MOLECULAR CHARACTERIZATION AND CONSTRUCTION OF POPULATION STRUCTURE OF SELECTED TOMATO GENOTYPES (*Solanum lycopersicum* L.) UNDER HIGH TEMPERATURE STRESS CONDITION

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Molecular characterization and construction of population structure of selected tomato genotypes (*Solanum lycopersicum* L.) under high temperature stress condition"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.

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LIST OF ABBREVIATIONS

IPCC	Intergovernmental Panel on Climate Change
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
APX	Ascorbate Peroxidase
SSR	Simple Sequence Repeats
DNA	Deoxyribo Nucleic Acid
LDL	Low Density Lipoprotein
HSR	Heat Stress Response
β-Gal	β-galactosidase
sRNAs	small RNAs
OH	Hydroxyl
GPX	Guaiacol Peroxidase
CAT	Catalase
DHAR	Dehydroascorbate reductase
AsA	Ascorbic Acid
DHA	Docosahexaenoic Acid
GSSG	Glutathione Disulfide
GSH	Glutathione
PAs	Polyamines
GB	Glycinebetaine
INV	Invertase Inhibitor
PCD	Programmed Cell Death
ABA	Abscisic Acid

ERFs	Ethylene Responsive Factors
ER genes	Ethylene-Responsive genes
SA	Salicylic Acid
BRs	Brassinsteroids
Rubisco	Ribulose-1, 5-biphosphate carboxylase/oxygenase
RuBP	Ribulose-1,5-bisphosphate
IAA	Indoleacetic Acid
SNP	Sodium Nitroprusside
NO	Nitric Oxide
cPTIO	(2-(4-carboxyphenyl)-4, 4, 5, 5-tetramethylimidazoline-1-oxyl-3-oxide)
HSPs	Heat Shock Proteins
Hsfs	Heat stress transcription factors
MHS	Moderate Heat Stress
RFLP	Restriction Fragment Length Polymorphism
RAPD	Random Amplification of Polymorphic DNA
AFLP	Amplified Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
REC	relative electrical conductivity
CC	chlorophyll content
QTL	Quantitative Trait locus
PSII	Photosystem II
cM	Centimorgans
PIC	Polymorphism information content
KAU	Kerala Agricultural University

IIHR	Indian Institute of Horticultural Research
IARI	Indian Agricultural Research Institute
UAS	University of Agricultural Sciences
HPAU	Himachal Pradesh Agricultural University
TNAU	Tamil Nadu Agricultural University
AVRDC	Asian Vegetable Research and Development Center
ICAR-IIVR	Indian Council of Agricultural Research-Institute of Vegetable Research
CTAB	Cetyltrimethyl Ammonium Bromide
TE	Tris-EDTA
TBE	Tris/Borate/EDTA
UPGMA	Un-weighted pair group method with arithmetic mean
NTSYSpc	Numerical Taxonomy SYStem for personal computer
SDS PAGE	Sodium Dodecyl Sulfate–Poly Acrylamide Gel Electrophoresis

1. INTRODUCTION

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belonging to the family Solanaceae was originated in South America and it is widely grown all over the world and the second most important vegetable crop next to potato worldwide (Golam *et al.*, 2012). India is currently the fourth largest tomato producer and the second-largest in the world after China in terms of acreage. Tomato has a quite compact genome within the family Solanaceae, characterized by its diploidy (2n = 2X = 24) (Shirasawa *et al.*, 2010).

Giovanelli and Paradiso (2002) identified that tomatoes are a reliable source of various essential nutrients and secondary metabolites for human health; which include minerals, vitamins C and E, beta-carotene, lycopene, flavonoids, organic acids, phenolics, and chlorophyll. Fresh tomatoes are the rich source of the antioxidant lycopene (Nguyen and Schwartz, 1999). Tomatoes are beneficial to human health and minimize the risks of cancer, cardiovascular disease, and osteoporosis (Waheed *et al.*, 2020).

Although tomatoes have great potential to grow anywhere in the universe, Hasanuzzaman *et al.* (2012) reported that the rapidly increasing ambient temperature is one of the most disruptive stresses of ever-changing atmospheric elements. The ideal condition for tomato production is around 25°C during the daytime and 20°C during night time (Somraj *et al.*, 2017). As per the Intergovernmental Panel on Climate Change (IPCC), average global temperature has risen by 0.3°C in each year (Jones *et al.*, 1999) and will reach approximately 1°C or even 3°C above the mean temperature by the year 2025. These increases in temperature hinder tomato growth and production by detrimentally influencing plant anatomy, biology, biochemistry, molecular processes and subsequently impacting yield (Bita and Gerats, 2013).

Temperature is one of the key factors for the fruit set in tomatoes. Miller *et al.* (2001) have reported that a temperature above 35°C was found to affect seed germination, seedling and vegetative development, flowering, fruit set, and fruit maturation in tomato. The optimum range of night temperature for tomato is 15-20°C

(Somraj *et al.*, 2017), however, pollen production and fruit set are likely to be inhibited above 18°C (Peet and Bartholemew, 1996).

In plants, high-temperature stress induces injury and is associated with excess production of reactive oxygen species (ROS) which causes cell damage. To protect against negative impacts of ROS, plants stimulate the production of antioxidant enzymes such as SOD (superoxide dismutase), catalase, and APX (ascorbate peroxidase). Until then, plants have antioxidant enzymes scavenging mechanisms, like proline and carotenoids, which work with the enzymatic scavenging framework (Hasanuzzaman *et al.*, 2012). Knowing the basic influence of high temperature on ROS and antioxidant function metabolism in the plant is a necessity for improving crops during high-temperature stress conditions (Zhou *et al.*, 2019).

However, genetic engineering and molecular breeding have alternate strategies to enhance tomatoes with higher temperature resistance. Advancement with crops depends on the extent of genetic diversity in economic characteristics, and hence the evaluation and exploitation of genetic diversity in an intended way are important in any initiative for advancement. The natural phenotypic variations found in the cultivated plants are linked to the molecular polymorphism by association genetics (Khan *et al.*, 2020).

Molecular markers are hence used to determine gene diversity in collections of germplasms and to recognize varieties within the population (Golam *et al.*, 2012), it is also used for the efficient selection of desirable agronomic traits since they are based on plant genotypes and are independent of environmental fluctuations (He *et al.*, 2003). Among the various available markers, the most commonly used types are simple sequence repeats (SSRs) or microsatellites.

SSR is considered an important method for studying biological variation and characterizing germplasm due to its reproductivity, co-dominance in nature, and sufficient distribution in genomes (Zhou *et al.*, 2015). For tomatoes, the genetic maps available at the moment have a small set of underlying SSR markers that were not spread evenly across the genome. The whole circumstances demonstrate a need for

more SSRs in genomic regions which lacked sufficient markers (Geethanjali *et al.*, 2010).

Recognizing whether gene expression is dispersed among and within populations is also critical for germplasm management, crop raising, and mapping relationships. Making DNA (Deoxyribonucleic acid) based markers provides a new method for genetic research at the population level. Together with advances in computer technology and computational resources can provide information in understanding populations (Excoffier and Heckel, 2006). Compared to certain other sub divisional quantification approaches, Pritchard *et al.* (2000) showed that using "Structure" it is possible to assess the amount of an individual's genome of each population asserted. The numerical clustering process used by the "Structure" program follows a Bayesian framework and was used in numerous studies of biological variation and able to research tomatoes (Mazzucato *et al.*, 2008).

With these backgrounds, the project titled "Molecular characterization and construction of population structure of selected tomato genotypes (*Solanum lycopersicum* L.) under high-temperature stress condition", focused on the molecular marker discovery related to high-temperature tolerance in tomatoes and to understand genetic variation among tomato genotypes using "structure" software.

2. REVIEW OF LITERATURE

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The actual home of the tomato appears to be along South America's western seaboard where the cold ocean currents balance the mean air temperatures, even near to the equator (Hobson and Grierson, 1993). It is botanically a fruit, yet horticultura lly a vegetable and is one among the most economically valuable vegetable consumed either fresh or processed. It is often used as a second vegetable in the world after potato and is undoubtedly the most popular garden plant. In the year 2012 global tomato production exceeded 161 million tons per annum (Zhou *et al.*, 2015). Tomato is a prominent model in plant genetic research, in addition to its global health and high economic significance as a crop (Benor *et al.*, 2008). On a global scale, tomatoes continue to rise its importance for fresh crop consumption, for inclusion as a major constituent in many packaged foods, and also for research into the fundamental principles of plant growth and development.

In the 16th century, the tomato was introduced to Europe and later distributed in the Mediterranean region. Subsequently, thousands of tomato varieties were produced through breeding and selection. They were grown through tropical regions to some locations of the Arctic Circle in a variety of locations in the universe. The largest countries developing tomatoes comprise China, Iran, the USA, Italy, India, Turkey, Egypt, Spain, Brazil, Mexico, Canada, Greece, and Russia.

Tomatoes are a significant source of lycopene, beta-carotene, folate, potassium, vitamin C, flavonoids and vitamin E. Tomato processing may have a significant effect on other nutrients bioavailability. Homogenization, preheating, and the use of oil in refined tomato products lead to increased lycopene bioavailability, with almost the same mechanisms affecting the substantial loss of many other nutrients. Many of the nutrients present in tomato functions individually and conjunction, it protects lipoproteins and vascular cells from oxidation and also appears to be effective in atherosclerosis. This hypothesis was accepted by both *in vitro* and *in vivo* multiple clinical kinds of research and suggests that a decreased rate of stroke disease will occur

through the intake of antioxidant-rich foods. Several cardioprotective roles provided by tomato nutrients usually involve; low-density lipoprotein (LDL) cholesterol control, platelet aggregation, blood pressure reduction, and homocysteine. Tomatoes provide several theoretical or verified effects and are widely consumed worldwide, they are considered as a key element of the cardioprotective diet. An essential argument is that since fresh tomatoes are high in nutrients, they lack saturated fatty acids and cholesterol. Fresh tomatoes are often low in sodium, but certain processed tomato products, such as tomato sauces and paste contains high sodium. Tomato's typical sweet-sour taste and total flavor intensity are dependent on the following elements: free acids (mainly citric acid), reducing sugars (fructose and glucose), their ratio, and also some unidentified volatile substances, and interactions between some groups of compounds listed above. Of the minerals, potassium (effecting the free acid content) and phosphate (owing to its buffering power) indirectly influence its taste. Primarily volatile substances decide the distinctive tomato-like taste. Volatile compounds evolve as a result of the activated enzymes partly during the maturation, partly during the comminution of the mature fruit and volatile substances which determines young tomato flavor is primarily derived through amino acids as well as fatty acids (Turza, 1986).

Moreover, the composition of a tomato is highly affected by the species, ripeness stage, development season, temperature, environmental conditions, soil, fertilizer application, watering, light, and several other factors. For cultivated tomatoes, approximately 50 percent of the dry mass consists of sugars, mostly glucose and fructose. Free amino acids on a total make up approximately 2 to 2.5 percent of tomatoes total dry matter content.

After fertilization, the percentage of cells in a tomato fruit rises significantly over 2 to 3 weeks. Afterward, the development would be almost entirely due to cell expansion, because the pericarp fruit as well as the seeds which emerge from the parent plant will accumulate carbohydrate. The shape is also largely controlled by genetic factors, but nutrition and the environment can also have an influence. The appearance is however predominantly genetically inherited, but minerals and climate also have an

impact on it. Consumers expect tomatoes in worlds most temperate regions to be relatively round, weighing approximately 75 grams, homogenous in shape, significantly wider than deep, and have a minimum of 3 locules with a multilocular one (Hobson and Grierson, 1993).

2.1 CHALLENGES IN TOMATO CULTIVATION

Salinity, drought, and extreme weather conditions are major adverse environmental factors that affect crop production. Abiotic stress can be described as adverse environmental conditions that reduce crop growth and yield thereby limiting crop productivity throughout the world. Either at physiological, biochemical, or molecular levels, abiotic stresses impact plant growth and yield.

Drought, salinity, wind, boiling, freezing, ozone, pathogens, and ultraviolet radiation are the significant environmental factors that impose stress on plants. These are the significant environmental factors that reduce crop production (Wani *et al.*, 2016.

During stress conditions to transmit the signal and activate nuclear transcription factors, sensors initiate a signaling cascade to induce the expression of specific sets of genes. Knowledge of the mechanisms through which plants perceive and transfer stress signals to activate adaptive mechanisms to the development of stress-tolerant crops is essential. Genetic engineering strategies are based on transmitting some or more genes involved in signaling and regulatory processes, or encoding enzymes present in processes involved in the synthesis of functional and structural proteins, or encoding the proteins which confer tolerance to high temperature (Rodríguez *et al.*, 2005).

The natural environment for plants is composed of a diverse range of abiotic and biotic stress. The response of plants to all these stresses is relatively complex. While it is difficult to get accurate evidence of the effects of abiotic stress on agricultural production, it is apparent that abiotic stress remains to have a significant effect on plants based on the percentage of affected land and the worldwide decline in crop production. In the post-genomic age, extensive analyses through three

management strategies or omics (transcriptomics, metabolomics, proteomics) have enhanced our understanding of the various molecular signaling pathways involved in stress adaptation as well as resistance (Cramer *et al.,* 2011).

2.1.1 High temperature stress

High temperature is described by a temperature rise above a threshold point for a while to induce irreversible damage to plant development (Wahid *et al.,* 2007). High-temperature stress is often regarded as one of the disastrous abiotic stresses causing significant yield losses worldwide. Owing to global climate change the extent and severity of heat stress are increased (Ahammed *et al.,* 2016).

Gaseous emissions through human activities make a significant contribution to the global greenhouse gas concentrations in particular CO_2, Methane, nitrous oxides, and chlorofluorocarbons. Diverse global circulation simulations predict that greenhouse gasses will raise the planets average atmospheric temperature gradually (Wahid *et al.*, 2007), and as per the IPCC report, the global average temperature is rising approximately 0.3°C every decade (Jones *et al.*, 1999) and the report also indicates that the average global temperature will be 1°C to 3°C higher than the current value by the years 2025 and 2100, and it will lead to climate change and global warming. High-temperature stress will adversely affect the RNA species, cytoskeletal structures, stability of various proteins, membranes, and alters the ability of cells enzymatic reaction and will generate a metabolic inconsistency in plants. The heat stress response (HSR) is highly conserved in plants and includes multiple pathways, regulatory networks, and cellular compartments. Recently at least four putative sensors were proposed to activate the HSR. It has a plasma membrane receptor that activates the inward calcium flow, two endoplasmic reticulcum, a histone sensor for the nucleus, and cytosol unfolded protein sensors. Each of these putative sensors is assumed to trigger a common collection of HSR genes resulting in higher thermal tolerance, however, the relationship between the two pathways and their hierarchical order is uncertain (Mittler *et al.,* 2012).

The sensation of heat stress in plants is mediated by various pathways. The specific sensor molecule is directly influenced by heat for example; due to temperature-induced alterations in its quaternary and tertiary structures. Likewise, due to the effect of temperature on several other cellular components this same sensor molecule may also be indirectly influenced by high temperatures. For example, temperature-induced alterations in membrane fluidity may significantly impact a membrane protein by high temperatures. Alterations in heat-related metabolic processes, including the deposition of ROS, abnormal metabolic fluxes, and release of ATP from cells, lower energy levels can also trigger the high-temperature sensor molecules (Mittler *et al.,* 2012).

Rising temperatures will alter the geographical distribution and growing season of several crops by inducing the threshold temperature at the start of the season and the maturity of the crops to hit earlier (Porter, 2005).

Yet most crop physiologists in general and contrary to popular beliefs expect global warming to reduce crop yields. High-temperature stress can reduce most of the plant's life cycle, reduce the time of its development, and also maximize the senescence of plants. Some crops could only withstand relatively narrow ranges of temperature. If a slight rise in temperature occurs during the flowering phase it will adversely affect fertile seed production and leads to yield loss.

2.2. OPTIMUM TEMPERATURE FOR PROPER GROWTH OF TOMATO

Went (1944) first studied the day and night time temperature range of tomato and reported the maximum development happens whenever the temperature drops during the dark time than during day time and termed this phenomenon as thermoperiodism.

2.2.1 Night time temperature

For the normal growth, fruit set, and reproduction in tomato the ideal night temperature was reported as 15-20°C (Somraj *et al.,* 2017). A research performed by Peet and Bartholemew (1996) showed that both the quantity of pollen produced and the percentage of normal occurring pollen grains is optimal at a temperature of about

20°C. At higher temperatures, during the night both the amount of pollen produced and the usual percentage dropped off.

2.2.2 Daily mean temperature

The optimal temperature for the development and growth of tomatoes is 20°C to 24°C. A threshold temperature refers to a daily average temperature at which development is significantly reduced. When the temperature increases above 35°C tomatoes vegetative development, seed germination, seedling, flowering, fruit setting, and plant maturation are adversely affected. Temperatures above 34°C are regarded as super optimal thermal stress (Somraj *et al.*, 2017).

2.3 ADVERSE EFFECTS OF HIGH TEMPERATURE ON TOMATO

Plant growth is temperature-dependent. Plants need a certain amount of heat to grow from one point in their life cycle to another for example from seeding to the four-leaf stage (Miller *et al.*, 2001). Heat stress due to high ambient temperatures is a serious threat to crop production worldwide (Hall, 2000). When the temperature exceeds more than the optimal range during day and night time the plant display signs of abnormal flower growth, fruit drop, abortion of ovules, reduced pollen viability, pollen production and which will leads to reduced yield (Hazra and Ansary, 2008). The reproductive portion of a flower is negatively influenced at extreme temperatures, poor germination of pollen, carbohydrate stress, stigma tube elongation, poor development of pollen tubes are the major cause of decreased fruit set in tomatoes during high-temperature stress (Saeed *et al.*, 2007).

Various responses of enzyme reactions to high-temperature stress reveal that the developing process requires so many independent biochemical events with variable extreme heat susceptibility one of such event was studied by Peet *et al.* (1998) and they found out that elevated temperatures around 40°C (2 days) and 40°C (2 days) + 36°C (2 days) had quite a higher incidence on tomato production rates for β-Gal (β-galactosidase); as well as the recovering of β Gal activity would be almost complete mostly on the transmission of tomatoes to 21°C. But from the other side regarding α-Galactosidase function researchers observed a slower pace of recovery, although heat

stress had very little effect on its chronological pattern. In certain situations, a sudden decline of Gal activity might arise from rapid clearance synthesis inhibition or inhibition of the in vivo enzymatic activity, or perhaps a mixture of mechanisms.

Pan *et al.* **(**2017) stated that autogamy in cultivated tomato varieties is a derived trait from wild type tomato plants, which are mostly allogamous. Nonetheless, environmental stress can cause phylogenetic abnormalities in tomato flowers as well as prevent autogamy. Tomato plants usually exhibit the phenotype of stigma exertion at elevated temperature with severely impeded self-pollination and fruit environment, whereas the underlying stigma exertion mechanism has been unknown to date.

Various small RNAs (sRNAs) were shown to perform significant roles in plant growth including stress responses. Tomato stamens and pistil libraries have a total of 69 and 30 heat retardant miRNAs reported in both. Similarities and discoveries of both the expression of miRNA as well as its targets in heat stress stamens and pistils have shown that specific target components of miRNA could play a significant role in regulating heat stress reactions as well as stigma exertion. These results help to determine how extreme temperatures induced the molecular mechanism of the stigma exertion of tomato plants.

Under certain conditions, it is important to grow varieties that might sustain high-temperature stress to ensure tomato production even during the warmer months. Before embarking on the breeding program for the production of temperature-tolerant varieties knowledge on the extent of genetic variability as well as the relationship of different temperature-tolerant characters is required (Saeed *et al.*, 2007).

2.3.1 Vegetative phase

In tomato seedlings, the impact of high temperatures has been mitigated by eliminating the first two leaves mostly during the initial vegetative cycle in slowing the shoot apex elongation thereby expanding the number of leaves developed before flowering. The first two leaves are not self-sustaining at this stage of seedling growth they grow faster at high temperatures and using an even larger percentage of the assimilate translocated from the cotyledons than they need at low temperature (Hussey,

1963). In plants which grow at 25°C, a rapid extension of the apex and earlier floral emergence is followed by the rapid loss of leaves, with the number of leaves produced prior flowering is reduced to that of crops grown at 15°C. This has been mentioned that even the contrary influence of temperature upon leaf growth and apex enlargement may also be justified based on competition of necessary assimilation between some of the apex and also the first two growing leaves. Whenever the cotyledons seem to be the primary photosynthetic organs throughout the initial stages during seedling development relatively more of the substrate produced in the cotyledons appears to be moved to some of these leaves also at the apex with increasing temperature. These findings clearly show the role of heat stress in slowing apex elongation as well as the early stages of the plant's vegetative process.

2.3.2 Tomatoes vegetative and fruiting reactions towards high temperature

Upon the advent of high temperatures, the plants are compromised by vegetative vigor and reproductive capacity to the degree that fruit-set fails. Nevertheless, this failure typically begins before temperatures were too high (95°-100°F) to induce pollen sterility. At the time of anther dehiscence also a high rate of flower abscission is initiated. Later exertion of style occurs and restricts fruit in flowers that have not abscised already (Johnson and Hall, 1953).

Went (1994) recorded carbohydrate translocation failure at high night temperatures in the tomato plant and discussed the impact of night temperature on growth in detail. He suggested that reduced carbohydrates under these conditions would limit the development of fruit in the tomato plant. Smith and Cochran, (1935) found that at 100°F pollen germination was substantially reduced and style exertion was observed with plants heavily fertilized with nitrogen at hot weather (Johnson and Hall, 1953). The development of the flowers during high temperatures is highly influenced and pollen viability, the quantity of pollen, and the growth rate of the pollen tubes were also affected.

There are 2 main changes in the structure of the cell wall during maturation in tomato fruit: (1) a substantial reduction of noncellulosic neutral sugars especially

galactose and arabinose (2) a rise in soluble polyuronides. Mitcham and McDonald, (1992) conducted a study to examine the impacts of high temperature on the components of modifications of the cell wall linked to the ripening of tomato fruit. They also demonstrated that the normal sugar loss rate and the aggregation of soluble polyuronides decreased after 4 days of treatment in mature green tomatoes at 40°C. Moreover, the quantity of uronide in the cell wall expanded in high temperature stress given tomatoes although stayed stable in the fruits which kept as control. The production of uronide-containing cell wall polymers tended to carry on to result in a slight improvement in uronides of the cell wall in high temperature applied fruit while regulation of uronides of tomatoes cell wall stayed the same. Heat stress therapies can be a beneficial method for researching cell wall turnover by distinguishing synthetic and degrading processes.

2.3.3 Bud drop

At high temperatures, the production of abscisic acid increases, and flowers begin to drop before pollination in tomato (Smith and Cochran 1935). The reduction in flowers is partly due to a reduction of fertilization which is often induced by various factors. Gametogenesis is interrupted during extreme temperatures, gamete viability is lowered and the flora produces lesser pollen (Iwahori, 1965). The germination as well as elongation of the pollen tube into the style could also be affected by the high temperatures and hence hinder pollination. Phenotypic changes in the composition of flowers were found at high temperatures like exertion of the style from the browning of the antheridial cone through drying of the stigma. Therefore any alteration can avoid or minimize self-pollination, resulting in reduced fruit set percentages. The transport of carbon was associated with the amount of starch in the buds, but with increasing temperatures sucrose levels in the source organ will increases while starch levels decrease and it will disrupt the transport of carbon (Dinar and Rudich, 1985).

2.3.4 Anthesis

Tomato flower buds from five to nine days before anthesis and flowers from one to three days after anthesis has been reported to be especially sensitive to high

temperatures and have failed to produce fruits. The anthesis was especially vulnerable to high temperatures and nearly all flowers did not bear fruit (Iwahori, 1966).

2.3.5 Pollen development

Peet *et al.* (1998) stated that the effects throughout synthesis and secretion of pollen on increased temperature are extremely harmful to seed growth and fruit set. Related stress therapies seem to be less detrimental while enforced during the development of female gametes and then after pollen development. Using male-sterile and male-fertile tomato lines lead to the finding that disruption of pollen production and release during high temperatures stress would be a contributory factor to reduced fruit set in tomatoes and probably less production of certain crops by global warming.

In tomato meiosis occurring 8-9 days before anthesis in both mega and microspore, mother cells were extremely vulnerable to excessive temperature (Iwahori, 1965). Microspore meiosis occurs around 9 days before anthesis and differentiation of the stamens, tapetum, middle layers, and endothecium provide carbohydrates for the development of pollen. Prolonged exposure of tomatoes to extreme temperatures (Day/ Night temperatures of 32/26°C) decreases the number of pollen grains as well as decreases pollen viability.

The impact of temperature stress on pollen viability has been linked to changes throughout the metabolism of carbohydrates in various parts of anther during its growth. The amount of total soluble sugars increased significantly in the anther walls as well as in pollen grains, approaching a maximum at anthesis. Prolonged exposure of plants to elevated temperature (32/26°C) prevents the temporary rise in starch levels and results in reductions mostly in concentrations of soluble sugars within anther walls including pollen grains (Pressman *et al.*, 2002).

2.3.6 Pollination

The optimum relative humidity is generally understood to be 50-70percent for tomato pollination but was not well tested under controlled conditions or in conjunction with high temperatures. Sato *et al.* (2000) demonstrated that pollen release could limit seeded fruit set in tomatoes under high temperatures. Temperature stress also induces

both delayed fruit production, parthenocarpic fruit, and abortion of flora in tomato plants.

2.3.7 Fruit set

Several factors can contribute to the reduction of tomato fruit set at high temperatures and can be considered as potential criteria for selection. These factors include reduced flower production, ovule, pollen viability, and pollen dehiscence. At high temperatures, fruit set in tomatoes is usually inhibited (Levy *et al.*, 1978). Lack of viable pollen is commonly believed as a significant cause of poor fruit set at high temperatures. Various studies showed that heat stress reduces the assimilate supply to the flower buds (Dinar and Rudich, 1985).

Additional sigma and style exertion in response to high temperature and reduced levels of photosynthetic translocation and assimilation will adversely affect the ability to set fruit. Reduction of pollen release and the viability of pollen are considered main fruit determining factors during persistent heat stress and it is also determined that the release of pollen could be a greater factor in testing varieties and in deciding its approach to extreme temperatures since pollen release deficiency would inhibit fruit set regardless of the viability of pollen grains (Sato *et al.*, 2000).

2.3.8 Oxidative metabolism

The tomato is a plant that requires optimum temperatures of 22–25°C for growth and development and exposure to higher temperatures can alter its oxidative metabolism significantly. High temperature may induce oxidative metabolism in plants affected by the overproduction of reactive oxygen (ROS) species such as singlet oxygen (O_2), hydroxyl (OH), superoxide radicals and hydrogen peroxide (H_2O_2). ROS is extremely decisive and capable of destroying proteins, nucleic acids chlorophylls, membrane lipids, and interferes with homeostasis in plants.

Many of the observed or detected symptoms are due to disrupted oxygen metabolism mainly associated with the transition from predominantly heterolytic (two-electron transmission) processes to enhanced homolytic (one-electron transfer). However, homolytic reactions throughout metabolic chains create free radicals and

must be counteracted by the increase of radical-scavenging processes or compounds thus ensuring sequences of metabolically regulated reactions.

The above impact is gradually lost throughout future episodes of stress and mostly disorderly progressive processes take around. Eventually, cellular decompartmentalizations induce lytic or necrotic reactions which are evident as the breakdown of darkening cells or tissues. Throughout this process, each step is controlled through a quite comprehensive balancing of proline and antioxidant capabilities particularly photosynthetic (intensely managed by metabolic and oxygen detoxification) or photodynamic processes (hardly controlled by scavenger and/or quencher). Like many instances, the (theoretical) course of activities could only be identified timely by precisely articulated (technical) marker responses and seems to be certainly unique between individuals including organs (Elstner and Osswald, 1994).

Reactive oxygen species (ROS) serve as a cellular signaling agent to accelerate the response of plants to abiotic stresses and it is the aggregation of ROS leads to oxidative stress.

To reduce these potential harms species have developed various mechanisms dependent on the production of defensive antioxidants. The superoxide dismutase (SOD) enzyme affects the inactivation of radical O_2 to H_2O_2 and is typically known as the first stage of cell protection. H_2O_2 can be detoxified by different enzymes in different cell compartments either through the activity of guaiacol peroxidase (GPX) or catalase (CAT) that results in the catalysis of H_2O_2 to H_2O and H_2O_2 accumulation in thermal stress plants is considered to be the primary cause of plant biomass reduction (Peters *et al.,* 1989).

Heat stress causes: (1) Reduced shoot weight (2) enhanced SOD activity (3) deposition of H_2O_2 (4) elevated levels of ascorbic acid (ASA) antioxidant compounds; Docosahexaenoic acid (DHA), Glutathione disulfide (GSSG), and Glutathione (GSH) and (5) reduced activity of CAT, GPX, ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR) (related to detoxifying H_2O_2). Besides, Rivero *et al.* (2004) demonstrate that increased temperature took place in tomato plants

at 35°C could immediately hinder the ascorbate or glutathione cycle and afterward activate an oxidative burst demonstrated by foliar H_2O_2 aggregation.

2.3.9 Photosynthesis

High temperature affects the function of photosynthetic enzymes, the integrity of membranes, photophosphorylation and electrostatic interactions in chloroplasts, stomatal conductance to CO_2 dissemination, and signal transduction of photoassimilates. Photochemical ability and ribulose, 1, 5-bisphosphate carboxylase activity can be influenced by excessive uncertainty over temperature and thereby the photosynthesis in tomatoes, its mesophyll resistance, and/or photoassimilate translocation (Dinar and Rudich, 1985).

High temperatures inhibit the photosynthetic fixation of CO_2 and harm photosynthetic electron transport specifically at the site of the PSII. PSII (water-oxidizing, quinone-reducing structure) is among the most heat-sensitive photosynthesis mechanisms involved in the photosynthetic transfer of electrons as well as ATP production of its chloroplast thylakoid-membrane protein complexes. The O_2 evolving complex proteins are often the most susceptible to heat stress within PSII (Heckathorn *et al.,* 1998).

Rubisco (Ribulose-1, 5-biphosphate carboxylase/oxygenase) activity was inhibited under moderately high temperatures by Rubisco activase Inhibition (Sato *et al.,* 2000). Activation of rubisco is quick and hindered transiently by high temperatures and proposed that inhibition was sensitive to variations in the structural properties of the activase (Feller *et al.,* 1998).

Thermal stress fastens membrane molecules kinetic energy and mobility within cellular membranes molecules and weakens its chemical bonds. This allows every lipid bilayer of its biological membranes to be rather liquid by either protein denaturation or even an increase in unsaturated fatty acids. These enhancements maximize the penetration of its membrane and it is noticeable through accelerated leakage of electrolytes. This increased solute release as just an indication of reduced thermal stability of the plasma membrane has often been used for an alternative

indicator for tolerance towards heat stress in different crops particularly tomatoes and potatoes (Golam *et al.*, 2012).

Giri *et al.* (2017) mentioned that sudden or short-term increased temperature could reduce root development (comparable to shooting), protein assimilation concentration, nutrient absorption, root biomass protein, and root nutrient absorption rates. High-temperature effects mostly on roots, as well as nutrient interactions, were often long lasting, with incomplete recovery even after seven days of post-heat recovery in severe stressed plants. The comparative impacts of modest versus extreme heat stress on crop nutritional quality and composition are being associated with a moderate effect on the root to root mass, root nutrient uptake rate per g, nutrient absorption, and N-assimilatory total proteins.

High temperature is often accompanied through water stress-induced through drought, high leaf transpiration, or reductions in xylem hydraulic conductivity and water stress could also contribute towards reductions in the absorption of nutrients during heat stress (e.g. reductions in water uptake or transfer from roots to shoots). Therefore potential increases in rapid or short-term extreme heat through global warming would possibly hurt plant nutrient interactions that become more severe increase in temperature leading to decreased agricultural production and nutrient content. Research is needed to identify crop genetic variants that maintain nutrient absorption and assimilation during heat stress would need to concentrate on protein production, root growth, and metabolism of nutrients.

2.4 HIGH TEMPERATURE STRESS TOLERANCE IN TOMATO

Plants will develop mechanisms to withstand certain stresses when continuously subjected to abiotic stress during their life cycles. Some of those mechanisms the tomato plants follow during high-temperature stress are as follows;

Polyamines (PAs) are small, ubiquitous compounds which have been involved in controlling many physiological processes and several plant stress responses. PAs, spermidine, spermine, and putrescine accumulate under abiotic stress conditions. The elevated concentration of polyamine plays a significant role throughout

the plant's defensive response to different abiotic and biotic stresses. This will improve the activity of antioxidant enzymes and prevents membrane lipid peroxidation and thereby improves the efficiency of CO_2 assimilation and prevents tomatoes from high-temperature stress (Cheng *et al.*, 2009).

Several of these pathways include the accumulation of compatible solutes that are low molecular metabolites, which are extremely water-soluble and non-toxic at high concentrations (Rhodes and Hanson, 1993). One of the stable solvent Glycine betaine (GB) accumulates quickly in plants during high drought, salt, and high-temperature circumstances. It enhances resistance to plants over elevated temperatures for both plant growth and development. Foliar application of GB often increases plant tolerance to different forms of environmental stress (Yang *et al.*, 2005).

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When plants experience heat stress during the vegetative growth stage it alters hormone homeostasis including hormone stability, content, biosynthesis, and compartmentalization and better thermotolerance may be achieved if hormonal control of plant processes in the thermotolerant state is optimized (Maestri *et al.*, 2002).

Rivero *et al.* (2001) conducted a study on thermal stress tolerance in tomato and watermelon and observed that tomatoes can produce an acclimatized process towards super optimal 35°C thermal stress, above the average temperature 25°C although watermelon plants show adaptation at 15°C. In both plants, their acclimatization process consists of the aggregation of polyphenols as a way of responding to the excessive heat to trigger acclimatized mechanisms in plants under stress caused by temperature by manipulating factors that are involved in the bioactivity of phenolic compounds.

Li *et al.* (2012) show evidence that high potential for import of sucrose and INV (Invertase Inhibitor) activity may lead to heat resistance in new tomato fruits, by enhancing glucose signalling pathway PCD (programmed cell death) repressive

activities. Such results suggest that INV controlled PCD process by sugar signaling is persisted in reproductive organs in between dicotyledonous tomato population and monocotyledonous maize respectively in response to heat and water stress.

2.4.1 Role of Antioxidant enzymes

Increased antioxidant enzyme activity (SOD, POD, CAT, and APX) reduces electrolyte leakage, enhances gaseous exchange variables (Pn, Gs, Ci, Tr, Ls), water quality, higher photosystem II quantity yield (Fv/Fm) and reduces oxidative damage by scavenging more reactive oxygen species (ROS) that significantly impact plants by preserving cell membranes against harm, higher chlorophyll content, lower lipid peroxidation deposition and H_2O_2. Biradar *et al.* (2019) investigate the reaction of tomato genotypes against the role of antioxidant enzyme and osmoprotectants. An increase in the activity of SOD, POD, and CAT was observed across the genotypes. The accumulation of sugars such as sucrose, inositol, fructose, and glucose was higher in all the tomato genotypes subjected to induction treatment.

2.4.2 Hormonal regulation during high temperature stress

Heat stress severely impedes plant growth and production by destroying the photosynthetic components and functions of antioxidant enzymes.

Stress hormones such as ethylene (C2H4) and abscisic acid (ABA) has a significant role in the regulation of various physiological properties by performing as signal molecules (Golam *et al.*, 2012).

ABA is a substance that originates naturally with great significance in controlling the growth of plants and so, therefore, it mediates one of the signaling mechanisms of intracellular dehydration. When tomato plants were exposed to constant stressful temperatures they experienced a rise in their ABA levels (Daie, 1980). ABA is engaged in a response syndrome some of which can contribute to environmental stress tolerance. In the case of temperature stress ABA may increase the plant's heat tolerance by altering its water balance.

ERFs (Ethylene Responsive Factors) are key regulators for the tolerance of abiotic stress in various species. Enhanced expression of ERF was documented following drought, salinity, light stress, cold, and heat stress (Muller *et al.*, 2015). Research of ethylene participation in plant stress tolerance has been clearly illustrated by Frank *et al.* (2009) and noted that the microarray study shows HS stimulation of several ethylene-responsive (ER) genes which includes LeJERF1, ER5, ER21 and ER24 throughout the development of pollen grains. In maturing tomato microspores HS-regulated expression of the gene coding for ACC synthase, the enzyme responsible for the biosynthesis of ethylene, was also observed.

Salicylic acid (SA) is an essential hormone regulator for mitigating heat stress. Salicylic acid (SA) functions well in basal thermotolerances. The SA-mediated mechanism increases heat tolerance across a wide variety of plants namely potatoes, mustards, beans, *Arabidopsis thaliana*, tobacco, and tomatoes (Szalai and Janda, 2007).

Furthermore, external SA treatment rises the proline content while also assigned to both the osmotic potential of plants to effectively absorb the water, which is a combination of photosynthetic devices triggered favorable impacts on stomatal aperture whereas chlorophyll pigment is lowered at extreme temperatures total carotenoids, osmotic potential, and leaf water potential were also cured. Collectively researchers claimed the pre-treated SA suppressed the adverse effects of heat stress by enhanced photosynthesis activity as well as antioxidative enzyme mechanisms.

External use of brassinosteroids (BRs) decreases high temperature stress through significantly increasing the total photosynthetic rate, stomatal conductivity, highest possible RuBisCO carboxylation frequency as well as greatest achievable electron transport rate in tomato leaves that attributed to ribulose-1,5-bisphosphate (RuBP) (Ogweno *et al.*, 2008). The reduction of photochemical efficiency caused by heat stress in BR-deficient mutant tomatoes are being improved through extracellular use of BR or ABA; this repression mostly in ABA-deficient tomato mutants could perhaps be ameliorated through ABA but not through BR implying whether stress ameliorative impact of BR is primarily based on endogenous ABA concentrations.

H_2O_2 has been required not only for BR as well as for the heat tolerance in tomatoes induced through ABA (Ahammed *et al.,* 2016).

Recent genetic work and efforts to persuade crop resistance at high temperatures using traditional guidelines and genetic engineering attributes showed that crop response to heat stress is a multifactorial trait. Specific resistance components regulated by various gene sets are critical for thermal stability at various crop stages or in different tissues (Witcombe *et al.,* 2008).

Siddiqui *et al.* (2017) conducted a study and showed that when supplied in conjunction with IAA (Indole 3-acetic Acid) the application of sodium nitroprusside (SNP) has a significant combined effect in the development of plant during heat stress. It may also be interpreted through accelerated growth features since this co-application of IAA and SNP has been more successful than a single application in improving the biosynthesis of photosynthetic pigments and proline both under non-stress and HS circumstances. A co-application of IAA and SNP enhances the activities of antioxidant enzymes as well as the development of nitric oxide (NO) in tomato seedlings thus resulting in the preventing of ROS and DNA damage in tomato plants and thereby enhances tolerance in plants to HS. Adding SNP scavenger (2-(4-carboxyphenyl)-4, 4, 5,5-tetramethylimidazoline-1-oxyl-3-oxide) cPTIO therefore confirms SNPs defensive position in conjunction with IAA.

2.4.3 Heat shock proteins with respect to tolerance for high temperature stress

Special protein development and accumulation are investigated when the heat stress is quick and those proteins are identified as Heat shock proteins (HSPs). Improved productivity of HSP develops whenever crops undergo often a gradual or a rapid increase in temperature (Nakamoto *et al.,* 1999).

HSP68 is located in mitochondria and usually expressed in corporately and found to have increased expression in barley and tomato under heat stress.

Tomato cloned the gene for a nuclear-encoded HSP, Hsa32 which encode proteins of 32kDa. Miroshnichenko *et al.* (2005) stated that during heat stress in tomato crops, HSPs will gather in a granular form in their cytoplasm and maintains the

protein bioproduction machinery. HSPs might suppress certain protein denaturation which may be influenced through extreme temperatures. Research pieces of evidence provide that the chloroplast HSP, HSP21, protects PSII under oxidative stress conditions but also includes plastid development (i.e., a transformation of chloroplast to chromoplast during tomato fruit maturation).

The heat stress transcription factors (Hsfs) control the expression of genes in response to environmental stress. At the transcription stage collaboration of different Hsf representatives as well as the interplay with chaperones regulates the Hsf channel in crops. Analysis of the various significant Hsfs, A1, A2, and B1, in tomato (*Solanum lycopersicum*), has identified two general mechanisms of chaperone Hsf control. Firstly, Hsp70 and Hsp90 control Hsf operate through real contact. Hsp70 suppresses HsfA1 function along with its DNA binding and also the role of HsfB1 coactivator in the HsfA2 complex whereas HsfB1 stimulates the binding DNA function through Hsp90. Secondly, Hsp90 influences the concentration of HsfA2 and HsfB1 by upregulating the destruction of the hsfA2 transcript that is included in HsfA2 production timing modulation. HsfB1 which binds to Hsp90 as well as DNA on the other hand is a prerequisite to aim the proteasomal destruction of this Hsf, which also relies on a sequencing component in its carboxyl-terminal domains. HsfB1 thus indicates Hsp90 client protein that is targeted for, rather than protected against degradation by interacting with the chaperone (Hahn *et al.,* 2011).

In such a trans-activation and trans-repression analysis transient expression assessments are being used in mutated tobacco (*Nicotiana plumbaginifolia)* mesophyll protoplast to test the impact of various tomato heat stress transcription factors, HSF24, HSF8, and HSF30. Different results report differences between various HSFs regarding their reaction to the configuration of the heat stress promoter element (HSEs) in the reporter construct (promoter specificity) as well as the stress regime used during activation. C-terminal deletion analysis recognized acid sequence elements including central tryptophan residue that are essential for the HSF activity control. Interestingly, transgenic HSFs by Drosophila including human cells were also active and not just

from yeast in this tobacco protoplast system as heat-induced transcription factors (Treuter *et al.,* 1993).

Lurie *et al*. (1996) observed that the heat-shock response of plant tissues is described mostly by numerous abundantly low-molecular HSP production. mRNAs associated with ripening, HSP17 mRNA increased substantially during heat treatment. The actual limit came after 1 day of thermal treatment and the level then decreases. This heat-shock protein activity has also been observed in other tissues. However, the HSP17 protein remained longer than the mRNA. When the protein disappears at 20°C within 3 days following heat treatment when the fruit was kept under 2°C it remained in the tissue for 14 days. Under high-temperature stress, certain low molecular weight HSPs have recently shown to defend proteins towards denaturation. The persistence of low-temperature protein HSP17 that imply that this keeps the tissues from freezing effect equivalent towards its premised function at high temperatures.

2.4.4 Gene regulation

Wang *et al*. (2020) reported that the SNAT enzyme is involved in melatonin biosynthesis which has been reported to regulate thermotolerance in many plants. The mechanistic basis for this regulation however remains unclear. Here they identified the SlSNAT gene responsible for the biosynthesis of melatonin in tomatoes. SlSNAT expression levels in SlSNAT over-expression lines OX-2 and OX-6 were three and five times higher respectively. The levels of melatonin were triple and fourfold higher than those of wild type. The levels of melatonin decreased by 50 percent when SlSNAT expression was reduced to 40 percent. SlSNAT over-expression in tomato plants provided significantly increased thermotolerance with better growth performance in the maximum photochemical quantum yield (Fv/Fm) of PS II and reduced heat injury. HSP40 functions as a chaperone for protecting the SNAT enzyme under heat stress during melatonin synthesis. Together HSP40 interacted with SlSNAT participated in the regulation of thermotolerance in tomato related to melatonin.

Yet another factor of plant reaction to unfavorable environments might just be the limited occurrence of polyphenol oxidase B and F in HT as found in different

research. The decreased expression of such enzymes simply prevents the oxidation of phenols which have a regulatory mechanism toward HT degradation in tomato precipitation. Such enzymes were also present in smaller amounts under CC in the tolerant genotype and also in similar quantities under HT in both genotypes implying their impact to basal tolerance for SAL. Similarly, the amount of 3-O-methyltransferase caffeic acid 5 and 6 reduced from both genotypes grown under HT. Such enzymes that are active in phenolic acid production are therefore essential for the methylation of flavonoids a major factor in the biosynthetic pathway of lignins. Leading to abiotic or biotic stresses or genetic modifications the down-regulation was correlated with a shift in the lignin structure resulting from a change in the ratio of guaiacyl (G) and syringyl (S) monolignol subunits that might affect the total lignin content. Therefore it was reported that HT may cause variations in the lignin biosynthetic pathway thereby influencing the thickness as well as stiffness in cell walls.

Use of genomics in tomatoes as just an indirect selection procedure to explore new and better-throughput genetic and molecular techniques provides the ability to improve the breeding performance by eliminating genotype relationship with the environment; Promoting the effective introduction into plants of superior wildlife alleles, supporting the gene-pyramid influence of quantitative traits and augmenting the development of higher-yielding stress-tolerant varieties. Developments in genetics and genomics, therefore, enhanced understanding of morphological and chemical dimensions of the plant genomes. The genes CBF/NHX1/DREB1 have been effectively used to develop tomato drought tolerance. Such genes are transcription factors that are active in managing drought and heat stress in tomatoes. Resistance to particular stress defined by many yield components regulated by the respective genes may provide a valuable tool to determine major genes for stress tolerance in tomatoes (Solankey *et al.*, 2015).

These lines of inquiry include the significance of advances employed by geneticists and plant breeders in various study programmes. In tropical and subtropical environments heat and drought-resistant tomatoes are required; a mixture of conventional breeding procedures including marker-assisted breeding would become a

practice in the development of heat-resistant tomatoes. Research discoveries have to be provided, effective strategies utilized, and efficient strategies retained to resolve the consequences of abiotic stress on tomatoes to be productive sufficient or deep-term improvement is required.

Plants modulate the expression of a plethora of genes in response to high-temperature stress. Such genes including its annotation may effectively separate mechanisms that are triggered or suppressed as well as those engaged in defense against acclimatization and temperature changes. Mostly during occurrence and recovery of heat stress, transcriptional profiling was conducted among unstressed, stressed plants and between variants which are heat resistant and heat sensitive. This investigation was carried out in many crops for example in rice and tomatoes (Driedonks *et al.*, 2016).

Bita *et al.* (2011) mentioned that temperature tolerant genotype had shown slight morphological alterations under moderate heat stress, unlike the heat-sensitive genotype. The heat-tolerant genotype frequently displays the distinct profile of fundamental expression of genes relative to the heat-sensitive genotype indicating genetic variations of high thermal tolerance. Many changes in gene expression are characterized by an upregulation in the heat-tolerant genotype while there is a general tendency in the heat-sensitive genotype at MHS (moderate heat stress) to down-regulate expression of genes. Several prospective functions correlated with genes detected by cDNA-AFLP or microarray indicate the presence of processes for heat shock, metabolism, plant development, and antioxidants. Depending mostly on variations found in response to MHS they established a variety of potential transcripts involved in heat tolerance.

Mazzeo *et al.* (2018) analysis highlight a profound cellular massive restructuring of both genotypes in tomato inflorescence to cope with HT economic expansion and directly leads to the detection of potentially temperature-tolerant proteins. Further proteomic studies of tropical plant anthers such as tomatoes may extend existing knowledge on main genes and biochemical factors specific to thermo-tolerance thus providing a new perspective for producing thermos tolerant genotypes using breeding models and theories to biotechnology. As global warming continues to

increase crops with improved resistance characteristics would certainly play a significant role in agricultural production in the future.

A thorough evaluation of crop tolerance for environmental stresses, therefore, needs to be conceived and implemented to meet future agricultural production requirements. The development of heat-tolerant cultivars is important for ensuring good crop production during climate change and variability cycles. The use of molecular markers to handle genotypes of heat-tolerant tomatoes is also seen as desperate.

2.5 MOLECULAR MARKERS

The latest advance in crop science analysis includes the production of massive quantities of molecular-genetic data an advancement of promising analytical capabilities in the research of molecular biology including model analyzes has paved the way for both the need towards approaches or ways to use the resources available to reinforce interdisciplinary attempts to find responses to the challenging targets of plant breeding activities (such as abiotic stress tolerance) that ultimately lead to successful crop enhancements. A decline from such discoveries and attempts was the recognition or development of yet another class of really valuable DNA markers called molecular markers using the ever-increasing archives of gene sequence knowledge which have been acquired for a huge variety of plants in current years as a result of EST sequencing projects (Varshney *et al.,* 2007).

Molecular markers are now commonly used in many crop breeding programs to track loci and genome regions as molecular markers are available in major crop species closely linked to plenty of the agronomic and disease resistance characteristics. The new high-precision maps can be produced comparatively easily and quickly (Philips and Vasil, 2001). They have a much higher marker density which allows more and more restricted segments of the genome to be revealed. This molecular markers usually involve (i) PCR (Polymerase Chain Reaction)-based markers: amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), and microsatellite or simple sequence repeat and (iii)

sequence-based markers: single nucleotide polymorphism (SNP), (iii) hybridization-based markers such as restriction fragment length polymorphism (RFLP). Certain applications of these molecular markers include gene introgression by backcrossing, genetic diagnosis, transformant characterization, genome structure research, and phylogenetic analysis. (Gupta *et al.*, 2002).

2.5.1 SSR markers

Microsatellites are a category of interspersed repetitive sequences that are prevalent in genomes of eukaryotes. Variation in the number of repeats results in length polymorphism at given loci between different individuals (Areshchenkova and Ganal, 2002).

Simple sequence repeat (SSR) motifs are common in plant genomes however molecular markers were commonly used during plant breeding. Among tomatoes, genetic maps presently offered to contain a small number of SSR markers that were not equally distributed across the genome. Such a situation warrants the need for more SSRs in the genomic regions which lack sufficient markers. There seem to be inadequate polymorphic markers among highly associated tomato species but within varieties of same species since most molecular markers were constructed on the basis of polymorphisms among domesticated tomatoes including wild species. This poses challenges in the creation and saturation of many linkage maps unique to the species.

SSR markers are always the molecular markers selected once required for marker-assisted plant breeding even though SSR markers possess favorable characteristics for high-throughput genotyping including high reproducibility, co-dominance, multi-allelic variability, simple evaluation, low distribution costs and simple automation (Edwards and McCouch, 2007). Alternatively, the DNA sequences collected in the public databases include a convenient and inexpensive origin for the advancement of SSR markers. SSR markers in tomatoes have been widely used for evaluating the variety and characterizing tomato germplasms.

SSR marker is a PCR-based technique that involves DNA fragment amplification among neighboring and inversely oriented microsatellites. This method

utilizes microsatellites as the primers, typically 16–25 bp long. These could be di-, quad-, tetra- or Penta nucleotides (Zhang *et al.*, 2014).

Crop enhancement relies on the degree of genetic diversity in economic factors therefore it is extremely necessary to evaluate and manipulate genetic diversity in the right direction in any yield enhancement program. It is important to establish PCR based markers developed to adapt wide-scale genotyping processes including certain SSR and SNP markers to promote genetic traits and genomics in tomato species.

The high polymorphism frequency and random genome distribution make genetic markers helpful to microsatellites for mapping population and evolutionary studies particularly in species with low DNA variability. Generating PCR primers for microsatellite markers remained a fairly complex procedure since the development and sequencing of various varieties of molecular libraries involves the identification of specific flanking sequences unique with each microsatellite locus. Vast quantities of genome sequence in gene repositories make SSR markers easier to establish (Areshchenkova and Ganal, 2002).

Wen *et al.* (2019) applied three Heat tolerance based physiological measures mainly; chlorophyll content (CC), relative electrical conductivity (REC), and maximum photochemical quantum efficiency (Fv/Fm) of PSII (photosystem II) as well as an index of heat injury (HII), phenotypic index, and conventional Quantitative Trait locus (QTL) coupled examination with QTL-seq technology for detailed identification of QTL heat tolerance in tomato seedlings. Between the two parental lines, 146 of the 516 SSR markers screened by them were polymorphic with a polymorphism level of 28.25%. The linkage map contains 137 mapped loci relating to the 12 tomato chromosomes in 12 linkage groups. The overall length of this map was 1503.82 centimorgans (cM) and the mean interval for the marker was 10.98 cM. Chromosome 8 would have the highest number of markers, with a total of 16; chromosomes 9 and 12 had a minimum number of markers (seven), and markers for other chromosomes varied from 9 to 15. These markers were usually evenly distributed allowing their appropriateness for evaluation of the QTL mapping. Between them, qCC-1-5 flanked by SSR134 and C01M86371 is valuable more analysis because it clarified far more

phenotypic variance (16.48 percent) and also had significant beneficial effects (0.22) between all the observed heat tolerance QTLs showing its economic benefit in breeding. Fv/Fm was related by SSR115 to two QTLs on chromosomes 5 and 12 with both the flanking markers SSR13, 6.94, and 8.80 percent respectively including its phenotypic variance. Regarding chromosome 2 with SSR96 and C02M4005 flanking markers traditional QTL study qREC-1-1, was the most important QTL would have the maximum LOD value of all QTL stress tolerance identified by traditional qCC-2-2 QTL mapping.

However, qREC-1-3 and qCC-1-5 that were traced to the same area (81.64–86.37 Mb) on chromosome 1 with both the flanking markers SSR134-C01M86371 and qCC-1-5 clarified far more phenotypic variance (16.48%) and also had a significant additive effect (0.22) between all the heat tolerance QTLs found by traditional QTL research indicating that QTL were strongly connected to heat tolerance and was worthy of gene mining.

QTLs were distributed in 8 chromosomes 1, 2,3,5,6,7,8,9. On chromosomes, 1,2,3,9 QTLs of relative electrical conductivity (REC) were detected. On chromosomes 1 and 2 QTLs for chlorophyll content (CC) and on chromosome 12 QTLs for High quantum photochemical performance (Fv/Fm) of PSII (photosystem II) has been observed.

Markers SSR-270, SSR-75, SSR-134 is located on chromosome 1. SSR-96, SSR-605, SSR-356 on chromosome 2. SSR-6 on chromosome 3. SSR 13, SSR 115 on chromosome 5. SSR 47 on chromosome 6. SSR 304, SSR 276 on chromosome 7. SSR 63 on chromosome 8. SSR 19 on chromosome 9. SSR 293 on chromosome 12. These primers are associated with high-temperature stress traits and can be useful in marker-assisted breeding for high-temperature stress in tomatoes. The SSR primers were picked from the Sol Genomics Network database (SGN, http:/solgenomics.net/).

Benor *et al*. (2008) performed a study of genetic variation of 39 defined and undefined inbred tomato lines obtained from S. Korea, China, Japan, and the United States. In these tomato lines, a total of 150 alleles have been discovered utilizing 35

polymorphic SSR markers with medium levels of inequality and a high number of distinct alleles. The mean number of alleles per locus was 4.3, as well as the mean PIC value was 0.31 and SSR75 has been reported.

Kwon *et al.* (2009) identified 33 SSR markers and studied the genetic composition of commercial tomato cultivars using 22 morphological traits. For 63 tomato varieties, thirty-three pairs of SSR primers were examined. The PIC values representation of the variability and frequency of both the alleles between the varieties weren't really incredibly strong for both the loci tested by the SSR. The PIC values ranged from 0.210 to 0.880 with a mean of 0.628. Extremely engaging SSR markers (PIC ~ O.1) such as SSR47, SSR63, SSR248 will be very useful for defect detection and genetic assessment of tomato varieties.

The SSR marker process is beneficial for studying genetic variation between inbred tomato lines of various locations. The mixture in polymorphism as well as the huge proportion of band in the experiment indicates that SSR has been the most useful marker method for tomato genotyping. From the data, Tom 57-58 (T10) had three alleles between the 150-175bp range with bands either present or absent at each locus. It had the maximum PIC value of 0.816 (81.6 %) (Ajenifujah *et al.,* 2018). Dhaliwal *et al.* (2011) observed SSR 70 with PIC value 0.57.

Xu *et al.* (2008) studied an SSR and RAPD marker correlated to tomato heat tolerance was evaluated by taking the permeability of plasma membranes in linkage groups (LGs) 3 and 7 accordingly. The standard QTL analysis qREC-1-3 and qCC-1-5 for chromosome 1 were traced to the very same region (SSR134) and qCC-1-5 explained far more phenotypic variation (16.48 percent) and also had a significant additive effect (0.22) observed among heat tolerance QTL.

2.6 POLYMORPHISM INFORMATION CONTENT

The value of polymorphic information content (PIC) is commonly used in genetics as a polymorphism measure for a locus marker used in the analysis of linkages. The degree of polymorphism is commonly measured in quantitative terms by two distinct quantities. One is called heterozygous and its unbiased estimator and variance

formula (Shete *et al.*, 2000). Two measures are used to calculate the consistency or informativeness of a polymorphism mostly as a genetic marker. They are; heterozygosity (H) and the polymorphic information content (PIC) (Botstein *et al.*, 1980). PIC has become the most frequently used method for genetic research to calculate the information content of various molecular markers. The heterozygosity of a locus is known as the probability of the locus being heterozygous for an individual in the population (Liu, 1997).

PIC refers to the significance of a marker for the identification of polymorphism within a population-based on the number of measurable alleles and its frequency distribution thus it provides an approximation of the specificity of the marker. PIC is defined as the probability that perhaps the marker genetic makeup of a provided individual would require to inference in the absence of crossing over about which of the two marker alleles of the impaired parents it had. In several other terms, PIC is a refinement of a heterozygosity measure that deducts an extra likelihood from the H value that even a genotype in such a linkage studies doesn't really add information for this study (Nagy *et al.*, 2012).

2.7 POPULATION GENETIC STRUCTURE ANALYSIS

To examine population structure utilizing multi-locus genotype information the free software package structure is used and it is applicable to most frequently used genetic markers including RFLPs, SNPs, microsatellites, and AFLPs. The applications involve interpreting the distribution of various populations investigating hybrid zones assigning different individuals to appropriate groups recognizing migrants, combined individuals, and determining levels of population in situations where multiple individuals were migrants or grouped together. Structural analyses are frequently used to determine genetic relatedness among varieties and can accurately identify genetic variations among diverse populations by combining both methods (Li *et al.*, 2010).

STRUCTURE software allocates different genotypes to various populations depending on the frequency of accession of the alleles. STRUCTURE software distributes various accessions to different populations depending on the accession

frequencies of the alleles. Variations in environmental stresses and environmental processes could be due to changes in genetic composition and genetic diversity dispersion between various populations.

Using model-based Bayesian clustering method- STRUCTURE was used to detect subpopulations and was used in various genetic variability and association mapping experiments of plant species including rice clustering was used to detect fundamental population structure in a set of individuals genotyped with multiple markers. The optimum number of clusters (K) was obtained using the evanno method based on the STRUCTURE HARVESTER system. Analysis benefit implemented with the STRUCTURE software (Pritchard *et al.*, 2000) has the ability to measure the proportion of an individual genome belonging to each assumed population (admixture) compared with several other subdivision quantification methods. Knowledge of population structure and genetic diversity is vital for the mapping of associations, genomic selection, and classification of individual genotypes into different groups.

3. MATERIALS AND METHODS

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The present study entitled "Molecular characterization and construction of population structure of selected tomato genotypes (*Solanum lycopersicum*L.) under high-temperature stress conditions" was conducted in the Department of Plant Physiology, College of Agriculture, Vellayani, Kerala Agricultural University during the years from 2019-2020. The main objective of the study is the molecular characterization of different tomato genotypes using SSR markers and to construct the population structure of tomato genotypes. The details of the materials used and methods adopted for this experiment as well as the procedures followed for laboratory analysis during the course of experimentation are described in this chapter.

3.1. PLANT MATERIALS

The material for this study contained 22 genetically diverse genotypes of tomato which were collected from different institutes of India which are given in Table 1.

Table 1. List of tomato genotypes used for study

Sl. No	Variety	Source
1.	Manuprabha	KAU
2.	Akshaya	KAU
3.	Pusa Ruby	IARI
4.	IC-45	IIHR
5.	Nandi	UAS
6.	IIHR-2200	IIHR
7.	IIHR-26372	IIHR

8.	Palam Pride	HPAU
9.	PKM-1	TNAU
10.	Manulakshmi	KAU
11.	Arka Samrat	IIHR
12.	Arka Rakshak	IIHR
13.	Arka Vikas	IIHR
14.	Pusa Rohini	IARI
15.	Arka Alok	IIHR
16.	Sakthi	KAU
17.	Vaibhav	AVRDC
18.	Vellayani Vijay	KAU
19.	Anagha	KAU
20.	Kashi Vishesh	ICAR-IIVR
21.	Arka Saurabh	IIHR
22.	Arka Abha	IIHR

3.2 MOLECULAR CHARACTERIZATIONS OF TOMATO ACCESSIONS WITH SSR MARKERS

3.2.1 Plant materials

All the above mentioned 22 tomato genotypes were sowed on 16th September 2019. About 30 days old plant was taken for further study (Plate 1, 2, 3).

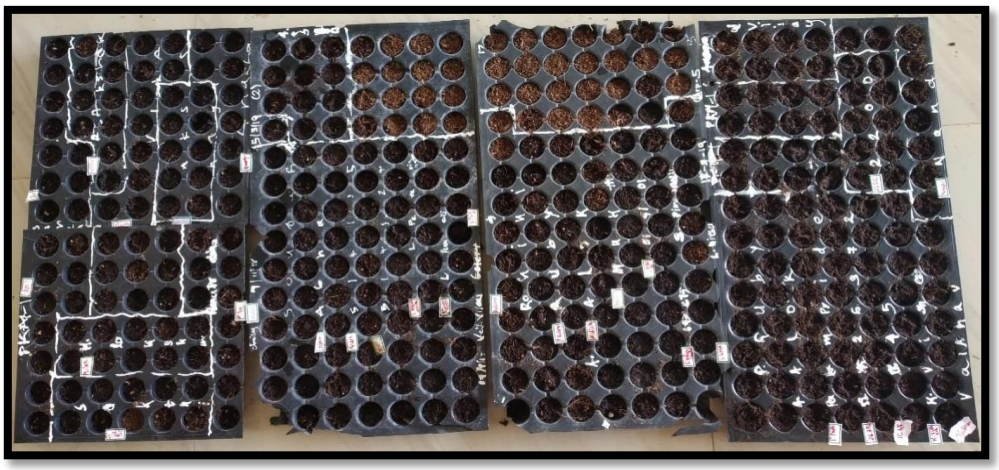


Plate 1. View of the pot trays in which seeds of tomato genotypes are sowed.

Plate 2. View of seedlings after 20 days of sowing.

Plate 3. View of seedlings after 1 month of sowing.

3.2.2. Experiment 1- Isolation of genomic DNA

The CTAB method is used for the isolation of DNA from new and stable leaves. The genomic DNA isolated from 22 Tomato varieties were analyzed and confirmed by agarose gel electrophoresis.

3.2.2.1 Protocol for extraction of genomic DNA

1. According to the procedure described by (Murray and Thompson, 1980), plant samples were grinded in a pre-chilled mortar pestle using liquid N2 at room temperature.
2. In a water bath, CTAB extraction buffer was preheated to 65ºC and measured the volume of sediment tissue in each sample.
3. Added 100µl CTAB extraction buffer to every sample taken, and then it was incubated at 65ºC for 10-30 minutes.
4. Following the incubation, centrifugation was performed at 14,000rpm. The supernatant was transformed into a new tube containing 5µl of Rnase solution and incubated at 32°C for 20 minutes

5. An equal volume of chloroform: isoamyl alcohol (24:1) was added to the tube, vortexed thoroughly, and centrifuged for 10 minutes at 13,000 rpm.
6. The aqueous layer was transferred to a sterile microcentrifuge tube and repeated the extraction process (step 5-6) with chloroform: isoamyl alcohol.
7. Added 0.7 volume of isopropanol, mixed by inversion and incubated at -20°C for 15 minutes.
8. The sample was centrifuged at 13,000rpm for 10 minutes and discarded the supernatant without disturbing the pellet.
9. Washed the pellet with 0.5 mL of cold 70% ethanol and then it was shortly centrifuged to obtain its pellet.
10. Removed the supernatant properly, as well as dried the pellet in vacuum or can be dried just in the air.
11. DNA was dissolved in water or TE buffer (20 µL)

3.2.3 Analysing quantity and quality of DNA

Nucleic acid quantification was carried out using UV-Vis Spectrophotometer (ELICO, SL218). The genomic DNA has been quantified spectrophotometrically at wavelengths of both 260 nm and 280 nm. Then absorbance at 260 nm allowed DNA concentration in the samples to be estimated. The purity of DNA was checked by reading at 260nm and 280nm (OD 260/OD 280). Pure DNA preparations have an OD ratio of 260 nm/ 280 nm from 1.7 to 1.8 (Moyo *et al.*, 2008). Quality can be measured employing gel electrophoresis on agarose gel with 5µl of crude DNA sample (0.8 percent) and stained by ethidium bromide.

The concentration of the DNA was calculated by the formula shown below: Concentration of the DNA µg/ml of sample = Optical density at 260nm X 50 X Dilution factor

The quality of DNA was checked by agarose gel electrophoresis. For agarose gel electrophoresis in 100 ml of 1X TBE electrophoresis buffer, 0.8 g of agarose was dissolved. The solution was heated until the agarose fully melted which is whenever the solution will become completely opaque. Through continuous mixing, this was cooled down to 60 ° C and added 4-5µl of ethidium bromide to the mixture. The agarose mixture was then transferred with combs into an already prepared gel mold and was forced to leave for solidification for 30-40 minutes. DNA samples were prepared by adding a loading dye (6X loading dye consisted mainly of 0.25% w/v bromophenol blue, 0.4% w/v sucrose, or 30% glycerol in sterile water) to that same DNA such that the final loading dye concentration was 1X. Just after solidification combs were carefully withdrawn and for electrophoresis, the gel was positioned in the gel tank. Genomic DNA has been loaded to the wells. After loading the gel underwent electrophoresis for about 1 hour at a constant voltage of 5V/cm. This same gel had been imaged under UV trans-illuminator after electrophoresis and photographed using a gel documentation system. Observations were taken mostly on the intactness of bands of DNA samples that showed the DNA quality.

3.2.4 Dilution of DNA samples

After quantification, the stock DNA samples were diluted to 30ng/µl of working solutions for PCR analysis. DNA dilutions were prepared by using the formula as follows:

$$M1\ V1 = M2\ V2$$

Where M1 is the stock DNA concentration, V1 is the volume of stock to be dissolved M2 is the working solution concentration and V2 is the volume of work solution to be prepared. The required volume from the stock was therefore converted to a micro-centrifuge tube of 0.5 ml and the volume was prepared to 100µl using TE buffer. Prepared sample of the DNA is maintained at-20oC before any further use.

3.3. PCR AMPLIFICATION USING SSR PRIMERS

3.3.1 Selection of primers

PCR screening was carried out using twenty-five microsatellite SSR markers and the sequence was taken from the database Sol Genomics Network. Their sequences are listed in Table 2.

Table 2. List of primers used for DNA polymorphism analysis.

Sl. No	Primer	Sequence	Expected product size
1.	SSR 134	F: CCCTCTTGCCTAAACATCCA	171
		R:CGTTGCGAATTCAGATTAGTTG	
2.	SSR 75	F:CCATCTATTATCTTCTCTCCAAC	155
		R:GGTCCCAACTCGGTACACAC	
3.	SSR 356	F:ACCATCGAGGCTGCATAAAG	259
		R:AACCATCCACTGCCTCAATC	
4.	SSR 605	F:TGGCCGGCTTCTAGAAATAA	196
		R:TGAAATCACCCGTGACCTTT	
5.	SSR 270	F:AGCTCAAGGCTTCTGTTGGA	131
		R:AACCACCTCAGGCACTTCAT	
6.	SSR 96	F:GGGTTATCAATGATGCAATGG	222
		R:CCTTTATGTCAGCCGGTGTT	
7.	SSR 47	F:TCCTCAAGAAATGAAGCTCTGA	191
		R:CCTTGGAGATAACAACCACAA	
8.	SSR 276	F:CTCCGGCAAGAGTGAACATT	148
		R:CGACGGAGTACTTCGCATTT	

9.	SSR 304	F:TCCTCCGGTTGTTACTCCAC R:TTAGCACTTCCACCGATTCC	186
10.	SSR 63	F:CCACAAACAATTCCATCTCA R:GCTTCCGCCATACTGATACG	250
11.	SSR 4	F:TTCTTCGGAGACGAAGGGTA R: CCTTCAATCCTCCAGATCCA	166
12.	SSR 13	F:GGGTCACATACACTCATACTA AGGA R:CAAATCGCGACATGTGTAAG A	104
13.	SSR 115	F:CACCCTTTATTCAGATTCCTCT R:ATTGAGGGTATGCAACAGCC	211
14.	SSR 19	F:CCGTTACCTTGGTCCATCAC R:GGGAGATGCCACATCACATA	188
15.	SSR 293	F:GCAAAGAGCTCGATCTCCAA R:TTCAGTTACTGGCCTTCGCT	129
16.	SSR 248	F:GCATTCGCTGTAGCTCGTTT R: GGGAGCTTCATCATAGTAACG	249
17.	SSR 124	F:TCAATCCATCACACCTTGGA R:GAGGAAGAAGACCACGCAAA	131
18.	SSR 70	F:TTTAGGGTGTCTGTGGGTCC R:GGAGTGCGCAGAGGATAGAG	120
19.	SSR 111	F:TTCTTCCCTTCCATCAGTTCT R:TTTGCTGCTATACTGCTGACA	188
20.	SSR 20	F:GAGGACGACAACAACAACGA R:GACATGCCACTTAGATCCACA A	157
21.	SSR 602	F:GGGTCACATACACTCATACTA AGGA	299

		R:GGCAATCATAGCCACTTGGT	
22.	SSR 450	F:AATGAAGAACCATTCCGCAC R:ACATGAGCCCAATGAACCTC	265
23.	SSR 341	F:TTTCTCTTGTGGGTGGCAAT R:AAGCCCTCGAATCTGGTAGC	292
24.	SSR 331	F:CGCCTATCGATACCACCACT R:ATTGATCCGTTTGGTTCTGC	178
25.	SSR 80	F: GGCAAATGTCAAAGGATTGG R: AGGGTCATGTTCTTGATTGTCA	180

3.3.2 Dilution of primers

Each primer was dissolved in 100µl of 1X Tris EDTA (TE) buffer and dissolved further through deionized water to the working concentration of 10µM. Dissolved the primers according to the formula given: µM of oligo in solution 100L = OD/10

3.3.3 Standardization of annealing temperature for SSR primers

For the research twenty five SSR markers are being used the annealing temperatures were optimized utilizing gradient PCR technique. Different annealing temperatures (Tm±5 ° C) were set between each block in this process and amplification was performed out according to the conditions of reaction described below. Because after annealing temperature was standardized the lines of the germplasm were expanded and use the same technique.

3.3.4 PCR Amplification

To study the parental polymorphism *In-vitro* amplification using Polymerase Chain Reaction (PCR) was undertaken in an Eppendorf Master cycler. PCR evaluation was carried using 25 primers SSR.

3.3.4.1 PCR reaction

PCR reaction was performed in a 20µl reaction mixture which consisted of;

Table 3**.** PCR reaction mixture

Components	Stock Concentration	Volume (µl)
Genomic DNA	25ng/µl	2.0
Taq assay buffer A	10X	2.0
dNTPs mix	2.5 mM	1.5
Taq DNA polymerase	1U	0.3
Forward primer	10 µM	0.75
Reverse primer	10 µM	0.75
Autoclaved distilled water	-	12.7
Total volume		20

PCR reaction was carried out using the Master Cycler gradient 5331-Eppendorf version 2.30.31-09, Germany. The thermal cycling was carried out with the following program

Table 4.Temperature profile used in PCR

Sl. No	**Cycling conditions**	**Temperature**	**Time**
1	Initial denaturation	94^{o}C	3 minutes
2	Denaturation	94^{o}C	1 minute
3	Primer annealing	50^{o}C - 55^{o}C	1 minute

4	Primer extension	72°C	1 minute
5	Go to step 2	-	35 cycles
6	Final extension	72°C	5 minutes
7	Incubation	4°C	∞

3.3.4.2 Visualization of PCR amplified product

After PCR, 2µl of 6X loading dye was added to each of the amplified products and mixed thoroughly. Agarose gel (1.5%) was prepared by dissolving agarose powder and after Ethidium bromide 4-5 µl/ 100 ml of gel in 1X TBE buffer. About 5µl of each sample mixed with loading dye was loaded into the wells in the gel.

A persistent default 100bp marker was filled with a sample containing fragments between 100bp and 1000bp. The gel was exposed to electrophoresis at a persistent voltage of 5 V/cm for approximately 1 hour after charging.

The PCR products were separated on agarose gel along with the marker (100bp ladder) and 1X TBE buffer. Ethidium bromide is used for staining purposes. The gel profile was visualized using a gel documentation system. The reported SSR patterns in the DNA strands between both the tomato genotypes have been thoroughly considered for the polymorphism.

3.4 PIC VALUE

The following Botstein *et al*. (1980) equation was used to predict the polymorphic information content (PIC) which gives an account of the sensitivity and specificity of a locus or loci keeping in mind not only of the number of alleles demonstrated but rather the frequency distribution of those alleles.

$$PIC = 1 - \sum (Pi)2$$

P_i depicts the proportion of samples carrying the i^{th} allele.

3.5. POPULATION STRUCTURE AND CLUSTER ANALYSIS

3.5.1 SSR alleles Scoring

The SSR allele dimensions were determined by the characteristics of bands comparative to the DNA ladder. In almost all of the genetic variants understudy the total number of alleles was measured for each microsatellite marker by having to give the quantity to amplified alleles as 0 for absence and 1 for allele presence.

3.5.2. Analysis of Population structure

Bayesian model-based scoring software STRUCTURE V2.3.4 had been used to evaluate the population structure of the 22genotypes (Pritchard *et al.*, 2000). The number of specific-populations data had been analyzed for a K value of 1–10 with performance parameters set as; burning time of 5000, MCMC reps 50,000, number of iterations as 5, the possibility of mixes and strongly linked allele frequency. In Structure Harvester the whole collection of findings acquired from that kind of initial analysis were further examined to determine the optimum K value by plotting the mean calculation of the data's posterior log probability [L (K)] against the given K value. There was also a calculation of an ad - hoc value is called ΔK, which further gave a significant peak at the appropriate K value.

3.5.3. Similarity coefficient and cluster analysis

The amplified gel pictures obtained from twenty-five primers were scored using binary codes. The presence of a band was scored as 1 and absence was scored as 0. The binary data generated for all the varieties for the polymorphic markers was entered in the NTedit program of NTSYSpc version 2.10 software. The similarity matrix was used to generate a dendrogram using the SHAN module for cluster analysis software. The SIMQUAL method is being used to measure the parameters of similarities for the Jaccard. UPGMA grouping was performed utilizing version 2.10 of the NTSYS-pc program. In the software, the individua ls are only clustering but there is no way for screening and selection. Yet the most popular use of NTSYSpc is to conduct different types of partitioning clustering algorithms of some kind of matrix of similarities and differences (Rohlf, 1998).

4. RESULTS

4. RESULTS

The experiment was conducted to characterize the molecular variation among twenty-two tomato genotypes using SSR markers and construction of population structure in the Department of Plant Physiology, College of Agriculture, Vellayani during 2019-20. Physiological, biochemical, and yield parameters of these twenty-two tomato varieties under normal and high-temperature stress conditions that were available in the Department of Plant Physiology were taken for the study.

In tomatoes, genetic maps presently available have a limited set of Simple Sequence Repeat (SSR) markers which are not distributed equally in the genetic material. One such circumstance justifies the need for further SSRs in gene sequences that lack the necessary markers (Geethanjali *et al*., 2010). For marker-assisted selection, molecular markers associated with high temperature should be used for high-temperature tolerance. Hence the present study was focused on the detection of molecular markers correlated to tomato tolerance at elevated temperatures.

Twenty-two tomato genotypes (Varieties released by KAU and NBPGR accessions include wild relatives of tomato) were raised in pot trays and the DNA was isolated from the fresh leaves using CTAB procedure. The quality and quantity of the DNA isolated from the twenty-two genotypes were analyzed and after checking the quality and quantity of DNA samples they were screened using 25 microsatellite markers.

Phenotypic data of the same genotypes available at the Department of Plant Physiology, College of Agriculture, Vellayani was utilized for interpretation of molecular data, construction of population structure of the genotypes, and cluster analysis. The data were analyzed and the results are presented in this chapter with suitable tables.

4.1 GENOMIC DNA EXTRACTION

The isolated genomic DNA of 22 tomato genotypes were analyzed and confirmed by agarose gel electrophoresis. All the DNA isolated were appeared on agarose gel ensuring good quality (Plate 4).

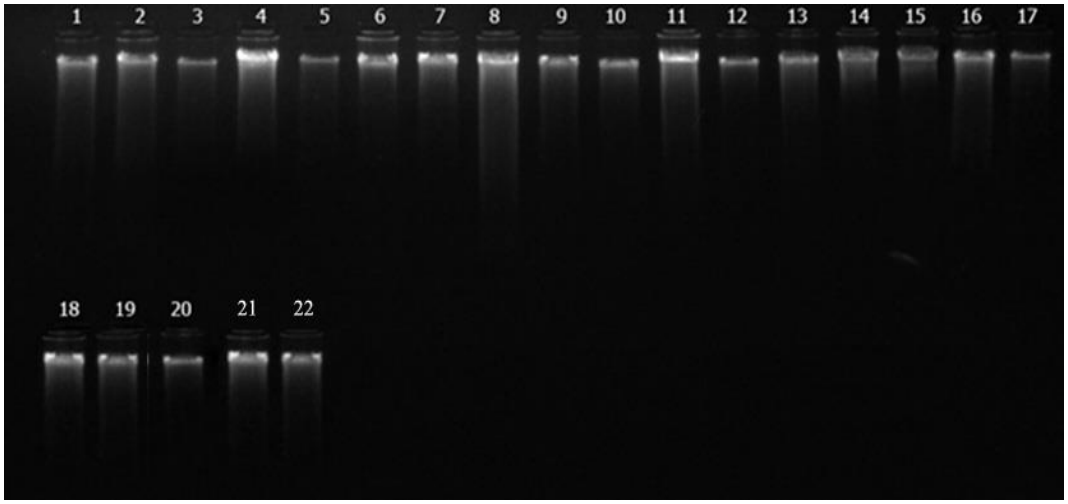


Plate 4. Gel profile with DNA bands of tomato (Lane 1-Manuprabha, Lane 2-Akshaya, Lane 3-Pusa Ruby, Lane 4-IC-45, Lane 5- Nandi, Lane 6-IIHR-2200,lane 7-IIHR-26372, lane 8-Palam Pride, lane 9-PKM-1, lane 10-Manulakshmi, lane 11-Arka Samrat, lane 12- Arka Rakshak, lane 13-Arka Vikas, lane 14-Pusa Rohini, lane 15-Arka Alok , lane 16-Sakthi, lane 17-Vaibhav,lane 18- Vellayani Vijay, lane 19-Anagha, lane 20-Kashi Vishesh, lane 21- Arka Saurabh, lane 22-Arka Abha).

4.1.1 Quality and quantity of DNA samples of tomato varieties selected for polymorphism analysis using SSR microsatellite markers.

Table 5. Concentration and purity of isolated DNA identified by spectrophotometric method

Sl. No	Variety	A_{260}/A_{280} value	DNA concentration (ng/µl)
1	Manuprabha	1.73	3060.0
2	Akshaya,	1.68	2070.0
3	Pusa Ruby	1.86	1230.0
4	IC-45	1.77	1380.0
5	Nandi	1.78	2880.0
6	IIHR-2200	1.85	3060.0
7	IIHR-26372	1.83	2970.0
8	Palam Pride	1.89	1740.0
9	PKM-1	1.72	2370.0
10	Manulakshmi,	1.73	3060.0
11	Arka Samrat	1.78	2610.0
12	Arka Rakshak	1.71	2820.0
13	Arka Vikas	1.76	2850.0
14	Pusa Rohini,	1.86	1230.0
15	Arka Alok	1.84	3090.0
16	Sakthi	1.85	2610.0
17	Vaibhav	1.82	2070.0
18	Vellayani Vijay	1.97	1710.0
19	Anagha	1.74	1980.0
20	Kashi Vishesh	1.84	1380.0
21	Arka Saurabh	1.71	2880.0
22	Arka Abha	1.61	2370.0

4.2 PCR AMPLIFICATION USING SSR PRIMERS

PCR reaction was carried out using selected primers. Out of 25 primers, 7 showed polymorphism in 3% agarose gel electrophoresis and all other primers were monomorphic. Out of the 25 primers 3 primers SSR 80, SSR 331, SSR 341 didn't show any amplification hence not used for further analysis. Out of twenty-two fifteen SSR markers, SSR450, SSR 602, SSR20, SSR111, SSR70, SSR 124, SSR 293, SSR 19, SSR115, SSR 304, SSR 276, SSR 47, SSR 75, SSR 134 and SSR-4 magnified monomorphic banding structures, therefore, no evaluation had been taken into consideration. Therefore seven markers have been used for population and cluster analysis.

The polymorphic markers for temperature tolerance were; SSR 96, showed a polymorphic band with size ~ 222bp, located in chromosome 1. Distinct polymorphism for high-temperature tolerance between high temperature tolerant and susceptible varieties (Arka Saurabh, Pusa Rohini, Palam Pride, and Arka Rakshak**)** was shown by SSR 96 (Plate 5).

SSR 63 showed polymorphic bands of size ~ 250bp, located in chromosome 8. Distinct polymorphism for high-temperature tolerance between high temperature tolerant (Vellayani Vijay, Anagha, Kashi Vishesh) and susceptible varieties were shown by SSR 63 (Plate 6).

SSR 13 showed polymorphic bands of size ~ 104 bp, located in chromosome 5. Distinct polymorphism for high-temperature tolerance between high temperature tolerant and susceptible varieties (Arka Rakshak and Pusa Rohini) was shown by SSR 13 (Plate 7).

SSR 270 with polymorphic bands of size ~ 131bp, located in chromosome 1. Distinct polymorphism for high-temperature tolerance between moderately temperature tolerant (Manuprabha, Akshaya, and IIHR-2200) and susceptible varieties were shown by SSR 270 (Plate 8).

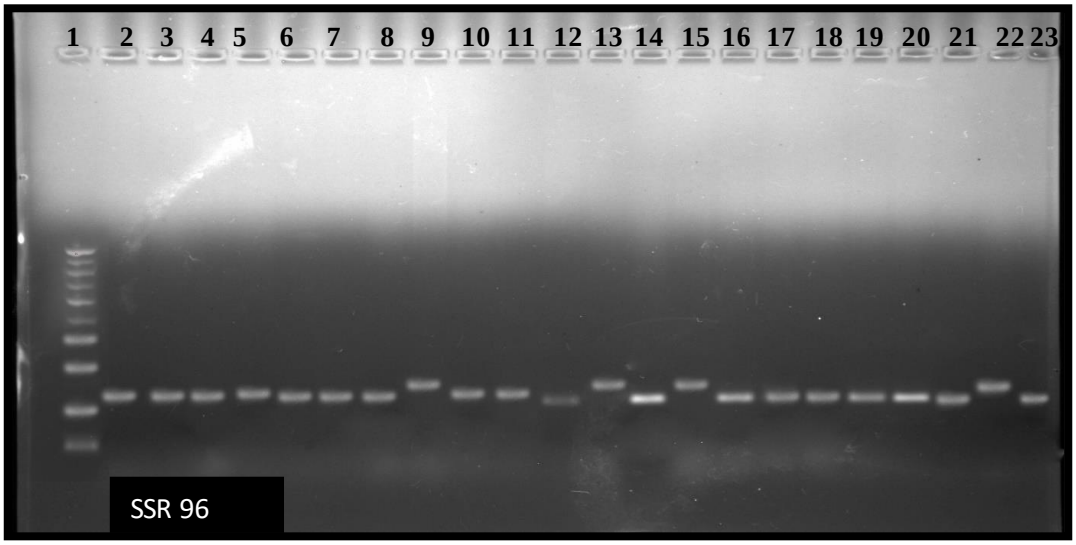
SSR 356 with polymorphic bands of size ~259 bp, located in chromosome 2. Distinct polymorphism for high-temperature tolerance between high temperature

tolerant and susceptible varieties (Pusa Rohini and Arka Saurabh) was shown by SSR 356 (Plate 9).

SSR 605 with polymorphic bands of size ~196 bp, located in chromosome 2. Distinct polymorphism for high-temperature tolerance between moderately tolerant (Akshaya) and tolerant variety (Vellayani Vijay) was shown by SSR 605 (Plate 10).

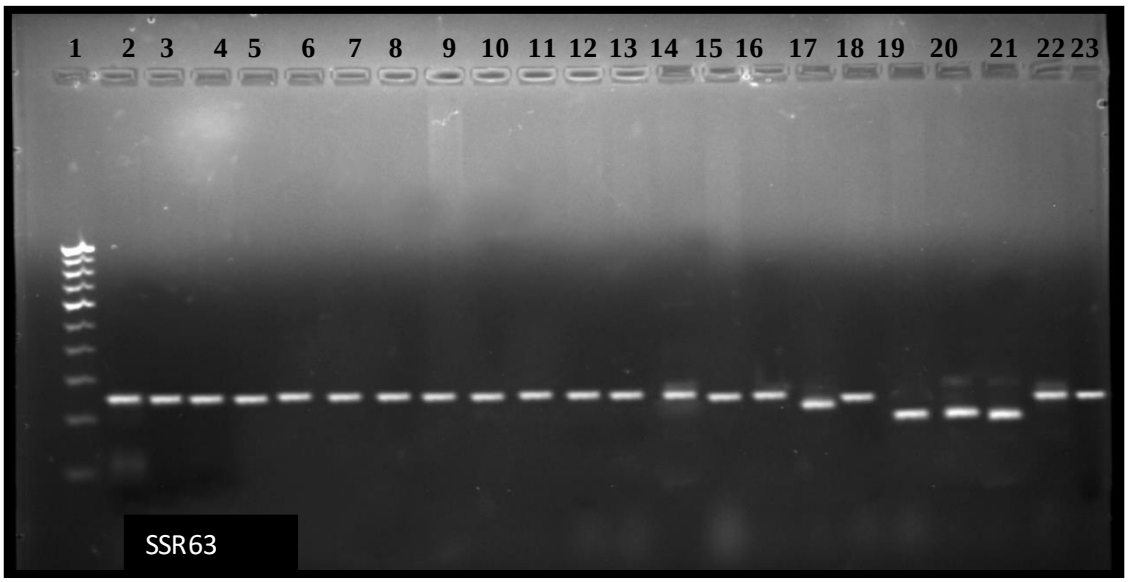
SSR 248 with polymorphic bands of size ~249 bp, located in chromosome 10. Distinct polymorphism for high-temperature tolerance between high temperature tolerant and susceptible varieties (Palam Pride, Pusa Rohini, and Arka Saurabh) was shown by SSR 248 (Plate 11). In some SSR loci (SSR 356 and SSR 248) 2 bands are demonstrated (Arka Saurabh and Pusa Rohini respectively) are found to be hybrid varieties. The bands in SSR 270 for Manuprabha is considered as nonspecific amplification.

Distinct polymorphism for high-temperature tolerance between high temperature tolerant and susceptible varieties was shown by SSR 63 and SSR 96.

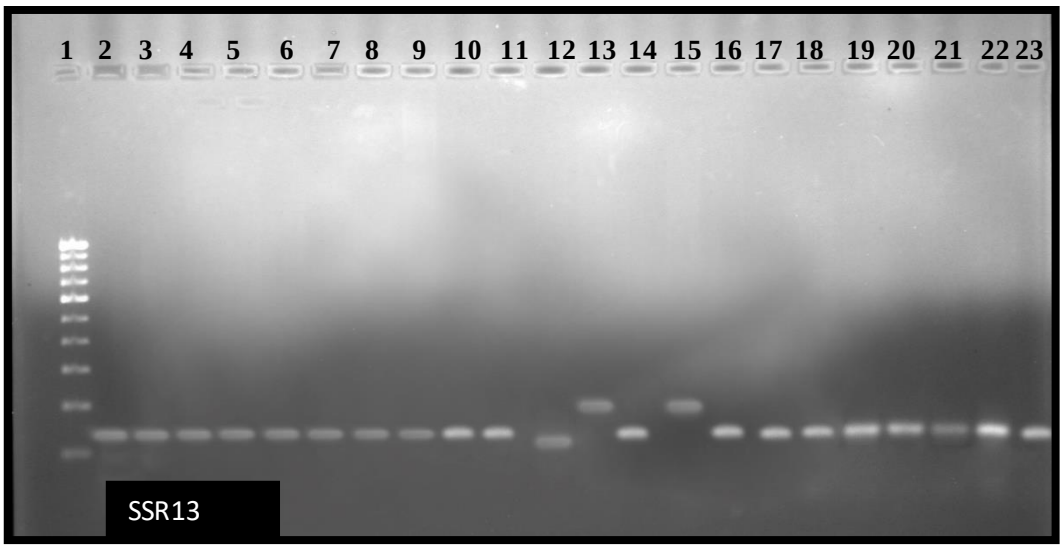


(Plate 5. Amplification pattern of 22 tomato genotypes obtained by SSR marker SSR 96. Lane 1- 100bp ladder, Lane 2-Manuprabha, Lane 3-Akshaya, Lane 4-Pusa Ruby, Lane 5-IC-45, Lane 6- Nandi, Lane 7-IIHR-2200, lane 8-IIHR-26372, lane 9-Palam Pride, lane 10-PKM-1, lane 11-Manulakshmi, lane 12-Arka

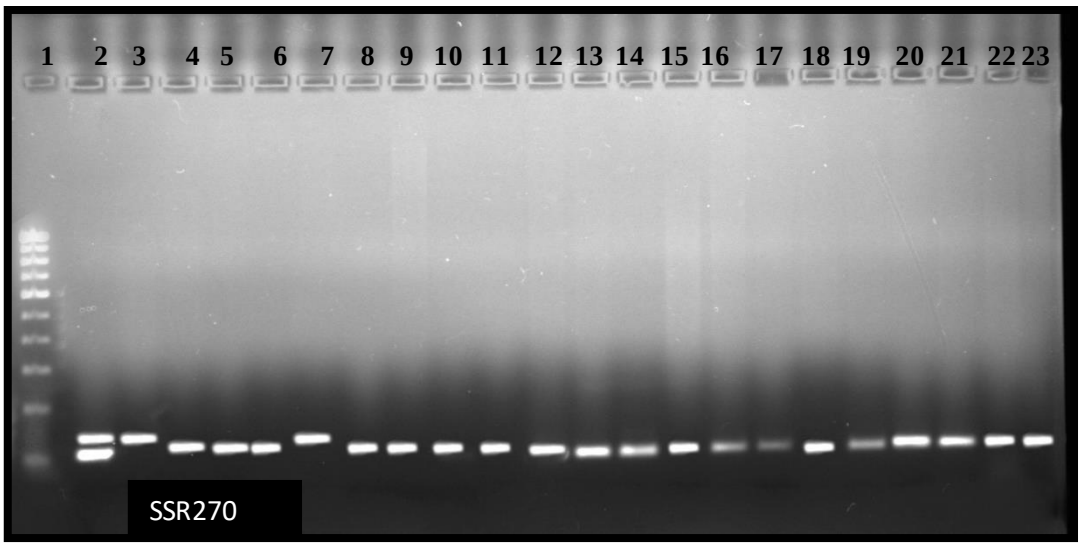
Samrat, lane 13- Arka Rakshak, lane 14-Arka Vikas, lane 15-Pusa Rohini, lane 16-Arka Alok , lane 17-Sakthi, lane 18-Vaibhav, lane 19- Vellayani Vijay, lane 20-Anagha, lane 21-Kashi Vishesh, lane 22- Arka Saurabh, lane 23-Arka Abha).



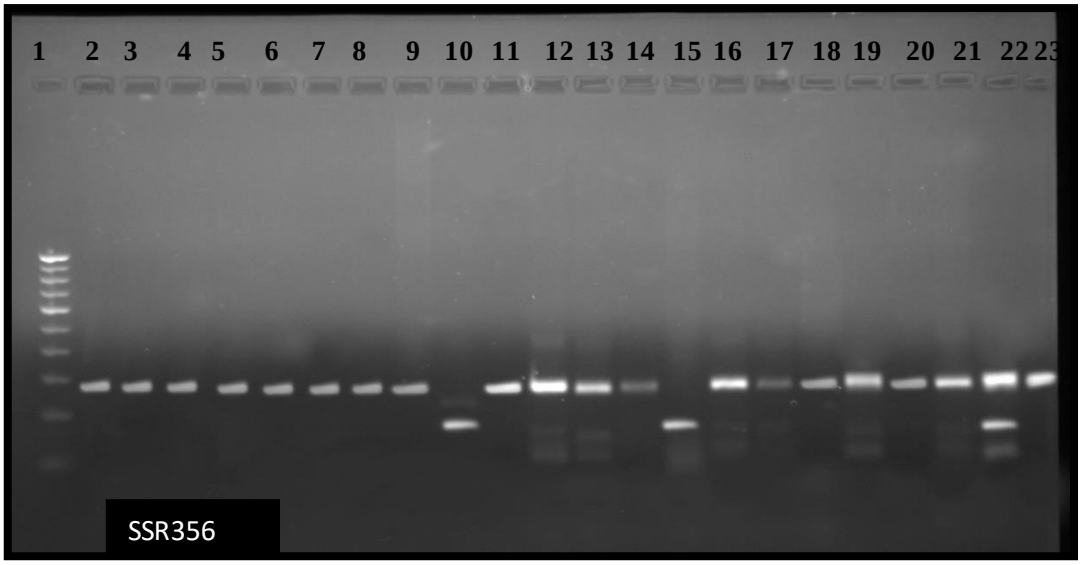
(Plate 6. Amplification pattern of 22 tomato genotypes obtained by SSR marker SSR 63.Lane 1- 100bp ladder, Lane 2-Manuprabha, Lane 3-Akshaya, Lane 4-Pusa Ruby, Lane 5-IC-45, Lane 6- Nandi, Lane 7-IIHR-2200, lane 8-IIHR-26372, lane 9-Palam Pride, lane 10-PKM-1, lane 11-Manulakshmi, lane 12-Arka Samrat, lane 13- Arka Rakshak, lane 14-Arka Vikas, lane 15-Pusa Rohini, lane 16-Arka Alok , lane 17-Sakthi, lane 18-Vaibhav, lane 19- Vellayani Vijay, lane 20-Anagha, lane 21-Kashi Vishesh, lane 22- Arka Saurabh, lane 23-Arka Abha).



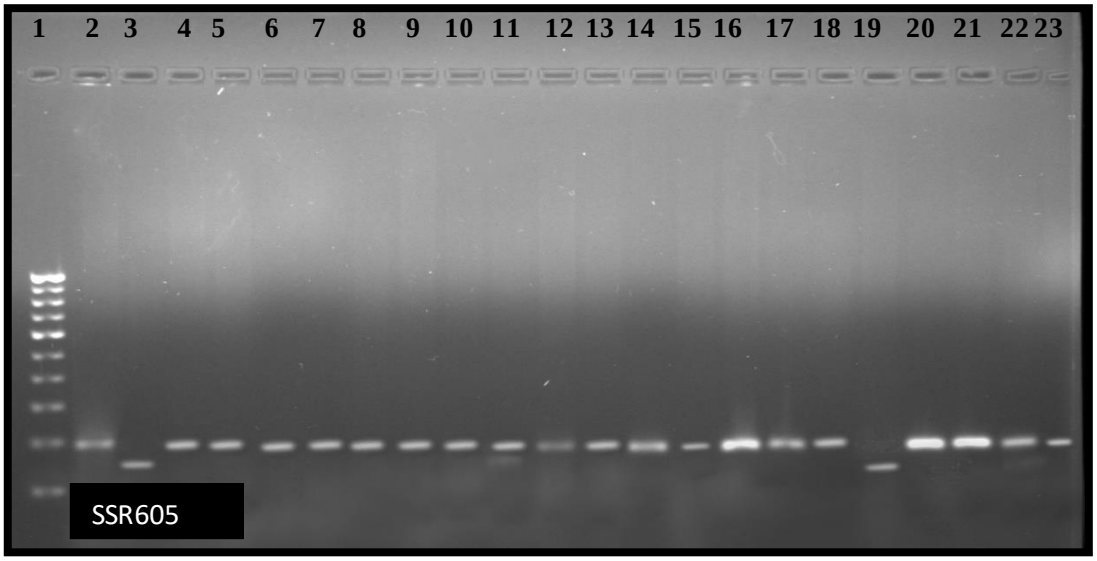
(Plate 7. Amplification pattern of 22 tomato genotypes obtained by SSR marker SSR 13. Lane 1- 100bp ladder, Lane 2-Manuprabha, Lane 3-Akshaya, Lane 4-Pusa Ruby, Lane 5-IC-45, Lane 6- Nandi, Lane 7-IIHR-2200, lane 8-IIHR-26372, lane 9-Palam Pride, lane 10-PKM-1, lane 11-Manulakshmi, lane 12-Arka Samrat, lane 13- Arka Rakshak, lane 14-Arka Vikas, lane 15-Pusa Rohini, lane 16-Arka Alok , lane 17-Sakthi, lane 18-Vaibhav, lane 19- Vellayani Vijay, lane 20-Anagha, lane 21-Kashi Vishesh, lane 22- Arka Saurabh, lane 23-Arka Abha).



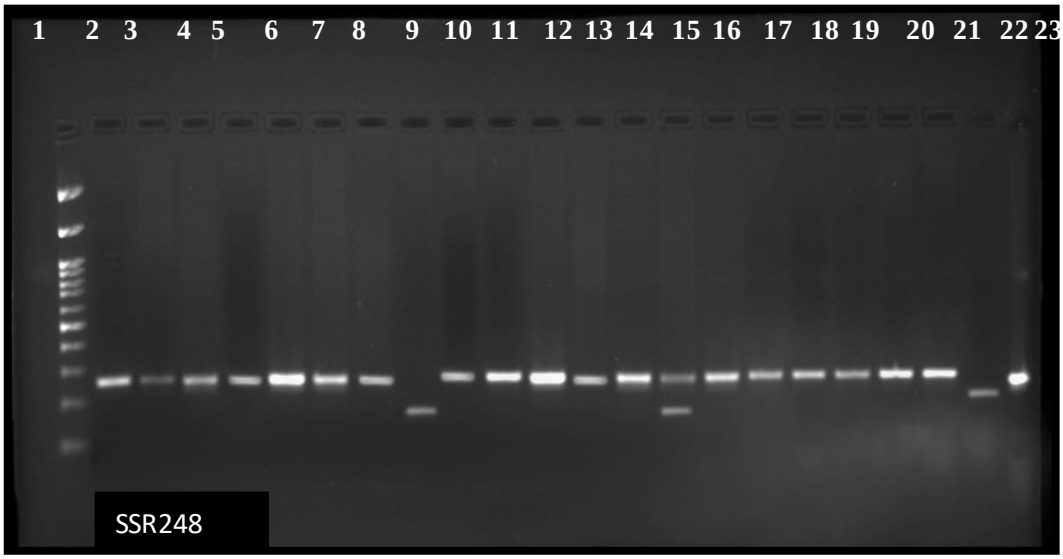
(Plate 8. Amplification pattern of 22 tomato genotypes obtained by SSR marker SSR 270. Lane 1- 100bp ladder, Lane 2-Manuprabha, Lane 3-Akshaya, Lane 4-Pusa Ruby, Lane 5-IC-45, Lane 6- Nandi, Lane 7-IIHR-2200, lane 8-IIHR-26372, lane 9-Palam Pride, lane 10-PKM-1, lane 11-Manulakshmi, lane 12-Arka Samrat, lane 13- Arka Rakshak, lane 14-Arka Vikas, lane 15-Pusa Rohini, lane 16-Arka Alok , lane 17-Sakthi, lane 18-Vaibhav, lane 19- Vellayani Vijay, lane 20-Anagha, lane 21-Kashi Vishesh, lane 22- Arka Saurabh, lane 23-Arka Abha).



(Plate 9. Amplification pattern of 22 tomato genotypes obtained by SSR marker SSR 356. Lane 1- 100bp ladder, Lane 2-Manuprabha, Lane 3-Akshaya, Lane 4-Pusa Ruby, Lane 5-IC-45, Lane 6- Nandi, Lane 7-IIHR-2200, lane 8-IIHR-26372, lane 9-Palam Pride, lane 10-PKM-1, lane 11-Manulakshmi, lane 12-Arka Samrat, lane 13- Arka Rakshak, lane 14-Arka Vikas, lane 15-Pusa Rohini, lane 16-Arka Alok , lane 17-Sakthi, lane 18-Vaibhav, lane 19- Vellayani Vijay, lane 20-Anagha, lane 21-Kashi Vishesh, lane 22- Arka Saurabh, lane 23-Arka Abha).



(Plate 10. Amplification pattern of 22 tomato genotypes obtained by SSR marker SSR 605. Lane 1- 100bp ladder, Lane 2-Manuprabha, Lane 3-Akshaya, Lane 4-Pusa Ruby, Lane 5-IC-45, Lane 6- Nandi, Lane 7-IIHR-2200, lane 8-IIHR-26372, lane 9-Palam Pride, lane 10-PKM-1, lane 11-Manulakshmi, lane 12-Arka Samrat, lane 13- Arka Rakshak, lane 14-Arka Vikas, lane 15-Pusa Rohini, lane 16-Arka Alok , lane 17-Sakthi, lane 18-Vaibhav, lane 19- Vellayani Vijay, lane 20-Anagha, lane 21-Kashi Vishesh, lane 22- Arka Saurabh, lane 23-Arka Abha).



(Plate 11. Amplification pattern of 22 tomato genotypes obtained by SSR marker SSR 248. Lane 1- 100bp ladder, Lane 2-Manuprabha, Lane 3-Akshaya, Lane 4-Pusa Ruby, Lane 5-IC-45, Lane 6- Nandi, Lane 7-IIHR-2200, lane 8-IIHR-26372, lane 9-Palam Pride, lane 10-PKM-1, lane 11-Manulakshmi, lane 12-Arka Samrat, lane 13- Arka Rakshak, lane 14-Arka Vikas, lane 15-Pusa Rohini, lane 16-Arka Alok , lane 17-Sakthi, lane 18-Vaibhav, lane 19- Vellayani Vijay, lane 20-Anagha, lane 21-Kashi Vishesh, lane 22- Arka Saurabh, lane 23-Arka Abha).

4.3 POLYMORPHISM INFORMATION CONTENT

Polymorphic information content (PIC) value calculated is shown in (Table 6). 25 SSR primers were used across twenty-two tomato accessions for the Polymorphic Information Content value detection in tomato. The PIC values for markers varied between 0 and 0.65. The primers which showed the highest PIC values were SSR96 (0.65) followed by SSR63 and SSR 248 (0.612).

Table 6: PIC values of primers

Sl.No	Primer	PIC value
1	SSR 63	0.62
2	SSR 13	0.58
3	SSR 248	0.62
4	SSR 270	0.58
5	SSR 356	0.58
6	SSR 605	0.58
7	SSR 96	0.65

4.4 POPULATION STRUCTURE ANALYSIS

The population structure of the 22 tomato genotypes based on banding results provided by SSR markers was used to analyze the genetic composition of various populations. K is the number of significant populations in each main group. FIVE runs for each *k* from 2 to 8 were performed, and results were analyzed using Evanno's method implemented in Structure HARVESTER. The total number of subpopulations was estimated to be four based on the highest delta K value obtained from STRUCTURE HARVESTER (Fig 1 a and Fig 1b)

Subpopulations (SP1) includes; 6-IIHR-2200, 1- Manuprabha. Based on the yield, physiological data (during high-temperature stress condition) and molecular characterization these varieties show similar characteristics and considered as moderately tolerant varieties. Pollen viability-45-50%, Thermo stability-40-50%, Starch <200 mg/g fresh weight, Photosynthetic rate-17-19 µmol CO_2 $m^{-2}sec^{-1}$, Stomatal conductance-55-65 mmol H_2O $m^{-2}sec^{-1}$, Chlorophyll fluorescence-0.6-0.8 Fv/Fm, yield-10-30 g/plant.

Subpopulations (SP2); 20- Kashi Vishesh, 19-Anagha, 18- Vellayani Vijay. Based on the yield, physiological data (during high-temperature stress condition) and molecular characterization these varieties show similar characteristics and considered tolerant varieties. Phenotypic data of genotypes are as follows; Pollen viability-50-70%, Thermo stability-60-70%, Starch-190-230 mg/g fresh weight, Photosynthetic rate-17-22 µmol CO_2 $m^{-2}sec^{-1}$, Stomatal conductance-47-68 mmol H_2O $m^{-2}sec^{-1}$, Chlorophyll fluorescence-0.6-0.8 Fv/Fm, yield-50-60 g/plant.

sub-populations 3(SP3) includes;5-Nandi, 17-Vaibhav, 3-Pusa Ruby, 10-Manulakshmi, 15-Arka Alok, 16- Sakthi,7-IIHR-26372,13-Arka Vikas, 22- Arka Abha, 4- IC-45, 11- Arka Samrat. Based on the yield, physiological data (during high-temperature stress condition) and molecular characterization these varieties show similar characteristics and considered as mixed varieties. Phenotypic data of genotypes are as follows; Pollen viability-36-88%, Thermo stability-45-55%, Starch-160-200 mg/g fresh weight, Photosynthetic rate-14-20µmol CO_2 $m^{-2}sec^{-1}$, Stomatal conductance-45-57 mmol H_2O $m^{-2}sec^{-1}$, Chlorophyll fluorescence-0.4-0.75 Fv/Fm, yield-20-35 g/plant.

Subpopulations 4(SP4) includes; Arka Saurabh, Pusa Rohini, Palam Pride, Arka Rakshak. Based on the yield, physiological data (during high-temperature stress condition) and molecular characterization these varieties show similar characteristics and considered as susceptible varieties. Phenotypic data of genotypes are as follows; Pollen viability-44-45%, Thermo stability-25-30, Starch -90-110 mg/g fresh weight, Photosynthetic rate-13-16 µmol CO_2 $m^{-2}sec^{-1}$, Stomatal conductance-30-37mmol H_2O $m^{-2}sec^{-1}$, Chlorophyll fluorescence-0.4-

0.5 Fv/Fm, yield- 0.1-0.8g/plant. The varieties 1-Manuprabha and 9- PKM-1 are considered as admixtures.

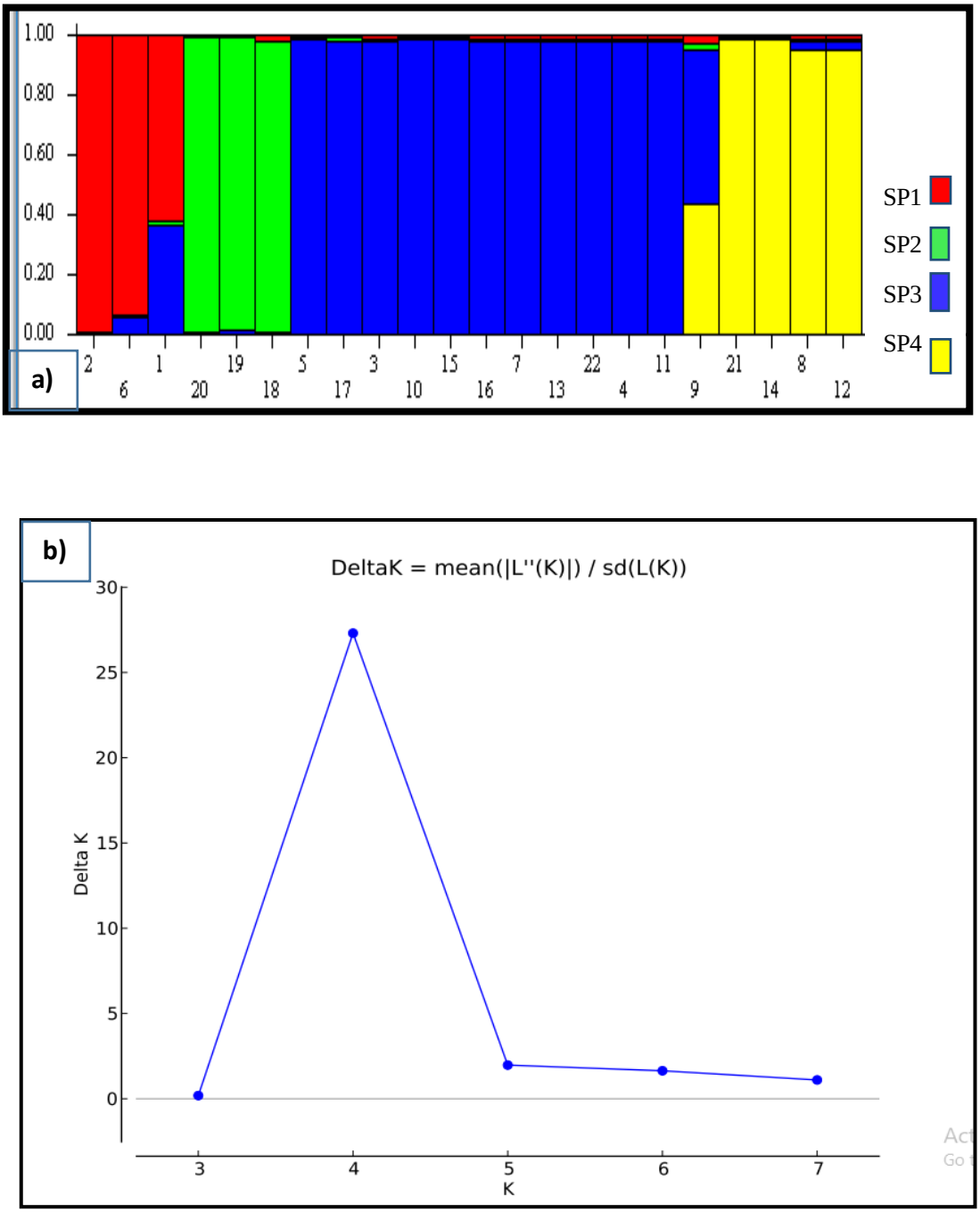


Fig. 1: (a) Population structure of 22 tomato genotypes based on 25 molecular markers (K = 4) and (b) Graph of estimated membership fraction for K = 4. The maximum of adhoc measure ΔK determined by structure harvester was found to

be K = 4, which indicated that the entire population can be grouped into four sub-populations (SP1, SP2, SP3 and SP3).

4.5 SIMILARITY COEFFICIENT AND CLUSTER ANALYSIS

Based on the DNA banding pattern of twenty-two tomato genotypes using 25 SSR markers, Jaccard's similarity coefficient was developed and displayed in (Fig. 2.) The genetic similarity coefficients of these tomato genotypes ranged from a minimum of 0.22 to a maximum of 1. The average genetic similarity range was 0.67.

Maximum genetic similarity (1) was shown by;

Pusa Ruby with 1C-45, IIHR-26372, Manulakshmi, Arka Samrat, Arka Alok, Sakthi, and Arka Abha with Nandi which belongs to same cluster and subpopulation (cluster 2 and SP-3). Based on the yield, physiological data, and molecular characterization these varieties showed similar characteristics.

The minimum genetic similarity coefficient (0.22) was showed by two pairs of genotypes viz. Pusa Rohini with Akshaya and Kashi Vishesh. Since they have low similarity they belong to different clusters and subpopulations.

Pusa Rohini (Susceptible Variety- belongs to SP-3 and cluster 4), Akshaya (Moderately tolerant variety belongs to SP1 and cluster 1), and Kashi Vishesh (tolerant variety belongs to SP-2 and cluster 3).

In the dendrogram (Fig. 3) the 22 genotypes were clustered into four distinct clusters. The phylogenetic tree was built employing UPGMA cluster analysis software NTSYSpc. UPGMA cluster evaluation of the matrix for genetic similarities culminated in a dendrogram and further divided into four major clusters. Cluster 1 consists of; Akshaya, IIHR-2200, Manuprabha. Cluster 2 includes; Nandi,

Vaibhav, Pusa Ruby, Manulakshmi, Arka Alok, Sakthi, IIHR-26372, Arka Vikas, Arka Abha, IC-45, Arka Samrat. Cluster 3-consists of Kashi Vishesh, Anagha, Vellayani Vijay. Cluster 4 includes; Arka Saurabh, Pusa Rohini, Palam Pride, Arka Rakshak.

Rows\	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22
G1	1																					
G2	0.66	1																				
G3	0.77	0.67	1																			
G4	0.77	0.67	1	1																		
G5	0.88	0.56	0.89	0.89	1																	
G6	0.77	0.89	0.78	0.78	0.67	1																
G7	0.77	0.67	1	1	0.89	0.78	1															
G8	0.56	0.44	0.78	0.78	0.67	0.56	0.78	1														
G9	0.67	0.56	0.89	0.89	0.78	0.67	0.89	0.67	1													
G10	0.78	0.67	1	1	0.89	0.78	1	0.78	0.89	1												
G11	0.78	0.67	1	1	0.88	0.78	1	0.78	0.89	1	1											
G12	0.56	0.44	0.78	0.78	0.67	0.56	0.78	0.78	0.67	0.78	0.78	1										
G13	0.77	0.67	0.78	0.78	0.89	0.78	0.78	0.56	0.67	0.78	0.78	0.56	1									
G14	0.33	0.22	0.56	0.56	0.44	0.33	0.56	0.78	0.67	0.56	0.56	0.78	0.33	1								
G15	0.78	0.67	1	1	0.89	0.78	1	0.78	0.89	1	1	0.78	0.78	0.56	1							
G16	0.78	0.67	1	1	0.89	0.78	1	0.78	0.89	1	1	0.78	0.78	0.56	1	1						
G17	0.67	0.78	0.89	0.89	0.77	0.67	0.89	0.67	0.78	0.89	0.89	0.67	0.67	0.44	0.89	0.89	1					
G18	0.56	0.44	0.78	0.78	0.67	0.56	0.78	0.78	0.67	0.78	0.78	0.56	0.56	0.56	0.78	0.78	0.67	1				
G19	0.67	0.56	0.89	0.89	0.78	0.67	0.89	0.67	0.78	0.89	0.89	0.67	0.67	0.44	0.89	0.89	0.78	0.89	1			
G20	0.67	0.56	0.67	0.67	0.78	0.67	0.67	0.44	0.56	0.67	0.67	0.44	0.89	0.22	0.67	0.67	0.56	0.67	0.78	1		
G21	0.67	0.33	0.67	0.67	0.78	0.44	0.67	0.67	0.78	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.56	0.44	0.56	0.56	1	
G22	0.89	0.56	0.89	0.89	1	0.67	0.89	0.67	0.78	0.89	0.89	0.67	0.89	0.44	0.89	0.89	0.78	0.67	0.78	0.78	0.71	1

Fig 2. Jaccard's similarity coefficient matrix for 22 tomato genotypes based on SSR data. Where, G1=Manuprabha, G2=Akshaya, G3=Pusa Ruby, G4= IC-45, G5=Nandi, G6=IIHR-2200,G7=IIHR-26372, G8=Palam Pride, G9=PKM-1, G10=Manulakshmi,G11=Arka Samrat, G12= Arka Rakshak, G13=Arka Vikas, G14=Pusa Rohini, G15=Arka Alok, G16=Sakthi,G17=Vaibhav, G18=Vellayani Vijay, G19=Anagha,G20=Kashi Vishesh, G21=Arka Saurabh, G22=Arka Abha.

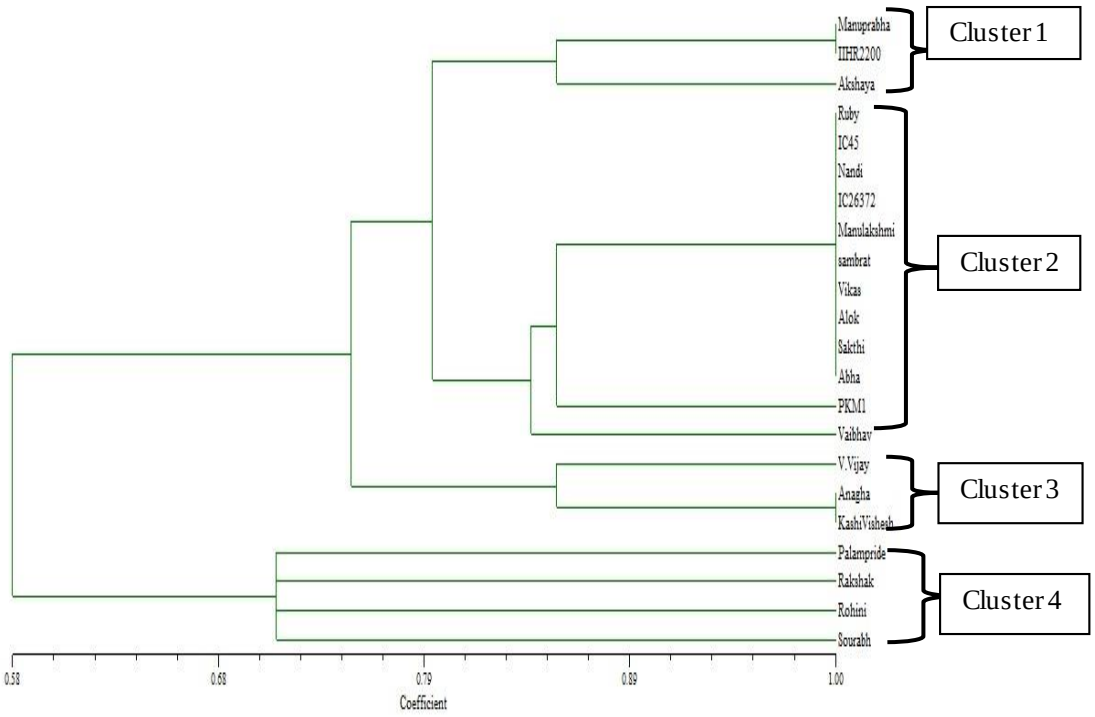


Fig. 3. Dendrogram showing genetic relationship of 22 genotypes of tomato based on Jaccard's coefficient of similarity.

5. DISCUSSION

5. DISCUSSION

Tomato (*Solanum lycopersicum* L., previously *Lycopersicon esculentum* Mill.) is now the fourth most commercially important vegetable in the Solanaceae family after rice, wheat, and soybean. Tomatoes have lately already become a prominent model system for genetic analysis in plants with limited genome size (about 950 Mb/haploid genome) a wide variety of high-density molecular maps DNA libraries (EST-expressed sequence tag and BAC-bacteria artificial chromosome), and microarrays (Kaushal *et al.*, 2017).

Global warming has become a problem around the world because of its drastic impact on crop output. Through the long term, mild temperature changes will interfere with the sexual reproduction thereby affecting fruit and seed. Extreme temperatures are one of the major abiotic stress harming tomatoes and significantly decrease their yield and quality of fruits. Since tomatoes can thrive in a wide variety of geographical situations their growth in vegetation and reproduction is severely impaired during high temperature and thus resulting in reduced yield and fruit quality (Pressman *et al.*, 2002). A successful approach to solve this issue is to recognize high-temperature stress-responsive (HSR) genes and breeding heat-tolerant varieties.

A number of specific methods like folding of proteins membrane stability refurbishment and photosynthesis, assimilation metabolism rate is seen to be triggered by temperature. When considering long-term mildly temperatures, indicative of heat waves, life cycle stages are distinctly different in terms of their vulnerability, with reproductive processes found to be more sensitive than vegetative ones (Bokszczanin and Fragkostefanakis, 2013).

Heat stress induces the denaturing of several heat-labile enzymes and hazardous reactive oxygen species significantly raise in living cells (Yang *et al.,* 2016). When faced with heat stress heat-shock gene expression rises exponentially resulting in a high content of heat-shock proteins (HSPs). HSP activity is induced primarily by transcription-level heat shock transcription factors (HSFs) and plays a vital role in addition to elevated-temperature stress (Lin *et al.,* 2011). Strengthening the thermal stability of crops by molecular imprinting is therefore essential; by identifying high-temperature resistant tomato genotypes and also to recognize the high-temperature stress involved molecular markers in the tomatoes.

Microsatellites are basic, tandem sequence motifs di- to tetra-nucleotide, flanking with known sequences. These remain useful as genetic markers although they co-dominate, identify the greater incidence of variation of the alleles are easily and economically checked by PCR analysis (McCouch *et al.,* 1997). Microsatellites (di-or tri-nucleotide repeat sequences) are widely used for the identification of varieties as well as for marker-assisted breeding due to their elevated polymorphism analysis and ease of use.

In the present study, 22 tomato genotypes released from; KAU, IIHR, IIHR, UAS & AVRDC, ICAR-IIVR, HPAU, TNAU, IARI were evaluated for the Molecular marker recognition associated with high-temperature tolerance in tomatoes. Phenotypic data of the same genotypes available at the Department of Plant Physiology, College of Agriculture, Vellayani was utilized for interpretation of molecular data, construction of population structure, cluster analysis of the genotypes and the results obtained are discussed in this chapter with appropriate support from previous studies of the genotypes.

5.1 PCR AMPLIFICATION USING SELECTED SSR PRIMERS.

In the present study out of 25 primers, 7 showed polymorphism in 3% agarose gel electrophoresis. Out of the 25 primers, 3 primers SSR 80, SSR 331, SSR 341 didn't show any amplification hence not used for further analysis. Out of twenty-two,

fifteen SSR markers, SSR450, SSR 602, SSR20, SSR111, SSR70, SSR 124, SSR 293, SSR 19, SSR115, SSR 304, SSR 276, SSR 47, SSR 75, SSR 134 and SSR 4 enhanced monomorphic banding trends but they were not taken into account for even more study. Therefore seven markers are being used for the ultimate section. The markers which showed polymorphism were; SSR 96, SSR 63, SSR 13, SSR 270, SSR 356, SSR 605, and SSR 248. The findings were also confirmed by Khan *et al.* (2020) using the markers SSR 13, SSR 47, SSR 63, SSR 248, SSR 110.

The polymorphic markers for temperature tolerance were SSR 96, showed a polymorphic band with size ~222bp, located in chromosome 1. Distinct polymorphism for high-temperature tolerance between high temperature tolerant and susceptible varieties (Arka Saurabh, Pusa Rohini, Palam Pride, and Arka Rakshak**)** was shown by SSR 96 (Plate 5). It was also reported by Wen *et al.* (2019) that 3 main indicators regarding heat resistance, The detection of heat tolerance were focused on relative electrical conductivity (REC), chlorophyll content (CC) and optimum photochemical efficiency (Fv/Fm) of PSII (photosystem II), and perhaps even the phenotypic index, heat injury index (HII), and traditional QTL examination associated with QTL-seq technology comprehensively. QTLs in tomato seedlings qCC-2-2 mostly with adjacent marker SSR96 have been reported on chromosome 2.

SSR 75 has amplified monomorphic band which was also reported by Benor *et al.* (2018) in which the analyzed SSR 75 in 39 varieties of tomatoes demonstrated a low polymorphism. SSR 111 has amplified monomorphic bands but Kaushal *et al.* (2017) reported that SSR 111 amplified 2 polymorphic bands and showed a polymorphism of 100 percent among 25 tomato genotypes.

SSR 13 showed polymorphic bands of size ~ 104 bp, located in chromosome 5. Distinct polymorphism for high-temperature tolerance between high temperature tolerant and susceptible varieties (Arka Rakshak and Pusa Rohini) was shown by SSR 13 (plate 7). Kwon *et al.* (2009) also reported that SSR 13 has shown a total of 5 alleles and the polymorphic band size was ~104bp. Wen *et al.* (2019) reported that

the Fv/Fm for high-temperature tolerance the adjacent marker SSR13 was connected to two QTLs on chromosomes 5 and 12 with 6% of the phenotypic variation.

SSR 63 showed polymorphic bands of size ~ 250bp, located in chromosome 8. Distinct polymorphism for high-temperature tolerance between high temperature tolerant (Vellayani Vijay, Anagha, Kashi Vishesh) and susceptible varieties were shown by SSR 63 (plate 6). Kwon *et al.* (2009) also identified 33 SSR markers and used 22 morphological traits to investigate the genetic characterization of commercial tomato varieties. Thirty-three pairs of SSR primers were screened for 63 tomato varieties and found that highly informative SSR markers (PIC ~ 0.1) are SSR47, SSR63, SSR248, are very useful for the identification of varieties and for the genetic evaluation of tomato germplasm, where SSR 63 and SSR 248 showed high polymorphism in the varieties as well. In SSR loci SSR 248, 2 bands were obtained for Pusa Rohini, which is found to be an hybrid variety. Kwon *et al.* (2009) also reported that SSR 63 showed a total of 8 alleles and the polymorphic band size was ~250bp. In the present study SSR 47 and SSR 75 has shown monomorphic banding pattern among the varieties. Benor *et al.* (2008) molecular characterization support this data.

SSR 248 exhibited polymorphic bands of size ~249 bp, in chromosome 10. SSR 248 showed distinct polymorphism for high-temperature tolerance between high-temperature tolerant varieties and susceptible varieties (Palam Pride, Pusa Rohini, and Arka Saurabh). Kwon *et al.* (2009) also reported that SSR 248 had amplified 5 alleles in total and the polymorphic band size was ~250bp. In SSR loci SSR 356, 2 bands were obtained for Arka Saurabh which was found to be a hybrid variety.

Identification of high-temperature stress-tolerant varieties is an essential requirement for the development of temperature tolerant varieties and The SSR markers were considered as valuable tools for the immediate selection of tolerant varieties rather than considering the phenotypic characters for the selection.

However, the analysis of genetic diversity with SSR markers will assist in selecting tolerant genotypes towards future crop breeding.

5.2 POLYMORPHISM INFORMATION CONTENT (PIC) VALUES

PIC value is the estimate of the diversity of the alleles and the frequency of the genetic variants. PIC value was determined to quantify the informativeness of each SSR marker. The PIC value is indeed a criterion for assessing the utility of DNA markers for functional genomics molecular breeding and genotypes assessment. Markers containing larger PIC values have more ability to show variability in alleles. The overall average PIC value of the various crop obtained by the evaluation of SSR markers was tested by different scientists and the value differed based on the number of SSR markers used and the number of varieties analyzed.

In the present study for the detection of the value of Polymorphic Information content in tomato, a total of 25 SSR primers were used across twenty-two tomato accessions. The PIC values varied between 0 and 0.65 for polymorphic markers. Primers that showed maximum PIC values was SSR96 (0.65) followed by SSR63 and SSR 248 (0.612). Kwon *et al.* (2009) also investigated commercial genetic characterization of tomatoes using 33 SSR markers and 22 morphological characteristics. For 63 tomato varieties, thirty-three pairs of SSR primers were screened. Insgesamt 132 polymorphic amplified fragments were acquired using 33 Markers of SSR. An estimate of PIC value given by 0.628, varying between 0.210 and 0.880. The highly informative SSR markers (PIC~0.1), such as SSR47, SSR63, SSR248, are considered to be very useful for various recognition and genetic evaluation of tomato germplasm. In which SSR 63 had a high PIC of 0.804, and SSR 248 showed 0.748, respectively. A lower PIC value from previous SSR research findings on tomatoes observed by He *et al.* (2003) (0.37), Bredemeijer *et al.* (2002) (0.40), Frary *et al.* (2005) (0.39) by evaluating 500 varieties of tomato with SSR markers. Such findings indicate that highly polymorphic markers are

suitable for conducting assessments designed to understand the genetic diversity of plant crops.

In this research work markers recorded an average PIC value of 0.65, demonstrated relatively high polymorphism these indicates that perhaps the SSR markers included in this research were highly informative towards genetic studies and are highly helpful in differentiating the polymorphic frequency of a marker at a particular locus.

5.3 POPULATION STRUCTURE ANALYSIS

A model-based analysis was carried out using the STRUCTURE software from the data from SSR markers. Model-based clustering was developed for the detection of Population structure comprising a variety of different genotypes with various markers. A benefit of the research carried out with the STRUCTURE program compared the potential to evaluate the quantity of an individual's genome (admixture) that belongs to each inferred group belongs to several other processes of categorizing subdivision. The quantitative clustering system used by STRUCTURE utilizes a Bayesian method that has been used in various plant species biological variation and association studies especially in including rice, wheat, sorghum, and tomatoes.

In the present study Population structure of the 22 tomato genotypes used to analyze different population's genetic structures depending on the banding pattern produced by SSR markers. The number of subpopulations was found to be four, by checking the maximum Delta K value developed by STRUCTURE HARVESTER software.

Sub- population-1 (SP1) includes; moderately tolerant varieties (IIHR-2200, Manuprabha). Based on the yield, physiological data during high-temperature stress conditions) and molecular pieces of information they show moderate tolerance to high temperature with a yield of 10-30.

Sub populations-2 (SP2) includes; tolerant varieties (Kashi Vishesh, Anagha, Vellayani Vijay. Based on the yield, physiological data (during high-temperature

stress condition) and molecular information's these varieties show high yield (50-60) during high-temperature stress conditions.

Sub-population-3(SP3) includes; Nandi, Vaibhav, Pusa Ruby, Manulakshmi, Arka Alok, Sakthi, IIHR-26372, Arka Vikas, Arka Abha, IC-45, Arka Samrat. Based on the yield, physiological data (during high-temperature stress condition) and molecular information's these varieties show similar characteristics and considered as mixed varieties with a yield of 20-35.

Sub-population-4(SP4) includes; Arka Saurabh, Pusa Rohini, Palam Pride, Arka Rakshak. Based on the yield, physiological data (during high-temperature stress condition) and molecular information's these varieties show similar characteristics and considered as susceptible varieties and show no yield during high-temperature stress. The varieties Manuprabha and PKM-1 are considered as admixtures. The varieties 1-Manuprabha and 9-PKM-1 are considered as admixtures. Admixed populations are populations that possess characters from multiple source groups. The "admixture model" of STRUCTURE assumes that each individual has similar characteristics from one or more genetically distinct populations. Therefore Manuprabha and PKM-1 showed similar characters between Population Structure and genetic diversity were evaluated in 70 genotypes of tomato by (Sim *et al.*, 2011).

5.4 SIMILARITY COEFFICIENT AND CLUSTER EVALUATION

Sample grouping was achieved through NTSYSpc software, based on a similarity matrix using the UPGMA algorithm. The cluster analysis divided the 22 tomato genotypes into four major clusters. Cluster 1 consisted of; Akshaya, IIHR-2200, Manuprabha. Cluster 2 included; Nandi, Vaibhav, Pusa Ruby, Manulakshmi, Arka Alok, Sakthi, IIHR-26372, Arka Vikas, Arka Abha, IC-45, Arka Samrat. Cluster 3-consists of Kashi Vishesh, Anagha, Vellayani Vijay. Cluster 4 Arka Saurabh, Pusa Rohini, Palam Pride, Arka Rakshak. Castellana *et al.* (2020) reported the total

average of alleles per locus as 3,158 and the median PIC value was 0.38 and Unweighted UPGMA grouped the tomato accessions into 4 groups.

The similarity coefficient between these twenty-two tomato genotypes predicted with SSR markers based on DNA amplification differed from 0.22 to 1. A vast array of the coefficient of similarity between these genotypes suggested the existence of strong genetic variation among genetic stocks researched. Similar to the present findings of similarity coefficients among tomato genotypes was earlier reported by Dhaliwal *et al.* (2011) among other varieties of tomatoes. The limited range of coefficient of similarity between these genotypes suggested the existence of limited genetic similarities among the analyzed genotype. The present findings are consistent with the structural analysis and based on the phenotypic and molecular details they belong to the same cluster and population.

The genotypes in cluster 1-Akshaya, IIHR-2200, Manuprabha are moderately tolerant varieties showed 89% similarity and they belong to the same subpopulation –SP 1

In cluster 2- Pusa Ruby, IC-26372, Manulakshmi, Arka Samrat, Arka Alok, Sakthi showed 100% similarity and they belong to SP- 3. Kaushal *et al.* (2017) reported a maximum of 96% similarity among tomato. A high level of similarity (95%) was revealed among 39 tomatoes by (Al-Abadi, 2007), the similarity of 100% was found among tomato by (Tam *et al.*, 2005).

In cluster 3- includes; Kashi Vishesh, Anagha, and Vellayani Vijay are tolerant varieties belong to SP- 2. Kashi Vishesh and Anagha showed 78% similarity. Kashi Vishesh and Vellayani Vijay showed 67% similarity. Vellayani Vijay and Anagha showed 89% similarity.

In cluster 4- includes susceptible varieties; Arka Saurabh, Pusa Rohini, Palam Pride, Arka Rakshak. Observed 78% similarity for three pairs of genotypes viz. Arka Saurabh with Pusa Rohini, Palam Pride, and Arka Rakshak. A 68% similarity observed for two pairs of genotypes viz. Pusa Rohini with Palam Pride and Arka Rakshak. They belong to SP-4.

NTSYSpc is a program system used for finding and displaying structure in multivariate data. For example, a set of data points indicates that the samples might have come from two or more distinct populations. This method was initially designed for biological use in the field of quantitative nomenclature. Many interventions, however, were also broadly used in the natural sciences, engineering, humanities, morphometry, environmental sciences as well as other fields of study. This field of application has also been described with the terms mathematical taxonomy and automatic classification. The approaches are already a branch of quantitative statistical analysis and are closely related to certain techniques of pattern recognition.

Kaushal *et al.* (2017) work on cluster assessment relies on Jaccard's coefficient of similarity and UPGMA and identified two clusters. There were a few phenotypic traits in both of these cases that also reported significant phenotypic and genetic patterns somewhat clustered collectively. Cluster A composed of the most complex genotypes (EC519821) corresponds to species of wild *Solanum pimpinellifolium* with a specific exponent of 0.65 percent and is distinct from other crops. Gonias *et al.* (2019) review of the clusters showed that the range could be divided into three clades with most landraces and moderate varieties differentiated from hybrids but also each other.

The molecular characterization study using SSR markers population structure and cluster analysis of tomato genotypes for high-temperature stress tolerance have classified the set of genotypes into four classes. The identification of heat-tolerant genotypes is an essential requirement for developing heat-tolerant varieties. In this study, more diversity was observed between heat-tolerant and susceptible genotypes in SSR analysis. The marker SSR 96 and SSR 63 was found as a functional marker associated with heat tolerance in tomato. This could be inferred that the SSR benefited in distinguishing tolerance and susceptibility in tomato at the molecular level as well as in establishing genetic relatedness among the studied genotypes. Analysis of populations and clusters help to effectively classify genotypes. In the current study, SSR was successfully employed on the grounds of

DNA strands for the molecular analysis of tomato varieties. The mixture of polymorphism and the large number of bands obtained per assay confirms that SSR is the most informative tomato genotyping marker system. The geneticists and plant breeders focused on the importance of such progress and many such exercises have been initiated by several projects. Considering that in the future heat-resistant tomatoes would be a challenging requirement in tropics and subtropics, a mix of multiple propagation techniques and marker-assisted breeding could be a standard heat-resistant tomatoes growth strategy.

SUMMARY

SUMMARY

The current study was conducted to do molecular characterization and construction of the population structure of selected tomato genotypes (*Solanum lycopersicum* L.) under high-temperature stress conditions. The findings are given below.

The materials for this research include 22 genetically diverse tomato genotypes obtained from various Indian institutes. Twenty-two tomato genotypes were grown in pot trays. On 16th September 2019, all the 22 genotypes of tomatoes were sowed. Approximately 30 days old plant has been taken for further study. DNA was isolated from the young and stable leaves by the genomic DNA isolation process using CTAB. The genomic DNA isolated from 22 Tomato varieties were analyzed and confirmed by agarose gel electrophoresis. Utilizing spectrophotometer, nucleic acid quantification was conducted. The genomic DNA was spectrophotometrically quantified at wavelengths of both 260 nm and 280 nm. The 260 nm absorbance allowed DNA concentration measurement in the sample. By reading at 260 nm, the purity of DNA was tested, and an assessment of the purity of the samples was made using 280 nm (OD 260/OD 280).

DNA quality was checked by agarose gel electrophoresis. PCR screening was performed using twenty-five SSR microsatellite markers, and the sequence was taken from the Sol Genomics Network database. Selected primers were used to conduct the PCR reaction.

In 1.5% gel electrophoresis, out of 25 primers, 7 showed polymorphism, and all other primers were monomorphic. Out of the 25 primers, 3 primers SSR 80, SSR 331, SSR 341 showed no amplification therefore they were not used for further analyses. Of the twenty-two SSR markers, SSR450, SSR 602, SSR20, SSR111, SSR70, SSR 124, SSR 293, SSR 19, SSR115, SSR 304, SSR 276, SSR 47, SSR 75, SSR 134 and SSR-4 amplified monomorphic banding patterns, no further analysis was therefore considered. Seven markers have been used for the

final study. The polymorphic temperature tolerance markers were SSR 96, showed polymorphic band size ~ 222bp, SSR 63 with polymorphic bands size ~ 250bp, SSR 13 with polymorphic bands size ~ 104bp, SSR 270 with polymorphic bands size ~ 231bp, SSR 356 with polymorphic bands size ~ 259bp, SSR 605 with polymorphic bands size ~ 196bp, SSR 248 with polymorphic bands size ~ 249bp.

SSR 63 and SSR 96 demonstrated distinct polymorphism for high-temperature tolerance between high temperature tolerant and susceptible varieties.

25 SSR primers were used for the detection of the polymorphic information content value in tomatoes among the twenty-two tomato accessions. PIC values varied between 0 and 0.65 for polymorphic markers. The primers which display the maximum PIC values were SSR96 (0.65) followed by SSR63 and SSR 248 (0.612).

The population structure of the 22 tomato genotypes used to evaluate the genetic makeup of various populations and was used for K= 8 depends on the data obtained from SSR markers. K is the number of significant populations in each major group. FIVE runs from 2 to 8 for each k were performed and results were analyzed using the Evanno method implemented in Structure HARVESTER. Four subpopulations were detected, depending on the maximum delta K value obtained by the STRUCTURE HARVESTER software.

Subpopulations (SP1) include moderately tolerant varieties; 6-IIHR-2200, 1- Manuprabha. Subpopulations (SP2) include tolerant varieties; Kashi Vishesh, Anagha, Vellayani Vijay. sub-populations 3(SP3) includes; mixed varieties. Nandi, Vaibhav, Pusa Ruby, Manulakshmi, Arka Alok, Sakthi, IIHR-26372, Arka Vikas, Arka Abha, IC-45, Arka Samrat. subpopulations 4(SP4) include; susceptible varieties. Arka Saurabh, Pusa Rohini, Palam Pride, Arka Rakshak. The varieties Manuprabha and PKM-1 are considered as admixtures.

Utilizing NTSYSpc cluster analytics tools, the phylogenetic tree was constructed with UPGMA. The UPGMA proposed approach of the matrix of genetic similarities has contributed to the dendrogram, the dendrogram revealed

that the 22 genotypes were divided into four distinct clusters. Cluster 1 consists of; Akshaya, IIHR-2200, Manuprabha. Cluster 2 includes; Nandi, Vaibhav, Pusa Ruby, Manulakshmi, Arka Alok, Sakthi, IIHR-26372, Arka Vikas, Arka Abha, IC-45, Arka Samrat. Cluster 3-consists of Kashi Vishesh, Anagha, Vellayani Vijay. Cluster 4; Arka Saurabh, Pusa Rohini, Palam Pride, Arka Rakshak. The examination of the structure and cluster revealed the same genotypical division.

Based on the interpretation of molecular data, STRUCTURE and cluster analysis 22 tomato genotypes were divided into 4 groups; Kashi Vishesh, Anagha, Vellayani Vijay are selected as tolerant varieties. IIHR-2200, Manuprabha, Akshaya are considered as moderately tolerant varieties. Varieties which has shown mixed characteristics include; Nandi, Vaibhav, Pusa Ruby, Manulakshmi, Arka Alok, Sakthi, IIHR-26372, Arka Vikas, Arka Abha, IC-45, Arka Samrat and PKM-1. Genotypes that are selected as susceptible varieties are Arka Saurabh, Pusa Rohini, Palam Pride, and Arka Rakshak.

Molecular markers linked to high temperatures are used for marker-assisted selection for high-temperature tolerance. Hence the present study helps for molecular marker detection which is associated with high-temperature tolerance in tomatoes.

Future line of work

The investigation was conducted in twenty-two tomato genotypes to characterize the molecular variation among different tomato genotypes using SSR markers and the construction of the population structure of tomato genotypes.

SDS PAGE (Sodium Dodecyl Sulfate–Poly Acrylamide Gel Electrophoresis) protein profile and native PAGE analysis of stress-related enzymes (Superoxide dismutase, Catalase, and Peroxidase) can be performed on the tolerant and susceptible varieties obtained from this study to explore the distinct response of ROS production and its scavenging system in tomatoes during high-temperature stress and to understand the role of antioxidant enzymes such as

SOD(superoxide dismutase) and APX(ascorbate peroxidase) in protecting against harmful ROS effect.

Nevertheless, further studies are required to accurately identify mechanisms of heat tolerance during reproductive development and interactions with the source or sink. Increasing climate variability requires the development of more efficient methods for germplasm screening and selection for the tolerance of high-temperature stresses.

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APPENDIX

APPENDIX-I

1. CHEMICALS FOR PLANT GENOMIC DNA ISOLATION

CTAB extraction buffer

Cetyltrimethyl ammonium bromide (CTAB)	3.0 ml
5 M NaCl	2.8 ml
0.5 M EDTA (pH 8.0)	0.4 ml
1 M Tris-Cl (pH 8.0)	1.0 ml
Polyvinylpyrrolidone (PVP) (MW 40 kDa)	0.3g
β-Mercaptoethanol	0.02 mL
H_2O	2.48 mL

1X TE Buffer (100 ml)

1M Tris-Hcl (pH-8)	1 ml
0.25 EDTA (pH-8)	0.4 ml

Final volume was adjusted to 100 ml and autoclaved.

II. CHEMICALS FOR AGAROSE GEL ELECTROPHORESIS

Gel loading dye

Formamide	50 ml
Xylene cyanol	50 mg
Bromophenol blue	50 mg
0.5 M EDTA	1 ml

10 X TBE Buffer (Tris-Borate-EDTA): 1000 ml

Tris base	107 g
Boric acid	55 g
Na_2EDTA	9.8 g

ABSTRACT

ABSTRACT

The study entitled "Molecular characterization and construction of population structure of selected tomato genotypes (*Solanum lycopersicum* L.) under high-temperature stress conditions" was undertaken during 2019-20 at the Department of Plant Physiology, College of Agriculture, Vellayani. The study was undertaken to evaluate the molecular variation between different tomato genotypes using simple sequence repeat (SSR) markers and the construction of the population structure of tomato genotypes.

Tomato (*Solanum lycopersicum*) is the second most important vegetable crop, cultivated worldwide in both temperate and tropical regions. High-temperature stress is one of the major abiotic stress affecting tomatoes and significantly reducing their fruit yield and quality. Molecular markers linked to high temperature can be used for marker-assisted selection for high-temperature tolerance in tomato genotypes. Hence the present study was focused on the identification of molecular markers linked to high-temperature tolerance in tomato.

The study included an experiment, in which twenty two tomato genotypes (KAU released varieties, NBPGR accessions including wild relatives of tomato) were raised in pot trays and the genomic DNA from one-month-old leaf samples was isolated by CTAB method. The quality and quantity of the isolated DNA from the twenty-two genotypes were analyzed. After checking the quality and quantity of DNA samples, they were screened using twenty-five microsatellite primers.

PCR reaction was carried out using 25 selected primers of which 3 primers SSR 80, SSR 331, SSR 341 did not show any amplification and hence they were not used for further analysis. Out of the twenty-two; fifteen SSR markers *viz.*, SSR450, SSR 602, SSR20, SSR111, SSR70, SSR 124, SSR 293, SSR 19, SSR115, SSR 304, SSR 276, SSR 47, SSR 75, SSR 134 and SSR 4 amplified monomorphic banding patterns, hence they were not considered for further analysis. Seven markers were thus selected for final analysis. The polymorphic

markers for temperature tolerance were SSR 96, SSR 63, SSR 13, SSR 270, SSR 356, SSR 605, and SSR 248. Among the 7 SSR markers, distinct polymorphic bands for temperature tolerance was shown by markers SSR 63 and SSR 96.

The value of polymorphic information content (PIC) is commonly used in genetics, which provides an estimate of the discriminatory power of a locus or loci, taking into account not only the number of alleles expressed but also the relative frequencies of those alleles, and is a measure of polymorphism for the locus marker used in linkage analysis. The PIC values for polymorphic markers ranged from 0 to 0.65. The primers which showed the highest PIC values were SSR96 (0.65) followed by SSR63 and SSR 248 (0.612).

The population structure of the 22 genotypes was performed using the Bayesian model-based scoring software STRUCTURE v2.3.4. Structure analysis divided the 22 genotypes into four subpopulations, in which tolerant genotypes were grouped into one sub-population, whereas the moderately tolerant, susceptible genotypes, genotypes which showed mixed characteristics were grouped into separate sub-populations.

The presence of a band was scored as 1 and absence was scored as 0. In the NTedit program of NTSYSpc (Numerical Taxonomy SYStem) version 2.10 software, binary data generated for all varieties for the polymorphic markers were entered. The phylogenetic tree was constructed using UPGMA (Un-weighted pair group method with arithmetic mean) using NTSYSpc cluster analysis software, resulted in the dendrogram and divided the 22 tomato genotypes into four major clusters. The pattern of grouping genotypes into the clusters was similar to that in the study of the population structure.

Phenotypic data of the same genotypes were collected from the Department of Plant Physiology, College of Agriculture, Vellayani was utilized for the interpretation of molecular data, the grouping of genotypes obtained from population structure and cluster analysis. Based on the interpretation of all the data obtained, among the 22 tomato genotypes; Kashi Vishesh, Anagha, Vellayani Vijay

were grouped as tolerant varieties. IIHR-2200, Manuprabha, Akshaya were categorized as moderately tolerant varieties. Varieties that showed mixed characteristics were Nandi, Vaibhav, Pusa Ruby, Manulakshmi, Arka Alok, Sakthi, IIHR-26372, Arka Vikas, Arka Abha, IC-45, Arka Samrat and PKM-1. Genotypes that were categorized as susceptible varieties were Arka Saurabh, Pusa Rohini, Palam Pride, and Arka Rakshak. Among the markers, distinct polymorphism for temperature tolerance between temperature tolerant (Kashi Vishesh, Anagha, and Vellayani Vijay) and susceptible varieties (Arka Saurabh, Pusa Rohini, Palam Pride, and Arka Rakshak) was shown by SSR 63 and SSR 96.