCT-l was found to be the most divergent and could be distinguished from others on the basis of two peptide markers i.e. 58 kDa and 15 kDa. All these differences in the seed storage proteins profiles of approved tomato cultivars, germplasm lines and hybrids provided proofs for genetic variability among the test material and usefulness of the technique for such studies.

Seed storage proteins of tomato consist of two major types; low molecular weight albumins and globulin. Globulins were further classified as vicillin and legumins (Bassler *et al.,* 2009). According to their molecular weight in kDa, in the present study, most of subunits have been identified. In the present study, we also detected a peptide of 8 kDa in all tested genotypes that seems to be albumin subunit. Oguri *et al.,* (2003) characterized and sequence tomato 2S seed albumin from tomato seed and it has two subunits having molecular weight of 8.125 kDa and 3.945 kDa. A vicilin subunit with a molecular weight 66.178 kDa has been reported with a role in the storage of nutritious substrates (Bassler *et al.,* 2009). In the current work, a peptide of 66 kDa was detected and that can be vicilin subunit. Previously, Bradford *et al.,* (2003) identified SNF-1 and SNF-4 belongs to SNF1/AMP-activated protein kinase subfamily having molecular weights of 58.824 kDa and 41.319 kDa, respectively. In the recent work, the peptides with molecular weight of 58 kDa and 40 kDa were identified in all tested genotypes and also reported earlies as SNF-1 and SNF-4. Moreover, Carmi *et al.,* (2003) identified alkaline alpha-galactosidase that belong to seed imbibition proteins (SIPs) having molecular weight of 83.798 kDa. In the present study, the peptide of 83 kDa could be the alkaline alpha-galactosidase.

Results revealed that a peptide with molecular weight of 64 kDa was observed as DELLA protein. DELLA protein with molecular weight of 64.526 kDa has been reported by Bassler *et al.,* (2009). Bradford *et al.,* (2002) identified BiP that belong to hsp70 family having molecular weight of 78 kDa. A peptide with the molecular weight of 78 kDa was also detected and that seems to be the reported as BiP protein. Galactinol synthase (GOLS) has been cloned from tomato seeds, and its expression was characterized as a protein having molecular weight of 36.402 kDa (Downie *et al.,* 2003). The peptide of 36 kDa in the seed storage protein profiles of tomato was also identified, and having MW of 36 kDa seems to be galactinol synthase. Xyloglucan endotransglycosylases (LeXET4) with molecular weight of 33.601 kDa has been reported along with a role in endosperm cap weakening, a key process regulating tomato seed germination (Chen and Bradford 2000). In the recent study, a peptide with the molecular weight of 31 kDa was noted in the seed storage protein profiles of all tested genotypes. Most probably, the above said peptide we found as seed storage protein and observed as LeXET4. Expansin having molecular weight of 26.821 kDa has been reported with a role in cell wall extension, possibly by disrupting hydrogen bonding between hemicellulosic wall components and cellulose microfibrils in tomato (Chen and Bradford 2000). In the present study, a peptide with the molecular weight of 26 kDa was also detected. Most probably this peptide can be earlier reported expansin.

In conclusion, the seed storage protein profiling based on SDS-PAGE can efficiently be used to assess the genetic variability among the tomato germplasm. This economical technique can differentiate approved cultivars, germplasm lines and commercial hybrids of tomato. Based on seed storage protein profiling using SDS-PAGE we were able to differentiate VCT-l from all other cultivars and a commercial hybrid Avinash-ll from all other hybrids. Based on molecular weight similarity, maximum variability was observed in expansin, BiP, DELLA and galactinol synthase. It is suggested that genotypes with similar banding patterns should be further analyzed by 2-D gel electrophoresis for its better resolution, and then by mass spectrometry to characterize the unknown protein bands to find out its function and phylogenetic relationship with other proteins, so that it can be classified in its most homologous protein family.

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(Received for publication 21 November 2012)