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Molecular characterisation in tomato (*solanum lycopersicum* L.) - A review

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Abstract

Tomato (*Solanum lycopersicum* L.) is most important fruit vegetable grown worldwide for its high nutritive value. Traditional genetic markers and breeding methods have several defects that reduce the ability to estimate genetic diversity in plants as it highly dependent on the environment for expression. Among the available genetic rnarkers *viz.,* morphological, cytological, biochemical and molecular (DNA), Molecular markers are an effective tool for efficient selection of desired agronomic traits because they are based on the plant genotypes and also are independent of environmental variations. Researchers have been calculated genetic variation in tomato landrace and cultivar collections using several molecular techniques including AFLP, RAPD, ISSR, SSR and SNP. An overview is conducted considering some useful above mentioned molecular markers to initiate systematic breeding programme on tomato improvement.

Highlights

- Genetic characterisation is first step of plant breeding for crop improvement.
- Molecular markers have beenextensively used for genotyping andcharacterization of genotypes.

Keywords: Tomato, molecular markers, RAPD, AFLP, polymorphism, genetic diversity

The genome of tomato plant is one of the most investigated plant genomes (Foolad 2007). Molecular markers have proven to be valuable tools in the evaluation of genetic variation both within and between species (Powell *et al*. 1996). The analysis of genetic diversity and relatedness between or within different species, populations and individuals is a prerequisite towards effective utilization and protection of plant genetic resources (Weising *et al.* 1995). In addition, the characterisation of much diversified materials with molecular markers offers a unique opportunity to define significant marker-trait associations of biological and agronomic interest.

Random amplified polymorphic DNA (RAPD) marker studies

El-Hady *et al*. (2010) carried out biochemical and molecular characterization of eight tomato varieties based on RAPD markers. A total number of 81 amplified DNA bands were generated across the studied genotypes with average of 11.57 bands / primer. 37 bands out of the total number were polymorphic and 19 were unique. Combination of the all data derived from the SDS-protein markers of both water soluble and non-soluble proteins produced a dendrogram almost similar to that obtained by the RAPD analysis. It could be concluded that, both of SDS-Protein and RAPD

markers are equally important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied varieties of tomato.

RAPD and sequence-related amplified polymorphism (SRAP) markers used to evaluate polymorphisms in 15 tomato genotypes. Eleven SRAP primer combinations were used and 66 bands were scored. The number of bands scored per primer combination ranged from three to 12, with a mean of six alleles per primer combination. All fragments scored for each primer combination were polymorphic. The percentage of polymorphic products ranged from 25 to 80%. The 15 tomato genotypes were screened for RAPD markers using 50 primers in a PCR-based DNA amplification procedure; 46 primers produced clear and good amplification. Ten of these 46 primers amplified monomorphic fragments in the tomato genotypes. A dendrogram was constructed by combining data from the RAPD and SRAP analyses. Similarity ratios of genotypes ranged from 0.87 to 0.99 (Comlekcioglu *et al*. 2010). Naz *et al*. (2013) accessed 15 polymorphic RAPD primers for the genetic distance calculation to find out the phylogenetic relationship among 25 tomato accessions. A total of 130 loci were generated out of which 98 were polymorphic by 15 primers with 05-14 loci/primer having fragment's size range from 400 to 2500bp maximum. The average genetic similarity observed across all the genotypes was 75.6% with 24.4% polymorphism in 25 tomato accessions. Although RAPD study supports the morphological characters but not upto 100%.

Mazzucato *et al*. (2008) characterized sixty one diversetomato genotypes by 15 morpho-physiological traits and 29 simple sequence repeat (SSR) loci. The low molecular polymorphism reported in tomato modern cultivars, author's data reveal a high level of molecular diversity in landraces. Such diversity has allowed the inference of the existence of a genetic structure that was factored into the association analysis. As the proportion of significant associations is higher between the Q-SSR subset of markers and the subset of traits related to fruit size and shape than for all of the other combinations. Finally author concluded that this approach is valid for establishing true positive marker-trait relationships in tomato.

19 Azerbaijan Tomato genotypes are analysed with RAPD markers. A total of 26 amplified products were revealed by 6 primers. The genetic similarity among evaluated genotypes ranged from 0.188 to 1.000. The lowest similarity was observed between cultivars 'Azerbaijan' and 'Shakar' (0.188), while the highest between 'El-nur' and 'Garatag' (1.000). The most polymorphic primer was OPB-18 that presented a genetic diversity index of 0.823, while the least informative was primer OPG-17 with an index of 0.349. The average genetic diversity calculated from RAPD data was 0.665 (Sharifova *et al*. 2013).

Pal and Singh (2013) analyzed *Solanum lycopersicum* F_1 hybrids and its parents (male and female) with 22 different decamer RAPD primers to identify the band pattern which could differentiate between the parents and hybrids. Out of these 22 primers only 5 primers, OPA-05, OPA-09, OPN-14, OPG-04 and OPK-02 showed polymorphic bands between male, female and hybrids. Since a lot of chances are there for contamination by selfing of female parental lines and out crossing with other plants during hybrid development programs. This seriously affects the hybrid production quality. So molecular marker tools can be effectively used to find out such contaminations in DNA polymorphism of respective hybrids.

Genetic diversity of 11 tomato varieties are assessed through RAPD analysis. Twenty arbitrary oligonucleotide primers produced a total of 584 different marker bands with an average of 29.2 bands per primer (Tabassum *et al*. 2013). Based on the banding pattern 94.168% polymorphism observed among the tomato varieties. Size range of amplified DNA bands varied from 0.1 - 10 kb. A total of 15 unique bands were amplified from the genome of the 11 tomato varieties. The values of pair-wise genetic distances ranged from 0.1838 - 0.9049, indicating the presence of wide genetic diversity. The highest genetic diversity (0.9049) found between the variety BARI Tomato 8 and RATAN whereas the lowest (0.1838) between the variety BARI Tomato 9 and MADHURI.

Genetic diversity in 19 tomato varieties using RAPD primers was analysed. Twenty seven primers produced 442 of main bands, out of which 312 were polymorphic bands (70.5%) and 70 were monomorphic (15.8%).DNA amplification products ranged in their size from 250 bp (OPA-01, OPU-14, OPX-15,OPX-19,OPT-08) to 2755 bp (OPX-18).

The highest number of polymorphic bands (21 bands) was produced by primer OPU-03 while, the lowest number of polymorphic bands (3 band) was produced by both primers OPA-14 and OPB-17. The primer efficiency ranged from 0.13 in (primer OPC-09) to 0.02 in (primer OPB-17). The lowest genetic distance was (0.2294) between varieties Oula and Shadylady, while, the highest genetic distance was (0.9459) between varieties Fotton and Special pack (Thamir *et al*. 2014).

Shah *et al*. (2015) studied molecular characterization of 21 tomato genotypes 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.

Simple Sequence Repeats (SSR) marker studies

The genetic diversity of 39 determinate and indeterminate tomato inbred lines collected from China, Japan, S. Korea, and USA. Using 35 SSR polymorphic markers, a total of 150 alleles were found with moderate levels of diversity, and a high number of unique alleles existing in these tomato lines. The mean number of alleles per locus was 4.3 and the average Polymorphism Information Content (PIC) was 0.31. Genetic similarity value of 0.85 grouped the inbred lines into four groups, where one USA cultivar formed a separate and more distant cluster, whereas the most different lines are from USA (Us-16) and Japan (Ja-2) with determinate and indeterminate growth habit, respectively (Benor *et al*. 2008).

El-Awady *et al*. (2012) screened ten cultivars of tomato with 20 simple sequence repeat (SSR) primers in order to determine genetic identities, genetic diversity and genetic relationships among these cultivars. On an average, 38 alleles were amplified using SSR primers with scorable fragment sizes ranging from approximately 75 to 275 bp. 23 alleles were polymorphic thus revealing 60.5% of polymorphism. The genetic similarity estimated according to SSR data was scaled between 17.6 and 93.2%, suggesting the potential of SSR markers in discriminating among plants of close or distant genetic backgrounds.The genetic diversity and relationship of 42 tomato varieties was examined with EST-SSR markers. The genetic diversity was between 0.18 and 0.77, with a mean of 0.49; the polymorphic information content ranged from 0.17 to 0.74, with a mean of 0.45. This indicates a fairly high degree of diversity among these tomato varieties.The high degree of polymorphism and the large number of bands obtained per assay shows that SSR is the most informative marker system for tomato genotyping for purposes of rights/protection and for the tomato industry in general (Korir *et al*. 2014).

Singh *et al*. (2014) screened a collection of twenty four determinate and indeterminate cultivars of tomato with twenty SSR (simple sequence repeat) primers and four lycopene gene specific primers in order to determine genetic identities, genetic diversity and genetic relationships. On the basis of resolving power, primer T-45, T-62 and T-106 were most significant as they are able to recognize all 24 genotypes. The gene diversity was varied from 0.65 to 0.97 values with a mean diversity of 0.84. On an average, 54 scorable and reproducible alleles were amplified using all primers. UPGMA based dendrogram grouped the cultivars into two main cluster with two individual separated at one end of the dendrogram. Cluster analysis clearly showed that some genotypes are closely related while some are significantly distinct.

Single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) marker

Hu *et al*. (2012) used Twenty-six morphological traits as well as 47 single nucleotide polymorphism and simple sequence repeat markers to investigate genetic variation in 67 tomato (*Solanum lycopersicum* L.) varieties. Approximately 65.0% of the morphological traits and 55.3% of the molecular markers showed polymorphisms in the 67 varieties. Average taxonomic distance between any two varieties

ranged from 0.6643 to 1.1776, while Nei's genetic distance varied from 0 to 0.2022. Cluster analysis indicated that 67 varieties could be grouped into three clusters.

Amplified fragment length polymorphism (AFLP) marker

Berloo *et al*. (2008) performed a diversity study on a diverse set of 94 cultivated tomato cultivars, representing a wide spectrum of phenotypes for quality related traits. on these cultivars, using information of 882 AFLP markers, of which 304 markers had a known map position. The AFLP markers were scored as much as possible in a co-dominant fashion. Mapped markers and unmapped markers were used to investigate population structure. A clear substructure was observed which seemed to coincide with a grouping based on fruit size. Linkage disequilibrium was observed over considerable (genetic) distances and amount of genetic variation in set of cultivars is limited, but that there exists scope for association studies.

ISSR (inter-simple sequence repeat) marker

Mansour *et al*. (2010) investigated three different molecular marker systems, namely RAPD (randomly amplified polymorphic DNA), ISSR (inter-simple sequence repeat) and IRAP (inter-retrotransposon amplified polymorphism) to detect genomic variation within 10 tomato cultivars. Different dendrograms constructed for the RAPD, ISSR and IRAP results individually and collectively reveal that similarity and clustering are highly dependent on the marker system used.

Ten ISSR primers were individually amplified to allow the differentiation of the 96 tomato accessions. All ten primers generated 144 DNA bands, 53 being polymorphic, with an average of 14.4 per primer. The profiles generated by primer 840-(GA)8YT contained the largest number of polymorphic bands (13 bands). The primer 855-(AC)8YT detected the greatest differentiation of the accessions (12 accessions) while the primer HBH-884(AG)7 did not detect any. DNA profiles based on ISSR markers revealed the potential of the digital fingerprints in the diagnosis of all the accessions. From the results obtained in this study, ISSR markers have a high efficiency to differentiate the germplasm of wild species (Aguilera *et al*. 2011).

SCoT and ISSR were used for genetic diversity analysis of 10 tomatoaccessesions. Using 10 selected SCoT primers 83 bands were generated, of which 30 (36.14%) were polymorphic. 10 selected ISSR primers amplified 86 bands with 20 (23.25%) being polymorphic. Average Polymorphic Information Content (PIC) values for SCoT and ISSR markers were 0.142 and 0.088 respectively. Mean Resolving Power (RP) values for SCoT and ISSR markers were 1.88 and 1.55 respectively. The 10 accessesion were clustered into 3 major groups based on the SCoT analysis and 2 major groups based on the ISSR analysis with UPGMA. The results also demonstrate that the SCoT marker system is useful for identification and genetic diversity analysis of tomato accessesions. In general, SCoT molecular marker was informative than ISSR molecular system (Shahlaei *et al*. 2014).

Conclusion

The analysis of genetic diversity between or within different species, populations and individuals is a prerequisite for effective breeding programme.Molecular markers associated with genes or QTLs have beenreported for numerous economically-important traits in tomato. Such marker information should be useful for improvingqualitative or quantitative traits in tomato via markerassistedbreeding. So these markers are effective tool for efficient selection of desired agronomic traits based on the plant genotypes and also are independent of environmental variations.

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