



Genetic Diversity of Some Tomato Cultivars and Breeding Lines Commonly Used in Pakistani Breeding Program

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ABSTRACT

Genetic diversity present in gene pool is an important determination for breeding programs, and characterization is useful of building crop plant collections primarily based on the knowledge of the presence of valuable genes and traits. Developing successful varieties for increasing the future yield and quality of tomato depend mainly on the genetic diversity of parents used in the breeding program. Molecular characterization of 21 tomato genotypes used in in Pakistani breeding program was studied using random amplified polymorphic DNA (RAPD) markers. Total 102 bands were amplified among 21 genotypes using 20 RAPD primers. Overall 73.5% polymorphism was shown as 75 out of 102 loci were polymorphic. High degree of divergence between varieties was indicated by low level of monomorphic bands. The number of PCR products per primer varied from 2-8 with an average of 5.1 bands per primer. Primer GL J-20 and GL C-09 produced maximum number of bands whereas the primers GL A-09 produced the lowest. The polymorphism per RAPD primer ranged from 50% to 100% with an average of 73.5%. The accumulative analysis of amplified products generated by RAPD's was enough to assess the genetic diversity among the genotypes. The information would be helpful for formulating future breeding and genome mapping programs. This study will also work as an indicator for tomato breeders to evolve varieties with genetic diverse back ground to achieve sustainability in tomato production in the country.

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Introduction

Tomato (*Solanum lycopersicum* L.) belongs to family Solanaceae. It is grown as summer vegetable in Pakistan. It is an excellent source of different vitamins like A, C and minerals like Ca, P and Fe (Dhaliwal et al., 2003). It is a rich source of lycopene antioxidant that reduces the risk of prostate cancer (Hossain et al., 2004). In terms of caloric value tomato does not rank high but because of its volume consumed in various forms such as cooking, salad, soup, pickles, ketchup and sauces etc. it contributes substantially to dietary intake of vitamins and essential minerals.

The cultivated tomato has recently been added to the world's most important food crop. Recently it has become one of the most famous and largely consumed vegetable grown on wide range of environmental conditions including field, green houses and plastic tunnels etc. The versatile nature of tomato as fresh and/or processed form make it widely adapted food commodity. Due to its

versatile nature tomato plant has been of keen interest to the plant breeders and biotechnologists (Georgelis et al., 2004; Mirshamsi et al., 2008 and Saliba et al., 2000). Tomato is commercially important all over the world because of its fresh fruit market and the processed food industries. Tomato is being produced in most of the countries of the world and United States, China, Turkey, Italy and India are the major producers. There has been an increase of 60% in the world tomato production over the recent many years. The wide adaptation of tomato in different environments, methods of production and versatility in its uses is attributed to existing genetic variation in the genus *Lycopersicon*. Due to its flowering behavior, genetic variability of tomato can easily be exploited for the developing high yielding hybrids having desirable and specific characteristics.

In Pakistan during 2011 tomato was cultivated on area of 52.3 thousand hectares which is 20% of total area

under vegetable cultivation. The average productivity of tomato in the country has been stagnant between 9.5 to 10.5 tons per hectare during the last decade (Anon, 2011) when compared with yield i.e. 33.6 tons per hectare of modern agricultural areas of the world (Anon, 2013). Tomato crop has a tremendous export potential due to its demand in the international market. Tomatoes are exported to Afghanistan, Iran, U.A.E, Saudi Arabia, Sri Lanka and India. Afghanistan, Iran and UAE are the main markets for tomato exports from Pakistan. Pakistan has the potential to increase share in these markets. In Pakistan, because of its secondary importance in crop sciences, little efforts have been made for the improvement in production and quality of tomato. Furthermore, tomato crop faces biotic stresses (early blight, late blight, cucumber mosaic virus etc.), abiotic stresses (heat, frost, drought etc.) and lack of quality seed (hybrids varieties) are major factors in Pakistan that limiting the yield. Due to the limited progress on the commercial production of tomato hybrid seed in Pakistan, a high quantity of seed is imported at very high cost. Issues of imported seed are adaptability to environment, risk of diseases, insects and pests. Therefore it is very important to estimate the genetic diversity before starting the breeding program to cope with above mentioned problem.

Modern plant breeding has succeeded spectacularly in raising crop productivity in line with the rising human population. International surveys have clearly shown the urgent need to save and manage local landraces, since these materials contain valuable genes for future cultivation practices and future breeding for higher yield and better quality (Comertpay et al., 2012; Alsaleh et al. 2014). It has been proposed that the knowledge about germplasm diversity and genetic relatedness among the available elite breeding material is a core element in plant breeding programs. Variations in genotype have been exploited to characterize and manage genetic diversity in germplasm collection. Molecular markers are frequently used to study genetic diversity, to develop conservation strategies, to facilitate their management (Rao, 2004; Sharma et al., 2011; Saeed et al., 2011). DNA fingerprinting techniques have been widely used to analyze the genetic variation and to differentiate of species and or populations (Baloch et al., 2010; Andeden et al., 2013). Recent developments in molecular marker technology and marker assisted selection (MAS) have made tomato breeding more efficient and productive. The developments of DNA markers have greatly facilitated genetic studies in plants, animals and prokaryotic organisms (Mullis, 1990; Erlich et al., 1991; Archak et al., 2002; Haishan et al., 2004; Yediay et al. 2010; Andeden et al. 2011).

Among the several DNA based techniques, random amplified polymorphic DNA (RAPD) was found simple and efficient (Welsh and McClelland, 1990; Williams et al., 1990) and it did not require any kind of sequence information (Gepts, 1993 and Karp et al., 1997). RAPD provides large number of descriptors for the comparison of individual plants and large population. With the availability of this genetic tool genetic diversity can be estimated (Demeke et al., 1996; Chapeo et al., 1992;

Landry et al., 1993; Klocke, 2004). RAPD marker technology can be very useful for tomato germplasm evaluation by providing plethora of information about available genetic variation in the existing gene pool. Ultimately this information will be very helpful in developing new breeding programs. RAPD markers are generated by using short, 10-mer oligonucleotides of arbitrary sequence. These markers are dominant and detect variation in coding as well as non-coding regions of the genome. RAPD analysis is technically simple and suitable for large scale germplasm characterization and can be performed even in a moderately equipped laboratory (Rafalski and Tingey, 1993).

Here RAPD analysis was carried out for the molecular characterization of 21 tomato breeding lines and cultivars. These breeding lines and cultivars were introduced from different countries and commonly used in the Pakistani tomato breeding program. Our objective was to understand the genetic diversity and genetic relationship among various tomato cultivars and breeding to be efficiently used in tomato breeding program.

Materials and Methods

Collection of plant material

Total number of 21 tomato genotypes including 11 breeding lines and 10 cultivars obtained from different countries was used as plant material in this study. Name of the breeding line or cultivars, their origin, some important morphological traits are given in Table 1. Seeds were kindly obtained from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Three seeds from each of cultivars were grown in pots. Leaf samples were collected from each genotype as bulk. Fresh leaves samples (8-10 days) from each tomato cultivars was collected two weeks after germination and immediately stored at -80°C for molecular studies. We use this plant material in RAPD study because it's widely used in the breeding material for research studies in Pakistan.

DNA extraction

Genomic DNA was extracted from leaves according to CTAB protocol of Doyle and Doyle (1990) according to some modification of (Baloch et al., 2010). The concentration of extracted DNA was estimated by comparing band intensity with λ DNA of known concentration, after 0.8% agarose gel electrophoresis and ethidium bromide staining. DNA was diluted to $5\text{ng}\mu\text{l}^{-1}$ for RAPD analysis.

RAPD analysis

PCR was performed in 25 μl reaction volume containing 75 mM Tris-HCl, pH 8.8; 20 mM $(\text{NH}_4)_2\text{SO}_4$; 2.0 mM MgCl_2 ; 0.2 μM Primer; 100 μM each of dATP, dGTP, dCTP, and dTTP; 1 unit of Taq DNA polymerase; and 10 ng of genomic DNA. PCR were performed in thermal cycler Eppendorf AG No. 533300839, Germany), using the following cycling program: one cycle (Hot Start) at 95°C for 5 minutes; 40 cycles of 95°C for 1 min; 34°C annealing temperature for 1 min; 72°C for 2 min followed by one cycle of 72°C for 10 min. A total of 35 polymorphic RAPD primers of GL series were used to amplify the genomic DNA.

Data analysis

Amplification products were analyzed by gel electrophoresis in 1.2% agarose in 0.5× TBE buffer, stained with ethidium bromide, and photographed under ultraviolet. RAPD bands were manually scored as present (1) or absent (0). Only clear and strong bands were recorded and used for analysis. Genetic similarity between all the 21 varieties was estimated by simple matching co-efficient. Genetic similarities were calculated according to the method developed by Jaccard (1908). A Jaccard genetics similarity matrix was used to build an unweighted pair-group method with arithmetic means (UPGMA) and principal component analysis was done using computer software program Palstat. Mean band frequency, gene diversity (H), and Shannon information index was calculated the computer software program Popgene (Yeh et al., 2000). For each RAPD primers the mean polymorphism information content (PIC) of each primer was calculated by using the following formula:

$$PIC = \sum (1 - p_i^2) / n,$$

Where p_i is the frequency of presence (1) for each band, n is the number of bands for each primer (Weir, 1990).

Results

In the present study twenty one tomato genotypes were assessed for the estimation of genetic diversity by using RAPD markers. Initially 21 genotypes were tested for amplification using 35 RAPD primers. 20 out of 35 RAPD primers with good/excellent PCR products were selected for genotyping the whole set of twenty one

tomato accessions. Rest of the primers was not used for diversity analysis as they produced monomorphic or faint banding pattern. Primers selected produced distinct, easily detectable bands of variable intensities. The bands reproducible over repeated runs with sufficient intensity to detect presence or absence with confidence were used for fingerprinting.

Considering all the primers and tomato genotypes, a total of 102 different loci were amplified among 21 genotypes, out of which 75 bands were polymorphic, showing 73.5% of overall polymorphism.

The number of amplification products produced per primer varied in between 2-8 with an average of 5.1 per primer. The maximum number of bands produced by the primer GL J-20 and GL C-09 while the minimum numbers of bands was produced by the primer GL A-09. The highest PIC value of 0.90 was obtained for primers GL A-12, GL B-07 and GL C-13, whereas the lowest was 0.26 for primer GL I-17 with a mean value of 0.66 shown in table 2. Gene diversity (H) and Shannon's Information Index (I) of primers were calculated. The highest value was observed in primer "GL A-12" i.e. 0.99 and 1.42 and the lowest values of 0.12 and 0.21 was noted in primer "GL A-09" with an average of 0.40 and 0.59 respectively as shown in table 2.

Dice genetic distance was calculated among tomato cultivars. Genetic distance between the genotypes ranged 0.19 to 0.83 with mean genetic distance is 0.41. RAPD analysis results showed that the highest genetic distance was found between CLN 1621 L 0.83 and CLN 2116 B 0.62 while lowest genetic distance was noted among BL 1078 0.19 and CLN 2498 A 0.23.

Table 1. Passport data of tomato germplasm used in present study.

Genotypes Name	Origin	Fruits Size and shape	Disease resistant traits
Breeding line			
CLN 2413	Taiwan	Large, Globe	Disease resistance (BW-TMV-F-1)
CLN 2418A	Taiwan	Large, Globe	Heat Tolerance
CLN 2498A	Taiwan		Disease Resistance
CLN 2443A	Taiwan	Long, Pear	Disease Resistance (WTG, BW, TMV, F-1, St)
CLN5915-93D4-1-0-3	Taiwan	Small, Globe	Heat Tolerance
CLN 2001A	Taiwan	Small, Globe	Heat Tolerance
CLN 1621L	Taiwan	Small	Heat Tolerance
CLN 2366A	Taiwan	Medium, Globe	Disease resistance (BW, TMV, F-1)
CLN1466 P	Taiwan	Large, Globe	Disease Resistance (BW, TMV, F-1, St)
CLN2116 B	Taiwan	Medium, Plum	Heat Tolerance
CLN2026D	Taiwan	Medium, Plum	Disease resistance (BW, TMV, F-1, F-2, St)
Cultivars			
BL 1076	Philippines	High-round	Salinity Tolerance
BL 1077	Philippines	Plum	Salinity Tolerance
BL 1078	Philippines	High-round	Salinity Tolerance
BL 1173	USA	Small, Circular	-
BL 1175	USA	Medium, Ovate	-
BL 109	USA	Pear	-
LA 2662	Unknown	High-round	Heat Tolerance
LA 2711	Egypt	Slightly flattened	Salinity Tolerance
LA 3120	USA	Cylindrical	Heat Tolerance
LA 3320	USA	Round,	Heat Tolerance

WTG= Resistance to white fly transmitted geminivirus. BW= Bacterial wilt tolerance. TMV= Resistance to tomato mosaic virus (Tm2a allele). F-1= Resistance to race 1 of *Fusarium oxysporum lycopersici*. F-2= Resistance to race 2 of *Fusarium oxysporum lycopersici*. St= Resistance to *Stemphylium solani* (cause of grey leaf spot) Further information about plant material is obtained from AVGRIS (AVRDC Vegetable Genetic Resources Information System) and TGRC (Tomato Genetic Resource Center)

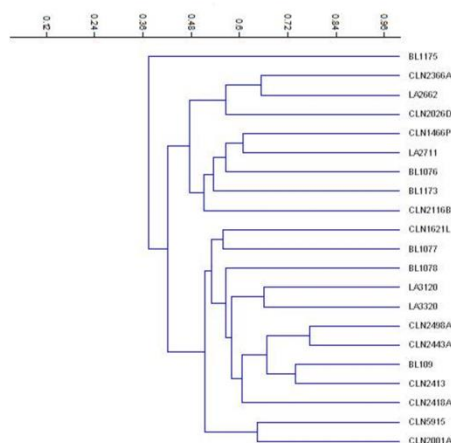


Figure 1 Association among 21 tomato breeding lines and cultivars as revealed by UPGMA cluster analysis of Jaccard genetic similarity coefficient calculated from RAPD data

Multivariate analysis was conducted to generate a similarity matrix using Popgen 32 software, version 1.44 (Yeh et al. 2000). Principle coordinate analysis was constructed as shown in Figure 2. Dendrogram drawn for the genetic distances is shown in the Figure 1. Cluster analysis defined two main groups group A and group B. Group A consisted only one cultivar 'BL1175' while group B consisted of rest of 20 cultivars. Group B again subdivided into two main subgroups B1, consisting of 8 genotypes and sub group B2 consisted of rest of 12 cultivars.

Discussion

Genetic diversity is characteristics of ecosystem and gene pools that describes an attribute which is commonly held to be advantageous for survival. Without genetic diversity it becomes difficult for a population to adapt to environmental changes creating a static population (Denis et al., 2005). DNA fingerprinting is valuable analysis due to its high sensitivity and ability to distinguished close related genotypes. Molecular markers support more detailed characterization of genetic resources. They have great potential to identify the structure of genetic diversity within and among accessions, which is important for optimization of collections; the planning of seed regeneration and the successful implementation of pre-breeding approaches (Yediay et al., 2010). They provide a base line for direct measurement of genetic diversity. Various types of DNA markers studies have been carried out to estimate genetic diversity among different tomato genotypes and phylogenetic relationship among different tomato cultivars. RAPD markers have been widely used for assessing genetic diversity and genome mapping (Welsh and McClelland 1990). It is also used to check the hybrid seed purity of tomato varieties (Liu et al., 2007). The advantages of RAPD are its simplicity, rapidity, requirement for only a small quantity of DNA and the ability to generate numerous polymorphisms (Cheng et al., 1997).

In this work we have reported a diversity analysis of collection of tomato genotypes at the genotypic level. In Pakistan, tomato is grown in Balochistan (Quetta Div),

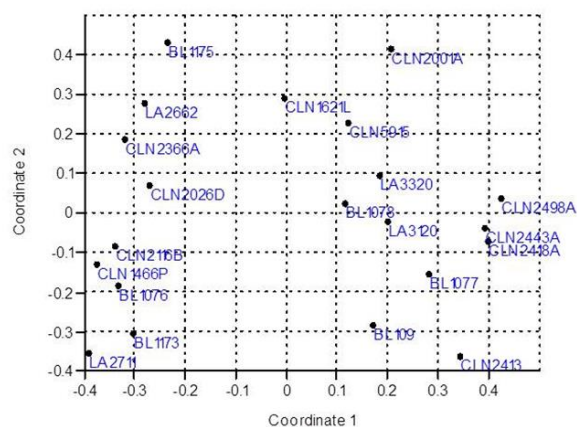


Figure 2 Principal coordinate analysis of 21 tomato breeding lines and cultivars based on RAPD data

KPK (Dargai area) and in Katha Saghral, Katha Misral and Kotli Dakhli villages of Khushab districts in Punjab. The farmers suffer heavy losses due to biotic and abiotic stresses. Tomato is heavily consumed in Pakistan but the breeding effort in Pakistan is negligible. Local hybrids are not available in Pakistan. Most of the tomato cultivars grown in Pakistan are introduced from abroad and there is urgent need to develop tomato cultivars adapted to local agroclimatic conditions of Pakistan and taste preference of the local consumers. For this, there is need to characterize the tomato germplasm available in Pakistan both at genotypic and phenotypic level in order to use them in breeding program effectively to develop cultivars with high yield and processing quality, resistant to insect, pest and diseases. One measure of genetic diversity at the genotypic level is the number of bands produced by each primer. The percentage of polymorphism yielded per RAPD primer ranged from 50% to 100% with an average of 73.5% shown in Table. 2 when compare with (Ercolano et al., 2005) who found average polymorphism of 78%.by using 15 tomato accession with 18 RAPD markers, (Carelli et al. 2006) found 78.6% of genetic diversity among Brazilian tomato cultivars and landraces. (Mirshamsi et al. 2008) analyzed total of 97 bands in 29 tomato lines showing 71% polymorphism using RAPD marker. (Egashira et al. 2000) obtained 435 RAPDs bands among 50 accessions of Lycopersicon using 10 random primers. (Archak et al., 2002) studied 27 tomato cultivars using RAPD marker generate 42 random primers and frequency of polymorphic markers was 63.8%, while (Tabassum et al., 2013) noted 94% of diversity among 11 tomato varieties using 20 RAPD primers and (Sharifova et al., 2013) reported 65.3% studying on 19 tomato genotypes using 6 RAPD primers.

In our study, the average band per RAPD primer was 5.1 compared with (Archak et al., 2002) who reported an average of 4.1 bands per primer in tomato. Kulkarni and Deshpande, (2006) observed 3.5 bands per primer using 12 RAPD markers among 10 genotypes, Huh et al., (2011) reported 6.3 bands per primer by using 80 RAPD primers among 36 cultivars. Naz et al., (2013) found 130 bands among 25 tomato genotypes by 15 RAPD primers.

Table 2 Mean band frequency and annealing temperature of tomato genotypes

Primer Name	Sequence	AT(°C)	TB	PB	P %	MBF	H	I	PIC
GL DecamerA-02	TGCCGAGCTG	34	5	4	80	0.22	0.32	0.49	0.81
GL DecamerA-08	GTGACGTAGG	34	4	3	75	0.16	0.47	0.67	0.88
GL DecamerA-09	GGGTAACGCC	34	2	2	100	0.12	0.12	0.21	0.68
GL DecamerA-12	TCGGCGATAG	34	5	3	60	0.16	0.99	1.42	0.90
GL DecamerA-15	TTCCGAACCC	34	4	2	50	0.25	0.48	0.67	0.68
GL DecamerB-07	GGTGACGCAG	34	4	3	75	0.14	0.31	0.56	0.90
GL DecamerC-03	GGGGGTCTTT	34	5	4	80	0.22	0.37	0.56	0.79
GL DecamerC-09	CTCACCGTCC	34	8	6	75	0.56	0.42	0.61	0.65
GL DecamerC-13	AAGCCTCGTC	34	4	4	100	0.13	0.49	0.68	0.90
GL DecamerC-15	GACGGATCAG	34	7	5	71	0.38	0.39	0.54	0.76
GL DecamerC-16	CACACTCCAG	34	5	4	80	0.19	0.37	0.55	0.84
GL DecamerD-10	GGTCTACACC	34	4	3	75	0.35	0.34	0.49	0.44
GL Decamer I-02	GGAGGAGAGG	34	6	4	66	0.59	0.38	0.56	0.28
GL Decamer I-03	CAGAAGCCCA	34	4	3	75	0.29	0.46	0.65	0.63
GL Decamer I-07	CAGCGACAAG	34	6	5	83	0.42	0.38	0.56	0.61
GL Decamer I-12	AGAGGGCACA	34	5	3	60	0.38	0.48	0.67	0.56
GL Decamer I-17	GGTGGTGATG	34	6	5	83	0.60	0.37	0.56	0.26
GL Decamer J-18	TGGTCGCAGA	34	4	4	100	0.23	0.40	0.59	0.83
GL Decamer J-20	AAGCGGCCTC	34	8	5	62	0.74	0.30	0.45	0.27
GL Decamer L-10	TGGGAGATGG	34	6	3	50	0.32	0.26	0.40	0.59

AT: Annealing Temperature (°C); TB: Total Bands; PB: Polymorphic Bands; MBF: Mean Band Frequency

Most cultivars or breeding lines produced unique amplification profiles sufficient to distinguish them from the other tested genotypes. These results confirm the efficiency of RAPD markers for the identification of plant genotypes (Weising et al., 1995) in particular *Lycopersicon* (Williams and Clair, 1993; Villand et al., 1998; Noli et al., 1999). The combined analysis of the amplification products generated by primer, A-02, A-08, A-09, A-12, A-15, B-07, C-03, C-09, C-13, C-15, C-16, D-10, I-02, I-03, I-07, I-12, I-17, J-18, J-20 and L-10 was enough to assess the genetic diversity among the genotypes. Different primers produced different mean band frequency. The highest mean band frequency was observed in primer GL J-20 (0.74) and lowest in primers GL A-09 (0.12). The mean band frequency for each population ranged from 0.12 to 0.74 with an average of 0.43 (Table 2). The mean band frequency was higher as compared with Phan et al., (2003).

PIC provides a somewhat better estimator of diversity than the raw number of band, because it takes account of the relative frequencies of each band present (Andeden et al., 2013). Here, the mean PIC was 0.76, with 12 of the 20 RAPD markers having a PIC >0.6. For comparison, average PIC value obtained from tomato cultivars and breeding lines were larger than the tomato from China that is 0.40 (Meng et al., 2010) landraces from Japan (AAA) using 10 RAPD primers, Ethiopia (BBB) using 12 RAPD primers and US tomato germplasm using 35 RAPD. The advantage of RAPD technique is easy, less labor required and cost effective. The disadvantages of this study are that the results are not reproducible. Further study is needed to investigate with other marker systems such as ISSR, AFLP, SSR and most recently single nucleotide polymorphism (SNP) markers.

Information deduced from the dendrogram showed that percentage of tomato genotypes did not contribute

significantly in the grouping pattern. Group B1 consisted of breeding lines mostly from Taiwan and group B2 consisted of varieties from different origin. This showed that due to its Latin American origin; similar varieties were introduced to Taiwan, USA and Egypt. It could be possible that same varieties introduced to USA then from USA same material could be used in Taiwan and then introduced to Pakistan by different sources. However, we need to careful investigation of genetic diversity among tomato varieties grown in Pakistan by using SNPs or SSR marker system using large germplasm size.

To have clearer picture, multivariate analyses were utilized to measure the variation in among the 21 tomato breeding lines and cultivars and results are depicted in Fig. 2. The first two PCA components accounted for 74.5% of the total variation, and two dimensional principal component graph was drawn, results of PCA was closely mirroring the cluster analysis. From the PCA graph, it was clear that some genotypes were clearly differentiated from the rest of genotypes and these could be used in tomato breeding program with specified objectives.

In spite of heavy consumption of tomato in Pakistani diet, tomato breeding lags behind than other crops. During the recent decade, scientists at several consultative groups have been investigating the genetic potential. Obviously, search for new genes and breeding for hybrid vigor would best be achieved with the use of new and more diverse materials. The more diversified material is important to start successful breeding programs and producing high yielding tomato varieties that is the need of present world. Here we had provided some information about tomato germplasm used in the breeding program in Pakistan. There is also urgent need to introduce foreign tomato germplasm from different countries particularly its center of diversity such as Latin America to improve tomato cultivars in Pakistan according to local demand.

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