DEVELOPING HIGH TEMPERATURE TOLERANCE IN TOMATO (*Solanum lycopersicum* L.) THROUGH SELECTIVE FERTILIZATION TECHNIQUE

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"DEVELOPING HIGH TEMPERATURE TOLERANCE IN TOMATO (*Solanum lycopersicum* L.) THROUGH SELECTIVE FERTILIZATION TECHNIQUE"** 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, associateship, fellowship or other similar title, of any other university or society.

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AMMU ALPHONSA JOHN

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LIST OF ABBREVIATIONS

A	Absorbance
ADP	Adenosine diphosphate
am	Anti meridiem
APX	Ascorbate peroxidase
BOD	Bio- oxygen demand
CD (0.05)	Critical difference at 5% level
cm	Centimeter
CRD	Completely randomized design
CO_2	Carbondioxide
DAT	Days after transplanting
DMSO	Dimethyl sulphoxide
E	East
et al.	Co-workers/ Co-authors
Fig.	Figure
Fv/ Fm	chlorophyll fluorescence
FYM	Farmyard manure
g	Gram
g^{-1}	Per gram
GST	Glutathione S- transferases
ha	Hactare
ha^{-1}	Per hactare
hrs	Hours
H_2O_2	Hydrogen peroxide
HSF	Heat stress transcription factors
HSP	Heat stress protien
HSR	Heat stress response
i.e.	That is
IPCC	Intergovernmental panel on climate change

K^+	Potassium ion
KAU	Kerala Agricultural University
kg	Kilogram
kg^{-1}	Per kilogram,
l	Litre
M	metre
m^2	square metre
m^{-2}	Per square metre
mg	Milligram
mm	Millimetre
mmolesH_2O m^{-2} s^{-1}	Milli moles H_2O $meter^{-2}$ $second^{-1}$
μ moles CO_2 m^{-2} s^{-1}	Micro moles $meter^{-2}$ $second^{-1}$
ml	Millilitre
M ha^{-1}	Million hactare
MSL	Mean sea level
N	North
Na^+	Sodium ion
NHB	National Horticultural Board
O_2	Oxygen
OTC	Open top chamber
pH	Potenz hydrogen
POP	Package of practices
ppm	Parts per million
PS II	Photosystem II
RNA	Ribonucleic acid
ROS	Reactive oxygen species
ROS	Rainout shelter
RuBisCO	Ribulose-1,5- biphosphate carboxylase oxygenase

RUBP	Ribulose biphosphate
rpm	Rotations per minute
RWC	Relative water content
SE m	Standard error of mean
SF	Selectively fertilized
SOD	Superoxide dismutase
t	Tonnes
sec	Second
V	Volume
viz.,	Namely
%	Percentage
°C	Degree Celsius
µ	Micro
µm	Micro molar

INTRODUCTION

1. INTRODUCTION

The global population is now growing at an alarming rate and is predicted to remain in this condition for at least 35 years more. But the food production is not plenty to meet the need of growing population. Global warming and related heat stress due to climate change is a major warning which affects the crop production adversely. IPCC predicted a temperature increase of 1-3 °C during the middle of 21st century and 2-5 ^{0}C during the late 21st century (IPCC, 2012).

Tomato (*Solanum lycopersicum* L.) is considered as an important and economic agricultural vegetable crop worldwide. Tomato belongs to the solanaceae family that comprises several other famous crops, such as potato, eggplant and pepper and is the second most consumed vegetable in the world after potato. The largest tomato producers are in Asia, which represents 60.3 % of tomato production. In India, tomato stands second in area and production among the vegetable crops.

Even though tomato stands second in consumption, the rate of production and productivity are not satisfactory, mainly due to detrimental effect of heat stress associated with global climate change. Heat stress due to high ambient temperature is a serious threat to crop production worldwide (Kaushal *et al.,* 2016). It is considered as the major abiotic stress in tomato with high potential impact on crop yield. Rise of few degrees above the optimal temperature can reduce the fruit production and seed set in tomato (Peet *et al.*, 1997).

The optimum temperature is between 25^{0}C and 30°C during day time and 20^{0} C during night in tomato (Camejo *et al.*, 2005), and the daily mean temperature above 34°C is considered as thermal stress (Ahmed *et al.,* 2013). Heat stress has high impact on plant physiological and molecular processes. It causes production of reactive oxygen species which leads to oxidative stress that results in reduced cellular redox potential, homeostasis, membrane integrity, and possibly cell death. So development of tomato cultivars which is tolerant to heat stress is highly worthy in tomato crop production. Flowers with reduced pollen production,

pollen shedding, pollen viability, ovule viability and stigma receptivity and increased distance between stigma and anther cone (Kinet and Peet, 1997), are some of the factors which result in reduced fruit set under long term temperature. Even though these factors respond differently at high temperature, lack of pollen viability is considered as the prime reason for reduced fruit set (Abdulbaki *et al.*, 1995).

Regarding the performance of tomato under heat stress, the pollen grain are vulnerable to high temperatures which results in a decrease of tomato fruit yield (Camejo et al., 2005). Hence for developing high temperature tolerance, the use of pollen viability as a screening tool will provide valuable information about the male gametophytic tolerance to high temperatures. If genes related to stress tolerance in the pollen phase were also expressed in the sporophyte, then by pollen selection, it should be possible to transfer these traits into useful cultivars. Thus selective fertilization technique would be useful in transferring heat tolerance into parental lines for hybrid development. Selection at the pollen level has been proposed as a strategy to enrich the frequencies of genes associated with useful agronomic traits. Selective fertilization is a new technique to develop hybrids upon imposing selection pressure artificially during pollen germination and fertilization. The pollen grains which are tolerant to selection pressure only will germinate and fertilize the ovule.

Considering the increased temperatures forecasted for the near future and the importance of the tomato crop worldwide, adaptation of tomato to high temperature condition is essential. Identification of genotypes tolerant to temperature stress and understanding of high temperature responses of crops to climate change, it is necessary to study the physiological and molecular responses of tomato to high temperature stress.

At this juncture the present study was attempted to identify the critical temperature for pollen selection and to carry out selective fertilization followed by evaluation of selectively fertilized hybrids.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Solanum lycopersicum (2n=24) is one of the most important solanaceae crops which can thrive well under tropical and subtropical regions and is native to Central and South America (Vavilov, 1951). It is a warm season crop which requires temperature from 15 to 30° C. After potato, tomato is mostly used as the second most important vegetable throughout the world. Tomatoes are important part of human diets and they contain about 94% water, 2.5% total sugars, 2% total fibre, 1% proteins, and other nutritional compounds (acids, lipids, amino acids, and carotenoids) (Koh *et al.,* 2012). Fresh fruits of tomato are in great demand round the year throughout the country. They are good source of potassium, folate, vitamin E, soluble and insoluble dietary fibers and rich source of lycopene and ascorbic acid (Kaur and Kapoor, 2008).

In India, tomato is grown as autumn-winter, winter and spring-summer crop all over the country in areas with warm temperature and rain. But it cannot be grown commercially in the North Indian plains from May to October (Pavan, 2015). In India, it occupies an area of 8.02 lakh hectares with a production of 19.69 million metric tonnes and an average productivity of 24.4 metric tonnes per hectare (NHB 2016-17). Madhya Pradesh is the highest tomato growing state covering an area of 100,000 hectare with a production of 3102,000 metric tonnes (NHB 2016-17). In Kerala, the area of tomato is only 230 ha with a production of 328 metric tonnes (NHB 2016-17). Even though tomato can be produced in most of the regions, it confronts many abiotic stress especially high temperature (Faruq *et al.*, 2012) which directly affects fruit set (Marine *et al.,* 2017).

IPCC predicted a temperature increase of 1-3°C during the middle 21st century and 2-5^{0} C during the late 21st century (IPCC, 2012). Crops require optimum climatic conditions for attaining genetic yield potential and hence the increase in temperature due to climate change drastically affect the overall growth and yield of crops (Laxman *et al.*, 2013). Rise of few degrees above the optimal

temperature can reduce the fruit production and seed set in tomato (Peet *et al.*, 1997). The optimum temperature is between 25^{0}C and 30°C during day time and 20^0 C during night in tomato (Camejo *et al.*, 2005). Temperature above 34°C is considered as thermal stress (Ahmed *et al.,* 2013). Heat stress adversely affect the vegetative and reproductive process in tomato (Abdulbaki, 1991; Dane *et al.*, 1991).

Heat stress has high impact on plant physiological and molecular processes. It causes production of reactive oxygen species which leads to oxidative stress, resulting in reduced cellular redox potential, ion homeostasis, membrane integrity, and possibly cell death (Mirza *et al.,* 2013). Polyamine accumulation in the transgenic plants increase the tolerance to high temperature stress (Cheng *et al.,* 2009). Accumulation of osmoprotectants plays crucial role in the adaptation of cell to various environmental condition (Nuccio *et al.,* 1999). Trehalose an osmoprotectant helps to induce photosynthesis, plant development and cell growth (Ponnu *et al.,* 2011). ROS production prompt transcriptional changes, and activate the production and signalling of abscisic acid, salicylic acid and ethylene hormone (Zinn *et al.,* 2010). The triggered transcriptional changes activate the expression of heat stress transcription factors (HSFs) (Baniwal *et al.,* 2004), resulted in the up-regulation of the genes encoding heat shock proteins (HSP) (Wang *et al.*, 2004).

Metabolic imbalance to physiological process also occurred due to the differential effect of temperature especially on the stability of proteins, membranes, RNA species and cytoskeleton structures (Suzuki *et al.,* 2012). Scorching and sunburns of leaves, twigs, stem and branches, leaf senescence and abscission, root and shoot growth inhibition, fruit discolouration and damage are some of the morphological symptoms occurred during heat stress (Rodriguez *et al.,* 2005). Programmed cell death in specific cells and tissues may occur within a minute at extreme high temperature condition due to protein denaturation and on other hand gradual death occurs at moderate temperature condition (Mirza *et al.,* 2013). Impaired meiosis in both male and female organs, lessened pollen germination and pollen tube growth, reduced number of pollen grains retained by the stigma, altered stigmatic and style positions, reduced ovule viability, distressed fertilization

processes, hindered growth of the endosperm, pro-embryo and unfertilized embryo also occurred, which are the main reason for sterility under heat stress (Cao *et al.*, 2008).

During summer season, the basic limiting factors of tomato production such as temperature and light intensity are very high in tropics (Abdulla *et al.*, 1968). Tomato has the ability to produce fruits and flowers in this season but the fruit setting percentage is very low (Mehraj *et al.*, 2014). So development of tomato cultivars which are tolerant to heat stress and improved fruit set is highly worthy in the tomato crop production regions where mean daily temperature range is $\geq 35^{\circ}C$ during the part of the growing season (Johnson and Hall, 1953; Stevens and Rudich, 1978).

The most basic criterion for the tolerance of tomato to heat stress is the ability to set fruits when exposed to high temperature but the field evaluation of germplasm for heat tolerance is highly laborious and costly (Abdulbaki *et al.*, 1995). A positive correlation between the pollen viability and temperature tolerance were observed by Weaver and Timm (1989) while exposing the flowering plants to high temperature in greenhouse. Polyhouse conditions favours tomato production as temperature plays a major role in the phenological development and early maturity of tomato (Awal *et al.*, 2003).

Ganesan, (2002) states that tomato grown under polyhouse condition have 5-8 days advancement in flowering and fruit setting than those at open condition. The air temperature and soil temperature in the polyhouse condition is higher than outside condition and the temperature difference is very small during early and late hours of the day and around $8^{\circ}C$ during mid-day condition (Parvej *et al.*, 2010). The better microclimate such as high temperature than the nearby open condition is responsible for the early flowering and fruit set of different vegetable crops during winter season (Cheema *et al.*, 2004). The total number of fruit harvest are more due to the prolonged fruit bearing period under polyhouse condition (Pandey *et al.*, 2004). Macglasson and Adata (1977) stated that during summer season *i.e.* April,

the day temperature in the greenhouse increases to 43°C which causes premature flowering and fruit drops.

Pollen selection has been proposed as an approach to increase the gene frequencies associated with useful agronomic traits, pollen which is exposed to thermal stress during its germination may represent a powerful means to alter the genetic structure of the resulting progeny by turning the allelic frequencies in favour of high tolerance (Zamir, 1983). The pollen from a cold tolerant variety *Lycopersicon hirsutum* L., had high germination and fertility and more functional pollen under cold condition than the commercial variety (Zamir *et al.*, 1981) and it is successfully transferred to the commercial cultivar, this indicated that the gene which is allowing the sporophyte to tolerate high temperature will also allowed the pollen to retain fertility after thermal stress (Benjamin *et al.*, 1988).

2.1 SELECTIVE FERTILIZATION

In flowering plants fertilization is a complex process, the male gametophyte or sperm cell loose their mobility during fertilization and requires pollen tube as a passive cargo to transport it to the female gametophyte. The released two sperm cells from the pollen tube fuse with the female dimorphic gametes *i.e.* the egg cell and central cell to form zygote and endosperm respectively. This process is known as double fertilization (Thomas *et al.*, 2016).

Mulachy (1979) proposed that microgametophytic selection may have strengthened the adaptive process of angiosperms. The genes which are expressed in the gametophytic stage may also express in the sporophytic stage and hence selecting these genes in the haploid phase may have a positive influence in the outcome of diploid phase. Zamir *et al.* (1981) observed that *Lycopersicon hirsutum* which is tolerant to low temperature at the gametophytic stage is tolerant to chilling at sporophytic stage also.

Peet *et al.* (1998) conducted an experiment where both male sterile and male fertile plants grown under heat stress ($32/26^0$C) and normal condition ($28/22^0$C).

The male sterile plants were crossed with pollen from both the stress condition and normal condition and the male sterile plant grown under stressed condition pollinated with pollens from control condition produced less fruit set and yield whereas male sterile plants under stressed condition produce no fruit set when crossed with pollen from stressed condition. The result showed that male gametophytic selection has effect on fruit set. More severe reduction in fruit set occurred by crossing the emasculated heat stressed flower with gametes of heat stressed pollen donor (Zinn *et al.*, 2010). Young *et al.* (2004) also studied the effect of heat stressed pollen in *Brassica napus*, where emasculated heat stressed female parent crossed with male parent from controlled condition results 37% reduction in fruit set, while emasculated female parent crossed with heat stressed male parent resulted in more severe reduction in fruit set *i.e.* 88% than control.

In plant species heat susceptibility varies among genotypes and the developmental stages of crop (Camejo *et al.*, 2005). Since heat tolerance is also a complex process, several genotypes need to be analysed to understand the physiological alterations (Sharma *et al.*, 2012). Male gametophyte is more sensitive to heat stress than female gametophyte, maximum impart is reflected in reproductive stage when the heat stress is applied to the male than to the female parent of a cross (Peet *et al.*, 1998). Various hypothesis have been modelled regarding the heat sensitivity of pollen, the anther cells grieve from a loss of male identity under long term mild heat stress is the most recent one (Muller *et al.*, 2016).

Male gametophytic tolerance of tomato genotypes to high temperature stress can be studied by using the pollen viability as a screening tool (Marine *et al.*, 2017). Selective fertilization technique has been reported successfully in different crops like coconut (Aisha *et al.*, 2015), cotton (Benjamin *et al.*, 1988), tomato (Zamir *et al.*, 1987). Selective fertilization is a new technique to develop hybrids upon imposing selection pressure artificially during pollen germination and fertilization and the pollen grains which are tolerant to selection pressure only will germinate and fertilize the ovule. Selective fertilization has been employed

successfully for imparting different abiotic stresses like water stress, salinity, temperature, pH, heavymetals etc.

2.2 HEAT TOLERANCE

The genetics of heat tolerance is complex trait (Bhattarai *et al.*, 2016) and recessive genes are responsible for the heat tolerance and controlled by multiple gene allies (Vilareal *et al.*, 1978). Tolerance to high temperature is not an easy trait to enhance due to its low heritability and sensitivity to the environmental conditions (Hazra *et al.,* 2009). Heat tolerant plants can be developed by the combined accumulation of heat tolerance from the yield attributing traits which is modified by the indirect selection in early generation (Bhattarai *et al.*, 2016). Heat tolerance is governed by both additive and non-additive genes (Gabry *et al.*, 2014). High level of heat tolerance is observed in parents having high general combining ability, so parents with high x high, and high x moderate general combining ability should be selected for breeding to improve heat tolerance (Bhattarai *et al.*, 2016). The assembly of gene-specific data concerning the male gametophyte has revealed approximately 150 different genes, assigned to 16 distinct functional groups, with strong evidence for pollen-specific expression in tomato (Twell, 2002). Pollen fertility and fruit set under high temperatures were primarily controlled by additive genes (Dane *et al.*, 1991) and the additive effect played more important roles than the dominant effect (Fengxia *et al.,* 2007).

2.3 GROWTH PARAMETERS

Tomato production under high temperature than the optimum temperature has adverse effect on plant growth (Zhang *et al.*, 2014) and will decrease productivity (Sato *et al.*, 2006). Basic physiological processes such as photosynthesis, assimilate partitioning, growth and development are adversely affected under high temperature (Bokszczanin *et al.*, 2013).

2.3.1 Pollen Germination Percentage

High temperature has great impact on pollen development (Rieu *et al.,* 2017). Pollen germination percentage is drastically reduced under high temperature (Ansary, 2006). The reproductive part of the crop is highly affected at high temperature and the poor pollen germination is one of the main reason for reduced fruit set (Saeed *et al.*, 2007). Pollen maturation, pollen viability, pollen germination and pollen tube growth are negatively correlated with high temperature, at 40^0 C (Zinn *et al.,* 2010). Pollen maturation requires the accumulation of starch, which provide energy for the germination and tube growth of pollen but the reduced assimilate translocation under heat stress results in poor germination (Filomena *et al.*, 2013). The energy sources for the development and germination of pollen are sucrose and hexose (Pressman *et al.,* 2012). Since sucrose concentration increased under heat stress and reduced breakdown of sucrose into soluble hexose results in the down regulation of invertase gene, silencing of *Lin*5 gene, which encodes an extracellular cell wall invertase, results in pollen malformation and abortion in tomato (Giorni *et al.,* 2013).

In heat tolerant genotypes, pollen tube growth was unaffected by heat stress (Zhou *et al.*, 2015), and shown high fruit set than control which was correlated with pollen tube growth (Zinn *et al.*, 2010). Abdulbaki *et al. (*1995) observed that the germination percentage and tube growth of pollens collected from non-stressed condition of both heat tolerant and sensitive genotypes are very high. Heat tolerant genotypes produced more number of pollen grains than the heat sensitive ones (Abdelmageed *et al.*, 2003). The number of pollen grains released were reduced under high temperature stress, in both heat sensitive and tolerant genotypes but it's scale was higher in susceptible one (Sato *et al.*, 2006). The tight closure of locules results in poor anther dehiscence and reduced pollen dispersal (Sato et al., 2002). In both monocots and dicots, pollen heat sensitivity is a conserved factor, and occurs under various levels of high temperature (Mesihovic *et al.,* 2016). The pollen heat sensitivity at its peak occur from pollen meiosis to pollen mitosis I (Rieu *et al.,* 2017). Exposure to heat at microspore stage results in microspore abortion, reduced

number of pollen grains at anthesis and proportion of mature viable pollen grains which are competent to germinate (Mesihovic *et al.*, 2016).

Heat stress transcription factors and heat shock proteins are induced in developing anthers, microspores and pollen during high temperature stress (Li *et al.*, 2015). Different types of HSPs are accumulated in anthers and pollens within a short exposure to high temperature stress (Rieu *et al.*, 2017). Fragkostefanakis *et al.* (2016) studied the heat stress response (HSR) in tomato microspores under high temperature. The HSR is regulated by heat stress transcription factors HSFA2 which forms a complex with HSFA1 proteins (Scharf *et al.*, 2012) and protects the microspores. The sensitivity of the tomato pollen to high temperature is increased by the down regulation of HSFA2 (Fragkostefanakis *et al.*, 2016). BAG proteins are also involved in the thermo-tolerance of pollen, which function as co-chaperons involved in the expression of HSPs (Frank *et al.*, 2009).

2.3.2 Pollen Viability

The major determinant of fruit production and the most sensitive process in plant under high temperature is pollen development phase (Dane *et al.*, 1991). The competition for nutrients in the locular fluid of anther during high temperature stress, results in small difference in the metabolic performance of microspores, which results in dead and fully nonviable pollen from same anther locule (Carrico *et al.*, 2017).

Reduced carbohydrate metabolism in the tomato anther during heat stress results in poor pollen development and viability (Pressman *et al.*, 2002). The pollens are highly sensitive to mild changes in the environment, can be used to study the whole plant changes under different conditions (Hebbar *et al.*, 2018). The genotypes which show tolerance to high temperature have a high pollen viability than the sensitive genotypes under high temperature (Dane *et al.*, 1991). So the high temperature tolerance in tomato can be correlated with the pollen viability under high temperature (Firon *et al.*, 2006). Xu *et al.* (2017) reported that heat tolerant varieties produced flowers with high pollen viability under long term moderate heat

condition. Heat stress will increases the accumulation of proline in tomato leaf tissues (Kuo *et al.*, 1986) and leads to the reduction of proline in reproductive tissues and thereby reducing the pollen viability (Abdelmageed *et al.*, 2003).

Continuous exposure of high temperature in tomato reduced the number of pollen grains per flower and pollen viability, due to the decreased starch and sugar content in pollen grains (Pressman *et al.*, 2002). Long term mild stress condition during reproductive phase had significant effect in pollen viability, pollen number and female fertility and the drop in pollen viability is more than that of female fertility (Xu *et al.*, 2017). The temperature induced changes in the transcriptomic and proteomic profiles can be monitored to give light on pollen thermo-tolerance mechanism (Frank *et al.*, 2009).The total number of pollen and the fraction of viable pollen are two independent pollen quality traits, and the product of both total number of pollen per flower and fraction of viable pollen (pollen viability) provide the degree of thermo tolerance (Marine *et al.*, 2017).

ROS has vital role in the formation of viable pollen. During tapetum degeneration and pollen maturity ROS levels in the anther will be at its peak (Rieu *et al.*, 2017). Heat induced up- regulation of GST and APX gene in tomato anther and pollen, resulted in increased level of corresponding proteins, associated with thermotolerance mechanism (Fragkostefanakis *et al.*, 2016). Ethylene plays an important role in pollen thermo-tolerance Ethylene insensitive tomato mutants recorded high pollen sensitivity towards mild heat stress, while external application of ethylene prior to heat stress reduced the sensitivity and improved the pollen thermo-tolerance (Firon *et al.*, 2012).

2.3.3 Plant Height

Temperature has a remarkable effect on plant height There will be a reduction in height at temperature stress condition than those at normal condition (Abdelmageed *et al.*, 2003). Under greenhouse condition plant height of tomato varied significantly (Mehraj *et al.*, 2014). Favourable ambient environmental condition increases plant height. Greenhouse temperature significantly influenced

node number and internodal length. The nodal number increases with increase in average temperature of greenhouse and the relationship between day and night temperatures regulate internodal length (Berghage, 1998). Erwin *et al.* (1989) studied the effect of day and night temperature interaction on stem length of Easter lily (*Lilium longiforum* Thumb).

2.3.4 Fruit Weight

Fruit weight and plant dry weight are correlated to each other across temperature levels (Alsamir *et al.,* 2017). Fruit length, fruit diameter and fruit weight showed significant changes in heat stress (Mehraj *et al.,* 2014). Rate of fruit growth also affected under temperature stress (Adams *et al.,* 2001). The weight of tomato fruits decreased during temperature stress due to accelerated fruit growth rate and hastened maturity (Hurd and Graves, 1985). Peet *et al.* (1998) studied that in tomato at daily mean temperature of 29°C reduced the fruit weight, fruit number and no. of seed per fruit compared to optimum condition (25°C).

The expansion of tomato fruit is highly correlated to temperature. The fruit growth rate is positively correlated to temperature from 10-30°C, after that it decreased every hour at a rate of 5 µm with the increase of each ^{O}C (Pearce *et al.,* 1993). In glasshouse condition fruit growth rate shown an asymmetric sigmoid curve, and the expansion rate is less than expected in temperature stress (Pearce *et al.,* 1993). Fruit size is also correlated with the fresh weight of plant and, 40 days after anthesis, the ultimate fruit size is proportional to the maximum rate of increase in fresh weight (Grange and Andrews, 1993).

2.3.5 Floral Characters

Tomato being a thermosensitive crop, temperature significantly makes impact on the production of flowers, fruits and ultimately yield (Zinn *et al.,* 2010). High temperature during the reproductive phase in tomato causes significant increase in flower drop (Hanna & Hernandez, 1982). Morphology of flower structure is altered under high temperature and cause significant flower drop (Sato *et al.,* 2006). Polyhouse plants had significantly more number of flower clusters per

plant, flowers per cluster, flowers per plant, than the open grown ones (Parvej *et al.*, 2010). Under high temperature condition total flower production reduced steadily (El Ahmedi and Stevens, 1979), but flower number was not affected under moderate heat condition (Sato *et al.*, 2006). Temperature stress results in early or delayed flowering and depends on environmental condition (Zinn *et al.,* 2010). Profusely flowered genotypes were less affected by temperature stress (Dane *et al.,* 1991).

The number of inflorescence is not affected under long term moderate temperature condition but the number of flowers per inflorescence will reduce (Adams *et al.*, 2001). The total flower number is compensated by longer flowering or higher inflorescence production under high temperature condition (Xu *et al.*, 2017). Heat tolerant genotypes had more flowers under control and high temperature condition (Abdulbaki, 1991). Under moderate temperature condition varieties with higher number of flower per inflorescence exhibited lower flower abortion and thereby smaller number of unfertilized flowers (Kugblenu *et al.*, 2013). Reproductive heat tolerance and flower per inflorescence had a correlation between each other (Xu *et al.*, 2017).

2.3.6 Total Yield per Plant

Heat stress is one of the important constraints in the crop production, it reduces the yield and fruit quality of crop due to its adverse effect on both vegetative and reproductive process (Abdul-Baki, 1991). A small rise in temperature above optimum have significant negative effects on yield (Mirza *et al.,* 2013). In tomato high temperatures can significantly reduce the yield mainly due to the inability of pollen to form elongation tube to reach the ovary for fertilization (Bhattarai *et al.*, 2016). In tomato, a shift of pollen mother cell to unicellular microspores, due to the pollen development impairment occurred in anther, specifixcally 8-13 days before anthesis and resulted in dramatic decrease in the fruit set under heat stress (Giorni *et al.,* 2013).

Reduction of reproductive success leads to yield loss is one of the major effects of high temperature (Asseng *et al.*, 2011), as irregular flower development,

reduction in pollen production and viability, fruit drop and ovule abortion are the reproductive processes which ultimately decreased yield (Hazara and Ansari, 2008). It also affects several physiological and biochemical processes in tomato which result in reduced yield (Dinar and Rudich, 1985) due to the marked inhibition of photosynthesis (Hazra *et al.,* 2007). Reduced photosynthesis leads to diminished assimilatory capacity by altered membrane stability and increased maintenance respiration cost in heat stress resulting in loss of productivity (Mirza *et al.,* 2013).

The significant rise of flower drop per plant at high temperature is the other main reason for reduced fruit yield (Rahman *et al.*, 1998). Yield is positively interrelated with no of branches per plant, no of fruit per plant (Kumar and Dudi , 2011), number of flowers per plant and number of fruiting clusters per plant at phenotypic and genotypic level. But it is negatively interrelated with days to flowering, days from flowering to fruit set, fruit maturity and percentage of flower abortion, this correlation of the secondary yield attributing traits to yield point out that the enhancement of these traits results in heat tolerance and eventually high yield (Bhattarai *et al.*, 2016).

Non additive genes governed the yield trait and additive genes governed the other most heat tolerant morphological traits (Shalaby, 2013). Bhattarai *et al.* (2016) studied that most yield attributing characters are governed by additive genes and through selection from the segregating population it can be improved. A controlled fruit yield can be obtained by a dominant and dominant x dominant gene action because yield is directed by non-additive genes (Biswas *et al.*, 2011).

2.4 PHYSIOLOGICAL PARAMETERS

2.4.1 Photosynthetic Rate

Photosynthesis is one of the most important growth factors which is highly sensitive to high temperature. The declines in assimilation occur with every single degree rise of temperature after 30^0 C where its rate is at its peak (Wise *et al.*, 2004). With increasing temperature photosynthetic rate and respiratory rate declines and the photosynthetic rate declines before respiratory rate (Taiz and zeiger, 2006).

Plants differ in respect to their heat tolerance and the threshold temperature varies with varieties itself in a group (Wahid *et al.,* 2007). In all plant types net photosynthetic rate significantly decreased when exposed to $38^{O}C$ for 4 days (Cheng *et al.,* 2009).

Exposure of tomato varieties for 30 days at 30° C led to the changes in the microstructure of leaf and chloroplast ultrastructure. Loss of grana stacking, swelling of grana and altered organization of thylakoids are the major alterations which occur in chloroplast under heat stress (Rodriguez *et al.,* 2005). Zhou *et al.* (2015) observed swollen chloroplast and degraded starch grain in heat susceptible plants under heat stress condition.

One of the main reason for the decline of net photosynthesis at elevated temperature might be due to the changes in the structural organisation of photosynthetic apparatus (Zhang *et al.,* 2014). Photosynthetic apparatus is very susceptible and photosystem II and oxygen evolving complex is very sensitive to high temperature (Mathur *et al.*, 2014).

Photochemical reactions in the thylakoid membrane and carbon metabolism in the thylakoid stroma are the primary target of damage at high temperature (Wise *et al.*, 2004). So the electron transport plays a pivotal role in limiting photosynthesis at high temperature as it damages the photosynthetic electron transport system especially the thylakoid proton conductance (Schrader *et al.*, 2004). The biochemical reactions of photosynthesis is affected by heat stress by irreversibly damaging RuBisCO, oxygen complexes, chloroplast ultrastructure, thylakoid membrane and PSII reaction centres (Chen *et al.,* 2012). Under stress condition maintenance of high transpiration rate maintained the leaf temperature status and protects photosystems from heat shock (Ilan *et al.,* 1995). Leaf water status, leaf stomatal conductance and intercellular CO_2 concentration is highly affected at heat stress, the reduction in the intercellular CO_2 concentration due to stomatal closure under heat stress impaired photosynthesis also (Mirza *et al.,* 2013).

Reduction in photosynthesis under heat stress is linked to the decrease in chlorophyll content also (Camejo *et al.*, 2006). Lipid peroxidation of chloroplast and thylakoid membrane are the main reason for the reduced chlorophyll content (Mirza *et al.*, 2013). Net photosynthesis is inversely correlated with relative water content and directly with leaf area, dry mass and total chlorophyll content (Nduwania *et al.*, 2017).

Polyamines also play a role in the structural and functional regulation of photosynthetic apparatus. The accumulation of polyamines increase the tolerance level of photosynthetic apparatus to heat stress by improving the thermo stability of thylakoid membrane (Kusano *et al.*, 2009). Cheng *et al.* (2009) studied that the introduction of ySAMDC gene from yeast into tomato increased the polyamine accumulation and carbon dioxide assimilation and thereby enhanced the tolerance to heat stress. The net photosynthetic rate was significantly higher in transgenic lines than that of wild type, and these lines showed recovery near to normal photosynthetic rate when exposed to optimal condition for 3 days after heat stress.

2.4.2 Fruit Setting %

Fruit setting is impaired by extreme temperature variations during hot summers (Bita and Gerats, 2013). Fruit set can be greatly hampered by the exposure of plants to high day and night temperature for successive days or weeks during reproductive phase (Sato *et al.*, 2000). During fruit set, temperature above 30^0 C is detrimental for yield (Ahmed *et al.*, 2013). The most sensitive phases during thermal stress are flowering and fruit set (Bhattarai *et al.*, 2016). Reduced flower production, ovule and pollen viability, pollen dehiscence, stigma and stylar exertion (El Ahmadi and Stevens, 1979), diminished photosynthetic and assimilate translocation rate (Dinar and Rudich, 1985) are some of the factors which reduce fruit set in tomato under high temperature.

Fruit setting is limited by impeding pollination due to style protruding out of the anther cone (Dane *et al.*, 1991). Fruit setting and pollen viability are positively correlated with the number of flowers per inflorescence (Xu *et al.*, 2017). Heat tolerance level of a genotype can be determined by fruit setting percentage

and fruit set ranged from 41% to 84% under optimum conditions (Abdulbaki *et al.*, 1995). Heat tolerant genotypes had higher fruit set under controlled and high temperature conditions (Abdulbaki, 1991). It ranged from 45% to 85% in heat tolerant but heat sensitive genotypes showed 45% fruit set in optimum condition, and no fruit set under high temperature condition (Abdulbaki *et al.*, 1995).

2.4.3 Stigma Exertion

Tomato is a self-pollinated plant. Stigma exertion at high temperature prevents pollination and reduce productivity (Rick & Dempsey, 1969). The exerted style (*i.e.* stigma is elongated beyond the anther cone) during reproductive stage is the most outstanding impact of high temperature in tomato, that may hinder selfpollination (Faruq *et al.*, 2012). The stigma and style exertion under high temperature affect fruit setting ability (EL Ahmadi and Stevens, 1979). Stigma tube elongation and cone splitting occurred in tomato under high temperature (Peet *et al.*, 1998).

Unnecessary elongation of style in most flowers reduced the pollen access to stigma in heat sensitive genotypes and reduce fertilization (Alsamir *et al.*, 2017). The exertion of stigma tube above 1mm results in the reduction of fruit yield in tomato (Rudich *et al.*, 1977). The genotypes producing flowers having no stigma exertion at high temperature is stable and produce high fruit yield (Saeed *et al.*, 2007). The viability of male and female gametes and style protrusion level are the major determinants of reproductive success under high temperature (Bhattarai *et al.*, 2016). The heritability of fruit setting is less, while the heritability of style exertion is relatively high (Levy *et al.*, 1978).

2.4.5 Carbohydrate Content

In both heat tolerant and heat sensitive tomato genotypes, under heat stress condition carbohydrate translocation and partitioning is highly inhibited and induce the accumulation of sucrose in leaves (Wahid *et al.*, 2007). Reduced reducing sugar concentration and increased sucrose phosphate synthase activity resulted in increased sucrose content in tomato leaf (Zhang et al., 2012).

Heat tolerance at the vegetative and reproductive stage in heat tolerant tomato is due to the maintenance of more available carbohydrate and accumulated biomass under stress condition (Zhou *et al.*, 2015). Both long and short term abiotic stress show decrease in starch content (Vinocur and Altman, 2005). Zhou *et al.* (2015) stated that in heat sensitive genotypes, starch content will decrease under stress condition, due to decreased net photosynthetic rate. Starch and sucrose synthesis is highly affected at high temperature by reduced activity of sucrose phosphate synthase, ADP-glucose pyrophosphorylase and invertase (Rodriguez *et al.,* 2005). Depletion of carbohydrate reserves and plant starvation occurred under extended heat stress (Djanaguiraman *et al.,* 2009).

At peak fruiting stage, total soluble sugar, reducing sugar and proline content augmented with increase in temperature (Laxman *et al.,* 2013). Total soluble sugars and proline and other osmolytes and enhanc the adaptive mechanism of plants to extreme temperature regimes (Wahid *et al.,* 2007). Carbohydrate content in the anther and pollen play a vital role in pollen development (Rieu *et al.,* 2017). Disruption of sugar metabolism and proline transport during male reproductive development phase reduce the fruit set in tomato (Sato *et al.,* 2006). Under normal conditions after pollen mitosis 1, the sucrose concentration remain stable, while the starch concentration reaches its peak, followed by breakdown of starch into soluble sugar at anthesis (Pressman *et al.,* 2012). Under stress condition, starch accumulation in the binucleate pollen and sucrose concentration in young microspores reducing and at anthesis stage soluble sugar content will be at its lowest. The tolerant genotypes maintain higher pollen starch and sugar level than the sensitive ones (Firon *et al.,* 2006).

2.4.6 Chlorophyll Content

Leaf chlorophyll content decreases significantly with stress (Manabendra *et al.*, 2000). The functional activity of the photosynthetic apparatus is affected by short time exposure of high temperature with respect to the temperature tolerance of two tomato cultivars (Camejo *et al.*, 2005) and the inactivation being related to the membrane integrity, chlorophyll content and carotenoid content of the plant

(Gerganova *et al.* 2016). Chlorophyll a, Chlorophyll b and total chlorophyll content significantly reduced under high temperature stress (Sang *et al.,* 2017).

Total chlorophyll content decreases at low and high temperature compared to ambient temperature conditions. At 35°C the chlorophyll activity decreases slightly but significantly due to the influence of temperature on the efficiency of excitation energy capture by photosystem II (Bacci *et al.*, 1996). Pushpalatha (2007) observed that the chlorophyll content of plants grown at high temperature condition showed less chlorophyll content than the control ones. Arun (2010) also observed that under stress condition chlorophyll a, chlorophyll b, total chlorophyll content and carotenoid content of chickpea reduced significantly, though heat tolerant genotypes showed higher values. Loss of chlorophyll content coincides with the grain filling in the crop due to its correlation with the leaf N status, photosynthetic capacity and RUBP carboxylase activity (Seeman *et al.*, 1987).

In tomato stay- green or delayed senescence is one of the traits of heat tolerance (Sharma *et al.*, 2014). Under high temperature conditions tomato genotype cannot stay green due to decrease in chlorophyll a, chlorophyll b and carotenoid content and it showed premature chlorosis and withered leaves (Zhou *et al.*, 2015). Porphyrins, particularly chlorophyll a content reduced with temperature at early green stage (Camejo *et al.,* 2001). Reduced antenna pigment under stress condition results in decrease in chlorophyll content (Camejo *et al.,* 2006).

2.4.7 Total Soluble Protein

Total protein content increased with increase in temperature, it has a protective role and change the gene expression for thermotelarance and acclimatization to high temperature (Camejo *et al.,* 2001). Heat shock proteins increased with increase in temperature, HSP63 a member of HSP60, facilitate proper folding of other proteins and in tomato at 40°C and HSP23 accumulated more in leaves (He *et al.,* 2005). Elevated temperature conditions produced active

oxygen species and imbalanced the homeostasis by damaging proteins, chlorophylls, nucleic acids and membrane lipids (Scandalios, 1993).

2.4.8 Relative Water Content (RWC)

Relative water content is a better indicator of water status of plant, as it express the absolute amount of water, which the plant requires to reach full saturation (Gonzales and Gonzales, 2001). The status of water potential and cell turgor determines the degree of plant stress (Nduwimana *et al.*, 2017) and optimum vegetative and reproductive development of tomato plants require high water potential (Torricellas *et al.,* 1995). Optimum water status is necessary for plant tolerance under heat stress (Jiang and Huang, 2000), as temperature stress results in water deficit and cell turgor loss (Ahmed et al., 1993).

Water loss from the plant increased with temperature due to the increase in demand of transpiration under high temperature (Rahman *et al.*, 2000). Loss of cell water content occurs at high temperature and leading to reduced cell size and growth (Rodriguez *et al.,* 2005). High relative water content increase the resistance to drought condition (Manabendra *et al.,* 2000). Net photosynthesis reduced with drop of water content in strawberry *i.e.* it is inversely correlated with the relative water content (Gratani *et al.,* 2002).

Osmotic stress and yield loss also occurred during temperature stress due to depletion in cellular water content and osmotic potential (Zhang *et al.,* 2011). Reduced water loss and increased water uptake, maintained the plant water relations under stress condition and increased the stress tolerance level (Arun, 2010). Liu and Huang (2000) observed that the increased tolerance under heat stress condition in bent grass was due to the maintenance of optimum water status under stress condition. Heat tolerant genotypes showed low membrane injury index and high RWC and had good metabolic activities (Deshmukh *et al.,* 2001).

2.4.9 Membrane Integrity

Temperature stress results in membrane disruption due to protein denaturation or melting of membrane lipids, due to membrane rupture and loss of cellular contents (Ahrens and Ingram, 1988). The primary symptom of heat stress is membrane collapse and the thermo stability of plasmalemma is considered as an effective pointer of thermo tolerance (Alsamir *et al.,* 2017). The varieties which are tolerant to high temperature maintain high membrane thermo-stability (Saeed *et al.*, 2007).

The level of ion leakage through the membrane is negatively correlated with the pollen viability, fruit setting and flower per inflorescence (Xu *et al.*, 2017). Fruit set is reduced as a result of membrane damage (Alsamir *et al.,* 2017). The percent of electrolyte leakage shows the extent of membrane damage (Laxman *et al.,* 2014) and heat sensitive genotypes showed more ion leakage than the tolerant ones (Camejo *et al.*, 2005).

Membrane thermo-stability has often been interrelated with photosynthetic and respiratory enactment in temperature condition (Wahid *et al.*, 2007). Cell membrane stability is measured by cell electrolyte leakage and the amount of leakage increased with heat stress exposure in all lines of tomato (Cheng *et al.*, 2009). Heat stress disrupted the ion homeostasis by altering ion transport and compartmentalization (Zhang *et al.,* 2006). Ion homeostasis specifically, Na^+ and K^+ homeostasis maintain the biological membrane potential and the activities of several enzymes and osmotic concentration help to cope with the cell volume (Conde *et al.,* 2011). Membrane lipid peroxidation and disruption of cell membrane stability by protein denaturation during heat stress cause oxidative stress (Camejo *et al.,* 2006).

2.4.10 Vitamin C Content

One of the factors determine the quality of tomato fruit is ascorbic acid (Vitamin C) content (Ward, 1964). The antioxidant property of ascorbic acid marks its key role in stress response of plant (Matteo, 2010). Temperature stress highly

affect the vitamin C content of fruits and vegetables (Babalola *et al.,* 2010) and its stability decreases with increase in temperature (Emese *et al.,* 2008).

Environmental factors and genetic constitution have marked effect on ascorbic acid content of tomato plants (Brown, 1955). Under stress condition, tomatoes grown under greenhouse condition had less ascorbic acid content than the open grown ones (Ward, 1964).

The position of the cluster on the plant also determines the ascorbic acid content of ripe fruit and it increases with increasing height of cluster. The clusters which are shaded by upper foliage have lower concentration of ascorbic acid content than the upper clusters (Kidson, 1943). Ascorbic acid content in the fruit is determined by the temperature condition in maturation stage (Liptay, 1986). Herandez *et al.* (2018) observed decreased vitamin C content in tomato plants when grown at high temperature stress condition.

2.4.11 Lycopene Content

Lycopene is a major carotenoid pigment accumulates highly in tomato as an orange- red pigment in the final stage of ripening (Klunklin *et al.,* 2017). Under high temperature condition lycopene content decreased significantly, decreased lycopene content in high temperature reduce the quality of fruit (Alsamir *et al.,* 2017). Lycopene present in the peripheral pericarp as small globules, fat soluble and has antioxidant properties (Lenucci *et al.,* 2006). Small sized cherry tomatoes showed more lycopene content than large cultivars (Pek *et al.,* 2014).

2.4.12 SOD Activity

Plants produce active oxygen species under stress conditions like high temperature, which impaire the plant metabolism and ultimately leads to death. To prevent this, antioxidants are produced as defence mechanism (Rivero *et al.,* 2004). Antioxidant enzymes play an important role in the defence mechanism to protect cellular membranes and organelles against ROS generated by environmental stresses in plants (Parvaiz *et al.* 2008). Overproduction of active oxygen species can

also trigger oxidative damage (Almeselmani *et al.* 2006). The first line of cell defence against oxidative stress is the activation of antioxidants (Rivero *et al.,* 2004).

Antioxidants exhibit a positive correlation between chlorophyll content and relative water content, while a negative correlation with membrane injury index under high temperature stress (Arun, 2010). Superoxide dismutase (SOD) act as an antioxidant enzyme to scavenge the active oxygen species, by catalysing the dismutation of O^{2-} to H_2O_2 and O_2 (Ushimaru *et al.,* 1997). Schoner and Krause (1990) reported the elevated SOD activity in spinach under high temperature condition, and high SOD activity is accompanied with the overproduction of O^{2-} (Rao *et al.,* 1996), by the electron transport chain of the photosynthetic apparatus (Scebba *et al.,* 1998).

Rivero *et al.* (2004) also observed seven fold higher SOD activity in tomato at 35°C than at 25 °C. The SOD activity is positively correlated with the H_2O_2 concentration. Under mild temperature stress itself the antioxidant enzyme activity boosts up in heat tolerant genotypes to cope up with the changing temperature (Laxman *et al.,* 2014). Tolerance to high temperature stress is associated with the increased activity of antioxidants, and heat tolerant genotypes showed higher activity of SOD in chickpea under heat stress condition (Arun, 2010).

Heat tolerant and heat sensitive tomato genotypes exposed to temperature stress showed that the SOD activity increased seven fold in heat tolerant ones while it decreased 83% in heat sensitive ones. The degree of heat tolerance is correlated with the antioxidant activity and genotypes exhibit highest SOD activity had highest yield and the genotypes exhibit lowest SOD activity had the lowest yield (Rainwater *et al.*, 1996). Under high temperature stress condition, the exogenous application of spermidine on antioxidant system of tomato increased the SOD activity (Sang *et al.,* 2017).

2.4.13 Chlorophyll Fluorescence

The most heat sensitive organelle which play a vital role in photosynthesis is chloroplast (Krause and santarius, 1975), and it become swollen with

decomposed starch in heat sensitive varieties and maintained normal shape in heat tolerant ones after heat stress (Zhou *et al.,* 2015). Heat stress decreases photosynthesis through disruption of photosynthetic apparatus (Ogweno *et al.*, 2008). As photosystem II (PSII) of the photosynthetic apparatus is the most sensitive element (Cajánek *et al.*, 1998), the damage to it is often the first response under heat stress (Mathur *et al.*, 2011).

Chlorophyll fluorescence was used as a tool to study the alterations of photosystem I and photosystem II activity (Gerganova *et al.*, 2016). The ratio between variable fluorescence and maximum fluorescence *i.e.* chlorophyll fluorescence (Fv/Fm) will give value of the maximum quantum efficiency of PSII (Butler, 1978) and is the best tool to understand phenotype variability different among tomato genotypes for heat tolerance (Zhou *et al.,* 2015).

Under abiotic stress condition especially heat stress, a decline in chlorophyll fluorescence was observed (Sharma *et al.*, 2012). Non-photochemical quenching under stress condition lead to decrease in Fm and the following increase in Fo, due to the photo-inactivation of PS II is the main reason for the decline of Fv/Fm (Baker, 2008). It is observed that in tomato, Fv/Fm under control condition is higher than Fv/Fm under stress condition (Zhou *et al.*, 2015).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The objective of the thesis entitled "Developing high temperature tolerance in tomato (*Solanum lycopersicum* L.) through selective fertilization technique" was to identify the critical temperature for pollen germination and to evaluate the selectively fertilized tomato hybrids for high temperature tolerance. The research work was conducted at Department of plant physiology, College of agriculture, Vellayani during the year from 2017-2019. Physiological and biochemical analysis were done at monthly intervals after transplantation of tomato crop.

3.1. EXPERIMENT 1: EVALUATION OF CRITICAL TEMPERATURE FOR POLLEN GERMINATION

3.1.1. Location

The experiment was conducted in the Department of Plant Physiology, College of Agriculture, Vellayani. The geographical co-ordinates of the location of Vellayani are 8^0 5'N Latitude and 76^0 9'E Longitude with an altitude of 29 M above Mean Sea Level.

3.1.2 Experimental Material

Five popular varieties of tomato *i.e.* Anagha, Manuprabha, Vellayani Vijay, Akshaya and Manulekshmi were selected for pollen germination study.

3.1.3 Experimental Details

3.1.3.1 Standardization of Critical Temperature

To standardize the critical temperature, mature pollen grains were incubated for 2 hrs at different temperatures in a pollen germination media proposed by Ravikumar *et al.* (2010). Critical temperature is assessed as the temperature where only 20-30% of the pollens germinate.

Table 1: Temperatures used to standardize the critical temperature

Sl. No.	Treatments	Temperature	Duration
1	T_1	34^0C	2hrs
2	T_2	36^0C	2hrs
3	T_3	38^0C	2hrs
4	T_4	40^0C	2hrs
5	T_5	42^0C	2hrs
6	T_6	44^0C	2hrs

3.1.4 Parameters Observed

3.1.4.1 Pollen Germination Percentage

Flowers having mature pollen grains were collected from the selected genotypes in the morning hours *i.e.* from 8.00 to 10.00 am. Collected pollens were incubated in the BOD incubator (Rotary shaker cum BOD, Rotek, ROSI-1) maintained by Department of Agricultural Entomology, at different temperatures for two hours in the standardized pollen germination medium in a petri plate. After incubation the pollen germination was assessed using compound microscope (Leica DC 7.5 V (10X)).

3.1.4.2 Pollen Viability

Acetocarmine dye method proposed by Shivanna and Rangaswamy (1992) was done to determine the pollen viability of tomato flowers and is expressed in percentage.

3.2 EXPERIMENT 2: EVALUATION OF THE SELECTIVELY FERTILIZED CROSSES

3.2.1 Location

The pot culture experiment was carried out in three locations

(1) Rainout shelter located in College of Agriculture, Vellayani, situated at 18^0 30' N latitude and 76^0 9' E longitudes at an altitude of 29 m above mean sea level

(2) Open Top Chamber located at College of Agriculture Vellayani, situated at 8^0 5'N latitude and 76^0 9' E longitude and an altitude of 29 m above mean sea level.

(3) Open field located at College of Agriculture Vellayani

3.2.2 Planting Material

The following crosses of tolerant and susceptible tomato varieties were made using selected pollens exposed to critical temperature, Susceptible X Tolerant, Susceptible X Tolerant (SF), Tolerant X Tolerant, Tolerant X Tolerant (SF), Susceptible X Susceptible and Susceptible X Susceptible (SF) and used for the study. The hybridisation was done in the Department of Plant Physiology, College of Agriculture, Vellayani.

3.2.2.1 Hybridisation Technique

The variety which was having high pollen germination percentage in the first experiment was selected as the tolerant one (male parent) and the variety having low pollen germination was selected as susceptible one (female parent). In selective fertilization (SF), the pollen from the male parent was collected and exposed to the specific critical temperature. After two hours of incubation, the pollens were used to pollinate the emasculated flowers of the female parent. Another set of cross was also made with the same parental combination without pollen selection. The selfing

within the male and female parents was also done with and without pollen selection for comparing the performance of selectively fertilized plants.

3.2.3 Layout of the Experiment and Design

The experiment was laid out in CRD with eighteen treatments and four replications. The six crosses were kept in three conditions *i.e.* in OTC, Open field and Rainout shelter.

3.2.4 Preparation and Planting

The selected tolerant *i.e.* Anagha (male parent) and susceptible one ie. Manuprabha (female parent) seeds were sown and maintained at ambient condition. At flowering stage the pollen from the male parent was collected and exposed to the critical temperature and after two hours of incubation, the pollens were used to pollinate the emasculated flowers of female parent and another set of cross was also made with same parental combination without pollen selection. The selfing within the male and female parents (with and without pollen selection) was also done. Then the seeds from the selectively fertilized and normal fruits were collected at maturing stage. The seeds were sown and the seedlings were raised at ambient temperature up to 30 days. They were transplanted to pots at 30 days after sowing and kept in different temperature conditions for evaluation

The potting mixture made up of FYM, sand and soil in 1:1:1 ratio was used for pot filling for the experiment. Nutrient application and pest control measures were given as per package of practices recommended by Kerala Agricultural University. The potted plants were kept in OTC, open field and Rainout shelter for a period of 3 months.

(a)

(b)

(c)

Plate 1. Plants at Open Top Chamber (a), ROS (b) and open field (c)

Table 2: Particulars of Experiment II

Crop	Tomato
Varietal crosses	V1- Tolerant X Susceptible V2- Tolerant X Susceptible (SF) V3- Tolerant X Tolerant V4- Tolerant X Tolerant (SF) V5- Susceptible X Susceptible V6- Susceptible X Susceptible (SF)
Conditions	Ambient temp. (control) Ambient temp.+3 ^{0}C (Rainout shelter- ROS) Ambient temp.+ 6 ^{0}C (OTC)
Treatments	T1: V1 X ambient temp. (control) T2: V1 X ambient temp. +3^{0}C (ROS) T3: V1 X ambient temp. + 6^{0}C (OTC) T4: V2 X ambient temp. (control) T5: V2 X ambient temp. +3^{0}C (ROS) T6: V2 X ambient temp. + 6^{0}C (OTC) T7: V3 X ambient temp. (control) T8: V3 X ambient temp. +3^{0}C (ROS) T9: V3 X ambient temp. + 6^{0}C (OTC) T10: V4 X ambient temp. (control) T11: V4 X ambient temp. +3^{0}C (ROS) T12: V4 X ambient temp. + 6^{0}C (OTC) T13: V5 X ambient temp. (control) T14: V5 X ambient temp. +3^{0}C (ROS) T15: V5 X ambient temp. + 6^{0}C (OTC) T16: V6 X ambient temp. (control) T17: V6 X ambient temp. +3^{0}C(ROS) T18: V6 X ambient temp. + 6^{0}C (OTC)

3.2.5 Observations

3.2.5.1 Biometrical Parameters

3.2.5.1.1 Plant height (cm)

Length from the ground level to the top most leaf bud of the plant was taken as plant height at 30 and 60 days after transplanting.

3.2.5.1.2 Fruit weight (g)

Fruit weight was taken in an electronic balance and recorded in grams at 60 days after transplanting.

3.2.5.1.3 No. of clusters

The number of clusters present in the plant was recorded at 30 and 60 days after transplanting.

3.2.5.1.4 No. of flower clusters per plant

The number of flower clusters present in the plant was counted and recorded at 30 and 60 days after transplanting.

3.2.5.1.5 No. of flowers per cluster

The number of flowers present per cluster was counted and recorded at 30 and 60 days after transplanting.

3.2.5.1.6 No. of fruits per cluster

The number of fruits per each cluster was recorded at 60 days after transplanting.

3.2.5.1.7 No. of flower with exerted stigma per cluster

The number of flowers having exerted stigma from the anther cone in each cluster was counted and recorded at 30 and 60 days after transplanting.

3.2.5.1.8 Total yield per plant

The weight of all the fruits collected per plant was taken and the total yield was calculated at 60 days after transplanting.

3.2.5.2 Physiological Parameters

3.2.5.2.1 Photosynthetic rate

Photosynthetic rate was measured using portable photosynthetic system (CIRAS-3 SW, PP System International, MA, USA) during day time between 9.00-10.30 am from the third leaves and were expressed in µmole CO_2 m^{-2} s^{-1}.

3.2.5.2.2 Fruit setting %

After transplanting the number of fruits present per cluster were recorded at 60 days after transplanting and percentage fruit set was calculated from the data on number of flowers per cluster.

$$\text{Fruit setting \%} = \frac{\text{Number of fruits per cluster}}{\text{Number of flowers per cluster}}$$

3.2.5.2.3 Stigma exertion

The stigma exertion of the tomato flower was taken by measuring the length of stigma exerted from anther cone using a scale. It was expressed in mm.

3.2.5.2.4 Estimation of carbohydrate content

The carbohydrate content in plants was estimated by Phenol-sulphuric acid method.

Plant sample of weight 0.1 g was homogenized in 80% ethanol to remove the sugars. The homogenate was centrifuged and 0.1 ml of supernatant was taken in a test tubes and made up the volume to 1 ml with water. 1 ml of phenol solution and 5 ml of 96% sulphuric acid was also added to the test tube and shaken well.

After 10 minutes the test tubes were kept in water bath at 25-30^0 C for 20 min and read the colour at 490 nm in spectrophotometer.

10 ml of stock (100 mg standard glucose in 100 ml water) diluted to 100 ml with distilled water was taken as the working standard. 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard diluted to 1 ml with water was used as standard.

3.2.5.2.5 Estimation of chlorophyll (DMSO method)

A known quantity of leaf sample was taken (0.5 g) and cut into small pieces. The samples were put in a test tube having 10 ml of DMSO:80% acetone mixture in a 1:1 v/v ratio. Incubated it overnight at room temperature. The extracted liquid was transferred to another tube and made up the volume to 10 ml using DMSO:80% acetone mixture. The absorbance was measured at 645 and 663 nm in spectrophotometer by using DMSO:80% acetone mixture as blank. The chlorophyll content in the plant sample was calculated by substituting the absorbance value in the given formula

Chlorophyll a = $(12.7 \times A_{663} - 2.69 \times A_{645}) \times V/1000 \times 1/$ Fresh weight

Chlorophyll b = $(22.9 \times A_{645} - 4.68 \times A_{663}) \times V/1000 \times 1/$ Fresh weight

Total chlorophyll (a+b) = $(8.02 \times A_{663} + 20.2 \times A_{645}) \times V/1000 \times 1/$ Fresh weight

3.2.5.2.6 Estimation of total soluble protein

The total soluble protein in the leaf sample was quantified by the Bradford method (1976).

In Bradford method the standard protein was extracted using phosphate buffered saline (PBS) buffer. Dye was prepared by adding 100 milligram of *Coomassin Brilliant Blue G*250 in 50 ml of 95% ethanol and 100 ml of 85 % (w/v) phosphoric acid and making up to 200 ml by adding water.

1 g of leaf sample was taken and homogenized with 10 ml of PBS buffer and centrifuged at 5000 rpm for 10 minutes. 1ml of the supernatant was taken in a

test tube and added 5 ml of diluted dye (1:4 ratio). The solution was mixed thoroughly by inversion and kept for developing blue colour for about 5 to 30 minutes and absorbance was read at 595 nm in spectrophotometer.

3.2.5.2.7 Estimation of relative water content

The relative water content of the plant sample was taken by taking the fresh weight, turgid weight and dry weight of the sample. 1g of the plant leaf sample was taken and cut into small pieces and taken the fresh weight. Then it was kept immersed in water for 3 hrs to obtain the turgid weight by blotting the leaf. After the estimate of turgid weight, the leaf samples were kept in hot air oven for 3 days at 80^0 C to obtain the dry weight. The relative water content was obtained by substituting the three values in the given formula

$$\text{RWC} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \text{ x } 100$$

3.2.5.2.8 Membrane integrity

About 10 number of leaf discs of the plant sample were immersed in 10 ml of water taken in a 50 ml beaker. The initial EC was measured to sense the small degree of leakage by the discs due to the punching treatment by the conductivity electrode (EC_a). EC_b *i.e.* the leakage of solute in the bathing medium was measured after 30 minutes of incubation. The beakers were then boiled for 10 minutes at 100^0C and the EC was measured (EC_c). The membrane integrity of the plant leaf sample was calculated by substituting the three EC values in the given formula and the membrane integrity was measured in terms of percentage leakage.

$$\% \text{ leakage} = \frac{ECb - ECa}{ECc} x\ 100$$

3.2.5.2.9 Estimation of Vitamin C content

The ascorbic acid content in the plant was estimated volumetrically by Harris and Ray (1935) method

The end point was considered as the appearance of pink colour by titrating mixture of 5ml of working standard solution and 10 ml of 4% oxalic acid against dye (V1ml). The dye consumed for obtaining the end point is equivalent to the amount of ascorbic acid. Sample (0.5 to 5 g) was taken and extracted with 4% oxalic acid. The extract was made up to 100 ml and centrifuged. 5ml of supernatant was pipetted out from that and added 10 ml of 4% oxalic acid. This solution was titrated against dye (V2ml) until the appearance of pink colour. The dye used was Dichlorophenol 2 indophenol (DCPIP) solution.

$$\text{Amount of ascorbic acid (mgper100g sample)} = \frac{0.5 \text{ mg}}{V1} \times \frac{V2}{15 \text{ ml}} \times \frac{100 \text{ ml}}{\text{Wt of the sample}} \times 100$$

3.2.5.2.10 Estimation of Lycopene

The lycopene content in the fruit was quantified by the method explained by Ranganna (1976).

A 5 to 10 g of the tomato fruit pulp sample was prepared by pulping 3-4 tomato fruits in a waring blender. The sample was extracted repeatedly with acetone using pestle and mortar until the residue is colourless. The acetone extracts pooled and transferred to a separating funnel containing about 20ml petroleum ether and mixed gently. 20ml of 5% sodium sulphate solution was added to the mixture in the separating funnel and shake gently. 20ml more of petroleum ether was added to the separating funnel for clear separation of two layers. Most of the colour was noticed in the upper petroleum ether layer. Two phases were separated and repeat the extraction of the lower aqueous phase again with petroleum ether, until it become colourless. Transferred the washed petroleum ether extract to an amber coloured bottle and added about 10g anhydrous sodium sulphate in it and kept it aside for

30min or longer. Decanted the petroleum ether extract through a funnel containing cotton wool into a 100ml volumetric flask. The washings of the sodium sulphate slurry with petroleum ether was transferred to the volumetric flask and the process continued until it become colourless. The volume was made up and absorbance at 503nm was noted in a spectrophotometer using petroleum ether as blank.

In spectrophotometer at 503 nm 1cm light path gives an absorbance of 17.2 x 10^4. Therefore, a concentration of 3.12 µg lycopene/ml gives unit absorbance.

Absorbance (1 unit) = 3.1206 µg lycopene/ml

$$\text{mg lycopene in 100g sample} = \frac{31.206 \times \text{Absorbance}}{\text{Wt of sample (g)}}$$

3.2.5.2.11 Estimation of Superoxide dismutase (SOD)

To estimate the SOD activity in plants the method described by Kakkar *et al.* (1984) was used. 0.5 g of sample was pulverized and homogenized with 3 ml of potassium phosphate buffer and centrifuged for about 10 minutes at 4500 rpm. Prepared a 3ml reaction mixture containing 50 mM potassium phosphate buffer, 13 mM methionine, 0.1 mM EDTA, 2 µM riboflavin, 75 µM NBT and 50 µl of crude enzyme extract and the total volume was made up with distilled water. The tubes were exposed to 400 W bulb for about 10 minutes, The absorbance was taken in spectrophotometer at 560 nm after 10 minutes.

3.2.5.2.12 Chlorophyll fluorescence

Chlorophyll fluorescence was measured using portable photosynthetic system (CIRAS -3 SW, PP System International, MA, USA). The leaves were covered with black cloth or aluminium foil about 3 hours before the observation taken to provide the dark adaption. The chlorophyll fluorescence was taken 3 hours after covering by clamping the leaf in the space provided in photosynthetic system.

RESULTS

4. RESULTS

The present study "Developing high temperature tolerance in tomato (*Solanum lycopersicum* L.) through selective fertilization technique" was done in two experiments in the Department of Plant Physiology, College of Agriculture, Vellayani. The objective of the study was to identify the critical temperature for pollen germination and to evaluate the selectively fertilized tomato hybrids for high temperature tolerance. The data obtained during the progression of study were statistically analysed and the results are presented in this chapter.

4.1 EVALUATION OF CRITICAL TEMPERATURE FOR POLLEN GERMINATION

Pollen germination % and pollen viability were recorded in five popular varieties of tomato Anagha, Manuprabha, Manulakshmi, Akshaya and Vellayani Vijay by exposing to temperature ranging from 34 – 44 ^{0}C and they showed varietal difference at each temperature.

4.1.1 Pollen Germination

Pollen germination percentage of five varieties of tomato after 2hrs incubation at different temperature treatments is depicted in Table 3. Significant varietal difference was observed among varieties at each treatment.

The pollen germination percentage decreased significantly with increase in temperature and after 36^0 C, the pollen germination percentage decreased below 20 %. So 36^0 C was identified as the critical temperature for pollen selection. At critical temperature Anagha recorded the highest pollen germination percentage (29.69 %) and Manuprabha recorded the least (11.48 %).

4.1.2 Pollen Viability

Data recorded for pollen viability of five varieties of tomato at different temperatures after 2hrs of incubation is depicted in Table 4.

Table 3: Pollen germination (%) after 2 hrs incubation at different temperatures

Treatments	Varieties					Mean
	Akshaya	Vellayani Vijay	Manuprabha	Manulakshmi	Anagha	
T1 (34^0C)	28.98	24.20	17.56	16.608	36.10	24.69
T2 (36^0C)	24.13	23.68	11.48	13.79	29.69	20.55
T3 (38^0C)	12.15	12.61	9.48	10.04	14.80	11.82
T4 (40^0C)	9.58	4.04	2.70	2.11	8.67	5.42
T5 (42^0C)	4.91	3.78	3.57	3.33	5.40	4.20
T6 (44^0C)	3.07	2.75	1.49	1.03	1.50	1.97
Mean	13.80	11.84	7.71	7.82	16.02	
	Temperature (T)	Variety (V)	TXV			
SE m±	0.91	0.83	2.03			
CD (0.05)	2.56	2.33	5.72			

Table 4 : Pollen viability (%) after 2 hrs incubation at different temperatures

Treatments	Varieties					Mean
	Akshaya	Vellayani Vijay	Manuprabha	Manulakshmi	Anagha	
T1 (34^0C)	96.92	97.25	98.51	98.97	98.50	98.03
T2 (36^0C)	95.09	96.22	96.43	96.66	94.60	95.80
T3 (38^0C)	90.41	95.96	91.33	97.88	96.97	94.51
T4 (40^0C)	91.87	93.24	84.48	77.65	93.46	88.14
T5 (42^0C)	88.71	89.95	87.39	87.85	90.52	88.88
T6 (44^0C)	85.20	87.81	81.32	85.86	89.51	85.94
Mean	91.37	93.40	89.91	90.81	93.93	
	Temperature (T)	Variety (V)	TXV			
SE m±	1.19	1.08	2.66			
CD (0.05)	3.34	3.05	NS			

Pollen viability decreased significantly with increase in temperature. The highest pollen viability was observed at 34 ^{0}C which was on par with pollen viability at 36 ^{0}C. Among the varieties Anagha recorded the highest pollen viability (93.93 %) which was on par with Akshaya and Vellayani Vijay and the lowest pollen viability was recorded in Manuprabha (89.91 %) which was on par with Manulakshmi.

4.2 EVALUATION OF SELECTIVELY FERTILIZED CROSSES

4.2.1. Biometrical Parameters

Height of the plant, fruit weight, number of clusters, number of flower clusters per plant, number of flowers per cluster, number of fruits per cluster, number of flower with exerted stigma per cluster and total yield per plant were recorded during the experiment, the result showed significant variation in selectively fertilized and normal crosses.

4.2.1.1 Height of plant

Effect of selective fertilization and different temperature condition on plant height is depicted in Table 5. The height increased with increase in temperature.

During first month after transplanting the height was recorded more in SXS in all the three temperatures and the highest was observed in T15 (65.20 cm) which is on par with selectively fertilized hybrid, T6 (63.70 cm). The lowest mean value was observed in T10 (32.62cm) which is on par with T11 (33.87cm) and T7 (34.42 cm).

During second month after transplanting, significantly higher value for plant height was observed in T15 (127.32 cm) and the lowest mean value was observed in T10 (71.9 cm). Among the crosses SXS showed the highest mean value (108.83 cm).

Table 5: Plant height of selectively fertilized tomato plants

Varietal crosses	Plant height (cm)							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	48.20	48.47	58.05	51.57	91.32	94.95	116.17	100.81
T X S (SF)	48.92	52.65	63.70	55.09	95.15	97.95	118.22	103.77
T X T	34.42	39.07	46.17	39.89	77.32	80.37	107.97	88.55
T X T (SF)	32.62	33.87	43.72	36.74	71.90	77.22	96.10	81.74
S X S	52.27	54.50	65.20	57.32	97.42	101.75	127.32	108.83
S X S (SF)	49.95	50.60	61.05	53.86	94.87	99.52	117.15	103.85
Mean	44.40	46.52	56.31		88.00	91.96	113.82	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.80	0.57	1.4		1.04	0.73	1.8	
CD (0.05)	2.29	1.62	4.00		2.96	2.09	4.83	

4.2.1.2 Fruit weight

Effect of selective fertilization and different temperature condition on fruit weight is depicted in Table 6.

The fruit weight was taken second month after transplanting. The fruit weight increased with increase in temperature and the maximum fruit weight was recorded in ROS (27.45 g). At OTC there was no fruit set due to high temperature. Among treatments, T17 (37.77 g) recorded the highest fruit weight followed by T5, T14 and T16 which were significantly differed from all other treatments and the lowest was recorded in T7 which was on par with T8. SXS (SF) showed highest fruit weight (24.11 g) among varietal crosses which was on par with SXS.

4.2.1.3. Number of clusters per plant

Effect of selective fertilization and different temperature condition on Number of clusters per plant is depicted in Table 7. The number of clusters increased with increase in temperature.

During first month after transplanting significantly higher number of clusters was observed in OTC (4.50) which was on par with ambient condition (4.25). Among the treatments significantly higher mean value was observed in T6 (7.75) which was on par with T4 (6.25). The lowest mean value for number of clusters was observed in T17 (1.5) which was at par with T14, T10, T15, T8 and T18. And among the varietal cross, TXS (SF) showed significantly higher number of cluster per plant (6.58).

During second month after transplanting significantly higher number of clusters was observed in OTC (34.17). Among the treatments T15 (45.50) showed significantly higher mean value and the lowest mean value was observed in T14 (9) which was on par with T17. Among the varietal crosses, highest number of clusters was observed in TXS (29.33) which was at par with TXT and TXT SF.

Table 6: Fruit weight of selectively fertilized tomato plants at 60 days after transplanting

Varietal crosses	Fruit weight (g)		
	Condition		Mean
	Ambient	ROS	
T X S	20.69	22.75	14.48
T X S (SF)	25.98	34.71	20.23
T X T	14.06	14.72	9.60
T X T (SF)	19.62	20.15	13.25
S X S	32.41	34.62	22.34
S X S (SF)	34.56	37.77	24.11
Mean	24.55	27.45	
	Variety (V)	Condition (C)	V X C
SE m±	0.87	0.61	1.51
CD (0.05)	2.48	1.75	4.30

Table 7: Number of clusters of selectively fertilized tomato plants

Varietal crosses	Number of clusters							
	30 DAT				60 DAT			
	condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	3.25	5.75	5.50	4.83	21.25	27.50	39.25	29.33
T X S (SF)	6.25	5.75	7.75	6.58	21.75	22.00	21.75	21.83
T X T	4.00	2.25	3.50	3.25	20.25	23.25	36.25	26.58
T X T (SF)	2.00	3.75	5.25	3.67	18.50	25.25	37.00	26.92
S X S	5.75	2.00	2.25	3.33	17.50	9.00	45.50	24.00
S X S (SF)	4.25	1.50	2.75	2.83	16.25	9.75	25.25	17.08
Mean	4.25	3.50	4.50		19.25	19.46	34.17	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.31	0.22	0.54		1.13	0.80	1.96	
CD (0.05)	0.89	0.63	1.54		3.22	2.28	5.58	

4.2.1.4 Number of flower clusters per plant

Effect of selective fertilization and different temperature condition on number of flower clusters per plant is depicted in Table 8. The number of flower clusters per plant was increased with increase in temperature.

During first month after transplanting significantly higher number of flower clusters was observed in OTC (4.50) which was on par with ambient condition (4.25). Among the treatments significantly higher flower clusters was observed in T6 (7.75) which was on par with T4 (6.25). The lowest number of flower clusters was observed in T17 (1.5) which was at par with T14 (2), T10 (2), T15 (2.25), T 8 (2.25) and T 18 (2.75). And among the varietal cross, TXS (SF) showed significantly higher number (6.58).

During second month after transplanting among the conditions significantly higher mean value for number of flower clusters was observed in OTC (34.17). Among the treatments T15 (45.50) showed significantly higher mean value and the lowest mean value was observed in T14 (9) which was on par with T17 (9.75). Among the varietal crosses, highest number of flower clusters was observed in TXS (29.33) which was at par with TXT and TXT SF.

4.2.1.5 Number of flowers per cluster

Effect of selective fertilization and different temperature condition on number of flowers per cluster is depicted in Table 9. The number of flower per cluster was increased with increase in temperature.

During first month after transplanting, among the conditions significantly higher no of flowers per cluster was observed in ROS (4.29) as compared to ambient and OTC. No significant difference was observed among the treatments. And among the varietal crosses, highest number of flowers was observed in TXT (SF) (4.92) which was on par with TXS (SF).

During second month after transplanting, significantly higher no of flowers per cluster was observed in OTC (6.54). Among the treatments significantly

Table 8: Number of flower clusters per plant of selectively fertilized tomato plants

Varietal crosses	Number of flower clusters							
	30 DAT				60 DAT			
	condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	3.25	5.75	5.50	4.83	21.25	27.50	39.25	29.33
T X S (SF)	6.25	5.75	7.75	6.58	21.75	22.00	21.75	21.83
T X T	4.00	2.25	3.50	3.25	20.25	23.25	36.25	26.58
T X T (SF)	2.00	3.75	5.25	3.67	18.50	25.25	37.00	26.92
S X S	5.75	2.00	2.25	3.33	17.50	9.00	45.50	24.00
S X S (SF)	4.25	1.50	2.75	2.83	16.25	9.75	25.25	17.08
Mean	4.25	3.50	4.50		19.25	19.46	34.17	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.31	0.22	0.54		1.13	0.80	1.96	
CD (0.05)	0.89	0.63	1.54		3.22	2.28	5.58	

Table 9: Number of flowers per cluster of selectively fertilized tomato plants

Varietal crosses	Number of flowers per cluster							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	4.00	4.25	3.25	3.83	3.25	4.00	6.00	4.42
T X S (SF)	4.25	4.00	4.50	4.25	4.25	5.50	6.50	5.42
T X T	4.00	5.00	3.50	4.17	6.00	5.50	7.25	6.25
T X T (SF)	4.25	4.75	5.75	4.92	7.00	6.25	11.00	8.08
S X S	2.25	3.75	2.50	2.83	4.00	3.25	4.00	3.75
S X S (SF)	3.00	4.00	3.00	3.33	2.75	4.00	4.50	3.75
Mean	3.63	4.29	3.75		4.54	4.75	6.54	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.25	0.17	0.43		0.38	0.27	0.66	
CD (0.05)	0.71	0.50	NS		1.08	0.76	1.8	

higher mean value was observed in T12 (11.00) and the lowest was observed in T16 (2.75) which was at par with T1, T2, T4, T13, T14, T15, T17 and T18. Among the varietal crosses, highest number of flowers was observed in TXT (SF) (8.08).

4.2.1.6 Number of fruits per cluster

Effect of selective fertilization and different temperature condition on number of fruits per cluster is depicted in Table 10.

The number of fruits per cluster was noted in second month after transplanting. The number of fruits per cluster was decreased with increase in temperature and among the conditions the highest number of fruits per cluster was recorded in ambient condition (3.71) and was on par with ROS. At OTC there was no fruit set. In treatments, significantly higher number of fruits per cluster was observed in T10 (6.00) which was at par with T7 and T11. The lowest number of fruits recorded in T14 followed by T1, T2, T13, T16 and T17. TXT (SF) (5.50) recorded the highest number of fruits per cluster among crosses which was on par with TXT.

4.2.1.7 Number of flowers with exerted stigma

Effect of selective fertilization and different temperature condition on percentage number of flowers with exerted stigma is depicted in Table 11. The number of flowers with exerted stigma was increased with increase in temperature.

During first month after transplanting significantly higher mean value for percentage number of flowers with exerted stigma was observed in OTC (68.94 %). Among the treatments significantly higher mean value was observed in T15 (91.66 %) which was at par with T3, T9 and T18. The lowest percentage of flowers with exerted stigma was observed in T10 (11.25 %) which was at par with T1, T4, T5, T7, T8, T10, T11 and T16. And among the varietal cross, TXT (SF) showed significantly lowest percentage (31.18).

During second month after transplanting among the conditions significantly higher mean value for percentage number of flowers with exerted stigma was

Table 10: Number of fruits per cluster in selectively fertilized tomato plants at 60 days after transplanting

Varietal crosses	Number of fruits per cluster		
	Condition		Mean
	Ambient	ROS	
T X S	2.75	2.50	2.63
T X S (SF)	3.75	4.75	4.25
T X T	5.25	4.25	4.75
T X T (SF)	6.00	5.00	5.50
S X S	2.50	1.75	2.13
S X S (SF)	2.00	2.75	2.38
Mean	3.71	3.50	
	Variety (V)	Condition (C)	V X C
SE m±	0.28	0.16	0.40
CD (0.05)	0.78	0.45	1.11

Table 11: Percentage of flowers with exerted stigma in selectively fertilized tomato plants

Varietal crosses	% of flowers with exerted stigma							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	19.58	41.25	68.75	43.19	33.33	51.66	90.83	58.61
T X S (SF)	17.50	40.41	45.00	34.30	17.50	36.66	70.83	41.66
T X T	28.75	20.41	62.91	37.36	13.57	35.41	78.82	42.60
T X T (SF)	11.25	22.50	59.79	31.18	11.87	32.14	75.32	39.78
S X S	58.33	65.41	91.66	71.80	30.83	39.58	87.50	52.63
S X S (SF)	37.50	49.58	85.41	57.50	24.99	43.75	77.50	48.74
Mean	28.82	39.93	68.92		22.01	39.87	80.13	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	6.24	4.41	10.81		4.05	2.86	7.02	
CD (0.05)	17.75	12.55	29.94		11.52	8.14	19.55	

Table 12: Total yield per plant in selectively fertilized tomato plants at 60 days after transplanting

Varietal crosses	Total yield (g)		
	Condition		Mean
	Ambient	ROS	
T X S	346.25	330.28	338.27
T X S (SF)	396.19	416.55	406.37
T X T	405.96	308.51	357.23
T X T (SF)	389.52	350.06	369.79
S X S	284.95	329.93	307.44
S X S (SF)	343.06	340.12	341.59
Mean	360.99	345.91	
	Variety (V)	Condition (C)	V X C
SE m±	11.78	6.80	16.67
CD (0.05)	33.94	20.3	48.01

observed in OTC (79.09 %). Among the treatments significantly higher mean value was observed in T15 (87.50 %) which was at par with T3, T6, T9, T12 and T18. The lowest mean value for percentage number of flowers with exerted stigma was observed in T10 (11.88 %) which was at par with T4, T7, T10, T13 and T16. And among the varietal cross, TXT (SF) showed significantly lowest percentage (39.78) which was at par with TXS (SF), TXT and SXS (SF).

4.2.1.8 Total yield

Effect of selective fertilization and different temperature condition on total yield is depicted in Table 12.

The total yield was noted second month after transplanting. The total yield was decreased with rise in temperature and among the condition the highest yield was recorded in ambient (360.99 g) and was on par with ROS also. At OTC there was no fruit set. In treatments, T5 (416.55 g) recorded the highest yield which was at par with T4 and T7. The lowest yield was observed in T13 followed by T2, T8 and T14. TXS (SF) given the highest yield (406.37 g) among crosses.

4.2.2. Physiological Parameters

Photosynthetic rate, fruit setting %, stigma exertion, carbohydrate content, chlorophyll content, total soluble protein, relative water content, membrane integrity, vitamin C content, lycopene content, SOD activity and chlorophyll fluorescence were recorded, they showed significant variation among selectively fertilized and normal crosses.

4.2.2.1 Photosynthetic rate (µmole CO_2 m^{-2} s^{-1})

Effect of selective fertilization and different temperature condition on photosynthetic rate of tomato is depicted in Table 13. The photosynthetic rate was decreased with increase in temperature.

During first month after transplanting, among the conditions significantly higher photosynthetic rate was observed in OTC (21.17 µmole CO_2 m^{-2} s^{-1}). Among

Table 13: Photosynthetic rate of selectively fertilized tomato plants

Varietal crosses	Photosynthetic rate (µmole CO_2 m^{-2} s^{-1})							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S (SF)	19.10	20.95	20.15	20.06	16.95	14.80	9.60	13.78
T X T	18.45	23.00	21.25	20.90	17.40	14.67	8.97	13.68
T X T (SF)	19.80	20.60	22.22	20.87	17.00	15.60	9.80	14.13
S X S	14.20	16.42	23.65	18.09	10.80	9.95	7.85	9.53
S X S (SF)	16.25	17.40	19.80	17.81	12.37	12.52	8.50	11.13
Mean	17.70	19.70	21.17		15.10	13.62	8.96	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.90	0.64	1.57		.45	0.32	0.78	
CD (0.05)	2.56	1.82	4.44		1.29	0.91	2.24	

the treatments significantly higher mean value was marked in T15 (23.65 µmole CO_2 m^{-2} s^{-1}) which was at par with T3, T4, T6, T7, T9, T10, T12, T15 and T18. The lowest mean value was observed in T13 (14.20 µmole CO_2 m^{-2} s^{-1}) which was at par with T1, T7, T14, T16 and T17. And among the varietal crosses, highest photosynthetic rate was observed in TXT (20.90 µmole CO_2 m^{-2} s^{-1}) which was at par with TXS (SF), TXS and TXT (SF).

During second month after transplanting, among the conditions significantly higher photosynthetic rate was observed in ambient condition (15.10 µmole CO_2 m^{-2} s^{-1}). Among the treatments significantly highest photosynthetic rate was observed in T7 (17.40 µmole CO_2 m^{-2} s^{-1}) which was at par with T1, T4, T10 and T11. The lowest mean value was observed in T15 (7.85 µmole CO_2 m^{-2} s^{-1}) which was at par with T3, T6, T9, T12, T14 and T18. And among the varietal crosses, highest photosynthetic rate was observed in TXT (SF) (14.13 µmole CO_2 m^{-2} s^{-1}) which was at par with TXS (SF), TXS and TXT.

4.2.2.2 Fruit setting percentage

Effect of selective fertilization and different temperature condition on fruit setting percentage is depicted in Table 14.

The fruit setting percentage was observed second month after transplanting. The fruit setting percentage was decreased with rise in temperature and ambient condition showed significantly higher fruit setting percentage (82.42 %). At OTC there was no fruit set. Among treatments the highest fruit setting percentage was exhibited in T7 (89.29 %) and which was at par with T1, T4, T5, T8, T10, T11, T13, T16 and T17. The lowest setting % was recorded in T14 (56.25 %). Among varies crosses, fruit setting percentage was noted more in selectively fertilized crosses than normal crosses, TXS (SF) recorded the highest (87.71%) which was at par with all other crosses except SXS.

Table 14: Fruit setting % of selectively fertilized tomato plants at 60 days after transplanting

Varietal crosses	Fruit setting %		
	Condition		Mean
	Ambient	ROS	
T X S	87.50	64.17	75.83
T X S (SF)	88.75	86.67	87.71
T X T	89.29	77.08	83.18
T X T (SF)	87.71	79.76	83.73
S X S	66.25	56.25	61.25
S X S (SF)	75.00	68.75	71.88
Mean	82.42	72.11	
	Variety (V)	Condition (C)	V X C
SE m±	6.01	3.47	8.50
CD (0.05)	17.32	10.00	24.29

4.2.2.3. Stigma exertion

Effect of selective fertilization and different temperature condition on length of exerted stigma is depicted in Table 15. The number of flowers with exerted stigma was increased with increase in temperature.

During first month after transplanting among the conditions significantly longer exerted stigma was observed in OTC (1.23 mm). Among the treatments significantly higher mean value was observed in T15 (1.88 mm) which was at par with T14 and T18. The lowest mean value for the exerted stigma length was observed in T4, T7 and T10 (0.06 mm). And among the varietal cross, TXS (SF) showed significantly lowest value (0.40 mm) which was at par with TXS, TXT, TXT (SF) and SXS (SF).

During second month after transplanting among the conditions significantly longer exerted stigma was observed in OTC (1.73 mm). Among the treatments significantly highest mean value was observed in T15 (2.50 mm). The lowest mean value for the exerted stigma length was observed in T7 and T10 (0.13 mm). And among the varietal cross, TXS (SF) showed significantly lowest value (0.52 mm) which was at par with TXS, TXT (SF) and TXT.

4.2.2.4 Carbohydrate content

Effect of selective fertilization and different temperature condition on carbohydrate content is depicted in Table 16. The carbohydrate content was decreased with increase in temperature.

During first month after transplanting, among the conditions significantly higher carbohydrate content was observed in ROS (69.86 mg g^{-1}). No significant difference was observed among the treatments. Among the varietal crosses, highest carbohydrate content was observed in TXT (SF) (71.79 mg g^{-1}) which was at par with TXS (SF) and TXT.

During second month after transplanting, among the conditions significantly higher carbohydrate content was observed in ROS (59.55 mg g^{-1}).

Table 15: Stigma exertion in selectively fertilized tomato plants

Varietal crosses	Stigma exertion (mm)							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	0.13	0.25	1.13	0.50	0.25	0.38	1.50	0.71
T X S (SF)	0.06	0.13	1.00	0.40	0.19	0.25	1.13	0.52
T X T	0.06	0.19	1.13	0.46	0.13	0.25	1.75	0.71
T X T (SF)	0.06	0.44	0.75	0.42	0.13	0.56	1.63	0.77
S X S	0.44	1.38	1.88	1.23	0.69	1.63	2.50	1.60
S X S (SF)	0.25	0.56	1.50	0.77	0.31	0.56	1.88	0.92
Mean	0.13	0.25	1.13	0.50	0.25	0.38	1.50	0.71
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.16	0.11	0.27		0.11	0.07	0.19	
CD (0.05)	0.45	0.32	0.78		0.31	0.22	0.55	

Table 16: Carbohydrate content in leaves of selectively fertilized tomato plants

Varietal crosses	Carbohydrate (mg g^{-1})							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	56.85	59.25	45.87	53.99	34.17	50.20	41.40	41.92
T X S (SF)	67.87	80.70	54.65	67.74	46.50	74.12	57.87	59.50
T X T	58.75	77.32	53.30	63.12	49.70	58.97	41.00	49.89
T X T (SF)	67.92	90.45	57.00	71.79	46.60	79.62	46.12	57.45
S X S	68.57	49.90	54.15	57.54	44.52	39.60	48.95	44.35
S X S (SF)	54.40	61.55	58.97	58.30	48.40	54.77	46.42	49.86
Mean	62.39	69.86	53.99		44.98	59.55	46.96	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	3.94	2.78	6.82		1.77	1.25	3.07	
CD (0.05)	11.20	7.92	NS		5.03	3.56	8.72	

Among the treatments significantly highest carbohydrate content was observed in T11 (79.62 mg g^{-1}) which was on par with T5. The lowest mean value was observed in T1 (34.17 mg g^{-1}) which was at par with T3, T9, and T14. And among the varietal crosses, highest carbohydrate content was observed in TXS (SF) (59.50 mg g^{-1}) which was on par with TXT (SF).

4.2.2.5 Chlorophyll content

Effect of selective fertilization and different temperature condition on chlorophyll content is depicted in Table 17. The chlorophyll content was decreased with increase in temperature.

During first month after transplanting, significantly higher chlorophyll content was observed in ambient condition (2.07 mg g^{-1}). Among the treatments significantly highest chlorophyll content was observed in T10 (3 mg g^{-1}). The lowest mean value was observed in T15 (0.77 mg g^{-1}) which was at par with T2, T3, T6, T9, T14, T16, T17 and T18. And among the varietal crosses, highest chlorophyll content was observed in TXT (SF) (2.17 mg g^{-1}) which was on par with TXT.

During second month after transplanting, significantly higher chlorophyll content was observed in ambient condition (1.61 mg g^{-1}). Among the treatments significantly highest chlorophyll content was observed in T7 (1.91 mg g^{-1}) which was at par with T1, T4, T8, T10 and T11. The lowest mean value was observed in T15 (0.67 mg g^{-1}) which was at par with T14, T15 and T18. And among the varietal crosses, highest chlorophyll content was observed in TXT (SF) (1.60 mg g^{-1}) which was on par with TXT.

4.2.2.6 Total soluble protein

Effect of selective fertilization and different temperature condition on total soluble protein is depicted in Table 18.

During first month after transplanting, among the conditions significantly higher accumulation of total soluble protein was observed in OTC (1.51 mg g^{-1}).

Table 17: Total chlorophyll content in leaves of selectively fertilized tomato plants

Varietal crosses	Chlorophyll content (mg g^{-1})							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	1.78	1.20	1.09	1.36	1.63	1.14	1.03	1.26
T X S (SF)	2.30	1.45	1.12	1.62	1.60	1.37	1.04	1.33
T X T	2.60	1.83	1.23	1.89	1.91	1.67	1.11	1.56
T X T (SF)	3.00	2.08	1.44	2.17	1.87	1.58	1.35	1.60
S X S	1.45	1.03	0.77	1.08	1.53	1.00	0.67	1.07
S X S (SF)	1.28	1.14	0.90	1.10	1.10	1.06	0.89	1.01
Mean	2.07	1.46	1.09		1.61	1.30	1.01	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.14	0.09	0.24		0.07	0.05	0.13	
CD (0.05)	0.39	0.28	0.62		0.21	0.15	0.35	

Table 18: Total soluble protein content in leaves of selectively fertilized tomato plants

Varietal crosses	Total soluble protein (mg g^{-1})							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	1.43	1.38	1.54	1.45	2.38	2.29	2.15	2.27
T X S (SF)	1.47	1.44	1.65	1.52	2.41	2.08	2.19	2.23
T X T	1.34	1.45	1.22	1.33	2.42	2.18	2.24	2.28
T X T (SF)	1.37	1.48	1.52	1.45	2.48	2.24	2.33	2.35
S X S	1.34	1.21	1.74	1.43	2.23	2.17	2.08	2.16
S X S (SF)	1.17	1.38	1.42	1.32	2.40	2.20	2.53	2.37
Mean	1.35	1.39	1.51		2.39	2.19	2.25	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.04	0.03	0.07		0.04	0.03	0.07	
CD (0.05)	0.12	0.08	0.21		0.12	0.08	NS	

Among the treatments significantly highest total soluble protein was observed in T6 (1.65 mg g^{-1}) which was at par with T3, T4, T5, T8, T11, T12 and T15. The lowest protein content was observed in T16 (1.17 mg g^{-1}) which was at par with T7, T9, T10, T13 and T14. And among the varietal crosses, highest total soluble protein was observed in TXS (SF) (1.52 mg g^{-1}) which was at par with TXT (SF), TXS and SXS.

During second month after transplanting, among the conditions significantly higher quantity of total soluble protein was observed in ambient condition (2.39 mg g^{-1}). No significant difference was observed among the treatments. And among the varietal crosses, highest total soluble protein was observed in SXS (SF) (2.37 mg g^{-1}) which was at par with TXT (SF), TXS and TXT.

4.2.2.7 Relative water content

Effect of selective fertilization and different temperature condition on relative water content is depicted in Table 19.

During first month after transplanting, tomato plant maintained at ambient condition given significantly higher relative water content (88.50 %) which was on par with OTC. Among the treatments significantly highest mean value was recorded in T10 (92.01 %) which was at par with T4, T6, T7, T9 and T12. T14 (67.75 %) which was on par with T17 given the lowest relative water content. Among the varietal crosses, highest relative water content was observed in TXT (SF) (84.69 %) which was at par with TXS (SF), TXS and TXT.

During second month after transplanting also ambient condition given significantly higher relative water content (75.80 %). But among the treatments significantly highest relative water content was recorded in T4 (78.30 %) which was at par with T1, T7 and T10. The lowest relative water content was observed in T15 (60.71 %). TXS (SF) was given highest relative water content (74.24 %) among the varietal crosses.

Table 19: Relative water content in leaves of selectively fertilized tomato plants

Varietal crosses	RWC %							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	87.01	72.86	88.49	82.78	76.97	72.04	64.80	71.27
T X S (SF)	89.74	74.34	89.84	84.64	78.30	74.80	69.61	74.24
T X T	90.96	71.30	89.00	83.75	75.99	69.91	66.83	70.91
T X T (SF)	92.01	72.44	89.62	84.69	76.45	71.68	67.26	71.80
S X S	84.74	67.75	84.04	78.84	73.05	65.27	60.71	66.34
S X S (SF)	86.55	70.88	85.47	80.97	74.01	68.48	64.21	68.90
Mean	88.505	71.60	87.74		75.80	70.36	65.57	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.71	0.50	1.24		0.67	0.47	1.18	
CD (0.05)	2.04	1.44	3.46		1.92	1.35	3.22	

4.2.2.8 Membrane integrity

Membrane integrity in terms of percentage leakage due to selective fertilization and different temperature condition is depicted in Table 20.

During first month, the percentage leakage was found to be highest in OTC (13.82 %). In treatment T4 percentage leakage was found to be the lowest (4.34 %). The highest percentage leakage was recorded in T15 (16.46 %) followed by T14 and T18 which were significantly different from all other treatments. Among crosses, highest was observed in SXS (14.13 %).

During second month after transplanting, percentage leakage was found to be highest in OTC (7.57 %) itself. In treatments the lowest percentage leakage was detected in T4 (1.40 %). The highest percentage leakage was noted in T15 (10.79 %) followed by T18 which were significantly different from all other treatments. Among crosses, lowest was observed in TXT (SF) (2.41 %).

4.2.2.9 Vitamin C content

Effect of selective fertilization and different temperature condition on vitamin C content on leaves is depicted in Table 21.

During first month after transplanting, vitamin C in leaves increased with increase in temperature after that a significant reduction in ascorbic content was obtained with increase in temperature. Highest mean value (552.72 μg g^{-1}) for vitamin C content was noticed in ROS. In treatments T5 and T11 recorded similar ascorbic acid content (578.23 μg g^{-1}) and were at par with T2, T6, T8 and T17. The lowest vitamin C content was obtained in T13 (357.15 μg g^{-1}) followed by T9 and T16. Among the varietal crosses, TXT (SF) (527.21 μg g^{-1}) given the highest vitamin C content and which was on par with TXS (SF).

During second month, vitamin C in leaves increased with increase in temperature. Highest mean value (327.98 μg g^{-1}) for vitamin C content was noticed in ROS and which was on par with OTC condition. In treatments T5 recorded highest vitamin C content (464.65 μg g^{-1}) and which was on par with T18 and the

Table 20: Membrane integrity (% leakage) of selectively fertilized tomato plants

Varietal crosses	Membrane integrity (% leakage)							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	9.32	13.16	14.88	12.45	3.83	4.45	7.63	5.30
T X S (SF)	7.31	10.90	11.58	9.93	3.10	3.63	4.95	3.89
T X T	6.33	10.81	11.86	9.67	2.46	2.50	5.69	3.55
T X T (SF)	4.34	9.77	10.80	8.30	1.40	2.42	3.40	2.41
S X S	9.58	16.35	16.46	14.13	5.62	8.51	10.79	8.30
S X S (SF)	9.03	15.28	16.33	13.55	4.55	7.64	10.52	7.57
Mean	7.65	12.71	13.65		3.49	4.86	7.16	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.13	0.09	0.23		0.08	0.05	0.14	
CD (0.05)	0.38	0.27	0.66		0.23	0.16	0.39	

Table 21: Vitamin C content in leaves of selectively fertilized tomato plant.

Varietal crosses	Vitamin C (µg g^{-1})							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	425.16	510.20	561.22	498.86	300.65	318.88	282.43	300.65
T X S (SF)	357.14	425.16	578.23	453.51	282.43	464.65	337.10	361.39
T X T	442.17	391.15	544.22	459.18	291.55	337.10	282.43	303.69
T X T (SF)	493.19	391.15	510.20	464.85	264.21	364.43	191.32	273.32
S X S	408.16	561.22	578.23	515.87	245.99	264.21	391.76	300.65
S X S (SF)	425.16	493.19	578.23	498.86	236.88	218.66	437.32	297.62
Mean	425.16	462.01	558.39		270.28	327.98	320.39	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	9.81	6.94	17.00		13.93	9.85	24.13	
CD (0.05)	27.97	19.73	48.35		39.62	28.01	68.62	

lowest was recorded in T12 (191.32 μg g^{-1}) and was at par with T13, T16 and T17. Among the varietal crosses, selectively fertilized TXS (SF) (361.39 μg g^{-1}) exhibited highest vitamin C content.

4.2.2.10 Lycopene content

Effect of selective fertilization and different temperature condition on lycopene content of fruit is depicted in Table 22.

The lycopene content was noted second month after transplanting. The lycopene content was decreased with rise in temperature and ambient condition recorded the highest lycopene content in fruits (17.87 μg g^{-1}). At OTC there was no fruit set. The highest lycopene content was recorded in T10 (24.86 μg g^{-1}) and the lowest was observed in T14 (11.60 μg g^{-1}) followed by T13 and T17. TXT (SF) (15.19 μg g^{-1}) recorded the highest lycopene content among crosses.

4.2.2.11. SOD activity

Data recorded for superoxide dismutase activity of selectively fertilized and normal crosses of tomato plants under different temperature treatments is presented in Table 23.

In the first month after transplanting plant grown under ROS recorded the highest SOD activity (666.42 mg^{-1} min^{-1}). Among the treatments, T5 recorded the highest SOD activity (818.94 mg^{-1} min^{-1}) followed by T8 (725.44 mg^{-1} min^{-1}) and the minimum activity was shown by T15 (297.75 mg^{-1} min^{-1}) and which was at par with T1, T4, T3, T7, T10, T13, T16 and T18. Maximum activity was observed in normal crossed plant TXT (563.21 mg^{-1} min^{-1}) which was at par with TXS (SF) and TXT (SF).

In the second month after transplanting plant grown under ROS recorded the highest SOD activity (269.73 mg^{-1} min^{-1}). Among the treatments, T5 recorded the highest SOD activity (337.50 mg^{-1} min^{-1}) followed by T2, T8 and T11 and the minimum activity was shown by T15 (90.88 mg^{-1} min^{-1}) and which was at par with

Table 22: Lycopene content in selectively fertilized tomato fruits at 60 days after transplanting

Varietal crosses	Lycopene content (µg g^{-1})		
	60DAT		
	Condition		Mean
	Ambient	ROS	
T X S	17.19	15.81	11.00
T X S (SF)	17.55	16.02	11.19
T X T	21.48	19.71	13.73
T X T (SF)	24.86	20.71	15.19
S X S	12.49	11.60	8.03
S X S (SF)	13.66	12.51	8.72
Mean	17.87	16.06	
	Variety (V)	Condition (C)	V X C
SE m±	0.28	0.20	0.49
CD (0.05)	0.81	0.57	1.40

Table 23: SOD activity in leaves of selectively fertilized tomato plants

Varietal crosses	SOD activity (activity mg^{-1} min^{-1})							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	331.38	588.19	377.94	432.50	202.50	311.75	166.19	226.81
T X S (SF)	397.63	818.94	396.56	537.71	215.00	337.50	241.81	264.77
T X T	363.13	725.44	601.06	563.21	230.00	291.94	172.88	231.60
T X T (SF)	362.63	594.69	653.56	536.96	245.00	302.50	225.00	257.50
S X S	336.38	579.50	297.75	404.54	217.31	156.31	90.88	154.83
S X S (SF)	368.00	692.13	361.81	473.98	145.50	218.38	113.69	159.19
Mean	359.85	666.48	448.12		209.22	269.73	168.41	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	13.7	33.6	13.64		9.64	23.63	19.4	
CD (0.05)	39.0	95.53	38.79		27.43	67.19	55.15	

T14 and T16. Maximum activity was observed in selectively fertilized TXS (SF) (264.77 mg^{-1} min^{-1}) which was at par with TXS, TXT and TXT (SF).

4.2.2.12. Chlorophyll fluorescence

Data recorded for chlorophyll fluorescence under different treatments is presented in Table 24.

During first month, observed mean value was maximum in ambient condition (0.73 Fv/Fm) and the fluorescence was decreased with increase in temperature. In treatments, T10 recorded maximum chlorophyll fluorescence (0.78 Fv/Fm) and which was at par with T1, T4 and T7 and the minimum chlorophyll fluorescence was observed in T15 (0.42 Fv/Fm) followed by T18. Also it was observed that selectively fertilized crosses under temperature stress showed higher fluorescence than normal crosses. Among the crosses, TXS (SF) recorded maximum chlorophyll fluorescence (0.68 Fv/Fm) followed by TXT and TXT (SF).

During second month chlorophyll fluorescence was maximum in ambient condition (0.60 Fv/Fm) and the fluorescence was decreased with increase in temperature. In treatments, T4 recorded maximum chlorophyll fluorescence (0.65 Fv/Fm) and which was at par with T1, T5, T7, T10 and T16 and the minimum chlorophyll fluorescence was observed in T15 (0.22 Fv/Fm) followed by T9. Also it is observed that selectively fertilized crosses under stress showed higher fluorescence than normal crosses. Among the crosses, TXS (SF) recorded maximum (0.54 Fv/Fm) followed by TXT (SF).

Table 24: Chlorophyll fluorescence (Fv/Fm) in selectively fertilized tomato plants

Varietal crosses	Chlorophyll fluorescence							
	30 DAT				60 DAT			
	Condition			Mean	Condition			Mean
	Ambient	ROS	OTC		Ambient	ROS	OTC	
T X S	0.73	0.60	0.51	0.61	0.59	0.51	0.28	0.46
T X S (SF)	0.76	0.64	0.62	0.68	0.65	0.57	0.41	0.54
T X T	0.77	0.64	0.53	0.65	0.62	0.47	0.30	0.46
T X T (SF)	0.78	0.62	0.57	0.66	0.63	0.54	0.35	0.51
S X S	0.68	0.61	0.42	0.57	0.53	0.46	0.22	0.40
S X S (SF)	0.66	0.65	0.50	0.60	0.57	0.40	0.33	0.43
Mean	0.73	0.63	0.53		0.60	0.49	0.32	
	Variety (V)	Condition (C)	V X C		Variety (V)	Condition (C)	V X C	
SE m±	0.01	0.01	0.03		0.01	0.01	0.03	
CD (0.05)	0.04	0.03	0.08		0.05	0.03	0.09	

DISCUSSION

5. DISCUSSION

The research project on "Developing high temperature tolerance in tomato (*Solanum lycopersicum L.*) through selective fertilization technique" was done with the objective to identify the critical temperature for pollen germination and to evaluate the selectively fertilized tomato hybrids for high temperature tolerance.

In the present programme, critical temperature for pollen germination in five varieties of tomato was standardized. The pollen grains that germinated under ambient temperature were used for selective fertilization to develop high temperature tolerance. Both selectively fertilized and normal crossed plants were exposed to different temperature conditions *i.e.* from ambient temperature to high temperatures in a ROS (ambient temp.+3^{0}C) and OTC (ambient temp.+ 6^{0}C). Observations on physiological and biometrical parameters were taken on selectively fertilized and normal crossed plants of one tolerant and one susceptible genotype under different temperatures.

5.1 EVALUATION OF CRITICAL TEMPERATURE FOR POLLEN GERMINATION

Pollens can be used as a selection tool for selecting genotypes on the basis of temperature tolerance. In general, genotypes which are tolerant to high temperature will have high pollen germination and viability than the sensitive ones (Dane *et al.*, 1991). So usage of pollen germination at critical temperature as a screening tool will provide information about tolerance level of genotypes at high temperatures.

In the first experiment, critical temperature for pollen germination was identified using five tomato genotypes, Anagha, Manuprabha, Vellayani Vijay, Manulakshmi and Akshaya. Results showed that as the temperature increased from its optimal temperature *i.e.* 32^0 C, the germination percent of the pollen got reduced markedly. After 36^0 C the pollen germination percentage decreased below 20 % in all the genotypes. Hence, 36°C was identified as critical temperature for pollen germination. On the basis of pollen germination at critical temperature, Anagha was

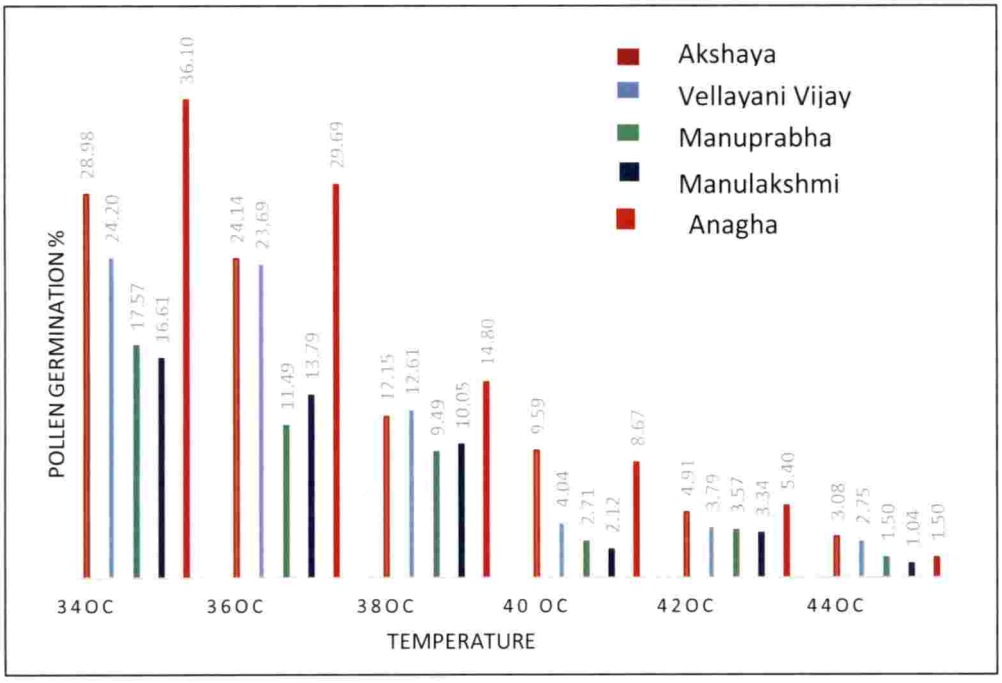


Figure 1: Pollen germination percentage of five tomato varieties after 2hr incubation in different temperatures

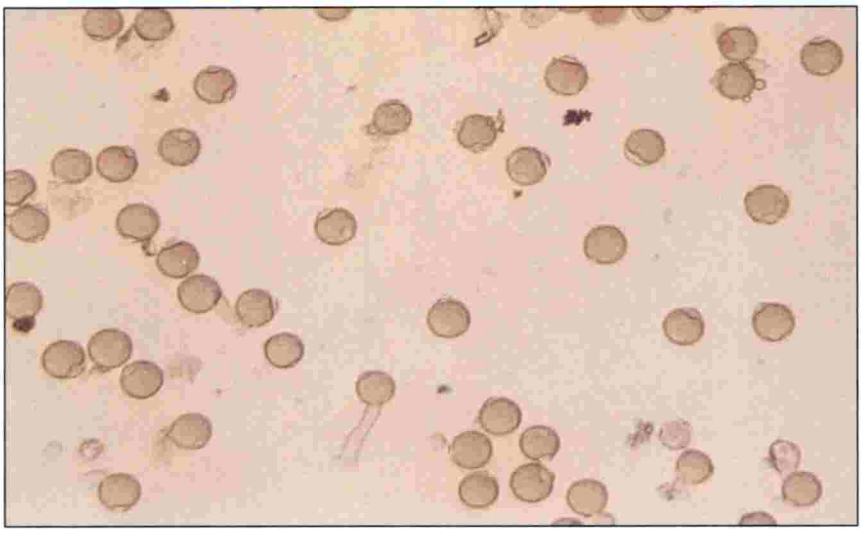


Plate 2: Pollen germination in Anagha at 36^0 C

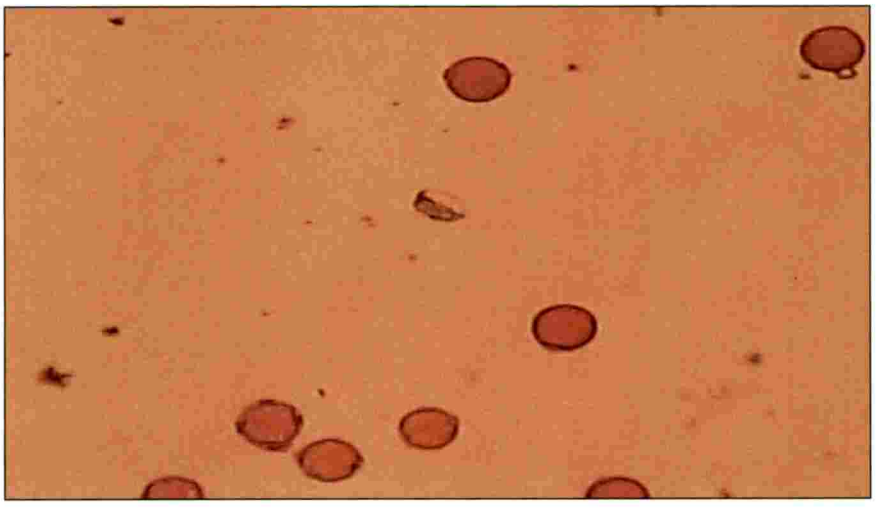


Plate 3: Pollen germination in Manuprabha at 36^0 C

selected as the tolerant genotype and Manuprabha with least pollen germination as the susceptible one. It was reported that germinated pollens were reduced 13 times when the temperature enhanced progressively from optimum (Pressman *et al.,* 2002). Marine *et al.* (2017) conducted a similar study in tomato to group different genotypes of tomato into tolerant and susceptible ones on the basis of pollen viability. A similar study was done in soybean also and confirmed that in vivo pollen germination study can be used to confirm the genotypic tolerance to high temperature where they classified different genotypes of soybean into tolerant, intermediate and susceptible one on the basis of pollen germination at different temperature (Salem *et al.,* 2007).

5.2 EFFECT OF SELECTIVE FERTILIZATION ON PHYSIOLOGICAL AND BIOMETRICAL PARAMETERS AT HIGH TEMPERATURE

The selective fertilization technique is a new method to develop hybrids by imposing a selection pressure like temperature on pollen during germination and the pollen which are tolerant to this selection pressure only will germinate and fertilize the ovule. Hence the resultant progeny may be tolerant to the selection pressure. In the second experiment selective fertilization technique was found to have a positive influence on the physiological and yield attributes as it increased the photosynthetic efficiency and high temperature stress tolerance. Peet *et al.* (1997) validated that in hand-pollinated tomatoes, relative seediness, fruitset percentage, and total number and weight of fruit per plant decreased linearly as mean daily temperature rose from 25 to 29 °C, even though pollen developed at low temperatures (26/22 °C). However selective fertilization of pollen grains increased the fruit weight, fruit setting percentage and pollen viability eventhough the mean daily temperature was very high (40/24 ^{0}C). Bhandari *et al.* (2017) also suggested that heterosis breeding can be used to improve the thermostability in tomato.

Temperature stress affected the stem growth and plant height in this experiment and an overall reduction in plant height was observed among the selectively fertilized plants under temperature stress. Alam *et al.* (2010) also

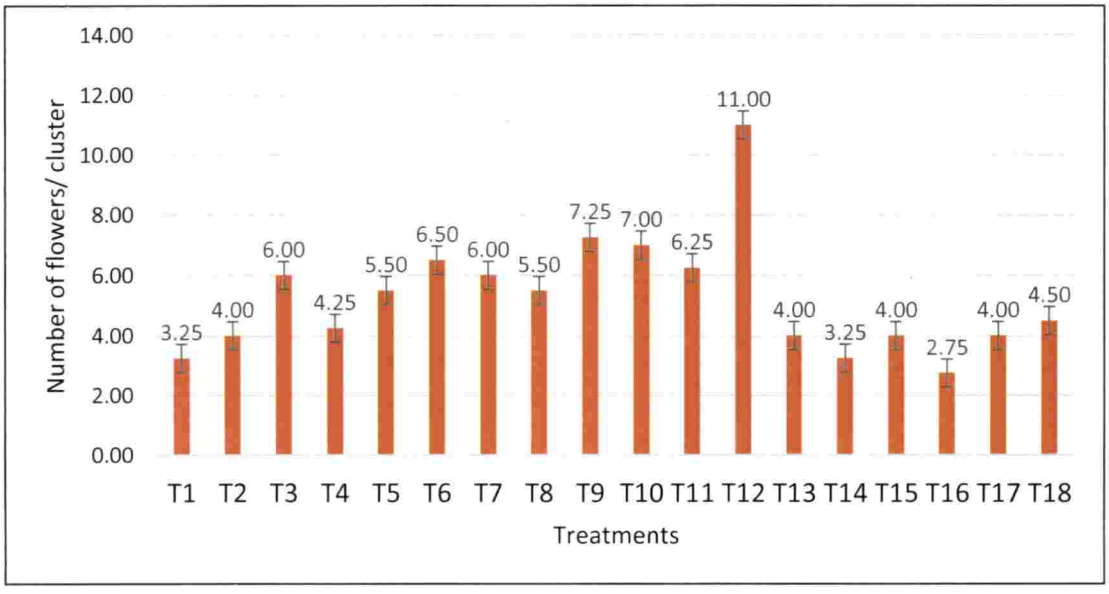


Figure 2: Number of flowers/ cluster of selectively fertilized tomato plants

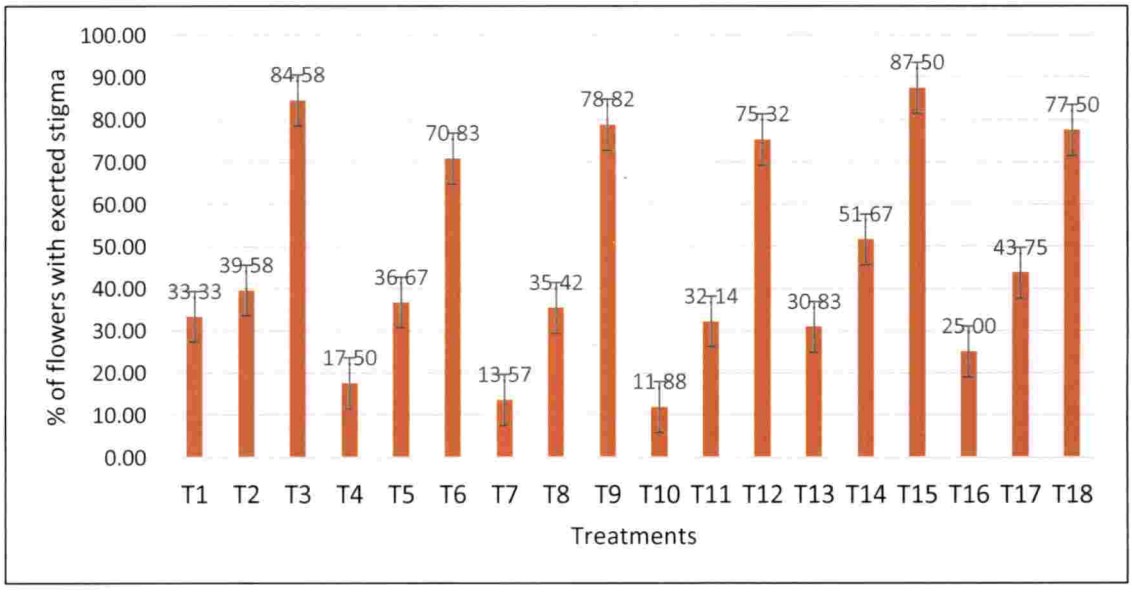


Figure 3: Percentage of flowers with exerted stigma in selectively fertilized tomato plants

reported that significant difference in plant height occurred among hybrids in tomato under different temperature condition.

Heat susceptible genotypes recorded lowest number of flowers and fruits than tolerant ones and there was significant difference among different conditions. This outcome was similar to that of Faruq *et al.* (2012) where heat susceptible cultivars produced less number of fruits and flowers under high temperature. Abdelmageed and Gruda (2009) also observed that number of fruits and flowers per crop, fruit fresh weight and fruit set were diverse in heat resistant and heat susceptible tomato lines and it differed in field and glasshouse environments. Dane *et al.* (1991) also reported that profusely flowered genotypes were less affected by temperature stress. Heat tolerant genotypes had more flowers under both control condition and high temperature condition (Abdulbaki, 1991).

Flowers with exerted stigma is a major problem in tomato under high temp as it decreases the self pollination due to the failure of pollen grain to stick on stigma. Less number of flowers with exerted stigma is a character of tolerant genotype as it increase fruit setting percentage. The exerted style (*i.e.*, stigma is elongated beyond the anther cone) in reproductive stage is the most owing impression of high temperature in tomato, that may hamper selfpollination (Faruq *et al.,* 2012). In this study tolerant genotypes showed less number of flowers with exerted stigma than susceptible genotypes and among them selectively fertilized plants are more tolerant. Saeed *et al.* (2007) also reported that genotypes generating flowers with no stigma exertion at high temperature is stable and produce high fruit yield.

Redundant elongation of style in most flowers abridge the pollen access to stigma in heat sensitive genotypes and reduce the fertilization (Alsamir *et al.,* 2017). Increased stigma exertion was observed in normal crosses without pollen selection and as a result, there was a reduction in fruit set in normal plants, than selectively fertilized plants and the stigma exertion was more than 1mm. Rudich *et al.* (1977) also reported that, exertion of stigma tube above 1mm results in the reduction of fruit yield in tomato.

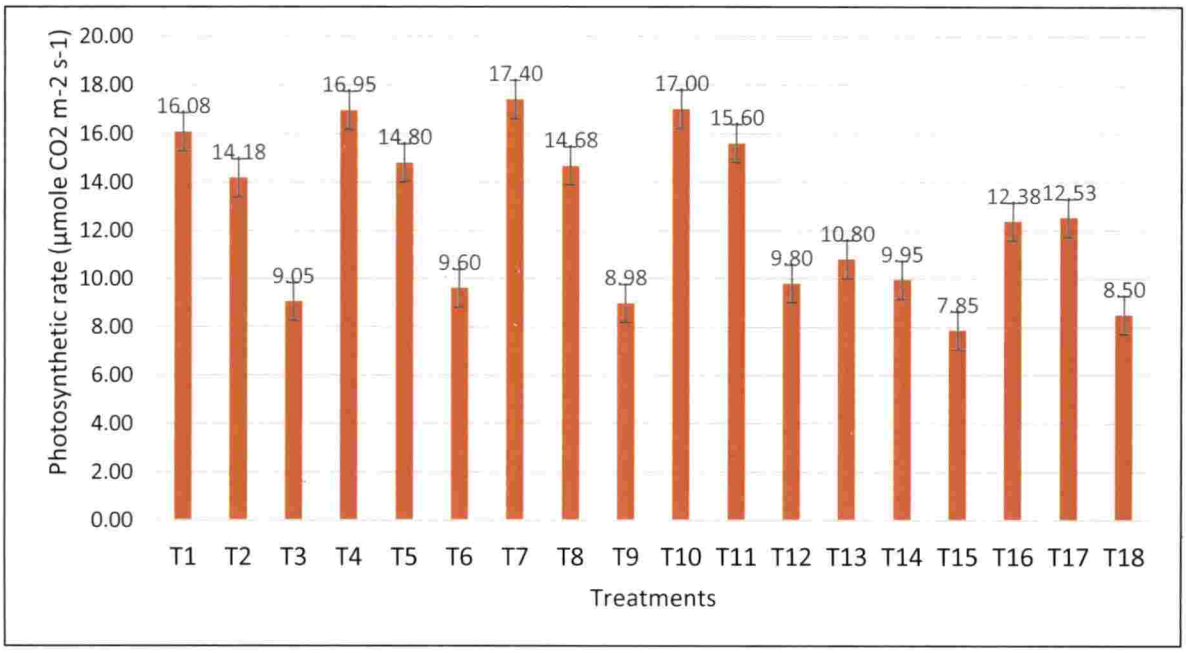


Figure 4: Photosynthetic rate of selectively fertilized tomato plants

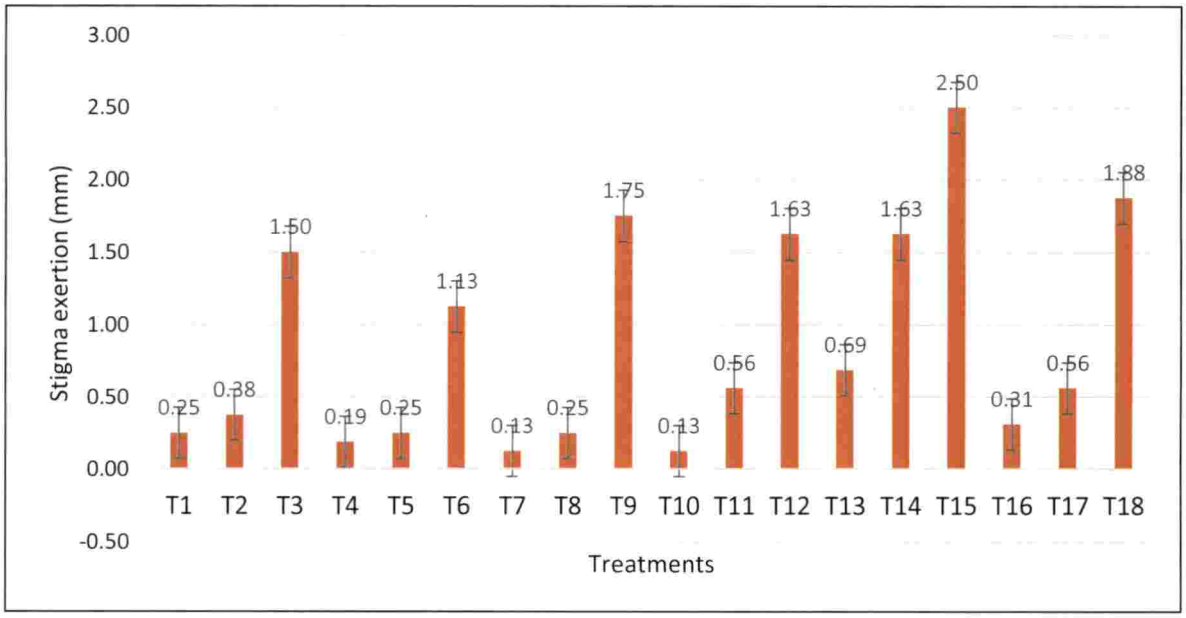
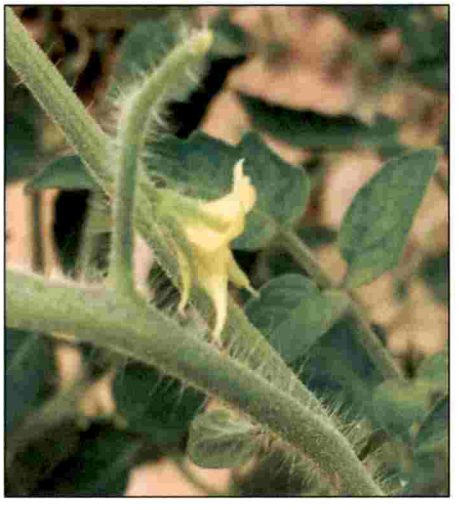


Figure 5: Stigma exertion in selectively fertilized tomato plants

(a)



(b)

Plate 4: Stigma exertion in selectively fertilized (a) and normal crossed (b) plants

Various physiological and biochemical processes were affected at high temperatures. This physiological parameters can be used as an indicator of thermo tolerance in tomato. The differential performance of physiological processes under heat stress can be the pointer for genotype selection under high temperatures. Physiological and biochemical processes affected by temperature are photosynthetic enzyme activity; membrane integrity, photophosphorylation and electron transport in chloroplast, stomatal conductance to CO_2 diffusion and photo assimilate translocation (Dinar and Rudich, 1985). Heat stress evidently limits photosynthesis, water balance, and cell membrane stability, and also disturbs metabolism in plants (Hemantaranjan *et al.,* 2014)

As the photosynthetic rate shows the proper index of the health status of plants, increased photosynthetic rate under heat stress shows healthier and resistant nature of plants. Highest photosynthetic rate was observed in selectively fertilized plants under heat stress than normal ones in the present experiment. Wahid *et al.* (2007) also stated that photosynthesis as a potential physiological index at high temperatures. In tomato, resistant varieties under high temperature had the capacity to maintain enhanced chlorophyll a:b proportion, showing the link of these alteration to thermo tolerance (Camejo *et al.,* 2005). Selectively fertilized plants showed comparably higher chlorophyll than the normal plants and which are more resistant to temperature. Improvement in photosynthetic aspects under heat stress are good indicators of thermo tolerance and any restriction in photosynthesis can diminish plant growth at high temperatures (Wise *et al.,* 2004). High temperature affects the photosynthetic capacity of C3 than C4 plants. The energy distribution, diminished carbon metabolism activities, particularly the rubisco, interruption of electron transport and PSII inactivation were reported under high temperature (Salvucci and Crafts-Brandner, 2004).

In the present experiment there was no significant difference in total soluble protein content among treatments. Individual effect of crosses showed significant difference and high protein content is reported in selectively fertilized crosses than normal ones. Selective fertilization of plant by giving a temperature stress to pollen

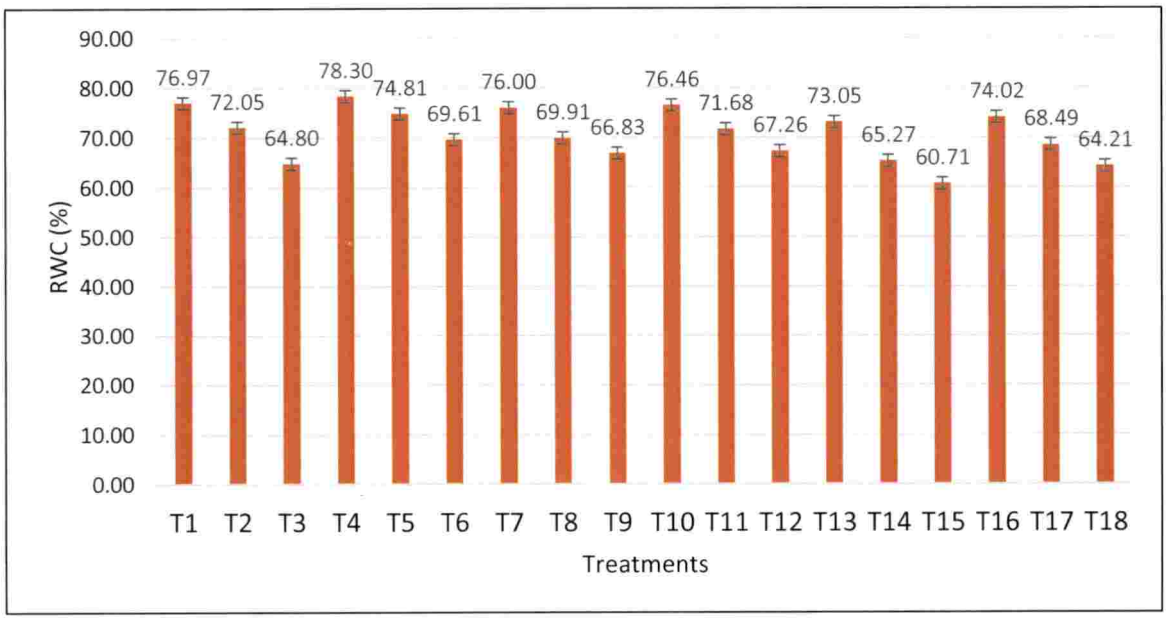


Figure 6: Relative water content (%) in leaves of selectively fertilized tomato plants

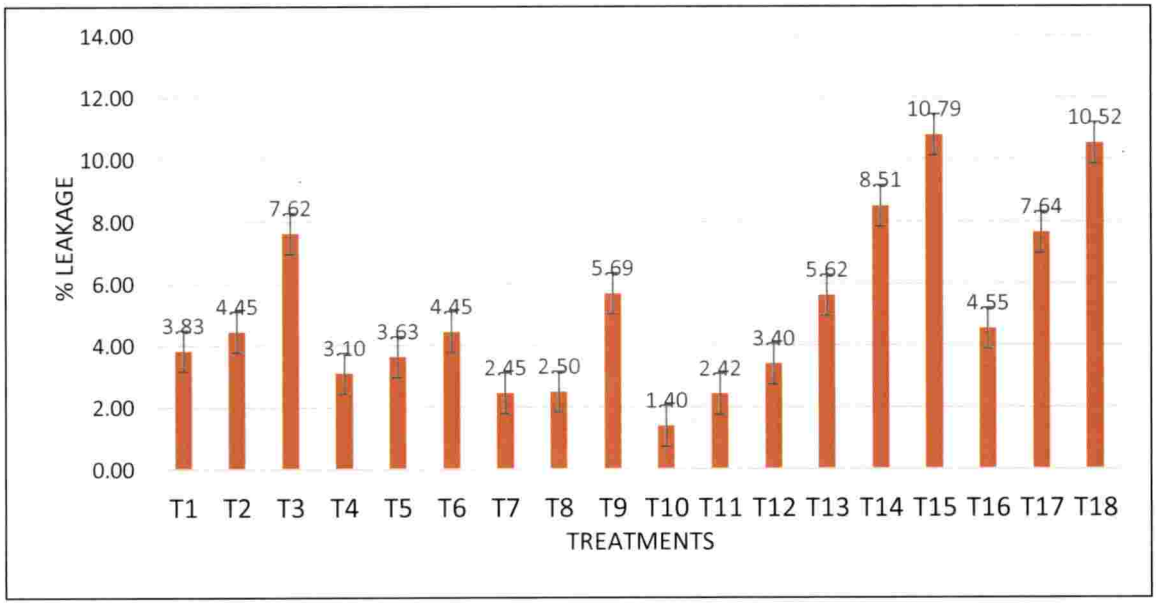


Figure 7: Membrane integrity (% leakage) of selectively fertilized tomato plants

may enhance the induction of some specialised HSPs and associated reduction in protein degradation which maybe the reason for thermo tolerance in selectively fertilized genotypes than normal crosses. He *et al.* (2005) also reported that in bent grass the protein degradation and induced expression of HSPs will occur under temperature stress and this induced expression and high protein content is associated with the enhanced thermo tolerance in genotypes.

Relative water content decreased in tomato under high temperatures and the selectively fertilized plants showed more water content than the normal crosses. Under changing environmental conditions, water content is an imperious variable (Mazorra *et al.,* 2002). Morale *et al.* (2003) also stated that in tomato, heat stress will disturb the hydraulic conductivity of roots and leaf water relationships. The increased water content under heat stress is an indicator of increased tolerance.

Membrane integrity has long been used as an indirect measure to evaluate the thermo tolerance in tomato (Chen *et al.,* 1982). Enhanced heat stress will damage the membrane stability, leading to an increased electrolyte leakage. Heat resistant genotypes show less leakage than the susceptible ones and selectively fertilized crosses showed a relatively higher membrane integrity or lesser leakage than the normal ones in this experiment. Liu and Huang (2000) also reported that generation of active oxygen species (AOS) like singlet oxygen (1 O_2), superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are symptoms of cellular injury due to high temperature. The increased production of reactive oxygen species at high temperature leads to the enhancement of antioxidant activity, like SOD and ascorbic acid, and tolerant genotypes show high antioxidant activity than the susceptible ones. Selectively fertilized genotypes show high ROS scavenging mechanism than the normal hybrids. Scandalios (1993) observed that, over expression of SOD in plants mark a number of physiological phenomena including H_2O_2 removal, toxic reductants oxidation, biosynthesis and degradation of lignin in cell walls, auxin catabolism and defensive responses.

The carbohydrate content decrease at elevating temperature and selectively fertilized plants show relatively more carbohydrate content than the normal ones in

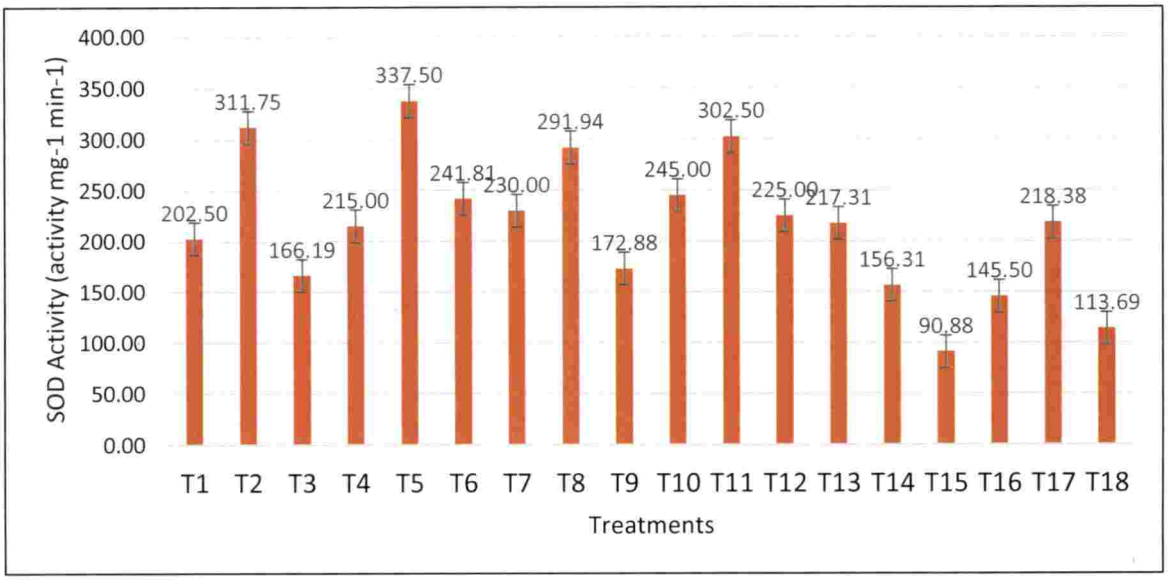


Fig 8: SOD activity (activity mg^{-1} min^{-1}) in leaves of selectively fertilized tomato plants

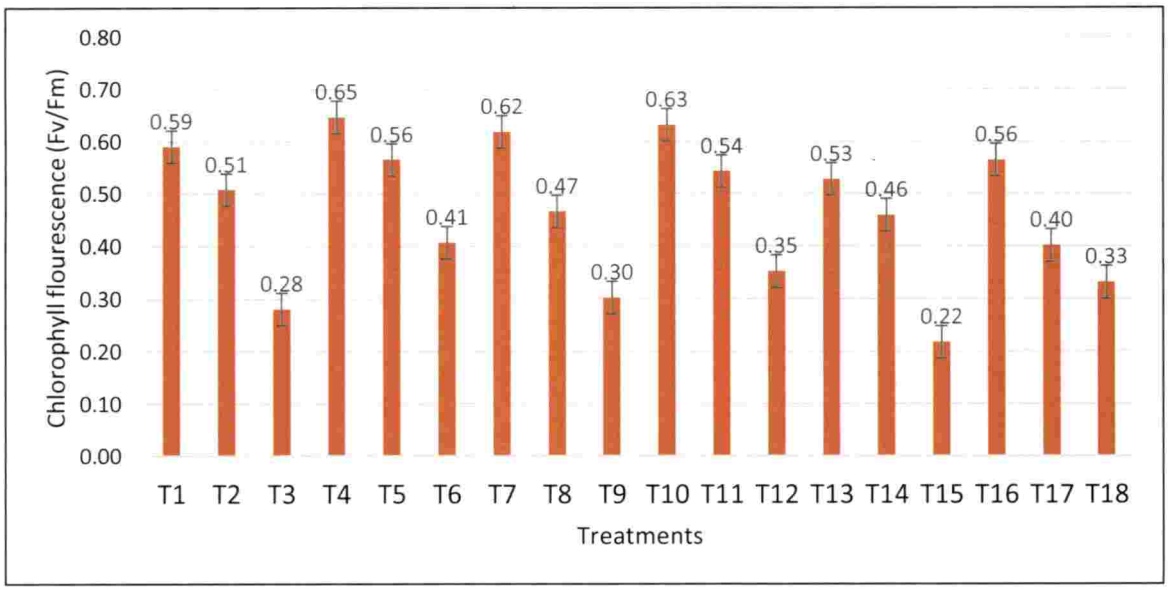
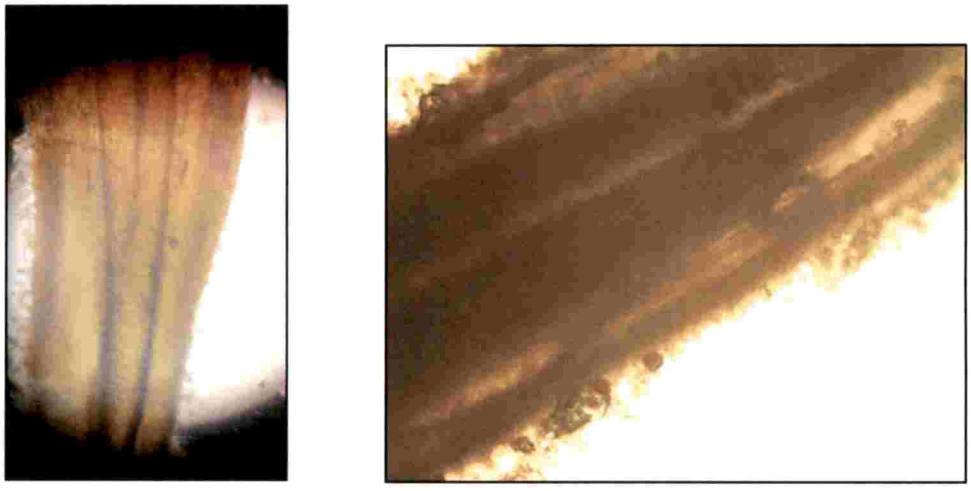


Figure 9: Chlorophyll fluorescence (Fv/Fm) in selectively fertilized tomato plants

the present study. Kinet and Peet (1997) also reported that decreased carbohydrate content under high temperature may be the reason for the associated increased poor fruit set in tomato. Well-organized carbohydrate metabolism is the essence of survival strategies of plants exposed to environmental impacts especially to higher temperatures (Krzysztof and Gabriela, 2007). Jie *et al.* (2011) reported that a moderately-high temperature triggered photosynthetic activity and carbohydrate metabolism in tomato leaves. The decrease in photosynthesis and modified carbohydrate contents in plant leaves, are correlated with each other.

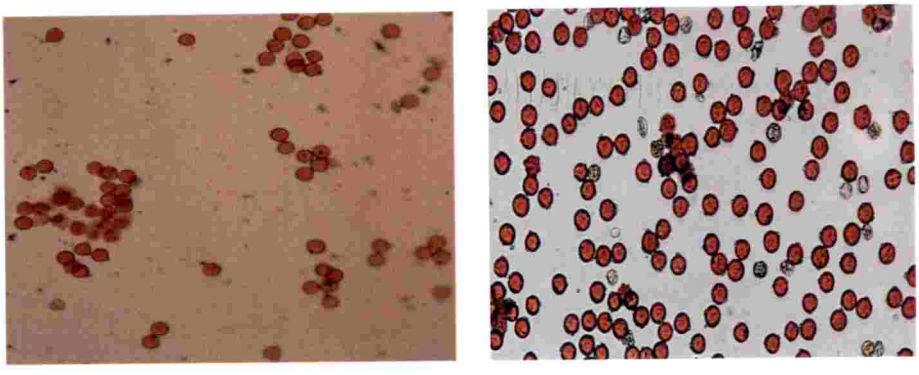
Chlorophyll fluorescence, the ratio of variable fluorescence to maximum fluorescence (Fv/Fm), are physiological constraints that are associated with heat tolerance (Yamada *et al.,* 1996). Hameed *et al.* (2015) also confirmed that in tomato, chlorophyll fluorescence fvperfm can be used as a forecaster of heat shock sensitivity in different genotypes and also observed that it decreased in heat sensitive genotypes. PSII is extremely thermo labile and its function is highly weakened or reasonably halted in high temperatures (Camejo *et al.,* 2005) which is the reason for decreased chlorophyll fluorescence under high temperatures.

In OTC, there was no fruit set and the pollens were also less in anthers. Among the crosses normal crosses were devoid of pollen as compared to the selectively fertilized crosses at very high temperature. Various scientists also discovered a decreased pollen release, viability, germination and fruit set in tomato at temperature above the medium levels (Peet *et al.,* 1997; Sato *et al.,* 2000; Pressman *et al.,* 2002). The decline in the fruit set of tomato under very high temperature stress is mainly because of the decreased pollen viability and release and not because of pollen generation (Sato *et al.,* 2006). Weaver and Trimm (1989) also reported a positive correlation between pollen viability maintenance and thermo tolerance. In tomato the reduced or no pollen viability under OTC condition may be due to the hindered sugar metabolism in pollen grains as it require high sugar content for germination and growth. Pressman *et al.* (2002) also stated that disrupted carbohydrate metabolism at high temperature impairs pollen development and viability in tomato.



(a) (b)

Plate 5: Anthers of normal crossed (a) and selectively fertilized (b) plants of tolerant and susceptible plant at OTC



(a) (b)

(c)

Plate 6: Pollen viability under different conditions viz. Control (a), ROS (b) and OTC (c)

Total yield, number of fruits per cluster and fruit setting percentage decreased and fruit weight increased with increase in temperature. Selectively fertilized crosses gave more yield than the normal crosses as the fruit setting percentage and number of fruits per cluster were higher in selectively fertilized plants. Dane *et al.* (1991) also reported a decline in fruit set in heat sensitive genotype under high temperature stress. Peet *et al.* (1998) also reported that decrease in total yield is mainly caused by the reduced number of fruits rather than the reduced fruit weight. Heat stress detrimentally let-down the vegetative growth and reproductive development of the tomato plants which eventually reduces yield and quality of tomato fruit. Mean daily temperature above 29 °C causes, decreased fruit number, fruit weight per plant and seed number per fruit in tomato compared with those at 25 °C (Amit *et al.*, 2017). Singh *et al.* (2002) reported that fruit weight should be taken into consideration to select genotypes for summer to compensate the low fruit set during hot conditions.

As the selectively fertilized cross of susceptible and tolerant parent resulted in higher number of fruits per cluster, fruit weight and yield shown the hybrid vigour in crossed varieties. Savale *et al.* (2017) reported that heterosis breeding in tomato will increase the fruit weight, number of clusters per plant and number of fruits per plants. From the experiment, it is evident that selective fertilization improved thermo tolerance and yield in tomato under high temperature and hence is a potential technique to impart high temperature tolerance in tomato.

SUMMARY

6. SUMMARY

The present programme "Developing high temperature tolerance in tomato (*Solanum lycopersicum* L.) through selective fertilization technique" was conducted in two experiments and the salient findings are given below.

In the first experiment critical temperature was standardized. 36^{O}C was identified as critical temperature for pollen selection where 20% pollen germination was observed. Beyond critical temperature, pollen germination percentage decreased below 20 %. Anagha with high pollen germination percentage at this critical temperature was selected as tolerant variety and the variety Manuprabha with least pollen germination was selected as susceptible variety.

In the second experiment selectively fertilized and normal crossed varieties of Anagha (male parent) and Manuprabha (female parent) were used to study the effect of selective fertilization in tomato under high temperature. The incubated pollen at critical temperature from the male parent was used to pollinate the emasculated flowers of female parent for selective fertilization. Normal cross without pollen selection was also done between the male and female parent. The selfing (with and without pollen selection) was also done within male and female parents. The 30 days old seedlings of the above crosses were transplanted and exposed to three different temperatures viz. ambient temp. (control), ambient temp.+3^{0}C (Rainout shelter), ambient temp.+ 6 ^{0}C (OTC).

Physiological, biometrical and yield parameters were studied in all the crosses under three conditions. Biometrical parameters such as plant height and floral characters such as number of clusters, numbers of flower clusters per plant and number of flowers were increased with temperature. Except plant height the floral characters were recorded highest in selectively fertilized plants compared to normal crosses. Plant height and the number of cluster were highest in SXS at OTC and number of flowers were highest in TXT under OTC. Floral characters like number of flowers with exerted stigma and the stigma exertion length recorded lowest in

selectively fertilized plants compared to normal plants and it was less in tolerant parent compared to susceptible one. In case of length of stigma exertion the crossed variety of parents showed more positive result than the selfed crosses of parents.

Physiological parameters like photosynthetic rate, chlorophyll content, total soluble protein and relative water content decreased significantly with increase in temperature. All the parameters were recorded highest in selectively fertilized crosses compared to their normal crosses. The photosynthetic rate and chlorophyll content was recorded highest in the selectively fertilized TXT than others. In case of total soluble protein there was no significant difference found. The relative water content was highest in the selectively hybridized cross than selfed crosses. Carbohydrate content decreased with increasing temperature and among the crosses selectively fertilized plants showed significantly higher content. The carbohydrate content was the highest in TXT SF.

The temperature tolerance characters like membrane integrity (% leakage), SOD activity and chlorophyll fluorescence were higher in selectively fertilized plants than the normal plants. The percentage leakage increased with increase in temperature and the selectively fertilized plants recorded the least. The SOD activity decreased in ROS and increased under OTC condition and the selectively fertilized cross of two parents had the highest activity under high temperature. The Fv/Fm which show PSII efficiency significantly decreased with increase in temperature, as the increased temperature damaged the photosystem of plants. Highest Fv/Fm was observed in the cross between two parents. Ascorbate content in leaves increased significantly with increase in temperature but decreased under OTC.

The yield characters like number of fruits, fruit setting percentage, fruit weight and lycopene content were high in selectively fertilized crosses than the normal crosses. The yield characters decreased significantly with increase in temperature and there was no fruit set at OTC due to very high temperature. The pollen grains were also observed less in OTC condition. Pollen viability decreased drastically under high temperature but pollen viability was observed only in

selectively fertilized crosses. All the yield parameters were significantly affected by temperature stress conditions in normal crossed plants compared to selectively fertilized ones. Selectively fertilized plants, recorded improved stress tolerance, which led to better performance under high temperature stress.

It is therefore, concluded that selective fertilization can improve the thermo tolerance in tomato. This technique not only improved better survival of plants under stress conditions but also improved yield parameters and pollen viability. It is also concluded that selective fertilization of cross of tolerant and susceptible parent TXS (SF) showed more hybrid vigour as well as more tolerance under high temperature condition.

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DEVELOPING HIGH TEMPERATURE TOLERANCE IN TOMATO (*Solanum lycopersicum* L.) THROUGH SELECTIVE FERTILIZATION TECHNIQUE

by

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ABSTRACT

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ABSTRACT

The study entitled "Developing high temperature tolerance in tomato (*Solanum lycopersicum* L) through selective fertilization technique", was undertaken with the objectives to identify the critical temperature for pollen germination and to evaluate the selectively fertilized tomato hybrids for high temperature tolerance at the Department of Plant Physiology, College of Agriculture, Vellayani during 2017-2019.

The first experiment was designed for the identification of critical temperature for pollen germination using five popular varieties of tomato namely Anagha, Vellayani Vijay, Manuprabha, Manulakshmi and Akshaya. Mature pollen grains from fully opened flowers were collected and incubated at different temperatures (34^0C to 44^0C) for two hours in the pollen germination medium. The design followed was CRD with 4 replications. The temperature of 36°C was identified as the critical temperature for pollen germination. Anagha and Manuprabha exhibited the highest (29.69 %) and the least (11.48 %) pollen germination percentage respectively at this critical temperature. Pollen viability decreased with increase in temperature in all the five varieties.

For the second experiment, the variety Anagha with the highest pollen germination at critical temperature was selected as tolerant (male parent) and the variety Manuprabha with least pollen germination at critical temperature was selected as the susceptible (female parent). Selective fertilization (SF) was done between Anagha and Manuprabha by pollinating the emasculated female parent with 2hr incubated pollen from male parent at critical temperature. Another set of cross was also made with same parental combination without pollen selection. The six crosses were made are Tolerant X Susceptible, Tolerant X Susceptible (SF), Tolerant X Tolerant, Tolerant X Tolerant (SF), Susceptible X Susceptible and Susceptible X Susceptible (SF). The seeds were collected from crosses and sown. The seedings were maintained at ambient temperature for 30 days and then transplanted in pots and exposed to three

different temperature conditions ie, at ambient temperature condition, Rainout shelter (ROS) and Open top chamber (OTC) to evaluate the growth performance of the crosses. The observations were taken at 30 and 60 days after transplanting. The experiment was laid out with 18 treatments in CRD with four replications.

Plant height and floral characters (number of flower clusters and number of flowers per cluster) were increased significantly with increase in temperature. Among the crosses, T15 recorded taller plants (127.32 cm). Selectively fertilized plants produced significantly more number of flowers per cluster than the normal crosses and T12 recorded maximum (11.00). However, the number of cluster was significantly higher in normal crosses and T15 had maximum number of cluster (45.50). Percentage of flowers with exerted stigma and length of stigma exertion were increased significantly with increase in temperature but the selectively fertilized crosses recorded lower stigma exertion. T15 showed the highest percentage stigma exertion (87.50%) which was at par with T3, T6, T9, T12 and T18. The length of exerted stigma was highest in T15 (2.50mm).

Physiological parameters like photosynthetic rate, chlorophyll content, total soluble protein and relative water content decreased significantly with increase in temperature and the selectively fertilized crosses showed higher value than the normal crosses under higher temperature. The photosynthetic rate (17.40 µmol CO_2 m^{-2} s^{-1}) and chlorophyll content (1.91 mg g^{-1}) was recorded higher in T7 which was at par with T1, T4, T10 and T11. In case of total soluble protein there was no significant difference. The relative water content was highest in T4 (74.80%) but at par with T1, T7 and T10. Carbohydrate content decreased with increasing temperature and among the crosses selectively fertilized plants showed significantly higher content. The carbohydrate content was the highest in T8 (79.62 mg g^{-1}) but at par with T5.

Temperature tolerance characters like membrane integrity (% leakage), SOD activity and chlorophyll fluorescence were higher in selectively fertilized plants than the normal plants. The percentage leakage increased with increase in temperature and

T10 had the least leakage (1.40 %). The SOD activity first decreased and then increased with temperature and T5 exhibited maximum SOD activity (337.50 activity mg^{-1} min^{-1}) which was at par with T2 and T8. The chlorophyll fluorescence significantly decreased with increase in temperature, the maximum fluorescence recorded in T4 (0.65 fv/fm) which was at par with T1, T7, T10, T16 and T5. Ascorbic acid content of leaves increased significantly with increase in temperature but decreased under OTC. T5 (464.65 µg g^{-1}) and T18 were on par with respect to ascorbic acid.

The yield characters like number of fruits, fruit setting %, fruit weight and lycopene content were high in selectively fertilized crosses than the normal crosses. The yield characters decreased significantly with increase in temperature and there was no fruit set at OTC due to very high temperature. T10 recorded highest number of fruits per cluster (6) which was at par with T7 and T1. The fruit setting percentage was the highest in T7 (89.29%) which was at par with all treatments except T2 and T14. The fruit weight increased in ROS condition compared to ambient condition and the highest fruit weight was recorded in T17 (37.77g) which was at par with T16, T5 and T14. Highest lycopene content was recorded in T10 (24.86 µg g^{-1}). The yield was decreased significantly with increase in temperature and the highest yield was obtained in T4 (402.15 g $plant^{-1}$) which was at par with T4, T7 and T10.

Critical temperature for pollen selection and selective fertilization was selected as 36^0 C. Anagha exhibited higher germination percentage and pollen viability at critical temperature among the five varieties of tomato tested and Manuprabha had the least pollen germination. Selective fertilization technique was found to have an advantageous influence on the physiological and yield attributes as it increased the photosynthetic efficiency and high temperature stress tolerance. Selective fertilization of cross of tolerant and susceptible parent TXS (SF) was found to be the best performing type under high temperature condition.