PHYSIOLOGICAL AND MOLECULAR BASIS OF SELECTIVE FERTILIZATION FOR HIGH TEMPERATURE STRESS TOLERANCE IN TOMATO (*Solanum lycopersicum* L.)

By
AMMU ALPHONSA JOHN
(2019-21-074)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE
Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695522
KERALA, INDIA
2024

DECLARATION

I, hereby declare that this thesis entitled **"PHYSIOLOGICAL AND MOLECULAR BASIS OF SELECTIVE FERTILIZATION FOR HIGH TEMPERATURE STRESS TOLERANCE IN TOMATO (*Solanum lycopersicum* L.)"** 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.

Vellayani

Date: 1/1/25

Ammu Alphonsa John

(2019-21-074)

CERTIFICATE

Certified that this thesis entitled **"PHYSIOLOGICAL AND MOLECULAR BASIS OF SELECTIVE FERTILIZATION FOR HIGH TEMPERATURE STRESS TOLERANCE IN TOMATO (*Solanum lycopersicum* L.)** is a record of research work done independently by Ms. Ammu Alphonsa John under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

1/1/25

Dr. Roy Stephen

Major Advisor
Professor
Department of Plant Physiology
College of Agriculture, Vellayani

Vellayani

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Ammu Alphonsa John (2019-21-074), a candidate for the degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURE** with major in Plant Physiology, agree that the thesis entitled **"PHYSIOLOGICAL AND MOLECULAR BASIS OF SELECTIVE FERTILIZATION FOR HIGH TEMPERATURE STRESS TOLERANCE IN TOMATO (*Solanum lycopersicum* L.)"** may be submitted by Ms. Ammu Alphonsa John in partial fulfilment of the requirement for the degree.

Dr. Roy Stephen
(Chairperson, Advisory Committee)
Professor
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram - 695 522

Dr. Manju R.V.
(Member, Advisory Committee)
Professor and Head
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram - 695 522

Dr. Beena R.
(Member, Advisory Committee)
Assistant Professor
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram - 695 522

Dr. Rafeekher M.
(Member, Advisory Committee)
Assistant Professor and Head
Department of Floriculture and Landscaping architecture
College of Agriculture, Vellayani
Thiruvananthapuram - 695 522

Dr. Sarada S.
(Member, Advisory Committee)
Assistant Professor and Head
Department of Vegetable Science
College of Agriculture, Vellayani
Thiruvananthapuram - 695 522

11/7/2025
EXTERNAL EXAMINER

Dr. SARAVANAN RAJU, Ph.D
Principal Scientist (Plant Physiology)
ICAR-Central Tuber Crops Research Institute
Sreekariyam, Pin: 695 017
Thiruvananthapuram, Kerala

ACKNOWLEDGMENT

And this is the time to look back on the path traversed during the endeavor and remember the faces and spirits behind the action with a huge sense of gratitude

First and foremost, I would like to praise ***The Almighty GOD****,* ***the most gracious and the most merciful****, for his blessings throughout the long five years. He made me courageous to complete this endeavor even the aggravate situation.*

Very politely and modestly I express my sincere gratitude to my major advisor ***Dr. Roy Stephen****,* ***Professor, Department of Plant Physiology*** *for his constant encouragement, worthy guidance and end fess support offered during the period of work. He never inculpated me for anything also gave advices when it was needed. I am in lacks of words to express my respect and thankfulness towards him.*

I also owe my deep sense of gratitude and sincere thanks to ***Dr. Manju R.V. Professor and Head Department of Plant Physiology****, College of Agriculture, Vellayani, member of my advisory committee for her timely help, critical scrutiny of the manuscript and valuable guidance for thesuccessful completion of my research work.*

My heartfelt thanks to ***Dr, Beena R, Assistant Professor, Department of Plant Physiology,*** *for her meticulous guidance, valuable suggestions, keen interest, wholehearted help and constructive criticism and also for the realization of the project.*

I am ineffable in expressing my deep sense of gratitude to my professor ***Dr. Viji, M.M. Professor, RARS Ambalavayal*** *for her inspiring guidance, critical comments, support and encouragement throughout the course of my research work.*

My sincere thanks to, ***Dr. Rafeekher M. Assistant Professor and Head, Department of Floriculture and Landscaping, College of Agriculture, Vellayani, member*** *of my advisory committee for the valuable guidance, timely suggestions and help rendered in the conduct of the experiment.*

I am extremely thankful to ***Dr. Sarada S, Assistant Professor and Head, Vegetable Science, College of Agriculture, Vellayani****, member of my advisory committee for his support, suggestions, valuable guidance and timely help rendered in the conduct of the experiment.*

I would like to acknowledge the help and support of my friends, Nalishma, Aisha chechi, Ponni, Elzu, Sappa, Sandy, PR, Sreena, Dorothy, Anju, Liz, Sree, RA, KK, Afnatha, Lakshmi and Sneha who offered their encouragement and camaraderie throughout this process. Your friendship has made this challenging journey more enjoyable.

Arya, I don't now know how to thank you. You were there with me from my PG days. I highly appreciate your friendship and thank you for being there during my ups and lows and also for always being there to listen.

I would also take this time to thank and express my gratitude to Vipin chettan and Navee. Without asking for and wanting anything in return they helped me wholeheartedly throughout my journey. Thank you for being there for me.

I would also thank some souls who were there with me, even though they are far away, my pbns, Swathy, Prathibha, Kavya, Gladu, Arathy and Justy. Thank you for your constant support.

A special thanks to my cousins, Achu, Vava, Amalu, Abi, Malu and Manikuttty, thank you for being an incredible support system and for always being there with a listening ear and a helping hand.

Last but certainly not least, My Family, Pappa, Amma, Anju and Appu, thank you for your unconditional love, unwavering support, and for always believing in me, even when I doubted myself. Your belief in me and your constant support have been my source of strength and motivation to complete this journey.

Thank you all for your contributions and support. This work would not have been possible without you

Ammu Alphonsa John

TABLES OF CONTENTS

CHAPTER	TITLE	PAGE NUMBER
1	INTRODUCTION	17
2	REVIEW OF LITERATURE	22
3	MATERIAL AND METHODS	53
4	RESULTS	74
5	DISCUSION	144
6	SUMMARY	161
7	REFERENCES	166
8	ABSTRACT	200

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
1	Components of PCR reaction	60
2	SSR Markers and details of annealing temperature	61
3	List of SSR primers used for selection	61
4	Temperatures used to standardize the critical temperature	62
5	Particulars of Experiment III	63
6	Pollen germination%, Photosynthetic rate, stomatal conductance & RWC in F2 generation	78
7	Chlorophyll content, CSI & % leakage of F2 generation	79
8	Pollen germination%, Photosynthetic rate, stomatal conductance & RWC in F3 generation	81
9	Chlorophyll content, CSI & % leakage of F3 generation	82
10	Pollen germination%, Photosynthetic rate, stomatal conductance & RWC in F4 generation	86
11	Chlorophyll content, CSI & % leakage of F4 generation	89
12	Pollen germination (%) of ten tomato varieties after 2 hrs incubation at different temperatures	93
13	Number of clusters in the selectively fertilized and normal crossed tomato plants	97 -98
14	Number of flower clusters per plant in the selectively fertilized and normal tomato plants	100-101
15	Number of fruits per clusters in the selectively fertilized and normal tomato plants	102-103
16	No. of days to first flowering after sowing in selectively fertilized and normal tomato plants	106-107
17	Percentage of flowers with exerted stigma in selectively fertilized and normal tomato plants	108-109
18	Stigma exertion length in selectively fertilized and normal tomato plants	112-113
19	Photosynthetic rate (µmole CO_2 m^{-2} s^{-1}) of selectively fertilized and normal tomato plants.	116-117
20	Relative water content (%) of selectively fertilized and normal tomato plants	119-120

21	Membrane integrity (% leakage) of selectively fertilized and normal tomato plants	122-123
22	Vitamin C (µg g^{-1}) in selectively fertilized and normal tomato plants	126-127
23	SOD activity (activity g^{-1} min^{-1}) of selectively fertilized and normal tomato plants	128-129
24	Pollen viability of selectively fertilized and normal tomato plants	131-132
25.	Total yield/ plant (g) in selectively fertilized and normal crossed tomato plants	134-135
26.	Pearson correlation matrix	138-139

LIST OF PLATES

PLATE NO.	TITLE OF PLATES	PAGE
1.	Selectively fertilized fruit	56
2.	Varieties used for pollen selection	65
3.	Plants grown for pollen selection	66
4.	Tolerant and susceptible varieties for selective fertilization	66
5.	Emasculated plants for selective fertilization	69
6.	Tagged plants for selective fertilization	69
7.	Seedlings planted for evaluation under ROS condition	70
8.	Evaluating the performance of selectively fertilized and normal crossed hybrids in rainout shelter	70
9.	Selectively fertilized plant and fruit of F_2 generation	77
10.	Gel image of SSR markers in selectively fertilized F_2 tomato plants	80
11.	Selectively fertilized plant and fruit of F_3 generation	83
12.	Gel image of SSR markers in selectively fertilized F_3 tomato plants	84
13.	Selectively fertilized plant and fruit of F_4 generation	87
14.	Gel image of SSR markers in selectively fertilized F_4 tomato plants	88
15.	Pollen germination of tolerant tomato genotypes	91
16	Pollen germination of susceptible tomato genotypes	92
17	a) Stigma exertion length in normal hybrid	115
	b)Stigma exertion length in selectively fertilized hybrid	115
18	Pollen viability of selectively fertilized (AN x MP) (SF)and normal (AN x MP) tomato plants	133

19	Pollen viability of PR x VV (normal) and PR x VV (SF) (selectively fertilized) tomato cross.	133
20	Sectional view of the anther of AN X MP and AN X MP (SF)	140
21	Sectional view of the anther of AN XAS and AN X AS (SF)	140
22	SEM of pollen sac of SF and normal hybrids	141
23	Sectional view of the anther of AS X AN and AS X AN (SF)	141
24	Pollen grains of selectively fertilized and normal tomato plants	142
25	Surface structure of anther in selectively fertilized and normal tomato plants	142

LIST OF FIGURES

FIG NO.	TITLE OF FIGURE	PAGE
1.	Pollen germination of tomato genotypes at different temperature	95
2.	Critical sterility temperature graph	96
3.	No of clusters in selectively fertilized and normal tomato plants	96
4.	No of flower per clusters in selectively fertilized and normal tomato plants.	105
5.	Percentage of flowers with exerted stigma (Stigma exertion %) of selectively fertilized and normal	110
6.	Photosynthetic rate of selectively fertilized and normal to	118
7.	RWC of selectively fertilized and normal tomato plants	121
8.	% Leakage in selectively fertilized and normal tomato plants	124
9.	Yield per plant in selectively fertilized and normal tomato plants	136

LIST OF ABBREVIATIONS

A	Absorbance
AOS	Active oxygen species
ATP	Adenosine triphosphate
AGL6	AGAMOUS-LIKE 6
AFLP	Amplified fragment length polymorphism
AN	Anagha
AOXs	Antioxidant enzymes
AP2	APETALA2
AS	Arka saurabh
APX	Ascorbate peroxidase
ASC	Ascorbic acid
BOD	Biochemical oxygen demand
Ca^{2+}	Calcium ion
CO_2	Carbondioxide
CAT	Catalase
CMT	Cell membrane thermostability
cm	Centimeter
CTAB	Cetyltrimethylammonium bromide
Fv/ Fm	chlorophyll fluorescence
CSI	Cholorophyll stability index
CLV-WUS	CLAVATA-WUSCHEL
CRD	Completely randomized design
COI 1	CORONATINE-INSENSITIVE 1
et al.	Co-workers/ Co-authors
CD (0.05)	Critical difference at 5% level
CTRI	Cumulative temperature response index
DAT	Days after transplanting
^{o}C	Degree celcious

dNTP	Deoxynucleotide triphosphate
DNA	Deoxyribonucleic acid
DCPIP	Dichlorophenol 2 indophenol
DMSO	Dimethyl sulfoxide
E	East
EC	Electrical conductivity
ER	Endoplasmic Reticulum
EDTA	Ethylenediamine tetraacetic acid
FYM	Farm yard manure
Fig.	Figure
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GWAS	Genome-wide association studies
G × E	Genotype by environment
GA_3	Gibberellic acid
GSH	Glutathione
GR	Glutathione reductase
g	Gram
GPX	Guaiacol peroxidase
HS	Heat stress
HSP	Heat stress protien
Hsfs	Heat stress transcription factors
hrs	Hours
H_2O_2	Hydrogen peroxide
IIHR	Indian Institute of Horticultural Research
IPCC	Intergovernmental Panel on Climate Change
JA	Jasmonic acid
KAU	Kerala Agricultural University

kV	Kilovolts
LHCII	Light harvesting complex II
Mg^{2+}	Magnesium ion
MDA	Malondialdehyde
MP	Manuprabha
MABC	Marker-assisted backcrossing
CH_4	Methane
MT	Metric tonne
µm	Micro molar
ml	Millilitre
mm	Millimetre
MAGIC	Multi parent advanced generation intercross
viz.,	Namely
N_2O	Nitrous oxide
N	North
OSMs	Osmolytes
O_2	Oxygen
O_3	Ozone
g^{-1}	Per gram
%	Percentage
POX	Peroxidase
PSI	Photosystem I
PSII	Photosystem II
PI	PISTILLATA
PEG	Poly ethylene glycol
PCR	Polymerase chain reaction
PR	Pusa Rohini
QTL	Quantitative Trait Locus

ROS	Rainout shelter
ROS	Reactive oxygen species
RWC	Relative Water Content
RFLP	Restricted fragment length polymorphism
Rubisco	Ribulose bisphosphate carboxylase oxygenase
rpm	Rotations per minute
RA	Rubisco activase
SEM	Scanning electron microscope
STK	SEEDSTICK
SF	Selective Fertilization
SEP1	SEPALLATA1
SHP1	SHATTERPROOF1
SMs	Signaling molecules
SNPs	Single nucleotide polymorphisms
SSR	Single sequence repeat
SOD	Superoxide dismutase
S X T	Susceptible genotype as male parent
i.e.	That is
T X S	Tolerant genotype as male parent
TGL11	TOMATO AGAMOUS LIKE11
TAP3	TOMATO APETALA3
TM6	TOMATO MADS BOX GENE6
TFs	Transcription factors
TTS	TRANSMITTING TISSUE SPECIFIC
TDA buffer	Tris-acetate-EDTA buffer
UPR	Unfolded protein response
VV	Vellayani vijay
V	Volume

INTRODUCTION

1. INTRODUCTION

The rise in ambient temperatures due to climate change is expected to have a significant impact on plant growth and development, leading to a sharp decrease in crop productivity and potentially causing severe famine. The continual increase in average surface temperature as a result of global warming poses a significant stress to all phases of plant growth and development, particularly affecting metabolism and productivity, especially in tropical and subtropical regions (Li *et al.,* 2018). According to the sixth assessment report from the IPCC (2021), temperatures are projected to increase by 1.5 ^{0}C in the twenty-first century over the next two decades, and could rise by 4.5 ^{0}C depending on greenhouse gas emission rates. Plants, being stationary organisms, are often negatively affected by unfavourable weather conditions such as drought and extreme temperatures that exceed their ideal range. The populations mostly reliant on agriculture for their livelihoods, constituting 75% of the rural population globally and nearly 50% of those in less developed countries. Furthermore, a 70% increase in food production will be necessary to meet future demands driven by a projected global population growth to 9 billion by 2050 and increasing food consumption in rapidly growing economies such as China and India (Hoshikawa *et al.,* 2021). The formidable challenge of increasing food production while anticipating significant crop losses due to climate change can only be addressed through the development of more sustainable agricultural production systems utilizing crop varieties that are more resilient to environmental stressors than those currently in use.

As immobile organisms, plants respond to Heat stress through avoidance mechanisms or programmed cell death (Zhang *et al.,* 2020). Each type of vegetable crop has its own temperature threshold for growth and development, with Heat stress occurring when the temperature exceeds the upper threshold (Wahid *et al.,* 2007). Generally, Heat stress is considered to occur when the ambient temperature exceeds the ideal temperature range for plant cultivation by 10-15 degrees Celsius (Wahid *et al.,* 2007). Heat stress, resulting from temperature increases caused by climate change, has detrimental effects on all stages of crop growth, leading to permanent damage. It can significantly impact plant morphology, development, physiology, biochemistry, and molecular pathways. Temperature fluctuations also have a significant influence on

anther and pollen development during flowering, leading to failures in reproduction and fertilization (Peet *et al.,* 1998; Erickson and Markhart, 2002). Consequently, there is reduced fruit set and lower-quality fruit and vegetable yields due to adverse effects on reproduction and fertilization processes (Bita and Gerats, 2013; Hasanuzzaman *et al.,* 2013).

The tomato (*Solanum lycopersicum* L.), a fruit vegetable crop, has enormous economic and cultural significance worldwide. It is grown in diverse climates worldwide, spanning from tropical regions to areas near the Arctic Circle. High temperature negatively affects fertilization and reproduction in tomato plants, leading to crop failure and reduced fruit quantity and quality (Prasad *et al.,* 1999; Sato *et al.,* 2000). During cultivation, tomato plants experience varying temperature conditions, resulting in different physiological and morphological changes based on genetic variations, growth stages, and duration of exposure to Heat stress. These changes are not limited to vegetative parts such as leaves only (Zhou *et al.,* 2017) but also affect reproductive organs like flowers and gametophytes (Firon *et al.,* 2006). Improving the Heat stress tolerance of crops is crucial for addressing climate change, as even mild Heat stress can impact fruit development and quality in crops like tomatoes.

According to Dane *et al.* (1991), the tolerant genotypes often maintain higher levels of pollen viability under high temperatures. Evaluating pollen viability can demonstrate the male gametophytic ability to endure high temperatures. If genes related to stress tolerance during the pollen phase also evident in the sporophyte, it may be possible to transfer these traits into usable cultivars through pollen selection. Selective fertilization using pollen viability as a screening method may aid in transmitting heat tolerance into parental lines for hybrid generation. Selective fertilization targets specific physiological or genetic traits at the fertilization stage and also involves controlled pollination, often between specific parental lines, to enhance desired traits such as heat tolerance, disease resistance, or yield. It also utilizes molecular or physiological markers to select the best combinations even before the seeds are fully developed. Regarding tomato performance under Heat stress, high temperatures can make pollen grains vulnerable, leading to reduced tomato fruit yield (Camejo *et al.,* 2005). Therefore, screening for pollen viability under high temperature can provide crucial

information on male gametophytic resistance to high temperatures, which is necessary for developing high-temperature tolerant genotypes. Selection at the pollen level has been proposed as a strategy to increase the frequency of genes associated with beneficial agronomic traits. Selective fertilization is a novel technique for producing hybrids by artificially applying selection pressure during pollen germination and fertilization, allowing only the pollen grains tolerant to the selection pressure to germinate and fertilize the ovule.

Understanding the genotypes possessing the necessary traits and comprehending how these genotypes handle stress are crucial for producing crops tolerant to high temperatures. The capacity of a genotype to promptly and effectively respond to stressful situations through molecular, biochemical, and physiological reactions which ultimately lead to overall plant responses is known as stress tolerance. The primary adaptive response of plants to high-temperature stress is through the production of heat shock proteins. The expression of HSPs during stress provides plants with tolerance by altering various plant functions, such as water and nutrient usage efficiency, membrane integrity, photosynthesis, and assimilate partitioning (Camejo *et al.,* 2005; Ahn and Zimmerman, 2006; Momcilovic and Ristic, 2007). These mechanisms enable plants to develop and grow under Heat stress. Analysing these processes in stress-tolerant genotypes is essential to establish any genotype as genuinely high-temperature stress-tolerant. Understanding the mechanisms that enable plants to thrive and produce under stressful conditions is key to breeding more stress-tolerant varieties.

Given the projected rise in temperatures and the global significance of tomato cultivation, it is imperative to facilitate the adaptation of tomato to high temperature conditions. In order to achieve this, it is essential to identify tomato genotypes that are tolerant to temperature stress and to comprehend how crops respond to high temperatures due to climate change, through the study of the physiological and molecular responses of tomato to high temperature stress. When producing high-yielding heat-tolerant varieties/cultivars, it is important to consider physiological traits such as the stay-green trait, canopy temperature depression, cell membrane thermostability, chlorophyll fluorescence, relative water content, and stomatal

conductance. Molecular approaches, including omics, molecular breeding, and transgenics, possess the potential to enhance heat tolerance. This can be achieved through the transfer of heat-tolerant genes/QTLs to elite cultivars using molecular markers or by elucidating tolerance mechanisms, ultimately leading to the identification and transfer of heat tolerance genes via genetic modifications. Alongside these approaches, simple agronomic strategies are crucial for mitigating the effects of Heat stress at the grassroots level. Therefore, it is critical to establish heat-tolerant plant varieties through physiological, molecular, and breeding-based strategies to ensure the sustainability of vegetable production systems and human health. Selective fertilization is a promising approach for developing high-temperature-tolerant crop varieties due to its ability to enhance specific genetic and physiological traits that confer resilience to Heat stress and also it bridges critical gaps in breeding strategies under Heat stress by addressing limitations in traditional and modern approaches, providing a targeted pathway to develop resilient varieties. Additionally, these approaches will provide valuable insights into the physiological and molecular mechanisms responsible for thermotolerance, thereby opening the door for the development of 'designer' vegetable crops to enhance health and nutritional security. Hence, the present study aims to evaluate the impact of selective fertilization in inducing heat tolerance and the physiological and molecular responses of tomato under high temperature conditions.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Tomato (*Solanum lycopersicum* L.) is one of the members of the Solanaceae and an important commercial crop around the world (Deuter *et al.,* 2012). The Food and Agriculture Organization (FAO) reported that in 2019, global tomato production reached 182 million tons on 4.85 million acres, with China, India, USA, Turkey, and Egypt leading production (FAOSTAT 2020). This makes tomato the second most crucial vegetable crop after potato. Tomatoes are highly valued for their nutritional and economic importance, containing vitamins, minerals, antioxidants, and phytochemicals that benefit human health (Shi *et al.,* 2019). Additionally, tomato serves as a model plant for studying various aspects of plant biology, including fruit development, ripening, disease resistance, and stress tolerance (Gupta *et al.,* 2019). However, tomato production and quality are negatively impacted by various abiotic stresses such as drought, salinity, cold, and heat (Gupta *et al.,* 2019). It is estimated that the world's harvested area increased by 10.4% from 2010 to 2014, increasing from 4.5 million hectares to 5.02 million hectares; yields from the harvested areas were 151.9 million kilotonne in 2010 to 1.71 billion kilotonne in 2014, yielding 34 MT (metric tonne) per hectare in 2014. Despite this, global tomato production per unit area has decreased. Tomato production is affected by both biotic and abiotic factors, with abiotic stresses often interconnected and occurring individually or in combination.

Abiotic stress leads to morphological, physiological, biochemical, and molecular changes that negatively affect plant growth, productivity, and yield. Rising temperatures (heat), drought, cold, and salinity are the primary abiotic stresses causing severe cell damage in wild and domesticated plant species, and are considered consequences of climate change (Bita and Gerats, 2013). Abiotic stressors, particularly high temperatures (Heat stress (HS)), significantly impact crop quantity, quality, nutritional status, and yield (Boote *et al.,* 2005; Aleem *et al.,* 2021). Climate change is expected to directly influence the incidence and severity of crop diseases, posing serious threats to food security (Gautam *et al.,* 2013; Kurukulasuriya and Rosenthal, 2013; Ali *et al.,* 2019). Potential climate change-related factors such as temperature fluctuations, precipitation changes, increased concentrations of CO2, CH4, nitrous

oxide (N2O), and O3, long-term water scarcity, unsuitable soil conditions, drought, desertification, and disease and pest outbreaks in crops and livestock are anticipated to significantly impact plant growth. The Fifth Assessment Report of the International Panel on Climate Change (IPCC) indicated that climate change over the past 30 years has resulted in a 1.5% per decade decline in global agricultural production compared to a baseline without climate change. Recent studies suggest that even a 2^{o} increase in global temperature will affect agricultural productivity, with a continuous and more pronounced effect in tropical regions.

Heat stress is a major limiting factor in plant productivity. Heat stress can be defined as a rise in temperature above a threshold level for a period of time that causes irreversible damage to plant growth and development (Wahid *et al.,* 2007). Based on multiple scenarios, global temperatures are projected to increase by the end of the 21st century (2081–2100) on average in the range of about 1°C to 3.7°C compared to their 1986–2005 levels [IPCC,2014]. Although an increase in temperature may be beneficial in some regions, crop yields are expected to decline unless adaptation strategies are implemented. Tomatoes are particularly sensitive to Heat stress, which can lead to a complete loss of yield. Heat stress can occur at different stages of plant life cycle, such as germination, vegetative growth, flowering, fruit development, and ripening. The threshold temperatures required for causing damages in reproductive tissues are lower than those required to harm vegetative tissues, making the reproductive stage of tomato and many other crops, the most susceptible to Heat stress (Sato *et al.,* 2006). Male gametophytes have lower threshold temperatures than vegetative tissues, making them more susceptible to HS than female gametophytes. Various aspects of tomato growth and development during the reproductive stage can be negatively affected by Heat stress. This can occur before or after pollination, impacting fertilization and reducing seed production (Sage *et al.,* 2015). Pre-pollination events that are particularly vulnerable to high temperatures include meiosis I and II of the microspore mother cell (Young *et al.,* 2004), the formation and subsequent disintegration of the tapetum layer (Farooq *et al.,* 2017), and the creation of exine and intine (Nahar *et al.,* 2016). Heat stress also influences post-pollination processes such as pollen load, germination, tube growth, and fertilization (Hedhly *et al.,* 2009; Sita *et al.,* 2017).

Numerous studies have examined the effects of Heat stress on the reproductive phases of different vegetative crops. In capsicum, Erickson and Markhart (2002) found that Heat stress resulted in vacant, small microspores lacking an exine layer. The appearance of shriveled pollen grains under Heat stress may be attributed to decreased starch accumulation in anther walls and pollen grains, which reduces soluble carbohydrates necessary for their development (Pressman *et al.,* 2002). Heat stress in soybean led to decreased pollen production, germination, and tube elongation, as well as abnormal pollen development characterized by missing apertures and disrupted exile ornamentation (Salem *et al.,* 2007; Nahar *et al.,* 2016). In tomato crops, Heat stress interfered with meiosis, causing deterioration in pollen germination and tube growth (Foolad, 2005). Additionally, Heat stress reduced microgametophyte fertility in brassica (Rao *et al.,* 1992). Heat stress can also induce physiological disorders in tomato fruits, including blossom-end rot, sunscald, cracking, and irregular ripening (Alsamir *et al.,* 2021). Furthermore, it can modify the biochemical composition of tomato fruits, affecting sugars, acids, pigments, aroma volatiles, and antioxidants (Shi *et al.,* 2019). Tomato plants employ a range of cellular, physiological, and molecular responses to cope with Heat stress. These mechanisms aim to safeguard cellular structures and functions from heat damage, maintain water and ion homeostasis, regulate the expression of heat-responsive genes, and enhance Heat stress tolerance (Hoshikawa *et al.,* 2021). Such responses encompass the production of heat shock proteins (HSPs), activation of antioxidant enzymes (AOXs), accumulation of osmolytes (OSMs), regulation of transcription factors (TFs), and involvement of signalling molecules (SMs).

Heat stress can be caused by different factors that affect the ambient temperature or the plant temperature. The ambient temperature is the temperature of the surrounding air, which can be influenced by the solar radiation, the wind speed, the humidity, and the cloud cover (Wahid *et al.,* 2007). The plant temperature is the temperature of the plant tissues, which can be influenced by the ambient temperature, the transpiration rate, the leaf size and shape, the leaf orientation and angle, and the leaf color and reflectance (Wahid *et al.,* 2007). The optimal temperature range for tomato growth and development is 20–25°C for day and 15–20°C for night (Dhaliwal*,* 2012). However,

the optimal temperature range may vary depending on the cultivar, the growth stage, and the duration of exposure. Generally, Heat stress can be classified into two types: chronic Heat stress and acute Heat stress (Wahid *et al.,* 2007). Chronic Heat stress is a long-term exposure to moderately high temperatures that exceed the optimal range but do not cause immediate damage to the plant tissues. Acute Heat stress is a short-term exposure to extremely high temperatures that exceed the critical threshold and cause irreversible damage to the plant tissues.

A rise of a few degrees (1°C) above an average daily temperature of 25°C can severely affect reproductive organs, particularly pollen viability and female fertility, resulting in drastic declines or even complete failure of fruiting (Ayanan *et al.,* 2019). As a result, Heat stress reduces the tomato growing window (the number of days per year with optimal temperatures for tomato production), particularly under outdoor and uncontrolled growing conditions, which are the dominant tomato growing systems in tropical regions. In these regions, Silva *et al.* (2017) predicted that over 2050-2100, heat and drought stress would negatively affect tomato growth and yield in open fields and reduce the optimal production area. Globally, there are calls for the development of heat-tolerant cultivars to adapt to current and projected increases in Heat stress (Bita *et al.,* 2013). However, breeding for heat tolerance in general has been hampered by the complexity of Heat stress and plant responses to the stress (Hedhly, 2011), as well as the limited understanding of the genetic basis of heat tolerance traits. The success of breeding for heat tolerance depends on the efficient identification and characterization of the component traits underlying fruit set under Heat stress and the depth of knowledge of their genetic architecture at both the vegetative and reproductive stages (Driedonks, 2018). Heat stress is determined by the intensity, duration and speed of the temperature increase (Wahid *et al.,* 2007). For effective screening of the germplasm for heat tolerance, the development of an appropriate technique to apply Heat stress is important. Mesihovic *et al.* (2016) identified four main thermotolerance regimes: short-term acquired thermotolerance, long-term acquired thermotolerance, basal thermotolerance, and thermotolerance to moderately high temperatures. Two categories of heat treatments are generally used in the screening of germplasm and in the study of the physiological responses of tomatoes under heat tolerance. The first is long-term

mild heat screening, in which plants are exposed to heat for a long period of time, which can span the entire development cycle. Mild Heat stress commonly applied to tomatoes consists of growing the plants at optimum temperatures until the first bud bud appears and then moving them to high temperature grow chambers (around 32°C/28°C, day/night temperatures). However, Heat stress from the flowering phase onwards may not cover the full range of plant responses to heat: although the reproductive stages are the most sensitive to Heat stress in many plants, vegetative stages are also negatively affected by Heat stress (Wahid *et al.,* 2007). For example, the rate of photosynthesis, maximum photosystem II quantum efficiency (Fv/Fm) and stomatal conductance decreased under heat which could result in decreased growth rate stress (Zhou *et al.,* 2018). So prolonged exposure to Heat stress (from pollen selection to maturity) may increase severity and trigger different response mechanisms than Heat stress applied at a particular developmental stage. In order to adapt to current and future Heat stress, there is an urgent need to develop heat tolerant cultivars as this is a major limiting factor in plant productivity.

2.1 HEAT TOLERANCE

Tolerance to high temperature is a difficult trait to enhance due to its low heritability and sensitivity to the environmental conditions (Hazra *et al.,* 2009). Crop adaptation or resistance to environmental pressures can be managed using several strategies in agricultural systems. In general, a combination of genetic development and cultural practices reduces the detrimental effects of abiotic stresses on agricultural productivity (Wahid *et al.,* 2007). Adjustment or changes in cultural practices, such as planting time, crop density, and soil and irrigation management, can reduce stress impacts. For example, Hanna *et al.* (1997) identified development and yield reactions of heat resistant tomato to depth of transplant, daily irrigation time, and polyethylene mulch color. Morning irrigation increased marketable and total yields, average fruit mass in 1994, and crop dry mass in 1995. White-surface mulch had an equivalent effect on fruit mass and yield. They concluded that deeper transplanting, morning irrigation, using white-surface polyethylene mulch, or a combination of all three can increase the yield of heat-resistant tomatoes.

The advancement of cultivars that are resilient to environmental stressors and produce economic yield is referred to as genetic improvement. But one economically stable way to produce plants under harsh conditions is for crops to genetically develop toward stress tolerance (Blum, 2018). Additionally, hybrid lines appeared to function consistently, even in stressful situations, as compared to ideal growing conditions (Yordanov, 1983). In the breeding of tomato heat resistance, both conventional and hybrid breeding technique, which benefit on genetic interactions and additively acting genes, should be helpful. The results of the previous study showed that around one-third of the diallel hybrid progenies had greater fruit set than their more heat-resistant parents, indicating the benefits of hybrid breeding (AVDRC, 1986). In a separate study, Opena *et al.* (1987) found that crosses made from heat-resistant stocks produced more fruit with greater setting capability and yield than those made from heat-sensitive parents in the diallel test. According to Bhattarai *et al.* (2016), Heat tolerant plants can be developed by the collective accumulation of heat tolerance from the yield attributing traits which is modified through indirect selection during the early generations. Being able to develop fruit when exposed to high temperatures is the most fundamental requirement for the resistance of tomato to heat stress, however evaluating germplasm in the field for heat tolerance is extremely time-consuming and expensive (Abdulbaki *et al.,* 1995).

Conventional breeding is the oldest but most widely used strategy, and it is mostly based on selecting phenotypic plant characteristics (Acquaah, 2015). Growing breeding materials in a hot target production environment and detecting individuals/lines with greater yield has been a traditional method of selecting crops for heat stress resistance (Ehlers and Hall, 1998). A proposed strategy has been discovered in crop growth selection criteria that can be linked to heat tolerance during reproductive stages. In tomato, there is a strong positive link between yield and fruit set at high temperatures. As a result, screening for fruit set under high temperature has been used to estimate germplasm to detect sources of heat resistance (Berry and Rafique-Uddin, 1988). Non-reproductive trends that are altered by high temperature include photosynthetic efficiency, assimilate translocation, mesophyll tolerance, and cellular membrane disintegration (Chen *et al.,* 1982). In recent decades, new procedures for

screening superior varieties have arisen that combine morpho-physiological plant traits with traditional breeding methods. These strategies take advantage of inherent plant capabilities to cope with HS and aid in the selection of heat-tolerant genotypes. Breeding to develop such properties under high temperatures can lead to improved cultivars with heat resistance techniques.

Heat tolerance in tomato could be significantly improved through genomic selection. Understanding the nature and magnitude of gene actions is important for the selection of suitable breeding strategies and parental lines to consider the extent and severity of gene behavior of heat resistance traits and subtraits in a breeding program. Based on the germplasm under consideration, heat tolerance traits were found to be regulated by additive, domination, and epistatic gene effects, with a predominance of one of these effects. Ahmed *et al.* (2013) observed that primarily additive gene effects dominated the inheritance of pollen fertility and fruit set. Similarly, additive gene effects were prominently regulated by the inheritance of fruit collection, fruit number per vine, brix, fruit weight, number of flowers per cluster. In fruit set inheritance, complete dominance was reported and fruit weight showed substantial heterosis under heat stress.

Tomato Heat stress transcription factors (Hsfs) are critical components that act either as activators or repressors in mediating heat tolerance. Tomato HsfA2 accumulates in response to increased temperatures and enhances the capacity of male reproductive tissues to cope with severe heat stress. HsfB1, on the other hand, acts as a repressor of heat tolerance mechanism and its suppression in transgenic tomato plants resulted in increased heat tolerance. These genes could potentially be candidates for site-directed mutation to improve heat tolerance in tomato. Once a candidate gene is cloned and a favorable allele identified, one can harness the large number of re-sequenced accessions to identify interesting lines carrying favorable alleles to be evaluated under heat stress. An integrated strategy involving plant breeding, genetic modification, and gene editing is expected to influence the production of food crops. Heat tolerance mechanisms increases with the dissection of the genetic architecture and genes that control heat tolerance, the potential of targeted mutation on these genes

would provide useful tools for rapid monitoring of heat tolerant variety growth. Also tomato heat stress transcription factors (Hsfs) are critical components that act either as activators or repressors in mediating heat tolerance. So these genes could potentially be candidates for sited directed mutation to improve heat tolerance in tomato (Ayenan *et al.,* 2019).

Pollen selection has also been proposed as a method to increase the gene frequencies associated with useful agronomic traits. Pollen that is exposed to thermal stress during germination may represent a potent means of altering the genetic structure of the resulting progeny by turning the allelic frequencies in favor of high tolerance (Zamir, 1983). In comparison to the commercial variety, the pollen from cold-tolerant variety of *Lycopersicon hirsutum* L. had higher germination and fertility, and it was more functional under cold conditions (Zamir *et al.,* 1981). This successfully transferred pollen to the commercial cultivar suggested that the gene that allows the sporophyte to tolerate high temperatures also allows the pollen to retain fertility after thermal stress (Rodriguez-Garay and Barrow, 1988). Weaver and Timm (1989) found that flowering plants exposed to high temperatures in greenhouses showed a positive link between pollen viability and temperature tolerance. To be successful in expanding agricultural productivity under stress conditions, both genetic advances and cultural practice adjustments must be made simultaneously (Wahid *et al.,* 2007).

2.2 SELECTIVE FERTILIZATION

Plants have two distinct stages in their lifecycle: the gametophyte stage and the sporophyte stage. Selection during the gametophyte phase of the plant life cycle has important effects on both gene and genome evolution and is likely to have important pleiotropic effects on the sporophyte. 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. Gametophytic selection and its fitness effect in sporophytes remains difficult despite over a century of research, there is a possibility of conflict between pollen and sporophytes.

As the pollen life cycle is simple it got attention of artificial selection for improving yield and there are findings indicating variations in offspring quality as a result of pollen competition. The notion that genes overexpressed in pollen are more likely to have fitness impacts on gametophyte transmission is consistent with a recent research in maize that combines knockout mutations with data on gene expression (Warman *et al.,* 2020). According to Hormaza and Herrero (1992), artificial selection on particular characteristics during pollen-tube growth has effectively altered the values of sporophyte traits under various environmental circumstances, including salt tolerance, toxin tolerance, and metal tolerance. More recent studies using gametophytic selection have created novel varieties resistant to two stressors in three domesticated species: Alternaria leaf blight resistance in cultivated sunflower (Shobha and Ravikumar, 2006) and cold tolerance in tomato and chickpea (Clarke *et al.,* 2004; Dominguez *et al.,* 2005). Genome-wide association studies (GWAS) and quantitative trait loci (QTL) were used to identify variant haplotypes of a cellulose synthase gene in cultivated varieties of chickpeas, which were highly related with increases in pod and seed number as well as quicker pollen-tube development (Kujur *et al.,* 2015). These chickpea gametophyte QTL had a major impact on sporophyte female fitness not because quicker gametophytes are typically more advantageous, because the gene is expressed in both pollen and seed pods. These results imply a shared genetic foundation.

Several studies have been conducted regarding the effect of gametophyte (pollen) selection in temperature stress. According to Zamir *et al.* (1981), *Lycopersicon hirsutum*, which can tolerate cold temperatures during the gametophytic stage, may also tolerate chilling during the sporophytic stage. In an experiment by Peet *et al.* (1998) under eat stress ($32/26^0$C) and under normal conditions ($28/22^0$C) studied that the male sterile plants grown in the stressed condition produced less fruit set and yield when pollinated with pollen from the control condition, whereas male sterile plants grown in the stressed condition produce no fruit set when crossed with pollen from the stressed condition. The outcome demonstrated that fruit set is impacted by male gametophytic selection and the highest impact occurs during the reproductive stage when the male parent of a cross is subjected to heat stress than the female parent because male

gametophytes are more susceptible to Heat stress than female gametophytes. The anther cells reflect on from a loss of male identity under long-term moderate Heat stress is the most current idea explaining the heat sensitivity of pollen (Muller *et al.,* 2016). Marine *et al.* (2017) also examined that the pollen viability can be used as a screening technique to examine the male gametophytic resistance of tomato genotypes to extreme temperature stress.

Selective fertilization is a modern breeding technique that involves targeted fertilization to combine specific traits, often under controlled conditions. It emphasizes the selection of desirable physiological, biochemical, or genetic traits during the fertilization stage, making the process highly precise. It 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. Abiotic stresses like water stress, salinity, temperature, pH, heavy metals etc. has been successfully deliberate using selective fertilization in many studies. Successful use of the selective fertilization approach has been documented in a variety of crops, including tomato (Zamir *et al.,* 1987), cotton (Rodriguez-Garay and Barrow, 1988), and coconut (Aisha *et al.,* 2015, Afna *et al.,* 2023). The selective fertilization is a best technique as it differ from conventional breeding approaches that relies on crossing two parent plants and selecting the offspring that express desirable traits over several generations. This method is less precise as it depends largely on phenotypic selection, which can be influenced by environmental factors and natural genetic variability.

2.3 GROWTH PARAMETERS

Environmental changes have a significant impact on the polygenic characteristic of heat tolerance (Blum, 2018). The response at one stage varies from the response at another, hence HS effects are stage-specific. Breeders use a variety of strategies to lessen the effects of an unpredictably changing environment on crops. An accurate understanding of the interactions between the crop and the stress atmosphere can be revealed by observing changes in the phenology of the crop in response to heat stress. The susceptibility of various phonological processes to high temperatures varies,

although this depends on the species and variations (Wollenweber *et al.,* 2003; Howarth, 2005).

2.3.1 Pollen Germination Percentage

The pollen development phase is the primary determinant of fruit production and the most sensitive process in plants. Elevated temperatures have a significant impact on pollen formation, leading to significantly lower pollen germination rates (Rieu *et al.,* 2017). Insufficient pollen germination is a major contributor to low fruit set. As temperatures rise progressively from the optimum, the germination of pollen grains decreases by 13 times (Pressman *et al.,* 2002). heat stress adversely affects pollen meiosis and germination, ovule development, and the improvement and viability of the embryo (Peet *et al.,* 1997). Heat stress impairs assimilate transfer in pollen, resulting in poor germination, as starch accumulation is necessary for pollen maturation to provide energy for germination and tube expansion (Filomena *et al.,* 2013). Additionally, sucrose and hexose, which also act as energy sources for pollen growth and germination, function as osmolytes (Pressman *et al.,* 2002). In addition, Foolad (2005) also mentioned that high temperatures in tomatoes improperly affect endosperm development, pre-embryo, and fertilized embryos, meiosis in male and female organs, pollen germination and pollen tube development, ovule viability, style and stigmatic situations, pollen grain number that is maintained by the stigma, fertilization and post-fertilization trends.

The ability of pollen to germinate and release under high temperatures is a crucial indicator of fruit set potential. Thus even if the pollen is viable, a failure in pollen germination or release can prevent the development of a fruit set (Sato *et al.,* 2000). Therefore, the ability to germinate and release pollen can serve as an appropriate benchmark for determining a crop's sensitivity to high temperatures, and this is used as a standard in breeding programs to choose heat-resistant varieties (Comlekcioglu and Soylu, 2010). According to Zhou *et al.* (2015), pollen tube growth in the heat-tolerant genotype was unaffected by heat stress and resulted in higher fruit set than the control. Heat-tolerant genotypes produce more pollen grains than those that are not heat-tolerant. The volume of released pollen grains decreases under high temperature stress

in both heat-sensitive and resistant genotypes, but the decrease is more significant in the susceptible ones (Sato *et al.,* 2006). Bita *et al.* (2011) observed that the germination percentage and tube growth of pollens collected from non-stressed conditions in both heat-tolerant and sensitive genotypes are very high. However, when exposed to mild heat stress conditions, heat-sensitive genotypes exhibit slightly smaller flowers with malformed anther cones and significantly reduced pollen germination.

2.3.2 Stigma Exertion

Tomato is an autogamous species with a floral structure suitable for self-pollination due to the anther cones (stamens) covering the style (also known as the stigma or pistil). Various abiotic stimuli, such as heat stress during bud development, significantly affect the position and maturity of the male (anther cone) and female (style) organs in tomato flowers, resulting in stigma (style) exertion (Pan *et al.,* 2019). High temperatures during the reproductive stage notably cause the style to extend beyond the anther cone, potentially hindering self-pollination (Faruq *et al.,* 2012; Giorno *et al.,* 2013). According to Pan *et al.* (2019), the exertion of tomato stigmas caused by heat stress is associated with JA signaling and various other variables and mechanisms. It was observed that stamens are more susceptible to heat stress than the pistil, leading to differences in cell morphology and resulting in stigma exertion in the presence of high temperature. Heat stress controls cell shape and number, affecting the divergent coregulation of pectin, sugar, expansion, and cyclin in both stamens and pistils. Both stamens and pistils require auxin to regulate growth inhibition induced by high temperature. The JA/JA receptor CORONATINE-INSENSITIVE 1 (COI1) signaling pathway plays a significant role in stigma exertion, and Jasmonic acid is crucial for protecting pistils against high temperature (Pan *et al.,* 2019).

Fruit setting ability is impacted by the stigma and style exertion in high temperatures. Under high temperatures, tomato cones split and the stigma tube grew longer. In most flowers, unnecessary style extension decreased pollen access to the stigma in genotypes that are susceptible to heat and decreased fertilization (Alsamir *et al.,* 2017). The genotypes that develop flowers at high temperatures with no stigma exertion are stable and provide a lot of fruit. The main factors affecting reproductive

success at high temperatures are the survival of male and female gametes and the degree of style protrusion (Bhattarai *et al.,* 2016). Fruit setting has a lower heredity than style exertion, which has a rather high inheritance.

2.3.3 Pollen Viability

The reproductive phase of tomato plants starts with pollen development, and this is more sensitive to high temperatures than female gametophytes and other vegetative parts (Bokszczanin *et al.,* 2013). Even a slight increase in temperature above average significantly affects pollen viability and production (Thomas and Prasad, 2003). Studies have observed a reduction in tomato pollen formation, release, viability, germination ability, fruit set, and production at temperatures higher than average levels (Peet *et al.,* 1997; Sato *et al.,* 2000; Pressman *et al.,* 2002). Reports indicate that the most heat-sensitive developmental stage in tomato plants is 7–15 days before flowering, as spindle formation during the meiosis phase is highly sensitive to high temperatures (Sato *et al.,* 2006). According to Pressman *et al.* (2002), the impact of heat stress on pollen viability is linked to carbohydrate metabolism during anther growth. Soluble sugar concentration in pollen slightly increases at moderate temperatures, but consistently high temperatures prevent starch concentration from rising and result in decreased soluble sugar in mature pollen, which likely reduces its viability.

According to Müller *et al.* (2016), the altered localization of two pistil-specific gene products, TRANSMITTING TISSUE SPECIFIC (TTS) and TOMATO AGAMOUS LIKE11 (TGL11), is responsible for the simultaneous decline in pollen viability and abnormal pistil-like anther production under high temperatures. This is associated with reduced expression of B-class genes in the anthers, including TOMATO APETALA3 (TAP3), TOMATO MADS BOX GENE6 (TM6), and PISTILLATA (PI) (Kramer *et al.,* 1998; Busi *et al.,* 2003; de Martino *et al.,* 2006). These studies showed that the downregulation of tomato B-class genes caused by high temperature stress leads to increased anther deformities and decreased male fertility (Müller *et al.,* 2016). A, B, C, D, and E are the five classes of the ABCDE model genes that encode MAD-box TFs (Rijpkema *et al.,* 2006; Smaczniak *et al.,* 2012), except for the class A gene APETALA2 (AP2) (Jofuku *et al.,* 1994). According to Wellmer *et al.*

(2014), the classes of Arabidopsis are as follows: AP1 is in class A, AP3 and PI are in class B, AGAMOUS (AG) is in class C, SEEDSTICK (STK), SHATTERPROOF1 (SHP1), and SHP2 are in class D, and SEPALLATA1 (SEP1), SEP2, SEP3, and SEP4 are in class E. Additionally, the AGAMOUS-LIKE 6 (AGL6)-clade genes, AGL6 and AGL13, are crucial for floral organ development, especially ovule production (Murai, 2013). Expression of the B-class PI, TAP3, and TM6 genes is reduced in the anthers of tomatoes under mild heat stress (Muller *et al.,* 2016). Temperature elevation causes TM6 to partially shut down, which decreases the occurrence of pistilloid anthers, pollen viability, and pollen production. Several factors directly or indirectly associated with pollen thermotolerance regulate the viability of tomato pollen. For example, secondary metabolites accumulated in mature pollen, such as flavonoids, may mitigate the damage caused by ROS scavengers (Paupiere *et al.,* 2017).

Both the early and late stages of pollen development were adversely impacted by HS. At different stages of pollen development, a complex network of metabolites and plant hormones is involved in the thermotolerance mechanism of tomato pollen; the early stage of pollen development involves the accumulation of unfolded protein response (UPR) in the endoplasmic reticulum (ER), cytoplasm, ROS homeostasis, metabolic reprogramming, carbohydrates, changes in histones, alternative splicing, plant hormones, and gibberellins and the later stage involves polyamines, flavonoids, UPRs, ROS, amino acids, plant hormones and carbohydrates. Additionally, successful fruit and seed development was also aided by compatible stigma and pollen (Raja *et al.,* 2019). Therefore, while creating strategies to increase tomato fruit production in high-temperature environments, these aspects need to be taken into account.

The pollens can be utilized to analyze the nature of the entire plant under various environmental conditions because they are very sensitive to even little environmental changes. In comparison to sensitive genotypes under high temperatures, genotypes that exhibit high temperature tolerance have higher pollen viability. So the high temperature tolerance in the tomato can be correlate with the pollen viability under high temperature (Pressman *et al.,* 2002).

2.3.4 Days to First Flowering

Heat stress has also been linked to early flowering and flower abortion, as reported in tomato (Sato *et al.,* 2004), pea (Guilioni *et al.,* 1997), mungbean (Sharma *et al.,* 2016) and common bean (Omae *et al.,* 2012). The critical period of vulnerability to high temperature (32/26 ^{o}C) is 7 to 15 days before anthesis (Sato *et al.,* 2002). Growth chamber and greenhouse trainings suggest that when flowers first appear, high temperatures are most detrimental and the susceptibility lasts 10 to 15 days.

2.3.5 Number of Flowers per Cluster

The environmental conditions, such as temperature, have an impact on the number of flowers in the inflorescence (David *et al.,* 1996). High temperatures during flowering can lead to reduced flower counts and lower yield due to damage to flower organs (Morrison and Stewart, 2002). Additionally, it decreases the number of flowering branches and the amount of flowers per plant (Harsant *et al.,* 2013).

Temperatures of 18.3-23.8C for squash, pumpkin, muskmelon, and cucumber, and 23.8-29.4C for watermelon, are optimum for crop growth and development. Temperatures above 35°C reduced flowers and sugar content in cucumber (*Cucumis sativus* L.) and watermelon (*Citrullus lanatus* L.) (Lai *et al.,* 2018). In many crop species, including tomato, high-temperature stress causes flower bud abortion and abscission of reproductive organs (Levy *et al.,* 1978; Pressman *et al.,* 2002; Sato *et al.,* 2002). High temperature (32/28^{o} C) has a negative impact on tomato flower initiation and development (Levy *et al.,* 1978; Sato *et al.,* 2002).

Abdelmageed and Gruda (2009), observed that heat-resistant and heat-susceptible tomato lines exhibited diverse morphological traits in terms of fruit and flower numbers, fruit weight percentage, and set. The outcomes varied in both field and glasshouse environments across 11 tomato lines. 'CLN-1-0-3' produced the most flowers in each plant, while 'Omdurman' and 'UC-82-B' produced the fewest. High temperatures primarily result in decreased bud or flower formation and flower drop. This finding was consistent with El-Ahmadi and Stevens (1979), who found that a heat-

sensitive cultivar only produced dropped flowers at high temperatures. Under high temperature conditions, the total flower number is compensated by prolonged flowering or larger inflorescence output (Xu *et al.,* 2017). Similarly, under moderate temperature conditions, cultivars with more flowers per inflorescence experienced lower flower abortion, resulting in fewer unfertilized flowers (Kugblenu *et al.,* 2013). There is a positive correlation between reproductive heat tolerance and the number of flowers per inflorescence (Xu *et al.,* 2017).

2.3.6 Number of Fruits per Cluster

The proportion of flowers that produce fruits (fruit set) is affected by high-temperature stress (Prasad *et al.,* 2000). The maintenance of stem cells in shoot and floral meristems is regulated by the CLV-WUS signaling system, which affects various agronomic parameters, including the number of flowers and fruits (Fletcher, 2018). In the early stages of tomato fruit development, the CLV-WUS feedback loop controls meristem activity and floral meristem size. In the later stages, it regulates carpel number in flowers and, consequently, seed locules in fruit (Rodrguez-Leal *et al.,* 2017).

Peet *et al.* (1998) discovered that high temperatures (29 ^{o}C) reduced fruit number (10%), total fruit weight/plant (6.4%), and seed number/fruit (16.4%) in male fertile tomatoes when compared to optimal temperatures (25^{o}C). Thuy and Kenji, 2015 also observed that, in comparison to normal temperature (33/21^{o}C), HS (38/30 ^{o}C) significantly reduced the weight (51.6%), diameter (25%), length (30%), and number of seeds per fruit (57%) of sweet peppers. Abdelmageed and Gruda (2009) observed that the outcome of fruit number per crop were diverse in heat resistant and heat susceptible tomato lines and the outcomes were differed in field and glasshouse environments in 11 lines of tomato. The heat resistant lines yielded the biggest fruits number compared to the susceptible line under open field conditions and yields average fruits number per crop in glasshouse; in contrast the fruits number was ‘zero’ in the heat susceptible line in glasshouse condition.

2.3.7 Total Yield

The yield of fruits in vegetable crops depends on both the number and size of the fruits. Variations in weather, such as temperature and rainfall, can decrease crop yields. According to research by Hansen *et al.* (2016), the rising temperatures due to climate change will greatly reduce crop yields and production. In high temperatures, the functions of gametes (pollen and ovule) play a critical role in fruit production. Prasad *et al.* (2017) found a strong positive correlation between fruit production and the viability of gametes. In tomato, Firon *et al.* (2006) also observed the correlation between fruit-set and pollen viability. High temperatures (>30°C) significantly affect the development of male and female gametophytes, leading to poor growth and abnormalities in reproductive tissues, which hinder fertilization in various plant species (Prasad *et al.,* 2017). In general, compared to heat susceptible genotypes, heat tolerant genotypes sustain better pollen viability (Dane *et al.,* 1991). In reality, tomatoes cultivated at 32/26°C day/night temperatures were unable to release enough pollen, which results in reduced fruit set (Sato *et al.,* 2000, 2006). HS has also been shown to impact the flower pollination rate in tomato, leading in low fruit set with reduced lycopene content and fruit quality (Alsamir *et al.,* 2021).

Gamete viability, which can be determined by viability assays such as staining, in-vitro and in-vivo pollen germination, and ovule function, determines how well a gamete performs. Under high temperature, gamete viability varies between genotypes. The fruit set decline under optimum high temperature stress may occur mainly because of the decline in pollen release and viability (Sato *et al.,* 2006). Previously, Sato *et al.* (2000) failed to find a clear correlation between the number of produced pollen grains and fruit set. Later, they came to the conclusion that the most crucial variables affecting fruit grown in high temperature conditions are pollen release and viability. The male-fertile tomato plants produced less fruit than the male-sterile lines due to the high temperature's impact on pollen formation and release. Similarly, fruit set and fruit size in tomato plants decreased at 29/23 ºC compared to 24/18 ºC (Saha *et al.,* 2010). A study by Vijayakumar *et al.* (2021) also found that yield per plant significantly decreased at high temperature (36 +/-2 ºC) in tomato genotypes compared to control

temperature. HS severely harmed fruit set in tomato exposed to 40°C for 4 hours before anthesis and lowered pollen germination from 79.5% (at 30/17 ºC) to 30% and pod set in peas from 63% (at 30/17 ºC) to 14.9% (Rudich *et al.,* 1977). Singh *et al.* (2015) concluded from their research that variables like fruit set and pollen viability might be utilized as a way to screen genotypes for heat stress in tomato. So in general, HS tolerance is provided by the combination of gamete viability and fruit set (Paupiere *et al.,* 2017; Pham *et al.,* 2020). Similarly, observations were also made on peppers (Reddy and Kakani, 2007).

2.4 PHYSIOLOGICAL PARAMETERS

Heat stress is known to adversely affect a number of important physiological, biochemical, and metabolic processes in plants as well as to disturb normal cellular homeostasis (Hasanuzzaman *et al.,* 2013). According to Hu *et al.* (2015), it encourages the overproduction and accumulation of reactive oxygen species (ROS), as well as the production of malondialdehyde (MDA) as a result of lipid peroxidation, photoinhibition, protein denaturation, and the buildup of suitable solutes. According to Wahid *et al.* (2007), the oxidative stress caused by heat stress results in cellular damage, membrane protein breakdown, lipid peroxidation, degradation of photosynthetic pigment, and denaturation of enzymes and nucleic acids. In addition, Heat stress also impairs plant respiration and photosynthesis, shortening the life cycle and lowering plant yield (Barnabás *et al.,* 2008).

Tomato cultivation at temperatures beyond the optimum range has a negative impact on plant growth and reduces productivity (Zhang *et al.,* 2014; Sato *et al.,* 2006). In tomato crop water relations, concentration of compatible osmolytes, cell membrane thermo integrity, photosynthesis, and alterations in hormones are important physiological reactions to heat stress.

2.4.1 Stomatal Conductance

Stomatal conductance measures the rate of carbon dioxide entering or water vapor exiting stomata. Changes in leaf temperature and water potential are made

possible by this variation in transpiration rate (Farquhar and Sharkey, 1982). Leaf stomatal conductance is frequently acknowledged as a crucial characteristic for assessing variations in responses to different environments. In order to respond to changes in the growing environment, leaves retain stomata machinery during primary synthesis and respiration processes. This machinery controls gas exchange and water vapor between the atmosphere and the intracellular space (Negi *et al.,* 2008). It can be used to analyze traits including water loss, leaf temperature, and photosynthetic CO2 uptake (Lawson and Vialet-Chabrand, 2019). The growth, development, and biomass of plants can be dramatically impacted by decreased stomatal activity in a changing environment (Way and Pearcy, 2012). Stomatal density and status are influenced by the temperature on the leaf surface; for instance, a complex network of stomata that regulates gas exchange and water vapor is formed to respond to abiotic stressors (Reynolds-Henne *et al.,* 2010). It was observed that in comparison to plants grown under control circumstances (25°C), tomato plants of the cultivar Campbell 28 had higher stomatal conductance after heat treatment (45°C), showing that stomatal closure was not responsible for the decrease in CO2 (Camejo *et al.,* 2005). While some studies have indicated that stomatal conductance decreases substantially in plants exposed to HS (Weston and Bauerle, 2007; Lahr *et al.,* 2015; Von caemmerer and Evans, 2015), others have found that stomatal conductance increases in plants exposed to HS (Radin *et al.,* 1994; Zhou *et al.,* 2015).

Stomatal conductance can be measured in vivo using a leaf porometer and gas exchange in a steady state. In comparison to the control, HS enhances in vivo adaxial stomatal conductance (Sharma *et al.,* 2016). According to Matthews *et al.* (2018), low stomatal responses under stress can reduce photosynthetic rate and lead to unnecessary transpiration, which decreases plant water use efficiency and productivity. This phenomenon has been utilized to choose heat-tolerant genotypes of tomato, chickpea, mungbean, and sweet pepper (Hanyingm *et al.,* 2001; Camejo *et al.,* 2005; Abdelmageed and Gruda, 2009). Even though numerous studies have used one of the aforementioned qualities to choose heat-tolerant genotypes, integrating multiple measures would more accurately indicate heat tolerance than depending on a single trait.

2.4.2 Electrical Conductivity of Leaf

Under stress, the continued function of cellular membranes is essential for processes like respiration and photosynthesis (Blum, 2018). Heat stress increases the kinetic energy and motion of molecules in membranes, which cause biological membrane molecules to loose chemical bonds. This results in either a denaturation of proteins or an increase in unsaturated fatty acids, which increases the fluidity of the lipid bilayer of biological membranes (Savchenko *et al.,* 2002). As a result of increased permeability or solute leakage from cells, HS disrupts the key processes of plant-like photosynthesis and respiration. Heat stress influences the change of membrane fluidity in plasma membrane in plants and activates the cyclic nucleotide-gated calcium channels, resulting in Ca^{2+} movement into the cytoplasm from the apoplastic space. The stability and roles of biological membranes are vulnerable to high temperature, as heat stress changes the tertiary and quaternary structures of membrane proteins, increasing membrane penetrance as evidenced by increased electrolyte loss (Savchenko *et al.,* 2002). Heat stress is measured by the amount of heat perceived by cell membranes in leaf tissues, which weakens cell membrane integrity/rigidity due to an increase in unsaturated fatty acids, which increases membrane fluidity. This may alter membrane permeability and disrupt the selective transit of molecules across the membrane, so interfering with cellular homeostasis (Marcum, 1998). Through photochemical changes during photosynthesis or ROS, HS can directly influence membrane integrity (Bita and Gerats, 2013).

Under stress circumstances, ionic leakage is linked to ROS buildup (Demidchik *et al.,* 2014). Drought stress has been linked to increased ion leakage in drought-sensitive tomato plants (Thirumalaikumar *et al.,* 2018). There are two popular methods for estimating heat tolerance in plants: (i) the common ion leakage measurement based on the total electrical conductivity released before and after heating, and (ii) the estimation of basal heat tolerance based on cell suspension or gradual (linear) heating of plant segments (Ilik *et al.,* 2018). Total ionic leakage is one of the most critical elements in determining plant responses to abiotic and biotic stimuli since it is linked to stress-induced injury in plants connected to programmed cell death (Zhu, 2016). The

efficiency of cell membrane thermostability experiments decides on the kind of tissue and stress utilized in plant adaptation. It's also unclear if membrane thermostability is related to other plant traits that confer heat resistance, such as growth and yield.

The screening for heat tolerance can be tested using an electrolyte leakage test to determine the cell membrane thermostability (CMT). The technique can be used to evaluate plant tissue responses at the vegetative stage and is easy, rapid, and affordable when compared to wholeplant screening (Yeh and Lin, 2003). A conductivity meter is used to monitor electrolyte leakage, and higher conductivity values indicate greater membrane degradation (Nyarko *et al.,* 2008). The CMT test has been used to screen heat-tolerant varieties and heat-sensitive genotypes of many crops, including tomato (Chen *et al.,* 1982), soybean (Martineau *et al.,* 1979), potato (Nagarajan and Bansal, 1986), cowpea (Ismail and Hall, 1999), cabbage (Nyarko *et al.,* 2008), chickpea (Kumar *et al.,* 2013), mungbean (Sharma *et al.,* 2016), cucumber (Ali *et al.,* 2019) and cauliflower (Aleem *et al.,* 2021).

Alsadon *et al.* (2006) studied heat resistance in twenty tomato lines at different steps of growth and perceived striking variability in heat resistance. Edkawi variety was found to have the highest average electrical conductivity values at the vegetative stage (63.12 mho/cm), and it was followed by Pakmore VF, Castle Rock, Chico, Pakmore, and Tnshet Star. They also discovered that these lines had higher susceptibility to heat stress at vegetative step. The markedly lowermost average value for EC was found in the Pearson, Super Strain-B, Queen, VFN-8, and Strain-B varieties, indicating that these lines had the best function and were resistant to heat stress at vegetative phase. The other nine genotypes detected as mild heat stress resistance as they have average mean values for EC. The heat resistant variety had the lowest average values in the following phase, whereas the heat susceptible types had the highest average values. The remaining kinds, which displayed average EC values, were found to be moderately heat resistant. In the fruiting step also same pattern were observed. The same findings were perceived in cowpea and wheat by Saadella *et al.* (1990), Kuo *et al.* (1993) and Ismail and Hall (1999).

2.4.3 Photosynthetic Rate

Photosynthesis is identified as a physiological marker which is highly sensitive to high temperatures. Temperature will rise as atmospheric CO_2 levels rise, which may have a significant impact on the yield and distribution of several crop genotypes in the future (Wahid *et al.,* 2007). Heat stress has a negative impact on respiration and photosynthesis, resulting in a shorter life cycle and a considerable loss in plant productivity (Barnabás *et al.,* 2008). The reaction to HS begins with structural changes in chloroplast protein complexes and decreased enzyme activity (Bita and Gerats, 2013), followed by cell membrane damage and microtubule organization. Because HS negatively affects membrane permeability, cytoskeleton can also be damaged, may result in changes in cell differentiation, elongation, and expansion (Smertenko *et al.,* 1997; Potters *et al.,* 2009). Under HS, maintaining cellular membrane function is critical for long-term and steady photosynthetic and respiratory continuation (Chen *et al.,* 2012). Swelling and aberration of grana stacks on photosynthetic membranes have been documented by some studies, resulting in changes in energy distribution to photosystems and ion leakage from leaf cells (Wahid and Shabbir, 2005; Allakhverdiev *et al.,* 2008).

Several studies have been conducted to investigate the affected photosynthetic pathways and mechanisms, which eventually diminish plant photosynthetic capability (Berry and Bjorkman, 1980; Sharkey, 2005). HS mostly disrupts thylakoid processes, Rubisco activity, and photosynthetic pigments. HS predominantly impacts the physical condition and structure of the thylakoid membrane by causing leakiness and unstacking thylakoids, hence harming the PSII D1 protein (Sharkey, 2005). To counteract these processes, zeaxanthin production increases, disrupting thylakoids' normal condition (Havaux, 1996). HS disrupts electron transfer between the two photosystems (PSI and PSII), lowering plant photosynthetic efficiency. Heat stress moreover accelerates the phosphorylation of the light-harvesting complex (LHCII) and disconnects it from the PSII core complex, lowering its turnover rate while raising PSI's (Wise *et al.,* 2004). HS dephosphorylates key proteins (D1, D2, and CP43), causing PSII to become inactive (Yamamoto *et al.,* 2016). The Fv/Fm ratio, which helps to assess the quantum efficiency

of PSII and determines the rate of linear electron transport and overall photosynthetic activity of plants, will altered by heat stress (Sharma *et al.,* 2015). In soybean, it was observed that high temperature condition reduced chlorophyll fluorescence, increased PII quantum yield, photochemical quenching, and increased respiration rate (Djanaguiraman *et al.,* 2013). Along with thylakoid reactions, HS causes Rubisco deactivation (Crafts-Brandner and Salvucci, 2000). Rubisco is a dual enzyme that catalyzes the carboxylation of ribulose1-5-bisphosphate in the photosynthetic Calvin cycle as well as oxygenation in the photorespiratory pathway; the ratio between the two reactions regulates the plant's photosynthetic efficiency. However, increased temperature slows CO_2 fixation, increases oxygenase activity, and decreases photosynthetic rate (CraftsBrandner and Salvucci, 2000). Rubisco activation is linked not only to stromal pH and Mg2+ concentrations, but also to Rubisco activase (RA), an ATPase. Rubisco activase stimulates Rubisco activation by raising the fraction of active sites and causing conformational changes that allow CO_2 and Mg^{2+} for activation and carbamylation. High temperatures can cause stroma pH and Mg^{2+} concentrations to fluctuate, interfering with the carbamylation stage of Rubisco activation, as well as rubisco activase dissociation due to its poor structural stability and heat lability (Demirevska-Kepova and Feller, 2004). Few studies have found that heat stress affects photosynthesis via heat sensitivity of Rubisco and rubisco activase activity. For example, heat stress (40°C for 8 hours for 6 days to 3 weeks old plant) decreased the accumulation of Rubisco enzyme isoforms in tomato (Parrotta *et al.,* 2020), like as in pea (Haldimann and Feller, 2005), potato (Cen and Sage, 2005), and spinach (Zhao Q. *et al.,* 2018). Changes in numerous photosynthetic processes in heat stress are accurate indicators of crop thermoresistance because they correlate with growth. Crop development can be hampered at high temperatures when photosynthesis is reduced.

2.4.4 Chlorophyll Content

The growth of plants and yield are impaired by decreasing chlorophyll under high temperature stress, which leads to a decrease in photosynthetic rate. In cucumber, heat stress reduced the activity of the first enzyme in the chlorophyll biosynthetic pathway, 5-aminolevulinate dehydratase (Tewari and Tripathy, 1998). Reduced

chlorophyll content, chlorophyll a/b ratio, and chlorophyll/carotenoid ratio have been documented in various crops under heat stress (Aien *et al.,* 2011). Aleem *et al.* (2021) reported that pea plants exposed to heat stress shows reduced chlorophyll production due to the loss of several enzymes involved in biosynthetic pathways. Under stress condition when the enzyme that forms carbon-carbon and carbon-nitrogen bonds in the pyrrole ring of porphobilinogen that is 5-aminolevulinic acid dehydratase (porphobilinogen synthase), is inactivated causes decreases in chlorophyll content (Taiz and zeiger, 2015).

The photosynthetic rate is related to the amount of chlorophyll in the leaves (Lin *et al.,* 2011). By boosting chlorophyllase activity and decreasing the amount of photosynthetic pigments, HS lowers photosynthesis and respiratory activity (Todorov *et al.,* 2003; Shar key and Zhang, 2010). Reduced photosynthesis under heat stress is associated with a decrease in chlorophyll content; lipid peroxidation of the chloroplast and thylakoid membranes is the primary cause of the decreased chlorophyll content (Camejo *et al.,* 2006). The negative impact of heat stress on chlorophyll and the photosynthetic machinery causes an increase in reactive oxygen species (ROS), which are involved in biotic and abiotic stress responses (Vara Prasad *et al.,* 2000; Shi *et al.,* 2015). Stay-green or delayed senescence is one of the heat resistance traits in tomatoes, and under high temperature conditions, tomato genotypes cannot stay green due to a decrease in chlorophyll a, chlorophyll b, and carotenoid content, resulting in early chlorosis and withered leaves (Zhou *et al.,* 2015).

2.4.5 SOD Activity

Plants develop active oxygen species in response to stress situations such as high temperature, which impairs plant metabolism and eventually leads to death. Antioxidants are created as a defense strategy to avoid this (Rivero *et al.,* 2004). Severe HS produces ROS as an outcome of aerobic metabolism, such as hydrogen peroxide (H_2O_2) and superoxide radical (O_2), which negatively affect cellular metabolism, such as lipid peroxidation, and damage nucleic acids and proteins (Bita and Gerats, 2013). Plants respond to ROS generation by activating ROS scavenging mechanisms, both enzymatic and non-enzymatic (Bita and Gerats, 2013). Superoxide dismutase (SOD),

catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), and glutathione reductase (GR) are the principal ROS scavenging enzymes, whereas ascorbic acid (ASC) and glutathione (GSH) are non-enzymatic chemicals (Suzuki *et al.,* 2012). SOD aids in the scavenging of O_2, whereas CAT and POX degrade H_2O_2. Elevated levels of these antioxidants are essential for plant thermotolerance (Awasthi *et al.,* 2014).

Antioxidant defense is critical in tomato plant response to a variety of abiotic stressors. To improve heat tolerance, tomato plants must regulate salicylic acid and activate other metabolic pathways; as heat stress causes serious damage to antioxidant enzymes function (Jahan *et al.,* 2019). Several antioxidative enzyme activities, such as SOD, POX, CAT, APX, guaiacol peroxidase (GPX), GR, etc., are generally reduced under heat stress (Balal *et al.,* 2016) and are upregulated in response to stress, particularly in tolerant species or in response to ameliorants (Balal *et al.,* 2016). Superoxide dismutase and ascorbic acid peroxidase are involved in the antioxidant defense mechanism in tomato plants in response to the detrimental effects of high temperature (Zhou *et al.,* 2019). In tomato, the relationship between antioxidant enzymes and HS is much more complex, where activity of SOD, APX increased and CAT activity decreased. where in chickpea, tolerant genotypes had higher SOD, CAT, APX, and GR activity than sensitive genotypes (Kumar *et al.,* 2013). In contrast to severe HS, moderate heat stress boosts the expression of a number of enzymatic antioxidants (Wilson *et al.,* 2014).

2.4.6 Relative Water Content

Under changing climatic temperatures, crop water situation is an imperative variable (Mazorra *et al.,* 2002). The relative water content of plants displays their hydration status and represents the balance between leaf water supply and transpiration rate. As a result, it can assess leaf water deficit and the extent of damage caused by HS (Mullan and Pietragalla, 2012). High transpiration causes an increase in water loss, which can lead to tissue dehydration and wilting (Mazorra *et al.,* 2002). Increased transpiration during the day influences crop water deficit, resulting in a loss in water potentiality and causes disturbance of many physiological parameters (Tsukaguchi *et al.,* 2003). High temperatures can cause crops to lose more water during

the day than at night (Wahid *et al.,* 2007). The degree of plant stress is determined by the status of water potential and cell turgor (Nduwimana *et al.,* 2017), and optimal vegetative and reproductive development of tomato plants requires high water potential (Torricellas *et al.,* 1995). As a result, genotypes that can retain turgid leaves will have less heat stress effects and have various physiological advantages. Gowda *et al.* (2011) proposed utilizing the relative water content as a selection criterion to increase yield under high temperature stress. High temperatures (40-42^{o}C) during the vegetative and reproductive stages gradually reduced the relative water content of capsicum genotypes, with the reproductive stage being the most affected (Puneeth, 2018). Heat stress disrupted the hydraulic conductivity of root and leaf water relationships in tomatoes (Morales *et al.,* 2003).

Temperature stress caused osmotic stress and yield loss due to a decrease in cellular water content and osmotic potential (Zhang *et al.,* 2011). Reduced water loss and enhanced water uptake, maintained plant water relations during stress, and boosted stress tolerance (Kumar, 2010). According to Liu and Huang (2000), the enhanced tolerance in bent grass under heat stress conditions was attributable to the maintenance of optimal water status under stress conditions. Heat tolerant genotypes exhibited a low membrane injury index, a high relative water content, and high metabolic activity (Deshmukh *et al.,* 2001). Relative water content has been employed to select heat-tolerant genotypes of mungbean (Sharma *et al.,* 2016), lentil (Sita *et al.,* 2017) , common bean (Chavez-Arias *et al.,* 2018), capsicum (Puneeth, 2018), tomato (Zhou *et al.,* 2018), cucumber (Ali *et al.,* 2019), and potato (Handayani and Watanabe, 2020).

2.4.7 Vitamin C content

Environmental conditions and genetic constitution have a significant impact on tomato plant ascorbic acid levels (Brown, 1955). Ascorbic acid's antioxidant capability highlights its importance in plant stress response (Matteo, 2010). It is one of the factors which determine the quality of tomato fruit (Ward, 1964).

According to Hernández *et al.* 2018, applying temperature stress during blooming and fruit set stages raised vitamin C content, and there may be a link between

vitamin C content increase and plant metabolism adaption to high-temperature stress. Dasgan *et al.* (2021) observed that vitamin C content of tomato genotypes increased significantly during high-temperature stress, with an average increase of 3.54% in all tomato genotypes. Another study of high-temperature stress in tomato genotypes found no significant difference in vitamin C content between sensitive genotypes under stress compared to the control, while all tolerant genotypes had increased vitamin C content (Lokesha *et al.,* 2019). It was studied that the quality parameters like lycopene content, total sugars, flavanol content decreases in both tolerant and susceptible genotypes but the extend of this reduction was considerate in tolerant ones (Vijayakumar and Beena, 2023).

2.5 ANATOMICAL STUDY OF ANTHER

Male gametophytes develop in specialized organs of the flowers called anthers, which are made up of epidermis, endothecium, tapetum, and connective tissues that surround the sporogenous cells (Goldberg *et al.,* 1993). The tomato androecium is composed of six anthers grouped in a ring around a central cavity through which the style protrudes. Individual anthers are tetrasporiangiate and bithecal. Pollen development happens within the four locules, which are bounded by the tapetum's sporophytic cell layers, the middle layer, and the epidermal layers. The endothecium may or may not be visible, but it is usually more visible in the wall enclosing the adaxial locules. Each theca's pair of locules is separated by four to six cell layers of connective tissue, with the interlocular septum separating the connective tissue from the stomium. With development, there is a steady loss of cells in the sporophytic layers (Brukhin *et al.,* 2003; Senatore *et al.,* 2009).

Temperature changes during the time of flowering can cause morphological changes in flower organs. The intensity (temperature degrees), duration, and rate of the temperature increase all influence these alterations. It has been discovered that phenotypic changes may occur in tomato flowers exposed to prolonged high temperature. Heat stress-induced changes in early buds and flowers during anthesis were characterized by aberrant anthers and style elongation (Giorni *et al.,* 2013). Male sterility caused by high temperatures appears to be linked to morphological changes in

sporophytic anther tissues such as the tapetum, epidermis, endothecium, and stomium (Sato *et al.,* 2002). Giorni *et al.* (2013) revealed the presence of significant modifications in tomato sporophytic and gametophytic tissues after 3 days at 36 °C/26 °C (day/night). Most major modifications occur in the tapetum layer, but mature microspores with altered vacuolization are also affected. Similar changes have been observed in other crop species (Oshino *et al.,* 2007). The development of male gametogenesis and, consequently, the proper formation of microspore cells, as reported in male sterile mutants, may be significantly impacted by Heat stress induced tapetal defects because the tapetum normally secretes callase to release the microspores from the tetrad wall and acts as a nutritional source during microspore development (Sakata *et al.,* 2008). Sato *et al.* 2002 also reported that analysis of mature anthers produced in long-term mild heat (*i.e.* several days at 32/26°C, day/night) revealed reduced stomium cell disintegration and structural anomalies of the anther wall, in addition to dead pollen.

2.6 MARKER-ASSISTED SELECTION

As previously stated, phenotype-based selection is susceptible to environmental factors, which can lead to erroneous results especially when the characteristic is complex and imparted by polygenes or QTLs. In such cases, genotype-based selection is more effective, precise, and rapid than phenotypic selection. Using markers connected to the gene of interest, genotype-based selection rather than phenotype-based selection is conceivable. Genotype-based selection employs DNA markers that are intimately connected to the desired gene(s) (Collard and Mackill, 2007). The initial stage in MAS is to locate markers connected to the gene or QTL utilizing mapping populations or association mapping, which uses a panel of genotypes to identify liked markers. Following that, these markers are utilized to determine gene transfer to progeny populations.

A DNA marker is often stemmed from a small section of DNA that shows sequence polymorphism among individuals within or between species. DNA markers that are phenotypically neutral and identically unconstrained in number have enabled the scanning of the entire genome and the assignment of landmarks in high density on

each chromosome in a variety of crop species, including tomato. Several types of molecular markers, including RFLP (restricted fragment length polymorphism), AFLP (amplified fragment length polymorphism), SSR (single sequence repeat), and SNPs (single nucleotide polymorphisms), have been improved and progressed over the last two decades, and the amount of variation in each marker can be determined. Gene mapping and discovering gene correlations with specific traits are useful for genetic crop improvement using this strategy (Ruane and Sonnino, 2007).

To define, any trait that is exhibited in several forms and inherited in a basic Mendelian method can be considered and used as a genetic marker. More than 1300 morphological, physiological (e.g., male sterility, fruit abscission, fruit ripening), and disease resistance genes have been identified in tomato (Kalloo, 1991), but only 400 have been mapped (Mutschler *et al.,* 1987; Tanksley, 1993; Chetelat, 2002). Isozymes, the second generation of genetic markers, became well-known in the 1970s and early 1980s. In tomato, 41 isozymic genes have been identified that correlate to 15 distinct enzymatic reactions, 36 of which have been mapped onto the 12 tomato chromosomes (Tanksley, 1993; Tanksley and Bernatzky, 1987). Despite their numerous advantages, isozyme markers are scarce and sometimes not polymorphic between closely related lines (Foolad *et al.,* 1993; Tanksley and Orton, 1983). With the introduction of DNA marker technology in the 1980s (Botstein *et al.,* 1980) and early 1990s, many limitations associated with isozyme and morphological markers were overcome, and genetic mapping entered a new exciting and developed era with the promise of dramatically improved crop breeding and genetics study.

High temperature tolerance is strongly linked to polymorphic markers. Because of the increased polymorphism rate, simple sequence repeats (SSR) are molecular markers that are significantly more efficient, cheaper, and easier to utilize in marker assisted selection for crop improvement (Gao *et al.,* 2016). Using gene pyramiding, identifying SSR markers associated with QTLs contributing to heat tolerance has enormous promise in breeding programs (Ali *et al.,* 2022). SSR markers are a useful tool for geneticists and breeders in correlating phenotypic and genotypic differences. Conti *et al.* (2019) used sixteen tomato-specific markers to investigate the effects of

drought stress on Italian tomato varieties. SSR248, SSR47, SSR70, and SSR603 were discovered to be drought-specific. SSR248 and SSR47 were utilized to assess other abiotic and biotic stressors as well as genetic diversity (Suliman- Polltschek *et al.,* 2002). Wen *et al.* (2019) found twelve possible QTLs associated with heat stress tolerance, six of which showed a positive additive effect, indicating that heat tolerant parents contributed to improved heat tolerance. Markers flanking the QTLs were also found; among the flanking markers identified were SSR134, SSR96, and SSR13, in which SSR134 being used in this study. SSR134 was discovered to have the most phenotypic variance as well as the highest positive additive impact, indicating its potential application in breeding programs.

Marker-assisted selection (MAS) provides major benefits in crop development, but it also presents several obstacles. Creating and validating molecular markers for qualities of interest can be costly and resource-intensive, and their effectiveness is frequently limited to traits controlled by a few key genes, rendering them unsuitable for complicated polygenic traits impacted by environmental factors. Furthermore, successful implementation necessitates specialized infrastructure, advanced laboratories, and professional workers, which may not be readily available in resource-constrained environments. There is also the possibility of a narrow genetic focus, as the emphasis on specific markers might overlook other important agronomic traits, thus limiting genetic diversity. Furthermore, verifying strong association between markers and traits via linkage or association mapping can be time-consuming. Despite these challenges, MAS remains a powerful tool in modern breeding programs when applied strategically.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The objective of the thesis entitled "Physiological and molecular basis of selective fertilization for high temperature stress tolerance in tomato (*Solanum lycopersicum* L.)" was to screen the critical temperature for pollen germination of tomato genotypes and to evaluate the thermotolerance of selectively fertilized tomato hybrids through physiological and molecular techniques. The research work was conducted at the Department of plant physiology, College of agriculture, Vellayani during the year from 2019- 2024. Physiological and biochemical analyses were done at monthly intervals after transplantation of tomato crop and the molecular analysis were also done.

3.1. EXPERIMENT 1: ADVANCING THE TEMPERATURE TOLERANT SELECTIVELY FERTILIZED HYBRID OF ANAGHA AND MANUPRABHA.

3.1.1. Location

The experiment was conducted in the open field located near Open Top Chamber 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.

3.1.2 Experimental Material

The temperature tolerant selectively fertilized tomato hybrid Anagha x Manuprabha was selected for advancing generations.

3.1.3 Experimental Details

3.1.3.1 Hybridisation Technique

The hybrid was advanced by selective fertilization technique. The F_1 generation of the cross Anagha and Manuprabha was selfed upto F_4 generation to study and improve the temperature tolerance in the hybrid. The F_1 hybrid seed *i.e.* the seeds from selectively fertilized Anagha x Manuprabha cross was obtained by pollinating the emasculated flower of Manuprabha with the pollen of Anagha after two hours of incubation at critical temperature (36^0 C). In selective fertilization (SF) to develop the F_2 generation, the pollen from the hybrid was collected and exposed to the specific

critical temperature (36^0 C). After two hours of incubation, the pollens were used to pollinate the emasculated flowers of the same plant (Plate 1).

3.1.4 Layout of the Experiment and Design

The experiment was laid out in a completely randomized design (CRD). The hybrids were kept in open condition.

3.1.4.1 *Preparation and Planting*

The seeds from the selectively fertilized fruits were collected at maturing stage. The seeds were sown and the seedlings were raised at ambient temperature (34^0C) as F_2 generation. This process was continued upto F_4 generation. At each stage the physiological and biochemical parameters were taken and molecular analysis was done to evaluate the best plant for the next generation. The lines with best physiological character and showing the selected markers associated with temperature were selected in each generation.

The potting mixture made up of FYM, sand and soil in 1:1:1 ratio was used for filling the pots for the experiment. The crop was raised according to the package of practices recommendations by Kerala Agricultural University (KAU, 2016). The potted plants were maintained in open field for a period of 90 days.

3.1.5 Observations

3.1.5.1 Physiological Parameters

3.1.5.1.1 Pollen germination percentage

In the morning hours *i.e.* from 8.00 to 10.00 am, mature pollen grains from the selected genotypes were collected. Collected pollens were incubated in the BOD incubator (Rotary shaker cum BOD, Rotek, ROSI-1) maintained by the Department of plant physiology, at 36^0 C 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)).

Plate 1. Selectively fertilized fruit

3.1.5.1.2 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

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

3.1.5.1.3 Membrane integrity (%)

The % leakage in the plant was estimated by the method explained by Sadasivam and Manickam (2008). The initial EC *i.e.* EC_a was measured to sense the slight degree of leakage by the discs due to the punching treatment by the conductivity electrode. After 30 minutes of incubation, EC_b *i.e.* the leakage of solute in the bathing medium was measured. The beakers were then boiled for 10 minutes at boiling temperature and the EC_c was measured. The membrane integrity of the leaf sample was calculated by substituting the three EC values in the known formula and the membrane integrity was measured in terms of percentage leakage.

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

3.1.5.1.4 Stomatal Conductance

The portable photosynthetic system (CIRAS-3 SW, PP System International, MA, USA) was used to measure stomatal conductance during daytime from 9:00 to 10:30 am. The measurements were taken from the 3rd leaves and expressed in mmol m^{-2} s^{-1}.

3.1.5.1.5 Chlorophyll content (mg/g)

Chlorophyll content of leaf samples was estimated as per the procedure described by Arnon (1949). A known quantity of leaf sample *i.e.* 0.5 g were taken and

cut into small pieces. The samples were put in a test tube having 5 ml of DMSO: 80% acetone mixture in a 1:1 volume per volume ratio. The samples in the mixture were incubated overnight at room temperature in dark condition. The extracted liquid was transferred to another tube and made up the volume to 5 ml using DMSO: 80% acetone mixture. The absorbance was measured in spectrophotometer at 645 and 663 nm 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 x A_{663}– 2.69 x A_{645}) x V/1000 x 1/ Fresh weight

Chlorophyll b = (22.9 x A_{645} – 4.68 x A_{663}) x V/1000 x 1/ Fresh weight

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

3.1.5.1.6 Chlorophyll stability index

A known quantity of leaf sample *i.e.* 1g were taken and put in two glass tubes having 10 ml distilled water. One tube kept in water bath at 56°C + 1°C for 30 minutes to imply heat stress. The other tube kept as control. Following the 30-minute period, the samples were ground with 20ml of 80% acetone, and the resulting slurry was filtered using Whatman No.1 filter paper. The absorbance of the extract was then measured in a spectrophotometer at 645 and 663 nm, using an 80% acetone mixture as the blank. The difference in readings between the samples heated and those not heated provided the chlorophyll stability index.
This is calculated using the formula:

$$CSI = \frac{\text{Total chlorophyll content of (treatment – control)}}{\text{Total chlorophyll content of control}} X\ 100$$

3.1.5.1.7 Photosynthetic rate

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

3.1.5.2 Molecular analysis

3.1.5.2.1 Isolation of DNA

The procedure described by Murray and Thompson (1980), was followed. The plant tissue was finely powdered by grinding 100 mg of it with liquid nitrogen in a mortar and pestle. Next, the ground samples were mixed with 750 µl of extraction buffer (CTAB + β mercapto-ethanol) and transferred to a 2 ml centrifuge tube. Then, the tube contents were thoroughly homogenized by inverting the tube and incubated in a water-bath at 65 ˚C for 30 minutes. Following the incubation, 750 µl of chloroform: isoamyl alcohol (24:1) solution was added to the centrifuge tube. The contents were gently mixed by inversion and then centrifuged at 13000 rpm for 10 minutes. The top phase obtained after centrifugation was carefully transferred to a new 2 ml centrifuge tube. The chloroform: isoamyl alcohol step was repeated, and the top phase was again moved to a fresh tube. After adding 450 µl of ice-cold isopropanol and mixing by inversion, the sample was centrifuged at 13000 rpm for 5 minutes. The supernatant was then discarded, and the DNA pellet obtained was washed with 500 µl of 70% ice-cold ethanol. Subsequently, the washed DNA pellet was centrifuged at 13000 rpm for 5 minutes, and the ethanol was carefully removed. The DNA pellet was air-dried at room temperature or in a speed vacuum. Once dried, the pellet was resuspended in 30 µl sterile water and stored at – 20 ˚C.

3.1.5.2.2 Confirmation of DNA quality

For electrophoresis, 0.8% agarose gel (melting 0.8g of agarose in 100mL of 1X TAE buffer) was made by adding ethidium bromide 10µL(1µg/mL). The following samples were loaded into separate wells.

1.0 µL 1kb ladder, 5µL sample+1µL 6X Loading Buffer

Electrophoresis was done for 30 min giving 70V. The obtained gel is further exposed to UV light and photographed in the gel documentation system. Highly resolved, high molecular weight band indicates good quality of DNA isolated.

3.1.5.2.3 Screening of Primers by Polymerase Chain Reaction

All samples were subjected to different PCR reaction with different primers. Depending upon the primer sequence annealing temperature for each reaction varied. Template concentration was changed according to the quantity of isolated DNA in each sample. The products were confirmed by visualizing the bands after Agarose Gel Electrophoresis.

The PCR reaction mix included the following: Template DNA, PCR buffer, dNTPs, forward and reverse primers and Taq polymerase. The components of PCR mix are listed in Table 1:

Table 1: Components of PCR reaction

PCR components	Stock Conc.	Units	Per Reaction (µL)
Water			13
PCR buffer with salt	10	X	2
dNTPs	2.5	mM	1
Primer- F	10	µM	1
Primer- R	10	µM	1
Taq DNA polymerase	1	U/µL	1
DNA template	25	ng/ µL	1
Total volume			20

PCR profile started initial denaturation at 94°C for 3 min, this followed by 40 cycles of denaturation at 94°C for 1 min, annealing for 1 min and extension at 72°C for 2 min. A final extension of 72°C for (7-10) min was also included. The annealing temperatures varied with primers. The various primers and their annealing temperature are listed out in Table.2.

Table 2: SSR Markers and details of annealing temperature

Markers	Annealing temperature
SSR 134	55º C
SSR 603	55.9º C

Markers specific for the heat stress tolerance genes were very few. Marker data of all accessions are available at https://solgenomics.net and are presented in Table 3.

Table 3: List of SSR primers used in the selection

Sl.No	Primer	Sequence	Map Position	Expected PCR product size
1	SSR 134	FP: CCCTCTTGCCTAAACATCCA RP: CGTTGCGAATTCAGATTAGTTG	Chr 1	171+172 bp
2	SSR 603	FP:GAAGGGACAATTCACAGAGTTTG RP: CCTTCAACTTCACCACCACC	Chr 4	251+251 bp

3.2. EXPERIMENT II: SCREENING FOR HIGH TEMPERATURE TOLERANCE USING THE CRITICAL TEMPERATURE FOR POLLEN GERMINATION.

3.2.1. 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.

3.2.2 Experimental Material

Ten popular varieties of tomato *i.e.* Anagha, Manuprabha, Vellayani Vijay, Akshaya, Pusa Rohini, Arka Saurabh, Arka Rakshak, Nandi, IIHR 26372 and Arka Vijay were selected for pollen germination study (Plate 2).

3.2.3 Experimental Details

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

Table 4: Temperatures used to standardize the critical temperature

Sl. No.	Treatments	Temperature	Duration
1	T_1	32°C	2hrs
2	T_2	34°C	2hrs
3	T_3	36°C	2hrs
4	T_4	38°C	2hrs
5	T_5	40°C	2hrs

3.2.4 Parameters Observed

3.2.4.1 Pollen Germination Percentage

In the morning hours *i.e.* from 8.00 to 10.00 am, mature pollen grains were collected from the selected genotypes (Plate 3). Collected pollens were incubated in the BOD incubator (Rotary shaker cum BOD, Rotek, ROSI-1) maintained by the Department of Plant physiology, at different temperatures for two hours in a petri plate in the standardized pollen germination medium. After incubation the pollen germination was assessed using compound microscope (Leica DC 7.5 V (10X)).

3.2.4.2 Critical sterility temperature

Critical temperature is assessed as the temperature at which only 20-30% of the pollens germinate.

3.3. EXPERIMENT III: EVALUATING THE SELECTIVELY FERTILIZED HYBRIDS OF THE TOLERANT AND SUSCEPTIBLE GENOTYPES.

3.3.1 Location

The pot culture experiment was carried out in Rainout shelter located in College of Agriculture, Vellayani, situated at 18º 30' N latitude and 76º 9' E longitudes at an altitude of 29 m above mean sea level

3.3.2 Planting Material

From the experiment II, based on the critical sterility temperature six genotypes were selected for pollen selection and selective fertilization. *i.e.* the three tolerant varieties Anagha, Vellayani Vijay and IIHR 26372 and three susceptible varieties Pusa Rohini, Manuprabha and Arka Saurabh.

The following 36 crosses between tolerant and susceptible tomato varieties were made using selected pollens exposed to critical temperature to confirm the thermotolerance of selectively fertilized hybrids.

Table 5: Particulars of Experiment III

Crop	Tomato
Varieties	T1 (Tolerant 1) T2 (Tolerant 2) T3 (Tolerant 3) S1 (Susceptible 1) S2 (Susceptible 2) S3 (Susceptible 3)
Conditions	Rainout shelter- ROS The temperature range was Max- 40-42 ºC Min- 28 ºC

Combination of varietal cross	T_1 X S1 T1 X S2 T1 X S3 T2 X S1 T2 X S2 T2 X S3 T3 X S1 T3 X S2 T3 X S3 T_1 X S1 (SF) T1 X S2 (SF) T1 X S3 (SF) T2 X S1 (SF) T2 X S2 (SF) T2 X S3 (SF) T3 X S1 (SF) T3 X S2 (SF) T3 X S3 (SF) S1 X T1 S2 X T1 S3 X T1 S1 X T2 S2 X T2 S3 X T2 S1 X T3 S2 X T3 S3 X T3 S1 X T1 (SF) S2 X T1 (SF) S3 X T1 (SF) S1 X T2 (SF) S2 X T2 (SF) S3 X T2 (SF) S1 X T3 (SF) S2 X T3 (SF) S3 X T3 (SF)

The hybridization was done in the Department of Plant Physiology, College of Agriculture, Vellayani.

Nandi **Anagha** **Vellayani Vijay** **Akshaya** **Manuprabha**

Pusa rohini **IIHR 26372** **Arka Vikas** **Arka Sourabh** **Arka Rakshak**

Plate 2. The varieties used for pollen selection

Plate 3. Plants grown for pollen selection to identify the critical sterility temperature for pollen germination

Plate 4. The tolerant and susceptible varieties selected for selective fertilization

3.3.3 Hybridization Technique

Reciprocal cross between the tolerant and susceptible genotypes was done by selective fertilization as well as normal cross for comparison. In selective fertilization (SF), the pollen from the male parent was collected and exposed to the specific critical temperature for two hours and the emasculated flowers of female parent was pollinated and tagged. In the first 18 crosses, the tolerant genotype was selected as the male parent and the normal and selectively fertilized crosses were done. In the next 18 crosses, the susceptible genotype was selected as male parent and crosses were done along with selective fertilization.

3.3.4 Layout of the Experiment and Design

The experiment was laid out in CRD with thirty-six treatments and three replications. The crossed plants were kept in Rainout shelter.

3.3.5 Preparation and Planting

The seeds of selected tolerant and susceptible genotypes were sown and maintained at ambient condition (Plate 4). 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 (Plate 5) of female parent and tagged (Plate 6). Another set of cross was also made with same parental combination without pollen selection. The reciprocal (susceptible genotype as male parent) crosses were also done. Then the seeds from the selectively fertilized and normal fruits were collected at maturity stage. The seeds were sown and the seedlings were raised at ambient temperature upto 30 days. They were transplanted to pots at 30 days after sowing and kept in high temperature condition *i.e.* ROS (40- 42 ^{o}C) for evaluation (Plate 7,8)

The potting mixture made up of FYM, sand and soil in 1:1:1 ratio was used for filling the pots for the experiment. The crop was raised according to the package of practices recommendations by Kerala Agricultural University (KAU, 2016). The potted plants were kept in Rainout shelter for a period of 120 days.

3.3.6 Observations

3.3.6.1 Biometrical Parameters

3.3.6.1.1 Number of flower clusters per plant

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

3.3.6.1.2 Number of flowers per cluster

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

3.3.6.1.3 Fruits per cluster

The number of fruits present in each cluster was recorded at 60 days after transplanting the seedling.

3.3.6.1.4 Number of flower with exerted stigma per cluster

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

3.3.6.1.5 Day to first flowering

The day of blooming of the first flower from the day of sowing.

3.3.6.1.6 Stigma exertion (mm)

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

3.3.6.1.7 Total yield per plant (g)

The total yield was calculated at 120 days after sowing by taking the weight of all the fruits collected, per plant.

Plate 5. Emasculated plants for selective fertilization

Plate 6. Tagged plants for selective fertilization

Plate 7. Seedlings planted for evaluation under ROS condition

Plate 8. Evaluating the performance of selectively fertilized and normal crossed hybrids in rainout shelter

3.3.6.2 Physiological Parameters

3.3.6.2.1 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 3rd leaves and were expressed in µmole CO_2 m^{-2} s^{-1}.

3.3.6.2.2 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 ^{O}C to obtain the dry weight. The relative water content was obtained by substituting the three values in the given formula

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

3.3.6.2.3 Membrane integrity

The % leakage in the plant was estimated by the method explained by Sadasivam and Manickam (2008).

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). After 30 minutes of incubation, the solute leakage in the bathing medium (EC_b) was measured. Subsequently, the beakers were boiled at 100^0 C for 10 minutes, and the EC was measured again (EC_c). The membrane's integrity was then calculated using the provided formula, and the percentage leakage was determined as a measure of the plant leaf sample's membrane integrity.

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

3.3.6.2.4 Estimation of Vitamin C content

The ascorbic acid content in the plant was estimated volumetrically by the method explained by Sadasivam and Manickam (2008).

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 amount of dye used to reach the end point is equivalent to the quantity of ascorbic acid present. A sample (0.5 to 5 g) was obtained and then extracted using 4% oxalic acid. The resulting extract was brought up to a volume of 100 ml and then subjected to centrifugation. 5ml of supernatant was then removed and combined with 10 ml of 4% oxalic acid. This solution was titrated against dye (V_2ml) until the pink color became visible. The dye used for titration was a solution of Dichlorophenol 2 indophenol (DCPIP).

$$\text{Amount of ascorbic acid (mg per100g sample)} = \frac{0.5 \text{ mg}}{V1} \times \frac{V2}{15 \text{ ml}} \times \frac{100 \text{ ml}}{\text{Wt of the sample}} \times 100$$

3.3.6.2.5 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.

3.3.6.2.6 Pollen viability

Acetocarmine dye method proposed by Shivanna and Rangaswamy (2012) was done to determine the pollen viability of tomato flowers and is expressed in percentage.

3.3.6.3 Anatomical study of anther

SEM analysis was done to study the anatomy of anther. A small substrate of 30 mm dimension was mounted on specimen stubs using carbon tape and was over coated with gold using JFC 1600. This ion sputtering device performs rapid and efficient gold coating on microscopic specimen, allowing surface visualization. The SEM measurements were performed at 20 kV accelerating voltage. Different magnifications were used as indicated on the images.

(Make/Model: JEOL, JSM—6390LV, Tokyo, Japan).

RESULTS

4.RESULTS

The present study "Physiological and molecular basis of selective fertilization for high temperature stress tolerance in tomato (*Solanum lycopersicum* L.)" was done in three 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 analyzed and the results are presented in this chapter.

4.1 ADVANCING THE TEMPERATURE TOLERANT SELECTIVELY FERTILIZED HYBRID OF ANAGHA AND MANUPRABHA.

First generation (F_1) hybrid seeds of selectively fertilized Anagha x Manuprabha were acquired from crosses conducted previously in the lab (Ammu, 2019). Parental genotypes were selected based on their performance under high temperature (42^0C) (Ammu, 2019). Subsequent selfing upto F_4 generation were continued by selective fertilization under ambient condition (34^{o}C).

4.1.1 F_2 generation

Individual plants were selected in each of the F_2 populations. Selections were made based on pollen germination %, relative water content, membrane integrity, stomatal conductance, chlorophyll stability index, chlorophyll content and photosynthetic rate. Molecular analysis using SSR markers were also done for selection. 14 F_2 lines were planted (labelled T1 to T14), their data were recorded as depicted in table 6 and table 7, superior plants were selected from F_2 population based on their performance, harvested individually, seeds were extracted and carried forwarded to the F_3 generation. The selectively fertilized plant and fruit of F_2 is depicted in plate 9.

The pollen germination %, photosynthetic rate, chlorophyll content and chlorophyll stability index were non-significant among the lines. The stomatal conductance was significant among the population and which was recorded highest in T5 line. The relative water content also showed significant difference among the lines and the

highest was observed in T10 which was at par with T11. Percent leakage is an important indicator of good performance, which was noted very less in T10 which was at par with T3.

SSR 134 and 603 were used for genotyping in each population (Plate 10). Based on the phenotyping and genotyping data, though the difference was not so prominent among the F_2 generation, T3 and T10 line showed comparatively better performance than the other lines. Hence both T10 and T3 lines were selected for developing F_3 generation.

4.1.2 F_3 generation

Superior plants were selected based on phenotypic and genotypic data from F_2 population and seeds were extracted. The F_3 seeds collected from F_2 lines T10 and T3 were sown and data were recorded for the parameters as discussed above in F_2 (Table: 8 & 9). 14 lines were again evaluated in the F_3 generation. Data were recorded from the 14 F_3 lines and better performed lines were selected for the F_4 generation. The selectively fertilized plant and fruit of F_2 is depicted in plate 11.

In case of F_3 generation, the lines did not differ significantly for germination percentage. The remaining parameters like photosynthetic rate, stomatal conductance, relative water content, % leakage, chlorophyll content and chlorophyll stability index were significantly different among the lines. Among the lines the photosynthetic rate was recorded high in T6 which was at par with T8. In addition to photosynthetic rate, relative water content and chlorophyll content were observed highest and % leakage was recorded lowest in T8 compared to other lines. In case of RWC, T8 was at par with T7. The stomatal conductance was significant among the population and was highest in T5 line. Chlorophyll stability index also showed significant difference among the lines, the highest was observed in T3.

SSR 134 and 603 were used for genotyping each population line in F_3 generation also (Plate 12). Based on the phenotyping and genotyping data, from the F_3 generation, T6 and T8 were selected for developing F_4 generation.

F2 plant

F2 Fruit

Plate 9. The selectively fertilized plant and fruit of F2 generation

Table 6: Pollen germination %, Photosynthetic rate, stomatal conductance & Relative water content of F_2 lines

F_2 lines	Pollen germination(%)	Photosynthetic rate (µmole CO_2m^{-2} s^{-1})	Stomatal conductance (mmole m^{-2} s^{-1})	RWC (%)
T1	94.16	18.40	394.00	81.13
T2	92.74	17.10	333.50	89.19
T3	93.20	18.85	455.25	94.21
T4	92.61	18.20	489.00	92.85
T5	89.96	10.05	602.50	91.54
T6	93.04	11.85	464.50	91.15
T7	88.38	16.20	388.00	94.80
T8	91.51	17.50	454.50	91.40
T9	95.97	16.95	425.00	92.42
T10	91.79	19.65	458.00	95.31
T11	93.22	13.20	467.50	95.10
T12	93.43	13.05	477.50	87.33
T13	91.45	15.15	482.50	94.82
T14	91.70	13.65	409.00	90.28
SE m±	2.46	3.11	4.60	1.11
CD (0.05)	NS	NS	13.95	3.37

Table 7: Chlorophyll content, CSI & % leakage of F_2 lines

F_2 lines	Total chlorophyll content (mg/g FW)	CSI (%)	% Leakage
T1	1.47	66.44	2.80
T2	1.99	49.37	4.00
T3	1.99	67.37	1.60
T4	2.05	60.04	2.28
T5	1.84	55.08	2.34
T6	1.70	71.40	5.86
T7	1.72	51.66	2.25
T8	1.89	60.77	6.51
T9	1.74	54.90	5.22
T10	1.92	65.64	1.21
T11	1.54	66.16	3.45
T12	1.55	50.97	2.31
T13	1.95	63.26	5.02
T14	2.23	68.96	2.39
SE m±	0.37	7.22	0.28
CD (0.05)	NS	NS	0.85

SSR 134

SSR 603

Plate 10. Gel image of SSR markers in selectively fertilized F2 tomato plants

Table 8: Pollen germination %, Photosynthetic rate, stomatal conductance & Relative water content of F_3 lines

F_3 lines	Pollen germination(%)	Photosynthetic rate (µmole CO_2m^{-2} s^{-1})	Stomatal conductance (mmole m^{-2} s^{-1})	RWC (%)
T1	93.83	15.50	331.0	84.15
T2	96.88	19.20	290.0	87.68
T3	97.72	18.15	405.0	90.17
T4	97.27	13.70	442.0	90.15
T5	94.04	21.15	572.5	81.02
T6	94.18	22.15	454.5	92.56
T7	96.59	19.80	366.0	95.65
T8	97.45	21.60	460.0	97.24
T9	96.66	14.10	429.5	90.83
T10	98.85	18.80	404.0	82.22
T11	97.62	19.55	407.5	90.17
T12	98.02	16.30	415.5	92.88
T13	96.20	12.35	437.5	73.80
T14	99.15	10.15	409.0	88.84
SE m±	1.87	2.31	16.18	3.32
CD (0.05)	NS	7.00	49.08	9.98

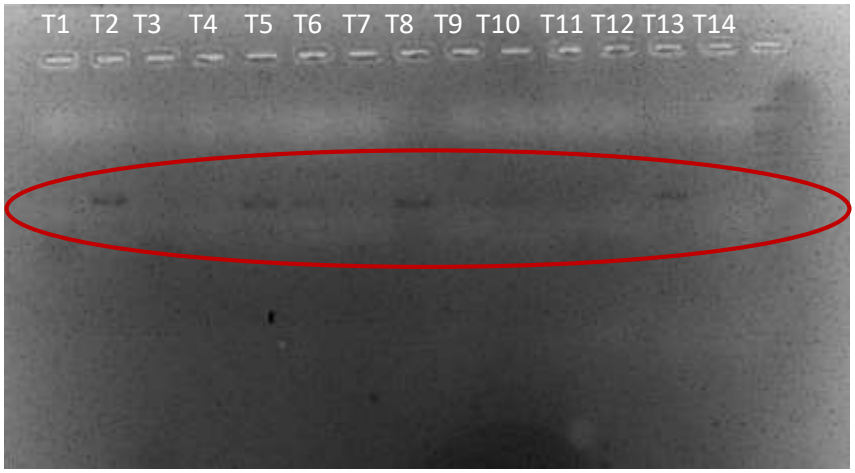
Table 9: Chlorophyll content, CSI & % leakage of F_3 lines

F_3 lines	Total chlorophyll content (mg/g FW)	CSI (%)	% Leakage
T1	1.26	82.207	1.70
T2	1.30	71.736	4.25
T3	1.19	84.390	2.66
T4	1.23	67.889	5.27
T5	1.02	73.700	1.78
T6	1.07	78.076	2.60
T7	1.14	65.410	2.33
T8	1.35	78.608	1.20
T9	1.11	70.631	2.50
T10	1.20	74.907	6.28
T11	1.24	73.597	2.77
T12	1.16	71.501	2.33
T13	0.78	72.962	2.93
T14	0.90	69.265	3.11
SE m±	0.01	1.42	0.19
CD (0.05)	0.05	4.33	0.6

F3 plant

F3 Fruit

Plate 11. The selectively fertilized plant and fruit of F3 generation



SSR 134

SSR 603

Plate 12. Gel image of SSR markers in selectively fertilized F3 tomato plants

4.1.3 F_4 generation

The F_4 generation was also developed similar to F_2 and F_3. The parameters taken in the previous generations were also recorded in F_4 as depicted in table 10 & 11 and the seeds from the best lines were collected and secured for further experiments. The selectively fertilized plant and fruit of F_2 is depicted in plate 13.

In F_4 generation, the pollen germination percentage, RWC and % leakage were not significantly different among the lines. The remaining parameters were significantly different among and showed differences among the lines. The photosynthetic rate was recorded high in T4 which was at par with T1 and the stomatal conductance was highest in T6. The chlorophyll content and chlorophyll stability index differ significantly in the lines and the highest chlorophyll content was observed in T6 which was at par with T4. In case of chlorophyll stability index, it was recorded high in T3 which was at par with T6. SSR 134 and 603 were used for genotyping each population line in F_3 generation also (Plate 14). Based on the phenotyping and genotyping data, in the F_4 generation, T4 and T6 were the best lines.

The mean performance of F_4 generation was higher than the F_3 and F_2 generation. The F_2, F_3 and F_4 lines showed no significant difference in germination percentage. However, there was an increase in germination percentage from the F_2's to the F_4's. The fruit setting % was also in the order of F_4, F_3 and F_2, when plants were selectively fertilized. It was observed that the plants are getting better by selfing through SF in each generation.

4.2 SCREENING FOR HIGH TEMPERATURE TOLERANCE USING THE CRITICAL TEMPERATURE FOR POLLEN GERMINATION.

The pollen germination and critical sterility temperature among ten tomato genotypes was quantified and recorded in order to identify differences in the tolerance of pollen to temperature variations. The effect of temperature on in vitro pollen germination was investigated in Anagha, Manuprabha, Akshaya, Vellayani Vijay, Nandi, Arka Saurabh, Pusa Rohini, IIHR 26372, Arka Vijay and Arka Rakshak by exposing to temperature ranging from 32 – 40^0C.

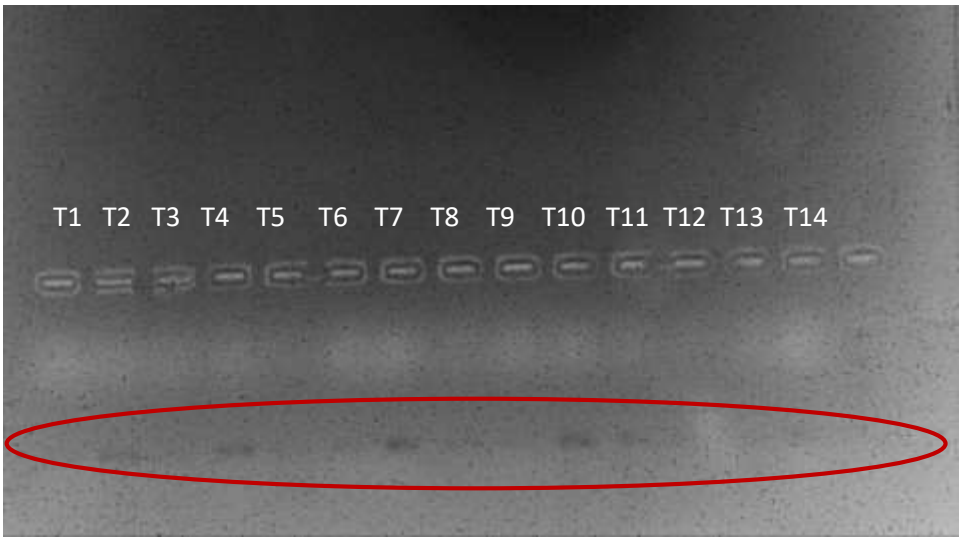
Table 10: Pollen germination %, Photosynthetic rate, stomatal conductance & Relative water content of F_4 lines.

F4 lines	Pollen germination(%)	Photosynthetic rate (µmole CO_2m^{-2} s^{-1})	Stomatal conductance (mmole m^{-2} s^{-1})	RWC (%)
T1	98.46	16.75	478.5	90.78
T2	95.51	15.78	387.0	92.48
T3	98.02	13.55	510.5	96.17
T4	96.17	17.05	535.5	94.99
T5	96.22	13.15	605.0	89.37
T6	93.83	11.78	470.5	93.97
T7	96.66	14.83	409.0	91.06
T8	93.38	13.08	465.0	93.19
T9	95.96	14.00	430.0	93.80
T10	94.27	13.90	518.0	86.18
T11	95.66	12.35	540.5	95.05
T12	97.76	11.90	543.5	93.87
T13	97.51	10.73	531.0	94.00
T14	94.98	10.10	450.5	94.85
SE m±	1.69	0.05	9.11	2.93
CD (0.05)	NS	1.60	27.64	NS

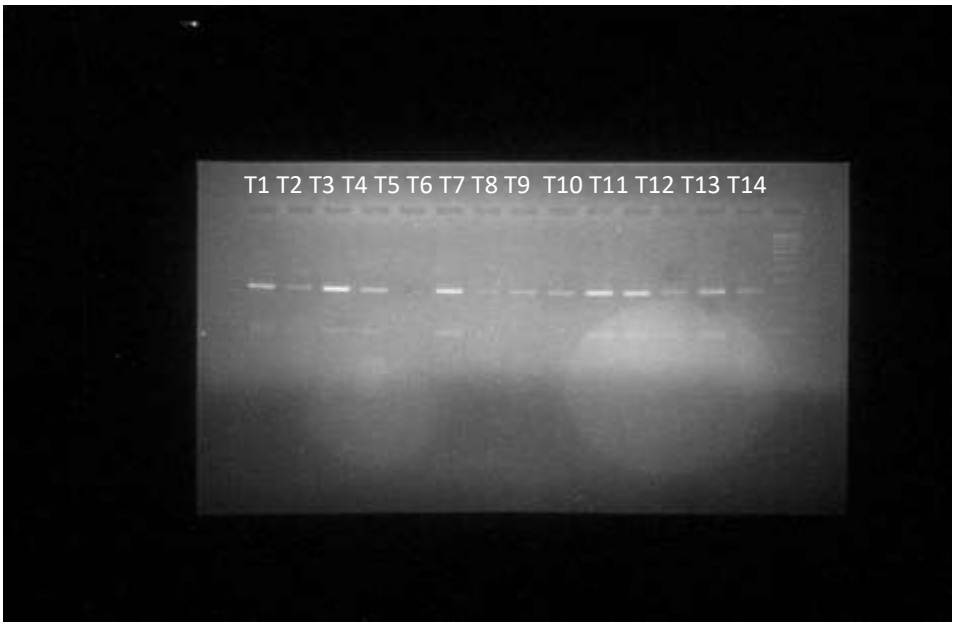
F4 Plant

F4 Fruit

Plate 13. The selectively fertilized plant and fruit of F4 generation



SSR 134



SSR 603

Plate 14. Gel image of SSR markers in selectively fertilized F4 tomato plants

Table 11: Chlorophyll content, CSI & % leakage of F_4 lines

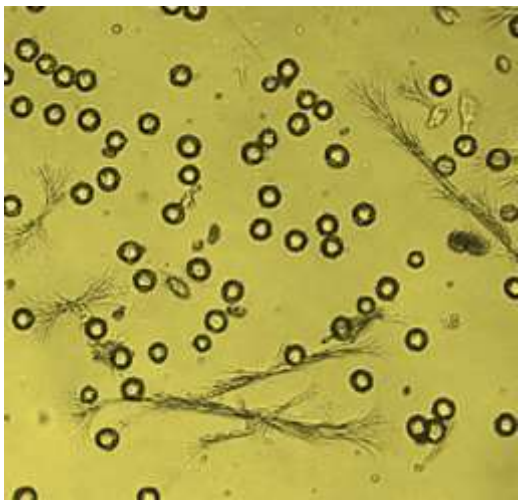
F_4 lines	Total chlorophyll content (mg/g FW)	CSI (%)	% Leakage
T1	1.34	76.31	2.88
T2	1.32	77.17	5.18
T3	1.41	85.42	2.65
T4	1.63	78.66	2.83
T5	1.49	78.42	5.36
T6	1.69	84.17	3.15
T7	1.42	83.19	2.88
T8	1.52	79.92	5.11
T9	1.53	82.45	2.65
T10	1.53	80.95	4.43
T11	1.52	83.48	3.81
T12	1.48	79.02	3.88
T13	1.41	78.70	3.52
T14	1.47	79.91	3.00
SE m±	0.021	0.87	0.96
CD (0.05)	0.06	2.66	NS

4.2.1 Pollen Germination

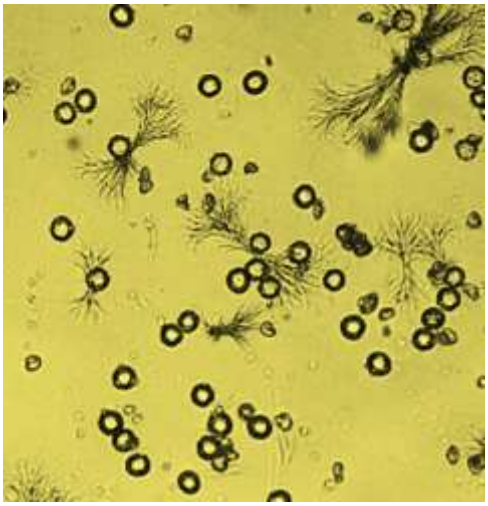
The effect of five constant temperature regimes from 32 to 40 ºC at 2 ˚C intervals on the in vitro pollen germination of ten tomato genotypes after 2hrs incubation was evaluated and expressed as the percentage of germinated pollen grains (Table 12). The fig 1 shows the graphical representation of the pollen germination of the genotypes. The pollen germination percentage decreased significantly with increase in temperature. Maximum germination percentage ranged from 24.57 % (Manuprabha) to 46.63% (Anagha), with a mean of 33.01 % were observed at 32ºC. After 36^0C, the mean pollen germination percentage decreased below 20 %. The pollen germination observed for the tomato genotypes at a temperature of 40 ºC was very low; and the germination rates were below 1% in three genotypes (Arka Saurabh, Arka Vikas and Pusa Rohini). Differences among pollen germination means were found statistically significant and the highest germination percentages were observed in Anagha (25.24 %) followed by Vellayani Vijay (23.66 %) and IIHR 26372 (20.92 %) (Plate 15). The lowest pollen germination was observed in Pusa Rohini (15.13%) which was at par with Manuprabha (15.37 %) and Arka Saurabh (16.04 %) (Plate 16).

4.2.2 Critical sterility temperature

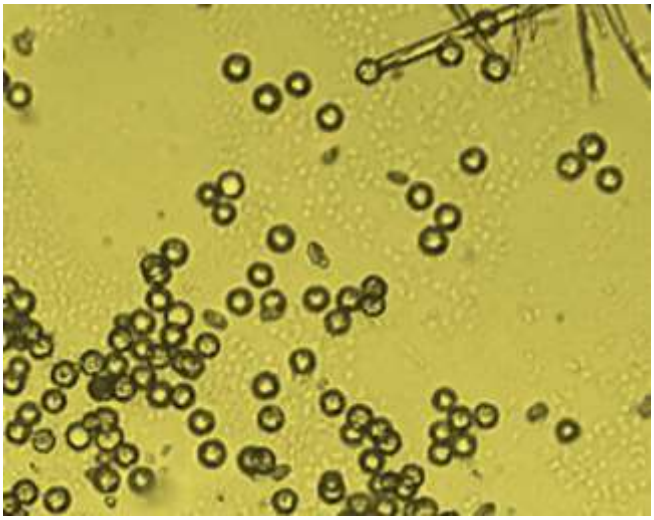
Critical temperature was assessed as the temperature where 20-30% of the pollen only germinates. Critical temperatures for pollen germination differed among genotypes. There was considerable variability in the critical temperatures for pollen germination among the genotypes. The genotypes Arka Saurabh, Arka Vikas, Pusa Rohini, Manuprabha and Akshay showed less than 20 % germination after 34º C while Vellayani Vijay, Anagha, Arka Rakshak, IIHR 26372 and Nandi has showed pollen germination percentage less than 20 only after 36ºC. The mean pollen germination % at each temperature were taken and was observed that germination percentage was 21.04 at 36ºC and decreased drastically to 9.29 % at 38^0C (Fig 2). So 36^0C was identified as the critical temperature for pollen selection. At the selected critical sterility temperature *i.e.* 36ºC, the pollen germination was highest in Anagha (25.88 %) and lowest in Arka Saurabh (16.34%), which was selected as tolerant and susceptible genotype respectively.



Anagha

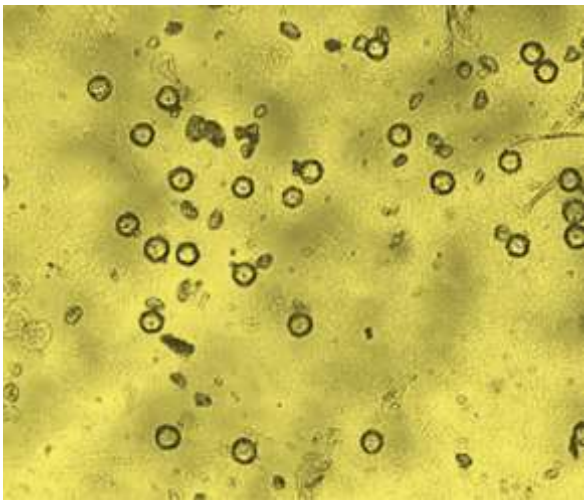


Vellayani Vijay

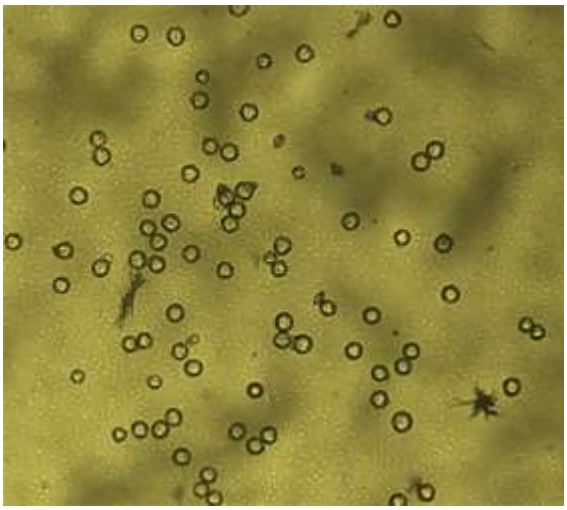


IIHR 26372

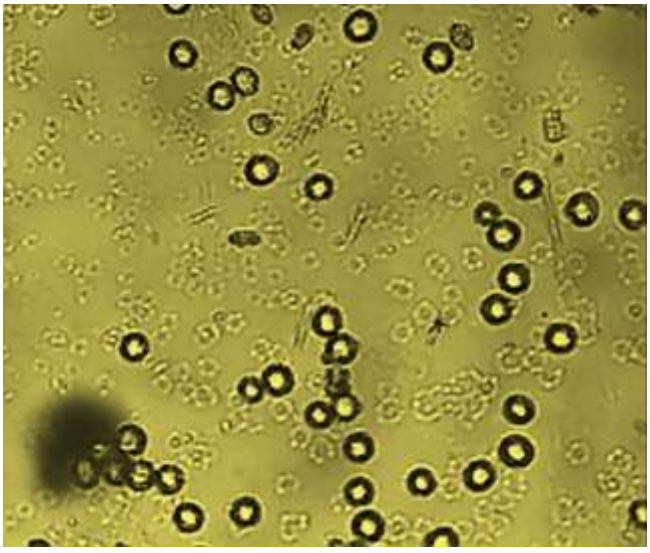
Plate 15. Pollen germination of tolerant tomato genotypes



Manuprabha



Pusa Rohini



Arka Saurabh

Plate 16. Pollen germination of susceptible tomato genotypes

Table 12: Pollen germination (%) of ten tomato varieties after 2 hrs incubation at different temperatures:

Treatment	Varieties										Mean
	Akshaya	**Vellayani Vijay**	**Manu prabha**	**Pusa Rohini**	**Anagha**	**Arka Rakshak**	**Arka Saurabh**	**Arka Vikas**	**IIHR 26372**	**Nandhi**	
T1 (32^0C)	33.60	42.33	24.57	31.13	46.63	32.64	26.15	25.10	34.96	32.99	33.01
T2 (34^0C)	29.89	33.9	21.01	22.11	34.96	26.13	26.26	32.01	29.79	22.11	28.70
T3 (36^0C)	19.97	25.63	17.94	19.03	25.88	21.27	16.34	19.69	22.74	21.89	21.04
T4 (38^0C)	14.24	12.18	10.33	0.147	12.66	10.63	11.29	8.9	12.453	0.117	9.30
T5 (40^0C)	5.74	4.26	2.99	0.121	6.11	9.836	0.172	0.02	4.663	4.09	4.47
Mean	20.68	23.66	15.37	15.13	25.24	20.10	16.04	17.01	20.92	16.24	
	Temperature (T)	Variety (V)	TXV								
SE m±	1.205	1.703	3.80								
CD (0.05)	3.38	4.781	5.72								

4.3. EXPERIMENT III: EVALUATING THE SELECTIVELY FERTILIZED HYBRIDS OF THE TOLERANT AND SUSCEPTIBLE GENOTYPES.

From the experiment II based on the critical sterility temperature and pollen germination percentage along with the tolerant genotype Anagha and susceptible genotype Manuprabha from the previous study, six genotypes were selected for pollen selection and selective fertilization. Number of clusters, Number of flowers per cluster, fruits per cluster, Number of flower with exerted stigma per cluster, Day to first flowering, Stigma exertion length (mm), Total yield per plant (g) were recorded in the normal and reciprocal crosses of tolerant and susceptible genotypes along with the selectively fertilized crosses of the same in the course of the experiment, the results exhibited significant variation in selectively fertilized crosses and normal crosses.

4.3.1. Biometrical Parameters

Number of clusters, number of flowers per cluster, number of fruits per cluster, number of flower with exerted stigma per cluster, days to first flowering, stigma exertion length and total yield per plant were recorded in the course of the experiment, the results exhibited significant variation in selectively fertilized crosses and normal crosses.

4.3.1.1. Number of clusters per plant

Effect of selective fertilization and high temperature condition on number of clusters per plant is depicted in Table 13. The difference among the varieties on number of clusters per plant was highly significant.

During first month after transplanting, among the varietal crosses the highest number of clusters were recorded from IIHR x MP(SF) (9.67), whereas IIHR x PR (SF) (2.67) gave the lowest number of clusters per plant. It shows that during first month after transplanting both highest and lowest number of clusters was observed among the selectively fertilized hybrids of the tolerant x susceptible (tolerant genotype as male parent) varieties than its normal crosses and reciprocal crosses (susceptible genotype as male parent) of the respected ones.

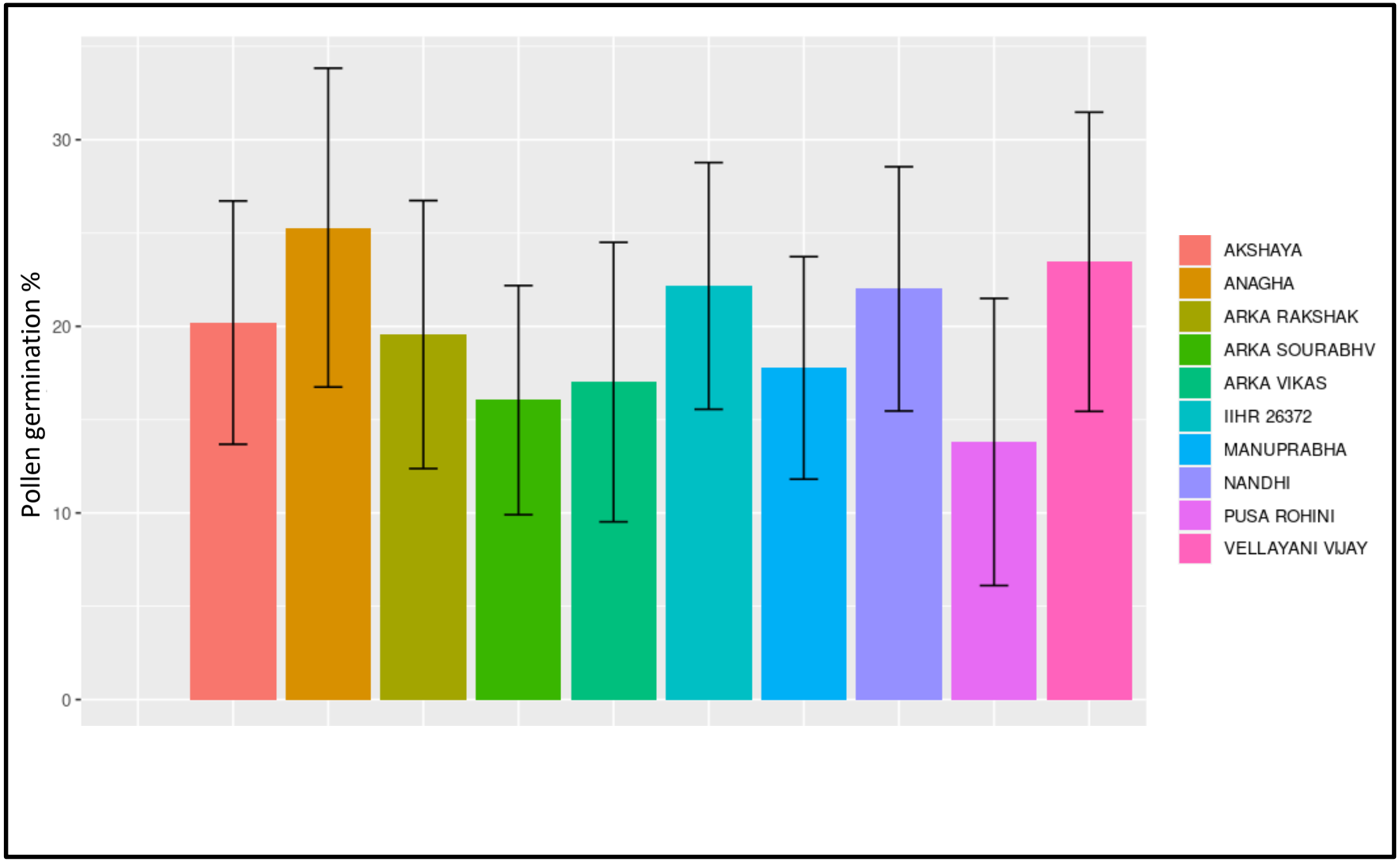


Figure 1. Pollen germination of tomato genotypes at different temperature

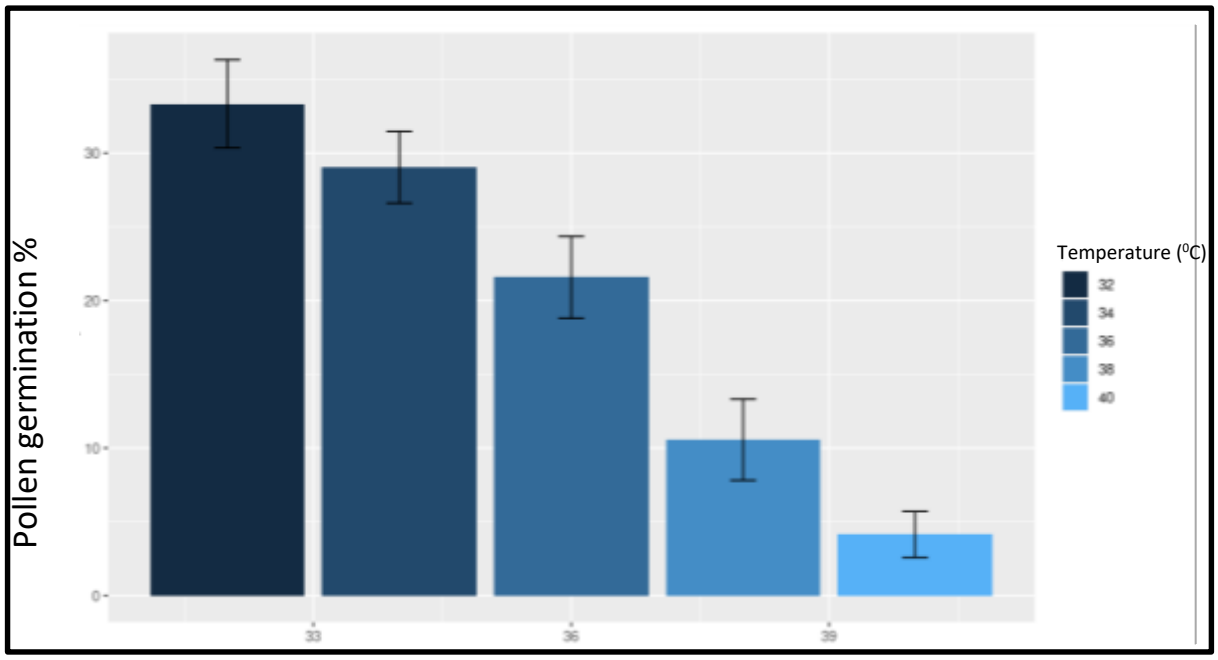


Figure 2. Critical sterility temperature

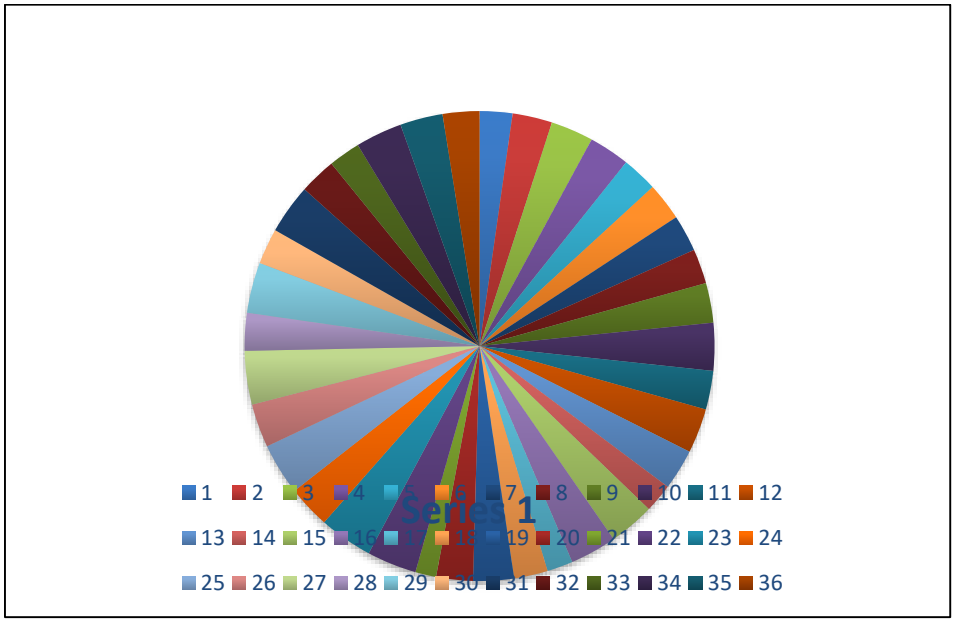


Figure 3. No of clusters in selectively fertilized and normal tomato plants (1-36 depicts the crosses from T1 to T36)

Table 13: Number of clusters in the selectively fertilized and normal crossed tomato plants

TREATMENTS	Varietal crosses	Number of clusters/ plant	
		30 DAT	60 DAT
T1	T1 x S1 (AN x MP)	5.67	11.00
T2	T1 X S2 (AN x PR)	7.00	13.33
T3	T1 X S3 (AN x AS)	7.33	14.33
T4	T2 X S1 (VV x MP)	7.67	13.67
T5	T2 X S2 (VV x PR)	5.33	12.00
T6	T2 X S3 (VV x AS)	6.67	12.67
T7	T3 X S1 (IIHR x MP)	8.33	12.33
T8	T3 X S2 (IIHR x PR)	5.67	11.67
T9	T3 X S3 (IIHR x AS)	4.67	13.33
T10	T1 X S1 (SF) (AN x MP (SF))	7.00	16.00
T11	T1 X S2 (SF) (AN x PR (Sf))	7.33	13.00
T12	T1 X S3 (SF) (AN x AS (SF))	8.33	15.00
T13	T2 X S1 (SF) (VV x MP(SF))	6.67	13.67
T14	T2 X S2 (SF) (VV x PR (SF))	3.67	9.67
T15	T2 X S3 (SF) (VV x AS (SF))	7.67	15.33
T16	T3 X S1 (SF) (IIHR x MP (SF))	9.67	15.67
T17	T3 X S2 (SF) (IIHR x PR (Sf))	2.67	8.67
T18	T3 X S3 (SF) IIHR x AS (SF))	5.00	11.33
T19	S1 X T1 (MP x AN)	9.33	13.67
T20	S2 X T1 (PR x AN)	6.00	12.33
T21	S3 X T1 (AS x AN)	4.33	7.00

T22	S1 X T2 (MP x VV)	6.33	16.67
T23	S2 X T2 (PR x VV)	8.33	18.00
T24	S3 X T2 (AS x VV)	4.67	14.33
T25	S1 X T3 (MP x IIHR)	6.33	17.33
T26	S2 X T3 (PR x IIHR)	3.67	14.67
T27	S3 X T3 (AS x IIHR)	7.67	18.00
T28	S1 X T1 (SF) (MP x AN (SF))	7.67	12.67
T29	S2 X T1 (SF) (PR x AN (SF))	7.33	17.00
T30	S3 X T1 (SF) (AS x AN(SF))	5.33	12.00
T31	S1 X T2 (SF) (MP x VV(SF))	6.00	16.67
T32	S2 X T2 (SF) (PR x VV (SF))	8.67	12.33
T33	S3 X T2 (SF) (AS x VV(SF))	3.33	10.67
T34	S1 X T3 (SF) (MP x IIHR(SF))	7.33	15.67
T35	S2 X T3 (SF) (PR x IIHR(SF))	5.67	14.33
T36	S3 X T3 (SF) (AS x IIHR(SF))	8.33	12.33
	SE m±	0.45	1.75
	CD (0.05)	1.28	4.94

In the second month after transplanting significantly higher number of clusters were recorded from PR x VV (18) which was at par with AS x IIHR (18). While the lowest number of clusters were recorded from AS x AN (7). During second month, the normal crosses of susceptible and tolerant (susceptible genotype as male parent) shows the highest and lowest number of clusters than other crosses (Fig 3). Overall it was observed that the reciprocal crosses (susceptible genotype as male parent) of the tolerant and susceptible varieties: *i.e.*, both normal and selectively fertilized crosses of S x T showed significantly higher number of clusters per plant than the tolerant x susceptible crosses (tolerant genotype as male parent).

4.3.1.2. Number of flowers per cluster

Effect of selective fertilization and high temperature condition on number of flowers per cluster is depicted in Table 14.

During first month after transplanting, no significant difference was observed among the crosses. While in the second month after transplanting, significantly higher no of flowers per cluster was observed in PR x VV (10.33) followed by IIHR x MP (9) and lowest number was observed in MP x IIHR (4.67). The highest and lowest mean value was noted in typical normally crossed reciprocal crosses (susceptible genotype as male parent) of tolerant and susceptible varieties than the selectively fertilized crosses (Fig 4).

4.3.1.3. Number of fruits per cluster

Effect of selective fertilization and high temperature condition on number of fruits per cluster is depicted in Table 15.

The number of fruits per cluster was noted in second month after transplanting. Majority of the selectively fertilized crosses recorded higher number of fruits/cluster under high temperature regime than the normal crossed ones. In genotypes, two crosses AN x MP (SF) (4.67) and in IIHR x MP (SF) (4.67) recorded significantly increased number of fruits under high temperature condition than that of normal crosses. The cross VV x PR (SF) (1.00) appeared to be most sensitive as it recorded the least.

Table 14: Number of flower clusters per plant in the selectively fertilized and normal tomato plants.

TREATMENTS	Varietal crosses	Number of flowers/ cluster	
		30 DAT	60 DAT
T1	T1 x S1 (AN x MP)	3.00	5.67
T2	T1 X S2 (AN x PR)	1.33	6.00
T3	T1 X S3 (AN x AS)	1.33	5.33
T4	T2 X S1 (VV x MP)	2.00	6.67
T5	T2 X S2 (VV x PR)	2.67	5.00
T6	T2 X S3 (VV x AS)	2.33	6.00
T7	T3 X S1 (IIHR x MP)	4.33	9.00
T8	T3 X S2 (IIHR x PR)	2.33	5.33
T9	T3 X S3 (IIHR x AS)	3.00	5.33
T10	T1 X S1 (SF) (AN x MP (SF))	3.67	7.67
T11	T1 X S2 (SF) (AN x PR (Sf))	4.00	6.67
T12	T1 X S3 (SF) (AN x AS (SF))	4.00	7.33
T13	T2 X S1 (SF) (VV x MP(SF))	4.67	7.33
T14	T2 X S2 (SF) (VV x PR (SF))	2.67	6.67
T15	T2 X S3 (SF) (VV x AS (SF))	3.00	7.33
T16	T3 X S1 (SF) (IIHR x MP (SF))	3.33	6.67
T17	T3 X S2 (SF) (IIHR x PR (Sf))	3.67	7.33
T18	T3 X S3 (SF) IIHR x AS (SF))	3.00	6.00
T19	S1 X T1 (MP x AN)	3.00	5.33
T20	S2 X T1 (PR x AN)	2.33	7.33
T21	S3 X T1 (AS x AN)	2.67	5.33

T22	S1 X T2 (MP x VV)	3.33	5.33
T23	S2 X T2 (PR x VV)	5.33	10.33
T24	S3 X T2 (AS x VV)	2.33	6.00
T25	S1 X T3 (MP x IIHR)	2.00	4.67
T26	S2 X T3 (PR x IIHR)	3.67	5.67
T27	S3 X T3 (AS x IIHR)	3.33	5.67
T28	S1 X T1 (SF) (MP x AN (SF))	4.33	5.67
T29	S2 X T1 (SF) (PR x AN (SF))	2.33	6.33
T30	S3 X T1 (SF) (AS x AN(SF))	5.00	7.33
T31	S1 X T2 (SF) (MP x VV(SF))	2.67	6.67
T32	S2 X T2 (SF) (PR x VV (SF))	3.00	7.67
T33	S3 X T2 (SF) (AS x VV(SF))	4.67	6.67
T34	S1 X T3 (SF) (MP x IIHR(SF))	2.33	5.33
T35	S2 X T3 (SF) (PR x IIHR(SF))	2.00	5.67
T36	S3 X T3 (SF) (AS x IIHR(SF))	3.00	7.33
	SE m±	0.79	0.60
	CD (0.05)	NS	1.71

Table 15: Number of fruits per clusters in the selectively fertilized and normal tomato plants.

TREATMENTS	Varietal crosses	fruits /cluster
T1	T1 x S1 (AN x MP)	3.67
T2	T1 X S2 (AN x PR)	3.00
T3	T1 X S3 (AN x AS)	1.67
T4	T2 X S1 (VV x MP)	2.67
T5	T2 X S2 (VV x PR)	3.00
T6	T2 X S3 (VV x AS)	1.67
T7	T3 X S1 (IIHR x MP)	2.33
T8	T3 X S2 (IIHR x PR)	3.67
T9	T3 X S3 (IIHR x AS)	3.00
T10	T1 X S1 (SF) (AN x MP (SF))	4.67
T11	T1 X S2 (SF) (AN x PR (Sf))	2.00
T12	T1 X S3 (SF) (AN x AS (SF))	3.67
T13	T2 X S1 (SF) (VV x MP(SF))	3.33
T14	T2 X S2 (SF) (VV x PR (SF))	1.00
T15	T2 X S3 (SF) (VV x AS (SF))	3.33
T16	T3 X S1 (SF) (IIHR x MP (SF))	4.67
T17	T3 X S2 (SF) (IIHR x PR (Sf))	1.67
T18	T3 X S3 (SF) IIHR x AS (SF))	3.00
T19	S1 X T1 (MP x AN)	3.33
T20	S2 X T1 (PR x AN)	3.67
T21	S3 X T1 (AS x AN)	2.67
T22	S1 X T2 (MP x VV)	3.33

T23	S2 X T2 (PR x VV)	2.33
T24	S3 X T2 (AS x VV)	1.67
T25	S1 X T3 (MP x IIHR)	2.33
T26	S2 X T3 (PR x IIHR)	2.67
T27	S3 X T3 (AS x IIHR)	2.00
T28	S1 X T1 (SF) (MP x AN (SF))	3.33
T29	S2 X T1 (SF) (PR x AN (SF))	3.33
T30	S3 X T1 (SF) (AS x AN(SF))	3.33
T31	S1 X T2 (SF) (MP x VV(SF))	4.33
T32	S2 X T2 (SF) (PR x VV (SF))	2.67
T33	S3 X T2 (SF) (AS x VV(SF))	2.00
T34	S1 X T3 (SF) (MP x IIHR(SF))	3.00
T35	S2 X T3 (SF) (PR x IIHR(SF))	1.33
T36	S3 X T3 (SF) (AS x IIHR(SF))	2.33
	SE m±	0.61
	CD (0.05)	1.73

4.3.1.4. Days to first flowering

Effect of selective fertilization and high temperature condition on time of anthesis in the crossed genotypes is depicted in Table 16. The difference among the varieties on days to flowering was highly significant.

AS x AN (SF) took the shortest period (37 days) for flowering, while AS x IIHR (SF) took the longest days for flowering (50 days). The normal and selectively fertilized crosses of T x S (tolerant genotype as male parent) flowered comparatively earlier than the reciprocal crossed (S x T) (susceptible genotype as male parent) ones. The number of days taken by the selectively fertilized and normal crosses of tolerant and susceptible were 37 to 44 days, with an exception of T1, T4, T9, T12 and T17, while reciprocal crosses took 45 to 50 days for first flowering.

4.3.1.5. Number of flowers with exerted stigma

Effect of selective fertilization and high temperature condition on percentage number of flowers with exerted stigma is depicted in Table 17. It was expressed in terms of percentage.

During first month after transplanting there was no significant difference observed among the crossed genotypes. While during the second month after transplanting the crosses shows significant difference in percentage number of flowers with exerted stigma. Among the crosses significantly higher mean value for number of flowers with exerted stigma was observed in PR x IIHR with a percentage of 73.49 which is a reciprocal crossed genotype of tolerant and susceptible (susceptible genotype as male parent). The lowest mean value for percentage number of flowers with exerted stigma was observed in IIHR x MP (SF) *i.e.* 20.63 %. Fig 5 represents the percentage of flowers with exerted stigma (Stigma exertion %) of selectively fertilized and normal tomato plants at 30 and 60 DAT.

Among the 36 crosses, the reciprocal crossed (susceptible genotype as male parent) genotypes *i.e.* both normal and selectively fertilized reciprocal crosses equally showed more stigma exertion percentage than the tolerant x susceptible (tolerant genotype as male parent) crosses (both SF and normal crosses). The selectively crossed

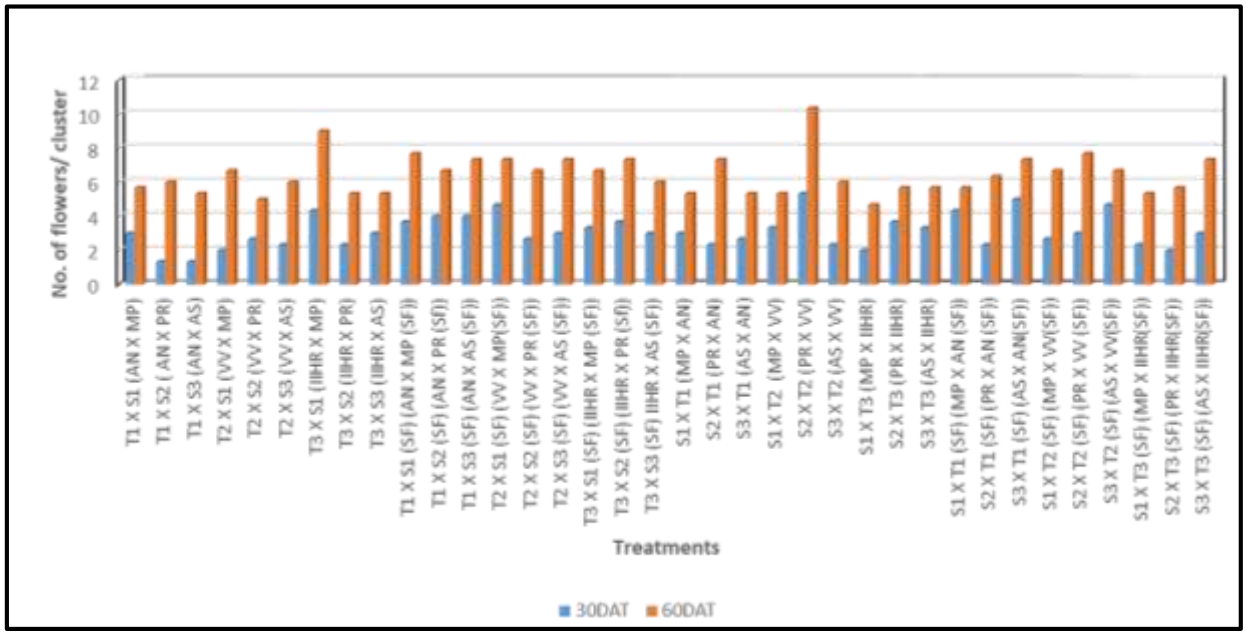


Figure 4: Number of flower per clusters in selectively fertilized and normal tomato plants.

Table 16: Number of days to first flowering in selectively fertilized and normal tomato plants.

TREATMENTS	Varietal crosses	Days to first flowering
T1	T1 x S1 (AN x MP)	45.5
T2	T1 X S2 (AN x PR)	41.5
T3	T1 X S3 (AN x AS)	43.0
T4	T2 X S1 (VV x MP)	47.5
T5	T2 X S2 (VV x PR)	42.0
T6	T2 X S3 (VV x AS)	39.0
T7	T3 X S1 (IIHR x MP)	41.0
T8	T3 X S2 (IIHR x PR)	39.5
T9	T3 X S3 (IIHR x AS)	44.5
T10	T1 X S1 (SF) (AN x MP (SF))	43.0
T11	T1 X S2 (SF) (AN x PR (Sf))	38.0
T12	T1 X S3 (SF) (AN x AS (SF))	45.0
T13	T2 X S1 (SF) (VV x MP(SF))	42.5
T14	T2 X S2 (SF) (VV x PR (SF))	42.5
T15	T2 X S3 (SF) (VV x AS (SF))	43.5
T16	T3 X S1 (SF) (IIHR x MP (SF))	38.0
T17	T3 X S2 (SF) (IIHR x PR (Sf))	44.5
T18	T3 X S3 (SF) IIHR x AS (SF))	41.5
T19	S1 X T1 (MP x AN)	42.0
T20	S2 X T1 (PR x AN)	44.0
T21	S3 X T1 (AS x AN)	44.5
T22	S1 X T2 (MP x VV)	44.0

T23	S2 X T2 (PR x VV)	38.0
T24	S3 X T2 (AS x VV)	46.5
T25	S1 X T3 (MP x IIHR)	45.0
T26	S2 X T3 (PR x IIHR)	47.5
T27	S3 X T3 (AS x IIHR)	44.0
T28	S1 X T1 (SF) (MP x AN (SF))	41.0
T29	S2 X T1 (SF) (PR x AN (SF))	46.5
T30	S3 X T1 (SF) (AS x AN(SF))	37.0
T31	S1 X T2 (SF) (MP x VV(SF))	42.5
T32	S2 X T2 (SF) (PR x VV (SF))	44.0
T33	S3 X T2 (SF) (AS x VV(SF))	46.0
T34	S1 X T3 (SF) (MP x IIHR(SF))	41.5
T35	S2 X T3 (SF) (PR x IIHR(SF))	43.5
T36	S3 X T3 (SF) (AS x IIHR(SF))	50.0
	SE m±	1.23
	CD (0.05)	3.55

Table 17: Percentage of flowers with exerted stigma in selectively fertilized and normal tomato plants.

TREATMENTS	Varietal crosses	Stigma exertion %	
		30 DAT	60 DAT
T1	T1 x S1 (AN x MP)	38.89	34.44
T2	T1 X S2 (AN x PR)	50.00	38.89
T3	T1 X S3 (AN x AS)	66.67	50.00
T4	T2 X S1 (VV x MP)	27.78	23.81
T5	T2 X S2 (VV x PR)	38.89	53.33
T6	T2 X S3 (VV x AS)	22.22	33.33
T7	T3 X S1 (IIHR x MP)	28.33	25.92
T8	T3 X S2 (IIHR x PR)	25.00	55.56
T9	T3 X S3 (IIHR x AS)	68.89	62.38
T10	T1 X S1 (SF) (AN x MP (SF))	16.67	30.55
T11	T1 X S2 (SF) (AN x PR (Sf))	86.67	50.79
T12	T1 X S3 (SF) (AN x AS (SF))	52.78	36.91
T13	T2 X S1 (SF) (VV x MP(SF))	19.44	45.77
T14	T2 X S2 (SF) (VV x PR (SF))	61.11	69.45
T15	T2 X S3 (SF) (VV x AS (SF))	55.56	31.94
T16	T3 X S1 (SF) (IIHR x MP (SF))	16.67	20.63
T17	T3 X S2 (SF) (IIHR x PR (Sf))	33.33	29.17
T18	T3 X S3 (SF) IIHR x AS (SF))	58.33	21.75
T19	S1 X T1 (MP x AN)	46.67	37.78
T20	S2 X T1 (PR x AN)	22.22	51.19
T21	S3 X T1 (AS x AN)	53.33	56.67
T22	S1 X T2 (MP x VV)	62.22	22.86

T23	S2 X T2 (PR x VV)	50.00	60.41
T24	S3 X T2 (AS x VV)	58.33	52.06
T25	S1 X T3 (MP x IIHR)	50.00	56.67
T26	S2 X T3 (PR x IIHR)	71.67	73.49
T27	S3 X T3 (AS x IIHR)	30.55	36.19
T28	S1 X T1 (SF) (MP x AN (SF))	48.89	34.44
T29	S2 X T1 (SF) (PR x AN (SF))	83.33	52.38
T30	S3 X T1 (SF) (AS x AN(SF))	37.22	41.67
T31	S1 X T2 (SF) (MP x VV(SF))	41.67	24.52
T32	S2 X T2 (SF) (PR x VV (SF))	70.00	55.56
T33	S3 X T2 (SF) (AS x VV(SF))	42.22	54.76
T34	S1 X T3 (SF) (MP x IIHR(SF))	25.00	56.67
T35	S2 X T3 (SF) (PR x IIHR(SF))	44.44	52.22
T36	S3 X T3 (SF) (AS x IIHR(SF))	68.89	50.00
	SE m±	21.55	10.42
	CD (0.05)	NS	29.39

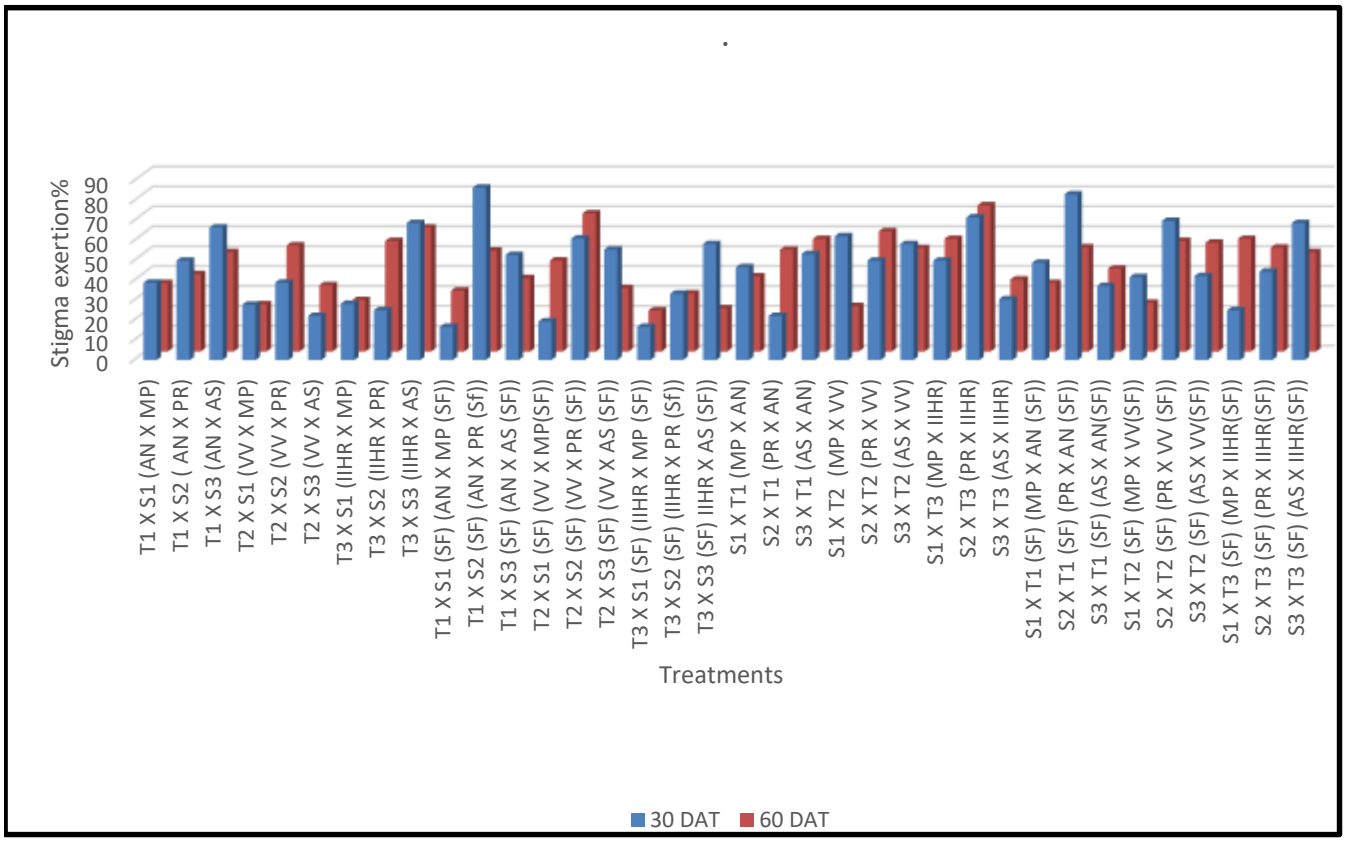


Figure 5: Percentage of flowers with exerted stigma (Stigma exertion %) of selectively fertilized and normal tomato plants

genotypes of tolerant and susceptible genotype (tolerant genotype as male parent) showed least stigma exertion percentage under high temperature condition than the other crosses, that shows the best performance under high temperature.

4.3.1.6 Stigma exertion (mm)

Effect of selective fertilization under high temperature condition on length of exerted stigma is depicted in Table 18. The number of flowers with exerted stigma was high under high temperature. During first month after transplanting among the crosses significantly longer exerted stigma was observed in the SF reciprocal crosses (susceptible genotype as male parent). Among the varietal crosses significantly higher mean value was observed in T33 (AS x VV(SF)) (1.98 mm) and the lowest mean value for the exerted stigma length was observed in T4 (VV x MP) and T10 (AN x MP (SF)) (0.02 mm).

During second month after transplanting among the crosses significantly longer exerted stigma was observed in normal hybrids of tolerant and susceptible (tolerant genotype as male parent). Among the varietal crosses significantly highest mean value was observed in T6 (VV x AS) (2.50 mm). The lowest mean value for the exerted stigma length was observed in T31 (MP x VV (SF)) (0.16 mm) which was at par with T1, T10, T25, T34, T13, T14, T33, T35, T32, T28, T16, T7, T2 and T17. Overall the SF crosses of both T x S (tolerant genotype as male parent) and S x T (susceptible genotype as male parent) showed less stigma exertion than its normal crosses (Plate 17a and 17b).

4.3.2. Physiological Parameters

Photosynthetic rate, relative water content, membrane integrity, vitamin C content, SOD activity and pollen viability per plant were recorded in the course of the experiment, the results exhibited significant variation in selectively fertilized crosses and normal crosses.

4.3.2.1. Photosynthetic rate

Effect of selective fertilization and high temperature condition on photosynthetic rate of tomato is depicted in Table 19. Photosynthetic rate will give the overall health figure of the plants. photosynthetic rate will be higher in tolerant ones as

Table 18: Stigma exertion length in selectively fertilized and normal tomato plants.

TREATMENTS	Varietal crosses	Stigma exertion (mm)	
		30 DAT	60 DAT
T1	T1 x S1 (AN x MP)	0.13	0.17
T2	T1 X S2 (AN x PR)	0.25	0.44
T3	T1 X S3 (AN x AS)	1.38	1.71
T4	T2 X S1 (VV x MP)	0.02	0.65
T5	T2 X S2 (VV x PR)	0.13	1.54
T6	T2 X S3 (VV x AS)	1.17	2.50
T7	T3 X S1 (IIHR x MP)	0.10	0.44
T8	T3 X S2 (IIHR x PR)	0.15	0.64
T9	T3 X S3 (IIHR x AS)	0.71	1.79
T10	T1 X S1 (SF) (AN x MP (SF))	0.02	0.21
T11	T1 X S2 (SF) (AN x PR (Sf))	0.56	0.46
T12	T1 X S3 (SF) (AN x AS (SF))	0.58	1.33
T13	T2 X S1 (SF) (VV x MP(SF))	0.48	0.23
T14	T2 X S2 (SF) (VV x PR (SF))	1.29	0.25
T15	T2 X S3 (SF) (VV x AS (SF))	1.29	1.71
T16	T3 X S1 (SF) (IIHR x MP (SF))	0.17	0.33
T17	T3 X S2 (SF) (IIHR x PR (Sf))	0.44	0.45
T18	T3 X S3 (SF) IIHR x AS (SF))	1.67	1.75
T19	S1 X T1 (MP x AN)	0.17	0.86
T20	S2 X T1 (PR x AN)	0.50	0.50
T21	S3 X T1 (AS x AN)	0.92	1.58
T22	S1 X T2 (MP x VV)	0.25	0.58

T23	S2 X T2 (PR x VV)	0.17	1.40
T24	S3 X T2 (AS x VV)	0.91	1.83
T25	S1 X T3 (MP x IIHR)	0.17	0.22
T26	S2 X T3 (PR x IIHR)	0.31	0.60
T27	S3 X T3 (AS x IIHR)	1.25	1.67
T28	S1 X T1 (SF) (MP x AN (SF))	0.25	0.33
T29	S2 X T1 (SF) (PR x AN (SF))	0.58	0.75
T30	S3 X T1 (SF) (AS x AN(SF))	1.00	1.17
T31	S1 X T2 (SF) (MP x VV(SF))	0.32	0.17
T32	S2 X T2 (SF) (PR x VV (SF))	1.25	0.33
T33	S3 X T2 (SF) (AS x VV(SF))	1.92	0.27
T34	S1 X T3 (SF) (MP x IIHR(SF))	0.25	0.22
T35	S2 X T3 (SF) (PR x IIHR(SF))	0.58	0.30
T36	S3 X T3 (SF) (AS x IIHR(SF))	1.20	1.17
	SE m±	0.3	0.25
	CD (0.05)	0.85	0.72

compared to susceptible parents under temperature stress.

During first month after transplanting, among the treatments significantly highest photosynthetic rate was observed in T12 (AN x AS (SF)) (18.03) and lowest in T26 (PR x IIHR) (8.30). While at 60 DAT, T13 (VV x MP (SF)) and T3 (AN x AS) showed the highest and lowest photosynthetic rate *i.e.*, 8.30 and 5.40 respectively. It was also observed from the experiment that the SF showed more photosynthetic rate than normal crossed ones. Among them also the selectively fertilized crosses having tolerant genotype as the male parent shows more photosynthetic rate than the crosses having susceptible genotype as male. Fig 6 shows the photosynthetic rate of selectively fertilized and normal tomato plants at 30 and 60 DAT.

4.3.2.2. Relative water content (RWC)

Effect of selective fertilization and high temperature condition on relative water content is depicted in Table 20. RWC is also an indicator of tolerance level of plant at high temperature. The tolerant plant can able to maintain plant water content at high temperature than the susceptible ones. Relative water content decreases at high temperature.

It was observed that among the crosses significantly highest mean value was recorded in the reciprocal crossed selective fertilized hybrids *i.e.*, the crosses which had susceptible genotype as male parent (Fig 7). At 30 DAT, T33 (AS x VV (SF)) (77.28 %) and T24 (AS x VV) (54.88%) recorded the highest and lowest RWC respectively. While at 60 DAT, T29 (PR x AN (SF)) recorded the highest & T10 (AN x MP (SF)) recorded the least RWC.

4.3.2.3. Membrane integrity (% Leakage)

Membrane integrity in terms of % leakage due to selective fertilization and high temperature condition is depicted in Table 21. Increased % leakage of electrolyte shows the decreased membrane stability. Decrease in % leakage at high temperature is an indicator of temperature tolerance.

Among the crosses, at 30 DAT, T27 (AS x IIHR) showed the least % leakage *i.e.* 3.69 % and T20 showed the highest leakage (62.54%). While at the time of 60 days

(a) Stigma exertion length in normal hybrid

(b) Stigma exertion length in selectively fertilized hybrid

Plate 17: Stigma exertion length in normal and selectively fertilized tomato genotypes.

Table 19: Photosynthetic rate (µmole CO_2 m^{-2} s^{-1}) of selectively fertilized and normal tomato plants.

TREATMENTS	Varietal crosses	Photosynthetic rate (µmole CO_2 m^{-2} s^{-1})	
		30 DAT	60 DAT
T1	T1 x S1 (AN x MP)	13.95	7.80
T2	T1 X S2 (AN x PR)	12.97	9.23
T3	T1 X S3 (AN x AS)	11.17	5.40
T4	T2 X S1 (VV x MP)	10.63	7.33
T5	T2 X S2 (VV x PR)	9.70	6.77
T6	T2 X S3 (VV x AS)	8.67	7.57
T7	T3 X S1 (IIHR x MP)	10.33	8.73
T8	T3 X S2 (IIHR x PR)	10.10	6.40
T9	T3 X S3 (IIHR x AS)	8.80	5.53
T10	T1 X S1 (SF) (AN x MP (SF))	11.53	7.20
T11	T1 X S2 (SF) (AN x PR (Sf))	13.47	10.40
T12	T1 X S3 (SF) (AN x AS (SF))	18.03	9.67
T13	T2 X S1 (SF) (VV x MP(SF))	16.80	10.73
T14	T2 X S2 (SF) (VV x PR (SF))	11.73	6.57
T15	T2 X S3 (SF) (VV x AS (SF))	13.63	9.57
T16	T3 X S1 (SF) (IIHR x MP (SF))	12.10	8.20
T17	T3 X S2 (SF) (IIHR x PR (Sf))	14.80	9.43
T18	T3 X S3 (SF) IIHR x AS (SF))	13.67	7.67
T19	S1 X T1 (MP x AN)	10.07	6.43
T20	S2 X T1 (PR x AN)	9.70	6.30
T21	S3 X T1 (AS x AN)	11.00	8.13
T22	S1 X T2 (MP x VV)	12.23	7.60

T23	S2 X T2 (PR x VV)	11.73	6.60
T24	S3 X T2 (AS x VV)	8.63	6.17
T25	S1 X T3 (MP x IIHR)	8.73	7.73
T26	S2 X T3 (PR x IIHR)	8.30	5.63
T27	S3 X T3 (AS x IIHR)	8.43	6.43
T28	S1 X T1 (SF) (MP x AN (SF))	8.47	5.50
T29	S2 X T1 (SF) (PR x AN (SF))	8.77	6.17
T30	S3 X T1 (SF) (AS x AN(SF))	11.67	8.20
T31	S1 X T2 (SF) (MP x VV(SF))	13.23	8.30
T32	S2 X T2 (SF) (PR x VV (SF))	12.27	7.27
T33	S3 X T2 (SF) (AS x VV(SF))	10.13	6.07
T34	S1 X T3 (SF) (MP x IIHR(SF))	12.30	8.37
T35	S2 X T3 (SF) (PR x IIHR(SF))	10.53	6.37
T36	S3 X T3 (SF) (AS x IIHR(SF))	10.77	6.90
	SE m±	0.981	0.415
	CD (0.05)	2.77	0.951

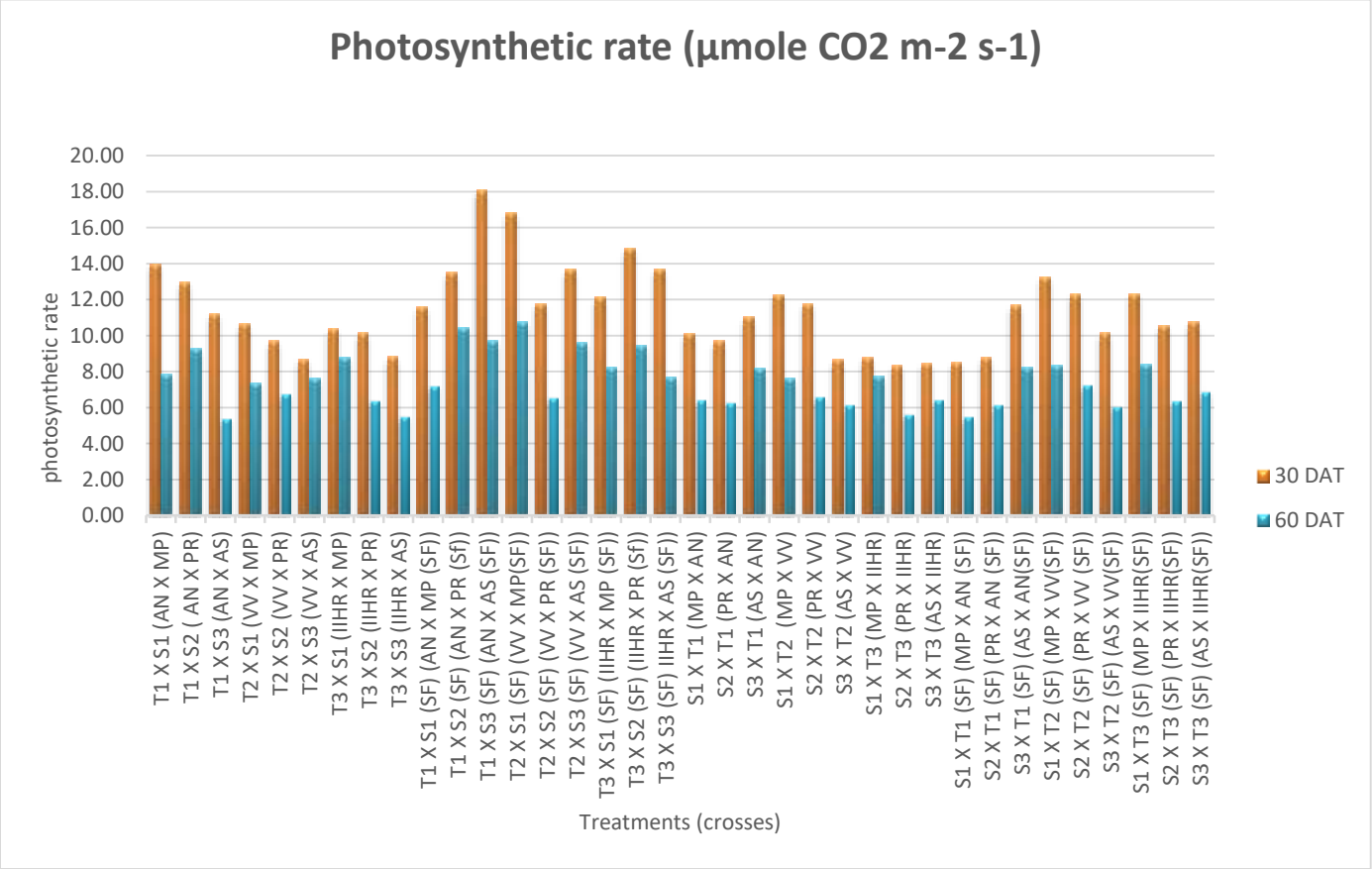


Figure 6: Photosynthetic rate of selectively fertilized and normal tomato plants

Table 20: Relative water content (%) of selectively fertilized and normal tomato plants.

TREATMENTS	Varietal crosses	RWC %	
		30 DAT	60 DAT
T1	T1 x S1 (AN x MP)	65.00	54.40
T2	T1 X S2 (AN x PR)	65.37	53.00
T3	T1 X S3 (AN x AS)	66.80	56.93
T4	T2 X S1 (VV x MP)	65.21	51.26
T5	T2 X S2 (VV x PR)	74.45	53.98
T6	T2 X S3 (VV x AS)	68.04	53.16
T7	T3 X S1 (IIHR x MP)	63.10	51.66
T8	T3 X S2 (IIHR x PR)	64.85	46.28
T9	T3 X S3 (IIHR x AS)	64.27	54.30
T10	T1 X S1 (SF) (AN x MP (SF))	63.93	44.01
T11	T1 X S2 (SF) (AN x PR (Sf))	67.09	47.87
T12	T1 X S3 (SF) (AN x AS (SF))	57.59	48.87
T13	T2 X S1 (SF) (VV x MP(SF))	57.60	46.66
T14	T2 X S2 (SF) (VV x PR (SF))	73.37	53.33
T15	T2 X S3 (SF) (VV x AS (SF))	60.43	49.67
T16	T3 X S1 (SF) (IIHR x MP (SF))	66.62	59.09
T17	T3 X S2 (SF) (IIHR x PR (Sf))	67.14	45.94
T18	T3 X S3 (SF) IIHR x AS (SF))	57.66	50.47
T19	S1 X T1 (MP x AN)	61.16	48.95
T20	S2 X T1 (PR x AN)	56.30	48.05
T21	S3 X T1 (AS x AN)	63.84	53.05
T22	S1 X T2 (MP x VV)	63.54	57.18

T23	S2 X T2 (PR x VV)	66.99	52.03
T24	S3 X T2 (AS x VV)	54.88	54.84
T25	S1 X T3 (MP x IIHR)	61.26	58.96
T26	S2 X T3 (PR x IIHR)	64.81	46.50
T27	S3 X T3 (AS x IIHR)	66.36	45.00
T28	S1 X T1 (SF) (MP x AN (SF))	70.39	56.14
T29	S2 X T1 (SF) (PR x AN (SF))	75.31	64.09
T30	S3 X T1 (SF) (AS x AN(SF))	67.04	48.62
T31	S1 X T2 (SF) (MP x VV(SF))	71.75	59.74
T32	S2 X T2 (SF) (PR x VV (SF))	73.17	59.44
T33	S3 X T2 (SF) (AS x VV(SF))	77.28	57.78
T34	S1 X T3 (SF) (MP x IIHR(SF))	70.84	57.48
T35	S2 X T3 (SF) (PR x IIHR(SF))	65.65	56.83
T36	S3 X T3 (SF) (AS x IIHR(SF))	75.15	55.71
	SE m±	1.61	1.31
	CD (0.05)	4.55	3.69

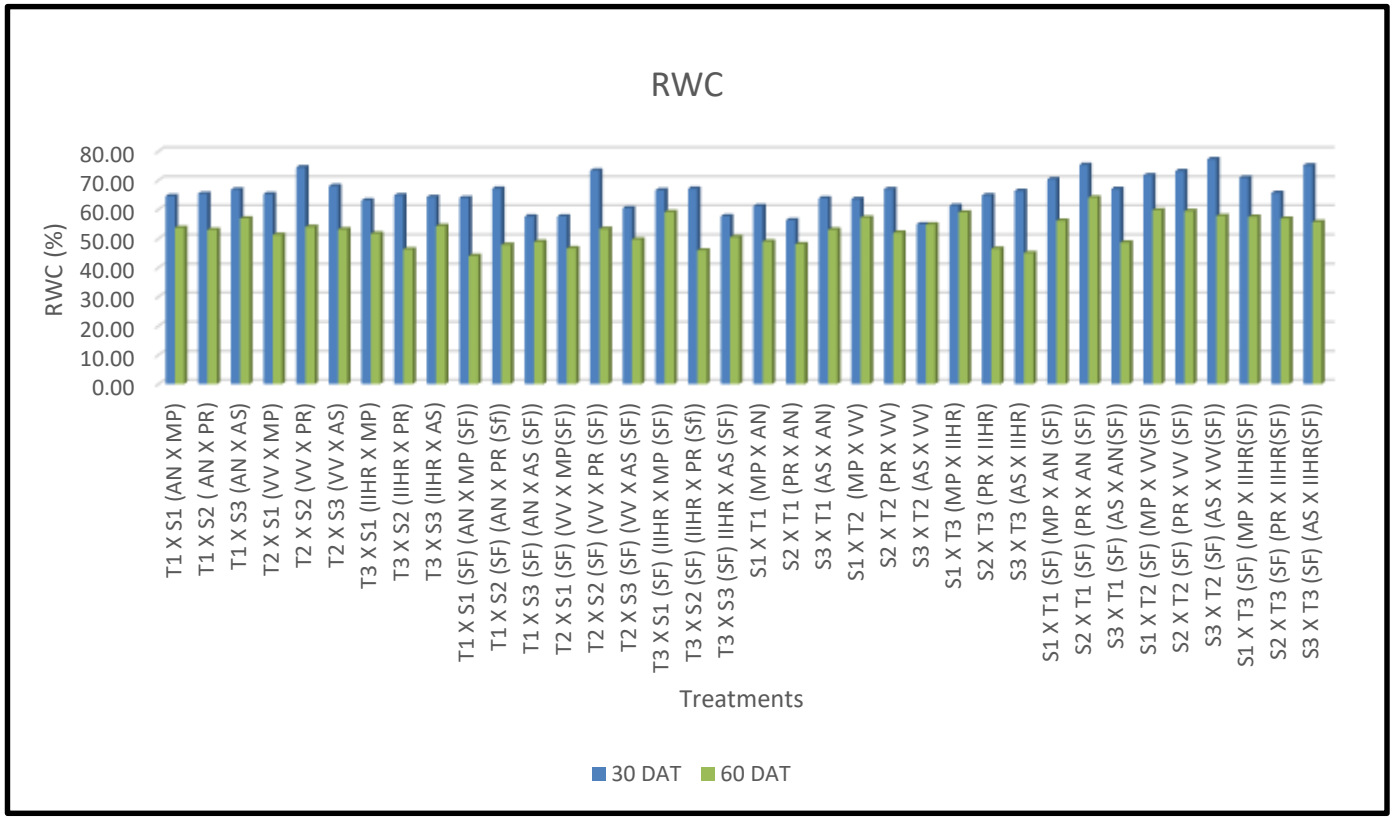


Figure 7: RWC of selectively fertilized and normal tomato plants

Table 21: Membrane integrity (% leakage) of selectively fertilized and normal tomato plants

TREATMENTS	Varietal crosses	% leakage	
		30 DAT	60 DAT
T1	T1 x S1 (AN x MP)	11.98	7.14
T2	T1 X S2 (AN x PR)	11.67	6.14
T3	T1 X S3 (AN x AS)	9.52	5.52
T4	T2 X S1 (VV x MP)	9.09	10.15
T5	T2 X S2 (VV x PR)	12.50	11.54
T6	T2 X S3 (VV x AS)	12.31	6.66
T7	T3 X S1 (IIHR x MP)	17.88	6.43
T8	T3 X S2 (IIHR x PR)	42.71	21.05
T9	T3 X S3 (IIHR x AS)	16.67	10.30
T10	T1 X S1 (SF) (AN x MP (SF))	15.08	8.94
T11	T1 X S2 (SF) (AN x PR (Sf))	11.14	7.46
T12	T1 X S3 (SF) (AN x AS (SF))	20.00	12.65
T13	T2 X S1 (SF) (VV x MP(SF))	16.41	9.61
T14	T2 X S2 (SF) (VV x PR (SF))	9.80	5.42
T15	T2 X S3 (SF) (VV x AS (SF))	11.00	6.45
T16	T3 X S1 (SF) (IIHR x MP (SF))	22.78	15.71
T17	T3 X S2 (SF) (IIHR x PR (Sf))	26.67	13.73
T18	T3 X S3 (SF) IIHR x AS (SF))	27.87	17.41
T19	S1 X T1 (MP x AN)	17.65	8.46
T20	S2 X T1 (PR x AN)	62.54	22.65
T21	S3 X T1 (AS x AN)	10.35	5.42
T22	S1 X T2 (MP x VV)	16.63	7.30

T23	S2 X T2 (PR x VV)	22.22	11.44
T24	S3 X T2 (AS x VV)	29.54	7.28
T25	S1 X T3 (MP x IIHR)	16.86	7.41
T26	S2 X T3 (PR x IIHR)	17.65	7.11
T27	S3 X T3 (AS x IIHR)	3.69	1.78
T28	S1 X T1 (SF) (MP x AN (SF))	54.51	16.31
T29	S2 X T1 (SF) (PR x AN (SF))	20.03	10.82
T30	S3 X T1 (SF) (AS x AN(SF))	8.36	2.56
T31	S1 X T2 (SF) (MP x VV(SF))	11.11	5.64
T32	S2 X T2 (SF) (PR x VV (SF))	25.42	8.72
T33	S3 X T2 (SF) (AS x VV(SF))	22.04	6.57
T34	S1 X T3 (SF) (MP x IIHR(SF))	22.35	8.79
T35	S2 X T3 (SF) (PR x IIHR(SF))	5.45	2.29
T36	S3 X T3 (SF) (AS x IIHR(SF))	8.08	4.47
	SE m±	2.64	0.29
	CD (0.05)	7.69	0.83

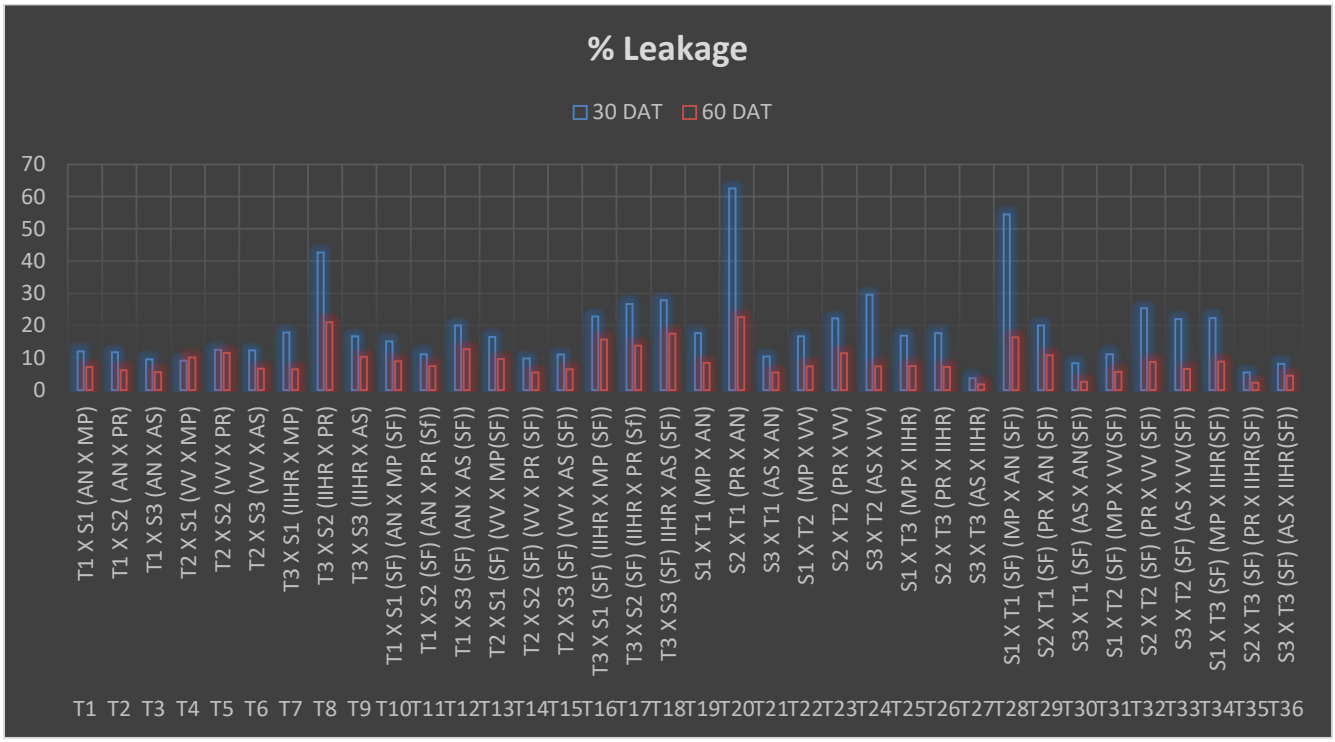


Figure 8: % Leakage in selectively fertilized and normal tomato plants

after transplanting, the lowest % leakage was detected in T27 (AS x IIHR) which was at par with T30 (AS x AN (SF)) and T35 (PR x IIHR (SF)) & highest in T20 (PR x AN).

In case of % leakage, it was observed that normal hybrids having tolerant genotype as male parent had less leakage than its SF crosses, but contradictory it was opposite in case of its reciprocal crosses (*i.e.* the hybrids having susceptible genotype as male parent), in which the SF hybrids shows less % leakage than the normal hybrids (Fig 8). Among the whole 36 crosses also the selectively fertilized crosses of hybrids having susceptible genotype as male parent showed better performance than the other crosses.

4.3.2.4. Vitamin C content

Effect of selective fertilization and high temperature condition on vitamin C content on leaves is depicted in Table 22.

Ascorbic acid (Vit C) is an antioxidant as it will help to scavenge ROS particles. The highest ascorbic acid content means it has more tolerance to temperature than the remaining ones. In this experiment the overall highest mean value was noticed in selectively fertilized crosses compared to normal crosses.

Among the varietal crosses, T12 (AN x AS (SF)) and T18 (IIHR x AS (SF)) recorded highest ascorbate content at 30 DAT (476.19 µg g^{-1}) and T9 (IIHR x AS) at 60 DAT (413.02 µg g^{-1}). The lowest was observed in T26 (PR x IIHR) (242.95 µg g^{-1}) at 30 DAT and in T5 (VV x PR) & T26 (PR x IIHR) (194.36 µg g^{-1}) at 60 DAT.

4.3.2.5. 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. SOD is also an antioxidant. Under high temperature stress, sod activity increases to scavenge the ROS particle.

In this experiment the mean activity of selectively fertilized hybrids recorded higher than the normal crosses. Among the SF hybrids, crosses having tolerant genotype as male parent recorded higher sod activity than the hybrids having susceptible

Table 22: Vitamin C (µg g^{-1}) in selectively fertilized and normal tomato plants

TREATMENTS	Varietal crosses	Vitamin C (µg g^{-1})	
		30 DAT	60 DAT
T1	T1 x S1 (AN x MP)	451.90	354.71
T2	T1 X S2 (AN x PR)	403.30	330.42
T3	T1 X S3 (AN x AS)	383.87	315.84
T4	T2 X S1 (VV x MP)	345.00	286.69
T5	T2 X S2 (VV x PR)	272.11	194.36
T6	T2 X S3 (VV x AS)	267.25	228.38
T7	T3 X S1 (IIHR x MP)	456.75	369.29
T8	T3 X S2 (IIHR x PR)	413.02	330.42
T9	T3 X S3 (IIHR x AS)	301.26	413.02
T10	T1 X S1 (SF) (AN x MP (SF))	374.15	238.10
T11	T1 X S2 (SF) (AN x PR (Sf))	417.88	301.26
T12	T1 X S3 (SF) (AN x AS (SF))	476.19	345.00
T13	T2 X S1 (SF) (VV x MP(SF))	383.87	374.15
T14	T2 X S2 (SF) (VV x PR (SF))	403.30	310.98
T15	T2 X S3 (SF) (VV x AS (SF))	432.46	354.71
T16	T3 X S1 (SF) (IIHR x MP (SF))	442.18	349.85
T17	T3 X S2 (SF) (IIHR x PR (Sf))	471.33	354.71
T18	T3 X S3 (SF) IIHR x AS (SF))	476.19	374.15
T19	S1 X T1 (MP x AN)	413.02	345.00
T20	S2 X T1 (PR x AN)	369.29	315.84
T21	S3 X T1 (AS x AN)	281.83	238.10
T22	S1 X T2 (MP x VV)	379.01	301.26

T23	S2 X T2 (PR x VV)	388.73	335.28
T24	S3 X T2 (AS x VV)	388.73	340.14
T25	S1 X T3 (MP x IIHR)	359.57	315.84
T26	S2 X T3 (PR x IIHR)	242.95	194.36
T27	S3 X T3 (AS x IIHR)	374.15	330.42
T28	S1 X T1 (SF) (MP x AN (SF))	417.88	349.85
T29	S2 X T1 (SF) (PR x AN (SF))	281.83	238.10
T30	S3 X T1 (SF) (AS x AN(SF))	413.02	359.57
T31	S1 X T2 (SF) (MP x VV(SF))	413.02	330.42
T32	S2 X T2 (SF) (PR x VV (SF))	398.45	340.14
T33	S3 X T2 (SF) (AS x VV(SF))	403.30	345.00
T34	S1 X T3 (SF) (MP x IIHR(SF))	306.12	267.25
T35	S2 X T3 (SF) (PR x IIHR(SF))	291.55	238.10
T36	S3 X T3 (SF) (AS x IIHR(SF))	276.97	218.66
	SE m±	7.85	6.67
	CD (0.05)	22.1	18.8

Table 23: SOD activity (activity g^{-1} min^{-1}) of selectively fertilized and normal tomato plants.

TREATMENTS	Varietal crosses	SOD activity **(activity g^{-1} min^{-1})**	
		30 DAT	**60 DAT**
T1	T1 x S1 (AN x MP)	0.16	0.06
T2	T1 X S2 (AN x PR)	0.20	0.07
T3	T1 X S3 (AN x AS)	0.08	0.04
T4	T2 X S1 (VV x MP)	0.18	0.09
T5	T2 X S2 (VV x PR)	0.23	0.18
T6	T2 X S3 (VV x AS)	0.15	0.13
T7	T3 X S1 (IIHR x MP)	0.25	0.14
T8	T3 X S2 (IIHR x PR)	0.36	0.08
T9	T3 X S3 (IIHR x AS)	0.22	0.04
T10	T1 X S1 (SF) (AN x MP (SF))	0.45	0.21
T11	T1 X S2 (SF) (AN x PR (Sf))	0.32	0.11
T12	T1 X S3 (SF) (AN x AS (SF))	0.29	0.11
T13	T2 X S1 (SF) (VV x MP(SF))	0.24	0.14
T14	T2 X S2 (SF) (VV x PR (SF))	0.26	0.22
T15	T2 X S3 (SF) (VV x AS (SF))	0.09	0.06
T16	T3 X S1 (SF) (IIHR x MP (SF))	0.22	0.09
T17	T3 X S2 (SF) (IIHR x PR (Sf))	0.14	0.16
T18	T3 X S3 (SF) IIHR x AS (SF))	0.12	0.09
T19	S1 X T1 (MP x AN)	0.20	0.05
T20	S2 X T1 (PR x AN)	0.27	0.04
T21	S3 X T1 (AS x AN)	0.25	0.11
T22	S1 X T2 (MP x VV)	0.20	0.12

T23	S2 X T2 (PR x VV)	0.19	0.08
T24	S3 X T2 (AS x VV)	0.27	0.09
T25	S1 X T3 (MP x IIHR)	0.26	0.07
T26	S2 X T3 (PR x IIHR)	0.18	0.03
T27	S3 X T3 (AS x IIHR)	0.24	0.03
T28	S1 X T1 (SF) (MP x AN (SF))	0.23	**0.35**
T29	S2 X T1 (SF) (PR x AN (SF))	0.25	0.02
T30	S3 X T1 (SF) (AS x AN(SF))	0.15	**0.01**
T31	S1 X T2 (SF) (MP x VV(SF))	0.10	0.05
T32	S2 X T2 (SF) (PR x VV (SF))	0.11	0.15
T33	S3 X T2 (SF) (AS x VV(SF))	0.20	0.09
T34	S1 X T3 (SF) (MP x IIHR(SF))	0.13	0.09
T35	S2 X T3 (SF) (PR x IIHR(SF))	0.21	0.14
T36	S3 X T3 (SF) (AS x IIHR(SF))	0.11	0.09
	SE m±	0.06	0.01
	CD (0.05)	0.18	0.03

genotype as male with an exception of T28 (MP x AN (SF)), in which the reciprocal crossed (susceptible as male parent) recorded more sod activity.

At 30 DAT, the observed highest sod activity was noted in T10 (AN x MP (SF)) and lowest in T3 (AN x AS), while which was highest in T28 (MP x AN (SF)) and lowest in T30 (AS x AN(SF)) at 60 DAT. The exceptional cases of normal hybrids having more sod activity than the SF hybrids also observed in the experiment, but the overall mean performance was always high in selectively fertilized ones.

4.3.2.6. Pollen viability

Effect of selective fertilization and high temperature condition in the pollen viability of each varietal crosses are depicted in Table 24.

Pollen viability decreases at high temp due to the deformity of pollen grains under heat stress. In case of pollen viability, the normal crosses showed slightly higher pollen viability than the SF ones. Among the 36 crosses, T1 (AN x MP) showed highest pollen viability followed by T10 (AN x MP (SF)) and T19 (MP x AN). The pollen viability of AN x MP and AN x MP (SF) is shown in plate 18.

While observing the hybrids it was seen that the pollen viability of SF hybrids of reciprocal crosses (susceptible genotype as male parent) drastically reduced under high temperature condition compared to other crosses with an exception of PR x VV, in which its SF cross showed more pollen viability than the normal cross (Plate 19).

4.3.2.7 Total yield

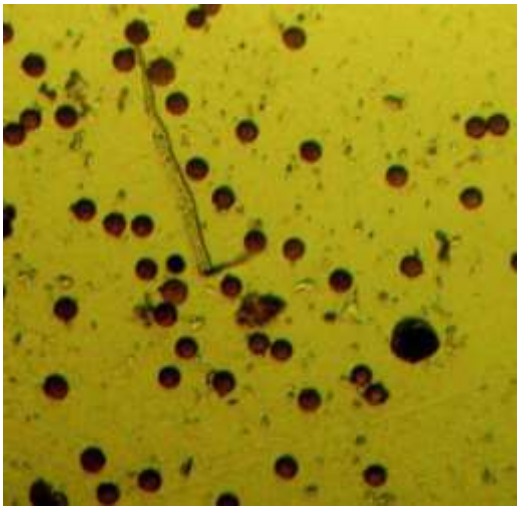
Effect of selective fertilization and high temperature condition on yield of each varietal crosses are depicted in Table 25. Yield per plant significantly decreased at high temperature in all tomato genotypes.

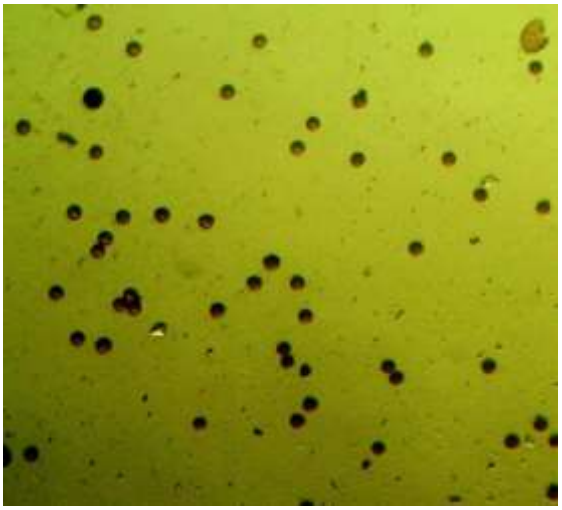
T10 (AN x MP (SF)) (226 g/plant) gave the maximum yield per plant under heat stress condition. The lowest yield was observed in T35 (PR x IIHR(SF)) (80.5 g/plant) which was at par with (PR x VV) (80 g/plant). The selectively fertilized hybrids given more yield than the normal crosses (Fig 9).

Table 24: Pollen viability of selectively fertilized and normal tomato plants

TREATMENTS	Varietal crosses	Pollen viability(%)
T1	T1 x S1 (AN x MP)	100.00
T2	T1 X S2 (AN x PR)	91.00
T3	T1 X S3 (AN x AS)	95.60
T4	T2 X S1 (VV x MP)	95.17
T5	T2 X S2 (VV x PR)	36.60
T6	T2 X S3 (VV x AS)	73.40
T7	T3 X S1 (IIHR x MP)	77.07
T8	T3 X S2 (IIHR x PR)	56.73
T9	T3 X S3 (IIHR x AS)	74.40
T10	T1 X S1 (SF) (AN x MP (SF))	98.53
T11	T1 X S2 (SF) (AN x PR (Sf))	91.03
T12	T1 X S3 (SF) (AN x AS (SF))	53.47
T13	T2 X S1 (SF) (VV x MP(SF))	86.97
T14	T2 X S2 (SF) (VV x PR (SF))	17.20
T15	T2 X S3 (SF) (VV x AS (SF))	65.82
T16	T3 X S1 (SF) (IIHR x MP (SF))	86.67
T17	T3 X S2 (SF) (IIHR x PR (Sf))	23.83
T18	T3 X S3 (SF) IIHR x AS (SF))	44.63
T19	S1 X T1 (MP x AN)	98.93
T20	S2 X T1 (PR x AN)	91.80
T21	S3 X T1 (AS x AN)	76.00
T22	S1 X T2 (MP x VV)	77.00
T23	S2 X T2 (PR x VV)	19.40

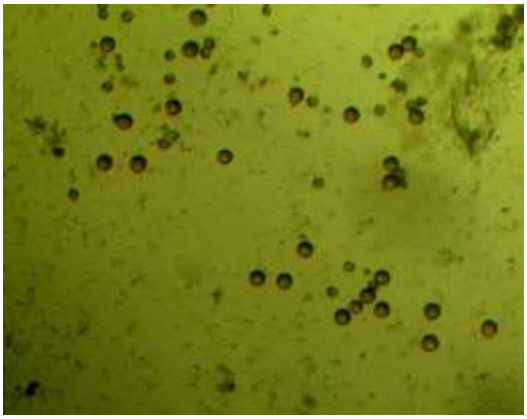
T24	S3 X T2 (AS x VV)	89.20
T25	S1 X T3 (MP x IIHR)	88.17
T26	S2 X T3 (PR x IIHR)	45.87
T27	S3 X T3 (AS x IIHR)	70.97
T28	S1 X T1 (SF) (MP x AN (SF))	56.97
T29	S2 X T1 (SF) (PR x AN (SF))	12.27
T30	S3 X T1 (SF) (AS x AN(SF))	14.17
T31	S1 X T2 (SF) (MP x VV(SF))	68.23
T32	S2 X T2 (SF) (PR x VV (SF))	52.10
T33	S3 X T2 (SF) (AS x VV(SF))	19.33
T34	S1 X T3 (SF) (MP x IIHR(SF))	45.47
T35	S2 X T3 (SF) (PR x IIHR(SF))	8.93
T36	S3 X T3 (SF) (AS x IIHR(SF))	22.73
	SE m±	1.65
	CD (0.05)	4.65





AN x MP **AN x MP (SF)**

Plate 18: Pollen viability of selectively fertilized and normal tomato plants



PR x VV **PR x VV (SF)**

Plate 19: Pollen viability of PR x VV (normal) and PR x VV (SF) (selectively fertilized) tomato cross.

Table 25: Total yield/ plant (g) in selectively fertilized and normal crossed tomato plants

TREATMENTS	Varietal crosses	Yield (g)
T1	T1 x S1 (AN x MP)	203.00
T2	T1 X S2 (AN x PR)	181.12
T3	T1 X S3 (AN x AS)	130.86
T4	T2 X S1 (VV x MP)	171.06
T5	T2 X S2 (VV x PR)	144.82
T6	T2 X S3 (VV x AS)	156.23
T7	T3 X S1 (IIHR x MP)	153.25
T8	T3 X S2 (IIHR x PR)	153.50
T9	T3 X S3 (IIHR x AS)	172.06
T10	T1 X S1 (SF) (AN x MP (SF))	226.00
T11	T1 X S2 (SF) (AN x PR (Sf))	189.50
T12	T1 X S3 (SF) (AN x AS (SF))	95.50
T13	T2 X S1 (SF) (VV x MP(SF))	155.00
T14	T2 X S2 (SF) (VV x PR (SF))	192.55
T15	T2 X S3 (SF) (VV x AS (SF))	167.15
T16	T3 X S1 (SF) (IIHR x MP (SF))	186.60
T17	T3 X S2 (SF) (IIHR x PR (Sf))	94.00
T18	T3 X S3 (SF) IIHR x AS (SF))	146.05
T19	S1 X T1 (MP x AN)	157.75
T20	S2 X T1 (PR x AN)	107.00
T21	S3 X T1 (AS x AN)	93.00
T22	S1 X T2 (MP x VV)	130.00

T23	S2 X T2 (PR x VV)	83.00
T24	S3 X T2 (AS x VV)	127.50
T25	S1 X T3 (MP x IIHR)	140.00
T26	S2 X T3 (PR x IIHR)	117.50
T27	S3 X T3 (AS x IIHR)	153.50
T28	S1 X T1 (SF) (MP x AN (SF))	105.00
T29	S2 X T1 (SF) (PR x AN (SF))	91.00
T30	S3 X T1 (SF) (AS x AN(SF))	113.86
T31	S1 X T2 (SF) (MP x VV(SF))	129.00
T32	S2 X T2 (SF) (PR x VV (SF))	128.00
T33	S3 X T2 (SF) (AS x VV(SF))	117.50
T34	S1 X T3 (SF) (MP x IIHR(SF))	121.00
T35	S2 X T3 (SF) (PR x IIHR(SF))	80.50
T36	S3 X T3 (SF) (AS x IIHR(SF))	105.00
	SE m±	27.54
	CD (0.05)	77.66

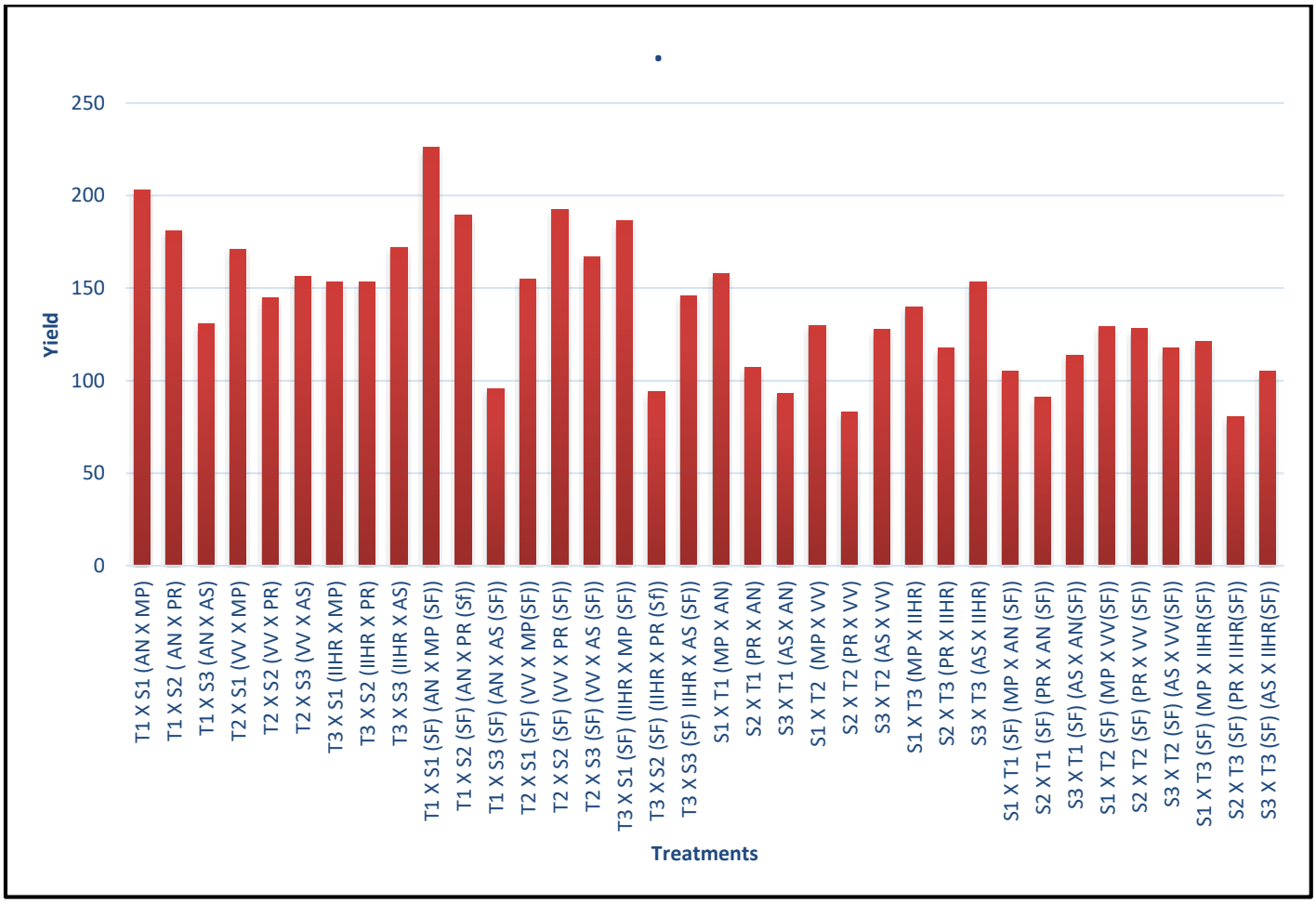


Figure 9: Yield per plant in selectively fertilized and normal tomato plants

The correlation analysis was done. The correlation between different yield attributes and thermotolerance characters with yield under stress condition is shown in the Table 26. Yield had a significant positive correlation with yield attributes like pollen viability (0.397**) and non-significant positive correlations with number of fruits per cluster (0.165). The traits no of flowers per cluster (-0.113) and number of flowers with exerted stigma (-0.133) were non-significant negative correlation with yield at the phenotypic level. The thermotolerance attributes like photosynthetic rate (0.139), Sod activity (0.134) showed positive but non-significant correlation with yield and the % leakage (-0.022) showed a negative correlation.

4.3.3. ANATOMY OF ANTHER

The SEM analysis of the sectional view of anther of 8 crosses were done to study the difference of anther surface and pollen grains abundance in selectively fertilized and normal hybrids. The one cross was AN x MP and its selectively fertilized line, as it was selected as best in the previous experiment. In case of Anagha and Manuprabha, in normal cross, the pollen grains are well developed and high in number compared to its selectively fertilized one. The pollen grains are well occupied in the normal cross while in SF, it is dispersed randomly in the anther (Plate 20).

The study of anther in the best tolerant (Anagha) and least susceptible (Arka Saurabh) varieties from the second experiment were also done. The whole four crosses among them were analysed. Both standard cross (tolerant as male parent) and its reciprocal cross (susceptible as male parent) with and without pollen selection were evaluated. In the first comparison *i.e.* tolerant as male parent, AN x AS and AN x AS (SF), the no of pollen grains was high in SF compared to normal hybrid (Plate 21). The anther walls and pollen grains were also well developed in SF compared to normal hybrid. Pollen sacs were also showed deformation in normal hybrid as it was not well developed and clumped together. But in SF the pollen sacs are well formed and have ample space for pollen grains (Plate 22).

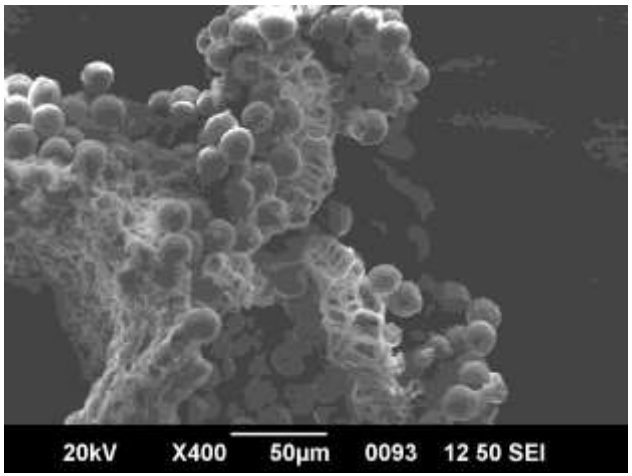
Secondly the reciprocal crossed (susceptible as male parent) hybrid of Anagha and Arka Saurabh was also done (*i.e.* AS x AN & AS x AN (SF)). In this one also the

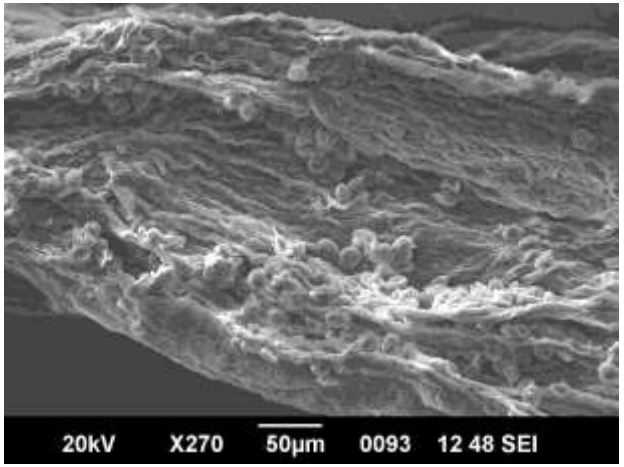
Table 26: Pearson correlation matrix

Correlations @ *P ≤ -0.05, **P≤ 0.01, ***P≤0.001

		1	2	3	4	5	6	7	8	9
		Yield	**No. of flowers per cluster**	**No .of fruits per cluster**	**No .of flowers with exerted stigma**	**No. of clusters per plant**	**Photosynthetic rate**	**Membrane integrity (%Leakage)**	**SOD activity**	**Pollen viability**
1	Yield	1	-0.113	0.165	-0.133	-0.12	0.139	-0.022	0.134	0.397***
2	No. of flowers per cluster	-0.113	1	0.033	-0.084	0.012	0.212*	0.044	0.074	-0.2*
3	No .of fruits per cluster	0.165	0.033	1	-0.261**	0.108	0.134	0.299**	-0.219*	0.225*
4	No .of flowers with exerted stigma	-0.133	-0.084	-0.261**	1	-0.027	-0.269**	-0.073	-0.059	-0.243*
5	No. of clusters per plant	-0.12	0.012	0.108	-0.027	1	-0.014	-0.084	0.065	0.114

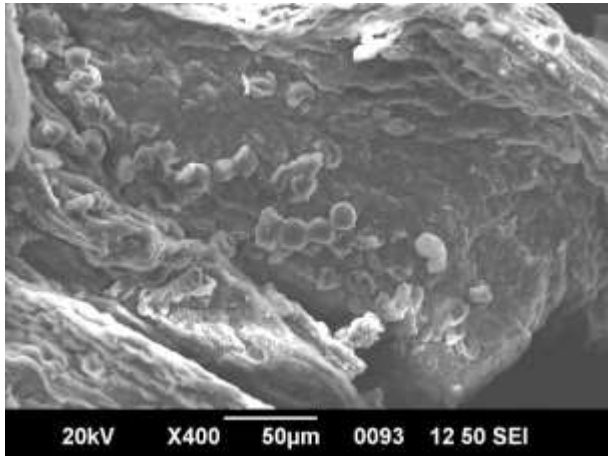
6	Photosynthetic rate	0.139	0.212*	0.134	-0.269**	-0.014	1	-0.076	0.111	0.156
7	Membrane integrity (%Leakage)	-0.022	0.044	0.299**	-0.073	-0.084	-0.076	1	-0.225*	0.062
8	SOD activity	0.134	0.074	-0.219*	-0.059	0.065	0.111	-0.225*	1	0.05
9	Pollen viability	0.397***	-0.2*	0.225*	-0.243*	0.114	0.156	0.062	0.05	1

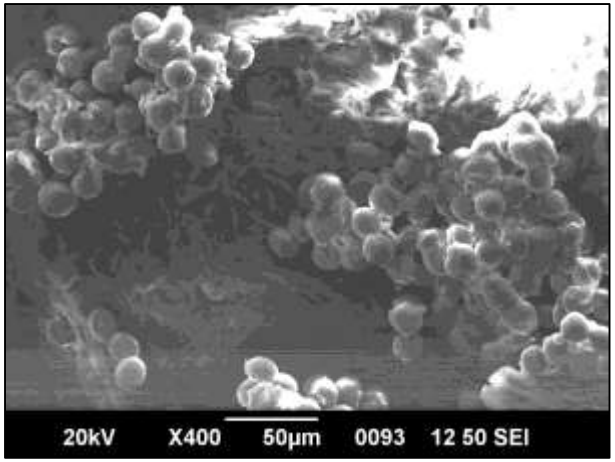




AN X MP **AN X MP (SF)**

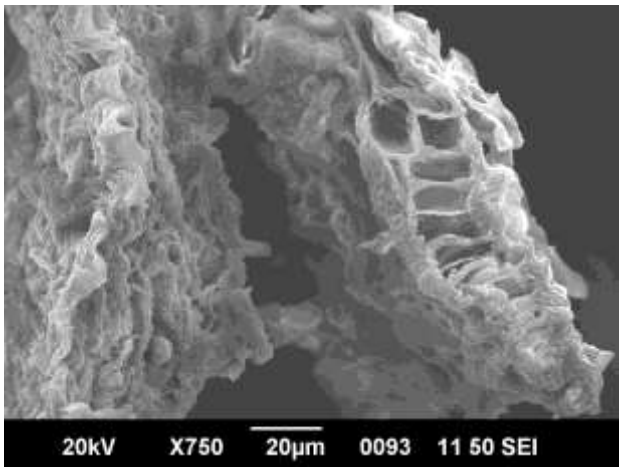
Plate 20. SEM of the anther of AN X MP and AN X MP (SF)

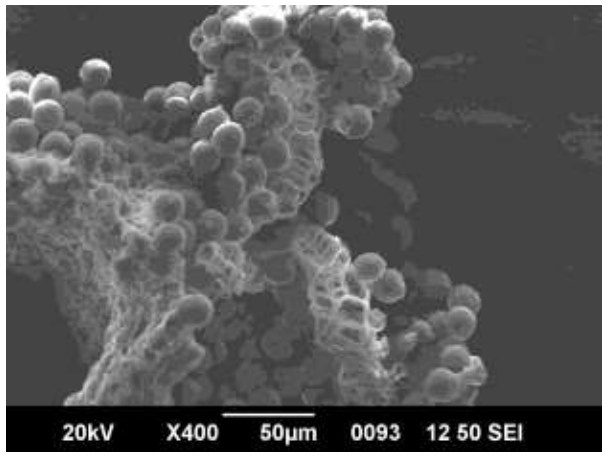




AN X AS **AN X AS (SF)**

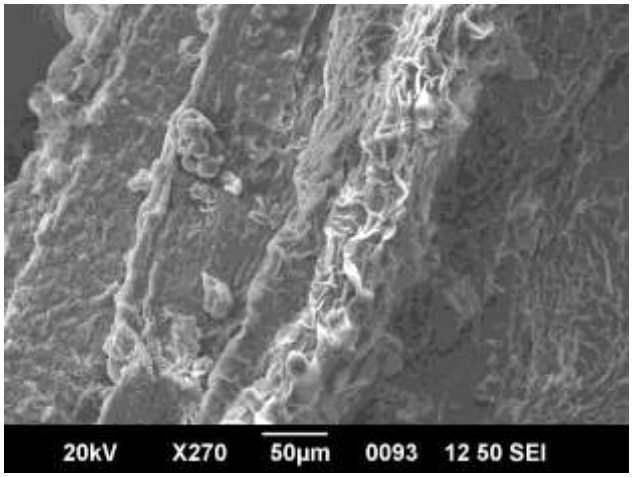
Plate 21. SEM of the anther of AN XAS and AN X AS (SF)

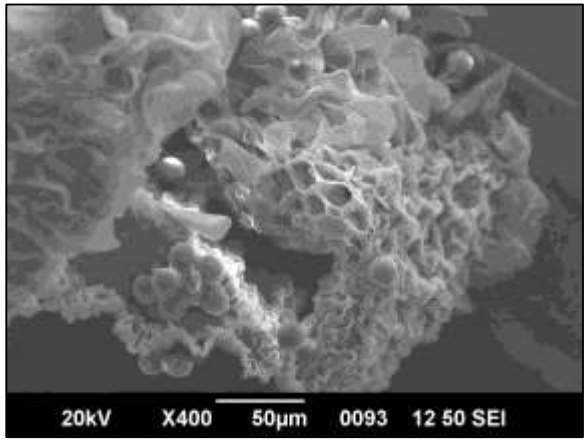




AN X AS **AN X AS (SF)**

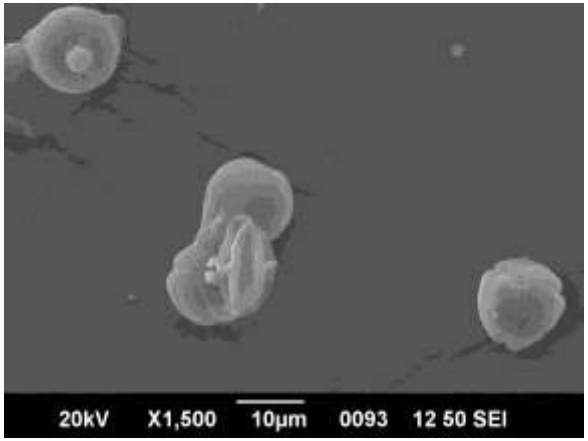
Plate 22. SEM of pollen sac of SF and normal hybrids

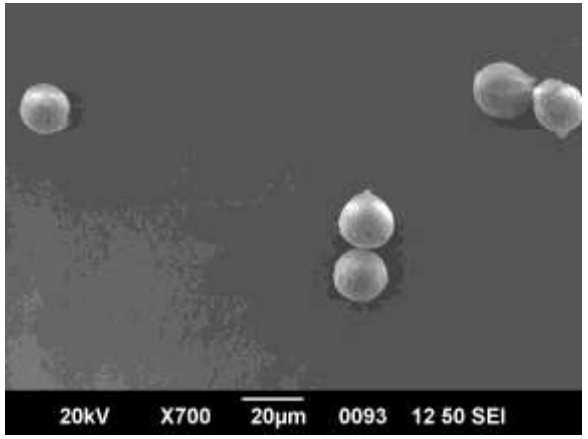




AS X AN **AS X AN (SF)**

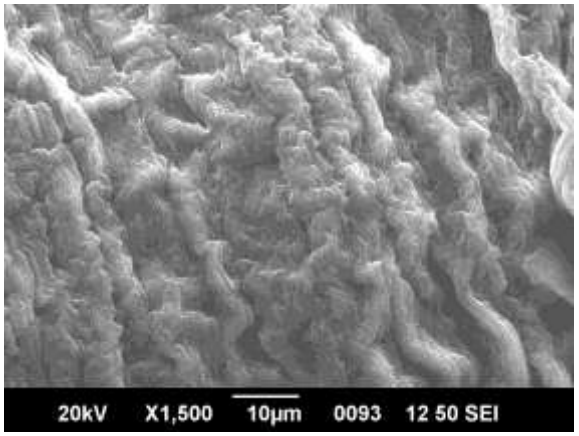
Plate 23. SEM of the anther of AS X AN and AS X AN (SF)

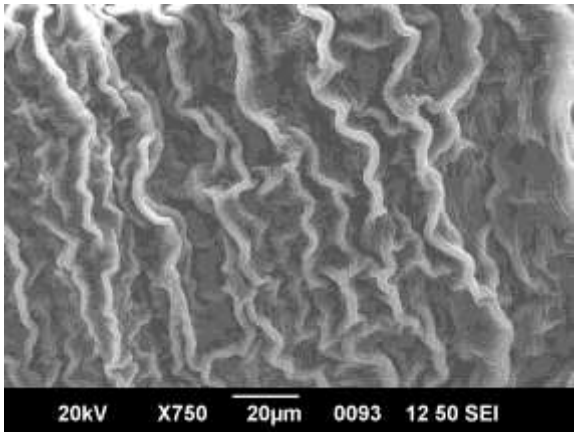




AS X AN **AS X AN (SF)**

Plate 24. Pollen grains of selectively fertilized and normal tomato plants





AS X AN **AS X AN (SF)**

Plate 25. Surface structure of anther in selectively fertilized and normal tomato plants.

cross section of anther of the SF were in upright form compared to normal hybrids (Plate 23). SEM micrographs showed that the tomato pollen exhibits feature of a typical dicot pollen grain, with a prolate spheroid shape, containing three radially equidistant elongated apertures. The pollen grains were high in number and well developed in selectively fertilized cross. But in normal hybrid, the deformation of pollen was observed (Plate 24). It was distorted and equidistant apertures were absent. The anther wall was well developed in SF, so it can trap pollen grains well. On the other hand, in normal hybrids, it is clustered together and distorted (Plate 25).

DISCUSSION

5. DISCUSSION

The research project on "Physiological and molecular basis of selective fertilization for high temperature stress tolerance in tomato (*Solanum lycopersicum* L.)" aimed to screen the critical temperature for pollen germination of tomato genotypes and to evaluate the thermotolerance of selectively fertilized tomato hybrids through physiological and molecular techniques.

In the present programme, critical temperature for pollen germination in ten varieties of tomato was standardized. The pollen grains that germinated under critical temperature were used for selective fertilization to develop high temperature tolerance. Both selectively fertilized and normal crossed plants of three best tolerant and three susceptible genotypes were exposed to high temperature condition *i.e.* in a ROS (ambient temp.+3^0C). Observations on physiological and biometrical parameters were done on the selectively fertilized and normal crossed plants in ROS (rainout shelter). The F1 hybrid developed in a previous experiment (Ammu, 2019) conducted in the Department of Plant Physiology were also advanced upto F_4 generation in ambient condition using selective fertilization technique to develop a temperature tolerant variety in this programme.

5.1 ADVANCING THE TEMPERATURE TOLERANT SELECTIVELY FERTILIZED HYBRID OF ANAGHA AND MANUPRABHA.

Afna *et al.* (2023) demonstrated the potential of selective fertilization in coconut hybrids, where pollen grains were selected under water stress conditions and used to produce hybrids with higher critical temperatures for pollen germination. This suggests that selective breeding can be effective in enhancing temperature tolerance in crops. However, the process is complex due to the polygenic nature of heat tolerance and the influence of genotype by environment (G × E) interactions, as noted in cowpea research (Jha *et al.,* 2020). The use of advanced breeding techniques, such as marker-assisted backcrossing (MABC) and multi parent advanced generation intercross (MAGIC), can accelerate the development of temperature-tolerant lines by enhancing genetic recombination and selection efficiency (Gaur *et al.,* 2018; Jha *et al.,* 2020). Advancing the temperature tolerance of selectively fertilized hybrids upto the F4 generation can

result in improved performance due to selective breeding, as evidenced by increased growth rates and genetic gains. But, the genetic architecture of temperature tolerance traits, which may include complex interactions and non-additive genetic effects, can influence the extent and predictability of performance improvements across generations (Alemu *et al.,* 2024; Reddy *et al.,* 2022). Therefore, while selective breeding can enhance temperature tolerance, the outcomes may vary depending on the specific genetic and environmental condition of the crop.

The advancement of temperature-tolerant hybrids from the F_1 to F_4 generation involves several breeding strategies and considerations. Initially, F_1 hybrids are created by crossing two genetically distinct inbred lines, which often exhibit heterosis or hybrid vigour (superior performance in the F_1 generation compared to the parent lines) (Labroo *et al.,* 2021). However, as the generations advance towards the F_4, the uniformity and heterosis may decline due to segregation and recombination of genes (Labroo *et al.,* 2021). Another significant obstacle to achieving genetic progress is the low heritability observed in most traits. This also suggests that selecting individual plants in the F_2 generation for heat tolerance will not be successful (Hazra *et al.,* 2009).

Heat tolerance is a complex trait influenced by multiple genes and environmental factors (Shanmugavel *et al.,* 2021). As the generations progressed from F_1 to F_4, selection of individuals that exhibit the desired temperature tolerance while also considering other physiological traits along with the use of molecular markers is important. As mentioned by Aswini *et al.* (2023), molecular markers and quantitative trait loci (QTL) mapping helps to identify and select for genes associated with heat tolerance. By the F_4 generation, the population may exhibit better performance compared to the F_1 due to the segregation of alleles. Similar phenotypic and genotypic selection practices were employed in the F_1, F_2 & F_3 generation, and F4 generation developed in this study. The performance of the F_3 generation was superior to that of the F_2 generation in the experiment. This corresponds with the findings of Barman & Borah (2012), wherein the F_3 generation exhibited higher mean performance and percentage of population mean than the F_2 generation for plant height, number of panicles per plant, grain density, and duration of flowering. They also noted that the maximum range value was higher in the F_4 generation for all parameters except fruit

weight and fruit diameter. In the current experiment, the lines performed better in the F_4 generation than in other generations, except for fruit size and weight. Barman & Borah (2012) also mentioned that the F_4 generation inherited and retained their parental characteristics.

There is no consistent trend of increasing or decreasing heritability from the F_3 to the F_4 generation, as observed in the present study, in accordance with Raval *et al.,* (2017). Most of the individual lines outperformed the whole population for the parameters. Upto the F_4 stage, the mean values of the parameters of interest for individual lines are more significant than the performance of the entire population. Rios (2015) noted that selection until the F_4 generation is based on individual plant assessment in the pedigree method. Making decisions based solely on the mean values of traits in early segregating populations is insufficient. Selecting based on mean values does not reveal the range and distribution pattern within each population (Welsh, 1981). Populations rejected solely on their mean value may result in the loss of low frequency of high yielding individuals. This statement supports our approach of focusing on the performance of individual plants rather than the performance of the entire population, which may decline at any level.

In summary, advancing temperature-tolerant hybrids from the F_1 to F_4 generation requires careful selection to maintain heterosis and desirable traits. The use of molecular breeding tools can facilitate the identification and selection of temperature-tolerant individuals.

5.2 SCREENING HIGH TEMPERATURE TOLERANCE USING THE CRITICAL TEMPERATURE FOR POLLEN GERMINATION

Pollens can be used as a tool to select genotypes based on temperature tolerance. Screening for high temperature tolerance in plants often involves assessing the critical temperature for pollen germination, as this is a key determinant of reproductive success under heat stress conditions. Studies have shown that pollen germination and viability are significantly affected by high temperatures, with genotypic differences observed in the response to heat stress (Singh *et al.,* 2016; Song *et al.,* 2014). In general, genotypes which are tolerant to high temperature will have high pollen germination and viability

than the sensitive ones (Dane *et al.,* 1991). For instance, in sorghum, genotypes differed in their response magnitude to high temperatures, but not in the duration of the critical period of sensitivity (Singh *et al.,* 2016). Similarly, in upland cotton, a critical temperature of 35 °C was identified for pollen viability, and genotypes with less decrease in pollen germination percentage at this temperature were considered more tolerant (Song *et al.,* 2014). Some studies have identified specific critical temperatures for pollen germination, while others have used a range of temperatures to determine the cardinal temperatures (minimum, optimum, and maximum) for pollen germination and tube growth, which can then be used to calculate a cumulative temperature response index (CTRI) for screening genotypes (Reddy & Kakani, 2007). This approach has been applied across various species, including coconut and capsicum, to identify genotypes with higher tolerance to heat stress (Ranasinghe *et al.,* 2012; Reddy & Kakani, 2007). Therefore, using pollen germination at critical temperatures as a screening tool will provide information about tolerance level of genotypes at high temperatures.

In the second experiment, critical temperature for pollen germination was identified using ten tomato genotypes, Anagha, Manuprabha, Vellayani Vijay, Akshaya, Arka Saurabh, Nandi, IIHR 26372, Pusa Rohini, Arka Rakshak and Arka Vikas. Results showed that as the temperature increased beyond the optimal 32°C, pollen germination percentage reduced markedly. After 36^{o} C the pollen germination percentage decreased below 20 % in all the genotypes except Manuprabha, Arka Saurabh, and Arka Vikas in which the percentage germination reduced below 20 after 34^{o} C itself. Hence, 36°C was identified as a critical temperature for pollen germination. Based on pollen germination at critical temperature, Anagha was selected as the tolerant genotype, and Arka Saurabh with the least pollen germination was the susceptible one. It was reported that germinated pollens were reduced 13 times when the temperature enhanced progressively from optimum (Pressman *et al.,* 2002).

Earlier studies also utilized an in vitro pollen germination assay to screen genotypes for high-temperature tolerance. In rice, Jagadish *et al.* (2008) highlighted significant genotypic differences in critical temperature for pollen germination, with some genotypes exhibiting germination at temperatures as high as 40°C, while others failed to germinate at temperatures above 35°C. Marine *et al.* (2017) conducted a

similar study and categorized various tomato genotypes as either tolerant or susceptible based on pollen viability. A parallel investigation in soybean validated the use of in vivo pollen germination study to identify genotypic tolerance to high temperatures. In this study by Salem *et al.* (2007), soybean genotypes were classified as tolerant, intermediate, or susceptible based on their pollen germination at different temperatures.

5.3 EFFECT OF SELECTIVE FERTILIZATION ON PHYSIOLOGICAL AND BIOMETRICAL PARAMETERS OF NORMAL AND RECIPROCAL CROSSED HYBRIDS UNDER HIGH TEMPERATURE

Selective fertilization is an innovative method to develop hybrids by imposing a selection pressure like temperature on pollen during germination. Only the pollen tolerant to these pressures will germinate and fertilize the ovule, potentially resulting in progeny that are also tolerant to the selection pressure. Results from the third experiment indicated that the selective fertilization technique had a positive impact on physiological and yield attributes, including increased photosynthetic efficiency, relative water content (RWC), scavenging systems for reactive oxygen species (ROS), and tolerance to high temperatures. Peet *et al.* (1997) validated that in hand-pollinated tomato, relative seediness, fruit set 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 even though the mean daily temperature was very high (40/24 ºC). Bhandari *et al.* (2017) also suggested that heterosis breeding can be used to improve the thermostability in tomato.

Ravikumar *et al.* (2003) provides evidence that selective fertilization using poly ethylene glycol (PEG) can improve moisture stress tolerance in sorghum progeny, indicating that a similar approach might affect pollen viability under heat stress. Additionally, Hazra *et al.* (2009) suggests that both additive and dominance genetic variance are important for traits such as pollen viability, and that non-additive genetic systems play a significant role in the expression of characters influencing heat tolerance (Hazra *et al.,* 2009). Contradictory or interesting facts emerge when considering that while selective fertilization has been shown to be effective in some cases, the overall

impact on pollen viability under heat stress may vary depending on the specific genotypes involved and the environmental conditions. For instance, Schoper *et al.* (1987) indicates that genotypic variability significantly affects pollen viability under heat stress, with some genotypes showing more desirable combining abilities than others. This suggests that the success of selective fertilization might be contingent upon the genetic makeup of the hybrids.

When Sato *et al.* (2006) compared reciprocal and normal tomato hybrids, they discovered that, reciprocal hybrids might display differential responses to high temperatures due to the interaction between the parental genetic backgrounds. Some reciprocal hybrids may show improved resilience to heat stress compared to normal hybrids, while others may not, depending on the specific genetic combinations. Normal tomato hybrids might show varying levels of sensitivity to heat stress, with some exhibiting moderate improvements in flower and cluster formation due to inherent hybrid vigour. However, their performance under heat stress is still limited compared to tolerant varieties (Sato *et al.,* 2006). The adaptability of agricultural systems to climate change can be improved by applying these strategies to additional crops, guaranteeing steady yields and food security in the face of rising global temperatures.

Genotypes having selectively fertilized exhibited an increased count of flowers and no. of clusters in comparison to their other counterparts even though the highest number were not observed in the selectively fertilized ones in both cases. In this experiment also the crosses having tolerant genotype as male parent showed better performance than the susceptible as male (reciprocal cross) with some exceptions, where reciprocal crosses showed better performance. Similar increased flower number in reciprocal crosses were seen in peppers, where the hybrid plants had differing responses to heat stress due to particular genetic interactions between the parental lines, reciprocal crosses showed improved heat tolerance and retain higher flower and cluster formation (Peet *et al.,* 2002). The number of flowers had the largest and lowest mean values in typical normal crossed reciprocal crosses (susceptible genotype as male parent) of tolerant and susceptible types, compared to selectively fertilized crosses in the study. The impact of heat stress on the number of flowers per cluster is somewhat debated as there have been reports of decreased flower numbers (Hanna, 1982; Adams

et al., 2001; Xu *et al.,* 2017), no observed change (Peet *et al.,* 1998; Sato *et al.,* 2006), and even an increase (Sherzod *et al.,* 2020) in flower number under heat stress.

The number of fruits were high in selectively fertilized crosses compared to normal crosses in both reciprocal and usual crosses in the present study. These observations align with the findings of Faruq *et al.* (2012), who reported diminished fruit and flower production in heat-susceptible cultivars under elevated temperatures, as in both cases in this experiment the selectively fertilized showed better performance than the normal crossed ones. Similarly, research by Abdelmageed and Gruda (2009) documented disparities in fruit and flower quantities, fruit fresh weight, and fruit set between heat-resistant and heat-susceptible tomato varieties, evident in both field and glasshouse environments. Dane *et al.* (1991) also find that genotypes with abundant flowers displayed increased resilience to temperature stress. c. Alasmir *et al.* (2021) found that heat stress had a significant impact on flower abortion, resulting in an 80% abscission rate and a subsequent decrease in fruit set. Additionally, exposure of tomato plants to moderately high constant stress (34°C/19°C, day/night) led to a 34% flower abscission and a 71% reduction in fruit set (Hazra *et al.,* 2009). Fruit set is determined by the ratio of fruits to flowers and can be significantly influenced by either a decrease (Xu *et al.,* 2017) or an increase in the number of flowers (Sherzod *et al.,* 2020). Thus, the changes in flower numbers in high-stress environments inevitably affect fruit set, impacting the correlation between fruit set and other characteristics, resulting in a weaker correlation with fruit yield compared to fruit number (Sherzod *et al.,* 2020). Therefore, breeders working on tomato genotypes with increased flower numbers per cluster under high stress should prioritize fruit number per cluster over fruit set to directly select for heat tolerance. This observation aligns with our study, which demonstrates higher flower numbers in normal hybrids compared to selectively fertilized hybrids, while the number of fruits shows the opposite trend.

In this study the number of days taken by the selectively fertilized and normal crosses of tolerant and susceptible (tolerant genotype as male parent) were less than the reciprocal crosses (susceptible genotype as male parent) for first flowering. The greater photosynthetic efficiency and reduced oxidative damage in heat-tolerant genotypes, traits potentially facilitating sustained reproductive development under high

temperatures (Hasanuzzaman *et al.,* 2013). Similarly, high temperatures have been observed to alter flowering characteristics and grain-setting rates in rice hybrids, with some showing adaptability through changes in flowering duration and timing (Tao *et al.,* 2008). The study on tomato hybrids indicate that fertilization treatments can influence flowering and fruit set under high temperatures (Moustafa *et al.,* 2019), although it does not specifically address the time to first flowering.

The presence of exerted stigma in tomato flowers presents a significant challenge in high-temperature conditions, resulting in reduced self-pollination due to the failure of pollen grains to adhere to the stigma. Tolerant genotypes exhibit a lower prevalence of flowers with exerted stigma, thereby positively impacting fruit setting percentages. The protrusion of the stigma beyond the anther cone during the reproductive stage serves as a distinct indicator of high-temperature stress in tomato plants, potentially impeding self-pollination (Faruq *et al.,* 2012). In this study, the selectively crossed genotypes of tolerant and susceptible genotype (tolerant genotype as male parent) showed least stigma exertion percentage under high-temperature conditions than the other crosses. Yadav *et al.* (2014) reported that under high temperature conditions (27/37°C), the susceptible genotype Pusa Rohini exhibited 100% stigma exertion, while the tolerant genotype Pusa Sadabahar showed 75% stigma exertion. This suggests that heat-tolerant genotypes may maintain lower stigma exertion under stress, potentially as a protective response. Additionally, it has been observed that genotypes that produce flowers without exerted stigma at elevated temperatures exhibit stability and yield higher fruit quantities (Saeed *et al.,* 2007). HT stress during anthesis triggered stigma exertion in tomato hybrids, consistent with previous reports (Dane *et al.,* 1991; Lohar and Peat, 1998; Saeed *et al.,* 2007; Kartikeya *et al.,* 2012). In one of the initial investigations into heat stress response in tomato also found that heat-susceptible cultivars exhibited more prominent bud abscission and style exertion, leading to decreased fruit set under heat stress (Levy *et al.,* 1978). Subsequent observations indicated that style exertion in various tomato genotypes varied between 25 to 55% under high-temperature conditions (Saeed *et al.,* 2007). More recent studies established a correlation between bud abscission and style exertion with reduced fruit set under field conditions as well (Kugblenu *et al.,* 2013; Singh *et al.,* 2015).

The elongation of the style in numerous flowers restricts pollen access to the stigma in heat-sensitive genotypes, consequently diminishing fertilization prospects (Alsamir *et al.,* 2017). Overall in the experiment, the SF crosses of both T x S (tolerant genotype as male parent) and S x T (susceptible genotype as male parent) showed less stigma exertion than its normal crosses. Notably, stigma exertion exceeding 1mm has been linked to diminished fruit yield in tomato plants (Rudich *et al.,* 1977). Ahmadi and Stevens (1978) also emphasized the significant role of stigma exertion in high-temperature field conditions, suggested that since genetic variance for this trait is mostly additive, selecting for low stigma position could be a quick and effective approach.

At elevated temperatures, a range of physiological and biochemical processes undergo changes. These physiological parameters serve as indicators of a tomato plant's ability to withstand heat. Differences in the performance of physiological processes under Heat stress can be used to select appropriate genotypes for high-temperature conditions. Elevated temperatures can have negative impacts on photosynthesis, respiration, water regulation, and membrane stability. Furthermore, they can influence the levels of hormones and various metabolites in plants (Wahid *et al.,* 2007). Hemantaranjan *et al.* (2014) also stated that heat stress will constrain photosynthesis, water balance, and cell membrane stability, while disrupting plant metabolism. Research indicates that factors like photosynthetic efficiency, water use efficiency (WUE), and the accumulation of osmoprotectants and antioxidants are crucial for determining a plant's ability to endure heat stress (Hasanuzzaman *et al.,* 2013, Panthee & Gotame, 2020). It is essential to thoroughly comprehend how plants respond physiologically to high temperatures, understand the mechanisms of heat tolerance, and explore potential strategies for enhancing crop thermotolerance (Wahid *et al.,* 2007).

When plants experience heat stress, one of the most crucial biochemical aspects affected is photosynthesis. The photosynthetic rate serves as an important indicator of plant health, and increased photosynthetic rate under heat stress reflects the plants' robust and resistant nature. In our current experiment, we observed the highest photosynthetic rate in selectively fertilized plants under heat stress compared to normal plants. Furthermore, among the selectively fertilized crosses, those with a tolerant

genotype as the male parent exhibited a higher photosynthetic rate than those with a susceptible genotype as the male parent. Wahid *et al.* (2007) also emphasized the significance of photosynthesis as a potential physiological indicator at high temperatures. In tomato, high-temperature-resistant varieties demonstrated the ability to maintain an enhanced chlorophyll a: b ratio, indicating the link between these alterations and thermotolerance (Camejo *et al.,* 2005). Improvements in photosynthetic aspects under heat stress are valuable indicators of thermotolerance, while any restrictions in photosynthesis can impede plant growth at high temperatures (Wise *et al.,* 2004). The heat-tolerant genotypes display greater photosynthetic efficiency and reduced oxidative damage under high temperatures, potentially enabling sustained reproductive development (Hasanuzzaman *et al.* 2013). High temperatures have a greater impact on the photosynthetic capacity of C_3 plants compared to C_4 plants. Under high temperatures, energy distribution, reduced carbon metabolism activities, particularly rubisco, interruption of electron transport, and PSII inactivation have been reported (Salvucci and Crafts-Brandner, 2004). The impact of Heat stress includes reducing leaf expansion, impairing the normal functioning of the photosynthetic apparatus, and ultimately promoting leaf senescence (Xu *et al.,* 2017). Yuan *et al.* (2017) also highlighted differences in heat tolerance between two genotypes of nonheading chinese cabbage, with the heat-tolerant genotype maintaining a higher photosynthetic capacity under stress. In tomato plants, under DAHS (45◦C, 2 h) compared to CK (25/20◦C, day/night), Pn, the CO2 assimilation rate, and PSII (Fv/Fm) were reduced in heat-susceptible cultivars (Camejo *et al.,* 2005). Rivero *et al.* (2021) also studied in tomato plants, and observed that genotypes tolerant to high temperatures maintain higher photosynthetic rates under heat stress compared to susceptible genotypes. They also stated that this difference is particularly pronounced during the first month after transplanting and around 60 days after transplanting (DAT), where tolerant genotypes exhibit superior photosynthetic performance under elevated temperatures.

Relative water content (RWC) is a critical physiological parameter indicating the water status of plants under stress conditions. Relative water content decreased in tomato under high temperatures and the selectively fertilized plants showed more water

content than the normal crosses. Among the crosses in this experiment significantly highest mean value was recorded in the reciprocal crossed selective fertilized hybrids *i.e*. the crosses which had susceptible genotype as male parent. Notably, under changing environmental conditions, water content is an imperative parameter (Mazorra *et al.,* 2002). Morale *et al.* (2003) also indicated that heat stress in tomato disrupts the hydraulic conductivity of roots and leaf water relationships. The observed increase in water content under heat stress indicates enhanced tolerance. In vitro gamete selection for heat-stress tolerance in maize resulted in progenies with higher RWC and reduced sensitivity to heat stress (Petolino *et al.,* 1990). Studies have shown that under high temperature conditions, tolerant genotypes generally maintain higher RWC compared to susceptible genotypes. In rice, tolerant genotypes exhibited a lesser decrease in RWC in response to low temperature stress, which is indicative of their ability to retain water under stress conditions (Ghosh *et al.,* 2016). Similarly, wheat genotypes that are tolerant to temperature stress maintained higher RWC and lower levels of oxidative damage when sown late in the season, which exposes them to higher temperatures (Sairam *et al.,* 2000). In soybean, drought-tolerant genotypes showed less reduction in RWC compared to susceptible genotypes under water stress, which often coincides with high temperature (Mondol *et al.,* 2018). Another study on tomato genotypes under controlled high temperature revealed significant variability in RWC among genotypes, with the most heat-tolerant genotypes maintaining higher RWC (Shaheen *et al.,* 2015).

Membrane integrity has long been utilized as an indirect measure to assess thermotolerance in tomato (Chen *et al.,* 1982). Enhanced heat stress can compromise membrane stability, leading to increased electrolyte leakage. In case of % leakage, it was observed that normal hybrids having tolerant genotype as male parent had less leakage than its SF crosses. But among the whole 36 crosses the selectively fertilized crosses of hybrids having susceptible genotype as male parent showed better performance than the other crosses. The measurement of ion or electrolyte leakage can serve as a reliable indirect indicator for heat tolerance and may be a suitable trait for selection purposes. In the case of tomato plants, various tomato genotypes displayed differing levels of ion leakage, which notably decreased when subjected to heat stress (Xu *et al.,* 2017). Heat-tolerant genotypes exhibited lower electrolyte leakage compared

to susceptible ones in tomato plants (Camejo *et al.,* 2005; Rajametov *et al.,* 2021). A study involving 13 tomato cultivars observed relatively strong correlation between ion leakage and fruit set or pollen viability, although these were not statistically significant (Xu *et al.,* 2017). Furthermore, an experiment utilizing a larger sample size of 43 cultivars revealed a significantly negative correlation between electrolyte leakage and fruit yield (Bhattarai *et al.,* 2021). Liu and Huang (2000) reported that the generation of active oxygen species (AOS), such as singlet oxygen (1 O2), superoxide radical (O2−), hydrogen peroxide (H2O2), and hydroxyl radical (OH−), are indicative of cellular injury due to high temperatures.

The increased production of reactive oxygen species at high temperatures leads to enhanced antioxidant activity, such as SOD and ascorbic acid, with tolerant genotypes exhibiting higher antioxidant activity than susceptible ones. Selectively fertilized genotypes exhibit a high ROS scavenging mechanism *i.e.*, high ascorbic acid and SOD activity compared to normal hybrids. The ability to maintain higher levels of ascorbic acid and SOD activity under stress conditions is indicative of a genotype's tolerance to high temperature (Goyal & Asthir, 2009; Sairam *et al.,* 2000). Scandalios (1993) noted that the overexpression of SOD in plants is linked to various physiological processes, including the removal of H_2O_2, oxidation of toxic reductants, biosynthesis and degradation of lignin in cell walls, auxin catabolism, and defensive responses. Goyal & Asthir (2009), observed same in wheat genotypes, SOD activity increased in response to high temperature stress across all genotypes, with temperature-tolerant genotypes such as C306 and C273 showing higher activities of SOD and other antioxidant enzymes, indicating a robust defense mechanism against oxidative stress. Genetic factors are known to play a significant role in the ascorbic acid content of plants, with different varieties showing large differences in vitamin C levels (Platenius, 1945). Additionally, high temperatures have been observed to affect the rate at which ascorbic acid accumulates in plants, with an inverse relationship reported at moderate temperatures (Platenius, 1945).

Pollen viability under high temperature conditions is a critical factor for crop fertility and yield, particularly in tomato (*Lycopersicon esculentum Mill*.) genotypes. Heat-tolerant genotypes exhibit a remarkable ability to maintain pollen viability and

fruit set under high temperature stress, as opposed to heat-sensitive genotypes which show a significant reduction in these traits (Abdul-Baki & Stommel, 1995; Dane *et al.,* 1991). In the experiment also crosses having tolerant genotype as male parent showed high pollen viability compared to its reciprocal crosses (susceptible genotype as male parent). A study in rice also shows the same results, in which the heat-tolerant lines exhibited only slight reductions in anther dehiscence, pollen viability, and germination, whereas susceptible lines showed severe effects on these parameters, leading to decreased seed set (Malumpong *et al.,* 2019). Similarly, in wheat, genotypes with high pollen viability under heat stress were identified, and these can serve as genetic resources for breeding heat-tolerant cultivars (Khan *et al.,* 2022). Studies showed that, heat-tolerant tomato genotypes maintained fruit set and pollen viability even under high temperature regimes, unlike their heat-sensitive counterparts (Abdul-Baki & Stommel, 1995). Additionally, genetic studies have indicated that pollen viability is a trait with moderate to high heritability, controlled by additive genetic effects, and can be improved through selection (Razaq *et al.,* 2017).

The anatomy of anthers in tomato genotypes under high temperature stress varies between heat-tolerant and heat-sensitive genotypes. Heat-tolerant genotypes maintain anther and pollen characteristics closer to those under optimal temperature conditions, while heat-sensitive genotypes exhibit morphological changes and reduced pollen viability when exposed to high temperatures (Porch & Jahn, 2001). Specifically, heat-tolerant genotypes show less disruption in pollen development and maintain the carbohydrate content of developing and mature pollen grains, which is crucial for pollen quality (Firon *et al.,* 2006). Interestingly, despite the general resilience of heat-tolerant genotypes, high temperature stress still causes some reduction in pollen release and germination in both tolerant and susceptible cultivars, although the impact is more pronounced in the susceptible ones (Sato & Peet, 2005). In the experiment, the number of pollen grains was higher in selectively fertilized genotypes compared to normal hybrids. The anther walls and pollen grains were also more developed in selectively fertilized genotypes compared to normal hybrids. Anatomically, pollen sacs in normal hybrids showed deformation and clumping, while in selectively fertilized genotypes, the pollen sacs were well formed, allowing ample space for pollen grains. According to

Barboza *et al.* (2016), the pollen shape in the Solanaceae family is commonly subspheroidal, ranging from suboblate to subprolate, with only a few genera having oblate or prolate grains. SEM micrographs showed that the tomato pollen exhibits feature of a typical dicot pollen grain, with a prolate spheroid shape, containing three radially equidistant elongated apertures. The pollen grains were high in number and well developed in selectively fertilized cross. But in normal hybrid, the deformation of pollen was observed. It was distorted and equidistant apertures were absent. Perveen and Qaiser (2007) reported that Solanaceae is a diverse family with pollen grains that are usually radially symmetrical, isopolar spheroidal or suboblate to subprolate, tricolporate, and mostly scabrate tectum. Male sterility induced by high temperature seems to be associated with several morphological alterations of sporophytic anther tissues such as tapetum, epidermis, endothecium, and stomium (Sato *et al.,* 2002; Oshino *et al.,* 2007). Giorni *et al.* (2013) corroborate the presence of remarkable alterations in sporophytic and gametophytic tissues of tomato after exposure to 3 days at 36 °C/26 °C (day/night), with most significant changes typically occurring in the tapetum layer, but also in mature microspores that show alterations in vacuolization.

In this experiment, selectively fertilized crosses resulted in a higher yield than normal crosses, as the number of fruits per cluster were higher in selectively fertilized plants. Selective fertilization may enhance the performance of hybrids by favoring the transmission of alleles conferring stress tolerance, which is critical for maintaining yields under adverse conditions (Ravikumar *et al.,* 2003; Hazra *et al.,* 2009; Kamara *et al.,* 2021; Khan *et al.,* 2022). Reciprocal hybrids in wheat exhibit variable responses to heat stress, with some hybrids showing better spike and grain development due to favorable genetic interactions. These hybrids can sometimes outperform normal hybrids in terms of maintaining yield stability under high temperatures (Liu *et al.,* 2009). Tolerant genotypes tend to maintain higher yields compared to susceptible ones under such stress (Abdul-Baki & Stommel, 1995). In the study by Dane *et al.* (1991), it was also noted that heat-sensitive genotypes experienced a reduction in fruit set during periods of high-temperature stress. Peet *et al.* (1998) similarly observed that the decline in overall yield is primarily due to a decrease in the quantity of fruits rather than a decrease in individual fruit weight. The adverse effects of Heat stress on tomato plants

include a negative impact on both vegetative growth and reproductive development, leading to decreased yield and fruit quality. Amit *et al.* (2017) found that average daily temperatures exceeding 29 °C result in a reduction in the quantity of fruits, fruit weight per plant, and seed number per fruit in tomato compared to those experiencing temperatures at 25 °C. In rice, heat-tolerant genotypes like Rasi and N22 maintained higher yields under high temperature stress, while susceptible genotypes like IR36 showed severe yield reduction (Veronica *et al.,* 2019). Similarly, in wheat, heat-tolerant genotypes exhibited less reduction in yield-related parameters and higher heat tolerance indices after treatment with sulfhydryl compounds (Agarwal *et al.,* 2017). Contradictions arise in the response of different genotypes to high temperature stress also there. Certain barley genotypes that were tolerant in vitro also performed better in the field under late sown Heat stress conditions (Bhagat *et al.,* 2024) but in contrast, some Cyclamen genotypes that were classified as heat-tolerant showed susceptibility under Heat stress even with GA_3 hormone treatment (Cornea-Cipcigan *et al.,* 2022). These discrepancies highlight the complexity of heat tolerance mechanisms and the influence of environmental conditions on genotype performance. In the study also some of the normal crosses and reciprocal crosses showed better performance than the selectively fertilized tolerant x susceptible genotypes (tolerant as male parent). In summary, while high temperature stress generally leads to a decrease in crop yield, genotypes with heat tolerance exhibit a relatively smaller decline in yield compared to susceptible genotypes.

The phenotypic correlation coefficient was compared to know the nature and magnitude of the relationship existing between yield and its component traits as well as the association among the component traits themselves. Yield had a significant positive correlation with pollen viability as observed in the study by Vijayakumar *et al.* (2021) and non-significant positive correlations with number of fruits per cluster (Raijadhav *et al.,* 1996). The trait number of flowers per cluster and number of flowers with exerted stigma has non-significant negative correlation with yield at the phenotypic level. The same was reported by Ramana *et al.* (2007) for number of flowers per cluster. This indicates the inverse relationship between per cent fruit set and number of flowers per cluster. Similar results were reported by Sharma and

Krishanswaroop (2000) in brinjal. In this field experiment, strong correlation of pollen viability with yield was observed under heat stress condition. Previous study also confirms that pollen viability can be used as a potential trait to screen genotypes for heat stress tolerance as it is positively correlated with yield (Masthigowda *et al.,* 2022).

The selectively fertilized cross of susceptible and tolerant parents resulted in more fruits per cluster, fruit weight, and yield, demonstrating hybrid vigor in crossed varieties under high temperature conditions. Correlation studies revealed that yield can be improved by selecting genotypes for better growth, more number of fruits per cluster and pollen viability and less number of flowers and also less stigma exertion. Savale *et al.* (2017) reported that heterosis breeding in tomato would increase fruit weight, number of clusters per plant, and number of fruits per plant. This experiment, however, goes a step further, showing that selective fertilization can significantly improve thermotolerance and yield in tomato under high temperatures. This finding underscores the practical potential of selective fertilization as a technique for imparting high-temperature tolerance in tomato, a key takeaway for breeders and agricultural professionals.

SUMMARY

6. SUMMARY

The present programme "Physiological and molecular basis of selective fertilization for high temperature stress tolerance in tomato (*Solanum lycopersicum* L.)" was conducted in three experiments and the salient findings are given below.

In the first experiment first generation (F_1) hybrid seeds of selectively fertilized Anagha x Manuprabha were acquired from crosses conducted previously in the lab (Ammu, 2019) between two tomato varieties Anagha and Manuprabha. Subsequent generations up to F_4 were produced through selective fertilization, with individual plant selections based on their performance in pollen germination %, relative water content, membrane integrity, stomatal conductance, chlorophyll stability index, chlorophyll content, and photosynthetic rate. Molecular analysis using SSR markers was also conducted for selection purposes. Throughout all generations, 14 lines were maintained, and the two best lines were chosen for the next generation. Despite the F_2 generation not showing significant differences based on phenotyping and genotyping data, the T3 and T10 lines exhibited relatively better performance compared to other lines. Consequently, both T10 and T3 lines were chosen for the development of the F_3 generation. From the F_3 generation, T6 and T8 were selected for the development of the F_4 generation. Based on phenotyping and genotyping data, the best lines in the F_4 generation were T4 and T6. The overall performance of the F_4 generation was superior to that of the F_3 and F_2 generations. The germination percentage did not differ significantly among the F_2, F_3, and F_4 lines, although there was an increase in germination percentage from the F_2 to the F_4 generation. Furthermore, fruit setting percentage followed the order of F_4, F_3, and F_2 when plants were selectively fertilized. It was observed that the plants improved through selfing in each generation. In conclusion, the successful advancement of temperature-tolerant selectively fertilized hybrids to the F_4 generation requires meticulous selection and breeding strategies that take into consideration the intricate genetics of heat tolerance

In the second experiment critical temperature was standardized by using ten tomato genotypes, Anagha, Manuprabha, Vellayani Vijay, Akshaya, Arka Saurabh, Nandi, IIHR 26372, Pusa Rohini, Arka Rakshak and Arka Vikas. $36^{O}C$ was identified as critical temperature for pollen selection where 20% pollen germination was

observed. Beyond critical temperature, pollen germination percentage decreased below 20 % except in Manuprabha, Arka Saurabh, and Arka Vikas in which the percentage germination reduced below 20 after 34^0 C itself. Anagha with high pollen germination percentage at this critical temperature was selected as tolerant variety and the variety Arka Saurabh with least pollen germination was selected as susceptible variety. Along with these two varieties two more tolerant and susceptible genotypes were selected for next experiment.

In the third experiment based on the critical sterility temperature and pollen germination percentage, the three tolerant varieties Anagha, Vellayani Vijay and IIHR 26372 and three susceptible varieties Pusa Rohini, Manuprabha and Arka Saurabh were used to study the effect of selective fertilization in tomato under high temperature. The incubated pollen at critical temperature from the male parent (tolerant genotype) was used to pollinate the emasculated flowers of female parent (susceptible genotype) for selective fertilization. Normal cross without pollen selection was also done between the tolerant and susceptible genotype. The reciprocal cross (with and without pollen selection) were also done within the tolerant and susceptible genotype to study the effect of male parent (*i.e.* the pollen grain) in temperature tolerance. The 30 days old seedlings of the above crosses were transplanted and exposed to high temperature condition viz. ambient temp.+3^0C (Rainout shelter).

Physiological, biometrical and yield parameters were studied in all the crosses under this condition. Biometrical parameters include floral characters like number of clusters, number of flowers and days to first flowering were observed in all crosses. Except in the days to first flowering, the floral characters were recorded highest in the reciprocal crosses compared to its opposite cross. In case of number of clusters, both normal and selectively fertilized crosses of S x T (susceptible genotype as male parent) showed significantly higher number of clusters per plant than the tolerant x susceptible crosses (tolerant genotype as male parent) while in case of number of flowers per cluster normal crossed reciprocal crosses (susceptible genotype as male parent) of tolerant and susceptible varieties showed better performance than the selectively fertilized crosses. The normal and selectively fertilized crosses of TxS (tolerant

genotype as male parent) flowered comparatively earlier than the reciprocal crossed (S x T) (susceptible genotype as male parent) ones.

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 the number of flowers with exerted stigma were less in the selectively crossed genotypes of tolerant and susceptible genotype (tolerant genotype as male parent) compared to its reciprocal crosses (susceptible genotype as male parent). In case of length of stigma exertion overall the SF crosses of both T x S (tolerant genotype as male parent) and S x T (susceptible genotype as male parent) showed less stigma exertion than its normal crosses.

Physiological parameters like photosynthetic rate, relative water content and ascorbic acid (Vit C) content were observed and all the parameters except ascorbic acid content were recorded highest in selectively fertilized crosses compared to their normal crosses. The photosynthetic rate was recorded highest in the selectively fertilized crosses having tolerant genotype as the male parent than others. In case of relative water content, the significantly highest mean value was recorded in the reciprocal crossed selective fertilized hybrids *i.e.,* the crosses which had susceptible genotype as male parent. Ascorbate content in leaves were highest in normal crosses compared to the selectively fertilized crosses.

The temperature tolerance characters like membrane integrity (% leakage) and SOD activity were recorded. The percentage leakage showed different responses in normal and reciprocal crosses. The normal hybrids having tolerant genotype as male parent had less leakage than its SF crosses, but contradictory it was opposite in case of its reciprocal crosses (*i.e.* the hybrids having susceptible genotype as male parent), in which the SF hybrids shows less % leakage than the normal hybrids. But overall, the selectively fertilized crosses of hybrids having susceptible genotype as male parent showed better performance than the other crosses. In case of SOD activity, the exceptional cases of normal hybrids having more sod activity than the SF hybrids also observed, but the overall mean performance was always high in selectively fertilized ones.

The yield characters like number of fruits per cluster and yield were high in selectively fertilized crosses than the normal crosses except pollen viability. In case of pollen viability, the normal crosses showed slightly higher pollen viability than the SF ones but the SF crosses having tolerant genotype as male parent showed better performance with high pollen viability. The pollen viability of SF hybrids of reciprocal crosses (susceptible genotype as male parent) drastically reduced under high temperature condition compared to other crosses with an exception of PR x VV, in which its SF cross showed more pollen viability than the normal cross. Majority of the selectively fertilized crosses recorded higher number of fruits/cluster under high temperature regime than the normal crossed ones. Yield was recorded high in selectively fertilized crosses compared to normal crosses. All the parameters with some exceptions 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.

The study's findings suggest that selective breeding has the potential to improve temperature tolerance in crops, but the results may differ based on the specific genetic and environmental factors involved. This breeding method not only enhanced the plants' ability to withstand stress, but it also had positive effects on yield parameters. Furthermore, it was observed that selectively fertilizing the cross between a tolerant and susceptible genotype, with the tolerant genotype as the male parent, resulted in greater hybrid vigour and improved tolerance to high temperatures compared to normal crosses and reciprocal crosses. In order to achieve success, it is crucial to utilize advanced breeding techniques and gain a deep understanding of the physiological and molecular mechanisms that contribute to heat tolerance. Integrating these approaches can ultimately lead to the development of crop varieties that are better equipped to handle the challenges posed by rising temperatures.

REFERENCES

7. REFERENCES

Abdelmageed, A. H. A. and Gruda, N. 2009. Influence of grafting on growth, development and some physiological parameters of tomatoes under controlled heat stress conditions. *Eur. J. Hortic. Sci.* 74: 16-20.

Abdulbaki, A. A. and Stommel, J. R. 1995. Pollen viability and fruit set of tomato genotypes under optimum and high temperature regimes. *Hortic. Sci.* 30(1): 115-117.

Alemu, A., Johanna, A., Montesinos-López, O. A., Julio, I. S., Fernández-Gónzalez, J., Wuletaw, T., Ramesh, R. V., Anders, S. C., Alf, C., Jose, C., Rodomiro, O., and Aakash, C. 2024. Genomic selection in plant breeding: Key factors shaping two decades of progress. *Mol. Plant.* 17(4):552-578

Acquaah, G. 2015. Conventional Plant Breeding Principles and Techniques. In: Al-Khayri, J., Jain, S., and Johnson, D. (eds), *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools*. Springer, pp. 115-158.

Adams, S., Cockshull, K., and Cave, C. 2001. Effect of temperature on the growth and development of tomato fruits. *Annu. Bot.* 88: 869-877.

Afna, P., Roy, S., Viji, M., Vanaja, T., Manju, R. V., and Vishnu, R. 2023. Physiological assessment of temperature stress tolerance in selectively fertilzed coconut hybrids. *Int. J. Environ. Clim.* 13: 391-398.

Agarwal, V., Gupta, N., Gupta, P. C., Rizwan, M., and Singh, G. 2017. Sulfhydral compounds mitigate the adverse effect of high temperature stress in contrasting wheat genotypes. *Vegetos.* 30: 87-96.

Ahmed, R., Iqbal, M. S., Ibrar, D., Mahmood, T., Naveed, M. S., and Naeem, M. K. 2013. A review on heat stress response in different genotypes of tomato crop (*Solanum lycopersicum* L.). *Intl. J. Modern Agric.* 2: 64-71.

Ahn, Y. J. and Zimmerman, J. L. 2006. Introduction of the carrot HSP17.7 into potato (*Solanum tuberosum* L.) enhances cellular membrane stability and tuberization in vitro. *Plant Cell Environ*. 29: 95-104.

Aien, A., Khetarpal, S., and Pal, M. 2011. Photosynthetic characteristics of potato cultivars grown under high temperature. *Am. Eurasian J. Agric. Environ. Sci*. 11: 633-639.

Aisha, R. N. A., Roy S., and Manju, R. V. 2015. Evaluation of selectively fertilized hybrids of coconut (*Cocos nucifera* L.) for water use efficiency under moderate water stress. *J. Plant Sci. Res*. 31(2): 197-200.

Aleem, S., Sharif, I., Tahir, M., Najeebullah, M., Nawaz, A., and Khan, M. I. 2021. Impact of heat stress on cauliflower (*Brassica oleracea* var. Botrytis): A physiological assessment. *Pak. J. Agric. Sci.* 34: 479-486.

Ali, F., Chen, W., Fiaz, S., Wang, Y., Wei, X., and Xie, L. 2022. QTL mapping for grain appearance quality traits using doubled haploid population of rice under different environments. *Pak. J. Bot.* 54: 1265-1275.

Ali, M., Ayyub, C. M., Amjad, M., and Ahmad, R. 2019. Evaluation of thermo-tolerance potential in cucumber genotypes under heat stress. *Pak. J. Agric. Sci.* 56: 53-61.

Allakhverdiev, S. I., Kreslavski, V. D., Klimov, V. V., Los, D. A., Carpentier, R., and Mohanty, P. 2008. Heat stress: An overview of molecular responses in photosynthesis. *Photosynth. Res.* 98: 541-550.

Alsadon, A., Wahb-Allah, M., and Khalil, S. 2006. In Vitro Evaluation of Heat stress Tolerance in Some Tomato Cultivars. *J. King Saudi Univ.* 19: 13-24.

Alsamir, M., Ahmad, N. M., Keitel, C., Mahmood, T., and Trethowan, R. 2017. Identification of high-temperature tolerant and agronomically viable tomato (*S. lycopersicum*) genotypes from a diverse germplasm collection. *Adv. Crop. Sci. Tech*. 5(4): 299-309.

Alsamir, M., Mahmood, T., Trethowan, R., and Ahmad, N. 2021. An overview of Heat stress in tomato (*Solanum lycopersicum* L.). *Saudi J. Biol. Sci*. 28: 1654-1663.

Amit, K. S., Maneesh, K. S., Vineet, S., Rajeev, S., Tushar, R., and Chandan S. 2017. Debilitation in tomato (*Solanum lycopersicum* L.) as result of Heat stress. *J. Pharmacogn. Phytochem.* 6(6): 1917-1922.

Ammu, A. J. 2019. Developing high temperature tolerance in tomato (*Solanum lycopersicum* L.) through selective fertilization technique. M.Sc.(Ag.) thesis, Kerala Agricultural University, Thrissur, 70p.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiol.* 24: 1-15.

Aswini, M. S., Kiran, K., Lenka, B., Shaniware, Y. A., Pandey, P., Dash, A. P., and Haokip, S. W. 2023. The role of genetics and plant breeding for crop improvement: Current progress and future prospects. *Int. J. Plant Soil Sci.* 35(20): 190-202.

Awasthi, R., Kaushal, N., Vadez, V., Turner, N. C., Berger, J., Siddique, K. H. M., and Nayyar, H. 2014. Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea. *Funct. Plant Biol*. 41: 1148-1167.

Ayenan, M. A. T., Danquah, A., Hanson, P., Ampomah-Dwamena, C., Sodedji, F. A. K., Asante, I. K., and Danquah, E.Y. 2019. Accelerating breeding for heat tolerance in tomato (*Solanum lycopersicum* L.): An integrated approach. *Agron. 9*(11): 720-729.

Balal, R. M., Shahid, M. A., Javaid, M. M., Iqbal, Z., Anjum, M. A., and Garcia-Sanchez, F. 2016. The role of selenium in amelioration of heat-induced oxidative damage in cucumber under high temperature stress. *Acta Physiol. Plant*. 38: 1-14.

Barboza, G. E., Hunziker, A. T., Bernardello, A. A., Cocucci, A. A., Moscone, A. E., Carrizo-Garcia, C., Fuentes, V., Dillon, M. O., Bittrich, V., Cosa, M. T., Sublis, R., Romanutti, A., Arroyo, S., and Anton, A. 2016. Solanaceae. In: Kadereit, J.

W. and Bittrich, V. (eds), *The Families and Genera of Vascular Plants*. Springer, Switzerland, pp.295-357.

Barman, D. and Borah, S. P. 2012. Effect of selection response on f3 and f4 generation for yield and yield component characters in mutant rice strain (*Oryza sativa* L.). *APCBEE Procedia*. 4: 183-187.

Barnabás, B., Jäger, K., and Fehér, A. 2008. The effect of drought and heat stress on reproductive. *Plant Cell Environ.* 31: 11-38.

Berry, J. and Björkman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol*. 31(1): 491-543.

Berry, S. and Uddin, M. R. Effect of high temperature on fruit set in tomato cultivars and selected germplasm. *Hort. Sci.* 23: 606-608.

Bhagat, M., Kaur, N., Goyal, P., and Kaur, S. 2024. Characterization for Heat stress tolerance in barley (*Hordeum vulgare* L.) using physiological and biochemical traits [on-line]. Available: https://doi.org/10.21203/rs.3.rs-4175744/v1 [23 Aug 2024.

Bhandari, H. R., Srivastava, K., and Reddy, G. E. 2017. Genetic variability, heritability and genetic advance for yield traits in tomato (*Solanum lycopersicum L.*). *Int. J. Curr. Microbiol. App. Sci*. 6(7): 4131-4138.

Bhattarai, S., Harvey, J. T., Djidonou, D., and Leskovar, D. I. 2021. Exploring morpho-physiological variation for heat stress tolerance in tomato. *Plants* 10: 347-362.

Bhattarai, U., Sharma, A., Das, R., and Talukdar, P. 2016. Genetic analysis of yield and yield-attributing traits for high temperature resistance in tomato. *Int. J. Veg. Sci*. 5260: 1-13.

Bita, C. E. and Gerats, T. 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Front. Plant Sci*. 4: 1-18.

Bita, C. E., Zenoni, S., Vriezen, W. H., Mariani, C., Pezzotti, M., and Gerats, T. 2011. Temperature stress differentially modulates transcription in meiotic anthers of heat-tolerant and heat-sensitive tomato plants. *BMC Genomics.* 12: 384-396.

Blum, A. 2018. *Plant Breeding for Stress Environments*. CRC Press Inc., Boca Raton, Florida, USA, 223p.

Bokszczanin, K. L. and Fragkostefanakis, S. 2013. Solanaceae pollen thermotolerance initial training network (SPOT-ITN) consortium perspectives on deciphering mechanisms underlying plant heat stress response and thermotolerance. *Front. Plant. Sci*. 4: 315-322.

Boote, K. J., Allen, L. H., Prasad, P. V. V., Baker, J. T., Gesch, R. W., Synder, A. M., Pan, D., and Thomas, J. M. G. 2005. Elevated temperature and CO_2 impacts on pollinaton, reproductve growth and yield of several globally important crops. *J. Agric. Meteorol.* 60: 469-474.

Botstein, D., White, R. L., Skolnick, M., and Davis, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Human Genet.* 32(3): 314-331.

Brown, G. B. 1955. The ascorbic acid content of tomatoes as related to illumination. *Am. Society. Hortic. Sci.* 65: 342-348.

Brukhin, V., Hernould, M., Gonzalez, N., Chevalier, C., and Mouras, A. 2003. Flower development schedule in tomato *Lycopersicon esculentum* cv. Sweet Cherry. *Sex. Plant Reprod*. 15: 311-320.

Busi, M. V., Bustamante, C., and Angelo, D. C. Hidalgocuevas M., Boggio S. B., Valle E. M., and Zabaleta E. 2003. MADS-box genes expressed during tomato seed and fruit development. *Plant Mol. Biol.* 52: 801-815.

Camejo, D., Jimenez, A., Alarcon, J. J., Torres, W., Gomez, J. M., and Sevilla, F. 2006. Changes in photosynthetic parameters and antioxidant activities following heat-shock treatment in tomato plants. *Func. Plant Biol.* 33: 177-187.

Camejo, D., Morales, M. A., Amico, J. M. D., Torrecillas, A., and Alarcon, J. J. 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol*. 162: 281-289.

Cen, Y. P. and Sage, R. F. 2005. The regulation of Rubisco activity in response to variation in temperature and atmospheric CO^2 partial pressure in sweet potato. *Plant Physiol*. 139: 979-990.

Chavez-Arias, C. C., Ligarreto-Moreno, G. A., and Restrepo-Díaz, H. 2018. Evaluation of heat stress period duration and the interaction of daytime temperature and cultivar on common bean. *Environ. Exp. Bot*. 155: 600-608.

Chen, H. H., Shen, Z. Y., and Li, P. H. 1982. Adapatability of crop plants to high temperature stress. *Crop Sci.* 22: 719-725.

Chen, L., Jia, H., Tian, Q., Du, L., Gao, Y., Miao, X., and Liu, Y. 2012. Protecting effect of phosphorylation on oxidative damage of D1 protein by down-regulating the production of superoxide anion in photosystem II membranes under high light. *Photosynthesis Res.* 112: 141-148.

Chetelat, R. T. 2002. Revised list of monogenic stocks. *Report Tomato Genet. Coop.* 52: 41-62.

Clarke, H. J., Khan, T. N., and Siddique, K. H. M. 2004. Pollen selection for chilling tolerance at hybridisation leads to improved chickpea cultivars. *Euphytica.* 139: 65-74.

Collard, B. C. and Mackill, D. J. 2007. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philos. Trans. R. Soc. Lond. Biol. Sci.* 363: 557-572.

Comlekcioglu, N. and Soylu, M. K. 2010. Determination of high temperature tolerance via screening of flower and fruit formation in tomato. *J. Agric. Sci.* 20: 32-37.

Conti, V., Mareri, L., Faleri, C., Nepi, M., Romi, M., Cai, G., and Cantini, C. 2019. Drought stress affects the response of italian local tomato (*Solanum*

lycopersicum L.) varieties in a genotype-dependent manner. *Plants* 8(9): 336-345.

Cornea-Cipcigan, M., Pamfil, D., Cordea, M. I., and Margaoan, R. 2022. Exogenously applied GA3 enhances morphological parameters of tolerant and sensitive *Cyclamen persicum* genotypes under ambient temperature and heat stress conditions. *Plants* 11(14): 1868-1880.

Dane, F., Hunter, A. G., and Chambliss, O. L. 1991. Fruit set, pollen fertility and combining ability of selected tomato genotypes under high temperature field conditions. *J. Am. Hortic. Sci.* 116(5): 906-910.

Dasgan, H. Y., Dere, S., Akhoundnejad, Y., and Arpaci, B. B. 2021. Effects of high-temperature stress during plant cultivation on tomato (*Solanum lycopersicum* L.) fruit nutrient content. *J. Food Qual.* 2021(1): 1-15.

David, F. D., Heidmann, J., Heinen, C., Lamp, B., Martin, J., Rost, T., Silady, R., Tong, K., and Tassel, D. V. 1996. Section of Plant Biology Division of Biological Sciences, University of California, 358p.

de Martino, G., Pan, I., Emmanuel, E., Levy, A., and Irish, V. F. 2006. Functional analyses of two tomato *APETALA3* genes demonstrate diversification in their roles in regulating floral development. *Plant Cell* 18(8): 1833-1845.

Demidchik, V., Straltsova, D., Medvedev, S. S., Pozhvanov, G. A., Sokolik, A., and Yurin, V. 2014. Stress-induced electrolyte leakage: The role of K+-permeable channels and involvement in programmed cell death and metabolic adjustment. *J. Exp. Bot.* 65(5): 1259-1270.

Demirevska-Kepova, K. and Feller, U. 2004. Heat sensitivity of Rubisco, Rubisco activase and Rubisco binding protein in higher plants. *Acta Physiol. Plant* 26: 103-114.

Deshmukh, P. S., Sairam, R. K., and Shukla, D. S. 2001. Measurement of ion leakage as a screening technique for drought resistance in wheat genotypes. *Indian J. Plant Physiol.* 34: 89-91.

Deuter, P., White, N., and Putland, D. 2012. Critical temperature thresholds case study: Agriscience Queensland.

Dhaliwal, M. S. 2012. Handbook of vegetable crops (2nd ed.) Kalyani Publishers, Ludhiana, India.

Djanaguiraman, M., Prasad, P. V., and Schapaugh, W. T. 2013. High day or night time temperature alters leaf assimilation, reproductive success, and phosphatidic acid of pollen grain in soybean [*Glycine max* (L.) Merr.] *Crop Sci.* 53(4): 1594-1604.

Domínguez, E., Cuartero, J., and Fernández-Munoz, R. 2005. Breeding tomato for pollen tolerance to low temperatures by gametophytic selection. *Euphytica.* 142: 253-263.

Driedonks, N. 2018. From flower to fruit in the heat reproductive thermotolerance in tomato and its wild relatives. Ph.D. thesis, Radboud University, Nijmegen, The Netherlands, 110p.

Ehlers, J. D. and Hall, A. E. 1998. Heat tolerance of contrasting cowpea lines in short and long days. *Field Crops Res.* 55: 11-21.

El Ahmadi, A. B. and Stevens, M. A. 1979. Reproductive responses of heat-tolerant tomatoes to high temperatures. *J. Am. Soc. Hortic. Sci.* 104: 686-691.

Erickson, A. and Markhart, A. 2002. Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. *Plant Cell Environ.* 25: 123-130.

FAOSTAT [Food and Agricultural Organization Statistics Division]. 2020. The United Nations Food and Agriculture Organization Database [on-line]. Available: http://faostat3.fao.org/home/ [23 Feb 2024].

Farooq, M., Nadeem, F., Gogoi, N., Ullah, A., Alghamdi, S. S., and Nayyar, H. 2017. Heat stress in grain legumes during reproductive and grain-filling phases. *Crop Pasture Sci*. 68: 985-1005.

Farquhar, G. D. and Sharkey, T. D. 1982. Stomatal conductance and photosynthesis. *Annu. Rev. Plant Physiol.* 33:317–345.

Faruq, G., Nezhadahmadi, A., Rahman, M. M., and Prodhan, Z. 2012. Heat tolerance in tomato. *Life Sci. J*. 99: 1936-1950.

Filomena, G., Wolters-Arts, M., Mariani, C., and Rieu, I. 2013. Ensuring reproduction at high temperatures: The Heat stress response during anther and pollen development. *Plants* 2: 489-506.

Firon, N., Shaked, R., Peet, M. M., Pharr, D. M., Zamski, E., Rosenfeld, K., Althan, L., and Pressman, E. 2006. Pollen grains of heat tolerant tomato cultivars retain higher carbohydrate concentration under heat stress conditions. *Sci. Hortic.* 109(3): 212-217.

Firon, N., Shaked, R., Peet, M., Pharr, D., Zamski, E., Rosenfeld, K., Althan, L., and Pressman, E. 2006. Pollen grains of heat tolerant tomato cultivars retain higher carbohydrate concentration under heat stress conditions. *Sci. Hortic.* 109: 212-217.

Fletcher, J. C. 2018. The CLV-WUS Stem Cell Signaling Pathway: A Roadmap to Crop Yield Optimization. *Plants* 7(4): 87-98.

Foolad, M. R. 2005. Breeding for abiotic stress tolerances in tomato. In: Ashraf, M. and Harris, P. J. C. (eds), *Abiotic Stresses: Plant Resistance through Breeding and Molecular Approaches*. The Haworth Press, New York, USA, pp.613-684.

Foolad, M. R., Jones, R. A., and Rodriguez, R. L. 1993. RAPD markers for constructing intraspecific tomato genetic maps. *Plant Cell Reports*. 12(5): 293-297.

Gao, B., Shen, D., Xue, S., Chen, C., Cui, H., and Song, C. 2016. The contribution of transposable elements to size variations between four teleost genomes. *Mob DNA.* 7(1): 4-16.

Gaur, P. M., Samineni, S., Vijayakumar, A. G., Fikre, A., Kimurto, P., Varshney, R. K., Girma, N., Ojiewo, C., Sajja, S. B., Jayalakshmi, V., Kileo, R. O., Chaturvedi, S. K., Tripathi, S., Mannur, D. M., Rao, N. V. P. R. G., Dixit, G. P., and Thudi, M. 2018. Integrated breeding approaches for improving drought and heat adaptation in chickpea (*Cicer arietinum* L.). *Plant Breed.* 138(4): 389-400.

Gautam, H. R., Bhardwaj, M. L., and Kumar, R. 2013. Climate change and its impact on plant diseases. *Curr. Sci. 105*(12): 1685–1691.

Ghosh, T., Rai, M., Tyagi, W., and Challam, C. 2016. Seedling stage low temperature response in tolerant and susceptible rice genotypes suggests role of relative water content and members of OsSNAC gene family. *Plant Signaling Behavior* 11(5): e1138192.

Giorni, F., Wolters-Arts, M., Mariani, C., and Rieu, I. 2013. Ensuring reproduction at high temperatures: The Heat stress response during anther and pollen development. *Plants* 2: 489-506.

Goldberg, R. B., Beals, T. P., and Sanders, P. M. 1993. Anther development: Basic principles and practical applications. *Plant Cell* 10: 1217-1229.

Gopinath, P.P., Parsad, R., Joseph, B., Adarsh, V.S. 2020. GRAPES: General Rshiny Based Analysis Platform Empowered by Statistics. https://www.kaugrapes.com/home. version 1.0.0. DOI: 10.5281/zenodo.4923220.

Gowda, D. S., Singh, G. P., and Singh, A. M. 2011. Relationship between canopy temperature depression, membrane stability, relative water content and grain yield in bread wheat (*Triticum aestivum*) under heat-stress environments. *Indian J. Agric. Sci*. 81: 197-202.

Goyal, M. and Asthir, B. 2009. Polyamine catabolism influences antioxidative defense mechanism in shoots and roots of five wheat genotypes under high temperature stress. *Plant Growth Regul.* 60(1): 13-25.

Guilioni, L., Wery, J., and Tardieu, F. 1997. Heat stress-induced abortion of buds and flowers in pea: is sensitivity linked to organ age or to relations between reproductive organs? *Ann. Bot*. 80: 159-168.

Gupta, A., Pandey, R., Sinha, R., Chowdhary, A., Pal, R. K., and Rajam, M. V. 2019. Improvement of post-harvest fruit characteristics in tomato by fruit-specific over-expression of oat arginine decarboxylase gene. *Plant Growth Regul.* 88: 61–71.

Haldimann, P. and Feller, U. R. S. 2005. Growth at moderately elevated temperature alters the physiological response of the photosynthetic apparatus to heat stress in pea (*Pisum sativum* L.) leaves. *Plant Cell Environ*. 28: 302-317.

Handayani, T. and Watanabe, K. 2020. The combination of drought and heat stress has a greater effect on potato plants than single stresses. *Plant Soil Environ*. 66: 175-182.

Hanna, H. Y. 1982. Response of six tomato genotypes under summer and spring conditions in Louisiana. *Hortic. Sci*. 17: 758-759.

Hanna, H. Y., Millhollon, E. P., Herrick, J. K., and Fletcher, C. L. 1997. Increased yield of heat tolerant tomatoes with deep transplanting, morning irrigation and white mulch. *Hortic. Sci.* 32(2): 224-226.

Hanna, H.Y., Millhollon, E. P., Herrick, J. K., and Fletcher, C. L. 1997. Increased yield of heat tolerant tomatoes with deep transplanting, morning irrigation and white mulch. *Hort Sci.* 32(2):224-226.

Hansen, J., Sato, M., Hearty, P., Ruedy, R., Kelley, M., and Masson-Delmotte, V. 2016. Ice melt, sea level rise and superstorms: evidence from paleoclimate data, climate modeling, and modern observations that 2C global warming could be dangerous. *Atmos. Chem. Phys.* 16: 3761-3812.

Hanyingm, W., Shenyanm, S., Zhujunm, Z., and Xintingm, Y. 2001. Effects of high temperature stress on photosynthesis and chlorophyll fluorescence in sweet pepper (*Capsicum fructescens* L.). *Acta Hortic. Sin.* 28: 517-521.

Harsant, J., Pavlovic, L., Chiu, G., Sultmanis, S., and Sage, T. L. 2013. High temperature stress and its effect on pollen development and morphological components of harvest index in the C3 model grass Brachypodiumdistachyon. *J. Exp. Bot*. 64: 2971-2983.

Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R., and Fujita, M. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci.* 14: 9643-9684.

Havaux, M. 1996. Short-term responses of photosystem I to Heat stress – Induction of a PS II-independent electron transport through PS I fed by stromal components. *Photosyn. Res.* 47: 85-97.

Hazra, P., Ansary, S. H., Dutta, A. K., Balacheva, E., and Atanassova, B. 2009. Breeding tomato tolerant to high temperature stress. *Acta Hortic*. 830: 241-248.

Hedhly, A. 2011. Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environ. Exp. Bot*. 74: 9-16.

Hedhly, A., Hormaza, J., and Herrero, M. 2009. Flower emasculation accelerates ovule degeneration and reduces fruit set in sweet cherry. *Sci. Hortic*. 119: 455-457.

Hemantaranjan, A., Nishant, B. A., Singh, M. N., Yadav, D. K., Patel, P. K., Singh, R., and Katiyar, E. 2014. Heat stress responses and thermo tolerance. *Adv. Plants Agric. Res.* 1(3): 62-70.

Hormaza, J. I., and Herrero, M. 1992. Pollen selection. *Theor. Appl. Genet.* 83: 663-672.

Hoshikawa, K., Pham, D., Ezura, H., Schafleitner, R., and Nakashima, K. 2021. Genetic and molecular mechanisms conferring heat stress tolerance in tomato plants. *Front. Plant Sci.* 12: 786688.

Howarth and Catherine. 2005. Genetic improvements of tolerance to high temperature. Abiotic Stresses: Plant Resistance Through Breeding and Molecular Approaches.

Hu, T., Liu, S., Amombo, E., and Fu, J. 2015. Stress memory induced rearrangements of HSP transcription, photosystem II photochemistry and metabolism of tall fescue (*Festuca arundinacea* Schreb) in response to high-temperature stress. *Front. Plant Sci.* 6: 1-13.

Ilík, P., Špundová, M., Šicner, M., Melkovičová H., Kučerová, Z., and Krchnák, P. 2018. Estimating heat tolerance of plants by ion leakage: a new method based on gradual heating. *New Phytol.* 218: 1278-1287.

IPCC (2014). Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press

IPCC (2021). *The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. United Kingdom: Cambridge University Press

Ismail, A. M. and Hall, A. E. 1999. Reproductive-stage heat tolerance, leaf membrane thermostability and plant morphology in cowpea. *Crop Sci.* 39: 1762-1768.

Jagadish, S. V. K., Craufurd, P. Q., and Wheeler, T. R. 2008. Phenotyping parents of mapping populations of rice for heat tolerance during anthesis. *Crop Sci.* 48(4): 1140-1146.

Jahan, M. S., Wang, Y., Shu, S., Zhong, M., Chen, Z., and Wu, J. 2019. Exogenous salicylic acid increases the heat tolerance in tomato (*Solanum lycopersicum* L) by enhancing photosynthesis efficiency and improving antioxidant defense system through scavenging of reactive oxygen species. *Sci. Hortic.* 247: 421-429.

Jha, U. C., Nayyar, H., Jha, R., Paul, P. J., and Siddique, K. H. M. 2020. Heat stress and cowpea: genetics, breeding and modern tools for improving genetic gains. *Plant Physiol*. 25(4): 645-653.

Jofuku, K. D., den Boer, B. G. W., Van Montagu, M., and Okamuro, J. K. 1994. Control of Arabidopsis flower and seed development by the homeotic gene APETALA2. *Plant Cell*. 6(9): 1211-1225.

Kakkar, P., Das, B., and Viswanathan, P. N. 1984. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys.* 21: 130–132.

Kalloo, G. 1991. *Genetic Improvement of Tomato*. Springer, Berlin, Germany, 458p.

Kamara, M. M., Motawei, M. I., El-Moneim, D. A., Germoush, M. O., Alhusays, A. Y., Kheir, A. M. S., Farid, M. A., Ibrahim, K. M., Mansour, E., and Rehan, M. 2021.

Combining ability and gene action controlling grain yield and its related traits in bread wheat under heat stress and normal conditions. *Agron. J.* 11(8): 1450-1462.

Kartikeya, S., Sunil, K., Surender, K., Pravin, P., and Vaishampayan, A. 2012.Screening of tomato genotypes for reproductive characters under high temperature stress conditions. *Sabrao J. Breed. Genet*. 44: 263-276.

KAU [Kerala Agricultural University]. 2016. Package of Practices Recommendations: Crops (15th Ed.). Kerala Agricultural University, Thrissur, 393p.

Khan, I., Sajjad, M., and Wu, J. 2022. Pollen viability-based heat susceptibility index (HSIpv): A useful selection criterion for heat-tolerant genotypes in wheat. *Front. Plant Sci.*13: 1-16.

Kramer, E. M., Dorit, R. L., and Irish, V. F. 1998. Molecular evolution of genes controlling petal and stamen development: Duplication and divergence within the *APETALA3* and *PISTILLATA* MADS-box gene lineages. *Genetics* 149(2): 765-783.

Kugblenu, Y. O., Oppong, D. E., Ofori, K., Andersen, M. N., Abenney-Mickson, S., Sabi, E. B., Plauborg, F., Abekoe, M. K., Ofosu-Anim, J., Ortiz, R., and Jorgensen, S. T. 2013. Screening tomato genotypes for adaptation to high temperature in West Africa. *Acta Agric. Scand. Sect. B. Soil. Plant. Sci.* 63: 516-522.

Kujur, A., Bajaj, D., Upadhyaya, H. D., Das, S., Ranjan, R., Shree, T., Saxena, M. S., Badoni, S., Kumar, V., and Tripathi, S. 2015. A genome-wide SNP scan accelerates trait-regulatory genomic loci identification in chick pea. *Sci. Rep.* 5: 11166.

Kumar, R. A. 2010. Physiological and biochemical basis of high temperatures stress tolerance in chickpea (*Cicer arietinum* L.) genotypes. Ph.D. thesis, Indian Agricultural Research Institute, New Delhi, 186p.

Kumar, S., Thakur, P., Kaushal, N., Malik, J. A., Gaur, P., and Nayyar, H. 2013. Effect of varying high temperatures during reproductive growth on reproductive

function, oxidative stress and seed yield in chickpea genotypes differing in heat sensitivity. *Arch. Agron. Soil Sci*. 59: 823-843.

Kuo, C. G., Chen, H. M., and Sun, H. C. 1993. Membrane Thermostability and Heat Tolerance of Vegetable Leaves. In: Kuo, C. G. (Ed), *Adaptation of Food Crops to Temperature and Water Stress*. Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp.160-168.

Kurukulasuriya, P. and Rosenthal, S. 2013. Climate Change and Agriculture : A Review of Impacts and Adaptations. Environment department papers; no. 91. Climate change series. © World Bank, Washington.

Labroo, M. R., Rutkoski, J. E., and Studer, A. J. 2021. Heterosis and hybrid crop breeding: A multidisciplinary review. *Front. Genet.* 12: 643761.

Lahr, E. C., Schade, G. W., Crossett, C. C., and Watson, M. R. 2015. Photosynthesis and isoprene emission from trees along an urban-rural gradient in Texas. *Glob. Change Biol.* 21: 4221-4236.

Lai, Y., Shen, D., Zhang, W., Zhang, X., Qiu, Y., and Wang, H. 2018. Temperature and photoperiod changes affect cucumber sex expression by different epigenetic regulations. *BMC Plant Biol.* 18: 268-282.

Lawson, T. and Vialet-Chabrand., S. 2019. Speedy stomata, photosynthesis and plant water use efficiency. *New Phytol.* 22: 93-98.

Levy, A., Rabinowitch, H. D., and Kedar, N. 1978. Morphological and physiological characters affecting flower drop and fruit set of tomatoes at high temperatures. *Euphytica* 27: 211-218.

Li, W., Mo, W., Ashraf, U., Li, G., Wen, T., Abrar, M., Gao, L., Liu, J., and Hu, J. 2018. Evaluation of physiological indices of waterlogging tolerance of different maize varieties in South China. *Appl. Ecol. Environ. Res.* 16: 2059-2072.

Lin, X., Zhu, D., and Lin, X. 2011. Effects of water management and organic fertilization with SRI crop practices on hybrid rice performance and rhizosphere dynamics. *Paddy Water Environ.* 9: 33-39.

Liu, J., Wang, Z., and Li, X. 2009. Effects of high temperature on the number of grains per spike and yield in wheat. *Field Crops Res.*113(2): 172-179.

Liu, X. and Huang, B. 2000. Heat stress injury in relation to membrane lipid peroxidation in creeping bent grass. *Crop Sci.* 40: 503-510.

Lohar, D. and Peat, W. 1998. Floral characteristics of heat-tolerant and heat-sensitive tomato (*Lycopersicon esculentum* Mill.) cultivars at high temperature. *Sci. Hortic*. 73: 53-60.

Lokesha, A. N., Shivashankara, K. S., Laxman, R. H., Geetha, G. A., and Shankar, A. G. 2019. Effect of high temperature on fruit quality parameters of contrasting tomato genotypes. *Int. J. Curr. Microbiol. Appl. Sci.* 8(3): 1019-1029.

Malumpong, C., Vanavichit, A., Detpittayanan, W., Cheabu, S., and Mongkolsiriwatana, C. 2019. Spikelet fertility and heat shock transcription factor (Hsf) gene responses to heat stress in tolerant and susceptible rice (*Oryza sativa* L.) genotypes. *J. Agric. Sci.* 157(4): 283-299.

Marcum, K. B. 1998. Cell membrane thermostability and whole-plant heat tolerance of Kentucky bluegrass. *Crop Sci*. 38: 1214-1218.

Marine, J. P., Haperen, P. V., Rieu, I., Richard, G. F. V., Yury, M. T., and Arnaud, G. B. 2017. Screening for pollen tolerance to high temperatures in tomato. *Euphytica* 213: 130-138.

Martineau, J. R., Specht, J. E., Williams, J. H., and Sullivan, C. Y. 1979. Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. *Crop Sci*. 19: 75-78.

Masthigowda, M., Sharma, D., Khobra, R., Krishnappa, G., Khan, H., Singh, S. K., and Singh, G. 2022. Pollen viability as a potential trait for screening heat-tolerant wheat (*Triticum aestivum* L.). *Funct. Plant Biol*. 49: 1-18.

Matteo, A. D., Sacco, A., Anacleria, M., Pezzotti, M., Delledonne, M., Ferrarini, A., Frusciante, L., and Barone, A. 2010. The ascorbic acid content of tomato fruits

is associated with the expression of genes involved in pectin degradation. *BMC Plant Biology*. 10: 163-169.

Matthews, J. S., Vialet-Chabrand, S., and Lawson, T. 2018. Acclimation to fluctuating light impacts the rapidity of response and diurnal rhythm of stomatal conductance. *Plant Physiol.* 176: 1939-1951.

Mazorra, L. M., Nunez, M., Hechavarria, M., and Manchado, F. C. 2002. Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures. *Biologia Plantarum* 45: 593-596.

Mesihovic, A., Iannacone, R., Firon, N., and Fragkostefanakis, S. 2016. Heat stress regimes for the investigation of pollen thermotolerance in crop plants. *Plant Reprod*. 29: 93-105.

Momcilovic, I. and Ristic, Z. 2007. Expression of chloroplast protein synthesis elongation factor, EF-Tu, in two lines of maize with contrasting tolerance to heat stress during early stages of plant development. *J. Plant Physiol.* 164:90-99.

Mondol, A. M., Chowdhury, J., Ahmed, A., Khaliq, Q., and Karim, M. 2018. Effect of drought stress on water relation traits of four soybean genotypes. *SAARC J. Agric.* 15(2): 163-175.

Morales, D., Rodriguez, P., Dellamico, J., Nicolas, E., Torrecillas, A., and Sanchez-Blanco, M. J. 2003. High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. *Biol. Plant* 47: 203-208.

Morrison, Malcolm, Stewart, and Doug. 2002. Heat stress during Flowering in Summer Brassica. *Crop Sci*. 42: 797-803.

Moustafa, A., Arisha, M., and El-Sawah, M. 2019. Response of some tomato hybrids to different fertilization sources under high temperature conditions. *Zagazig J. Agric. Res.* 46(6): 1847-1861.

Mulachy, D. L. 1979. The rise of angiosperms: A genecological factor. *Sci.* 206: 20-23.

Mullan, D. and Pietragalla, J. 2012. Leaf relative water content. In: Pask, A. J. D., Pietragalla, J., Mullan, D., and Reynolds, M. (eds), *Physiological Breeding II: A Field Guide to Wheat Phenotyping*. CIMMYT, Mexico, pp.25-27.

Muller, F. and Rieu, I. 2016. Acclimation to high temperature during pollen development. *Plant Reprod.* 29: 107-118.

Müller, F., Xu, J., Kristensen, L., Wolters-Arts, M., de Groot, P. F., Jansma, S. Y., Mariani, C., Park, S., and Rieu, I. 2016. High-temperature-induced defects in tomato (*Solanum lycopersicum*) anther and pollen development are associated with reduced expression of B-Class floral patterning genes. *PloS ONE* 11(12): 234-237.

Murai, K. 2013. Homeotic genes and the ABCDE model for floral organ formation in wheat. *Plants* 2(3): 379-395.

Murray, M. G. and Thompson, W. F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic acids Res*. 8(19): 4321-4326.

Mutschler, M. A., Tanksley, S. D., and Rick, C. M. 1987. Linkage maps of the tomato (*Lycopersicon esculentum*). *Report Tomato Genet. Coop.* 37: 5-34.

Nagarajan, S. and Bansal, K. C. 1986. Measurement of cellular membrane thermostability to evaluate foliage heat tolerance of potato. *Potato Res.* 29: 163-167.

Nahar, K., Hasanuzzaman, M., and Fujita, M. 2016. Heat stress responses and thermotolerance in soybean. In: Miransari, M. (Ed), *Abiotic and Biotic Stresses in Soybean Production*, Academic Press, Cambridge, MA, pp.261-284.

Nduwimana, A. and Wei, S. M. 2017. A comparative study of the effects of high temperature regime on cherry tomato plant water status when cultivated in different growing substrates systems. *Biol. Chem. Res.* 4: 173-186.

Negi, J., Matsuda, O., Nagasawa, T., Oba, Y., Takahashi, H., and Kawai-Yamada, M., 2008. CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. *Nat*. 452: 483-486.

Nyarko, G., Alderson, P. G., Craigon, J., Murchie, E., and Sparkes, D. L. 2008. Comparison of cell membrane thermostability and chlorophyll fluorescence parameters for the determination of heat tolerance in ten cabbage lines. *J. Hortic. Sci. Biotechnol*. 83: 678-682.

Omae, H., Kumar, A., and Shono, M. 2012. Adaptation to high temperature and water deficit in the common bean (*Phaseolus vulgaris* L.) during the reproductive period. *J. Bot*. 23: 1-6.

Opena, R. T., Kuo, G. C., and Yoon, J. Y. 1987. Breeding for stress tolerance under tropical conditions in tomato and heading Chinese cabbage. ASPAC-FFTC, Taipei, Taiwan, pp.88-109.

Oshino, T., Abiko, M., Saito, R., Ichiishi, E., Endo, M., Kawagishi-Kobayashi, M., and Higashitani, A. 2007. Premature progression of anther early developmental programs accompanied by comprehensive alterations in transcription during high-temperature injury in barley plants. *Mol. Genet. Genomics.* 278: 31-42.

Pan, C., Yang, D., Zhao, X., Jiao, C., Yan, Y., and Lamin-Samu, A. T. 2019. Tomato stigma exsertion induced by high temperature is associated with the jasmonate signalling pathway. *Plant Cell Environ*. 42: 1205-1221.

Panthee, D. R. and Gotame, T. P. 2020. Improving heat stress tolerance in tomato. *CABI Rev.* 2020(60): 1-15.

Parrotta, L., Aloisi, I., Faleri, C., Romi, M., Duca, S. D., and Cai, G. 2020. Chronic heat stress affects the photosynthetic apparatus of *Solanum lycopersicum* L. cv Micro-Tom. *Plant Physiol. Biochem*. 154: 463-475.

Paupière, M. J., van Haperen, P., Rieu, I., Visser, R. G. F., Tikunov, Y. M., and Bovy, A. G. 2017. Screening for pollen tolerance to high temperatures in tomato. *Euphytica.* 213: 1-8.

Peet, M. M., Sato, S., and Gardner, R. G. 1998. Comparing heat stress effects on male-fertile and male-sterile tomatoes. *Plant Cell Environ.* 21: 225-231.

Peet, M. M., Willits, D. H., and Gardner, R. 1997. Response of ovule development and post- pollen production processes in male-sterile tomatoes to chronic, sub-acute high temperature stress. *J. Exp. Bot.* 48(1): 101-111.

Peet, M. M., Willits, D. H., and Gardner, R. 2002. Response of ovule development and post-pollen production processes in tomato to chronic, sub-acute high temperature stress. *J. Experiment. Bot. 53*(373):1187–1195.

Perveen, A. and Qaiser, M. 2007. Pollen morphology of family Solanaceae from Pakistan. *Pak. J. Bot*. 39(7): 2243-2256.

Petolino, J. F., Cowen, N. M., Thompson, S. A., and Mitchell. J. C. 1990. Gamete selection for heat-stress tolerance in maize. *Plant Physiol.* 136(2): 219-224.

Pham, D., Hoshikawa, K., Fujita, S., Fukumoto, S., Hirai, T., Shinozaki, Y., and Ezura, H. 2020. A tomato heat-tolerant mutant shows improved pollen fertility and fruit-setting under long-term ambient high temperature. *Environ. Exp. Bot.*178: 104150.

Platenius, H. 1945. Diurnal and seasonal changes in the ascorbic acid content of some vegetables. *Plant Physiol.* 20(1): 98-105.

Porch, T. G. and Jahn, M. 2001. Effects of high-temperature stress on microsporogenesis in heat-sensitive and heat-tolerant genotypes of *Phaseolus vulgaris*. *Plant Cell Environ.* 24(7): 723-731.

Potters, G., Pasternak, T. P., Guisez, Y., and Jansen, M. A. 2009. Different stresses, similar morphogenic responses: integrating a plethora of pathways. *Plant Cell Environ.* 32: 158-169.

Prasad, P. V. V., Craufurd, P. Q., Summerfield, R. J., and Wheeler T. R. 2000. Effects of short episodes of heat stress on floral production and fruit-set of groundnut (*Arachis hypogaea* L.). *J. Exp. Bot.* 51: 777-784.

Prasad, P. V., Bheemanahalli, R., and Jagadish, S. K. 2017. Field crops and the fear of heat stress-opportunities, challenges and future directions. *Field Crops Res*. 200: 114-121.

Prasad, P. V., Craufurd, P., and Summerfield, R. 1999. Fruit number in relation to pollen production and viability in groundnut exposed to short episodes of heat stress. *Annu. Bot.* 84: 381-386.

Pressman, E., Peet, M. M., and Pharr, D. M. 2002. The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrates concentration in the developing anthers. *Annu. Bot.* 90: 631-636.

Puneeth, P. 2018. Evaluation of hot pepper (*Capsicum annuum* L.) genotypes for Heat stress tolerance Ph.D. thesis, ICAR-Indian Agricultural Research Institute, New Delhi, India, 194p.

Radin, J. W., Lu, Z., Percy, R. G., and Zeiger, E. 1994. Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. *Proc. Natl. Acad. Sci.* 91: 7217-7221.

Raijadhav, S. B., Choudhari, K. G., and Kale, P. N. 1996. Correlation studies in tomato. *J. Maharashtra Agri. Univ.* 21(2): 252-253.

Raja, M. M., Vijayalakshmi, G., Naik, M. L., Basha, P. O., Sergeant, K., Hausman, J. F., and Khan, P. S. S. V. 2019. Pollen development and function under heat stress: from effects to responses. *Acta Physiol. Plant.* 41(4): 47-55.

Rajametov, S. N., Yang, E. Y., Jeong, H. B., Cho, M. C., Chae, S. Y., and Paudel, N. 2021. Heat treatment in two tomato cultivars: A study of the effect on physiological and growth recovery. *Hortic*. 7: 119-129.

Ramana, C. V., Shankar, V. G., Kumar, S. S., and Rao, P. V. 2007. Trait interrelationship studies in tomato (*Lycopersicon esculentum* Mill.). *Res. Crops* 8(1): 213-218.

Ranasinghe, C. S., Pradeep, A. P. C., Waidyarathna, K. P., and Meneripitiya, M. S. K. 2012. Approach to screen coconut varieties for high temperature tolerance by in-vitro pollen germination. *COCOS*. 19(1): 13-26.

Rao, M. V. 1992. Cellular detoxifying mechanisms determine age dependent injury in tropical plants exposed to high temperature. *J. Plant Physiol*. 140: 733-740.

Raval, L. J., Pithia, M. S., Mehta, D. R., and Ribadiya, A. H. 2017. Estimation of inter-generation parameters in various selection procedures in desi chickpea (*Cicer arietinum* L.) crosses. *Int. J. Pure Appl. Biosci.* 5(2): 536-540.

Ravikumar, R. L. and Satish, D. 2010. Standardization of in vitro pollen germination media in selected varieties of cotton and tomato. *J. Agric. Sci*. 23(2): 317-319.

Ravikumar, R. L., Salimath, P. M., and Patil, B. S. 2003. Drought tolerance in sorghum by pollen selection using osmotic stress. *Euphytica.* 133(3): 371-376.

Razaq, K., Shahzad, M., Ashraf, E., Rauf, S., and Shah, F. 2017. Genetic analysis of pollen viability: an indicator of heat stress in sunflower (*Helianthus annuus* L.). *Int. J. Innov. Approaches Agric. Res.* 1(1): 40-50.

Reddy, K. R., and Kakani, V. G. 2007. Screening Capsicum species of different origins for high temperature tolerance by in vitro pollen germination and pollen tube length. *Sci. Hortic.* 112(2): 130-135.

Reddy, R. K. B., Reddy, H. K., Sudhakar, P., Reddy, R. B., and Reddy, M. D. 2022. Evaluation of P1, P2, F1, F2, B1 and B2 generations of LM 95 × EC 362096 cross of mung bean for yield and yield attributes along with water use efficiency and heat stress tolerance related traits using generation mean analysis. *Int. J. Farm Sci*. 12(4): 145-151.

Reynolds-Henne, C. E., Langenegger, A., Mani, J., Schenk, N., Zumsteg, A., and Feller, U. 2010. Interactions between temperature, drought and stomatal opening in legumes. *Environ. Exp. Bot*. 68: 37-43.

Rieu, I., Twell, D., and Firon, N. 2017. Pollen development at high temperature: From acclimation to collapse. *Plant Physiol*. 173: 1967-1976.

Rijpkema, A., Gerats, T., and Vandenbussche, M. 2006. Genetics of floral development in petunia. *Adv. Bot. Res.* 44: 237-278.

Rios, R. O. 2015. *Plant Breeding in Omics Era*. Springer, Switerzland, 252p.

Rivero, R. M., Ruiz, J. M., and Romero, L. 2004. Oxidative metabolism in tomato plants subjected to heat stress. *J. Hortic. Sci. Biotechnol.* 79: 560-564.

Rivero, R. M., Ruiz, J. M., García, P. C., Lopez-Lefebre, L. R., Sanchez, E., and Romero, L. 2021. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci.* 160(2): 315-321.

Rodriguez-Garay, B. and Barrow, J. R. 1988. Pollen selection for heat tolerance in cotton. *Crop Sci*. 28(5): 857-859.

Rodríguez-Leal, D., Lemmon, Z. H., Man, J., Bartlett M. E., and Lippman, Z. B. 2017. Engineering quantitative trait variation for crop improvement by genome editing. *Cell* 171: 470-480.

Ruane, J. and Sonnino, A. 2007. Marker-assisted selection as a tool for genetic improvement of crops, livestock, forestry and fish in developing countries: an overview of the issues. In: Guimaraes, E. P., Ruane, J., Scherf, B. D., Sonnino, A., Dargie, J. D. (eds), *Marker-Assisted Selection*. FAO, Rome, pp.3-13.

Rudich, J., Zamski, E., and Regev, Y. 1977. Genotype variation for sensitivity to high temperature in the tomato: pollination and fruit set. *Et. Gaz.* 138: 448-452.

Saadella, M. M., Shanahan, J. F., and Quick, J. S. 1990. Heat tolerance in winter wheat: Hardining and genetic effects on membrane thermostability. *Crop Sci.* 30: 1243-1247.

Sadasivam, S. and Manickam, A. 2008. Biochemical methods. New Age International Pvt. Ltd, New Delhi, 270p.

Saeed, A., Hayat, K., Khan, A. A., and Iqbal, S. 2007. Heat tolerance studies in tomato (*Lycopersicon esculentum* Mill.). *Int. J. Agric. Biol.* 9: 649-652.

Sage, T. L., Bagha, S., Lundsgaard-Nielsen, V., Branch, H. A., Sultmanis, S., and Sage, R. F. 2015. The effect of high temperature stress on male and female reproduction in plants. *Field Crops Res*. 182: 30-42.

Saha, S., Hossain, M., Rahman, M., Kuo, C., and Abdullah, S. 2010. Effect of high temperature stress on the performance of twelve sweet pepper genotypes. *Bangladesh J. Agric. Res.* 35: 525-534.

Sairam, R. K., Saxena, D. C., and Srivastava, G. C. 2000. Increased antioxidant activity under elevated temperatures: A Mechanism of heat stress tolerance in wheat genotypes. *Biol. Plant* 43(2): 245-251.

Sakata, T. and Higashitani, A. 2008. Male sterility accompanied with abnormal anther development in plants-genes and environmental stresses with special reference to high temperature injury. *Int. J. Plant Dev. Biol.* 2: 42-51.

Salem, A. M., Kakani, V. G., Koti, S., and Reddy, K. R. 2007. Pollen-based screening of soybean genotypes for high temperatures. *Crop Sci*. 47: 219-226.

Salvucci, M. E. and Crafts-Brandner, S. J. 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Plant Physiol.* 120: 179-186.

Crafts-Brandner, S. J., & Salvucci, M. E. (2000). Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO2. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(24), 13430–13435.

Sato, S. and Peet, M. M. 2005. Effects of moderately elevated temperature stress on the timing of pollen release and its germination in tomato (*Lycopersicon esculentum* Mill.). *J. Hortic. Sci. Biotechnol.* 80: 23-28.

Sato, S., Kamiyama M., and Iwata, T. 2006. Moderate increase of mean daily temperature adversely affects fruit set of *Lycopersicon esculentum* by disrupting specific physiological processes in male reproductive development. *Annu. Bot.* 97: 731-738.

Sato, S., Peet, M. M., and Thomas, J. F. 2002. Determining critical pre- and post-anthesis periods and physiological processes in *Lycopersicon esculentum* Mill exposed to moderately elevated temperatures. *J. Exp. Bot*. 53: 1187-1195.

Sato, S., Peet, M., and Thomas, J. 2000. Physiological factors limit fruit set of tomato (*Lycopersicon esculentum* Mill.) under chronic, mild heat stress. *Plant Cell Environ*. 23: 719-726.

Sato, S., Peet, M. M., and Gardner, R. G. 2004. Altered flower retention and developmental patterns in nine tomato cultivars under elevated temperature. *Sci. Hort.* 101: 95–101.

Savale, S. V., Patel, A. I., and Sante, P. R. 2017. Study of heterosis over environments in tomato (*Solanum lycopersicum* L.). *Int. J Chem. Stu.* 5(3): 284-289.

Savchenko, G. E., Stupak, A. P., and Klyuchareva, E. A. 2002. Study of dynamic properties of the lipid layer of plastid inner membranes using lipophilic fluorescent probes, Zh. *Prikl. Spektr*. 69: 497-501.

Scandalios, J. G. 1993. Oxygen stress and superoxide dismutases. *Plant Physiol.* 101: 7-12.

Schoper, J. B., Lambert, R. J., Vasilas, B. L., and Westgate, M. E. 1987. Plant factors controlling seed set in maize: The influence of silk, pollen, and ear-leaf water status and tassel heat treatment at pollination. *Plant Physiol.* 83(1): 121-125.

Senatore, A., Trobacher, C. P., and Greenwood, J. S. 2009. Ricinosomes predict programmed cell death leading to anther dehiscence in tomato. *Plant Physiol.* 149(2): 775-790.

Shaheen, M. R., Ayyub, C. M., Amjad, M., and Waraich, E. A. 2015. Morpho-physiological evaluation of tomato genotypes under high temperature stress conditions. *J. Sci. Food Agric.* 96(8): 2698-2704.

Shanmugavel, P., Rajaprakasam, S., Chockalingam, V., Ramasamy, G., Thiyagarajan, K., and Marimuthu, R. 2021. Breeding mechanisms for high temperature tolerance in crop plants. In: Abdurakhmonov, I. V. (Ed), *Plant Breeding - Current and Future Views*, Intechopen [on-line]. Available: http://dx.doi.org/10.5772/intechopen. 94693 [25 Mar 2024].

Sharkey, T. D. 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell Environ*. 28: 269-277.

Sharkey, T. D. and Zhang, R. 2010. High temperature effects on electron and proton circuits of photosynthesis. *J. Integr. Plant Biol.* 52: 712-722.

Sharma, D. K., Andersen, S. B., Ottosen, C. O., and Rosenqvist, E. 2015. Wheat cultivars selected for high Fv /Fm under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. *Physiologia Plantarum* 153(2): 284-298.

Sharma, L., Priya, M., Bindumadhava, H., Nair, R. M., and Nayyar, H. 2016. Influence of high temperature stress on growth, phenology and yield performance of mungbean [*Vigna radiata* (L.)] under managed growth conditions. *Sci. Hortic*. 213: 379-391.

Sharma, T. V. R. S. and Kishanswaroop. 2000. Genetic variability and character association in brinjal (*Solanum melongena* L.). *Indian J. Hortic.* 57(10): 59-65.

Sherzod, R., Yang, E. Y., Cho, M. C., Chae, S. Y., and Chae, W. B. 2020. Physiological traits associated with high temperature tolerance differ by fruit types and sizes in tomato (*Solanum lycopersicum* L.). *Hortic. Environ. Biotechnol.* 61: 837-847.

Shi, G. J., Li, Y., Cao, Q. H., Wu, H. X., Tang, X. Y., Gao, X. H., Yu, J. Q., Chen, Z., and Yang, Y. 2019. In vitro and in vivo evidence that quercetin protects against diabetes and its complications: A systematic review of the literature. *Biomed. Pharmacother.* 109: 1085-1099.

Shi, H., Jiang, C., Ye, T., Tan, D. X., Reiter, R. J., and Zhang, H. 2015. Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [*Cynodon dactylon* (L). *Pers.] by exogenous melatonin. J. Exp. Bot.* 66: 681-694.

Shivanna, K. R. and Rangaswamy, N. S. 2012. *Pollen biology: a laboratory manual*. Springer Science & Business Media.

Shobha, R. T. and Ravikumar, R. L. 2006. Sporophytic and gametophytic recurrent selection for improvement of partial resistance to *Alternaria* leaf blight in sunflower (*Helianthus annuus* L.). *Euphytica.* 147: 421-431.

Silva, R. S., Kumar, L., Shabani, F., and Picanço, M. C. 2017. Assessing the impact of global warming on worldwide open field tomato cultivation through CSIRO-Mk3.0 global climate model. *J. Agric. Sci.* 155: 407-420.

Singh, U., Patel, P. K., Singh, A. K., Tiwari, V., Kumar, R., Rai, N., Bahadur, A., Tiwari, S. K., Singh, M., and Singh, B. 2015. Screening of tomato genotypes underhigh temperature stress for reproductive traits. *Veg. Sci*. 42: 52-55.

Singh, V., Van Oosterom, E. J., Nguyen, C. T., Yang, Z., Hammer, G. L., and Chapman, S. C. 2016. Genotypic differences in effects of short episodes of high-temperature stress during reproductive development in sorghum. *Crop Sci.* 56(4): 1561-1572.

Sita, K., Sehgal, A., Kumar, J., Kumar, S., Singh, S., Siddique, K. H. M., and Nayyar, H. 2017. Identification of high-temperature tolerant lentil (*Lens culinaris* Medik.) genotypes through leaf and pollen traits. *Front. Plant Sci.* 8: 1-27.

Smaczniak, C., Immink, R. G., Angenent, G. C., and Kaufmann, K. 2012. Developmental and evolutionary diversity of plant MADS-domain factors: Insights from recent studies. *Development* 139: 3081-3098.

Smertenko, A., Dráber, P., Viklický, V., and Opatrný, Z. 1997. Heat stress affects the organization of microtubules and cell division in *Nicotiana tabacum* cells. *Plant Cell Environ.* 20: 1534-1542.

Song, G., Chen, Q., and Tang, C. 2014. The effects of high-temperature stress on the germination of pollen grains of upland cotton during square development. *Euphytica* 200(2): 175-186.

Stevens, M. A. and Rudich, J. 1978. Genetic potential for overcoming physiological limitations on adaptability, yield and quality in the tomato. *Hortic. Sci.* 13: 673-678.

Suliman-Pollatschek, S., Kashkush, K., Shats, H., Hillel, J., and Lavi, U. 2002. Generation and mapping of AFLP, SSRs and SNPs in *Lycopersicon esculentum. Cellular Mol. Biol. Lett.* 7(2): 583-597.

Suzuki, N., Koussevitzky, S., Mittler, R., and Miller, G. 2012. ROS and redox signalling in the response of plants to abiotic stress. *Plant Cell Environ.* 35: 259-270.

Vara Prasad, P. V., Craufurd, P. Q., Summerfield, R. J., Wheeler, T. R. 2000. Effects of short episodes of heat stress on flower production and fruit-set of groundnut (*Arachis hypogaea* L.). *J. Exp. Bot.* 51:777–784.

Taiz, L., Zeiger, E., Moler, I. M., and Murphy, A. 2015. *Plant Physiology and Development*. Sinauer Associates, Massachusetts, 761p.

Tanksley, S. D. 1993. Linkage map of the tomato (*Lycopersicon esculentum*) (2N = 24) In: O'Brian, S. J. (Ed), *Genetic Maps: Locus Maps of Complex Genomes.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA, pp. 3-15.

Tanksley, S. D. and Bernatzky, R. 1987. Molecular markers for the nuclear genome of tomato. *Plant Biol.* 4: 37-44.

Tanksley, S. D. and Orton, T. J. 1983. *Isozymes in Plant Genetics and Breeding.* Elsevier, Amsterdam, The Netherlands, 356p.

Tao, L. X., Tan, H. J., Wang, X., Cao, L. Y., Song, J., and Cheng, S. H. 2008. Effects of high temperature stress on flowering and grain-setting characteristics for Guodao 6. *Acta Agron. Sin.* 34: 609-674.

Tewari, A. K. and Tripathy, B. C. 1998. Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucumber and wheat. *Plant Physiol*. 117: 851-858.

Thirumalaikumar, V. P., Devkar, V., Mehterov, N., Ali, S., Ozgur, R., and Turkan, I. 2018. NAC transcription factor JUNGBRUNNEN 1 enhances drought tolerance in tomato. *Plant Biotechnol. J.* 16: 354-366.

Thomas, J. M. G. and Prasad, P. V. V. 2003. *Plants and the Environment /Global Warming Effects*. University of Florida, Gainesville, FL, USA, 152p.

Thuy, T. L. and Kenji, M. 2015. Effect of high temperature on fruit productivity and seed-set of sweet pepper (*Capsicum annuum* L.) in the field condition. *J. Agric. Sci. Technol*. 5: 515-520.

Todorov, D. T., Karanov, E. N., Smith, Aileen, Hall, and Mike. 2003. Chlorophyllase activity and chlorophyll content in wild and mutant plants of *Arabidopsis thaliana*. *Biol. Plant.* 46: 125-127.

Torrecillas, A., Guillaume, C., Alarcon, J. J., and Ruiz-Sanchez, M. C. 1995. Water relations of two tomato species under water stress and recovery. *Plant Sci.* 8: 105- 110

Tsukaguchi, T., Kawamitsu, Y., Takeda, H., Suzuki, K., and Egawa, Y. 2003. Water status of flower buds and leaves as affected by high temperature in heat tolerant and heat-sensitive cultivars of snap bean (*Phaseolus vulgaris* L.). *Plant Prod. Sci*. 6: 4-27.

Veronica, N., Ashoka, Y., Subrahmanyam, D., Narasimha, K. L., Ahamed, M. L., Prasuna, P., and Yugandhar, P. 2019. Evaluation of stress indices for screening of rice cultivars for high temperature tolerance. *Oryza* 56: 341-351.

Vijayakumar, A. and Beena, R. 2023. Response of Tomato Quality and Yield to Elevated Temperatures under Controlled Environment. *Int. J. Environ. Clim.* 13. 256-271

Vijayakumar, A., Shaji, S., Beena, R., Sarada, S., Rani, T. S., Stephen, R., and Viji, M. M. 2021. High temperature induced changes in quality and yield parameters of tomato (*Solanum lycopersicum* L.) and similarity coefficients among genotypes using SSR markers. *Heliyon* 7(2): 35-45.

Von Caemmerer, S., and Evans, J. R. 2015. Temperature responses of mesophyll conductance differ greatly between species. *Plant Cell Environ*. 38: 629-637.

Wahid, A. and Shabbir, A. 2005. Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. *Plant Growth Regul.* 46: 133-141.

Wahid, A., Gelani, S., Ashraf, M., and Foolad, M. R. 2007. Heat tolerance in plants: an overview. *Environ. Exp. Bot.* 61: 199-203.

Ward, G. M. 1964. Greenhouse tomato nutrition- a growth analysis study. *Plant Soil* 21: 125-133.

Warman, C., Panda, K., Vejlupkova, Z., Hokin, S., Unger-Wallace, E., Cole, R. A., Chettoor, A. M., Jiang, D., Vollbrecht, E., and Evans, M. M. S. 2020. High expression in maize pollen correlates with genetic contributions to pollen fitness as well as with coordinated transcription from neighboring transposable elements. *Plos Genet.* 16: e1008462.

Way, D. A. and Pearcy, R. W. 2012. Sunflecks in trees and forests: from photosynthetic physiology to global change biology. *Tree Physiol.* 32: 1066-1081.

Weaver, M. L. and Timm, H. 1989. Screening tomato for high temperature tolerance through pollen viability tests. *Hortic. Sci.* 24: 493-495

Wellmer, F., Bowman, J. L., Davies, B., Ferrándiz, C., Fletcher, J. C., Franks, R. G., Graciet, E., Gregis, V., Ito, T., and Jack, T. P. 2014. Flower development: open questions and future directions. In: Riechmann, J. L. and Wellmer, F. (eds), *Flower Development, Methods and Protocols.* Humana Press, New York, pp.103-124.

Welsh, J. R. 1981. *Fundamentals of Plant Genetics and Breeding*. John Willy and Sons. Inc., pp.181-195.

Wen, J., Jiang, F., Weng, Y., Sun, M., Shi, X., Zhou, Y., Yu, L., and Wu, Z. 2019. Identification of heat-tolerance QTLs and high-temperature stress-responsive genes through conventional QTL mapping, QTL-seq and RNA-seq in tomato. *BMC Plant Biol.* 19: 1-17.

Weston, D. J. and Bauerle, W. L. 2007. Inhibition and acclimation of C_3 photosynthesis to moderate heat: a perspective from thermally contrasting genotypes of *Acer rubrum* (red maple). *Tree Physiol.* 27: 1083-1092.

Wilson, R. A., Sangha, M. K., Banga, S. S., Atwal, A. K., and Gupta, S. 2014. Heat stress tolerance in relation to oxidative stress and antioxidants in *Brassica juncea*. *J. Environ. Biol.* 35(2): 383-387.

Wise, R. R., Olson, A. J., Schrader, S. M., and Sharkey, T. D. 2004. Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. *Plant Cell Environ*. 27: 717-724.

Wollenweber, B., Porter, J. R., and Schellberg, J. 2003. Lack of interaction between extreme high-temperature events at vegetative and reproductive growth stages in wheat. *J. Agron. Crop Sci.*189(3): 142-150.

Xu, J., Mieke, W. A., Celestina, M., Heidrun, H., and Ivo, R. 2017. Heat stress affects vegetative and reproductive performance and trait correlations in tomato (*Solanum lycopersicum*). *Euphytica* 213: 156-168.

Yadav, R. K., Kumar, R., Kalia, P., Jain, V., and Varshney, R. 2014. Effect of high day and night temperature regimes on tomato (*Solanum lycopersicum*) genotypes. *Indian J. Agric. Sci.* 84(2): 287-290.

Yamamoto, E., Matsunaga, A., Onogi, A., Kajiya-Kanegae, H., Minamikawa, M., and Suzuki, A. 2016. A simulation-based breeding design that uses whole-genome prediction in tomato. *Sci. Rep*. 6: 19454.

Yeh, Der-Ming, and Lin, H. 2003. Thermostability of Cell Membranes as a Measure of Heat Tolerance and Relationship to Flowering Delay in Chrysanthemum. *J. Am. Soc. Hortic. Sci.* 128: 32-33.

Yordanov, M. 1983. Heterosis in tomato. *Monogr. Theor. Appl. Genet*. 6: 189-219.

Young, L. W., Ron, W., and Wilen, P. C. B. 2004. High temperature stress of Brassica napus during flowering reduces micro- and megagametophyte fertility, induces fruit abortion and disrupts seed production. *J. Exp. Bot. 55*(396): 485-495.

Yuan, L., Chen, G., Liu, S., Zhu, S., Hou, J., Wang, J., Yuan, Y., and Wang, C. 2017. Influence of high temperature on photosynthesis, antioxidative capacity of

chloroplast, and carbon assimilation among heat-tolerant and heat-susceptible genotypes of nonheading chinese cabbage. *Hortic. Sci.* 52(11): 1464-1470.

Zamir, D. 1983. Pollen gene expression and selection: applications in plant breeding. In: Tanksley, S. D. and Orton, T. S. (eds), *Isozymes in Plant Genetics and Breeding*. Elsevier, Amsterdam, pp.313-329.

Zamir, D. and Gadish, I. 1987. Pollen selection for low temperature adaptation in tomato. *Theory. Appl. Genet*. 74: 545-548.

Zamir, D., Tanksley, S. D., and Jones, R. A. 1981. Low temperature effect on selective fertilization by pollen mixtures of wild and cultivated tomato species. *Theory. Appl. Genet*. 59: 235-238.

Zhang, C., Liu, J., Zhang, Y., Cai, X., Gong, P., Zhang, J., Wang, T., Li, H., and Ye, Z. 2011. Overexpression of SlGMEs leads to ascorbate accumulation with enhanced oxidative stress, cold, and salt tolerance in tomato. *Plant Cell Rep.* 30: 389-398.

Zhang, J., Jiang, X. D., Li, T. L., and Cao, X. J. 2014. Photosynthesis and ultrastructure of photosynthetic apparatus in tomato leaves under elevated temperature. *Photosynthesis* 52: 430-436.

Zhang, X., Li, Y., and Sun, Y. 2020. Regulatory effect of heat shock transcription factor-1 gene on heat shock proteins and its transcriptional regulation analysis in small abalone *Haliotis diversicolor*. *BMC Mol. Cell Biol.* 21: 83-84.

Zhao, Q., Chen, W., Bian, J., Xie, H., Li, Y., and Xu, C. 2018. Proteomics and phosphoproteomics of heat stress-responsive mechanisms in spinach. *Front. Plant Sci*. 9: 800-812.

Zhou, R., Kjaer, K., Rosenqvist, E., Yu, X., Wu, Z., and Ottosen, C. O. 2017. Physiological response to heat stress during seedling and anthesis stage in tomato genotypes differing in heat tolerance. *J. Agron. Crop Sci.* 203: 68-80.

Zhou, R., Wu, Z., Wang, X., Rosenqvist, E., Wang, Y., and Zhao, T. 2018. Evaluation of temperature stress tolerance in cultivated and wild tomatoes using

photosynthesis and chlorophyll fluorescence. *Hort. Environ. Biotech.* 59:499–509.

Zhou, R., Kong, L., Yu, X., Ottosen, C. O., Zhao, T., and Jiang, F. 2019. Oxidative damage and antioxidant mechanism in tomatoes responding to drought and heat stress. *Acta Physiol. Plant.* 41: 20-32.

Zhou, R., Xiaqing, Y., Katrine, H. K., Eva, R., Carl-Otto, O., and Zhen, W. 2015. Screening and validation of tomato genotypes under heat stress using Fv/Fm to reveal the physiological mechanism of heat tolerance. *Environ. Exp. Bot.* 118: 1-11.

Zhu, J. K. 2016. Abiotic stress signalling and responses in plants. *Cell* 167: 313-324.

PHYSIOLOGICAL AND MOLECULAR BASIS OF SELECTIVE FERTILIZATION FOR HIGH TEMPERATURE STRESS TOLERANCE IN TOMATO (*Solanum lycopersicum* L.)

By

AMMU ALPHONSA JOHN
(2019-21-074)

ABSTRACT

Submitted in partial fulfilment of the requirements for the degree of

Doctor of philosophy in agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2024

ABSTRACT

The study entitled “Physiological and molecular basis of selective fertilization for high temperature stress tolerance in tomato (*Solanum lycopersicum* L.)”, was undertaken with the objective to screen the critical temperature for pollen germination of tomato genotypes and to evaluate the thermotolerance of selectively fertilized tomato hybrids through physiological and molecular techniques. The experiments were conducted using the Open field & Rainout shelter (ROS) facility at the Department of Plant Physiology, College of Agriculture, Vellayani during 2019-2024.

The first experiment was devised to advance the selectively fertilized hybrid of Anagha and Manuprabha by the method of selective selfing by pollen selection up to F4 to stabilize the thermotolerance. Pollen germination %, photosynthetic rate, RWC, percentage leakage, chlorophyll content, CSI and stomatal conductance were observed in all generations. In F2 generation, T3 and T10 line showed comparatively better performance than the other lines on the basis of phenotypic evaluation and the molecular markers SSR136 and SSR630 were also present in these lines. The seeds from T3 and T10 line selected for next generation and F3 was developed. In F3 generation, T6 and T8 line were selected for next generation *i.e.,* F4 and the molecular markers SSR134 and SSR630 were present in these lines. In F4 generation, T4 and T6 were the best lines.

The second experiment was designed as a confirmation study of the selective fertilization technique in which ten popular varieties of tomato namely Anagha, Vellayani Vijay, Manuprabha, Nandi, Arka Saurabh, Arka Rakshak, Pusa Rohini, IIHR 26372, Arka Vikas and Akshaya were used for the identification of critical temperature for pollen germination. Mature pollen grains from the fully opened flowers were collected and incubated at different temperatures (32^0C to 40^0C) for two hours in the pollen germination media. The temperature of 36^oC identified as the critical temperature for pollen germination and Anagha showed high pollen germination percentage (25.88 %) and Arka Saurabh showed least pollen germination percentage (16.34 %) at this critical temperature.

In the third experiment, based on the critical sterility temperature six varieties were selected for pollen selection and selective fertilization. The variety Anagha, Vellayani Vijay and IIHR26372 with highest critical temperature for pollen germination percentage were selected as tolerant genotypes and the variety Manuprabha, Arka Saurabh and Pusa Rohini with low critical temperature were selected as the susceptible genotype. Selective fertilization (SF) was done between the six genotypes. Reciprocal cross between the tolerant and susceptible genotypes was also done by selective fertilization as well as normal cross for comparison. Growth performance of the selectively fertilized tomato plants was evaluated in ROS. The experiment was laid out in CRD with three replications.

Floral characters (number of clusters and number of flowers per cluster) were noted significantly high in crosses with susceptible genotype as male parent. Among the crosses, T23 recorded the highest no. of clusters (18) and number of flowers per cluster (10). The flower characters like percentage of flowers with exerted stigma and length of stigma exertion were found high under high temperature but the selectively fertilized crosses recorded less value than the normal crosses. T16 (20.63%) showed lesser stigma exertion. The length of exerted stigma was recorded least in T7 (0.13 mm) which was on par with T2, T11, T17, T16, T23, T28, T26, T24, T19, T5, T4, T25, T34, T10, T1, T22 and T8. The no. of days to first flowering after sowing were recorded, T30 took the shortest period (37 days) for flowering, and T36 took the longest (50 days). The Normal and SF crosses with tolerant as male parent flowered comparatively earlier than its reciprocal crossed ones.

Physiological parameters like photosynthetic rate and relative water content were observed high in selectively fertilized crosses than the normal crosses under higher temperature, the crosses of SF plants with tolerant genotype as male parent show significantly higher photosynthetic rate while it was SF plants with susceptible genotype as male parent in case of RWC. The photosynthetic rate was recorded higher in T13 (10.73 µmol CO_2 m^{-2} s^{-1}) and the relative water content was recorded highest in T29 (64.09%).

Among the temperature tolerance characters like membrane integrity (% leakage), SOD activity and pollen viability, % leakage and SOD activity recorded higher in selectively fertilized plants than the normal plants while the pollen viability recorded high

in normal crosses. The T27 (1.78 %) recorded the least percentage leakage, which was on par with T35 and T30. The highest value of SOD activity was recorded in T28 (0.35 activity g^{-1} min^{-1}) and pollen viability was observed highest in T1 (100 %). Vitamin C content of leaves were recorded highest in normal crossed hybrid with tolerant as male parent, T9 (413.02 µg g^{-1}) showed highest value. The number of fruits/cluster and yield were high in selectively fertilized crosses than the normal crosses, specifically the SF crosses with tolerant genotype as male parent. T10 (4.67) recorded highest number of fruits per cluster which was on par with T16. The yield was very less in ROS condition and the highest value was also observed in the treatment T10 (226 g $plant^{-1}$).

In the first experiment, the selfing of plant through SF improves its performance in each generation. The mean performance of F4 plants were better than F2 and F3. In the second experiment among the ten varieties of tomato, Anagha was found as the best with high germination percentage with critical temperature 36^0 C and Arka saurabh as the susceptible one with lowest pollen germination percentage. In the third experiment 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 mechanism. Among the various cross combinations, selectively fertilized cross of tolerant and susceptible parent having tolerant genotype as male parent was found to be the best at high temperature. Hence this study has importance in improving the high temperature tolerance mechanism in tomato under increasing daily mean temperature.

സംഗ്രഹം

“തക്കാളിയിലെ ഉയർന്ന താപനില സമ്മർദ പ്രതിരോധത്തിന് വേണ്ടിയുള്ള തിരഞ്ഞെടുക്കപ്പെട്ട ബീജസങ്കലനത്തിന്റെ സസ്യശരീരശാസ്ത്ര ജനിതക അടിസ്ഥാനം” എന്ന വിഷയത്തിൽ വെള്ളായണി കാർഷിക കോളേജില് 2019-2024 കാലഘട്ടത്തിൽ സസ്യശരീരശാസ്ത്ര വിഭാഗത്തിൽ ഒരു പഠനം നടത്തുകയുണ്ടായി. ഈ പഠനത്തിന്റെ പ്രധാന ലക്ഷ്യങ്ങൾ തക്കാളിയിൽ പൂമ്പൊടി മുളയ്ക്കുന്നതിന് ഏറ്റവും അനുയോജ്യമായ താപനില കണ്ടെത്തുക, തിരഞ്ഞെടുക്കപ്പെട്ട ബീജസങ്കലനം ചെയ്ത സങ്കരയിനം തക്കാളിയുടെ താപനില പ്രതിരോധശേഷി വിവിധ സസ്യശരീരശാസ്ത്ര ജനിതക സാങ്കേതിക വിദ്യകളുപയോകിച്ച് വിലയിരുത്തുക.

ആദ്യത്തെ പരീക്ഷണത്തിൽ അനഘ, മനുപ്രഭ തക്കാളിയിനങ്ങളുടെ തിരഞ്ഞെടുക്കപ്പെട്ട ബീജസങ്കലനം ചെയ്ത സങ്കരയിനത്തെ F4 തലമുറ വരെ “പോളെൻ സെലെക്ഷൻ” എന്ന രീതിയിലൂടെ തിരഞ്ഞെടുത്ത ഉയര്ന്ന താപനില പ്രതിരോധശേഷി സ്തിരപ്പെടുത്തി. F2 തലമുറയിൽ T3, T10 ലൈനുകൾ മികച്ച പ്രതിരോധശേഷി പ്രകടിപ്പിക്കുകയും SSR136, SSR 630 എന്നീ ജനിതക മാർക്കറുകൾ ഉണ്ടായിരിക്കുകയും ചെയ്തു. T3, T10 ലൈനുകളുടെ വിത്തുകൾ തിരഞ്ഞെടുത്ത് F3 രൂപപ്പെടുത്തി. F3 തലമുറയിൽ T6, T8 ലൈനുകൾ F4 തലമുറക്കായി തിരഞ്ഞെടുത്തു. SSR136, SSR 630 എന്നീ ജനിതക മാർക്കറുകൾ ഇവയിൽ ഉണ്ടായിരിന്നു. F4 ലും ഇതേ മാർക്കറുകൾ കാണപ്പെട്ടു. എന്നാൽ F4 തലമുറയിൽ T4,T6 എന്നിവയായിരുന്നു മികച്ച ലൈനുകൾ.

രണ്ടാമത്തെ പരീക്ഷണത്തിൽ പ്രസിദ്ധമായ പത്തു തക്കാളിയിനങ്ങൾ തിരഞ്ഞെടുത്തതു പൂമ്പൊടി മുളക്കുന്നതിനു ഏറ്റവും അനുയോജ്യമായ താപനില കണ്ടുപിടിക്കാനായിരുന്നു പഠനം നടത്തിയത്. അനഘ, വെള്ളായണി വിജയ്, മനുപ്രഭ, അർക്ക സൗരഭ്, അർക്ക രക്ഷക്, പൂസ രോഹിണി, IIHR 26372, അർക്ക വികാസ്, അക്ഷയ എന്നിവയായിരുന്നു തിരഞ്ഞെടുക്കപ്പെട്ട തക്കാളിയിനങ്ങൾ. 36^0C പൂമ്പൊടി മുളക്കുന്നതിനു ഏറ്റവും അനുയോജ്യമായ താപനിലയായി കണ്ടെത്തി. ഈ നിർണായക ഊഷ്മാവിൽ അനഘ ഉയർന്ന പൂമ്പൊടി മുളയ്ക്കൽ ശതമാനവും (25.88 %) അർക്ക സൗരഭ് ഏറ്റവും കുറഞ്ഞ പൂമ്പൊടി മുളയ്ക്കൽ ശതമാനം (16.34 %) കാണിച്ചു.

മൂന്നാമത്തെ പരീക്ഷണത്തിൽ ഏറ്റവും അനുയോജ്യമായ വന്ധ്യതാ താപനിലയുടെ അടിസ്ഥാനത്തിൽ 6 തക്കാളിയിനങ്ങളെ പൂമ്പൊടി തിരഞ്ഞെടുക്കുന്നതിനും ബീജസങ്കലനത്തിനുമായി തിരഞ്ഞെടുത്തു. പൂമ്പൊടി മുളക്കുന്നതിനാവശ്യമായ ഉയർന്ന ക്രിടിക്കൽ താപനിലയുള്ള അനഘ, വെള്ളായണി വിജയ്, IIHR 26372 എന്നീയിനങ്ങൾ പ്രതിരോധശേഷിയുള്ളവയായി തിരഞ്ഞെടുത്തു. മനുപ്രഭ, അർക്ക സൗരഭ്, പൂസ രോഹിണി എന്നിവ പ്രതിരോധശേഷിയില്ലാത്തവയായും രേഖപ്പെടുത്തി. ബീജസങ്കലനത്തിനു വിധേയമാക്കിയ ചെടികളെ മഴമറക്കലിൽ വളർത്തി.

പൂവിന്റെ ഗുണങ്ങൾ വിലയിരുത്തിയപ്പോൾ ഏറ്റവും കൂടുതൽ പൂക്കളുടെ ക്ലസ്റ്ററുകളും (18) ഒരു ക്ലസ്റ്ററിലെ പൂക്കളുടെ എണ്ണവും T23 ൽ രേഖപ്പെടുത്തി. പ്രകാശസംശ്ലേഷണ നിരക്ക്, ആപേക്ഷിക വെള്ളത്തിന്റെ അളവ് എന്നിവ തിരഞ്ഞെടുക്കപ്പെട്ട ബീജസങ്കലനം നടത്തിയ ക്രോസ്സുകൾ കൂടുതലായി കണ്ടു. താപനിലയെ പ്രതിരോധിക്കുന്ന ഗുണങ്ങൾ പഠനം നടത്തിയതിൽ കോശസ്തരങ്ങളുടെ സമഗ്രത, SOD യുടെ പ്രവർത്തനങ്ങൾ, പൂമ്പൊടിയുടെ പ്രവർത്തനക്ഷമത എന്നിവയും തിരഞ്ഞെടുക്കപ്പെട്ട ബീജസങ്കലനം നടത്തിയ ക്രോസ്സുകളിൽ കൂടുതലായി കണ്ടു. വിളവു കൂടുതലും ഇതേ വിഭാഗത്തിലെ ക്രോസ്സിലാണ് രേഖപ്പെടുത്തിയത്. T10 ആണ് ഏറ്റവും കൂടുതൽ തക്കാളിയുടെ ക്ലസ്റ്റർ ഉള്ളതായി രേഖപ്പെടുത്തിയത്.

ആദ്യത്തെ പരീക്ഷണത്തിൽ, തിരഞ്ഞെടുക്കപ്പെട്ട ബീജസങ്കലനത്തിലൂടെ സസ്യങ്ങളെ സെൽഫിങ് ചെയ്തതിലാണ് മികച്ച പ്രകടനം രേഖപ്പെടുത്തിയത്. F4 തലമുറയിലാണ് F2 നെക്കാളും F3 യെക്കാളും മികച്ച പ്രകടനം ലഭിച്ചത്. രണ്ടാമത്തെ പരീക്ഷണത്തിൽ 36^0C ക്രിട്ടിക്കൽ താപനിലയിൽ അനഘ ഉയർന്ന പൂമ്പൊടി മുളക്കൽ ശതമാനം രേഖപ്പെടുത്തി. മൂന്നാമത്തെ പരീക്ഷണത്തിൽ തിരഞ്ഞെടുക്കപ്പെട്ട ബീജസങ്കലനം നടത്തിയ ചെടികൾ കൂടുതൽ താപനില സമ്മർദ്ദത്തെ പ്രതിരോധിക്കുന്നതായും കണ്ടെത്തി. വിവിധ ക്രോസ് കോമ്പിനേഷനുകളിൽ,സഹിഷ്ണുതയുള്ളതും സാധ്യതയുള്ളതുമായ മാതാപിതാക്കളുടെ തിരഞ്ഞെടുത്ത ബീജസങ്കലനം ഉയർന്ന താപനിലയിൽ പുരുഷ മാതാപിതാക്കൾ മികച്ചവരാണെന്ന് കണ്ടെത്തി. അതിനാൽ ശരാശരി താപനില ദിനംപ്രതി വർദ്ധനവ് രേഖപ്പെടുത്തുന്ന ഈ സാഹചര്യത്തിൽ തക്കാളിയിലെ ഉയർന്ന താപനില സഹിഷ്ണുത സംവിധാനം മെച്ചപ്പെടുത്തുന്നതിൽ ഈ പഠനത്തിന് പ്രാധാന്യമുണ്ട്.