STUDIES ON IDENTIFICATION OF MOLECULAR MARKERS FOR BUCKEYE ROT (*Phytophthora nicotianae* Breda de Hann. var. *parasitica* (Dastur) Waterhouse) RESISTANCE IN TOMATO (*Solanum lycopersicum* L.)

Thesis

by

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(H-2012-20-D)

Submitted to

Dr YASHWANT SINGH PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY
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CERTIFICATE-I

This is to certify that the thesis entitled, **"Studies on identification of molecular markers for buckeye rot (*Phytophthora nicotianae* Breda de Hann. var. *parasitica* (Dastur) Waterhouse) resistance in tomato (*Solanum lycopersicum* L.)"** submitted in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY MOLECULAR BIOLOGY AND BIOTECHNOLOGY** in the discipline of **BIOTECHNOLOGY** to Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP)-173 230 is a record of bonafide research work carried out by **Ms Shilpa (H-2012-20-D)** daughter of Sh. Gurdev Singh under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations has been fully acknowledged.

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CERTIFICATE-II

This is to certify that the thesis entitled, **"Studies on identification of molecular markers for buckeye rot (*Phytophthora nicotianae* Breda de Hann. var. *parasitica* (Dastur) Waterhouse) resistance in tomato (*Solanum lycopersicum* L.)"** submitted by **Ms Shilpa (H-2012-20-D)** daughter of Sh. Gurdev Singh to Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP)-173 230 India in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY MOLECULAR BIOLOGY AND BIOTECHNOLOGY** in the discipline of **BIOTECHNOLOGY** has been approved by the Advisory Committee after an oral examination of the same in collaboration with the External Examiner.

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"Think positive, God is always with you"

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Place:
Date: **Shilpa**

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LIST OF ABBREVIATIONS/ACRONYMS

ºC	-	Degree celsius
µg	-	Micrograms
µl	-	Microlitre
%	-	Per cent
ADW	-	Autoclaved distilled water
AFLP	-	Amplified fragment length polymorphism
bp (s)	-	Base pair (s)
BLASTx	-	Basic local alignment search toolx
CIM	-	Composite interval mapping
cM	-	centiMorgan
CMA	-	Corn meal agar
CTAB	-	Cetyl trimethyl ammonium bromide
cv.(s)	-	Cultivar (s)
dNTP	-	Deoxynucleotide-5-triphosphate
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylene diamine tetraacetic acid
E	-	Effective multiplex ratio
EST-SSR	-	Expressed sequence tag-simple sequence repeat
F_1	-	First filial generation
F_2	-	Second filial generation
F_3	-	Third filial generation
$F_{2:3}$	-	F_2 derived F_3 generation
Fig.	-	Figure
gm	-	Grams
HCl	-	Hydrogen chloride
ISSR	-	Inter simple sequence repeat
kb	-	Kilobase pairs
l	-	Litres
LOD	-	Logarithm of the odds
MI	-	Marker index
mg	-	Milligrams
ml	-	Millilitres

mM	-	Millimolar
mA	-	Milliampere
M	-	Molar
NCBI	-	National centre for biotechnology information
ng	-	Nanograms
nm	-	Nanometres
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
O.D.	-	Optical density
pMoles	-	Picomoles
PCA	-	Principal component analysis
PCR	-	Polymerase chain reaction
PIC	-	Polymorphism information content
QTL	-	Quantitative trait loci
RAPD	-	Random amplified polymorphic DNA
rpm	-	Revolutions per minute
RNase	-	Ribonuclease
SAHN	-	Sequential agglomerative hierarchical and nested clustering
sp.	-	Species
subsp.	-	Subspecies
SSRs	-	Simple sequence repeats
TAE buffer	-	Tris acetate EDTA buffer
Taq	-	*Thermus aquaticus*
TE	-	Tris EDTA
U	-	Unit
UPGMA	-	Unweighted pair group method with arithmetic averages
UV	-	Ultra-violet
V	-	Volts
var.	-	Variety
v/v	-	Volume/volume
ver.	-	Version

Chapter-1

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most economically important and widely grown vegetable crops in family Solanaceae. It is the second most consumed vegetable crop after potato. Wild tomato species are native to diverse habitats in South America and show great morphological and ecological diversity that have been proven useful in breeding programmes (He *et al.,* 2003). Tomato is grown worldwide for its edible fruits. It is a self-pollinated crop but in some cases 30 per cent cross-pollination also occurs (Gomez and Maloof, 2009). Tomato species are cultivated as annuals in colder regions while they are perennial in warmer regions. China is the leading producer of tomato in the world. India ranks second in the area as well as in production of tomato with an area of 8.84 million hectares, production of 17.87 million tonnes and an average yield of 20.22 metric tonnes per hectare. Himachal Pradesh is the major off-season tomato growing state covering an area of 9.93 lakh hectares with a production of 413.71 lakh metric tonnes and an average yield of 41.7 metric tonnes per hectare (Tiwari *et al.*, 2014).

Scientific classification:

Kingdom	:	Plantae
Order	:	Solanales
Family	:	Solanaceae
Genus	:	*Solanum*
Species	:	*S. lycopersicum*

Tomato cultivars produce red, yellow, pink, green, black or white fruit. It is extensively used in salad, for culinary purposes and also for various processed forms including pastes, sauces, pulps, juices, ketchup and as flavoring ingredients in soups, meat or fish dishes (Gosselin and Trudel, 1984). The fruit contains significant amounts of lycopene, beta-carotene, magnesium, iron, phosphorus, potassium, riboflavin, niacin, sodium and thiamine. It is a good source of vitamin A and C in significant amount (Zhang *et al.,* 2009). Tomato intake reduces cholesterol and triglyceride levels which decreases cardiovascular

risk, diabetes, cancer and is strongly protective against neurodegenerative diseases (Freedman *et al.,* 2008).

One of the problems of tomato cultivation is the damage caused by pathogens, including virus, bacteria, nematode and fungi, which causes severe production losses. Many diseases and disorders like early blight, late blight, bacterial spot, bacterial speck, septoria leaf spot, anthracnose, fusarium wilt and buckeye rot can affect tomatoes during the growing season. Among these buckeye rot is the most devastating disease causing huge crop losses.

Buckeye rot is a major constraint in tomato cultivation and has been reported from all tomato growing areas of the world where high relative humidity along with warm weather conditions prevail. Sherbakoff in 1917 reported buckeye rot for the very first time from Florida (Wani, 2011). In India, this disease has been reported for the first time from Solan area of Himachal Pradesh (Jain *et al.,* 1961). In India, losses from this disease range between 35 and 40 per cent, which may rise with the prevalence and severity of disease depending upon the favourable weather conditions (Gupta *et al*., 2005).

Buckeye rot of tomato fruit is caused by soilborne fungus *Phytophthora nicotianae* Breda de Hann. var. *parasitica* (Dastur) Waterhouse. The disease can result in large losses in the yield of harvestable fruit. Initial symptoms consist of a brownish, water-soaked spot that usually appears near the blossom end, or at the point of contact between the fruit and soil. The spot further enlarges and develops into a lesion with a characteristic target like pattern of concentric rings of narrow dark brown and wide light brown bands. Buckeye fruit rot lesions are, at first, firm and smooth. Buckeye fruit rot may produce a white, cottony fungal growth on the lesion under moist conditions. The buckeye rot fungus can affect both green and ripe fruit. Diseased fruit are usually located nearest the ground in staked tomatoes. The disease most commonly occurs under prolonged warm and wet conditions. Large amounts of rainfall or frequent irrigation may also result in sudden appearance of buckeye rot. The buckeye rot fungus may be introduced through infected seeds or transplants, by contact with infested soil or through plants from the previous crop. Ideal temperature for disease development ranges between 23° and 30°C.

As till date no variety with resistance to this disease is available, thus, the only management options are cultural and chemical control which include proper soil drainage, avoiding soil compaction, use of soil fumigation to disinfect heavily infested soils, rotation

on a three to four year basis with crops other than those belonging to the tomato family, use of stakes and/or mulches (plastic, straw, etc.) to reduce fruit and soil contact, applying fungicides that contain chlorothalonil, maneb, mancozeb,or metalaxyl as their active ingredients on a regularly scheduled spray programme. Mainly used fungicide is mancozeb. But in case of severe infection by the pathogen these controls no more prove to be effective. Therefore, biotechnology interventions seem to be appropriate alternates for the control of this disease by the development of resistant varieties by first identifying the good susceptible commercial variety and resistance source. The wild cherry tomatoes are supposed to contain resistant genes for this disease. The identified source can then be used for breeding purpose.

Tomato is a well-studied crop species for breeding, genetics and genomics. It is one of the initial crop plants for which a genetic linkage map was constructed and presently there are several molecular maps based on crosses between cultivars and many wild species of tomato (Kumar *et al.,* 2003). Characterization of genetic diversity is important for parental selection in breeding and genetic improvement programmes to broaden the genetic base in base populations by crossing cultivars with a high level of genetic distance (Lefebvre *et al*., 2001). Developing successful varieties for increasing the future yield and quality depends mainly on the genetic diversity of parents used in breeding programme. Thus, a sound knowledge of the genetic diversity among germplasm is vital for strategic germplasm collection, maintenance, conservation and utilization.

The genetic diversity within and between populations of tomato varieties can be measured by using morphological, biochemical and molecular characterization (Garcia *et al.,* 2004). Morphological markers have several defects that reduce the ability to estimate genetic diversity in plants as these are highly dependent on the environment for expression. However, molecular markers can give 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 (Franco *et al.,* 2001).

Conventional plant breeding is primarily based on phenotypic selection of superior individuals among segregating progenies resulting from hybridization. It is often time consuming as breeding a new cultivar takes between eight and 12 years and even then, the release of improved cultivar is not guaranteed. Hence, breeders are extremely interested in new technologies that could make this procedure more efficient. Molecular markers offer such a possibility by improving the selection strategies in crop breeding

(Alvarez, 2011). Molecular markers can be used for variety identification, genetic mapping/ quantitative trait loci (QTL) mapping and phylogenic analysis.

QTL mapping is based on the principle that genes and markers segregate via chromosome recombination (called crossing-over) during meiosis, thus, allowing their analysis in the progeny. Genes or markers that are close together or tightly-linked will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart. Markers that have a recombination frequency of 50 per cent are described as 'unlinked' and assumed to be located far apart on the same chromosome or on different chromosomes. The construction of a linkage map requires a segregating population (i.e. known as mapping population derived from sexual reproduction) (Bohra *et al*., 2012). The parents selected for the mapping population will differ for one or more traits of interest. Population sizes generally range from 50 to 250 individuals, however for high-resolution mapping larger populations are required (Mohan *et al*., 1997). Various types of mapping populations viz., F_1, F_2, F_2 derived $F_{2:3}$, recombinant inbred lines (RILs), backcross populations, near iso-genic lines (NILs), doubled haploid etc. are used for genetic mapping, QTL analysis and construction of linkage map, among which F_2 population derived by selfing of F_1 hybrids is the simplest type of mapping population developed for self pollinating species. The main advantages are that it is easy to construct and requires a short time to produce. Further the choice of mapping population may vary depending upon the objectives, the time frame, as well as resources available for undertaking mapping analysis (Collard *et al*., 2005).

The use of molecular markers associated to genes for resistance helps to identify new resistant varieties. This will reduce intake of pesticides, assuring consumers of a more healthy food (Barone and Monti, 2011). Thus, it is suggested that DNA markers will play a vital role in enhancing global food production by improving the efficiency of conventional plant breeding programmes.

To date, several kinds of DNA marker systems have been employed for diversity analysis, marker identification and QTL analysis such as random amplified polymorphic DNA (RAPD) (Sarkhosh *et al.,* 2006; Zamani *et al.,* 2010), amplified fragment length (AFLP) (Yuan *et al.,* 2007; Jbir *et al.,* 2008), simple sequence repeat (SSR) (Vaidya *et al*., 2012), inter simple sequence repeat (ISSR) (Vaidya, 2014; Samriti, 2014) etc.

Microsatellites or SSRs (Litt and Luty; 1989), considered as oneof the most powerful Mendelian markers, have been widely used in germplasm identification, population genetics, evolutionary studies and crop improvement in a variety of plant species like rice (Mackill *et al*., 1999), wheat (Koebner and Summers, 2003), maize (Tuberosa *et al*., 2003), barley (Thomas, 2003), tuber crops (Barone, 2004), pulses (Kelly *et al*., 2003), oilseeds (Snowdon and Friedt, 2004) and horticultural crop species (Baird *et al*., 1996; Saxena *et al*., 2011; Vaidya *et al*., 2012; Vaidya, 2014; Kaur *et al*., 2015a,b). SSRs are polymorphic loci with 1-6 base pairs repeating units present in nuclear and organelle DNA. They are typically neutral, co-dominant and hypervariable. Tandem repeats number of 60-100 gives high level of inter- and intra-specific polymorphism. On the basis of origin SSR markers are of two types: genomic SSRs which are developed from enriched DNA libraries, and genic or expressed sequence tags (EST)-SSRs, derived from EST sequences originating from the expressed region of the genome which are submitted, in public domain, as cDNA clones (Arnold *et al*., 2002; Chagne *et al*., 2004). The development of genomic SSRs is a time consuming job while genic-SSRs are easier to be searched *in silico* for a particular organism.

ISSRs are arbitrary multiloci markers produced by PCR amplification using microsatellites marker (Zietkiewicz *et al.,* 1994). A unique PCR primer composed of a few SSR repeats (di-, tri-, tetra-, penta-nucleotide) is used to amplify DNA sequences between two inverted SSRs. ISSR exhibit the specificity of microsatellite markers but need neither sequence information nor prior genetic studies for these analyses, so enjoying the advantage of random markers. ISSR markers are more in demand, because they are known to be abundant, very reproducible, highly polymorphic and highly informative.

During the last about two decades, molecular markers have been used for genetic improvement in a wide range of horticultural crops. The markers and maps have been used to locate and tag genes or QTL for disease resistance and many other horticultural characteristics (Foolad and Sharma, 2005). Inspite of development of genetic maps for most of the important fruit and vegetable species, and a number of horticulturally important gene loci being tagged, there are only a few reports of application of molecular markers for progeny selection. New, easy to perform allele-testing methods are needed to bridge this large gap between marker development and application (Rajapakse, 2003). In case of tomato, genes have been tagged for various characters including growth characters and disease resistance, but there is no report of QTL identification for buckeye rot resistance. Thus, it is

the need of the hour to first identify resistant source followed by gene identification through molecular breeding. Once the genes are located, they can easily be used to perform marker assisted studies.

Keeping in mind the above introduction, the present thesis was taken up with the following objectives:

1) Evaluation of genetic diversity amongst tomato genotypes using molecular markers
2) To study genetics of inheritance of resistance to *Phytophthora nicotianae* var. *parasitica*
3) Identification of molecular markers/ quantitative trait loci (QTL) for buckeye rot resistance in tomato (*Solanum lycopersicum* L.)

Chapter-2

REVIEW OF LITERATURE

Review of literature is discussed in the light of available literature relevant to the research on "Studies on identification of molecular markers for buckeye rot (*Phytophthora nicotianae* Breda de Hann. var. *parasitica* (Dastur) Waterhouse) resistance in tomato (*Solanum lycopersicum* L.)" under the following headings:

2.1 Evaluation of genetic diversity in tomato

2.2 Evaluation of genetic diversity in other crops

2.3 To study genetics of inheritance of resistance to various diseases

2.4 Identification of molecular markers/ QTL for disease resistance and other characters in tomato

2.5 Identification of molecular markers/ QTL for disease resistance and other characters in other crops

2.1 Evaluation of genetic diversity in tomato

Genetic variability of a species is essential for its survival and adaptation in different environments and studies of genetic diversity using molecular markers are necessary to understand the genetic structure of a population. A good knowledge of the genetic diversity among germplasm is vital for strategic germplasm collection, maintenance, conservation and utilisation. Many successful attempts have been made till date to analyze diversity among crops.

For conducting diversity studies, He *et al*. (2003) developed and characterized SSR primers in tomato. They searched 500 EST sequences, of which 158 SSR primer pairs were designed. These 158 pairs of SSR primers were then used to screen 19 tomato cultivars. Out of these, 129 pairs produced amplification on PCR analysis, and 65 of them were polymorphic with the polymorphism information content (PIC) ranging from 0.09 to 0.67. Among the polymorphic primers, two to six SSR alleles were detected for each primer with an average of 2.7 alleles per primer. Majority (93.8%) of the microsatellite loci contained di- or tri-nucleotide repeats and only 6.2% had tetra- and penta-nucleotide repeats. The tomato cultivars were clustered into two main groups based on the banding patterns generated by the

65 polymorphic SSR primers. The clustering was found congruent with their genetic background. Since the markers developed in this study are from expressed sequences, they can be used for molecular mapping, cultivar identification, marker-assisted selection and identifying gene-trait relations in tomato.

Tam *et al*. (2005) used four retrotransposon-based sequence-specific amplification polymorphism (SSAP), nine AFLP and 29 SSR primers to assess the genetic diversities of collections of 34 tomato and 35 pepper lines. On the basis of their results, SSAP was found most informative of the three systems for studying genetic diversity in tomato and pepper. SSAP showed about four to nine fold more diversity than AFLP and had the highest number of polymorphic bands per assay ratio. For tomato, SSAP was found more suitable for revealing overall genetic variation and relationships, while SSR has the ability to detect specific genetic relationships. All three marker systems results for pepper showed general agreement with pepper types. Additionally, retrotransposon sequences isolated from one species can be used in related Solanaceae genera. These results suggest that different marker systems are suited for studying genetic diversity in different contexts depending on the group studied, where discordance between different marker systems can be very informative for understanding genetic relationships within the group.

In a study conducted by Benor *et al*. (2008) the genetic diversity of 39 determinate and indeterminate tomato inbred lines collected from China, Japan, S. Korea, and USA was determined. They used 60 SSR primers, of which 19 (31.7%) did not amplify the DNA and six (10%) amplified monomorphic banding patterns. The remaining 35 (58.3%) SSR primers, revealed polymorphic banding pattern. A total of 150 alleles were detected by these polymorphic primers. The mean PIC for the 35 SSR primers was 0.31. The average number of SSR alleles per primer for the 35 polymorphic primers was 4.3. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering at genetic similarity value of 0.85 grouped the inbred lines into four groups, where one USA cultivar formed a separate and more distant cluster. Clustering was consistent with the known information regarding geographical location and growth habit. The genetic distance information reported in this study might be used by breeders when planning future crosses among these inbred lines.

Characterization of 63 tomato varieties was done by Kwon *et al*. (2009) using 33 SSR primers and 22 morphological traits. A total of 132 polymorphic amplified fragments were obtained using 33 SSR primers. The average PIC was 0.62 ranging from 0.21 to 0.88.

UPGMA cluster analysis divided the genotypes in two main groups. The relationship between morphological and molecular data was analyzed using Mantel matrix correspondence test. The co-relation value between two methods was 0.644. SSR based dendrogram also showed good extent of similarity with morphological traits. Thus, SSR primers can be used for varietal identification and pre-screening for distinctiveness test of tomato varieties.

Parmar *et al.* (2010) used 23 SSR primers to measure genetic diversity of 25 cultivars of tomato. Three primers (13%) did not show any amplification and eight primers (34.8%) revealed monomorphism, while remaining 12 primers (52.2%) generated polymorphism. In the phylogenetic analysis, most of the tomato lines were clustered according to their geographical locations, and thus, might have a similar genetic background and those which were distantly grouped, were from different geographical locations. UPGMA clustering analysis almost completely separated American lines from Indian lines. The present study has established that the SSR primers are useful to study genetic diversity among tomato lines collected from diverse geographical locations.

Bae *et al.* (2010) developed Single Nucletide Polymorphism (SNP) primers based on conserved ortholog set II (COSH) and intron-based primers derived from pepper EST sequences for cultivar identification in tomato. Out of 628 primers sets tested on 32 cultivars, 417 primer sets amplifying single bands were selected. Of the 417 primer sets, 70 primer sets showing polymorphism among four inbred lines, were selected, from which 11 primers were used for cultivar identification analysis. These selected 11 primers successfully identified 32 commercial tomato cultivars. Hence it was concluded from present study that cultivar identification can be done effectively using SNP primers based on COSH and intron-based primers.

Three different molecular marker systems viz., Inter-retrotransposon amplified polymorphism (IRAP), ISSR and RAPD were used by Mansour *et al*. (2010) to assess genetic variation among ten tomato cultivars. A total of four IRAP, 20 ISSR and 20 RAPD primers were used, of which all IRAP, RAPD and 15 ISSR primers generated polymorphism. Three marker systems produced different type of clustering of cultivars. But on combined analysis of these marker systems, though different results, but in agreement with individual marker system dendrograms were obtained confirming their individual results. This study for the first

time in tomato revealed that it is inadequate to use only one marker system for assessing genetic diversity.

Miskoska *et al*. (2011) evaluated genetic relationships among six morphologically different tomato varieties (var. *grandifolium*, var. *cerasiforme* (red), var. *cerasiforme* (yellow), var. *pruniforme*, var. *pyriforme* and var. *racemigerum*) using nine SSR primers. The results showed that the lowest genetic distance (16.741) was identified between var. *cerasiforme* (yellow) and var. *cerasiforme* (red), and the largest (34.985) between var. *pyriforme* and var. *grandifolium*. On the other hand, the lowest genetic distance (22.144) was observed between subsp. *subspontaneum* and subsp. *spontaneum*, and the highest (29.714) between subsp. *subspontaneum* and subsp. *cultum*.

Asgedom *et al*. (2011) analysed the diversity among farmers' varieties of tomato from Eritrea and compared these varieties with other African and Italian varieties. A total of 15 SSR primers were used, out of which 13 were polymorphic with two to five alleles per primer. A high degree of diversity was observed among the Eritrean varieties. The dendrogram showed two major types of varieties: San-Marzano and Marglob. Eritrean varieties were closely related to old Italian varieties in both types. Analysis of the within-variety variation showed that the Eritrean tomato genotypes were less uniform than the other varieties, probably because of mixing. A survey among farmers showed that some of them purposely mixed seeds to prolong the harvesting period for yield stability and stress tolerance.

Aguilera *et al.* (2011) used ten ISSR primers to evaluate the genetic variability of 96 tomato accessions. All ten primers generated 144 bands, 53 being polymorphic (36.80% polymorphism), with an average of 14.4 bands per primer. The dendrogram obtained by UPGMA and Tocher' grouping method differentiated all the accessions. Accession 'BGH-980' was located in a separate group, being the most divergent amongst the accessions tested by both methods. From the results obtained in this study, ISSR primers demonstrated a high efficiency to differentiate the germplasm.

In a study conducted by Hwang *et al.* (2012), assessment of genetic similarity and relationships among inbred lines for selecting recurrent parent for the development of tomato cultivars was done using a total of 303 polymorphic SSR primers. Similarity coefficient ranged from 0.33 to 0.80; the highest similarity coefficient (0.80) was found between

bacterial wilt-resistant donor lines '10BA333' and '10BA424', and the lowest (0.33) between a late blight resistant-wild species 'L3708' (*S. pimpinelliforium* L.) and '10BA424'. UPGMA analysis grouped the inbred lines into three clusters based on the similarity coefficient. Parent combinations (donor parent x recurrent parent) showing appropriate levels of genetic distance and SSR primer polymorphism were selected based on the dendrogram. These combinations included 'TYR1' x 'RPL1' for tomato yellow leaf curl virus (TYLCV), '10BA333' or '10BA424' x 'RPL2' for bacterial wilt, and 'KNU12' x 'AV107-4' or 'RPL2' for powdery mildew and 'L3708' x 'AV107-4' for late blight.

Hu *et al*. (2012) used 26 morphological traits, 37 SNP and ten SSR primers to investigate genetic variation in 67 tomato varieties collected from Argentina between 1932 and 1974. 65.0% of the morphological traits and 55.3% of the molecular markers showed polymorphism. Nei's genetic distance varied from 0 to 0.202. Cluster analysis grouped 67 varieties into three clusters at both morphological and molecular levels. Higher genetic variation was observed among varieties collected before 1960 than those collected after 1960.Thus, it was concluded that SNP and SSR primers can be successfully used for diversity studies.

Joshi *et al*. (2012) assessed the diversity of 32 tomato genotypes released from North Carolina State University based on their coefficient of parentage (COP) and RAPD primers. The COP analysis revealed that a single ancestor 'Walter' constituted 25.17% of the North Carolina tomato gene pool. Out of 96 RAPD primers used, 18 were polymorphic revealing 19% polymorphism. Similarity coefficients based on the RAPD molecular markers ranged from 0.461 to 0.935, with an average of 0.770. The similarity coefficient of genotype 'NC3Grape' and 'NCEBR8' was the lowest (0.461), whereas it was the highest (0.935) between genotypes 'NC1CS' and 'NC946'. A significant positive correlation existed between two similarity matrices based on the RAPD and the pedigree. Cluster analysis was performed using neighbor joining method which grouped the genotypes in two main clusters. This information may be useful for selecting the parents in breeding programs, particularly to widen the genetic base for designing future breeding strategies.

Genetic diversity among 47 tomato varieties was carried out by Sardaro *et al.* (2013) using 11 microsatellite primers. Among the primers used, a total of 48 alleles were detected showing three to nine alleles per primer with a mean of 4.36. PIC ranged from 0.23 to 0.78, with an average of 0.50. UPGMA clustering grouped the 47 varieties into two major clusters

at 0.75 similarity coefficient, differentiating the modern varieties from tomato landraces. The DNA based markers confirmed the possibility to support the genotype identification all along the tomato production chain.

Naz *et al.* (2013) characterized 25 tomato accessions using both morphological and molecular markers. A total 25 RAPD primers were used, out of which 15 were polymorphic. A total of 130 loci were generated, out of which 98 were polymorphic, revealing 75.3% polymorphism. The Nie and Lie's coefficients were used to calculate the genetic similarity which showed sequence similarity index of 75.6% with dissimilarity index of 24.4%. Phylogenetic tree was constructed by DNAMANN software which divided the studied accessions into four main groups. It was concluded that although RAPD study supported the clustering by morphological characters, but the correlation was not upto 100%.

Sharifova *et al.* (2013) used ten RAPD primers for genetic diversity studies among 19 tomato genotypes. Out of ten primers used, six were polymorphic which produced 65.3% polymorphism. The genetic similarity among genotypes ranged from 0.188 to 1.000. The lowest similarity was observed between cultivars Azerbaijan and Shakar (0.188), while the highest between 'Elnur' and 'Garatag' (1.000). The UPGMA cluster analysis based on Jaccard's similarity coefficient divided genotypes into four main groups. The grouping pattern matched with the place of collection of genotypes confirming the reliability of RAPD primers for genetic diversity analysis.

Pal and Singh (2013) studied identification and purity of F_1 hybrids and their parents of tomato and Chilli (*Capsicum annuum*) by using 22 and 15 RAPD primers, respectively. Out of 15 RAPD primers used for analysis in chilli, three primers yielded polymorphic bands. For tomato, out of 22 RAPD primer only five primers showed polymorphic bands between male, female and hybrids. Since there are many chances of contamination by selfing of female parental lines and out crossing with other plants during hybrid development programs, which 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 was analyzed among 42 tomato varieties sourced from different geographic regions by Korir *et al*. (2014) with 50 EST-SSR primers. 14 (28 %) primers did not produce any amplification, seven (14 %) were monomorphic and remaining 29 (58 %) primers produced polymorphism. The genetic diversity was between 0.18 and 0.77, with a

mean of 0.49; the PIC value ranged from 0.17 to 0.74, with a mean of 0.45. This indicated a fairly high degree of diversity among these tomato varieties. Based on the cluster analysis using UPGMA, all the tomato varieties were divided into five groups. The Principal Component Analysis (PCA) supported the clustering result. This information about the genetic relationships between these tomato lines helped to distinguish these 42 varieties and will be useful for tomato breeding and selection. The polymorphism reports of this study shows that SSR is one of the most informative marker systems for tomato genotyping.

Shahlaei *et al*. (2014) used two molecular marker systems viz., Start Codon Targeted (SCoT) and ISSR for genetic diversity analysis of ten tomato accessesions. Using ten SCoT primers, 83 bands were generated, of which 30 (36.14%) were polymorphic, while ten ISSR primers amplified 86 bands with 20 (23.25%) being polymorphic. Average PIC values for SCoT and ISSR primers were 0.142 and 0.088, respectively. The ten accessesion were clustered into three major groups based on the SCoT analysis and two major groups based on the ISSR analysis with UPGMA. The PCA confirmed the results of the clustering. This study demonstrated that the SCoT marker system was more informative than ISSR marker system.

Genetic variation in two Bulgarian tomato varieties and six breeding lines was assessed using a highly efficient and low cost fluorescent SSR (5' end of primer labeled with florescent dye) genotyping platform (Todorovska *et al*., 2014). Genotyping was conducted with 165 publicly available microsatellite primers among which only five (3.03%) failed to amplify DNA of any of the types. Of the remaining primers, 81 (50.62%) were polymorphic. The total number of amplified alleles was 299 with a mean of 1.86 and the average PIC value was 0.19. The genetic diversity within the collection was relatively low (0.222). Nei's genetic distance varied from 0.095 to 0.399. Cluster analysis using UPGMA method indicated four main clusters, which is to some extent consistent with the morphotypes of the studied tomatoes. The genetic distance information from this study might be useful for further implementation of breeding strategies and crosses among these inbred lines.

Shah *et al*. (2015) characterized 21 tomato genotypes using RAPD primers. A total of 102 bands were produced using 20 RAPD primers, of which 75 out of 102 bands were polymorphic revealing 73.5% polymorphism. High level of polymorphism indicated high degree of divergence between varieties. The number of bands per primer ranged from two to eight with an average of 5.1 bands per primer. The polymorphism per RAPD primer ranged from 50% to 100% with an average of 73.5%. UPGMA cluster analysis, two clusters were

observed which divided the genotypes according to place of their collection. This study indicated the use of diversity analysis for tomato breeders to evolve varieties with genetically diverse back ground to achieve sustainability in tomato production.

Zhou *et al*. (2015) evaluated morphological traits (growth habit, plant height, stem diameter, hypocotyl color, leaf shape, leaf area, leaf length, leaf width and pubescence of stem and leaf) and molecular markers to assess the genetic diversity of 29 cultivated tomatoes, 14 wild tomatoes and seven introgression lines. 15 genomic SSR and 13 EST-SSR primers were used and all were found polymorphic, with an amplification of 1115 and 780 fragments, respectively. Genomic SSRs detected a total of 64 alleles, with a mean of four alleles per primer, while EST-SSRs detected 52 alleles, with a mean of 4 alleles per primer. The PIC value was slightly higher in genomic-SSRs (0.49) than in EST-SSRs (0.45). The dendrogram based on genetic distance divided the 50 tomato genotypes into eight clusters. To assess morphological variations, PCA was used which revealed 78.54% morphological variations in the tomato genotypes used. Based on these morphological traits, a three-dimensional PCA plot separated the genotypes into distinct groups, and a dendrogram divided them into six clusters. High variability of the tomato genotypes was observed at the morphological and molecular level, indicating valuable tomato germplasm, especially in the wild tomatoes, which could be used for further genetic studies.

Metwali *et al*. (2015) conducted a study to identify tomato cultivars with improved drought tolerance. Several sensitivity and tolerance indices were computed based on morphological markers along with use of 16 ISSR primers. Only ten primers were found informative which amplified 83 fragments. The highest value of the effective multiplex ratio (E) and marker index (MI) was detected for primer ‘INC7’ followed by ‘INC1’. Based on Jaccard's similarity coefficients, the genetic distance varied from 0.702 to 0.942 with a mean value of 0.882. The results showed a clear-cut separation of the 15 tomato cultivars due to their genetic variability, making them a valuable genetic source for their incorporation into potential breeding programs.

2.2 Evaluation of genetic diversity in other crops

Varshney *et al*. (2007) used 16 EST-SSR, 15 SNP and four AFLP primers to analyze 43 wild, 35 cultivated and 12 elite barley lines. SSR primers were found most polymorphic with an average PIC value of 0.59 and eight alleles per primer, while AFLP markers showed

26.4 as the highest effective multiplex ratio (26.4) and marker index was recorded to be 5.042. 0.486 was recorded the highest E value for AFLP primers and the lowest E 0.341 was for SNP markers while the SSR markers had an intermediate E (0.442). Cluster analysis on combined set of SSR, SNP and AFLP genotyping data classified wild, cultivated and elite barley lines in three distinct groups. In present study SNP markers were suggested as the best class of markers for characterizing and conserving the genebank materials and AFLP and SSR markers were found more suitable for diversity analysis and fingerprinting studies.

Rocha *et al*. (2010) evaluated the genetic diversity and identified 16 potato cultivars by using 25 RAPD and 20 SSR primers. RAPD primers generated 92 polymorphic bands whereas SSR primers produced 136 polymorphic bands. The dendrograms generated by cluster analysis distinguished the cultivars genetically although the dendrograms were not correlated for the comparison of the two marker systems used. The PIC values demonstrated the high information content of the primers used and 16 potato varieties were identified based on six RAPD primers and three SSR primer pairs. Thus, RAPD and SSR primers successfully assessed genetic diversity and identified commercial potato cultivars in this study. In another study for identification of 38 accessions of potato Favoretto *et al*. (2011) used ten SSR primers. A total of 46 bands were generated, of which only five were monomorphic and rest were polymorphic, revealing 89.1% polymorphism. The PIC value varied from 0.13 to 0.86, with an average of 0.54. The Jaccard´s coefficient performed using software NTSYSpc ranged from 0.410 to 0.930, showing high genetic variability among accessions. Two possible duplicates, collected from different regions i.e. Canada and Chile were identified, which proved the efficiency of SSR markers for identification and solving disputes based on forensic studies.

Demir *et al*. (2010) conducted molecular characterization of eggplant genotypes collected from different geographical regions of Turkey using five SSR and 11 RAPD primers. With SSR primers, the number of alleles per microsatellite primer ranged from two to ten, with a total of 24 alleles with an average of 4.8 alleles per primer. RAPD primers amplified 100 bands, of which 29 were polymorphic. The number of bands per primer ranged from seven to 14. RAPD primer 'OPB07' was the most polymorphic, generating 64% polymorphic bands, while rest of the primers gave less than 50% of polymorphism. UPGMA dendrograms were used to examine the genetic relatedness of the genotypes which divided the genotype in two main clusters by analyzing data separately from SSR and RAPD primers. The present study revealed the genetic diversity within a collection of eggplant germplasm

representing different geographical regions of Turkey. Mahmoud and El-Mansy (2012), Verma *et al.* (2012) and Ansari and Singh (2014) also conducted diversity studies on eggplant and reported the usefulness of ISSR, RAPD and SSR markers for assessing genetic relationship in eggplant.

Efficiency of the use of single versus multiple marker system was evaluated by Leal *et al*. (2010), by studying genetic diversity among ten inbred popcorn lines using both RAPD and SSR primers. The nine RAPD primers used yielded 126 bands, of which 104 were reported to be polymorphic, with an average of 11.6 polymorphic bands per primer. 14 SSR primers gave 47 alleles with two to five alleles per locus. When comparing the groups formed using SSR and RAPD primers, there were similarities in the combinations of genotypes from the same pedigree. Correlation between genetic distances obtained through RAPD and SSR primers was relatively high (0.5453), indicating that both techniques are efficient for evaluating genetic diversity in the genotypes of popcorn that were evaluated.

Kaur *et al*. (2010) characterized 15 walnut accessions (seven exotic and eight indigenous) using 36 RAPD primers. Of total primers used, 21generated amplification. A total of 190 bands were generated, out of which 157 were polymorphic, revealing 82.6% polymorphism. Upon cluster analysis using NTSYS software, exotic and indigenous accessions were clustered in two different groups. The cluster analysis agreed with geographical origin of studied germplasm indicating the usefulness of RAPD primers in genetic characterization studies.

Genetic relationship and diversity among seven cabbage cultivars were analyzed using RAPD and SSR primers by Saxena *et al*. (2011). A total of 17 selected RAPD primers generated 90 bands, 76 of which were polymorphic (84.44%). In addition, 27 selected SSR primers generated 67 amplified bands with 59 of which were polymorphic (87.6%). Though both the marker techniques were able to discriminate the cultivars effectively, analysis of combined data of both marker types resulted in better distinction of cultivars. By combining both the marker systems, a total of 157 bands were detected of which 135 bands (85.98%) were polymorphic, i.e. an average of 5.95 bands per primer. High level of polymorphism (> 85%) recorded with two different marker systems indicated a high level of genetic variation existing among the cultivars. Genetic relationship estimated using similarity co-efficient (Jaccard's) values between different pairs of cultivars varied from 0.21 to 0.77 in RAPDs, 0.42 to 0.82 in SSRs, and 0.43 to 0.89 with combined data from both marker types. A high

correspondence had been recorded between the values of genetic variations generated by UPGMA clustering and scatter plot diagrams. The cultivars January King Sel. Improved and Golden Acre are highly divergent cultivars as demonstrated by both the marker systems.

In an investigation, Vaidya *et al*. (2012) *in silico* extracted EST-SSR primers in cauliflower and used them to characterize different genotypes alongwith genomic SSRs. Out of the 18 genomic SSRs, 16 gave amplification, eight of which were found to be polymorphic, revealing 65.85% polymorphism among the cauliflower genotypes. On the other hand, 13 out of 16 dbEST – SSRs produced amplification, 11 primers being polymorphic, revealing 52.35% polymorphism. The dendrograms and rooted trees generated using NTSYS ver.2.02h and DARwin5 ver.5.0.155, respectively, generated for both the SSR marker sets, divided the cauliflower genotypes into two main clusters, while genotype 'US Agri Seeds' was singled out from rest of the genotypes. From this study it was concluded that both genomic as well as EST-SSRs produced high polymorphism and are suitable for diversity analysis studies.

Thul *et al*. (2012) investigated diversity among 22 accessions of six *Capsicum* species using 27 RAPD and eight ISSR primers. Congruence was reported between molecular marker analysis and floral characteristics. RAPDs and SSRs have been reported useful for genetic relationships between 13 genotypes of chilli and paprika collected from different places of India. In another study, genomic SSR and random amplified microsatellite polymorphism (RAMPO) primers were used by Rai *et al*. (2013) to analyze diversity and relationships among 48 pepper genotypes originating from nine countries. The markers used in this study could clearly separate non-*annuum* genotypes and *annuum* genotypes. In a study Tilahun *et al*. (2013) demonstrated that both RAPD and SSR markers analyzed as powerful tools for estimating genetic similarities and diversity. Hybridity was also studied between six hybrids of *C. annuum* and *C. frutescens* by Ahmed (2013) with ten ISSR primers. 52 polymorphic bands were produced out of total 87 bands revealing polymorphism of 60% between two parents. Molecular markers matched five hybrids with *C. frutescens* and sixth one with *C. annuum.* Gaikwad *et al.* (2013) carried out DNA fingerprinting studies for 30 *Capsicum* accessions using six AFLP and four ISSR primers. The high level of heterozygosity detected by the ISSR markers indicated that this technique may be used effectively for diversity analysis and DNA fingerprinting of *Capsicum.* Hazarika and Neog (2014a, b) studied genetic diversity of *Capsicum* accessions collected from three states of North- This study discovered

wide ranges of genetic variation among the accessions used revealing that these varieties were derived from different origin and could be utilized in future breeding programmes.

Usaizan *et al.* (2014) analyzed 130 accessions of *Physalis minima* using eight ISSR primers. 900 bands were amplified with 98.78% polymorphic loci. Cluster analysis was done using Dice's coefficient similarity which divided the accessions into four main clusters differentiated according to the geographical regions. This finding validated the use of ISSR marker to elucidate the genetic variation between populations. Therefore, improving the knowledge about genetics of weed can provide good information for the development of innovative control options for demonstration of *P. minima* L.

Samriti (2014) data mined 20 genic SSRs for *Rubus ellipticus* which were used in genetic diversity studies alongwith 35 ISSR primers among 21 collections of *Rubus ellipticus*. All EST-SSR and ISSR primers showed amplification in all the collections. Jaccard's similarity matrix was developed and dendrograms were generated using NTSYSpc ver.2.02h to establish the similarity among the 21 collections of *R. ellipticus*. Both the markers systems i.e EST-SSR and ISSR separately demonstrated more closeness between those collections which have actually been collected either from close sites or same site. EST-SSRs and ISSRs used in the present study exhibited a high level of diversity in the 21 colllections of *Rubus ellipticus* revealing their efficiency for diversity analysis studies.

EST-SSR primers were also developed for *Prunus persica* by Kaur *et al*. (2015a) by using 2000 EST sequences, from which 43 EST-SSR primer pairs were developed which were custom synthesized and used for polymorphism study in six genotypes of peach. 38 primers showed scorable amplification, 20 being polymorphic. These 20 polymorphic primers of peach were then further used to carry out transferability studies in apricot, apple, rose and strawberry revealing 50%, 95%, 95% and 45% transferability, respectively. Dendrograms were generated using NTSYS ver.2.02h. The coefficient values were found to range from 0.483 to 0.711 in six peach genotypes and 0.451 to 0.975 in all 22 genotypes under study. It was concluded from the present study that EST-SSRs of peach produce high polymorphism in different Rosaceous species, indicating their cross-transferability to different members of Rosaceous family.

For *in silico* development of EST-SSRs in stevia, Kaur *et al*. (2015b) downloaded 5548 stevia EST sequences from NCBI database, of which 18 primers were synthesized. Di-

nucleotide motif was reported to be the dominant repeat type (62.5%), followed by tri-nucleotide (37.5%). Upon diversity studies among 16 genotypes of stevia these primers produced 61.11% polymorphism. *In silico* mined primers, in this study, facilitated genome analysis in stevia, which had not been performed previously. Additionally, the EST-SSRs can be used for molecular work in related plant species since they generally exhibit cross species transferability, making further work cost effective and simple.

Kaur and Vaidya (2015) developed 107 EST-SSR primers from 2564 EST sequences of cauliflower, brussels sprout, cabbage and broccoli. Di-nucleotide repeat motif was found to be most common (68.22%) followed by tri-nucleotide repeat motif (31.78%). BLASTx analysis was carried out for the 100 EST sequences. Based on this analysis, a putative function could be assigned to 82 of the sequences (82%). Out of the designed primer, 14 were custom synthesized and were used for marker validation on a set of 20 cauliflower genotypes. The primers gave scorable bands and revealed 52% polymorphism among the tested cauliflower genotypes. This study demonstrated that *in silico* mining of microsatellite loci is an efficient means for EST– SSR marker development. Since EST–SSRs form part of transcribed regions of genome, therefore, they make a valuable resource both in structural as well as functional genomics in *Brassica oleracea* varieties and also in related members of Brassicaceae.

In another study performed by Vaidya *et al*. (2015), 4575 EST-SSRs were developed in apple. 25 primers were synthesized and were tested on 48 apple accessions. All the markers gave strong amplification, generating 93% polymorphism. These findings indicate the usefulness of EST-SSRs in genome analysis. This study further emphasizes the importance of assembly of the vast amounts of data submitted in public databases.

2.3 To study genetics of inheritance of resistance to various diseases

Inheritance of any character in an organism depicts the order in which the genes are transmitted from parents to offsprings. It is mainly of two types: Mendelian and non-Mendelian. In Mendelian inheritance a typical pattern of 3:1, in case of monogenic characters, and 9:3:3:1, in case of oligogenic characters, is followed. This inheritance pattern is also known as qualitative inheritance. The traits which follow Mendelian ratio are kwon as qualitative traits. But in some cases ratio in the next generation deviates from these ratio. This condition is kwon as non-Mendelian inheritance. In such cases inheritance is controlled by

many genes which have cumulative/ additive effect. This condition is also known as polygenic/ quantitative inheritance and such traits are called quantitative traits. Pattern of inheritance is necessary to uncover to facilitate further breeding experiments for crop improvement. Various studies have been conducted in past on genetic inheritance some of which are discussed in the next given examples.

Fruit rot disease caused by *Phytophthora nicotianae* var. *parasitica* is a limiting factor in tomato production in Himachal Pradesh. 30 to 60 per cent fruits are damaged by this disease (Rattan and Saini, 1979). Crosses were made between 'EC 54725' (*L. pimpinellifolium*), a small fruited type, resistant to fruit rot and four highly susceptible tomato commercial cultivars (Gola, Sioux, S12, and Lalmani). Resistant and susceptible F_2 plants showed ratio of 3:1. Studies of F_1's, F_2's and back crosses indicated that 'EC 54725' carries a dominant gene imparting resistance to fruit rot. Another study conducted by Chauhan (1988) also aimed at finding out genetic basis of inheritance to buckeye rot resistance in tomato. The crosses were made between two resistant lines 'EC 130035' and 'EC 129603' and four susceptible parents viz. Solan Gola, Solan Surkha, A-2, Gold Maker and F_2, BC_1 and BC_2 generations were developed. Qualitative analysis showed resistance to buckeye rot under one dominant gene control but the generation mean analysis depicted the presence of significant additive gene effects in all crosses.

Sharma (1987) crossed two susceptible parents 'California Wonder' and 'Yolo Wonder' and two resistant sources 'Perennial' and 'S 41-1'. The resistance to a strain of potato virus Y (PVY-sbp) in pepper was found to be under one recessive gene control. The biochemical studies revealed the dominance of low values over high values of phenolics, enzymatic activity and chlorophyll contents and the high values of each biochemical constituent were under the control of one recessive gene, as was with inheritance of resistance to mosaic virus disease. Thomas and McGrath (1988) further confirmed these results.

The inheritance of resistances to race 2 and race 3 of *Fusarium oxysporum* f. sp. *lycopersici* was investigated by McGrath *et al*. (1987) in crosses involving parents 'Contender', 'Rouge de Marmande' and 'PI414773'. Segregation in F_2 population of the crosses 'Rouge de Marmande' x 'PI414773' and 'Contender' x 'PI414773' revealed that resistance to race 2 was controlled by two independent dominant genes. Segregations in F_2 derived from 'Contender' x 'PI414773' indicated that a single dominant gene conferred

resistance to race 3. The results indicated that the selected parents prove reliable resources to provide resistant genes against *F. oxysporum* f. sp. *lycopersici* which can further be used in breeding programmes to develop resistant varieties. This single gene controlled inheritance was also confirmed by Vakalounakis (2007).

Crosses were made by Lynch *et al*. (1997) between resistant genotype of *S. chacoense* 'PI 472819' and a susceptible genotype 'PI 472810', to determine genetic inheritance to Verticillium wilt (*Verticillium alboatrum*). Segregation ratios of 3:1 in F_2 population indicated that resistance was controlled by a single dominant gene which was named as '*Vc*'. Transfer of this '*Vc*' gene to commercial germplasm can provide effective and economical control of Verticillium wilt.

Ciccarese *et al*. (1998) performed inheritance studies for powdery mildew (caused by *Oidium lycopersici*) resistance. 'LC-95', resistant parent, was crossed with cv. Marmande (susceptible parent) and F_1, F_2 progenies were derived. Plants of F_2 population were grown in glasshouses at 23^{o}C and 95–100% relative humidity and inoculated with *O. lycopersici*. The F_2 segregants' inoculation results fitted in 1:3 Mendelian ratio. It was concluded from the results that powdery mildew is controlled by single recessive gene.

Pharintanun (2001) used two resistant lines (LO-3850-3 and LO-0302-1) and two susceptible varieties (Tabtimhauysai and Sedahauysai) to leaf mold disease, caused by *Cladosporium fulvum* Cooke, for producing crosses. F_2 hybrid of LO-3850-3 x Tabtimhauysai and LO-0302-1 x Sedahauysai were tested for the inheritance of their resistance to *C. fulvum*. These plants at two to three leaf stage were inoculated with pathogen. Ratio of resistant and susceptible plants of F_2 plant was found to be 3:1. This indicated that resistance to *C. fulvum* was controlled by single dominant gene.

Kumar (2002) conducted experiment which consisted of bacterial wilt resistant lines viz. 'BL-342-1' and 'EC 191536' and two susceptible lines 'UHF 265' and 'UHF 120', their F_1, BC_1, BC_2 and F_2 generations. The disease screening was done under artificial epiphytotic conditions created by root dip inoculation method. Results revealed that resistance to bacterial wilt in both lines was governed by single recessive gene.

Vikram (2003) used *Phytophthora* blight resistant line 'UHF-1', three susceptible parents 'California Wonder', 'Arka Mohini' and 'HC 201', their advanced progenies i.e. F_2,

BC_1 and BC_2 to work out inheritance of *Phytophthora* blight resistance. The disease screening was carried out using artificial inoculatioin under conditions favourable for disease development. Resistance to *Phytophthora* blight was found to be governed by single dominant gene. In another study conducted by Abreu *et al*. (2008), genetics of inheritance was found out for same disease by making crosses between *S. lycopersicum* cv. Santa Clara which is susceptible to late blight and *S. habrochaites* accession BGH 6902, resistance source. Further F_1, F_2, BC_1 and BC_2 progenies were analyzed for inheritance. Analysis of the area under the disease progress curve (AUDPC) indicated that inheritance is polygenic. But in a study conducted by Elsayed *et al*. (2012) the genetic analysis revealed two recessive genes controlling the resistance. The scaling test of additive-dominance model showed authenticity of data confirming the absence or neglect of epistasis.

Castro *et al*. (2007) carried out a breeding program for selection for resistance to TYLCV and Tomato yellow leaf curl Sardinia virus (TYLCSV). For this, cross was developed between *S. lycopersicum* × *S. pimpinellifolium* UPV16991, followed by several selfing generations. One partially resistant F_6 plant (L102) was chosen to form progeny to study the genetic control of resistance to TYLCV. Crosses between four breeding lines susceptible to TYLCD and L102 were also performed to study the dominance of the resistance in *S. lycopersicum* genetic backgrounds. Response to TYLCV infection of P_1, P_2, F_1, F_2, BC_1, and BC_2 generations fitted, for this line, a monogenic control with partial recessiveness and incomplete penetrance. Partial resistance derived from 'UPV16991' will be useful in homozygosis or combined with resistance genes from other sources.

Kozik and Sobiczewski (2008) made crosses between tomato cv. Ontario 7710 (sensitive) and A100 (insensitive) to reveal inheritance pattern for bacterial speck (*Pseudomonas syringae* pv. *tomato*) resistant. Genetic analysis in F_2 segregants was characterized into 3:1 Mendelian ratio indicating that resistance to bacterial speck in tomato is governed by one completely dominant gene.

Resistance of tomato to bacterial spot race T4 (*Xanthomonas perforans*) was characterized by Hutton and Scott (2010) using generation means analysis (GMA) in three advanced breeding lines: 'Fla. 8326', 'Fla. 8233', and 'Fla. 8517'. GMA of 'Fla. 8326' for two of three seasons (Fall 2006 and Summer 2007). Results indicated that resistance is mostly dominant with significant additive and epistatic effects. GMA of 'Fla. 8233' in Spring 2007 and of 'Fla. 8517' in Summer 2007 also showed dominance to be the main effect in

addition to additive and epistatic effects. Duplicate dominance or recessive suppressor type epistasis was indicated in each breeding line. In F_2 populations of crosses between resistant parents, suggested that these lines have quantitative trait loci in common and the inheritance is polygenic.

Four tomato lines viz. 'Hawaii-7998', 'CRA-66', 'FT-5' and 'Solan Vajr' were screened by Sharma *et al*. (2011) for bacterial spot resistance under artificial inoculation conditions. The experiment on the genetics of resistance to bacterial spot on lines 'Hawaii-7998' (resistant) and 'CRA-66' (moderately resistant), susceptible lines, 'FT-5' and 'Solan Vajr' and their F_1s', BC_1, BC_2 and F_2s were carried out under field conditions. Results revealed that resistance to bacterial spot in both the lines was governed by polygenes. The additive and dominance effect of genes played an important role in the inheritance of this disease.

Calis and Topkaya (2011) tested the responses of six cultivated and three wild tomato accessions with three isolates of the fungal pathogen. To investigate inheritance of the resistance, resistant plants of 'NCEBR2' and 'NCEBR4' genotypes were crossed with susceptible 'NC84173' tomato line, and their F_1, F_2 and BC_1 populations were established. In the established populations, resistance differences were significant (P>0.05) for 'NCEBR2' × 'NC8413' and 'NC84173' × 'NCEBR4' parents, F_1 and BC_1. However, no significance (P>0.05) was obtained in their F_2 population to early blight pathogen. The data from these populations revealed that early blight resistance in 'NCEBR2' and 'NCEBR4' was quantitatively controlled by more than one gene or quantitative trait locus.

Studies have been conducted by Sen *et al*. (2015) to analyze genetics of resistance to bacterial canker in tomato. Backcross and F_2 populations between resistant and susceptible *S. arcanum* accessions revealed two or three recessive genes that are involved in resistance.

2.4 Identification of molecular markers/ QTL for disease resistance and other characters in tomato

Many agriculturally important traits such as yield, quality and some forms of disease resistance are controlled by many genes which have cumulative effect. They are known as quantitative traits. The regions within genomes that contain genes associated with a particular quantitative trait and identified by molecular markers are known as QTL. The identification of QTL based only on conventional phenotypic evaluation is not possible. A major

breakthrough in the characterization of quantitative traits that created opportunities to select for QTL was initiated by the development of DNA (or molecular) markers in the 1980s (Botstein *et al*., 1980). DNA markers have many uses in agricultural research, of which main is the construction of linkage maps for diverse crop species. Linkage maps have been utilised for identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis. The process of constructing linkage maps and conducting QTL analysis is known as QTL mapping. DNA markers that are tightly linked to agronomically important genes (called gene 'tagging') may be used as molecular tools for marker-assisted selection (MAS) in plant breeding.

In early 90s a study was carried in search for QTL by Balint-Kurti *et al*. (1994). They developed a population consisting of F_2 progeny from the interspecific cross *L. esculentum* × *L. pennellii.* Disease resistance genes to the fungus *Cladosporium fulvum* were identified using RFLP markers. The two closest markers identified were 'CP46', 2.6 cM distal, and 'TG236', 3.7 cM proximal to genes '*cf-4/9'*. These markers can be used in selection of progenies resistant to fungus *C. fulvum.*

Danesh *et al*. (1994) studied resistance to bacterial wilt in tomato by analyzing 71 F_2 individuals from a cross between a resistant parent 'L285' and a susceptible parent 'C286' with 280 RFLP and 80 RAPD primers, of which 67 RFLP and 12 RAPDs generated polymorphism. Statistical comparisons using MAPMAKER-QTL analysis between DNA marker genotypes and disease phenotypes identified three genomic regions correlated with resistance i.e. on chromosomes 6, 7 and 10. On further validation of linked markers, six markers were successfully scored on F_3 population revealing their promising use in marker assisted selection.

The '*hero*' gene confers resistance to a wide spectrum of pathotypes of the potato cyst nematode *Globodera rostochiensis*. This gene has been introgressed from the wild tomato species *L. pimpinellifolium* into the cultivated tomato. Ganal *et al*. (1995) used RFLP and RAPD analysis for the targeted search of the *L. pimpinellifolium* into the cultivated tomato. It was found that the resistant line 'LA 1792' contains a single introgressed segment on chromosome 4, which is characterized by three RFLP markers from the high-density RFLP map of tomato. The locating map position of the '*hero*' gene in large populations, four additional markers were identified in the introgressed region. After analyzing more than 800 gametes for recombination, it was found that one marker is only 0.4 cM away from the '*hero*'

gene. YAC clones isolated from a region near the '*hero*; gene indicate that in this area of the genome, the kb/cM ratio is relatively low (<450 kb/cM) and chromosome walking should be feasible in order to isolate this gene.

Chague *et al*. (1997) used Bulked Segregant Analysis (BSA) to identify RAPD markers linked to a QTL involved in resistance to the TYLCV. 11 F_4 lines were distributed into two pools, each consisting of the most resistant and the most susceptible individuals, which were then screened using 600 primers. Four RAPD primers were linked to a QTL responsible for up to 27.7% of the resistance. These markers, localized in the same linkage group within a distance of 17.3 cM, were mapped to chromosome 6 on the map. Four QTL linked primers were identified which can further be employed in marker assisted selection for late blight resistance.

Stommel and Zhang (1998) identified RAPD and AFLP markers linked to QTL involved in tomato anthracnose resistance in F_2 population of tomato developed from cross of an unadapted and small-fruited, but highly anthracnose-resistant *L. esculentum* accession and an adapted, but anthracnose-susceptible processing type tomato. 1000 RAPD primers and 64 AFLP primers were screened for polymorphisms between the parental lines. Primers which differentiated the anthracnose resistant and susceptible parents were utilized to screen the F_2 population for detection of QTL. Using single-factor analysis of variance, a number of markers, including six unmapped RAPD markers were identified that were significantly associated with resistance.

Genetic analysis of resistance to late blight was performed by Moreau *et al*. (1998) on F_2 progeny of 200 plants derived from a cross between the tomato line *L. esculentum* var. Hawaii7996, susceptible to late blight, and the resistant wild relative *L. pimpinellifolium* var. WVa700. Marker analysis by using 62 RFLPs and 13 RAPDs of the genome showed that this resistance was controlled by a single, incompletely dominant allele, '*Ph-2',* present on the distal part of the long arm of chromosome 10 in an interval of 8.4 cM flanked by markers 'CP105' and 'TG233'. Genetic analysis of F_2 progeny from a second cross between an *L. esculentum* introgression line IL10-3 carrying a homozygous *L. pennellii* segment spanning the distal part of the long arm of chromosome 10 and WVa700 confirmed the map location. A high-resolution genetic linkage map of the chromosomal region surrounding '*Ph-2'* was initiated to permit future map-based cloning of this gene.

Crosses were made between 'Hawai 7996', resistant bacterial wilt parent, and 'WVa700', susceptible parent, by Mangin *et al*. (1999). 200 F_3 individuals were used to carry out marker studies with 13 RFLP markers. Marker maps were established with both MAP-MAKER and with JoinMap, using Haldane mapping function. In this study map of chromosome 6 was developed. Several markers showed significant association with resistance. Two regions of the chromosome, between 'Cf-2' and 'TG153', and between 'CP18' and 'TG406', showed clear association with resistance, with LOD score peaks ranging from 4.3 to 5.9, respectively. Further it was shown that at least two separate loci approximately 30 cM apart on chromosome 6 are most likely involved in the resistance.

Griffiths and Scott (2001) crossed tomato mottle virus (ToMoV) resistant inbred '7324' with a susceptible tomato inbred 7613. Based on 12 RAPD markers in F_2 population two linkage groups were observed, designated R1 and R2, using join scaling test. It was found that resistant line linked to the morphological markers self-pruning (*sp*) and potato leaf (*c*) on chromosome 6. R1 and R2 regions were about 40 cM apart either side of the morphological markers '*sp'* and '*c'* on chromosome 6. Region R2 was linked to the '*c'* gene on the long arm of chromosome 6 at a distance of 29.3 cM and region R1 was at 5.3 cM from '*sp*' gene. Molecular studies suggested that the action of at least two additive regions controlled ToMoV resistance.

To study the genetic control of fruit quality traits, a RIL population of 144 plants was developed by Causse *et al*. (2002) from an intraspecific cross between a cherry tomato line with a good aroma intensity and an inbred line with medium flavour but bigger fruits. A total of 38 traits involved in organoleptic quality were evaluated including flavour, aroma, texture. Molecular markers including 84 RFLPs, two RAPDs, and 16 AFLPs were used to construct a map which covered 965 cM, which corresponded to about 85% of the genome map. A total of 130 QTL for 38 traits were identified. Major QTL (>30% contribution) were detected for fruit weight, diameter, colour, firmness, meltiness, and for six aroma volatiles.

Zhang *et al*. (2003) identified QTL for early blight resistance using total of 820 BC_1 plants of a cross between an susceptible tomato breeding line 'NC84173' (maternal and recurrent parent) and resistant parent 'PI126445'. A genetic linkage map, spanning approximately 1298 cM of the 12 tomato chromosomes with an average marker distance of 7.3 cM, was constructed. Seven QTL were detected for early blight resistance one on each of chromosomes 3, 4, 5, 6, 8, 10 and 11. Of these, all but the QTL on chromosome 3 were

contributed from the resistant wild parent, 'PI126445'. These QTL can be used for marker-assisted breeding for early blight resistance.

Ammiraju *et al*. (2003) identified and mapped a novel heat-stable nematode resistance gene by screening 216 F_2 individuals derived from an intraspecific cross between susceptible parent 'LA392' and resistant parent 'LA2157'. Further mapping of recombinants with both RFLP and PCR-based markers localized '*Mi-9*' to the short arm of chromosome 6.

Bai *et al*. (2003) developed 104 F_2 individuals from a cross of *L. esculentum* cv. Moneymaker x *L. parviflorum* G1.1601 which were used to map powdery mildew resistance by using 318 AFLP markers. JoinMap3.0 was used to perform QTL mapping applying Kosambi's mapping function. A LOD threshold value of 3 was set for declaring a QTL. The resistance was reported to be controlled by three QTL. Ol-qtl1 was located on chromosome 6, while Ol-qtl2 and Ol-qtl3 were placed on chromosome 12.

Brouwer *et al*. (2004) mapped QTL for resistance to late blight using reciprocal backcross populations derived from cultivated *L. esculentum* x wild *L. hirsutum* (BC-E, backcross to *L. esculentum*; BC-H, backcross to *L. hirsutum*). Linkage maps were constructed for each BC population with RFLPs. Resistance QTL were identified on all 12 tomato chromosomes using composite interval mapping. Six QTL in BC-E (lb1a, lb2a, lb3, lb4, lb5b, and lb11b) and two QTL in BC-H (lb5ab and lb6ab) were detected. These resistant QTL detected in tomato coincided with chromosomal locations of previously mapped R genes and QTL for resistance to *P. infestans* in potato, suggesting functional conservation of resistance within the Solanaceae family.

Hai-Shan (2006) studied the genetics of resistance and identification of molecular markers for late blight resistance. F_2 progeny of 241 plants was derived from a cross between inbred line susceptible to late blight and a resistant accession 'CLN2037E'. The late blight responses of F_2 plants were tested *in vitro* and natural infection assayed under greenhouse conditions. Both methods showed that the resistance is dominant and inherited as monogenic trait. Genetic mapping and linkage analysis was done using 41 SSRs which showed that the late blight resistance gene '*Ph-rol'* was located on chromosome 9 with a genetic distance of 5.7 cM to the SSR marker 'TOM236'.

A study conducted by Grilli *et al*. (2007) aimed to detect QTL by using fAFLP (Fluorescent AFLP) markers associated to the trait tomato fruit set at high temperatures. A

cross between line ‘Jab-95’ (heat-tolerant) and cultivar Caribe (heat-susceptible) was made. A total of 192 plants of the F_2 generation were evaluated by using 172 polymorphic markers. The map covered 191.46 cM of the genome. Six trait-linked QTL were identified on chromosome 1, 3, 4, 6, 7 and 9. These results could be highly useful in improvement programs, since heat-tolerant plants can be selected rapidly which improves tomato fruit set.

Sharma *et al*. (2008) have developed a linkage map based on an F_2 population of a cross between ‘LA2093’ and a tomato breeding line, using 115 RFLP, 94 EST, and 41 resistance gene analog (RGA) markers. The map spanned 1002.4 cM of the 12 tomato chromosomes with an average marker distance of 4.0 cM. Locations of several ESTs and RGAs coincided with locations of several known tomato resistance genes and quantitative resistance loci (QRL), suggesting that candidate-gene approach may be effective in identifying and mapping new R genes. This map will be useful for marker-assisted exploitation of desirable traits in ‘LA2093’ and other *S. pimpinellifolium* accessions, and possibly for utilization of genetic variation within *S. lycopersicum.*

Nascimento *et al*. (2009) distinguished resistant homozygotes and heterozygotes from susceptible plants in crossing populations of Stevens tomato cultivar and advanced backcrossing populations by using ‘Sw421’ SCAR marker which is located at 1.0 cM from the ‘Sw-5’ allele (which confers resistance to the tomato spotted wilt virus, TSWV). 57 plants from the isogenic progenies were characterized based on banding pattern: 18 plants (31.6%) were identified as resistant homozygotes, eight plants (14.0%) as resistant heterozygotes and 31 plants (54.4%) were characterized as susceptible. Thus, it was concluded that SCAR ‘Sw-421’ marker is an important tool for selection against TSWV.

Robbins *et al.* (2010) crossed tomato lines ‘NC592’ containing *Phytophthora infestans* (late blight) resistant gene ‘*Ph-3*’ and ‘NC946’ containing tomato spotted wilt virus (TSWV) gene ‘*Sw-5*’ to develop an F_2 population and subsequent inbred generations. These two genes were linked within 5 cM on several maps near the telomere of the long arm on chromosome 9. MAS using three PCR-based codominant markers (‘TG328’, ‘TG591’, and ‘SCAR421’) was used in F_2 progeny to select recombinant lines. From 1152 F_2 plants, 11 were identified with potential recombination events between ‘Ph-3’ and ‘Sw-5’. F_3 progeny were generated from the remaining eight F_2 recombinants and resistance to both pathogens was confirmed in three of those. Thus, MAS was an efficient tool for selecting the desirable recombination events for these two pathogen resistance genes.

He *et al*. (2010) used F_2 population of 125 plants generated from F_1 plant of tomato hybrid 'DRW4409' for genetic analysis of powdery mildew resistance using 158 SSR primers. The computer program MAPMAKER/EXP 3.0 was used for linkage analysis with a minimum LOD threshold of 3.0 based on the Kosambi mapping function. SSR marker 'LEat014' was found to be linked to resistant gene at a map distance of 8.0 cM. This was the first report of an SSR marker linked to the resistance gene in 'DRW4409'. Because of its co-dominant nature, this SSR should be useful to breeders in screening tomato plants for resistance to powdery mildew when DRW4409 is used as the resistance gene source.

Li *et al.* (2011) crossed tomato cultivars 08086 and 08085 contrasting for macro calyx (*mc*) gene to obtain a segregating F_2 population and analyzed it using 488 AFLP primers. Three markers ('E32M36-D', 'E65M63-D' and 'E47M75-A') were found to be closely linked to '*mc*' gene, with distance of 7.2, 5.1 and 12.3 cM, respectively. The results showed that this trait was controlled by single gene. Further 48 germplasm resources were detected by using AFLP marker 'E65M63-D' along with identification in the fields and the results showed high degree of fitness and provided the basis for molecular breeding and gene cloning.

Zou *et al.* (2012) used BSA to find out RAPD markers linked to late blight resistance gene '*Ph-3*', using an F_2 population of 147 individuals derived from a cross of tomato lines 'CLN2037' (resistant) and 'T2-03' (susceptible). 230 RAPD primers were used for PCR amplification. One RAPD marker 'CCPB272-03740' was found to be tightly linked to the resistance gene '*Ph-3*' and was located 5.8 cM from the resistance gene. Marker 'CCPB272-03740' is the first marker of gene '*Ph-3*' based on PCR reaction.

A RIL population of tomato was developed by Foolad (2015) from a cross between resistant tomato breeding line 'NC 84173' and susceptible accession 'LA 0722' of the tomato wild species *S. pimpinellifolium*. The RIL population of F_9 generation consisted of 145 individuals was used for further marker studies. A genetic linkage map of the population was developed with 191 molecular markers, including 129 RFLPs and 62 RGAs. The genetic map covered 1505 cM of the 12 tomato chromosomes with an average inter-marker distance of 7.9 cM. This genetic map can be utilized for identification, characterization and exploitation of important genes or QTL and for introgression of useful traits into the cultivated tomato via marker-assisted breeding.

Genetic basis of yield was investigated by Bautista *et al*. (2015) in 159 plants of F_2 population derived from a cross between *S. lycopersicum* and its most closely related wild species *S. pimpinellifolium*. A total of 60 SSR markers were used for parental polymorphism survey, of which 31 were polymorphic and were further used in genotyping of F_2 population individuals. It was found that average fruit weight, fruit diameter, and fruit length had a strong effect on yield. In addition, small effects on yield due to soluble solids content and locule number were also observed. A total of 25 different significant QTL were detected for six traits (fruit length and diameter, fruit weight, yield, locule number, and Brix degrees), of which 17 were directly linked to fruit size traits. The percentage of phenotypic variation associated with single QTL ranged from 4.19% to 12.67%. QTL with a major effect were identified on fruit size traits on linkage groups 1 and 3. A strong co-location of QTL among yield and fruit size traits was observed, suggesting that these QTL play a role in the same expression process controlling yield. This result then may suggest that yield in tomato is mainly formed by fruit size QTL, whereas the remaining factors may play a complementary role in the expression of tomato yield.

2.5 Identification of molecular markers/ QTL for disease resistance and other characters in other crops

Two RAPD markers linked to resistance to stalk rot (*Sclerotinia sclerotiorum*) in cauliflower were identified by Saxena *et al*. (2009) using a mapping population of 200 F_2 individuals (Olympus × PSB) with 'Olympus' being the resistant parent. Stalk rot resistance in cauliflower found to be governed by many genes. A total of 222 RAPD primers were used to survey parental polymorphism. The primers which showed polymorphism in parental lines were used for BSA. RAPD markers '$D\text{-}3_{450}$' and '$C\text{-}20_{350}$' flanking the stalk rot resistance gene with a distance of 2.7 cM, and 4.2 cM, respectively, were identified. These two markers are close enough to the stalk rot resistance gene to allow a dependable marker-assisted selection for stalk rot resistance.

In another study conducted by Saxena (2010), QTL for blackrot resistance were identified in cabbage. To carry out this study 200 F_2 individuals were used which were raised from cross between resistant parent 'January King Sel' and susceptible parent 'Golden Acre'. Phenotyping of parents and mapping population was done by spraying with bacterial suspension, which showed inheritance ratio of 9:7 revealing polygenic control of resistance. For parental polymorphism survey, 80 RAPDs and 120 SSRs were used, out of which two

RAPDs and 30 SSRs were found polymorphic and were further used to carry out genotyping of mapping population. Linkage map was constructed using MAPMAKER/EXP ver 2.0. A total of five linkage groups were constructed spanning a distance of 1538 cM, with an average distance of 48.06 cM. Four QTL were obtained for black rot resistance. The molecular markers flanking QTL can be used for further validation of cabbage germplasm.

Lu *et al.* (2012) integrated 41 SNP markers developed from comparative transcriptomes into a previous linkage map and mapped 12 agronomic and morphological traits into the integrated map in red pepper. A total of 39 markers were assigned to 13 linkage groups (LGs). 23 QTL from 11 traits were detected using the composite interval mapping (CIM) algorithm. An interval between markers 'a035_1' and '170_1' on LG5 was detected as a main-effect locus among the resistance QTL to *P. capsici.* 17 QTL for another eight traits were located on LG3, 4 and 12. Furthermore, the locus for corolla color was mapped to LG10 as a marker. The integrated map and QTL identified would be helpful for current genetics research and crop breeding, especially in the Solanaceae family.

Genetic linkage map of stevia were constructed by Sharma (2013) by using multiple marker systems. For linkage map construction, F_2 population was used as a mapping population. To survey the polymorphism among contrasting parents 170 RAPDs, 26 ISSRs and 89 EST-SSRs were employed and it was observed that 36 RAPD, 10 ISSR and 33 EST-SSR primers were found to be polymorphic. These primers were then used for the genotyping of the mapping population. Phenotyping was carried out by using high performance liquid chromatography (HPLC) of parents as well as segregating population. Both genotypic and phenotypic data were used to construct a linkage map using MAPMAKER/EXP ver 3.0b. A total of four linkage groups were constructed spanning a distance of 927.3 cM with an average distance between loci as 16.29 cM. On QTL identification, a total of 53 QTL locations were found for both trait 1 (rebaudioside-A) and trait 2 (stevioside). Among 53 QTL locations of trait 1, two major QTL were found for rebaudioside-A on linkage group 3 at LOD of 2.5 and 2.7, respectively. No similar work on linkage map construction and QTL identification with multiple marker system has been reported before in stevia. Thus, the map will greatly facilitate further genetic studies with practical impact of this work for the advancement of stevia breeding and genome analysis.

In an investigation carried out by Vaidya (2014), QTL for two vegetative characteristics, vegetative bud break and crotch angle in apple were identified. For this,

cultivars ‘Red Delicious’ and ‘Maharaji’ were used as parents. 120 F_1 individuals were raised by crossing the parental plants. Parental polymorphism survey carried out using 164 SSR primers, both genomic and EST derived, revealed 97 polymorphic primers. These 97 primers were used for genotyping the mapping population. The individuals of the mapping population as well as the parental plants were scored for the two phenotypic traits. A linkage map consisting of 74 markers grouped into four linkage groups and covering 971.6 cM was generated using MAPMAKER/EXP ver 3.0b. QTL analysis was carried out using QTL Cartographer. Two significant QTL for bud burst were identified, one on chromosome 1, and other on chromosome 3. Six significant QTL for crotch angle were detected on chromosome 1. Seventh QTL for crotch angle was located on chromosome 3. This study lead to efficient identification of QTL, which can further be used to select promising populations using marker assisted breeding.

Sun *et al*. (2015) conducted QTL analysis for resistance to *Colletotrichum acutatum* in pepper. For this crosses were made between susceptible parent ‘77013’ and resistant parent ‘PBC932’, from which BC_1 population of 186 individuals was prepared by backcrossing the interspecific F_1 to parent ‘77013’. Resistance test was performed on detached mature green fruits under *in vitro* conditions by evaluated in disease incidence, true lesion diameter and overall lesion diameter. Based on a linkage map with 14 linkage groups using 385 markers (349 SSR, one InDel and 35 CAPS), 1310.2 cM in length, a total of nine QTL were located on chromosome 3, 5, 7, 10 and 12 associated with resistance. CIM revealed main effect QTL located in a close marker interval on chromosome 5. Identification of recombinant individuals suggested that resistance in pepper fruits may be controlled by distinct genes within the QTL interval on chromosome 5.

Chapter-3

MATERIALS AND METHODS

The present study entitled **"Studies on identification of molecular markers for buckeye rot (*Phytophthora nicotianae* Breda de Hann. var. *parasitica* (Dastur) Waterhouse) resistance in tomato (*Solanum lycopersicum* L.)"** was undertaken by following the given steps.

3.1 Evaluation of genetic diversity amongst tomato genotypes using molecular markers

3.1.1 Isolation of genomic DNA

3.1.2 Qualitative and quantitative assessment of DNA

3.1.3 *In silico* build up of tomato EST-SSRs

3.1.3.1 Searching and assembly of EST sequences

3.1.3.2 Finding out SSR sequences

3.1.3.3 Frequency of SSRs

3.1.3.4 Designing of primers

3.1.3.5 Putative annotation using BLASTx tool

3.1.4 PCR-Amplification of genomic DNA using molecular markers

3.1.4.1 PCR-Amplification using ISSR primers

3.1.4.2 PCR-Amplification using genomic SSR primers

3.1.4.3 PCR-Amplification using EST-SSR primers

3.1.5 Statistical analysis

3.2 To study genetics of inheritance of resistance to *Phytophthora nicotianae* var. *parasitica*

3.2.1 Raising of mapping population

3.2.2 Isolation and morphological characterization of *Phytophthora nicotianae* var. *parasitica*

3.2.3 Maintenance of pure culture

3.2.4 Preparation of fungal inoculum for screening

3.2.5 Pathogenicity test of segregants using fungal inoculum

3.2.6 Genetics of inheritance study

3.3 Identification of molecular markers/ QTL for buckeye rot resistance in tomato

3.3.1 Phenotyping of parents using fungal inoculum

3.3.2 Phenotyping of mapping population using fungal inoculum

3.3.3 Parental polymorphism survey using molecular markers

3.3.3.1 Using ISSR primers

3.3.3.2 Using genomic SSR primers

3.3.3.3 Using EST-SSR primers

3.3.4 Genotyping of mapping population

3.3.5 Software analysis to identify markers/ QTL for resistance to buckeye rot

3.1 Evaluation of genetic diversity amongst tomato genotypes using molecular markers

3.1.1 Isolation of genomic DNA

Source plant material:

Plant material is comprised of different lines, accessions and varieties of tomato (Table 1). The seeds were procured from Department of Vegetable Science, Dr YS Parmar university of Horticulture and Forestry, Nauni-Solan. Seeds were germinated in autoclaved mixture of vermicompost : soil (1:1). The seedlings were then transplanted to pots after one month of seed germination. The purpose of this objective was to study genetic diversity at molecular level so that the diverse lines can further be used in breeding programmes.

DNA from leaves taken from a single plant of each genotype was isolated using the CTAB method (Doyle and Doyle, 1987) with some modifications.

Table 1: List of tomato genotypes used for evaluation of genetic diversity

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

Reagents used for DNA isolation were as following:

Reagents:

a)	10% CTAB	10 gm of CTAB was dissolved in 100 ml of distilled water by warming the solution at 65^0C.
b)	0.5M EDTA (pH 8.0)	18.61 gm of EDTA was dissolved in 80 ml distilled water. pH of the solution was adjusted to 8.00. Final volume of the solution was made to 100 ml with distilled water and the solution was sterilized by autoclaving.
c)	4M NaCl	23.37 gm of NaCl was dissolved in minimum amount of distilled water and the final volume was made to 100 ml using distilled water and was then sterilized by autoclaving.
d)	1M Tris HCl (pH 8.0)	15.76 gm of Tris HCl was dissolved in 80 ml distilled water. The pH was adjusted to 8.00. The final volume was made to 100 ml with distilled water and the solution was sterilized by autoclaving.
e)	DNA extraction buffer	100 ml of the extraction buffer contained 1. 10 ml 1M Tris HCl 2. 4 ml 0.5M EDTA 3. 20 ml 10% CTAB 4. 35 ml 4M NaCl 5. 31 ml distilled water 6. 0.2% β-mercaptoethanol
f)	Chloroform : Isoamyl	96 ml of chloroform and 4 ml of isoamyl alcohol were mixed (24:1,v/v) together and the mixture was kept in a closed container at room temperature (25^0C).
g)	70% ethanol	70 ml of absolute alcohol was mixed with 30 ml of distilled water to make it 100 ml.
h)	TE buffer	0.1576 gm of Tris HCl and 0.0372 gm of EDTA were dissolved in 100 ml of distilled water. The pH was adjusted to 8.00.

Procedure of DNA isolation:

Step 1 Collected approximately 2 gm of young and healthy leaves and homogenized completely to fine powder with liquid nitrogen using prechilled pestle and mortar.

Step 2 Transferred leaf powder to 50 ml tube containing 10 ml pre-warmed (at 65^0C) DNA extraction buffer. Leaf powder should not get moist because under wet condition DNase digests total DNA.

Step 3 Incubated the tubes for one-two hours at 65^oC in a water bath. During incubation the samples were mixed well by inverting the tubes every five minutes.

Step 4 To each tube 10 ml of chloroform : isoamyl alcohol (24:1, v/v) was added and the contents were mixed gently by hand inversions till the colour in the lower portion of the tube turned dark green.

Step 5 Centrifuged the above suspension at 12000 rpm for ten minutes at room temperature.

Step 6 Transferred the aqueous phase gently without disturbing the inter phase to fresh autoclaved centrifuge tubes.

Step 7 Added 2/3rd volume of prechilled isopropanol, mixed gently by hand inversions and incubated at -20^0C for one hour or overnight so that DNA precipitated out.

Step 8 Precipitated DNA was pelleted by centrifugation at 10000 rpm for ten minutes at 4^0C.

Step 9 Washed the DNA with 500µl of 70% ethanol and centrifuged at 5000 rpm for five minutes at 4^0C.

Step 10 Decanted off the supernatant and dried the pellet overnight to completely evaporate the alcohol.

Step 11 Dissolved the DNA pellet in 500 µl TE buffer.

Isolated DNA was then purified following below given reagents and steps.

Reagents

a) RNase (10mg/ml) Dissolved 10 mg lyophilized RNase in 1 ml autoclaved distilled water. The mixture was kept overnight to completely dissolve the powder.

b)	Phenol : chloroform (1:1v/v)	Mixed 50 ml phenol and 50 ml chloroform properly. Stored at 4^0C in a covered container.
c)	Chloroform : isoamyl alcohol (24:1v/v)	As described earlier in reagents used for DNA isolation
d)	3M sodium acetate	Dissolved 24.609 gm of sodium acetate in 80 ml distilled water and adjusted the pH to 4.8 using glacial acetic acid. Made the final volume to 100 ml with distilled water.
e)	Absolute ethanol (95%)	95 ml absolute ethanol was mixed properly with 5 ml distilled water so that final volume is 100 ml.
f)	70% ethanol	As described earlier in reagents used for DNA isolation

Procedure of DNA purification:

Step 1 Added 10 µg/µl of RNase to the isolated DNA samples and incubated at 37^0C for one hour.

Step 2 Added equal volume of phenol : chloroform and mixed gently.

Step 3 Centrifuged at 11000 rpm for two minutes at room temperature and transferred the aqueous phase to fresh eppendorf tubes.

Step 4 Extracted twice with equal volume of chloroform : isoamyl alcohol (24:1, v/v) and spun at 11000 rpm for two minutes.

Step 5 Separated the aqueous phase. Added $1/10^{th}$ volume of 3M sodium acetate and 2.5 volume of absolute ethanol. Mixed gently and incubated at 4^0C.

Step 6 Pelleted the DNA by centrifugation at 11000 rpm for five minutes.

Step 7 Decanted the supernatant. Washed the pellet with 70% ethanol, and air dried the pellet.

Step 8 Resuspended the pellet in 100 µl TE buffer.

3.1.2 Qualitative and quantitative assessment of DNA

Quality of DNA was assessed by running DNA on 0.8% agarose gel supplemented with ethidium bromide (10 µg/ul).

The DNA quantity was accessed by running it on 0.8% agarose gel alongwith λ (lambda) DNA marker of known concentration. Alternatively the quantity was also assessed spectrophotometrically using the following formula:

$$\text{DNA } (\mu g/ml) = \frac{\text{OD260 x dilution factor x 50}}{1000}$$

The ratio of absorbance at 260 nm and at 280 nm was measured to check the purity of DNA (Table 2).

Table 2: Purity check of DNA on the basis of A_{260}:A_{280} ratio

S. No.	A_{260}:A_{280} ratio (Absorbance ratio)
1.	Above 1.8
2.	1.4 to 1.8
3.	Below 1.4

3.1.3 *In silico* build up of tomato EST-SSRs

3.1.3.1 Searching and assembly of EST sequences

A total of 4200 EST sequences of tomato were downloaded from NCBI website (www.ncbi.nih.gov/nucest) in FASTA format.

EGassembler webserver (Masoudi-Nejad *et al.,* 2006) was used to assemble the downloaded sequences by creating contigs and singletons. Contigs were created to remove the overlapping sequences, as a result of which contiguous sequences without any overlap were obtained. Singletons which represented unique sequences without any repetition were also obtained. Both contigs and singletons were obtained in zipped format which were downloaded to personal computer.

3.1.3.2 Finding out SSR sequences

The online interface of simple sequence repeat identification tool (SSRIT; Temnykh *et al*., 2001) available on website www.gramene.org/db/searches/SSRtool was used to find out SSR sequences among the contigs and singleton sequences. The following parameters were used to find out desirable SSR motifs:

1) Maximum length of the motif was set as decamer

2) Minimum length of repeat was set as five

The desired SSR motifs were then saved in a table form containing sequence ID, SSR repeat, length of SSR motif, start site of SSR and end site of SSR.

3.1.3.3 Frequency of SSRs

Frequency of occurrence of different repeat motifs with variability in their length was calculated by using the given formula:

$$\text{Frequency of repeat} = \frac{\text{Number of times particular repeat occured}}{\text{Total number of repeats of same length}} \text{ x } 100$$

The frequency of SSRs was converted into pie charts.

3.1.3.4 Designing of primers

Primers were then designed using Primer3 software (www.frodo.wimit.edu/primer3/) (Rozen and Skaletsky, 2000). For this, the EST sequence containing desired SSR motif was pasted in the area provided, followed by filling up the other criteria such as sequence ID, SSR repeat motif length with start site. After this the pick primer button was clicked. As a result of this primers were designed which were copied along with desired information like sequence of forward and reverse primer, GC content, primer Tm. These primer sequences were then custom synthesized from recognized firm and further used for molecular marker studies.

3.1.3.5 Putative annotation using BLASTx tool

Basic local alignment search toolx (BLASTx) tool of Uniprot database (http://www.uniprot.org/) was used for putative annotation of *in silico* designed EST-SSRs, assuming an E value of ≤1E-5 as a significant criterion of homology. In this database comparative analysis and assignment of functions is done on the basis of EST sequence homology with other sequences already submitted in the databank. For this, the EST sequences having EST-SSR primers were pasted in the provided area followed by clicking on button. The most significant matches for each sequence were recorded.

3.1.4 PCR-Amplification of genomic DNA using molecular markers

Isolated DNA was subjected to PCR for amplification for studying genetic diversity using different molecular marker systems such as ISSR, genomic and EST-SSRs.

3.1.4.1 PCR-Amplification using ISSR primers

A total of 25 ISSR primers synthesized from Metabion International (AG, Deutschland, Germany) through Hysel India Pvt. Ltd., New Delhi, India were used to carry out genetic diversity studies of tomato genotypes. List of primers used is provided in Table 3.

Table 3: List of ISSR primers used in present study

S. No.	Primer Name	Primer sequence (5'→3')	GC Content* (%)	Tm** (^{0}C)	Length (base pairs)
1	Primer 1	CACACACACACACACAAGG	52.6	56	19
2	ISSR-2	CAGAGAGAGAGAGAGAYT	47.2	53	18
3	UBC-840	GAGAGAGAGAGAGAGACTC	52.6	57	19
4	IISRS-3-E	ACACACACACACACACG	52.9	52	17
5	IISRS-3-G	GTGGTGGTGGTGGTG	66.7	52	15
6	IISRS-3-H	GACGACGACGACGAC	66.7	52	15
7	IISRS-3-F	GACAGACAGACAGACA	50.0	48	16
8	IISRS-3-L	GACAGACAGACAGACA	50.0	48	16
9	ISSR-4	AGAGAGAGAGAGAGAGYT	47.2	53	18
10	ISSR 844B	CTCTCTCTCTCTTGC	53.3	46	15
11	ISSR-5	AGAGAGAGAGAGAGAGYC	52.8	55	18
12	ISSR-7	ACACACACACACACACYC	52.8	55	18
13	ISSR-3	GAGAGAGAGAGAGAGAYC	52.8	55	18
14	UBC-855	ACACACACACACACACCTT	47.4	55	19
15	UBC-848	CACACACACACACACAAGG	52.6	57	19
16	ISSR17898A	CACACACACACAAC	50.0	41	19
17	ISSR178998B	CACACACACACAGT	50.0	41	14
18	UBC-894	TGGTAGCTCTTGTCAGGCAC	55.0	60	20
19	ISSR-3-I	CACACACACACACACACG	55.6	56	18
20	UBC-854	TCTCTCTCTCTCTCTCCAGG	55.0	60	20
21	UBC-841	GAGAGAGAGAGAGAGACTC	52.6	57	19
22	ISSR-HB-12	CACCACCACGC	72.7	38	11
23	ISSR-HB-15	GTGGTGGTGGC	72.7	38	11
24	ISSR-3-0	CACACACACACACACAGC	55.6	56	18
25	ISSR-3-M	ACACACACACACACAC	49.2	50	16

GC Content*: Guanine Cytosine Content; Tm**: Annealing temperature

Standardization of PCR-ISSR protocol

A reaction volume of 20µl was standardized using suitable concentrations of the reaction components (Table 4).

Table 4: Concentrations of different reaction components used for PCR-ISSR amplification

S. No.	Component	Variable Concentrations
1	PCR buffer A (10 X) (containing 1.5 mM $MgCl_{2)}$	1 X
2	dNTP (10 mM)	0.2 mM to 1.0 mM
3	Primer	10 to 35 pMoles
4	Taq DNA polymerase	0.5 to 2.5 U
5	DNA	30 to 50 ng
6	Autoclaved Distilled Water (ADW)	To make up final volume of 20 µl

After preparation of reaction volume (without DNA), vortexing was done for few seconds. Then equal amount of reaction volume i.e. 17 µl was separately distributed in each PCR tube of 0.2 ml capacity (Axygen Scientific Pvt. Ltd., New Delhi, India). Finally 3 µl DNA (50 ng) from each genotype was separately added. These tubes were then kept in thermal cycler (Applied Biosystems, Foster city, California, USA) for amplification.

The thermal profile was standardized following three steps of PCR cycle set at different temperatures as given below:

ISSR thermal profile			
Step		**Temperature (^{0}C)**	**Time duration**
Initial denaturation		94^0C	2 minutes
Denaturation	40 cycles repeat	94^0C	10 seconds
Annealing		Annealing temperature according to primer Tm	30 seconds
Extension		72^0C	65 seconds
Final extension		72^0C	10 minutes

Visualization of PCR product

Amplified product was mixed thoroughly with 6X loading dye (Appendix I) followed by electrophoresis in 1.2% agarose gel (Appendix IIa) prepared in 1X TAE buffer (Appendix III) supplemented with 10 µg/µl ethidium bromide. The gel was then run at constant voltage rate of 5V/cm under submerged conditions for about two hours.

Co-electrophoresis of standard molecular weight marker (Hind III/ EcoRI, double digest, Bangalore Genei, India) was used to determine the size of amplified product. DNA profiles were visualized on UV transilluminator and the images of the amplified products on gels were documented on Gel-Documentation System (Syngene, Cambridge, UK).

3.1.4.2 PCR-Amplification using genomic SSR primers

25 genomic SSR primers (Table 5) synthesized from Genaxy Scientific (New Delhi, India) through International Scientific and Surgicals Pvt. Ltd. (New Delhi, India) were used for diversity studies.

Table 5: List of genomic SSR primers used in present study

S. No.	Primer Name	Primer sequence (5'→3')	GC Content (%)	Tm (^{0}C)	Length (base pairs)
1	G1	F: TCTTTCATTTCATGTCACGA R: AGGAGACCTTATGATTCAAGG	35.0 42.9	51.2 55.9	20 21
2	G2	F: GTGTTTTTATGCAGGGTTTG R: CACACTTATACCTCACCCGT	40.0 50.0	53.2 57.3	20 20
3	G3	F: ACAAACTCAAGATAAGTAAGAGC R: GTGAATTGTGTTTTAACATGG	34.8 33.3	55.3 52.0	23 21
4	G4	F: GGATTGTAGAGGTGTTGTTGG R: TTTGTAATTGACTTTGTCGATG	47.6 31.8	57.9 52.8	21 22
5	G5	F: CTCTCTCAATGTTTGTCTTTC R: GCAAGGTAGGTAGCTAGGGA	38.1 55.0	54.0 59.4	21 20
6	G6	F: AATGTAACAACGTGTCATGATTC R: AAGTCACAAACTAAGTTAGGG	34.8 38.1	55.3 54.0	23 21
7	G7	F: AGCATGGGAAGAAGACACGT R: TTGAGCAAAACATCGCAATC	50.0 40.0	57.3 53.2	20 20
8	G8	F: CGCTCCCTTTTTGAATTGAG R: TTGCTGTTGTGGTTTTCGAT	45.0 40.0	55.3 53.2	20 20
9	G9	F: CCTTGCAGTTGAGGTGAATT R: TCAAGCACCTACAATCAATCA	45.0 38.1	55.3 54	20 21
10	G10	F: CGGCGTATTCAAACTCTTGG R: GCGGACCTTTGTTTTGGTAA	50.0 45.0	57.3 55.3	20 20

Table 5. Cont…..

S. No.	Primer Name	Primer sequence (5'→3')	GC Content (%)	Tm (^{0}C)	Length (base pairs)
11	G11	F: TTCGGTTTATTCTGCCAACC R: GCCTGTAGGATTTTCGCCTA	45.0 50.0	55.3 57.3	20 20
12	G12	F: AATTTCGGACCCGCCGAG R: TTCAACGCCATCGATGC	61.1 52.9	58.2 52.8	18 17
13	G13	F: CCTCCAAATCCCAAAACTCT R: TGTTTCATCCACTATCACGA	45.0 40.0	55.3 53.2	20 20
14	G14	F: TGTATCCTGGTGGACCAATG R: TCCAAGTATCAGGCACACCA	50.0 50.0	57.3 57.3	20 20
15	G15	F: GAAAAATCTGGCTCCAGCAC R: TCGTTCTACTTTCTCCCCAAC	50.0 47.6	57.3 57.9	20 21
16	G16	F: GCACGAGACCAAGCAGATTA R: GGGCCTTTCCTCCAGTAGAC	50.0 60.0	57.3 61.4	20 20
17	G17	F: TTCCTCACTATTTTGAATTGCG R: TGTACTTCTCTGCAGATTCCA	36.4 42.9	54.7 55.9	22 21
18	G18	F: AGCCACCCATCACAAAGATT R: GTCGCACTATCGGTCACGTA	45.0 55.0	55.3 59.4	20 20
19	G19	F: TTCAAGGTTTATTCGAAAATCC R: TTTGGGCCTATCACCTTGTC	31.8 50.0	52.8 57.3	22 20
20	G20	F: TGATGGCAGCATCGTAGAAG R: GGTGCGAAGGGATTTACAGA	50.0 50.0	57.3 57.3	20 20
21	G21	F: TTGTCGCTTCAGTTTTGGC R: TTCACCTTGCCACTGTGAAG	47.4 50.0	54.5 57.3	19 20
22	G22	F: GCGCACCCAAAGTTGAAG R: CCTCATAGGGACGCACATAC	55.6 55.0	56.0 59.4	18 20
23	G23	F: TGTTGGTTGGAGAAACTCCC R: AGGCATTTAAACCAATAGGTAGC	50.0 39.1	57.3 57.1	20 23
24	G24	F: TGTGTTGGATGTTTGGCACT R: GCCATTGAAACTTGCAGAGA	45.0 45.0	55.3 55.3	20 20
25	G25	F: GAAACCGCCTCTTTCACTTG R: CAGCAATGATTCCAGCGATA	50.0 45.0	57.3 55.3	20 20

Standardization of PCR-SSR protocol

Standardization of different reaction components viz., PCR buffer A (10X) containing 1.5 mM $MgCl_2$, dNTP mix (10 mM), primer (both forward and reverse) and taq DNA polymerase was done to prepare 20µl reaction volume (Table 6).

Table 6: Concentrations of different reaction components used for PCR-SSR amplification

S. No.	Component	Variable Concentrations
1	PCR buffer A (10 X) (containing 1.5 mM $MgCl_2$)	1 X
2	dNTP (10 mM)	0.2 mM to 1.0 mM
3	Primer-Forward	10 to 35 pMoles
4	Primer-reverse	10 to 35 pMoles
5	Taq DNA polymerase	0.5 to 2.5 U
6	DNA	30 to 50 ng
7	ADW	To make up final volume of 20 µl

The prepared reaction volume (without DNA) was vortexed for few seconds followed by equal distribution of reaction volume i.e. 17 µl in each PCR tube of 0.2 ml capacity. Then 3 µl DNA (50 ng) from each sample was added separately. The following thermal profile was used for amplification of DNA in thermal cycler (Applied Biosystems, Foster city, California, USA):

PCR-SSR thermal profile			
Step		**Temperature**	**Time duration**
Initial denaturation		94^0C	3 minutes
Denaturation	45 cycles repeat	94^0C	1 minute
Annealing		Annealing temperature according to primer Tm	1 minute
Extension		72^0C	2 minutes
Final extension		72^0C	10 minutes

Visualization of PCR product

Amplified product was mixed thoroughly with 6X loading dye (Appendix I) followed by electrophoresis in 3.5% agarose gel (Appendix IIb) prepared in 1X TAE buffer (Appendix III) supplemented with 10 µg/µl ethidium bromide. The gel was then run at constant voltage rate of 5V/cm under submerged conditions for about two hours.

Co-electrophoresis of standard molecular weight marker (Hind III/ EcoRI, double digest, Bangalore Genei, India) was used to determine the size of amplified product. DNA profiles were visualized on UV transilluminator and the images of the amplified products on gels were documented on Gel-Documentation System (Syngene, Cambridge, UK).

3.1.4.3 PCR-Amplification using EST-SSR primers

20 *in silico* synthesized primers were custom synthesized from Genaxy Scientific (New Delhi, India) through International Scientific and Surgicals Pvt. Ltd. (New Delhi, India) and used for genetic analysis studies. List of primers used along with sequence ID, primer sequence, GC content, Tm and sequence length is provided in Table 7.

Table 7: List of EST-SSR primers used in present study

S. No.	Primer Name	Sequence of primer (5' ⟶ 3')	Tm (^{o}C)	GC content (%)	Length (base pairs)
1	Contig7	F:GGGGAGATAGCACGGATTTT	57.36	50.00	20
		R:GTGGGCGGCAAATTAAGGAT	58.89	50.00	20
2	Contig143	F:GTCATCGACGAAACAAAGCA	57.06	45.00	20
		R:CTTGCCTCTTGATCTTCGCC	58.99	55.00	20
3	Contig162	F:TAATGCTGGACCTGGAACCA	58.63	50.00	20
		R:GTCAGCAATAACCACAGGCT	58.17	50.00	20
4	Contig265	F:GGTGGAGGTGGGCATAATGA	59.45	55.00	20
		R:GTAATTGATCCACCGGCGTC	59.06	55.00	20
5	Contig340	F:GCCCTCTTGAGGACTTGGAG	59.75	60.00	20
		R:ACCTTCAAAAGCAGTGCAGC	59.61	50.00	20
6	Contig352	F:GAACGCTCCCTCTACCTTTT	57.24	50.00	21
		R:TAACTTTCAGCTGGCTCACC	57.81	50.00	21
7	gi|116644211|	F:AAAAGATGCAACGCTGGAAC	57.58	45.00	20
		R:ACTGAAACGCGCACATGTAA	58.78	45.00	20
8	gi|4387244|	F:CGCAACTCCTTCTGCTGATG	59.27	55.00	20
		R:GCAACTGAGTCCTTCGATGT	57.91	50.00	20
9	gi|4386975|	F:TCTCCATCTCACCGTCGATG	58.97	55.00	20
		R:AGCACGGACAGGGGAATTTA	59.01	50.00	20
10	gi|4386907|	F:TTGTGGACTATCGGACCCTG	58.81	55.00	20
		R:ACCATGGTTCCTGCAGATGA	59.01	50.00	20
11	gi|4386813|	F:TGGGGTTTTGTTGTGAGGAA	57.47	45.00	20
		R:ATATCCGGTGGCCTCGAAAT	58.94	50.00	20
12	gi|4386782|	F:ATCAATTTCACTCCACCGCC	58.54	50.00	20
		R:TCGGCATCCATCTCTCCTTC	58.96	55.00	20
13	gi|4386589|	F:GGATTTCTCGCCGGTTAACC	58.99	55.00	20
		R:TGGAGGATCTGTCAGCTTCG	59.18	55.00	20
14	gi|4386576|	F:GGTCACGTGTCAACCATCAT	58.19	50.00	20
		R:AGGGGAAAGGGAAAGAGTCG	59.02	55.00	20
15	gi|4386543|	F:ACTCCTGAGATGTCGTGCAA	59.03	50.00	20
		R:TGCCCCACAAAACTCAAACA	58.44	45.00	20
16	gi|4386508|	F:GCCAGCTGAGAAGAAACCAG	58.84	55.00	20
		R:ACCCATAGACTTGCTGGAGA	57.75	50.00	20
17	gi|4386388|	F:CCTTACTCTCTCCCCTGCTC	58.59	60.00	20
		R:TTTCTCGAGTGCAGCAATGG	58.84	50.00	20
18	gi|4386332|	F:GGCACGAGTCGATTTGTCTG	59.28	55.00	20
		R:TTGGTTGATGATGCGGAGGA	59.38	50.00	20
19	gi|4386229|	F:ACGAGCTTCCTTCTACCACA	58.37	50.00	20
		R:GCGGGTGAATGGAGGAAAAG	59.19	55.00	20
20	gi|76572221|	F:ATCACCATCTTCCTCTGCCT	58.11	50.00	20
		R:CCCGTTGAAGTTGATCGCAA	59.13	50.00	20

Standardization of PCR-SSR protocol

Same protocol as discussed in section 3.1.4.2 i.e. concentrations of reaction volume components, thermal profile and visualization of PCR product, was used in case of EST-SSR primers.

3.1.5 Statistical analysis

Polymorphism and DNA fingerprinting studies

Polymorphism study was carried out separately for ISSRs, genomic SSRs and EST-SSRs to find out variability among the genotypes by using the following formula:

$$\text{Per cent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Position of unique bands was used for fingerprinting the genotypes.

Polymorphism Information Content (PIC)

The PIC values provide an estimate of the discriminative power of a marker by taking into account not only the number of alleles at a locus but also relative frequencies of those alleles in the genotypes (Anderson *et al.*, 1993). The PIC value was calculated using the formula:

$$PIC = 1 - \sum pi^2$$

where 'pi' is the frequency of the *i*th allele

Effective multiplex ratio (E)

Effective multiplex ratio denotes the number of polymorphic loci in the germplasm, analyzed per experimentation (Varshney *et al.*, 2007). It was calculated by the following formula:

$$E = n\beta$$

Where, 'β' is the fraction of polymorphic markers and is estimated as β = np/(np + nnp), indicating 'np' as polymorphic loci and 'nnp' as non-polymorphic loci

'*n*' is the multiplex ratio, measured as the average number of DNA fragments amplified/detected per genotype using a marker system.

Marker Index (MI)

The utility of a given marker system is a balance between the level of polymorphism detected and the extent to which an assay can identify multiple polymorphisms. Marker index provides a convenient estimate of any marker system utility (Varshney *et al.*, 2007). It was estimated as follows:

$$MI = PIC \times E$$

Where, 'PIC' is polymorphism information content and 'E' is effective multiplex ratio

Data analysis

Binary matrix was prepared in Microsoft Excel by indicating the presence of band by 1 and absence by 0. NTSYS-pc version 2.0 (Rohlf, 1998) was used for combined analysis of results obtained through three different molecular marker systems. Similarity matrix and dendrogram showing relationship between different genotypes was constructed based on the Jaccard coefficient from SimQual function of similarity module followed by UPGMA clustering method of SAHN module.

3.2 To study genetics of inheritance of resistance to *Phytophthora nicotianae* var. *parasitica*

3.2.1 Raising of mapping population

For raising mapping population following parental lines were used:

Resistant lines: Hawai 7998, EC-520075, EC-251649, EC-126902, EC-5283373

Susceptible variety: Solan Lalima

Different cross combinations were tried by crossing susceptible variety 'Solan Lalima' with above mentioned resistant lines. Cross between 'Solan Lalima' and 'EC-251649' produced maximum number of F_1 seeds. So this cross combination was used for further studies. This F_1 population was then selfed to raise the F_2 population which was used as mapping population for the present study.

On the basis of inoculation of segregating population (F_2) genetics of inheritance was studied.

To carry out this study first of all fungal inoculums was prepared as follows:

3.2.2 Isolation and morphological characterization of *Phytophthora nicotianae* var. *parasitica*

For isolation of pathogen, buckeye rot infected fruit of tomato, obtained from fields of Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) showing brownish pattern of concentric rings, was used. After this the fruit was washed with autoclaved distilled water. Then part of fruit around infected portion was cut using sterile blade followed by culturing on corn meal agar (CMA) (Appendix IV). Morphological characterization of fungus was carried out under microscope.

3.2.3 Maintenance of pure culture

The culture of *Phytophthora nicotianae* var. *parasitica* was maintained on CMA medium (Appendix-IV) in the petriplates by culturing a single bit of previously grown culture to obtain pure culture of pathogen. Then the culture was incubated at 25°C for 7-10 days till uniform fluffy growth was obtained. Thereafter the culture plates with pathogen were covered properly and kept at low temperature (4°C) to stop further growth.

3.2.4 Preparation of fungal inoculum for screening

After morphological confirmation, a dilute suspension of fungal cells was prepared on Corn Meal broth (Appendix-V). After ten days of inoculum growth, the density of fungal hyphae was standardized using haemocytometer for inoculating fruits. Optimum density of 15-20 hyphae/ cm^3 in haemocytometer was obtained by mixing 1 gm of fungal hyphae in 80 ml distilled water. Then the fungal inoculum was used to infect the fruits of tomato at different concentrations viz., 2 ml, 5 ml, 10 ml and 15 ml. Inoculation with 10 ml of inoculum was found most effective. Too low concentrations i.e. 2 ml and 5 ml did not cause much damage, while too high concentration i.e. 15 ml cause early and complete damage of fruit.

Screening was done by using two methods:

1) Detached fruit method
2) Intact fruit method

The fruits were injected with 10 ml of inoculum. Infected material was observed periodically for the appearance of symptoms like formation of brownish spot and pattern of concentric ring of brown bands on the fruits. The disease incidence was calculated using following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased fruits per plant}}{\text{Total number of fruits per plant}} \text{ X } 100$$

After calculation of disease incidence, the scale given in Table 8 was used for assessing disease reaction on fruits, depending on it the fruits of each plant were grouped in different categories.

Table 8: Scale for assessing disease reaction on fruits

Category/ Disease reaction	Infected Fruits (%)
Resistant	0-5.0
Moderately Resistant	5.1-15.0
Moderately Susceptible	15.1-30.0
Susceptible	30.1-45.0
Highly Susceptible	45.1 or above

3.2.5 Pathogenicity test of segregants using fungal inoculum

Parents and 100 F_2 individuals were screened for their reaction to buckeye rot using fungal culture by injecting 10 ml inoculum on detached fruits (under *in vitro* conditions) and intact fruits (under *in vivo* conditions). Disease reaction was assessed as given in section 3.2.4.

3.2.6 Genetics of inheritance study

For calculating genetics of inheritance, ratio of susceptible and resistant fruits was calculated. Then the observations were recorded for the pattern of inheritance by noting whether the ratio fits in Mendalian ratio of 3:1 or deviates from it. Then Chi-square (χ^2) test was performed to determine whether the observed ratio fits in expected one or not.

3.3 Identification of molecular markers/ QTL for buckeye rot resistance in tomato

3.3.1 Phenotyping of parents using fungal inoculum

For phenotyping, the parental lines were inoculated both under *in vitro* as well as *in vivo* conditions to test their susceptibility and resistance to pathogen.

3.3.2 Phenotyping of mapping population using fungal inoculum

Phenotypic data recorded on 100 F_2 individuals as given in section 3.2.6 was also used to further conduct molecular marker/ QTL studies.

3.3.3 Parental polymorphism survey using molecular markers

Parental polymorphism survey was done to find out polymorphic primers which were further used to carry out mapping population genotyping for generating informative polymorphism data for QTL mapping. To carry out polymorphism studies among parental lines DNA isolation was done using the method given in 3.1.1 and for PCR amplification three different types of molecular markers viz., ISSR, genomic and EST-SSRs were used by following the procedure described in section 3.1.4. In detail the information of different primers used is provided in following steps.

3.3.3.1 Using ISSR primers

A total of 44 ISSR primers were used to conduct parental polymorphism survey. List of 25 primers is already provided in Table 3 and that of remaining is given in below Table 9.

Table 9: List of ISSR primers used for parental polymorphism survey

S. No.	Primer Name	Primer sequence (5'→3')	GC Content (%)	Tm (^{0}C)	Length (base pairs)
1	Primer 2	TGTGTGTGTGTGTGTGC	52.9	52	17
2	ISSR-HB-10	GAGAGAGAGAGACC	57.1	44	14
3	ISSR-HB-11	GTGTGTGTGTGTCC	57.1	44	14
4	ISSR-6	AGAGAGAGAGAGAGAGYG	52.8	55	18
5	ISSR-8	CACACACACACACACA	50.0	48	16
6	ISSR 844-B	CTCTCTCTCTCTTGC	53.3	46	15
7	UBC-808	AGAGAGAGAGAGAGAGC	52.9	52	17
8	UBC-829	TGTGTGTGTGTGTGTGC	52.9	52	17
9	UBC-850	GTGTGTGTGTGTGTGTCTC	52.6	57	19
10	UBC-876	GATAGATAGACAGACA	37.5	43	16
11	UBC-880	GGAGAGGAGAGGAGA	60.0	49	15
12	UBC-886	ACGAGTACGCTCTCTCTCTCTCT	52.2	65	23
13	UBC-890	ACGACTACGGTGTGTGTTTGTGT	47.8	63	23
14	IISRS-3-A	CTCTCTCTCTCTCTCTT	47.1	50	17
15	IISRS-3-B	CACACACACACACACAT	47.1	50	17
16	IISRS-3-C	TCTCTCTCTCTCTCTCA	47.1	50	17
17	IISRS-3-D	TCTCTCTCTCTCTCTCG	52.9	52	17
18	IISRS-3-N	CACACACACACACACATG	50.0	53	18
19	IISRS-3-P	GAGAGAGAGAGAGATA	43.8	46	16

3.3.3.2 Using genomic SSR primers

A total of 89 genomic SSR primers were used to conduct parental polymorphism, of which, information of 25 primers used is given in previous Table 5 and list of rest primers is given in Table 10.

Table 10: List of genomic SSR primers used for parental polymorphism survey

S. No.	Primer Name	Primer sequence (5'→3')	GC Content (%)	Tm (^{0}C)	Length (base pairs)
1	G26	F: CCGTCCAGAAGACGATGTAA	50.0	57.3	20
		R: CAAAGTCTTGCCAACAATCC	45.0	55.3	20
2	G27	F: ATTGCTCATACATAACCCCC	45.0	55.3	20
		R: GGGACAAAATGGTAATCCAT	40.0	53.2	20
3	G28	F: TTCTATCTCATTTGGCTTCTTC	36.4	54.7	22
		R: TTACCTTGAGAATGGCCTTG	45.0	55.3	20
4	G29	F: TTGGTCTAGAACGATGAGCA	45.0	55.3	20
		R: GCCATGCATCACTGAATGAC	50.0	57.3	20
5	G30	F: ACATGACCGGTTGACGACTA	50.0	57.3	20
		R: AAATTGTCCACATGGTGGGT	45.0	55.3	20
6	G31	F: CATTTGTTGTATGGCATCGC	45.0	55.3	20
		R: CAGTGACCTCTCGCACAAAA	50.0	57.3	20
7	G32	F: GGGTTAATAAAGCAATGTAGCG	40.9	56.5	22
		R: CTCTTCATTAAAGTTGCCGC	45.0	55.3	20
8	G33	F: ACTCCTTGTCATTGGGAAGC	50.0	57.3	20
		R: AGCTTCACCAAAAGGCATTC	45.0	55.3	20
9	G34	F: ATGGTCGTCCTTCCAGTTTT	45.0	55.3	20
		R: AAATCAGCCCAGCTCACCTA	50.0	57.3	20
10	G35	F: CATCACACTCAGACCCCACT	55.0	59.4	20
		R: GGGGGTTTAAAATCCACCTT	45.0	55.3	20
11	G36	F: TGGGTTTCGTTTCAGTAGCA	45.0	55.3	20
		R: TGGGCGTATCTAGGGTTGAG	55.0	59.4	20
12	G37	F: TTGCACAAGGGTAGACGTGA	50.0	57.3	20
		R: GCCAACATTCAAGTGATTCCA	42.9	55.9	21
13	G38	F: TTCGGGGACGAAACTAATGA	45.0	55.3	20
		R: TTCGGGCATAGATTGAGGAT	45.0	55.3	20
14	G39	F: CGCGATATATAAAGAGCGAACA	40.9	56.5	22
		R: AATTCTCATCCCAAGGCAAC	45.0	55.3	20
15	G40	F: GATCTCAAAGGATGAACAATAC	36.4	54.7	22
		R: TCATTAGGAGATTCTTTGTATCA	30.4	53.5	23
16	G41	F: ATGCAGTTCCAAGCATCATT	40.0	53.2	20
		R: TTGCCACATTAATGTTGAAGT	33.3	52.0	21
17	G42	F: TTCTGGCAATCCACATTCAA	40.0	53.2	20
		R: TTATCGCAACGGAATCTGAA	40.0	53.2	20
18	G43	F: TCCGGGGTCAAATTAAGAGG	50.0	57.3	20
		R: TCAAAATGGCTCCACAAATG	40.0	53.2	20
19	G44	F: ATTCCATCTCCACCACCAAG	50.0	57.3	20
		R: TGGAGTTGCCACATTCAAAA	40.0	53.2	20

Table 10. Cont…..

S. No.	Primer Name	Primer sequence (5'→3')	GC Content (%)	Tm (^{0}C)	Length (base pairs)
20	G45	F: ACCGCCCTAGCCATAAAGAC	55.0	59.4	20
		R: TGCGTCGAAAATAGTTGCAT	40.0	53.2	20
21	G46	F: AATTGCCACGTGGATTGACT	45.0	55.3	20
		R: TGCAAGCTGTTCTTTTCAGA	40.0	53.2	20
22	G47	F: ATCCGTTTGATTGCTCGAAT	40.0	53.2	20
		R: TCTTCTGTTGCAGTCCCAAA	45.0	55.3	20
23	G48	F: CACCACAATCATTGCTGTCC	50.0	57.3	20
		R: TTTAAGGCACTGGAATGTGC	45.0	55.3	20
24	G49	F: GCAGGTACGCACGCATATAT	50.0	57.3	20
		R: GCTCCGTCAGGAATTCTCTC	55.0	59.4	20
25	G50	F: AAGCGAACGGGTACTGTCAC	55.0	59.4	20
		R: GCTTTGTGGGTTCGAAACAT	45.0	55.3	20
26	G51	F: TGACATAGAGTCGAAGGCAATC	45.5	58.4	22
		R: AACCGAGTCGTGGAAACAAC	50.0	57.3	20
27	G52	F: CTGATTGCAGACAACGAGGA	50.0	57.3	20
		R: CGCTTTGTCGAAGCTCTGAT	50.0	57.3	20
28	G53	F: TCTTAGGCTGGGTGGGAGTA	55.0	59.4	20
		R: GAGGCCTTGCTCATGTGATT	50.0	57.3	20
29	G54	F: ATCTCATTCAACGCACACCA	45.0	55.3	20
		R: AACGGTGGAAACTATTGAAAGG	40.9	56.5	22
30	G55	F: GTCTTGGCTCATCAGCATCA	50.0	57.3	20
		R: TGCAACGGCAATATTACGAG	45.0	55.3	20
31	G56	F: GAAAAATCTGGCTCCAGCAC	50.0	57.3	20
		R: TCGTTCTACTTTCTCCCCAAC	47.6	57.9	21
32	G57	F: GTTCTCGATCTTTGGCTTCG	50.0	57.3	20
		R: GGCTTACAGGGCATACCAAA	50.0	57.3	20
33	G58	F: GTTCTCGATCTTTGGCTTCG	50.0	57.3	20
		R: TTTTCTTACGGGGCATACCA	45.0	55.3	20
34	G59	F: AAGCGAACGGGTACTGTCAC	55.0	59.4	20
		R: GCTTTGTGGGTTCGAAACAT	45.0	55.3	20
35	G60	F: TCGTAGCTTCTTTCACGTTGT	42.9	55.9	21
		R: CCGAATGAAAAGGACAAGGA	45.0	55.3	20
36	G61	F: ATCCTCTGGTCTTTGCCAAC	50.0	57.3	20
		R: TCATCCTGAACCACATGTCC	50.0	57.3	20
37	G62	F: TCTTGGCTCTGCTCAACTCA	50.0	57.3	20
		R: GCTCATGTTGATGGTTGTCG	50.0	57.3	20
38	G63	F: CCTCGACATGACAAATCACA	45.0	55.3	20
		R: CAGAAATAGTGGAATGGGATCA	40.9	56.5	22
39	G64	F: CACCACAATCATTGCTGTCC	50.0	57.3	20
		R: TTTAAGGCACTGGAATGTGC	45.0	55.3	20
40	G65	F: AAGGAAAGGGAAAGGGGAAT	45.0	55.3	20
		R: CCTTGGTGAAAATCCTGCAT	45.0	55.3	20
41	G66	F: AAGATAGCTGGGCCTTTGGT	50.0	57.3	20
		R: CTCTCTCTCACACACGCACA	55.0	59.4	20
42	G67	F: ATCCTCTGGTCTTTGCCAAC	50.0	57.3	20
		R: TCATCCTGAACCACATGTCC	50.0	57.3	20
43	G68	F: TCTTGGCTCTGCTCAACTCA	50.0	57.3	20
		R: GCTCATGTTGATGGTTGTCG	50.0	57.3	20
44	G69	F: ATCCGTTTGATTGCTCGAAT	40.0	53.2	20
		R: TCTTCTGTTGCAGTCCCAAA	45.0	55.3	20

Table 10. Cont…..

S. No.	Primer Name	Primer sequence (5'→3')	GC Content (%)	Tm (^{0}C)	Length (base pairs)
45	G70	F: GAGAGGGTGGCACAGCTACT R: CCTGTCATCCCCATTAGTCC	60.0 55.0	61.4 59.4	20 20
46	G71	F: CCGAACATTGATCCATTCGT R: CACCCAACCCTTTCTAACCC	45.0 55.0	55.3 59.4	20 20
47	G72	F: CCTTCGAAATTCAGGGATGA R: GGGTAAGGCTGGGTACATCA	45.0 55.0	55.3 59.4	20 20
48	G73	F: CATAGCAAACCAACATGGGA R: TAGCCAATGGCCTATCAGGA	45.0 50.0	55.3 57.3	20 20
49	G74	F: GTTTGATACAGGCCACACCC R: AGGTCTACCCTGGACCCACT	55.0 60.0	59.4 61.4	20 20
50	G75	F: CTTTCTCGGCGAGTTTGTTC R: GCTCCCTCGTACTCCGTATG	50.0 60.0	57.3 61.4	20 20
51	G76	F: CTTGCAGCTTGTTTGACATTG R: CAGGGAATGCATATCTGGGT	42.9 50.0	55.9 57.3	20 20
52	G77	F: TCCACCATTCTTGGGTTTTC R: TTATTGCCAAGAATCGGACC	45.0 45.0	55.3 55.3	20 20
53	G78	F: GCAGACGAGATTGCTAGCTG R: ACTGTCATATTTCGACCGCC	55.0 50.0	59.4 57.3	20 20
54	G79	F: TACTGGAATTGTCGAAGGGC R: ATACCTCGCGGAAGTCCTTT	50.0 50.0	57.3 57.3	20 20
55	G80	F: AATTGACCACCCAATTCAGC R: GTTGTGCCCATGCCTAATTC	45.0 50.0	55.3 57.3	20 20
56	G81	F: GCTCCCATTTCAGCAGAATC R: ATGGCTTCTCCACCTCTTCA	50.0 50.0	57.3 57.3	20 20
57	G82	F: TACACCCTCTCCTCCACCAC R: ACCACCACCACTACCACCAT	60.0 55.0	61.4 59.4	20 20
58	G83	F: AAAAACTCAAACGGGGTTCC R: TGGCTGAAGAGGAGGATCTG	45.0 55.0	55.3 59.4	20 20
59	G84	F: TGCCTGCAACACTACTCTGC R: GAGCCAGAGGAGAAAAAGGT	55.0 50.0	59.4 57.3	20 20
60	G85	F: GCACCACCAGTGTATCGAAG R: GAGGTAGTGGCCTGGTCTCA	55.0 60.0	59.4 61.4	20 20
61	G86	F: CCATGAAGCTTGTCGAGGAC R: GCTTGATGGTTGTTTTGTGG	55.0 45.0	59.4 55.3	20 20
62	G87	F: TGATTACCTTGGCTTTGCTG R: ACCCAAATGGGGTTTTTCTC	45.0 45.0	55.3 55.3	20 20
63	G88	F: GCCTTGAATGCAAAACATGA R: ACCCCACCCTTATGAGATCC	40.0 55.0	53.2 59.4	20 20
64	G89	F: TCCATATCGAACACCGAAAA R: GGGATGGGTTCATTGACTTG	40.0 50.0	53.2 57.3	20 20

3.3.3.3 Using EST-SSR primers

20 *in silico* developed EST-SSR primers were used for polymorphism study among parental lines. The details of these primers are provided in Table 7 of section 3.1.4.3.

3.3.4 Genotyping of mapping population

F_2 population was used as mapping population to carry out genotyping studies. 100 F_2 individuals constituted the mapping population. The DNA was isolated as described in

section 3.1.1. The primers found polymorphic with parents were short listed from section 3.3.3 and further used for genotyping of F_2 population. Data was recorded and designated as 'A', 'B' and 'H' for the band corresponding to susceptible parent 'Solan Lalima', resistant parent 'EC-251649' and heterozygote, respectively.

3.3.5 Software analysis to identify markers/ QTL for resistance to buckeye rot

In next step, pairwise recombination frequencies between markers were calculated to establish linkage groups, to estimate map distance and map order by suitable statistical programmes (Semagn *et al*., 2006). Segregation data was analyzed using suitable software such as MAPMAKER/EXP version 3.0b (Lincoln *et al*., 1992). This software performs full multipoint linkage analyses i.e., estimation of all recombination frequencies from the marker data for dominant, recessive and co-dominant markers. Following steps were followed for construction of linkage map:

i) A '.raw' (dot raw) file containing information of mapping population type, genotypic data, number of primers, phenotypic data of trait and coding scheme of the data set was prepared.
ii) After loading this file to MAPMAKER, map function was set as Kosambi. The Kosambi mapping function converts the recombination values into genetic distances.
iii) Triple error detection was set on to know error probabilities and logarithm of the odds (LOD) error values.
iv) Minimum LOD and maximum centiMorgan (cM) distance to declare linkage between markers was set.
v) By using 'GROUP' command the markers were separated into linkage groups.
vi) 'ORDER' command was used to build map orders.
vii) 'LOD' command was used to print all the two point data, the results obtained were LOD score and cM distance.
viii) 'MAP' command then calculated and displayed the maximum likelihood map for the order of markers specified.

Using these commands all the primers were placed into a genetic linkage map. The linkage maps were obtained in '.ps' (dot ps) files that could be viewed with '.psviewer' (dot psviewer). At the end .data (dot data) and .trait (dot trait) output was obtained.

Genome coverage by the map was calculated according to the formula given by Chakravarti *et al*. (1991) as follows:

$$\frac{Map\ Length}{Map\ Length \times (\frac{No.of\ Loci+1}{No.of\ loci-1})}$$

where map length is the length of linkage group(s) in cM.

After identification of markers QTL analysis was performed for disease buckeye rot. QTL mapping was used to estimate the presence of QTL for each position on the linkage map using programme Windows QTL Cartographer Version 2.5 by following below given steps.

i) The map file generated in MAPMAKER was imported into QTL Cartographer.

ii) The map function was selected to Kosambi by Clicking on Basic Info and selecting Map Function Kosambi

iii) Then by selecting ANALYSIS command statistical method Composite Interval Mapping was activated and analysis was done using both forward and backward regression method.

iv) On starting the analysis a result file was generated and the identified QTL peaks were obtained as a graphic output file.

The QTL map was constructed following Kosambi mapping (Kosambi, 1944) function. Firstly the map distances were converted into Morgans, (Chhatre, 2013) using following formula:

$$d = \frac{d\ cM}{1000}$$

Then Kosambi function was used to estimate the recombination frequency using the formula given below.

$$r = \frac{1}{2} \times \frac{e^{4d}-1}{e^{4d}+1}$$

where ‘r’ is the recombination frequency, ‘d’ is the distance in Morgans, and ‘e’ is the inverse of natural logarithm ln.

Chapter-4

RESULTS AND DISCUSSION

Plant breeders mainly rely on phenotypic selection for the improvement of plant varieties. However, with the advent of molecular markers, it became possible to select desirable traits directly. Molecular markers, primarily DNA markers, are sections of the genome of the organisms that are used for recognition of a larger area of the genome. A molecular marker can either be located within the gene of interest or be linked to a gene determining a trait of interest. In addition, DNA markers are also used for germplasm evaluation, genetic diagnostics, phylogenetic analysis and study of genome organization. Keeping in view the utility of DNA based molecular markers the present study aimed at finding out genetic diversity, genetics of inheritance and identification of disease resistance linked molecular markers/ QTL.

The results obtained in the study entitled "Studies on identification of molecular markers for buckeye rot (*Phytophthora nicotianae* Breda de Hann. var. *parasitica* (Dastur) Waterhouse) resistance in tomato (*Solanum lycopersicum* L.)" are presented in the following heads:

4.1 Evaluation of genetic diversity amongst tomato genotypes using molecular markers

4.1.1 Isolation of genomic DNA

4.1.2 Qualitative and quantitative assessment of DNA

4.1.3 *In silico* build up of tomato EST-SSRs

4.1.3.1 Searching and assembly of tomato EST sequences

4.1.3.2 Finding out SSR sequences

4.1.3.3 Frequency of SSRs

4.1.3.4 Designing of primers

4.1.3.5 Putative annotation using BLASTx tool

4.1.4 PCR-Amplification of genomic DNA using molecular markers

4.1.4.1 PCR-Amplification using ISSR primers

4.1.4.2 PCR-Amplification using genomic SSR primers

4.1.4.3 PCR-Amplification using EST-SSR primers

4.1.5 Statistical analysis

4.2 To study genetics of inheritance of resistance to *Phytophthora nicotianae* var. *parasitica*

4.2.1 Raising of mapping population

4.2.2 Isolation and morphological characterization of *Phytophthora nicotianae* var. *parasitica*

4.2.3 Maintenance of pure culture and preparation of fungal inoculum for screening

4.2.4 Pathogenicity test of parents and segregants using fungal inoculum

4.2.5 Genetics of inheritance study

4.3 Identification of molecular markers/ QTL for buckeye rot resistance in tomato

4.3.1 Phenotyping of parents using fungal inoculum

4.3.2 Phenotyping of mapping population using fungal inoculum

4.3.3 Parental polymorphism survey using molecular markers

4.3.4 Genotyping of mapping population

4.3.5 Software analysis to identify markers/ QTL for resistance to buckeye rot

4.1 Evaluation of genetic diversity amongst tomato genotypes using molecular markers

4.1.1 Isolation of genomic DNA

DNA isolation method given by Doyle and Doyle (1987) was followed for isolating genomic DNA of 32 tomato genotypes and 100 individuals of F_2 population along with two parents. Some modifications were done wherever required.

Isolation of good quality genomic DNA is the most important step to carry out molecular marker studies. This is because the presence of any kind of impurity in DNA will hinder further molecular studies either through poor amplification or no amplification. Keeping this in mind, in present study DNA isolation was carried out very carefully. A variety of protocols have been developed for extracting plant genomic DNA of both good quality and yield (Dellaporta *et al.,* 1983; Doyle and Doyle, 1987; Wang and Taylor, 1993; Li *et al.*, 2001; Pirttila *et al.*, 2001; Drabkova *et al.*, 2002; Shepherd *et al.*, 2002; Mogg and Bond, 2003; Kang and Yang, 2004). But the protocol given by Doyle and Doyle (1987) is most widely used and results in obtaining high quality and yield of DNA (Parmar *et al.,* 2010; Miskoska *et al.,* 2011; Sharifova *et al.,* 2013; Kaur *et al.*, 2015a,b; Vaidya *et al.*, 2015). Some

points like gentle inversions, separation of aqueous phase very gently, smooth pipetting and no shocks to DNA pellet were taken care of during DNA isolation process. Some modifications were also done in DNA isolation procedure which included increase in incubation and inversion time. All these steps helped to obtain good quality of DNA.

4.1.2 Qualitative and quantitative assessment of DNA

DNA quality check done using 0.8% agarose gel indicated high quality of isolated DNA by presence of sharp band without any smear in all samples.

DNA quantity was assessed by running it on 0.8% agarose gel alongwith λ (lambda) DNA marker of known concentration and also on the basis of absorbance at 260 nm by using UV/VIS Spectrophotometer, the results of which showed DNA concentration range of 500-700 ng/µl. The DNA was diluted to obtain 50 ng/µl concentration, which was further used to carry out PCR amplification. DNA purity assessment was done by calculating absorbance ratio at 260 nm and 280 nm, which ranged between 1.4 and 1.8, indicating good quality of DNA as given below.

Purity check results of DNA on the basis of A_{260}:A_{280} ratio

S. No.	A_{260}:A_{280} ratio (Absorbance ratio)	Indication
1.	Above 1.8	Protein contamination
2.	1.4 to 1.8	**Good quality DNA**
3.	Below 1.4	RNA contamination

Assessment of both quality as well as quantity of DNA is necessary to obtain good quality of amplification during further studies. Presence of single sharp and compact band on 0.8% agarose gel is indicated as good quality DNA. Presence of smears or peaks indicate broken DNA which is not suitable for further DNA studies. Quantity of DNA is another important criterion to obtain good amplification because too high and low quantity will not produce desired amplification.

4.1.3 *In silico* build up of tomato EST-SSRs

4.1.3.1 Searching and assembly of tomato EST sequences

A total of 4200 EST sequences of tomato were downloaded from NCBI website in FASTA format. Further, for assembly of these sequences, EGassembler software was used

which resulted in assembly of 352 contigs and extraction of 1918 singletons. In total 45.95 % redundancy in data was observed (Table 11).

Table 11: EST sequence assembly results

Name of crop	Total Number of EST Sequences	Number of Singletons	Number of Contigs	Non redundant data set (singletons + contigs)	Reduction in Redundancy
S. lycopersicum	4200	1918	352	2270	45.95 %

4.1.3.2 Finding out SSR sequences

SSRs were searched from contigs and singletons. A total of 94 SSRs were found, out of which 19 SSRs were obtained from contigs and 75 SSRs from singletons.

4.1.3.3 Frequency of SSRs

The SSRs obtained were of di- and tri-nucleotide repeats. Di-nucleotide repeats were recorded to be in abundance with a frequency of 58.51%, while tri-nucleotide repeats contributed 41.48% (Table 12; Fig. 1). Five different types of di-nucleotide motifs were observed, of which AT/TA (36.36%) repeat motif was most common and GT/TG (7.27%) was least repeated motif (Fig. 2). In case of tri-nucleotide repeats 13 different motifs were found, among which TCT/CTT/TTC (17.94%) was most common, while both GGC and ATC (2.56%) contributed the least (Fig. 3).

Table 12: Summary of SSRs found in EST sequences

SSR type	Total Number	Frequency (%)
Di-nucleotides	55	58.51
Tri-nucleotides	39	41.48
Total	94	

4.1.3.4 Designing of primers

A total of 55 EST-SSR primer pairs were designed from 94 SSR containing EST sequences using Primer3 software. Out of these, 20 primer pairs were custom synthesized and were further used to carry out molecular marker studies. The details of these primers have already been provided in section 3.1.3.3 (Table 7).

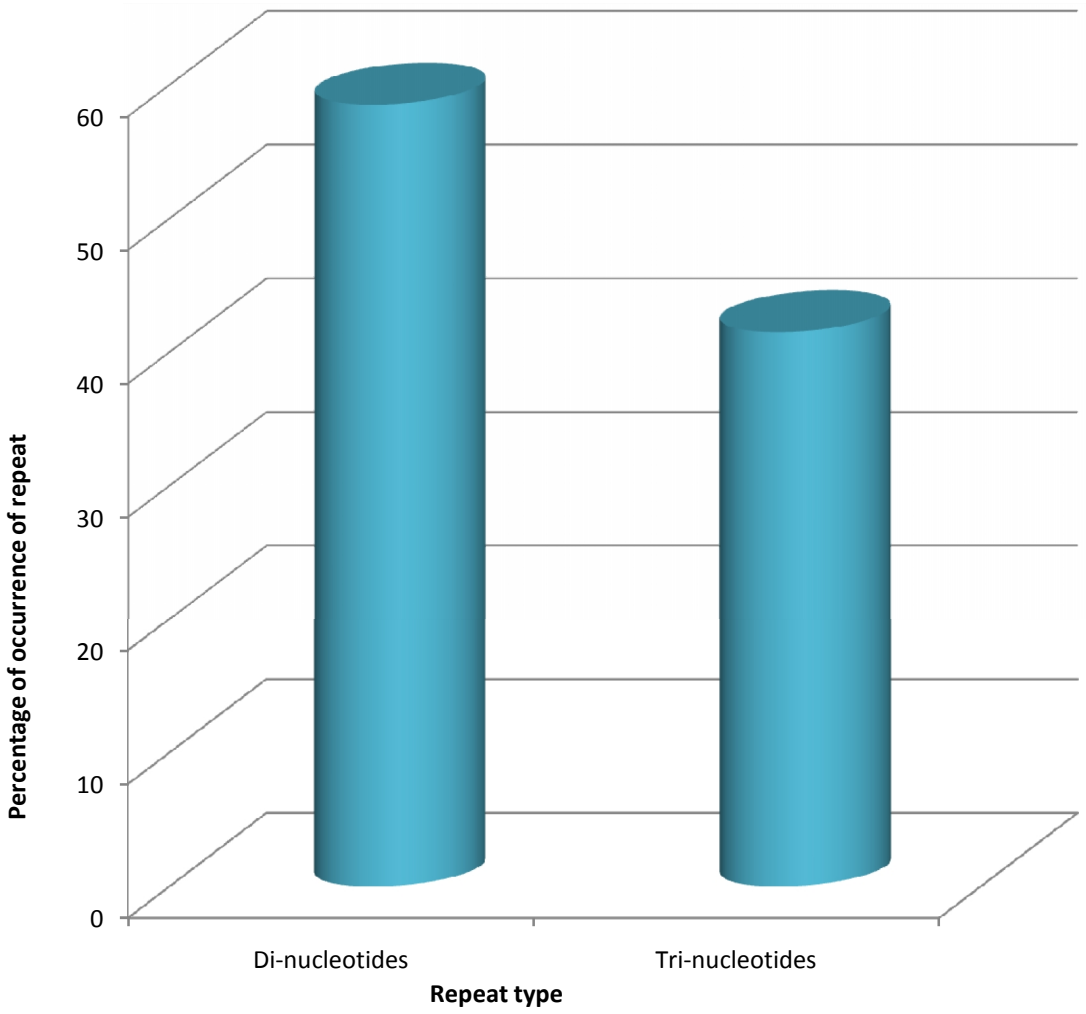
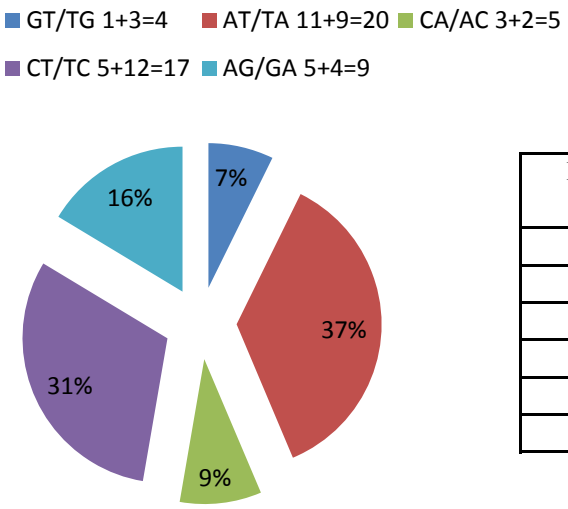
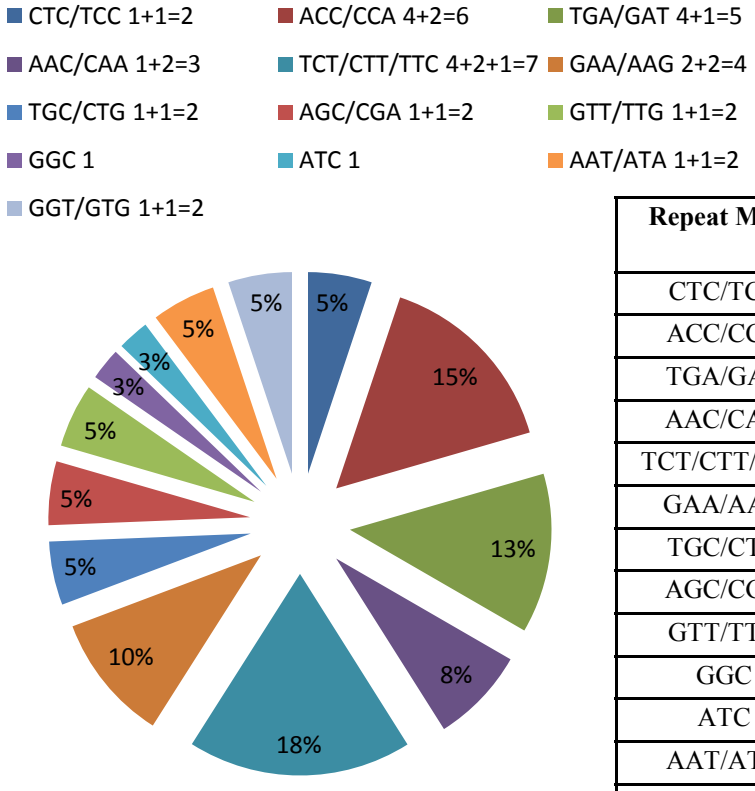


Fig. 1: Per cent distribution of different repeats of SSRs



Repeat Motif	Number	Frequency (%)
GT/TG	1+3=4	7.27
AT/TA	11+9=20	36.36
CA/AC	3+2=5	9.09
CT/TC	5+12=17	30.90
AG/GA	5+4=9	16.36
Total	55	

Fig. 2. Per cent frequency of di-nucleotide repeat motif occurrence



Repeat Motif	Number	Frequency (%)
CTC/TCC	1+1=2	5.12
ACC/CCA	4+2=6	15.38
TGA/GAT	4+1=5	12.82
AAC/CAA	1+2=3	7.69
TCT/CTT/TTC	4+2+1=7	17.94
GAA/AAG	2+2=4	10.25
TGC/CTG	1+1=2	5.12
AGC/CGA	1+1=2	5.12
GTT/TTG	1+1=2	5.12
GGC	1	2.56
ATC	1	2.56
AAT/ATA	1+1=2	5.12
GGT/GTG	1+1=2	5.12
Total	39	

Fig. 3. Per cent frequency of tri-nucleotide repeat motif occurrence

EST-SSRs represent exonic regions of genome which will transcribe into proteins and remain conserved increasing their importance as markers for various molecular studies. Also, due to ease of their development, they are considered advantageous over the other type of SSR markers i.e. genomic SSRs. For their development first and foremost requirement is the availability of EST sequences. In present study, 4200 EST sequences were downloaded from NCBI website, in which 45.95% reduction in redundancy was observed by using EG assembler software, the working of which relies on finding out the contigs and singletons. The use of this software deducted overlapping sequences and also provided the unique sequences without any duplication, which is necessary to screen out and discard unwanted sequences to increase further precision of primer designing. Kaur *et al.* (2015b) and Vaidya *et al.* (2015) also reported significant reduction in redundancy of 22.2% and 80.33%, respectively, by using EG assembler. Further the occurrence of repeats i.e. 58.51% di-nucleotide and 41.48% tri-nucleotide-repeats show that di-nucleotide repeats are more abundant than tri-nucleotide repeats. These results are supported by previous findings of Parmar *et al.* (2010), Vaidya *et al.* (2012) and Kaur *et al.* (2015b).

4.1.3.5 Putative annotation using BLASTx tool

On using BLASTx tool of Uniprot database putative functions were assigned to 20 custom synthesized primer pairs. High homology was achieved with four plants species: 14 sequences were homologous with *S. lycopersicum*, four showed homology with *S. tuberosum*, while rest two sequences were found homologous with *Nicotiana tabacum* and *Coffea canephora* (Table 13).

High homology of SSR containing EST sequences with members of Solanaceae family was revealed on BLASTx analysis. Annotation analysis was also conducted by this tool which assigned different functions to EST sequences. Resemblance with various functional domains showed that the EST sequences used for EST-SSR primer designing in this study code for different functions and also revealed their direct involvement in metabolic pathways. As observed in earlier studies, relevant transcripts were detected using functional annotation (Sharma, 2013; Vaidya, 2014; Kaur *et al.,* 2015a). High homology with various plant species was obtained.

Table 13: BLASTx putative annotation of EST sequences selected for primer designing

Sequence ID	Annotation	Species	E-value	Polymorphic/ monomorphic
Contig7	Uncharacterized protein	*S. lycopersicum*	7.5e-30	Polymorphic
Contig143	Uncharacterized protein	*S. lycopersicum*	1.2e-95	Polymorphic
Contig162	Alpha-tubulin	*S. tuberosum*	2.6e-129	Polymorphic
Contig265	Hop-interacting protein THI041	*S. lycopersicum*	5e-172	Polymorphic
Contig340	Hydrogen peroxide-induced 1	*Nicotiana tabacum*	4.5e-68	Polymorphic
Contig352	Uncharacterized protein	*S. lycopersicum*	9.5e-90	No amplification
gi|116644211|	*Coffea canephora* DH200=94 genomic scaffold, scaffold_26	*Coffea canephora*	2.1e-90	Polymorphic
gi|4387244|	Uncharacterized protein	*S. lycopersicum*	1e-90	Polymorphic
gi|4386975|	Glutamate-1-semialdehyde 2,1-aminomutase, chloroplastic	*S. lycopersicum*	1.6e-127	Polymorphic
gi|4386907|	Uncharacterized protein	*S. tuberosum*	1.6e-72	Polymorphic
gi|4386813|	Uncharacterized protein	*S. lycopersicum*	4.6e-46	No amplification
gi|4386782|	Uncharacterized protein	*S. tuberosum*	4.6e-48	Polymorphic
gi|4386589|	Transcription factor JERF1	*S. lycopersicum*	2e-122	Polymorphic
gi|4386576|	Transcription factor JERF1	*S. lycopersicum*	2e-123	Polymorphic
gi|4386543|	Chloroplast-specific ribosomal protein	*S. lycopersicum*	2.3e-83	No amplification
gi|4386508|	Histone H2B.1	*S. lycopersicum*	8.6e-92	Polymorphic
gi|4386388|	Carbonic anhydrase	*S. lycopersicum*	1.3e-69	Polymorphic
gi|4386332|	Ketol-acid reductoisomerase	*S. lycopersicum*	3.1e-15	No amplification
gi|4386229|	Elongation factor Tu	*S. tuberosum*	3.2e-96	Polymorphic
gi|76572221|	Phospholipase D	*S. lycopersicum*	7.7e-22	Polymorphic

4.1.4 PCR-Amplification of genomic DNA using molecular markers

4.1.4.1 PCR-Amplification using ISSR primers

Out of total 25 ISSR primers used, 20 produced polymorphism, one being monomorphic and four ('ISSR-HB-12', 'ISSR-HB-15', 'IISRS-3-O' and IISRS-3-M') did not

produce any amplification. A total of 151 bands were generated, of which seven were monomorphic (4.63%) and 144 revealed polymorphism (95.36%). Primer 'UBC-894' produced maximum number of bands i.e. 11, while primer 'UBC-854' generated single band. The bands produced amplification in a range of <100 – >1000 bp (Plate 1-16). Further details of amplification produced by ISSR primers are provided in Table 14.

Table 14: Details of amplification produced by ISSR primers

S. No.	Primer name	Total scorable bands	Polymorphic bands	Monomorphic bands	Position of bands (bp)
1	Primer 1	6	6	-	100-300
2	ISSR 2	6	6	-	100-800
3	UBC840	8	7	1	100->1000
4	IISRS-3-E	9	7	2	100->1000
5	IISRS-3-G	8	8	-	100->1000
6	IISRS-3-H	8	8	-	100->1000
7	IISRS-3-F	9	8	1	100->1000
8	IISRS-3-L	7	7	-	100->1000
9	ISSR-4	9	8	1	100-900
10	ISSR 844B	5	5	-	100-700
11	ISSR-5	9	8	1	100- >1000
12	ISSR-7	10	10	-	100-.>1000
13	ISSR-3	7	7	-	100-600
14	UBC-855	2	2	-	400-700
15	UBC-848	5	5	-	200-500
16	ISSR17898A	10	10	-	100- >1000
17	ISSR178998B	10	10	-	100- >1000
18	UBC-894	11	11	-	100- >1000
19	ISSR-3-I	8	8	-	100-900
20	UBC-854	1	-	1	100
21	UBC-841	3	3	-	200-400
	TOTAL	**151**	**144**	**7**	

4.1.4.2 PCR-Amplification using genomic SSR primers

In case of 25 genomic SSR primers, 16 were polymorphic, one was monomorphic and eight failed to amplify ('G18-G25'). Out of total 76 bands, two were reported to be

monomorphic (2.63%) and 74 were polymorphic (97.36%). Maximum ten bands were produced by 'G3' primer, while 'G14' and 'G17' primers generated single band (Table 15). Position of amplified bands ranged from <100 – >1000 bp (Plate 17-26).

Table 15: Details of amplification produced by genomic SSR primers

S. No.	Primer name	Total scorable bands	Polymorphic bands	Monomorphic bands	Position of bands (bp)
1	G1	8	8	0	<100-900
2	G2	6	6	0	100-500
3	G3	10	9	1	<100-400
4	G4	7	7	0	<100-700
5	G5	5	5	0	<100-400
6	G6	8	8	0	<100-400
7	G7	2	2	0	<100-200
8	G8	5	5	0	<100-400
9	G9	5	5	0	<100-500
10	G10	2	2	0	<100-500
11	G11	3	3	0	<100-300
12	G12	5	5	0	100-1000
13	G13	3	3	0	<100-200
14	G14	1	0	1	<100
15	G15	2	2	0	<100
16	G16	3	3	0	<100-200
17	G17	1	1	0	<100
	TOTAL	**76**	**74**	**2**	

4.1.4.3 PCR-Amplification using EST-SSR primers

Out of total 20 EST-SSR primers used, 16 produced polymorphism and four ('Contig352', 'gi|4386813|', 'gi|4386543|' and 'gi|4386332|') did not produce any amplification. In total, 62 bands were produced, of which only two were monomorphic (3.33%) and remaining 60 revealed polymorphism (96.77%) (Table 16). Amplified bands, position ranged from <100 – >1000 bp (Plate 27-38).

Table 16: Details of amplification produced by EST-SSR primers

S. No.	Primer name	Total scorable bands	Polymorphic bands	Monomorphic bands	Position of bands (bp)
1	Contig7	5	5	0	100-500
2	Contig143	1	1	0	400
3	Contig162	4	4	0	<100-200
4	Contig265	1	1	0	<100
5	Contig340	5	5	0	<100-600
6	gi|116644211|	2	2	0	100-300
7	gi|4387244|	5	5	0	<100-400
8	gi|4386975|	4	4	0	<100-400
9	gi|4386907|	2	1	1	<100-200
10	gi|4386782|	5	5	0	<100-1000
11	gi|4386589|	2	2	0	200-300
12	gi|4386576|	2	2	0	<100-300
13	gi|4386508|	15	15	0	<100-1000
14	gi|4386388|	3	3	0	<100-100
15	gi|4386229|	3	2	1	<100-200
16	gi|76572221|	3	3	0	100-300
	TOTAL	**62**	**60**	**2**	

Relatively high level of polymorphism was revealed in this study by three marker systems viz., ISSRs, genomic SSRs and EST-SSRs, confirming their promising use in genetic diversity analysis. In a few of the previous studies high level of polymorphism was recorded in plum cultivars by Goulao (2001), strawberry (Carrasco *et al.,* 2007; Nunes *et al.,* 2013) and apple (Vaidya, 2014), which supported high polymorphism results of ISSR primers in the present study. Further genomic SSR polymorphism results obtained by Parmar *et al.* (2010) in tomato (52.2%), Ansari and Singh (2014) in brinjal (83%) and Sharma and Nandineni (2014) in potato (100%) supported the high polymorphism results of present study. High level of polymorphism in various studies i.e. 58% by Korir *et al.* (2014) in tomato, 96.7% by Sharma (2013) in stevia, 97.81% by Vaidya (2014) in apple and 100% by Samriti (2015) in *Rubus elliptics* with EST-SSR primers is in agreement with the present findings.

4.1.5 Statistical analysis

DNA fingerprinting studies

In total, 16 unique bands were produced by 14 ISSR primers namely, 'Primer 1', 'ISSR-2', 'IISRS-3-E', 'IISRS-3-G', 'IISRS-3-F', 'IISRS-3-L', 'ISSR-4', 'ISSR 844B', 'ISSR-5', 'ISSR-7', 'UBC-855', 'ISSR17898A' and 'ISSR-3-I' (Table 17), whereas, genomic SSR primers produced five unique bands by four different primers viz., 'G3', 'G10', 'G12' and 'G16' (Table 17).

A total of 16 unique bands were produced by six EST-SSR primers i.e. 'Contig 7', 'Contig 340', 'gi|4387244|', 'gi|4386782|', 'gi|4386508|' and 'gi|4386388|' (Table 17).

PIC value

PIC value for ISSR primers ranged from 0.27 to 0.89 with an average of 0.67 (Fig. 4). This range varied from 0.14 to 0.82 in case of genomic SSR primers with an average of 0.52 (Fig. 5) and with EST-SSR primers value of PIC ranged from 0.15 to 0.92 with an average of 0.47 (Table 17; Fig. 6).

Effective multiplex ratio (E)

The E values for three marker systems i.e. ISSR, Genomic SSR and EST-SSR were recorded to be 4.47, 2.32 and 1.85, respectively (Table 18).

Marker Index (MI)

MI values were found to be 3.08, 1.20 and 0.86 for ISSR, Genomic SSR and EST-SSR primers, respectively (Table 18).

Unique bands represent the portion of genome that is present at a specific location in particular individual and act as DNA fingerprints. The primers which are able to uncover these unique positions in the genome are considered best and help to further identify the individual. These unique bands prove very helpful in identifying a particular individual from a mixture of many. Present study resulted in finding out many unique bands with different primers in different genotypes. Some earlier studies conducted by Vaidya *et al.* (2012), Samriti (2015) and Kaur *et al.* (2015b) also demonstrated number of unique bands in many plant species.

Table 17: Description of PIC values and unique bands produced by three marker systems

ISSR primers				
Primer name	**PIC**	**Unique band (s)**	**Position of unique bands**	**Genotype name showing unique bands**
Primer 1	0.74	1	200 bp	EC-25265
ISSR-2	0.78	1	900 bp	EC-126902
UBC-840	0.79	-	-	-
IISRS-3-E	0.80	1	1000 bp	EC-36883
IISRS-3-G	0.74	2	1000 bp, 900bp	EC-10662
IISRS-3-H	0.76	-	-	-
IISRS-3-F	0.80	1	> 1000 bp	EC-126902
IISRS-3-L	0.60	1	900 bp	EC-521041
ISSR-4	0.75	2	1000 bp, 900 bp	EC-168283, EC-36883
ISSR 844B	0.69	1	700 bp	EC-6486
ISSR-5	0.81	2	> 1000 bp 1000 bp	EC-501074 EC-25265
ISSR-7	0.85	1	> 1000 bp	EC-528373
ISSR-3	0.79	-	-	-
UBC-855	0.32	1	500 bp	EC-520075
UBC-848	0.72	-	-	-
ISSR17898A	0.77	1	> 1000 bp	EC-25265
ISSR178998B	0.89	-	-	-
UBC-894	0.89	-	-	-
ISSR-3-I	0.83	1	900 bp	Solan Vajr
UBC-854	0	-	-	-
UBC-841	0.27	-	-	-

Table 17. Cont…..

Genomic SSR primers					EST-SSR primers				
Primer Name	**PIC**	**Unique bands**	**Position of unique bands**	**Genotype name showing unique band**	**Primer Name**	**PIC**	**Unique bands**	**Position of unique band**	**Genotype name showing unique band**
G1	0.81	-	-	-	Contig7	0.77	4	500 bp, 250 bp & 150 bp 400 bp	EC-10304 EC-528373
G2	0.79	-	-	-	Contig143	0	-	-	-
G3	0.70	1	< 100 bp	EC-36883	Contig162	0.69	-	-	-
G4	0.82	-	-	-	Contig265	0	-	-	-
G5	0.69	-	-	-	Contig340	0.71	3	300 bp 200 bp 100 bp	EC-2798 EC-251649 EC-114375
G6	0.82	-	-	-	gi|116644211|	0.50	-	-	-
G7	0.39	-	-	-	gi|4387244|	0.77	3	300 bp 200 bp 100 bp	EC-521054 EC-528373 EC-168283
G8	0.57	-	-	-	gi|4386975|	0.66	-	-	-
G9	0.79	-	-	-	gi|4386907|	0.15	-	-	-
G10	0.14	1	200 bp	EC-2798	gi|4386782|	0.26	3	500 bp 400 bp 300 bp	EC-521
G11	0.47	-	-	-	gi|4386589|	0.50	-	-	-
G12	0.73	2	400 bp 300 bp	Hawai 7998, EC-168283	gi|4386576|	0.41	-	-	-
G13	0.64	-	-	-	gi|4386508|	0.92	2	1000 bp 200 bp	EC-114375 EC-6486
G14	0	-	-	-	gi|4386388|	0.55	1	100 bp	EC-29914
G15	0.30	-	-	-	gi|4386229|	0.24	-	-	-
G16	0.25	1	100 bp	EC-521041	gi|76572221|	0.53	-	-	-
G17	0	-	-	-					

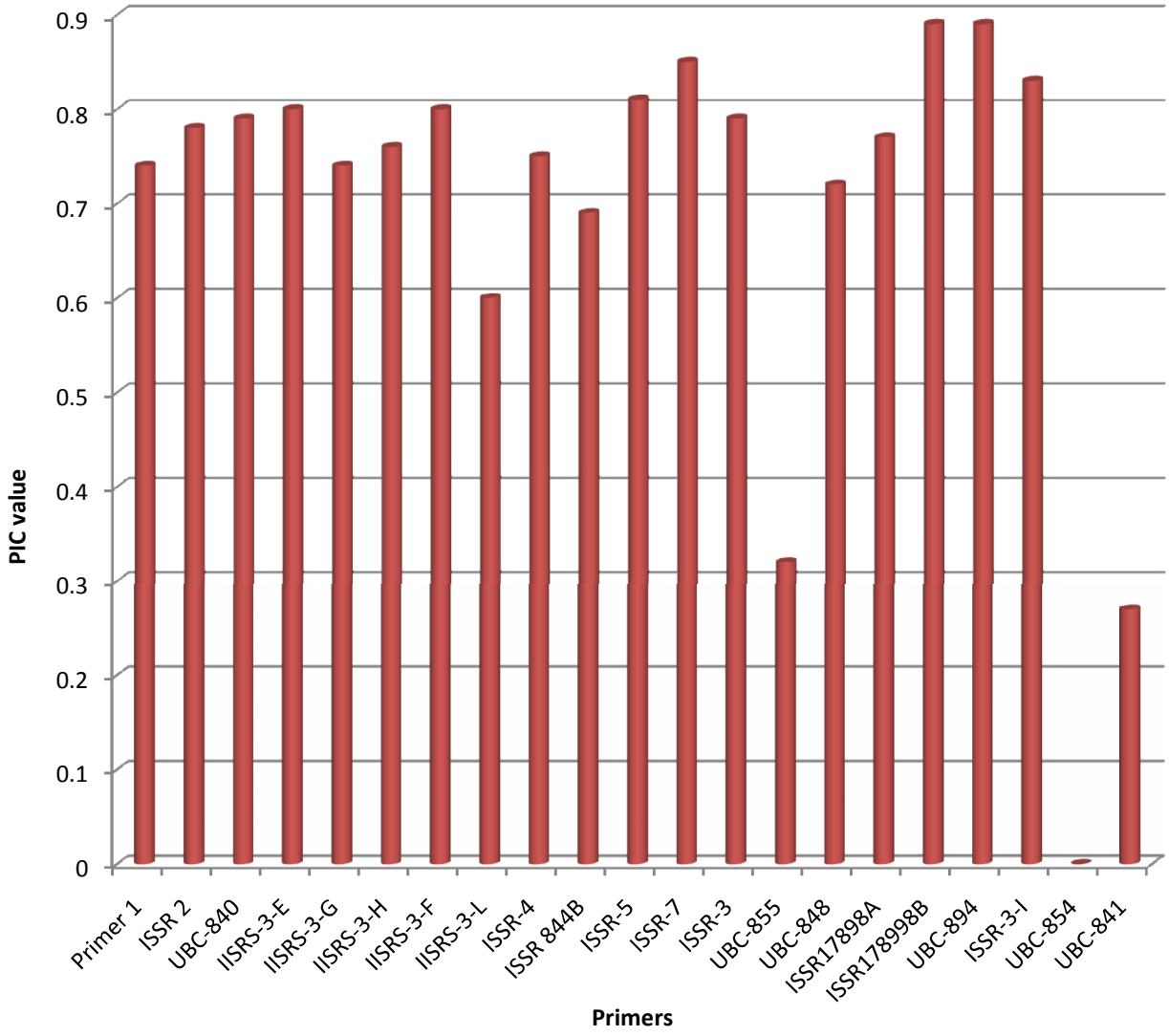


Fig. 4: Bar diagram of PIC values of ISSR primers

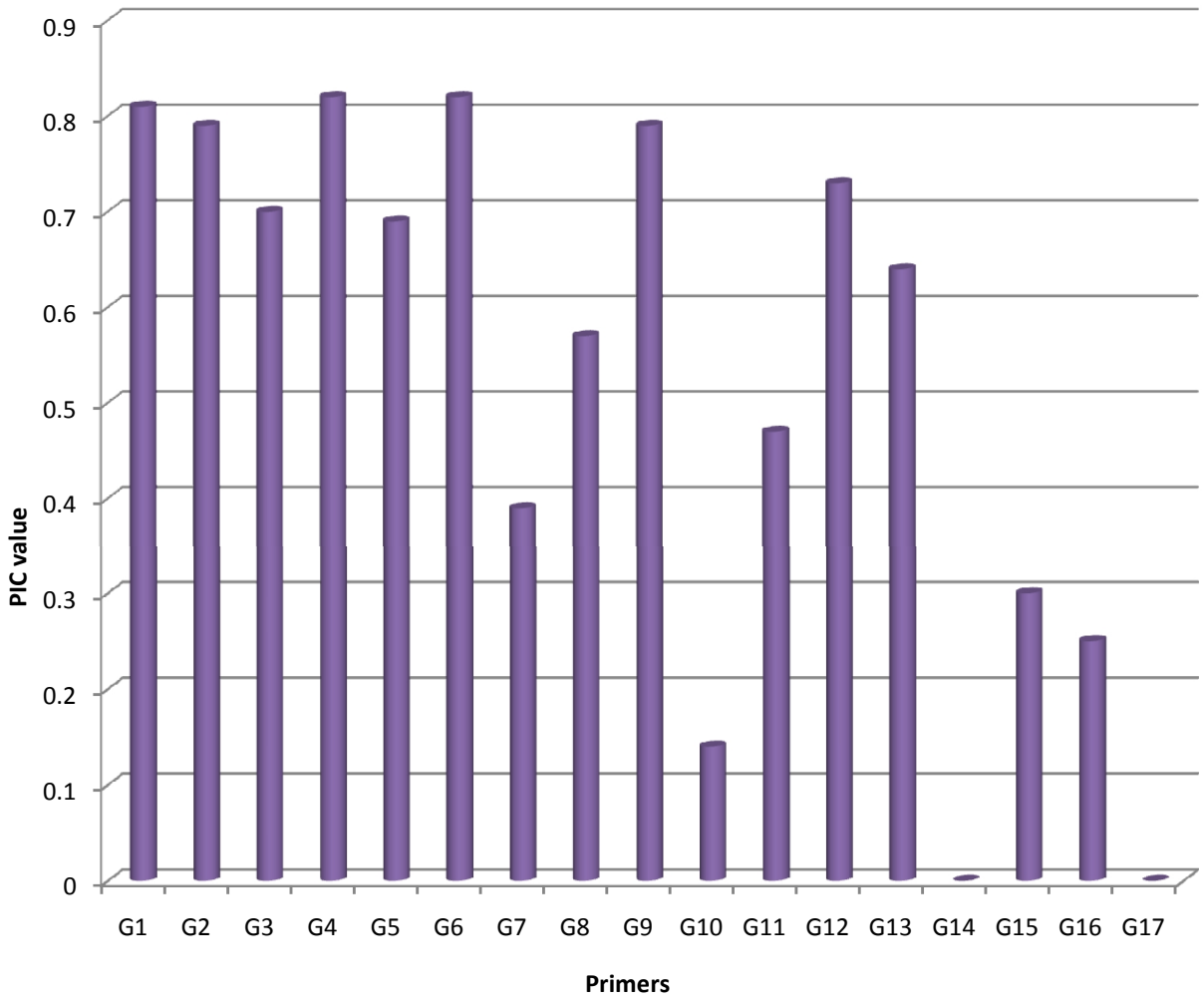


Fig. 5: Bar diagram of PIC values of genomic SSR primers

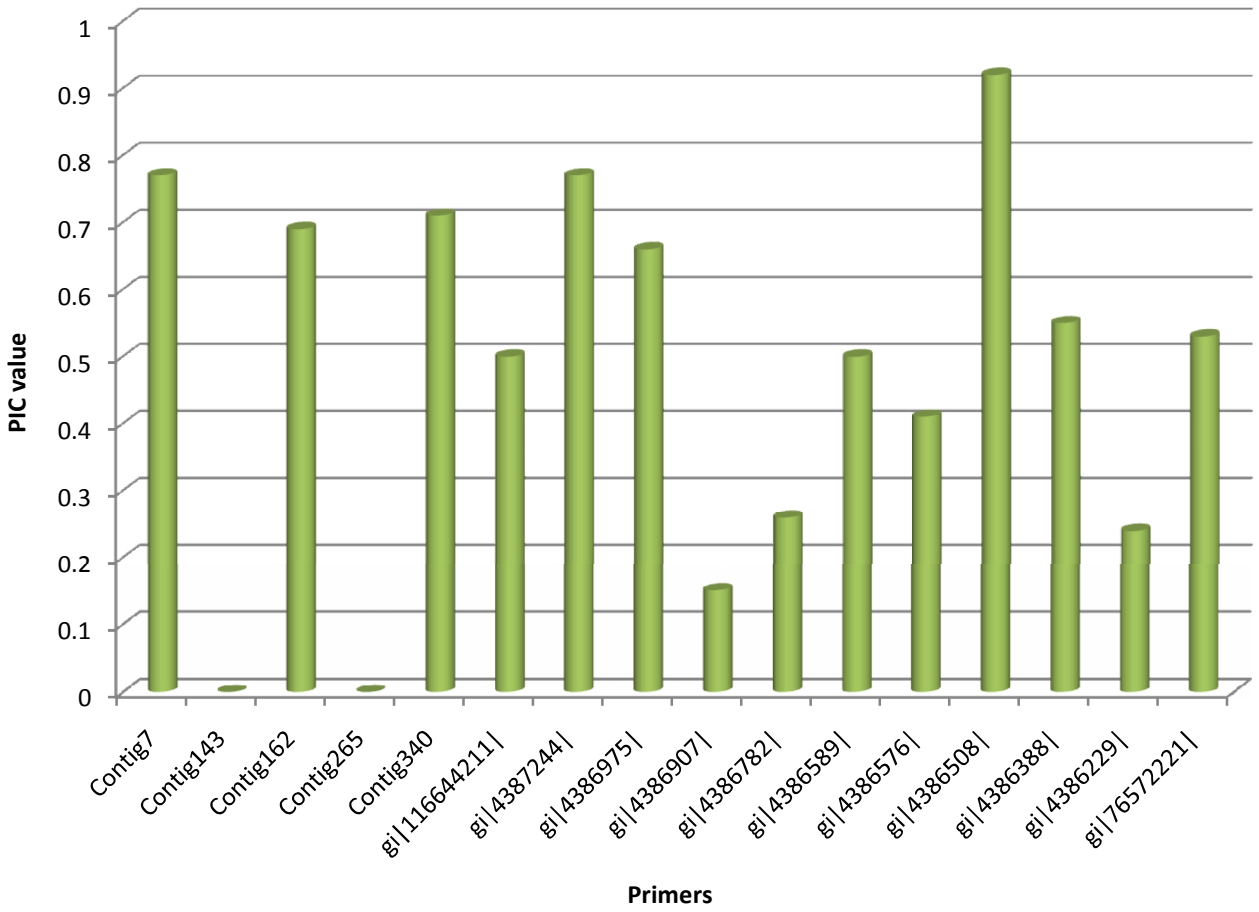


Fig. 6: Bar diagram of PIC values of EST-SSR primers

PIC value represents the polymorphism information of a primer and high level of polymorphism reveals the usefulness of that primer to conduct further molecular marker studies. In present study high PIC range with three marker systems along with high average values with individual primer showed the usefulness of primers used in further DNA fingerprinting as well as marker studies. High PIC values have also been reported earlier by Varshney *et al.* (2007), Sharma (2013), Dhaliwal *et al.* (2014), Vaidya (2014) and Samriti (2015) with ISSRs, genomic and EST-SSRs.

The effective marker ratio considers all the possible attributes such as information content, fraction of polymorphic fragments and multiplex ratio. For determining the overall utility of a given marker system, the marker index (MI) was calculated for all the three marker systems examined. Present study investigations with high E and MI values were in congruence with earlier findings of Varshney *et al.* (2007) and Vaidya (2014).

Comparison among three marker systems

Detailed comparison among three different marker systems used in the present study viz., ISSR, genomic SSR and EST-SSR is presented in Table 18. On comparing average number of bands per primer, ISSR primers gave maximum value of 7.19 followed by genomic SSRs with 4.47 value and EST-SSRs produced least value of 3.87. A total of 1405, 885 and 440 fragments were generated by ISSRs, genomic SSRs and EST-SSRs, respectively. On calculating average number of fragments per polymorphic primer, ISSR primers were noted to produce 70.25 fragments per primer followed by genomic SSR primers with value of 55.31 and then by EST-SSR primers with 29.33 fragments. Average number of amplified fragments per accession were recorded to be maximum in ISSRs (43.90), followed by genomic SSRs (27.65) and then EST-SSRs (13.75). Maximum number of unique bands i.e. 16 were generated by both ISSR and EST-SSR primers, while genomic SSR primers were recorded with minimum of five unique bands. ISSRs also exceeded in average PIC (0.67), E (4.47) and MI (3.08) values, followed by genomic SSRs with 0.52, 2.32, 1.20 and EST-SSRs with 0.47, 1.85 and 0.86 for same parameters. Genomic SSRs produced maximum polymorphism percentage of 97.36, followed by EST-SSRs with 96.77% and then by ISSRs with percentage of 95.36.

Table 18: Summary of comparison among three marker systems

Description	ISSR primers	Genomic SSR primers	EST-SSR primers
Total number of primers	25	25	20
Total number of informative primers	21	17	16
Number of polymorphic primers	20	16	16
Per cent of total polymorphic primers	80%	64%	80%
Total number of bands	151	76	62
Total number of polymorphic bands	144	74	60
Average number of bands per primer	7.19	4.47	3.87
Per cent of total polymorphic bands	95.36%	97.36%	96.77%
Total number of fragments scored	1405	885	440
Average number of fragments per polymorphic primer	70.25	55.31	29.33
Average number of amplified fragments per accession	43.90	27.65	13.75
Number of unique bands	16	5	16
Average PIC value	0.67	0.52	0.47
Effective multiplex ratio (E)	4.47	2.32	1.85
Marker Index (MI)	3.08	1.20	0.86

From comparison of three marker systems it was concluded that genomic SSRs produced highest polymorphism percentage followed by EST-SSRs and ISSRs. This may be because genomic SSRs are based on both intronic as well as exonic regions of the genome. Thus, they tend to generate more polymorphism as compared to EST-SSRs which are based only on exonic regions. The detection of a higher level of polymorphism using genomic SSRs was in agreement with some earlier reports that genomic library-derived SSRs exhibited higher levels of polymorphism as in rice (Cho *et al.*, 2000), sugarcane (Pinto *et al*., 2004), wheat (Eujayl *et al.*, 2001), cauliflower (Vaidya *et al.*, 2012). ISSRs produced less polymorphism as compared to SSRs in spite of producing more number of bands. This may be because of generation of more monomorphic bands as compared to SSRs. To finally conclude, three marker systems produced high polymorphism and the difference between polymorphism percentages is very low. Thus, it is recommended to use these marker systems in combination to attain more precise results.

Data analysis

Data analysis was done using NTSYS-pc version 2.0 by combined analysis of results obtained through three different molecular marker systems. For this collective similarity matrix and dendrogram were created by joining the data from three marker systems.

Similarity range of 0.252 to 0.615 was observed depicting medium base of genotypes studied. Maximum similarity of 61.5% was observed between 'EC-521' and 'EC-8591' while minimum similarity of 25.2% was obtained between 'EC-528373' and 'EC-528367' (Table 19).

In the dendrogram all genotypes were divided in two main clusters 'A' and 'B'. Cluster 'A' contained 25 genotypes with further sub-clustering into groups 'A1' and 'A2', while cluster 'B' consisted of seven genotypes with sub-clustering into 'B1' and 'B2'. Sub-cluster 'A1' is comprised of 22 genotypes 'Solan Vajr', 'EC-13904', 'EC-168283', 'EC-25265', 'EC-501074', 'EC-528367', 'EC-521059', 'EC-362949', 'EC-521054', 'EC-35322', 'EC-29914', 'EC-10304', 'EC-251649', 'EC-528374', 'EC-10662', 'EC-36883', 'EC-520075', 'EC-251649', 'EC-528373', 'EC-2517', 'EC-2798', 'Hawai 7998', while cluster 'A2' contained three genotypes namely, 'EC-521051', 'EC-12699', 'EC-2791'. In sub-cluster 'B1' six genotypes viz., 'EC-126902', 'EC-6486', 'EC-521041', 'EC-251646', 'EC-521', 'EC-8591' were clustered and in 'B2' 'EC-114375' remained a singlet (Fig. 7). It was found that 'Solan Vajr' which is a commercial variety, was present on the top of cluster separating it from rest of the genotypes.

The genotypes under this study have not been undertaken earlier for any marker studies. Thus, for their further use in breeding programmes, it was necessary to find out the broadness of genetic base. Combined use of data produced by three marker systems resulted in more authenticated and precise results. This may be because of different targeting of genome by different marker systems. In various previous studies different marker systems have been individually used on different sets of tomato germplasm (Kwon *et al.*, 2009; Parmar *et al*., 2010; Miskoska *et al*., 2011; El-Awady *et al.*, 2012; Sharifova *et al*., 2013). However, in some of the other reports such as Tam *et al.* (2005), Varshney *et al.* (2007), Bae *et al.* (2010), Mansour *et al.* (2010), Hu *et al.* (2012), Joshi *et al.* (2012), it was reported that it is inadequate to use only one marker system for assessing genetic diversity. Present study also reveals that combined use of different marker systems leads to better genome coverage. Also, in present study relatively high level of polymorphism was revealed by three marker systems viz., ISSR, genomic and EST-SSRs, confirming their promising use in genetic diversity analysis. This led to the conclusion that ISSRs, genomic and EST-SSRs are promising to assess relationship of tomato germplasm.

Table 19: Jaccard's similarity matrix coefficient based on combined ISSR, genomic and EST-SSR analysis

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24	G25	G26	G27	G28	G29	G30	G31	G32
G1	1.000																															
G2	0.357	1.000																														
G3	0.343	0.355	1.000																													
G4	0.338	0.339	0.465	1.000																												
G5	0.301	0.345	0.445	0.519	1.000																											
G6	0.327	0.287	0.262	0.284	0.302	1.000																										
G7	0.357	0.295	0.269	0.292	0.333	0.460	1.000																									
G8	0.389	0.338	0.366	0.408	0.390	0.379	0.431	1.000																								
G9	0.394	0.348	0.337	0.371	0.375	0.294	0.338	0.442	1.000																							
G10	0.375	0.284	0.394	0.372	0.387	0.277	0.294	0.377	0.429	1.000																						
G11	0.395	0.325	0.335	0.340	0.375	0.289	0.368	0.348	0.506	0.355	1.000																					
G12	0.365	0.344	0.363	0.371	0.423	0.271	0.268	0.388	0.402	0.385	0.354	1.000																				
G13	0.380	0.279	0.286	0.238	0.283	0.394	0.451	0.360	0.340	0.300	0.309	0.325	1.000																			
G14	0.335	0.347	0.427	0.429	0.446	0.297	0.325	0.440	0.393	0.437	0.404	0.396	0.271	1.000																		
G15	0.395	0.369	0.320	0.352	0.319	0.255	0.348	0.386	0.503	0.412	0.439	0.404	0.331	0.385	1.000																	
G16	0.446	0.407	0.365	0.396	0.425	0.330	0.336	0.421	0.453	0.407	0.458	0.432	0.392	0.398	0.456	1.000																
G17	0.398	0.344	0.363	0.338	0.365	0.376	0.392	0.430	0.442	0.345	0.374	0.353	0.346	0.345	0.384	0.432	1.000															
G18	0.406	0.414	0.414	0.439	0.432	0.290	0.389	0.460	0.439	0.382	0.432	0.439	0.323	0.460	0.496	0.463	0.439	1.000														
G19	0.396	0.416	0.404	0.393	0.398	0.348	0.416	0.396	0.401	0.426	0.392	0.373	0.377	0.374	0.402	0.466	0.418	0.487	1.000													
G20	0.369	0.307	0.290	0.292	0.298	0.460	0.478	0.383	0.328	0.325	0.357	0.268	0.372	0.314	0.338	0.358	0.392	0.377	0.378	1.000												
G21	0.465	0.350	0.358	0.401	0.394	0.321	0.305	0.383	0.418	0.412	0.442	0.403	0.341	0.381	0.462	0.525	0.462	0.483	0.486	0.375	1.000											
G22	0.418	0.414	0.403	0.391	0.432	0.324	0.414	0.355	0.450	0.362	0.476	0.427	0.387	0.426	0.463	0.512	0.416	0.521	0.426	0.377	0.508	1.000										
G23	0.458	0.388	0.380	0.366	0.359	0.370	0.349	0.373	0.340	0.414	0.358	0.393	0.373	0.338	0.434	0.431	0.442	0.438	0.428	0.430	0.504	0.504	1.000									
G24	0.367	0.440	0.377	0.425	0.418	0.301	0.333	0.338	0.407	0.377	0.443	0.413	0.292	0.413	0.419	0.477	0.426	0.473	0.504	0.274	0.500	0.473	0.480	1.000								
G25	0.421	0.403	0.416	0.444	0.423	0.296	0.292	0.396	0.432	0.500	0.425	0.418	0.277	0.441	0.435	0.508	0.418	0.464	0.481	0.292	0.518	0.464	0.471	0.587	1.000							
G26	0.323	0.322	0.267	0.303	0.350	0.329	0.430	0.275	0.352	0.362	0.338	0.288	0.380	0.293	0.330	0.351	0.375	0.321	0.414	0.430	0.355	0.358	0.428	0.364	0.357	1.000						
G27	0.370	0.349	0.427	0.403	0.435	0.317	0.324	0.418	0.321	0.392	0.348	0.417	0.338	0.370	0.380	0.431	0.468	0.477	0.403	0.349	0.437	0.401	0.515	0.411	0.485	0.382	1.000					
G28	0.338	0.387	0.338	0.322	0.371	0.333	0.316	0.323	0.398	0.360	0.330	0.328	0.310	0.350	0.324	0.404	0.403	0.388	0.401	0.305	0.421	0.388	0.412	0.421	0.426	0.342	0.412	1.000				
G29	0.320	0.318	0.263	0.300	0.306	0.425	0.428	0.304	0.317	0.336	0.291	0.284	0.377	0.312	0.306	0.300	0.386	0.368	0.412	0.411	0.339	0.342	0.426	0.333	0.340	0.507	0.395	0.393	1.000			
G30	0.317	0.287	0.283	0.271	0.303	0.419	0.390	0.268	0.284	0.321	0.300	0.270	0.373	0.309	0.314	0.321	0.410	0.339	0.339	0.423	0.299	0.314	0.438	0.343	0.297	0.500	0.376	0.349	**0.615**	1.000		
G31	0.400	0.297	0.347	0.330	0.311	0.329	0.350	0.330	0.372	0.361	0.360	0.301	0.280	0.327	0.384	0.412	0.411	0.381	0.409	0.336	0.392	0.345	0.452	0.418	0.424	0.356	0.422	0.432	0.402	0.468	1.000	
G32	0.339	0.284	0.280	0.269	**0.252**	0.288	0.311	0.245	0.292	0.318	0.341	0.267	0.292	0.273	0.322	0.318	0.352	0.336	0.349	0.270	0.359	0.336	0.417	0.397	0.389	0.415	0.329	0.359	0.341	0.371	0.500	1.000

G1-G32 description is provided in Table1where S. No.1-32 indicates G1-G32

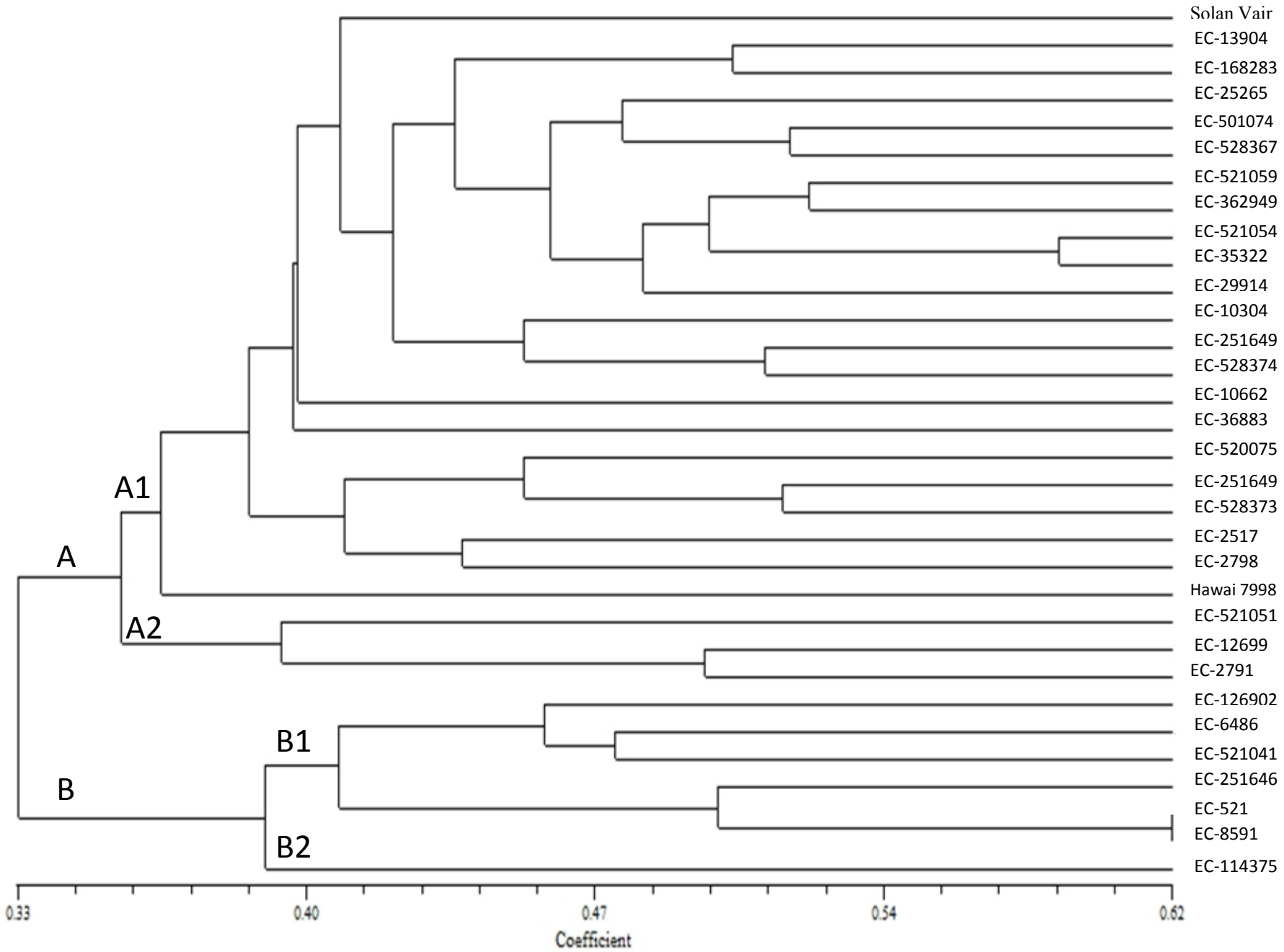


Fig. 7: Dendrogram of 32 tomato genotypes based on combined ISSR and genomic and EST-SSR analysis

Plate 1: ISSR pattern of 32 tomato genotypes generated by Primer 1

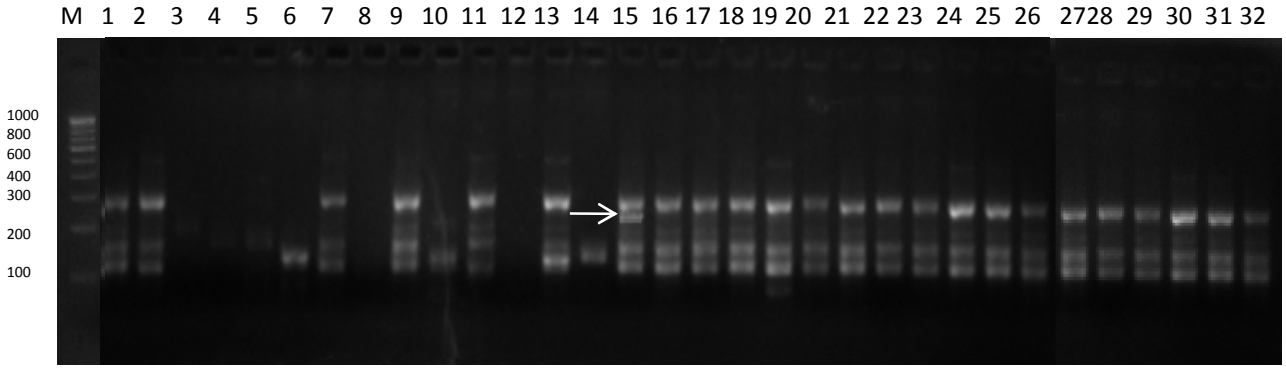
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

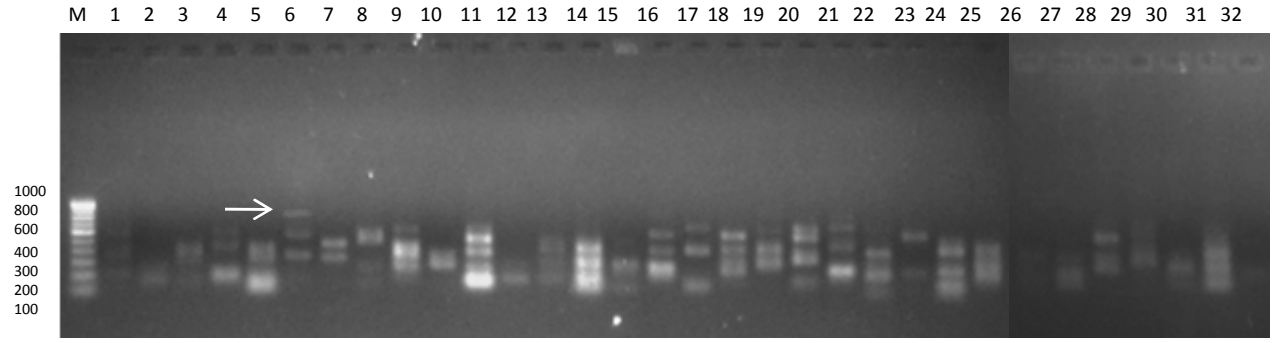
Plate 2: ISSR pattern of 32 tomato genotypes generated by ISSR-2

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



ISSR primer Primer 1
Arrow indicates unique band
Plate 1



ISSR primer ISSR-2
Arrow indicates unique band
Plate 2

Plate 3: ISSR pattern of 32 tomato genotypes generated by UBC-840

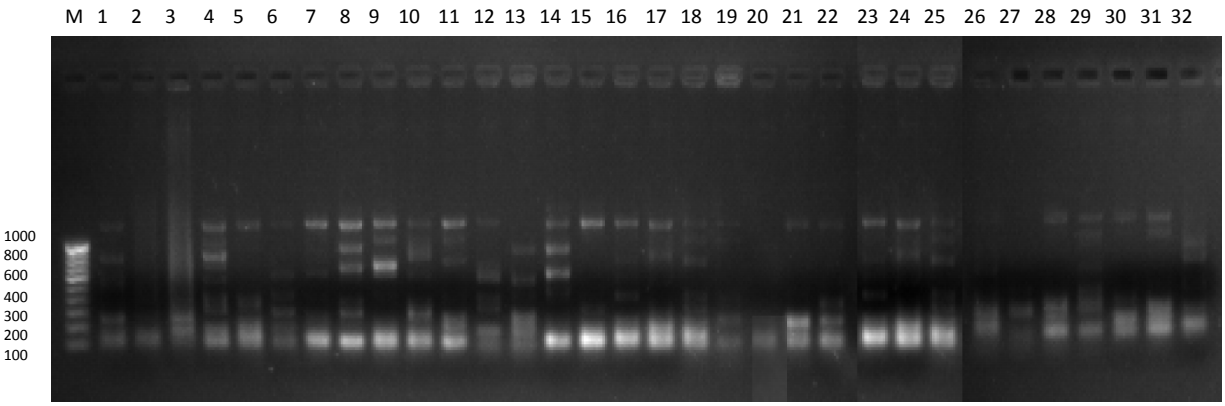
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

Plate 4: ISSR pattern of 32 tomato genotypes generated by IISRS-3-E

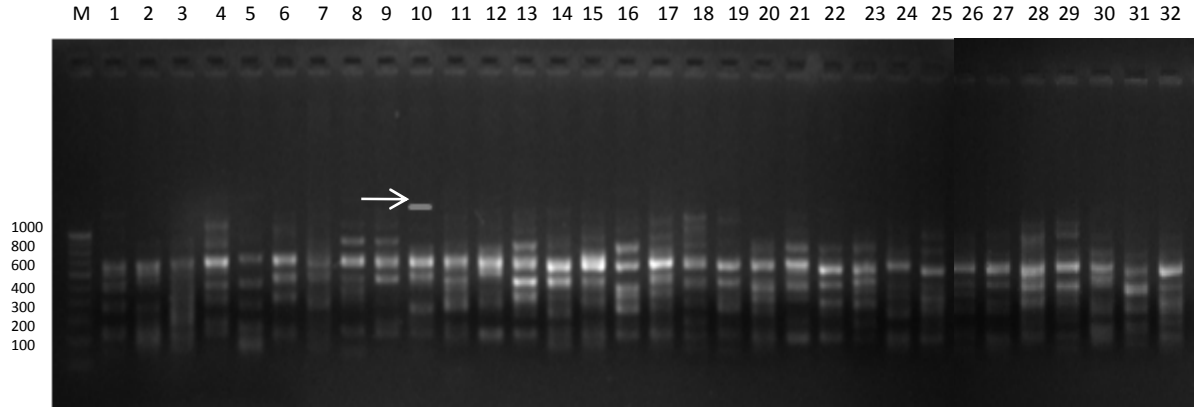
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



ISSR primer UBC-840

Plate 3



ISSR primer IISRS-3-E

Arrow indicates unique band

Plate 4

Plate 5: ISSR pattern of 32 tomato genotypes generated by IISRS-3-G

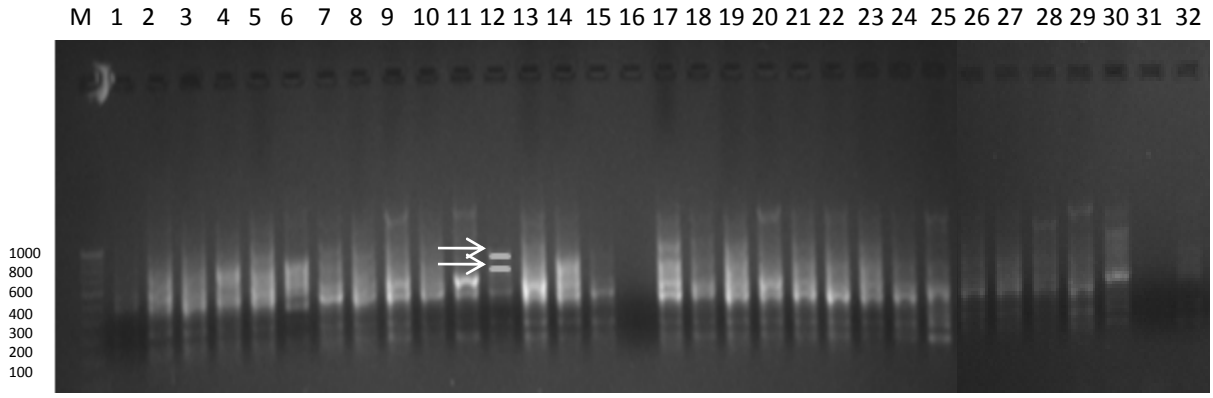
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

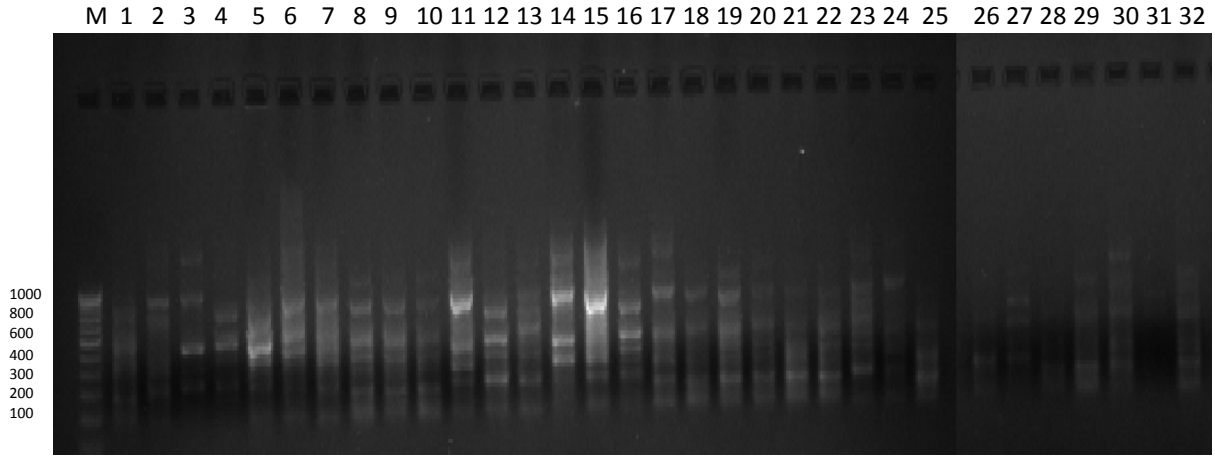
Plate 6: ISSR pattern of 32 tomato genotypes generated by IISRS-3-H

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



ISSR primer IISRS-3-G
Arrow indicates unique band
Plate 5



ISSR primer IISRS-3-H
Plate 6

Plate 7: ISSR pattern of 32 tomato genotypes generated by IISRS-3-F

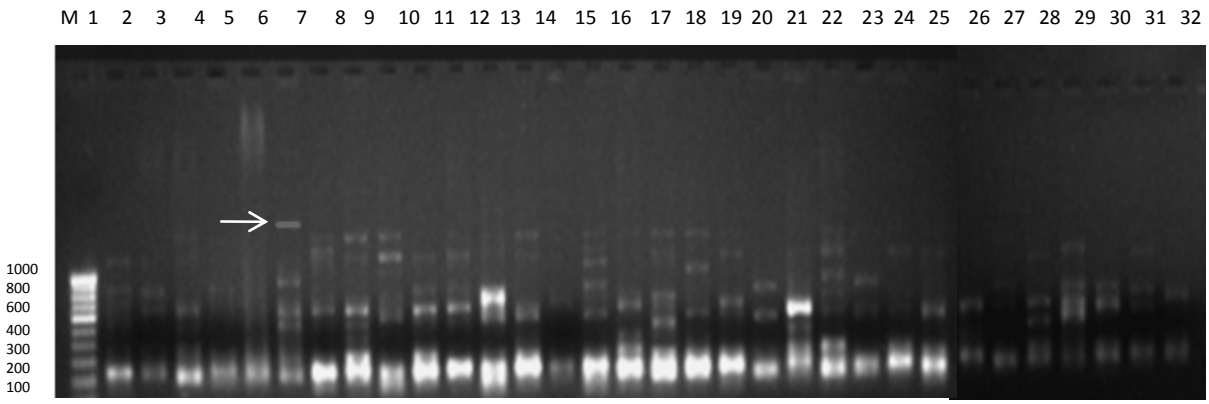
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

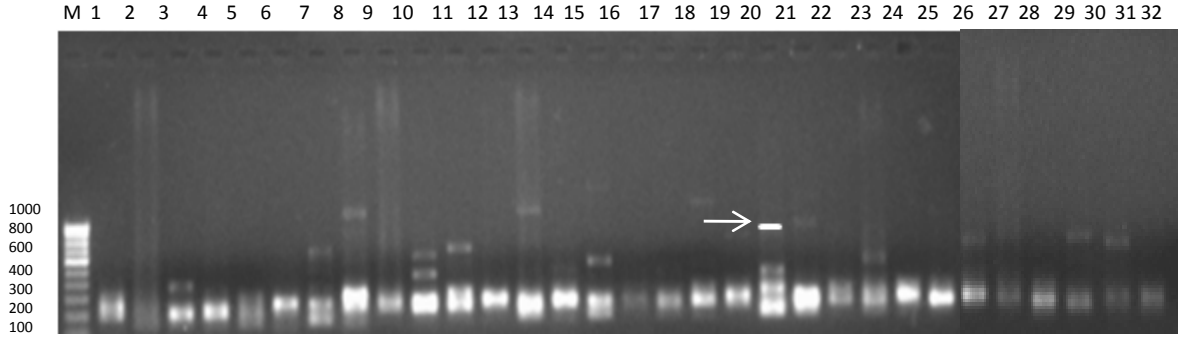
Plate 8: ISSR pattern of 32 tomato genotypes generated by IISRS-3-L

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



ISSR primer IISRS-3-F
Arrow indicates unique band
Plate 7



ISSR primer IISRS-3-L
Arrow indicates unique band
Plate 8

Plate 9: ISSR pattern of 32 tomato genotypes generated by ISSR-4

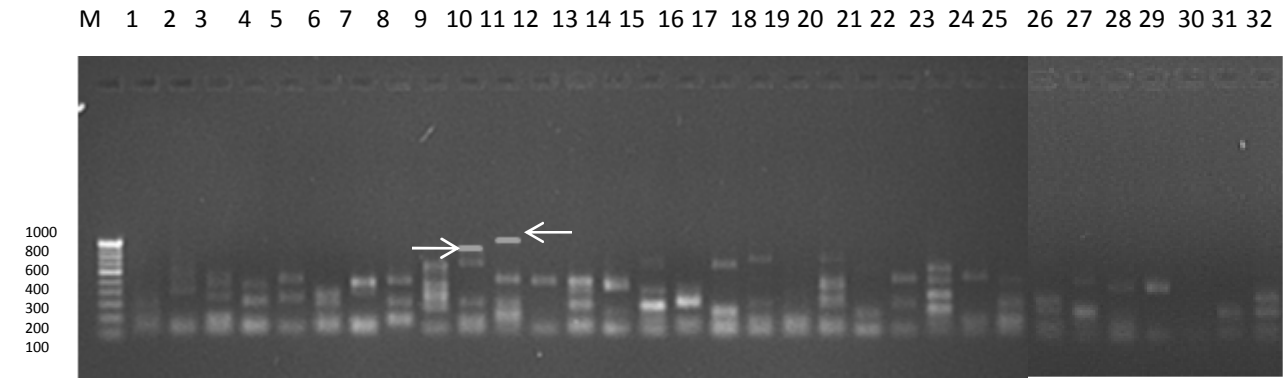
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

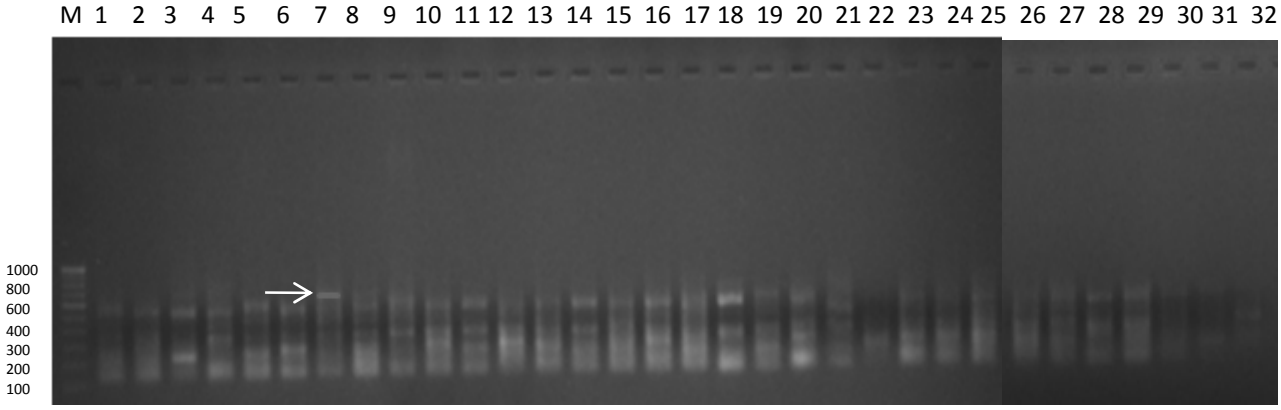
Plate 10: ISSR pattern of 32 tomato genotypes generated by ISSR 844 B

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



ISSR primer ISSR 4
Arrow indicates unique band
Plate 9



ISSR primer ISSR 844 B
Arrow indicates unique band
Plate 10

Plate 11: ISSR pattern of 32 tomato genotypes generated by ISSR-5

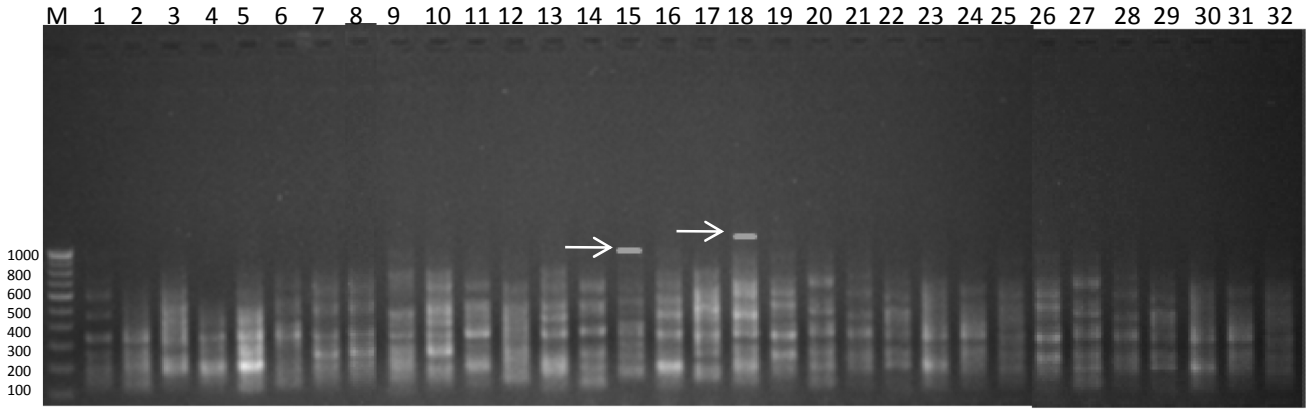
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

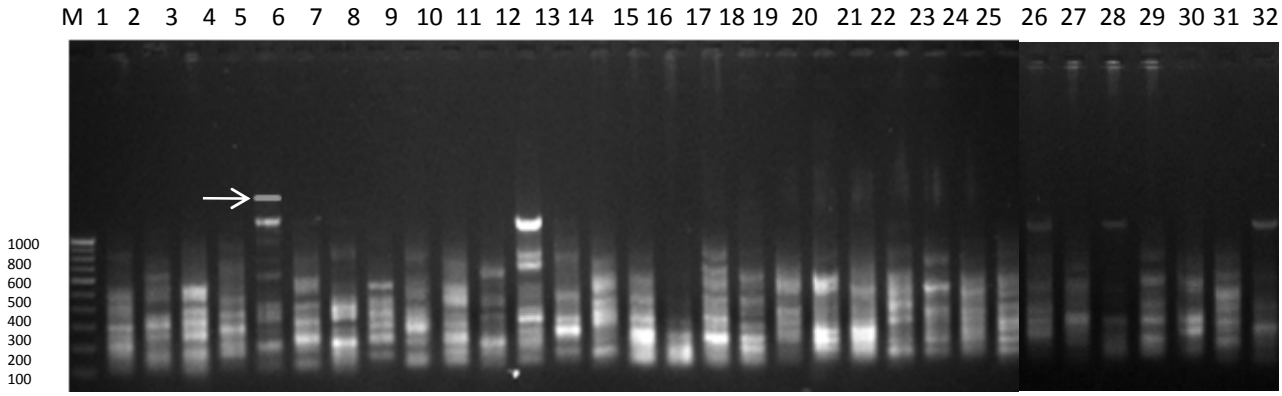
Plate 12: ISSR pattern of 32 tomato genotypes generated by ISSR-7

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



ISSR primer ISSR-5
Arrow indicates unique band
Plate 11



ISSR primer ISSR-7
Arrow indicates unique band
Plate 12

Plate 13: ISSR pattern of 32 tomato genotypes generated by ISSR 17898A

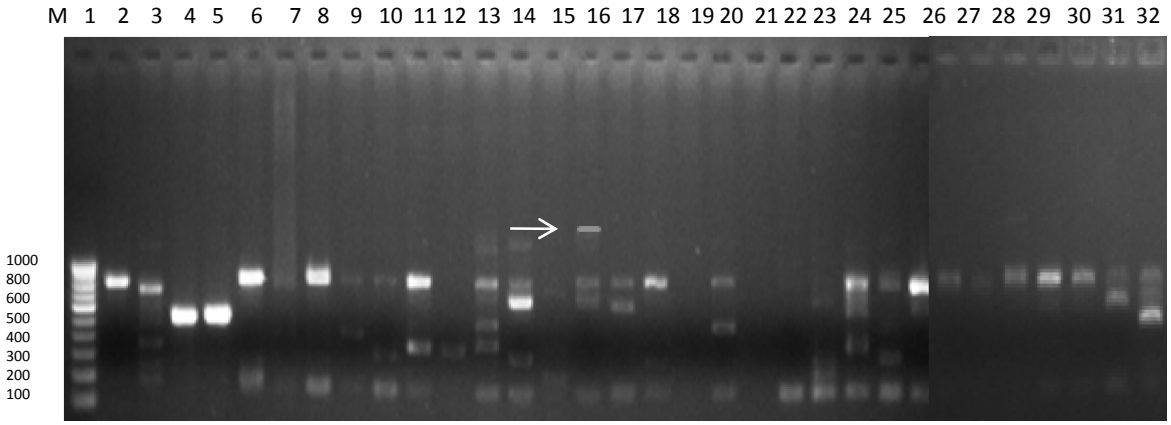
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

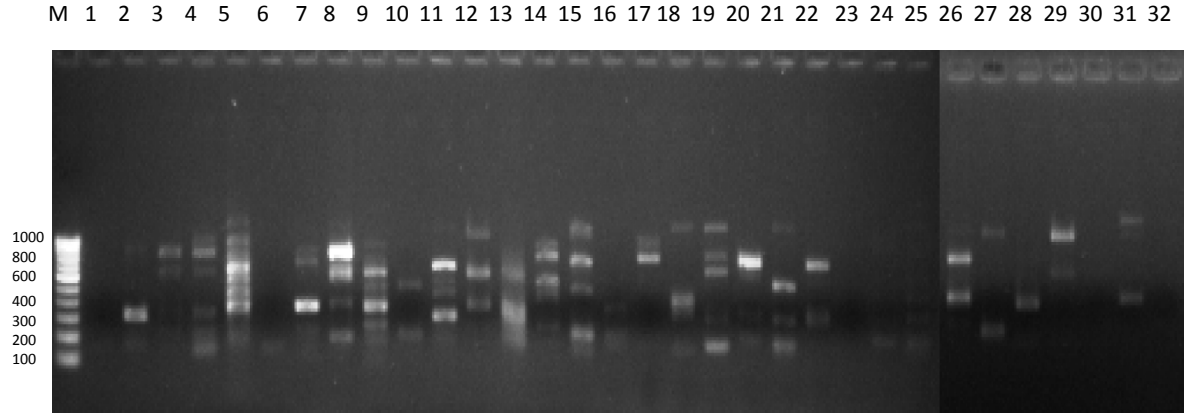
Plate 14: ISSR pattern of 32 tomato genotypes generated by ISSR 17898B

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



ISSR primer ISSR 17898A
Arrow indicates unique band
Plate 13



ISSR primer ISSR 17898B
Plate 14

Plate 15: ISSR pattern of 32 tomato genotypes generated by UBC-894

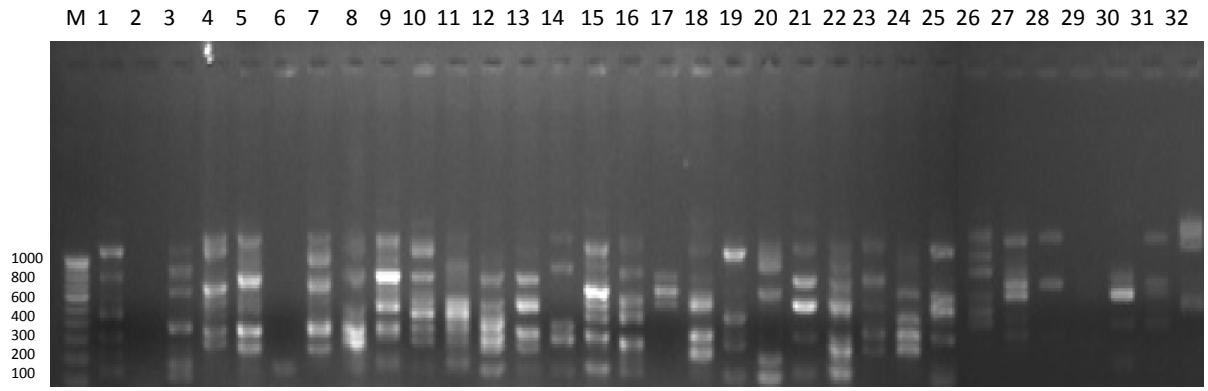
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

Plate 16: ISSR pattern of 32 tomato genotypes generated by IISRS-3-I

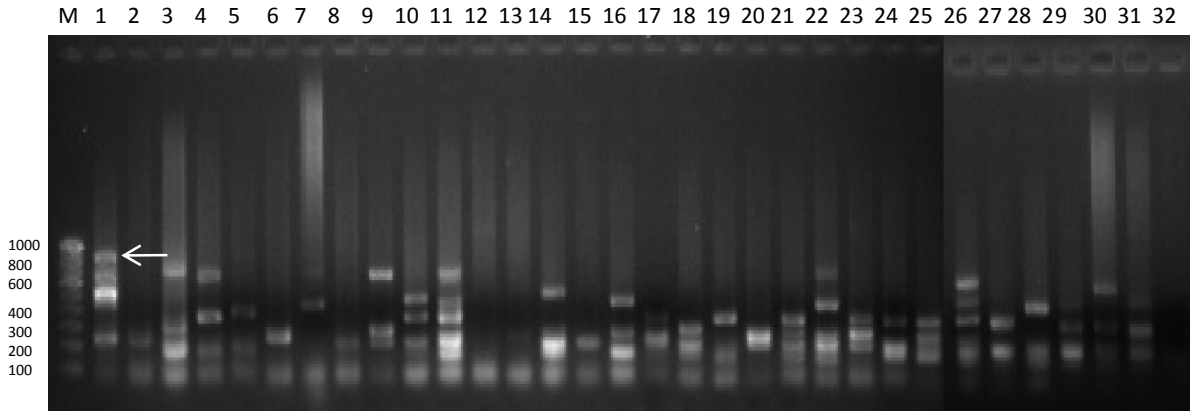
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



ISSR primer UBC-894

Plate 15



ISSR primer IISRS-3-I
Arrow indicates unique band

Plate 16

Plate 17: Genomic SSR pattern of 32 tomato genotypes generated by G1

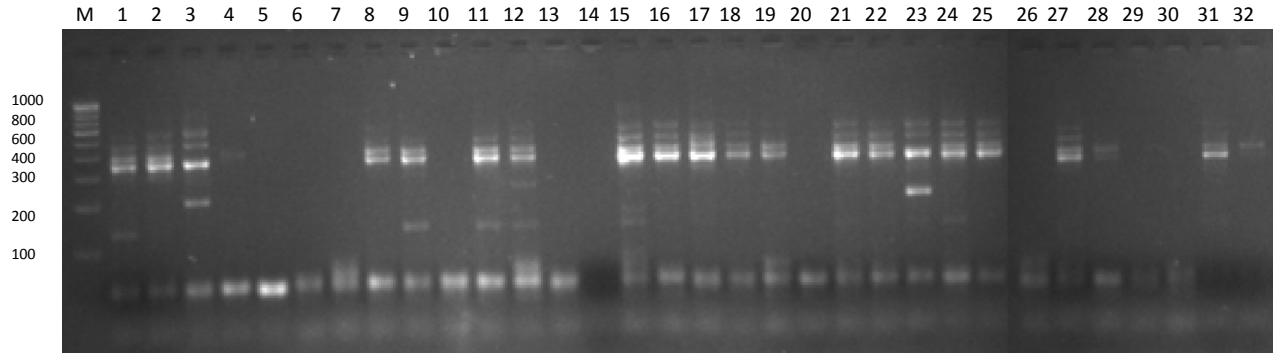
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

Plate 18: Genomic SSR pattern of 32 tomato genotypes generated by G2

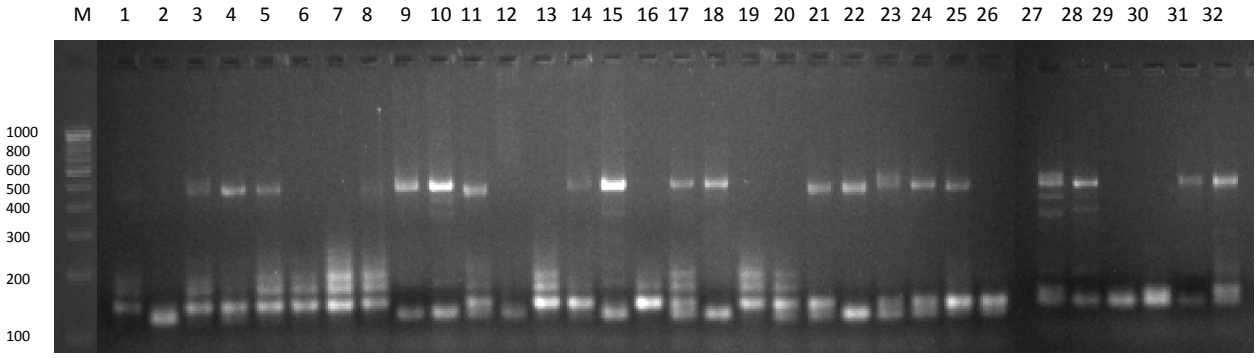
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



Genomic SSR primer G1

Plate 17



Genomic SSR primer G2

Plate 18

Plate 19: Genomic SSR pattern of 32 tomato genotypes generated by G3

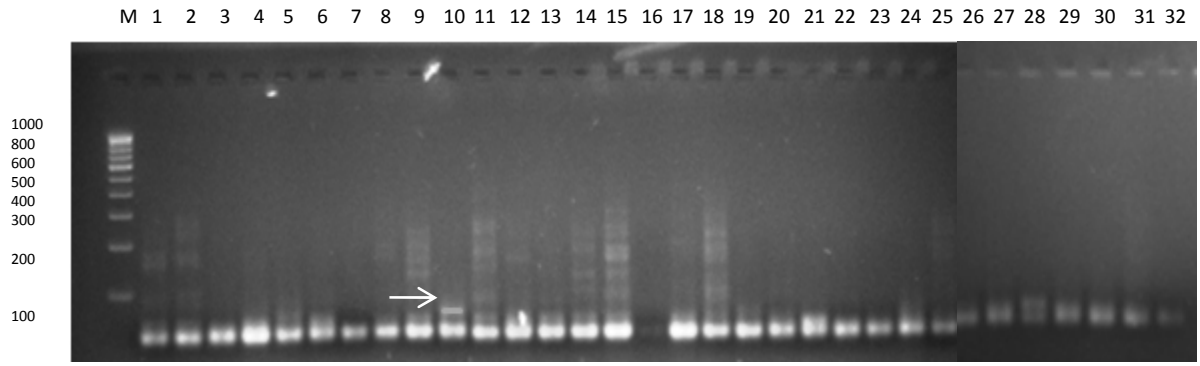
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

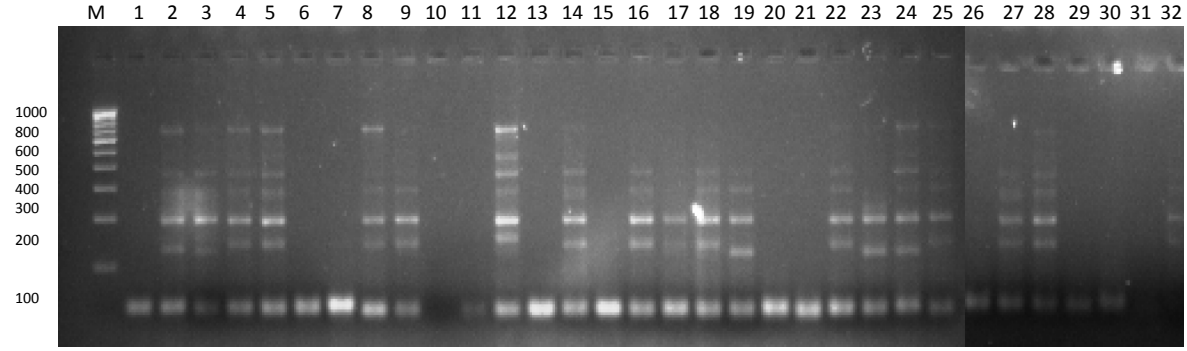
Plate 20: Genomic SSR pattern of 32 tomato genotypes generated by G4

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



Genomic SSR primer G3
Arrow indicates unique band
Plate 19



Genomic SSR primer G4
Plate 20

Plate 21: Genomic SSR pattern of 32 tomato genotypes generated by G5

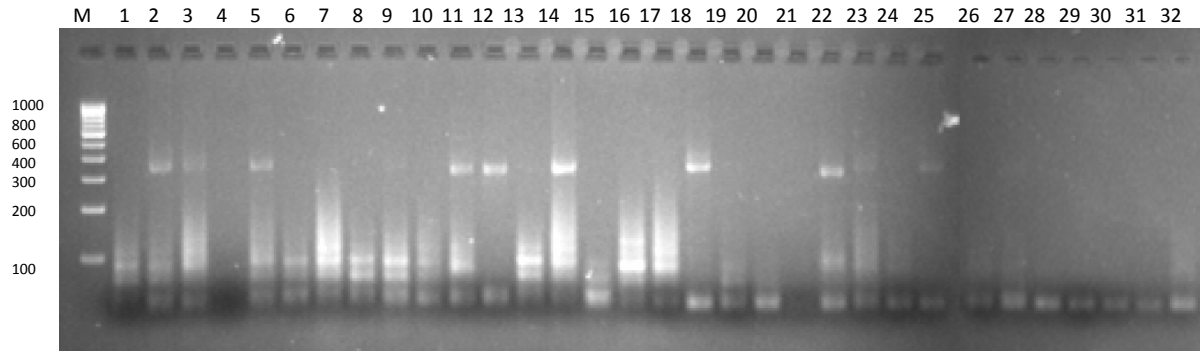
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

Plate 22: Genomic SSR pattern of 32 tomato genotypes generated by G6

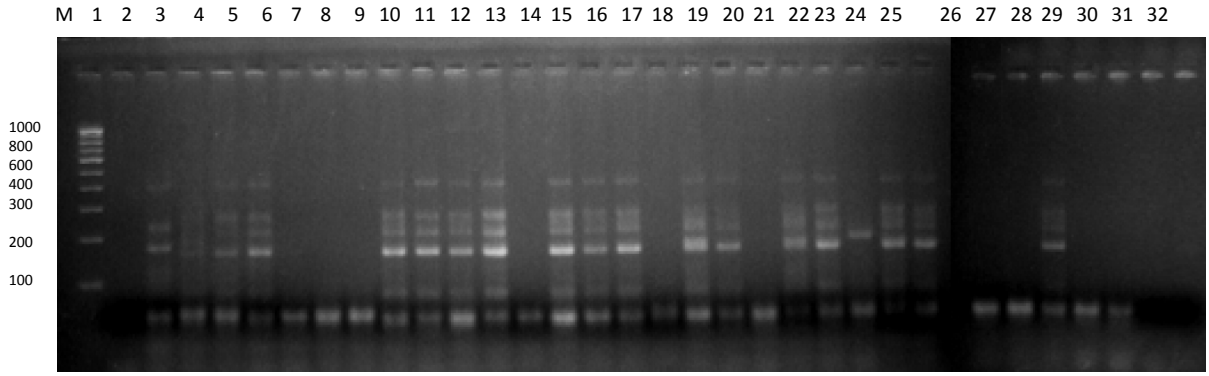
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



Genomic SSR primer G5

Plate 21



Genomic SSR primer G6

Plate 22

Plate 23: Genomic SSR pattern of 32 tomato genotypes generated by G8

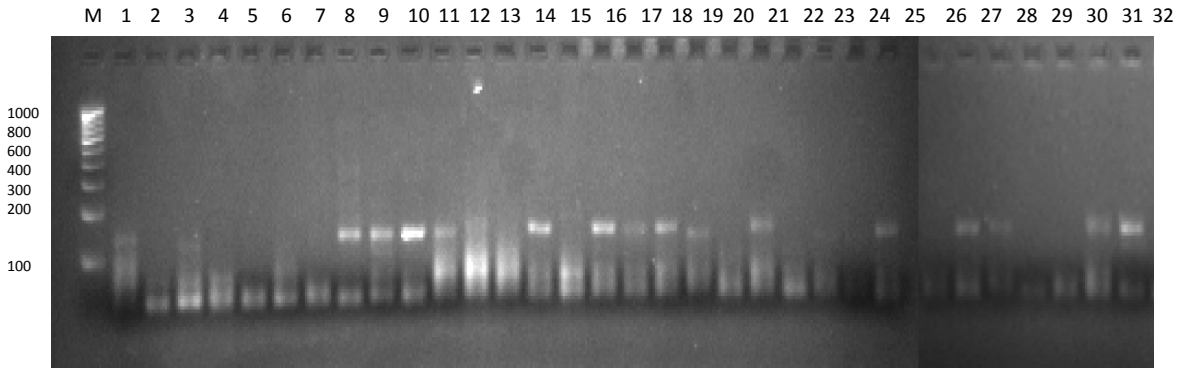
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

Plate 24: Genomic SSR pattern of 32 tomato genotypes generated by G9

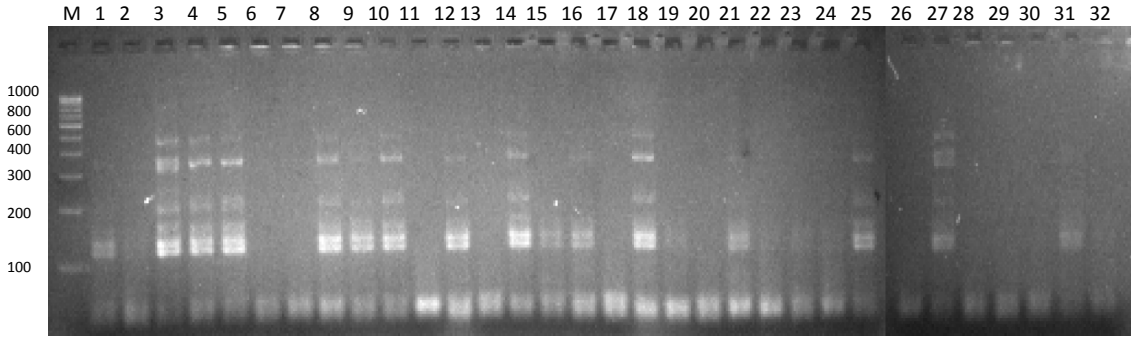
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



Genomic SSR primer G8

Plate 23



Genomic SSR primer G9

Arrow indicates unique band

Plate 24

Plate 25: Genomic SSR pattern of 32 tomato genotypes generated by G13

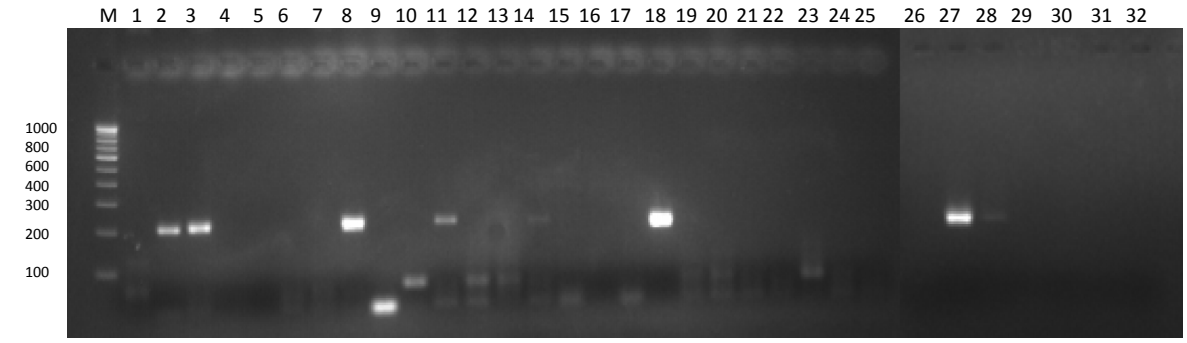
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

Plate 26: Genomic SSR pattern of 32 tomato genotypes generated by G15

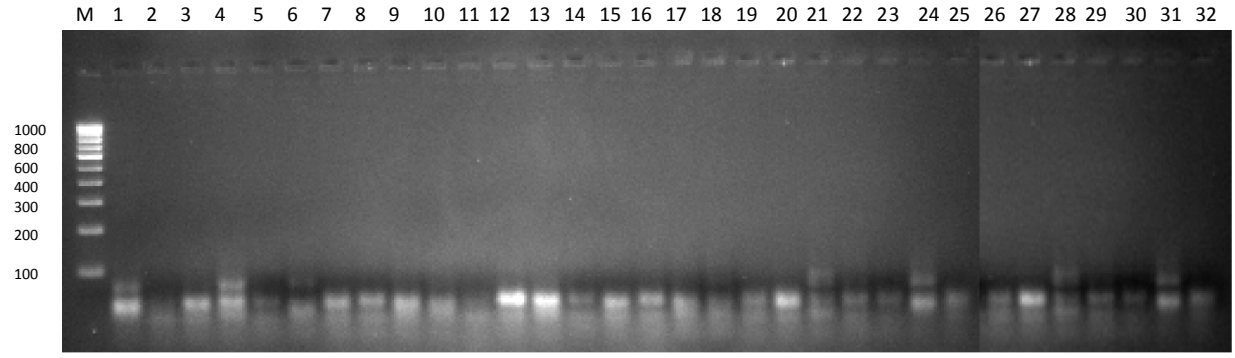
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



Genomic SSR primer G13

Plate 25



Genomic SSR primer G15

Plate 26

Plate 27: EST-SSR pattern of 32 tomato genotypes generated by Contig 7

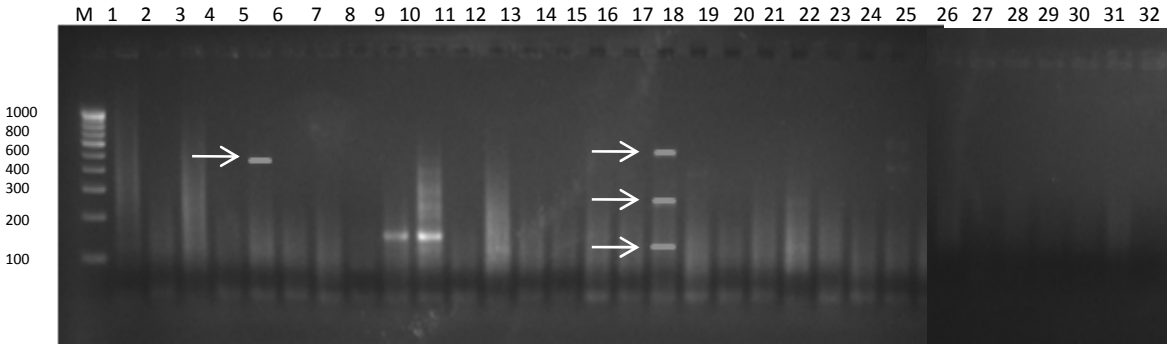
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

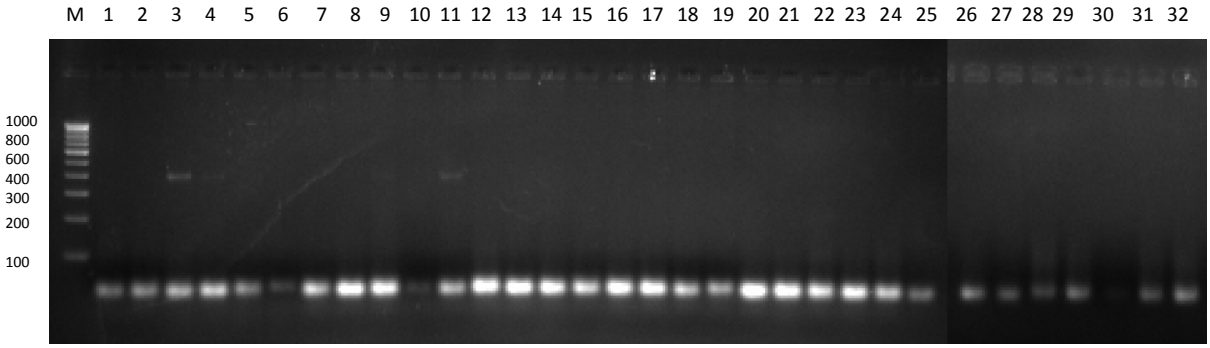
Plate 28: EST-SSR pattern of 32 tomato genotypes generated by Contig 143

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



EST-SSR primer Contig 7
Arrow indicates unique band
Plate 27



EST-SSR primer Contig 143
Plate 28

Plate 29: EST-SSR pattern of 32 tomato genotypes generated by Contig 162

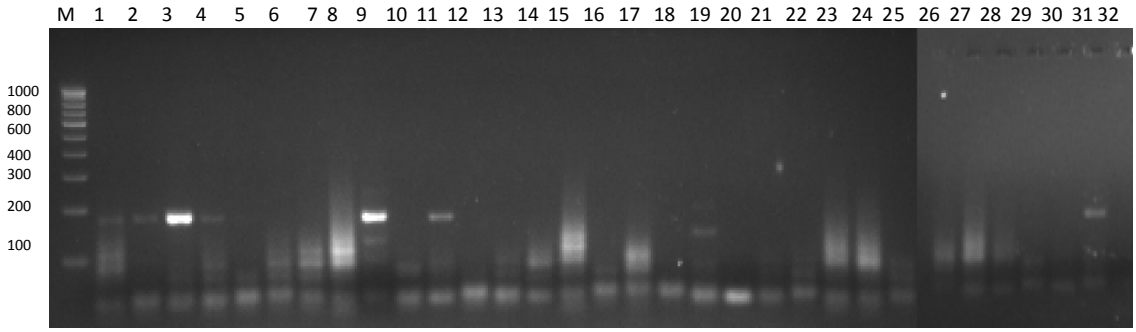
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

Plate 30: EST-SSR pattern of 32 tomato genotypes generated by Contig 265

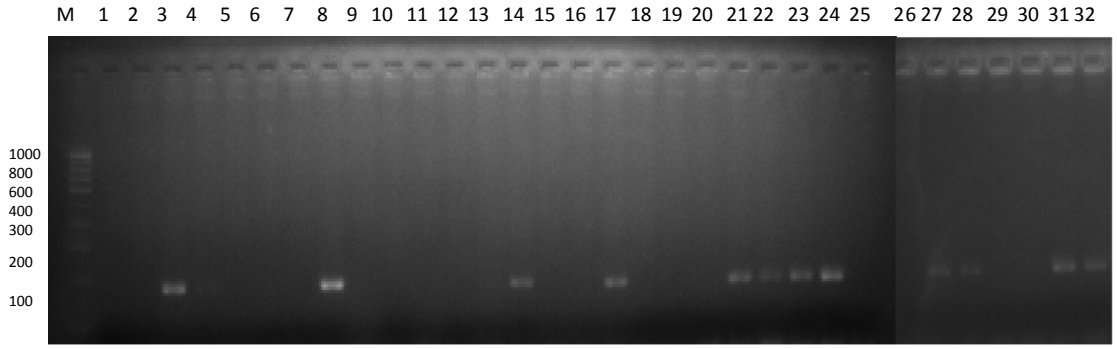
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



EST-SSR primer Contig 162

Plate 29



EST-SSR primer Contig 265

Plate 30

Plate 31: EST-SSR pattern of 32 tomato genotypes generated by Contig 340

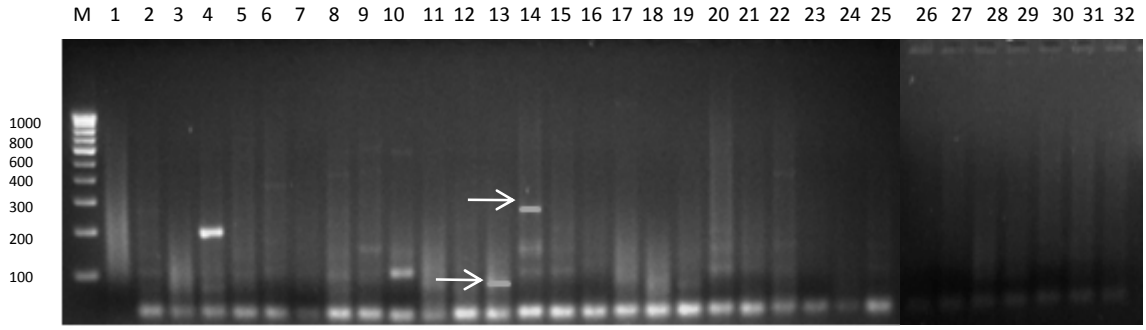
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

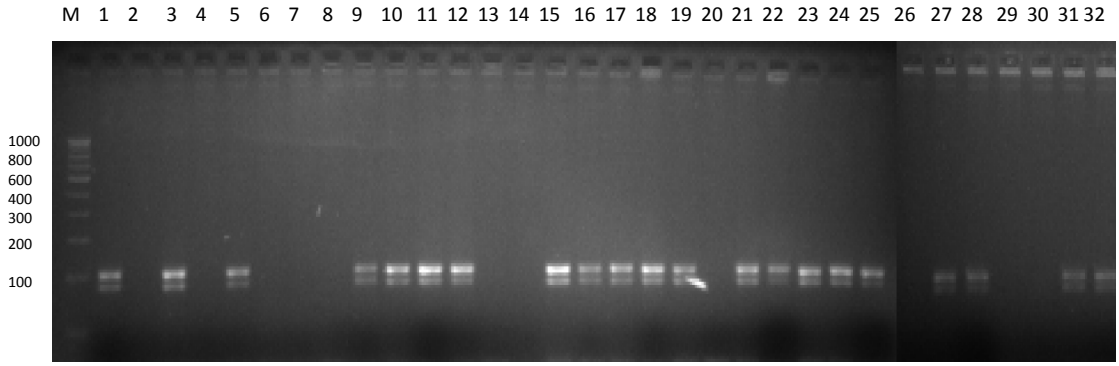
Plate 32: EST-SSR pattern of 32 tomato genotypes generated by gi|116644211|

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



EST-SSR primer Contig 340
Arrow indicates unique band
Plate 31



EST-SSR primer gi|116644211|
Plate 32

Plate 33: EST-SSR pattern of 32 tomato genotypes generated by gi|4387244|

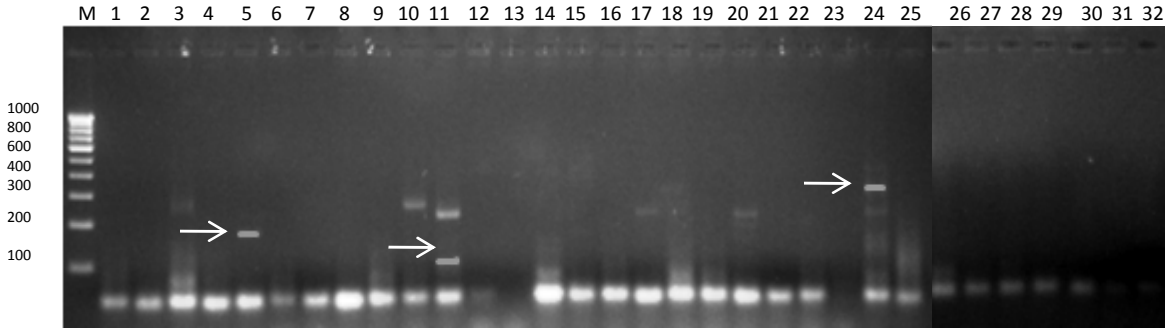
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

Plate 34: EST-SSR pattern of 32 tomato genotypes generated by gi|4386975|

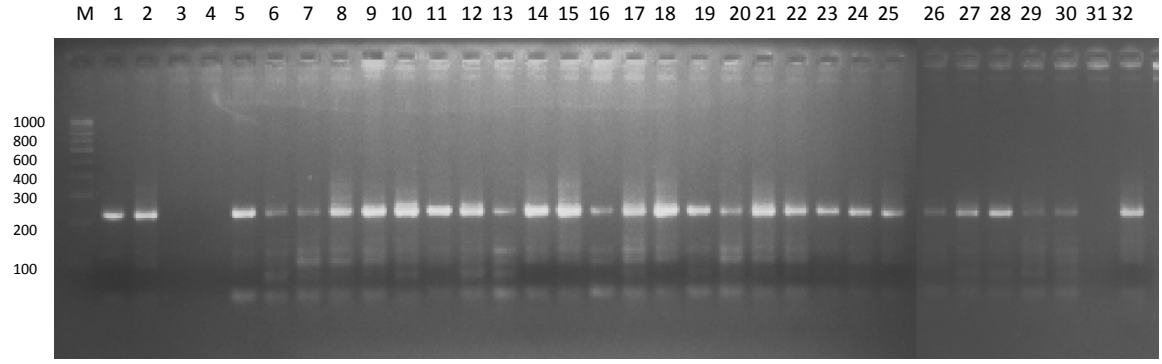
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



EST-SSR primer gi|4387244|
Arrow indicates unique band

Plate 33



EST-SSR primer gi|4386975|

Plate 34

Plate 35: EST-SSR pattern of 32 tomato genotypes generated by gi|4386589|

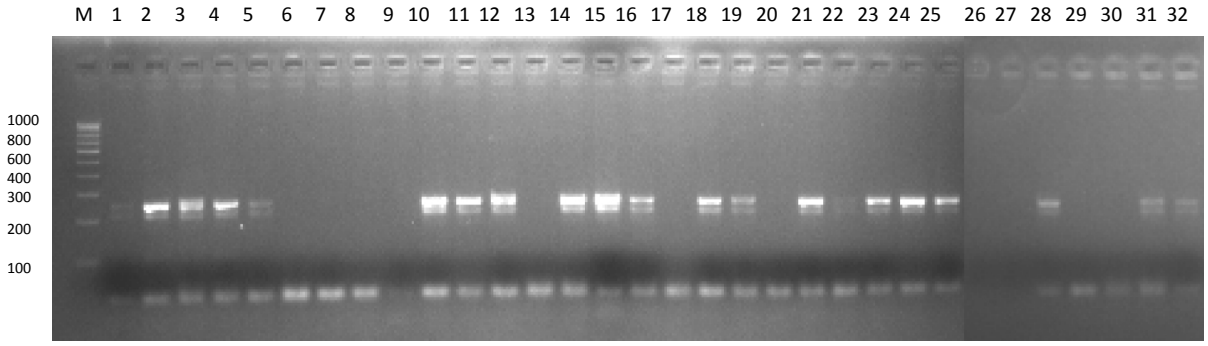
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

Plate 36: EST-SSR pattern of 32 tomato genotypes generated by gi|4386576|

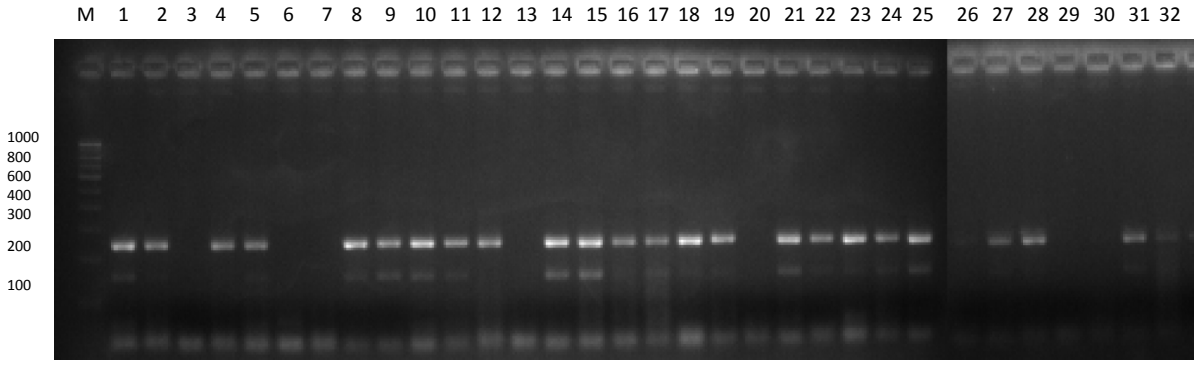
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



EST-SSR primer gi|4386589|

Plate 35



EST-SSR primer gi|4386576|

Plate 36

Plate 37: EST-SSR pattern of 32 tomato genotypes generated by gi|4386508|

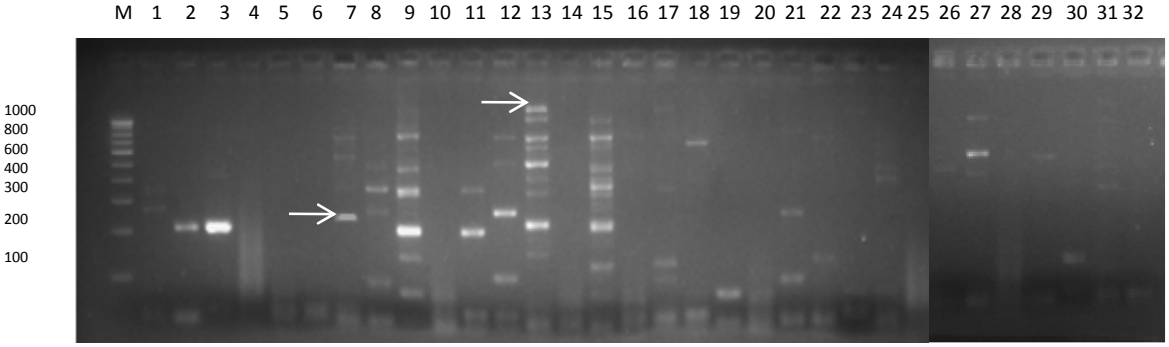
S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker

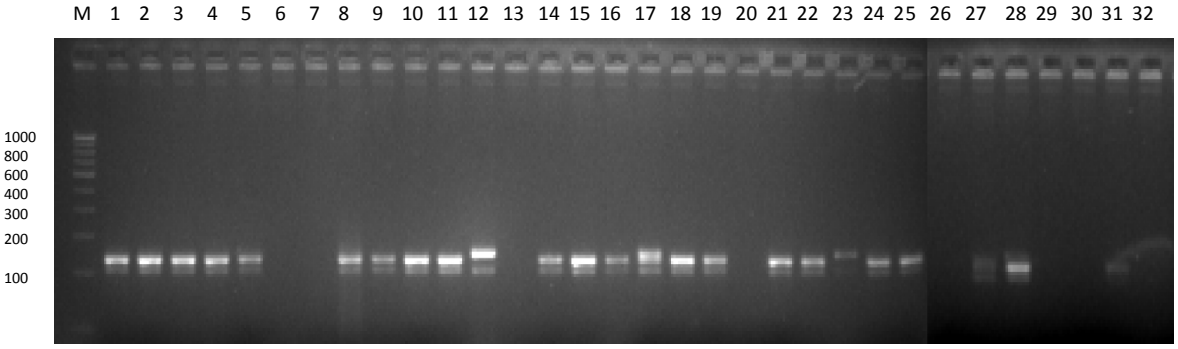
Plate 38: EST-SSR pattern of 32 tomato genotypes generated by gi|76572221|

S. No.	Name/Accession Number	S. No.	Name/Accession Number	S. No.	Name/Accession Number
1.	Solan Vajr	12.	EC-10662	23.	EC-251649
2.	Hawai 7998	13.	EC-114375	24.	EC-521054
3.	EC-520075	14.	EC-2798	25.	EC-35322
4.	EC-251649	15.	EC-25265	26.	EC-251646
5.	EC-528373	16.	EC-521059	27.	EC-528374
6.	EC-126902	17.	EC-10304	28.	EC-521051
7.	EC-6486	18.	EC-501074	29.	EC-521
8.	EC-2517	19.	EC-29914	30.	EC-8591
9.	EC-13904	20.	EC-521041	31.	EC-12699
10.	1EC-36883	21.	EC-362949	32.	EC-2791
11.	EC-168283	22.	EC-528367		

M denotes 100 bp Molecular Size Marker



EST-SSR primer gi|4386508|
Arrow indicates unique band
Plate 37



EST-SSR primer gi|76572221|
Plate 38

4.2 To study genetics of inheritance of resistance to *Phytophthora nicotianae* var. *parasitica*

4.2.1 Raising of mapping population

F_1 seeds produced from cross combination of susceptible variety 'Solan Lalima' and resistant line 'EC-251649' were selfed to raise F_2 population (Plate 39-41). A total of 100 F_2 individuals constituted the mapping population.

4.2.2 Isolation and morphological characterization of *Phytophthora nicotianae* var. *parasitica*

Isolation of the pathogen from infected fruit was successfully achieved on CMA medium (Plate 42-43). For proper growth of fungal isolate, the culture room temperature was maintained at 25^0C.

Morphological characterization was done under microscope. For this the slide was prepared by placing a single bit of culture in a drop of distilled water followed by covering it with cover slip. Then the slide was examined under microscope. The pathogen/ fungus was identified based on the following features: the mycelium is hyaline and coenocytic, branching at right angles. The sporangium arises from hyphal threads and produced sporangiospore (Plate 44).

According to Flores *et al*. (2013) *Phytophthora* genus is a group whose isolation and conservation is laborious. Thus, the work on search of better options for both mycelial growth and sporulation for different isolates of *Phytophthora* is a continuous process. In the studies conducted earlier various media were tried which included CMA, lima bean agar (LBA), modified lima bean agar (MLBA), malt extract agar (MEA), oat meal agar (OMA) and potato dextrose agar (PDA), but CMA medium was recorded as one of the best media for *Phytophthora nicotianae* growth (Dodan and Shyam, 1996; Hossain and Banik, 1999). The growth conditions were also same with those used in present study. The identifying features matched with standard keys given by Ribeiro (1978).

4.2.3 Maintenance of pure culture and preparation of fungal inoculum for screening

Once the fungus was isolated, further sub-culturing was done to maintain pure culture devoid of any other contaminant's growth. This was done by placing a single bit from previously isolated culture on CMA medium. The cultured plates were then kept at a temperate of 25^0C.

After seven to ten days of growth of pure culture a loopful of fungal hyphae was transferred to corn meal broth. The liquid culture was then kept at 100 rpm at 25^0C in incubator shaker. After ten days white flocculent growth appeared in broth (Plate 45- 46).

Fungal hyphae were separated from the liquid medium by filtration through filter paper. The fungal mass then wighed to 1 gm followed by crushing to obtain small sized hyphae. Final volume of this fungal inoculum was maintained 80 ml to obtain optimum density of 15-20 hyphae/ cm^3 in haemocytometer.

4.2.4 Pathogenicity test of parents and segregants using fungal inoculum

The fruits of susceptible and resistant parents along with 100 F_2 individuals were screened for their reaction to buckeye rot using fungal culture by injecting 10 ml inoculum in detached fruits (under *in vitro* conditions) as well as in intact fruits (under *in vivo* conditions) (Plate 47-53). Disease reaction was assessed as given in section 3.2.4.

On the basis of screening the parent which was considered susceptible was characterized in highly susceptible category, while the resistant parent was characterized as moderately resistant one. Nine fruits of each F_2 plant for *in vitro* and nine fruits for *in vivo* pathogenicity assays were inoculated. Screening results of 100 F_2 segregants are presented in Table 20. The data obtained for *in vitro* verses *in vivo* pathogenicity assays on 100 F_2 plants was similar. Thus the data given in Table 20 represents both *in vitro* and *in vivo* pathogenicity results. The highest and lowest percentage of disease incidence was 0% and 100%, respectively, with an average of 63.66%.

Table 20: Disease incidence per cent and score in F_2 plants after eight days of inoculation

S. No.	F_2 Plant No.	Disease Incidence (%)	Symptom Score
1	1*	0	Resistant
2	2	100.00	Highly Susceptible
3	3	33.33	Susceptible
4	4	33.33	Susceptible
5	5	66.66	Highly Susceptible
6	6	33.33	Susceptible
7	7	66.66	Highly Susceptible
8	8	100.00	Highly Susceptible
9	9	33.33	Susceptible
10	10	33.33	Susceptible
11	11*	0	Resistant
12	12	33.33	Susceptible
13	13	33.33	Susceptible
14	14	33.33	Susceptible

Table 20. Cont…..

S. No.	F_2 Plant No.	Disease Incidence (%)	Symptom Score
15	15	66.66	Highly Susceptible
16	16	33.33	Susceptible
17	17*	0	Resistant
18	18*	0	Resistant
19	19	33.33	Susceptible
20	20	33.33	Susceptible
21	21	100.00	Highly Susceptible
22	22	100.00	Highly Susceptible
23	23	100.00	Highly Susceptible
24	24	33.33	Susceptible
25	25	33.33	Susceptible
26	26	100.00	Highly Susceptible
27	27	100.00	Highly Susceptible
28	28	100.00	Highly Susceptible
29	29	33.33	Susceptible
30	30	100.00	Highly Susceptible
31	31	66.66	Highly Susceptible
32	32	33.33	Susceptible
33	33	66.66	Highly Susceptible
34	34	66.66	Highly Susceptible
35	35	100.00	Highly Susceptible
36	36	66.66	Highly Susceptible
37	37	66.66	Highly Susceptible
38	38	33.33	Susceptible
39	39	66.66	Highly Susceptible
40	40	100.00	Highly Susceptible
41	41	100.00	Highly Susceptible
42	42	33.33	Susceptible
43	43	100.00	Highly Susceptible
44	44	100.00	Highly Susceptible
45	45	66.66	Highly Susceptible
46	46	100.00	Highly Susceptible
47	47	100.00	Highly Susceptible
48	48	100.00	Highly Susceptible
49	49	100.00	Highly Susceptible
50	50	100.00	Highly Susceptible
51	51	100.00	Highly Susceptible
52	52	66.66	Highly Susceptible
53	53	66.66	Highly Susceptible
54	54	33.33	Susceptible
55	55	33.33	Susceptible
56	56	33.33	Susceptible
57	57	66.66	Highly Susceptible

Table 20. Cont…..

S. No.	F_2 Plant No.	Disease Incidence (%)	Symptom Score
58	58	100.00	Highly Susceptible
59	59	66.66	Highly Susceptible
60	60	100.00	Highly Susceptible
61	61	100.00	Highly Susceptible
62	62	33.33	Susceptible
63	63	33.33	Susceptible
64	64	66.66	Highly Susceptible
65	65	66.66	Highly Susceptible
66	66	100.00	Highly Susceptible
67	67	100.00	Highly Susceptible
68	68	33.33	Susceptible
69	69	66.66	Highly Susceptible
70	70	33.33	Susceptible
71	71	66.66	Highly Susceptible
72	72	100.00	Highly Susceptible
73	73	100.00	Highly Susceptible
74	74	100.00	Highly Susceptible
75	75	100.00	Highly Susceptible
76	76	66.66	Highly Susceptible
77	77	66.66	Highly Susceptible
78	78	66.66	Highly Susceptible
79	79	66.66	Highly Susceptible
80	80	100.00	Highly Susceptible
81	81	33.33	Susceptible
82	82	100.00	Highly Susceptible
83	83	66.66	Highly Susceptible
84	84*	0	Resistant
85	85	33.33	Susceptible
86	86	66.66	Highly Susceptible
87	87	33.33	Susceptible
88	88	66.66	Highly Susceptible
89	89	100.00	Highly Susceptible
90	90	100.00	Highly Susceptible
91	91	66.66	Highly Susceptible
92	92	33.33	Susceptible
93	93	33.33	Susceptible
94	94	33.33	Susceptible
95	95	33.33	Susceptible
96	96	66.66	Highly Susceptible
97	97	33.33	Susceptible
98	98	66.66	Highly Susceptible
99	99	66.66	Highly Susceptible
100	100	66.66	Highly Susceptible

*indicates phenotypically resistant individual

Screening against buckeye rot disease under laboratory conditions has been done earlier by Oliva-Risco (1983) following different methods *viz.* fruit dip followed by inoculation, fruit dip-injury-inoculation and injury-fruit dip-inoculation. The data of disease incidence were recorded for ten days when 100% disease appeared in one of the treatments. Among these methods injury-fruit dip-inoculation was found most effective.

4.2.5 Genetics of inheritance study

From screening results of 100 F_2 segregants it was shown that 95 were susceptible and five were resistant giving a ratio of 3.8:0.2. Chi-square (χ^2) test was performed to determine whether the observed ratio fits in expected one or not (Table 21).

$$\chi^2 = \sum (O-E)^2 / \sum E$$

where; O= Observed value and E= Expected value

Table 21: Chi-square (χ^2) test

	Observed (O)	Expected (E)	O-E	$(O-E)^2$	χ^2 value
Resistant	5	25	-20	400	16
Susceptible	95	75	20	400	5.33
Total	100	100	0	800	21.33

Table value of χ^2 at 1 degree of freedom at 5% probability is 3.841. Since the calculated value χ^2 (Table 21) is more than the tabulated value, thus, the observed F_2 ratio did not fit into the expected Mendelian ratio for monogenic inheritance of buckeye rot in tomato leading to the conclusion that this disease is not under control of single gene and may be controlled by polygenes.

It is very important to study the inheritance pattern of any character because this study will lead to a final conclusion about how the character is controlled at genetic level and in which manner it transmits to the next generation. This will facilitate further breeding experiments for crop improvement. In tomato various inheritance studies have been conducted for disease resistance including viruses (Thomas and McGrath, 1988; Castro *et al.*, 2007), bacteria (Kumar, 2002; Kozik and Sobiczewski, 2008; Sharma *et al.*, 2011) and fungus (Vakalounakis, 2007; Abreu *et al.*, 2008; Calis and Topkaya, 2011). In case of buckeye rot which is a fungal disease of tomato, inheritance studies conducted in past were not in agreement with each other. So it was necessary to confirm the accurate inheritance pattern of this disease. In present study quantitative/ polygenic inheritance for disease resistance was revealed. But according to Rattan and Saini (1979) buckeye rot resistance is under control of single dominant gene. Qualitative analysis conducted by Chauhan (1988) showed resistance

to buckeye rot under one dominant gene control but the generation mean analysis depicted the presence of significant additive gene effects in all crosses. Thus, the results of Chauhan (1988) supported the findings of the present study.

4.3 Identification of molecular markers/ QTL for buckeye rot resistance in tomato

4.3.1 Phenotyping of parents using fungal inoculum

Phenotyping of both susceptible and resistant parents was carried out as earlier explained in section 4.2.4 (Plate 47-48). This experiment confirmed the contrast (susceptibility and resistance) between parents at phenotypic level which is the first requisite to carry out marker/ QTL identification studies.

4.3.2 Phenotyping of mapping population using fungal inoculum

Phenotyping of 100 F_2 individuals which constituted mapping population of present study is explained in section 4.2.4 (Plate 49-53). On the basis of disease reaction the individuals were characterized on scale (Table 20).

4.3.3 Parental polymorphism survey using molecular markers

To conduct polymorphism studies of parents a total of 153 primers were used which consisted of 44 ISSRs, 89 genomic SSRs and 20 EST-SSRs. Out of these 93 primers were found polymorphic between parents including 36 ISSRs, 41 genomic SSRs and 16 EST-SSRs (Table 22). This study confirmed variations between parents at genomic level. These polymorphic primers were then further used to carry out genotyping study of mapping population.

4.3.4 Genotyping of mapping population

100 F_2 individuals were used as mapping population to carry out genotyping studies. The short listed polymorphic primers with parents were used to conduct genotyping studies among individuals of mapping population. Data coding indicates ‘A’, ‘B’ and ‘H’ for the band corresponding to susceptible parent ‘Solan Lalima’, resistant parent ‘EC-251649’ and heterozygote, respectively (Table 22).

Plate 39: Susceptible parent 'Solan Lalima'

Plate 40: Resistant parent 'EC-251649'

Plate 41: F_2 population derived from cross between susceptible parent 'Solan Lalima' and resistant parent 'EC-251649'

Plate 39

Plate 40

Plate 41

Plate 42: Buckeye rot infected fruit sample of tomato used for isolation of *Phytophthora nicotianae* var. *parasitica*

Plate 43: *Phytophthora nicotianae* var. *parasitica* on CMA medium after one week of culturing

Plate 44: Microscopic view of *Phytophthora nicotianae* var. *parasitica*

Plate 42

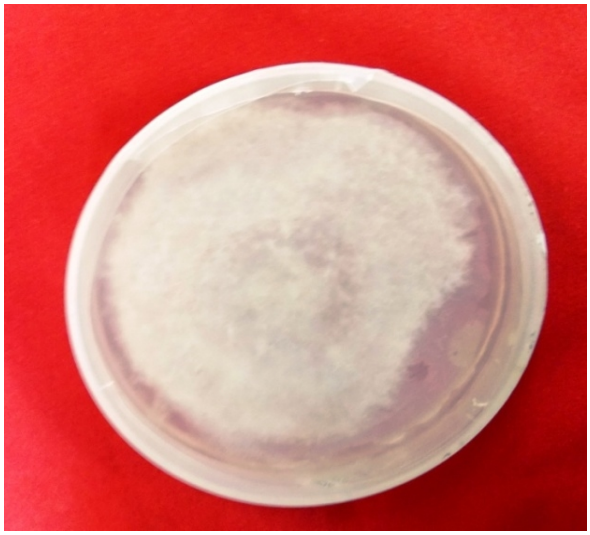


Plate 43

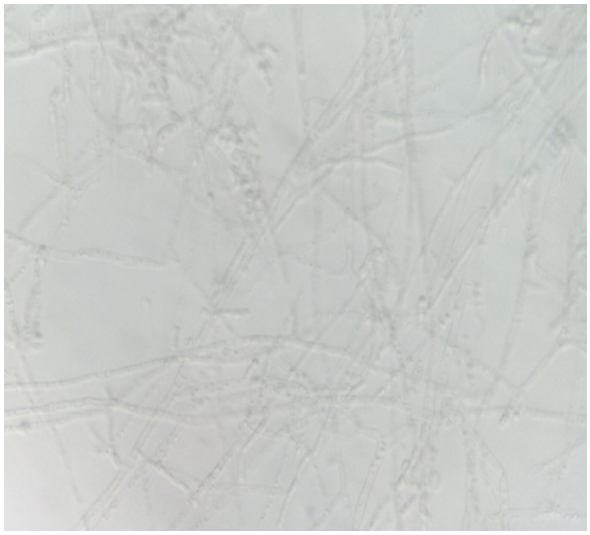


Plate 44

Plate 45: Control flask having the Corn Meal broth without *Phytophthora nicotianae* var. *parasitica.*

Plate 46: Flask inoculated with the *Phytophthora nicotianae* var. *parasitica* after ten days of inoculation

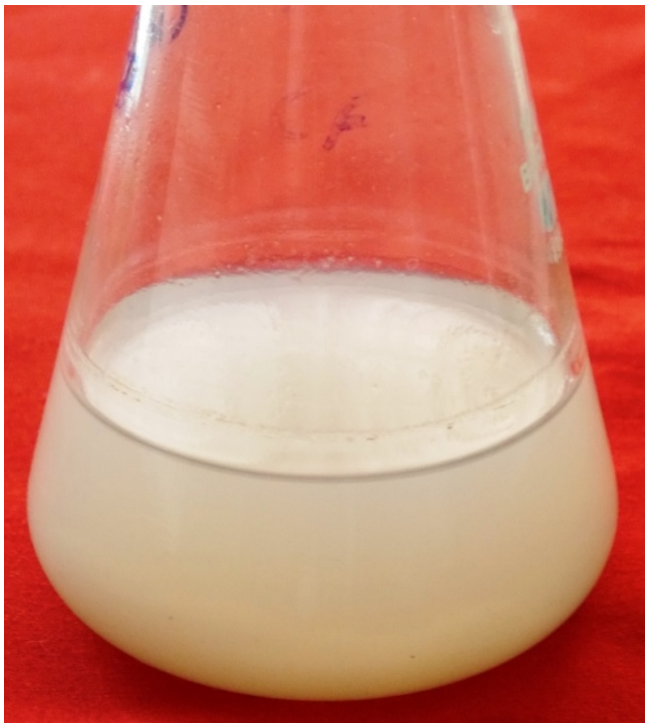


Plate 45

Plate 46

Plate 47a: Detached fruit of susceptible parent 'Solan Lalima' inoculated with fungus (*Phytophthora nicotianae* var. *parasitica*)

Plate 47b: Detached fruit of susceptible parent 'Solan Lalima' showing brown rings after four days of inoculation

Plate 47c: Detached fruit of susceptible parent 'Solan Lalima' showing more infection after eight days of inoculation

Plate 48a: Detached fruit of resistant parent 'EC-251649' inoculated with fungus (*Phytophthora nicotianae* var. *parasitica*)

Plate 48b: Detached fruit of resistant parent 'EC-251649' showing no symptoms of disease after four days of inoculation

Plate 48c: Detached fruit of resistant parent 'EC-251649' showing no symptoms of disease after eight days of inoculation

Plate 47a

Plate 48a

Plate 47b

Plate 48b

Plate 47c

Plate 48c

Plate 49a: Detached fruit of susceptible F_2 plant number 3 inoculated with fungus (*Phytophthora nicotianae* var. *parasitica*)

Plate 49b: Detached fruit of susceptible F_2 plant number 3 showing brown rings after four days of inoculation

Plate 49c: Detached fruit of susceptible F_2 plant number 3 showing more infection after eight days of inoculation

Plate 49a

Plate 49b

Plate 49c

Plate 50a-e: Detached fruits of resistant F_2 plants showing no symptoms of disease after eight days of inoculation with fungus (*Phytophthora nicotianae* var. *parasitica*)

Plate 50a-e

Plate 51: Intact fruit of susceptible parent 'Solan Lalima' showing brown ring after eight days of inoculation

Plate 52: Intact fruit of resistant parent 'EC-251649' showing no symptoms of disease after eight days of inoculation

Plate 51

Plate 52

Plate 53a-e: Intact fruits of resistant F_2 plants showing no symptoms of disease after eight days of inoculation with fungus (*Phytophthora nicotianae* var. *parasitica*)

Plate 53a-e

Table 22: Genotypic data of 100 F_2 individuals obtained with 93 primers

Primer Name	Primer Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Primer1	L1	B	A	B	A	A	B	A	B	B	B	B	A	B	H	H	H	H	H	B	B	A	B	B	B	A	A	A	H	B	B	B	B	B	B	H	B	H	B	B
UBC-841	L2	H	A	B	H	A	A	A	A	A	A	A	A	A	H	A	A	H	A	A	H	A	H	H	H	H	H	H	A	H	A	H	H	H	H	H	H	A	H	H
UBC-808	L3	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	H	H
ISSR-HB-12	L4	H	B	H	H	A	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	B	B	H	H	H	H	H	H	H	H	H	H	H	H	H
ISSR-HB-15	L5	H	B	A	B	B	H	H	B	B	B	B	B	B	B	A	H	A	B	A	B	B	B	B	B	H	H	B	B	H	B	B	B	B	B	B	B	H	B	A
ISSR-HB-10	L6	H	A	A	B	B	A	A	A	A	A	A	B	B	B	B	A	A	A	B	A	H	A	A	A	A	A	A	A	H	H	A	A	A	B	A	A	B	A	A
ISSR-HB-11	L7	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	H	H	A	H	H	B	H	A	A
UBC-840	L8	H	H	B	H	H	H	H	B	H	H	B	H	H	H	B	A	B	H	A	B	A	H	A	B	H	H	H	H	H	H	H	B	H	H	H	H	B	H	B
IISRS-3-M	L9	H	H	A	A	B	B	B	H	H	H	A	A	B	B	H	H	H	H	H	H	H	B	B	B	B	H	H	H	B	B	B	B	B	B	H	H	B	B	H
IISRS-3-N	L10	H	H	B	H	H	H	H	B	H	H	B	H	H	H	B	A	B	H	A	B	A	H	A	B	H	H	H	H	H	H	H	B	H	H	H	H	B	H	B
ISSR-7	L11	H	H	H	H	H	B	H	H	H	H	H	H	H	H	A	A	B	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H	A	B	H	B	B	H	H
Primer2	L12	H	B	A	B	B	H	H	B	B	B	B	B	B	B	A	H	A	B	A	B	B	B	B	B	H	H	B	B	H	B	B	B	B	B	B	B	H	B	A
UBC-854	L13	B	A	B	A	A	B	A	B	B	B	B	A	B	H	H	H	H	H	B	B	A	B	B	B	A	A	A	H	B	B	B	B	B	B	H	B	H	B	B
UBC-855	L14	B	H	H	H	H	H	H	H	A	H	B	H	H	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H
UBC-880	L15	A	A	B	A	B	A	A	A	A	H	A	A	A	A	B	A	B	H	A	A	A	A	A	A	A	B	B	H	B	B	H	H	H	B	B	H	H	B	H
UBC-894	L16	B	B	H	B	B	B	B	A	A	A	A	A	B	A	A	B	A	B	A	B	B	B	B	B	B	B	B	B	B	H	B	B	B	B	B	A	B	H	H
ISSR-4	L17	H	B	H	H	A	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	B	B	H	H	H	H	H	H	H	H	H	H	H	H	H
ISSR-6	L18	H	B	A	B	B	H	H	B	B	B	B	B	B	B	A	H	A	B	A	B	B	B	B	B	H	H	B	B	H	B	B	B	B	B	B	B	H	B	A
UBC-829	L19	A	A	A	A	H	H	H	A	A	H	A	A	H	H	H	H	H	H	H	A	H	A	H	A	A	H	A	A	H	H	A	H	A	H	H	A	H	H	H
IISRS-3-C	L20	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A
IISRS-3-F	L21	H	A	A	B	B	A	A	A	A	A	A	B	B	B	B	A	A	A	B	A	H	A	A	A	A	A	A	A	H	H	A	A	A	B	A	A	B	A	A
IISRS-3-I	L22	A	A	H	A	H	A	A	A	A	A	B	H	B	H	A	A	H	A	A	A	A	A	A	A	A	A	B	A	B	H	A	A	A	B	H	H	A	H	H
UBC-850	L23	H	H	H	H	H	H	H	H	H	H	H	B	B	B	A	H	A	A	H	H	H	H	A	H	H	B	A	A	A	B	H	A	H	B	H	H	H	H	B
UBC-848	L24	H	H	A	B	H	A	H	H	A	A	H	H	H	B	H	H	B	H	H	H	A	H	H	H	A	H	A	A	B	H	H	A	B	A	A	A	H	H	H
UBC-890	L25	H	H	H	A	A	A	A	H	A	H	H	A	A	H	A	A	A	H	A	A	A	A	A	A	A	A	A	A	H	H	A	A	A	H	H	H	H	H	H
UBC-886	L26	H	H	H	H	H	H	A	H	H	A	H	H	A	H	H	H	A	H	H	H	A	H	H	H	H	H	H	A	H	H	H	H	B	H	H	H	A	H	B
ISSR-5	L27	H	H	A	H	H	H	H	H	H	H	H	B	A	A	H	H	H	H	H	H	H	H	H	H	A	A	A	H	H	H	H	H	A	H	A	A	H	H	H
ISSR17898A	L28	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	H	H	A	H	H	B	H	A	A
ISSR17898B	L29	B	H	H	H	H	H	H	H	A	H	B	H	H	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H
IISRS-3-E	L30	H	H	A	H	H	H	H	H	H	H	B	H	H	A	A	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	A	B	H	H	B	H	H
IISRS-3-G	L31	H	H	A	A	B	B	B	H	H	H	A	A	B	B	H	H	H	H	H	H	H	B	B	B	B	H	H	H	B	B	B	B	B	B	H	H	B	B	H

Table 22. Cont…..

Primer Name	Primer Code	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
Primer1	L1	H	B	H	H	H	B	A	B	A	B	B	A	H	H	A	H	H	B	B	A	H	A	B	B	A	H	H	H	B	H	A	A	B	A	H	H	H	H	H	A
UBC-841	L2	H	A	A	H	H	H	H	H	A	H	H	H	H	A	H	B	B	H	H	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	A
UBC-808	L3	A	A	A	A	A	A	A	A	A	B	A	H	A	H	B	A	A	A	A	A	B	A	A	A	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A
ISSR-HB-12	L4	A	H	H	H	H	H	H	H	A	H	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H
ISSR-HB-15	L5	B	B	B	B	B	A	B	B	A	B	H	B	H	H	H	B	B	H	B	A	B	B	B	A	H	H	A	H	H	A	H	B	B	H	B	B	B	H	H	A
ISSR-HB-10	L6	B	A	A	B	A	B	B	A	B	H	A	B	A	H	H	H	B	H	H	H	A	B	B	B	A	H	B	H	H	A	B	H	H	H	H	B	B	H	H	A
ISSR-HB-11	L7	A	A	A	H	A	A	A	B	A	A	A	H	A	A	A	A	A	H	B	H	A	B	A	A	A	A	A	H	H	H	B	H	B	B	B	A	A	A	A	A
UBC-840	L8	H	A	H	A	A	B	H	H	A	H	A	A	B	H	H	A	H	B	H	H	A	A	A	A	H	A	H	B	H	H	H	H	A	A	A	B	A	H	H	H
IISRS-3-M	L9	H	H	B	H	H	H	A	H	A	H	H	B	B	H	H	H	H	A	H	H	H	H	H	H	H	H	A	B	H	H	H	H	B	B	H	B	B	B	A	A
IISRS-3-N	L10	H	A	H	A	A	B	H	H	A	H	A	A	B	H	H	A	H	B	H	H	A	A	A	A	H	A	H	B	H	H	H	H	A	A	A	B	A	H	H	H
ISSR-7	L11	A	H	A	B	H	H	B	H	A	H	H	H	H	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Primer2	L12	B	B	B	B	B	A	B	B	A	B	H	B	H	H	H	B	B	H	B	A	B	B	B	A	H	H	A	H	H	A	H	B	B	H	B	B	B	H	H	A
UBC-854	L13	H	B	H	H	H	B	A	B	A	B	B	A	H	H	A	H	H	B	B	A	H	A	B	B	A	H	H	H	B	H	A	A	B	A	H	H	H	H	H	A
UBC-855	L14	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H		
UBC-880	L15	H	H	B	B	H	B	H	H	H	A	H	A	B	H	H	A	A	A	A	A	H	H	A	H	A	H	A	H	H	B	H	H	H	B	H	A	A	H	A	H
UBC-894	L16	A	H	A	A	B	A	B	A	A	H	A	A	A	A	H	B	A	A	B	H	B	H	A	A	A	B	A	A	A	A	A	B	A	A	B	B	A	B	B	B
ISSR-4	L17	A	H	H	H	H	H	H	H	A	H	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H	
ISSR-6	L18	B	B	B	B	B	A	B	B	A	B	H	B	H	H	H	B	B	H	B	A	B	B	B	A	H	H	A	H	H	A	H	B	B	H	B	B	B	H	H	A
UBC-829	L19	H	H	H	H	H	H	H	H	H	B	B	B	B	B	B	H	H	H	H	A	A	A	H	H	H	H	H	H	H	A	H	A	A	A	A	H	A	H	H	B
IISRS-3-C	L20	A	B	A	A	H	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A
IISRS-3-F	L21	B	A	A	B	A	B	B	A	B	H	A	B	A	H	H	H	B	H	H	H	A	B	B	B	A	H	B	H	H	A	B	H	H	H	H	B	B	H	H	A
23.12.15 4	L22	B	H	H	H	A	H	H	A	H	H	A	A	A	A	A	A	A	A	A	A	H	A	A	H	H	A	A	H	H	A	H	H	H	H	B	A	A	A	A	H
UBC-850	L23	B	A	H	H	A	H	H	A	H	B	H	H	H	H	H	H	H	H	H	H	H	A	A	B	H	H	H	H	H	B	H	H	B	H	H	B	H	H	H	H
UBC-848	L24	A	H	A	H	A	H	H	A	A	A	H	H	H	H	H	H	A	A	A	A	B	A	H	H	A	A	A	H	H	H	H	H	H	H	H	A	A	H	H	A
UBC-890	L25	A	A	A	B	B	H	B	B	H	B	H	H	H	A	A	A	B	B	A	A	A	A	H	H	B	B	B	H	B	H	A	H	B	H	B	B	B	H	A	H
UBC-886	L26	H	H	H	H	H	B	H	H	H	A	H	H	H	H	H	H	H	H	B	H	B	H	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H	A	A	A	B
ISSR-5	L27	H	H	H	H	H	A	H	H	A	H	H	A	H	H	A	H	H	H	A	A	A	H	H	H	H	H	A	H	H	H	H	H	A	A	H	H	H	H	H	B
ISSR17898A	L28	A	A	A	H	A	A	A	B	A	A	A	H	A	A	A	A	A	H	B	H	A	B	A	A	A	A	A	H	H	H	B	H	B	B	B	A	A	A	A	A
ISSR17898B	L29	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	
IISRS-3-E	L30	H	H	A	A	H	A	B	H	H	H	H	A	H	H	H	H	H	H	H	H	A	H	H	A	H	H	A	A	A	B	A	A	H	A	B	H	H	H	H	H
IISRS-3-G	L31	H	H	B	H	H	H	A	H	A	H	H	B	B	H	H	H	H	A	H	H	H	H	H	H	H	H	A	B	H	H	H	H	B	B	H	B	B	B	A	A

Table 22. Cont…..

Primer Name	Primer Code	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Primer1	L1	H	B	A	H	H	H	H	A	B	A	H	B	A	H	A	A	A	A	A	A	A
UBC-841	L2	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
UBC-808	L3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
ISSR-HB-12	L4	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H
ISSR-HB-15	L5	B	A	H	H	H	B	H	H	H	B	B	H	A	A	A	A	A	H	H	A	H
ISSR-HB-10	L6	B	A	A	B	A	A	H	H	A	A	A	B	A	A	A	A	H	A	A	B	B
ISSR-HB-11	L7	A	A	A	A	B	H	B	A	A	A	A	A	A	A	H	A	A	A	A	A	A
UBC-840	L8	B	A	B	B	H	A	A	B	H	H	A	H	A	B	A	H	B	B	B	A	A
IISRS-3-M	L9	A	A	A	A	H	H	H	H	H	B	H	H	H	H	A	H	A	H	A	H	A
IISRS-3-N	L10	B	A	B	B	H	A	A	B	H	H	A	H	A	B	A	H	B	B	B	A	A
ISSR-7	L11	H	H	H	H	H	H	A	H	H	H	H	H	A	H	H	H	B	H	H	H	B
Primer2	L12	B	A	H	H	H	B	H	H	H	B	B	H	A	A	A	A	A	H	H	A	H
UBC-854	L13	H	B	A	H	H	H	H	A	B	A	H	B	A	H	A	A	A	A	A	A	A
UBC-855	L14	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
UBC-880	L15	H	H	H	B	B	B	B	A	A	H	B	H	B	A	B	B	A	A	A	A	A
UBC-894	L16	B	B	B	B	B	A	A	A	A	H	A	B	B	B	A	A	A	A	A	A	B
ISSR-4	L17	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H
ISSR-6	L18	B	A	H	H	H	B	H	H	H	B	B	H	A	A	A	A	A	H	H	A	H
UBC-829	L19	H	A	A	A	A	A	A	A	B	H	H	H	H	A	B	A	A	B	B	B	B
IISRS-3-C	L20	A	A	A	B	A	A	A	A	A	A	A	A	B	A	B	A	H	A	H	H	H
IISRS-3-F	L21	B	A	A	B	A	A	H	H	A	A	A	B	A	A	A	A	H	A	A	B	B
23.12.15 4	L22	H	A	H	H	A	A	H	A	H	H	H	A	H	H	H	H	B	A	A	A	H
UBC-850	L23	H	A	H	H	H	H	H	H	B	B	H	H	H	H	H	H	H	A	H	H	H
UBC-848	L24	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	B
UBC-890	L25	H	H	H	H	H	A	H	H	H	A	H	H	H	H	H	H	H	H	H	H	A
UBC-886	L26	B	H	H	B	H	H	H	H	H	H	A	A	A	H	A	H	H	H	H	H	H
ISSR-5	L27	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A
ISSR17898A	L28	A	A	A	A	B	H	B	A	A	A	A	A	A	A	H	A	A	A	A	A	A
ISSR17898B	L29	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
IISRS-3-E	L30	H	B	H	A	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H
IISRS-3-G	L31	A	A	A	A	H	H	H	H	H	B	H	H	H	H	A	H	A	H	A	H	A

Table 22. Cont…..

Primer Name	Primer Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
IISRS-3-L	L32	H	H	H	H	H	B	H	H	H	H	H	H	H	H	A	A	B	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H	A	B	H	B	B	H	H
IISRS-3-D	L33	H	H	A	H	H	H	H	H	H	H	H	B	A	A	H	H	H	H	H	H	H	H	H	H	A	A	A	H	H	H	H	H	A	H	A	A	H	H	H
IISRS-3-O	L34	H	H	H	A	A	A	A	H	A	H	H	A	A	H	A	A	A	H	A	A	A	A	A	A	A	A	A	H	H	A	A	A	H	H	H	H	H	H	H
ISSR-3	L35	H	A	A	A	A	B	H	H	A	A	H	H	H	A	A	B	B	A	A	A	H	A	A	A	H	A	H	A	A	H	A	H	H	A	A	H	B	H	A
UBC-876	L36	H	H	H	H	H	H	A	H	H	A	H	H	A	H	H	H	A	H	H	H	A	H	H	H	H	H	H	A	H	H	H	H	B	H	H	H	A	H	B
Contig 143	L37	A	A	A	A	A	A	A	A	A	A	A	A	A	H	B	A	B	A	A	A	A	A	A	A	A	A	B	A	B	B	A	A	A	B	A	B	A	B	H
Contig 162	L38	A	A	B	H	B	A	H	A	A	A	H	B	B	H	B	H	B	A	H	H	H	A	A	A	A	A	B	A	H	B	A	A	H	B	B	B	A	B	B
Contig 265	L39	H	A	B	A	H	A	A	H	A	A	H	H	H	B	B	A	H	H	H	A	H	A	A	H	A	A	B	A	H	H	A	A	A	H	H	H	A	H	B
Contig 340	L40	H	H	A	H	A	H	H	H	H	H	A	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	B	B
Contig 352	L41	H	H	A	A	H	A	A	A	A	A	H	A	B	A	H	A	H	A	H	A	A	A	A	A	A	A	A	A	A	H	A	A	A	H	A	H	H	H	H
gi|116644211|	L42	A	A	H	A	H	A	A	H	A	A	B	A	H	H	H	A	H	A	H	A	A	A	A	H	A	A	A	A	A	H	H	H	H	B	H	H	A	H	H
gi|4387244|	L43	A	A	A	A	A	A	A	A	A	A	A	A	A	H	B	A	B	A	A	A	A	A	A	A	A	A	B	A	B	B	A	A	A	B	A	B	A	B	H
gi|4386975|	L44	A	A	B	H	B	A	H	A	A	A	H	B	B	H	B	H	B	A	H	H	H	A	A	A	A	A	B	A	H	B	A	A	H	B	B	B	A	B	B
gi|4386907|	L45	H	A	B	A	H	A	A	A	A	A	B	A	A	H	H	A	B	H	H	H	H	A	A	A	A	A	H	A	A	B	A	A	B	A	A	A	A	H	H
gi|4386813|	L46	H	A	H	A	H	A	A	A	A	A	A	A	A	A	H	A	H	A	H	A	A	A	A	A	A	A	H	A	A	H	A	A	H	A	H	H	A	H	H
gi|4386589|	L47	H	A	B	A	H	A	A	H	A	A	H	H	H	B	B	A	H	H	H	A	H	A	A	H	A	A	B	A	H	H	A	A	A	H	H	H	A	H	B
gi|4386543|	L48	H	H	A	H	A	H	H	H	H	H	A	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	B	B
gi|4386388|	L49	H	H	A	A	H	A	A	A	A	A	H	A	B	A	H	A	H	A	H	A	A	A	A	A	A	A	A	A	A	H	A	A	A	H	A	H	H	H	H
gi|4386508|	L50	A	A	B	A	B	A	A	A	A	A	B	A	A	A	A	A	B	A	B	A	A	A	A	A	A	A	B	A	A	B	A	A	B	A	B	A	A	A	A
gi|4386229|	L51	H	H	H	A	H	H	B	H	A	H	H	H	H	B	H	H	H	A	A	H	H	H	H	H	H	H	B	A	B	B	H	H	B	H	H	A	H	H	H
gi|76572221|	L52	H	H	H	H	H	A	H	B	A	H	A	H	H	H	H	H	A	H	H	H	H	A	H	H	H	A	A	H	H	A	A	H	H	H	H	H	A	H	H
G5	L53	H	H	H	H	H	H	A	A	B	H	A	H	H	H	H	H	H	H	H	H	H	B	B	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H
G17	L54	H	H	A	H	A	A	A	A	A	A	H	H	A	B	H	B	H	A	A	A	B	A	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A	H	A
G3	L55	H	H	H	H	A	A	A	A	A	A	H	B	A	A	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	H	A	H	A	H	A
G21	L56	H	A	H	A	H	A	A	H	A	H	H	H	H	H	H	H	A	H	A	A	A	H	B	A	A	A	H	H	A	A	H	H	H	H	B	A	A	H	A
G12	L57	H	H	H	H	H	H	A	A	B	H	A	H	H	H	H	H	H	H	H	H	H	B	B	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H
G7	L58	H	H	H	H	A	B	B	H	H	H	H	H	A	A	H	A	B	H	B	H	B	H	H	H	H	H	A	H	H	H	H	H	H	H	H	B	B	H	B
G22	L59	H	H	H	H	H	A	A	H	H	H	H	B	H	H	B	H	H	A	H	H	A	H	H	H	H	H	H	B	H	H	A	H	H	H	H	H	A	H	A
G30	L60	A	H	B	H	H	H	H	B	H	A	H	H	H	A	H	H	H	A	A	A	A	A	A	H	A	A	A	A	B	A	A	H	A	H	A	A	A	H	A
G36	L61	H	H	A	H	A	A	A	A	A	A	H	H	A	B	H	B	H	A	A	A	B	A	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A	H	A
G37	L62	H	H	H	H	A	A	A	A	A	A	H	B	A	A	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	H	A	H	A	H	A

Table 22. Cont…..

Primer Name	Primer Code	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
IISRS-3-L	L32	A	H	A	B	H	H	B	H	A	H	H	H	H	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
IISRS-3-D	L33	H	H	H	H	H	A	H	H	A	H	H	A	H	H	A	H	H	H	A	A	A	H	H	H	H	H	A	H	H	H	H	H	A	A	H	H	H	H	H	B
IISRS-3-O	L34	A	A	A	B	B	H	B	B	H	B	H	H	H	A	A	A	B	B	A	A	A	A	H	H	B	B	B	H	B	H	A	H	B	H	B	B	B	H	A	H
ISSR-3	L35	A	A	A	A	A	H	A	A	A	A	H	H	A	B	B	A	B	B	H	A	H	A	B	A	A	B	H	H	A	B	A	A	A	H	A	A	H	B	H	B
UBC-876	L36	H	H	H	H	H	B	H	H	H	A	H	H	H	H	H	H	H	B	H	B	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H	A	A	A	B
Contig 143	L37	B	H	B	B	A	H	A	A	A	A	A	B	A	B	A	A	B	A	H	A	B	A	A	B	B	B	B	B	B	H	B	B	B	B	B	A	A	A	A	B
Contig 162	L38	B	B	B	B	A	B	B	A	B	H	A	H	B	B	B	A	A	A	B	B	B	A	A	B	B	H	B	B	H	H	B	A	B	B	B	A	A	B	A	H
Contig 265	L39	H	A	H	H	A	H	H	B	H	A	H	H	A	B	B	A	B	A	H	H	H	A	A	H	H	B	B	B	B	A	B	A	B	A	B	A	A	B	A	H
Contig 340	L40	H	B	B	H	H	B	H	H	B	H	H	H	H	B	H	H	B	H	A	A	H	H	H	H	A	H	B	B	H	H	A	H	H	H	H	H	H	H	H	A
Contig 352	L41	H	A	A	H	A	A	A	A	H	A	A	A	A	H	H	A	H	A	H	H	H	A	A	H	H	A	H	H	H	A	H	H	H	H	A	A	A	A	A	H
gi|116644211|	L42	H	H	H	B	A	A	A	H	H	A	H	A	A	H	H	A	B	A	B	H	H	A	A	H	H	H	B	H	H	H	H	B	H	B	A	A	H	B	A	B
gi|4387244|	L43	B	H	B	B	A	H	A	A	A	A	A	B	A	B	A	A	B	A	H	A	B	A	A	B	B	B	B	B	B	H	B	B	B	B	B	A	A	A	A	B
gi|4386975|	L44	B	B	B	B	A	B	B	A	B	H	A	H	B	B	B	A	A	A	B	B	B	A	A	B	B	H	B	B	H	H	B	A	B	B	B	A	A	B	A	H
gi|4386907|	L45	A	H	H	B	A	H	A	A	H	A	A	B	A	H	B	H	H	H	H	H	H	H	H	H	H	A	A	B	H	A	A	A	H	A	A	A	A	A	A	H
gi|4386813|	L46	A	H	H	A	A	H	A	A	H	A	A	H	A	H	H	H	H	A	H	H	H	H	H	H	H	A	A	H	A	A	H	H	A	H	A	A	A	A	H	A
gi|4386589|	L47	H	A	H	H	A	H	H	B	H	A	H	H	A	B	B	A	B	A	H	H	H	A	A	H	H	B	B	B	B	A	B	A	B	A	B	A	A	B	A	H
gi|4386543|	L48	H	B	B	H	H	B	H	H	B	H	H	H	H	B	H	H	B	H	A	A	H	H	H	H	A	H	B	B	H	H	A	H	H	H	H	H	H	H	H	A
gi|4386388|	L49	H	A	A	H	A	A	A	A	H	A	A	A	A	H	H	A	H	A	H	H	H	A	A	H	H	A	H	H	H	A	H	H	H	H	A	A	A	A	A	H
gi|4386508|	L50	B	H	B	B	A	B	A	A	H	A	A	B	A	B	B	H	A	B	B	B	B	A	A	B	A	A	A	B	B	A	A	A	A	B	B	A	A	A	A	A
gi|4386229|	L51	B	H	A	A	A	H	H	H	H	H	A	H	H	H	H	H	A	H	A	H	B	B	B	B	H	B	H	H	H	B	H	H	H	H	H	H	H	H	H	B
gi|76572221|	L52	H	A	H	H	H	H	H	A	A	H	A	A	H	H	A	H	H	H	H	H	H	A	H	H	A	H	A	B	A	A	H	A	A	A	A	A	A	H	A	H
G5	L53	H	H	H	H	H	A	H	B	H	H	B	B	H	H	H	H	H	H	H	H	H	H	B	H	H	B	H	H	H	B	H	H	H	H	H	H	H	H	H	H
G17	L54	B	H	A	B	B	A	A	A	A	A	A	A	B	A	A	A	A	B	B	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
G3	L55	A	H	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	H	B	A	H	A	A	A	A	A	H	A	A	A	A	A	H	A	A	A	A	A	A	A
G21	L56	H	H	H	H	H	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A	H
G12	L57	H	H	H	H	H	A	H	B	H	H	B	B	H	H	H	H	H	H	H	H	H	H	B	H	H	B	H	H	H	B	H	H	H	H	H	H	H	H	H	H
G7	L58	B	A	B	B	B	A	B	B	B	A	A	H	B	H	H	A	H	H	H	H	H	H	H	H	H	A	A	A	H	H	B	B	B	B	B	B	B	B	B	A
G22	L59	B	H	H	H	A	H	H	H	B	H	H	H	H	H	H	H	H	H	H	H	B	H	A	H	H	B	H	B	H	A	H	H	H	H	B	H	H	H	H	A
G30	L60	A	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	B	A	H	H	H	H	A	H	H	H	H	H	H	H	H	H	A	A	A	A	A
G36	L61	B	H	A	B	B	A	A	A	A	A	A	A	B	A	A	A	A	B	B	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
G37	L62	A	H	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	H	B	A	H	A	A	A	A	A	H	A	A	A	A	A	H	A	A	A	A	A	A	A

Table 22. Cont…..

Primer Name	Primer Code	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
IISRS-3-L	L32	H	H	H	H	H	H	A	H	H	H	H	H	A	H	H	H	B	H	H	H	B
IISRS-3-D	L33	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A
IISRS-3-O	L34	H	H	H	H	H	A	H	H	H	A	H	H	H	H	H	H	H	H	H	H	A
ISSR-3	L35	H	H	H	H	H	H	B	H	H	H	H	A	H	H	A	H	H	H	H	H	H
UBC-876	L36	B	H	H	B	H	H	H	H	H	H	A	A	A	H	A	H	H	H	H	H	H
Contig 143	L37	A	A	A	A	A	A	H	H	A	B	B	A	B	B	A	A	A	A	A	A	A
Contig 162	L38	B	B	H	B	H	A	B	A	H	H	H	H	H	H	B	B	A	A	A	H	B
Contig 265	L39	H	H	A	A	A	H	H	A	A	A	H	H	H	H	H	H	A	A	A	A	A
Contig 340	L40	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H	H	H
Contig 352	L41	H	A	A	H	A	A	H	A	H	H	A	A	H	H	H	H	A	A	A	A	H
gi|116644211|	L42	B	A	A	B	A	A	B	H	B	A	B	A	H	H	H	B	B	A	A	H	B
gi|4387244|	L43	A	A	A	A	A	A	H	H	A	B	B	A	B	B	A	A	A	A	A	A	A
gi|4386975|	L44	B	B	H	B	H	A	B	A	H	H	H	H	H	H	B	B	A	A	A	H	B
gi|4386907|	L45	A	A	A	A	A	A	A	H	H	H	A	A	B	H	A	A	A	A	A	H	A
gi|4386813|	L46	H	A	A	A	A	A	H	H	H	A	H	A	A	H	H	H	A	A	A	H	A
gi|4386589|	L47	H	H	A	A	A	H	H	A	A	A	H	H	H	H	H	H	A	A	A	A	A
gi|4386543|	L48	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H	H	H
gi|4386388|	L49	H	A	A	H	A	A	H	A	H	H	A	A	H	H	H	H	A	A	A	A	H
gi|4386508|	L50	B	A	A	B	A	A	B	A	A	A	A	A	H	A	A	A	A	A	A	A	B
gi|4386229|	L51	H	H	H	H	H	H	H	H	B	H	B	B	B	B	A	H	A	H	H	A	H
gi|76572221|	L52	A	A	H	A	A	H	A	A	H	A	A	A	A	H	A	A	A	H	A	H	A
G5	L53	A	H	H	H	H	H	H	A	A	A	A	B	A	H	H	H	H	A	H	H	H
G17	L54	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A
G3	L55	A	A	A	A	A	H	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A
G21	L56	H	A	A	A	H	A	A	A	A	A	A	A	A	A	B	A	H	H	H	H	A
G12	L57	A	H	H	H	H	H	H	A	A	A	A	B	A	H	H	H	H	A	H	H	H
G7	L58	H	B	H	B	B	B	H	A	A	H	B	B	A	B	H	H	B	H	H	H	A
G22	L59	H	A	H	B	A	H	H	A	A	B	H	A	B	A	H	H	H	A	H	H	H
G30	L60	A	A	H	A	H	A	H	A	H	A	A	A	H	H	H	H	H	A	H	H	A
G36	L61	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A
G37	L62	A	A	A	A	A	H	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A

Table 22. Cont…..

Primer Name	Primer Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
G38	L63	H	A	H	A	H	A	A	H	A	H	H	H	H	H	H	H	A	H	A	A	A	H	B	A	A	A	H	H	A	A	H	H	H	H	B	A	A	H	A
G53	L64	H	H	H	H	A	B	B	H	H	H	H	H	A	A	H	A	B	H	B	H	B	H	H	H	H	H	A	H	H	H	H	H	H	H	H	B	B	H	B
G54	L65	A	A	B	H	B	A	H	H	H	A	B	B	B	B	B	H	B	H	A	A	A	A	H	A	A	A	A	A	A	H	H	H	H	B	H	A	H	B	H
G55	L66	H	H	H	H	H	A	A	H	H	H	H	B	H	H	B	H	H	A	H	H	A	H	H	H	H	H	H	B	H	H	A	H	H	H	H	H	A	H	A
G56	L67	A	H	B	H	H	H	H	B	H	A	H	H	H	A	H	H	H	A	A	A	A	A	A	H	A	A	A	A	B	A	A	H	A	H	A	A	A	H	A
G57	L68	A	A	B	H	B	A	H	H	H	A	B	B	B	B	B	H	B	H	A	A	A	A	H	A	A	A	A	A	A	H	H	H	H	B	H	A	H	B	H
G58	L69	H	H	A	A	A	B	B	B	B	B	A	H	B	H	A	B	A	B	H	H	H	H	B	H	H	H	H	H	B	B	B	B	B	A	B	B	B	B	B
G59	L70	A	A	A	H	A	H	H	H	H	H	B	A	H	H	A	H	H	H	H	H	A	A	B	H	H	A	A	B	H	H	A	A	H	B	H	A	H	B	H
G60	L71	H	H	A	H	A	A	H	H	H	H	H	H	H	A	H	H	H	H	A	H	A	A	H	H	B	H	H	H	B	H	H	A	H	H	H	H	H	H	H
G61	L72	H	H	A	A	A	B	B	B	B	B	A	H	B	H	A	B	A	B	H	H	H	H	B	H	H	H	H	H	B	B	B	B	B	A	B	B	B	B	B
G65	L73	H	H	H	B	B	H	H	A	B	A	H	H	A	A	A	A	H	B	B	B	A	B	A	A	A	A	A	H	A	A	A	A	A	H	A	A	A	A	A
G66	L74	A	A	A	H	A	H	H	H	H	H	B	A	H	H	A	H	H	H	H	H	A	A	B	H	H	A	A	B	H	H	A	A	H	B	H	A	H	B	H
G32	L75	H	H	H	H	A	H	B	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
G33	L76	A	A	A	A	A	H	A	A	A	A	A	B	A	B	A	A	A	A	H	A	A	A	A	A	A	H	A	A	A	H	A	A	A	A	B	H	B	B	A
G69	L77	H	H	H	B	B	H	H	A	B	A	H	H	A	A	A	A	H	B	B	B	A	B	A	A	A	A	A	H	A	A	A	A	A	H	A	A	A	A	A
G74	L78	H	H	H	H	A	H	B	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
G75	L79	H	A	H	A	H	A	A	A	A	A	B	A	B	A	A	A	A	H	H	H	H	A	A	A	A	A	A	A	B	H	A	A	A	A	A	B	H	A	A
G76	L80	H	A	H	H	A	A	A	H	A	A	B	A	H	H	A	A	A	B	B	A	A	B	A	A	A	A	H	A	B	A	A	A	B	A	A	H	B	H	A
G77	L81	H	A	H	A	H	A	A	A	A	A	B	A	B	A	A	A	A	H	H	H	H	A	A	A	A	A	A	A	B	H	A	A	A	A	A	B	H	A	A
G78	L82	H	B	A	H	A	B	A	H	A	H	H	H	B	H	H	H	H	A	H	B	H	H	H	A	H	H	A	H	A	B	H	A	H	A	A	H	A	H	H
G80	L83	H	A	H	H	A	A	A	H	A	A	B	A	H	H	A	A	A	B	B	A	A	B	A	A	A	A	H	A	B	A	A	A	B	A	A	H	B	H	A
G81	L84	H	H	A	H	A	A	H	H	H	H	H	H	H	A	H	H	H	H	A	H	A	A	H	H	B	H	H	H	B	H	H	A	H	H	H	H	H	H	H
G83	L85	H	H	A	H	A	A	H	H	H	H	H	H	H	A	H	H	H	H	A	H	A	A	H	H	B	H	H	H	B	H	H	A	H	H	H	H	H	H	H
G84	L86	A	A	B	B	H	H	H	H	H	H	H	H	A	H	H	A	H	B	H	B	H	H	B	A	B	B	B	B	H	B	B	H	H	B	H	B	H	H	H
G85	L87	B	A	B	B	A	A	A	A	B	A	H	A	A	A	A	H	A	B	B	A	B	H	A	H	H	B	H	A	H	B	B	B	B	A	A	A	A	B	A
G86	L88	A	A	B	B	H	H	H	H	H	H	H	H	A	H	H	A	H	B	H	B	H	H	B	A	B	B	B	B	H	B	B	H	H	B	H	B	H	H	H
G87	L89	H	H	H	H	H	H	H	H	B	H	A	H	H	B	H	H	H	H	A	H	H	H	H	H	H	H	H	H	B	H	H	H	B	H	H	H	H	H	H
G88	L90	B	A	B	B	A	A	A	A	B	A	H	A	A	A	A	H	A	B	B	A	B	H	A	H	H	B	H	A	H	B	B	B	B	A	A	A	A	B	A
G71	L91	H	B	H	H	A	H	H	H	H	H	H	A	H	H	H	A	H	H	H	A	H	H	A	A	A	A	A	A	H	A	A	B	A	A	H	A	A	A	B
G73	L92	H	H	H	H	A	B	H	H	H	A	H	H	A	H	B	H	H	A	A	B	H	H	H	B	A	H	A	A	A	B	H	H	A	A	A	A	A	A	A
G89	L93	H	H	A	A	H	A	H	A	A	A	H	B	B	A	H	A	H	H	H	A	H	B	B	A	H	A	A	A	B	H	A	A	A	A	H	H	B	A	H

Table 22. Cont…..

Primer Name	Primer Code	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
G38	L63	H	H	H	H	H	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A	A	A	A	A	A	A	A	A	A	A	H
G53	L64	B	A	B	B	B	A	B	B	B	A	A	H	B	H	H	A	H	H	H	H	H	H	H	H	H	A	A	A	H	H	B	B	B	B	B	B	B	B	A	A
G54	L65	H	B	A	H	H	H	H	H	H	B	A	H	H	A	A	H	H	H	H	H	H	A	H	B	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	A
G55	L66	B	H	H	H	A	H	H	H	B	H	H	H	H	H	H	H	H	H	H	H	B	H	A	H	H	B	H	B	H	A	H	H	H	H	B	H	H	H	H	A
G56	L67	A	H	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	B	A	H	H	H	H	A	H	H	H	H	H	H	H	H	H	A	A	A	A	A
G57	L68	H	B	A	H	H	H	H	H	H	B	A	H	H	A	A	H	H	H	H	H	H	A	H	B	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	A
G58	L69	H	A	H	H	H	H	H	H	H	H	A	H	H	H	H	H	B	H	H	H	A	A	B	H	B	H	H	B	B	H	H	H	A	B	H	H	H	H	H	H
G59	L70	H	B	A	H	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	A	B	H	H	H	B	H	H	A	H	A	H	A	A	A	A	A	A	A	A	A
G60	L71	H	H	H	A	A	A	H	B	H	A	A	A	B	A	B	H	B	B	A	H	H	B	H	H	H	H	H	H	H	H	H	H	H	B	A	H	B	A	A	B
G61	L72	H	A	H	H	H	H	H	H	H	H	A	H	H	H	H	H	B	H	H	H	A	A	B	H	B	H	H	B	B	H	H	H	A	B	H	H	H	H	H	H
G65	L73	A	H	A	B	A	A	A	A	A	A	H	A	H	A	H	A	A	A	A	B	H	A	A	A	A	A	A	H	H	A	A	A	A	A	A	H	A	H	H	A
G66	L74	H	B	A	H	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	A	B	H	H	H	B	H	H	A	H	A	H	A	A	A	A	A	A	A	A	A
G32	L75	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H
G33	L76	H	A	B	A	A	H	A	A	A	A	B	A	A	A	A	A	A	A	B	B	H	A	A	A	A	A	A	H	H	A	A	A	A	A	H	A	H	H	H	A
G69	L77	A	H	A	B	A	A	A	A	A	A	H	A	H	A	H	A	A	A	A	B	H	A	A	A	A	A	A	H	H	A	A	A	A	A	A	H	A	H	H	A
G74	L78	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H
G75	L79	A	A	H	A	A	B	B	A	B	A	A	A	H	B	B	A	A	A	H	B	B	A	A	A	A	B	B	H	B	A	A	B	B	H	H	A	A	B	A	H
G76	L80	A	H	H	A	A	H	H	A	H	B	A	A	H	A	H	A	H	B	B	H	A	A	A	A	A	B	B	H	H	A	A	A	A	B	H	A	A	H	A	B
G77	L81	A	A	H	A	A	B	B	A	B	A	A	A	H	B	B	A	A	A	H	B	B	A	A	A	A	B	B	H	B	A	A	B	B	H	H	A	A	B	A	H
G78	L82	H	H	H	H	A	A	A	H	H	A	A	A	H	A	A	A	A	H	H	H	H	A	H	H	A	B	A	H	H	H	A	H	A	H	H	A	A	A	A	A
G80	L83	A	H	H	A	A	H	H	A	H	B	A	A	H	A	H	A	H	B	B	H	A	A	A	A	A	B	B	H	H	A	A	A	A	B	H	A	A	H	A	B
G81	L84	H	H	H	A	A	A	H	B	H	A	A	A	B	A	B	H	B	B	A	H	H	B	H	H	H	H	H	H	H	H	H	H	H	B	A	H	B	A	A	B
G83	L85	H	H	H	A	A	A	H	B	H	A	A	A	B	A	B	H	B	B	A	H	H	B	H	H	H	H	H	H	H	H	H	H	H	B	A	H	B	A	A	B
G84	L86	H	H	H	H	H	H	H	H	H	H	A	H	A	A	A	A	A	H	A	H	A	H	B	B	H	H	B	H	A	A	A	A	A	A	A	A	A	A	A	H
G85	L87	A	A	A	B	A	B	A	A	A	B	B	A	A	B	H	A	A	H	B	A	B	B	H	B	H	B	H	H	H	H	H	H	B	A	B	A	B	B	H	H
G86	L88	H	H	H	H	H	H	H	H	H	H	A	H	A	A	A	A	A	H	A	H	A	H	B	B	H	H	B	H	A	A	A	A	A	A	A	A	A	A	A	H
G87	L89	H	H	H	H	H	H	H	H	B	H	B	H	H	H	H	H	H	B	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H	H	B	H	H	B	H
G88	L90	A	A	A	B	A	B	A	A	A	B	B	A	A	B	H	A	A	H	B	A	B	B	H	B	H	B	H	H	H	H	H	H	B	A	B	A	B	B	H	H
G71	L91	H	A	H	A	A	A	H	H	H	H	B	H	B	H	B	B	B	B	H	A	H	H	B	B	B	A	B	H	B	H	H	H	H	H	H	H	H	H	H	B
G73	L92	H	A	A	A	H	A	A	A	A	A	H	B	H	B	A	H	H	A	A	H	H	A	A	H	H	H	H	H	H	A	B	H	H	H	A	H	B	H	H	A
G89	L93	A	H	B	A	H	H	H	H	H	H	A	A	H	H	H	A	A	H	H	H	H	H	H	H	H	B	H	H	B	H	H	B	B	A	A	A	H	H	H	B

Table 22. Cont…..

Primer Name	Primer Code	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
G38	L63	H	A	A	A	H	A	A	A	A	A	A	A	A	A	B	A	H	H	H	H	A
G53	L64	H	B	H	B	B	B	H	A	A	H	B	B	A	B	H	H	B	H	H	H	A
G54	L65	A	A	A	A	H	H	H	A	B	B	H	H	H	A	H	H	H	H	A	B	A
G55	L66	H	A	H	B	A	H	H	A	A	B	H	A	B	A	H	H	H	A	H	H	H
G56	L67	A	A	H	A	H	A	H	A	H	A	A	A	H	H	H	H	H	A	H	H	A
G57	L68	A	A	A	A	H	H	H	A	B	B	H	H	H	A	H	H	H	H	A	B	A
G58	L69	H	H	H	H	H	H	B	B	A	H	H	H	H	H	H	H	A	H	H	A	H
G59	L70	A	A	A	A	H	A	A	A	A	B	A	A	A	H	A	A	A	A	A	A	A
G60	L71	H	B	B	H	A	H	H	A	A	A	H	H	H	B	B	H	A	A	A	B	B
G61	L72	H	H	H	H	H	H	B	B	A	H	H	H	H	H	H	H	A	H	H	A	H
G65	L73	A	H	H	B	B	B	B	B	A	A	H	A	A	H	H	A	A	H	A	H	H
G66	L74	A	A	A	A	H	A	A	A	A	B	A	A	A	H	A	A	A	A	A	A	A
G32	L75	B	H	H	H	B	H	H	H	H	H	H	H	B	H	H	H	H	H	H	A	A
G33	L76	H	H	H	H	H	H	H	B	A	A	H	H	A	A	H	H	A	B	H	A	H
G69	L77	A	H	H	B	B	B	B	B	A	A	H	A	A	H	H	A	A	H	A	H	H
G74	L78	B	H	H	H	B	H	H	H	H	H	H	H	B	H	H	H	H	H	H	A	A
G75	L79	H	B	H	B	A	A	A	B	B	A	B	A	B	A	H	A	H	A	A	B	H
G76	L80	H	H	H	H	H	A	A	H	A	A	H	B	H	A	H	A	H	H	H	A	A
G77	L81	H	B	H	B	A	A	A	B	B	A	B	A	B	A	H	A	H	A	A	B	H
G78	L82	A	A	H	H	H	H	A	A	A	H	H	H	H	H	B	A	A	A	B	H	A
G80	L83	H	H	H	H	H	A	A	H	A	A	H	B	H	A	H	A	H	H	H	A	A
G81	L84	H	B	B	H	A	H	H	A	A	A	H	H	H	B	B	H	A	A	A	B	B
G83	L85	H	B	B	H	A	H	H	A	A	A	H	H	H	B	B	H	A	A	A	B	B
G84	L86	H	H	H	H	B	H	H	A	H	H	A	A	B	A	H	H	H	A	A	B	B
G85	L87	B	H	H	H	H	A	A	A	A	A	A	A	H	H	A	H	A	A	A	A	A
G86	L88	H	H	H	H	B	H	H	A	H	H	A	A	B	A	H	H	H	A	A	B	B
G87	L89	H	H	H	H	H	H	H	H	H	H	H	H	H	B	H	H	H	H	H	B	H
G88	L90	B	H	H	H	H	A	A	A	A	A	A	A	H	H	A	H	A	A	A	A	A
G71	L91	B	H	H	H	H	H	H	H	A	A	B	A	A	A	H	B	B	B	B	B	B
G73	L92	A	A	B	A	B	A	H	A	A	A	H	A	A	H	B	B	B	B	B	B	B
G89	L93	B	B	B	A	A	A	H	A	A	H	A	A	B	B	B	B	B	H	B	B	A

4.3.5 Software analysis to identify markers/ QTL for resistance to buckeye rot

The genotyping data of 100 F_2 individuals generated by 93 primers was given in Table 22. This data was then entered in computer based programme MAPMAKER/EXP version 3.0b to generate linkage map. The linkage between markers was calculated using logarithm of odds ratio (i.e. the ratio of linkage versus no linkage), also known as LOD value or LOD score. LOD value of 2 was used to group the linked primers into linkage groups. Kosambi map function was used to generate distance between the primers (Kosambi, 1944) in order to convert recombination values to genetic distances.

In total 12 linkage groups were obtained (Fig. 8). Two markers each were placed on linkage groups LG1, LG4, LG5, LG8, LG9, LG11 and LG12. On linkage group LG2 57 markers were grouped. Each of the linkage groups LG3, LG7 and LG10 spanned three markers. Whereas linkage group LG6 was constituted of four markers. LG2 was the largest group spanning a distance of 4584.9 cM followed by LG6 which covered a distance of 39.3 cM (Table 23). All markers covered total genetic map distance of 4660.7 cM. The average distance between adjacent markers in the linkage map was observed to be 50.11 cM.

Table 23: Summary of markers assigned to different linkage groups of tomato linkage map

Sr. No.	Marker Code	Marker Name	Linkage Group Name	Distance between markers (cM)
1	L2	UBCB-841	LG2	44.1
2	L3	UBC-808	LG2	111.6
3	L4	ISSR-HB-12	LG2	106.5
4	L7	ISSR-HB-11	LG2	72.4
5	L11	ISSR-7	LG2	18.3
6	L14	UBC-855	LG2	190
7	L15	UBC-880	LG2	190
8	L17	ISSR-4	LG2	112.2
9	L20	IISRS-3-C	LG2	31.3
10	L22	IISRS-3-I	LG2	42.9
11	L24	UBC-848	LG2	190
12	L25	UBC-890	LG2	68.5
13	L26	UBC-886	LG2	39.5
14	L27	ISSR-5	LG2	79.8
15	L28	ISSR17898A	LG2	190
16	L29	ISSR17898B	LG2	17.4
17	L30	IISRS-3-E	LG2	23.2
18	L32	IISRS-3-L	LG2	30.6
19	L33	IISRS-3-D	LG2	54
20	L34	IISRS-3-O	LG2	68.5
21	L36	UBC-876	LG2	83.6
22	L37	contig 143	LG2	41.3

Table 23. Cont…..

Sr. No.	Marker Code	Marker Name	Linkage Group Name	Distance between markers (cM)
23	L38	contig 162	LG2	37.5
24	L39	contig 265	LG2	110.5
25	L40	contig 340	LG2	190
26	L41	contig 352	LG2	25.5
27	L42	gi|116644211|	LG2	40.6
28	L43	gi|4387244|	LG2	41.3
29	L44	gi|4386975|	LG2	65.3
30	L45	gi|4386907|	LG2	18.3
31	L46	gi|4386813|	LG2	36
32	L47	gi|4386589|	LG2	110.4
33	L48	gi|4386543|	LG2	190.1
34	L49	gi|4386388|	LG2	37
35	L50	gi|4386508|	LG2	64.1
36	L52	gi|76572221|	LG2	190
37	L53	G5	LG2	190
38	L54	G17	LG2	16.9
39	L55	G3	LG2	27.8
40	L56	G21	LG2	111.5
41	L57	G12	LG2	190
42	L60	G30	LG2	36
43	L61	G36	LG2	16.9
44	L62	G37	LG2	27.8
45	L63	G38	LG2	33.6
46	L67	G56	LG2	46.4
47	L70	G59	LG2	64.8
48	L73	G65	LG2	64.8
49	L74	G66	LG2	142.8
50	L75	G32	LG2	99.3
51	L76	G33	LG2	36.9
52	L77	G69	LG2	190
53	L78	G74	LG2	190
54	L79	G75	LG2	34.7
55	L80	G76	LG2	34.7
56	L81	G77	LG2	67.7
57	L82	G78	LG2	0
	Total map length of LG2			**4584.9 cM**
58	L5	ISSR-HB-15	LG3	0
59	L12	Primer2	LG3	0.1
60	18	ISSR-6	LG3	0
	Total map length of LG3			**0.1 cM**
61	L9	IISRS-3-M	LG6	0
62	L31	IISRS-3-G	LG6	39.3
63	L69	G58	LG6	0
64	L72	G61	LG6	0
	Total map length of LG6			**39.3 cM**
65	L23	UBC-850	LG7	36.2
66	L65	G54	LG7	0.1
67	L68	G57	LG7	0
	Total map length of LG7			**36.3 cM**
68	L71	G60	LG10	0
69	L84	G81	LG10	0.1
70	L85	G83	LG10	0
	Total map length of LG10			**0.1 cM**
	Total			**4660.7 cM.**

The markers namely 'L1 and L13' on LG2, 'L6 and L21' on LG4, 'L8 and L10' on LG5, 'L58 and L64' on LG8, 'L59 and L66' on LG9, 'L86 and L88' on LG11, 'L87 and L90' on LG12 were not covered in Table 23 as the distance between two markers was 0 cM. Nine markers viz., L16, L19, L35, L51, L83, L89, L91, L92 and L93 were not assigned any linkage group and were considered unlinked. Deviation of observed frequency of alleles at a given locus from its expected Mendelian frequencies within a segregating population has been defined as segregation distortion. Segregation distortion was calculated for three types of markers used and was found to be 8.33% for ISSR markers, 12.19% for genomic SSR markers and 6.25% for EST-SSR markers (Table 24).

Table 24: Distribution of markers showing segregation distortion

Marker Type	Markers polymorphic in parents (P)	Markers appeared on linkage groups	Unlinked markers (D)	% segregation distortion = $\frac{D}{P}$ X 100
ISSR	36	33	3	8.33%
Genomic SSR	41	36	5	12.19%
EST-SSR	16	15	1	6.25%
Total	93	84	9	

The genome coverage was also calculated for individual linkage group as well as for entire map length. The genome coverage was found to be 96.55% for LG2, 60% for LG6 and 0.5% each for LG3, LG7, LG10 and 97.18% for the total map length (Table 25).

Table 25: Genome coverage by the markers

	Map length	No. of Loci	Genome coverage
LG2	4584.9 cM	57	96.55%
LG3	0.1cM	3	0.5%
LG6	39.3 cM	4	60%
LG7	36.3 cM	3	0.5%
LG10	0.1cM	3	0.5%
Entire Map	4660.7 cM	70	97.18%

QTL identification

QTL cartographer software was used to perform QTL analysis for disease trait. Composite interval mapping (CIM) was used as statistical approach to calculate the presence of QTL for each position on the linkage map.

In total 49 peaks were obtained for putative QTL locations contributing to disease resistance (Fig. 9, 10, 11). 22 QTL were considered more significant because of their positive

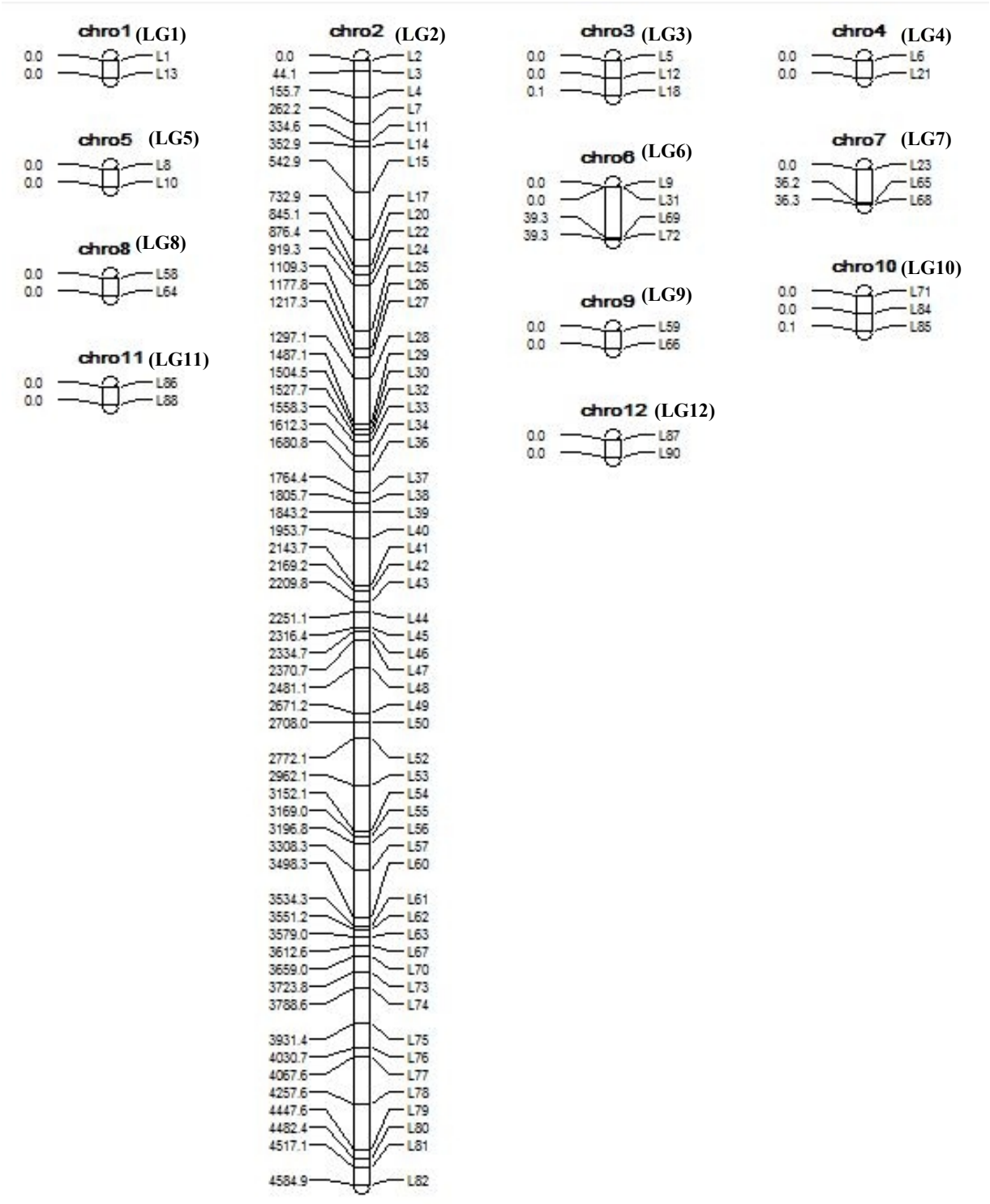


Fig. 8: Linkage groups of tomato on F_2 population
Loci names are listed on the right of the linkage groups and map distances between markers in centiMorgan (cM) is mentioned on the left of the linkage groups

effect (lower curve in second part of Fig. 9, 10,11 indicates which allele has positive effect), of which 20 were identified for disease resistance on chromosome 2 lying between markers namely 'L4-L7', 'L11-L14', 'L14-L15', 'L15-L17', 'L24-L25', 'L25-L26', 'L28-L29', 'L34-L36', 'L36-L37', 'L39-L40', 'L40-L41', 'L47-L48', 'L48-L49', 'L52-L53', 'L53-54', 'L56-L57', 'L57-L60', 'L75-L76', 'L76-L77', 'L78-L79', while chromosome 6 and 7 contained single QTL each between markers 'L31-L69' and 'L23-L65', respectively (Table 26; Fig. 9, 10, 11, 12).

Table 26: QTL detected for buckeye rot resistance in tomato genome

QTL ID	Linkage Group	Flanking markers' code	LOD
A1	2	L4-L7	12
A2	2	L11-L14	20
A3	2	L14-L15	12.6
A4	2	L15-L17	12.6
A5	2	L24-L25	12
A6	2	L25-L26	12
A7	2	L28-L29	12
A8	2	L34-L36	12
A9	2	L36-L37	12
A10	2	L39-L40	15
A11	2	L40-L41	15
A12	2	L47-L48	15
A13	2	L48-L49	15
A14	2	L52-L53	13.5
A15	2	L53-54	13.5
A16	2	L56-L57	13
A17	2	L57-L60	13.5
A18	2	L75-L76	16.8
A19	2	L76-L77	20
A20	2	L78-L79	12
B1	6	L31-L69	14
C1	7	L23-L65	10

Molecular markers provide an effective approach for studying complex disease resistances (Young, 1996). In addition to various breeding applications, molecular markers tightly linked to a target gene can be used to construct linkage map and can also act as starting points for physical mapping and subsequent cloning of the gene (Tanksley *et al.,* 1995). Linkage maps indicate the position and relative genetic distances between markers along chromosomes (Paterson, 1996). The most important use of linkage maps is to identify chromosomal locations containing genes and QTL associated with traits of interest, such maps are referred to as 'QTL maps' and procedure of construction of QTL maps is known as 'QTL mapping'.

QTL mapping is based on the principle that genes and markers segregate via chromosome recombination (called crossing-over) during meiosis (i.e. sexual reproduction), thus, allowing their analysis in the progeny (Paterson, 1996). Genes or markers that are close together or tightly-linked will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart. In a segregating population (mapping population), there is a mixture of parental and recombinant genotypes. The frequency of recombinant genotypes can be used to calculate recombination frequencies, which is then used to infer the genetic distance between markers. By analyzing the segregation of markers, the relative order and distances between markers can be determined. The lower the frequency of recombination between two markers, the closer they are situated on a chromosome and vice-versa. Mapping functions are used to convert recombination frequencies into map units called cM. Linkage maps are constructed from the analysis of many segregating markers.

With the development of saturated linkage maps, all regions of the genome can be assayed for the presence of a locus contributing for a particular trait (Tanksley, 1993). In tomato, first RFLP linkage map was constructed by Tanksley *et al.* (1992). For linkage analysis of markers various software programs are used including MAPMAKER/ EXP (Lander *et al.*, 1987; Lincoln *et al.*, 1993) and MapManager QTX (Manly *et al.*, 2001), of which MAPMAKER/ EXP is most commonly used. MAPMAKER/ EXP was used to construct linkage groups in current study. The present study resulted in construction of tomato linkage map which consisted of 12 linkage groups. Only five linkage groups (LG2, 3, 6, 7, 10) were considered significant as they covered some portion of genome, while other linkage groups (LG1, 4, 5, 8, 9, 11, 12) were not considered significant as the distance between markers was 0 cM. In these groups only the presence of markers was shown and there is further need to cover these linkage groups with more markers. Entire map covered a total of 4660.7 cM which represents a good coverage of genome and much higher than the marker densities of the genetic linkage maps of some other tomato cultivars in a few of the earlier studies (Grilli *et al*., 2007; Sharma *et al*., 2008; Foolad, 2015). The genome coverage was also calculated and found to be 96.55% for LG2, 60% for LG6 and 0.5% each for LG3, LG7, LG10 and 97.18% for the total map length. Sharma (2013) and Vaidya (2014) also reported high genome coverage of 96.5% in stevia and 98.03% in apple, respectively.

Many agriculturally important traits such as yield, quality and disease resistance are controlled by many genes and are known as quantitative traits (also ‘polygenic,’ ‘multifactorial’ or ‘complex’ traits). The regions within genomes that contain genes

associated with a particular quantitative trait are known as QTL. DNA markers that are tightly linked to agronomically important genes may be used as molecular tools for MAS in plant breeding (Ribaut and Hoisington, 1998). The construction of a linkage map requires a segregating population derived from sexual reproduction of contrasting parents (Bohra *et al*., 2012). The parents selected for the mapping population will differ for one or more traits of interest. In present study 'Solan Lalima' was used as susceptible parent and 'EC-251649' acted as resistant parent for buckeye rot disease of tomato which were used to produce F_2 population of 100 individuals. Population sizes generally range from 50 to 250 individuals (Mohan *et al*., 1997), however for high-resolution mapping larger populations are required. F_2 population is the mapping population of choice because it is the simplest type of mapping populations developed for self pollinating species. Their main advantages are that they are easy to construct and require a short time to produce. On this population phenotyping and genotyping studies were carried out which were further used for QTL mapping using QTL Cartographer software. For mapping analysis many statistical programmes viz., simple interval mapping (SIM), interval mapping (IM) and CIM are used. More recently, CIM has become popular for mapping QTL (Collard *et al*., 2005). This method combines interval mapping with linear regression and includes additional genetic markers in the statistical model in addition to an adjacent pair of linked markers for interval mapping (Jansen, 1993; Jansen and Stam, 1994; Zeng, 1993, 1994). The main advantage of CIM is that it is more precise and effective at mapping QTL compared to single-point analysis and interval mapping, especially when linked QTL are involved. Many researchers have used QTL Cartographer (Basten *et al.*, 1994, 2001; Saxena, 2010; Vaidya, 2014) for QTL mapping. A total of 22 QTL were identified for disease resistance in present study on chromosome 2 between markers namely 'L4-L7', 'L11-L14', 'L14-L15', 'L15-L17', 'L24-L25', 'L25-L26', 'L28-L29', 'L34-L36', 'L36-L37', 'L39-L40', 'L40-L41', 'L47-L48', 'L48-L49', 'L52-L53', 'L53-54', 'L56-L57', 'L57-L60', 'L75-L76', 'L76-L77', 'L78-L79', and single QTL each on chromosome 6 and 7 between markers 'L31-L69' and 'L23-L65', respectively. In tomato various QTL mapping studies have been conducted earlier for resistance to different diseases (Ji *et al.*, 2009; Robbins *et al.,* 2010; He *et al.*, 2010; Sun *et al.,* 2011; Yang *et al.,* 2012; Zhang *et al.*, 2013; Foolad, 2015), but present study is the very first study which found QTL for buckeye rot resistance. No other study has been reported for QTL identification for this disease. Thus, this study will significantly contribute toward buckeye rot resistance and facilitate the development of new resistant varieties through marker assisted selection (MAS) in near future.

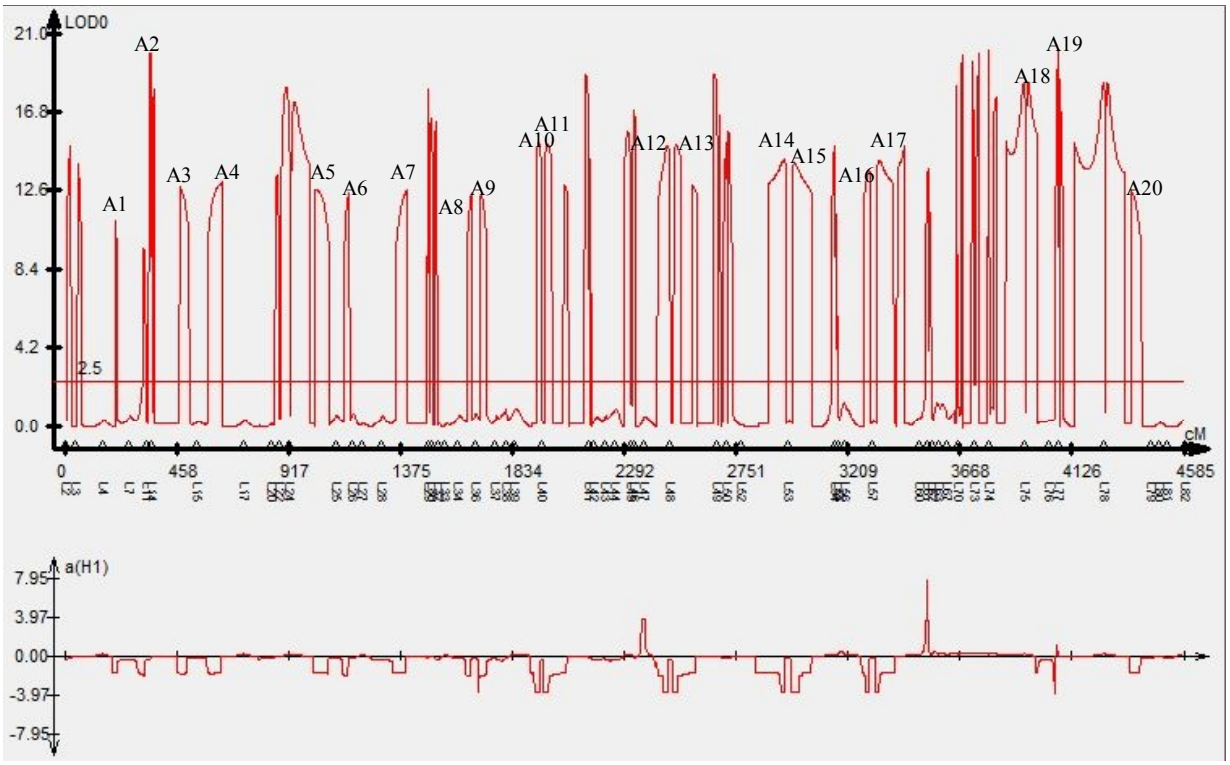


Fig. 9: Chromosome 2 having 20 significant QTL (A1-A20) for buckeye rot resistance

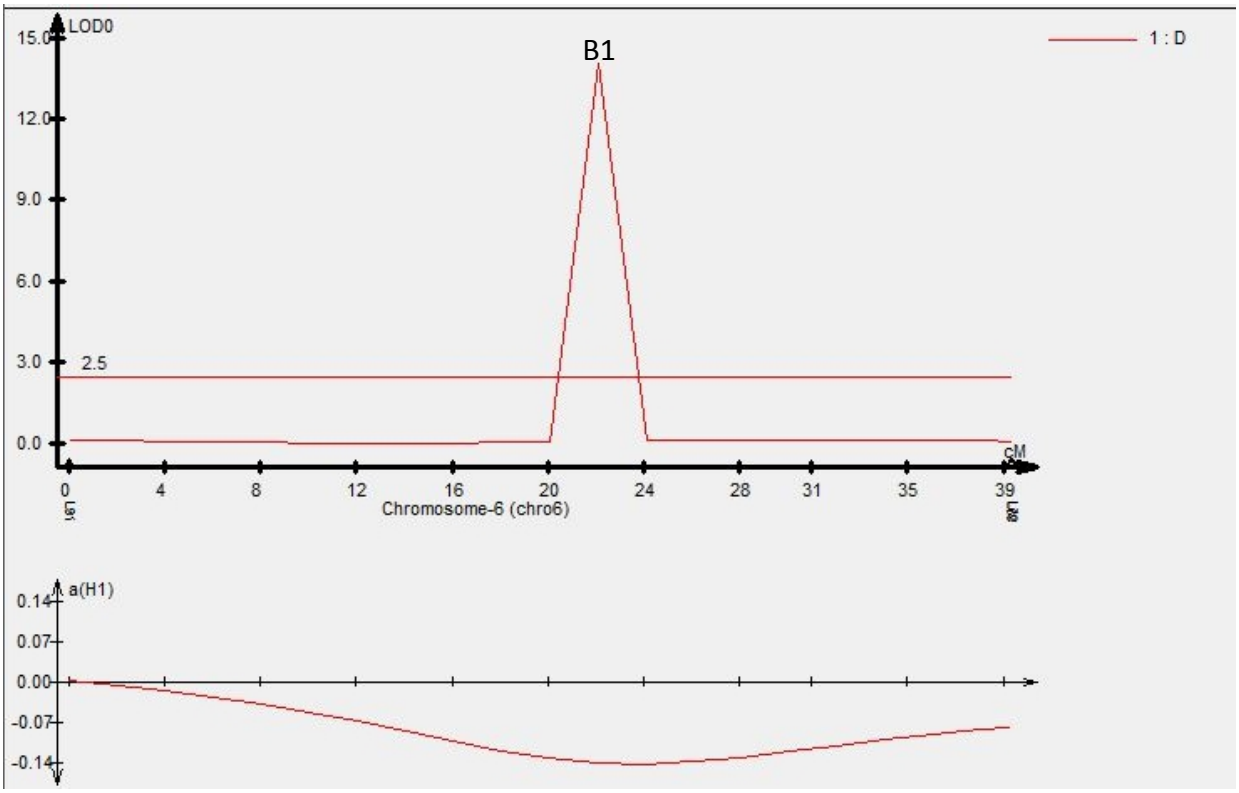


Fig. 10: Chromosome 6 having one significant QTL (B1) for buckeye rot resistance

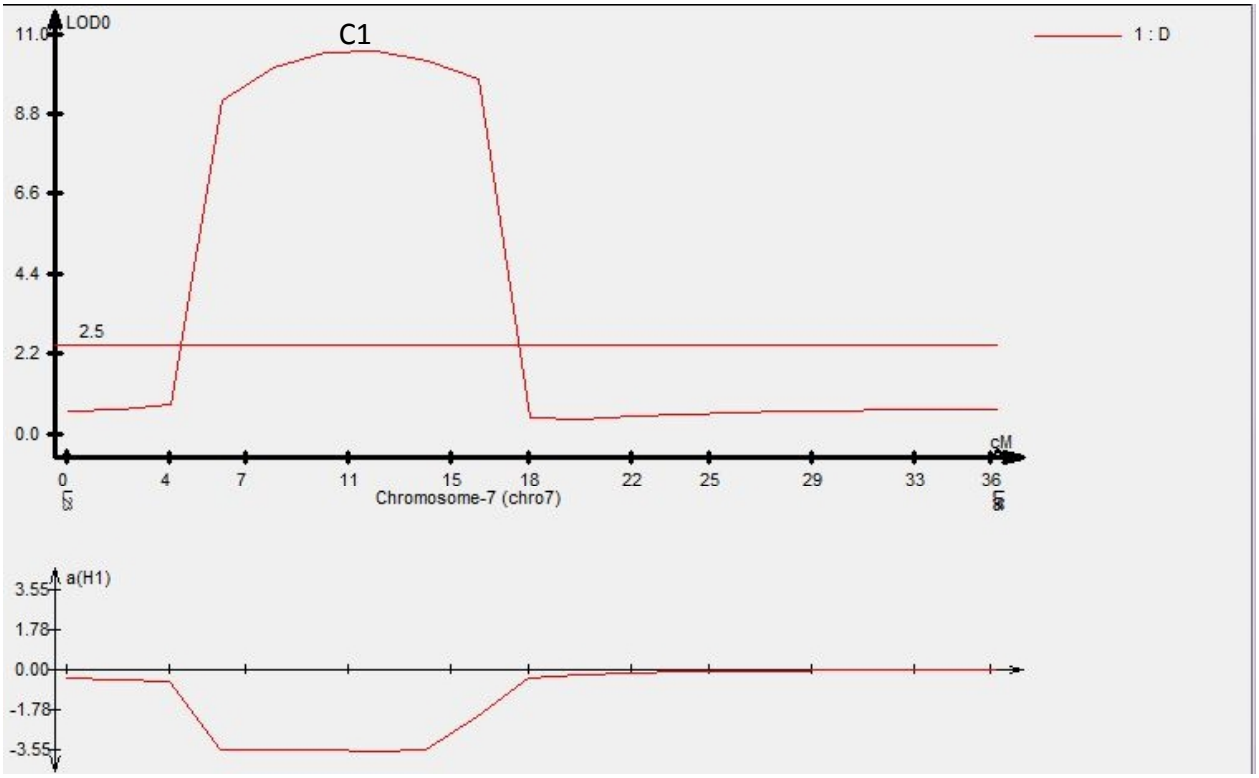


Fig. 11: Chromosome 7 having one significant QTL (C1) for buckeye rot resistance

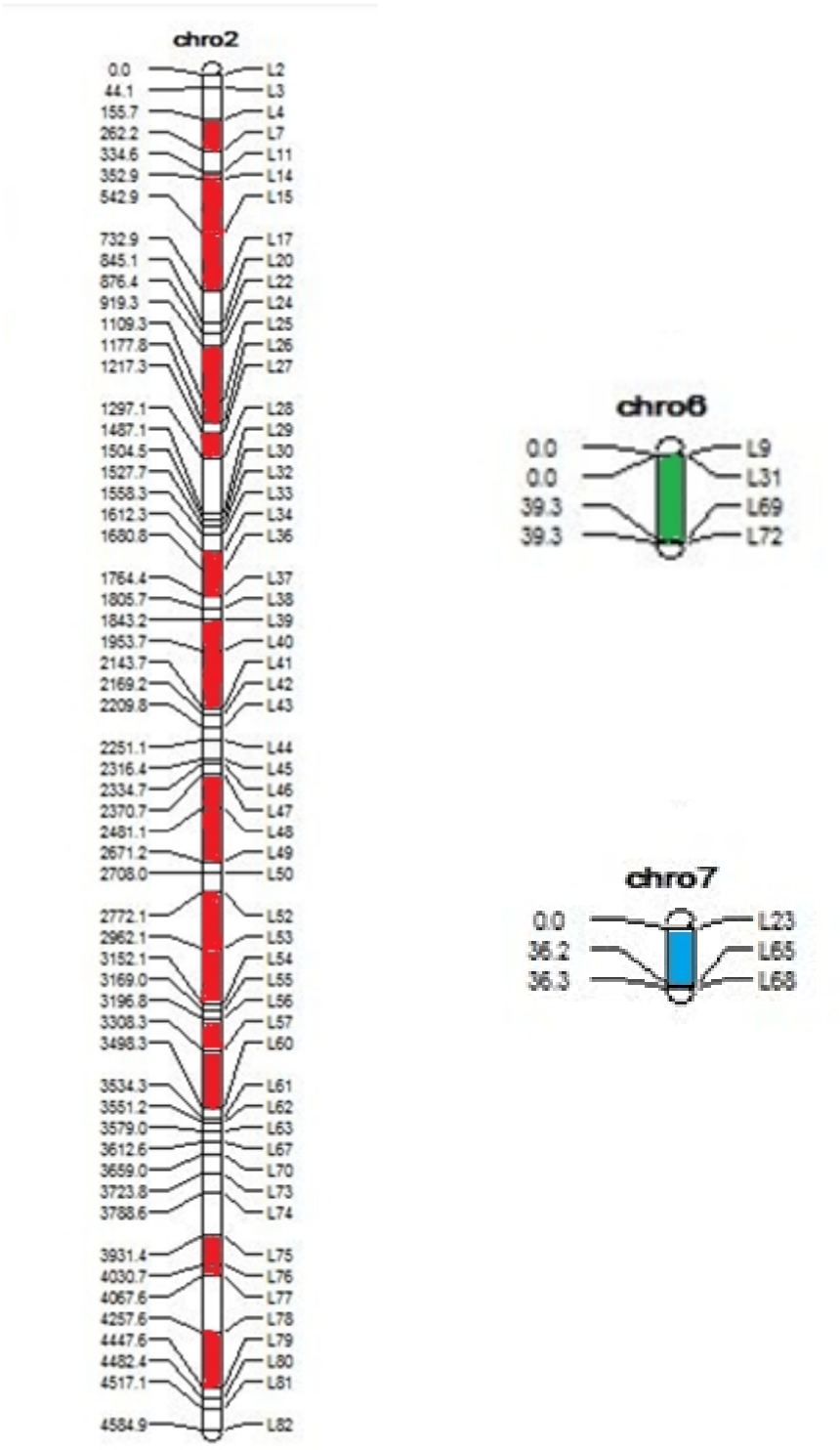


Fig. 12: QTL mapping for buckeye rot resistance on linkage groups
Coloured portions on chromosomes show the position of QTL

Chapter-5

SUMMARY AND CONCLUSION

Important findings of the study entitled **"Studies on identification of molecular markers for buckeye rot (*Phytophthora nicotianae* Breda de Hann. var. *parasitica* (Dastur) Waterhouse) resistance in tomato (*Solanum lycopersicum* L.)"** are summarized below:

1. Evaluation of genetic diversity amongst tomato genotypes using molecular markers

- DNA isolation method given by Doyle and Doyle (1987) was followed for isolating genomic DNA of 32 tomato genotypes.
- Quantitative and qualitative assessment indicated high quality of isolated DNA.
- 4,200 EST sequences of tomato were *in silico* downloaded from NCBI website (www.ncbi.nih.gov/ nucest).
- EGassembler software was used for assembly of these sequences which resulted in assembly of 352 contigs and extraction of 1918 singletons with 45.95% redundancy in data.
- SSR motifs were screened using SSRIT (www.gramene.org/db/ searches/SSRtool) which detected a total of 94 SSRs, out of which 19 SSRs were obtained from contigs and 75 SSRs from singletons.
- Di-nucleotide repeats were recorded to be in abundance with a frequency of 58.51%, followed tri-nucleotide repeats of 41.48%.
- From these 94 SSR containing EST sequences 55 primer pairs were designed using Primer3 software (www.frodo.wimit.edu/primer3/), of which 20 primer pairs were custom synthesized and were further used to carry out molecular marker studies.
- Further BLASTx tool of Uniprot database was used to assign putative functions to 20 custom synthesized primers which revealed high homology with four plant species: 14 sequences were homologous with *S. lycopersicum*, four showed homology with *S. tuberosum*, while rest two sequences were found homologous with *Nicotiana tabacum* and *Coffea canephora*

- Then genetic diversity was analyzed using ISSRs, genomic SSRs 25 of each and 20 EST-SSRs which produced polymorphism of 95.36%, 97.36% and 96.77%, respectively.
- 16 unique bands were generated by ISSR and EST-SSR primers, while genomic SSR primers produced five unique bands. ISSRs exceeded in average PIC (0.67), E (4.47) and MI (3.08) values, followed by genomic SSRs with values of 0.52, 2.32, 1.20 and EST-SSRs with values of 0.47, 1.85 and 0.86 for same parameters.
- Data analysis was done using NTSYS-pc version 2.0 by combined analysis of results obtained through three different molecular marker systems. Similarity range of 0.252 to 0.615 was observed. Maximum similarity of 61.5% was observed between 'EC-521' and 'EC-8591' while minimum similarity of 25.2% was obtained among 'EC-528373' and 'EC-528367'.
- In the dendrogram all genotypes were divided into two main clusters 'A' and 'B'. Cluster 'A' contained 25 genotypes, while cluster 'B' was consisted of seven genotypes.
- It was found that 'Solan Vajr' which is a commercial variety, was present on the top of cluster 'A' separating it from the rest of the genotypes.

2. To study genetics of inheritance of resistance to *Phytophthora nicotianae* var. *parasitica*

- F_2 population was raised by selfing F_1 population produced from cross combination of susceptible variety 'Solan Lalima' and resistant line 'EC-251649'. A total of 100 F_2 individuals constituted the mapping population.
- Isolation of *Phytophthora nicotianae* var. *parasitica* from buckeye rot infected fruit was successfully achieved on CMA medium.
- Morphological characterization of fungus was done under microscope. The pathogen/ fungus was identified based on the following features: hyaline and coenocytic mycelium along with branching at right angles. The sporangium arises from hyphal threads and produced sporangiospore.
- Further a loopfull of fungal hyphae was transferred to Corn Meal broth from which one gm hyphae was taken out by filtration through filter paper followed by suspending in 80 ml distilled water to obtain optimum density of 15-20 hyphae/ cm^3 in haemocytometer.

- Screening the results according to the scale revealed that the parent which was considered susceptible was characterized in highly susceptible category while the resistant parent was characterized as moderately resistant one.
- Upon screening of 100 F_2 individuals, 95 segregants were recorded to be susceptible and five were found to be resistant giving a ratio of 3.8:0.2.
- The calculated value of χ^2 is more than the tabulated value. Thus, the observed F_2 ratio did not fit into the expected Mendelian ratio for monogenic inheritance of buckeye rot in tomato leading to the conclusion that this disease is not under control of single gene and may be controlled by polygenes.

3. Identification of molecular markers/ QTL for buckeye rot resistance in tomato

- In total 153 primers were used for parental polymorphism survey which consisted of 44 ISSRs, 89 genomic SSRs and 20 EST-SSRs. Out of these 93 primers were found to be polymorphic which included 36 ISSRs, 41 genomic SSRs and 16 EST-SSRs.
- The polymorphic primers were further used to conduct genotyping studies among 100 individuals of mapping population.
- Screening results of parents and segregating population from inheritance experiment were used as phenotyping outcome.
- Both phenotypic and genotypic data generated was then entered in computer based programme MAPMAKER/EXP version 3.0b to construct linkage groups.
- In total 12 linkage groups were obtained.
- LG2 was the largest group spanning a distance of 4584.9 cM followed by LG6 which covered a distance of 39.3 cM.
- All primers covered total genetic map distance of 4660.7 cM. The average distance between adjacent markers in the linkage map was observed to be 50.11 cM.
- QTL Cartographer was used to construct QTL map which resulted in detection of total 22 QTL with placement of 20 QTL on chromosome 2 and single QTL each on chromosome 6 and 7.

Conclusion

The genotypes under this study have not been undertaken earlier for any marker studies. For their further use in breeding programmes, it was necessary to find out the broadness of genetic base. Combined use of data produced by three marker systems resulted

in more authenticated and precise results. In present study relatively high level of polymorphism was revealed by three marker systems viz., ISSR, genomic and EST-SSRs confirming their promising use in genetic diversity analysis. This led to the conclusion that ISSRs, genomic and EST-SSRs are promising to assess relationship of tomato germplasm.

It is very important to study the inheritance pattern of any character because this study will lead to a final conclusion about how the character is controlled at genetic level and in which manner it transmits to the next generation. This will facilitate further breeding experiments for crop improvement. So it was necessary to confirm the accurate inheritance pattern of this disease. In present study quantitative/ polygenic inheritance for disease resistance was revealed.

Present study is the very first study which found QTL for buckeye rot resistance in tomato. In this study several significant QTL were successfully identified for buckeye rot resistance in tomato. Three different marker systems viz., ISSR, genomic and EST-SSRs were efficiently used to map the location of detected QTL. These markers can be used further in studies on map based cloning of these genes as well as in marker assisted selection for disease resistance against buckeye rot.

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DEPARTMENT OF BIOTECHNOLOGY
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Major Advisor	**:**	Dr (Mrs) Rajinder Kaur

ABSTRACT

Present study was carried out for identification of molecular markers for buckeye rot resistance in tomato. In this study first of all genetic diversity was assessed among 32 tomato genotypes. For this EST-SSR primers were *in silico* synthesized by downloading 4200 EST sequences of tomato from NCBI website followed by finding out of contigs and singletons using EGassember server with further extraction of SSR containing sequences using SSRIT software with final designing of 55 EST-SSR primers with Primer3 software of which 20 EST-SSRs were custom synthesized. These EST-SSRs along with ISSRs and genomic SSRs 25 each were used to conduct genetic diversity studied among 32 tomato genotypes revealing polymorphism of 96.77%, 95.36% and 97.36%, respectively. On data analysis with NTSYS-pc version 2.0 similarity matrix with range of 0.252 to 0.615 was observed with maximum similarity of 61.5% between 'EC-521' and 'EC-8591' and minimum similarity of 25.2% between 'EC-528373' and 'EC-528367'. Dendrogram divided all genotypes in two main clusters 'A' and 'B' with 25 and seven genotypes, respectively. Genetics of inheritance was found out by screening 100 F_2 segregants, derived from a cross between susceptible parent 'Solan Lalima' and resistant parent 'EC-251649', with fungal inoculum which showed that 95 were susceptible and five were resistant giving a ratio of 3.8:0.2. The calculated value of χ^2 was found more than the tabulated value showing that observed F_2 ratio did not fit into the expected Mendelian ratio for monogenic inheritance of buckeye rot in tomato revealing polygenic control of this disease. 153 primers were used for parental polymorphism survey which consisted of 44 ISSR, 89 genomic SSR and 20 EST-SSR primers. Out of these 93 primers were found polymorphic which included 36 ISSRs, 41 genomic SSRs and 16 EST-SSRs which were used for genotyping studies of F_2 population. The phenotypic and genotypic data of F_2 population was used for construction of linkage map using MAPMAKER/EXP version 3.0b which resulted in construction of 12 linkage groups. LG2 was the largest group spanning a distance of 4584.9 cM followed by LG6 which covered a distance of 39.3 cM. All primers covered total genetic map distance of 4660.7 cM. QTL were constructed using software QTL Cartographer which detected total 22 QTL with placement of 20 QTL on chromosome 2 and single QTL each on chromosome 6 and 7. This is the first study for identification of QTL for buckeye rot resistance in tomato which can further be used for marker assisted selection and other molecular studies.

Signature of Student
Name: Shilpa
Date:

Signature of the Major Advisor
Name: Dr (Mrs) Rajinder Kaur
Date:

Head of the Department

APPENDIX-I

6 X LOADING DYE

S. No	Constituents	Quantity
1.	Bromophenol blue	0.25%
2.	Sucrose	40%

Added autoclaved distilled water (ADW) to make the final volume 100 ml

APPENDIX-II

GEL ELECTROPHORESIS

a) Agarose gel 1.2%

2.4 gm agarose was added to 200 ml 1 X TAE buffer and dissolved. The molten gel was cooled to 40^{o}C and 5 µl of ethidium bromide solution was added and mixed well. The molten gel was casted in a gel tray with comb containing 26 teeth to produce wells.

b) Agarose gel 3.5%

7 gm agarose was added to 200 ml 1 X TAE buffer and dissolved. The molten gel was cooled to 40^{o}C and 5 µl of ethidium bromide solution was added and mixed well. The molten gel was casted in a gel tray with comb containing 26 teeth to produce wells.

APPENDIX-III

1X TAE BUFFER

Stock: 50 X TAE buffer

S. No	Constituents	Quality/l
1.	Tris Base	242 gm
2.	Glacial acetic acid	57.1 ml
3.	0.5 M EDTA (pH 8.0)	100 ml

Make final volume 1 litre using ADW.
From this stock 1X TAE Buffer was prepared by adding 2ml 50X TAE Buffer in 98 ml of ADW.

APPENDIX –IV

COMPOSITION OF CORN MEAL AGAR MEDIUM

S. No.	Constituents	Quantity (g/l)
1.	Corn meal	19.0
2.	Agar	3.0
3.	Dextrose	5.0

pH of the medium was adjusted to 6.0.

APPENDIX –V

COMPOSITION OF CORN MEAL MEDIUM

S. No.	Constituents	Quantity (g/l)
1.	Corn meal	19.0
2.	Dextrose	5.0

pH of the medium was adjusted to 6.0.

APPENDIX – VI

SOURCE OF CHEMICALS AND INSTRUMENTS

1. Chemicals

Tris, NaCl (molecular biology grade), chloroform, ethanol, EDTA, agarose were obtained from Qualigens and Bangalore Genei. The dNTP and Taq DNA polymerase kits were obtained from Bangalore Genei.

2. Instruments and other accessories

i) Instruments used in DNA extraction

Waterbath	:	Popular Traders
Deep Freezer (-20^{0}C)	:	Vestfrost
Refrigerator	:	Godrej
pH meter	:	Systronics
Refrigerated Centrifuge	:	Eppendorf
Vortex Shaker	:	Indosati Scientific Lab Equipments
Spectrophotometer	:	UV/VIS spectrophotometer Model UV-5704SS ECIL, Hyderabad, India

ii) Instruments used in PCR analysis

Thermal Cycler	:	Applied Biosystems
Laminar flow	:	Microsil India
Weighing balance	:	Sartorius
Gel electrophoresis unit	:	Genei
UV transilluminator	:	Pharmacia
Gel Documentation system	:	Syngene
Microwave oven	:	Samsung
Spinner	:	Tarsons

BRIEF BIODATA OF THE STUDENT

Name	**:**	Shilpa
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Mother's Name	**:**	Smt. Suman Bala
Date of Birth	**:**	5th September, 1989
Permanent address	**:**	V.P.O Nakroh (Ramnagar) Teh. Amb, Distt. Una, Himachal Pradesh, 177 213, India

Academic Qualifications:

Certificate/ Degree	Class/Grade	Board/University	Year
10th class	First	HP Board	2005
12th class	First	HP Board	2007
B.Sc. (Biotechnology-E)	First	Panjab University, Chandigarh	2010
M.Sc. (Molecular Biology and Biotechnology)	First	Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP	2012

(**Shilpa)**