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Original Article

Genetic structure of Pakistani tomato accessions based on morphological traits and RAPD markers

Usman Ahmed[1], Dawood Ahmad[1], Abdullah Jalal[1], Hala Rajab[1], Syed Sartaj Alam[2], and Muhammad Sayyar Khan[1*]

[1] *Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, 25130 Pakistan*

[2] *Department of Plant Pathology, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, 25130 Pakistan*

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Abstract

Genetic variations among eight Pakistani tomato (*Solanum lycopersicum* L.) accessions were investigated using morphological traits and randomly amplified polymorphic DNA (RAPD) primers. Significant differences among accessions were observed for most of the quantitative and qualitative morphological traits. The RAPD-PCR assay amplified a total of 39 different alleles with 33 polymorphic alleles resulting in an 84.25% rate of polymorphism among eight tomato accessions. The most informative primers were B06 and H05 by revealing the maximum number of bands in the germplasm. The dendrogram for the morphological traits classified the germplasm into a single larger cluster except for genotype 17880. The dendrogram constructed for the molecular data clearly segregated the germplasm into two clusters. The accessions 17862 and 17870 were placed in the small cluster while the rest of the accessions were grouped together in the larger cluster. Genetic similarity ranged from 0.31 to 0.83 with an average of 0.68 for all evaluated accessions.

Keywords: tomato, RAPD, diversity, molecular markers, morphological traits

1. Introduction

Cultivated tomato (*Solanum lycopersicum* L.) is one of the most essential and widely grown plants of the Solanaceae family. It is a good source of different vitamins such as A, C, and minerals including calcium (Ca), phosphorous (P), and iron (Fe) (Dhaliwal, Singh, & Cheema, 2003). It has become one of the most renowned and largely consumed vegetable grown under a wide range of environmental conditions including field, green houses, and plastic tunnels. Due to its versatile nature, plant breeders and biotechnologists have a keen interest in exploring the full potential for tomato improvement (Georgelis, Scott, & Baldwin, 2004; Mirshamsi, Farsi, Shahriari, & Nemati, 2008; Saliba, Causse, Gervais, & Philouze, 2000). The knowledge of germplasm diversity and characterization is fundamental for their potential use in plant breeding programs. In this context, morphology is of great practical importance as the characterization of plant germplasm has traditionally relied on morphological characters. Morphological characters are easy to study, relatively cheap to evaluate and can be easily observed visually. They are potentially useful for clonal identification and genetic distance estimation. Genotypic and environmental variance components of each character should also be estimated in order to design breeding strategies for character improvement.

Although phenotypic traits are the basic and central selection tools used in breeding programs, they do not always provide accurate information necessary to distinguish different genotypes as they are highly affected by environmental factors. Therefore, phenotypic variations of plants do not always follow the genetic pattern of variation and diversity of

*Corresponding author
Email address: sayyar@aup.edu.pk

plant populations as documented by the lack of congruence between morphological and genetic data (Ayele, Gailing, & Finkeldey, 2011; Ruisi *et al.*, 2011; Smissen & Heenan, 20 10). Therefore, further assessment of the collected germplasms at the molecular level is required (Carmen de Vicente, Felix Alberto, Engels, & Ramanatha, 2006; Ferreira, 2006). Different types of molecular markers, such as amplified fragment length polymorphism (AFLP), restricted fragment length polymorphism, simple sequence repeat (SSR), and expressed sequence tag data, have been developed and mapped onto the 12 chromosomes of tomato (Broun & Tanksley, 1996; Frary *et al.*, 2005; Saliba *et al.*, 2000). Since the successful application of randomly amplified polymorphic DNA (RAPD) markers and gene mapping in tomato by Klein-Lankhorst, Vermunt, Weide, Liharska, and Zabel (1991), these markers have been successfully used in tomato genotypes to estimate genetic diversity and polymorphism (Egashira, Ishihara, Takashina, & Imanishi, 2000; Nawaz *et al.*, 2015; Naz, Zafrullah, Shahzadhi, & Munir, 2013) and for the identification of markers related to important traits such as drought tolerance (El-Sayed, El-Aref, Taghian, & Hashad, 2002), virus resistance (Smiech, Rusinowski, Malepszy, & Niemirowicz-Szczytt, 2000), callus induction (Mansour, Zaki, Ahmed, & Ahmed 2005) and purity of hybrid seeds (Singh *et al.*, 2007). RAPD analysis is simple, quick, robust, and does not require the information of sequence in the flanking region (Karp *et al.*, 1997; Molnar, James, & Kasha, 2000). The present study was conducted to examine the genetic diversity of tomato accessions collected from the Plant Genetic Resources Institute (PGRI) at the National Agricultural Research Centre (NARC), Islamabad, Pakistan using morphological descriptors and RAPD markers. The data provided useful background information that can be useful for potential use in indigenous tomato improvement breeding programs.

2. Materials and Methods

2.1 Plant material

In this study eight tomato accessions named as 17862, 17870, 17880, 17879, 17873, 17872, 17863, and 178 60 were used for morphological and genetic variation analysis. The seeds of these eight different tomato accessions were kindly provided by the gene bank of the PGRI, NARC, Islamabad, Pakistan.

2.2 Growth conditions

Seeds (five seeds per pot) of each tomato accession were sown in five pots under green house conditions. The pots were properly checked and watered as required. Sprouting started in almost all accessions in two weeks. After one month, the thinning of seedlings was done and each seedling was transferred to a separate pot.

2.3 Morphological parameters

Twenty morphological traits including qualitative and quantitative characters were scored under green house conditions for each genotype following the tomato descriptor published by the International Plant Genetic Resources Institute (Darwin, Knapp, & Peralta, 2003; International Plant Genetic Resources Institute [IPGRI], 1996) (Tables 1 and 2). The data for the traits were scored using either binary recording (absence=0, presence=1) or a scale (1–9).

Table 1. Variability in 10 qualitative morphological characters scored for the evaluated tomato germplasm.

Character	Description	No. of accessions	% occurrence
Hypocotyl: anthocyanin coloration	Absent	8	100
Stem: anthocyanin coloration	Absent	2	25
	very weak	4	50
	weak	2	25
Growth type	Indeterminate	7	87.5
	Determinate	1	12.5
Leaf type	Regular	7	87.5
	Potato type	1	12.5
Leaf attitude (in middle third of plant)	Horizontal	2	25
	Semierect	6	75
Division of blade	Bipinnate	7	87.5
	Pinnate	1	12.5
Attitude of leaflet petiole in relation to main axis	Semi erect	4	50
	horizontal	4	50
Leaf intensity of green color	Light	4	50
	Medium	3	37.5
	Dark	1	12.5
Inflorescence type	Uniparous	2	25
	Biparous	4	50
	Triparous	2	25
Flower color	Pale Yellow	3	37.5
	Yellow	5	62.5

Table 2. Variability in 10 quantitative characters scored for the evaluated tomato germplasm.

Morphological Parameters	Range	Mean	Variance*	P-value	SE
Stem: length of internode (cm)	0.733-6.47	3.60	8.227432	0.0000**	±0.3363
Plant height (inches)	14.3-35	24.65	107.1225	0.0011**	±2.2779
No. of leaves	18.33-33.66	26.00	58.75223	0.0979	±6.96
No. of leaflets	129.66-300	214.83	7253.929	0.0782	±38.581
No. of branches	2-6.33	4.17	4.687225	0.0024**	±0.5893
No. of Flowers	7.33-87.66	47.50	1613.227	0.0000**	±5.114
No. of inflorescence	2.0-19	10.50	72.25	0.0000**	±1.1278

** Significance at 1% level of probability

2.4 DNA extraction

Total genomic DNA was isolated from three different young and healthy plants of each accession using the CTAB method (Murray & Thompson, 1980) with slight modifications. The concentration and quality of total genomic DNA was measured by NanoDrop by measuring the optical density at wavelengths of 260/280 nm.

2.5 RAPD assay

Eight RAPD primers were used for the characterization and genetic diversity analysis of these accessions (Table 3). These RAPDs primers were initially screened and according to the reproducibility of the primer, they were used for the final analysis. PCR amplification was performed using Bio-Rad Thermal cycler (T-100). The total PCR reaction was 12.5 µL containing 6.25 µL of PCR mix, 1 µL (50 ng) DNA, 0.5 µL 20 pmol primer, and 0.3 µL Taq polymerase. The thermal cycler profile which was set for PCR reactions consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 50 s, 36 °C for 45 s and 72 °C for 1 min. An additional cycle of 10 min at 72 °C was used for final extension. The amplified product of PCR was then run on 1.5% agarose gel stained with ethidium bromide. The gel documentation system was used for visualization of the DNA bands.

2.6 Data analysis

The amplified DNA fragments were scored as present (1) or absent (0) and used for the data analyses. Genetic similarities among the accessions of the germplasm were determined based on the Jaccard (1908) coefficients. Using the data matrix of similarity coefficients, a dendrogram was then constructed using the unweighted pair group arithmetic mean (UPGMA) method. The genetic similarities and dendrogram were computed using the Numerical Taxonomy & Multivariate Analysis System (NTSYSpc; Rohlf, 2000).

3. Results

3.1 Morphological characterization of the germplasm

Phenotypic variations among the eight different accessions of tomato (*S. Lycopersicum* L.) were evaluated using various qualitative and quantitative characters (Tables 1 and 2). Data recorded for 10 qualitative morphological traits revealed a differential profile for most of the germplasm (Table 1). Anthocyanin coloration of the hypocotyl was absent in all accessions while stem anthocyanin coloration was mostly weak. The growth type was indeterminate in the majority of the accessions (87.5%). Leaf shape observed in the majority of the accessions was a regular type except for accession 17880 which showed a different leaf shape compared to the other accessions (Table S1a). Dark green leaves were observed only in accession 17880, whereas the other 50% of the accessions showed light green leaves and 37.5% of the accessions showed medium green leaves. Leaf attitude (in the middle third of plant) was semi-erect in most of the accessions (75%), while in the others it was horizontal. The flowers were yellow in the majority of the accessions (62.5%). The blade was both bipinnate (87.5%) and pinnate (12.5%). Uniparous (25%), biparous (50%), and trypasarous (25%) inflorescence type were observed in different accessions (Table S1a).

The quantitative characters revealed that plant height ranged from 14.3 to 35 inches. Maximum plant height and internodes lengths were observed for accessions 17862 whereas accession 17880 showed minimum values for these traits (Table S1b). The average number of leaves, leaflets, branches, flowers, and inflorescences were 26, 214.83, 4.17, 47.5, and 10.5, respectively (Table 2). Significant differences among the accessions were observed for most of the quantitative characters including plant height, internode length, number of branches, flowers, and inflorescence (Table 2).

The dendrogram, based on morphological traits, displayed clustering of seven accessions in a larger cluster while genotype 17880 was displayed as an operational taxonomic unit. Among the seven genotypes grouped together in the larger cluster, genotypes 17837 and 17863 were most similar to each other. The larger cluster, if observed closely, was further divided into two sub-clusters showing variation of the morphological characters based on the scored traits of the tomato accessions used in this study (Figure 1).

3.2 RAPD assay

Based on the amplification efficiency and reproducibility of the primers, eight different RAPD primers were used to amplify the genomic DNA extracted from the leaves of eight different accessions. A differential profile was produced by these primers among the eight accessions (Figure

Table 3. Scoring of number of bands, polymorphic bands and polymorphism rate of each primer among eight different accessions of tomato.

Primer name	Primer seq (5'→3')	Annealing temp (°C)	Total bands (polymorphic)	Polymorphism percentage (%)	Average allele frequency	Average band No.	Allele size range (bp)
B06	TGCTCTGCCC	37	9(8)	89	0.64	5.8	300-1100
B12	CCTTGACGCA	34	2(2)	100	0.81	1.6	150-200
E12	TTATCGCCCC	34	2(2)	100	0.88	1.8	120-250
H05	AGTCGTCCCC	37	9(9)	100	0.44	4.1	300-1200
H09	TGTAGCTGGG	34	4(1)	25	0.78	3.1	200-1500
H11	CTTCCGCAGT	34	6(6)	100	0.33	2.0	400-1100
H16	TCTCAGCTGG	34	2(2)	100	0.56	1.1	400-900
H18	GAATCGGCCA	34	5(3)	60	0.90	4.5	400-1300
Total			39 (33)				
Average			4.9(4.1)	84.25	0.67	3.1	

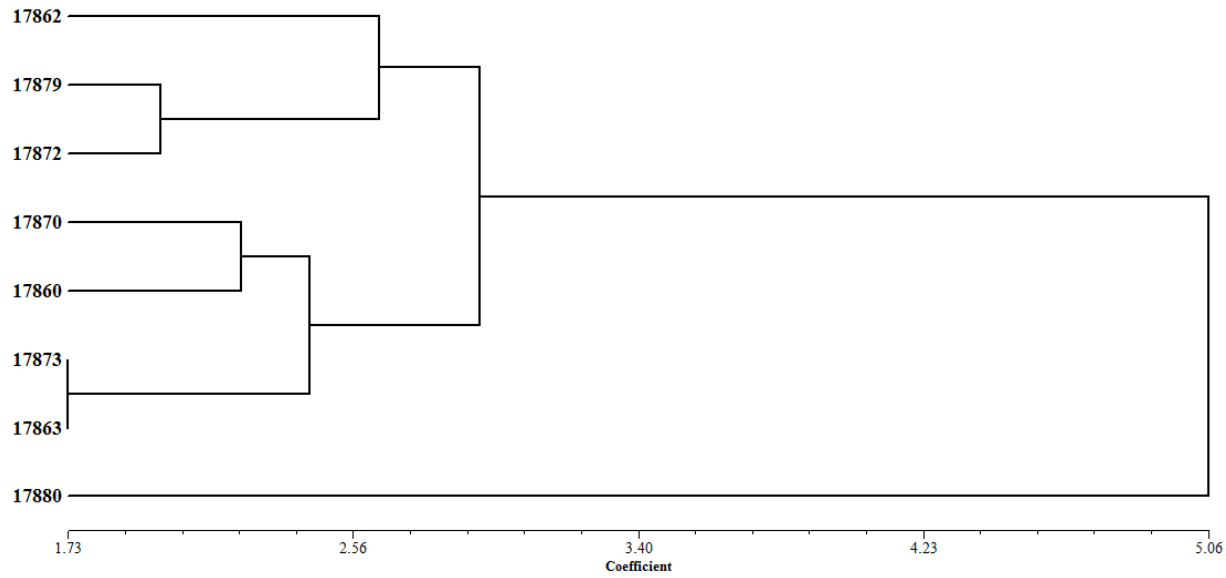

Figure 1. Dendrogram generated from morphological traits of eight different tomato accessions using Euclidian coefficient.

S1). The RAPD-PCR assay amplified a total of 39 different alleles with 33 polymorphic alleles that resulted in an 84.25% rate of polymorphism among the eight tomato accessions used (Table 3). An average of 3.1 bands per primer was produced in each accession (Table 3). The size of the amplified bands ranged from 120 to 1500 bp. The average allelic frequency of bands amplified ranged from 0.33 to 0.9 (Table 3).

To determine the genetic relationship of the germplasm, a dendrogram was constructed based on genetic similarity matrices. The dendrogram displayed a differential grouping pattern that clearly segregated the germplasm into two sub-clusters. Two accessions including 17862 and 17870 were placed in the small sub-cluster while the remainder of the accessions were grouped together in the larger sub-cluster (Figure 1). According to the similarity matrix, the genetic distances ranged from 0.31 between 17860 and 17862 to 0.83 between 17872 and 17879 with an average of 0.68 for all the accessions (Table 4).

4. Discussion

Characterization using genetic diversity of horticultural crops is critical and provides invaluable information regarding the planning of future breeding strategies (Wicaksana, Gilani, Ahmad, Kikuchi, & Watanabe, 2011; Cooper, Spillane, & Hodgkin, 2001). Genetic diversity assessments using a combination of morphological and molecular markers were shown to give more reliable results (Acosta-Quezada, Vilanova, Martínez-Laborde, & Prohens, 2012; Zhou, Wu, Cao, & Jiang, 2015). Here we have documented the genetic diversity and relationships of the Pakistani tomato accessions based on morphological and molecular markers. Even though the number of genotypes was less in this study, high variability was revealed by both marker systems.

A high range of variation was found within 14 traits across 17 investigated morphological characters that included qualitative and quantitative traits which showed the existence of a high degree of morphological variation among the tomato germplasm. Anthocyanins are naturally occurring antioxidants found in plants that are known as anti-inflammatory, anticancer, and anti-microbial agents (Faramarzi, Yadollahi, & Soltani, 2014; Himeno *et al.*, 2014). These compounds are not limited to fruits. They are also found in the leaves and stems as well. Our data revealed the absence of anthocyanin coloration from hypocotyls in all of the accessions while stem anthocyanin coloration was mostly weak in the accessions.

Table 4. Genetic distance between 8 tomato accessions based on RAPD primers data using Jaccard's coefficient. The boxed values indicate the most similar accessions (0.83) and the least similar accessions (0.31).

	17862	17870	17880	17879	17873	17872	17863	17860
17862	1.00							
17870	0.58	1.00						
17880	0.41	0.44	1.00					
17879	0.47	0.59	0.72	1.00				
17873	0.52	0.59	0.72	0.80	1.00			
17872	0.39	0.47	0.69	0.83	0.66	1.00		
17863	0.44	0.53	0.67	0.81	0.69	0.71	1.00	
17860	0.31	0.41	0.63	0.66	0.66	0.62	0.59	1.00

Since anthocyanin production is regulated by developmental, hormonal, and light signals (Faramarzi *et al.*, 2014), the smaller production of anthocyanins in our study could be attributed to the differential environmental conditions of the green house.

Knowledge of the plant growth type, leaf shape, and inflorescence type is important as plant architecture can influence yield (Jones, Rick, Adams, Jemstedt, & Chetelat, 20 07). In our study both determinate and indeterminate growth type and mostly regular type leaf shape were observed. According to the IPGRI standards, four types of growth are expected in tomatoes: dwarf, determinate, semi-determinate, and indeterminate. Most of our germplasm showed indeterminate growth (87.5%) while the others showed determinate growth (12.5%). Bhattarai, Louws, Williamson, and Panthee (2016) also noted only two types of growth (i.e. determinate and indeterminate) in tomato genotypes collected from eastern USA. Leaf attitude in the middle third of the plant was semi-erect in most of the accessions (75%) while in the others it was horizontal. The blade was both bipinnate (87.5%) and pinnate (12.5%). Uniparous, biparous, and trypasarous inflorescence type and both pale yellow and yellow flower colours were scored.

The data measured for quantitative characters revealed that the plant height ranged from 14.3 to 35 inches. Recently, Naz *et al.* (2013) scored 7.9–31.5 inches for the plant height in PGRI-Islamabad acquired tomato plants while Fehmida and Ahmad (2007) recorded an average of 54 inches for the plant height for three Azad Kashmir derived genotypes. The average number of leaves, leaflets, branches, flowers, and inflorescences were 26, 218.83, 4.17, 47.5, and 10.5, respectively. Significant differences among the accessions were observed for most of the quantitative characters including plant height, internode length, number of branches, flowers, and inflorescence. The dendrogram drawn based on morphological traits also displayed variations and a diverse profile of the traits observed in the germplasm.

To see the larger picture of the genetic bases of our germplasm revealed by morphological traits, molecular characterization was carried out using RAPD primers. A total of 39 different alleles with 33 polymorphic alleles with an average of 3.1 bands per primer were produced in each accession. In a similar study Ezekiel, Nwangburuka, Ajibade, and Odebode (2011) amplified 14 Nigerian market derived tomatoes using 10 RAPD primers that revealed an average of 7.4 fragments per primer and showed 62.2% polymorphism in the products that ranged from 200 to 3100 bp. In another study, Naz *et al.* (2013) obtained 130 bands with a 72.6 polymophism rate giving 8.6 bands per primer that ranged in size from 400 to 2500 bp using 15 RAPD primers with 25 tomato accessions. In spite of the fewer number of accessions in our study compared to the two studies mentioned above, our germplasm showed a more diverse genetic base of 84.25% polymorphism.

Genetic similarity is one of the measures to study genetic relatedness among the taxa (Ahmad, Kikuchi, Jatoi, Mimura, & Watanabe 2009; Sharma, Jones, Young, & French, 2001). One of our aims was to investigate the genetic relationships of the tomato germplasm using genetic similarities based on the RAPD marker system. The cluster analysis showed clear intraspecific discrimination and genetic relationships of the germplasm distributing them into two groups. The 17879 and 17872 accessions appeared to be identical. These might be the clones of each other or might be the result of adulterated sampling. A relatively low (0.68) genetic similarity was revealed by our accessions compared to genetic similarity (0. 825) of Indian tomato accessions (Archak, Karihaloo, & Jain, 2002), that showed a high resolution power of the RAPD primers used in our study. The current genetic diversity analysis depicted high resolution of the RAPD markers for tomato germplasm. The morphological traits also showed distinguishing features to describe the accession. Since morphological traits provide basic and rapid selection tools but are also highly influenced by the environment, a combination of morphological descriptors with easy and robust molecular markers like RAPD for characterization of tomato provides more reliable tools for assessing genetic diversity to design future breeding strategies. Apart from RAPD, other molecular

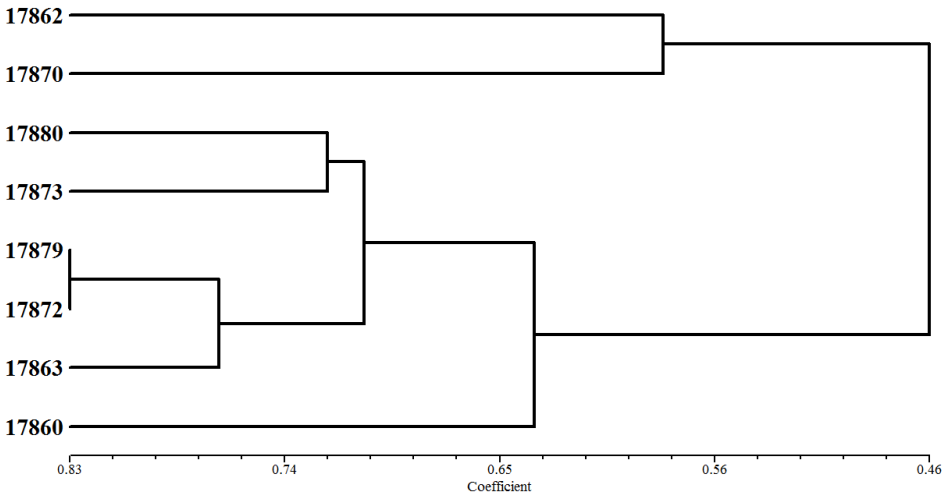

Figure 2. Genetic relationships of eight tomato accessions constructed by the UPGMA method on the basis of RAPD data analysis. The dendrogram shows the separation of the germplasm into different larger and smaller groups.

tools, such as AFLP, SSR, and single nucleotide polymerphisms, which require more sophistication and prior information of the genome of interest may also be employed for future genetic diversity assessments in tomato.

5. Conclusions

Out of 17 qualitative and quantitative morphological traits, a high range of variation was found in the 14 investigated traits. A total of 39 different alleles with 33 polymorphic alleles resulted in an 84.25% rate of polymorphism among the eight tomato accessions. The morphological descriptors complemented by the RAPD molecular markers in the current study provided valuable information for the exploitation of these accessions in future breeding programs.

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Appendix

Table S1a. Qualitative date of morphological parameters in all accessions of tomato.

Anthocyanin coloration of hypocotyls	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Growth type	Indeterminate	Indeterminate	Determinate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate
Stem: anthocyanin coloration	Weak	Very weak	Absent	Absent	Very weak	Very weak	Weak	very weak
leaf type	Regular	Regular	Potato type	Regular	Regular	Regular	Regular	Regular
Leaf attitude (in middle third of plant)	Semi erect	Horizental	Semi erect	Semi erect	Semi erect	Horizental	Semi erect	Semi erect
Division of blade	Bipinnate	Bipinnate	Pinnate	Bpinnate	Bpinnate	Bpinnate	Bpinnate	Bpinnate
attitude of petiole of leaflet in relation to main axis	Semi erect	Horizental	Horizntal	Semi erect	Semi erect	Horizental	Semi erect	Horizental
Leaf intensity of green color	Light	Medium	Dark	Light	Medium	Light	Light	Medium
Inflorescence type	Biparous	Biparous	Triparous	Uniparous	Bioparous	Triparous	Bioparous	Uniparous
Flower color	Pale yellow	Yellow	Pale yellow	Yellow	Yellow	Yellow	Pale yellow	Yellow

Table S1b. Quantitative data of morphological parameters in all accessions of tomato.

Morphological Parameters	17862	17870	17880	17879	17873	17860	17872	17863
Stem: length of internodes (cm)	6.47	5.58	0.7333	4.93	3.98	4.51	3.93	3.28
Plant height (inches)	35	23.8	14.3	27.3	24.66	22.6	20	26.66
No. of leaves	30	18.6	30.666	19.33	49	24.66	18.33	33.66
No. of leaflets	231.3	150.3	170.66	129.66	300	241.33	144	228.6
No. of branches	3.33	3	6.33	2	5	3.66	2.3	4.33
No. of Flowers	9	11.33	87.66	8.33	16.33	14.66	9	7.33
No. of inflorescence	3	3	19	2.33	4.66	2.66	2.33	2

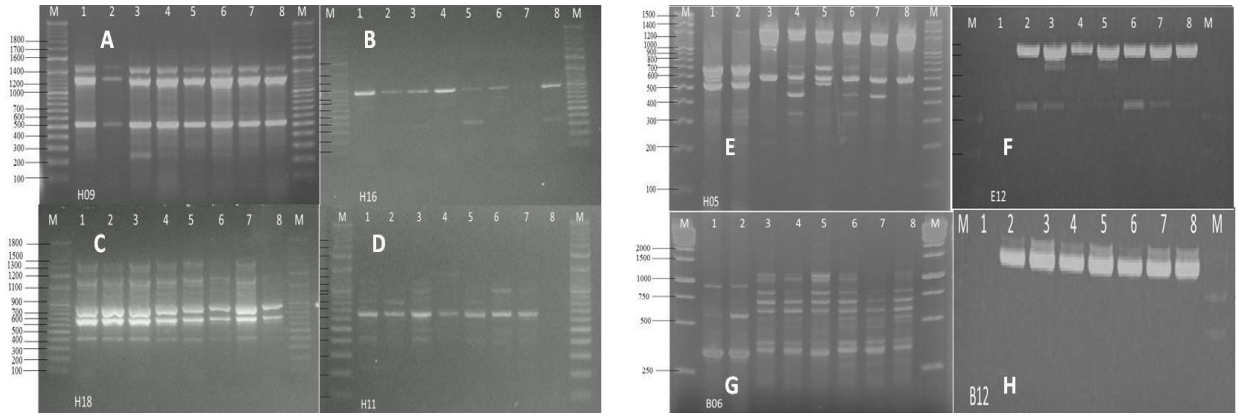

Figure S1. Separation of amplification product of eight different accessions of tomato on 1.5% agarose gel using different RAPD primers. A: H09 primer, B: H16 primer, C: H18primer, D: H11 primer, E: H05 primer, F: E12 primer, G: B06 primer and H: B12 primer. M,1 Kbp Ladder; 1,17862; 2,17870; 3, 17880; 4, 17879; 5, 17873; 6, 17872; 7,17863; 8, 17860.