

Assessment of genetic diversity in cultivated tomato (*Solanum lycopersicum* L.) genotypes using morphological and molecular markers

By

Supneet Kaur
(J-14-MB-21)

A Thesis submitted to
Faculty of Post Graduate Studies
in partial fulfillment of the requirements
for the degree of

**MASTER OF SCIENCE
IN
BIOTECHNOLOGY**



SCHOOL OF BIOTECHNOLOGY

Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu
Main Campus, Chatha, Jammu 180009

2016

CERTIFICATE – I

This is to certify that the thesis entitled “**Assessment of genetic diversity in cultivated Tomato (*Solanum lycopersicum* L.) genotypes using morphological and molecular markers**” submitted in partial fulfillment of the requirements for the degree of **Master of Science** in subject of **M.Sc. Biotechnology** to the Faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu is a record of bonafide research, carried out by **Ms. Supneet Kaur**, Registration Number **J-14-MB-21** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that such help and assistance received during the course of thesis investigation have been duly acknowledged.

Dr. A.K. Singh
(Major Advisor)

Place: Jammu.

Date:

Endorsed:

Head

Division of School of Biotechnology

Date:

CERTIFICATE – II

We, the members of Advisory Committee of **Ms. Supneet Kaur**, Registration No. **J-14-MB-21**, a candidate for the degree of **Master of Science in Biotechnology** have gone through the manuscript of the thesis entitled “**Assessment of genetic diversity in cultivated Tomato (*Solanum lycopersicum* L.) genotypes using morphological and molecular markers**” and recommend that it may be submitted by the student in partial fulfillment of the requirements for the degree.

Dr. A.K. Singh
Major Advisor

Advisory Committee

Place: Jammu

Date:

Advisory Committee Members:

Dr. R.K Salgotra
Coordinator School of Biotechnology
Member from Major Subject

Dr. Manmohan Sharma
Assoc. Prof
School of Biotechnology
Member from Minor Subject

Dr. Vikas Sharma
Asstt. Prof
Division of Biochemistry and Plant Physiology
Director Education Nominee

CERTIFICATE – III

This is to certify that the thesis entitled **“Assessment of genetic diversity In cultivated Tomato (*Solanum lycopersicum* L.) genotypes using morphological and molecular markers”** submitted by **Ms. Supneet Kaur**, Registration No. **J-14-MB-21** to the Faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, in partial fulfillment of the requirement for the degree of **Master of Science in Biotechnology** was examined and approved by the Advisory Committee and External Examiner(s) on _____

External Examiner

(Dr. A.K Singh)
Major Advisor

Coordinator
School of Biotechnology

Dean, FoA

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LIST OF ABBREVIATIONS

cm	-	Centimeter
cTAB	-	cetyl-trimethyl ammonium bromide
DNA	-	Deoxyribonucleic acid
dNTP's	-	Deoxynucleotide triphosphate
EDTA	-	Ethylene diamine tetra acetic acid
fig	-	Figure
ha	-	hectare
kg/ha	-	Kilogram per hectare
L	-	Litre
mg	-	milligram
ml	-	millilitre
ng	-	nanogram
°C	-	Degree Celsius
OD	-	Optical Density
pmol	-	Picomole
RNA	-	Ribonucleic acid
RNase	-	Ribonuclease enzyme
rpm	-	Rotations per minute
μ g	-	Microgram
μ l	-	Microlitre
%	-	Percentage

ACKNOWLEDGEMENT

Writing this thesis was my life-long ambition, but would never have been accomplished and brought to a favorable conclusion without the help of those to whom I would express my heart-felt thanks.

*I would like to express my deep and sincere gratitude to my major advisor, **Dr. A.K.Singh**, Associate Professor of School of Biotechnology, SKUAST-J, Chatha for giving me the opportunity to do research and providing invaluable guidance throughout this research. His dynamism, vision, sincerity and motivation have deeply inspired me.*

*I would like to express my deepest gratitude to the worthy members of my advisory committee, **Dr. R.K.Salgotra** (Coordinator, School of Biotechnology), **Dr.Manmohan Sharma** (Assoc. Prof School of Biotechnology), **Dr. Vikas Sharma** (Asstt. Prof, Division of Biochemistry) Director Education Nominee for their constant help, encouragement and valueable suggestions during the investigation and preparation of manuscript.*

*I equally reiterate my gratitude and indebtedness to **Dr. G.K.Rai**, Assistant Professor, School of Biotechnology, **Dr, Susheel Sharma**, Assistant Professor, School of Biotechnology for their help when approached.*

*I am very thankful to **Hon'ble Vice Chancellor** for allowing me to undertake the study and for providing necessary facilities and financial support. It is rarest to thank **Dr T.S. Ganai**. (Director Education) for his extraordinary help and timely advice throughout the course of the study.*

*I am highly thankful to **Dr. Prashant Bakshi** (Associate Professor, Division of Fruit Science) for extending their helping hand during the investigation.*

*I am highly thankful to my friends namely **Deeksha, Riya, Deepika** for their whole hearted support and constant inspiration during the investigation.*

*Next I would like to thanks my senior **Sreshiti bagati, Rohini Bhat, Reetika Mahajan, Abida Parveen, Nancy Gupta, Sonika Sharma, Ramandeep Kaur, Meenakshi Raina** for their immense help, thoughts and suggestions have been invaluable throughout my candidature.*

*This note of acknowledgement will be incomplete without a profound delineation of the scene commitment, enlightening love and path showing inspiration which I was rewarded by my parents, **Mr. Ranbir Singh** and **Mrs. Harwant Kour**, my sister **Pavneet**, my brother **Harsh** and my grandmother **Smt. Harbans Kaur** whose inspirations, countless blessings, love and affection stood by me throughout my academic carrier and were always a source of strength for me and their everlasting desire brought me up here up to.*

I feel highly privileged when I award them with the epithet of peerless asset, who revitalized my dream to live and theme to struggle with an eternal touch of dedication.

It is my pleasure to mention the help provided by the non teaching staff of my division during research work,

Above all my humble and whole prostrations before Almighty for sprinkling his unprecedented favors upon me.

*Last but not least, I feel pleasure in thanking **Mahavir internet Café** for intense endeavor and giving final shape to this manuscript.*

This thesis of mine is dedicated to my parents. They had been a source of great inspiration for me.

None is forgotten but everyone is not included.

Supneet Kaur
Place: Jammu
Date:

ABSTRACT

Title of Thesis/Dissertation	:	Assessment of genetic diversity in cultivated tomato (<i>Solanum lycopersicum</i> L.) using morphological and molecular markers
Name of Student	:	Supneet Kaur
Admission No	:	J-14-MB-21
Major subject	:	Biotechnology
Name and Designation of		Dr. A. K. Singh
Major Advisor	:	Associate Professor
Degree to be awarded	:	M.Sc. Biotechnology
Year of award of Degree	:	2016
Name of the University	:	Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

ABSTRACT

Assessment of genetic diversity in any crop species provides a basis for devising future strategies for crop improvement; conservation and sustainable use. An experiment consisting of 24 genotypes of Tomato was conducted during the year 2016 at the Research Farm and Molecular Biology Laboratory of School of Biotechnology, SKUAST-J, Chatha. The experiment was conducted in Randomised Block Design (RBD) with three replications in 2 rows of 5m length with spacing of 45 x 90 cm. The extent of genetic divergence /relatedness was estimated among 24 genotypes by using 11 traits viz. plant height (cm), number of branches, number of fruits per bunch, total soluble solids, flesh thickness (mm), number of locules, fruit width (cm), fruit length (cm), yield per plant (g), average fruit weight (g), number of fruits per plant. The maximum number of fruits/bunch was recorded in “Utkal Pragyan” (3.66) and the minimum number was recorded in “Swarna Sampada” (2.03). Maximum TSS(%) was recorded in DCT-1 (8.06%) and minimum TSS was recorded in “Dhanshri” (2.83%). Maximum number of fruits and yield/plant was recorded in “DCT-1” (115.33) and “Hisar Lalit” (2507.36g), respectively. The minimum number of fruits and yield/plant was recorded in “NDT-4” (23.20) and “DCT-1” (861.40g), respectively. Based on squared Euclidean distance and Unweighted pair group method with arithmetic mean (UPGMA) clustering the genotypes were grouped into two main clusters with various sub-clusters using SSR and RAPD markers. For molecular characterization, 13 RAPD and 9 SSR (Simple Sequence repeats) markers were used. Polymorphic information Content (PIC) value for SSR markers was high (0.303) as compared to RAPD (0.29). The information obtained from hierarchical clustering, RAPD and SSR markers may well assist tomato breeders in identifying a limited number of highly differentiated genotypes to be selected for further use in developing suitable variety/genotypes with good quality potential.

Keywords: Genetic diversity analysis, Morphological traits, SSR, RAPD.

Signature of Major Advisor

Signature of Student

INTRODUCTION

Tomato is a herbaceous crop and belongs to the family Solanaceae, which consists of approximately 100 genera and 2500 species, including several other plants of agronomic importance such as potato, eggplant, pepper, and tobacco (Olmstead *et al.*, 2008). *Solanum Lycopersicum* has a relatively compact genome among the Solanaceae species, characterized by its diploid chromosome number ($2n = 2X = 24$). It is approximately 950 Mb in size, and is one of the most intensively characterized Solanaceae genomes (Arumuganathan *et al.*, 1991). The tomato is grown successfully under a wide range of agro-climatic conditions. It is a warm season crop and requires a relatively long growing season to produce profitable yield.

It is one of the significant vegetable crops of special economic importance in the horticulture industry, originating in South America and its many varieties are now commonly grown in greenhouse in cooler climates (He *et al.*, 2003). It is the most popular garden vegetable belonging to the genus *Lycopersicon*, the resemblance between leaves and flowers of potato and tomato plants seems to certify this taxonomic grouping (Wang *et al.*, 2005 and Shidfar *et al.*, 2011).

There are approximately twelve species within genus *Lycopersicon*. On the basis of fruit colour, all these species have been classified into sub genera, viz., *Eulycopersicon* (characterized by red fruits with carotenoid pigmentation and annual growth habit) and *Eriopersicon* (characterized by green fruits with anthocyanin pigmentation). The cultivated species of Tomato (*L. esculentum*) and one wild species (*L. pimpinellifolium*) belong to *Eulycopersicon* and these two species were classified into five botanical varieties. There are 16 wild species of tomato, including *S. habrochaites*, *S. pennellii*, *S. pimpinellifolium*, *S. cheesmaniae*, *S. galapagense*, *S. peruvianum*, *S. corneliomulleri*, *S. chilense*, *S. chmielewskii*, *S. arcanum*, *S. neorickii*, *S. huaylasense*, *S. lycopersicoides*, *S. ochranthum*, *S. jugandifolium*, and *S. sitiens* (Knapp *et al.*, 2009; Bedinger *et al.*, 2011). One of these varieties, namely, *L. esculentum* var. *ceraseform* (cherry tomato) is considered as the immediate ancestor of present day cultivated tomato (kalloo, 1986).

These species in the tomato clade are considered to have evolved primarily by genic changes rather than large-scale chromosomal rearrangements (Anderson *et al.*, 2010).

Because of its high per capita consumption, tomatoes are nutritionally valuable for their high pro-vitamin A and vitamin C content and rank first in their nutrient contribution to the diet. Tomato fruits are a rich source of ascorbic acid and the flavour of fruits is controlled by various volatile components and balance of sugar: acid ratio

It is extensively used in salad as well as for culinary purposes and also used for various processed forms include pastes, sauces, pulps, juices, ketchup and as flavouring 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 has antioxidant properties and potential beneficial health effects (Zhang *et al.*, 2009).

In the year 2010, the annual production of tomato was more than 145.5 million tons, harvested from over 4.3 million hectares at an average yield of 336 hg/ha (FAO, 2012). There are more than 7500 tomato varieties successfully bred and grown for various purposes worldwide, and plant variety registration bodies in different countries keep records of most of these varieties. Although each tomato variety has unique characters of interest to breeders and other researchers as well as growers, cases of synonyms and homonyms abound in the tomato industry just like in other crops, and this is an important problem that should not be overlooked but rather should be addressed (Korir *et al.*, 2012).

The tomato genetic resources are important materials for breeding and biotechnology, and determination of their relationships has valuable potential in the tomato industry. The success of tomato genetic resource collection, preservation, exploitation, and utilization in both present and long-term breeding, and production programs depend largely on the knowledge and understanding of the genetic background, diversity, relationships, and identification of these resources. Tomato is the 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, presently there are several molecular maps based on crosses between the cultivars and many wild species of tomato. Genetic analysis of tomato is essential to enhance the genetic yield potential and maximum utilization of the desirable characters for synthesizing of any ideal genotypes (Kumar *et al.*, 2003). The assessment of genetic diversity within and between populations of tomato varieties is measured by using morphological, biochemical and molecular characterization (Garcia *et al.*, 2004).

One of the primary needs of the crop industry is the estimation of genetic diversity between cultivated accessions for identification and breeding purposes. To this end,

various marker techniques have been successfully applied, either individually or in combination, to study the genetic diversity of various plant species. Molecular genetic diversity estimates are extremely useful for intellectual property protection, particularly in the determination of essential derivation. Measurements of genetic diversity can be used in breeding programs to increase the genetic variation in base populations by crossing cultivars with a high level of genetic distance as well as for the introgression of exotic germplasm. The genetic diversity estimates based on molecular marker data may be compared to a minimum genetic distance which indicates that two cultivars are not essentially derived (Lefebvre *et al.*, 2001). Study of phenotypic and genetic diversity in landrace collections is important for germplasm conservation. In addition, the characterization of much diversified materials with molecular markers offers a unique opportunity to define significant marker-trait associations of biological and agronomic interest.

Molecular characterization is now the favored means to quantify variation within large germplasm samples. New DNA sequencing and genotyping technologies provide the power to interrogate thousands to millions of diagnostic polymorphisms, across hundreds to thousands of genotypes, thus facilitating the analysis of genetic structure and providing a rationale basis to identify and select among the underlying lineages. Such approaches not only resolve genetic relationships at fine scale, but they also provide important measures of genetic divergence between and genetic diversity within the major genetic clusters that comprise crop germplasm. Numerous studies have been undertaken with a range of molecular marker technologies, focusing principally on nuclear markers. The precision and robustness of the patterns thus revealed now principally rest on a pertinent choice of materials, implying that a sufficient number of accessions are analyzed. DNA markers also allow access to cytoplasmic (i.e. mitochondrial or chloroplastic) variation, which is usually maternally inherited and not affected by recombination. This provides another view of genetic diversity, which is very helpful in highlighting the role of hybridization in the overall crop evolution process (Glaszmann *et al.*, 2010). The genetic diversity of tomato has been investigated in several studies using isozymes, restriction fragment length polymorphisms (RFLPs), oligonucleotide fingerprinting and simple sequence repeats (SSRs). With the exception of SSRs, limited information was obtained due to a lack of variability that was ascribed to the self pollinating nature of modern tomato cultivars combined with their narrow genetic base (Alvarez *et al.*, 2001). Genetic diversity in the cultivated tomato is generally low, due to the occurrence of population bottlenecks during the domestication and generation of modern varieties (Rick, 1976). The

cultivated tomato (*Lycopersicon esculentum*) is known to be highly monomorphic at the molecular level although it is phenotypically very diverse. The information on genetic diversity within and among closely related varieties is essential for rational use of genetic resources. The analysis of genetic variability within and among breeding materials is of fundamental concern for plant breeders (Chakravarthi and Naravaneni, 2006). During and following domestication, the tomato has undergone intensive selection, and cultivated varieties have narrow genetic diversity relative to other crops. This narrow diversity makes it difficult to identify molecular markers that are polymorphic in modern breeding material. However, a number of polymorphic microsatellite markers generated from database sequences have been successfully used for genotyping tomato cultivars and accessions (He *et al.*, 2003; Smulders *et al.*, 1997; Bredemeijer *et al.*, 1998).

Microsatellite or simple sequence repeat (SSR) marker has been used in plant diversity analysis; the popularity of these markers is due to their ease of amplification by polymerase chain reaction (PCR), their co-dominant nature and their typically high levels of allelic diversity at different loci. There are numerous reports suggesting the usefulness of microsatellite markers for measuring the genetic variability in a wider taxonomic range (Ryberg *et al.*, 2002; Li *et al.*, 2007; Kawka *et al.*, 2007; Chan *et al.*, 2008; Banhos *et al.*, 2008). SSR markers tend to be among the most polymorphic genetic marker types and have been introduced into the process of cultivar and variety identification as well as in pedigree reconstruction and genetic mapping (Holton *et al.*, 2002; Yu *et al.*, 2004a; Celucia *et al.*, 2009), to analyze functional diversity (Senior *et al.*, 1998; Leigh *et al.*, 2003; Dreisigacker *et al.*, 2004), and for comparative mapping (Yu *et al.*, 2004b; Varshney *et al.*, 2005a). It is widely accepted that SSR techniques are expensive if the sequence information for designing the primers has not yet been developed. However, for the tomato, the primer sets for SSR analysis have already been developed (Smulders *et al.*, 1997; Bredemeijer *et al.*, 1998; He *et al.*, 2003). Besides, SSR has recently produced highly informative genotyping sets in other crops, such as leafy brassicas (Celucia *et al.*, 2009), among others. Furthermore, Jones *et al.*, (1997) and Wang *et al.*, (2011) indicated the reproducibility of SSRs in closely related species and cultivars.

The present study entitled “**Assessment of genetic diversity in cultivated tomato (*Solanum lycopersicum* L.) genotypes using morphological and molecular markers**” shall be undertaken with the following objectives:

1. To study the genetic diversity of tomato cultivars using morphological traits.
2. To assess the genetic diversity using RAPD and SSR markers.

REVIEW OF LITERATURE

Tomato (*Solanum lycopersicum* L.) is an annual classified in family Solanaceae. Fleshy berry of cultivated tomato has been ranked third in global production, after potato and sweet potato (Hsueh-Li tan *et al.*, 2010). Other than fresh fruit consumption, processed forms as canned tomatoes, ketchup, and variety of sauces, juice, soups, paste and powder are equally popular. Around the world cultivation of the crop is attributed to its adaptation in diverse environments. Tomatoes are being grown globally with a world production of 130 million tons.

The tomato plants show ample morphological variation. The plants may be in form of bushes (determinate) or vines up to six feet tall (indeterminate). The stem and leaves are pubescent having non glandular and glandular trichomes with unpleasant odour. The stem hair may develop into roots when in contact with soil. The leaves display spiral phyllotaxy i.e. one leaf at each node and are petiolate, compound, imparipinnate. Tomato shoots show sympodial branching with apical meristems. Cultivated tomato is autogamous and style is enclosed by the staminal cone to assure self pollination.

2.2 GENETIC DIVERSITY

Genetic diversity is a study undertaken to classify an individual or population compared to other individuals or populations. This is a relative measure, as the distance between any pair of entries in the study is greater or lesser depending on all pair wise comparisons that can be made in the study. However, genetic fingerprinting is the unambiguous identification of an individual (based on the presence or absence of alleles at different markers) or a population (based on frequencies of alleles of the markers). This is an absolute measure and does not change depending on other individuals or populations under study. Both Genetic diversity and fingerprinting studies are done using molecular markers. Determining genetic diversity can be based on morphological, biochemical, and molecular types of information (Mohammadi & Prasanna 2003; Sudre *et al.*, 2007; Goncalves *et al.*, 2009). However, molecular markers have advantages over other kinds, where they show genetic differences on a more detailed level without interferences from environmental factors, and where they involve techniques that provide fast results detailing genetic diversity (Binneck *et al.*, 2002; Garcia *et al.*, 2004; Saker *et al.*, 2005; Goncalves *et al.*, 2008; Souza *et al.*, 2008). Moreover, the discovery of high throughput

platforms increases number of data per run and reducing the cost of the data and increasing map resolution.

According to State of the Worlds Plant Genetic Resources (PR 96/9), among other crops 81% of tomato varieties have been lost in USA. Studies of cultivated tomato for estimates of genetic diversity have revealed that cultivated tomato retains less than 5% allelic diversity as compared to its weedy and wild relatives (Miller and Tanksley, 1990). One reason for low diversity level is observed when a crop loses its ability to out breeding as happened with tomato which became autogamous during domestication (Rick, 1976). Added to it are successive bottle necks which the crop had gone through during domestication in different parts of the world other than at the centre of origin respectively. Tomato is rich in germplasm resources. Cultivated tomato and 12 species of wild and weedy relatives have been identified by Peralta *et al.* (2006) and named while traditionally recognized species were 9 (Rick, 1990). The wild relatives though with small sized, round and green fruits except *S. pimpinellifolium* with red fruits exhibit a low level of morphological diversity but genotypically these are highly diverse (Tanksley, 2004).

Natural interspecific hybridization and gene flow among wild self compatible tomato species have been reported in *S. pimpinellifolium* and *S. lycopersicum* in Ecuador and Peru. Mutations, recombination (Campbell, 1946), independent assortment and natural hybridization resulting in gene flow within and between species are natural way of generating genetic diversity. Revitalizing cultivated tomato by using the natural genetic variation of wild species is expected to be of great economic value. Resistance against various diseases in wild relatives of tomato have been reported (Kabelka *et al.*, 2002; Bai *et al.*, 2004; and Ammiraju *et al.*, 2003) and many introgressed in cultivated tomato. Resistance against various insects (Sanchez-pena *et al.*, 2006, Simmons *et al.*, 2005; Frelichowski and Juvic, 2005; and Talekar *et al.*, 2006) and abiotic stresses as extreme temperatures (Venema *et al.*, 2000; Dolstra *et al.* and Foolad, 2004) have been discovered in wild and weedy germplasm). *Solanum cheesmaniae* has been reported to have high sugar and β -carotene content which can be exploited for quality improvement of tomato (Nuez *et al.*, 2004). Introduction of distinctive aroma from *Solanum peruvianum* into germplasm of cultivated tomato has been reported (Kamal *et al.*, 2001). Introgression of genes from *S. Habrochaites* to cultivated tomato resulted in many fold increase in total yield, soluble solids content and better colour respectively (Foolad, 2007). Plants wild and weedy relatives are the only resources for future food security against large scale disastrous starvation (Harlan, 1972b). The tomato land races in Myanmar were highly

diverse however replacement of this germplasm by high yielding genetically uniform varieties posed a threat of extinction (San-San-Yi, 2008).

2.3 MORPHOLOGICAL MARKERS

Morphological characters have for a long time remained the means of studying genetic variations in plant species. Morphological data are affected by ecological interactions; thus explanations must be made with suitable replication (Beckmann & Soller (1986). Valid comparisons are only possible for accounts taken at the same location and during the same 13 season (Smith & Smith, 1988). Beckmann & Soller (1986) stated that related to morphological and biochemical characteristics, the DNA genome provides a meaningful and more influential source of genetic variances. Phillipp *et al.* (1994) also pointed out that the benefit of DNA fingerprinting over morphological markers is the dominance and the lack of pleiotropic effects. Among these morphological properties, length, width, thickness, mass, volume, projected areas and centre of gravity are the most important factors in sizing systems (Mohsenin, 1986). Determining relations between mass and dimensions and projected areas may be beneficial and applicable.

Nawab *et al.* (2008) conducted study at Vegetables Research Institute, AARI, Faisalabad, Pakistan during 2005-06, 12 pea genotypes were planted in a randomized complete block design with three replications. Analysis of variance revealed highly significant differences among genotypes for days to emergence, days to 50 percent flowering, number of pods per plant, weight of pods per plant (g), pod length (cm), number of seeds per pod, 100-seed weight (g) and green pod yield (kg/plot). The correlation studies revealed that in general, estimates of genotypic correlation coefficient were higher than corresponding phenotypic correlation coefficient. The results suggested that these traits could be considered as major green pod yield contributing characters in garden peas. 100-seed weight, number of pods per plant, number of seeds per pod and days to 50 percent flowering exhibited maximum positive direct effect on green pod yield per plot, respectively. It indicated that these are main contributors towards yield.

Akinfasoye *et al.* (2011) conducted field experiment at National Horticultural Research Institute of Nigeria (NIHORT), Ibadan during wet and dry seasons of 2009 and 2010 respectively to investigate the relationships among agronomic traits of 10 tomato hybrids. The data obtained on vegetative and fruit parameters were analyzed combined by analysis of variance and Least Significant Difference at 5% probability was used to test significance. Both the vegetative growth and fruit yield of tomato were superior in the

drier season. It was found that selection cannot be reliably inferred from fruit width. Tomato with fewer leaves can be selected for high fruit producers. These varieties are also likely to flower earlier. Based on this, number of leaves can be used to select early maturing varieties. Tall tomato plants can be assumed to branch more and produce heavier fruits. Conclusively, number of leaves, days to 50% flowering, number of fruits plant , number of branches and 1 fruit weight can to a large extent be used to select high yielding varieties.

Hu *et al.* (2012) Studied twenty-six morphological traits as well as 47 single nucleotide polymorphism and simple sequence repeat markers to investigate genetic variation in 67 tomato (*Solanum lycopersicum* L.) varieties collected from Argentina between 1932 and 1974. Approximately 65.0% of the morphological traits and 55.3% of the molecular markers showed polymorphisms in the 67 varieties. Average taxonomic distance between any two varieties ranged from 0.6643 to 1.1776, while Nei's genetic distance varied from 0 to 0.2022. Cluster analysis indicated that 67 varieties could be grouped into three clusters at both morphological and molecular levels. The varieties collected before 1960 had larger genetic variation than those collected after 1960.

Dufera. (2013) evaluated twenty one tomato (*Lycopersicon esculentum* Mill.) germplasms for agronomic performances, lycopene variability and some quality parameters to identify the genotypes having high yielding and better quality performances. The genotypes Roma VF, Cochoro/Pace setter), Pirson, Melkashola/Red pear and Fetan/Picador showed over all superior yield and quality performances to the other genotypes, thus they could be recommended as promising varieties for production in the area. The result indicates the presence of enough variability among the genotypes to select parents with desirable performances and combine with varieties having better lycopene contents for further genetic improvement.

Reddy *et al.* (2013) conducted study on nineteen exotic collections of tomato. Genetic divergence analysis following Mahalanobis D^2 statistics revealed considerable genetic diversity among 19 genotypes of tomato (*Solanum lycopersicum* L.) for all the eighteen quantitative characters which was pertaining to the growth, earliness, yield and quality. Fruit weight, plant height and number of fruits per plant contributed 92.40% to the total divergence. Appreciable diversity within and between the clusters was observed. The characters fruit weight, number of fruits per plant and plant height were the potent factors in differentiating the germplasm of tomato under study. Five clusters were fanned from the D^2 analysis using Tocher's method. Cluster II topped with maximum number of

genotypes among cluster fanned, while maximum inter-cluster distance was observed between cluster III and IV followed by cluster IV and V.

Nwosu *et al.* (2014) studied the extent of genetic variability among 19 accessions of tomato (*Solanum* spp.) using genetic variability parameters as a basis for harnessing of the crop. High significant differences among the accessions for all attributes studied. Cluster analysis based on 37 agro-morphological attributes separated accessions into two distinct groups according to the fruit types i.e. cherry and classic fruit types. Values for genotypic and phenotypic coefficients of variation showed variability among the accessions. Correlation analysis showed fruit per plant is positively and significantly correlated to plant height, number of branches per plant and leaf length. Very high genetic advance and heritability estimates for leaf length, leaf width, days to flower, days to 50% flowering, number of fruits per plant, fruit length, fruit diameter, fruit weight and 1000 seed weight suggest simple inheritance system and thus amenability for these attributes to selection in tomato improvement.

Meitei *et al.* (2014) investigation was carried out to study the genetic variability for yield and yield attributes and to screen genotypes for resistance to early blight disease of tomato (*Solanum lycopersicum* L.). The pooled analysis of variance revealed significant variation among the genotypes studied for all the characters except primary branches plant. The genotype H-24 was found to be the best among the genotypes 1 for yield (1309.50g plant) and fruit cluster plant (37.33) and ultimately resulted in highest yield/ha (401.64q). The genotype 2012/TOLCVRES-3 had significantly highest for single fruit weight (71.33g). Another genotype, NDT-3 was the best for fruit diameter (6.47cm). Sel-35 had more fruits and fruit clusters plant. High GCV were observed for fruit cluster and fruits plant, single fruit weight, fruit yield plant and fruit yield /ha

Henareh. (2015) worked on thirty tomato landraces collected from North West of Iran with three commercial cultivars “Korall, Fauna and Peto Early CH” were studied in completely randomized design in four replications at the Kahriz Station of Agriculture and Natural Resources Research Centre of West Azerbaijan. Analysis of variance has shown the significant difference among genotypes for all the evaluated characters. The highest and lowest of heritability were observed for number of seeds per fruit and number of flowers per inflorescence respectively. In principal component analysis, the first five components clarified 77% of total variations in tomato germplasm. These five components were entitled fruit pH, yield, vegetative growth, fruit size and fruit maturing respectively.

In component yield, characters of fruit weight and fruit diameter had high positive coefficients. The cluster analysis located genotypes into five groups.

Sacco *et al.* (2015) experimented a collection of 123 genotypes from different geographical areas which was established with the aim of capturing a wide diversity. Eighteen morphological traits were evaluated, mainly related to the fruit. About 45% of morphological variation was attributed to fruit shape, as estimated by the principal component analysis, and the dendrogram of relatedness divided the population in subgroups mainly on the basis of fruit weight and locule number. Genotyping was carried out using the tomato array platform SolCAP able to interrogate 7,720 SNPs. In the whole collection 87.1% markers were polymorphic but they decreased to 44–54% when considering groups of genotypes with different origin. The neighbour-joining tree analysis clustered the 123 genotypes into two main branches. The STRUCTURE analysis with $K = 3$ also divided the population on the basis of fruit size. Six regions were evidenced in which candidate genes co-localized with 19 associated SNPs. In addition, 17 associated SNPs were localized in genomic regions lacking candidate genes. The identification of these markers demonstrated that novel variability was captured in our germplasm collection. They might also provide a viable indirect selection tool in future practical breeding programs.

Lekshmi *et al.* (2016) evaluated forty tomato genotypes in randomized block design with three replications at College of Agriculture, Vellayani, Kerala during October 2013 to April 2014. A wide range of variation was observed among the characters studied which have a great interest for polyhouse tomato breeding. Genetic divergence analysis was carried out using Mahalanobis D² statistics and the 40 tomato genotypes were grouped into eight clusters. Cluster I was the largest cluster with twenty four genotypes followed by cluster II with ten genotypes and all other clusters were solitary. The highest intracluster distance was noticed in cluster II followed by cluster I. The highest intercluster distance was observed between clusters VII and VIII, followed by clusters IV and VIII. Lycopene content and truss per plant had maximum contribution towards total divergence followed by fruit length, fruit weight and yield per plant.

2.4 MOLECULAR MARKERS

A genetic marker is a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait. With the advent of molecular markers, a new generation of markers has been introduced over the last two decades,

which has revolutionized the entire scenario of biological sciences. DNA-based molecular markers have acted as versatile tools and have found their own position in various fields like taxonomy, physiology, embryology, genetic engineering, etc. They are no longer looked upon as simple DNA fingerprinting markers in variability studies or as mere forensic tools. Ever since their development, they are constantly being modified to enhance their utility and to bring about automation in the process of genome analysis. The discovery of PCR (polymerase chain reaction) was a landmark in this effort and proved to be a unique process that brought about a new class of DNA profiling markers. This facilitated the development of marker-based gene tags, map-based cloning of agronomically important genes, variability studies, phylogenetic analysis, synteny mapping, marker-assisted selection of desirable genotypes, etc. Thus giving new dimensions to concerted efforts of breeding and marker-aided selection that can reduce the time span of developing new and better varieties and will make the dream of super varieties come true. These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analysed objectively.

RFLP

Restriction fragment length polymorphism. In molecular biology, the term restriction fragment length polymorphism, or RFLP, (commonly pronounced “rif-lip”) refers to a difference between two or more samples of homologous DNA molecules arising from differing locations of restriction sites, and to a related laboratory technique by which these segments can be distinguished. In RFLP analysis the DNA sample is broken into pieces (digested) by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis. Although now largely obsolete, RFLP analysis was the first DNA profiling technique cheap enough to see widespread application. In addition to genetic fingerprinting, RFLP was an important tool in genome mapping, localization of genes for genetic disorders, determination of risk for disease, and paternity testing.

AFLP

Amplified Fragment Length Polymorphism PCR (or AFLP-PCR or just AFLP) is a PCR-based tool used in genetics research, DNA fingerprinting, and in the practice of genetic engineering. Developed in the early 1990’s by Keygene, AFLP uses restriction enzymes to cut genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments are then amplified using

primers complementary to the adaptor and part of the restriction site fragments (as described in detail below). The amplified fragments are visualized on denaturing polyacrylamide gels either through autoradiography or fluorescence methodologies. AFLP-PCR is a highly sensitive method for detecting polymorphisms in DNA.

RAPD

RAPD (pronounced "rapid") stands for Random Amplification of Polymorphic DNA. It is a type of PCR reaction, but the segments of DNA that are amplified are random. The scientist performing RAPD creates several arbitrary, short primers (8-12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction. No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. This makes the method popular for comparing the DNA of biological systems that have not had the attention of the scientific community, or in a system in which relatively few DNA sequences are compared (it is not suitable for forming a DNA databank). Because it relies on a large, intact DNA template sequence, it has some limitations in the use of degraded DNA samples. Its resolving power is much lower than targeted, species specific DNA comparison methods, such as short tandem repeats. In recent years, RAPD has been used to characterize, and trace, the phylogeny of diverse plant and animal species.

Rajput *et al.* (2006) did experiment involving screening of 60 RAPD primers and was used to confirm the reproducibility. Every experiment was performed 50 times by 10 candidates in which the reproducibility of two popular molecular marker techniques was examined: random amplified fragment length polymorphism (RAPD), and simple sequence repeat (SSR) For each technique, an optimal system was chosen, which has been standardized and routinely used by one individual. This system (genetic screening package) was distributed to different candidates in laboratory and the results obtained were compared with those of the original generator or sender. Different experiences were gained in exchange with this experiment with the different techniques. RAPDs proved difficult to reproduce. Whilst SSR alleles were amplified by all candidates, but small difference in their sizing were obtained.

Filimon *et al.* (2011) used RAPD markers to evaluate genetic diversity among 8 common bean cultivars (*Phaseolus vulgaris* L.) that included six developed in Romania and two in CIAT Colombia. Only 4 out of 8 random primers were used. Four primers (OPD-08, OPG-03, OPG-12, OPY-20) generated 33 DNA bands, of which 17 bands showed polymorphism (48.84%). On an average, each primer generated 8.25 bands, of which 4.25 were polymorphic. Cluster analysis based on RAPD amplification products, divided genotypes in two main groups according to their geographical origin. Genetic similarity varied greatly (60% - 96%), depending on the pairs of genotypes and on the groups.

Ahmad *et al.* (2012) assessed the genetic diversity of *Cicer arietinum* 70 accessions from Pakistan and USA using morphological traits, seed protein and molecular markers. Based on four morphological traits, the average coefficient of variation was calculated as 56.8% with significant correlation among yield traits. The analysis revealed that the accessions 1898, 2819, 3022, 3037, 3040, 3043, 3054, 3059 and 3063 were best in performance with a total of 12% environmental error. The cluster analysis based on protein data revealed 50% genetic diversity among accessions. The clustering pattern did not show any grouping that could be attributed to either the geographic distribution or the field performance. For molecular characterization of germplasm 5 PCR based RAPD primers, OPA4, OPA9, OPG13, UBC181 and UBC733b used were found to be polymorphic with 37% genetic diversity among local and exotic accessions. Whereas, 3 SSR primers viz., CaSTMS2, CaSTMS15 and CaSTMS21 scored the genetic variability up to 55% by cluster analysis through UPGMA percent disagreement. The primers, TA72 and TA130 were linked with yield related traits, indicated highest dissimilarity index value (0.69) and notable variation in the identified promising lines. The Morphometric, Biochemical and Molecular markers reported here, are helpful to assess the extent of genetic diversity among Chickpea accessions and can be used to identify the unreported cultivars with desirable quantitative traits for improving Chickpea yield in Pakistan. Based on the study, the accessions 3043 and 3054 have been recommended to the breeders for their future use in multiplication and screening against various diseases.

Naz *et al.* (2013) selected a total of 25 high yielding tomato accessions for the characterization that helped in the reliable varietal selection programme for breeding. All tomato accessions were analyzed by two parameters e.g. morphological and molecular parameters. For morphological characterization the plant height, shape, size, leaf length and width, and fruit size, colour and shape were selected and for molecular

characterization RAPD markers were used. A total 25 RAPD decamer primers were selected for the genetic analysis of all tomato accessions. Only 15 polymorphic RAPD primers were accessed for the genetic distance calculation to find out the phylogenetic relationship among 25 tomato accessions under study. A total of 130 loci were generated out of which 98 were polymorphic by 15 primers with 05-14 loci/primer having fragment's size range from 400 to 2500bp maximum. The Nie and Lie's Coefficients was used to calculate the genetic similarity. The average genetic similarity observed across all the genotypes was 75.6% with 24.4% polymorphism in 25 tomato accessions. Although RAPD study supports the morphological characters but not upto 100%.

Ariyaratna *et al.* (2013) investigated the genetic diversity of 30 bean germplasm (*Phaseolus vulgaris* L.) using Random Amplified Polymorphic DNA (RAPD) markers. A total of 5 random primers were used which identified 18 polymorphic bands, having an average of 3.6 bands/primer. The germplasm were divided into two main clusters on the basis of the Jaccard similarity coefficient and Unweighted Pair Group Method and Arithmetic Average (UPGMA). The average genetic distances between germplasm were calculated on the basis of marker frequencies. These distances were used for grouping the germplasm by UPGMA resulting in two main groups in dendrogram. These two groups may have a relationship with two centres of origin of bean namely Middle American and Andean. It was concluded that RAPD markers could be used as an alternative or supplementary tool to prevailing methods for the evaluation and classification of bean genetic resources in Sri Lanka.

Pal *et al.* (2013) analyzed *Solanum lycopersicum* F1 hybrids and its parents (male and female) with 22 different decamer primers to identify the band pattern which could differentiate between the parents and hybrids. Out of these 22 RAPD primers only 5 primers, OPA-05, OPA-09, OPN-14, OPG-04 and OPK-02 showed polymorphic bands between male, female and hybrids. So molecular marker tools can be effectively used to find out such contaminations in DNA polymorphism of respective hybrids.

Sharifova *et al.* (2013) carried out Random Amplified Polymorphic DNA (RAPD) analysis on 19 Azerbaijan tomato genotypes, both cultivars and local populations. A total of 26 amplified products were revealed by 6 primers. The genetic similarity among evaluated genotypes ranged from 0.188 to 1.000. The lowest similarity was observed between cultivars 'Azerbaijan' and 'Shakar' (0.188), while the highest between 'Elnur' and 'Garatag' (1.000). The first group was the largest and consisted of 12 genotypes, while the fourth group was the smallest consisted of 1 genotype only. The most

polymorphic primer was OPB-18 that presented a genetic diversity index of 0.823, while the least informative was primer OPG-17 with an index of 0.349. The average genetic diversity calculated from RAPD data was 0.665.

Vishwanath *et al.* (2013) applied various marker techniques, either individually or in combination to study the genetic diversity of this crop. They revealed the usefulness of different markers system for analyzing the genetic diversity and relation between different varieties and to find out correlation between marker systems revealed that all tested tomato cultivars could be differentiated from each other based on either morphological/protein/RAPD markers individually, and can be applied for grouping of cultivars, pedigree analysis and genetic diversity analysis. However, markers system used in this study showed variations in understanding the genetic relation between studied varieties. In a study conducted to evaluate reproducibility of two marker systems by Rajput *et al.* (2006) the SSR markers were reported to be reproducible while RAPDs were difficult to reproduce.

Thamir *et al.* (2014) conducted a study to evaluate the genetic diversity among 19 tomato varieties (determinate and indeterminate) cultivated in Iraq using polymerase chain reaction based DNA markers (PCR based DNA markers) ; Random Amplified Polymorphic DNA (RAPDs). The first small major clusters included four (4 varieties) while the second large major cluster included (15 varieties). The overall analysis of the result showed that RAPDs markers are powerful tool in fingerprinting and revealing the genetic relationships among tomato varieties. The relationship among varieties was not concern to their morphological characters and geographical origins.

Shah *et al.* (2015) did molecular characterization of 21 tomato genotypes using random amplified polymorphic DNA (RAPD) markers. Total 102 bands were amplified among 21 genotypes using 20 RAPD primers. Overall 73.5% polymorphism was shown as 75 out of 102 loci were polymorphic. High degree of divergence between varieties was indicated by low level of monomorphic bands. The number of PCR products per primer varied from 2-8 with an average of 5.1 bands per primer. Primer GL J-20 and GL C-09 produced maximum number of bands whereas the primers GL A-09 produced the lowest. The polymorphism per RAPD primer ranged from 50% to 100% with an average of 73.5%. The accumulative analysis of amplified products generated by RAPD's was enough to assess the genetic diversity among the genotypes. The information would be helpful for formulating future breeding and genome mapping programs. This study will

also work as an indicator for tomato breeders to evolve varieties with genetic diverse background to achieve sustainability in tomato production in the country.

Aida *et al.* (2015) carried out work in the Agricultural Genetic Engineering Research Institute (AGERI) in Cairo, Egypt to study genetic relationship between three landraces of tomato (*Solanum lycopersicum*) from Sudan in relation to a heat sensitive commercial genotype, RAPD markers using 16 primers were used to detect DNA polymorphism. The 16 primers gave polymorphism percentage for each single primer range between 0 –83% with a total polymorphism percentage reaching 39%. Primers OPB-0 2, OPA-10 and OPB-20 gave the highest polymorphism percentage in a range of 71-83% while OPC-07 did not give any polymorphic fragments. The dendrogram showed that Toktuk and Abu Zarif belong to the same group with a genomic DNA similarity reaching 91%. While similarity between this group and Strain B was 88%. HSD 0977 (The cherry tomato) belongs to separate group related to the main group that correlates the three other genotypes with a percentage of 85%.

Rai *et al.* (2016) carried out experiment to study the phylogenetic relationships of 10 selected tomato genotypes using random amplified polymorphic DNA analysis. Significant differences ($P \leq 0.05$) were observed among the tomato lines for the principal antioxidants phytonutrients, viz. total carotenoids, lycopene and vitamin C. Vitamin C content ranged from 15.82-31.93 mg/100 g in fresh weight, the total carotenoid content ranged from 4.92-7.66 mg/100 g, and lycopene content ranged from 3.33-5.66 mg/100 g. Significant variation ($P \leq 0.05$) was also observed for pH and anhydrous citric acid (acidity). The pH varied from 3.70-4.46 and anhydrous citric acid ranged from 0.267-0.56%. The total soluble solids varied from 2.50-4.66%. The maximum Vitamin C content, Acidity was recorded in 2012/TOMATO Hyb DET AVT-3 (31.93 mg/100 g) whereas maximum total carotenoid content were recorded in 2012/TOMQTO AVT DET-3 (7.66 mg/100 g). Maximum lycopene content was estimated in 2012/TOMQTO AVT DET-8 (5.68 mg/100 g). Out of 10 primers screened, only four random primers gave reproducible polymorphic DNA bands. A total number of 35 amplified DNA bands were generated across the studied line with average of 8.75 bands/primer. Out of 35 bands, 26 bands were polymorphic. Cluster analysis based on UPGMA divided the tomato lines into 3 distinct clusters. In cluster I five tomato lines, in cluster II two tomato lines and in cluster III three tomato lines were observed. It could be concluded that, RAPD markers are important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied varieties of tomato lines.

SSR

The term microsatellites, invented by Litt & Luty (1989) and also known as Simple Sequence Repeats (SSR), are sections of DNA that consist of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units. These are arranged throughout the genomes of most eukaryotic species (Powell *et al.*, 1996). SSRs are short cycle repeats with 1 to 10 bp, most classically, 2-3 bp. They are extremely variable and consistently circulated throughout the genome. SSR polymorphisms can be visualised by agarose or polyacrylamide gel electrophoresis (Dean *et al.*, 1999). Microsatellite alleles are one of alternative forms of a gene that can exist at a single locus which can be noticed, using numerous methods: ethidium bromide, silver staining, radioisotopes or fluorescence. These markers are simply automated, highly polymorphic, and have good analytical determination, thus making them a preferred choice of markers (Matsuoka *et al.*, 2002).

Benor *et al.* (2008) conducted study to determine the genetic diversity of 39 determinate and indeterminate tomato inbred lines collected from China, Japan, S. Korea, and USA. Using 35 SSR polymorphic markers, a total of 150 alleles were found with moderate levels of diversity, and a high number of unique alleles existing in these tomato lines. The mean number of alleles per locus was 4.3 and the average polymorphism information content (PIC) was 0.31. 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. The most similar inbred lines are from USA, both with determinate type, whereas the most different lines are from USA (Us-16) and Japan (Ja-2) with determinate and indeterminate growth habit, respectively. 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.

Mazzucato *et al.* (2008) studied 50 tomato landraces (mainly collected in central Italy), nine vintage and modern cultivars, and two wild out groups grown at two locations in central Italy and characterized for 15 morpho-physiological traits and 29 simple sequence repeat (SSR) loci. The markers were selected to include a group of loci in regions harbouring reported quantitative trait loci (QTLs) that affect fruit size and/or shape (Q-SSRs) and a group of markers that have not been mapped or shown to have a prior known linkage (NQ-SSRs). As revealed by univariate and multivariate analyses of

morphological data, the landraces grouped according to vegetative and reproductive traits, with emphasis on fruit size, shape and final destination of the product.

Pritesh *et al.* (2010) employed twenty three SSR primers to scan 25 tomato cultivars with determinate, indeterminate habit and different geographical locations to determine genetic similarity, unraveling of genetic diversity and genetic relationship.

Milevska *et al.* (2011) did experimental studies to evaluate 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 markers (LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LE20592, TMS9, LE2A11 and LECHSOD). Fragment analysis was conducted using an Applied Biosystems DNA analyzer (ABI 3130) and GeneMapper® Software program. The data were analyzed using Power Marker Software and MEGA3 programs. The results showed that the lowest genetic distance (16.7415) was identified between var. cerasiforme (yellow) and var. cerasiforme (red), and the largest (34.9859) between var. pyriforme and var. grandifolium. On the other hand, the lowest genetic distance (22.1446) was observed between subsp. subspontaneum and subsp. spontaneum, and the largest (29.7147) between subsp. subspontaneum and subsp. cultum. Precise dendrograms were created based on the statistical data.

Awady *et al.* (2012) amplified 38 alleles of Tomato cultivars using SSR primers with scorable fragment sizes ranging from approximately 75 to 275 bp. 23 alleles were polymorphic thus revealing 60.5% of polymorphism. The genetic similarity estimated according to SSR data was scaled between 17.6 and 93.2%, suggesting the potential of SSR markers in discriminating among plants of close or distant genetic backgrounds. The genetic distance information obtained in this study might be useful to breeder for planning crosses among these cultivars.

Hu *et al.* (2012) did experiment on twenty-six morphological traits as well as 47 single nucleotide polymorphism and simple sequence repeat markers to investigate genetic variation in 67 tomato (*Solanum lycopersicum* L.) varieties collected from Argentina between 1932 and 1974. Approximately 65.0% of the morphological traits and 55.3% of the molecular markers showed polymorphisms in the 67 varieties. Average taxonomic distance between any two varieties ranged from 0.6643 to 1.1776, while Nei's genetic distance varied from 0 to 0.2022. Cluster analysis indicated that 67 varieties could be

grouped into three clusters at both morphological and molecular levels. The varieties collected before 1960 had larger genetic variation than those collected after 1960.

Parmar *et al.* (2013) introduced a new SSR marker (TOM-144) which was deduced after evaluation of eight microsatellite loci amongst the twenty-one different tomato cultivars. The marker selected was inherited and segregated in mendelian fashion as demonstrated in successive generation of a cross between parents H-24 x GT-2.

Shah *et al.* (2013) evaluated the genetic diversity within in a diverse collection of rice accession for determining the differences in the pattern of diversity within aromatic and non aromatic varieties. Forty two rice accessions were evaluated by means of 24 SSR markers and 66 alleles were detected at 24 SSR loci. Dendrogram based on cluster analysis grouped the 40 varieties into three groups effectively differentiating aromatic and non- aromatic cultivars which could be useful for monitoring purity, genotype identification and for plant variety protection.

Shabib *et al.* (2013) in their study selected eleven bean varieties, nine of *P. vulgaris* L., one of *Phaseolus coccineus*, and one of *Phaseolus lunatus* namely: Bean CORA, Judia cilena, Diamant, palati, Distinction, Contender, Fagiolo Nano, Ambra, Bronco, Merit, Lima bean were selected SSR markers that were relatively more efficient in detecting similarities among Pharsalus genotypes studied with similarities up to 95%. SSR profiling provides useful information on the level of polymorphism and genetic diversity in common bean.

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

Korir *et al.* (2014) studied the genetic diversity and relationship of 42 tomato varieties sourced from different geographic regions with EST-SSR markers. The genetic

diversity was between 0.18 and 0.77, with a mean of 0.49; the polymorphic information content ranged from 0.17 to 0.74, with a mean of 0.45. This indicates a fairly high degree of diversity among these tomato varieties. Based on the cluster analysis using unweighted pair-group method with arithmetic average (UPGMA), all the tomato varieties fell into 5 groups, with no obvious geographical distribution characteristics despite their diverse sources. The principal component analysis (PCA) supported the clustering result; however, relationships among varieties were more complex in the PCA scatter plot than in the UPGMA dendrogram. This information about the genetic relationships between these tomato lines helps distinguish these 42 varieties and will be useful for tomato variety breeding and selection. We confirm that the EST-SSR marker system is useful for studying genetic diversity among tomato varieties. The high degree of polymorphism and the large number of bands obtained per assay shows that SSR is the most informative marker system for tomato genotyping.

Zhou *et al.* (2015) studied morphological traits and molecular markers to assess the genetic diversity of 29 cultivated tomatoes, 14 wild tomatoes and seven introgression lines. The three components of the principal component analysis (PCA) explained 78.54% of the total morphological variation in the 50 tomato genotypes assessed. Based on these morphological traits, a three-dimensional PCA plot separated the 50 genotypes into distinct groups, and a dendrogram divided them into six clusters. Fifteen polymorphic genomic simple sequence repeat (genomic-SSR) and 13 polymorphic expressed sequence tag-derived SSR (EST-SSR) markers amplified 1115 and 780 clear fragments, respectively. Genomic-SSRs detected a total of 64 alleles, with a mean of 4 alleles per primer, while EST-SSRs detected 52 alleles, with a mean of 4 alleles per primer. The polymorphism information content was slightly higher in genomic-SSRs (0.49) than in EST-SSRs (0.45). The mean similarity coefficient among the wild tomatoes was lower than the mean similarity coefficient among the cultivated tomatoes. The dendrogram based on genetic distance.

MATERIALS AND METHODS

The present investigation entitled "**Assessment of genetic diversity in cultivated tomato (*Solanum lycopersicum* L.) genotypes using morphological and molecular markers.**" was undertaken at the Experimental Farm and Molecular Biology Laboratory of School of Biotechnology, SKUAST-J, Main Campus, Chatha, Jammu during 2016.

A replicated experiment consisting of 24 genotypes of Tomato was conducted in Randomized Block Design (RBD) with three replications in which 21 days old seedlings were transplanted in 2 rows of 5m length with a spacing of 45 x 90 cm. All the agronomic and plant protection practices as applicable for commercial tomato crop were adopted. In each genotype, 5 plants were selected for various observations while molecular markers were used to assess the prevalent diversity.

Materials and methodology adopted in this study are elucidated under the following headings:

- 3.1 Material used
- 3.2 Methodology adapted
 - 3.2.1 Experimental Data
 - Morpho-physiological characters
 - Phenological characters
 - Yield components
 - Quality attributes
- 3.3 Statistical Analysis
 - 3.3.1 Analysis of variance
 - 3.3.2 Genetic parameters
 - 3.3.3 Multivariate and clustering analysis
- 3.4 Molecular characterization
 - 3.4.1 Diversity analysis
 - 3.4.1.1 Genomic DNA Isolation
 - 3.4.1.2 DNA Quantification
 - 3.4.1.3 PCR amplification
 - 3.4.1.3.1 Primers used for DNA amplification
 - 3.4.1.3.2 Components used for PCR reaction
 - 3.4.1.3.3 PCR amplification program

3.4.1.4 SSR-PCR Banding Profile

3.4.1.5 Scoring of Bands

3.4.1.6 Diversity Analysis

3.1 Materials used

The experimental material for the study comprised of 24 genotypes of Tomato (*Solanum lycopersicum* L.), which were grown in a Randomized Block Design (RBD) with three replications in 2 rows of 5m length with plot spacing of 45 x 90 cm.

The material used in this study were taken from Indian Institute of Vegetable Sciences (IIVR), Varanasi and list is given in table below:

Table 1: Genotypes of Tomato used for study

S.No	NAME OF GENOTYPES	SOURCE
1.	UTKAL PRAGYAN	IIVR, VARANASI
2.	HISAR LALIT	IIVR, VARANASI
3.	KASHI HEMANT	IIVR, VARANASI
4.	FEB-2	IIVR, VARANASI
5.	DCT-1	IIVR, VARANASI
6.	CO-3	IIVR, VARANASI
7.	ARKA ABHAY	IIVR, VARANASI
8.	SWARNA SAMPADA	IIVR, VARANASI
9.	ANGHA(L-E415)	IIVR, VARANASI
10.	DHANSHRI	IIVR, VARANASI
11.	PUNJAB RATTA	IIVR, VARANASI
12.	PANT –T-5	IIVR, VARANASI
13.	HISAR ANMOL	IIVR, VARANASI
14.	AZAD-T-2	IIVR, VARANASI

15.	PT-11	IIVR, VARANASI
16.	NDTUR-73	IIVR, VARANASI
17.	BT-136	IIVR, VARANASI
18.	SEL-12	IIVR, VARANASI
19.	NDT-9	IIVR, VARANASI
20.	ANGHA-1	IIVR, VARANASI
21.	ANGHA	IIVR, VARANASI
22.	NDT-1	IIVR, VARANASI
23.	ANAND TOMATO-3	IIVR, VARANASI
24.	NDT-4	IIVR, VARANASI

3.2 Methodology adopted

Recommended package of practices were followed for raising a good crop. Observations were recorded on the various morphological, agronomical, yield and quality traits in order to study the magnitude of variability and level of genetic divergence in the material. Five plants per plot per replication were randomly selected and tagged for recording the characters. Mean values for all the characters were worked out.

3.2.1 Morpho-physiological characterization

Five plants were randomly selected from each plot in each replication for observing the data. The characters included were:

1. Plant height (cm)

Plant height was measured just before last harvesting in centimeters from the ground level to the top of the primary branch on 5 random plants from the middle of the plot and data averaged.

2. Number of branches per plant

The numbers of branches which originate from the main stem were considered as primary branches. Total number of primary branches was counted at the time of last fruit harvest on 5 random plants from the middle of the plot and data averaged.

3. No of fruits per bunch

Number of fruits per bunch were counted at the time of first harvesting on 5 random plants.

4. Fruit length (cm)

Fruit length was measured in centimeters by the help of vernier calliper. The data were recorded on 10 randomly selected fruits in a plot and averaged.

5. Fruit width(cm)

Fruit width was measured in centimetre by the help vernier calliper. The data were recorded on 10 randomly selected fruits in a plot and average.

6. No. of fruit per plant

The number of fruits counted at each harvest and cumulative total of all the harvest were known as number of fruits per plant. The data were recorded on 5 randomly selected plants in a plot and averaged.

7. No. of locules per fruit

The number of locules were counted after cutting the fruit transversely and counting the locules isolated by septae. The data were recorded on 5 randomly selected fruits in a plot and averaged.

8. Total soluble solids (⁰Brix)

One drop of fresh tomato juice from fruit of each of 5 randomly selected plants from each replication was used to examine the TSS by hand refractometer in °Brix. The data recorded was averaged.

9. Flesh thickness(cm)

Flesh thickness was measured in centimetres from equatorial section of the fruit per by help of vernier caliper. The data were recorded on 5 randomly selected fruit bin plot and averaged.

10. Yield per plant(g)

Yield per plant was recorded from randomly selected plants from each replication and average was calculated.

11. Average fruit weight (g)

Average fruit weight was measured in gram. The data were recorded on 10 randomly selected fruits in a plot and averaged.

3.3 STATISTICAL ANALYSIS

The data recorded during the investigation was subjected to the statistical analysis as follows:

3.3.1 Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) for all characters was carried out separately (Table 2).

Table 2: Components of variance and their expectations

Source of variation	D.f.	Mean sum of squares (M.S.S)	Expected values of M.S.S.
Replication (r)	r-1	M_1	
Genotypes (g)	g-1	M_2	$\sigma_e^2 + \sigma_g^2$
Error	(r-1) (g-1)	M_3	σ_e^2
Total	rg-1	$M_1 + M_2 + M_3$	

Where, r = number of replications

g = number of genotypes

M_1 = mean sum of squares due to replications

M_2 = mean sum of squares due to genotypes

M_3 = mean sum of squares due to error

3.3.2 Genetic parameters

i) Genotypic and phenotypic coefficient of variation

Both genotypic and phenotypic coefficients of variability were computed as per the method suggested by Burton and De vane (1953).

Genotypic coefficient of variability (GCV)

$$\text{GCV (\%)} = \frac{\sigma_g}{\bar{X}} \times 100$$

Phenotypic coefficient of variability (PCV)

$$\text{PCV (\%)} = \frac{\sigma_p}{\bar{X}} \times 100$$

Where,

σ_g = Genotypic standard deviation

σ_p = Phenotypic standard deviation

$\bar{X}$ = General mean of the character

GCV and PCV values were categorized as low, moderate and high as indicated by Sivasubramanian and Menon (1973). It is as follows

0-10% : Low

10-20% : Moderate

20% and above : High

ii) Heritability

It was estimated as per the procedure presented by Burton and De Vane (1952), Jhonson *et al.* (1955) and Hanson *et al.* (1956).

$$\hat{h}^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_p^2}$$

Where,

$\hat{h}^2$ = Estimate of heritability in broad sense,

$\hat{\sigma}_g^2$ = Estimate of genotypic variance, and

$\hat{\sigma}_p^2$ = Estimate of phenotypic variance

The heritability was categorized as low, moderate and high :

0-30% : Low

30-60% : Moderate

60% and above : High

iii) Genetic advance

Genetic advance at 5 per cent selection intensity was worked out using the formula given below:

$$G.A. = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_p^2} \times (\hat{\sigma}_p^2)^{0.5} \times K$$

Where, G.A. = Genetic advance of the trait,

$\hat{\sigma}_g^2$ = Genotypic variance of the trait,

$\hat{\sigma}_p^2$ = Phenotypic variance of the trait, and

K = Selection differential, with value of K at 5 percent selection intensity being 2.06

3.4 Molecular characterization

3.4.1 Methods adopted

The present investigation was carried out in under different stages of experimentation.

3.4.1.1 Assessment of genetic diversity using SSR and RAPD markers

3.4.1.1.1 Genomic DNA isolation

The total genomic DNA was isolated from each genotype and is done by cetyl trimethyl ammonium bromide (CTAB) method, (Clarke, 2009) with certain modifications. The genotypes were grown at the Experimental Farm of School of Biotechnology, SKUAST -J, Chatha for 2-3 weeks and fifteen days old fresh leaves were taken for genomic DNA extraction. 5gm of the plant tissue were taken and grinded in liquid nitrogen by using pestle and mortar for fine powder and then put this fine powder into 2 ml of eppendorf tube which contain 800µl of extraction buffer (CTAB buffer). Put the eppendorf tubes in water bath which should be set at 65°C and shake it well after 10 minutes. An equal volume of Chloroform:Isoamylalcohol (24:1) was added to the tube and slowly mixed by inverting the tubes for 10 minutes. The samples were centrifuged at 8,000 rpm for 10 minutes. Transfer the supernatant (upper phase) into fresh tubes and again treated with Chloroform: Isoamyl alcohol (24:1), mixed slowly for 10 minutes and centrifugation was done. 0.6 volume of chilled Isopropanol was added to the supernatant and stored at 4°C for 1-2 hours. Centrifugation was done at 10,000 rpm for 10 minutes at 4°C. The supernatant was discarded and the pellets were washed with 70% ethanol and then centrifuged at 10,000 rpm for 5 minutes and then air dried the pellet. 200µl of 1xTEbuffer was added to dissolve the pellet and stored at 4°C. RNase treatment was given by added 2µl of RNase (10mg/ml) was added to the samples and incubated at 37°C for 1 hour in water bath. For purification of genomic DNA, an equal volume of Phenol: Chloroform : Isoamyl alcohol (25:24:1) was added and gently mixed for 10 minutes and spined at 13,000 rpm for 10 minutes, Then supernatant was collected in another tube and again added equal volume of C :I (Chloroform: Isoamyl alcohol). Spin for 10 minutes at 13,000 rpm and then added 0.6 volume of ice chilled pure ethanol and kept it at refrigerator for 10 minutes. Centrifuged at 7000 rpm for 5 minutes pelleting. The DNA pellet was washed with 70 % ethanol and then spined at 7000 rpm for 5 minutes, air dried and then dissolved in 100µl of 1xTE (Tris-cl, EDTA) buffer and stored at 4°C for further use.

3.4.1.1.2: DNA Quantification

DNA was quantified by using nanodrop and agarose gel electrophoresis.

3.4.1.1.2.1: Nanodrop method

The DNA was quantified by using thermoscientific Nano drop spectrophotometer. Firstly, set the instrument blank with DEPC water. Put 2µl of DNA sample into the receiving fibre of the instrument and then source fibre brought in contact with the end of receiving fibre and then measure for taking the reading. Note down the concentration (ng/µl) at OD_{260/280} value of the sample. If the OD value is between 1.8-2.0, then the DNA is pure. If the OD value is above 2.0, then there is protein contamination and if it is below 1.8 then RNA contamination.

3.4.1.1.2.2 Agarose gel electrophoresis

The DNA was quantified by loading 2µl of DNA mixed with 2µl of loading dye (Bromophenol blue) and 6µl of ddH₂O into separate wells on 0.8% agarose gel. For the preparation of agarose gel, weigh 0.8gm of agarose and put it in 100 ml TBE (Tris, Borate EDTA, 1x) buffer and heat it in microwave for 3 minutes. The mixture was allowed to cool for few minutes and then add 6 µl of ethidium bromide for visualization and stirred for some time. The gel was then poured into the casting tray with combs in it and then allowed it to polymerize at room temperature for 20-25 minutes. DNA ladder were also loaded to estimate the concentration of DNA in each sample. The electrophoresis was carried out at 75V for 1 hour. The gel was then viewed under Bioimaging system. The concentration of DNA was determined by comparing the intensity of bands with that of 100 bp molecular ladder quality was indicated by having intact band.

3.4.1.1.3 SSR amplification:

3.4.1.1.3.1 Primers used for DNA Amplification

Nine SSRs primers were selected from the map of tomato genome for the amplification of genomic DNA.

Table 3. List of SSRs primers used for DNA amplification

PRIMER	SEQUENCE	ANNEALING TEMPERATURE
AI773078	F:GATGGACACCCTTCAATTTATGGT R:TCCAAGTATCAGGCACACCAGC	54
AI778183	F:GCGAAGAAGATGAGTCTAGAGCATAG R: CTCTCTCCCATGAGTTCTCCTCTTC	54
AW037347	F: GCCACGTAGTCATGATATACATAG R: GCCTCGGACAATGAATTG	49
Y09371	F: TGAGAACAACGTTTAGAGGAGCTG R: CGGGCAGAATCTCGAACTC	53
SSR 241	F: TCCTCGCTAATTGATCCACC R: TCAACAGCATAGTGGAGGAGG	52
X90770	F: TGTAGATAACTTCCTAGCGACAATC R: ACGGACGGATGGACAAATG	51
SSR20	F: GAGGACGACAACAACAACGA R:GACATGCCACTTAGATCCACAA	55
SSR74	F: ACTCACCATGGCTGCTTCTT R:TTTCTTGAAGGGTCTTTCCC	52
X90937	F:TGCCCATGACGTTCCATC R:GACAGACAGAGAGACAGATTTAGAG	55
SSR 9	F:CCCTTTGCAAGTTCTTCTTCA R:TTCATGAGCCAACATAGGAGG	51

Primer Dilution

For primer dilution, we used double distilled and autoclaved water to dilute the primers at a concentration of 10pmol.

3.4.1.1.3.1 Components used for PCR Reaction

DNA amplification was carried out in 0.2ml PCR tubes containing 25 µl reaction mixture. The components which were used for PCR reaction are 3µl of template DNA (25ng/µl), 1.5 µl of 10x PCR buffer without MgCl₂, 1.5 µl of MgCl₂(mM/ µl), 2.5 mM of each dNTPs (dTTPs, dGTPs, dCTPs, dATPs), primer (0.1Mm) concentration, 5 units Taq DNA polymerase. The quantity of these components used in a reaction is given in Table 3

Table 4: Reagents with their concentration and quantity used for single PCR reaction

S.No.	Reagents	Concentration	Quantity
1.	DNA template	25 ng/μl	3μl
2.	Sterile water		10.5 μl
3.	PCR Buffer	10 X	2.5 μl
4.	MgCl ₂	50Mm/ μl	1.5
5.	DNTPs	2.5 mM/ μl	2μl
6.	Primer	10pmole	5μl
7.	Taq polymerase	5 U/μl	0.5μl
Total			25 μl

3.4.1.1.3.2 PCR Amplification Program

PCR tubes containing master mix and DNA template were thoroughly mixed and subjected to the thermal profile given in Table 4. The amplification reaction was carried out in a gradient Thermocycler. An initial denaturation step of 4 minutes was programmed in the thermocycler, followed by a loop of 35 cycles each consisting of denaturation (at 94⁰C for 1 minute), annealing (49°C-55°C for 1 minute) and extension (at 72⁰C for 2 minutes). The final extension was performed at 72⁰C for 7 minutes. The PCR products were then stored at 4°C for 10 minutes.

Table 5: Thermal profiles used for DNA amplification

Steps	Cycles	Temperature	Duration
Initial Denaturation	1	94 ⁰ C	4 min
Denaturation		94 ⁰ C	1 min
Annealing	35	49-55°C	1 min
Extension		72 ⁰ C	2 min
Final Extension	1	72 ⁰ C	7 min

3.4.1.1.4 SSR-PCR banding Profile

PCR products were separated on 3% agarose gel electrophoresis. For the preparation of 3% gel, weighed 3gm of agarose with the help of weighing balance and put it in the flask containing 100ml 1xTE buffer and this mixture was heated in microwave for 2-3 minutes and allowed it to cool for some time and then add 5μl of ethidium bromide

(EtBr) which is used for visualization. The mixture was poured into casting tray and allowed it to polymerise at room temperature for 20-25 minutes. 5µl of loading dye (Bromophenol blue) was added in each PCR tubes and mixed it gently. Then 10 µL of PCR product was loaded in each well of the gel. 100bp DNA ladder were also loaded which is used as the molecular marker for determining the product size of SSR primers. Electrophoresis were carried out at 80v for 3-4 hours and then viewed under Biometra gel documentation unit.

3.4.1.1.5 Scoring of Bands

The SSR-PCR bands were examined under ultra violet transilluminator and photographed under gel documentation unit. The SSR bands were counted and scored as 1 for their presence or 0 for their absence. The sizes of the bands were estimated by using 100bp standard marker. The presence and absence of bands in all genotypes for 22 primers were used to generate Bi-nomial data using excel sheet. Bands were marked as present only if the DNA amplification produced the fragment of a particular sequence and absent if the DNA amplification lacked that fragment. The banding patterns of all genotypes against each primer were compared. Bands present in one genotype and absent in another genotype, were regarded as variable and used to score for polymorphism. In order to check the informativeness and discriminatory power of SSR primers utilized in this study, certain parameters like polymorphism percentage, polymorphic information content and number of alleles were calculated.

(a) Percentage Polymorphism

It was calculated by dividing the polymorphic bands by the total number of scored bands:

$$\frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

(b) Polymorphism Information Content (PIC value)

The markers with more alleles have larger polymorphism information content.

It was calculated as proposed by Roldan-Ruiz *et al.*, (2000)

$$\text{PIC} = 2f_i(1-f_i)$$

where, f_i = frequency of bands present

$1-f_i$ = frequency of bands absent

3.4.1.1.6 Diversity Analysis

The collected data were aligned for the construction of cluster analysis and similarity matrix. The cluster analysis of 24 genotypes was constructed with the help of NTSYS software based on Unweighted paired group of arithmetic mean average

(UPGMA). A tree like dendrogram was constructed using NTSYS software. Genotypes were divided in various clusters, sub-cluster and sub-sub clusters based on genetic diversity among them and linkage distance was calculated.

3.4.2 RAPD Amplification

3.4.2.1 Primers used for DNA amplification

A set of 13 arbitrary random 10-mer primers were selected for use in DNA amplification of genomic DNA. Based on earlier studies, RAPD's were selected. They were synthesized from IDT (Integrated DNA Technologies, USA).

Primer Dilution: Double distilled, autoclaved and deionized water was used to dilute the primers at a concentration of 5pmol as shown in Table 5.

TABLE 6: Arbitrary 10 mer primers with their sequence selected for DNA amplification

S.NO	PRIMER	SEQUENCE	ANNEALING TEMPERATURE
1	OPA-07	5' GAAACGGGTG '	31
2	OPA-13	5' CAGCACCCAC 3'	34
3	OPA-19	5' CAAACGTCGG 3'	31
4	OPA-02	5' TGCCGAGCTG 3'	37
5	OPC-04	5' CCGCATCTAC 3'	31
6	OPC-14	5' TGC GTGCTTG 3'	34
7	OPC-20	5' ACTTCGCCAC 3'	32
8	OPN-15	5' CAGCGACTGT 3'	32
9	OPO-20	5' ACCCGGTCAC 3'	36
10	OPQ-20	5' TCGCCCAGTC 3'	36
11	OPU-16	5' CTGCGCTGGA 3'	37
12	OPY-15	5' AGTCGCCCTT 3'	33
13	OPU-17	5' ACCTGGGGAG 3'	33

3.4.2.2 Components used for pcr reaction

DNA amplification was carried out in a PCR tubes containing 25 μ L reaction mixture. Reaction mixture contained 3 μ L of template DNA (25 ng/ μ L), 2.5 μ L of 10X PCR Buffer, $MgCl_2$ (2mM), 0.2 Mm of each dNTPs(dTTPs, dGTPs, dCTPs, dATPs), primer (5 pmol) concentration , 5 unit Taq DNA polymerase. The quantity of these components used in a reaction is given in Table 6.

Table 7. Reagents with their concentration and quantity used for single PCR reaction

S.No.	Reagents	Concentration	Quantity
1.	DNA template	25 ng/ μ l	3 μ l
2.	Sterile water		10.5 μ l
3.	PCR Buffer	10 X	2.5 μ l
4	$MgCl_2$	50Mm/ μ l	1.5
5.	DNTPs	2.5 mM/ μ l	2 μ l
6.	Primer	10pmole	5 μ l
7.	Taq polymerase	5 U/ μ l	0.5 μ l
Total			25 μl

3.4.2.3 PCR Amplification Program

PCR tubes containing master mix and DNA template were thoroughly mixed and subjected to the thermal profile given in table 4. The amplification reaction was carried out in a gradient Thermocycler. An initial denaturation step of 4 minutes was programmed in the thermocycler, followed by a loop of 35 cycles each consisting of denaturation (at 94⁰C for 1 minute), annealing (at 36-38⁰C for 1 minute) and extension (at 72⁰C for 2 minutes). The final extension was performed at 72⁰C for 10 minutes. The PCR products were then stored at 4⁰C for 10 minutes.

Table 8. Thermal profiles used for DNA amplification

Steps	Cycles	Temperature	Duration
Initial Denaturation	1	94 ⁰ C	4 min
Denaturation		94 ⁰ C	1 min
Annealing	35	36-38 ⁰ C	1 min
Extension		72 ⁰ C	2 min
Final Extension	1	72 ⁰ C	10 min

After the completion of PCR, the products were stored at 4⁰ C until the gel electrophoresis was done.

3.4.2.4 RAPD-PCR banding Profile

The amplification products were then subjected to electrophoretic separation using horizontal agarose gel electrophoresis. Agarose gel was prepared in 1X TBE buffer stained with ethidium bromide. 5µl of loading dye (Bromophenol blue) was added in each PCR tubes and mixed it gently and then loaded in separate wells. Then 10 µL of PCR product was loaded in each well of the gel. 100bp DNA ladder was also loaded which is used as the molecular marker for determining the molecular weight of RAPD based PCR bands. Electrophoresis was carried out at 80v for 2-3 hours. The gel was visually examined under UV and documented using gel documentation system.

3.4.3 Scoring Of Bands

RAPD-PCR bands were detected in the gel using gel documentation system. Bands were seen for their presence (1) or absence (0). All the scorable bands were considered as single locus/allele. Their sizes were estimated using 100bp standard marker or 1 kb ladder. The presence and absence of bands in all genotypes for 13 primers were used to generate bi-nomial data using excel sheet. Bands were marked as present only if the DNA amplification produced the fragment of a particular sequence and absent if the DNA amplification lacked that fragment. The banding patterns of all genotypes against RAPD primer were compared. Bands present in one genotype and absent in another genotype, were regarded as variable and used to score for polymorphism. In order to check the informativeness and discriminatory power of RAPD primers utilized in this study, certain parameters like polymorphism percentage, polymorphic information content, resolving power and marker index were calculated.

(a) Polymorphism percentage

It was calculated by dividing the polymorphic bands by the total no of scored bands and multiplied by 100.

$$\frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

(b) Polymorphism Information Content (PIC)

Average PIC indicates the ability of utilized markers to differentiate the genotypes. Maximum Polymorphic information content in case of dominant markers as RAPD is 0.5 when half of the accessions have band and the other half does not have the band.

It was calculated as proposed by Roldan-Ruiz *et al.*, (2000)

$$PIC = 2f_i(1-f_i)$$

where, f_i = frequency of bands present

$1-f_i$ = frequency of bands absent

(c) Resolving Power

It was calculated according to Prevost and Wilkinson, (1999) as elucidated below:

$$R_p = \sum I_b$$

Where R_p is the resolving power and

I_b is the band informativeness

$$I_b = 1 - [2 \times |0.5 - p|]$$

where p is the proportion of genotypes containing the band

(d) Marker Index (MI)

Marker index for each primer was calculated as proposed and used by Maras *et al.*, (2008)

$$MI = PIC \times \frac{\text{sum of polymorphic bands} \times \text{total bp}}{\text{Genotypes} \times \text{bp}}$$

3.4.4 Diversity analysis

Bi-nomial data matrix of all genotypes generated from 9 primers was subjected to the UPGMA (Un-weighted Pair Group Method with Arithmetic Averages) analysis and a dendrogram was constructed using NTYSISpc 2.0 software. Genotypes were divided in various clusters, sub-cluster and sub-sub clusters based on genetic diversity among them and their similarity co-efficient was calculated.

3.5 Reagents and Solutions

3.5.1 Reagents

(a) **Primers** : Arbitrary decamer primer were got synthesized from IDT (Integrated DNA Technologies, USA).

(b) **dNTPs** : (dTTPs, dGTPs, dCTPs, dATPs)

100 mM stock of each dNTP was diluted to 2.5 mM of dNTPS.

(c) **PCR Buffer 10 X**

3.5.2 Stock solutions

(A) Preparation of Stock solutions (500ml) for DNA extraction

a) 0.5 M EDTA 93.05 g

b)	1 M Tris-cl	60.55 g
c)	5 M Nacl	146.1 g

The pH of 0.5 M EDTA and 1 M Tris-cl was adjusted to 8.0. The solutions were autoclaved after making the final volume upto 500ml.

d) CTAB Buffer

0.5 M EDTA	20ml
1 M Tris-cl	50ml
5 M Nacl	140ml
ddH ₂ O	
β - mercaptoethanol	0.2%

The final volume of CTAB buffer was made up to 500ml using ddH₂O. It was then autoclaved after adjusting the pH to 8.0. β - mercaptoethanol (0.2%) was added immediately before use.

e) TE Buffer (10X)

1 M Tris-cl	100ml
0.5 M EDTA	20ml

880ml ddH₂O was added to make 1L TE Buffer. pH was adjusted to 8.0 and autoclaved.

f) RNase (10mg/ml) – 10ml

5 M Nacl	30 μ l
1 M Tris-cl	100 μ l
ddH ₂ O	9870 μ l
RNase	10mg

The solutions were boiled for 15 minutes, allowed to cool at room temperature and stored at 4°C.

(B) Preparation of Stock solutions for electrophoresis

a) Preparation of Buffers

1. TBE Buffer (10 X)

Tris Base	108g
Boric acid	55g
0.5 M EDTA (pH 8)	40ml

Double distilled water was used to make the final volume upto 1L, mixed with the help of magnetic stirrer and then autoclaved.

2. DNA loading Dye (6X)

Glycerol	30 % (v/v)
Bromophenol Blue	0.25 % (w/v)
Xylene cyanol	0.25 % (w/v)

3. Tank Buffer (1X TBE)

100 ml 10X TBE was dissolved in 900 ml double distilled water.

RESULTS

The present study “**Assessment of genetic diversity in cultivated tomato (*Solanum lycopersicum* L.) genotypes using morphological and molecular markers**” was conducted to estimate the genetic diversity /relatedness among the 24 genotypes of tomato using morphological and molecular markers. Information regarding genetic diversity and genetic relationships among different genotypes is very valuable in crop improvement. Analysis of genetic diversity is useful in selecting diverse parental combinations, reliable classification of accessions, and for exact identification of variety. The experimental results of the present study are presented below:

4.1. Phenotypic characters

In this study, 24 cultivars were evaluated for morphological characters as per the standard procedure. The description of the genotypes with respect to 11 characters viz. (1) Plant height (cm), (2) No. of branches, (3) No. of fruits per bunch, (4) Number of locules, (5) Fruit length (cm), (6) Fruit width (cm), (7) No. of fruits per plant, (8) Total soluble solids (⁰Brix), (9) Flesh thickness (mm), (10) Average fruit weight (gm) and (11) Yield per plant (gm) is presented in the Table 10.

4.1.1. Plant Height (cm)

The plant height data revealed significant variation among the tomato genotypes. The plant height ranged from 35.10 cm to 82.02 cm amongst the 24 tomato genotypes (Table 11) with the mean of 55.59 cm (Table 10). The maximum plant height was recorded in Punjab Ratta (82.02cm) followed by Hisar Anmol (79.55cm) (Table 10, Figure 1). The minimum plant height was recorded in Anand Tomato-3 (35.10cm) (Table 10).

4.1.2. Number of branches per plant

The number of branches per plant ranged from 4.53 to 9.66 amongst the 24 tomato genotypes (Table 11). The maximum number of branches per plant was recorded in NDT-4 (9.66) followed by Punjab Ratta (9.20) (Table 10, Figure 2). The minimum number of Branches per plant was recorded in Swarna Sampada (4.53), the mean of number of branches among the genotypes was observed i.e. 6.96 (Table 10).

4.1.3 Number of fruits per bunch

The number of fruits /bunch ranged from 2.03 to 3.66 amongst the 24 tomato genotypes. The maximum number of clusters per plant was recorded in Utkal Pragyan (3.66) followed by PT-11 (3.44). The minimum number of clusters per plant was recorded in Swarna Sampada (2.03) (Table 10, Figure 3). Mean value of number of fruits per bunch among the tomato genotypes was noted as 2.74 (Table 10).

4.1.4 Fruits per plant

It is evident from the data (Table 11) that the number of fruits per plant varied from 23 to 122 amongst the 24 tomato genotypes. The maximum number of fruits per plant was recorded in DCT-1 (115.33) (Table 10, Figure 4) while minimum number of fruits per plant was recorded in NDT-4 (23.00). Average number of fruits per plant was observed as 40.94 (Table 10).

4.1.5 Yield per plant

Data presented in Table 10 indicated large variation in yield per plant among tomato genotypes. The total yield per plant ranged from 861.40-2507.36g amongst 24 tomato genotypes (Table 11). The maximum total yield per plant was recorded in Hisar Lalit (2507.36) (Table 10, Figure 5). The minimum total yield per plant was recorded in DCT-1 (861.40) (Table 10).

4.1.6 Average fruit weight (g)

Data showing fruit characters of 24 tomato genotypes in Table 10 revealed that there was significant variation in tomato genotypes. The average fruit weight ranged from 7.43-80.00g (Table 11). The maximum single fruit weight was recorded in NDT-4 (80.00) (Table 10, Figure 6). The minimum single fruit weight was recorded in DCT-1 (7.43) (Table 10).

4.1.7 Number of locules per fruit

Perusal of data table 10 indicated that the number of locules per fruit ranged from 2.18 to 5.5 amongst the 24 tomato genotypes (Table 11). The maximum number of locules per fruit was recorded in Angha-1 (5.50) followed by Angha (5.33), Anand Tomato-3(3.33) NDT-4(3.33) and Pant-T-5(3.33) (Table 10, Figure 7) genotypes showed similar number of locules in tomato fruits. The minimum number of locules per fruit was recorded in Utkal Pragyan (2.18) (Table 10, Figure 7). Mean value of number of locules per fruit among the tomato genotypes was observed i.e. 3.57 (Table 10).

4.1.8 Flesh thickness (mm)

The pericarp thickness per fruit ranged from 1.21 to 4.31 amongst the 24 tomato genotypes (Table 11). The maximum pericarp thickness per fruit was recorded in Hisar Lalit and Dhanshri (4.31) followed by Utkal Pragyan (4.08). The minimum pericarp thickness per fruit was recorded in DCT-1 (1.21) (Table 10, Figure 8). The mean value of pericarp thickness was noted as 2.67mm (Table 10).

4.1.9 Fruit length (cm)

The fruit length ranged from 2.90-5.63 cm amongst the 24 tomato genotypes. The maximum fruit length was recorded in Hisar Lalit (5.63) followed by Utkal Pragyan (4.88). The minimum fruit length was recorded in DCT-1 (2.90) (Table 10, Figure 9). The average fruit length was recorded as 3.86 (Table 10).

4.1.10 Fruit width (cm)

The Fruit width ranged from 2.85-4.98cm amongst the 24 tomato genotypes (Table 11). The maximum fruit width was recorded in Dhanshri (4.98) followed by Arka Abhay (4.78cm). The minimum fruit width was recorded in DCT-1(2.85) (Table 10, Figure 10). The mean value of pericarp thickness was noted as 4.24cm among the 24 tomato genotypes (Table 10).

4.2 BIOCHEMICAL CHARACTERS

4.2.1 Total Soluble Solids (⁰Brix)

In present study, the total soluble solids (TSS) ranged between 2.83 to 8.06 ⁰Brix amongst the 24 tomato genotypes (Table 11). Maximum total soluble solids (TSS) was recorded in DCT-1 (8.06 ⁰Brix) followed by Anand Tomato-3 (7.00 ⁰Brix) (Table 10, Figure 11). The minimum total soluble solids (TSS) i.e.2.83 ⁰Brix was recorded in Dhanshri (Table 10).

4.3 Analysis of variance (ANOVA)

Analysis of variance was carried on various morpho-physiological, phenological, yield components and quality traits for studying the variation. ANOVA showed highly significant variation among the genotypes for all the characters. The analysis of variance for the data recorded on various traits viz. plant height, number of branches, number of fruits per bunch, total soluble salts, pericarp thickness, fruit length, fruit width, number of locules, average fruit weight, number of fruits per plant and yield per plant are presented in the Table 9.

Table 9: Analysis of Variance (ANOVA) for morphological traits in tomato genotypes

	DF	Plant Height (cm)	No. of branches	No. of fruits/ bunch	Total soluble salts (brix)	Flesh thickness (mm)	No. of locules	Fruit length (cm)	Fruit width (cm)	Average fruit weight (g)	No. of fruits/plant	Yield/plant (g)
Treatment	23	9443.27**	121.29**	12.17**	94.30**	46.23**	53.274**	22.99**	17.26**	12344.45**	24225.11**	11765066.78**
Replication	2	63.02	1.85	0.27	0.08	0.45	1.46	0.23	0.05	2.12	70.11	109133.20
Error	46	958.22	53.81	7.55	7.78	9.193	15.78	7.30	9.29	248.94	866.55	1497909.25

* $P \leq 0.01\%$ level of significance ** $P \leq 0.05\%$ level of significance

Table 10: Mean values of morphological traits

Genotype	Plant height (cm)	No. of branches	No. of fruits/bunch	Total soluble salts (brix)	Flesh thickness (mm)	No. of locules	Fruit length (cm)	Fruit width (cm)	Average fruit weight (g)	No. of fruits/plant	Yield/plant (g)
UTKAL PRAGYAN	57.55	6.43	3.66	6.20	4.08	2.18	4.88	3.91	36.26	34.00	1233.40
HISAR LALIT	59.50	6.99	2.48	3.20	4.31	2.66	5.63	4.66	54.10	46.23	2507.36
KASHI HEMANT	52.19	5.44	2.40	5.40	3.00	2.50	4.13	4.42	28.66	38.33	1092.00
FEB-2	55.00	5.32	2.61	5.20	2.00	3.50	3.52	3.50	31.80	32.66	1039.20
DCT-1	52.15	6.33	3.10	8.06	1.21	2.33	2.90	2.85	7.43	115.33	861.40
CO-3	48.29	6.20	2.66	4.83	1.83	4.33	3.63	4.45	46.00	28.33	1304.33
ARKA ABHAY	60.12	7.33	2.42	5.86	3.10	3.50	3.71	4.78	44.50	46.00	2049.33
SWARNA SAMPADA	47.97	4.53	2.03	6.00	2.10	4.66	4.03	4.36	40.83	28.33	1157.66
ANGHA(L-E415)	43.43	7.09	2.51	6.36	2.75	4.00	3.90	4.53	46.00	41.00	1883.00
DHANSHRI	58.23	6.99	2.10	2.83	4.31	3.00	4.48	4.98	47.33	25.00	1182.66
PUNJAB RATTA	82.02	9.20	2.72	5.86	1.91	4.16	3.62	3.76	32.30	52.00	1676.80
PANT –T-5	66.72	8.44	2.55	4.66	2.66	3.33	3.69	4.03	45.56	41.00	1873.06
HISAR ANMOL	79.55	9.00	2.51	4.20	3.13	3.13	3.76	4.50	35.56	31.66	1123.63
AZAD-T-2	52.34	5.99	2.50	5.73	2.41	3.16	3.36	4.23	27.80	36.00	995.66
PT-11	74.80	8.00	3.44	5.43	3.03	3.16	3.51	4.36	33.50	65.66	2198.96
NDTUR-73	65.63	5.75	3.32	6.13	2.83	2.66	3.44	3.87	33.26	46.00	1530.10
BT-136	57.45	6.22	2.42	5.03	3.41	3.66	4.29	4.60	47.80	37.00	1764.76
SEL-12	45.00	6.66	3.00	5.80	2.46	3.83	3.51	4.11	31.86	43.00	1373.63
NDT-9	43.31	5.44	2.35	3.56	2.33	4.66	4.55	4.76	55.05	25.66	1415.40
ANGHA-1	51.40	8.33	2.95	4.76	2.76	5.50	3.35	4.48	38.36	38.66	1480.76
ANGHA	50.02	7.23	2.87	5.80	2.66	5.33	3.55	4.57	41.26	33.66	1389.30
NDT-1	55.28	7.96	2.91	6.26	2.61	3.83	3.57	4.34	36.86	48.33	1745.83
ANAND TOMATO-3	35.10	6.66	3.00	7.00	1.50	3.33	3.83	3.33	54.00	25.66	1394.66
NDT-4	41.24	9.66	3.33	5.30	1.66	3.33	3.98	4.45	80.00	23.00	1834.33
Mean	55.59	6.96	2.74	5.39	2.67	3.57	3.86	4.24	40.67	40.94	1504.4710
C.V.	8.20	15.51	14.75	7.62	16.72	16.38	10.30	10.58	5.71	10.60	11.99
S.E.	2.63	0.62	0.23	0.23	0.25	0.33	0.23	0.25	1.34	2.50	104.18
C.D. 5%	7.50	1.77	0.66	0.67	0.73	0.96	0.65	0.73	3.82	7.13	296.57
C.D. 1%	10.01	2.37	0.88	0.90	0.98	1.28	0.87	0.98	5.10	9.52	395.90

Table 11: Descriptive statistics for morphological traits in tomato genotypes

Traits	Mean±SD	Standard error	Maximum	Minimum	Range	Variance
Plant height (cm)	55.59±12.14	1.43	82.02	35.10	35.10-82.02	147.38
No. of branches	6.96±1.57	0.18	9.66	4.53	4.53-9.66	2.49
No. of fruits/bunch	2.74±0.53	0.06	3.66	2.03	2.03-3.66	0.28
Total soluble solids (brix)	5.39±1.19	0.14	8.60	2.83	2.83-8.60	1.43
Flesh thickness (mm)	2.67±0.88	0.10	4.31	1.21	1.21-4.31	0.78
No. of locules	3.57±0.99	0.11	5.5	2.18	2.18-5.5	0.99
Fruit length (cm)	3.86±0.65	0.07	5.63	2.90	2.90-5.63	0.43
Fruit width (cm)	4.24±0.61	0.07	4.98	2.85	2.85-4.98	0.37
Average fruit weight (g)	40.67±13.31	1.56	80	7.43	7.43-80	177.40
No. of fruits/plant	40.94±18.82	2.21	122	21	21-122	354.39
Yield/plant (g)	1504.47±433.98	51.14	2507.36	861.40	861.40-2507.36	188339.56

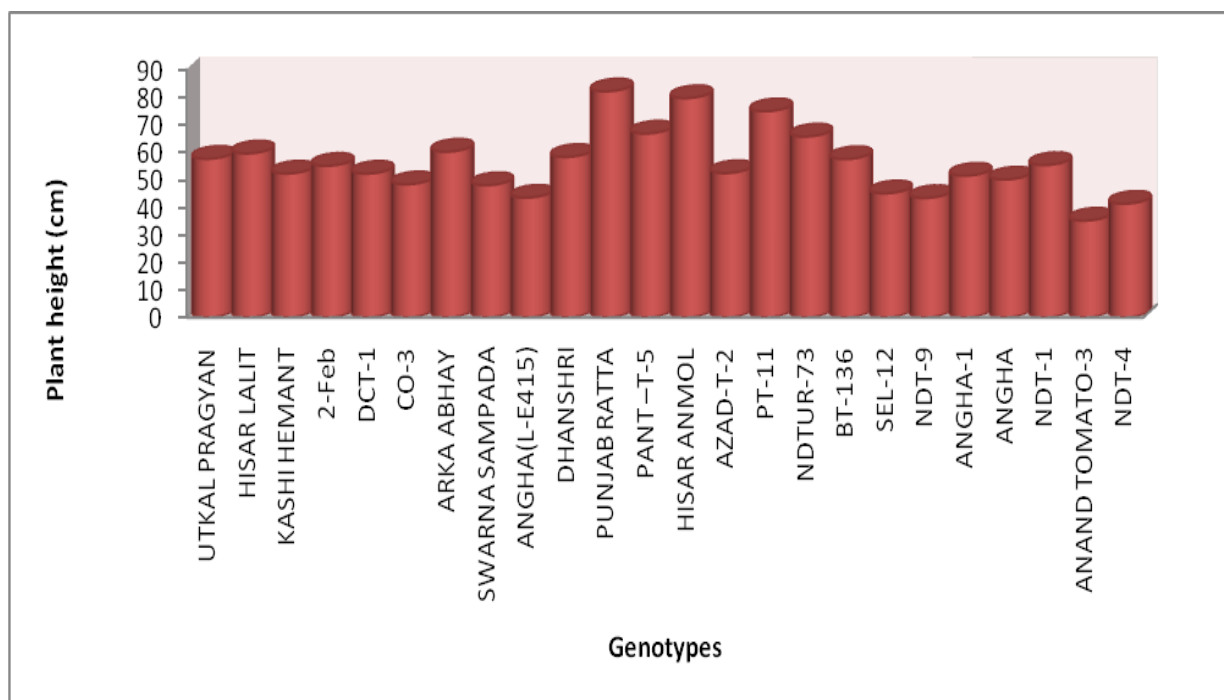


Figure1: Graphical representation of plant height in tomato genotypes

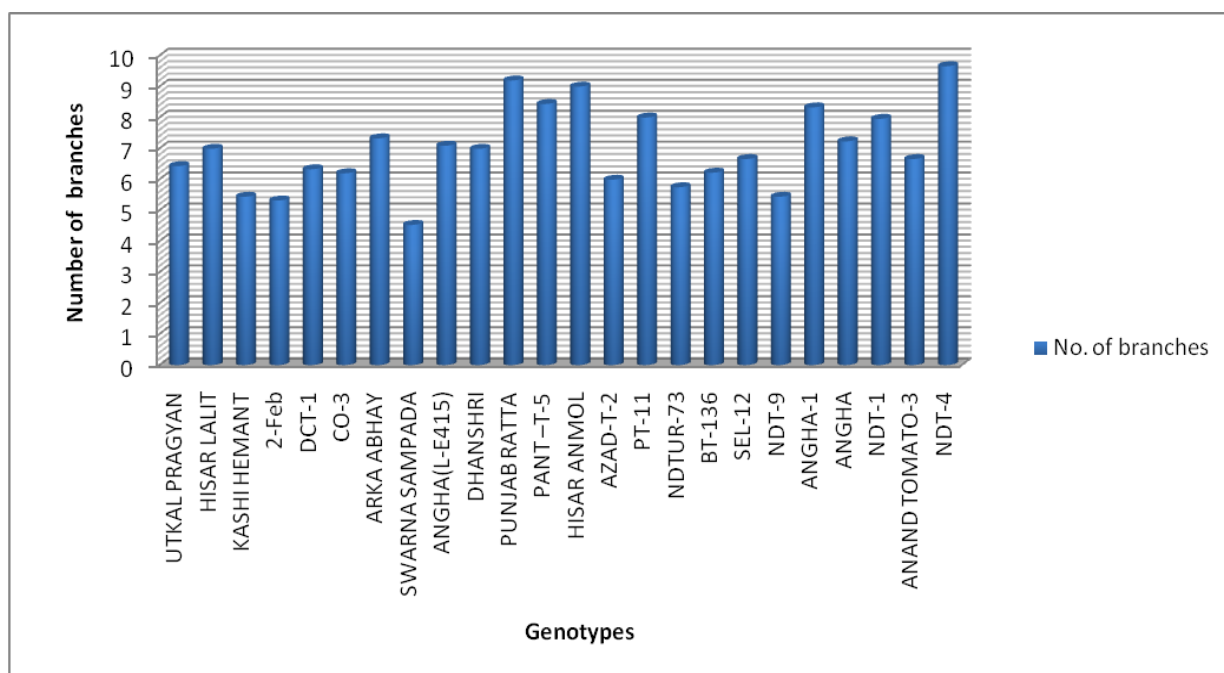


Figure 2: Graphical representation of Number of branches per plant in tomato genotypes

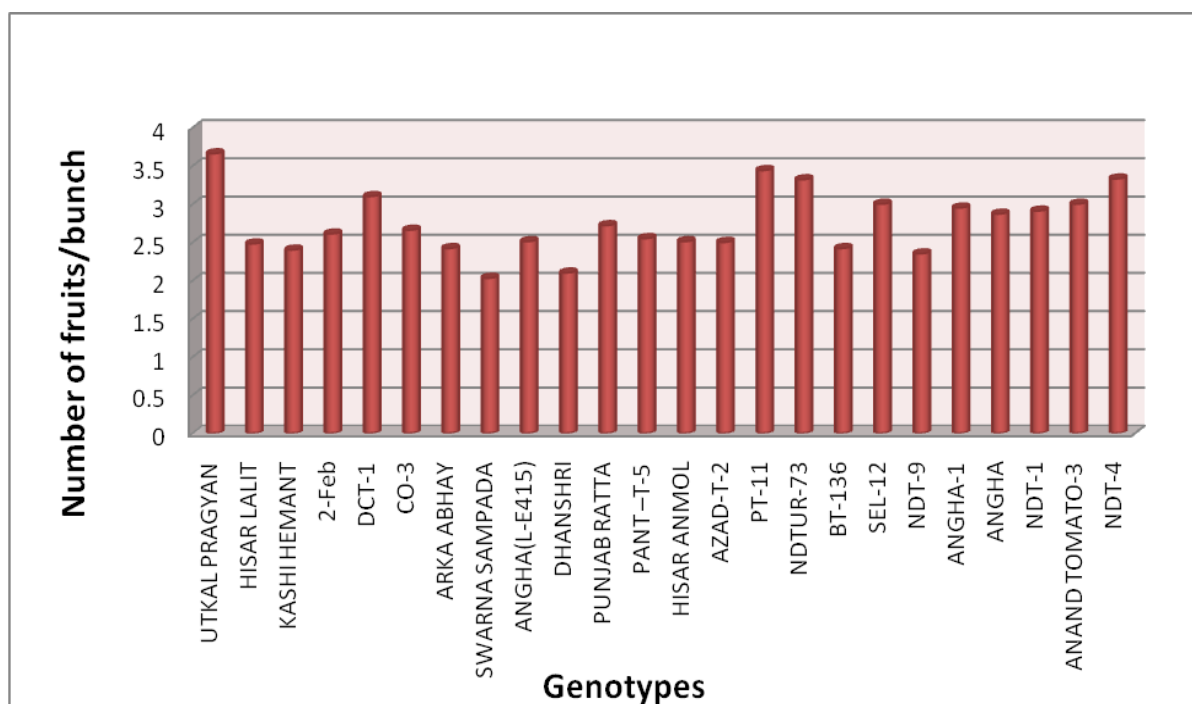


Figure 3: Graphical representation of Number of fruits/ bunch in tomato genotypes

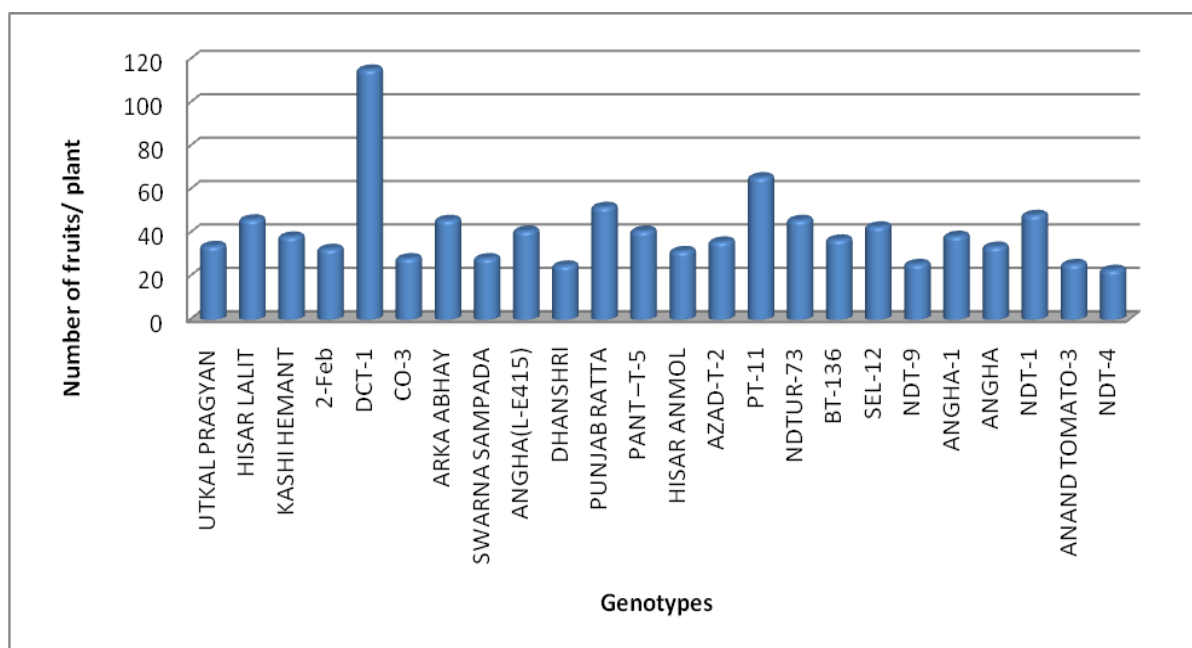


Figure 4: Graphical representation of Number of fruits per plant in tomato genotypes

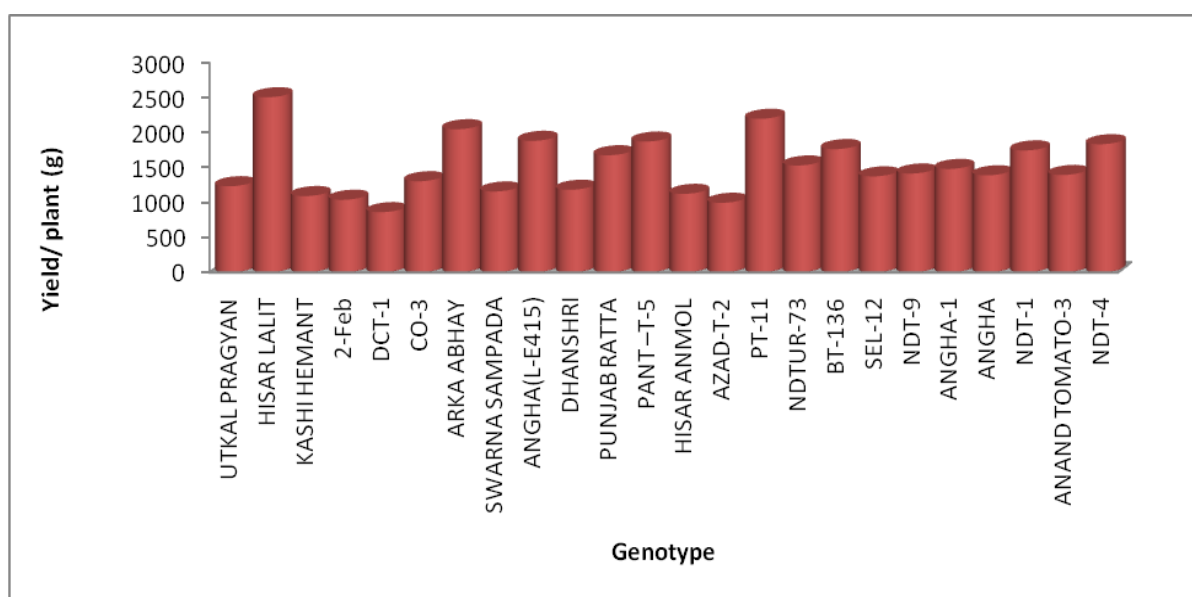


Figure 5: Graphical representation of Yield per plant (g) in tomato genotypes

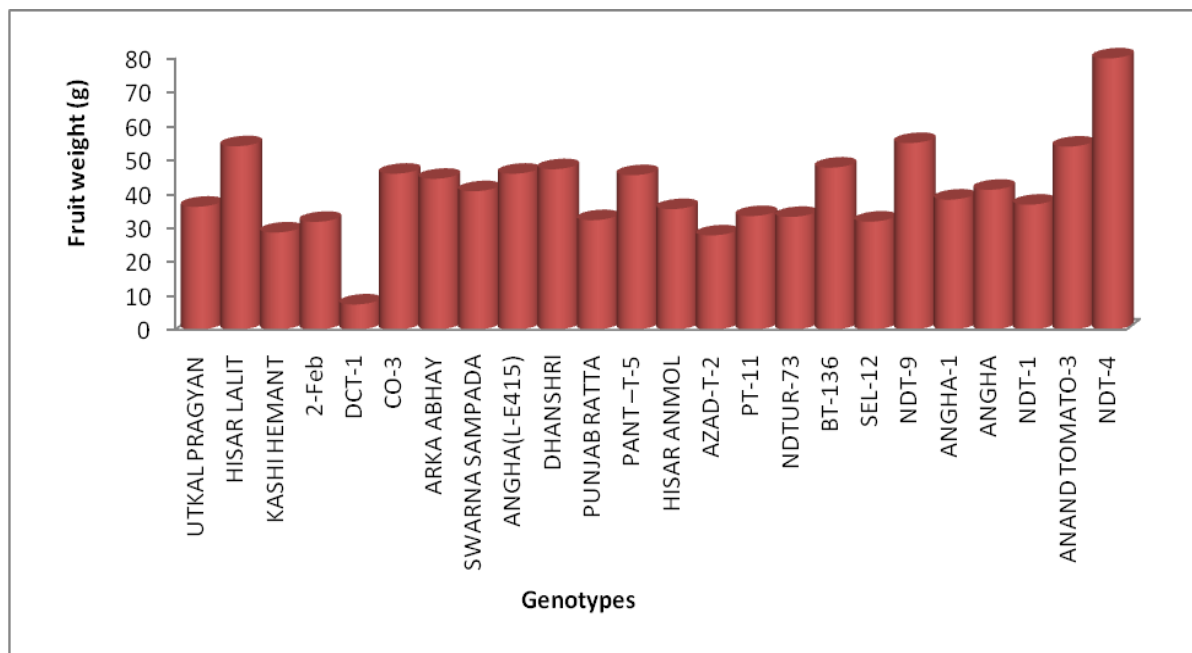


Figure 6: Graphical representation of Average fruit weight (g) in tomato genotypes

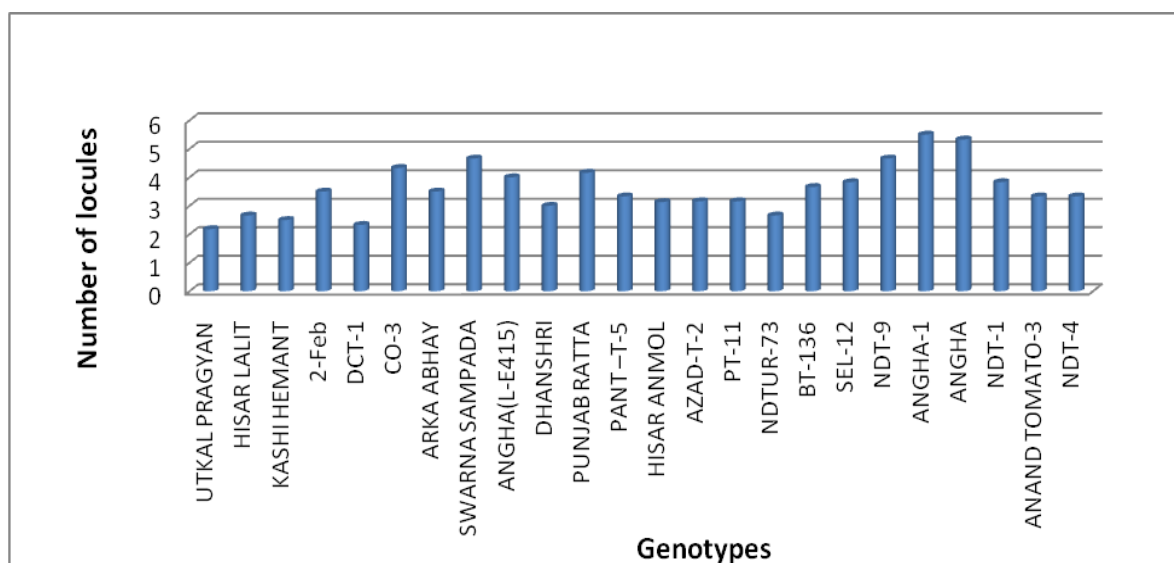


Figure 7: Graphical representation of Number of locules in tomato genotypes

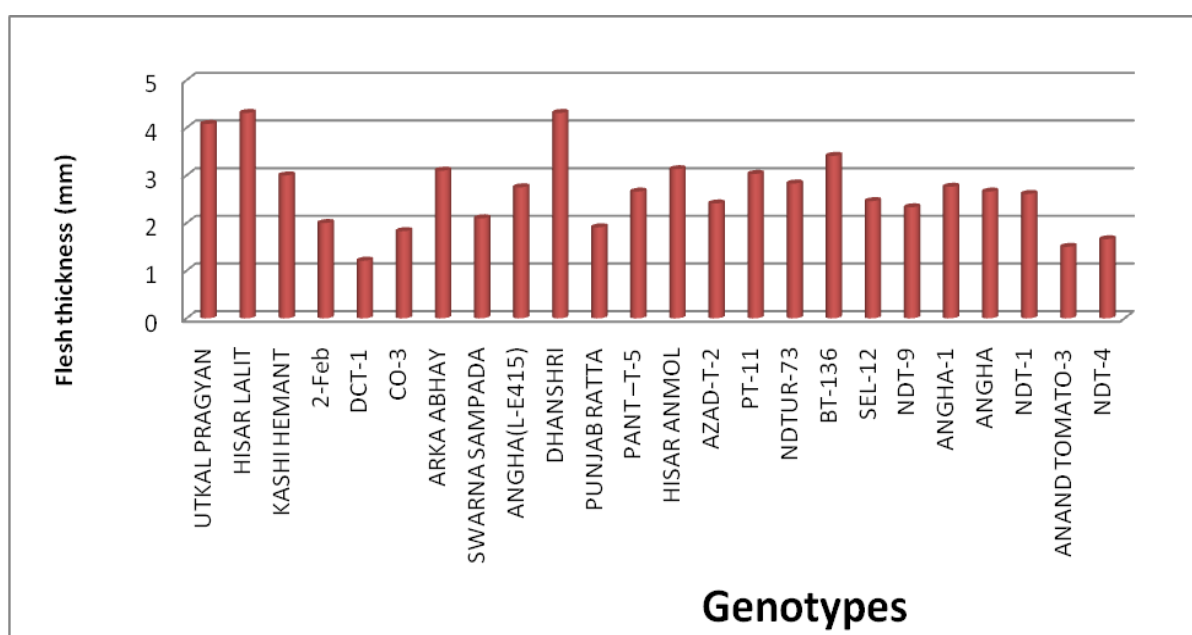


Figure 8: Graphical representation of Flesh thickness (mm) in tomato genotypes

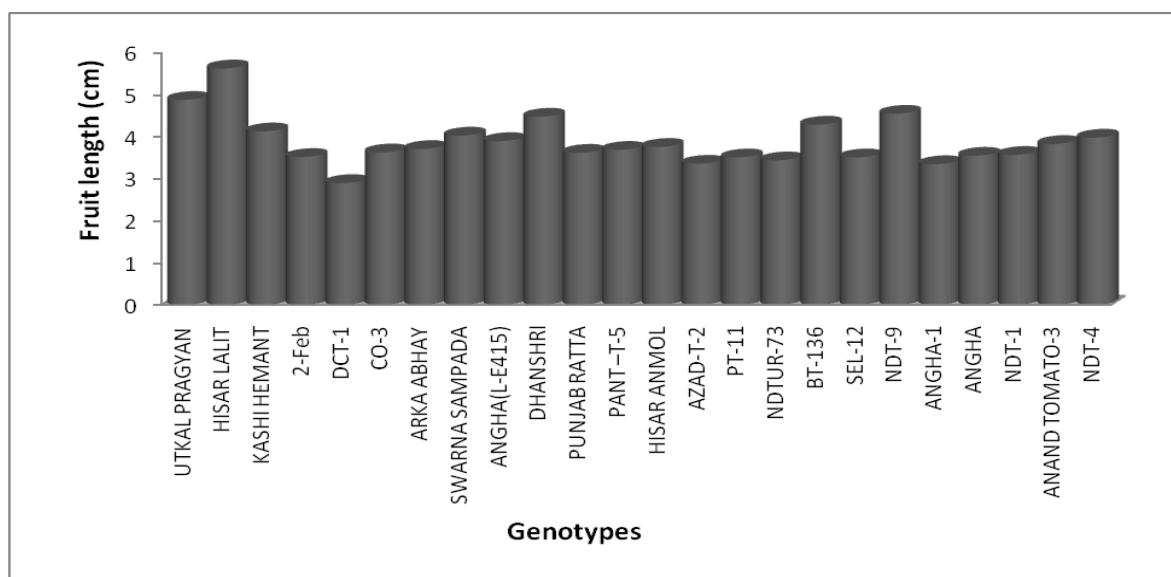


Figure 9: Graphical representation of Fruit length (cm) in tomato genotypes

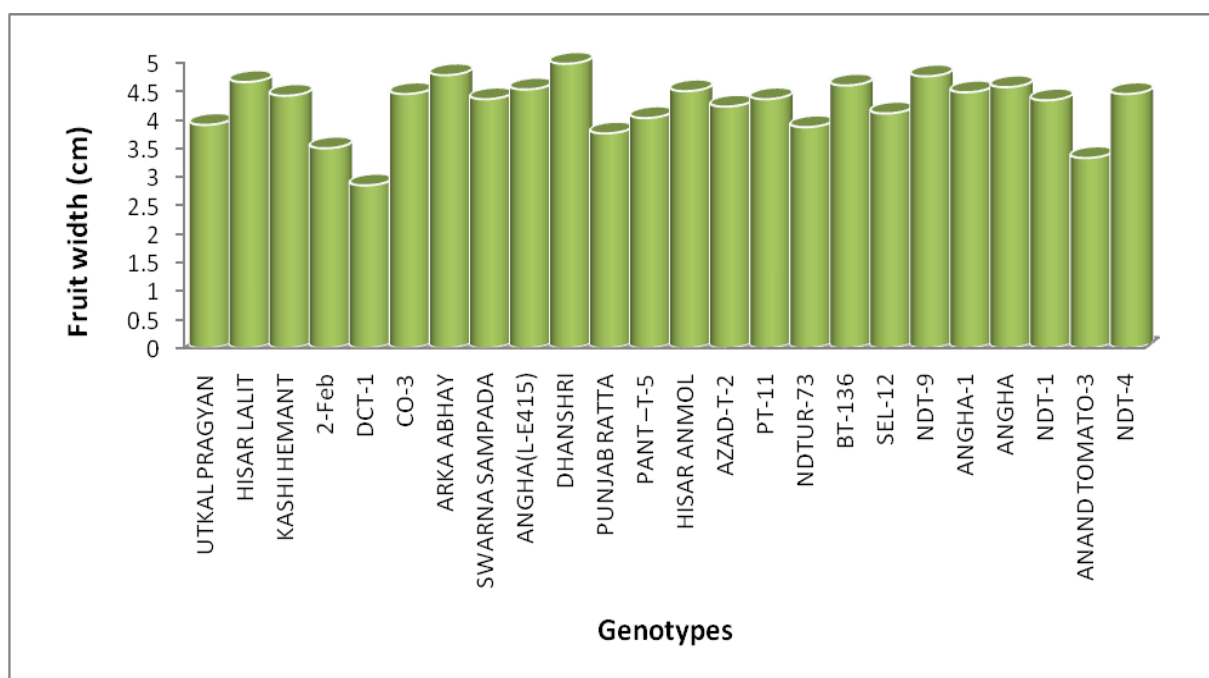


Figure 10: Graphical representation of Fruit width (cm) in tomato genotypes

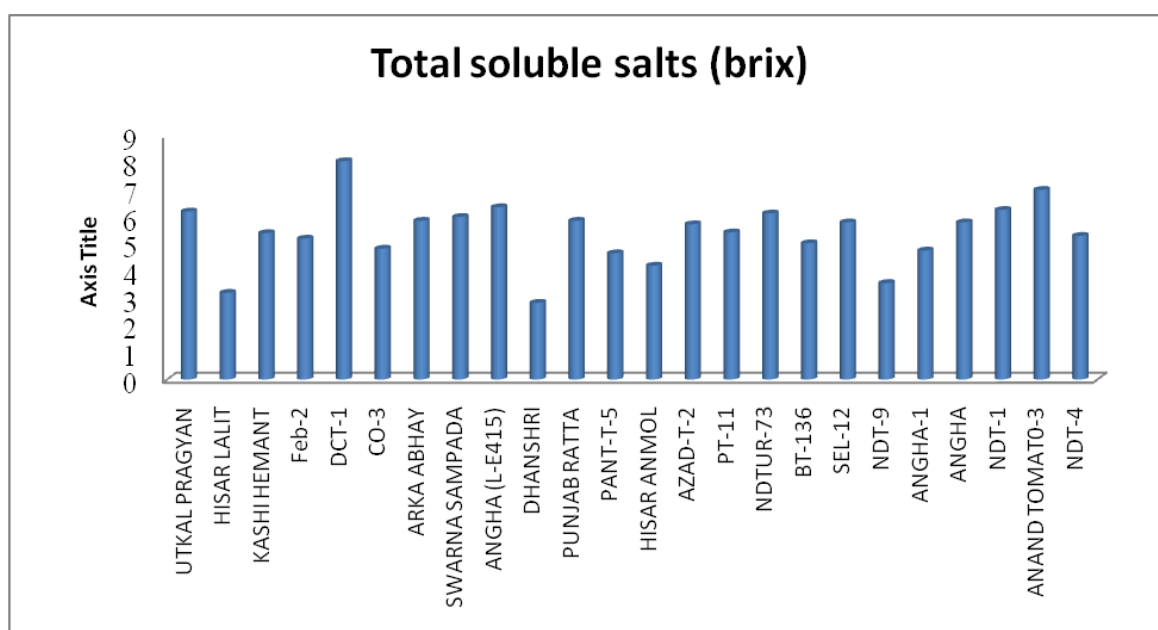


Figure 11: Graphical representation of Total soluble salts ⁰Brix) in tomato genotypes

4.4 Genetic parameters for various morphological, phenological, yield components and quality traits in tomato genotypes

The estimation of these variances showed that genotypic variations contributed maximum to the phenotypic variations for all the traits (Table 13).

4.4.1 Phenotypic coefficient of Variation (PCV)

The phenotypic variance ranged from 14.61 to 46.57 and the lowest variance was recorded for fruit width (14.61) and maximum was recorded for number of fruits per plant (46.57) followed by flesh thickness (33.53) and average fruit weight (33.21) (Table 12).

4.4.2 Genotypic coefficient of Variation (GCV)

The genotypic coefficient of variance (GCV) ranged from 10.07 to 45.35. High GCV was observed in number of fruits per plant (45.35) and is followed by average fruit weight (32.71) and flesh thickness (29.06). Lowest GCV was recorded in fruit width (10.07) (Table 12)

4.4.3 Heritability

The heritability for the various phenotypic traits ranged from 42.60 per cent for number of fruits per bunch to 97.00 per cent for average fruit weight (Table 12).

Table 12: Genetic parameters for various morphological, phenological, yield components and quality traits in tomato genotypes

Trait	GCV (%)	PCV (%)	ECV (%)	Heritability (h^2)	GA (@ 5%)	GA (@ 1%)
Plant height (cm)	20.50	22.08	8.20	86.20	21.79	27.93
No. of branches/ Plant	16.78	22.85	15.51	53.90	1.76	2.26
No. of fruits/bunch	12.70	19.47	14.75	42.60	0.46	0.60
Total soluble salts ($^{\circ}$ brix)	21.20	22.53	7.62	88.60	2.21	2.84
Flesh thickness (mm)	29.06	33.53	16.72	75.10	1.38	1.77
No. of locules/Fruit	22.68	27.97	16.38	65.70	1.35	1.73
Fruit length (cm)	13.68	17.13	10.30	63.80	0.87	1.11
Fruit width (cm)	10.07	14.61	10.58	47.50	0.60	0.77
Average fruit weight (g)	32.71	33.21	5.72	97.00	27.00	34.60
No. of fruits/plant	45.35	46.57	10.60	94.80	37.24	47.73
Yield/plant (g)	26.55	29.14	11.99	83.06	75.01	96.13

4.5 Diversity analysis in tomato genotypes based on morphological markers:

All the 24 genotypes of tomato genotypes were grouped into two main clusters by Ward Linkage method (1963). The data related to the number of clusters and genotypes are presented in the Table 13 and Figure 12. The results showed that the cluster A had two sub clusters; i.e. sub cluster A₁ and sub cluster A₂. Sub cluster A₁ had 8 genotypes (BT-136, SEL-12, NDT-9, ANGHA-1, ANGHA, NDT-1, ANAND TOMATO-3 and NDT-4) followed by Sub cluster A₂ which again had 8 genotypes (ANGHA (L-E415)), DHANSHRI, PUNJAB RATTA, PANT-T-5, HISAR ANMOL, AZAD-T-2, PT-11 and NDTUR-73). Cluster B had further two sub clusters; i.e. Sub cluster B₁ and B₂. Sub cluster B₁ had 4 genotypes (UTKAL PRAGYAN, HISAR LALIT, KASHI HEMANT and

FEB-2). Sub cluster B₂ had 4 genotypes (DCT-1, CO-3, ARKA ABHAY and SWARNA SAMPADA).

Table 13: Distribution of 24 tomato genotypes into two main clusters

Cluster	Sub clusters	Total entries	Genotypes
A	A ₁	8	BT-136 SEL-12, NDT-9, ANGHA-1, ANGHA, NDT-1, ANAND TOMATO-3 NDT-4
	A ₂	8	ANGHA(L-E415)) DHANSHRI PUNJAB RATTA PANT-T-5 HISAR ANMOL AZAD-T-2 PT-11 NDTUR-73
B	B ₁	4	UTKAL PRAGYAN, HISAR LALIT KASHI HEMANT FEB-2
	B ₂	4	DCT-1 CO-3 ARKA ABHAY SWARNA SAMPADA

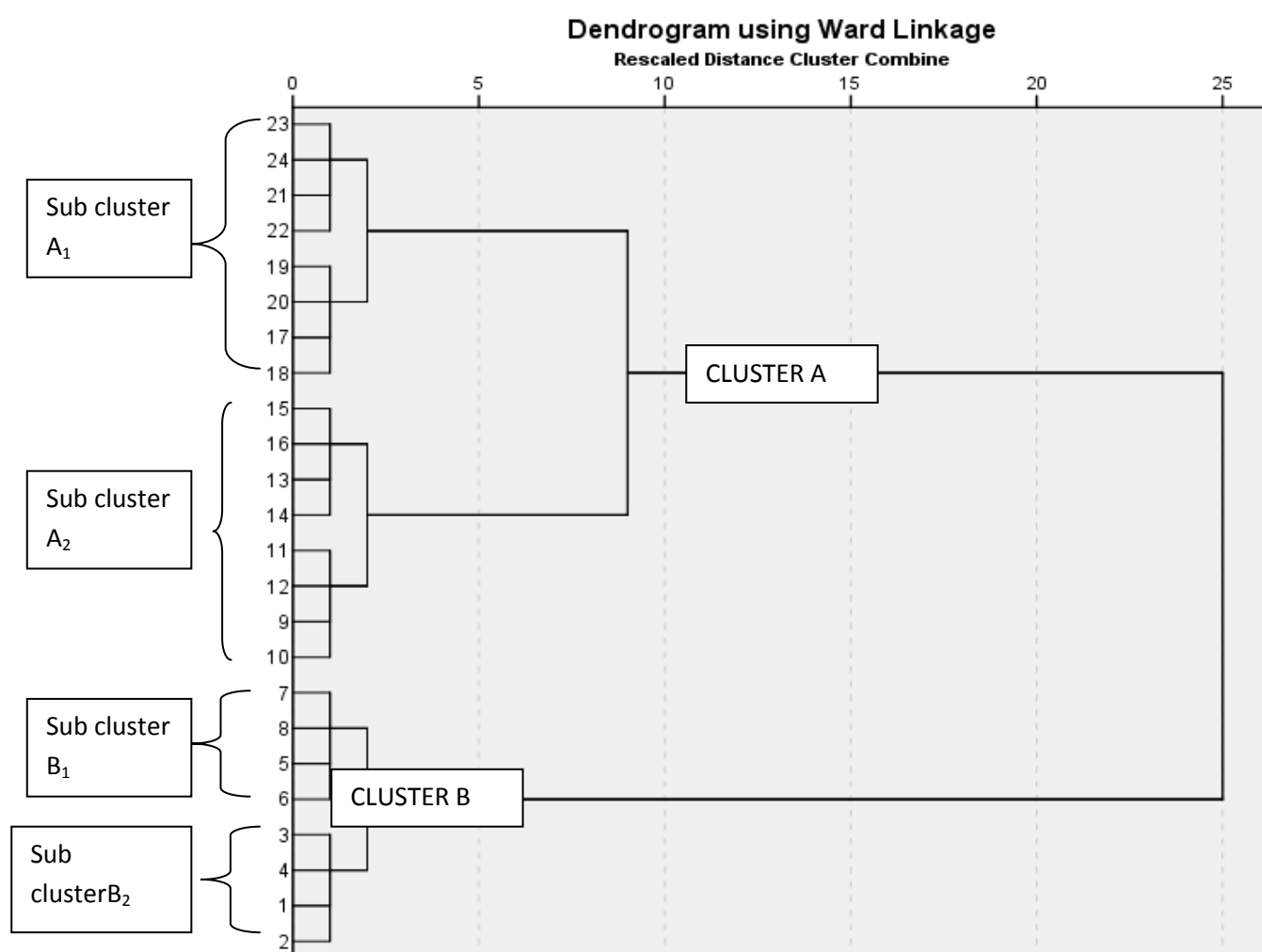


Figure 12: Dendrogram showing Genetic diversity in Tomato genotypes based on morphological markers. Using Ward linkage.

1-Utkal Pragyan, 2- Hisar Lalit, 3- Kashi Hemant, 4- FEB-2, 5- DCT-1, 6-CO-3, 7- Arka Abhay, 8- Swarna Sampada, 9- ANGHA (L-E415), 10- Dhanshri, 11- Punjab Ratta, 12- PANT-T-5, 13- Hisar Anmol, 14- AZAD-T-2, 15- PT-11, 16- NDTUR-73, 17- BT-136, 18- SEL-12, 19- NDT-9, 20- ANGHA-1, 21- ANGHA, 22-NDT-1, 23- Anand Tomato-3, 24- NDT-4

4.6 Molecular characterization

In the present study, twenty four genotypes of tomato (*Solanum lycopersicum* L.) were used to evaluate genetic diversity at the molecular level using SSR and RAPD primers. For molecular characterization, genomic DNA from each genotype was extracted and quantified using Nanodrop and agarose gel electrophoresis. Then PCR amplification by using 13 RAPD and 9 SSR markers was done.

4.6.1 DNA quantification

DNA quantification was done to know the concentration of genomic DNA and is done by two methods

- ❖ Agarose gel electrophoresis
- ❖ Nanodrop

The concentration of genomic DNA varied from 200 ng to 2,000 ng and OD260/280 ratio ranged between 1.5 to 2.1 (Table 14), indicating the purity of DNA. DNA quantification is followed by the dilution of concentrated DNA and the concentration of diluted DNA is 50 ng/μl.

Table 14: DNA concentration as per nanodrop

S.No	Genotypes	Concentration(μg/μl)	A260/280
1.	UTKAL PRAGYAN	2096.48	2.1
2.	HISAR LALIT	2266.546	2.0
3.	KASHI HEMANT	1880.14	2.0
4.	FEB-2	1246.125	2.1
5.	DCT-1	2185.24	1.8
6.	CO-3	228.959	1.8
7.	ARKA ABHAY	1402.97	1.8
8.	SWARNA SAMPADA	461.985	2.1

9.	ANGHA(L-E415)	1103.15	1.8
10.	DHANSHRI	1725.26	1.9
11.	PUNJAB RATTA	143.336	1.5
12.	PANT –T-5	765.987	1.8
13.	HISAR ANMOL	803.347	1.9
14.	AZAD-T-2	939.424	2.1
15.	PT-11	690.465	1.5
16.	NDTUR-73	1109.567	1.9
17.	BT-136	1070.929	2.0
18.	SEL-12	1195.846	1.9
19.	NDT-9	1570.108	1.7
20.	ANGHA-1	416.125	2.1
21.	ANGHA	474.284	2.0
22.	NDT-1	1493.456	1.6
23.	ANAND TOMATO-3	542.662	2.1
24.	NDT-4	413.820	1.9

4.6.2 SSR and RAPD amplification

9 SSR and 13 RAPD primers were selected from the linkage map of tomato genome and were used for the amplification of genomic DNA of tomato genotypes.

4.6.3 Scoring of bands

The bands were scored and then compared against each other and ladder for assessing the genetic diversity based on the polymorphism (band present in one genotype and absent in another genotype). Genetic diversity/relatedness among the genotypes was

assessed on the basis of Polymorphic information content (PIC) value and Percentage polymorphism (%age polymorphism).

All 9 SSR primers used in the study were found to be polymorphic. A total of 45 alleles were found and out of which, 43 alleles were polymorphic with different product sizes. Similarly 13 RAPD primers used in our study showed polymorphism. A total of 203 alleles were found and out of which 191 alleles were polymorphic with different product sizes.

4.6.4 Polymorphism information content (PIC)

Polymorphic information content (PIC Value) of each SSR and RAPD primer was calculated. It ranged from 0.16 (AI773078 and AI778183) to 0.50 (SSR 241) with an average of 0.303 for SSR primers. For RAPD primers it ranged from 0.243(OPC-04) to 0.432(OPU-16) with an average of 0.29. High PIC value for SSR marker was observed for SSR 241 (0.50) and SSR 74 (0.469) and for RAPD marker high PIC value was observed for OPC-16 (0.432) followed by OPU-17(0.375). Low PIC value was observed for AI773078 (0.16) for SSR markers and for RAPD markers low PIC was observed for OPC-04(0.243). PIC values of all the genotypes are listed in the Table 16.

Percentage polymorphism (%age polymorphism) was calculated. 100 percent polymorphism has been observed for all the markers used in the study.

Table 15: Parameters used for the evaluation of polymorphism in SSR markers

S.NO.	PRIMER	SEQUENCE 5'→ 3'	PIC	MI	RP	NB	NPB	NM B	NU B	PP B
1	AI773078	F:GATGGACACCCTTCA ATTTATGGT R:TCCAAGTATCAGGC ACACCAGC	0.16	1.28	1.58	8	8	0	4	100
2	AI778183	F:GCGAAGAAGATGAG TCTAGAGCATAG R:CTCTCTCCCATGAGT TCTCCTCTTC	0.26	1.12	1.58	7	7	0	4	100
3	AW037347	F:GCCACGTAGTCATG ATATACATAG R:GCCTCGGACAATGA ATTG	0.21	1.68	2	8	8	0	2	100
4	Y09371	F:TGAGAACAACGTTT AGAGGAGCTG R:CGGGCAGAATCTCG AACTC	0.16	1.34	1.58	8	8	0	4	100

5	SSR 241	F:TCCTCGCTAATTGAT CCACC R:TCAACAGCATAGTG GAGGAGG	0.50	0.99	1.91	2	2	0	0	100
6	X90770	F:TGTAGATAACTTCCT AGCGACAATC R:ACGGACGGATGGAC AAATG	0.32	1.29	2.41	4	4	0	0	100
7	SSR74	F:ACTCACCATGGCTGC TTCTT R:TTTCTTGAAGGGTCT TTCCC	0.46	0.93	1.75	2	2	0	0	100
8	X90937	F:TGCCCCATGACGTTCC ATC R:GACAGACAGAGAGA CAGATTTAGAG	0.29	0.59	1.91	2	2	0	0	100
9	SSR 9	F:CCCTTTGCAAGTTCT TCTTCA R:TTCATGAGCCAACA TAGGAGG	0.33	0.13	1.75	4	4	0	0	100
	AVERAGE		0.30	1.04	1.83	5.00	5.00	5.00	0.00	100

* NB: number of bands, NPB: number of polymorphic bands, NMB: number of monomorphic bands, NUB: number of unique bands, PPB: percentage of polymorphic bands, PIC: polymorphism information content, MI: marker index, Rp: resolving power.

Table 16: Parameters used for the evaluation of polymorphism in RAPD markers

S.NO	PRIMER	SEQUENCE	PIC	MI	RP	NB	NPB	NMB	NUB	PPB
1	OPA-07	5' GAAACGGGTG3'	0.25	2.52	3.25	10	10	0	2	100
2	OPA-13	5' CAGCACCCAC 3'	0.26	1.93	18	11	9	2	0	81.82
3	OPA-19	5' CAAACGTCGG 3'	0.28	4.62	6.83	16	16	0	3	100
4	OPA-02	5' TGCCGAGCTG 3'	0.27	0.67	44.58	10	5	5	0	50
5	OPC-04	5' CCGCATCTAC 3'	0.24	3.88	6.25	16	16	0	2	100
6	OPC-14	5' TGC GTGCTTG 3'	0.29	7.78	24.83	28	27	1	1	96.42

7	OPC-20	5' ACTTCGCCAC 3'	0.27	5.52	7.75	20	20	0	4	100
8	OPN-15	5' CAGCGACTGT 3'	0.30	1.84	7.16	6	6	0	0	100
9	OPO-20	5' ACCCGGTCAC 3'	0.26	11.02	9.75	41	41	0	7	100
10	OPQ-20	5' TCGCCCAGTC 3'	0.30	3.32	9.75	11	11	0	1	100
11	OPU-16	5' CTGCGCTGGA 3'	0.43	6.48	22.17	15	15	0	0	100
12	OPY-15	5' AGTCGCCCTT 3'	0.25	1.34	11.75	12	8	4	3	66.67
13	OPU-17	5'ACCTGGGGAG 3'	0.37	2.62	4.5	7	7	0	0	100
AVERAGE			0.29	4.12	13.58	15.62	14.69	0.92	1.77	191.92

* NB: number of bands, NPB: number of polymorphic bands, NMB: number of monomorphic bands, NUB: number of unique bands, PPB: percentage of polymorphic bands, PIC: polymorphism information content, MI: marker index, Rp: resolving power.

4.6.5 Data Analysis

The data was analysed using NTYSYS software based on UPGMA (Unweighted pair group method with arithmetic mean) and dendrogram was constructed. The dendrogram revealed a peculiar picture showing the different clusters of different genotypes and grouped genotypes based on genetic similarity.

4.6.5.1 Cluster analysis done using SSR markers

Clustering analysis of the genotypes is depicted in Figure 13. The genotypes were grouped into two main clusters, cluster A and cluster B. Cluster A and cluster B was further divided into two sub clusters i.e. sub cluster A₁, sub cluster A₂, sub cluster B₁ AND sub cluster B₂. Sub cluster A₁ had 11 genotypes (NDT-1, NDT-4, NDTUR-73, PT-11, BT-136, SEL-12, AZAD-T-2, PANT-T-5, Hisar Anmol, Punjab Ratta and Dhanshri) and sub cluster A₂ had 9 genotypes (Utkal Pragyan, Arka Abhay, Swarna Sampada, ANGHA-(L-E415), Kashi Hemant, FEB-2, DCT-1, Hisar Lalit and CO-3). Cluster B₁ consisted of 1 genotype (NDT-9) and sub cluster B₂ consisted of 3 genotypes (ANGHA, Anand Tomato-3 and ANGHA-1). The similarity coefficients among 24 genotypes ranged from 0.24 to 0.72. Scored data were used for the estimation of Jaccard's similarity coefficient using NTSYS-pc version 2.02e (Rohlf, 1998) package to compute pair-wise Jaccard's similarity coefficient (Jaccard, 1908). Among all the pair-wise combinations, Arka Abhay and CO-3 (0.72) showed the highest similarity index, while genotypes FEB-2 and NDT-1 (0.24) showed the lowest similarity index (Table 17). The clustering done using UPGMA was further confirmed by Principal component analysis (PCA) as represented in Figure 14. The observations of Principal Component Analysis revealed similar genetic differentiation among 24 genotypes of Tomato as revealed by UPGMA dendrogram.

4.7.5.2 Clustering analysis done using RAPD markers

Clustering analysis of the genotypes is depicted in Figure 14. The genotypes were grouped into two main clusters, cluster A and cluster B. Cluster A was further divided into two sub clusters i.e. sub cluster A₁ and sub cluster A₂. Sub cluster A₁ had 13 genotypes (Utkal Pragyan, Hisar Lalit, DCT-1, Kashi Hemant, CO-3, Arka Abhay, ANGHA(L-E415), Dhanshri, Punjab Ratta, PANT-T-5, Hisar Anmol, FEB-2 and Swarna Sampada) and sub cluster A₂ had 6 genotypes (AZAD-T-2, PT-11, NDTUR-73, BT-136, SEL-12 and NDT-9). Cluster B consisted of further two sub clusters B₁ and B₂. Sub cluster B₁ had 2 genotypes (ANGHA-1 and ANGHA) while Sub cluster B₂ had 3 genotypes (NDT-1, AZAT-T-2 and NDT-4). The dendrogram showed genetic variation among the 24

genotypes of Tomato. The similarity coefficients among 24 genotypes ranged from 0.26 to 0.77. Among all the pair-wise combinations, Arka Abhay and CO-3 (0.77) showed the highest similarity index, while genotypes FEB-2 and NDT-9 (0.26) showed the lowest similarity index (Table 18). The clustering done using UPGMA was further confirmed by Principal component analysis (PCA) as represented in Figure 15. The observations of Principal Component Analysis revealed similar genetic differentiation among 24 genotypes of tomato as revealed by UPGMA dendrogram.

4.7.5.3 Comparative Clustering analysis done using SSR and RAPD markers

Comparative clustering analysis of the genotypes using SSR and RAPD markers is depicted below in Figure 17. The genotypes were grouped into two main clusters, cluster A and cluster B. Cluster A was further divided into two sub clusters i.e. sub cluster A₁ and sub cluster A₂. Sub cluster A₁ had 6 genotypes (ANGHA (L-E415), Dhanshri, Punjab Ratta, PANT-T-2, Hisar Anmol and PT-11). Sub cluster A₂ had 8 genotypes (Utkal Pragyan, Hisar Lalit, Kashi Hemant, CO-3, Arka Abhay, FEB-2, Swarna Sampada and DCT-1). Cluster B consisted of further two sub clusters B₁ and B₂. Sub cluster B₁ had 4 genotypes (NDTUR-73, BT-136, SEL-12 and NDT-9). Sub cluster B₂ had 5 genotypes (ANGHA-1, ANGHA NDT-1, Anand Tomato-3 and NDT-4). The dendrogram showed genetic variation among the 24 genotypes of Tomato. The similarity coefficients among 24 genotypes ranged from 0.37 to 0.72. Among all the pair-wise combinations, Arka Abhay and CO-3(0.72) showed the highest similarity index, while genotypes FEB-2 and NDT-1(0.37) showed the lowest similarity index (Table 19). The clustering done using UPGMA was further confirmed by Principal component analysis (PCA) as represented in Figure 18. The observations of principal component analysis revealed similar genetic differentiation among 24 genotypes of tomato as revealed by UPGMA dendrogram.

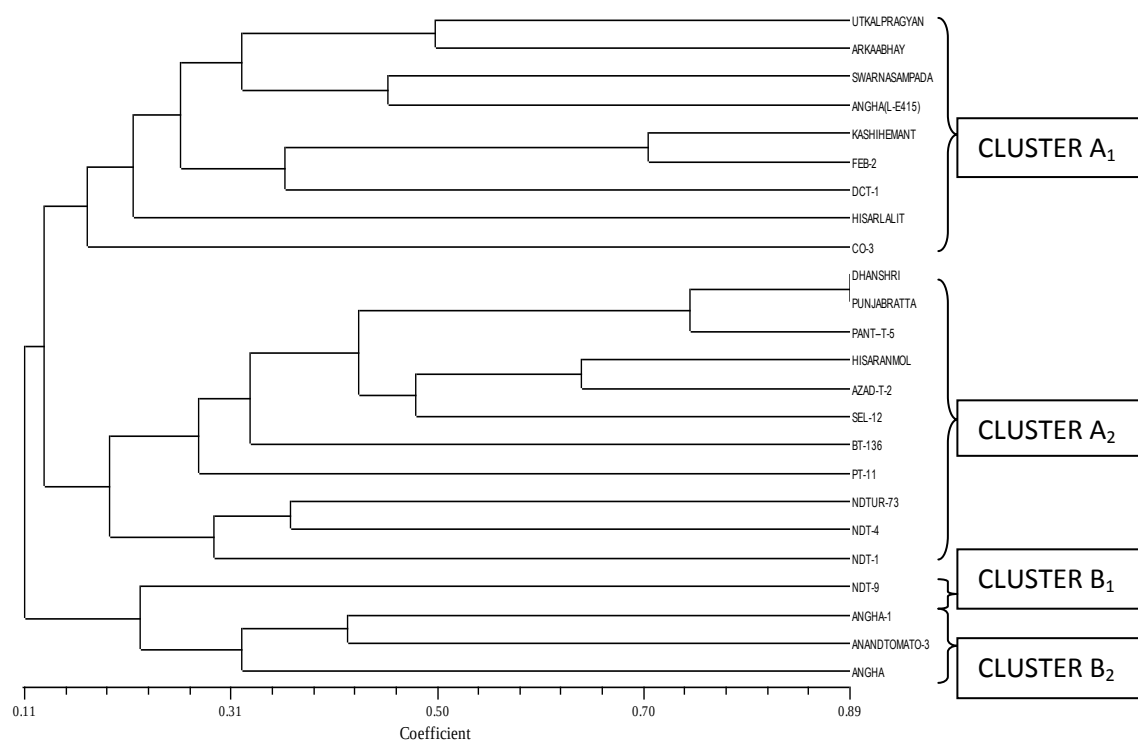


Figure 13: Clustering analysis of Tomato Genotypes based on 9 SSRs marker.

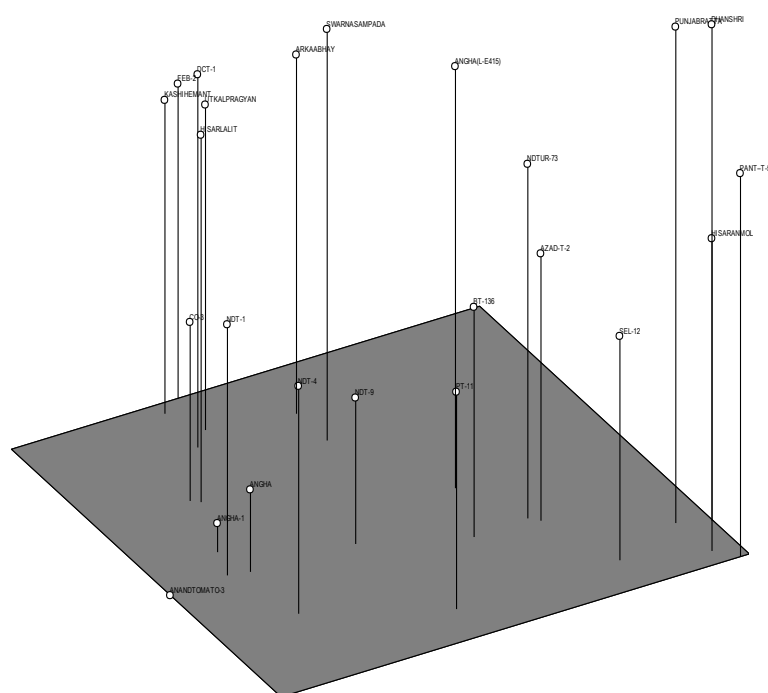


Figure 14: PCA analysis based results of tomato genotypes using SSR markers.

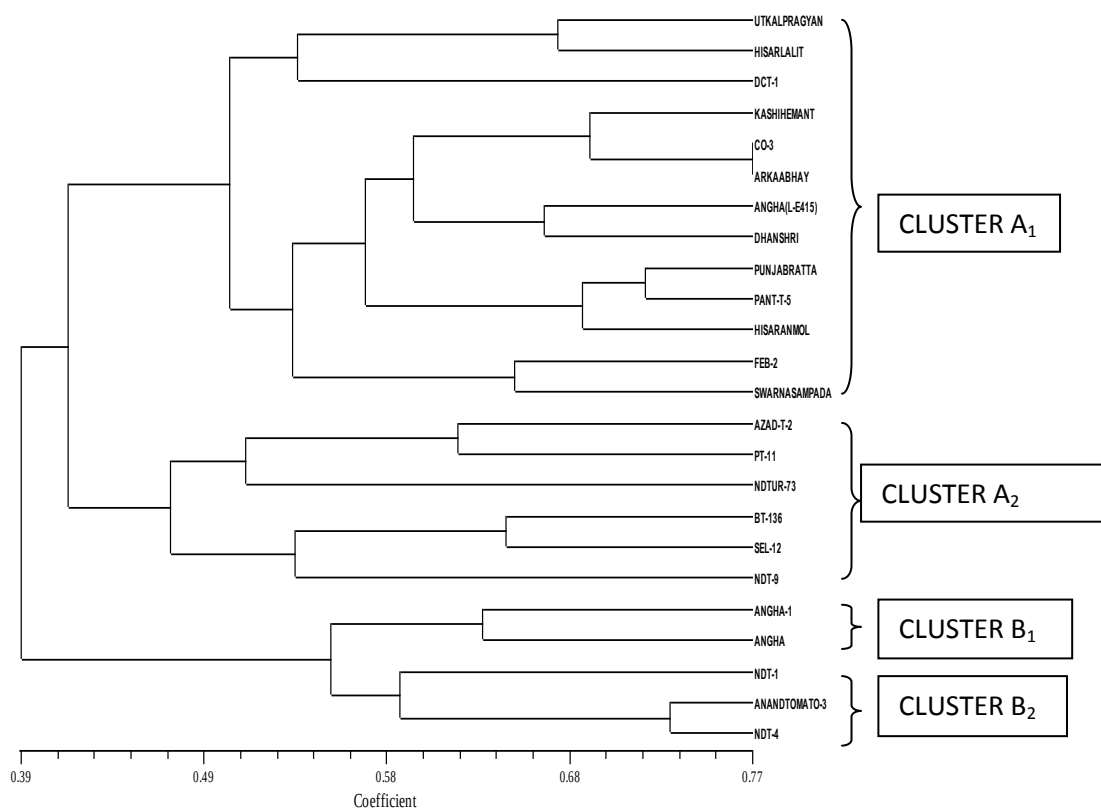


Figure 15: Cluster analysis of tomato based on UPGMA using RAPD markers.

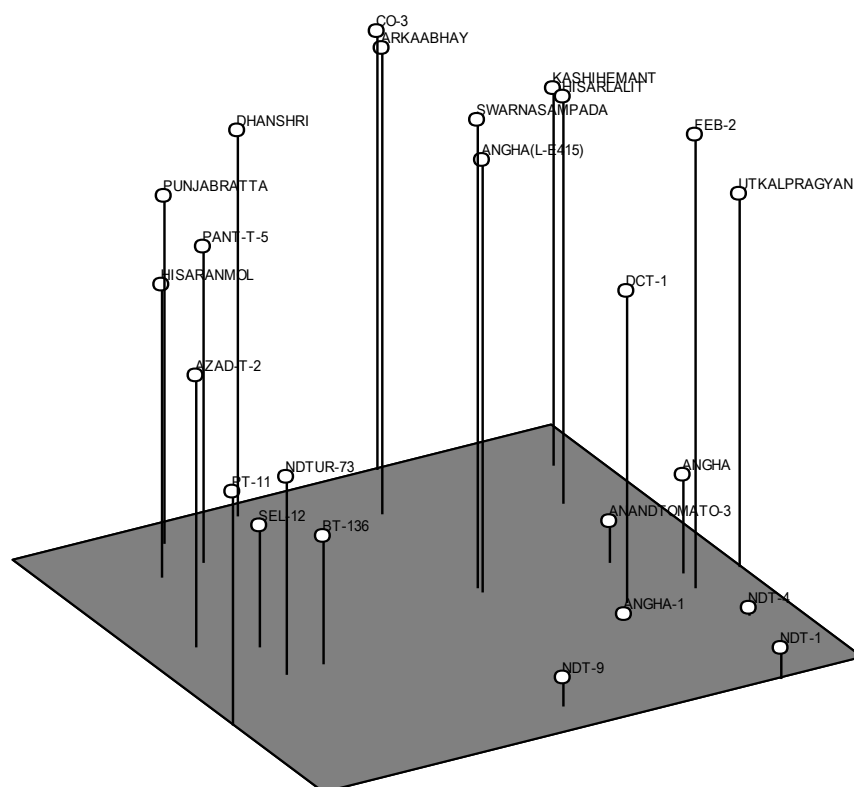


Figure 16: Principal component analysis based clustering using RAPD markers

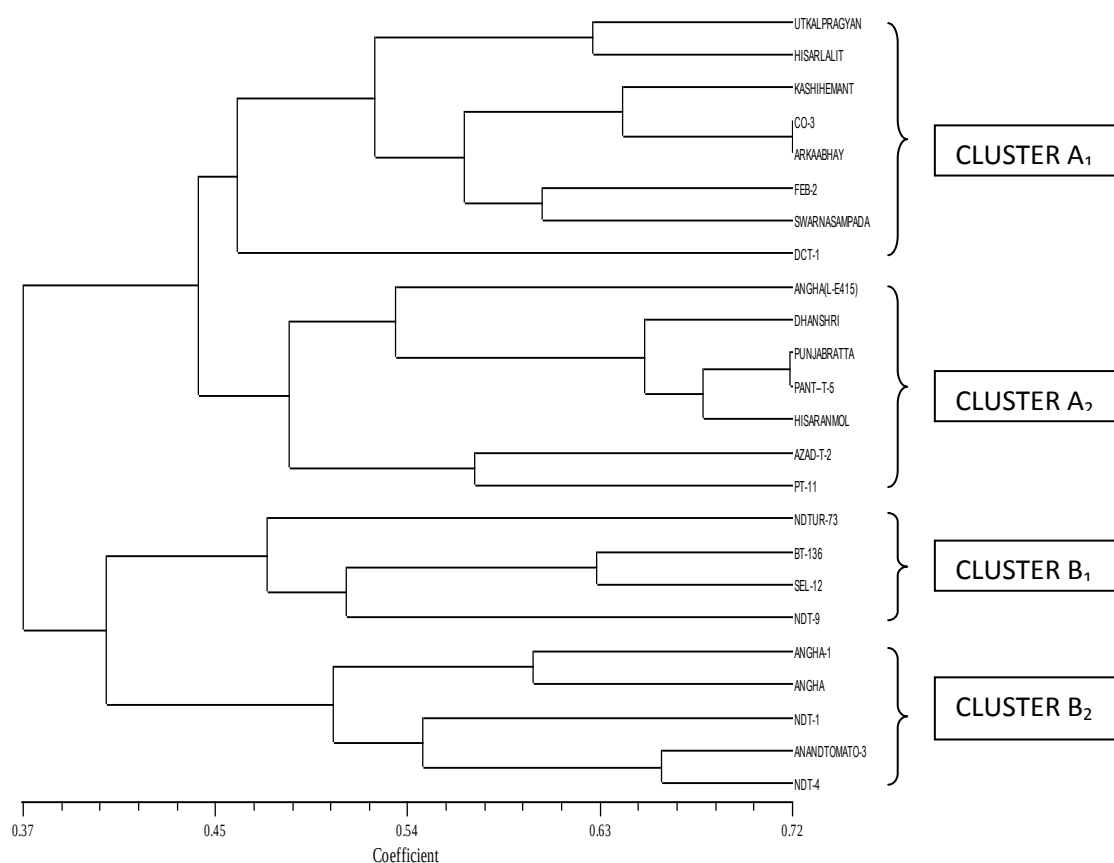


Figure 17: Cluster analysis of 24 genotypes of tomato based on UPGMA based on 13 RAPD and 9 SSR primers.

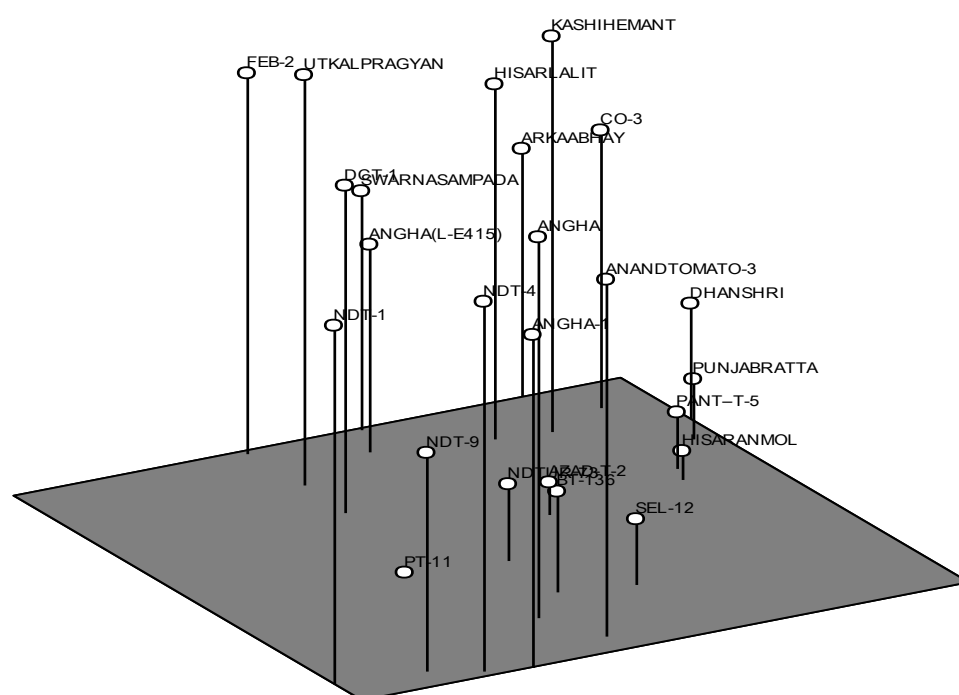


Figure 18: Principal component based analysis using RAPD and SSR markers.

SSR- PCR amplification profile of 24 genotypes produced by 9 primers

S.No.	Primer
1.	AI773078
2.	AI778183
3.	AW037347
4.	Y09371
5.	SSR 241
6.	X90770
7.	SSR 74
8.	X90937
9.	SSR 9

RAPD- PCR amplification profile of 24 genotypes produced by 13 primers

S.No.	Primer
1.	OPA-07
2.	OPA-13
3.	OPA-19
4.	OPA-02
5.	OPC-04
6.	OPC-14
7.	OPC-20
8.	OPN-15
9.	OPO-20
10.	OPQ-20
11.	OPU-16
12.	OPY-15
13.	OPU-17

Wells 1 to 24 represent 24 genotypes and M represent Ladder:

- | | |
|-----|----------------|
| 1. | UTKAL PRAGYAN |
| 2. | HISAR LALIT |
| 3. | KASHI HEMANT |
| 4. | FEB-2 |
| 5. | DCT-1 |
| 6. | CO-3 |
| 7. | ARKA ABHAY |
| 8. | SWARNA SAMPADA |
| 9. | ANGHA(L-E415) |
| 10. | DHANSHRI |
| 11. | PUNJAB RATTI |
| 12. | PANT –T-5 |
| 13. | HISAR ANMOL |
| 14. | AZAD-T-2 |
| 15. | PT-11 |
| 16. | NDTUR-73 |
| 17. | BT-136 |
| 18. | SEL-12 |
| 19. | NDT-9 |
| 20. | ANGHA-1 |
| 21. | ANGHA |
| 22. | NDT-1 |
| 23. | ANAND TOMATO-3 |
| 24. | NDT-4 |

DISCUSSION

In the present study, 24 genotypes of Tomato were characterized at the morphological and as well as at the molecular level. Estimation of genetic diversity and relationships between germplasm collections are important for facilitating efficient germplasm collection, evaluation and utilization (Rafalski, 2009). Significant improvement in tomato along with basic information has been reported (Kamenetzky *et al.*, 2010). Using a combination of morphological traits and molecular markers has been shown to lead to more reliable conclusions in assessments of genetic diversity (Khadiji-Khub *et al.*, 2008; Nikoumanesh *et al.*, 2011; Acosta-Quezada *et al.*, 2012). The use of molecular markers serves as a modern and suitable approach to cultivar and variety identification, since it is more rapid and cost-effective (Korir *et al.*, 2012). Information from present study will be helpful in the study of genetic relationships, representation of genetic diversity and morphological evaluation.

5.1 Morphological Traits

Present study was carried out to assess the variability among the 24 tomato genotypes with respect to 11 traits. Significant variation for all the 11 traits in tomato genotypes was observed which indicated presence of sufficient amount of diversity in the genotypes of tomato undertaken for the study. The widest range (861.40-2507.36 g) was recorded for yield per plant (gm/plant) followed by Number of fruits per plant (21.00-122.00 fruits/plant), Average fruit weight (7.43-80.00 gm/ fruit), Plant height (35.10-82.702 cm), Number of branches per plant (4.53-9.66), Total Soluble Solids (2.83- 8.06 °Brix), Number of locules per plant (2.18-5.5), Fruit length (2.9-5.63cm), Fruit width (2.85- 4.98), Number of fruits per bunch (2.03-3.66), and Flesh thickness (1.21-4.8 mm). The maximum plant height was recorded in Punjab Ratta (82.02cm) and minimum plant height was recorded in Anand Tomato-3 (35.10cm). The maximum number of branches per plant was recorded in NDT-4 (9.66) and minimum number of Branches in Swarna Sampada (4.53). The maximum number of clusters per plant was recorded in Utkal Pragyan (3.66) and minimum number of clusters per plant was recorded in Swarna Sampada (2.03). The maximum number of fruits per plant was recorded in DCT-1 (115.33) while minimum number of fruits per plant was recorded in NDT-4 (23.00). The maximum total yield per plant was recorded in Hisar Lalit (2507.36) and minimum total yield per plant was recorded in DCT-1 (861.40). The maximum single fruit weight was recorded in NDT-4 (80.00) and minimum single fruit weight was recorded in DCT-1

(7.43). The maximum number of locules per fruit was recorded in Angha-1 (5.50) and minimum number of locules per fruit was recorded in Utkal Pragyan (2.18). The maximum flesh thickness per fruit was recorded in Hisar Lalit and Dhanshri (4.31) while minimum flesh thickness per fruit was recorded in DCT-1 (1.21). The maximum fruit length was recorded in Hisar Lalit (5.63) and minimum fruit length was recorded in DCT-1 (2.90). The maximum fruit width was recorded in Dhanshri (4.98) while minimum fruit width was recorded in DCT-1(2.85).

The significant variation in tomato genotypes with respect to yield and quality characters may be due to the genetic makeup, status of water and oxygen during the growing period of these genotypes. The oxygen deficiency restricts root respiration and negatively affects water and nutrient uptake. This eventually reduces the yield and its quality. Raviv *et al.* (2004) reported that low yield and quality may also be attributed to the poor rainfall and high day and night temperatures during the experimental period.

The range of the mean values defines the genetic potential of different genotypes for various characters studied. The results showed that there was significant genetic distance between the genotypes for some of the characters like yield and its attributing traits. These results indicate that if the genotypes having larger value for range of variability for various characters, there will be better chance to improve the exiting cultivars by different breeding procedures. It can be used in selection or hybridization programme for the respective characters. The result regarding mean and range values obtained in the present investigation are in accordance with the results obtained by Golani *et al.* (2007).

5.2 Biochemical Characters

The Total Soluble Solids are composed of all fruit components except water and those volatilized during drying. About 50 percent of the dry matter is composed of sugars, primarily reducing sugars, glucose and fructose and the quantity of sucrose is negligible. Also, minute quantities of saccharose, raffinose, arabinose, xylose, galactose and sugar alcohol myoinositol have been reported. In present study, the total soluble solids (TSS) ranged between 2.83 to 8.06. Maximum total soluble solids (TSS) was recorded in DCT-1 (8.06 °Brix) and minimum total soluble solids (TSS) i.e.2.83 °Brix was recorded in Dhanshri. The results are in agreement with the results of Ruggieri *et al.* (2014).

5.3 The extent of genetic variability

The analysis of variance revealed significant mean square estimates for all the characters indicating sufficient diversity among the genotypes. The variation in the genotypes would be helpful in the development of superior varieties. The results are in agreement with the observations of Golani *et al.* (2007).

The present investigation revealed that the phenotypic variation was contributed maximum to the genotypic variations. The phenotypic variation was high as compared to genotypic variation for all traits under study and maximum for Yield per plant, average fruit weight and plant height. These results are in agreement with the observations of Henareh . (2015) and Golani *et al.* (2007).

Phenotypic coefficient of variation (PCV) was more than genotypic coefficient of variation (GCV) for all studied 11 traits. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were high for Number of fruits/plant (45.35, 46.57%), Average fruit weight (32.71, 33.21 %), flesh thickness (29.06, 33.53 %), Yield per plant (26.55, 29.14%) and Number of locules/ Fruit (22.68, 22.97 %), which suggested greater phenotypic and genotypic variability among the accessions and sensitiveness of the attributes for making further improvement by selection. Wide difference between GCV and PCV for Number of branches and Number of fruits/bunch implied its susceptibility to environmental fluctuation, whereas narrow difference between GCV and PCV for other traits suggested their relative resistance to environmental alteration. The PCV was higher than the respective GCV for all the characters denoting environmental factors influencing their expression to some degree or other. These results are in agreement with the observations of Henareh. (2015) who showed that high PCV and GCV was observed for plant height followed by average fruit weight estimated. In present study highest estimates of GCV and PCV were recorded for number of fruits per plant (45.35 and 46.57 per cent respectively), the results of these observations are in close conformity with the results of Nair and Thamburaj (1995b), which is an important yield component.

5.4 Heritability and genetic advance

Heritability is the heritable variation in the experimental material. Genetic variability is due to additive and non- additive gene action.

In the present study, the broad sense heritability estimates were high for all the parameters. Such high values of heritability for Average fruit weight, Number of fruits per plant, Yield per plant, Plant height and Total soluble solids clarified that they were least affected by environmental modification and selection based on phenotypic performance

would be reliable. In traits with high heritability, genotypic variance is more than environmental variance and these characters could be considered and exploited for selection in earlier generations. Whereas, in the traits with low heritability, influence of environmental factors is strong for their expression and genotype selection based on these characters should be postponed to the later generations. The results are in close conformity with Golani *et al.* (2007) who observed high heritability for average fruits weight, fruit length, number of locules/fruit and fruit yield.

High genetic advance was observed for yield per plant, number of fruits per plant, average fruit weight and plant height. The results are in close conformity with Golani *et al.*, (2007). High heritability (94.80%) with low genetic advance (47.73%) was reported for number of fruits per plant. These characters also exhibited high values of GCV which portrayed that these are controlled by additive gene and phenotypic selection for their improvement could be achieved by simple selection.

5.5 Genetic divergence based on Ward's linkage:

In the present investigation, 24 tomato genotypes were evaluated to estimate the nature and magnitude of genetic divergence. Cluster analysis was conducted for morphological characters. Distance between all pairs of genotypes was calculated using Squard Euclidean Distance method and genotypes were clustered based on Ward's method (1963). All the 24 genotypes were grouped into two main clusters with sub clusters. The results showed that the cluster A had two sub clusters; i.e. sub cluster A₁ and sub cluster A₂. Sub cluster A₁ had 8 genotypes (BT-136, SEL-12, NDT-9, ANGHA-1, ANGHA, NDT-1, Anand Tomato-3, NDT-4) followed by cluster Sub cluster A₂ which again had 8 genotypes (ANGHA (L-E415), Dhanshri, Punjab Ratta, PANT-T-5, Hisar Anmol, AZAD-T-2, PT-11 and NDTUR-73). Cluster B had further two sub clusters; i.e. Sub cluster B₁ and B₂. Sub cluster B₁ had 4 genotypes (DCT-1, CO-3, Swarna Sampada and ANGHA (L-E415)). Sub cluster B₂ had 4 genotypes (Utkal Pragyan, Hisar Lalit, Kashi Hemant and FEB-2). Anand Tomato-3 and Hisar Lalit were found to be highly dissimilar among 24 genotypes. The results of this study are in agreement with the results of Henareh. (2015) which could be exploited for breeding new tomato varieties or development of hybrid genotypes.

5.6 Molecular characterization

5.6.1 Estimation of genetic diversity using SSR and RAPD markers

Genetic diversity determines the inherent potential of a cross for heterosis and frequency of desirable recombinants in advance generations. Genetic diversity can be

assessed at the phenotypic, genotypic, physiochemical and molecular level which is helpful for the selection of diverse parents for hybridization. Genetic diversity assessed by the phenotypic traits is quite easy than that done with molecular markers but the disadvantage of phenotypic traits are that they get affected by the environment. DNA markers are widely used and are not affected by the different growth stages and the environment, due to which they are usually preferred than morphological and biochemical markers (Mc couch *et al.*, 1997).

Adaptation of narrowly based advanced varieties for intensive cultivation is one main reason of depletion for genetic variation. Assessment of diversity at inter as well as intra specific levels in crops is essential for sustained agriculture. The autogamy in crops like tomato results in uniformity. To evaluate diversity in such species which are expected to have low diversity level, DNA markers provide a good substitute due to limitations of morphological and biochemical markers or these may be used in association. In current study 9 microsatellite markers and 13 RAPD markers were used to assess the genetic diversity of tomato germplasm. The results indicated significant genetic variation among 24 tomato genotypes studied. In this study, while assessing the genetic diversity using SSR markers, the number of alleles detected varied from 2 to 8. The average number of polymorphic alleles per marker was 5 alleles per locus with an average PIC 0.303. Hu *et al.* (2012) reported 26 polymorphic markers out of 47 markers in the 67 Argentina tomato varieties. Twenty polymorphic markers had 2 alleles and the remaining 6 markers had 3 alleles each. PIC for most polymorphic markers ranged from 0.029 to 0.528. Glogovac *et al.* (2013) reported 3-5 alleles per locus while PIC value ranged from 0.12 to 0.73. The results of this study indicated that average number of alleles per locus was higher and PIC value was lesser than the results reported in the two studies referred here.

In the present study, 24 tomato genotypes were used to study the genetic diversity at the molecular level. Percentage polymorphism, number of alleles and PIC value showed the level of genetic divergence. A total of 45 alleles were observed. Out of which, 2 alleles were monomorphic and 43 alleles were polymorphic which range of 2 (SSR 74 and X90937) to 8 (AI773078, AWO37347 and Y09371) and an average of 5 allele per locus.

PIC value among the genotypes were varied from 0.16 (AI773078) to 0.50 (SSR 241). PIC value was recorded up to 0.50 for all the 9 SSR markers. PIC of > 0.5 is highly informative for genetic studies. These results are in agreement with the observations of Jamil *et al.* (2013) who used 24 SSR markers for the estimation of genetic diversity among 25 genotypes. They identified a total of 76 alleles with an average of 13.09 and

PIC value ranged from 0.012 to 0.45. Shah *et al.* (2013), Benor *et al.* (2008) Zhou *et al.* (2015) Korir *et al.* (2014) also reported similar results while studying the PIC of genomic SSRs and EST-SSR markers in various tomato lines.

In the present study, the genetic diversity has been assessed using RAPD marker. The number of alleles detected varied from 6 to 41. The average number of polymorphic alleles per marker was 15.62 with an average PIC of 0.290. The results of this study are in close conformity with the results of Sharifova *et al.* (2013)

Percentage polymorphism, number of alleles per locus and PIC values depicted the level of genetic divergence. A total of 203 alleles were produced, Out of which, 12 alleles were monomorphic and 191 alleles were polymorphic which ranged from 6 (OPN-15) to 41 (OPO-20) with an average of 15.62 allele per locus.

PIC value among the genotypes varied from 0.243 (OPC-04) to 0.432 (OPU-16) with an average of 0.29 allele per primer. PIC value less than 0.50 was recorded for all the 13 RAPD markers. The results are in conformity with the findings of Dhaliwal *et al.* (2009), Thamir *et al.* (2014) and Zargar *et al.* (2014).

5.6.2 Cluster analysis using SSR and RAPD markers based on UPGMA and diversity pattern

5.5.2.1 Cluster analysis using SSR markers

The Jaccard similarity coefficients among 24 genotypes ranged from 0.24 to 0.72. The lowest genetic distance was (0.24) between genotypes NDT-1 and FEB-2, while, the highest genetic distance was (0.72) between varieties C0-3 and ARKA ABHAY. Among all the pair-wise combinations, Arka Abhay and CO-3 showed the highest similarity index, while genotypes FEB-2 and NDT-1 showed the lowest similarity index. The dendrogram revealed a peculiar picture showing the different clusters of different genotypes. The genotypes were grouped into two main clusters, cluster A and cluster B (Figure 14). Among 24 tomato genotypes Dhanshri and Punjab Ratta showed highest similarity as revealed by clustering using SSR markers. Similarity coefficient of 24 genotypes using SSR markers ranged from 0.11 to 0.89. The clustering done using UPGMA was further confirmed by Principal component analysis (PCA) as represented in figure 15. The observations of principal component analysis revealed similar genetic differentiation among 24 genotypes of Tomato as revealed by UPGMA dendrogram. The results obtained match the findings of Singh *et al.* (2014).

5.6.2.1 Cluster analysis using RAPD markers

Cluster analysis of the genotypes is depicted in Figure 17. The genotypes were grouped into two main clusters, cluster A and cluster B. Cluster A was further divided into two sub clusters i.e. sub cluster A₁ and sub cluster A₂. Sub cluster A₁ had 3 genotypes and cluster B consisted of two sub clusters B₁ and B₂. Dendrogram showed genetic variation among the 24 genotypes of Tomato. Among 24 tomato genotypes CO-3 and Arka Abhay showed highest similarity as revealed by clustering using RAPD markers. Similarity coefficient of 24 genotypes using RAPD markers ranged from 0.39 to 0.77. . The lowest genetic distance was (0.26) between genotypes NDT-4 and FEB-2, while, the highest genetic distance was (0.77) between varieties C0-3 and ARKA ABHAY. The clustering done using UPGMA was further confirmed by Principal component analysis (PCA) as represented in Figure 18. The observations of principal component analysis revealed similar genetic differentiation among 24 genotypes of Tomato as revealed by UPGMA dendrogram. Similar results have been reported earlier by Sharifova *et al.* (2013).

5.6.2.1 Comparative Cluster analysis done using SSR and RAPD markers

Comparative cluster analysis of the genotypes done using SSR and RAPD markers is depicted in Figure 19. Dendrogram showed genetic variation among the 24 genotypes of Tomato. Among 24 tomato genotypes CO-3 and Arka Abhay and Punjab Ratta and PANT-T-5 showed highest similarity as revealed by comapartive clustering using SSR and RAPD markers. Similarity coefficient of 24 genotypes using RAPD markers ranged from 0.37 to 0.72. The lowest genetic distance was (0.24) between genotypes NDT-1 and FEB-2, while, the highest genetic distance was (0.72) between varieties C0-3 and ARKA ABHAY. The clustering done using UPGMA was further confirmed by Principal component analysis (PCA) as represented in Figure 20. The observations of principal component analysis revealed similar genetic differentiation among 24 genotypes of Tomato as revealed by UPGMA dendrogram. The results obtained match the findings of Zargar *et al.* (2014).

Therefore, in the present study, it has been revealed that genetic diversity exists among the tomato genotypes both at the genotypic and phenotypic level. High genetic diversity between the genotypes i.e. HISAR LALIT and ANAND TOMATO-3 (based on morphological traits), UTKAL PRAGYAN and ANGHA (based on SSR markers) and UTKAL PRAGYAN and NDT-4 (based on RAPD markers) could be exploited through hybridisation to recover the seggregants possessing high yield potential with improved

grain quality characteristics. Genetic diversity analysis at molecular level by SSR markers that cover the whole genome would be helpful to identify the diverse parents.

High, medium, and low locus polymorphism is defined as $PIC > 0.5$, $0.5 > PIC > 0.25$, and $PIC < 0.25$, respectively (Xie *et al.*, 2010). Therefore, in our case, SSR and RAPD markers detected medium locus polymorphism among the 24 tomato genotypes, indicating that both markers are of great utility for genetic diversity studies of tomatoes which can further be utilized in strengthening tomato breeding programmes.

SUMMARY AND CONCLUSION

The present investigation entitled “**Assessment of genetic diversity in cultivated tomato (*Solanum lycopersicum* L.) genotypes using morphological and molecular markers**” was carried out to assess the magnitude and nature of genetic variability at the morphological level and as well as at the molecular level among 24 genotypes of tomato. In this study, 9 SSR and 13 RAPD markers were used to study the genetic diversity at the molecular level. An experiment consisting of 24 genotypes of Tomato was conducted during Rabi season, 2015-16 at the Experimental Farm and Molecular Biology Laboratory of School of Biotechnology, SKUAST-J, Chatha. The experiment was conducted in randomised block design (RBD) with three replications and spacing of 45x90 cm. The data was recorded on 11 traits viz. Plant height (cm), Number of branches, Number of fruit per plant, Number of fruits per bunch, Fruit length (cm), Fruit width (cm), Number of locules, Total soluble solids (⁰Brix), Flesh thickness (mm), Average fruit weight (g) and Yield per plant (g).

Characterization of the collected 24 tomato genotypes on the morphological basis showed significant variation for studied 11 traits of different genotypes with respect to their plant height, number of branches, Fruit length (cm), Fruit width (cm), No. of fruit per plant, No. of locules, Total soluble solids (⁰Brix), No. of fruits per bunch, Flesh thickness (mm), Average fruit weight (g) and Yield per plant (g). The widest range (861.40-2507.36 g) was recorded for Yield per plant (g/plant) followed by Number of fruits per plants (21.00-122.00), Plant height (35.10-82.02 cm), Average fruit weight (7.43-80 g/fruit), Number of branches (4.53-9.66), Total Soluble solids (2.83-8.6⁰Brix), Number of locules (2.18-5.5), Fruit Length (2.90-5.63 cm), Fruit width (2.85-4.98 cm), Number of fruits per bunch (2.03-3.66), Flesh thickness (1.21-4.31 mm). Maximum plant height (82.02 cm) was recorded in Punjab Ratta and Number of branches (9.66) was recorded in NDT-4. Utkal Pragyan recorded maximum number of fruits/bunch (3.66), Maximum Total soluble solids was recorded in DCT-1 (8.06 ⁰Brix), Maximum flesh thickness was recorded in Hisar Lalit and Dhanshri (4.31mm). Maximum number of locules per fruit (5.50) was recorded in Angha-1. Highest Fruit length was recorded in Hisar Lalit (5.63 cm) and Highest Fruit width was recorded in Dhanshri (4.98 cm). DCT-1 recorded highest number of fruits per plant (115.33). Maximum average fruit weight was recorded in NDT-4 (80.00 g). Maximum yield/Plant (2507.36 gm/plant) was observed in Hisar Lalit.

Minimum Yield per plant (861.40 gm) was noted in DCT-1. Hisar Lalit showed large fruit size with reference to Single fruit weight, Flesh thickness, Fruit length and Fruit width and Yield per plant. Poor size of fruits was recorded in DCT-1.

The analysis of variance revealed significant variation for all the characters for all the traits viz., Plant height (cm), Number of branches/plant, Fruit length (cm), Fruit width (cm), Number of fruit per plant, Number of locules/per plant, Total soluble solids (⁰Brix), Number of fruits per bunch, Flesh thickness (mm), Average fruit weight (g) and Yield per plant (g) indicating sufficient diversity among the genotypes.

The variation was maximum for yield/plant (861.40-2507.36 g) followed by Number of fruits per plant (21 to 122 /plant), Average fruit weight (7.43 to 80 g/ fruit), Plant height (35.10 to 82.02 cm) and minimum for Number of fruits/ bunch (2.03 to 3.66). The characters showing wide range of variation provide ample scope for selecting the desirable types.

Phenotypic coefficient of variation (PCV) was more than genotypic coefficient of variation (GCV) for all studied 11 traits. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were high for Number of fruits/plant (45.35, 46.57%), Average fruit weight (32.71, 33.21%), Flesh thickness (29.06, 33.53%), Yield per plant (26.55, 29.14%) and Number of locules (22.68, 22.97%). which suggested greater phenotypic and genotypic variability among genotypes and sensitiveness of the attributes for making further improvement by selection. The PCV was higher than the respective GCV for all the characters denoting environmental factors influencing their expression to some degree or other.

The heritability in broad sense ranged from 42.60% for Number of fruits/bunch to 97.00 for Average fruit weight. Such high values of heritability for Average fruit weight, fruit length, number of locules/ fruit and fruit clarified that they were least affected by environmental modification and selection based on phenotypic performance would be reliable.

The genetic advance as per cent of mean (genetic gain) varied from 34.60 percent to 96.13 percent. High estimates of genetic gain were obtained for Yield per plant (96.13 %) followed by number of fruits per plant (47.73%) and Average fruit weight (34.60%). High estimates of genetic gain coupled with high values of GCV portrayed that these are controlled by additive gene and phenotypic selection for their improvement could be achieved by simple selection. High estimates of heritability coupled with low GCV and

genetic gain were observed for Total Soluble Solids which might be attributed to non-additive gene action controlling its expression and simple selection would not be rewarding. Nevertheless this could be improved by development of hybrid varieties or utilization of transgressive segregants in heterosis breeding programme.

Morphological traits grouped all the 24 genotypes of tomato into two main clusters (Cluster A and Cluster B). Cluster A had two sub clusters (cluster A₁ and sub cluster A₂). Cluster B had further two sub clusters (Sub cluster B₁ and B₂). The genotypes HISAR LALIT and ANAND TOMATO-3 were found to be the most diverse among the 24 genotypes.

For molecular characterization, 24 genotypes of tomato were used and subjected to banding profiling by using 9 SSR markers and 13 RAPD markers. SSR and RAPD markers with highest Polymorphic information content (PIC) value i.e. 0.50 (SSR 241) and 0.43 (OPU-16) respectively, can be used further for carrying out genetic diversity and polymorphism studies.

Cluster analysis done using SSR, RAPD and comparative clustering analysis of the genotypes using SSR and RAPD markers UTKAL PRAGYAN and ANGHA, UTKAL PRAGYAN and NDT- 4 and UTKAL PRAGYAN and NDT-4 respectively were found to be the most diverse and hence based on cluster analysis the identified diverse tomato lines can be can be effectively selected for carrying out various breeding and crop improvement programmes.

The broad conclusions could be drawn as under:

- Considerable genetic diversity among the cultivated 24 tomato genotypes was observed at both the morphological and molecular levels, which is of importance for germplasm classification, management, and further utilization.
- SSR marker proved to be better than RAPD marker as they showed higher values for most of the parameters that determine the potential of markers in diversity analysis.
- For better germplasm characterization and development of potential markers for agronomically important traits, more SSR markers should be included in the analysis.
- This type of research, coupled with the results of field observations, can present a starting point for MAS (Marker Assisted Selection) in tomato breeding.

- High level of variability between the genotypes could further be exploited through hybridization and selection among identified lines for the development of superior tomato genotypes.
- All the observations made in this study will provide valuable evidence for decision making in choosing of markers for future work, characterization of germplasm, breeding and Tomato germplasm management.

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VITA

Name of the student	Supneet Kaur
Father's name	S. Ranbir Singh
Mother's name	Smt. Harwant Kaur
Nationality	Indian
Date of birth	13-12-1991
Permanent home Address	F/339, Sainik Colony, Jammu

EDUCATIONAL QUALIFICATION

Bachelors degree	B.Sc. Biotechnology
University and year of award	SKUAST-JAMMU, 2014
OGPA	7.34/10.00
Master's degree	M.Sc. Biotechnology
OGPA	7.72/10.00

CERTIFICATE – IV

Certified that all the necessary corrections as suggested by the external examiner/evaluator and the Advisory committee have been duly incorporated in the thesis entitled **“Assessment of genetic diversity in cultivated Tomato (*Solanum lycopersicum* L.) genotypes using morphological and molecular markers”**, submitted by **Ms. Supneet Kaur**, Registration No. **J-14-MB-21**.

(Dr. A. K. Singh)
Major Advisor

Place: Jammu

Date:

Head

Division of School of Biotechnology

Table 17: Jaccard Similarity Coefficient Matrix obtained using SSR

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1.00																							
2	0.63	1.00																						
3	0.58	0.60	1.00																					
4	0.52	0.53	0.63	1.00																				
5	0.48	0.54	0.51	0.42	1.00																			
6	0.49	0.55	0.67	0.53	0.48	1.00																		
7	0.51	0.55	0.61	0.50	0.45	0.72	1.00																	
8	0.42	0.50	0.50	0.60	0.37	0.57	0.67	1.00																
9	0.43	0.52	0.47	0.44	0.42	0.55	0.54	0.50	1.00															
10	0.42	0.51	0.52	0.48	0.45	0.58	0.59	0.56	0.64	1.00														
11	0.38	0.48	0.47	0.43	0.42	0.55	0.61	0.53	0.53	0.71	1.00													
12	0.39	0.46	0.45	0.38	0.41	0.53	0.52	0.48	0.48	0.63	0.71	1.00												
13	0.37	0.43	0.46	0.39	0.43	0.52	0.48	0.43	0.50	0.61	0.66	0.69	1.00											
14	0.36	0.41	0.42	0.34	0.38	0.48	0.48	0.47	0.39	0.50	0.53	0.56	0.60	1.00										
15	0.29	0.39	0.32	0.27	0.34	0.37	0.36	0.35	0.38	0.45	0.48	0.46	0.52	0.57	1.00									
16	0.32	0.40	0.38	0.33	0.38	0.40	0.42	0.37	0.39	0.52	0.49	0.47	0.48	0.44	0.53	1.00								
17	0.35	0.37	0.37	0.31	0.35	0.40	0.38	0.36	0.33	0.47	0.43	0.47	0.46	0.49	0.45	0.51	1.00							
18	0.34	0.38	0.39	0.31	0.34	0.41	0.39	0.36	0.34	0.46	0.52	0.47	0.53	0.52	0.51	0.50	0.63	1.00						
19	0.31	0.31	0.35	0.25	0.31	0.35	0.30	0.25	0.28	0.36	0.35	0.39	0.39	0.36	0.33	0.43	0.48	0.55	1.00					
20	0.33	0.34	0.40	0.29	0.36	0.34	0.29	0.26	0.29	0.36	0.36	0.38	0.42	0.37	0.32	0.39	0.42	0.46	0.52	1.00				
21	0.41	0.43	0.44	0.36	0.37	0.41	0.34	0.32	0.37	0.38	0.37	0.41	0.38	0.36	0.34	0.38	0.41	0.41	0.44	0.60	1.00			
22	0.26	0.30	0.36	0.24	0.35	0.33	0.31	0.28	0.28	0.33	0.35	0.34	0.34	0.30	0.30	0.33	0.36	0.34	0.37	0.48	0.45	1.00		
23	0.33	0.38	0.42	0.33	0.33	0.43	0.38	0.35	0.31	0.39	0.40	0.41	0.41	0.39	0.34	0.37	0.44	0.44	0.46	0.57	0.52	0.55	1.00	
24	0.34	0.36	0.36	0.26	0.34	0.33	0.30	0.30	0.29	0.37	0.38	0.37	0.34	0.33	0.33	0.37	0.37	0.40	0.43	0.51	0.53	0.55	0.66	1.00

Table 18: Jaccard Similarity Coefficient Matrix obtained using RAPD

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1.00																							
2	0.67	1.00																						
3	0.63	0.64	1.00																					
4	0.56	0.58	0.62	1.00																				
5	0.51	0.57	0.54	0.42	1.00																			
6	0.53	0.62	0.72	0.56	0.53	1.00																		
7	0.51	0.59	0.65	0.53	0.47	0.77	1.00																	
8	0.43	0.53	0.53	0.65	0.38	0.64	0.69	1.00																
9	0.46	0.57	0.51	0.48	0.46	0.63	0.58	0.51	1.00															
10	0.46	0.55	0.57	0.53	0.48	0.65	0.64	0.59	0.66	1.00														
11	0.41	0.51	0.52	0.46	0.45	0.62	0.65	0.57	0.55	0.69	1.00													
12	0.44	0.50	0.51	0.42	0.45	0.59	0.58	0.52	0.5	0.62	0.72	1.00												
13	0.42	0.47	0.50	0.42	0.47	0.58	0.53	0.46	0.52	0.61	0.67	0.69	1.00											
14	0.38	0.45	0.46	0.36	0.40	0.51	0.50	0.49	0.42	0.52	0.56	0.58	0.60	1.00										
15	0.33	0.43	0.37	0.31	0.39	0.40	0.41	0.39	0.39	0.47	0.51	0.48	0.54	0.62	1.00									
16	0.33	0.41	0.42	0.36	0.41	0.45	0.44	0.38	0.41	0.53	0.50	0.49	0.51	0.46	0.55	1.00								
17	0.37	0.39	0.41	0.34	0.36	0.43	0.40	0.37	0.36	0.50	0.45	0.48	0.48	0.49	0.47	0.53	1.00							
18	0.38	0.41	0.43	0.34	0.37	0.44	0.42	0.38	0.36	0.48	0.54	0.47	0.53	0.53	0.52	0.51	0.64	1.00						
19	0.33	0.33	0.37	0.26	0.34	0.37	0.32	0.27	0.29	0.38	0.37	0.41	0.40	0.38	0.34	0.46	0.50	0.56	1.00					
20	0.36	0.38	0.44	0.31	0.40	0.37	0.32	0.29	0.33	0.40	0.41	0.42	0.46	0.40	0.36	0.44	0.45	0.50	0.54	1.00				
21	0.46	0.47	0.49	0.40	0.40	0.45	0.38	0.35	0.42	0.42	0.41	0.44	0.41	0.38	0.37	0.40	0.44	0.44	0.47	0.63	1.00			
22	0.29	0.32	0.40	0.27	0.38	0.37	0.35	0.29	0.29	0.35	0.37	0.38	0.38	0.33	0.32	0.33	0.39	0.37	0.42	0.55	0.48	1.00		
23	0.38	0.42	0.48	0.36	0.38	0.45	0.42	0.39	0.36	0.45	0.45	0.45	0.45	0.42	0.36	0.42	0.49	0.48	0.50	0.59	0.55	0.60	1.00	
24	0.38	0.39	0.41	0.30	0.38	0.37	0.34	0.32	0.30	0.39	0.41	0.39	0.37	0.36	0.34	0.38	0.41	0.42	0.48	0.58	0.57	0.57	0.73	1.00

Table 19: Jaccard Similarity Coefficient Matrix obtained using RAPD and SSR

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1.00																							
2	0.63	1.00																						
3	0.58	0.60	1.00																					
4	0.52	0.53	0.63	1.00																				
5	0.48	0.54	0.51	0.42	1.00																			
6	0.49	0.55	0.67	0.53	0.48	1.00																		
7	0.51	0.55	0.61	0.50	0.45	0.72	1.00																	
8	0.42	0.50	0.50	0.60	0.37	0.57	0.67	1.00																
9	0.43	0.52	0.47	0.44	0.42	0.55	0.54	0.50	1.00															
10	0.42	0.51	0.52	0.48	0.45	0.58	0.59	0.56	0.64	1.00														
11	0.38	0.48	0.47	0.43	0.42	0.55	0.61	0.53	0.53	0.71	1.00													
12	0.39	0.46	0.45	0.38	0.41	0.53	0.52	0.48	0.48	0.63	0.71	1.00												
13	0.37	0.43	0.46	0.39	0.43	0.52	0.48	0.43	0.50	0.61	0.66	0.69	1.00											
14	0.36	0.41	0.42	0.34	0.38	0.48	0.48	0.47	0.39	0.50	0.53	0.56	0.60	1.00										
15	0.29	0.39	0.32	0.27	0.34	0.37	0.36	0.35	0.38	0.45	0.48	0.46	0.52	0.57	1.00									
16	0.32	0.40	0.38	0.33	0.38	0.40	0.42	0.37	0.39	0.52	0.49	0.47	0.48	0.44	0.53	1.00								
17	0.35	0.37	0.37	0.31	0.35	0.40	0.38	0.36	0.33	0.47	0.43	0.47	0.46	0.49	0.45	0.51	1.00							
18	0.34	0.38	0.39	0.31	0.34	0.41	0.39	0.36	0.34	0.46	0.52	0.47	0.53	0.52	0.51	0.50	0.63	1.00						
19	0.31	0.31	0.35	0.25	0.31	0.35	0.30	0.25	0.28	0.36	0.35	0.39	0.39	0.36	0.33	0.43	0.48	0.55	1.00					
20	0.33	0.34	0.40	0.29	0.36	0.34	0.29	0.26	0.29	0.36	0.36	0.38	0.42	0.37	0.32	0.39	0.42	0.46	0.52	1.00				
21	0.41	0.43	0.44	0.36	0.37	0.41	0.34	0.32	0.37	0.38	0.37	0.41	0.38	0.36	0.34	0.38	0.41	0.41	0.44	0.60	1.00			
22	0.26	0.30	0.36	0.24	0.35	0.33	0.31	0.28	0.28	0.33	0.35	0.34	0.34	0.30	0.30	0.33	0.36	0.34	0.37	0.48	0.45	1.00		
23	0.33	0.38	0.42	0.33	0.33	0.43	0.38	0.34	0.37	0.44	0.44	0.46	0.57	0.52	0.35	0.31	0.39	0.40	0.41	0.41	0.39	0.55	1.00	
24	0.34	0.36	0.36	0.26	0.34	0.33	0.30	0.30	0.29	0.37	0.38	0.37	0.34	0.33	0.33	0.37	0.37	0.40	0.43	0.51	0.53	0.55	0.66	1.00

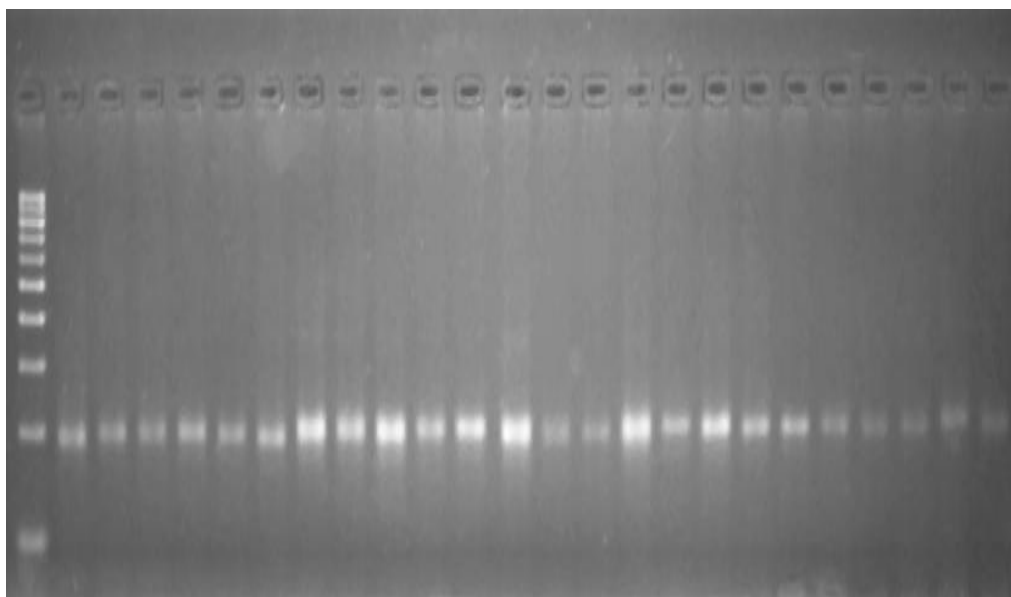


Plate 1: Banding pattern of SSR markers SSR 241

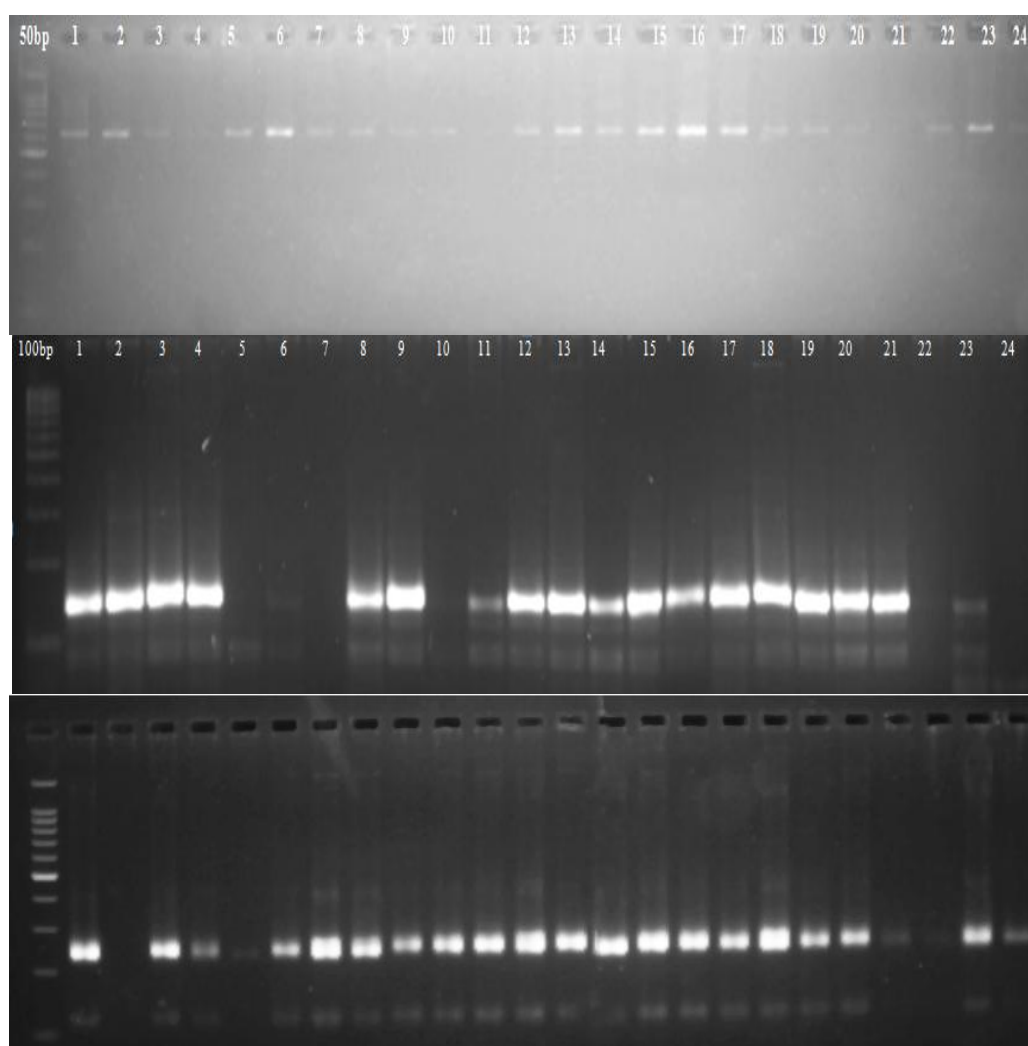


Plate 2: Banding pattern of SSR markers X90937, AI773078, X90770

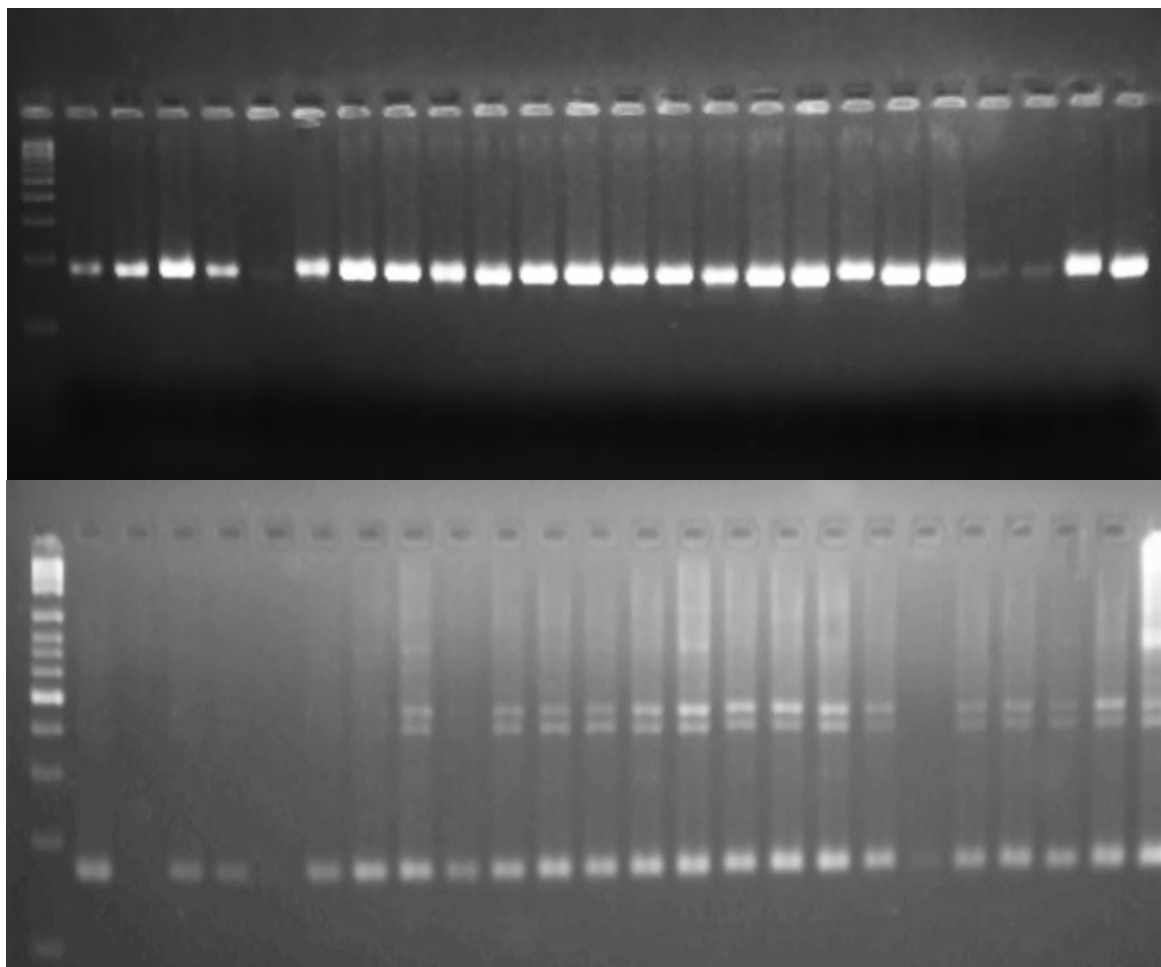


Plate 3: Banding pattern of SSR markers AW03747, SSR 9

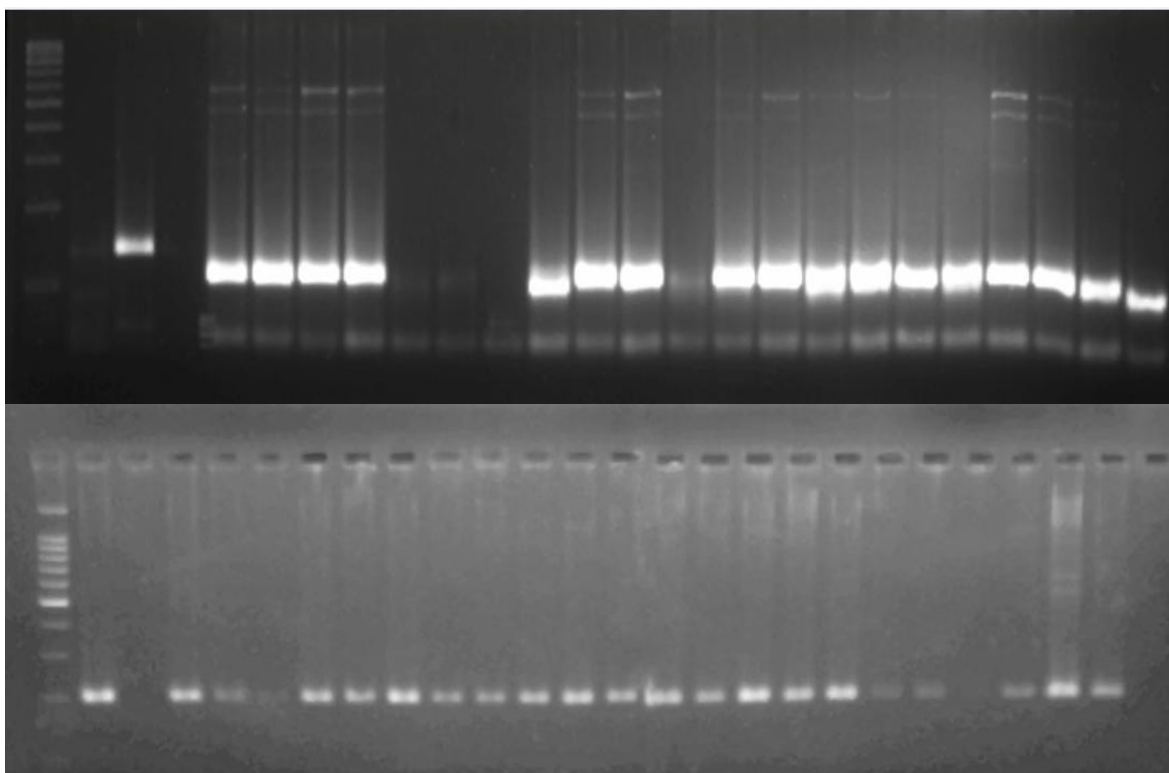


Plate 4: Banding pattern of SSR markers AI778183, SSR-74

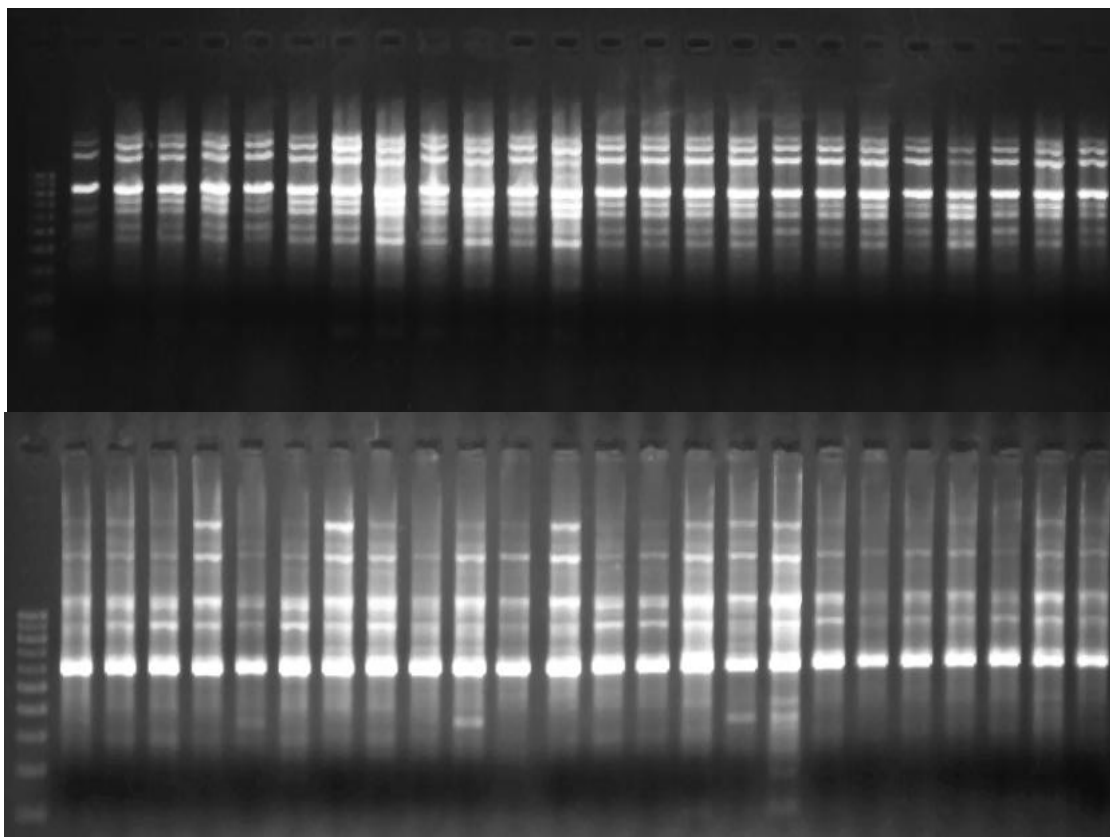


Plate 5: Banding pattern of RAPD markers OPA-02, OPY-15

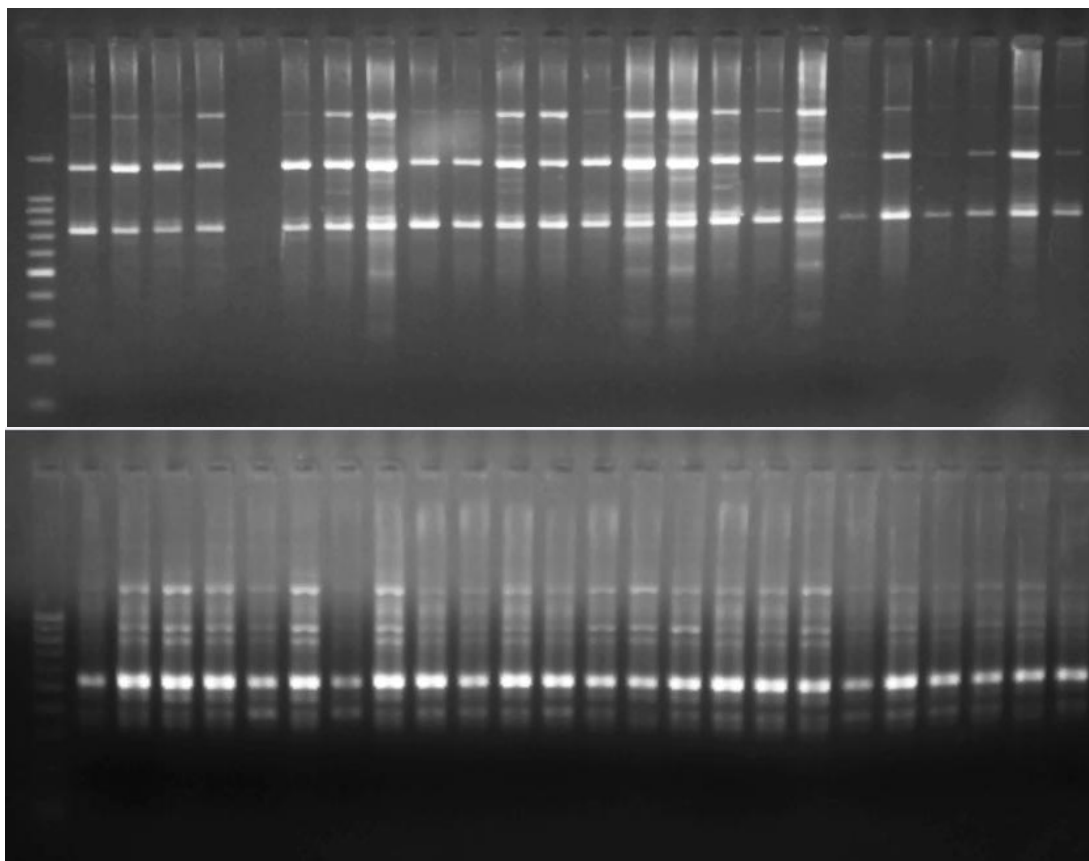


Plate 6: Banding pattern of RAPD Markers OPC-20,OPN-15

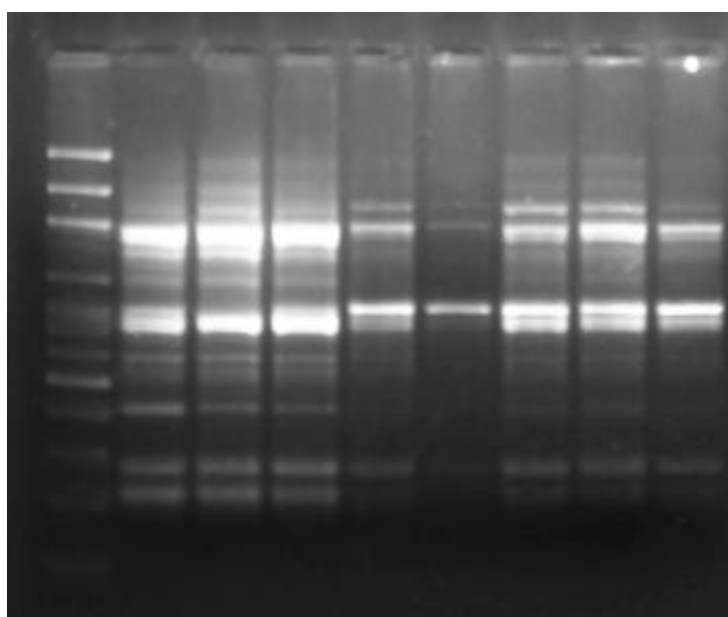
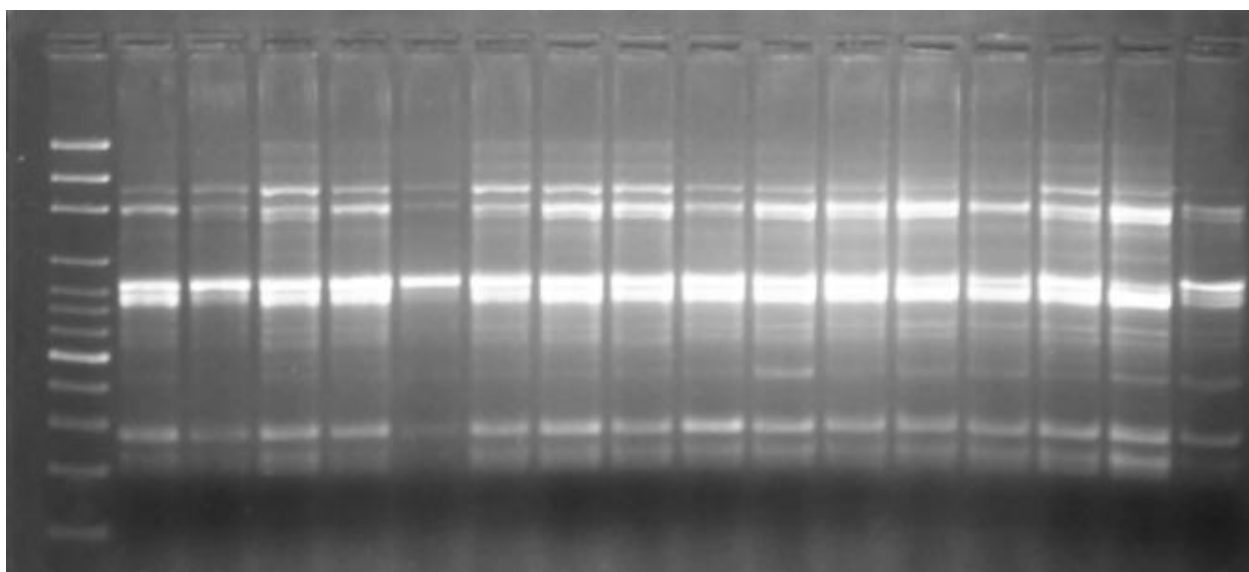


Plate 7: Banding pattern of RAPD Marker OPO-20

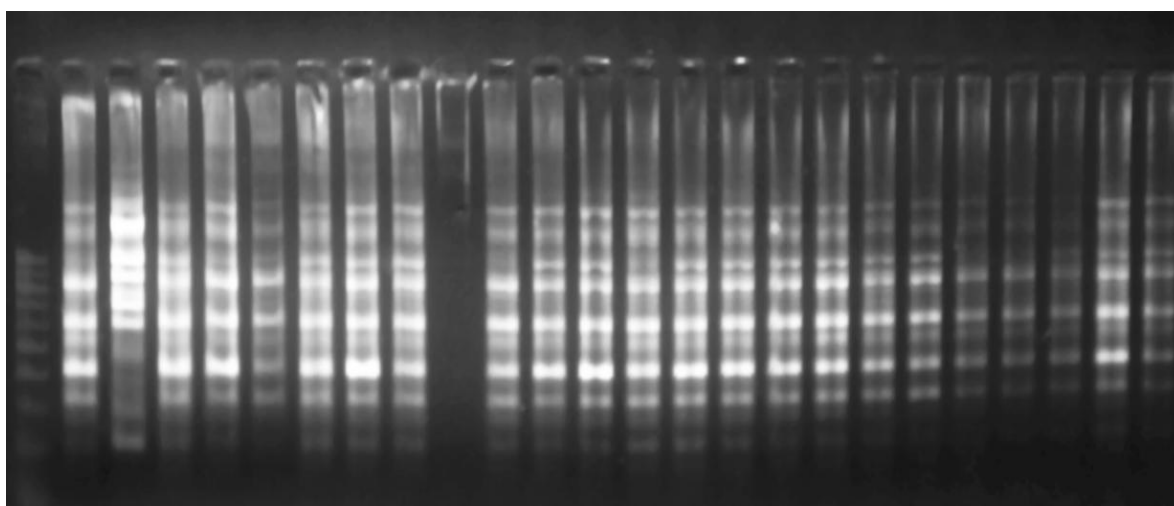
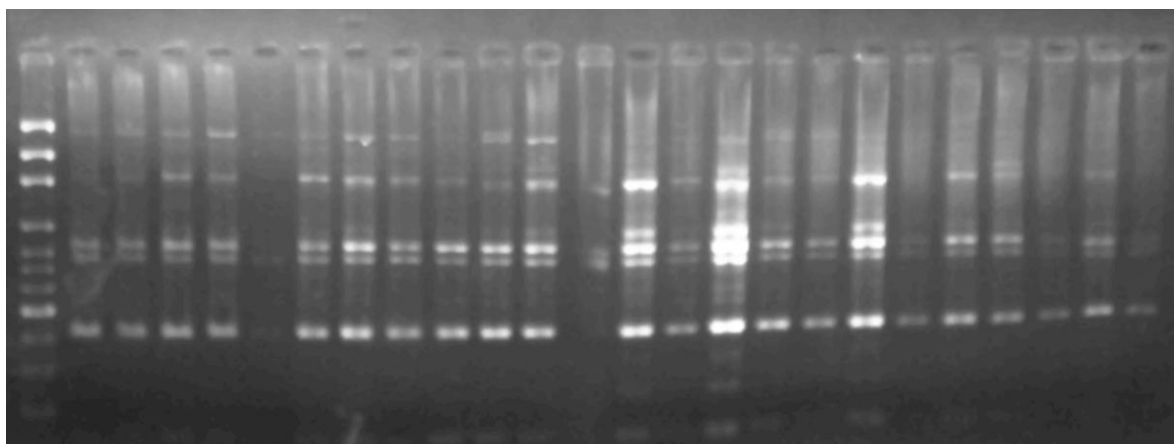


Plate 8: Banding pattern of RAPD Markers OPQ-20, OPA-13

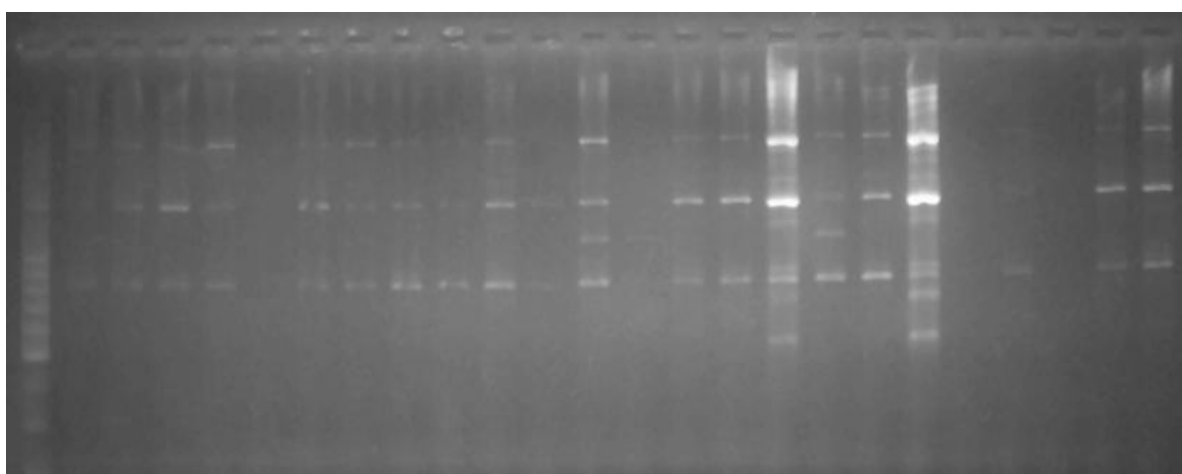


Plate 9: Banding pattern of RAPD Marker OPC-04