EXPLOITING GENETIC VARIATION FOR HEAT STRESS TOLERANCE IN TOMATO

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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December 2020

Major Subject: Horticulture

ABSTRACT

Tomato production is vulnerable to extreme heat during the spring-summer cropping season, exacerbated by a lack of superior genetic materials that can perform well in such environments. The selection of resilient varieties through an improved understanding of morpho-physio-biochemical traits is imperative to sustain tomato production under high-temperature conditions. Thus, this study was conducted to determine heat-tolerant tomato varieties through an improved understanding of their physio-biochemical basis of heat-tolerance.

The first study was conducted in three stages to select heat-tolerant varieties under persistent heat-stress conditions in controlled and open-field conditions. In the first stage, varieties were screened based on yield responses. Then, eighteen varieties were chosen and exposed to control (green-house: 26/20 ℃) and constant heat-stress (growth-chamber: 34/24 ℃) conditions. The last stage was executed in an open field with twenty-four varieties selected from the first two experiments. Plant morphology and physio-biochemistry were assessed under different environments. From this study, we concluded that heat-tolerant genotypes selected through chlorophyll fluorescence measurements and heat injury index rank in controlled heat-stress conditions exhibited heat-tolerance in open-field conditions as well. Electrolyte leakage and heat injury index distinguished the varieties best in open-field conditions as the plants with low electrolyte leakage and heat injury index had higher marketable yield. ‘Heat Master,’ ‘New Girl,’ ‘HM-1823’, ‘Rally,’ ‘Valley Girl,’ ‘Celebrity,’ and ‘Tribeca’ were selected as high heat-tolerant varieties.

In the second study, selected ten tomato varieties were exposed to three temperature conditions (26/18℃ as control, 38/28℃ for seven days as heat-stress, and 40 ℃ for 7 hours as

heat-shock), and plant physio-biochemical traits were assessed. Electrolyte leakage, malondialdehyde content, and heat injury index most efficiently discriminated the varieties under both heat-stress and heat-shock conditions. ‘Heat Master’ under heat-shock and ‘Celebrity,’ ‘Heat Master,’ ‘Valley Girl,’ ‘New Girl,’ and ‘Picus’ under heat-stress conditions were identified as the most heat-tolerant varieties.

‘Celebrity,’ ‘Heat Master,’ ‘Valley Girl,’ and ‘New Girl’ were established as heat-tolerant under all environments in the study. Adopting these promising varieties could potentially enhance tomato production in the areas prone to extreme temperatures during the cropping period.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisor, Dr. Leskovar, for inspiring my interest in defining my research path. Observing your research expertise and passion for your profession has instilled a fervor in me to continue to grow academically and professionally. Words fall short to explain how grateful I am for your great deal of assistance, insightful feedback, and opportunities that have shaped me into who I am today.

I would like to thank my committee members, Dr. Patil and Dr. Joshi, for their invaluable suggestions and guidance during the research period. Further, I extend my acknowledgment to my team, Josh Harvey, Dr. Desire Djidonou, Dr. Madhumita Joshi, Chungkeun Lee, Kuan Qin, Manuel Figueroa, Juan Hernandez, and Irwin Plata, for offering their helping hands in the research. I would also like to thank Dr. Amit Kumar Mishra for his help in executing the laboratory analyses.

I would like to express my heartfelt gratitude to Dr. Dong and Dr. Malla for allowing me to use their lab instruments, which were crucial for completing my research. Similarly, I would like to thank all the faculty, staff, and my friends in the Department of Horticultural Sciences for making my stay at the Texas A&M University a great experience.

I am forever grateful to my family for their unconditional love, support, patience, and encouragement throughout the journey.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Professor Daniel I. Leskovar as advisor and Professor Bhimnagouda Patil as co-advisor of the Department of Horticultural Sciences and Professor Vijay Joshi of the Department Molecular and Environmental Plant Sciences.

All the work for the thesis was completed by the student, under the guidance of Daniel I. Leskovar of the Department of Horticultural Sciences.

Funding Sources

The first year's graduate study was supported by an excellence fellowship from the College of Agricultural and Life Sciences, Texas A&M University.

The research was conducted through funds from the Texas Department of Agriculture and TAMU Seed Grant.

NOMENCLATURE

C	Control
CF	Chlorophyll fluorescence
Ci	Intercellular CO_2 concentration
EL	Electrolyte leakage
gs	Stomatal conductance
HII	Heat injury index
HS	Heat stress
MDA	Malondialdehyde
Pn	Net photosynthesis rate
SDW	Shoot dry weight
SFW	Shoot fresh weight
SHS	Heat shock
WUEins	Instantaneous water use efficiency
WUEintr	Intrinsic water use efficiency
LT	Leaf Temperature
Ht	Plant Height
D	Stem Diameter

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1. INTRODUCTION AND LITERATURE REVIEW

Tomato (*Solanum lycopersicum* L.), the second-most valuable crop globally, originated in the Andean region of South America (Rick, 1973). Consuming fresh or processed tomatoes has many health benefits as they are highly nutritious; they are a rich source of minerals, vitamins, antioxidants, and fibers (Foolad, 2007). Tomato is consumed fresh and ripe as salad and juice, used for various culinary purposes, and processed into sauces and soups. Due to its broad uses, there is an ever-growing demand for tomato in the world market.

Worldwide, China is the leading producer of tomato, representing 31% of the total production, followed by India(11%), the United States (9%), Turkey(7%), and Egypt(5%) (Heuvalink et al., 2020). In the United States, domestic tomato production accounts for about 42% of the total consumption volume. The rest of the demand is supplied by imports, with about 90% of monthly imports from Mexico (USDA-ERS, 2019). In 2019, California and Florida constituted about two-thirds of the U.S. total tomato production (USDA-ERS, 2019). The fresh tomato import value in 2019 (2616 million pounds) increased by 6.6% in comparison to that of 2017 (2454 million pounds) (USDA-ERS, 2019). Despite the large numbers of tomato growers, more than 80% of tomato demand in Texas markets is met via Mexico imports. In Texas, tomato production decreased by 85% from 1982 to 2015, i.e., from 80 million pounds to 12 million pounds, respectively. One of the main reasons behind the low production in Texas is erratic high-temperature extremes during the spring-summer cropping season (averaged over seven years, Figure 1) exacerbated by the lack of superior genetic materials that can perform well in such environments. To overcome this challenge, there is an urgent need to select superior cultivars that can adapt to the Texas environments and exhibit high yield stability under heat stress conditions.

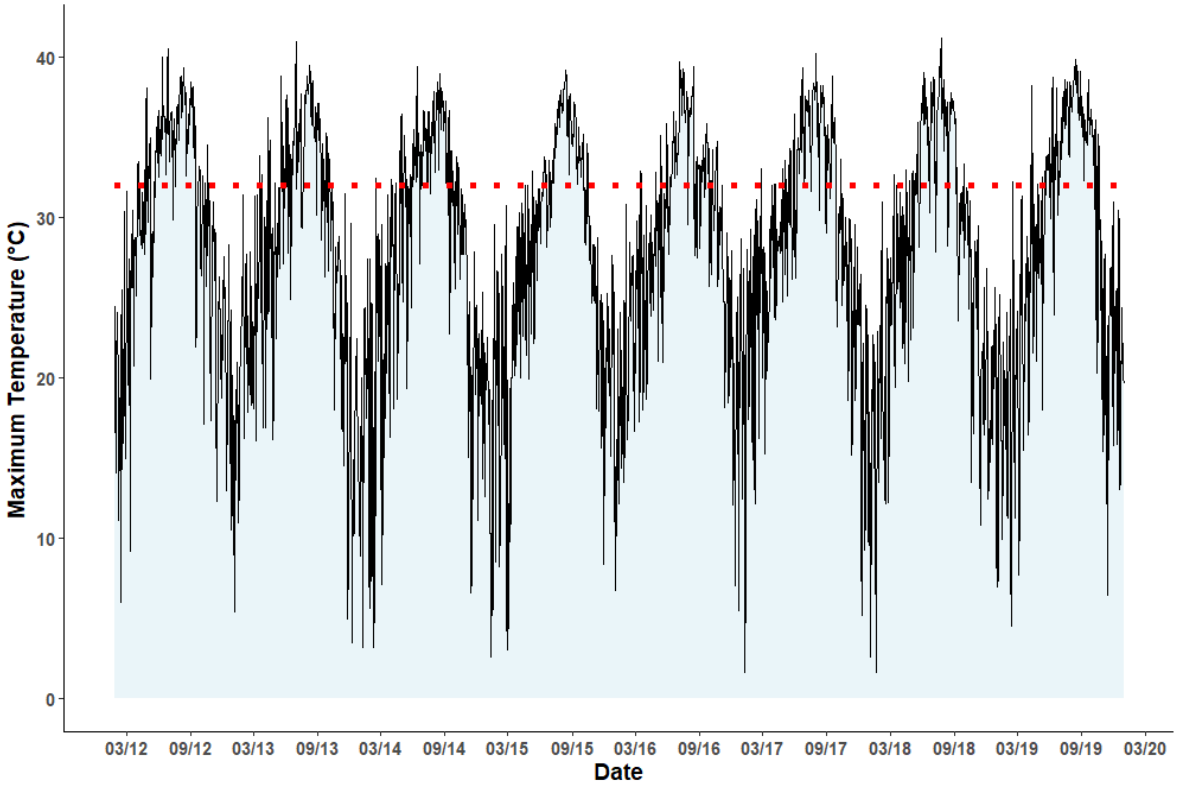

Figure 1.1 Daily maximum temperature from 2012-2019 in Uvalde, Texas. The dotted line indicates 32 °C as the threshold heat stress temperature for tomato.

1.1. Heat stress in plants

The earth's temperature is predicted to rise between 1.5℃ - 11℃ by the next century (Stainforth et al., 2005). This increase will pose severe consequences for food production, especially in the areas characterized by hot and humid summers like the tropics. An increase in temperature beyond the threshold limit resulting in a negative impact on plant growth and productivity is termed heat stress. The heat stress level in plants depends on the intensity, frequency, duration, and rise rate in a day or night temperature (Blum, 2018; Wahid et al., 2007). Plants exposed to heat stress generally show multiple symptoms such as stunted growth, reduced

the size of fruits, drying, wilting, and in severe cases, plant death. Besides, heat stress can inhibit seed germination, alter photosynthesis, decreasing fruit quality, and induce oxidative stress.

Some of the innate responses that plants show to high temperatures can be avoidance or tolerance mechanisms. Early maturation, increased transpirational cooling, and morphological changes like leaf rolling are avoidance mechanisms that a plant uses to protect itself from heat stress (Hasanuzzaman et al., 2013). Plants with tolerance mechanisms might exhibit changes in membrane lipid composition, induction of heat-shock proteins, accumulation of osmoprotectants, activation of antioxidants, and stress-inducible genes (Rivero et al., 2003).

1.2. Responses of heat stress in plants

Heat stress in plants can bring about many changes in morphological, biochemical, physiological, and molecular responses, depending on the developmental stage and genotypes (Wahid et al., 2007). These changes do not occur independently; instead, they involve multiple and complex interactions that plants use to cope with extreme conditions.

1.2.1. Germination and stand establishment

Germination and stand establishment are directly affected when the temperature exceeds the optimum threshold temperature, which for tomato is 35℃. However, the percentage and rate of seed germination depend upon species and the environment to which they are exposed. For instance, tomato seed emergence is adversely affected when exposed to a temperature above 30 ℃ (Camejo et al., 2005). Although it is well established that heat stress response in plants depends on their developmental stage, it is still vague if the damages at a particular stage relate to the damage in other stages of plant growth (Wollenweber et al., 2003).

Irreversible changes in plant growth and physiology imposed by transient or persistent elevation in intensity and frequency of temperature extremes reduce crop yield and quality

drastically. During the vegetative phase, high temperature mainly distorts the leaf gas exchange mechanisms in heat-sensitive tomato plants, whereas, in the reproductive phase, it primarily affects plant yield through flower wilting and abscission, deformation of the anther, loss of pollen viability, low pollen germination, and reduction in fruit (Müller et al., 2016). In tomato, day and night temperature above 32 ℃ and 21 ℃ respectively, adversely affects growth and fruit set (Sato et al., 2004), whereas exposure to an acute temperature of 45℃ for 3 hours drastically reduces chlorophyll content, photosynthesis, and stomatal conductance (Camejo et al., 2006).

1.2.2. General physiological responses

Tomato plants exposed to high temperatures under non-limited water conditions tend to transpire more to dissipate heat. However, when high temperature coincides with transient drought conditions, they actively activate stomata closure to conserve water for critical physiological functions (Zhou et al., 2019). Duan et al. (2017) reported that heat-tolerant tomato varieties have higher stomatal conductance than sensitive plants, providing more diffusion of CO_2 into leaves and higher leaf water potential, which leads to an improved rate of photosynthesis as compared to sensitive ones. Protective compounds, called osmolytes, like proline and glycine betaine, are actively involved in regulating water-related functions in heat-tolerant tomato varieties. The accumulation of these compounds in cells increases the water potential, thus, maintaining the cell turgidity and cellular functions (Golam et al., 2012).

1.2.3. Oxidative stress

Most plant responses to heat stress are secondary, which are caused by oxidative stress. Oxidative stress is caused because of impaired balance between production and removal of reactive oxygen species (ROS) like H_2O_2 (hydrogen peroxide), $^{\cdot}OH$ (hydroxyl radical), $^{\cdot}O_2$ (superoxide anion), and $^1O_2$ (singlet oxygen) in plant cells (Miller et al., 2008). Accumulation of a high

concentration of ROS causes oxidative burst, resulting in programmed cell death. The fate of each ROS is different and depends on the place of its production. For instance, $^1O_2$ actively reacts with polyunsaturated fatty acids (e.g., linolenic acid and arachidonic acid) by destroying their double bonds, $\cdot O_2$ alters protein Fe-S centers, and $^{\cdot}OH$ affects all cellular components (Moller et al., 2007). To repress the adverse effects of ROS, plants produce antioxidant enzymes like superoxide dismutase (SOD), catalases (CAT), glutathione peroxidases (GPX), and ascorbate peroxidases (APX) (Kapoor et al., 2015) in the chloroplasts. These antioxidants actively scavenge and detoxify ROS when their production exceeds the equilibrium. It has been shown that heat-tolerant tomato plants have a higher level of antioxidants that alleviate ROS-mediated damage in plant cells (Camejo et al., 2006).

1.2.4. Photosynthetic responses

In cultivated crops like tomato, photosynthesis is the foremost process affected by high temperatures (Nankishore and Farrel, 2016). The targets of high temperature on tomato plants photosynthetic machinery are reduction in photosynthetic pigments like chlorophyll a and b, disorganization of thylakoid membranes, production of ROS, decrease in stomatal conductance, and inhibition of photosystem II, electron transport chain, oxygen-evolving complex, photosystem I, and carbon dioxide fixation (Zhou et al., 2015). In response to these effects, plants develop various tolerance mechanisms to drive photosynthesis normally, which are changes in membrane structure by altering lipid composition, induction of heat-shock proteins (HSPs) and stress-inducible genes, and production of antioxidants, osmolytes, and other novel protective agents (Bita et al., 2013).

Photosystem II is considered the most thermosensitive component of the photosynthetic apparatus (Čajánek et al., 1998). In tomato plants, the chemical nature of the chloroplasts seems

to be the reason for the high sensitivity of the electron transport of PSII to high-temperature values (Zhou et al., 2015). The viscosity of lipids forming the thylakoid membrane in tomato plants changes rapidly when leaf temperature rises above an upper threshold (35°C), which increases fluidity leading to dislodgement of PSII light-harvesting complexes (LHC) (Berry and Bijorkman, 1980). When PSI and PSII are not functioning in balance, excess electrons can actively produce reactive oxygen species (ROS).

Decrease in ATP production due to light reaction inhibition results in reduced CO_2 fixation by Rubisco (ribulose 1,5- biphosphate carboxylase/oxidase) in C_3 plants. Heat stress also decreases maximum quantum efficiency by increasing non-photochemical quenching and photoinactivation of PSII reaction centers (Baker, 2008).

Many enzymes are thermolabile and denatured at high temperature. RuBisCo activase (RCA) is one of the significant targets of heat stress (Morales et al., 2003). RCA is crucial to activate closed RuBisCo. The activity of RuBisCo activase is likely insufficient to keep pace with the faster rates of RuBisCo inactivation at high temperatures. Expression of heat-stable RCA or an increase in RCA at high temperatures could maintain an activation state of RuBisCo and confer heat acclimation (Yamori et al., 2014).

1.2.5. Membrane stability response

At high temperatures, protein composition and structure are modified because of a decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of the protein. This modification leads to an increase in ion leakage and a reduction in the activity of the membrane-associated electron carriers; thus, attenuating the rate of photosynthesis and respiration (Wahid et al., 2007). Electrolyte leakage has been linked with heat tolerance or sensitivity of many crops. As a measure of membrane thermostability, electrolyte leakage has been studied in various

crops, including tomato. Heat tolerant tomato plants have higher thermostability and low electrolyte leakage than heat-sensitive tomato genotypes (Wahid et al., 2007).

Membrane stability under heat stress is maintained through lipid composition changes (Djanaguiraman et al., 2018). During high-temperature stress, some plants have an increased ratio of saturated to unsaturated fatty acids in phospholipids that increases the melting point and thus, prevents the increase of fluidity of the cell membrane and ensures proper functioning of membrane protein component (Zhang et al., 2005; Maienza et al., 2013).

1.2.6. Molecular responses

Expression of heat shock proteins (HSPs) has a crucial role in regulating thermotolerance to plants and improving their survival under heat extremes (Vierling, 1991). HSPs are synthesized intensively when plants are exposed to rapid heat stress. HSPs can protect proteins from denaturation under high-temperature conditions and subsequently protect the plants from heat-induced damages (Miroshnichenko et al., 2005). In tomato, thermotolerance is controlled by 21 heat stress transcription factors (HSFs) (Scharf et al., 1998).

1.2.7 Yield responses

Heat stress causes loss of production in many heat-sensitive crops, including tomato. In heat-sensitive tomato varieties, loss in productivity is mainly due to heat-induced reproductive damage to the plants like flower abortion, deformation of the anther, loss of pollen viability, low pollen germination, and fruit set (Müller et al., 2016). Reduction in carbon assimilation due to damage to photosynthetic machinery and enhanced respiration leads to the poor performance of heat-sensitive plants under elevated temperatures. Extreme heat during the tomato production stage causes fruit disorders like sun-scorching, cat-face, blossom-end rot, and cracking.

For tomato production, the optimum temperature for growth and development is between 25-30 ℃ during the daytime and 20 ℃ at night (Camejo et al., 2005). However, its production is increasing in the areas where the temperature often exceeds this optimal range. Human intervention is imperative to sustain food production in these unfavorable conditions to fulfill the ever-increasing population's demand. One of the most effective ways to overcome potential losses in tomato production due to heat stress is through exploiting a wide range of genetic variations and adopting resilient cultivars in production.

1.3. Research questions

This project is intended to answer the following questions:

a) Are there tomato genotypes that exhibit tolerance to heat stress in open field conditions?
b) How tomato heat-tolerant genotypes differ in morpho-physio-biochemical traits based on the degree and duration of heat condition?
c) Are there any correlations between the phenotypical, physiological, and biochemical mechanism of heat stress tolerance in tomato plants?

1.4. Objectives

a) Identify agronomically superior heat-tolerant tomato genotypes in open field conditions.
b) Evaluate selective physio-biochemical traits determining tolerance or sensitivity of selected genotypes to different temperature regimes.
c) Establish correlations between morphological performance, physiological-biochemical response, and yield components in heat-stressed tomato plants.

2. DETERMINATION OF HEAT TOLERANT TOMATO GENOTYPES THROUGH MORPHOLOGICAL AND PHYSIO-BIOCHEMICAL STUDIES IN CONTRASTING ENVIRONMENTS

2.1. Introduction

Tomato (*Solanum lycopersicum* L.), the second-most valuable crop globally, originated in the Andean region of South America (Rick, 1973). Being a nutritious food with a multitude of uses, tomato demand is escalating in the world market (FAOSTAT, 2017). In terms of world production, China is the leading producer of tomato, representing 31% of the total volume, followed by India(11%), the United States (9%), Turkey(7%), and Egypt (5%) (Heuvalink et al., 2020). The US tomato demand is supplied through imports from other countries, with 90% of monthly imports from Mexico (USDA-ERS, 2019). In the US, tomato production is mainly concentrated in California, representing 96% of the US total tomato production (USDA-ERS, 2020). Despite the large numbers of tomato growers, more than 80% of tomato demand in Texas markets is met via Mexico imports. A major limiting factor of the low production in Texas is due to erratic high-temperature extremes during the spring-summer cropping season, which is exacerbated by a lack of genetic materials that can perform well in such environments. Thus, it is vital to determine tomato varieties that can sustain yield under high-temperature conditions.

Exposure of tomato plants to elevated temperatures invites numerous alterations in plant physiology and morphology due to heat stress. Generally, tomato plants under heat-stress show symptoms like wilting, reduction of growth, improper development, alteration of photosynthesis, and reduction in crop yield and quality (Wahid et al., 2007). However, the sensitivity of tomato to high temperature differs among different genotypes (Poudyal et al., 2019; Zhou et al., 2015; Zhou

et al., 2017; Zhou et al., 2019), which opens an opportunity to explore, select and adopt tomato varieties with heat-tolerance in areas which experience elevated temperatures during the cropping period.

For tomato production, the optimum temperature for growth and development is between 25-30 ℃ during the daytime and 20 ℃ at night (Camejo et al., 2005). An increase in temperature beyond 32 ℃ significantly reduces tomato fruit production (Nankishore and Farrell, 2016). At high temperature, degradation of proteins, chlorophyll content and membrane stability, and increase in electrolyte leakage results in attenuation of maximal photochemical efficiency of PSII in heat-sensitive tomato plants (Cajanek et al., 1998). Heat stress also affects the structural organization of the thylakoid membrane, dislodgement of PSII light-harvesting complexes, stimulates the synthesis of reactive oxygen, species and inhibits the functionality of PSII that ultimately leads to suppression of CO2 assimilation (Berry and Bjorkman, 1980). In addition to the reduced efficiency of PSII in heat-sensitive tomato plants, elevated temperature induces reproductive damage to plants like flower abortion, deformation of the anther, loss of pollen viability, low pollen germination, and fruit set (Müller et al., 2016).

The tomato plant's response to heat stress is exacerbated by limited water availability. Plants exposed to high temperatures under non-limited water conditions tend to transpire more to dissipate heat. However, when high temperature coincides with transient drought conditions, they actively close stomata to conserve water for critical physiological functions (Zhou et al., 2019). Heat tolerant plants have higher stomatal conductance than that of sensitive plants, providing more diffusion of CO2 into leaves and higher leaf water potential, leading to an improved rate of photosynthesis compared to sensitive plants (Duan et al., 2017). Protective compounds, called osmolytes, like proline and glycine betaine, are actively involved in regulating water-related

functions in heat-tolerant plants. Their accumulation in cells increases the water potential, thus, maintaining the cell turgidity and cellular functions (Golam et al., 2012). `

Various studies have been conducted to select the heat-tolerant tomato varieties, among which most of the experiments have been executed in controlled heat stress environments like growth chambers and greenhouse (Zhou et al., 2017; Camejo et al., 2005). Only a few studies have been conducted in natural heat stress conditions in open-field (Poudyal et al., 2019). While screening in controlled heat stress conditions provides specific heat-related responses of plants, screening in open-field still remains important as plants are exposed to a combination of different environmental conditions and heat. The selection of heat-tolerant varieties under controlled heat stress conditions alone does not necessarily imply that these varieties will be performing well in open-field conditions. Thus, an integrated approach to screen tomato varieties using controlled and open-field conditions should be followed to select the most heat-tolerant varieties.

The predicted rise in the earth's temperature, between 1.5℃ up to 11℃ by the next century, will pose severe consequences for food production (Stainforth et al., 2005). Crop yield is estimated to decrease by 17% with every one-degree increase above the optimum threshold in the average temperature of the growing season (Lobell and Asner, 2003). Texas tomato production is already vulnerable to extreme heat in the spring-summer cropping period, which will be exacerbated by the predicted rise in the temperature in the following three decades. It is imperative to sustain food production in such unfavorable conditions. Thus, production systems in these areas should be inured with vigorous tomato cultivars that can enhance production under such unfavorable conditions, meet the local demand, and potentially add to the US economy through improved export values. This study hypothesizes that there are significant differences in morpho-physiological responses between different tomato genotypes under high-temperature conditions in

open-field and controlled chambers. This study aims to determine varieties that exhibit heat tolerance in the South-Texas environment and understand the basis of heat-tolerance in the superior varieties.

2.2. Materials and methods

Three experiments were conducted to screen the heat-tolerant tomato varieties on exposure to long-term heat stress conditions. The first study was conducted in 2019 in an open-field condition, followed by a second screening in a controlled heat stress environment, and the last one consisted of screening in an open-field in 2020.

2.2.1. Plant materials and field growing conditions in 2019

Forty-three different commercial and TAMU (Texas A&M University) tomato breeding lines (Table 2.1), heirloom and hybrid, were grown in the open-field located at Texas A&M AgriLife Research and Extension Center at Uvalde, TX (29.21°N, 99.79°W) in 2019. Uvalde's climate is classified as warm and temperature (retrieved from climate- data.org) or as a Cfa climate in Köppen-Geiger classification (Peel et al., 2007). The maximum, average, and minimum temperatures for the whole growth period are given in Figure 2.1.

Table 2.1 List of varieties for the first open-field experiment.

SN	Variety	Source	Growth habit	Description
1	Amelia*	Seeds n Such	Determinate, 75 d	Hybrid, heat/humidity tolerant, Large, 8 to 10-oz., firm, bright red
2	Arkansas Traveler*~	Seeds n Such	Indeterminate, 87 d	Hybrid, reddish-pink, smooth to a bit rough, weigh 6 to 8-oz
3	Better Bush	Syngenta	Determinate, 68 d	Hybrid, medium to large, 8 oz.
4	BHN 589*~	BHN Seed	Semi-determinate, 75 d	Hybrid, large, 8 to 10-oz., crack-resistant fruits
5	BHN-1021*~	Johnny's Seed	Determinate, 76 d	Hybrid, 8-16 oz. bright-red slicers
6	Big Beef	Johnny's Seed	Indeterminate, 73 d	Hybrid, Large, 10-12 oz. fruit
7	Black Krim	Johnny's Seed	Indeterminate, 80 d	Heirloom, Deep brown/red, 8-16 oz.
8	Black Prince	Johnny's Seed	Indeterminate, 74 d	Heirloom, 3–5 oz
9	Bolseno F1	Johnny's Seed	Indeterminate, 75 d	Hybrid, 7–12 oz. fruits
10	Carbon	Johnny's Seed	Indeterminate, 76 d	Heirloom, 10-14 oz. fruit
11	Dixie Red	Seeds n Such	Determinate, 70 d	Hybrid, Large, 10-oz. fruits
12	Estiva*	Johnny's Seed	Indeterminate, 70 d	Hybrid, 7-9 oz. fruits
13	FL 91*~	Seminis	Determinate, 72 d	Hybrid, Very large, 10-oz.
14	Fall 2018-253	TAMU-WES	Early Determinate	Inbreed line, large cherry type, sweet, pink fruits
15	TAM-FLW1	TAMUWES	Determinate, 75 d	Hybrid, large beefsteak, red fruits
16	TAM-FLW3~	TAMU-Carlos	Determinate, 75 d	Hybrid, large beefsteak, red fruits
17	Heat Master*~	Seeds n Such	Determinate, 75 d	Hybrid, 7 to 8-oz., deep red fruits
18	HM-1823*~	Clifton Seed	Determinate,	Hybrid, large to X-large
19	Homestead*	Seeds n Such	Determinate, 80 d	Hybrid, 8 oz., bright red fruits

Table 2.1 Continued

SN	Variety	Source	Growth habit	Description
20	HT 1*~	TAMU-CS	Determinate, 75 d	Heirloom, 10 oz fruits
21	HT 2*	TAMU-Crosby	Determinate,75 d	Heirloom, 8-10 oz fruits
22	LaF44*	TAMU-Crosby	Determinate, 75d	Hybrid, 6 oz fruits
23	LaF77	TAMU-Crosby	Determinate, 75d	Hybrid, 6-7 oz fruits
24	LaF66*	TAMU-Crosby	Determinate, 75 d	Hybrid, 6-7 sized fruits
25	Manalucie FSt	Seeds n Such	Indeterminate, 82 d	Hybrid, Large, bright red, globe-shaped, 1-lb. fruits
26	New Girl*~	Johnny's Seed	Indeterminate, 62 d	Hybrid, 4–6 oz. fruit
27	Phoenix*~	Seminis Vegetable Seed	Determinate, 72 d	Hybrid, Bright red, 8-oz. fruits
28	Picus*~	Seeds n Such	Determinate, 79 d	Hybrid, 4 to 5-oz., deep red, roma-shaped, blocky fruits
29	Porter	Seeds n Such	Indeterminate, 78 d	Hybrid, small, 2-oz., plum-shaped, smooth, dark pink fruits
30	Pruden's Purple*~	Johnny's Seed	Indeterminate, 67 d	Heirloom, Large to very large (many over 1 lb.) fruits
31	Rally*	Sakata Seed America	Determinate, 72 d	Hybrid, 9-10 oz. large red fruits
32	RS 1	TAMU	Determinate, 75d	Hybrid, 5-7 oz fruits
33	RS 8	TAMU	Determinate, 75d	Hybrid, 6-8 oz fruits
34	Shourouq*~	Seminis Vegetable Seed	Determinate, 75 d	Hybrid, 8-12 oz fruits
35	Skyway	Johnny's Seed	Determinate, 78 d	Hybrid, 8-12 oz. fruits
36	Summerpick	Syngenta	Determinate, 75 d	Hybrid, extra-large to jumbo, 11.3 oz. fruit
37	Tasti-Lee*~	Bejo Seeds, Inc	Determinate,	Hybrid
38	Tribeca*~	Vilmorin	Determinate, 70 d	Hybrid, 9-10 oz.

Table 2.1 Continued

SN	Variety	Source	Growth habit	Description
39	Valley Girl*~	Johnny's Seed	Determinate, 65 d	Hybrid, 7–8 oz. globe-shaped red fruits
40	Wisconsin 55	Johnny's Seed	Indeterminate, 80 d	Hybrid, 6–8 oz. fruits
41	Bella Rosa*~	Sakata Seed America, Inc.	Determinate, 74 d	Hybrid, Large, 10 to 12-oz., round, deep red, firm fruits
42	Celebrity*~	Clifton Seed	Determinate, 70-75 d	Hybrid, 7-8 oz, globe-shaped, firm red fruits
43	Tonopah	Seeds n Such	Determinate, 67 d	Hybrid, 10 oz, globe-shaped red fruits

*genotypes used in open field experiments in both 2019/2020, ~ genotypes used in control environment experiment.

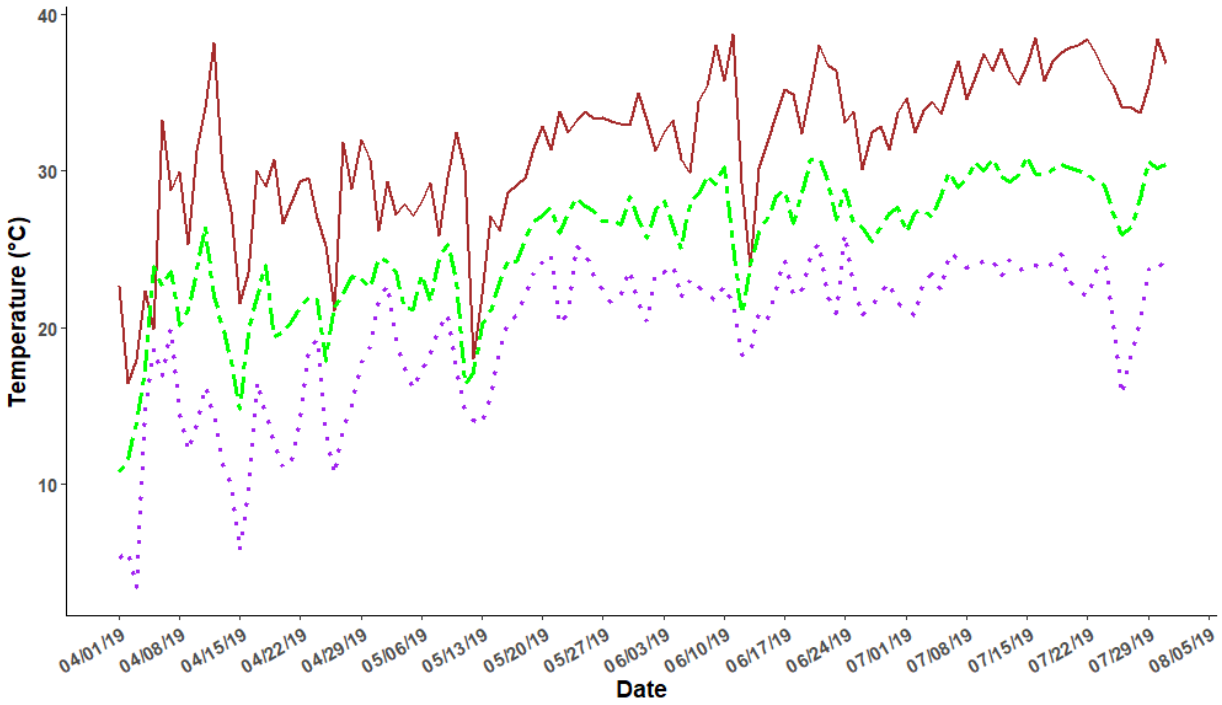

Figure 2.1 Temperature graph for Uvalde, TX from April 2019 - July 2019, when the tomato plants were grown in the open-field for heat-stress tolerance screening. The brown, green, and purple lines indicate daily maximum, average, and minimum temperatures, respectively.

The seeds were sown in polystyrene 200-cell trays (2.5 × 2.5 × 7.6 cm3; Speedling, Ruskin, FL, USA) filled with LM-GPS (Lambert Germination, Plugs, and Seedlings, Lambert, Quebec, Canada) media, which constitutes 90% sphagnum peat moss and 10% perlite, and vermiculite. The trays were saturated with water, incubated in the dark at 25 ℃ for two days, and transferred to a greenhouse. The trays were uniformly irrigated daily with an overhead motorized spraying boom system in the greenhouse. At the four true leaf stage, seedlings were transplanted to the field on April 12, 2019. The experiment was set up in a randomized complete block design (RCBD) with 43 varieties, three blocks, and seven replications. Plants were spaced 0.6 m apart in rows and 1.8 m between rows. They were grown with subsurface drip irrigation (0.12 m deep), and white plastic mulch and tomato stems were staked and strung three times (every 12 inches). The screening was done based on the average fresh weight of fruits recorded from six harvests performed on 6/27/2019, 7/2/2019, 7/11/2019, 7/17/2019, 7/25/2019, and 7/31/2019. Fruits from pink to the red ripe stage were picked during each harvest. Fruits were sorted into extra-large, large, medium, and cull based on USDA standards. The fruits with deep cracks or any other disorders were excluded from the total weight.

2.2.2. Plant material and growth chamber and green-house conditions 2019

Eighteen varieties selected from the first field experiment were used for screening in the growth chamber and green-house. Sowing and seedling management were done as in the first open-field experiment. Plants were transplanted to 0.8-L square pots (10.66 cm top outside, 8.68 cm bottom outside, 9.19 cm depth; TO Plastics, Clearwater, MN) filled with farm soil (28% sand, 47% clay, and 25% silt) on 11/01/2019, and kept in the greenhouse for five days. Half of the pots were then transferred to growth chambers (Conviron Gen 1000) set at 26/18 ℃ (day/night) and allowed to acclimate for five days. The plants kept in the growth chamber were subjected to a

ramping regime from 24-34℃ with 34℃ as maximum day temperature for 4 hours and constant 24 ℃ at dark in the growth chamber for 8 hours. The pots were equally irrigated in both environments every morning to avoid desiccation. The experiment was set up in factorial CRD (Complete Randomized Design) with eighteen varieties (described in Table 2.1), two temperature conditions (26/18℃ in the greenhouse as control, and 34/24℃ in growth-chamber as heat stress), and four replications. The growth chamber was set to a photoperiod of 16/8 hours (light/dark), PAR of 350 $\mu molm^{-1}s^{-1}$, and RH of 65-75%. Data were collected for plant height, stem diameter, chlorophyll fluorescence, chlorophyll content, and heat injury index (HII) starting at 25 DAT.

2.2.2.1. Chlorophyll content

The non-destructive chlorophyll content index was measured as an average of three leaves per plant using a spad meter (SPAD-502 Plus, Minolta, Japan) at 25, 45, and 65 DAT. The average of the measurements was used for analysis.

2.2.2.2. Chlorophyll fluorescence

Chlorophyll fluorescence (CF) was measured using the OS30p Chlorophyll Fluorometer (Opti-Sciences Inc., Hudson, NH) after dark adaptation for 30 minutes. The value obtained was an average of two leaves per plant at 25, 45, and 65 DAT. The average of the measurements was used for analysis.

2.2.2.3. Plant height and stem diameter

Plant height (cm) and diameter (mm) was measured at the end of the experiment (65 DAT). Stem diameter was measured 2 cm above the covered area of the plant using a caliper.

2.2.2.4. Heat Injury Index (HII)

Plants were scored between 1 and 5 as follows, according to Hong et al. (2009).

1= no injury

2= yellow and mildly dehydrated margins of old leaflets

3= mildly dehydrated plants with the middle and crinkled bottom leaflets

4= severely dehydrated plants with upper leaflets crinkled

5= plants with most leaves withered

2.2.3. Plant material and field growing conditions in 2020

Twenty-four varieties (described in Table 2.1) were chosen from a controlled environment and open-field screening conducted in 2019. Activities from sowing (02/15/2020) to transplanting (04/13/2020) in the field was done exactly like in the first open-field screening. The experiment design was set up in a randomized complete block design (RCBD) with 24 varieties, four blocks, and eight replications. Plants were spaced 0.6 m apart in rows and 2.03 m between rows. They were grown with subsurface drip irrigation (0.12 m deep), and white plastic mulch and tomato stems were staked and strung three times (every 12 inches). Measurements were recorded for chlorophyll content, leaf gas exchange, chlorophyll fluorescence, intrinsic and instantaneous water use efficiency, maximum and minimum chlorophyll fluorescence, electrolyte leakage, HII, and yield. All the measurements were performed at stage-1(51 DAT, 34℃) and stage-2 (86 DAT, 41℃), except for yield. The plants were grown in the field until 07/28/2020. The temperature graph for the growth period is given in figure 2.2.

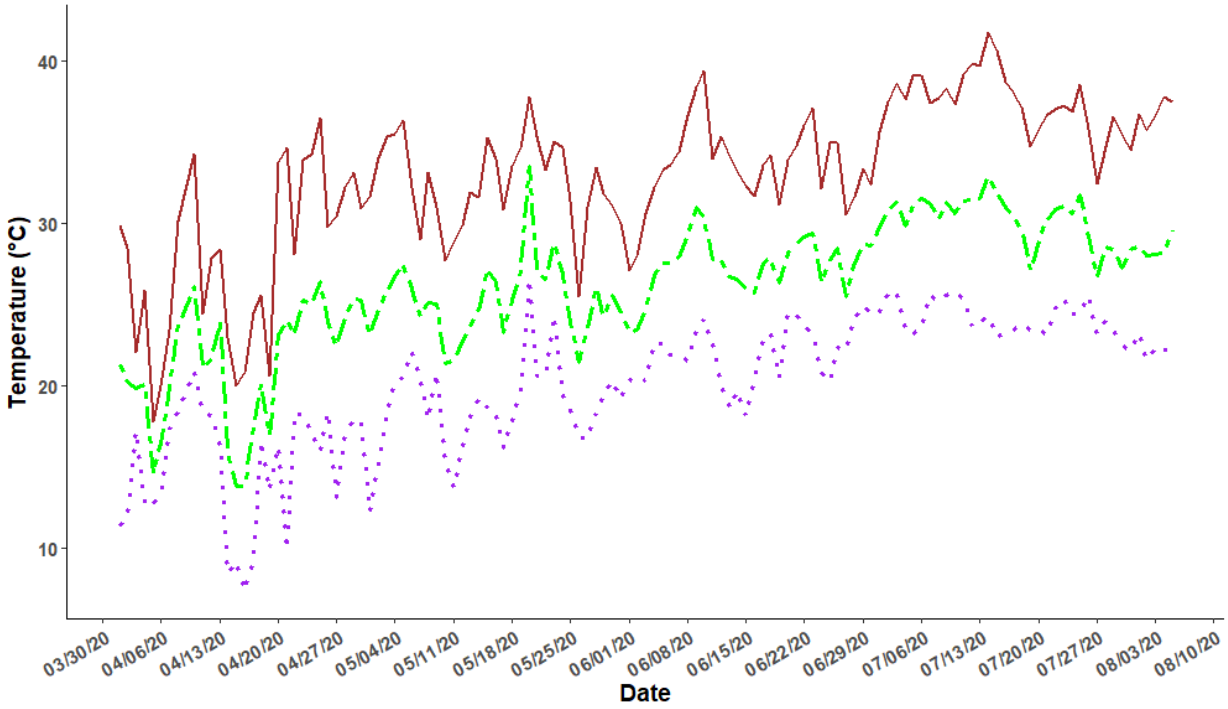

Figure 2.2 Temperature graph for Uvalde, TX from April 2020 - July 2020, when the tomato plants were grown in the open-field for heat-stress tolerance screening. The brown, green, and purple lines indicate daily maximum, average, and minimum temperatures, respectively.

2.2.3.1. Chlorophyll content

The non-destructive chlorophyll content index was measured as an average in two plants per variety and three leaves per plant using a spad meter (SPAD -502 Plus, Minolta, Japan) between 9:00 – 11:00 am. The average of the measurements was used for analysis.

2.2.3.2. Leaf gas exchange, chlorophyll fluorescence, and leaf temperature

The penultimate leaf was taken for gas exchange and chlorophyll fluorescence measurements at 56 and 86 DAT, which was performed between 10:00 am to 2:00 pm. A portable photosynthesis system (LI-6400 XT, LICOR Biosciences, NE, USA) was used to measure the net photosynthetic rate ($P_{N,}$ µmol CO2 $m^{-2}s^{-1}$), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$), and leaf temperature (°C). Chlorophyll fluorescence (CF)

was measured using the OS30p Chlorophyll Fluorometer (Opti-Sciences Inc., Hudson, NH) between 9:00 am – 12:00 pm.

2.2.3.3. Intrinsic and instantaneous leaf water use efficiency

Intrinsic leaf water use efficiency (WUEintr, µmol CO_2 mmol H_2O^{-1}) was calculated as the ratio between P_N and gs, and instantaneous leaf water use efficiency (WUEins, mol CO2 $mmol^{-1}$ H_2O) was obtained as the ratio between P_N and E (Sun et al., 2018).

2.2.3.4. Electrolyte Leakage

Electrolyte leakage (EL, %) was measured using Shinohara and Leskovar (2014) methods. Three leaf discs were extracted from four plants of each variety and placed in sealed culture tubes (25 * 150mm) with 10 ml of distilled water, maintained in a shaking water bath at 25℃ for 24 h, and electrical conductivity (EC) of the supernatant (EC_1) was measured. The tubes were then autoclaved at 120 ℃ for 20 min. The second EC (EC_2) was measured after allowing it to cool to room temperature. The EL was determined with the equation given below:

EL (%) = (EC_1 / EC_2) * 100

2.2.3.5. Heat Injury Index (HII)

Plants were scored between 1 and 5 as follows, according to Hong et al. (2009).

1= no injury

2= yellow and mildly dehydrated margins of old leaflets

3= mildly dehydrated plants with the middle and crinkled bottom leaflets

4= severely dehydrated plants with upper leaflets crinkled

5= plants with most leaves withered

2.2.3.6. Average Marketable Yield

The average yield from a total of four harvests was recorded. The harvesting was done on

07/01/2020, 07/11/2020, 01/22/2020, and 07/28/2020. Fruits from pink to the red ripe stage were picked during each harvest. Fruits were sorted into extra-large, large, medium, and cull based on USDA standards. The fruits with deep cracks or any other disorders were excluded from the total weight.

2.2.4. Statistical analysis

The data collected were analyzed in R software using two-way ANOVA. The correlation among the variables was analyzed using Pearson's correlation, and a correlogram was constructed for each temperature treatment in the controlled heat-stress experiment and total plant responses from two stages for the field experiment. T he multiple comparisons of means were made using Tukey's HSD (Honestly Significant Difference) under $P \leq 0.05$. For only significant main effects of a stage, mean separation for that two stages was done within each level of varieties using Tukey's HSD (Honestly Significant Difference) at $P \leq 0.05$. Clusters of varieties were obtained along with a heatmap based on observed value for each parameter. Correlation distance was employed in clustering analysis.

2.3. Results

2.3.1. First open-field screening

Forty-three different heirloom and hybrid types tomato varieties were screened in an open-field in Uvalde, TX, to determine their heat tolerance for fruit set and yield in a natural heat environment (Table 2.1). These varieties demonstrated high variability in their ability to produce high yield under the study's environmental conditions. The average mean yield was 39 Ton ha^{-1}, which was similar to the yield of LaF7 (Figure 2.3). A total of 21 varieties had lower yields than this average mean, ranging from 9 to 38ton ha^{-1}. MANA had the lowest yield and was the most

susceptible to heat stress. In contrast, CELE had the highest yield, being 95% higher than the average mean yield. These 22 varieties seemed more promising to ensure enhanced marketable yields under an open-field production system, especially in the regions more prone to frequent stressful temperatures, such as south Texas.

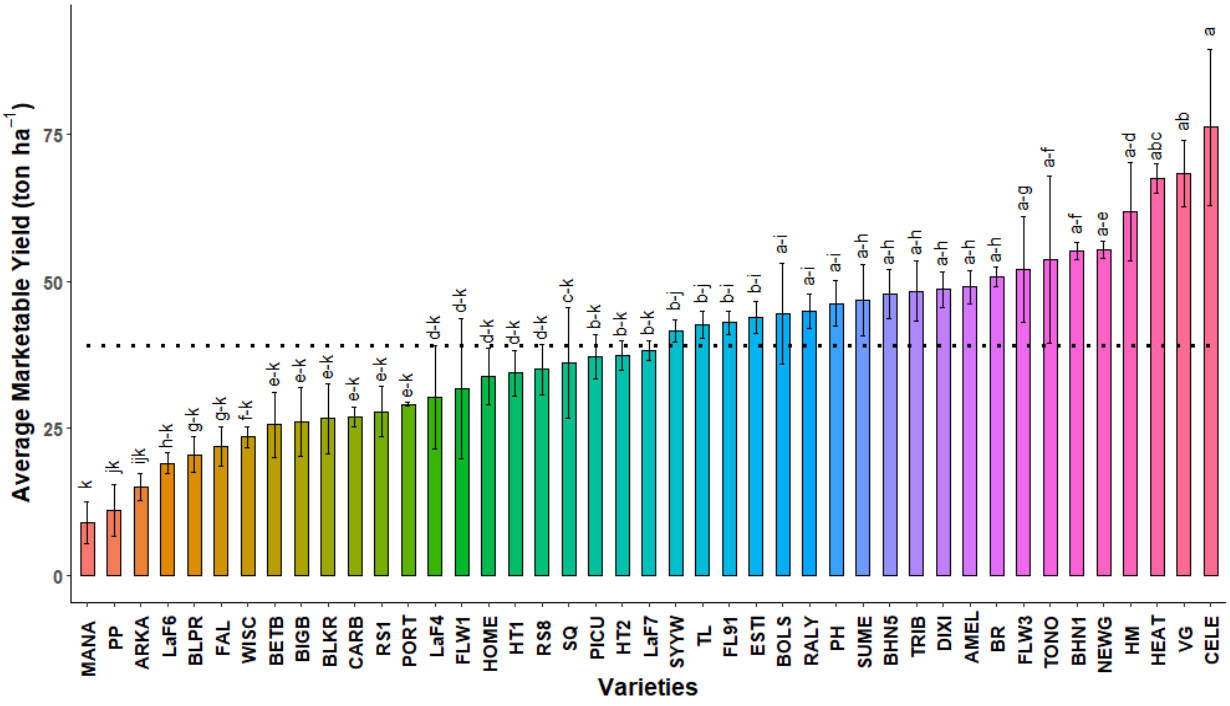

Figure 2.3 Average marketable yield (ton ha-1) of 43 genotypes obtained in 2019. The dotted line indicates the total mean yield (39 ton ha^{-1}).

2.3.2. Growth chamber and green-house screening

Eighteen varieties with high, medium, and low yield were chosen from the initial field screening to study their growth and physiological heat stress responses in growth chamber environments and contrast those with plants grown in greenhouse conditions, considered control.

There were significant interaction effects of variety and temperature treatment for all the parameters assessed. (Table 2.2).

Table 2.2 ANOVA of different parameters measured in the second experiment (greenhouse and growth-chamber) as influenced by varieties (var) and heat-treatments (trt).

Parameter	SOV	P-value	Significance
Chlorophyll Fluorescence (CF)	var	5.98E-06	***
	trt	1.88E-11	***
	var*trt	3.48E-06	***
SPAD Value	var	1.69E-11	***
	trt	< 2e-16	***
	var*trt	0.00428	**
Plant Height (Ht)	var	2.21E-08	***
	trt	< 2e-16	***
	var*trt	0.000347	***
Stem Diameter (D)	var	5.83E-08	***
	trt	5.86E-09	***
	var*trt	9.21E-07	***
Heat Injury Index (HII)	var	<2e-16	***
	trt	<2e-16	***
	var*trt	<2e-16	***

***, **,* show significant difference at P≤ 0.001, 0.01,0.05, respectively.
NS means not significant at P≤0.05.

2.3.2.1. Chlorophyll fluorescence

The average chlorophyll fluorescence for all varieties under the control condition was 0.795, which was 5.37% higher than that under heat-stress conditions (Figure 2.4). The lowest chlorophyll fluorescence values under heat stress were measured for ARKA, BHN1, HM, and PP. These varieties had chlorophyll fluorescence lower than 0.78 under heat-stress, which indicated that their PSII functioning was altered due to heat treatment.

2.3.2.2. SPAD value

The average SPAD value in all 18 varieties under heat stress was 58.77, which was 37.56% higher than the control value (Figure 2.5). Between the varieties, only significant differences in SPAD values were observed for HT1 and NEWG under HS, where the latter had lower value.

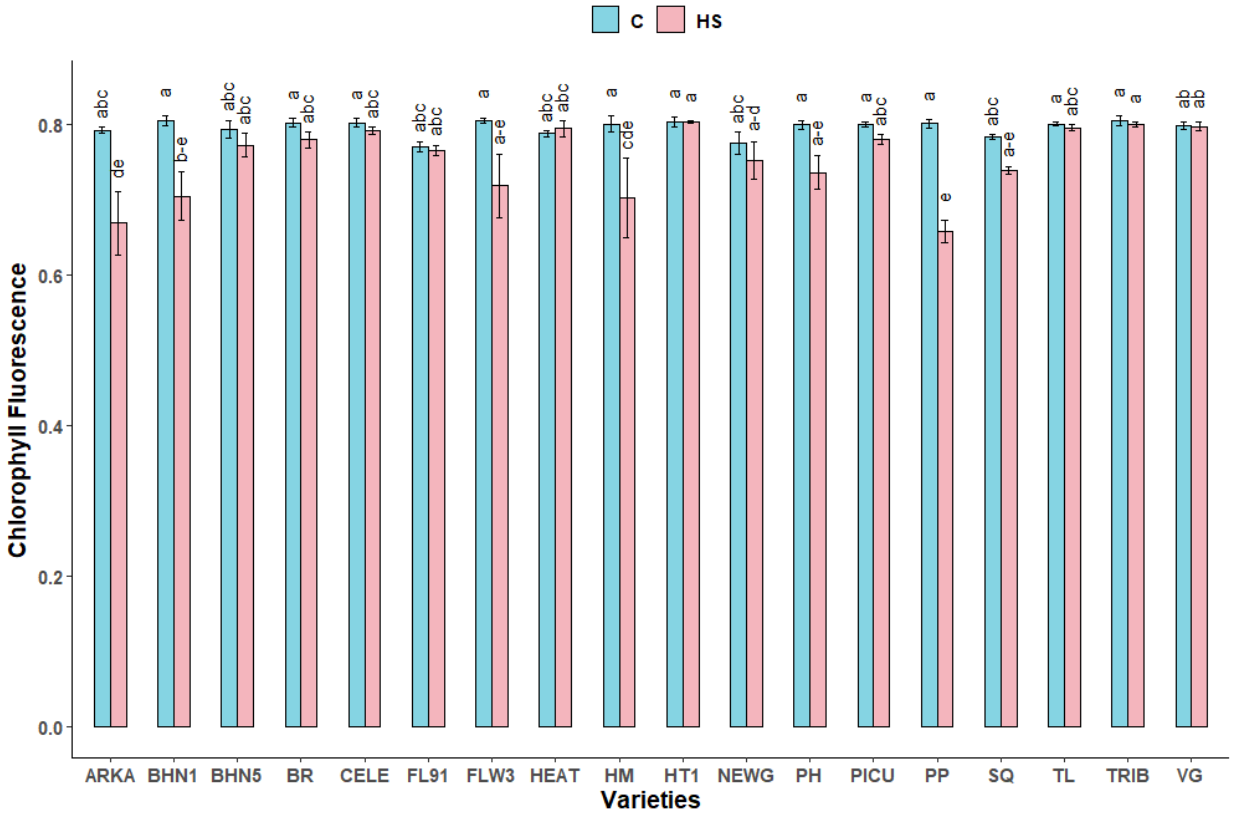

Figure 2.4 Chlorophyll Fluorescence (CF) of different tomato varieties when exposed to two different temperature treatments: Control (C, 26/18 °C) and Heat-stress (HS, 34/24 °C). Different small letters signify significant differences between variety-temperature combinations based on Tukey's HSD test (P≤0.05). Each bar represents mean ± standard error values.

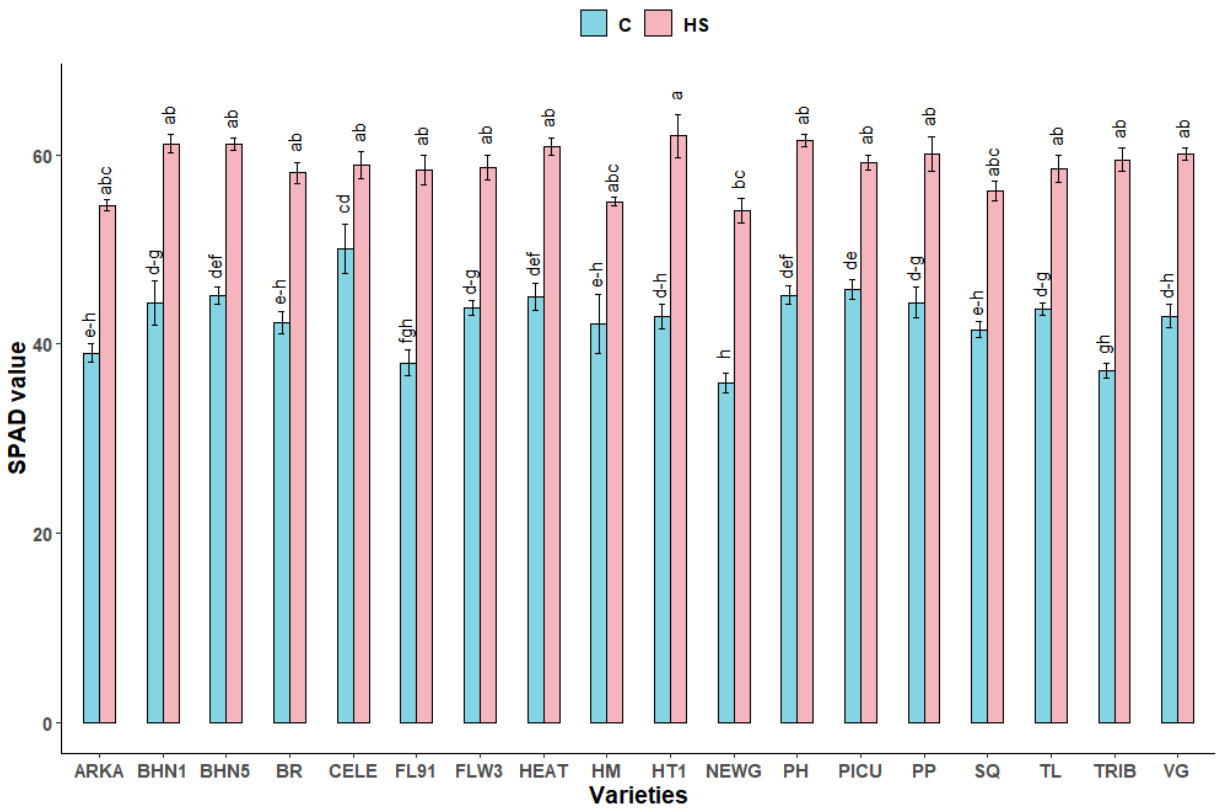

Figure 2.5 SPAD values of different tomato varieties when exposed to two different temperature treatments: Control (C, 26/18 ℃) and Heat-stress (HS, 34/24 ℃). Different small letters signify significant differences between variety-temperature combinations based on Tukey's HSD test (P≤0.05). Each bar represents mean ± standard error values.

2.3.2.3. Plant Height

The plant height was significantly reduced under heat-stress for all varieties (Figure 2.6). On average, plant height was 24.3% higher under control compared to heat stress conditions. Under HS, the height reduction was lowest for TL (15.9%), whereas it was highest for PP (31.07%).

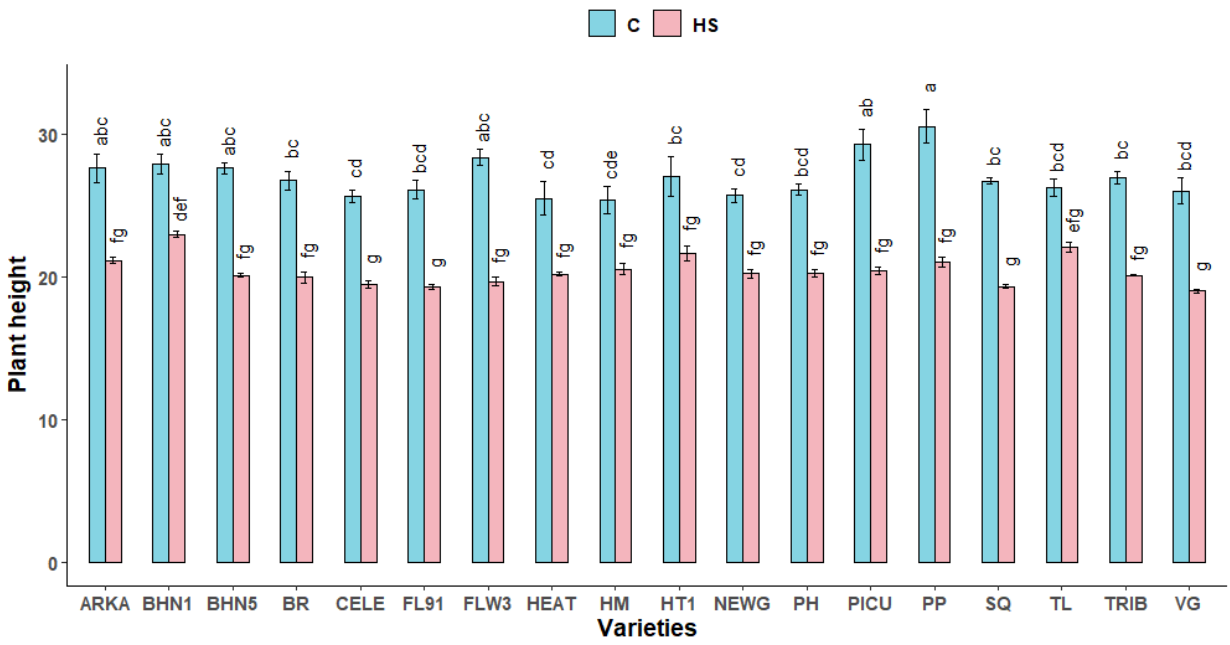

Figure 2.6 Height (cm) of different tomato varieties when exposed to two different temperature treatments: Control (C, 26/18 ℃) and Heat-stress (HS, 34/24 ℃). Different small letters signify significant differences between variety-temperature combinations based on Tukey's HSD test (P≤0.05). Each bar represents mean ± standard error values.

2.3.2.4. Stem Diameter

The average stem diameter of all varieties was 7.3% lower than the control in heat-stress (Figure 2.7). However, there were differences in the response of different varieties to HS. While almost all the varieties had lower diameter under HS, SQ and NEWG had 1.14% and 28.56% higher diameter, respectively, compared to control.

2.3.2.5. Heat Injury Index

The varieties under study varied in showing macroscopic thermal injury symptoms (Figure 2.8). Under heat-stress, the lowest HII was observed in BR, CELE, HEAT, HT1, PICU, and VG, whereas the highest HII was observed in PP, SQ, PH, ARKA, and BHN1.

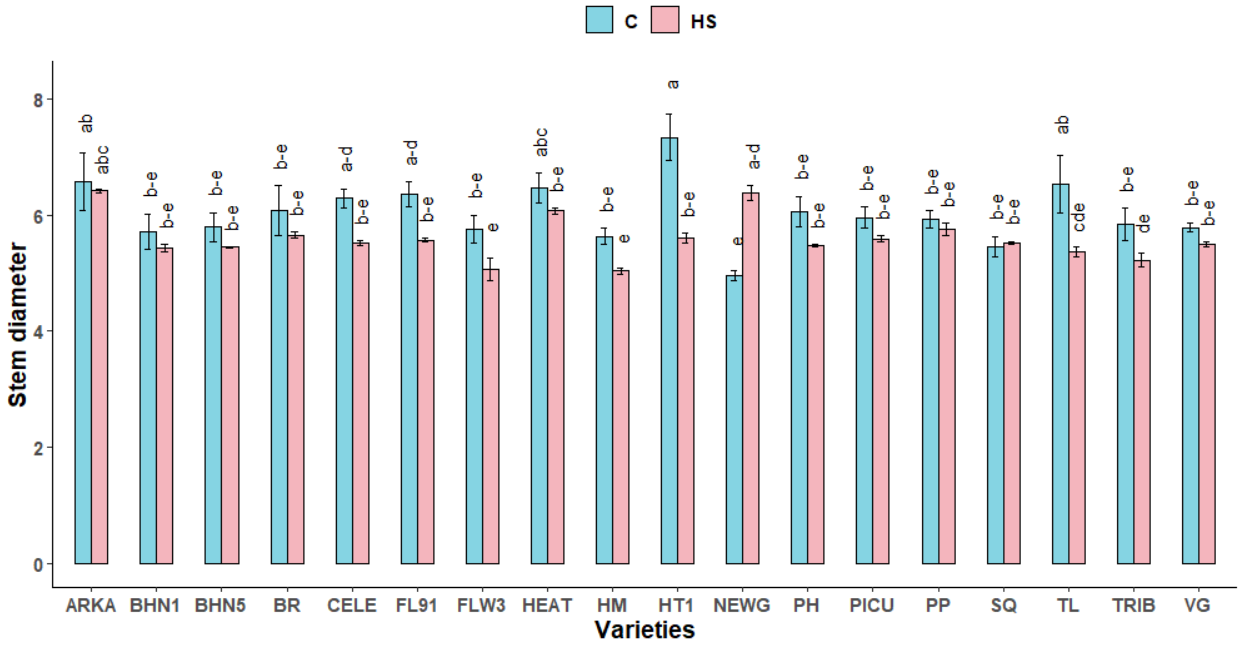

Figure 2.7 Stem diameter (mm) of different tomato varieties when exposed to two different temperature treatments: Control (C, 26/18 ℃) and Heat-stress (HS, 34/24 ℃). Different small letters signify significant differences between variety-temperature combinations based on the Tukey's HSD test (P≤0.05). Each bar represents mean ± standard error values.

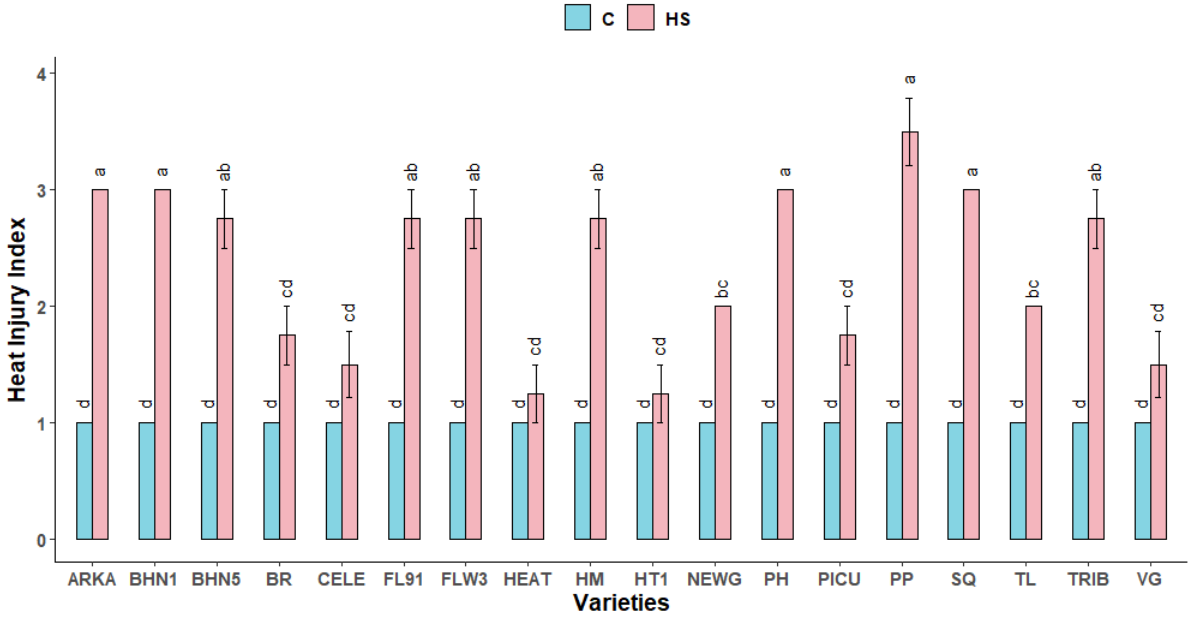

Figure 2.8 Heat Injury Index (HII) of different tomato varieties when exposed to two different temperature treatments: Control (C, 26/18 ℃) and Heat-stress (HS, 34/24 ℃). Different small letters signify significant differences between variety-temperature combinations based on Tukey's HSD test (P≤0.05). Each bar represents the mean ± standard error values.

2.3.2.6. Correlation analysis

To better understand the correlation among the variables under control and heat-stress treatments, correlograms were constructed. There was a significant positive correlation (blue dot, 0.55) between the SPAD index and chlorophyll fluorescence under control (Figure 2.9, left), but the relationship did not hold under heat stress (Figure 2.9, right). A significant positive correlation was found under heat-stress between chlorophyll fluorescence and heat injury index (red dot, 0.9).

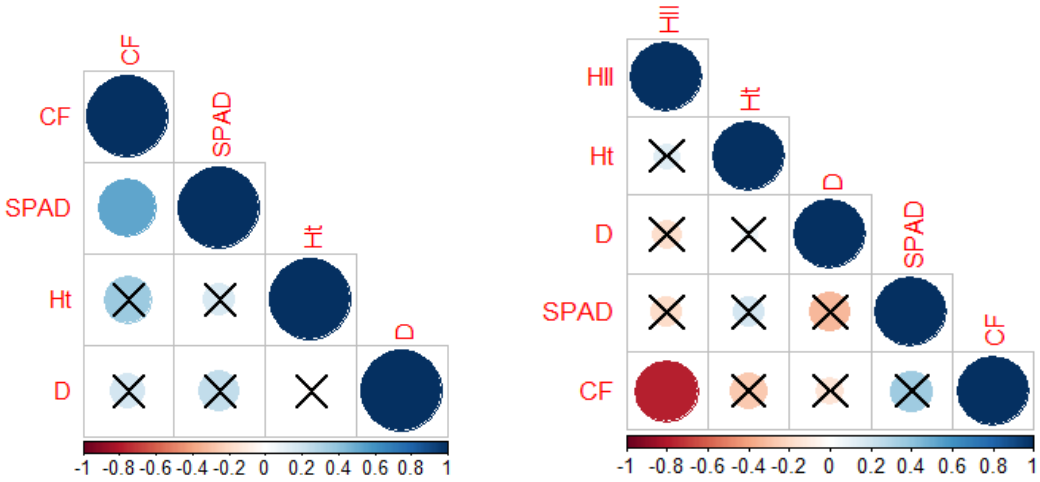

Figure 2.9 Correlogram showing the relationship between variables in the control treatment (26/18 ℃, left figure) and heat-stress treatment (34 ℃, right figure). The intensity of color and size of the circle increases with an increase in the significance of correlation. Dark red denotes a high negative correlation, whereas dark blue denotes a high positive correlation. The cells with cross marks denote no significant correlation between the variables.

2.3.3. Second open-field screening

A second open-field screening was carried out in Uvalde, TX, with 24 varieties selected from the controlled environment heat stress screening and initial open-field screening. There were significant interaction effects of variety and stage (of growth and temperature exposure) on leaf transpiration rate, maximum fluorescence, SPAD values, electrolyte leakage, heat injury index,

and yield (Table 2.3). Only stage (of growth and temperature exposure) had significant effects on stomatal conductance, intercellular carbon dioxide concentration, leaf temperature, initial fluorescence, chlorophyll fluorescence, and intrinsic and instantaneous water use efficiency. There was no effect of varieties, stage, or their interaction in the net photosynthesis rate.

Table 2.3 ANOVA of different parameters as influenced by varieties (var) and stages (Stage-1: 56 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃).

SN	Parameter	SOV	P-value	Significance
1.	Net Photosynthesis Rate (Pn)	Var	0.8253	NS
		Stage	0.0605	NS
		Block	<2e-16	***
		Var × Stage	0.804	NS
2.	Stomatal Conductance (gs)	Var	0.1892	NS
		Stage	<2e-16	***
		Block	0.0786	NS
		Var × Stage	0.0833	NS
3.	Intercellular CO_2 Concentration (Ci)	Var	0.771	NS
		Stage	<2e-16	***
		Block	1.31E-13	***
		Var × Stage	0.892	NS
4.	Transpiration rate (E)	Var	0.00752	**
		Stage	5.88E-06	***
		Block	2.76E-05	***
		Var × Stage	0.02974	*
5.	Leaf Temperature (LT)	Var	1	NS
		Stage	< 2e-16	***
		Block	3.36E-09	***
		Var × Stage	0.999	NS
6.	Initial Fluorescence (Fo)	Var	0.0977	NS
		Stage	<2e-16	***
		Block	2.46E-09	***
		Var × Stage	0.1675	NS
7.	Maximum Fluorescence (Fm)	Var	0.00143	***
		Stage	< 2e-16	***

Table 2.3 Continued

SN	Parameter	SOV	P-value	Significance
		Block	< 2e-16	***
		Var × Stage	0.00337	**
8.	SPAD value	Var	7.31E-11	***
		Stage	< 2e-16	***
		Block	0.58358	NS
		Var × Stage	0.00545	**
9.	Chlorophyll Fluorescence (CF)	Var	0.131	NS
		Stage	< 2e-16	***
		Block	7.02E-07	***
		Var × Stage	0.134	NS
10.	Instantaneous Water Use Efficiency (WUE_{inst})	Var	0.836	NS
		Stage	8.30E-08	***
		Block	< 2e-16	***
		Var × Stage	0.97	NS
11.	Intrinsic Water Use Efficiency WUE_{intr}	Var	0.682	NS
		Stage	< 2e-16	***
		Block	6.15E-12	***
		Var × Stage	0.796	NS
12.	Electrolyte Leakage (EL)	Var	< 2e-16	***
		Stage	< 2e-16	***
		Block	0.2	NS
		Var × Stage	7.68E-13	***
13.	Heat Injury Index (HII)	Var	< 2e-16	***
		Stage	1.11E-06	***
		Block	0.92813	NS
		Var × Stage	0.00262	**
14.	Marketable Yield	Var	3.4e-12	***
		Block	0.124	

***, **,* show significant difference at P≤ 0.001, 0.01,0.05, respectively.
NS means not significant at P≤0.05.

2.3.3.1. SPAD value

SPAD value decreased significantly in the second stage in TRIB, TL, RALY, LaF6, HT2, HM, BR, BHN5, BHN1, ARKA, and AMEL than in the first stage (Figure 2.10). At stage-1, the highest SPAD value was observed in BHN1, followed by AMEL, BHN5, BR, HEAT, HM. HT2, PH, PICU, and TL.

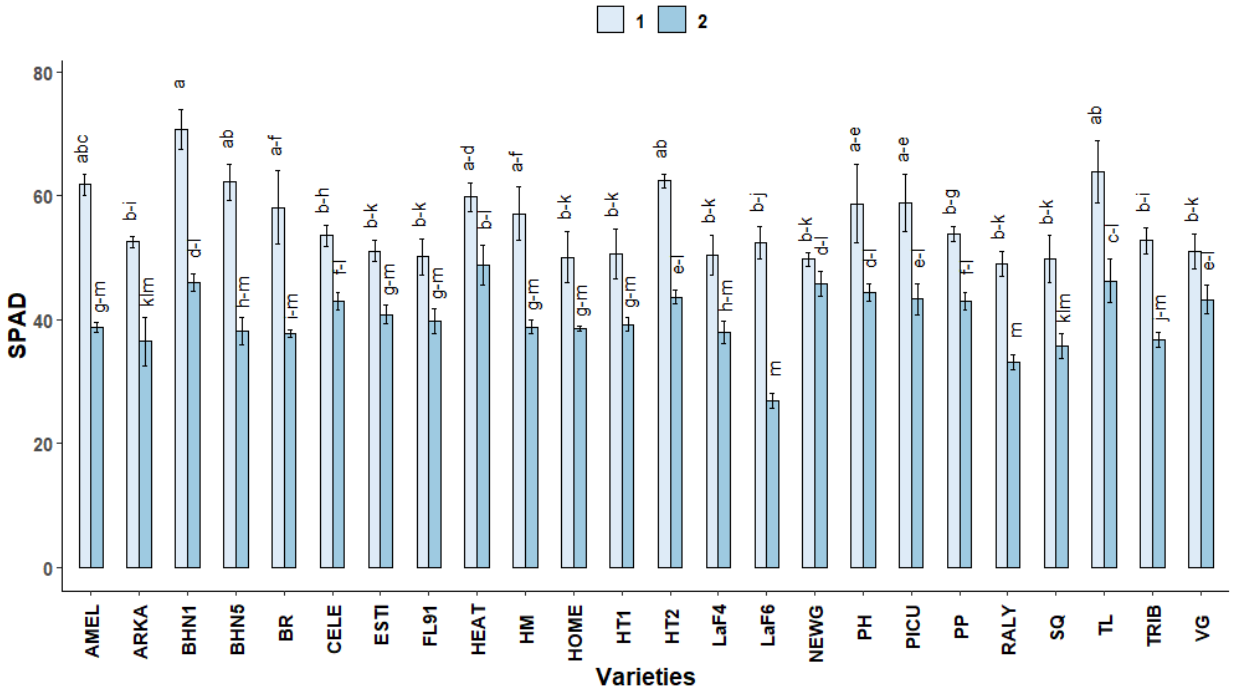

Figure 2.10 SPAD values of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. Different small letters signify significant differences between variety-stage combinations based on the HSD test (P≤0.05). Each bar represents mean ± standard error values.

2.3.3.2. Chlorophyll Fluorescence

Initial fluorescence (Fo) increased significantly for all varieties, except for CELE, HEAT, NEWG, SQ, LaF4, RALY, HOME, and TL at stage-2 (Table 2.4). PH had the highest increase, which was 93.9% higher than stage-1. The lowest increase was observed in HT1 (37.9%).

Maximum fluorescence (Fm) decreased at stage-2 (Figure 2.11), but the only significant difference was observed in LaF6. All the varieties had similar Fm values at stage-1, as well as stage-2.

Chlorophyll fluorescence decreased significantly at stage-2 for all varieties (Table 2.5), the highest decrease being in LaF6 (53.4%), whereas the lowest being in HT1(22.2).

Table 2.4 Initial/minimum chlorophyll fluorescence of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. The values represent the mean ± standard error. Different small letters within each row indicate significant differences between the observed values under different temperature treatments within each variety. The varieties have been ordered from high to low percentage increase of initial chlorophyll fluorescence from stage-1 to stage-2.

	Initial chlorophyll fluorescence (Fo)		
Variety	**Stage-1**	**Stage-2**	**Increase (%)**
PH	40.7 ± 2.4 b	79.0 ± 8.30 a	93.8
BR	42.7 ± 1.4 b	80.5 ± 9.10 a	88.3
PICU	39.7 ± 1.7 b	74.7 ± 1.40 a	88.0
BHN1	40.5 ± 1.3 b	73.7 ± 8.60 a	82.0
LaF6	41.7 ± 2.6 b	73.2 ± 7.40 a	75.4
ESTI	43.5 ± 2.0 b	74.7 ± 11.7 a	71.8
AMEL	44.5 ± 0.6 b	75.5 ± 7.50 a	69.6
ARKA	36.2 ± 4.1 b	61.5 ± 7.50 a	69.6
CELE	42.7 ± 1.8 a	72.2 ± 12.0 a	
HT2	44.0 ± 1.2 b	74.2 ± 3.50 a	68.7
FL91	40.0 ± 1.0 b	66.7 ± 5.40 a	66.8
PP	41.5 ± 0.9 b	66.7 ± 4.50 a	60.8
HEAT	41.2 ± 1.4 a	65.5 ± 10.5 a	
NEWG	45.0 ± 2.0 a	71.2 ± 12.2 a	
BHN5	42.7 ± 1.4 b	66.2 ± 7.70 a	54.9
SQ	43.5 ± 1.1 a	65.2 ± 11.2 a	
VG	41.0 ± 1.8 b	61.5 ± 3.30 a	50.0
HM	45.2 ± 1.2 b	65.7 ± 6.40 a	45.3
LaF4	43.2 ± 0.9 a	62.7 ± 11.1 a	
TRIB	41.2 ± 1.2 b	59.0 ± 4.60 a	43.0
RALY	43.0 ± 2.9 a	59.5 ± 13.5 a	
HT1	40.2 ± 2.0 b	55.5 ± 3.30 a	37.9
HOME	45.2 ± 1.8 a	60.0 ± 8.40 a	
TL	43.2 ± 1.6 a	57.0 ± 9.40 a	

Table 2.5 Chlorophyll fluorescence of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. The values represent the mean ± standard error. Different small letters within each row indicate significant differences between the observed values under different temperature treatments within each variety. The varieties have been ordered from high to low percentage decrease of chlorophyll fluorescence from stage-1 to stage-2.

	Chlorophyll fluorescence (CF)		
Variety	**Stage-1**	**Stage-2**	**Decrease (%)**
LaF6	0.79 ± 0.01 a	0.37 ± 0.10 b	53.4
PH	0.79 ± 0.01 a	0.40 ± 0.04 b	49.1
HT2	0.81 ± 0.01 a	0.42 ± 0.08 b	48.6
ESTI	0.79 ± 0.01 a	0.41 ± 0.07 b	48.4
BR	0.79 ± 0.01 a	0.43 ± 0.02 b	46.5
PICU	0.79 ± 0.01 a	0.47 ± 0.09 b	41.1
NEWG	0.78 ± 0.01 a	0.47 ± 0.06 b	39.7
HOME	0.79 ± 0.01 a	0.48 ± 0.10 b	38.9
BHN5	0.79 ± 0.02 a	0.49 ± 0.05 b	38.5
LaF4	0.78 ± 0.01 a	0.48 ± 0.06 b	38.0
AMEL	0.79 ± 0.02 a	0.49 ± 0.01 b	37.6
TRIB	0.80 ± 0.00 a	0.51 ± 0.01 b	36.5
FL91	0.79 ± 0.02 a	0.50 ± 0.07 b	36.0
SQ	0.79 ± 0.01 a	0.51 ± 0.08 b	35.9
RALY	0.77 ± 0.02 a	0.50 ± 0.06 b	35.1
HEAT	0.80 ± 0.01 a	0.53 ± 0.03 b	34.5
ARKA	0.80 ± 0.01 a	0.53 ± 0.05 b	34.0
HM	0.79 ± 0.01 a	0.56 ± 0.06 b	29.9
TL	0.78 ± 0.01 a	0.56 ± 0.05 b	28.1
BHN1	0.79 ± 0.01 a	0.58 ± 0.01 b	26.6
VG	0.79 ± 0.02 a	0.59 ± 0.04 b	25.5
PP	0.78 ± 0.02 a	0.59 ± 0.02 b	23.3
CELE	0.79 ± 0.01 a	0.61 ± 0.02 b	23.3
HT1	0.80 ± 0.01 a	0.62 ± 0.03 b	22.2

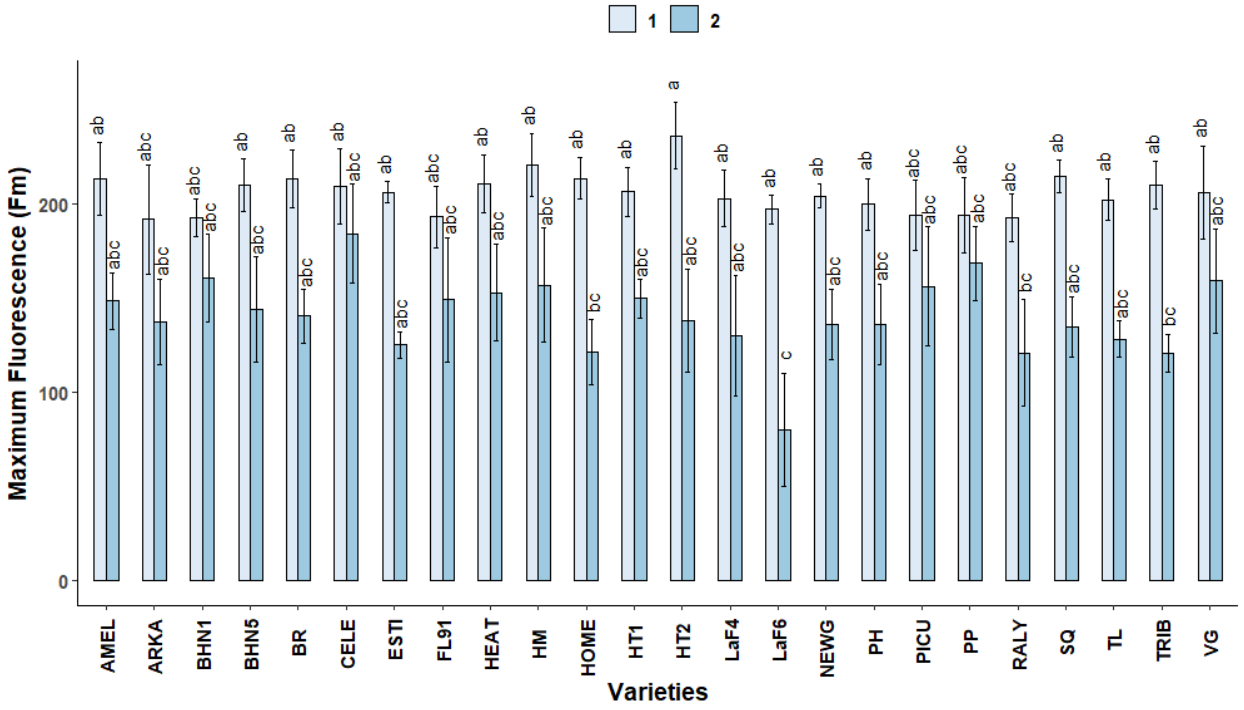

Figure 2.11 Maximum chlorophyll fluorescence (Fm) of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. Different small letters signify significant differences between variety-stage combinations based on the HSD test ($P \leq 0.05$). Each bar represents the mean ± standard error value.

2.3.3.3. Electrolyte Leakage

Overall, there was a subtle increase in electrolyte leakage at stage-2, but the increase was significant only for ARKA, HT2, and PP (Figure 2.12). ARKA had the highest electrolyte leakage among the varieties, whereas HEAT had the lowest electrolyte leakage at both stages.

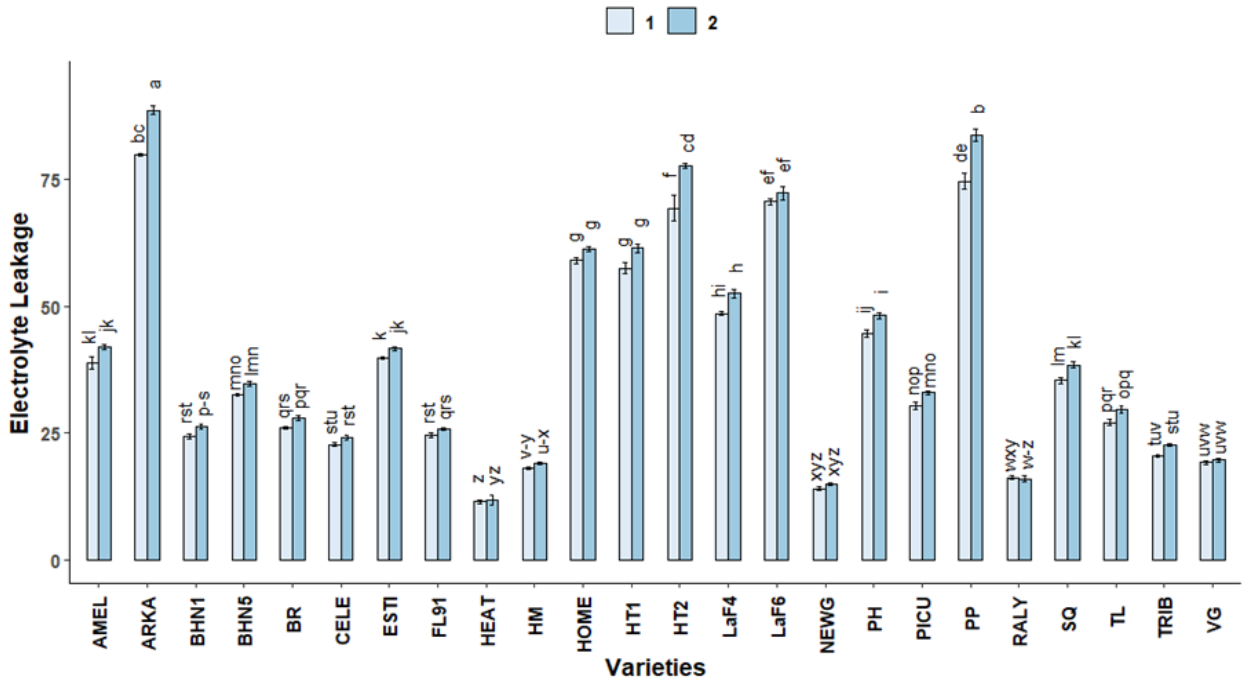

Figure 2.12 Electrolyte leakage (EL, %) of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. Different small letters signify significant differences between variety-stage combinations based on the HSD test (P≤0.05). Each bar represents the mean ± standard error values.

2.3.3.4. Heat Injury Index

There were no significant differences between the heat injury index within varieties at two different stages (Figure 2.13). At stage-1, ARKA and LaF6 had the highest heat injury index, which was statistically different from CELE, HEAT, HM, HT1, NEWG, PH, RALY, TRIB, and VG. At stage-2, ARKA and LaF6 had the highest heat injury index, which was statistically similar to HT2 and PP. HEAT had the lowest heat injury index, which was statistically at par with NEWG at both stages.

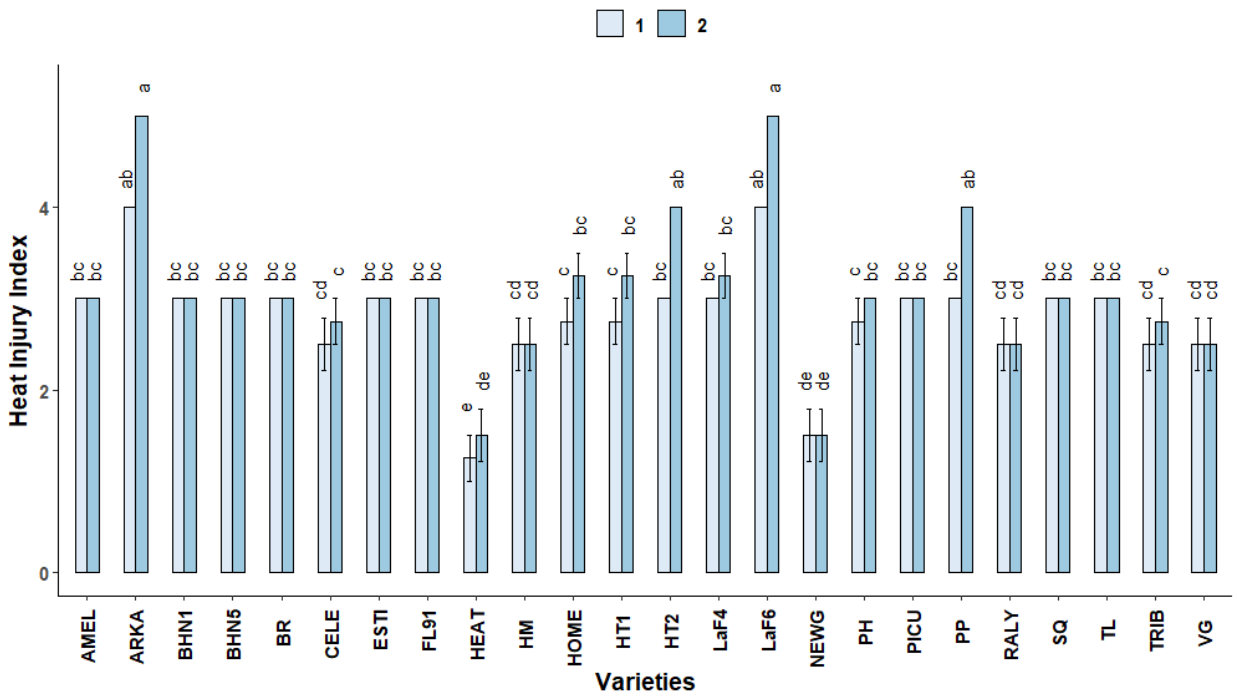

Figure 2.13 Heat Injury Index (HII) of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 °C and Stage-2: 86 DAT, 41°C. Different small letters signify significant differences between variety-stage combinations based on the HSD test ($P \leq 0.05$). Each bar represents the mean ± standard error values.

2.3.3.5. Gas exchange

Stomatal conductance decreased drastically at stage-2 for most of the varieties (Table 2.6). LaF4 had the highest decrease, which was 83.3% less than at stage-1. In contrast, PP had the lowest decrease (30.1%) in stomatal conductance.

Intercellular CO_2 concentration decreased at stage-2 for LaF4, ESTI, RALY, PH, NEWG, PICU, HT2, BHN1, and AMEL (Table 2.7). The highest significant decrease was observed in LaF4 (74.4%), whereas the lowest decrease was observed in AMEL (31.1%).

The transpiration rate was different among different varieties under the two given stages (Figure 2.14), with some varieties having higher transpiration rates at stage-1, while others had

higher values at stage-2. However, within varieties, the differences between the transpiration rates at stage-1 and stage-2 were not significantly different. The only difference was observed at stage-2 between LaF4 and PICU.

Table 2.6 Stomatal conductance (gs) of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. The values represent the mean ± standard error. Different small letters within each row indicate significant differences between the observed values under different temperature treatments within each variety. The varieties have been ordered from high to low percentage decrease of stomatal conductance from stage-1 to stage-2.

Stomatal conductance (gs, mol H_2O m^{-2} s^{-1})			
Variety	**Stage-1**	**Stage-2**	**Decrease (%)**
LaF4	0.72 ± 0.0813 a	0.1195 ± 0.0114 b	83.340
CELE	0.72 ± 0.0239 a	0.1405 ± 0.0333 b	80.372
RALY	0.79 ± 0.1043 a	0.1579 ± 0.0356 b	80.093
BHN1	0.80 ± 0.0307 a	0.1749 ± 0.0127 b	78.112
ESTI	0.67 ± 0.0361 a	0.1496 ± 0.0268 b	77.762
VG	0.70 ± 0.0402 a	0.1611 ± 0.0313 b	77.067
TRIB	0.71 ± 0.0379 a	0.1673 ± 0.0287 b	76.393
FL91	0.78 ± 0.0245 a	0.1948 ± 0.0458 b	74.910
HEAT	0.69 ± 0.0492 a	0.1756 ± 0.0565 b	74.654
LaF6	0.70 ± 0.0437 a	0.1782 ± 0.0443 b	74.599
ARKA	0.63 ± 0.0708 a	0.1820 ± 0.0670 b	70.984
HT1	0.67 ± 0.0434 a	0.1991 ± 0.1023 b	70.175
HOME	0.72 ± 0.0261 a	0.2338 ± 0.0507 b	67.725
TL	0.73 ± 0.0357 a	0.2343 ± 0.0782 b	67.719
NEWG	0.80 ± 0.0507 a	0.2588 ± 0.0657 b	67.582
PH	0.69 ± 0.0351 a	0.2478 ± 0.0611 b	63.842
BR	0.60 ± 0.0322 a	0.2216 ± 0.0712 b	62.894
BHN5	0.64 ± 0.1465 a	0.2458 ± 0.0665 b	61.858
HT2	0.74 ± 0.0342 a	0.2979 ± 0.0716 b	59.619
AMEL	0.79 ± 0.0513 a	0.3262 ± 0.1036 b	58.436
SQ	0.71 ± 0.0455 a	0.3249 ± 0.0895 b	54.073
HM	0.72 ± 0.0329 a	0.3497 ± 0.1195 b	51.246
PICU	0.71 ± 0.0370 a	0.3678 ± 0.0333 b	48.307
PP	0.54 ± 0.0437 a	0.3768 ± 0.0554 b	30.103

Table 2.7 Intercellular CO2 concentration of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. The values represent the mean ± standard error. Different small letters within each row indicate significant differences between the observed values under different temperature treatments within each variety. The varieties have been ordered from high to low percentage decrease of intercellular CO2 concentration from stage-1 to stage-2.

Intercellular CO2 concentration (Ci, µmol CO_2 mol^{-1})			
Variety	**Stage-1**	**Stage-2**	**Decrease (%)**
HOME	320.9 ± 3.80 a	36.30 ± 167.4 a	
HT1	320.9 ± 3.80 a	36.30 ± 167.4 a	
ARKA	318.9 ± 17.1 a	75.00 ± 121.9 a	
LaF4	323.1 ± 7.40 a	82.60 ± 69.80 b	74.4
LaF6	333.2 ± 9.70 a	93.10 ± 123.7 a	
CELE	330.3 ± 12.3 a	105.0 ± 93.40 a	
ESTI	322.0 ± 5.90 a	109.4 ± 83.40 b	66.0
VG	316.0 ± 8.50 a	109.9 ± 90.10 a	
RALY	331.7 ± 7.50 a	124.1 ± 63.80 b	62.5
TL	328.5 ± 13.2 a	131.1 ± 102.8 a	
HEAT	322.3 ± 7.75 a	132.5 ± 83.80 a	
BR	322.6 ± 10.6 a	146.6 ± 92.40 a	
TRIB	316.5 ± 5.91 a	145.4 ± 84.10 a	
FL91	322.5 ± 10.0 a	161.1 ± 67.70 a	
BHN5	330.3 ± 9.81 a	169.4 ± 80.10 a	
PH	318.9 ± 12.5 a	173.5 ± 50.20 b	45.6
SQ	330.7 ± 9.45 a	200.6 ± 80.70 a	
NEWG	340.6 ± 10.6 a	213.4 ± 22.30 b	37.3
PICU	316.7 ± 7.00 a	202.7 ± 43.30 b	35.9
HM	324.7 ± 7.70 a	208.9 ± 74.30 a	
HT2	332.9 ± 6.00 a	217.1 ± 18.30 b	34.7
BHN1	328.7 ± 8.20 a	221.1 ± 32.30 b	32.7
AMEL	336.5 ± 7.30 a	232.0 ± 29.80 b	31.1
PP	325.2 ± 10.8 a	236.4 ± 40.20 a	

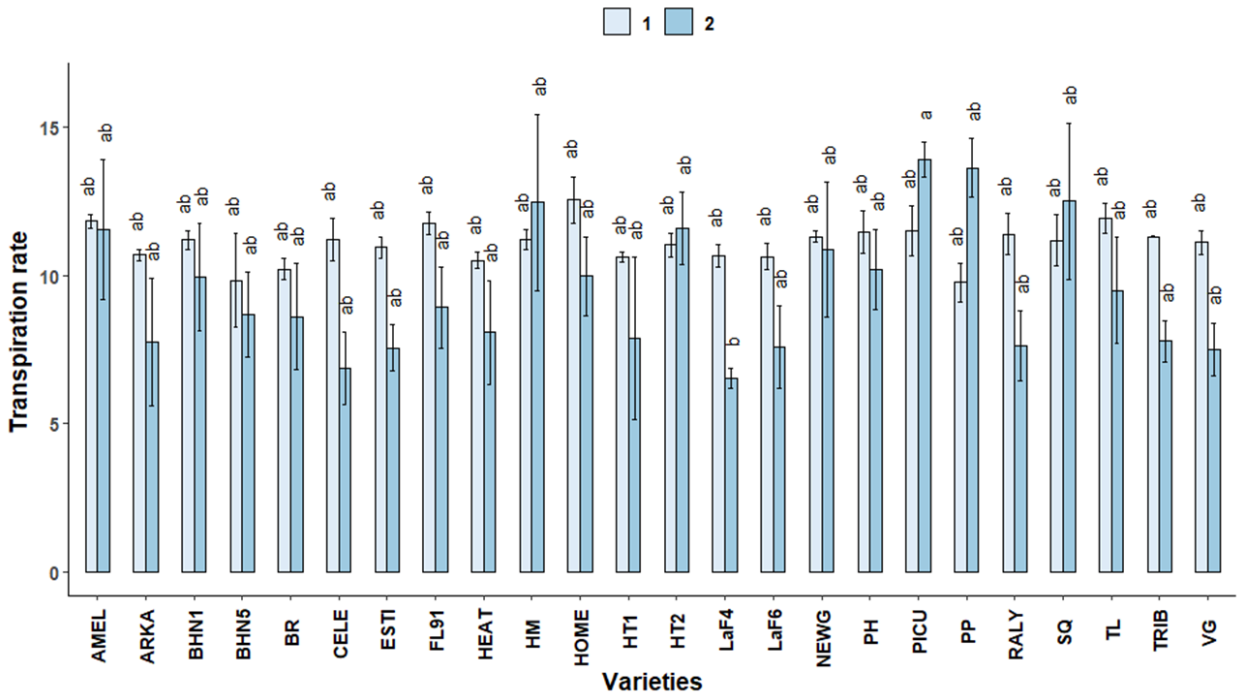

Figure 2.14 Transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$) of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. Different small letters signify significant differences between variety-stage combinations based on the HSD test ($P \leq 0.05$). Each bar represents the mean ± standard error values.

2.3.3.6. Leaf Temperature

Leaf temperature increased significantly for all varieties at stage-2 (Table 2.8). The highest increase was observed in LaF4 (38.1%), whereas the lowest increase was observed in PP (22.1%).

2.3.3.7. Water use efficiency

Instantaneous water use efficiency did not differ significantly between and within the varieties at both stages in the mean separation test. Nevertheless, intrinsic water use efficiency significantly increased in half of the varieties at stage-2 (Table 2.9). ARKA showed the highest increase, which was 564.7% higher than stage-1, whereas PICU showed the lowest increase, which was 181 % higher than the stage-1.

Table 2.8 Leaf temperature of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. The values represent the mean ± standard error. Different small letters within each row indicate significant differences between the observed values under different temperature treatments within each variety.

	Leaf temperature (℃)		
Variety	**Stage-1**	**Stage-2**	**Increase (%)**
LaF4	29.1 ± 0.7 b	40.2 ± 1.8 a	38.1
BR	28.8 ± 1.2 b	39.6 ± 1.6 a	37.5
CELE	28.8 ± 1.2 b	39.6 ± 1.6 a	37.5
BHN1	28.4 ± 0.6 b	39.0 ± 1.8 a	37.5
HEAT	28.6 ± 0.9 b	39.2 ± 2.1 a	36.9
RALY	29.1 ± 0.8 b	39.6 ± 2.1 a	36.0
FL91	29.2 ± 0.5 b	39.3 ± 2.2 a	34.3
ESTI	29.6 ± 0.2 b	39.7 ± 2.2 a	33.9
VG	29.1 ± 1.0 b	39.1 ± 2.2 a	33.9
HT1	29.5 ± 0.4 b	39.4 ± 1.8 a	33.2
NEWG	28.6 ± 0.8 b	38.0 ± 2.1 a	32.9
LaF6	29.1 ± 0.8 b	38.6 ± 2.3 a	32.5
TRIB	29.4 ± 0.6 b	39.0 ± 2.4 a	32.5
ARKA	29.8 ± 0.9 b	39.3 ± 2.6 a	31.6
HT2	29.0 ± 0.9 b	38.1 ± 1.7 a	31.4
BHN5	29.4 ± 1.1 b	38.2 ± 1.5 a	29.7
TL	30.0 ± 0.4 b	38.9 ± 2.4 a	29.3
PH	29.8 ± 0.9 b	38.3 ± 1.9 a	28.5
AMEL	29.1 ± 0.8 b	37.3 ± 2.5 a	28.3
HM	29.1 ± 0.9 b	37.2 ± 2.8 a	27.9
HOME	30.3 ± 0.5 b	38.6 ± 2.2 a	27.1
PICU	29.6 ± 0.2 b	37.5 ± 1.5 a	26.5
SQ	29.5 ± 0.1 b	36.9 ± 2.4 a	25.3
PP	30.1 ± 0.7 b	36.8 ± 1.9 a	22.1

Table 2.9 Intrinsic water use efficiency of different field-grown tomato varieties at two different stages: Stage-1: 51 DAT, 34 ℃ and Stage-2: 86 DAT, 41℃. The values represent the mean ± standard error. Different small letters within each row indicate significant differences between the observed values under different temperature treatments within each variety. The varieties have been ordered from high to low percentage increase of intrinsic water use efficiency from stage-1 to stage-2.

Intrinsic water use efficiency (WUEinst, µmol CO_2 mmol H_2O^{-1})			
Variety	**Stage-1**	**Stage-2**	**Increase (%)**
LaF6	27.0 ± 4.6 a	192.2 ± 98.50 a	
HT1	33.2 ± 1.8 a	225.1 ± 113.9 a	
ARKA	34.8 ± 8.9 b	231.2 ± 123.2 a	564.6
CELE	28.2 ± 5.7 a	163.8 ± 59.01 a	
RALY	27.2 ± 4.0 b	150.5 ± 39.29 a	453.1
LaF4	32.3 ± 4.2 b	176.6 ± 43.62 a	446.8
TL	28.3 ± 6.7 a	146.0 ± 65.66 a	
ESTI	32.5 ± 2.8 a	160.3 ± 52.74 a	
VG	35.3 ± 4.0 a	159.7 ± 55.97 a	
HEAT	32.8 ± 3.9 a	145.9 ± 51.74 a	
HOME	32.7 ± 5.3 a	145.1 ± 57.50 a	
BHN5	28.1 ± 4.5 a	122.9 ± 50.79 a	
BR	32.8 ± 5.3 a	137.2 ± 59.11 a	
NEWG	22.9 ± 5.1 b	93.00 ± 13.21 a	305.1
FL91	31.4 ± 4.6 a	126.2 ± 41.32 a	
TRIB	34.8 ± 3.0 a	137.7 ± 51.55 a	
SQ	28.4 ± 4.9 a	104.7 ± 48.72 a	
PH	34.0 ± 6.0 b	118.0 ± 31.30 a	246.8
AMEL	24.5 ± 3.4 b	82.50 ± 16.93 a	235.9
HT2	26.8 ± 2.7 b	89.60 ± 10.99 a	233.8
HM	30.9 ± 3.7 a	100.2 ± 46.00 a	
BHN1	28.5 ± 3.7 b	90.60 ± 19.56 a	217.4
PICU	34.7 ± 3.8 b	97.70 ± 25.20 a	181.0
PP	31.9 ± 5.6 a	79.10 ± 24.01 a	

2.3.3.8. Average Marketable Yield

There were significant variations in marketable yield among varieties (Figure 2.15). The average yield was 22 t ha^{-1}. A total of 17 cultivars had yield lower than the average value, ranging from 1.3 to 21.5 t ha^{-1}. ARKA had the lowest yield, and together with PP, HT2, LaF6, and HOME were deemed more susceptible to heat stress. In contrast, HEAT had the highest yield, followed by NEWG, RALY, and HM.

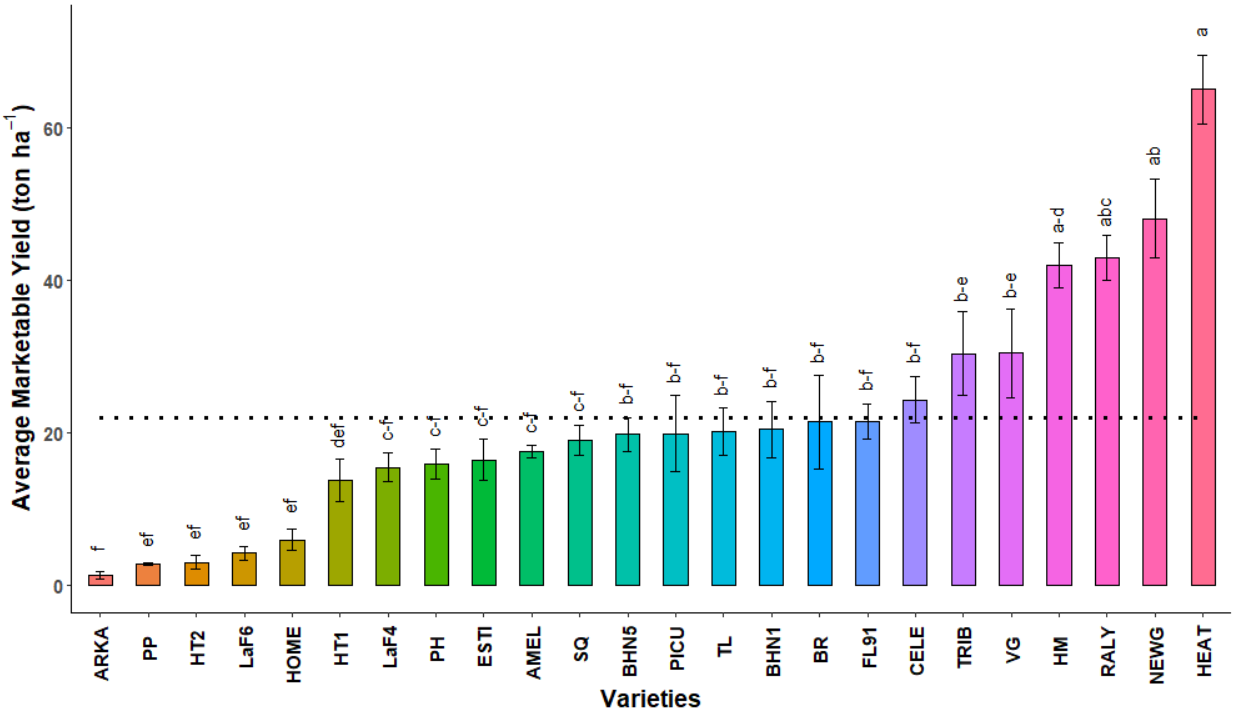

Figure 2.15 Average marketable yield (ton ha^{-1}) of 24 genotypes obtained in 2020. The dotted line indicates the total mean yield (22 ton ha^{-1}).

2.3.3.9. Correlation analysis

Correlogram (Figure 2.16) depicts the correlation between all parameters measured in 24 varieties in open-field conditions. Intercellular CO_2 concentration was significantly positively correlated to the transpiration rate (blue dot, 0.75), stomatal conductance (blue dot, 0.8), initial fluorescence (blue dot, 0.55), and SPAD values (blue dot, 0.5). However, there was a significant negative correlation of the parameters intercellular CO_2 concentration, transpiration, and stomatal conductance with leaf temperature (red dots, -0.9, -0.6, -0.7), instantaneous water use efficiency (red dots, -1, -0.8, -0.8) and intrinsic water use efficiency (red dots, -1, -0.8, -0.8).

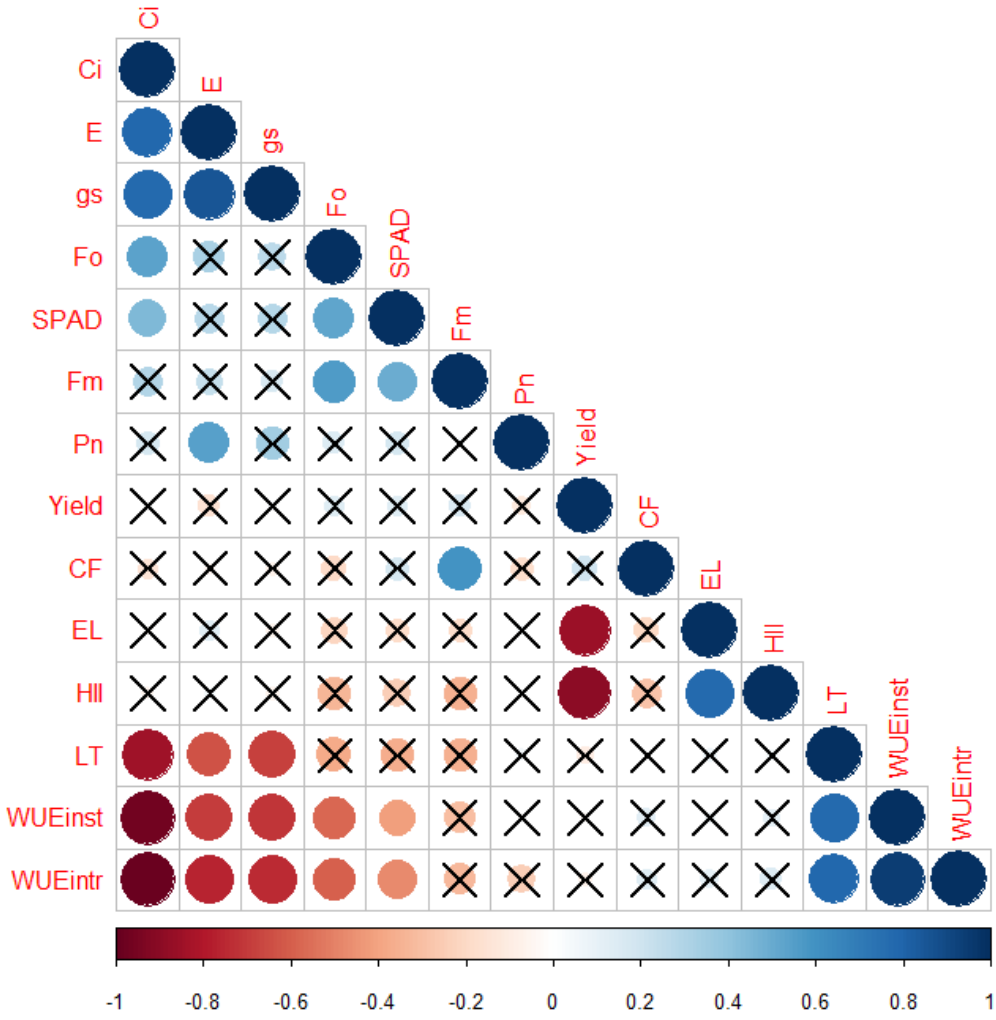

Figure 2.16 Correlogram showing the relationship between average values of the variables in open-field conditions. The intensity of color and size of the circle increases with an increase in the significance of correlation. Dark red denotes a high negative correlation, whereas dark blue denotes a high positive correlation. The cells with cross marks denote no significant correlation between the variables.

The transpiration rate was positively correlated to stomatal conductance (blue dot, 0.9) and net photosynthesis rate (blue dot, 0.55). Additionally, there was a significant positive correlation between initial fluorescence, maximum fluorescence, and spad value (blue dots, 0.55 for all). Chlorophyll fluorescence was positively correlated with maximum fluorescence (blue dot, 0.55).

Marketable yield was significantly negatively correlated to electrolyte leakage (red dot, 0.9) and heat injury index (red dot, 0.9), while these two parameters were positively correlated (blue dot, 0.8). Intrinsic water use efficiency, instantaneous water use efficiency, and leaf temperature were positively correlated (blue dots, 0.8).

2.4. Discussion

2.4.1. Growth chamber and green-house experiment

Chlorophyll Fluorescence has been widely used for heat-stress screening under controlled heat-stress conditions (Zhou et al., 2015; Poudyal et al., 2019). A decrease in chlorophyll fluorescence under heat stress is attributable to an increase in initial fluorescence or a decrease in maximum fluorescence, or both. A decrease in maximum fluorescence is observed due to an increase in non-photochemical quenching and an increase in initial fluorescence due to photoinhibition of PSII (Baker, 2008; Maxwell and Johnson, 2000). Heat stress affected the PSII functionality of PP, ARKA, BHN1, HM, FLW3, PH, and SQ, as shown by lower chlorophyll fluorescence in these varieties.

Determining non-destructive chlorophyll content through spad value has been employed by many researchers based on the direct proportional relation between absolute chlorophyll content and spad values (Ling et al., 2011). Our results demonstrated an increase in leaf chlorophyll content in HS conditions in all varieties, as measured by spad values. The increase in spad value may be an acclimation response of plants to high temperatures. Plant leaves under heat-stress were greener, smaller, and thicker than control, which may have increased chlorophyll content per unit area and, thus, spad value (Tang et al., 2018).

A reduction in plant growth under high temperatures might occur, depending on the varietal response (Shaheen et al., 2016). Tomato plant height differs among different varieties.

Indeterminate varieties tend to grow more than determinate plants. Our study saw differences among the varieties, and these differences may be attributable to their growth habit. However, the differences in height within varieties under heat stress and control may be significant. Heat stress reduced tomato plant height (Heuvelink, 1989). Lower height reduction in heat-stress compared to control signifies that the plant could maintain their growth properly under stressed conditions. Plant diameter changes under heat stress may be related to changes in stem tissue hydration (Kleeper et al., 1971).

When the plant physiology is disturbed by heat-stress, plants show visual symptoms of injury (Zhou et al., 2015). The plants which have a higher injury index are more sensitive to heat stress, and vice-versa. In our study, HEAT and NEWG had the lowest heat injury index. They may be potentially heat-tolerant varieties.

Based on correlation analysis, we found that as injury to plants due to heat-stress increased, chlorophyll fluorescence decreased. Similarly, plants with a low heat injury index showed higher chlorophyll fluorescence. Thus, chlorophyll fluorescence could be used as a useful tool to assess plant sensitivity or tolerance under extended heat-stress conditions.

2.4.2. Field experiments

At high-temperature conditions, the heat-tolerant plants had increased transpiration (Hasanuzzaman et al., 2013). The loss of heat from the leaf surface due to enhanced transpiration led to decreased leaf temperature. An increase in transpiration also facilitated an increase in plants' stomatal conductance, which subsequently led to an increase in CO_2 diffusion into the leaves, thereby increasing intercellular CO_2 concentration (Zhou et al., 2020). An increase in intercellular CO_2 concentration means an increase in the substrate for photosynthesis, which directly improves the plants' net photosynthesis rate. At low leaf temperature, unaltered membrane stability in

chloroplasts prevented higher electrolyte leakage (Hameed et al., 2015) and thus, the PSII functionality was not affected, which was evident by higher chlorophyll fluorescence((Jahan et al., 2019; Li et al., 2015). The decrease in electrolyte leakage and low leaf temperature also prevented the degradation of chlorophyll molecules, as shown by the higher spad values, and did not cause many injuries to the plants, evidenced by lower heat injury index. Lower injury and sustained chlorophyll production further added to sustained photosynthesis in the plants at a high temperature, which led to higher yield from the heat-tolerant plants (Li et al., 2015).

A heatmap (Figure 2.17) was generated to establish better relationships between the variables under different heat stresses and cluster the variables based on their responses. The heatmap clearly distinguished three cluster groups that separated the varieties into highly heat-tolerant (first cluster from top), heat-sensitive (second cluster from top), and moderately heat-tolerant (last cluster). The clusters were clearly distinguished based on the yield, electrolyte leakage, and heat injury index. The highly heat-tolerant group consisted of HEAT, NEWG, HM, RALY, VG, CELE, and TRIB. Notably, these varieties were heat-tolerant under long heat-stress treatment for a more extended period in the green-house and growth-chamber experiment, mainly distinguished by chlorophyll fluorescence HII. The highly heat-tolerant group had the lowest electrolyte leakage, lowest heat injury index, and highest yield, whereas the heat-sensitive group had the highest electrolyte leakage, highest heat injury index, and lowest yield. Our study concludes that in open field screening for heat tolerance in plants, assessing leaf electrolyte leakage and yield obtained from the plants, and observing the macroscopic heat injury symptoms could help select heat-tolerant varieties.

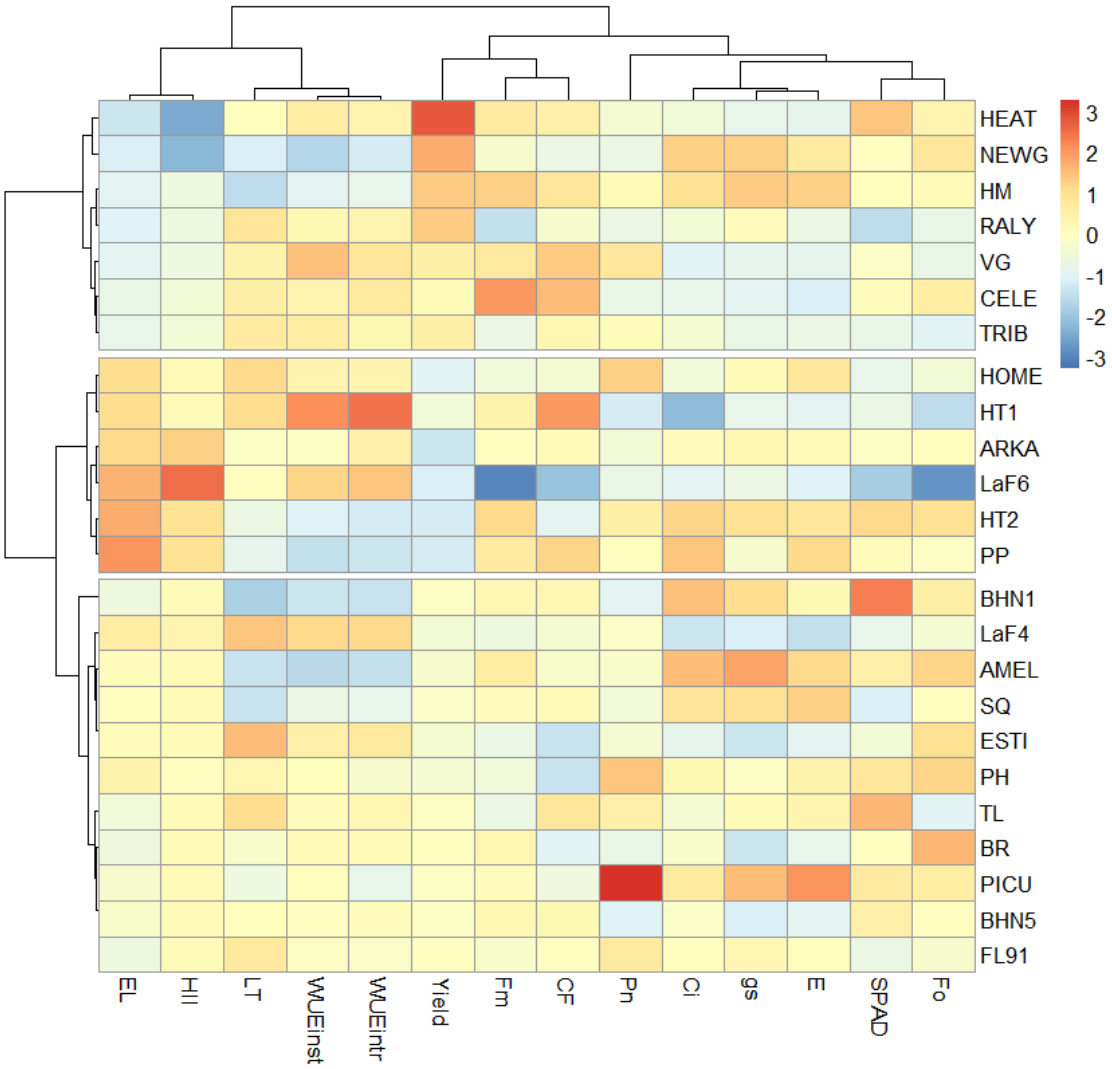

Figure 2.17 Heatmap and clustering of varieties based on the measured variables' standardized values obtained under open-field conditions. Each row represents a variety, and each column indicates a measured parameter. Treatments are clustered based on their measured variables, and variables are clustered based on their correlation. The variables that are clustered together have a high positive correlation. Cells with red and blue color have high and low relative expression, respectively.

2.5. Conclusion

Efforts to sustain crop production under steadily increasing earth's temperature remains imperative for food security. Exploring genetic variation to determine the variety that can perform best under temperature extremes is of high priority to avoid significant food production losses in

the following years. Thus, this study was conducted to explore different tomato varieties' potential to sustain yield under high-temperature conditions in the Texas environment.

In our experiment, we obtained three significant outcomes which are based on a) methods to screen tomato varieties under heat-stress conditions in two contrasting environments (open-field and controlled environment), b) determination of heat-tolerant varieties and c) establishment of the general mechanism of heat-tolerance in tomato varieties in open-field conditions based on correlation analysis.

Under a controlled heat-stress environment, chlorophyll fluorescence is the most effective method to determine heat-tolerance or heat-sensitivity in the varieties. Similarly, in open-field conditions, electrolyte leakage is the best method as it is negatively correlated to the yield. Also, it is essential to note any injury symptoms in the plants as they indicate how a plant responds to the environment they are exposed to. Based on the values of the variables measured, the varieties that were clustered as heat-tolerant were Heat Master, New Girl, HM-1823, Rally, Valley Girl, Celebrity, and Tribeca.

The general mechanism of heat-tolerance as observed from correlation analysis is that as air temperature rises, the heat-tolerant varieties have increased transpiration rate compared to other varieties that facilitate higher stomatal conductance, higher diffusion of carbon dioxide into the leaves, and ultimately enhances photosynthesis and yield. Higher transpiration also causes loss of heat from the plant leaves. Low leaf temperature does not stimulate the overproduction of reactive oxygen species, and hence, there is no alteration of the membrane integrity. The stable cell membrane indicates low electrolyte and, thus, no changes to the structural stability of PSII. Chlorophyll production is normal in stable PSII, which maintains chlorophyll fluorescence, which

again depicts that the photosystem is functioning well in the high-temperature condition in heat-tolerant varieties compared to other varieties.

From our study, we conclude that adopting varieties like Heat Master, New Girl, HM-1823, Rally, Valley Girl, Celebrity, and Tribeca could increase local tomato production in Texas, especially in South-West areas, during the spring-summer cropping season. Further exploration of their potential under exposure to heat combined with other abiotic stresses like drought could offer new insights into their sustainable production in other environments as well.

3. DETERMINATION OF HEAT TOLERANT TOMATO GENOTYPES THROUGH PHYSIO-BIOCHEMICAL STUDIES UNDER HEAT-STRESS AND HEAT-SHOCK CONDITIONS

3.1. Introduction

For tomato production, the optimum temperature for growth and development is between 25-30 ℃ during the daytime and 20 ℃ at night (Camejo et al., 2005). However, its production is vulnerable to temperature extremes, which exceeds this optimal range during spring-summer production in many areas of the world, adversely affecting the plant performance. An increase in temperature beyond the threshold limit resulting in a negative impact on plant growth and productivity is termed heat stress. The heat stress level in plants depends on the intensity, frequency, duration, and speed of day or night temperature rise (Blum, 2018; Wahid et al., 2007).

Irreversible changes in plant growth and physiology imposed by transient or persistent elevation in intensity and frequency of temperature extremes reduce crop yield and quality drastically. During the vegetative phase, high temperature affects the plants primarily by distorting the gas exchange mechanisms. In the reproductive phase, heat stress causes flower wilting and abscission, deformation of the anther, loss of pollen viability, low pollen germination, and reduced fruit set in heat-sensitive plants (Müller et al., 2016). In tomato plants, day and night temperature above 32 ℃ and 21 ℃ respectively, adversely affects growth and fruit set (Sato et al., 2004), whereas exposure to an acute temperature of 45℃ for 3 hours drastically reduces chlorophyll content, photosynthesis, and stomatal conductance (Camejo et al., 2006).

Plants exposed to high temperatures under non-limited water conditions tend to transpire more to dissipate heat. However, when high temperature coincides with transient drought conditions, they actively activate stomata closure to conserve water for critical physiological functions (Zhou et al., 2019). Heat tolerant plants have higher stomatal conductance than that of sensitive plants, providing more diffusion of CO_2 into leaves and higher leaf water potential, leading to an improved rate of photosynthesis compared to sensitive plants (Duan et al., 2017). Protective compounds, called osmolytes, like proline and glycine betaine, actively regulate water-related functions in heat-tolerant plants. Their accumulation in cells helps increase the water potential and maintain cell turgidity and cellular functions (Golam et al., 2012).

Most plant responses to heat stress are secondary, which are caused by oxidative stress. Oxidative stress is caused because of impaired balance between production and removal of reactive oxygen species (ROS) like H_2O_2 (hydrogen peroxide), $^{\cdot}OH$ (hydroxyl radical), $^{\cdot}O_2$ (superoxide anion), and $^1O_2$ (singlet oxygen) in plant cells (Miller et al., 2008). Accumulation of a high concentration of ROS causes oxidative burst, resulting in programmed cell death. To repress the adverse effects of ROS, plants produce antioxidant enzymes like superoxide dismutase (SOD), catalases (CAT), glutathione peroxidases (GPX), and ascorbate peroxidases (APX) (Kapoor et al., 2015). These antioxidants actively scavenge and detoxify ROS when their production exceeds the equilibrium. It has been shown that heat-tolerant plants have a higher level of antioxidants that alleviate ROS-mediated damage in plant cells (Camejo et al., 2006).

In tomato plants, photosynthesis is the foremost process affected by high temperatures (Nankishore and Farrel, 2016). The targets of high temperature on photosynthetic machinery are reduction in photosynthetic pigments like chlorophyll a and b, disorganization of thylakoid membranes, production of ROS, decrease in stomatal conductance, and inhibition of photosystem

II, electron transport chain, oxygen-evolving complex, photosystem I, and carbon dioxide fixation (Zhou et al., 2015). In response to these effects, plants develop various tolerance mechanisms to drive photosynthesis normally, which are changes in membrane structure by altering lipid composition, induction of heat-shock proteins (HSPs) and stress-inducible genes, and production of antioxidants, osmolytes, and other novel protective agents (Bita et al., 2013).

Photosystem II is considered the most thermosensitive component of the photosynthetic apparatus (Čajánek et al., 1998). The viscosity of lipids forming the thylakoid membrane in tomato plants changes rapidly when leaf temperature rises above an upper threshold (35°C), which increases fluidity leading to dislodgement of PSII light-harvesting complexes (LHC) (Berry and Bijorkman, 1980). When PSI and PSII are not functioning in balance, excess electrons can actively produce reactive oxygen species (ROS). Heat stress also decreases maximum quantum efficiency by increasing non-photochemical quenching and photoinactivation of PSII reaction centers (Baker, 2008).

Membrane stability under heat stress is maintained through lipid composition changes (Djanaguiraman et al., 2018). During high-temperature stress, some plants have an increased ratio of saturated to unsaturated fatty acids in phospholipids that increases the melting point and thus, prevents the increase of fluidity of the cell membrane and ensures proper functioning of membrane protein component (Zhang et al., 2005; Maienza et al., 2013). Electrolyte leakage as a measure of membrane thermostability has been studied in various crops, including tomato. Heat tolerant tomato plants have higher thermostability and low electrolyte leakage than heat-sensitive tomato genotypes (Wahid et al., 2007).

Heat stress in plants can bring about many changes in morphological, biochemical, physiological, and molecular responses, depending on the developmental stage and genotypes

(Wahid et al., 2007). These changes do not occur independently; they involve multiple and complex interactions that plants use to cope with extreme conditions. Improved understanding of the physio-biochemical traits and their correlation under high-temperature regimes will enable the selection, development, and adoption of resilient varieties in tomato production. Adhering with this concept, we experimented to identify physio-biochemical mechanisms that distinguish the genetic potential of ten tomato genotypes exposed to transient heat shock and persistent heat stress treatments. We also attempt to elucidate critical traits that facilitate the determination of elite genetic materials for production under extreme heat conditions and provide new insights into genetic engineering and breeding heat-tolerant tomato varieties.

3.2. Material and methods

3.2.1. Experimental design

Seeds of ten tomato heirloom and hybrid breeding lines (Table S1) were sown in polystyrene 200-cell trays (2.5 × 2.5 × 7.6 cm^3; Speedling, Ruskin, FL, USA) filled with LM-GPS (Lambert Germination, Plugs, and Seedlings, Lambert, Quebec, Canada) media, which constitutes of 90% sphagnum peat moss and 10% perlite, and vermiculite. The trays were saturated with water, incubated in the dark at 25 ℃ for two days, and transferred to the greenhouse at the Texas A&M AgriLife Research and Extension Center at Uvalde, TX (29.21 °N, 99.79 °W). The trays were uniformly irrigated daily with an overhead motorized spraying boom system suspended in the greenhouse. At four true leaf stage, the seedlings were transplanted to 0.8-L square pots (10.66 cm top outside, 8.68 cm bottom outside, 9.19 cm depth; TO Plastics, Clearwater, MN) and kept in the greenhouse for five days. The pots were then transferred to three growth chambers (Conviron Gen 1000) set at 26/18 ℃ (day/night) and allowed to acclimate for 15 days. The experiment was set up in factorial CRD (Complete Randomized Design) with ten varieties, three temperature conditions

(26/18℃ as control, 38/28℃ for seven days as heat stress, and 40 ℃ for 7 hours as heat shock treatment), and eight replications. The growth chambers were set to a photoperiod of 16/8 hours (light/dark), PAR of 350 µmol$m^{-1}s^{-1}$, and 65-75% RH. During development, data were collected for chlorophyll fluorescence, chlorophyll content, heat injury index (HII), gas exchange measurements, malondialdehyde content, electrolyte leakage (EL), leaf water potential (LWP), and relative water content (RWC). Each measurement was performed 30 days after transplanting (DAT).

3.2.2. Plant biomass

Fresh and dry shoot weight (after oven-dried at 70 ℃ for three days) was measured per plant at the end of the experiment.

3.2.3. Chlorophyll content

The non-destructive chlorophyll content index was measured as an average of three leaves per plant using a SPAD meter (SPAD-502 Plus, Minolta, Japan) from 9:00 am- 11:00 am at the end of the experiment.

3.2.4. Leaf gas exchange and chlorophyll fluorescence

The penultimate leaf was taken for gas exchange and chlorophyll fluorescence measurements to be performed between 10:00 am to 2:00 pm. A portable photosynthesis system (LI-6400 XT, LICOR Biosciences, NE, USA) was used to measure the net photosynthetic rate ($P_{N,}$ µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (Ci, µmol CO_2 mol^{-1}) and transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$). Chlorophyll fluorescence (CF) was measured using the OS30p Chlorophyll Fluorometer (Opti-Sciences Inc., Hudson, NH) after dark adaptation for 30 minutes.

3.2.5. Intrinsic and instantaneous leaf water use efficiency

Intrinsic leaf water use efficiency (WUEintr, µmol CO_2 mmol H_2O^{-1}) was calculated as the ratio between P_N and gs, and instantaneous leaf water use efficiency (WUEins, mol CO_2 $mmol^{-1}$ H_2O) was obtained as the ratio between P_N and E (Sun et al., 2018).

3.2.6. Plant-water relations

Relative water content (RWC, %) and leaf water potential (LWP, MPa) were measured to determine plant-water relations. Leaf water potential was estimated by cutting a second leaflet from the top and placing it in the Scholander-type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA). For determination of RWC, three leaf discs (8 mm diameter) taken from the base, middle, and apex portions of the plants were weighed for the fresh weight (W_f) and submerged in distilled water in darkness overnight. They were reweighed to determine the turgid weight (W_t) and dried for 48 hours at 70 ℃ to get the dry weight (W_d). The RWC was calculated using the following equation:

$$RWC= [(Wf - W_d)/ (W_t - W_d)] *100$$

3.2.7. Membrane lipid peroxidation

Malondialdehyde (MDA, µmol g^{-1} fresh weight), as a degradation product of lipid peroxidation, was determined as described by Heath and Packer (1968). A leaf from each plant was harvested and immediately frozen in liquid nitrogen and stored at -80℃ for later use. The samples (0.1 g) were homogenized in a 0.5 ml of 1% (m/v) trichloroacetic acid (TCA) solution and centrifuged (15,000 × g, 4℃ for 10 min), and 1.5 ml of 20% TCA containing 0.5% (m/v) thiobarbituric acid (TBA) was added to the supernatant aliquot, heated at 95℃ for 25 min, and allowed to cool in an ice bath. The absorbance of the solution at 532 and 600 nm will be recorded

after centrifugation (15,000 × g, 10 min). MDA concentration will be quantified using the following equation:

MDA (µmol g^{-1}) = [(A_{532} – A_{600})/ 155] * 1000

3.2.8. Electrolyte Leakage

Electrolyte leakage (EL, %) was measured using methods described by Shinohara and Leskovar (2014). Three leaf discs were extracted from each plant and placed in sealed culture tubes (25 * 150mm) with 10 ml of distilled water, maintained in a shaking water bath at 25℃ for 24 h, and electrical conductivity (EC) of the supernatant (EC_1) be measured. The tubes were then autoclaved at 120 ℃ for 20 min. The second EC (EC_2) was measured after allowing it to cool to room temperature. The EL was determined with the equation given below:

EL (%) = (EC_1 / EC_2) * 100

3.2.9. Heat Injury Index (HII)

Plants were scored between 1 and 5, according to Hong et al. (2009).

1= no injury

2= yellow and mildly dehydrated margins of old leaflets

3= mildly dehydrated plants with the middle and crinkled bottom leaflets

4= severely dehydrated plants with upper leaflets crinkled

5= plants with most leaves withered

3.2.10. Statistical analysis

The data collected were analyzed in SAS (SAS Institute Inc., Cary, NC) and R using two-way ANOVA. The correlation among the variables was analyzed using Pearson's correlation, and a correlogram was constructed for each temperature treatment. The multiple comparisons of means were made using Tukey's HSD (Honestly Significant Difference) under $P \leq 0.05$. For only

significant main factor effects, mean separation for that factor was done within each level of another factor using Tukey's HSD (Honestly Significant Difference)at $P \leq 0.05$. Clusters of varieties were obtained along with a heat-map based on observed value for each parameter. Correlation distance was employed in clustering analysis.

3.3. Results

There were significant fixed effects of varieties and heat-treatment for the SPAD index, relative water content, net photosynthesis rate, transpiration, stomatal conductance, and shoot dry weight among the parameters measured (Table 3.1). Only varieties had a significant effect on intrinsic and instantaneous water use efficiency. Similarly, only heat-treatments had a significant effect on the minimum fluorescence (F_o), maximum fluorescence F_m), the maximum potential quantum efficiency of PSII (Fv/Fm, CF), and intercellular CO_2 concentration (Ci). There was a significant interaction effect of varieties and heat-treatments on electrolyte leakage, malondialdehyde content, heat injury index (HII), leaf water potential, and shoot fresh weight.

Table 3.1 ANOVA of different parameters as influenced by varieties (var) and heat-treatments (trt).

S.N	Parameter	SOV	P-value	Significance
1.	SPAD value	Var	0	***
		Trt	0	***
		Var × T	0.0702	NS
2.	Initial Fluorescence	Var	0.9835	NS
	(F_o)	Trt	0	***
		Var × Trt	0.9999	NS
3.	Maximum Fluorescence	Var	0.9217	NS
	(F_m)	Trt	0	***
		Var × Trt	0.9993	NS
4.	Chlorophyll Fluorescence	Var	0.7006	NS
	(CF)	Trt	0	***
		Var × Trt	0.9706	NS
5.	Leaf Water Potential	Var	0.0219	*
	(LWP)	Trt	0	***
		Var × Trt	6e-04	***
6.	Relative Water Content	Var	0.0024	**
	(RWC)	Trt	0	***
		Var × Trt	0.2808	NS
7.	Electrolyte Leakage	Var	0	***
	(EL)	Trt	0	***
		Var × Trt	0	***
8.	Net Photosynthesis Rate	Var	0.0223	*
	(Pn)	Trt	0.0016	**
		Var × Trt	0.8354	NS
9.	Transpiration rate	Var	0	***
	(E)	Trt	0	***
		Var × Trt	0.3045	NS
10.	Stomatal Conductance	Var	0	***
	(gs)	Trt	0	***
		Var × Trt	0.4055	NS
11.	Intercellular CO_2	Var	0.2786	NS
	Concentration (Ci)	Trt	0.0013	**
		Var × Trt	0.6533	NS
12.	Intrinsic Water Use	Var	0.0023	**
	Efficiency (WUEintr)	Trt	0.1366	NS
		Var × Trt	0.5654	NS
13.	Instantaneous Water Use	Var	0.0013	**
	Efficiency (WUEins)	Trt	0.056	NS

Table 3.1 Continued

S.N	Parameter	SOV	P-value	Significance
		Var × Trt	0.4856	NS
14.	Shoot Fresh Weight (SFW)	Var	3e-04	***
		Trt	0	***
		Var × Trt	0.0367	*
15.	Shoot Dry Weight (TDW)	Var	0	***
		Trt	0	***
		Var × Trt	0.0768	NS
16.	Heat Injury Index (HII)	Var	< 2e-16	***
		Trt	< 2e-16	***
		Var × Trt	4.6e-15	***
17.	Malondialdehyde Content (MDA)	Var	<2e-16	***
		Trt	< 2e-16	***
		Var × Trt	< 2e-16	***

***, **,* show significant difference at P≤ 0.001, 0.01,0.05, respectively.
NS means not significant at P≤0.05.

3.3.1. SPAD

Between varieties within heat-treatments: When plants were grown under normal conditions, CELE had the highest, whereas NEWG had the lowest spad value (Table 3.2). Under heat-stress conditions, PICUS and CELE had the highest, whereas ARKA had the lowest spad value. Similarly, CELE had the highest, and ARKA, BR, and NEWG had the lowest spad value under heat-shock conditions.

Table 3.2 SPAD of different tomato varieties under control (C), heat-stress (HS), and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different small letters within each column show significant differences between the varieties. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

	SPAD		
	Control (26/18℃)	**HS (38/28℃-7 days)**	**SHS (40℃-7hrs)**
ARKA	48.0 ± 1.7 Ade	28.1 ± 1.0 Bd	46.5 ± 1.6 Ac
BR	50.3 ± 1.2 Acd	32.3 ± 1.9 Cbcd	46.1 ± 0.8 Bc
CELE	57.0 ± 1.6 Aa	41.4 ± 2.7 Ba	52.9 ± 1.6 Aa
HEAT	55.3 ± 1.8 Aab	35.6 ± 1.8 Cabc	50.8 ± 0.5 Bab
HT1	47.0 ± 1.3 Ade	33.4 ± 1.7 Bbcd	47.5 ± 0.8 Abc
NEWG	45.2 ± 1.5 Ae	30.6 ± 1.9 Bcd	47.0 ± 0.9 Ac
PICUS	50.9 ± 1.8 Abcd	41.8 ± 3.2 Ba	49.0 ± 1.3 Abc
PP	52.8 ± 1.3 Aabc	37.9 ± 2.6 Bab	48.2 ± 1.5 Abc
TL	52.9 ± 1.5 Aabc	33.0 ± 2.0 Bbcd	49.3 ± 1.3 Abc
VG	51.4 ± 1.7 Abcd	37.4 ± 2.8 Bab	47.4 ± 1.2 Abc

Between heat-treatments within varieties: SPAD decreased under heat-stress for all varieties, whereas it declined only in HEAT and BR when heat-shocked. BR and HEAT had significantly different values under three different temperature regimes, the highest being in control and the lowest in the heat-stress treatment. For these varieties, spad decreased by 36% in heat-stress and 8% in heat-shock conditions. The greatest decrease was observed in ARKA under heat-stress, which was 41% lower than that of control, followed by TL with a 37% decrease, while the lowest decrease was seen in PICUS with 17% decrement from that of control.

3.3.2. Relative water content

Between varieties within heat-treatments: HEAT and HT1 had the highest relative water content under heat-stress whereas PICUS had the lowest value (Table 3.3). Similarly, HEAT had the highest value under heat-shock whereas BR had the lowest relative water content.

Table 3.3 Relative Water Content (RWC) of different tomato varieties under control (C), heat-stress (HS) and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different small letters within each column show significant differences between the varieties. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

	Relative Water Content (%)		
	Control (26/18°C)	**HS (38/28°C-7 days)**	**SHS (40°C-7hrs)**
ARKA	74.0 ± 1.0 a	72.1 ± 3.7 ab	76.3 ± 1.7 ab
BR	67.0 ± 1.9 bc	67.8 ± 2.6 ab	70.2 ± 1.7 b
CELE	72.1 ± 1.2 ABab	67.9 ± 2.2 Bab	74.3 ± 2.4 Aab
HEAT	68.8 ± 1.4 Babc	75.5 ± 2.8 Aa	77.7 ± 1.8 Aa
HT1	71.6 ± 1.7 ab	75.3 ± 3.4 a	73.3 ± 3.3 ab
NEWG	59.9 ± 1.7 Bd	68.0 ± 3.3 Aab	71.9 ± 2.5 Aab
PICUS	68.6 ± 3.8 ABabc	64.5 ± 4.0 Bb	75.8 ± 2.1 Aab
PP	64.6 ± 2.2 Bcd	68.3 ± 2.6 ABab	73.3 ± 1.2 Aab
TL	69.6 ± 3.4 abc	70.7 ± 2.0 ab	73.6 ± 3.0 ab
VG	66.5 ± 0.8 bc	73.2 ± 3.4 ab	72.4 ± 2.3 ab

Between heat-treatments within varieties: HEAT had 10% and 12% increase in relative water content under heat-stress and heat-shock compared to control. NEWG had 14% and 17% higher relative water content than control in heat-stress and heat-shock, respectively. Likewise, it increased significantly only under heat-shock in PP. CELE an PICUS had different relative water content under heat-stress and heat-shock, the later having higher mean values.

3.3.3. Net photosynthesis rate

Between varieties within heat-treatments: BR and TL had the highest and lowest photosynthesis values under heat-stress, respectively (Table 3.4). Under heat-shock, HEAT had the highest following closely by PICUS and HT1, while PP had the lowest photosynthesis rate.

Table 3.4 Net photosynthesis rate (Pn) of different tomato varieties under control (C), heat-stress (HS) and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different small letters within each column show significant differences between the varieties. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

	Net photosynthesis rate (P_N, µmol CO_2 $m^{-2}s^{-1}$)		
	Control (26/18°C)	**HS (38/28°C-7 days)**	**SHS (40°C-7hrs)**
ARKA	5.1 ± 0.5	3.8 ± 0.5 cd	5.6 ± 1.0 ab
BR	5.6 ± 1.0	6.6 ± 0.6 a	6.3 ± 0.6 ab
CELE	5.1 ± 0.6	4.4 ± 0.9 bcd	6.4 ± 1.0 ab
HEAT	6.2 ± 0.8	5.7 ± 0.7 ab	7.8 ± 0.7 a
HT1	5.0 ± 0.3	5.0 ± 0.6 abc	6.9 ± 0.9 ab
NEWG	5.6 ± 1.5	5.6 ± 0.3 abc	6.2 ± 1.2 ab
PICUS	4.8 ± 0.8	6.1 ± 0.5 ab	7.1 ± 0.9 ab
PP	4.7 ± 0.2	5.7 ± 0.6 ab	4.7 ± 0.8 b
TL	4.3 ± 0.2 B	3.0 ± 0.5 Cd	5.6 ± 0.3 Aab
VG	5.6 ± 1.0	5.7 ± 0.4 ab	6.5 ± 0.8 ab

Between heat-treatments within varieties: Only TL had significantly different photosynthesis values under different temperature regimes, highest being under heat-shock and lowest under heat-stress. It increased by 29% in heat-shock whereas it decreased by 30% in heat-stress from that of control.

3.3.4. Stomatal conductance

Between varieties within heat-treatments: NEWG and VG had the highest stomatal under both heat-stress and heat-shock whereas the lowest value was observed for HT1 in heat-stress and PP in heat-hock (Table 3.5).

Table 3.5 Stomatal conductance (gs) of different tomato varieties under control (C), heat-stress (HS) and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different small letters within each column show significant differences between the varieties. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

	Stomatal conductance (gs, mol H_2O m^{-2} s^{-1})		
	Control (26/18°C)	**HS (38/28°C-7 days)**	**SHS (40°C-7hrs)**
ARKA	0.19 ± 0.01 Aab	0.12 ± 0.02 Bbc	0.17 ± 0.03 ABbc
BR	0.14 ± 0.02 c	0.15 ± 0.01 abc	0.19 ± 0.04 abc
CELE	0.17 ± 0.02 ABbc	0.13 ± 0.01 Bbc	0.17 ± 0.03 Aabc
HEAT	0.21 ± 0.01 ab	0.17 ± 0.02 ab	0.22 ± 0.04 abc
HT1	0.13 ± 0.01 Bc	0.12 ± 0.01 Bc	0.23 ± 0.03 Aab
NEWG	0.22 ± 0.02 a	0.20 ± 0.02 a	0.26 ± 0.03 a
PICUS	0.14 ± 0.01 Bc	0.15 ± 0.02 Babc	0.21 ± 0.03 Aabc
PP	0.15 ± 0.01 c	0.15 ± 0.03 abc	0.15 ± 0.01 c
TL	0.13 ± 0.02 Bc	0.13 ± 0.01 Bbc	0.20 ± 0.03 abc
VG	0.20 ± 0.01 ABab	0.19 ± 0.02 Ba	0.25 ± 0.02 Aa

Between heat-treatments within varieties: PICUS and HT1 had significantly higher stomatal conductance under heat-shock than control. ARKA had the highest value under control which was statistically different from that of heat-stress. However, other varieties did not have significant differences among the different heat levels.

3.3.5. Transpiration

Between varieties within heat-treatments: NEWG had the highest transpiration under all treatments whereas ARKA and PP had the lowest value in heat-shock and ARKA and TL had the lowest value under heat-stress (Table 3.6).

Table 3.6 Transpiration rate (E) of different tomato varieties under control (C), heat-stress (HS) and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different small letters within each column show significant differences between the varieties. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

	Transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$)		
	Control (26/18°C)	**HS (38/28°C-7 days)**	**SHS (40°C-7hrs)**
ARKA	4.8 ± 0.4 ab	3.7 ± 0.3 d	4.9 ± 0.7 c
BR	3.7 ± 0.4 cde	4.6 ± 0.4 bcd	5.4 ± 0.8 abc
CELE	4.3 ± 0.5 Aba-d	4.0 ± 0.3 Bcd	5.5 ± 0.5 Aabc
HEAT	5.0 ± 0.3 a	5.0 ± 0.4 abc	5.9 ± 0.8 abc
HT1	3.4 ± 0.2 Bde	3.7 ± 0.2 Bb	6.1 ± 0.6 Aabc
NEWG	5.1 ± 0.4 Ba	5.8 ± 0.6 ABa	7.1 ± 0.7 Aa
PICUS	3.7 ± 0.3 Bde	4.6 ± 0.4 ABbcd	5.9 ± 0.6 Aabc
PP	3.8 ± 0.2 b-e	4.7 ± 0.5 bcd	4.5 ± 0.2 c
TL	3.2 ± 0.4 Be	4.0 ± 0.3 Bd	5.4 ± 0.5 Abc
VG	4.7 ± 0.4 Babc	5.6 ± 0.5 ABab	6.8 ± 0.5 Aab

Between heat-treatments within varieties: Under heat-shock, NEWG, VG, PICUS, TL and HT1 had significantly higher transpiration rate for that of control. Between heat-stress and heat-shock, TL, CELE and HT1 had significantly different transpiration values.

3.3.6. Shoot dry weight

Shoot dry weight was highest in CELE and lowest in HEAT under heat-stress (Table 3.7). CELE and VG had the highest whereas PP and ARKA had the lowest dry weight under heat-shock. The mean dry weight was similar for control and heat-shock treatments whereas it was highest under heat-stress.

Table 3.7 Shoot Dry Weight (SDW) of different tomato varieties under control (C), heat-stress (HS) and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different small letters within each column show significant differences between the varieties. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

	Shoot dry weight (SDW, g)		
	Control (26/18°C)	**HS (38/28°C-7 days)**	**SHS (40°C-7hrs)**
ARKA	1.6 ± 0.1 Bab	2.1 ± 0.2 Aab	1.4 ± 0.1 Be
BR	1.5 ± 0.1 Bbc	2.1 ± 0.1 Aab	1.5 ± 0.1 Bb-e
CELE	1.6 ± 0.1 Cab	2.4 ± 0.1 Aa	1.9 ± 0.2 Ba
HEAT	1.5 ± 0.1 abc	1.6 ± 0.2 c	1.4 ± 0.0 cde
HT1	1.3 ± 0.1 Bc	1.9 ± 0.1 Abc	1.7 ± 0.2 Aab
NEWG	1.7 ± 0.1 Bab	2.0 ± 0.2 Aabc	1.7 ± 0.1 Bab
PICUS	1.7 ± 0.1 Bab	2.2 ± 0.1 Aab	1.7 ± 0.1 Ba-d
PP	1.6 ± 0.1 abc	2.1 ± 0.2 ab	1.3 ± 0.1 e
TL	1.8 ± 0.1 Ba	2.3 ± 0.1 Aab	1.7 ± 0.1 Babc
VG	1.7 ± 0.1 Bab	2.3 ± 0.1 Aab	1.9 ± 0.1 Ba

3.3.7. Initial/minimum and maximum fluorescence

There were no differences in Fo across varieties under control or both heat temperature conditions (Table 3.8). Comparing treatments within varieties, a significant increase in initial fluorescence was observed for ARKA under heat-stress, which was statistically at par with the value obtained in heat-shock. Across all varieties, initial fluorescence was the highest in heat-shock and lowest for control.

There were no differences in F_m across varieties under control or both heat temperature conditions (Table 3.9). BR, NEWG, PICUS, PP and VG had no differences in maximum fluorescence when exposed to different temperature regimes. ARKA, CELE, HEAT, HT1 and TL had lowest F_m under heat-stress and no significant changes under heat-shock in comparison to control.

Table 3.8 Initial/Minimum fluorescence (Fo) of different tomato varieties under control, heat-stress (HS) and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

Initial/Minimum fluorescence (F_o)			
	Control (26/18°C)	**HS (38/28°C-7 days)**	**SHS (40°C-7hrs)**
ARKA	36.9 ± 5.0 B	50.5 ± 4.4 A	55.9 ± 0.8 A
BR	39.0 ± 5.9 B	47.6 ± 3.9 AB	57.0 ± 1.6 A
CELE	38.6 ± 5.6 B	45.4 ± 3.8 B	61.8 ± 2.0 A
HEAT	36.3 ± 5.2 B	41.5 ± 3.9 B	55.8 ± 1.6 A
HT1	38.8 ± 6.4 B	47.3 ± 5.1 AB	57.8 ± 1.7 A
NEWG	38.1 ± 5.6 B	49.0 ± 5.7 AB	57.6 ± 2.4 A
PICUS	38.6 ± 5.6 B	50.0 ± 5.0 AB	56.3 ± 2.5 A
PP	39.0 ± 5.7 B	45.0 ± 3.9 B	59.5 ± 1.4 A
TL	39.0 ± 5.5 B	49.1 ± 5.9 AB	59.6 ± 2.0 A
VG	38.0 ± 4.9 B	45.6 ± 4.2 B	57.3 ± 2.0 A

Table 3.9 Maximum fluorescence (Fm) of different tomato varieties under control (C), heat-stress (HS) and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

Maximum fluorescence (Fm)			
	Control (26/18°C)	**HS (38/28°C-7 days)**	**SHS (40°C-7hrs)**
ARKA	262.1 ± 12.9 A	221.9 ± 16.8 B	244.0 ± 6.5 AB
BR	268.8 ± 13.8	230.8 ± 21.6	261.6 ± 5.6
CELE	271.4 ± 13.8 A	219.3 ± 16.2 B	274.3 ± 8.6 A
HEAT	257.6 ± 12.2 A	216.9 ± 18.9 B	266.6 ± 7.5 A
HT1	280.9 ± 17.9 A	226.8 ± 19.5 B	272.8 ± 8.5 AB
NEWG	264.8 ± 13.9	231.6 ± 20.8	269.9 ± 6.9
PICUS	271.3 ± 14.5	245.6 ± 23.2	259.4 ± 5.7
PP	270.4 ± 17.0	233.1 ± 17.1	263.8 ± 7.9
TL	279.6 ± 15.9 A	226.5 ± 23.1 B	272.9 ± 6.4 AB
VG	266.0 ± 6.61	232.6 ± 21.2	262.3 ± 3.9

3.3.8. Chlorophyll fluorescence

Chlorophyll fluorescence was significantly reduced under high-temperature treatments for all varieties (Table 3.10). There were no differences between chlorophyll fluorescence values under heat-stress and heat-shock for all varieties except for PP, which showed lowest value under heat-shock treatment.

Table 3.10 Chlorophyll Fluorescence (CF) of different tomato varieties under control (C), heat-stress (HS) and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

	Chlorophyll Fluorescence (CF)		
	Control (26/18°C)	**HS (38/28°C-7 days)**	**SHS (40°C-7hrs)**
ARKA	0.86 ± 0.01 A	0.77 ± 0.001 B	0.77 ± 0.08 B
BR	0.86 ± 0.02 A	0.79 ± 0.007 B	0.78 ± 0.01 B
CELE	0.86 ± 0.01 A	0.79 ± 0.010 B	0.77 ± 0.08 B
HEAT	0.86 ± 0.02 A	0.81 ± 0.005 B	0.79 ± 0.00 B
HT1	0.87 ± 0.02 A	0.79 ± 0.008 B	0.79 ± 0.00 B
NEWG	0.86 ± 0.02 A	0.79 ± 0.009 B	0.79 ± 0.01 B
PICUS	0.86 ± 0.01 A	0.80 ± 0.002 B	0.78 ± 0.01 B
PP	0.86 ± 0.01 A	0.81 ± 0.006 B	0.77 ± 0.00 C
TL	0.86 ± 0.01 A	0.79 ± 0.009 B	0.78 ± 0.01 B
VG	0.86 ± 0.02 A	0.80 ± 0.005 B	0.78 ± 0.01 B

3.3.9. Intercellular CO_2

The varieties did not show any difference under control and high temperature treatments for intercellular CO_2 concentration (Ci) except for TL (Table 11), which had a significantly increased in Ci under high temperature treatments (Table 3.11).

Table 3.11 Intercellular CO_2 concentration (Ci) of different tomato varieties under control (C), heat-stress (HS) and heat-shock (SHS) treatments. The values represent the mean ± standard error. Different capital letters point to the significant differences between the observed values under different temperature treatments within each variety.

Intercellular CO_2 concentration ((Ci, µmol CO_2 mol^{-1})			
	Control (26/18°C)	**HS (38/28°C-7 days)**	**SHS (40°C-7hrs)**
ARKA	300.5 ± 17.5	325.9 ± 7.00	322.3 ± 7.40
BR	302.2 ± 7.80	302.1 ± 10.4	317.3 ± 9.40
CELE	290.2 ± 22.4	324.3 ± 8.60	324.0 ± 7.70
HEAT	318.8 ± 8.80	317.2 ± 10.5	309.0 ± 11.6
HT1	305.5 ± 8.00	306.7 ± 9.80	325.6 ± 8.60
NEWG	302.8 ± 24.6	325.8 ± 5.50	342.3 ± 5.60
PICUS	301.8 ± 15.7	303.6 ± 11.4	323.3 ± 5.30
PP	325.8 ± 3.90	312.7 ± 10.1	324.9 ± 9.10
TL	305.5 ± 6.80 B	336.7 ± 7.90 A	328.2 ± 7.70 A
VG	321.9 ± 13.8	327.2 ± 6.64	337.8 ± 5.60

3.3.10. Electrolyte Leakage

Electrolyte leakage (EL) was generally higher under heat-shock stress for all varieties and lowest for control (Figure 3.1). Under heat-shock, ARKA, PP and TL had the highest electrolyte leakage values whereas, VG, HEAT and CELE had the lowest values. Similarly, under heat stress, VG, HEAT and CELE had the lowest electrolyte leakage, while ARKA and PP had the highest values.

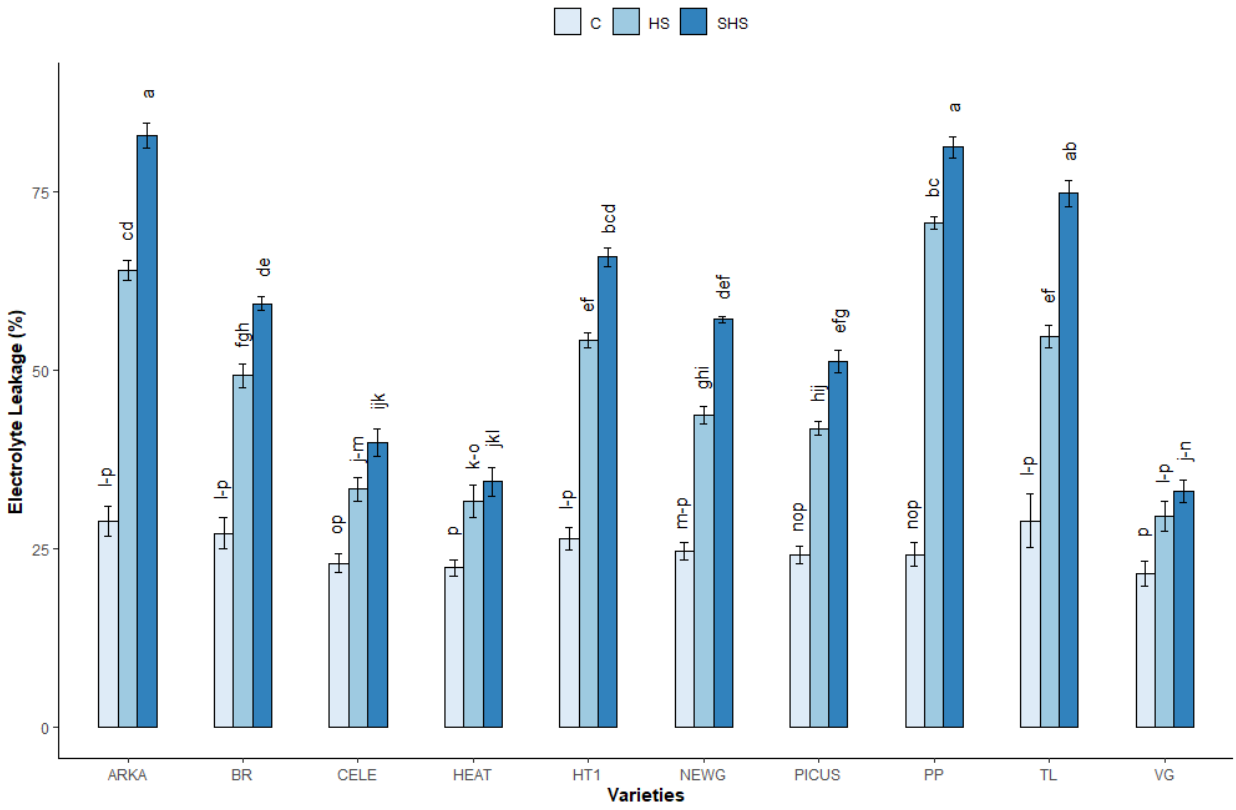

Figure 3.1 Electrolyte leakage (EL, %) of different tomato varieties when exposed to three different temperature treatments: Control (C), Heat-stress (HS) and Heat-shock (SHS). Different small letters signify significant differences between variety-temperature combinations based on HSD test ($P \leq 0.05$). Each bar represents mean ± standard error values.

3.3.11. Leaf Water Potential

Leaf water potential varied among the heat treatments among the given varieties (Figure 3.2). Heat-stress and heat-shock had a significant reduction in leaf water potential as compared to control in PICUS, TL and VG. For BR and PP, LWP was the lowest under heat shock and significantly different from that of control.

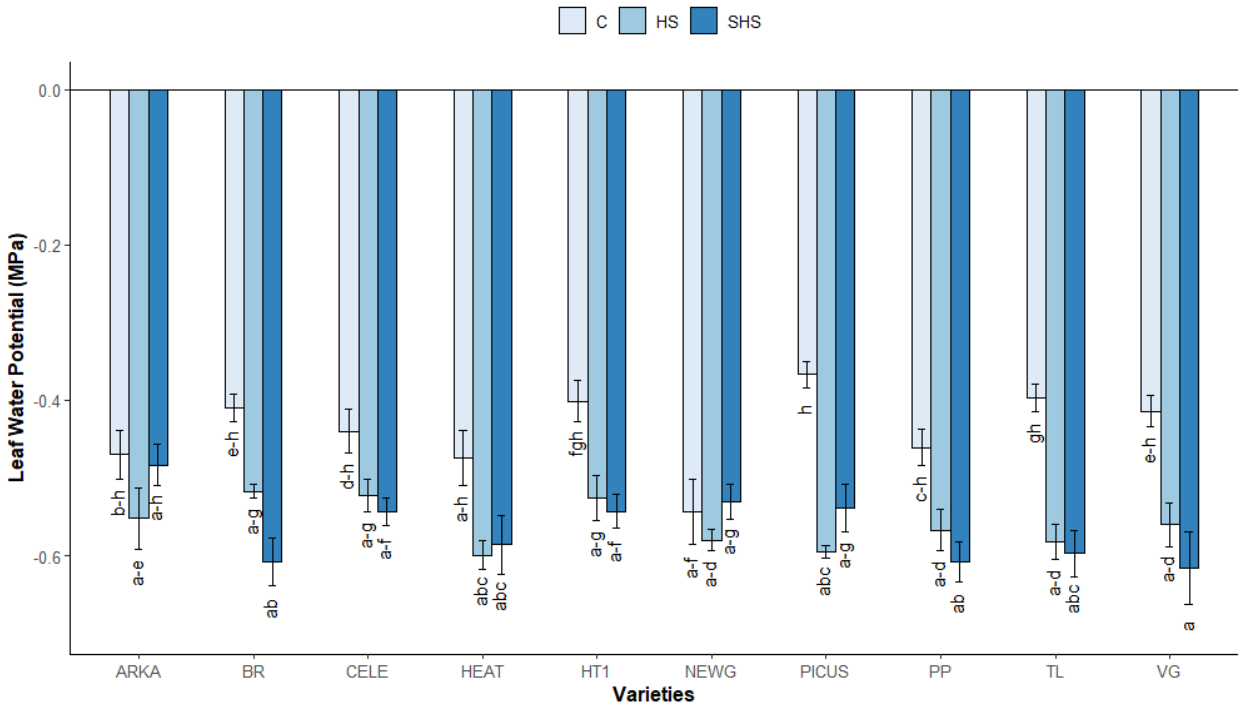

Figure 3.2 Leaf water potential (LWP, MPa) of different tomato varieties when exposed to three different temperature treatments: Control (C), Heat-stress (HS) and Heat-shock (SHS). Different small letters signify significant differences between variety-temperature combinations based on HSD test ($P \leq 0.05$). Each bar represents mean ± standard error values.

3.3.12. Malondialdehyde

Malondialdehyde content is used as an indicator of membrane damage and is highly correlated to electrolyte leakage (Figure 3.3). ARKA, PP and TL had the highest malondialdehyde content under heat-stress as well as heat-shock. However, HEAT, CELE, NEWG and VG had the lowest values under heat-shock.

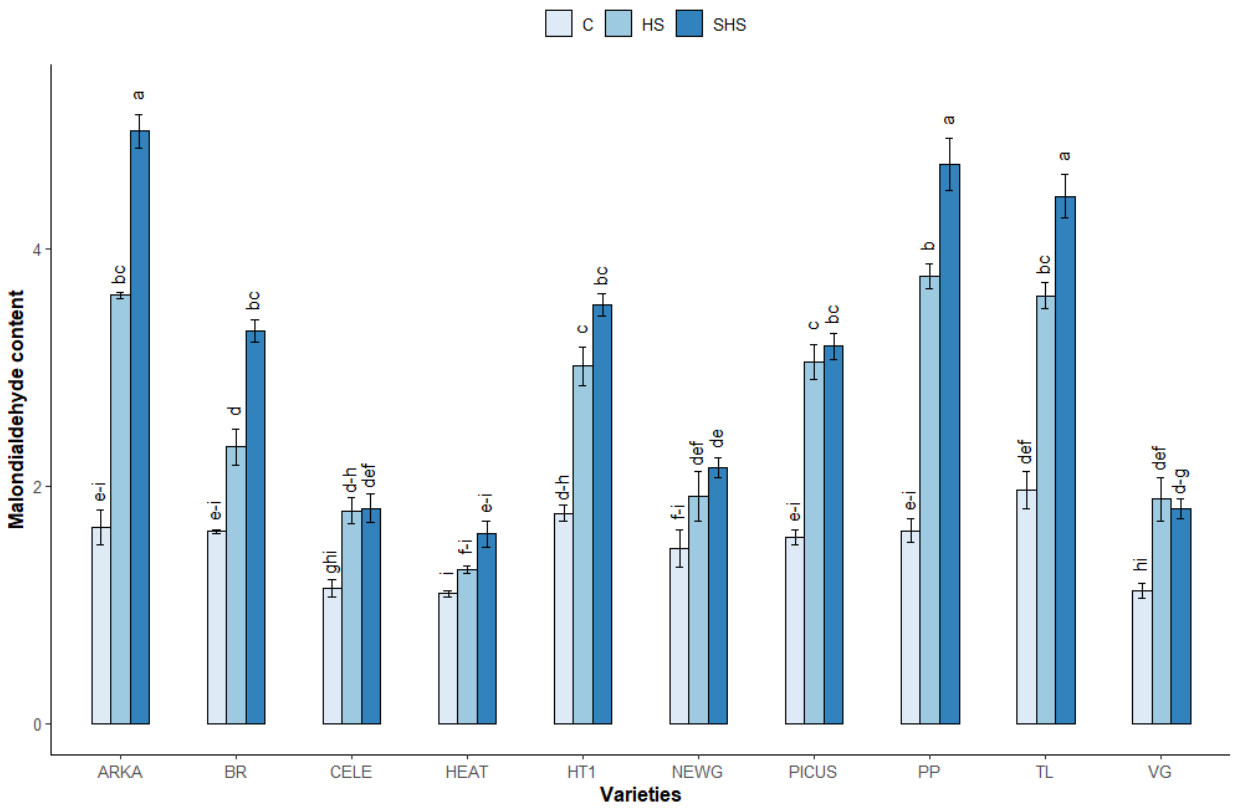

Figure 3.3 Malondialdehyde Content (MDA, µmol g^{-1} fresh weight) of different tomato varieties when exposed to three different temperature treatments: Control (C), Heat-stress (HS) and Heat-shock (SHS). Different small letters signify significant differences between variety-temperature combinations based on HSD test ($P \leq 0.05$). Each bar represents mean ± standard error values.

3.3.13. Heat Injury Index

Heat injury index was generally highest under heat-stress for all varieties (Figure 3.4). Underheat-stress, ARKA, PP and TL had significantly highest values, whereas lowest index values were observed for VG, PICUS, CELE, NEWG and HEAT. Under heat-shock, the highest HII were obtained for ARKA, PP and TL but these values were lower than those under heat-stress.

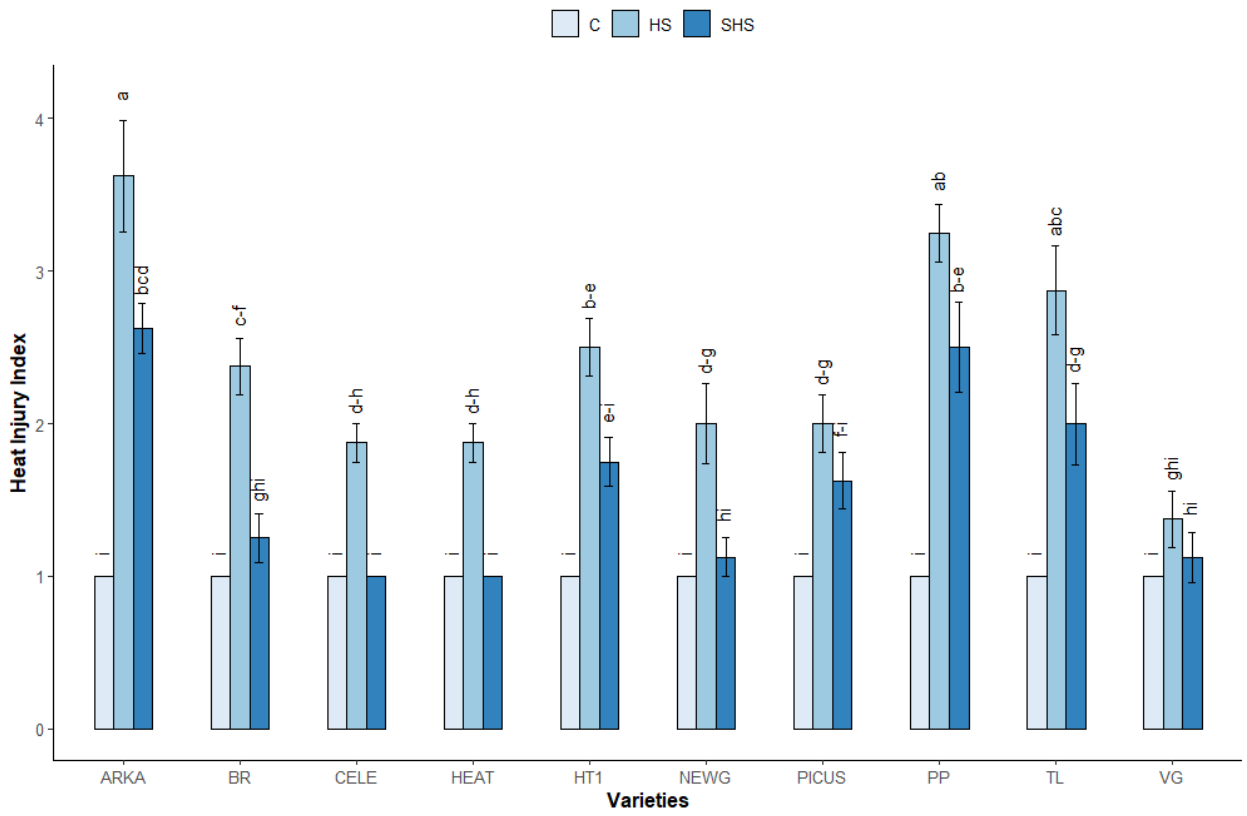

Figure 3.4 Heat Injury Index (HII) of different tomato varieties when exposed to three different temperature treatments: Control (C), Heat-stress (HS) and Heat-shock (SHS). Different small letters signify significant differences between variety-temperature combinations based on HSD test ($P \leq 0.05$). Each bar represents mean ± standard error values.

3.3.14. Shoot Fresh Weight

Shoot Fresh Weight was similar for all varieties among all heat treatments with some exceptions (Figure 3.5). Under heat-stress, highest fresh weight was measured in CELE whereas lowest in HEAT. Under heat-shock, lowest fresh weight was measured for PP and BR but was similar to that of control.

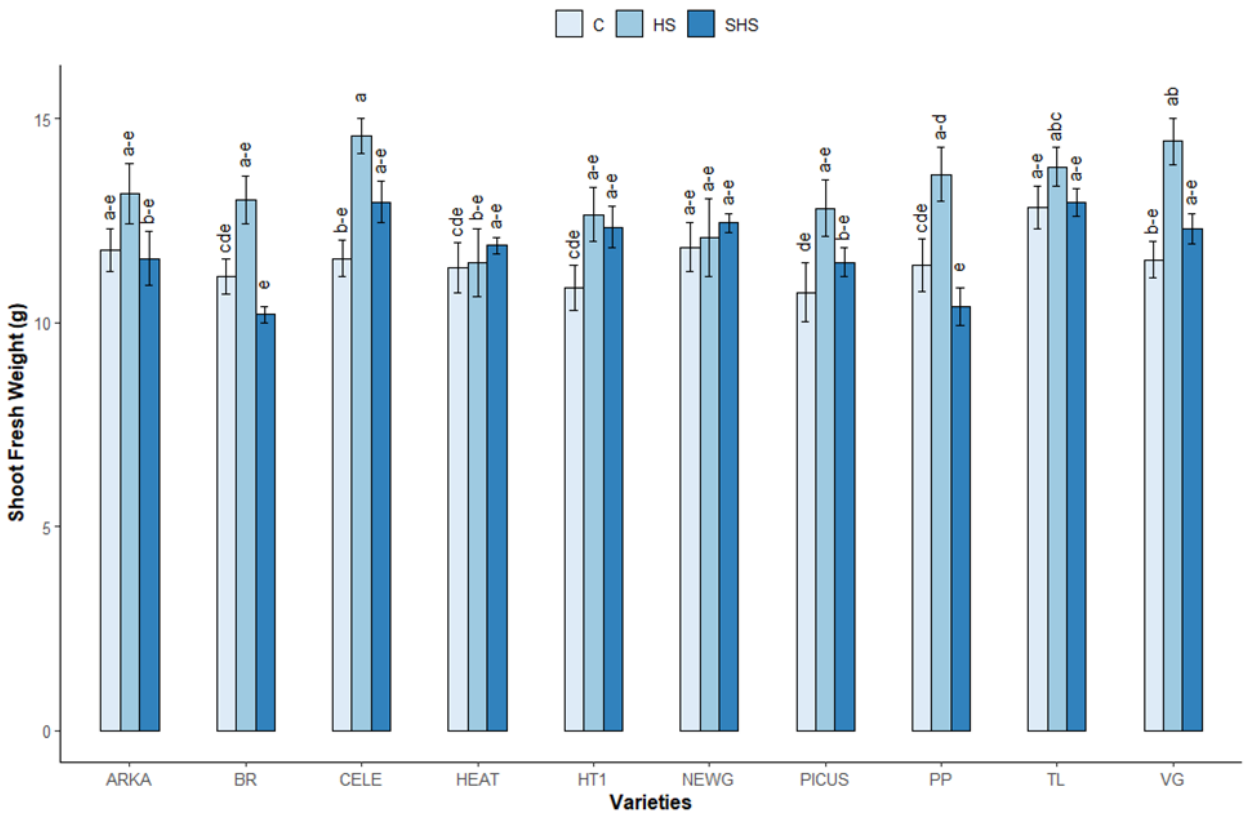

Figure 3.5 Shoot Fresh Weight (SFW, g) of different tomato varieties when exposed to three different temperature treatments: Control (C), Heat-stress (HS) and Heat-shock (SHS). Different small letters signify significant differences between variety-temperature combinations based on HSD test ($P \leq 0.05$). Each bar represents mean ± standard error values.

3.4. Discussion

A decrease in the SPAD index under high-temperature conditions suggests a reduction in the synthesis and/or rapid degradation of the chlorophyll pigments (Zhou et al., 2015). In this study, CELE had the highest SPAD values than other varieties in each temperature treatment, which means that it is robust against photosynthetic pigments' degradation under heat stress. In contrast, ARKA had the lowest values under high-temperature stress treatments suggesting that it might be sensitive to increased temperature during the vegetative stage.

A reduction in chlorophyll fluorescence under heat-stress indicates damage to PSII through reactive oxygen species production, leading to increase in non-photochemical quenching and subsequent photoinhibition (Maxwell & Johnson, 2000). However, it has been reported that this damage could be alleviated by efficient repair mechanisms in PSII, leading to sustained photosynthesis under high-temperature treatments (Nath et al., 2013). In addition to efficient repair mechanisms, increased stomatal conductance and transpiration led to even higher photosynthesis under SHS (Duan et al., 2017). Despite that heat-stressed plants had lower photosynthesis than SHS, they had a higher biomass. This suggests that there were more leaf area and vegetative growth for heat-stressed plants despite lower photosynthetic rate. Biomass was measured at the end of the experiment just at one growth point, which might not represent the whole growing cycle during stress.

High temperature treatments increased electrolyte leakage, malondialdehyde content and heat injury index. It is notable that electrolyte leakage and malondialdehyde content were highest under heat-shock for PP, ARKA and TL, but heat injury index was lower than heat-stress treatment for these varieties. This indicates that plants' heat stress can be assessed through electrolyte leakage and malondialdehyde analysis before plants show any visual injury symptoms.

Based on this study's data, electrolyte leakage, malondialdehyde content, heat injury index, stomatal conductance, transpiration rate and net photosynthesis rate appear as important plant variable traits to be considered for the selection of heat-tolerant varieties. To better understand the relationship of variables, correlograms were constructed for each temperature treatment.

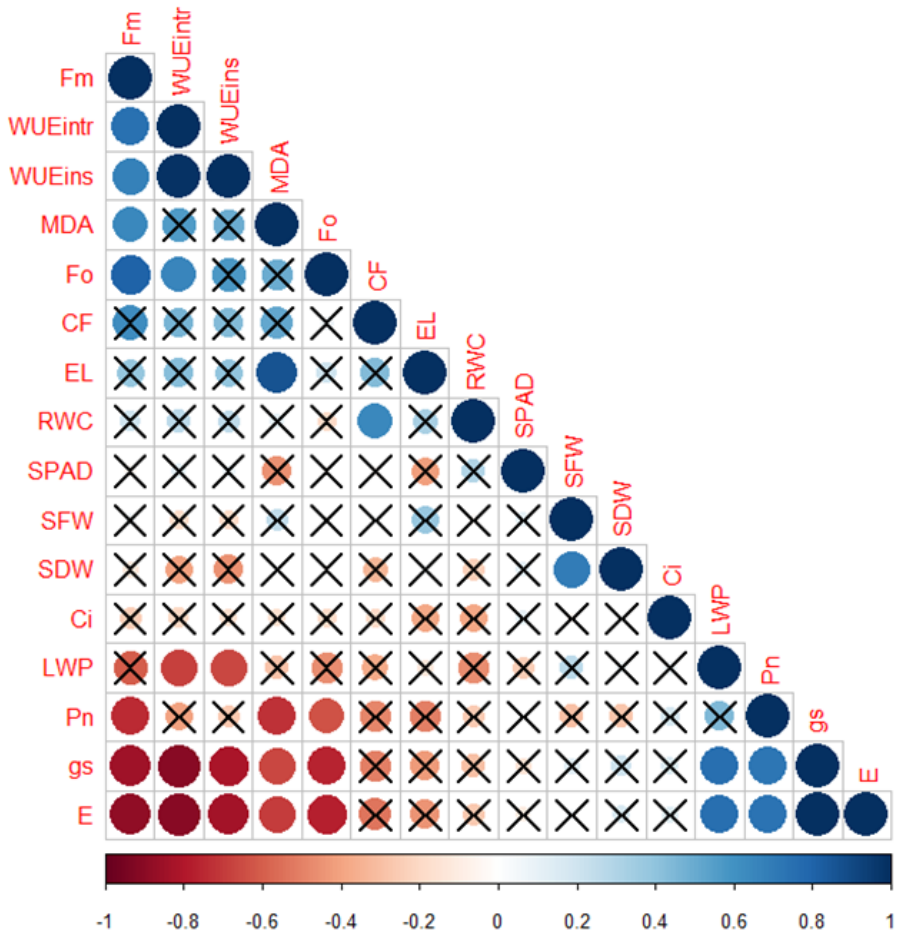

Figure 3.6 Correlogram showing the relationship between variables in control treatment (26/18 °C). The intensity of color and size of circle increases with increase in significance of correlation. Dark red denotes high negative correlation whereas dark blue denotes high positive correlation. The cells with cross marks denote not significant correlation between the variables.

Under optimum growing temperatures (control), there was a significant positive correlation between the parameters net photosynthesis rate, stomatal conductance and transpiration rate (Figure 3.6, 0.7, blue dots). When soil moisture is not limited, increase in transpiration due to stomatal opening facilitates gas exchange, leading to better diffusion of CO_2 with the resulting increase in net photosynthesis rate (Zhou et al., 2020). The transpiration rate and stomatal conductance were highly correlated with the leaf water potential (Figure 6, 0.7, blue dots).

Chlorophyll fluorescence was highly correlated with relative water content which signifies that the chlorophyll fluorescence values were high in plants with higher relative water content (Figure 6, 0.6, blue dots). Both electrolyte leakage and malondialdehyde content had lower values under control conditions, and they have a significant positive correlation (Figure 6, 0.9, blue dots), meaning that plants with lower malondialdehyde content had lower electrolyte leakage.

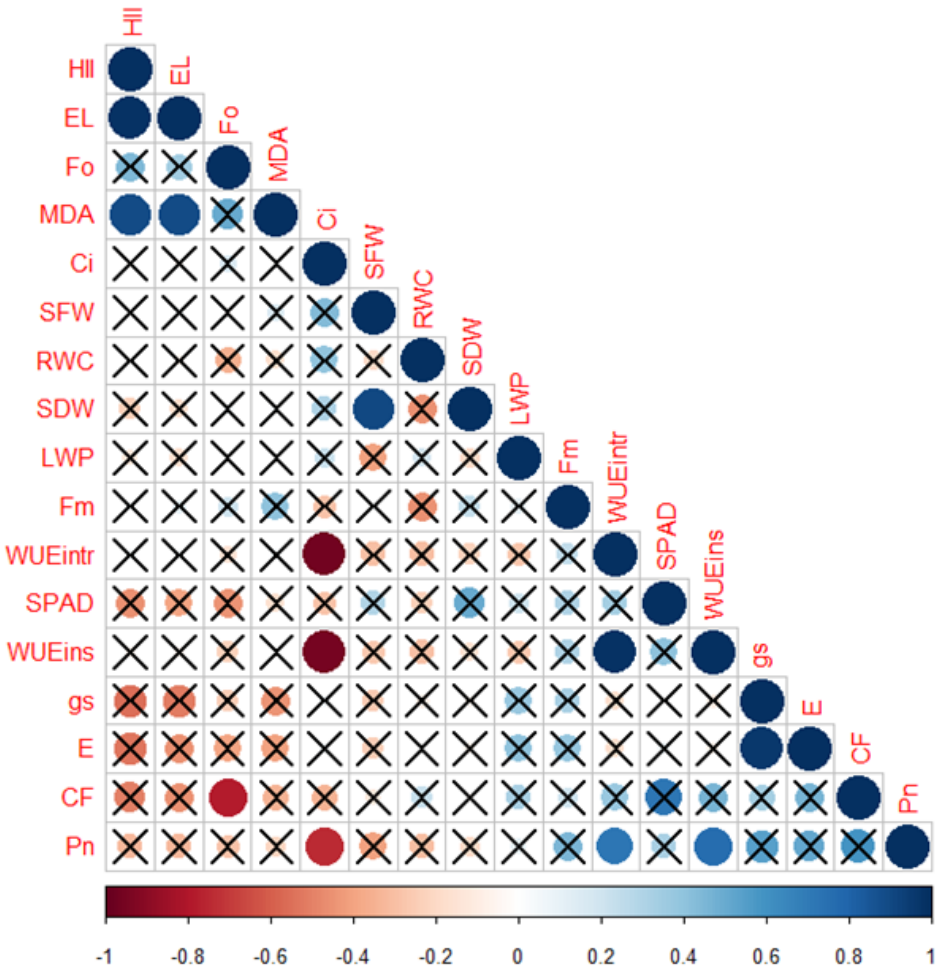

Figure 3.7 Correlogram showing the relationship between variables in heat-stress treatment (38/28 ℃- 7 days). The intensity of color and size of circle increases with increase in significance of correlation. Dark red denotes high negative correlation whereas dark blue denotes high positive correlation. The cells with cross marks denote not significant correlation between the variables.

Under the heat-stress treatment, net photosynthesis rate was significantly positively correlated to both intrinsic and instantaneous water use efficiency (Figure 3.7, 0.6, blue dots) but was negatively correlated with intercellular CO_2 concentration (Figure 7, -0.8, red dots). Plants have higher water use efficiency when they have reduced stomatal conductance and transpiration, and higher net photosynthesis rate. Increased intercellular CO_2 concentration under heat-stress results from a decrease in photochemical efficiency due to non-stomatal factors; reduction of CO_2 assimilation due to alterations in mesophyll capacity that depends on Rubisco's activity and on the capacity of the photosynthetic electron transport to regenerate Rubisco (Abdelmageed and Gruda, 2009). Thus, under heat-stress conditions, when photosynthesis decreased, there was an increase in intercellular CO_2 concentration. There was a negative correlation between initial fluorescence and chlorophyll fluorescence (Figure 7, -0.8, red dots), which means that plants with higher initial fluorescence have lower chlorophyll fluorescence. It has been noted that an increase in initial fluorescence led to a decrease in chlorophyll fluorescence, which signifies that photoinhibition occurred in plants exposed to heat-stress treatments (Poudyal et al., 2019). A highly significant correlation between malondialdehyde content, electrolyte leakage and heat injury index (Figure 7, 0.9, blue dots) indicates that plants with damaged membranes had high permeability, leading to higher leakage of ions and water on exposure to high temperature, which is evident by macroscopic heat injury (Jahan et al., 2019). The higher the malondialdehyde content and electrolyte leakage, the more expression of heat injury symptoms by plants. Thus, this study prompts that heat-tolerant plants will have lower intercellular CO_2 concentration, electrolyte leakage, malondialdehyde content and heat injury index, despite decreases in net photosynthesis rate and chlorophyll fluorescence when exposed to heat-stress conditions.

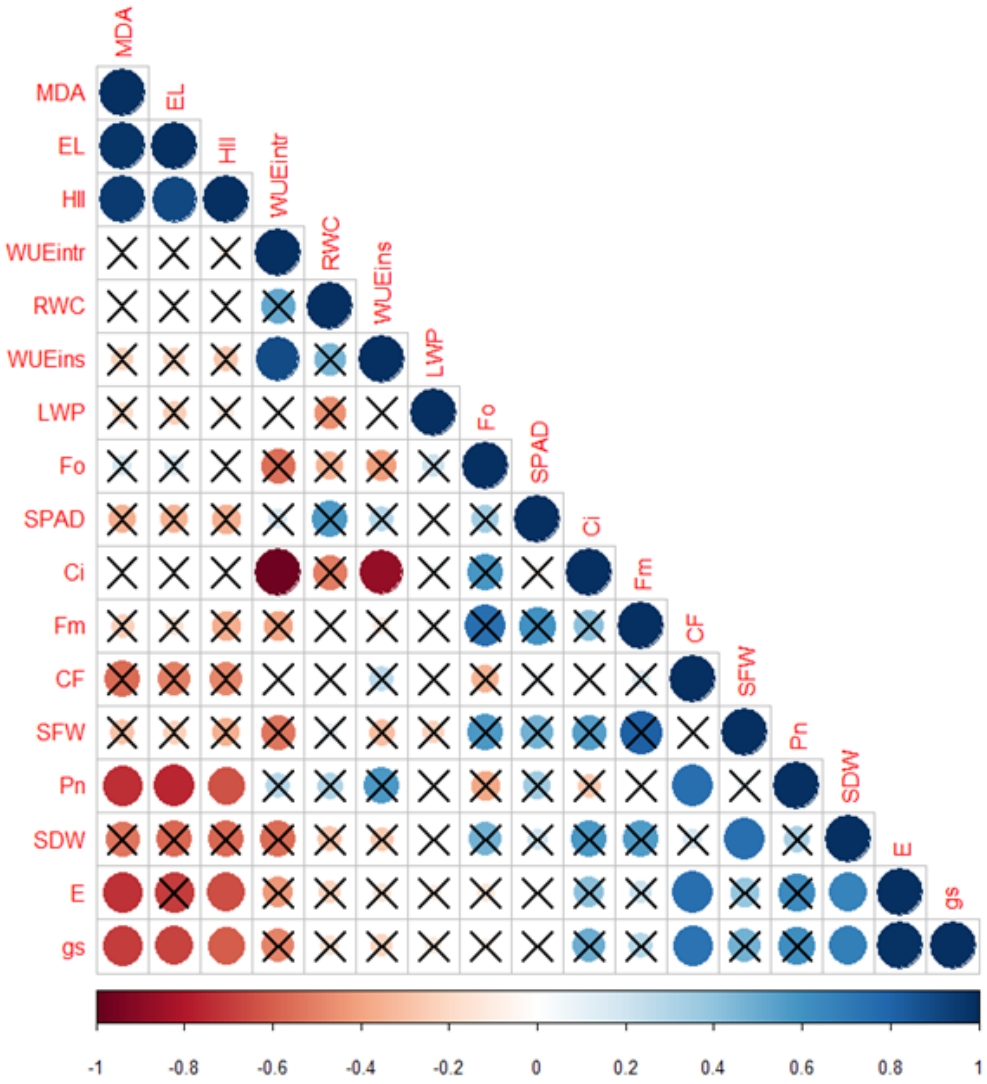

Figure 3.8 Correlogram showing the relationship between variables in heat-shock treatment (40℃- 7 hrs). The intensity of color and size of circle increases with increase in significance of correlation. Dark red denotes high negative correlation whereas dark blue denotes high positive correlation. The cells with cross marks denote not significant correlation between the variables.

There was a significant positive correlation of net photosynthesis rate, stomatal conductance and transpiration rate with chlorophyll fluorescence (Figure 3.8, 0.8, blue dots) under heat-shock condition. This indicates that heat-shock tolerant plants have improved net photosynthesis rate, stomatal conductance, transpiration rate and chlorophyll fluorescence.

Improved photosynthesis under heat-shock is related to improved photochemical efficiency. As in heat-stressed plants, heat-shock also depicted a high positive correlation between malondialdehyde content, heat injury index and electrolyte leakage (Figure 8, 0.95, blue dots). Notably, net photosynthesis rate, transpiration rate and stomatal conductance had a significant negative correlation with electrolyte leakage, malondialdehyde content and heat injury index (Figure 8, -0.6 to -0.8, red dots). It has been established that an increase in electrolyte leakage due to alterations in the permeability of membrane by lipid peroxidation damages the functionality of PSII, and directly hampers carbon assimilation (Li et al., 2015). This suggests that under heat-shock, plants with lower electrolyte leakage, malondialdehyde content and heat injury index are superior in terms of heat-tolerance, as shown by lower injuries to the plants. Moreover, maintenance of the functionality of PSII under lower electrolyte leakage and malondialdehyde content is suggested by the negative correlation of net photosynthesis rate with electrolyte leakage and malondialdehyde content (Hameed et al., 2015). Highly significant correlations of shoot dry weight with stomatal conductance and transpiration rate (Figure 8, 0.6, blue dots) signifies that improved conductance and transpiration could increase shoot dry weight through improvements in net photosynthesis rate.

A heatmap was generated to establish better relationships between the variables under different heat stresses and cluster the variables based on their responses (Figure 3.9). The heatmap clearly distinguished four cluster groups that separated for the variety-temperature treatments. The first, cluster included VG-C to TL-C, the second CELE-SHS to PICUS-SHS, the third TL-HS to TL-SHS and fourth group NEWG-HS to VG-HS, respectively. The treatments were clearly distinguished mainly because of EL, MDA and HII values. The relative expression values of these

variables for different clusters could be observed as: First cluster < Fourth cluster < Second cluster < Third cluster.

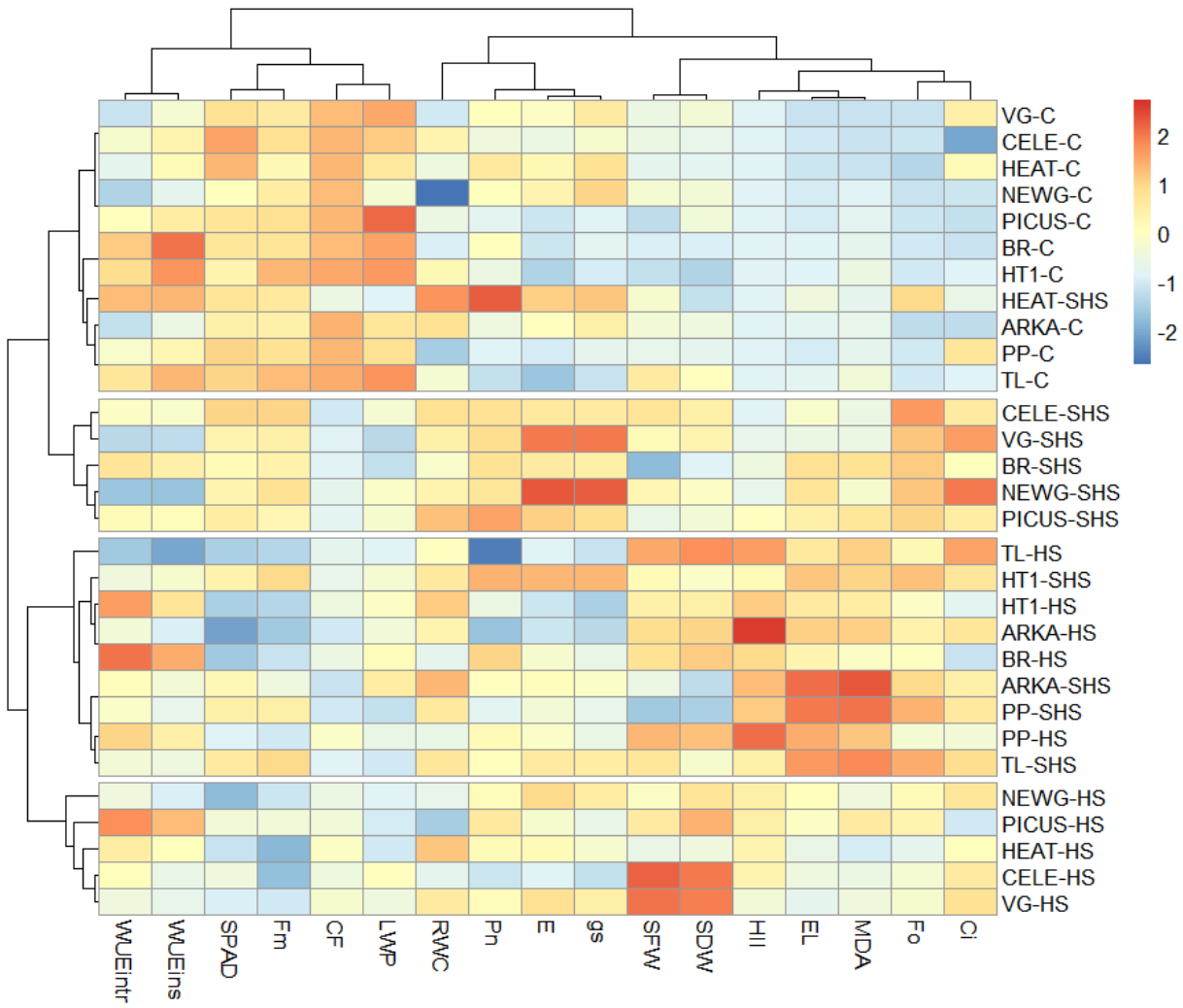

Figure 3.9 Heatmap and clustering of varieties based on the standardized values of the measured variables obtained under exposure to three different temperature regimes: Control (C), Heat-Stress (HS) and Heat-Shock (SHS). Each row represents a cultivar-temperature treatment, and each column indicates a measured parameter. Treatments are clustered based on their measured variables, and variables are clustered based on their correlation. The variables that are clustered together have a high positive correlation. Cells with red and blue color have high and low relative expression, respectively.

From the heat map, we can conclude that the third cluster varieties were the most sensitive ones. They are TL, HT1, ARKA, and PP under heat-stress and heat-shock conditions plus BR under heat-stress conditions. HEAT was found to be the most tolerant under heat-shock as it has been grouped together with the control, which means that it had minimum effects to heat-shock conditions. Likewise, VG, CELE, HEAT, PICUS and NEWG were the superior varieties under heat-stress conditions. For screening tomato varieties for adoption in production systems under high-temperature conditions, it is recommended to assess electrolyte leakage and malondialdehyde content in plant leaves along with observing macroscopic injury symptoms.

3.5. Conclusion

Enough genetic variation was determined for the measured physiological and biochemical parameters, which helped identify elite tomato varieties with heat-stress tolerance. Complex relationships were unraveled between the variables that could potentially create new prospects to be considered for plant heat tolerance studies. Responses of plants differ under transient and persistent heat conditions which makes selection of heat-tolerant plants feasible. Under both heat-stress and heat-shock conditions, assessing electrolyte leakage and MDA contents was the most efficient way to determine heat tolerance or tomato plants' sensitivity. Determining water relations might not aid to the varietal selection when enough moisture is provided; it is decoupled from other physiological mechanisms under high-temperature conditions. In our study, ‘Heat Master’ was the most heat-tolerant variety under heat-shock and ‘Celebrity’, ‘Heat Master’, ‘Valley Girl’, ‘New Girl’ and ‘Picus’ were most tolerant under heat-stress conditions. Similarly, ‘Pruden’s Purple’, ‘Tasti-Lee’, ‘Arkansas Traveler’ and ‘Bella Rosa’ were found to be the most heat-sensitive varieties. This controlled environment study revealed the genetic potential of tomato

plants to a single stress (heat), but under field conditions, plants are exposed to multiple stresses at a time. Thus, further validation under variable field environments should be considered to explore the varieties' true production potentials.

4. SUMMARY AND CONCLUSIONS

This study was conducted to distinguish tomato genetic variation for heat-stress tolerance by short or long-term exposure to varying temperature degrees in contrasting environments. The tomato genotypes used in this study exhibited variable physiological and morphological responses to different heat stress intensities.

Notably, exposure to high temperature in both short-term in a controlled environment and long-term in open-field experiments suggested that tomato plants were undergoing photorespiration, as an increase in intercellular CO_2 concentration was not favoring the increase in net photosynthesis rates. Photorespiration has been established as a wasteful energy cycle in C_3 plants, but it has not been explored as a potential stress indicator for heat-stress tolerance studies, especially in tomato plants grown under open-field conditions. There is a need to adopt a novel technology that can predict photorespiration to explain changes in plant photosynthesis, especially when other physiological functions are normal under abiotic stress conditions like heat stress.

We also observed that tomato plants could be effectively selected for heat-tolerance in a controlled environment, thereby saving time and resources compared to field screening studies. In our study, plants were given enough moisture to avoid combined drought stress and high temperature. Thus, this result holds valid only when sufficient moisture is provided to plants, but might vary based on the environmental conditions plants are exposed in the field. In areas where plants suffer from combined abiotic stress conditions (heat and water stress), it is best to screen plants in field conditions where they are expected to adapt and sustain yield.

In this research study, electrolyte leakage was the best determinant that best distinguished heat-tolerant varieties in any environment or duration of heat exposure. While observing visual

injury symptoms in plants was deemed helpful in screening the varieties for high-temperature tolerance, selecting plants based on only the morphological performance might lead to invalid conclusions as the growth traits of tomato varieties differ and might not relate to their tolerance potential. Therefore, it is imperative to look into the physiological variables that best predict heat-tolerance in the varieties by performing correlation analysis among the variables so that the relationship could be explained well.

Summarizing the results in all environments, the most tolerant varieties were Heat Master, Valley Girl, New Girl, and Celebrity. Adopting these varieties in production systems of South-West Texas is highly recommended to enhance tomato yield in the extreme spring-summer cropping season. Furthermore, using these varieties to explore their potential in combined stress conditions might offer new insights into their production in other stress-prone areas, particularly in southern regions of the US. Understanding the genetic makeup of these varieties to determine the basis of heat tolerance might assist in breeding more heat-tolerant tomato varieties.

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