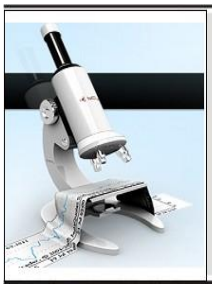

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Studies on genetic diversity using SSR marker associated traits in tomato genotypes (*Lycopersicum esculentum* L.)

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ABSTRACT
Tomato (*lycopersicum esculentum L.*), an economically important crop, the research work carried with 18 genotypes for studying its genetic diversity using five SSR markers. The result suggested the existence of high diversity among the tomato genotypes. High genetic diversity was registered between the genotypes *i.e*., LE-150 and LE-22. Among the 18 tomato genotypes LE-150 and LE-22 showed highest similarity as revealed by clustering using SSR markers. Similarity coefficient of 18 genotypes using SSR markers ranged from 0.1 to 0.4. The clustering done using UPGMA was further confirmed by DARWIN (6.0 version). These genotypes may be crossed among themselves to obtain high yielding F_1 hybrids and best segregants in the later generations. SSR markers detected medium locus polymorphism among the 18 tomato genotypes, which could further be utilized in strengthening tomato breeding programme.

Keywords: genetic diversity, molecular marker, polymorphism, SSR markers.

Introduction
Tomato (*Solanum lycopersicum* L.), (2n = 2x = 24) is an important and widespread vegetable in the world for fresh consumption and processed products. However, the reduction of genetic variation in tomato (*Solanum lycopersicum* L.) through domestication and breeding has resulted in the need for conservation and utilization of all existing genetic resources. In Tamil Nadu, tomato landraces were widely cultivated till the introduction of hybrids. Currently, very few farmers still grow them only for their own or local consumption.

The genetic diversity of tomato has been investigated using Simple Sequence Repeats (SSRs). With the exception of SSRs, limited information was obtained due to a lack of variability that was ascribed to the self-pollinating nature of modern tomato cultivars combined with their narrow genetic base (Alvarez *et al.*, 2001) [2]. Due to domestication and generation of modern varieties, the tomato has undergone intensive selection, and cultivated varieties have narrow genetic diversity relative to other crops. This narrow diversity makes it difficult to identify molecular markers that are polymorphic in modern breeding material. However, a number of polymorphic microsatellite markers generated from database sequences have been successfully used for genotyping tomato cultivars and accessions (He *et al.*, 2003). Microsatellite or simple sequence repeat (SSR) marker has been used in plant diversity analysis; the popularity of these markers is due to their ease of amplification by polymerase chain reaction (PCR), their co-dominant nature and their typically high levels of allelic diversity at different loci. There are numerous reports suggesting the usefulness of microsatellite markers for measuring the genetic variability in a wider taxonomic range (Banhor S *et al.*, 2008) [4]. Also, recently there are few reports regarding the utilization of SSR to study the genetic relationships among the tomato varieties (Bebeli, 2008). The objective of the present study was to analyze the genetic diversity of some tomato varieties from Tamil Nadu and to compare the effectiveness of SSR.

Materials and Methods
Molecular characterization of eighteen tomato cultivars (*Lycopersicon esculentum* L.) accessions were used in the present investigation and were collected from different geographical regions of Tamil Nadu.

Isolation of Genomic DNA

The fresh vegetative leaves were collected for the isolation of DNA. Total genomic DNA was isolated from leaf samples by using CTAB following the standard protocol of Doyle and Doyle (1990) with some modification. DNA was further purified by RNase digestion followed by extraction with phenol / chloroform / iso-amyl alcohol and ethanol precipitation.

The mortar and pestle were precooled by adding liquid nitrogen and the leaf sample was transferred to the mortar and ground to find powder. Immediately the powder was transferred into into a 15ml plastic conical tube containing 5ml 2% CTAB buffer (2% w/v CTAB; 1.4 M NaCL;20 Mm EDTA; 10mM Tris- HCL, pH 8.0; 1% sodium sulphite; 2% v/v- ß-mercaptoethanol). The mixture was incubated in a water bath at 65˚C for 20 min with occasional manual shaking. An equal volume(5ml) of chloroform: Iso-amylalcohol (C:IAA=24:1) mixture was added to each tube and the samples were centrifuged at 3000 rpm for 15 min. After centrifugation aqueous layer equal volume of cold (kept at -20˚C) isopropanol was added and the content were gently mixture by inverting and tubes were kept at -20˚C for 10 min. The samples were centrifuged at 8000 rpm for 15 min. supernatant was decanted.

The pellet was washed in 1 ml 70% ethanol 3 times and air dried. In order to remove RNA, 200µl of low salt TE buffer and 3 µl RNase (stock 10 mg/ml) were added to each tube containing dry pellet and mixed properly. The solution was incubated at 37˚C for 30 min. At the end of incubation, 200µl of chloroform: isoamyl alcohol (24:1) mixture was added to each tube and centrifuged at 3000 rpm for 15 minutes. The aqueous layer was transferred to the fresh tubes, 15µl (approximately $1/10^{th}$ volume) 3M sodium acetate and 300 µl (2 µl vol.) 100% ethanol was added and subsequently placed in freezer for 5 minutes. Tubes were centrifuged at 8000 rpm for 15 minutes. Supernatant was decanted. Pellets were allowed to air dry for one hour. Completely dried pellets were resuspended in 400 µl of 1XTE buffer, and dissolved DNA samples were kept in 4˚C.

DNA quality and quantity estimation

The concentration of DNA was estimated spectrophotometrically and also by gel electrophoresis using 0.8 per cent agarose with known concentrations of DNA. In spectophotometric analysis, 5µl of DNA was diluted to 3000 µl of TE buffer. Spectrophotometer reading were recorded at 260 nm. A good DNA preparation generally exhibits the following spectral properties A260 / A280 = 1.80. DNA concentration was calculated using OD values at 260 nm using the following formula. Concentration of DNA (µl / ml)=OD at 260 nm x 50 To test the quality of DNA, samples were run on 0.8 per cent agarose gel in 1 X TAE buffer and stained with ethidium bromide. DNA was evaluated by comparing it with a standard digested DNA sample.

Amplification of SSR markers

PCR reactions were performed in 25 µl reaction volume containing 1 U of Taq DNA polymerase (Thermo), 2 mM dNTPs (Thermo), 1.5 µl MgCl2 (25 mM), 2 µl primer (100 pm, Bioron Co.) and 1.0 µl DNA (100 ng). The reaction mixture was vortexed and centrifuged briefly. PCR was initiated by an initial de-naturation step for 4 min at 94ºC followed by 35 cycles (94ºC/45 sec, 40ºC/30 sec, 72ºC/2 min), and then a final extension cycle at 72ºC for 5 min. The PCR prod-ucts were separated on 2.5% agarose gel in 1X TBE buffer containing ethidium bromide and visualized on a UV transilluminator and photographed using a Gel Documentation System (Alpha Innotech). The list of 5 SSR primers were listed in Table 1.

Table 1: List of SSR primers used is Study

Primer code	Primer sequence
T-7	F:5'GTGGATTCACTTACCGTTACAAGTT-3' R:5'CATTCGTGGCATGAGATCAA-3'
T-57	F:5'GTGGACCATTTCAAGTTCAACA-3' R:5'TGAATGACATCCATCCATGA-3'
T-62	F:5'-GTGACCACATGAGATATCCAGA-3' R:5'-CAGTTGTCCATATTGTGTGGG-3'
T-70	F:5'-AACATGCGGAGAAAAATT-3' R:5'-GGAACACGTCCCAAAAATGT-3'
T-107	F:5'-GCACAAATAATTTTTCAAGACCAA-3' R:5'-AAAAACGGACATAGCTTTGTACT-3'

Results and Discussion

The studies was carried out to assess the magnitude and nature of genetic variability at the morphological level and as well as at the molecular level among 18 genotypes of tomato using 5 SSR markers. The broad sense heritability estimates were high for all the parameters. In traits with high heritability, genotypic variance is more than environmental variance and these characters could be considered and exploited for selection in earlier generations. Whereas, in the traits with low heritability, influence of environmental factors is strong for their expression and genotype selection based on these characters should be postponed to the later generation Table-2.

Table 2: SSR primers used for assessment of genetic diversity among tomato genotypes

Primer code	Primer sequence	Ta (°C)	Number of scorable bands per primer	No. of Monomorphic bands	No. of Polymorphic bands	Exact amplicon size (bp)
T-7	F:5'GTGGATTCACTTACCGTTACAAGTT-3' R:5'CATTCGTGGCATGAGATCAA-3'	55	15	11	7	100-700
T-57	F:5'GTGGACCATTTCAAGTTCAACA-3' R:5'TGAATGACATCCATCCATGA-3'	58	16	10	8	100-600
T-62	F:5'-GTGACCACATGAGATATCCAGA-3' R:5'-CAGTTGTCCATATTGTGTGGG-3'	58	18	0	0	200-500
T-70	F:5'-AACATGCGGAGAAAAATT-3' R:5'-GGAACACGTCCCAAAAATGT-3'	54	12	10	2	350-650
T-107	F:5'-GCACAAATAATTTTTCAAGACCAA-3' R:5'-AAAAACGGACATAGCTTTGTACT-3'	55	15	13	2	150-200

Genetic diversity is the basic requirement for successful breeding programme. Collection and evaluation of genotypes of any crop is pre-requisite for any programme, which provides a greater scope for exploiting genetic diversity. The results indicated significant genetic variation among 18 tomato genotypes of interest. In the present inquiry, the genetic diversity using SSR markers, revealed that the number of alleles detected varied from 1.00-2.00 alleles per locus with average PIC value 0.3623 for SSR Table 3.

Table 3: Parameters used for the evaluation of polymorphism in SSR markers

S. No	Primer	Sequence5′→3′	PIC	Major allele Frequency	No. of Alleles	Genetic Diversity	Heterozygosity
1	T-7	F:5'GTGGATTCACTTACCGTTACAAGTT-3' R:5'CATTCGTGGCATGAGATCAA-3'	0.3742	0.5278	2.0000	0.4905	0.7222
2	T-70	F:5'GTGGACCATTTCAAGTTCAACA-3' R:5'TGAATGACATCCATCCATGA-3'	0.3742	0.5278	2.0000	0.4905	0.7222
3	T-107	F:5'-GTGACCACATGAGATATCCAGA-3' R:5'-CAGTTGTCCATATTGTGTGGG-3'	0.3623	0.6111	2.0000	0.4640	0.5556
4	T-57	F:5'-AACATGCGGAGAAAAATT-3' R:5'-GGAACACGTCCCAAAAATGT-3'	0.2392	0.8333	2.0000	0.2714	0.3333
5	T-62	F:5'-GCACAAATAATTTTTCAAGACCAA-3' R:5'-AAAAACGGACATAGCTTTGTACT-3'	0.0000	1.0000	1.0000	0.0000	0.0000
Mean			0.2700	.0.7000	0.7000	0.3433	0.4667

PIC: Polymorphism Information Content.

Percentage polymorphism, number of alleles and PIC value showed the level of genetic divergence. A total of 9 alleles were observed in SSR. PIC value among the genotypes were varied from 0.2392 (T-57) to 0.3742 (T-70). PIC value was recorded up to 0.2700 for all the 5 SSR markers. Among 18 tomato genotypes LE-150 and LE-22 showed highest similarity as revealed by clustering using SSR markers. Similarity coefficient of 18genotypes using SSR markers ranged from 0.1 to 0.4. The clustering done using UPGMA was further confirmed by DARWIN(6.0 version). It is inferred that genetic diversity exists among the tomato genotypes both at the genotypic and phenotypic level. High genetic diversity between the genotypes *i.e.*, LE-150 and LE-22(based on SSR markers) could be exploited through hybridisation to recover the seggregants possessing high yield potential with improved fruit quality characteristics Table-4.

Table 5: Distribution of 18 tomato genotypes into two main clusters-SSR Marker

Cluster No.	Sub cluster		Number of genotypes	Genotypes			Source
				A1		A2	
A	A1	A2	7	LE-104, LE-115, LE-118, LE-11, LE-6.	LE-116	LE-19	TNAU
				B1	B2		
B	B1	B2	5	LE-23, LE-14, LE-355	LE-105, LE-10.		
C	C1	C2	6	LE-13, LE-150, LE-7.	LE-3, LE-22, LE-15.		

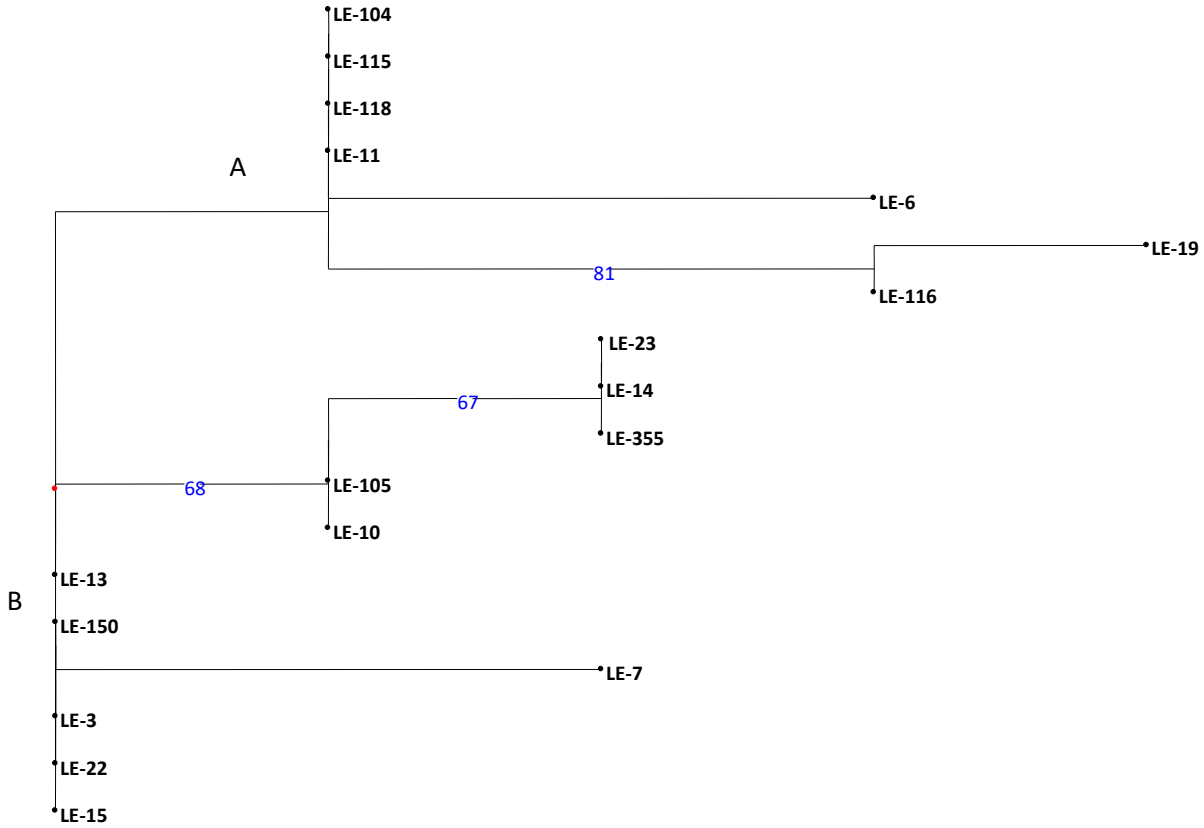

Fig 1: Cluster analysis of tomato based on UPGMA using SSR marker

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