Available Online at ESci Journals
Journal of Plant Breeding and Genetics
ISSN: 2305-297X (Online), 2308-121X (Print)
http://www.escijournals.net/JPBG

GENETIC DIVERSITY ANALYSIS OF RIPENING SPECIFIC GENOTYPES USING POTENTIAL PUBLIC DOMAIN SSR MARKERS IN TOMATO (*SOLANUM LYCOPERSICUM*)

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A B S T R A C T

Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops belongs to the family *Solanaceae* and acts as a model system for genetic and genomic studies. It is highly monomorphic at molecular level due to intensive selection during domestication. However, introgressions from wild species for potential traits led to increased diversity in the present day cultivars. Eventually, it resulted in difficulty in identifying potential breeding material for crop improvement. In the present study, molecular genetic diversity analysis of 29 inbred lines and 3 natural mutant lines, with respect to fruit ripening, was carried out using 245 microsatellite markers derived from sol genomics network database. A UPGMA based genetic tree was constructed using 68 polymoprhic loci, which differentiated the genotypes into two main branches. PCA also supported the UPGMA based genetic diversity analysis with minor exceptions.

Keywords: Tomato, Ripening, Genetic diversity, SSR.

INTRODUCTION

Solanum lycopersicum generally called as tomato is one of the most important vegetable crops, originated in South America and later domesticated into various parts of the world (Bolger *et al*. 2014). Tomato belongs to the family *solanaceae*, which comprises of 100 genera and 2500 species including various plants of agronomic importance (Olmstead *et al*. 2008). Tomato genome is relatively small in size, i.e. 950mb, and it is one of the most intensively studied genome among all other *solanaceae* species (Ichihashi and Sinha 2014). Over 7500 landraces and varieties of tomato have been successfully bred and grown for different purposes across the world. Plant variety registration organizations in different countries maintain the record of most of these genetic resources. This material is important for genetic and genomic studies which in turn has potential value for tomato industry. Understanding the genetic background, diversity and relationships will help in successful collection, preservation and utilization of the genetic resources (Korir *et al*. 2014).

Genetic diversity in the cultivated tomato is considerably very low due intensive selection during and after the domestication process. This narrow diversity makes it difficult to identify the molecular markers that can differentiate the present day breeding material. However, sequence based markers like Simple Sequence Repeat (SSR) markers have been applied successfully for genotyping tomato cultivars and accessions (Benor *et al*. 2008a).

Although a variety can be distinguished by its morphological characters, genotypic difference cannot be quantified as it can be altered by environmental factors. In contrast, molecular markers such as SSRs can be used effective tool in identifying a variety since they are independent of environmental factors (Korir *et al*. 2012). Among all other markers, SSR markers have an advantage in variety identification because of their co-dominance nature, reproducibility, multi allelic variation, abundance and genome coverage. The tandem repeats can vary in number in a given sequence and

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many of them can exist in a population, which are termed as alleles (Barchi *et al*. 2011). SSR markers are considered as most polymorphic markers and have been used in the genetic diversity analysis as well as pedigree construction and genetic mapping and for comparative mapping (Shirasawa *et al*. 2013). Although SSRs invented in early 90's, their application was limited by the amount of sequence data, therefore, only few of them were reported (Varshney *et al*. 2005). With the advent of novel sequence technologies, increase in the amount of sequence data from Expressed Sequence Tag (EST) projects in tomato and several other plant species made it easier in identification of genomic SSRs in large number (Zhao *et al*. 2011). Over 18,208 SSRs have been submitted to sol genomics network and made it available to public use by June 2012. In addition, many more markers found in other data bases and laboratories across the world (Korir *et al*. 2012).

The generation and characterization of EST based SSR markers in tomato and their cross species amplification in its closely linked species and varieties have been done with a total of 7599 microsatellite markers, which are being generated through data mining (Shirasawa *et al*. 2010). For instance, only 20 SSR markers differentiated 468 of 521 European tomato verities (Bredemeijer *et al*. 2002). Confirmation of SSR applicability in genetic diversity analysis was carried out by screened against a set of 19 diverse tomato cultivars (He *et al*. 2003). Although they are expensive without sequence information for primer designing, the developed primer sets for tomato makes their utilization cheaper (Shirasawa *et al*. 2013). Furthermore, studies indicated the reproducibility of SSR markers in closely related species & cultivars (Zhao *et al*. 2011). In the present study we investigated 32 tomato genotypes including 29 inbred lines and 3 natural mutant genotypes specific for fruit ripening to establish the genetic relationships through diversity analysis using SSR markers.

MATERIALS AND METHODS

Plant materials: Seed from a total of 32 tomato genotypes showing contrasting phenotype with respect to fruit ripening were collected from in house germplasm and planted in a glass house. Leaf samples were collected at true leaf stage for extraction of total genomic DNA used in this study. The genotypes comprised both inbred lines and natural mutant lines for delayed ripening (supplementary file 1). Total genomic DNA of each genotype was extracted from young leaves using the modified cetyl tri-methyl ammonium bromide method (Murray and Thompson 1980; Bousquet *et al*. 1990) and 0.8% agarose gels were used to determine the quality of DNA. The isolated DNA was then diluted to a final concentration (20 ng/ul) with 1X TE buffer for further use.

Genotyping and data analysis: Total 245 microsatellite markers were chosen on the basis of their chromosomal location and annealing temperature. Originally these markers were screened from the Solgenomics database (http://www.sgn.cornell.edu). The oligos were commercially synthesized by SIGMA-ALDRICH CO. LLC, Hyderabad and used for genotyping. The 245 pairs of tomato SSR primers were used to perform PCR amplification in a 10-μL reaction system containing 1μL 20 ng/μL genomic DNA, 0.1μL 10 pmol of each primer, 0.1μL 5U/μL Taq DNA polymerase, 1μL 10X buffer and 0.75μL 2.0 mM dNTPs and the final volume was adjusted to 10μL. Amplification was performed in Kbio-Sciences Hydro-cycler using initial denaturation for 4 min at 94°C and 35 cycles of denaturation at 94°C for 30 s, corresponding annealing temperature for 45 s, extension at 72°C for 1 min 30 s, followed by a final extension step at 72°C for 7 min. PCR products were subjected to electrophoresis using 3% agarose gels to check the DNA banding patterns. Polymorphic SSRs were scored using binary coding system. Population genetic parameters estimation and phylogenetic analysis were carried out using the PowerMarker V3.25 software(Liu and Muse 2005), including the number of alleles (NA), genotype, genetic diversity and PIC. All genotypes were subjected to calculate a frequency based distance matrix using shared allele method. Tree building was carried out sing unweighted pair group method with arithmetic mean (UPGMA) via bootstrapping. Principle coordinates were calculated using GenAlEx 6.502 (Peakall and Smouse 2012) application in MS Excel.

RESULTS AND DISCUSSION

Genetic diversity between tomato genotypes was investigated on the basis of SSR marker polymorphisms and genotypes were classified based on allele frequencies at each locus examined. Out of the original 245 microsatellite markers used to test the genetic diversity of 32 tomato genotypes, 68 (27.75%) markers were polymorphic, 147 (57.95%) were monomorphic while 35 (14.28 %) primers failed to amplify the expected PCR fragments. All the polymorphic markers yielded amenable and reproducible amplicons to the

detection of a total of 155 alleles (Supplementary file 2). Surprisingly, polymorphic markers covered all the 12 chromosomes with minimum number of 2 markers on Chromosome 8 and 11, and maximum number of 9 markers on Chromosome 9. This supports the statement that chromosome 9 harbors most of the disease resistance and fruit quality related genes (Doganlar *et al*. 2000; Labate and Robertson 2012; Sim *et al*. 2012a). The number of alleles at each SSR locus ranged from 2 to 11, with an average of 2.87, which is comparable to the polymorphisms at SSR loci reported in tomato (Benor *et al*. 2008b). In addition, the average PIC in this study was 0.34 compared to 0.06 (TES1802) and 0.87(TES1724) reported in similar studies in other tomato populations (Tam *et al*. 2005; Benor *et al*. 2008b). A genetic tree constructed by using UPGMA differentiated the genotypes into two conspicuous branches (Figure 1).

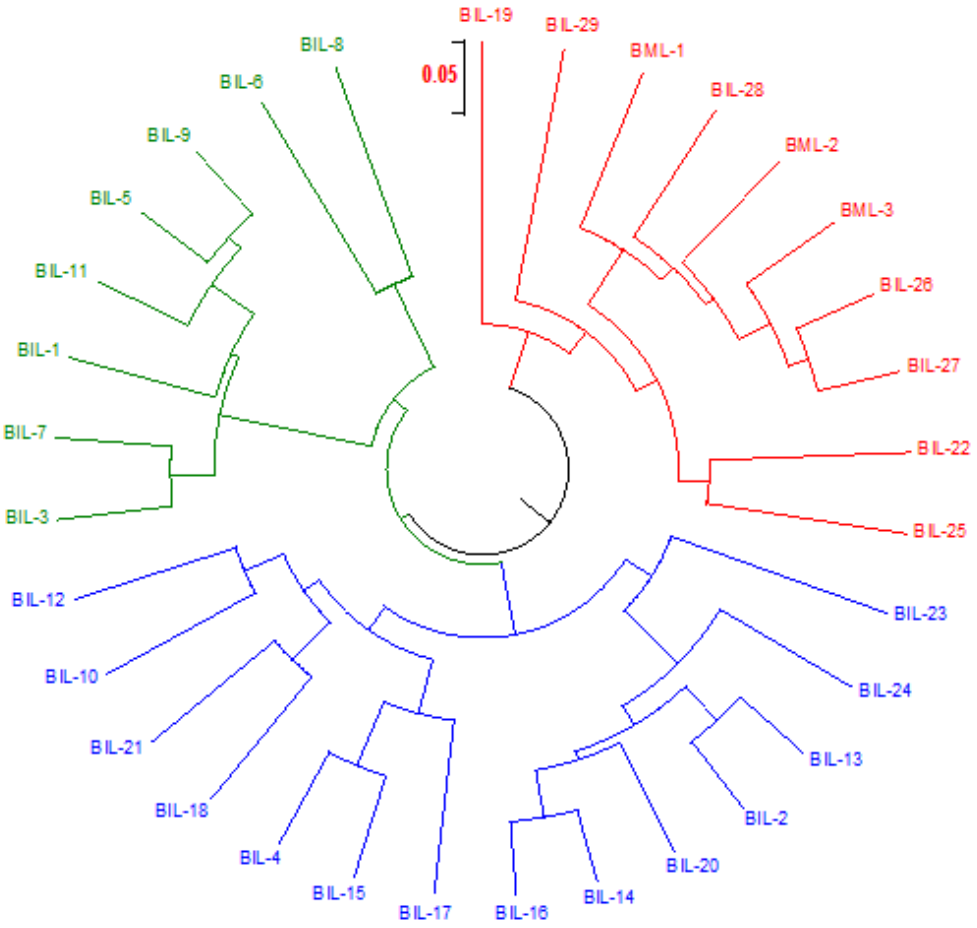

Figure 1. Genetic tree of 32 tomato genotypes based on SSR data as clustered using UPGMA algorithm.

As shown in the tree plot, the first branch consists of 3 mutant lines BML-1, BML-2 and BML-3 grouped together with estimated hierarchy along with inbred lines BIL-19, BIL-22, BIL-25, BIL-26, BIL-28 and BIL-29. Which indicates 3 mutant lines along with 7 inbred lines shared the similar genetic background and derived from the common ancestor. On the other hand, the second branch formed by the 22 inbred lines is sub divided into two minor branches. Minor branch I consists inbred lines BIL-8, BIL-6, BIL-9, BIL-5, BIL-11, BIL-1, BIL-7 and BIL-3. The minor branch II consisting inbred line BIL-12, BIL-10, BIL-21, BIL-18, BIL4, BIL-15, BIL-17, BIL-16, BIL-14, BIL-20, BIL-2, BIL-13, BIL-24 and BIL23. This suggests that, even though the 22 lines grouped as a major cluster, genome introgression from the other genetic background might diversified the cluster in to two minor branches (Sim *et al*. 2012b). SSR based genetic tree building for 32 genotypes is very distinct, which is in agreement with the general degree of diversity in cultivated tomato varieties. The results showed that the tomato genotypes tested have a relatively good genetic diversity in contrast to the inbreeding depression(Aflitos *et al*. 2014).

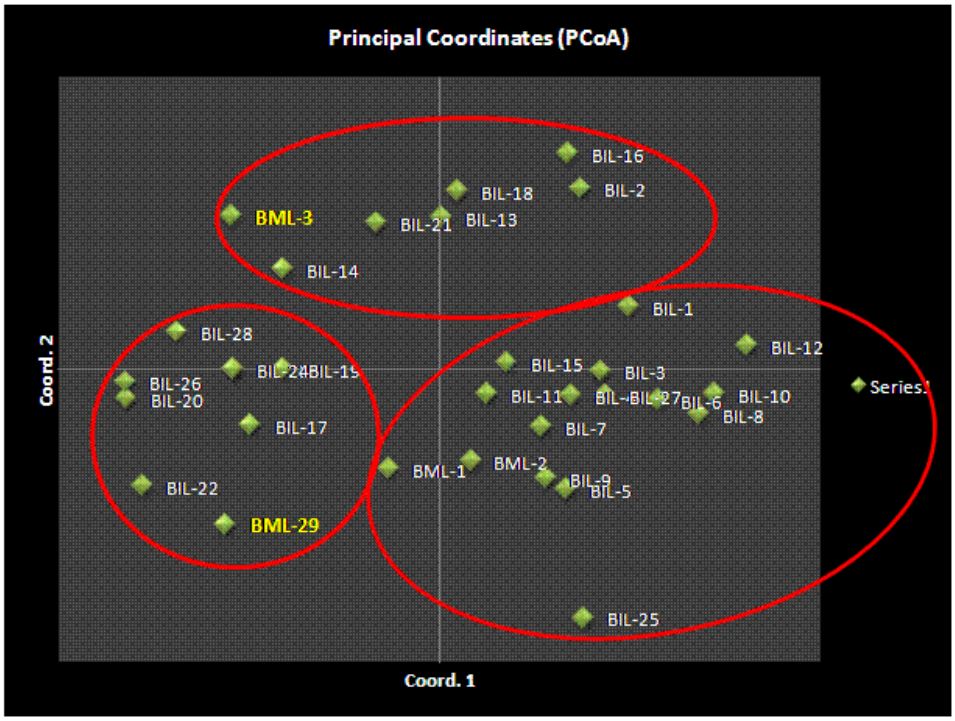

Figure 2: Relationships between tomato genotypes revealed by PCA based on SSR data.

Principal component analysis (PCA) also supported the UPGMA based tree plot with minor exceptions. 15 components contributed to the total variation among the given genotypes. Out of which, three components were extracted based on their contribution. The amount of contribution for each was 21.60, 16.63 and 13.24 for PC1, PC2 and PC3 respectively. The cumulative contribution for the variation was about 51.47. A 3D scattered plot was generated using first three components to visualize the clustering of the 32 genotypes (Figure 2). BIL-12, BIL-16 and BIL-6 shown positive correlation towards variation, while 26, 25, 29 shown least negative correlation -0.206, -0.254, -0.218 at PC1, PC2 and PC3 respectively. Total 3 clusters were formed along with the 2 coordinates representing the UPGMA tree plot. While some genotypes were consistent with the genetic tree some genotypes were shuffled to the other groups in response to their geometric distribution based on the variation component. Genotypes BIL-29, BML-2, BIL-27 and BIL-25 of Branch-I of tree plot moved to Cluster II of the PCA scattered plot, while BIL-3 of Minor branch-I of tree plot moved to cluster II of the scattered plot. Similarly, genotypes BIL-12, BIL-10, BIL-4, BIL-5, BIL-17, BIL-14, BIL-20, BIL-24 and BIL-23 of Minor branch II of genetic tree moved to the Cluster II and cluster I of the PCA scattered plot respectively.

There were, however, some outstanding materials such as BML-3, BIL-26, BIL16, BIL-1, BIL-11, BIL-5 and BIL-9 which were comparatively distant from other genotypes in both the genetic tree and PCA scatter plot. The reasons for this shuffling of the genotypes needs further research as it may point to special tomato lines with unique breeding and production value. Classification of genotypes via diversity analysis based on SSR markers will have a greater advantage because, SSRs have higher polymorphisms and are more discriminative due to co-dominant inheritance. Although single nucleotide polymorphisms (SNPs) and insertion-deletion (In-Del) markers are also informative markers, the phylogenic analysis using SSRs in tomato has been shown to be consistent with known pedigrees and previous marker evaluation (Tam *et al*. 2005). When PCA and Genetic diversity analysis of the SSR results are compared, the results bring out the complexity in the relationship between the genotypes. Consequently, studies on the genetic relationships between species should integrate the use of these 2 complementary methods as well as additional strategies to give mutual authentication and subsequently more accurate and reliable results.

CONCLUSION

Even though the polymorphism level in tomato is very less the genotypes analyzed in the present study were

showing good level of polymorphism. A combined strategy of UPGMA clustering and PCA have been successfully applied in estimating the diversity and pattern of distribution 32 genotypes. Based on these results BML-3, BIL-26, BIL16, BIL-1, BIL-11, BIL-5 and BIL-9 found to be excellent breeding material and can be exploited to develop mapping population specific for fruit ripening.

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