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Original article

Genetic variation among Saudi tomato (*Solanum lycopersicum* L.) landraces studied using SDS-PAGE and SRAP markers

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ABSTRACT

Genetic diversity among seven Saudi tomato landraces collected from different regions of the country was assessed using SDS-PAGE and molecular (sequence-related amplified polymorphism- SRAP) markers. A total of 19 alternative protein bands with different mobility rates were identified within a molecular weight range of 9.584–225 KDa, with 53% polymorphism. Specific protein bands were observed in the "Hail 548" and "Qatif 565" landraces. Genetic similarity based on Jaccard's coefficient ranged from 0.53 to 1.00, with an average of 0.72. For molecular evaluation, 143 amplicons (fragments) were generated using 27 SRAP primer pair combinations, of which 88 were polymorphic across all the landraces. The PIC values ranged from 0.46 to 0.90, with an average of 0.76. All landraces showed an average of 0.66 similarity coefficient value. The UPGMA dendrogram supported by principal coordinate analysis (PCoA) revealed clusters of the landraces that almost corresponded to their geographical origin. Thus, seed storage protein profiling based on SDS-PAGE and SRAP markers can efficiently be used to assess genetic variability among tomato germplasms. The information obtained in the analysis will be of great interest in the management of *ex situ* collections for utilization in breeding programs or for direct use in quality markets.

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1. Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae, which consists of approximately 100 genera and 2500 species, including several plants of agronomic importance such as potato, eggplant, pepper, and tobacco. *S. lycopersicum* has a relatively compact genome among the Solanaceae, characterized by its diploidy (2n = 2X = 24). It is approximately 950 Mb in size, and is one of the most intensively characterized Solanaceae genome (Arumuganathan and Earle, 1991). More than 7500 tomato landraces and varieties are successfully bred and grown for various purposes worldwide (Korir et al., 2014). These tomato genetic resources are important materials for breeding and biotechnology, and determination of their relationships has valuable potential for the tomato industry. The success of tomato genetic resource collection, preservation, exploitation, and utilization in both present and long-term breeding and production programs depends largely on the knowledge and understanding of the genetic background, diversity, relationships, and identification of these resources.

Tomato is one of the most important widely grown vegetables and the second most consumed vegetable after potato, with a worldwide production of more than 170 million tons covering more than 5 million ha (FAOSTAT, 2014). It was probably domesticated in Mesoamerica and introduced to Europe in the sixteenth century. Italy and Spain have been recognized as secondary centers of diversification (Bauchet and Causse, 2012), and since the early days of cultivation, a large number of tomato varieties with different fruit shapes have been documented in these countries (Grandillo et al., 1999). As a consequence of selecting for a limited

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set of traits, including fruit color and size, domestication for tomato has been clearly distinct from species divergence by natural selection. As a result, its genetic basis was seriously narrowed, a phenomenon known as the 'domestication syndrome' (Rick, 1976; Doebley et al., 2006; Bauchet and Causse, 2012).

The term "landraces" includes local varieties, local populations, traditional cultivars, farmer varieties and populations (Zeven, 1998), and traditional and primitive varieties (Negri et al., 2009). Landraces have a certain genetic integrity; are recognizable morphologically; differ in adaptation to soil type, time of seeding, date of maturity, height, nutritive value, and use; and are genetically diverse (Harlan, 1975). A review of the various definitions of a landrace by Zeven (1998) suggested that an autochthonous landrace is a variety with a high capacity to tolerate biotic and abiotic stress, resulting in high yield stability and an intermediate yield level under a low-input agricultural system. Tomato landraces are less sensitive to environmental stresses and are still grown on small farms due to quality issues and the special demands of consumers. Tomato can contain valuable alleles that are not common in modern germplasms (Sardaro et al., 2013); therefore, these landraces are valuable sources of genetic traits, and plant breeders can use breeding programs for improvement of this crop. One of the primary requirements for crop improvement is analysis of genetic variability using agro-morphological traits, biochemical protein markers (SDS-PAGE), and DNA levels. The cultivated tomato reveals a wide range of morphological variation both in its vegetative and reproductive parts. Consequently, numerous true breeding lines and aggregates of landraces have evolved in this crop.

Both biochemical and molecular marker technologies are used for analyzing genetic diversity and genetic basis since they provide abundant information, are highly efficient, and unlike morphological data, are not sensitive to environmental factors. Seed protein patterns have been successfully used for analysis of genetic diversity within and between accessions/genotypes including tomato, for plant domestication in relation to genetic resource conservation and breeding, for establishment of genome relationships, and as a tool in crop improvement (Iqbal et al., 2005; Nisar et al., 2007; Hameed et al., 2009, 2012a, 2012b). Hameed et al. (2014) concluded that seed storage protein profiling based on SDS-PAGE can be efficiently used to assess genetic variability in the tomato germplasm. It can differentiate approved cultivars, germplasm lines, and commercial hybrids of tomato. However, a weak polymorphism in SDS-PAGE banding patterns of *Lycopersicon esculentum* Mill. ecotypes have been reported (Mennella et al., 2001), and differences in seed protein profiles of tomatoes are not sufficient for use in identification of different tomato lines, hybrids, and cultivars (Miskoska-Milevska et al., 2008).

Various DNA-based marker systems have been used to study genetic diversity and phylogenetic relationships among tomato genotypes, including restriction fragment length polymorphism (RFLP) (Miller and Tanksley, 1990; Williams and Clair, 1993; Stevens et al., 1995), simple sequence repeat (SSR) (Bredemeijer et al., 2002; He et al., 2003; Al-Qadumii et al., 2012; Korir et al., 2014; Todorovska et al., 2014), amplified fragment length polymorphism (AFLP) (Park et al., 2004; van Berloo et al., 2008), sequence-related amplified polymorphism (SRAP) (Comlekcioglu et al., 2010; Ruiz et al., 2005; Mane et al., 2013), and SNP (Yang et al., 2004; Labate and Baldo, 2005). However, Bredemeijer et al. (2002) characterized 500 cultivated European lines and showed that it was possible to distinguish them using a set of 20 SSR markers. Comparing old varieties (or landraces) to modern hybrids, a higher level of molecular diversity in landraces is usually observed (Mazzucato et al., 2008; van Berloo et al., 2008).

The Saudi National Gene Bank initiated an effort to collect landraces from all the areas practicing cultivation of these landraces. In this context, the evaluation of Saudi tomato landraces seems to be a good model for analyzing variation in tomato landraces. A morphological and molecular characterization of this germplasm is needed to make this collection useful for breeders and farmers. This study is focused on characterization of tomato landraces collected from diverse environments and conserved at the National Gene Bank of the National Research Center for Agriculture and Animals Resources based on two marker systems: seed storage protein profiles using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Sequence-Related Amplified Polymorphism (SRAP) molecular markers.

2. Materials and methods

2.1. Plant material

Seeds from seven tomato landraces were provided by the Saudi Genetic Resources Gene Bank (Table 1). The seeds were stored at 8 °C until protein extraction.

2.2. SDS-PAGE protein electrophoresis

About 250 mg seeds from each genotype were ground in liquid nitrogen with a mortar and pestle. Next, 100 mg of finely powdered seed flour and 500 μL protein extraction buffer (10 mM Hepes, pH 7.5, 0.65 M NaCl, 1 mM EDTA, 0.34 M sucrose, protease inhibitor cocktail 20) were mixed thoroughly with a small glass rod. For clarification of the extraction, the homogenized seed flour samples were carefully mixed by vortexing and then spun at 12,000 × *g* for 5 min in a centrifuge at room temperature. Through centrifugation, the crude protein, in the form of a clear supernatant on the upper portion of the tube, was recovered, transferred to another centrifuge tube of 1.5 mL, and stored for gel electrophoresis at −20 °C, while the pellet was discarded.

Extracted soluble proteins were fractionated by one-dimensional SDS-PAGE by using 10% and 5% running and stacking gels with a Mini-PROTEAN system (Bio-Rad, California, USA) according to Laemmli (1970). Electrophoresis was conducted at a constant current of 150 V (30–40 A) run in SDS running buffer MOPS (50 mM MOPS, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, pH 7.7) until the tracking dye reached the bottom of the gel. A ladder with molecular weight markers of 10–250 kDa was used. After electrophoresis, the gels were stained overnight in 0.25% Coomassie brilliant blue-R250, followed by destaining in methanol and acetic acid for 45 min. The gels were further destained until the background was clear enough for band scoring. In order to eliminate differences in electrophoretic conditions as a cause of variation in the protein profiles, each genotype protein sample was separated from three independent electrophoretic runs and two separate extractions.

2.3. Molecular characterization using SRAP markers

Genomic DNA was extracted from the germinated seedlings using a Promega Wizard Genomic DNA Purification Kit (Promega,

Table 1
The list and regions of tomato landraces used in the current study.

Landrace code	Geographical origin	Latitude	Longitude
Al-Ahsa-308	East- Al-Ahsa	E 50° 40′	N 22° 17′
Qateef-365	East- Qateef	E 50° 00′	N 26° 35′
Hail-548	North- Hail	E 41° 41′	N 27° 30′
Hail-747	North- Hail	E 41° 41′	N 27° 30′
Najran-934	South- Najran	E 44° 14′	N 17° 33′
Taif-1018	West- Taif	E 40° 30′	N 21° 26′
Hail-1072	North- Hail	E 41° 41′	N 27° 30′

Wisconsin, USA) according to instructions provided by the manufacturer. Then, 200 mg of leaf tissue was processed by freezing with liquid nitrogen and ground into a fine powder. A total of 50 mg of powder was transferred to a 2.0 mL microcentrifuge tube. Next, 600 μL of nuclei lysis solution was added to the fine powder and mixed and incubated at 65 °C for 15 min. After that, 3 μL of RNase was added to the cell lysate and mixed and incubated at 37 °C for 15 min. Next, 200 μL of protein precipitation solution was added and mixed vigorously for 20 s. The samples were centrifuged for 3 min at 13,000 × *g*. The supernatant containing the DNA was carefully removed from the tubes and transferred to clean 1.5 mL microcentrifuge tubes containing 600 μL of cold isopropanol. The solution then was centrifuged at 13,000 × *g* for 1 min at room temperature. The supernatant was carefully decanted; leaving the DNA pellet adhered to the bottom of the tube. Then, 600 μL of room temperature 70% ethanol was added to the tube containing the DNA pellet and gently inverted several times to wash the DNA. The samples were then centrifuged at 13,000 × *g* for 1 min at room temperature. The ethanol was carefully aspirated and the tubes inverted on the cleaned absorbent paper. The pellet was left to air-dry for 15 min. Then, 100 μL of DNA rehydration (TE buffer) solution was added to each tube and it was incubated at 65 °C in a water bath with gentle shaking for 1 h. The DNA was stored at −20 °C in the freezer. Genomic DNA quality and concentration were detected by 0.8% agarose gel electrophoresis and spectrophotometry using a NanoDrop 2000 (Fisher Scientific, USA). Dilution with TE was carried out, and DNA concentration was adjusted to 50 ng/μL.

DNA samples were tested using combinations of 32 SRAP (4 ∗ 8) primers (Li and Quiros 2001). Primers that were amplified with consistently reproducible polymorphisms were selected and used to analyze all seven tomato landraces (Table 2). For each PCR, the 20 μL reaction volume contained 1 × GoTaq Green Master mix (Promega Corporation, Madison, USA), 0.1 μM of each forward and reverse primer, 50 ng DNA templates, and nuclease-free water to a volume of 20 μL. The thermal cycler profile for PCR amplification was set on a TC-5000 Thermal Cycler (Bibby Scientific, U.K.) as follows: denaturation at 94 °C for 5 min, followed by five cycles of denaturation at 94 °C for 1 min, annealing at 35 °C for 1 min, and elongation at 72 °C for 1 min. For the remaining 30 cycles, the annealing temperature was increased to 50 °C for 1 min, with a final elongation step at 72 °C for 7 min. Amplified products from the SRAP reactions were separated by a horizontal gel electrophoresis unit using 1.5% agarose gel in 1X TBE buffer and stained with acridine orange (10 mg/mL). The run was performed at 80 V for 60 min. A DNA ladder with a 100 base pair (bp) molecular size ladder (Fermentas, Germany) was used, and the gel was photographed using a Gel Doc EZ Gel Documentation System (Bio-Rad California, USA). Each PCR was repeated twice in order to ensure that banding patterns were consistent and reproducible and only stable products were scored for further data analysis.

Table 2
The forward and reverse sequence-related amplified polymorphism primer information for this study.

	Forward primers (5′–3′)		Reverse primers (5′–3′)
SRAP1	TGAGTCCAAACCGGATA	SRAP1	GACTGCGTACGAATTAAC
SRAP2	TGAGTCCAAACCGGAGC	SRAP2	GACTGCGTACGAATTAAT
SRAP3	TGAGTCCAAACCGGACA	SRAP3	GACTGCGTACGAATTACA
SRAP4	TGAGTCCAAACCGGAGA	SRAP4	GACTGCGTACGAATTACG
		SRAP5	GACTGCGTACGAATTACT
		SRAP6	GACTGCGTACGAATTAGC
		SRAP7	GACTGCGTACGAATTATG
		SRAP8	GACTGCGTACGAATTATT

2.4. Data analysis

The electrophoretic patterns of proteins and the reproducible banding patterns of each primer produced by SRAP were chosen for analysis. Each gel was scored as present (1) or absent (0). The data generated from the SRAP and protein analysis were analyzed by using the similarity coefficients and pairwise comparisons between individuals were made to calculate the Jaccard's (1908) coefficient of genetic similarity. The similarity coefficients were used to construct the dendrogram by using the unweighted pair group method with the arithmetic average (UPGMA). The cophenetic correlation coefficient) COPH) was calculated to measure the goodness-of-fit between the cophenetic matrix and the original similarity matrix using PAST (version 3.15) program (Hammer et al., 2001). The PAST software was also used to generate the phylogenetic trees based on the unweighted pair-group method with the arithmetic mean algorithm (UPGMA). The variation among the landraces was also studied through the Principal Coordinate Analysis (PCoA) of genetic diversity in thirty-six of lentil genotypes. The polymorphism information content (PIC) for each primer was calculated to estimate its allelic variation as follows: $PIC = 1 - \sum_{j=1}^{n} Pij^2$, where Pij is the frequency of the ith allele for the marker j, and the summation extends over n alleles, being calculated for each SRAP marker (Anderson et al., 1993). Discrimination power was calculated by dividing the number of polymorphic markers amplified for each primer by the total number of polymorphic bands obtained (Brake et al., 2014). The possible correlation between SDS-PAGE and SRAP patterns was evaluated by a Mantel test (Mantel 1967) based on Pearson's correlation (XLSTAT Pearson edition, version 2017).

3. Results

The SDS protein banding patterns produced 19 bands distributed in all landraces with molecular weights ranging from 9.6 KDa to 225 KDa. Ten of the 19 bands were polymorphic (53% polymorphism). For convenient description, the bands were classified into three zones according to the mobility of their proteins as follows: designated as; zone A contained proteins with a molecular mass of more than 52 kDa, zone B contained proteins between 24 and 50 kDa, and zone C contained proteins of less than 24 kDa according to the protein markers. All tomato landraces were clearly identifiable from their protein banding patterns (Fig. 1). Moreover, some landraces possessed bands that were absent in other landraces (Table 3). For instance, bands 11, 16, 17, and 19 were unique for two landraces, Hail548 and Qatif365, while band 2 was absent in Altaif1018 and band 7 was absent in Najran 934. The maximum number of bands (19) was found in Hail548 and Qatif365 and the minimum number (13) in the Alahsa308 landrace. The molecular weight protein band of 225 KDa was recorded in all landraces that were studied, whereas the lowest one (9.6 KDa) was detected in two landraces, Hail548 and Qatif365. Genetic similarity based on Jaccard's coefficient ranged from 0.53 to 1.00, with an average of 0.72. UPGMA clustered all landraces in two main clusters at 0.72 (overall mean) similarity coefficient and further sub-clustered them into three sub-clusters at 0.82 similarity coefficient (Fig. 2). The first sub-cluster contained the Altaif1018 and Najran934 landraces. Landraces Hail747, Hail1072, and Alahsa308 were grouped in the second sub-cluster, and the third sub-cluster gathered the Hail548 and Qatif365 landraces, which showed the highest degree of genetic similarity.

Of the 32 SRAP primer pair combinations screened, 27 SRAP primer pair combinations showed the ability to prime PCR amplification of the seven landraces that were selected. The characteristics of the primers across the landraces tested are summarized in

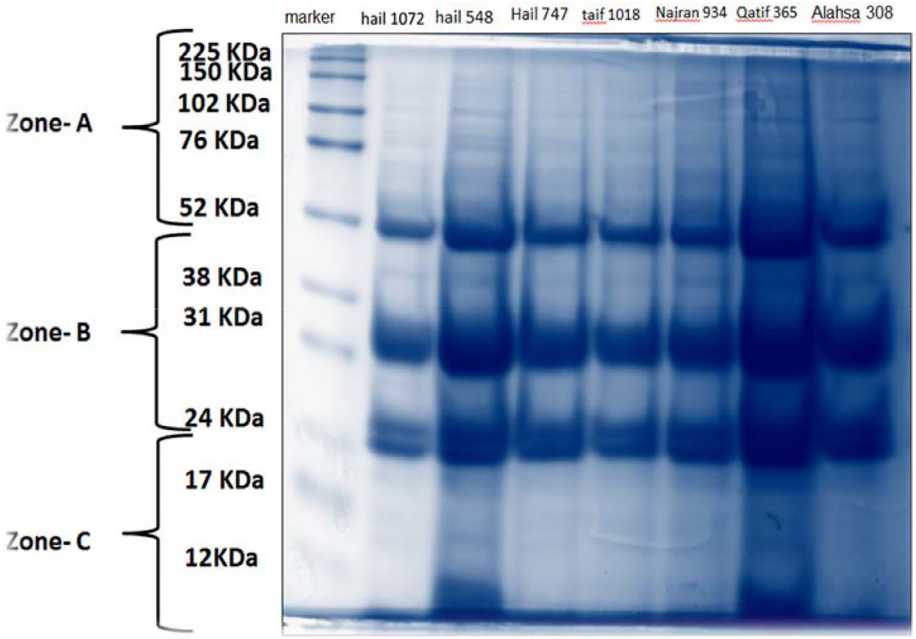

Fig. 1. Protein banding patterns of the seven tomato landraces, protein molecular weight marker, M. W. 12–225 KDa.

Table 3
Distribution of protein bands across landraces with their molecular weight sizes.

Band No.	Tomato Landraces								
	Marker Mw (kDa)	Hail 1072	Hail 548	Hail 747	Altaif 1018	Najran 934	Qatif 365	Alahsa 308	Zones
Band1	225	1	1	1	1	1	1	1	A
Band2	150	1	1	1	0	1	1	1	A
Band3		1	1	1	0	0	1	1	A
Band4		1	1	1	0	0	1	0	A
Band5	102	1	1	1	1	1	1	1	A
Band6		1	1	1	1	1	1	1	A
Band7		1	1	1	1	0	1	1	A
Band8	76	1	1	1	1	1	1	1	A
Band9		1	1	1	0	0	1	1	A
Band10	52	1	1	1	1	1	1	1	A
Band11		0	1	0	0	0	1	0	B
Band12	38	1	1	1	1	1	1	1	B
Band13	31	1	1	1	1	1	1	1	B
Band14		1	1	1	1	1	1	1	B
Band15	24	1	1	1	1	1	1	1	B
Band16	17	0	1	0	0	0	1	0	C
Band17		0	1	0	0	0	1	0	C
Band18	12	0	1	1	0	0	1	0	C
Band19		0	1	0	0	0	1	0	C
Total of bands		14	19	15	10	10	19	13	

Table 4. A total of 143 amplicons (fragments) were generated using the 27 SRAP primer pair combinations, of which 88 were polymorphic across all the landraces. In total, 739 data points (bands) were scored, with an average of 27.4 bands per primer pair across all genotypes, thereby confirming the high multiplex ratio expected for SRAPs. The capability of different primer pairs to generate SRAP fragments varied significantly, ranging from two fragments in primer pair Pr22 to 10 in primer pair Pr19, with an average of 5.3 fragments per primer pair. The average number of bands per primer pair ranged from 11 in Pr22 to 68 in Pr19, with an average of 27.4 bands per primer per landrace. The polymorphic percentage of primers across landraces ranged from 10% in Pr19 to 100% in primers Pr10, Pr13, and Pr17, with an average of 64.74%. Primers generated polymorphic information content (PIC) values ranging from 0.46 for primer pair Pr22 to 0.90 for primer pair Pr19, with an average of 0.76. Primers Pr14 and Pr27 had the highest discrimination power with a value of 6.8%, and primers Pr5, Pr19, and Pr22 had the lowest value (1.1%). All primers showed an average DP of 3.7%.

Genetic similarity estimates of the SRAP data based on Jaccard's (1908) similarity coefficients were used to assess the genetic relatedness among the seven tomato landraces. The mean similarity indices presented by the seven landraces ranged from 0.58 for Najran934 and Hail1072 to 0.89 for Hail747 and Altaif1018. All landraces showed an average of 0.66 similarity coefficient value. The UPGMA dendrogram revealed clusters that almost corresponded to the geographical origin of the landraces. Two main clusters at 0.66 with high bootstrap values were formed, and the Najran934 landrace showed the lowest degree of genetic similarity, failing to make clusters and separating individually (Fig. 3). Landraces from eastern parts of the country (Qatif365 and Alahsa308) formed

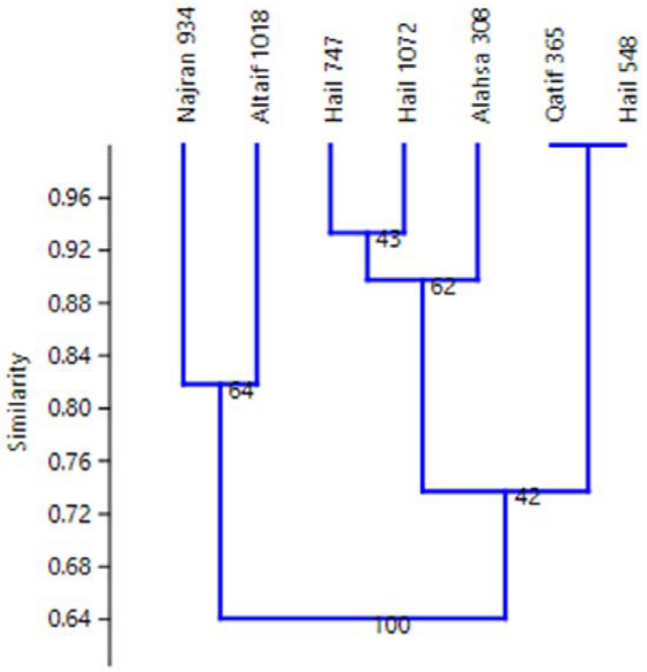

Fig. 2. Dendrogram generated using Unweight Pair Group Method with Arithmetic average (UPGMA) analysis, showing relationships between seven tomato landraces using SDS-PAGE data based on Jaccard genetic similarity coefficient.

the first cluster. The second cluster contained all landraces collected from the northern region of the country and one landrace from the western region (Altaif1018).

PCoA further validated the results of cluster analysis (Fig. 4). In the PCoA, the first three axes explained more than 87% of the variation in the estimates of genetic similarity. The first axis contributed 51.6% and the second axis 26.24%. Altogether, two distinct groups were revealed by the first two principal coordinates. The first axis separated landraces from the eastern parts of the country (Qatif365 and Alahsa308) from the others, and the second axis clearly differentiated the Najran934 and Hail1072 landraces from Altaif1018 and the two landraces from Hail (747 and 548). To compare the variability among tomato landraces and the extent of agreement between dendrograms that were derived from the proteins and DNA profiles, the correlation among the matrices of the datasets was generated using Mantel's test based on Pearson's correlation (Mantel, 1967). The *p*-value was calculated from the distribution of r (SRAP/SDS) using 10,000 permutations. A weak correlation between the proteins and SRAP profiles (r = 0.01, *p* = 0.300) was recorded.

4. Discussion

Tomato landraces are characterized by genetic diversity, local adaptation, and specific cultural uses that may be subject to selection. They are still grown on small farms owing to quality issues and the special demands of consumers. These landraces are valuable sources of genetic traits that can be used for crop improvement and the preservation of native biodiversity. Estimation of genetic diversity is one of the primary requirements for crop improvement. Furthermore, a knowledge of genetic diversity is a useful tool in gene bank management, tagging of germplasm, identification and/or elimination of duplicates in gene stock, establishment of core collections, and sorting of populations for genome mapping experiments (Kaga et al., 1996). However, it is extremely time-consuming to identify, characterize, evaluate, and conserve available precious genetic resources to improve the yield and fruit quality of currently available tomato germplasm. Therefore,

Table 4
Primers used for SRAP analyses, total number and polymorphic amplicons, % of polymorphism obtained, total number of bands across landraces, polymorphism information content (PIC) and discrimination power (DP).

SRAP primers	Total number of amplicons	Polymorphic amplicons	Polymorphism%	Total bands	PIC	DP
Pr1($SR_{1F}X_{1R}$)	4	3	75	19	0.71	3.4
Pr2($SR_{1F}X_{2R}$)	5	2	40	31	0.80	2.3
Pr3($SR_{1F}X_{3R}$)	7	4	60	41	0.85	4.5
Pr4($SR_{1F}X_{4R}$)	5	3	60	23	0.75	3.4
Pr5($SR_{2F}X_{1R}$)	4	1	25	26	0.75	1.1
Pr6($SR_{2F}X_{2R}$)	8	3	38	42	0.85	3.4
Pr7($SR_{2F}X_{3R}$)	3	2	70	13	0.59	2.3
Pr8($SR_{2F}X_{4R}$)	3	2	70	17	0.66	2.3
Pr9($SR_{3F}X_{2R}$)	5	4	80	15	0.68	4.5
Pr10($SR_{3F}X_{3R}$)	5	5	100	25	0.80	5.7
Pr11($SR_{3F}X_{4R}$)	3	2	70	14	0.56	2.3
Pr12($SR_{4F}X_{1R}$)	6	5	80	29	0.82	5.7
Pr13($SR_{4F}X_{3R}$)	5	5	100	26	0.80	5.7
Pr14($SR_{1F}X_{5R}$)	9	6	70	45	0.88	6.8
Pr15($SR_{1F}X_{6R}$)	6	4	70	35	0.83	4.5
Pr16($SR_{1F}X_{7R}$)	5	3	60	26	0.76	3.4
Pr17($SR_{1F}X_{8R}$)	4	4	100	15	0.66	4.5
Pr18($SR_{2F}X_{5R}$)	5	4	80	25	0.78	4.5
Pr19($SR_{2F}X_{6R}$)	10	1	10	68	0.90	1.1
Pr20($SR_{2F}X_{7R}$)	5	2	40	28	0.78	2.3
Pr21($SR_{2F}X_{8R}$)	5	3	60	23	0.75	3.4
Pr22($SR_{3F}X_{5R}$)	2	1	50	11	0.46	1.1
Pr23($SR_{3F}X_{6R}$)	5	4	80	25	0.79	4.5
Pr24($SR_{3F}X_{7R}$)	5	3	60	26	0.78	3.4
Pr25($SR_{4F}X_{6R}$)	5	2	40	31	0.79	2.3
Pr26($SR_{4F}X_{7R}$)	6	4	85	26	0.81	4.5
Pr27($SR_{4F}X_{8R}$)	8	6	75	34	0.85	6.8
Total	143	88	–	739	–	
Average	5.30	3.26	64.74	27.4	0.76	3.7
Min	2	1	10	11	0.46	1.1
Max	10	6	100	68	0.9	6.8

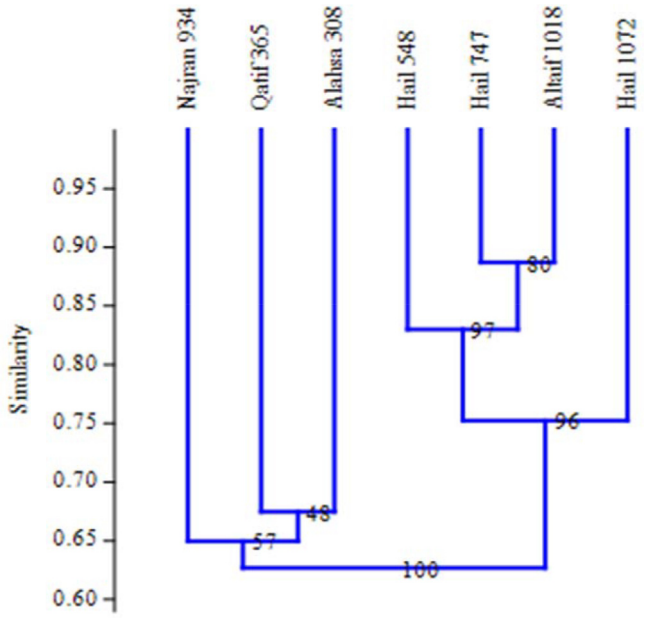

Fig. 3. Dendrograms generated using unweighted pair group method with arithmetic average (UPGMA) analysis, showing relationships between seven tomato landraces using SRAP data based on Jaccard genetic similarity coefficient.

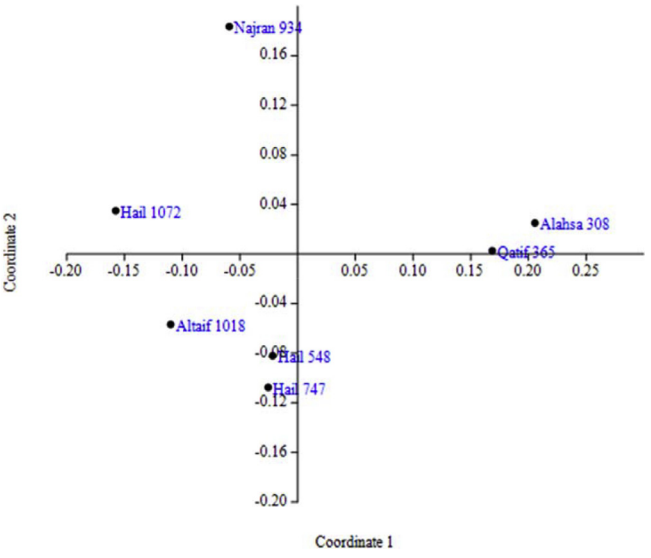

Fig. 4. Principal coordinate analysis (PCoA) of seven tomato landraces using SRAP data based on Jaccard genetic similarity coefficient.

several methodologies and procedures have been reported for the identification and detailed description of tomato genotypes based on agronomic, morphological, biochemical, and genetic traits. Genetic markers and protein profiling (particularly SDS-PAGE) can be successfully used not only to resolve the taxonomic and evolutionary problems of several crop species but also to distinguish cultivars of a particular crop species, including tomato.

Seed protein patterns produced by SDS-PAGE have been used for the identification of Solanaceae crops including tomato (Bhat and Kudesia, 2011; Hameed et al., 2014; Miskoska-Milevska et al., 2008), pepper (Odeigah et al., 1999; Kumar and Tata, 2010; Anu and Peter, 2003), eggplant (Karihaloo et al., 2002), and potato (El-Banna and Khatab, 2016). In this study, 19 bands with 53% polymorphism generated by SDS protein banding patterns distributed in all landraces were classified into three zones according to the mobility of the proteins. These results were in agreement with Miskoska-Milevska et al. (2008), who reported that three zones of tomato seed proteins separated by SDS-PAGE were detected. Zone A contained protein fragments with the highest molecular weight (114 kDa), followed by zone B, and the smallest protein fragments were detected in zone C. Patra and Chawla (2010) also detected 10 polymorphic polypeptide bands in 24 varieties using SDS-PAGE electrophoresis. They showed a moderate degree of polymorphism, and UPGMA cluster analysis was able to individually distinguish 9 of 18 tomato varieties. However, a weak polymorphism in SDS-PAGE banding patterns of *L. esculentum* Mill. ecotypes was detected by Mennella et al. (2001). In another study, nearly 42 peptides were resolved by SDS-PAGE and peptides of different molecular weights ranging from 8 kDa to 114 kDa were identified in 24 tomato genotypes (Hameed et al., 2014).

Genetic diversity of seed storage proteins has been reported for many crops in *Solanaceae* and other important crop families. In the present study, we were able to differentiate the tomato landraces from one another using seed storage protein profiles. The Hail 548 and Qatif 365 landraces displayed a similar banding pattern that differentiated them from the other landraces. The explanation might be that these two landraces are identical but are known under two different designations, or that these two landraces have a common ancestor that is very close in time. The two landraces, Hail 1072 and Hail 747, that were collected from the same region displayed a similar banding pattern except for one peptide marker (12 kDa), which was absent in Hail 1072 and present in Hail 747. Moreover, Altaif 1018 and Najran 934 displayed a similar banding pattern except for two peptide markers. Band 2 (150 kDa) was present in Najran 934 and absent in Altaif 1018, while band 7 was present in Altaif 1018 and absent in Najran 934. It has been reported that geographic relationship based on total seed protein profiles provides clues as to the introduction of the same germplasm in different areas and transgression of genes into different landraces (Nisar et al., 2007). Hameed et al. (2014) reported that distinguishing tomato hybrids, approved cultivars, and germplasm lines is possible based on peptide differences, and they were able to separate one tomato hybrid from another in the study. They concluded that differences in the seed storage protein profiles provided proof of genetic variability among the test materials and the usefulness of the technique for such studies. In 10 breeding lines in each of the six varieties of peppers (*Capsicum annuum* and *Capsicum frutescens*), Odeigah et al. (1999) found that 12 polypeptide bands with 22–98 kDa were generated and seven polypeptide bands could be characterized as the six varieties, making it possible to identify genotype duplicates in the collection of Nigerian Capsicum germplasm. In contrast, studies revealed either weak polymorphism in the SDS-PAGE banding patterns of *L. esculentum* Mill. Ecotypes (Mennella et al., 2001) or not enough to use them in identification of different tomato lines, hybrids, and cultivars (Miskoska-Milevska et al., 2008). The results of this study coincide with those of El-Banna and Khatab (2016), who recorded 28 bands generated by SDS–PAGE patterns distributed in all potato cultivars, with molecular weights ranging from 21.30 to 112.6 KDa and 50% polymorphism. All nine potato cultivars were clearly identifiable and almost all bands differed in their intensity among the studied cultivars. Moreover, some cultivars possessed some bands that were absent in other cultivars. Furthermore, Khan et al. (2014) found that out of 21 protein sub-units ranging from 6 to 180 kDa observed among 136 rapeseed (*Brassica napus* L.) genotypes, 16 (76.19%) were polymorphic, and a low level of genetic divergence was found using SDS-PAGE of total seed proteins. Seed protein patterns (Yüzbaşıoğlu et al., 2008) revealed 24 polypeptide bands with molecular masses ranging from 14.4 to 116 kDa. Five polymorphic

polypeptide bands were not sufficient to distinguish 14 lentil cultivars, whereas band patterns obtained from RAPD markers (Yüzbaşıoğlu et al., 2006) differentiated those not distinguished by SDS-PAGE. The authors concluded that commercial lentil cultivars grown in Turkey come from a restricted gene pool.

DNA-based markers have been developed to carry out genetic differentiation and fingerprinting of tomato populations and genotypes. For instance, the works of Smulders et al. (1997), Alvarez et al. (2001), Bredemeijer et al. (2002), and He et al. (2003) confirm the utility of SSRs for the study of genetic diversity and variability in genus Solanum and for tomato cultivar identification. Several other molecular markers, RFLP (Miller and Tanksley, 1990) and RAPD (Williams and Clair, 1993; Paran et al., 1995; Carelli et al., 2006), have been used to identify varieties and their relationships in wild and cultivated tomato.

The 27 SRAP primer pair combinations generated a total of 143 amplicons (fragments), of which 88 (64.74%) were polymorphic, and the PIC values ranged from 0.46 to 0.90 with an average of 0.76. These results were in agreement with those of Ruiz et al. (2005), who used 26 SRAP primer combinations to generate 348 fragments, of which 242 (66%) were polymorphic among Lycopersicon accessions and 58 (15.1%) were polymorphic among *L. esculentum* cultivars, and all cultivars could be distinguished with the SRAP markers used. Previous studies conducted by He et al. (2003) found PIC values ranging from 0.09 to 0.67 and Al-Abadi (2007) recorded a value of 0.98. Tam et al. (2005) reported that the average polymorphism information content was 0.39 in collections of tomato industrial lines. However, PIC values ranging from 0.62 to 0.97 were recorded by Al-Qadumii et al. (2012). Park et al. (2004) reported that 74 tomato cultivars, primarily from California, were genotyped using a minimum of seven AFLP primer combinations with a low level (9.3%) of polymorphism. Genetic similarity values varied from 0.16 to 0.98 among cultivars, and clustering and PCoA revealed that most modern hybrid cultivars were grouped separately from the landraces. Similarly, Sardaro et al. (2013) found, based on microsatellite polymorphism, that 47 Italian varieties were grouped into two major clusters, differentiating the modern varieties from the tomato landraces.

The high and significant cophenetic correlation coefficient value (0.95) of the SRAP data in this study indicates that the dendrogram fitted the observed data very well. The investigated landraces showed an intermediate level of genetic variability, as indicated by similarity coefficients (0.58–0.89). Genetic diversity among and within 75 populations of 29 Spanish tomato landraces evaluated using agro-morphological characteristics, quality parameters, and AFLP markers revealed a wide range of variation for all traits except DNA marker levels (Cebolla-Cornejo et al., 2013). In a study of comparative analyses of genetic diversity within tomato collections using retrotransposon-based sequence-specific amplification polymorphism (SSAP), AFLP, and SSR were conducted (Tam et al., 2005). SSAP was the most informative of the three systems for studying genetic diversity in tomato. It showed about four- to ninefold more diversity than AFLP and had the highest number of polymorphic bands per assay ratio and the highest marker index, while SSR had the ability to detect specific genetic relationships. Moreover, unique SSR profiles were observed for 468 of 508 European tomato cultivars (Bredemeijer et al., 2002). Furthermore, using a combination of SSR and AFLP, markers showing unique fingerprints of even closely related tomato cultivars were successfully demonstrated (García-Martínez et al., 2006). Another study using EST-SSR markers to study the genetic diversity and relationships of 42 tomato varieties sourced from different geographic regions revealed a high degree of diversity (0.18–0.77; mean 0.49) among these tomato varieties and an average PIC value of 0.45. Despite their diverse sources, all the tomato varieties fell into five groups, with no obvious geographical distribution characteristics (Korir et al., 2014). Todorovska et al. (2014) tested 160 SSR primers on a set of eight Bulgarian tomato genotypes, which generated 50.62% polymorphism with a mean PIC value of 0.196 and 1.869 alleles per locus. The mean genetic diversity was found to be relatively low (22.22%), which might indicate specific selection strategies for yield and resistance to biotic and abiotic stress as well as fruit quality.

In the present study, the UPGMA dendrogram validated by PCoA revealed clusters that almost corresponded to the geographical origin of the landraces. Clear separation of Eastern landraces (which had the overall highest genetic diversity) was found when compared with accessions from the northern part of the kingdom. Thus, SRAP markers have good potential to provide valuable information for tomato breeding and germplasm management and will be helpful for establishment of a core collection. These results were in agreement with those of Aflitos et al. (2014), who reported that habitat and mating type correlated with phylogenetic relationships and pointed to the occurrence of geographical races within these groups, which are of practical importance for introgressive hybridization breeding. Moreover, there is evidence that mating system shifts have a large effect on complex multigene-based traits such as floral and fruit development (Moyle, 2008), which might further account for large intra-species variations. The results of Todorovska et al. (2014) indicated that morpho- and hemo-types and common origin were behind the grouping of the Bulgarian tomatoes studied.

In the present study, weak and insignificant correlations between SRAP markers and the SDS-PAGE profiles were recorded based on Mantel correlation analysis, indicating that each marker type has importance in the analysis of variability among landraces. Moreover, both classes reflected the various patterns of genetic diversity and validated the use of the data to calculate the different diversity statistics. The genetic relationships observed using molecular markers may provide information about the history and biology of genotypes; however, they may unnecessarily reflect phenotypic variability among the genotypes, even in the case of autogamous species, where correlations between traits are generally the strongest. Thus, the establishment of a representative collection of species needs to be evaluated using different traits (Cattan-Toupance et al., 1998). Furthermore, molecular diversity was not necessarily related to gene expression, and it was very likely that the observed polymorphism was owing to both non-coding and coding regions (Khan et al., 2009). Moreover, diversity revealing variation at the small portion of the genome was expressed (phenotypic traits); therefore, the combination of phenotypic and molecular-based analysis of the genetic diversity assessment appears to be of great importance in developing any breeding program (Pandey et al., 2015; Parsaeian et al., 2011). It has also been suggested that the convergent evolution and complex genetic structure of traits, which result in environmental effects on trait expression, are the main causes of the limited linkage of molecular markers with morphological traits (Tabatabaei et al., 2011).

5. Conclusion

Seed storage protein profiling based on SDS-PAGE and SRAP markers can be efficiently used to assess genetic variability in tomato germplasm. The information obtained in this analysis will be of great interest in the management of *ex situ* collections, for utilization in breeding programs, and for direct use in quality markets.

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References

Aflitos, S., Schijlen, E., Jong, H., Ridder, D., Smit, S., Finkers, R., Wang, J., Zhang, G., Li, N., Mao, L., 2014. Exploring genetic variation in the tomato (Solanum section Lycopersicon) clade by whole-genome sequencing. Plant J. 80, 136–148.

Al-Abadi, B., 2007. Genetic variations among Jordanian tomato landraces using simple sequence repeat (SSR) and performance of their seedling to salinity stress. Master Thesis. Jordan University of Science and Technology, Irbid-Jordan.

Al-Qadumii, L.W., Sadder, M.T., Migdadi, H., 2012. Assessment of in silico BAC-based simple sequence repeat (SSR) marker development for tomato (*Solanum lycopersicum* L.). African J. Biotechnol. 11, 13938–13946.

Alvarez, A.E., Van de Wiel, C.C.M., Smulders, M.J.M., Vosman, B., 2001. Use of microsatellites to evaluate genetic diversity and species relationships in the genus Lycopersicon. Theor. Appl. Genet. 103, 1283–1292.

Anderson, J., Churchill, G., Autrique, J., Tanksley, S., Sorrells, M., 1993. Optimizing parental selection for genetic linkage maps. Genome 36, 181–186.

Anu, A., Peter, K.V., 2003. Analysis of seed protein of 29 lines of *Capsicum annuum* L. by polyacrylamide gel electrophoresis. Genet. Resour. Crop Evol. 50, 239–243.

Arumuganathan, K., Earle, E.D., 1991. Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol. Biol. Report. 9, 229–241.

Bauchet, G., Causse, M., 2012. Genetic diversity in tomato (*Solanum lycopersicum*) and its wild relatives. Genetic Diversity in Plants. InTech.

Bhat, T.M., Kudesia, R., 2011. Evaluation of genetic diversity in five different species of family Solanaceae using cytological characters and protein profiling. Genet. Eng. Biotechnol. J. 20, 20–25.

Brake, M., Migdadi, H., Al-Gharaibeh, M., Ayoub, S., Haddad, N., El Oqlah, A., 2014. Characterization of Jordanian olive cultivars (Olea europaea L.) using RAPD and ISSR molecular markers. Sci. Hort. 176, 282–289.

Bredemeijer, G., Cooke, R., Ganal, M., Peeters, R., Isaac, P., Noordijk, Y., Rendell, S., Jackson, J., Röder, M., Wendehake, K., 2002. Construction and testing of a microsatellite database containing more than 500 tomato varieties. Theor. Appl. Genet. 105, 1019–1026.

Carelli, B.P., Gerald, L.T.S., Grazziotin, F.G., Echeverrigaray, S., 2006. Genetic diversity among Brazilian cultivars and landraces of tomato *Lycopersicon esculentum* Mill. revealed by RAPD markers. Genet. Resour. Crop Evol. 53, 395–400.

Cattan-Toupance, I., Michalakis, Y., Neema, C., 1998. Genetic structure of wild bean populations in their South-Andean centre of origin. Theor. Appl. Genet. 96, 844–851.

Cebolla-Cornejo, J., Roselló, S., Nuez, F., 2013. Phenotypic and genetic diversity of Spanish tomato landraces. Sci. Hortic. 162, 150–164.

Comlekcioglu, N., Simsek, O., Boncuk, M., Aka-Kacar, Y., 2010. Genetic characterization of heat tolerant tomato (*Solanum lycopersicon*) genotypes by SRAP and RAPD markers. Genet. Mol. Res. 9, 2263–2274.

Doebley, J.F., Gaut, B.S., Smith, B.D., 2006. The molecular genetics of crop domestication. Cell 127, 1309–1321.

El-Banna, A.N., Khatab, I.A., 2016. Assessing genetic diversity of some potato (*Solanum tuberosum* L.) cultivars by protein and RAPD markers. Egypt. J. Genet. Cytol. 42 (1), 89–101.

FAOSTAT, F., 2014. Statistics of the Food and Agriculture Organization of the United Nations [WWW Document]. URL <http://faostat.fao.org/site/567/default.aspx#ancor>. [access 27 sept 2014].

García-Martínez, S., Andreani, L., Garcia-Gusano, M., Geuna, F., Ruiz, J.J., 2006. Evaluation of amplified fragment length polymorphism and simple sequence repeats for tomato germplasm fingerprinting: utility for grouping closely related traditional cultivars. Genome 49, 648–656.

Grandillo, S., Ku, H.M., Tanksley, S.D., 1999. Identifying the loci responsible for natural variation in fruit size and shape in tomato. Theor. Appl. Genet. 99, 978–987.

Hameed, A., Gul, A., Gulzar, T., 2014. Characterization of tomato germplasm through seed storage protein profiling by SDS-PAGE. Pak. J. Bot. 46, 827–832.

Hameed, A., Qureshi, M., Nawaz, M., Iqbal, N., 2012a. Comparative seed storage protein profiling of mung bean genotypes. Pak. J. Bot. 44, 1993–1999.

Hameed, A., Saddiqa, A., Nadeem, S., Iqbal, N., Atta, B.M., Shah, T.M., 2012b. Genotypic variability and mutant identification in Cicer arietinum L. by seed storage protein profiling. Pak. J. Bot. 44, 1303–1310.

Hameed, A., Shah, T.M., Atta, B.M., Iqbal, N., Haq, M.A., Ali, H., 2009. Comparative seed storage protein profiling of Kabuli chickpea genotypes. Pak. J. Bot. 41, 703–710.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. Paleontological statistics software: package for education and data analysis. Palaeontol. Electron.

Harlan, J.R., 1975. Our vanishing genetic resources. Science 80 (188), 618–621.

He, C., Poysa, V., Yu, K., 2003. Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. Theor. Appl. Genet. 106, 363–373.

Iqbal, S.H., Ghafoor, A., Ayub, N., 2005. Relationship between SDS-PAGE markers and Ascochyta blight in chickpea. Pak. J. Bot. 37, 87–96.

Jaccard, P., 1908. Nouvelles researches sur la distribution florale. Bull Soc. Vaud. Sci. Nat. 44, 223–270.

Kaga, A., Tomooka, N., Egawa, Y., Hosaka, K., Kamijima, O., 1996. Species relationships in the subgenus Ceratotropis (genus Vigna) as revealed by RAPD analysis. Euphytica 88, 17–24.

Karihaloo, J.L., Kaur, M., Singh, S., 2002. Seed protein diversity in *Solanum melongena* L. and its wild and weedy relatives. Genet. Resour. Crop Evol. 49, 533–539.

Khan, M.A., von Witzke-Ehbrecht, S., Maass, B.L., Becker, H.C., 2009. Relationships among different geographical groups, agro-morphology, fatty acid composition and RAPD marker diversity in safflower (*Carthamus tinctorius*). Genet. Resour. Crop Evol. 56, 19–30.

Khan, S., Iqbal, J., Khurshed, H., Zia, M., Shinwari, Z., Rabbani, M., 2014. Intra-specific genetic divergence in rapeseed (BRASSICA NAPUS L.) genotypes estimated through SDS-PAGE of total seed proteins. Int. J. Basic Appl. Sci. 3, 110–117.

Korir, N.K., Diao, W., Tao, R., Li, X., Kayesh, E., Li, A., Zhen, W., Wang, S., 2014. Genetic diversity and relationships among different tomato varieties revealed by EST-SSR markers. Genet. Mol. Res. 13, 43–53.

Kumar, O.A., Tata, S.S., 2010. SDS-PAGE seed storage protein profiles in chili peppers (Capsicum L.). Not. Sci. Biol. 2, 86.

Labate, J.A., Baldo, A.M., 2005. Tomato SNP discovery by EST mining and resequencing. Mol. Breed. 16, 343–349.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Li, G., Quiros, C.F., 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor. Appl. Genet. 103, 455–461.

Mane, R., Sridevi, O., Nishani, S., Salimath, P.M., 2013. Evaluation of genetic diversity and relationships among tomato genotypes using morphological parameters and SRAP markers. Indian J. Hortic. 70, 357–363.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.

Mazzucato, A., Papa, R., Bitocchi, E., Mosconi, P., Nanni, L., Negri, V., Picarella, M.E., Siligato, F., Soressi, G.P., Tiranti, B., 2008. Genetic diversity, structure and marker-trait associations in a collection of Italian tomato (*Solanum lycopersicum* L.) landraces. Theor. Appl. Genet. 116, 657–669.

Mennella, G., Onofaro Sanajà, V., D'Alessandro, A., 2001. Tomato ecotype characterization by anionic exchange¿ high performance liquid chromatography analysis of endosperm seed proteins, pp. 453–457.

Miller, J.C., Tanksley, S.D., 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus Lycopersicon. Theor. Appl. Genet. 80, 437–448.

Miskoska-Milevska, E., Dimitrievska, B., Poru, K., Popovski, Z.T., 2008. Differences in tomato seed protein profiles obtained by SDS-PAGE analysis. J. Agric. Sci. 53, 13–23.

Moyle, L.C., 2008. Ecological and evolutionary genomics in the wild tomatoes (*Solanum Lycopersicon*). Evolution 62, 2995–3013.

Negri, V., Maxted, N., Veteläinen, M., 2009. European landrace conservation: an introduction. Eur. Landrac. on-farm Conserv. Manag. use, 1–22.

Nisar, M., Ghafoor, A., Khan, M.R., Ahmad, H., Qureshi, A.S., Ali, H., 2007. Genetic diversity and geographic relationship among local and exotic chickpea germplasm. Pak. J. Bot. 39, 1575–1581.

Odeigah, P.G.C., Oboh, B., Aghalokpe, I.O., 1999. The characterization of Nigerian varieties of pepper, *Capsicum annuum* and *Capsicum frutescens* by SDS-polyacrylamide gel electrophoresis of seed proteins. Genet. Resour. Crop Evol. 46, 127–131.

Pandey, S.K., Das, A., Rai, P., Dasgupta, T., 2015. Morphological and genetic diversity assessment of sesame (*Sesamum indicum* L.) accessions differing in origin. Physiol. Mol. Biol. Plants 21, 519–529.

Paran, I., Goldman, I., Tanksley, S.D., Zamir, D., 1995. Recombinant inbred lines for genetic mapping in tomato. Theor. Appl. Genet. 90, 542–548.

Park, Y.H., West, M.A.L., St. Clair, D.A., 2004. Evaluation of AFLPs for germplasm fingerprinting and assessment of genetic diversity in cultivars of tomato (*Lycopersicon esculentum* L.). Genome 47, 510–518.

Parsaeian, M., Mirlohi, A., Saeidi, G., 2011. Study of genetic variation in sesame (*Sesamum indicum* L.) using agro-morphological traits and ISSR markers. Russ. J. Genet. 47, 314.

Patra, N., Chawla, H.S., 2010. Biochemical and RAPD molecular markers for establishing distinctiveness of basmati rice (Oryza sativa L.) varieties as additional description for plant variety protection. Indian J. Biotechnol. 9, 371–377.

Rick, C.M., 1976. Evolution of Crop Plants. Longman group, London.

Ruiz, J.J., García-Martínez, S., Picó, B., Gao, M., Quiros, C.F., 2005. Genetic variability and relationship of closely related Spanish traditional cultivars of tomato as detected by SRAP and SSR markers. J. Am. Soc. Hortic. Sci. 130, 88–94.

Sardaro, M.L.S., Marmiroli, M., Maestri, E., Marmiroli, N., 2013. Genetic characterization of Italian tomato varieties and their traceability in tomato food products-Sardaro-2012-Food Science and Nutrition-Wiley Online Library. Food Sci. Nutr. 1, 54–62. https://doi.org/10.1002/fsn3.8.

Smulders, M.J.M., Bredemeijer, G., Rus-Kortekaas, W., Arens, P., Vosman, B., 1997. Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor. Appl. Genet. 94, 264–272.

Stevens, M.R., Lamb, E.M., Rhoads, D.D., 1995. Mapping the Sw-5 locus for tomato spotted wilt virus resistance in tomatoes using RAPD and RFLP analyses. Theor. Appl. Genet. 90, 451–456.

Tabatabaei, I., Pazouki, L., Bihamta, M.R., Mansoori, S., Javaran, M.J., Niinemets, U., 2011. Genetic variation among iranian sesame (*Sesamum indicum* L.) Accessions

vis-a-vis exotic genotypes on the basis of morpho-physiological traits and rapd markers. Aust. J. Crop Sci. 5, 1396.
Tam, S.M., Mhiri, C., Vogelaar, A., Kerkveld, M., Pearce, S.R., Grandbastien, M.A., 2005. Comparative analyses of genetic diversities within tomato and pepper collections detected by retrotransposon-based SSAP, AFLP and SSR. Theor. Appl. Genet. 110, 819–831.
Todorovska, E., Ivanova, A., Ganeva, D., Pevicharova, G., Molle, E., Bojinov, B., Radkova, M., Danailov, Z., 2014. Assessment of genetic variation in Bulgarian tomato (*Solanum lycopersicum* L.) genotypes, using fluorescent SSR genotyping platform. Biotechnol. Equip. 28, 68–76.
Van Berloo, R., Zhu, A., Ursem, R., Verbakel, H., Gort, G., van Eeuwijk, F.A., 2008. Diversity and linkage disequilibrium analysis within a selected set of cultivated tomatoes. Theor. Appl. Genet. 117, 89–101.
Williams, C.E., Clair, D.A.S., 1993. Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum*. Genome 36, 619–630.
Yang, W., Bai, X., Kabelka, E., Eaton, C., Kamoun, S., van der Knaap, E., Francis, D., 2004. Discovery of single nucleotide polymorphisms in *Lycopersicon esculentum* by computer aided analysis of expressed sequence tags. Mol. Breed. 14, 21–34.
Yüzbaşioğlu, E., Acik, L., Özcan, S., 2008. Seed protein diversity among lentil cultivars. Biol. Plant. 52, 126–128.
Yüzbaşıoğlu, E., Özcan, S., Açık, L., 2006. Analysis of genetic relationships among Turkish cultivars and breeding lines of *Lens culinatis* Mestile using RAPD markers. Genet. Resour. Crop Evol. 53, 507–514.
Zeven, A.C., 1998. Landraces: a review of definitions and classifications. Euphytica 104, 127–139.