

**SCREENING OF TOMATO (*Solanum lycopersicum* L.) GENOTYPES FOR
HIGH TEMPERATURE TOLERANCE**

by
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(2021-11-076)

THESIS
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DEPARTMENT OF PLANT BREEDING AND GENETICS
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KERALA, INDIA
2024

DECLARATION

I, hereby declare that this thesis entitled “**Screening of tomato (*Solanum lycopersicum* L.) genotypes for high temperature tolerance**” is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis, for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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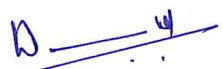
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CERTIFICATE

Certified that this thesis, entitled “**Screening of tomato (*Solanum lycopersicum* L.) genotypes for high temperature tolerance**” is a record of research work done independently by **Ms. Anjali Joy S. L. (2021-11-076)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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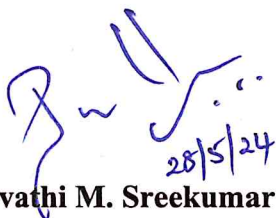
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LIST OF ABBREVIATIONS AND SYMBOLS USED

°C	Degree Celsius
CD	Critical Difference
cm	Centimeter
<i>et al.</i>	And others
Fig.	Figure
g	Gram
GA	Genetic advance
GAM	Genetic advance as per cent of mean
GCV	Genotypic coefficient of variation
ha	Hectare
<i>ie.</i>	That is
KAU	Kerala Agricultural University
kg	Kilogram
mg	Milligram
Mha	Million hectare
mt	Metric ton
mL	Millilitre
mm	Millimeter
nm	Nanometer
No.	Number
PCV	Phenotypic coefficient of variation
SE	Standard Error
Sl.	Serial
t	Ton
µg	Microgram
µL	Microlitre
<i>viz.</i>	Namely
%	Per cent

INTRODUCTION

1. INTRODUCTION

Green revolution was intended to increase productivity of food crops in order to feed the growing population. To attain this goal, various breeding techniques were employed, to improve yield and quality. The effects of climate change are now more severe, and adaptation must be incorporated along with yield and quality. Abiotic stresses, including heat, drought, cold and salinity, can cause severe cellular damage to plant species, affecting growth and yield. High temperature is a significant abiotic element that negatively impacts the vegetative development and reproduction in plants. High temperature is seen as a key threat to agriculture and allied industries in the current scenario of global warming, with major ramifications for food production and quality. The frequency of high temperature extremes has increased in tandem with the changes in the mean temperature, whereas, the frequency of cold extremes has decreased (Gourdji *et al.*, 2013). The Intergovernmental Panel on Climate Change (IPCC, 2007) issued its Fourth Assessment Report, which stated that air temperature increased by 0.2 °C every decade. World Meteorological Organisation (WMO, 2023) reported that there is a 66 per cent chance that the annual average global temperature will be more than 1.5 °C exceeding pre-industrial levels for at least one year between 2023 and 2027.

The NOAA National Center for Environmental Information's 2022 Global Climate Report shows that, 2022 had among the ten warmest months on record, with November being the coolest month. From January to October, every month was among the ten warmest on record. The year ranked sixth warmest since 1880. Asia recorded its second warmest year on record, and 2022 was the 35th consecutive year with temperatures above average.

India's average temperature has risen by 0.15 (annual mean), 0.15 (maximum), and 0.13 (minimum) degrees Celsius per decade between 1986 and 2015 (Sanjay *et al.* 2020). India experienced its fifth warmest year on record in 2022, with an annual mean land surface air temperature of +0.51 °C above the 1981–2010 average. The past decade was the warmest on record, with the pre-monsoon season experiencing more warmth. India experienced above-normal temperatures for ten months, with heat waves affecting nearly 70 per cent of the country (IMD, 2022).

Heat stress affects plant growth, affecting morphology, physiology, biochemistry and molecular pathways. It can hinder seed germination, affect photosynthesis, respiration, water relations, membrane stability, hormone levels and hormone expression. Plants respond to heat stress by maintaining membrane stability, scavenging reactive oxygen species, producing antioxidants, adjusting solutes, triggering enzymes and chaperone signaling and transcriptional activation (Wahid *et al.*, 2007). Recent global warming episodes have ended up in significant economic losses averaging around US\$125 billion (Oyebamiji *et al.*, 2023). It is expected that yield loss of vegetables due to heat stress would rise by 40 per cent in coming decades (Ali *et al.*, 2020).

Tomato (*Solanum lycopersicum* L., $2n=24$), a flowering plant of the nightshade family (Solanaceae), originated in Peru-Ecuador region and is widely cultivated for its culinary fruits. India is the second largest producer of tomato. Tomatoes are a good source of lycopene and are classified as a vegetable for nutritional reasons. The fruits are frequently consumed fresh in salads, cooked as a vegetable, added to a variety of prepared foods and pickled. Tomatoes can be cultivated in a variety of climates, although they are particularly delicate to hot and humid circumstances.

The current cultivars bear fruit in a limited temperature range of 15 °C to 21 °C at night and 30 °C to 35 °C during the day. The ideal daily mean temperature range for tomatoes is between 21 and 24 °C, depending on their stage of growth. While the ideal low and high temperatures for tomatoes have been shown to range from 22 to 28 °C, a few degrees above the ideal temperature range can reduce fruit set and consequently, fruit production (Ayankojo and Morgan, 2020). Above these temperatures, there is insufficient viable pollen production, and pollination and fertilization are challenging, so typically there is no fruit set.

The fertilization of tomato flowers is substantially reduced in high temperature conditions, which causes flower drop and poor fruit set. When exposed to multiple days of daily temperatures over 29 °C and night temperatures over 21 °C, tomato plants lose their blooms, while reproduction is significantly hampered at daytime temperatures over 35 °C. Within 4 hours of tomato exposure to mid-day temperatures

above 40 °C, flower drop can happen (Berry and Uddin, 1988). The optimal air temperature range varies across studies and developmental stages.

Several morphological traits like pollen viability, style protrusion, fruit set and yield are known to be associated with heat tolerance in tomatoes. Molecular markers linked to heat tolerance traits have also been identified. These markers can be effectively utilised for the identification of heat tolerant tomato genotypes. However, a large number of tomato germplasm accessions or varieties must be evaluated for high temperature tolerance and yield characteristics, for utilisation in breeding programmes to develop heat tolerant tomatoes.

Hence, screening of tomato genotypes for high temperature tolerance was taken up with the objective of evaluating tomato genotypes under laboratory field and polyhouse conditions, along with molecular marker screening for the identification of heat tolerant genotypes for tomato breeding programme.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

A review of the importance of tomato as a crop, and the effect of temperature on the growth and yield of tomato is dealt with in this chapter.

2.1 Botany

Tomato is an important vegetable crop that is mostly farmed in tropical areas. Tomato is a warm-season, day neutral crop. Plants typically have many branches, with a spread of 60 and 180 cm and droop when they are fruiting, although some varieties are compact and upright. The leaves can be up to 45 cm long, are slightly hairy, and have a pungent smell. Yellow, pendant, five-petaled flowers measure 2 cm across and are found in clusters. Fruits are berries with a diameter ranging from 1.5 to 7.5 cm.

2.2 Tomato cultivation: Significance and constraints

The tomato is the most consumed vegetable, whether raw or processed, with year round demand for the produce and is cultivated worldwide (Table 1). They are also grown as an ornamental plant in a few parts of the globe (Gatahi, 2020). Tomatoes are useful to maintain a healthy, well-balanced diet. Minerals, vitamins, vital amino acids, carbohydrates, and dietary fibers are abundant in them. Tomatoes are high in vitamin B and C, as well as iron and phosphorus. Yellow tomatoes have more vitamin A than red tomatoes, but red tomatoes have lycopene, an anti-oxidant that provides protection against carcinogens. Suitable for cultivation in tropical as well as sub-tropical regions, they require a moderately cold, dry climate for maximum yield and quality.

Table 1. Statistics of tomato cultivation (FAOSTAT, 2021)

Parameter	India (Official figure)	World (Estimated value)
Area harvested	0.084 Mha	0.52 Mha
Yield	25.066 t/ha	36.60 t/ha
Production	2.12 mt	18.91mt

Tomato production is severely constrained by biotic and abiotic stresses. Many diseases affect the crop, including damping off, bacterial wilt, early blight, leaf spot, and others. Pests such as *Spodoptera*, whiteflies, *Helicoverpa*, etc. are also significant biotic constraints affecting crop production. Abiotic factors such as

drought, frost, salinity, heat, increasing CO₂ concentration, etc. hinder the crop growth and development. Since climate change is a major concern, emphasis is placed on incorporating climate resilience traits in addition to biotic stress resistance/tolerance. High temperature is a significant abiotic factor that has a negative impact on plant vegetative development and reproduction. Tomato crop is extremely sensitive to temperature fluctuations and its reproductive ability is determined by the duration and intensity of the day and night temperatures.

2.3 Temperature requirement of tomato

The crop requires an optimal temperature range of 18 to 25 °C for growth and development (Hurd and Cooper, 1970). High temperatures have been shown to negatively impact tomato yield by 28 to 70 per cent depending on the degree and duration of stress at different phases of crop growth (Alsamir *et al.*, 2019; Ro *et al.*, 2021). During various stages of plant growth and development, each 1 °C temperature increase above 30-35 °C has been found to cause 10-15 per cent crop loss in solanaceous vegetables (Faiz *et al.*, 2020). Every 1 °C increase in temperature above 25 °C (optimum) reduces tomato production significantly (Alsamir *et al.*, 2021). Ajithkumar (1999) reported that the ideal temperature range for tomato crop growth is 30.6 °C to 33.7 °C, and the minimum is 22.1 °C to 24.3 °C, with 24.1 °C to 24.3 °C being the optimum for blooming. Tomatoes prefer cool and dry weather, with ideal temperature ranging from 21 to 28 °C during the day and 15 to 20 °C at night, with night temperature being more crucial than day temperature (Malavika, 2017).

Table 2. Temperature requirement at different growth stages of tomato (Ahmad *et al.*, 2013)

Stages	Temperature (°C)		
	<i>Minimum</i>	<i>Optimum</i>	<i>Maximum</i>
Seed germination	11	16-29	24
Seedling growth	18	21-24	32
Fruit set	18	20-24	30
Red colour development	10	20-24	30

2.4 Effect of high temperature

The maximum and minimum temperature have a higher influence on yield or fruit set than the average temperature during the growing season. This is because exposure to above-optimal temperatures even for a short period during the sensitive reproductive stages of the crop development can significantly influence reproductive traits (particularly gamete viability), resulting in significant yield losses in many crops, and response is dependent on heat stress timing, intensity, and duration (Ro *et al.*, 2021; Giorno *et al.*, 2013). In tomato, day and night temperatures above 38.4 °C and 19.7 °C respectively affect the physiological processes involved in flowering and fruit development, thus reducing fruit set (Hameed *et al.*, 2015). High temperature has major effects on various floral characteristics, which in turn have an impact on fruit set and production. Temperature over 35 °C reduces total dry mass, and root-to-shoot ratio in tomato (Giri *et al.*, 2017).

2.4.1 Effect on plant growth

High temperature affects plant architecture at the tissue, cellular, and subcellular levels, resulting in poor crop development and production (Wahid *et al.*, 2007). Some factors including stomatal closure and water loss, decreased cell size, increased stomatal number, and a greater number of root and shoot xylem vessels are also observed (Anon *et al.*, 2004). Photosynthesis, assimilation, growth, and development are the physiological processes affected by heat stress (Bokszczanin *et al.*, 2013). Depending on the varietal response, plant growth may be reduced at high temperatures (Bhattarai *et al.*, 2021).

According to findings of Guo *et al.* (2022), tomato plants exposed to heat showed a reduced rate of net photosynthesis when compared to plants grown at normal temperature. Hazra *et al.* (2007) showed that high temperature inhibits photosynthesis at temperatures above the optimum, resulting in a significant drop in yield. Rubisco synthesis (Calvin cycle) is a critical step in photosynthesis that was suppressed at temperatures ranging from 35 to 40 °C, leading to decreased net photosynthetic adaptation and carbohydrate generation. Plant biomass accumulation is regulated by the movement of the cyclin-dependent kinase enzyme, which decreases as temperature rises. Heat stress had a substantial influence on the length, and fresh and dry weight of shoot and root.

2.4.2 Effect on reproductive physiology

High temperature negatively impacts processes such as meiosis, stigma position, endothecium development, pollen grain retention on stigma, pollen germination, pollen tube growth, ovule viability, fertilization, post-fertilization, and growth of endosperm, proembryo, and fertilized embryo in plants (Peet *et al.*, 2002). A study examining the effects of high temperatures on ovule development and pollen production in male-sterile tomatoes was conducted by Peet *et al.* (1997) over a 9 month period. The primary parameter affecting the reproductive responses was mean daily temperature, with day temperature playing a secondary role.

Hazra *et al.* (2007) showed that fruit set failure at high temperatures is caused by bud drop, irregular flower development, poor pollen synthesis and viability, ovule abortion, and lack of carbohydrates in tomato. According to Abdul-Baki (1991), blooming in tomato was reduced by 38 per cent and 68 per cent, in the high-temperature resistant and sensitive cultivars respectively, due to heat stress.

Amrutha (2020) reported that yield was positively and significantly correlated with membrane stability index, photosynthetic rate, stomatal conductance, starch content, soluble sugar content, chlorophyll fluorescence (Fv/Fm), pollen viability, total chlorophyll content, number of fruits per plant, fruit set (%) and average fruit weight, and negatively correlated with intensity of flower drop under heat stress conditions.

Vijayakumar *et al.* (2021) found that under heat stress, the content of starch and soluble sugar in the leaves of tomato genotypes decreased significantly. Titrable acidity (TA), total soluble solids (TSS), and ascorbic acid content of tomato fruits were higher at high temperature, while lycopene concentration dropped as temperature increased.

To understand tomato thermotolerance, it is critical to investigate many contributing qualities, such as pollen thermotolerance, style traits, and physiological traits such as membrane stability.

2.4.3 Effect on pollen grain development

Pollen grains, once freed from anthers, function independently and are exposed to the environment. As a result, high-temperature occurrences during flowering would have a bigger impact on pollen than on ovules (Kakani *et al.*, 2005; Weaver, 1989).

Anther and pollen formation during anthesis are particularly sensitive to temperature changes, causing failure of reproduction and fertilization processes (Hoshikawa *et al.*, 2021). Earlier studies have shown that heat stress significantly affects the male reproductive organs and leads to a decrease in the number of pollen grains, pollen viability, and germination (Alsamir *et al.*, 2021). Pollen release and pollen viability may be the key limiting factors for fruit set when exposed to high-temperature stress, especially for longer duration. Pollen release may be a better criterion for screening cultivars or predicting responsiveness to high temperatures, since failure in pollen release will inhibit fruit development regardless of pollen grain viability (Sato *et al.*, 2001).

According to Pressman *et al.* (2002), prolonged exposure of tomato to high temperature significantly reduced pollen viability and the number of pollen grains produced per flower. Based on their evaluation of tomato accessions under high temperatures (32 °C during the day and 26 °C during the night) for total pollen number and pollen viability, Paupière *et al.* (2017) suggested that different accessions may use different strategies for successful pollination under high temperature conditions. Strategies include increasing the total number of pollen grains produced, keeping the fraction of viable pollen high under high temperature, or a combination of both.

Heat stress also causes early abortion of tapetal cells, causing pollen mother cells to quickly move into meiotic prophase and eventually undergo programmed cell death, resulting in pollen sterility. Diminished dehiscence of tomato anther during heat stress is associated with closure of the locules and hence reduced pollen distribution. Continuing heat stress after a successful fertilization might potentially impede embryonic growth (Bita and Gretas, 2013).

Heat stress reduces tomato fruit set significantly, mostly due to impaired pollen development process 8-13 days before anthesis. This sensitivity was demonstrated after both brief and long-term exposure to 40 °C and 32 °C/28 °C (day/night) respectively (Giorno *et al.*, 2013). According to Sato *et al.* (2001), heat stress between 7–15 days before anthesis inhibits pollen grain release, though the sensitive time varies with cultivar. They found higher rates of pollen release after 11 days of high-temperature relief than in controls (optimum temperature). This might be due to a lack

of carbohydrate competition from previously set fruits in relief treatments, compared to plants remaining at 28/22 °C (optimum temperature). Longer durations of respite increased the quantity of pollen grains released, and linear regression analysis revealed a significant association between the percentage of fruit set and the number of pollen grains released.

According to Peet and Bartholemew (1996), the percentage of normal pollen grains was higher when the night time temperature was between 18 and 22 °C compared to 24 and 26 °C. They observed that pollen produced at 18–22 °C (night temperature) had a high germination rate, even when the day temperature reached 26 °C and plants raised at a night temperature of 18 °C also had significant seed content. However, under 26 °C at night, fewer flowers and fruits were present.

Ng and Yanofsky (2001) reported that the tomato flowers that developed under constant mild heat, had a decrease in pollen amount and viability, as well as anther deformations that looked like a partial conversion to pistil was observed. The study conducted by Muller *et al.* (2016) revealed that high temperature can reduce pollen viability and the production of pistil-like anthers in tomato plants.

2.4.4 Effect on pollen germination

In vitro pollen germination is a reliable method for testing pollen viability. However, pollen germination media differs between species and even between crop varieties (Jayaprakash, 2018). Ginoya *et al.* (2022) found that medium having 1 per cent agar, 12 per cent sucrose, and 300 ppm boron resulted in the best germination of fresh pollen in brinjal. Guo *et al.* (2016) reported the germination of tomato pollen, in the media containing sucrose (120 g/L), boric acid (120 mg/L), gibberellin (4 mg/L), thiamine (0.5 mg/L), and agar (1%). When the length of the germinated pollen tube is equal to or greater than the diameter of the pollen grain, it was considered to have germinated (Kakani *et al.*, 2002).

The study conducted by Maisonneuve and Den Nijs (1984) compared *in vitro* pollen germination of four tomato varieties at different temperatures and their plant growth under controlled conditions. Pollen germination was slow at 10 °C, but after 6 hours of exposure, the percentage of germination was comparable to that at 22 °C. Maximum germination under 14 °C was attained after 4 hours of exposure. Differences in pollen tube growth rate were greater at 22 °C than at 10 °C.

Charles and Harris (1972) observed that, when pollen originated and germinated at 18.3 °C, there was no difference between pollen sampled at the intermediate and late stages of development; however, when pollen developed at 26.7 °C, germination of pollen from the late stage was inferior to that from the intermediate stage. Pollen grown at 10 °C did not form tubes. Pollen grown at 26.7 °C exhibited a lower percentage of germination at 10 °C than pollen developed at 18.3 °C.

Sato *et al.* (2001) reported that in tomato, at 32/26 °C, there was no germination of released pollen grains for NC 8288, and that pollen germination was significantly reduced even in the tolerant cultivar, FLA 7156. Pollen grain germination was reduced in high-temperature grown plants. However, under chronic, mild heat stress, the amount of pollen grains generated, photosynthesis, and night respiration did not affect fruit set. This revealed that cultivar variations in pollen release and germination under heat stress are the most critical determinants affecting fruit set.

2.4.5 Effect on stigma exsertion

Tomatoes are prone to stigma exsertion at high temperature. Although inserted stigma is a well-established phenotype in modern tomatoes, exserted stigma is still found in a few landraces and vintage varieties. Furthermore, exsertion of the stigma is a common response to high temperature stress, and as a cause of reduced fertility, is becoming increasingly important (Riccini *et al.*, 2021). In contrast to the stigma exsertion seen in wild tomato species, Pan *et al.* (2019) showed that the stigma exsertion generated by high temperature in cultivated tomatoes is caused by more severely reduced stamen length than pistils. The study showed that tomato flowers exposed to high temperatures had elevated expression of the genes involved in jasmonate production and signalling, and that under normal temperature settings, exogenous jasmonic acid administration may cause tomato flowers to exert their stigma.

Saeed *et al.* (2007) discovered that elevated temperatures increased the length of the style of different tomato genotypes by 25–55 per cent. Different temperature and time gradients could affect the elongation of exserted stigma, and the functional style male sterility of stigma exserted tomato, could reach a 95 per cent infertility rate in the field exposed to 20 °C and higher temperature (Yu *et al.*, 2015).

2.4.6 Effect on fruit development

Under heat stress, the heat-tolerant genotypes maintain normal truss growth, while, the heat-sensitive genotypes exhibit bud and blossom drop. At high temperatures, the floral organ growth of the heat-tolerant genotype was found to be superior to that of the heat-sensitive genotype. Heat stress inhibited tomato fruit set when administered prior to anthesis but not immediately after anthesis (Zhou *et al.*, 2017).

Iwahori (1966) discovered that ovules which were subjected to high temperature 18 hours after pollination aborted, which could be attributed to slowing or stopping pollen tube elongation at high temperature. Endosperm degeneration was observed in ovules that had been subjected to high temperature for 24 to 96 hours after pollination.

There is a tendency towards the development of small parthenocarpic fruits at both high (26 °C) and low (14 °C) temperature regimes, coupled with a low flower count and fruit set (Malavika, 2017). According to Abdul-Baki (1991), regardless of the level of heat tolerance displayed by the line or cultivar, the production of viable seeds under high-temperature regime was drastically reduced or completely inhibited. Since even the most heat-tolerant and heat-sensitive cultivars or lines were unable to produce viable seeds under high heat conditions (39 °C day/28 °C night), he suggested the utilization of a lower heat stress to evaluate thermotolerance of varieties ensuring production of viable seeds.

According to Zhou *et al.* (2017) reduction in chlorophyll content, photosynthesis, and carbohydrate content in mature tomato leaves might be linked to fruit set failure at high temperatures. Their findings revealed that physiological responses to heat stress at the seedling stage matched with the reactions at the anthesis stage, suggesting that heat stress sensitivity may be tested in young plants. Fruit set was selected by Firon *et al.* (2006) as the final indicator of a genotype's resistance to high temperature, and it was found to be not related to pollen viability in tomato (Gonzalo *et al.*, 2020).

2.4.7 Effect on plant physiology

High temperature increases membrane permeability and causes membrane disintegration, which can be used to detect heat stress in tomato. Thermostability of

plasmalemma, an indicator of thermotolerance, is measured as electrolyte leakage from leaf discs over a range of temperatures and is a sensitive and rapid method to evaluate heat tolerance in plants. It is highly correlated to fruit set and yield under high temperature conditions in tomato (Alsamir *et al.*, 2017).

Mittler *et al.* (2012) investigated how plants responded to extreme temperatures by varying the fluidity of the cell membrane. Calcium channels are activated when the fluidity of the cell membrane changes. Calcium uptake into cells creates transduction signals for ROS production and transcription factor activation, most prominently heat shock factors (HSFs). According to Mittler *et al.* (2012) the heat stress response is triggered by a plasma membrane channel that begins an inward calcium flow, a histone sensor in the nucleus, and two unfolded protein sensors in the endoplasmic reticulum and the cytoplasm. Each of these potential sensors is considered to activate a comparable collection of heat stress resistance genes, resulting in increased thermotolerance.

Reactive Oxygen Species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$) acts as signal transduction molecules to enhance plant response under abiotic stress conditions, and their buildup generates oxidative damage (Miller *et al.*, 2008). Understanding the underlying effect of combined stress on plant ROS metabolism and antioxidant mechanisms is essential for crop development in a changing environment.

According to Zhou *et al.* (2019) tomato stress tolerance under drought and heat is directly related to its ability to respond to oxidative damage and the control of the ROS scavenging system, which has a unique ROS response caused by the combined stress.

2.5 Genetics of high temperature tolerance

According to Villareal *et al.* (1978), many genes and environmental factors, as well as recessive genes with low heritability, play a role in controlling heat tolerance (Table 3).

Bhattarai *et al.* (2016) showed that the majority of yield-related variables were controlled by additive gene action, and selection in a segregating population might boost these features. The improvement of tomato heat resistance features by indirect

selection for yield utilizing yield-attributing morphological qualities was suggested for increasing yield.

Heat stress tolerance is a difficult trait to improve due to its low heritability and sensitivity to the surrounding environment (Hazra *et al.*, 2009). Although desired features from wild sources can be added to cultivated tomatoes, the offspring frequently exhibit undesirable agronomic traits. The main issues are brought on by the quantitative nature of the traits associated with heat tolerance, with numerous genes involved (Alsamir *et al.*, 2021).

Ayenan *et al.* (2021) found that *Solanum pimpinellifolium* demonstrated significant pollen quantity and viability characteristics, making it a potential source to widen the genetic base for high pollen viability and inflorescence numbers per plant in cultivated tomatoes. Despite this, they had a low fruit set. Characterization of tomato germplasm to assess diversity, and to find new sources of heat tolerance is required in order to accelerate the production of heat-resistant lines (La Pena and Hughes, 2007).

Using suppression subtractive hybridization, Shen *et al.* (2014) identified a gene encoding ureidopropionase (SIUPB1, EC 3.5.1.6) from the stamen of a heat-tolerant tomato (CL5915), which catalyzes the synthesis of alanine. SIUPB1 expression was 2.16 and 2.93 times higher in CL5915 stamens grown at 35/30 °C (day/night) than in a heat-sensitive tomato (L4783) grown at 30/25 °C or 25/20 °C, respectively, during the anthesis stage.

A cell's acquisition of thermotolerance is associated with increased production of a group of proteins known as heat-shock proteins (Hsps) (Nakamoto *et al.*, 2000). The SNAT (5-hydroxytryptamine-N-acetyltransferase) enzyme is involved in the production of melatonin, which has been linked to thermotolerance regulation in several plants. Wang *et al.* (2020) discovered the SISNAT gene, which is responsible for melatonin production in tomatoes. Overexpression of *SISNAT* in tomato plants improved thermotolerance and growth, performance in Photosystem II (PSII) with maximal photochemical quantum yield (Fv/Fm) and reduced heat damage

2.6 Breeding for high temperature tolerance

Heat tolerance has been transferred to cultivated varieties using both traditional and modern breeding methods. AVRDC screened thousands of tomato genotypes in

arid environments and found less than 1 per cent to be tolerant to heat stress (Oyebamiji *et al.*, 2023).

According to Hazra *et al.* (2009), both additive and dominance genetic variance were important for fruit yield and floral characters; however, the dominance component was larger in magnitude for other characters. Most of the characters showed over-dominance, with partial dominance for days to flowering, trusses/plant, and pollen viability, indicating the importance of a non-additive genetic system for the expression of characters influencing heat tolerance. They observed low narrow sense heritability estimates for a majority of the characters; single plant selection will be ineffective in such a condition. They reported that selection for high temperature fruit set should be based primarily on replicated family testing in F₃ and later generations.

Hall (1992) opined that in order to transfer traits controlled by major genes that contribute to heat tolerance, the pedigree method, backcross method, and recurrent selection methods can be used.

Hybridization is a useful breeding strategy to incorporate heat tolerance. By crossing two distinct genotypes, Summertime (heat tolerant) and Campbell 28 (susceptible to heat stress), a heat tolerant tomato variety, L72, was developed by Hazra *et al.* (2007). Due to the significant role of dominance genetic variance in influencing different fruit set characters under high temperature, as well as the large negative genetic correlation between heat tolerance and fruit size, heterosis breeding may be a viable strategy to develop heat tolerant hybrids with better fruit size and quality (Oyebamiji *et al.*, 2023).

Genetic diversity tends to decrease with continuous screening and selection, so variability needs to be created. HT7 is a mutant tomato variety from the Micro Tom tomato mutant collection that produces fruit with seeds, as well as increased fruit number, pollen number, pollen viability, and heat-related gene expression under heat stress conditions (Pham *et al.*, 2020).

Heat tolerance is a quantitative trait involving a number of genes with smaller effects. Only a few reports exist on the fine mapping of heat tolerance QTL and the identification of HSR genes in tomato. SSR markers were used to identify five major QTLs for heat tolerance in tomato from a cross between LA1698 and LA2093 (Wen *et al.*, 2019). After thorough validation and determination of their function, identified

QTLs can be introgressed into cultivated varieties using marker assisted breeding to improve heat tolerance (Oyebamiji *et al.*, 2023).

Gene editing techniques such as CRISPR-Cas9, RNAi, and others have been used to improve the tolerance to high temperature in crops. Mitogen-activated protein kinases (MAPKs) are highly conserved serine and threonine protein kinases that play a role in plant stress response. In tomato, 16 putative SIMAPK genes have been identified, with *SIMAPK3* being one of the most extensively studied. The CRISPR-Cas9-mediated *SIMAPK3* mutant of tomato demonstrated greater resistance to elevated temperature than the control, confirming the role of *SIMAPK3* gene as a negative regulator of thermotolerance (Yu *et al.*, 2019). Under stress conditions, the *SIMAPK3* mutant showed less membrane damage, less wilting, lower ROS levels, and high activity and abundance of antioxidant enzymes as well as transcripts (Kim *et al.*, 2021).

The SIAGAMOUS-LIKE (SIAGL6) gene encodes for the parthenocarpic phenotype in tomato (Klap *et al.*, 2017). Knockout of the SIAGL6 gene using CRISPR-Cas9 leads to increased fruit setting under high temperature (Ahmar *et al.*, 2020).

To meet the food demand of the growing population, challenges of changing environmental conditions, and consumer preferences, the breeding cycle must be shortened. Incorporating biotechnological approaches (such as genome editing and omics) into speed breeding will help to improve plant traits and confer tolerance to heat stress (Oyebamiji *et al.*, 2023).

Table 3. Key genes related to heat stress mechanism in tomato

Gene/locus symbol	Origin	Defined function	Related trait/phenotype	References
SOD	Tomato	Antioxidant enzyme	Antioxidant defence	Zhou <i>et al.</i> , 2019
APX	Tomato	Antioxidant enzyme	Antioxidant defence	Zhou <i>et al.</i> , 2019
SENU2	Tomato	Senescence-associated cysteine proteinase	Leaf senescence	Drake <i>et al.</i> , 1996
HsfA1, a, b, c, e	Tomato	Transcriptional activators to HS	Transcription regulatory network	El-Shershaby <i>et al.</i> , 2019
HsfA1b	Tomato	Later response gene in transcription regulatory network	Transcription regulatory network	El-Shershaby <i>et al.</i> , 2019
ERF.C1/F4/F5	Tomato	Ethylene-responsive transcription factors	HS regulation	Balyan <i>et al.</i> , 2020
HSFA7, HSFA6b, HSFA4c, HSFB1, HSFB2b	Tomato	Downstream targets of HSFA	Transcription regulatory network	Rao <i>et al.</i> , 2021
TTS, TGL 11	Tomato	Pistil-specific expression	Flower morphology	Muller <i>et al.</i> , 2016
TAP3, TM6, PI	Tomato	Class B activity	Flower morphology	Muller <i>et al.</i> , 2016
AGL6	Tomato	MADS-box transcription factor, fruit parthenocarp	Flower morphology, fruit parthenocarp	Klap <i>et al.</i> , 2017
CLV	Tomato	Signal peptide, shoot, and floral meristem regulation	Shoot and floral meristem	Somssich <i>et al.</i> , 2016
WUS	Tomato	Homeodomain transcription factor, shoot and floral meristem regulation	Shoot and floral meristem	Somssich <i>et al.</i> , 2016
TAG1, TAGL 1	Tomato	MADS-box transcription factor	Fruit size	Gimenez <i>et al.</i> , 2016
CCS52A, WEE1	Tomato	Cell cycle switch protein	Fruit size	Gonzalez <i>et al.</i> , 2007
miRNA 172	Tomato, apple	miRNA	Fruit size	Jose Ripoll <i>et al.</i> , 2015
FAS, LC	Tomato	Flattening and fruit locule number	Fruit size	Rodriguez <i>et al.</i> , 2011
SUN, OVATE	Tomato	Fruit elongation	Fruit size	Rodriguez <i>et al.</i> , 2011
LIN5	Tomato	Cell wall invertase	Fruit sugar	Fridman <i>et al.</i> , 2000.
SUT or SUC	Tomato, Arabidopsis, potato	Sucrose transporter	Fruit sugar	Barker <i>et al.</i> , 2000.
SIVPEs	Tomato	Vascular processing enzymes, negative regulators	Fruit sugar	Ariizumi <i>et al.</i> , 2011

2.7 Screening for thermotolerance

Several criteria, including membrane thermostability, floral features (stigma exertion and antheridial cone splitting), flower number, and yield per plant, have been shown to be reliable in assessing tomato heat stress resistance (Alsamir *et al.*, 2021). Ayenan *et al.* (2019) observed that considering the complexity in plant response to heat tolerance, a combination of physiological and morphological traits will be required to select thermo-tolerant lines in tomato.

2.7.1 Screening for morphological traits

Sherzod *et al.* (2020) grew 38 tomato accessions with three fruit sizes (cherry, medium, and large) in greenhouses at 25 °C (normal temperature) and 40 °C (high temperature). They discovered that heat boosts plant height and stem diameter, while has minimal influence on leaf length and breadth. High temperature has a beneficial influence on the number of flowers but a negative effect on the number of fruits, fruit set, weight and yield, pollen germination, and tube length.

Solankey *et al.* (2017) identified days to first flowering, days to 50% flowering, days to first fruit set, fruit setting (%) and number of fruits per plant as the major yield contributing morphological characters under heat stress based on correlation analysis.

Huimin *et al.* (2019) investigated the impact of high temperature stress on tomato seedling growth and flower bud differentiation. The ‘Zhongza 9’ tomato seedlings were subjected to 37 °C/27 °C treatment, while 25 °C/15 °C was used as control. Results showed higher plant height and internode length, smaller stem diameter, lower leaf area and root surface area, and accelerated flower bud differentiation under high temperature conditions. High temperature treatment significantly accelerates flower bud differentiation from the first day, decreases on the 9th day, and increases flower bud size from the 5th day to the 7th day. The study concluded that high temperatures inhibited vegetative growth, accelerated flower bud differentiation, and reduced bud size, ultimately leading to decreased yield.

Ro *et al.* (2021) examined eleven tomato genotypes under ideal and high temperature in the field, and high temperature in greenhouse settings. Tomato genotypes performed better under ideal temperature field circumstances compared to high temperature field and greenhouse environments. Under ideal temperature-field conditions, fruit yield, fruit setting, and plant biomass were higher. Under high

temperature field and greenhouse conditions, the genotypes showed a reduction in fruit production, fruit setting, and plant biomass.

2.7.2 Marker assisted screening

Although marker assisted breeding can accelerate the identification of genotypes, the success of marker assisted breeding depends on reliable QTL information. Two QTLs have been reported by Kadirvel (2010) on chromosomes 6 (TES0111- SLM6-5) and 12 (SLM12-31-SLM12-50) involved in heat tolerance in tomato.

Kamel *et al.* (2010) assessed the heat tolerance of 23 local lines and cultivars. They reported three heat-tolerant markers: two RAPD markers (A16-100 bp and Z13-500 bp) and one ISSR marker (650 bp).

Shaji *et al.* (2020) through traditional QTL analysis found qCC-2-2 on chromosome 2 with the SSR 96 flanking marker as a QTL for heat tolerance. SSR 63 and SSR 96 revealed a distinct polymorphism for high temperature tolerance between high temperature tolerant and susceptible types.

Makhadmeh *et al.* (2022) found that the ABA/WDS-induced protein gene (Solyc10g011690), which encodes the protein ABA/WDS-induced, was linked to the Asr2 SSR marker. This protein is associated with water deficit stress (WDS) or abscisic acid (ABA) stress. In addition, this gene helps other plant species, including durum wheat, barley, and *Pinus taeda* L., to tolerate a variety of abiotic conditions like dryness, heat, and salt (Makhadmeh *et al.*, 2022).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled ‘Screening of tomato (*Solanum lycopersicum* L.) genotypes for high temperature tolerance’ was carried out during 2021 - 2023. Experiments were carried out at the field (Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara) and polyhouse (Department of Plant Physiology, College of Agriculture, Vellanikkara). The objective of the study was to evaluate tomato accessions to identify high temperature tolerant genotypes, which can be further utilized in the development of resistant or tolerant varieties or hybrids. Materials used and methodologies adopted are presented in this chapter.

3.1 Materials

3.1.1 Experimental site

The field experiments were conducted at the experimental field plot of the Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, Thrissur, Kerala. The area is situated between 10°32'47" N and 76°16'43" E at an altitude of 97 m above MSL. The weather parameters at Vellanikkara during the period of study is presented in Table 4.

The polyhouse experiments were conducted at the experimental polyhouse of the Department of Plant Physiology, College of Agriculture, Vellanikkara, Thrissur, Kerala. Selected genotypes were subjected to heat stress under the polyhouse for morphological and physiological evaluation. Polyhouse of size 21.37 m² was cladded with 0.15 mm thick polyethylene sheet with roofing of 0.25 mm thick poly filter which transmit 80 per cent of full spectrum radiation and had a 0.25 mm thick polyethylene sheet on all four sides for inducing high temperature.

The laboratory screening was conducted at the laboratories of the Department of Plant Breeding and Genetics and the Department of Plant Physiology, College of Agriculture, Vellanikkara, Thrissur, Kerala.

3.1.2 Experimental material

The material consisted of 30 tomato genotypes including thirteen NBPGR (National Bureau of Plant Genetic Resources) accessions, four hybrids from IIHR, Bengaluru, eight breeding lines from World Vegetable Center, Taiwan and five varieties of Kerala Agricultural University. The details of genotypes used are

presented in Table 5. Laboratory screening was done during September–December (2022) season; polyhouse and field screening was done during April–June (2023) season.

Table 4. Weather parameters at Vellanikkara

Month	Temperature (°C)				Mean Relative humidity (%)
	Mean maximum	Highest maximum	Mean minimum	Lowest minimum	
August (2022)	29.9	32.6	23.6	22.2	84
September (2022)	31.1	33.3	23.7	22.9	81
October (2022)	32.0	33.8	23.6	21.6	77
November (2022)	32.4	34.1	23.0	21.0	73
December (2022)	32.2	34.4	22.6	18.6	66
January (2023)	33.3	35.0	22.6	18.9	56
February (2023)	34.8	36.2	23.3	19.5	84
March (2023)	57.0	38.0	36.1	24.2	57
April (2023)	36.8	40.0	25.5	23.6	67
May (2023)	34.7	36.2	25.7	23.5	74
June (2023)	31.8	35.1	24.4	22.8	83

Table 5. Genotypes screened for tolerance to high temperature

Sl. No.	Genotypes	Source
1	AVTO 0301	World Vegetable Centre, Taiwan
2	AVTO 0922	"
3	AVTO 1314	"
4	AVTO 1702	"
5	AVTO 1706	"
6	AVTO 1707	"
7	AVTO 1725	"
8	AVTO 1726	"
9	EC 315489	NBPGR
10	EC 523851	"
11	EC 528360	"
12	EC 528368	"
13	EC 538153	"
14	EC 549819	"
15	EC 567305	"
16	EC 620428	"
17	EC 620486	"
18	EC 620488	"
19	EC 620494	"
20	EC 631354	"
21	EC 636872	"
22	Arka Abhed	IIHR, Bengaluru
23	Arka Rakshak	"
24	Arka Samrat	"
25	Arka Vishesh	"
26	Akshaya	KAU
27	Anagha	"
28	Manuprabha	"
29	Manulakshmi	"
30	Vellayani Vijay	"

3.2 Methods

3.2.1 Laboratory screening for high temperature tolerance

The seeds of 30 selected genotypes were treated with *Pseudomonas flourescence* (8 g/kg seeds for 8 hours) and were sown in pro trays. The potting mixture comprised of coir pith, vermiculite, and perlite in a 3:1:1 ratio. Pro trays helped in proper germination as well as reduced mortality rate, and 30 days old seedlings were transplanted. Seedling root dip with streptomycin (1 g/10 L) was given before transplanting to prevent bacterial wilt.

Plants were raised during September- December in Completely Randomised Design (CRD) with three replications. The crop was grown in 35 x 20 x 20 cm UV-stabilized polybags with a gauge of 600 (150 microns). To keep animals and birds out of the field, a net was erected around it. To stop weed growth, a weed mat was laid out on the ground. Agronomic and cultural practices were carried out in accordance with the Package of Practices Recommendations: Crops (KAU, 2016).

3.2.1.1 Pollen viability (%)

For the pollen viability screening, newly opened flowers were collected in the morning from 9 to 11 a.m. under natural conditions. In total, two flowers per plant were collected from all the plants per accession. Flowers were collected into a polythene cover with wet tissue. Anthers were cut into pieces with a razor blade and put into a 1.5 mL Eppendorf tube. A common method to assess pollen viability is by staining and direct counting (Heslop-Harrison, 1992). For this purpose, anthers from open flowers were collected and placed in 1% acetocarmine solution (Singh, 2003). After that, the pollen was dispersed on the slide, and the number of stained (viable) and unstained (nonviable) grains was counted.

$$\text{Pollen viability} = \frac{\text{Number of viable pollen}}{\text{Total number of pollen}} \times 100$$

3.2.1.2 Pollen germination (%)

For the pollen germination test, pollen grains from two flowers of each plant under ambient conditions were spread uniformly on the germination medium on a glass slide. Germination media used in this study contained sucrose (120 g/L), boric acid (120 mg/L), gibberellin (4 mg/L), thiamine (0.5 mg/L) and agar (1%) (Zhou *et al.*, 2015). Glass slides were incubated at room temperature, 35 °C and 40 °C for 8 hours under dark conditions. Pollen grains were considered germinated when pollen tube length was greater than pollen diameter.

$$\text{Pollen germination (\%)} = \frac{\text{Number of pollen germinated}}{\text{Total number of pollen}} \times 100$$

3.2.1.3 Style protrusion (%)

Style is considered as protruded when the style is elongated and stigma protrudes above the anther cone. Style protrusion (%) was calculated using the following formula:

Style protrusion (%)

$$= \frac{\text{Number of flowers with protruded style in a cluster}}{\text{Total number of flowers in a cluster}} \times 100$$

3.2.1.4 Electrolyte leakage and membrane stability

Electrolyte leakage (%) was estimated using leaf tissue following the method suggested by Yeh and Lin (2003). Five to ten leaf discs of 9 mm in diameter were made using a paper punching machine from young fully expanded leaves. Leaf discs were rinsed three times thoroughly in distilled water to remove dirt and dust and immersed in 10 mL of distilled water taken in a test tube, and incubated at room temperature for 1 hour, and the initial EC was recorded. Subsequently they were autoclaved for 15 minutes, and the final EC was recorded. Electrolyte leakage (%) was calculated using the following formula:

$$\text{Electrolyte leakage (\%)} = \frac{\text{Initial EC}}{\text{Final EC}} \times 100$$

Membrane stability index (%) was calculated using the initial and final EC obtained as described above and applied to the formula proposed by Saleh (2013).

$$\text{Membrane stability index (\%)} = \frac{\text{Final EC} - \text{Initial EC}}{\text{Final EC}} \times 100$$

3.2.1.5 Style length (cm)

The length of the style, from the tip of ovary to the tip of stigma, was recorded on the day of flower opening.

3.2.1.6 Temperature (°C)

The temperature and relative humidity inside the chamber were properly monitored and recorded using a field thermometer at every one-hour interval for 24 hours. The temperature and relative humidity under ambient conditions were obtained from the Department of Agricultural Meteorology, College of Agriculture, Vellanikkara.

3.2.2 Polyhouse screening for thermotolerance

Screening was conducted in a set up with no ventilation so that temperature build up was ensured (Plate 1). The minimum, maximum and average temperature and relative humidity data inside the polyhouse and open condition during screening is given in Table 6.

Table 6. Weather parameters inside the polyhouse and in open condition

	Temperature (°C)			Mean relative humidity (%)
	Maximum	Minimum	Average	
Open	30.6	23.5	27	81.85
Polyhouse	34.4	25.5	28.7	86.3

Plants were raised during April-June in Completely Randomised Design (CRD) with three replications. The crop was grown in 35 x 20 x 20 cm UV-stabilized polybags with a gauge of 600 (150 microns) at a spacing of 0.6 m X 0.6 m. Agronomic and cultural practices were carried out in accordance with the Package of Practices Recommendations: Crops (KAU, 2016).

3.2.2.1 Observations

Electrolyte leakage and membrane stability, pollen viability, pollen germination, style length and style protrusion were recorded as described in section 3.2.1.4. Pollen collected from heat stress condition (polyhouse) was incubated only at room temperature for assessment of pollen germination.

3.2.3 Field screening for thermo-tolerance

The seeds of 30 selected genotypes were treated with *Pseudomonas fluorescens* (8 g/kg seeds for 8 hours) and were sown in pro trays. The potting mixture comprised coir pith, vermiculite, and perlite in a 3:1:1 ratio. Pro trays helped in proper germination as well as reduced mortality rate, and 30 days old seedlings were transplanted to the main field (Plate 1).

Plants were raised during March to May in Completely Randomised Design (CRD) with two replications having 15 plants/replication. The crop was grown in 35 x 20 x 20 cm UV-stabilized polybags with a gauge of 600 (150 microns) at a spacing of 0.6 m X 0.6 m. To keep animals and birds out of the field, a net was erected around it. To stop weed growth, a weed mat was laid out on the ground. Agronomic and cultural practices were carried out in accordance with the Package of Practices Recommendations: Crops (KAU, 2016). Seedling root dip with streptocycline (1 g/10 L) was given before transplanting to prevent bacterial wilt.

3.2.3.1 Pollen and Style characters

Observations on pollen viability, style protrusion (%) and style length (cm) was recorded from two clusters/plant as described in section 3.2.1.4.

3.2.3.2 Fruit set (%)

Fruit set per cent accounts for the number of flowers that have been converted into fruits which was observed at the initial stages of fruit development. Fruit set (%) was calculated using the following formula suggested by Dahal *et al.* (2015):

$$\text{Fruit set (\%)} = \frac{\text{Number of flowers set/cluster}}{\text{Total number of flower buds/cluster}} \times 100$$

3.2.3.3 Deformed fruits (%)

Accounts for the number of flowers that failed to get converted into mature fruits. Deformed fruits (%) was calculated using the following formula:

$$\begin{aligned} \text{Deformed fruits (\%)} \\ = \frac{\text{No. of flower buds} - \text{No. of fruits harvested}}{\text{No. of flower buds}} \times 100 \end{aligned}$$

3.2.3.4 Number of fruits/plant

The total number of fruits produced by each plant was recorded.

3.2.3.5 Fruit yield/plant (g)

The weight of fruits produced by each plant was recorded in grams.

3.2.3.6 Average fruit weight (g)

The average weight of fruits produced by each genotype was recorded.

3.2.3.7 Plant height (cm)

The height of the plant from ground level to the topmost leaf bud was recorded.

3.2.3.8 Days to 50 per cent flowering

The number of days after transplanting until 50 per cent of the plants in a replication had at least one open flower was recorded.

3.3 Statistical analysis

3.3.1 Analysis of variance

KAU GRAPES software was used to perform analysis of variance (ANOVA) (Gopinath *et al.*, 2020). The mean value of traits of each genotype in each replication was used to test for the differences between genotypes (Table 7).

Table 7. Analysis of variance

Source of variation	Degrees of freedom	Mean squares	F value
Treatment	(t-1)	TrMS	TrMS/EMS
Error	t(r-1)	EMS	
Total	(rt-1)		

r = Number of replications

t = Number of treatments

TrMS = Treatment mean square

EMS = Error mean square

Standard Error of Mean (SEm) = $\sqrt{2EMS/r}$

Critical difference (CD=0.05) = $SEm \times t_{(0.05, \text{error d.f})}$

where, $t_{(0.05, \text{error d.f})}$ is the student's t table value at error degrees of freedom and at five per cent level of significance.

3.3.2 Estimation of genetic parameters

For each character, genotypic and phenotypic variances, genotypic and phenotypic coefficient of variation, heritability, and genetic advance as a per cent of mean were calculated.

3.3.2.1 Genotypic variance (σ_g^2)

$$\sigma_g^2 = \sigma_p^2 - \sigma_e^2$$

where, σ_p^2 – Phenotypic variance

σ_e^2 – Error mean square

3.3.2.2 Phenotypic variance (σ_p^2)

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

where, σ_g^2 – Genotypic variance

σ_e^2 – Error mean square

3.3.2.3 Genotypic Coefficient of Variation (GCV)

$$GCV = \frac{\sigma_g}{\bar{X}} \times 100$$

Where, σ_g - Genotypic standard deviation

$\bar{X}$ - Grand mean

A)



B)



**Plate 1. View of screening of tomato genotypes under polyhouse and field conditions
A) Polyhouse screening; B) Field screening**

3.3.2.4 Phenotypic Co-Efficient of Variation (GCV)

$$PCV = \frac{\sigma_p}{\bar{X}} \times 100$$

Where, σ_p - Phenotypic standard deviation

$\bar{X}$ - Grand mean

According to Sivasubramanian and Menon (1973), GCV and PCV were categorised as low (<10%), moderate (10-20%) or high (>20%).

3.3.2.5 Heritability (H^2)

Broad sense heritability was calculated and expressed in percentage.

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where, σ_g^2 – Genotypic variance

σ_p^2 – Phenotypic variance

Robinson *et al.* (1949) grouped heritability (%) as low (0-30%), moderate (31-60%) and high (>60%).

3.3.2.6 Genetic advance as per cent of mean

Genetic advance was estimated by the method formulated by Johnson *et al.* (1955).

$$GA = K. \sigma_p.h^2$$

where, K – Selection differential at 5% selection intensity (2.06)

σ_p - Phenotypic standard deviation

H^2 – Heritability

GA was also expressed as mean (GAM)

$$GAM = \frac{\text{Genetic advance}}{\text{Mean}} \times 100$$

GAM was categorized as low (<10%), moderate (10-20%) and high (>20%) by Johnson *et al.* (1955).

3.3.3 Estimation of correlation

Correlation coefficient is the statistical measure which indicates the degree and direction of association between two variables. The correlation

between various traits was assessed using KAU GRAPES software (Gopinath *et al.*, 2020) as per the method suggested by Pearson (1905).

3.4 Molecular marker analysis

3.4.1. Laboratory chemicals, glassware and equipment

In this study, Sisco Research Laboratories' (SRL) AR (analytical reagents) grade chemicals (extra pure) and Genei were used. The primers were synthesized by Sigma Aldrich Chemical Pvt. Ltd. in Bangalore. For centrifugation, a high-speed refrigerated centrifuge (Eppendorf 5804 R) was used. The Implen NanoPhotometer N80 was used to estimate the quality and quantity of DNA. For amplification, an Eppendorf Mastercycler nexus gradient PCR machine was used. For agarose gel electrophoresis, a horizontal gel electrophoresis unit from Bio-Rad, United States, was used.

3.4.2 Isolation of DNA

DNA isolation was done by the method suggested by Rogers and Benedich (1994) with slight modification.

Reagents used:

- a. CTAB buffer (2X)
 - 2 per cent CTAB (w/v)
 - 100 mM Tris base (pH 8.0)
 - 20 mM EDTA (pH 8.0)
 - 1.4 M NaCl
 - 1 per cent Polyvinyl pyrrolidone (PVP)
- b. 0.2 per cent 2- β mercaptoethanol
- c. Chloroform: Isoamyl alcohol (24:1 v/v)
- d. Chilled isopropanol (100%)
- e. Ethanol 100% and 70%
- f. Autoclaved distilled water

Procedure:

300 mg of tender leaf tissue were ground in a pre-chilled mortar and pestle with 1 mL of pre-warmed 2X CTAB extraction buffer. A pinch of PVP and 10 μ L of β -mercaptoethanol were added to the mortar before grinding. The homogenised material was transferred into a 2.0 mL sterile microcentrifuge tube. After

thoroughly mixing the contents, the mixture was incubated for at 65 °C, for man hour gentle inversions. An equal volume of isoamyl alcohol (24:1) was added and mixed by inversion. centrifuged for 15 minutes at 4 °C at 12,000 rpm. After transferring the uppermost aqueous layer to a microcentrifuge tube, gently mix in 0.6 volume of chilled isopropanol. Centrifuged for five minutes at 4 °C and 8,000 rpm. The pellet was not disturbed as the supernatant was carefully removed. Added 200 µL of absolute alcohol, and centrifuged for 5 minutes at 4°C at 8,000 rpm. The pellet was left to air dry for half an hour after the alcohol was removed. Pellet was kept at -20 °C after being dissolved in 50 µL of distilled water.

3.4.3 Estimation of quality of DNA by agarose gel electrophoresis

Agarose gel electrophoresis was carried out to determine the quality of the isolated DNA.

Reagents used:

- a. Agarose (0.8%)
- b. TAE buffer 50X (pH 8.0)
2M Tris buffer
Glacial acetic acid
0.5M EDTA
- c. Ethidium bromide (stock 10 mg/mL: working concentration 0.5 µg/ml).
- d. Loading dye (6X)

Procedure:

After wiping the gel tray and comb with 70 per cent ethanol, the ends were sealed. The comb was inserted into the gel tray vertically, with the teeth positioned between one and two millimeters above the tray's surface, and about an inch from one end of the tray. In a conical flask with 250 mL of 1X TAE buffer, 0.8 per cent agarose (0.8 g in 100 mL) was prepared. Agarose was dissolved in a microwave for 45 to 60 seconds, or until the solution turned clear. Once the solution was cooled down to between 42 °C and 45 °C, 4 µl of the working solution of ethidium bromide was added and mixed well. After the gel solution was added to the tray, it was left to solidify at room temperature for thirty to forty-five minutes. The tray was put inside the electrophoresis chamber, the comb was carefully taken out, and 1X TAE buffer was added until the wells were completely

submerged. In order to get samples ready for electrophoresis, 1µL of 6X gel loading dye was added, thoroughly mixed, and loaded for every 5µL of DNA solution. The electrophoresis process was carried out at 80 volts until the dye migrated for two-thirds of the gel length.

Gel documentation

Gel documentation was done using the BioRad molecular imaging system using Imagelab software. The gel was captured using the controls in the imaging device window.

3.4.4 Assessing the quality and quantity of DNA

The purity of DNA was further checked using 1 µL of DNA sample in the Implen NanoPhotometer N80 as per the manufacturer's protocol. Proteins show maximum absorption at 280 nm, whereas nucleic acid shows peak absorption at 260 nm. Absorbance was recorded at both wavelengths, and purity was indicated by A_{260}/A_{280} . The values between 1.8 and 2.0 are indicative of pure DNA free from proteins. The quantity of DNA was calculated using the relation,

$$\text{Quantity of DNA in } \mu\text{g/mL} = A_{260} \times 50$$

3.4.5 Molecular markers used for the study

Attempt was made to detect temperature tolerance genes by screening with reported molecular markers. The study used two types of markers: SSR (Simple Sequence Repeats) and RAPD (Random Amplified Polymorphic DNA). In this study, thirty genotypes were screened using four reported markers. Table 8 contains a list of the markers used in this study.

Table 8. Molecular markers for high temperature tolerance.

Marker	Sequence (5'- 3')	Reference
SSR 96	F: GGGTTATCAATGATGCAATGG R: CCTTTATGTCAGCCGGTGTT	Shaji <i>et al.</i> , 2020; Wen <i>et al.</i> , 2019
SSR 63	F: CCACAAACAATTCCATCT CA R: GCTTCCGCCATACTGATA CG	Shaji <i>et al.</i> , 2020; Oladokun <i>et al.</i> , 2022
Z13 (RAPD)	GACTAAGCCC	Kamel <i>et al.</i> , 2010
A16 (RAPD)	AGCCAGCGAA	Kamel <i>et al.</i> , 2010

3.4.6 DNA amplification by PCR

The PCR conditions required for effective amplification in analysis include an appropriate proportion of the reaction mixture components and a temperature profile of the thermal cycle. 10X *Taq* assay buffer A (with KCl and 15 mM MgCl₂), dNTPs, *Taq* DNA polymerase, primers, and autoclaved distilled water were used in the reaction. Genomic DNA was added to aliquots of this master mix in PCR microcentrifuge tubes. The PCR was done in an Eppendorf Mastercycler Nexus gradient. Based on the nature of the primer used and the size of the product, the thermocycler was programmed for the appropriate temperature and duration for denaturation, annealing, and extension.

The reaction mixture consisted of,

Genomic DNA (50 ng)	- 1.0 µL
10X <i>Taq</i> assay buffer	- 2.5 µL
dNTP mix (200 µM)	- 0.5 µL
<i>Taq</i> DNA polymerase (1U)	- 0.3 µL
Forward primer (5 picomole)	- 2.0 µL
Reverse primer (5 picomole)	- 2.0 µL
Autoclaved distilled water	- 16.7 µL
Total reaction volume	- 25.0 µL

PCR thermal regime for SSR 96 and SSR 63

Initial denaturation	: 94 °C 3 min.	
Denaturation	: 94 °C 1 min.	35 cycles
Primer annealing	: 50 °C 1 min.	
Primer extension	: 72 °C 1 min.	
Final extension	: 72 °C 5 min.	
Cold storage	: 4 °C ∞	

PCR thermal regime for Z13 and A16 (RAPD primer)

Initial denaturation	: 94 °C 3 min.	
Denaturation	: 94 °C 1 min.	35 cycles
Primer annealing	: 35 °C 1 min.	
Primer extension	: 72 °C 2 min.	
Final extension	: 72 °C 5 min.	

Cold storage : 4 °C ∞

3.4.7 Marker analysis

Using the four selected primers, all 30 genotypes were screened. The PCR products were separated on a two per cent agarose gel with a suitable DNA ladder (100 bp ladder). The amplification profile was visualized and documented using the BioRad Gel Documentation system, and relevant products were scored for amplification.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 Laboratory screening for thermotolerance

Thirty tomato genotypes were raised for laboratory screening during September - December 2022. Crop was raised in the field during September - December when the mean maximum temperature and mean minimum temperature ranged between 31 to 32.5 °C and 22.5 to 23.7 °C. Average relative humidity ranged between 66 to 81 per cent during the period. Electrolyte leakage, pollen viability, pollen germination, style length and style protrusion were studied. Genotypes with higher pollen viability and germination, lower style protrusion, and low electrolyte leakage were identified.

4.1.1 Electrolyte leakage and membrane stability

Electrolyte leakage is the consequence of membrane damage and is an important factor in heat tolerance. It has been used to assess heat tolerance in a variety of different crops, including wheat (Saadallah *et al.*, 1990; Blum *et al.*, 2001), soybean (Martineau *et al.*, 1979), potato and tomato (Chen *et al.*, 1982), and cowpea (Ismail and Hall, 1999).

AVTO 0301 (79.50%) and EC 636872 (62.12%) showed the highest electrolyte leakage, which were significantly different from every other genotype (Table 9). Arka Samrat (18.47%) recorded the lowest electrolyte leakage. EC 538153, AVTO 1314, Arka Vishesh and Manuprabha were on par with Arka Samrat. AVTO 0301, EC 636872, EC 528368, Akshaya and Manulakshmi were significantly different, while all other genotypes were on par with Vellayani Vijay. Superior genotypes with respect to electrolyte leakage were Arka Samrat, Arka Vishesh, EC 538153, AVTO 1314 and Manuprabha which had lower electrolyte leakage compared to other genotypes.

Alsamir *et al.* (2017) observed a mean electrolyte leakage of 27.5±0.79 µmhos/cm under control conditions (day/night temperature 26/20°C) in tomato. Electrolyte leakage of 57.77 per cent was reported by Ullah and Ayub (2021) in tomato genotypes under field conditions at Peshawar, India. In the present study, the observed mean electrolyte leakage was 33.51 per cent, which was lower than electrolyte leakage reported by Ullah and Ayub (2021). Electrolyte leakage ranging from 18 to 52 per cent was observed by Srivastava *et al.* (2012), while evaluating 34 tomato genotypes under average day/night temperature of 38/22.2 °C. Electrolyte

leakage ranged between 18.47 and 79.50 per cent in the present study indicating higher variability among the genotypes.

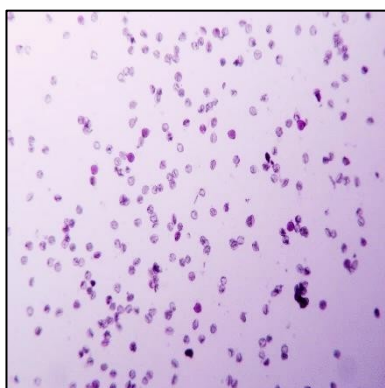
Arka Samrat (81.53%) had the highest and AVTO 0301 (20.50%) showed the lowest membrane stability (Table 9). Vellayani Vijay, the heat tolerant variety released from KAU, showed high membrane stability (70.43%). Saeed *et al.* (2007) as well as Vijayakumar and Beena (2023) reported that tomato membrane thermostability was decreased under heat stress and resistant genotypes had higher membrane thermostability. Arka Samrat, Arka Vishesh, EC 538153, AVTO 1314 and Manuprabha showed significantly higher membrane stability than every other genotype tested. Akshaya, AVTO 0301, EC 528368, EC 636872 and Manulakshmi had significantly lower membrane stability than Vellayani Vijay. All other genotypes were on par with Vellayani Vijay. In our study, membrane stability ranged between 20.50 and 81.53 per cent. Membrane thermostability in tomato reported by Vijayakumar and Beena (2023) ranged between 78.48 and 30.48%, which is comparable with our study.

Arka Samrat, Arka Vishesh, EC 538153, AVTO 1314 and Manuprabha were the superior genotypes, which recorded lower electrolyte leakage and higher membrane stability.

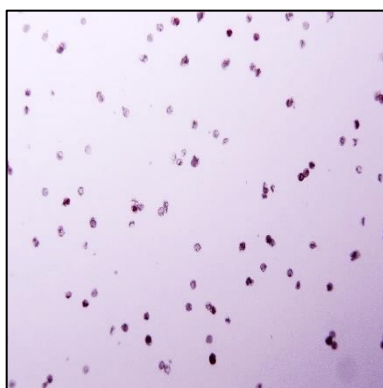
4.1.2 Pollen viability

EC 523851 (81.64%) and EC 631354 (21.16%) recorded highest and lowest pollen viability respectively (Table 11). EC 523851 (81.64%) was significantly superior than Vellayani Vijay (59.47%), while, EC 549819, EC 620428 and EC 631354 were significantly inferior to Vellayani Vijay with respect to pollen viability. All other genotypes were on par with Vellayani Vijay.

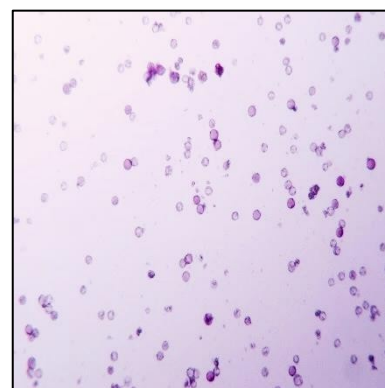
Laxman *et al.* (2018) reported pollen viability ranging from 30.8 to 58.5 per cent under ambient temperature conditions (30 ± 0.5 °C) in tomato. Srivastava *et al.* (2012) reported pollen viability between 66.36 to 95.48 per cent under average day/night temperature of 27.12/15.15 °C. These findings were comparable to our observations, in which pollen viability ranged from 21.16 to 81.64 per cent at 32 °C. Fresh pollen collected from open field condition recorded a pollen viability of 32.29 per cent in tomato using acetocarmine staining method (Laxman *et al.*, 2021) which was lower than in our study.



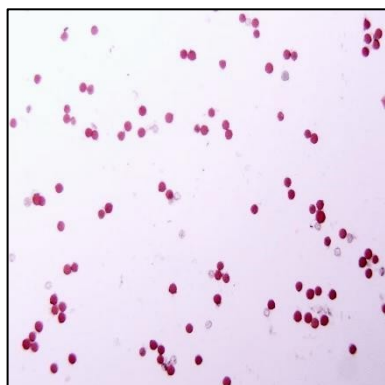
EC 523851



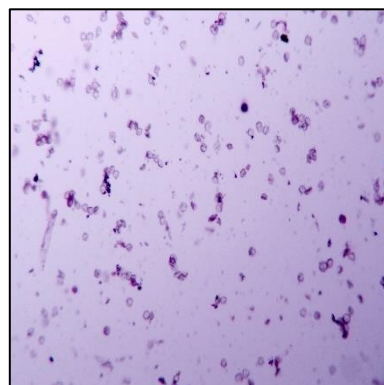
EC 567305



EC 538153



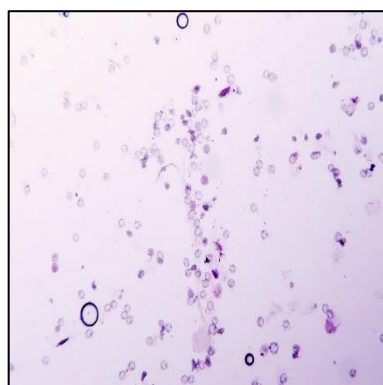
EC 528360



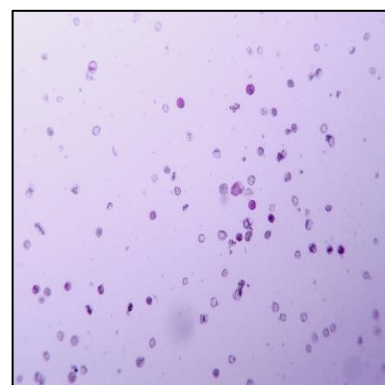
AVTO 0922



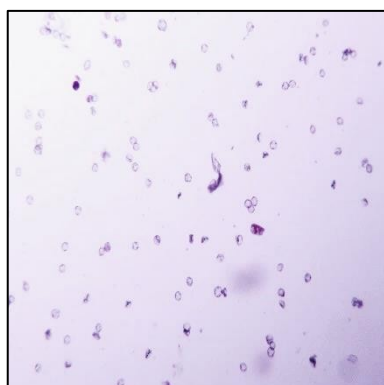
Arka Vishesh



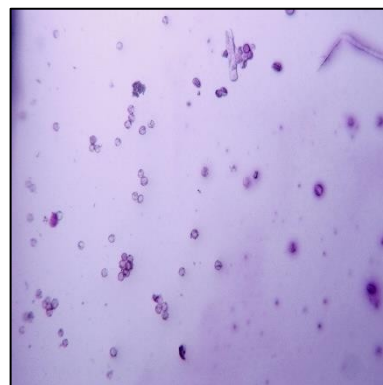
AVTO 1314



EC 549819



EC 620428



EC 631354

Plate 2. Pollen viability of genotypes under laboratory screening

Note: Magnification 40X

Table 9. Performance of genotypes for physiological traits

Sl. No.	Accessions	Membrane thermostability (%)	Electrolyte leakage (%)
1	Akshaya	41.92 ^{gh}	58.08 ^{bc}
2	Anagha	73.90 ^{abc}	26.10 ^{ghi}
3	Arka Abhed	74.33 ^{abc}	25.67 ^{ghi}
4	Arka Rakshak	58.73 ^{cdef}	41.27 ^{defg}
5	Arka Samrat	81.53 ^a	18.47 ⁱ
6	Arka Vishesh	78.58 ^a	21.42 ⁱ
7	AVTO 0301	20.50 ⁱ	79.50 ^a
8	AVTO 0922	69.43 ^{abcd}	30.57 ^{fghi}
9	AVTO 1314	79.78 ^a	20.22 ⁱ
10	AVTO 1702	71.53 ^{abcd}	28.47 ^{fghi}
11	AVTO 1706	77.22 ^{ab}	22.78 ^{hi}
12	AVTO 1707	72.32 ^{abcd}	27.68 ^{fghi}
13	AVTO 1725	77.21 ^{ab}	22.78 ^{hi}
14	AVTO 1726	75.34 ^{ab}	24.66 ^{hi}
15	EC 315489	69.67 ^{abcd}	30.33 ^{fghi}
16	EC 523851	61.83 ^{bcdef}	38.16 ^{defgh}
17	EC 528360	72.31 ^{abcd}	27.69 ^{fghi}
18	EC 528368	48.54 ^{fgh}	51.46 ^{bcd}
19	EC 538153	78.73 ^a	21.27 ⁱ
20	EC 549819	56.40 ^{defg}	43.60 ^{cdef}
21	EC 567305	65.93 ^{abcde}	34.07 ^{efghi}
22	EC 620428	66.01 ^{abcde}	33.99 ^{efghi}
23	EC 620486	72.03 ^{abcd}	27.96 ^{fghi}
24	EC 620488	71.58 ^{abcd}	28.42 ^{fghi}
25	EC 620494	68.85 ^{abcd}	31.15 ^{fghi}
26	EC 631354	70.60 ^{abcd}	29.40 ^{fghi}
27	EC 636872	37.88 ^h	62.12 ^b
28	Manulakshmi	51.06 ^{efgh}	48.94 ^{bcde}
29	Manuprabha	80.41 ^a	19.59 ⁱ
30	Vellayani Vijay	70.43 ^{abcd}	29.57 ^{fghi}
	CD	16.40	16.40
	SE(d)	8.20	8.20

4.1.3 Pollen germination

Pollen germination was recorded after incubating pollen grains at three temperature conditions, viz., room temperature (27 to 30 °C), 35 °C, and 40 °C, for 8 hours. AVTO 1706 (39.56%) recorded highest and EC 636872 (3.69%) recorded lowest pollen germination under room temperature (Table 11). Arka Samrat, AVTO 0301, AVTO 0922, AVTO 1702, AVTO 1706, AVTO 1726, AVTO 1725, EC

523851, EC 528360, EC 620486, EC 620488, EC 631354, and Manuprabha were on par with Vellayani Vijay (32.37%) for pollen germination under room temperature condition.

Highest and lowest pollen germination per cent was exhibited by AVTO 1706 (32.48%) and Anagha (3.57%) respectively when incubated at 35 °C (Table 11). Arka Samrat, AVTO 0301, AVTO 0922, AVTO 1702, AVTO 1706, AVTO 1725, AVTO 1726, EC 523851 and EC 528360 were on par with Vellayani Vijay (24.52%), which ranked third for pollen germination under 35°C. All other genotypes were inferior to Vellayani Vijay.

When incubated at 40 °C, Vellayani Vijay (17.85%) was significantly superior to all other genotypes, and recorded the highest pollen germination (Table 11). Vellayani Vijay (17.85%) recorded highest pollen germination per cent, while pollen of AVTO 1314 and Manulakshmi failed to germinate when incubated at 40 °C.

Pollen germination across room temperature, 35 °C and 40 °C showed a decreasing trend (Table 11). Manulakshmi, EC 549819 and EC 538153 exhibited inferior pollen germination than Vellayani Vijay at all the three temperature conditions. EC 549819 was significantly inferior to Vellayani Vijay for pollen viability as well, indicating its low thermotolerance.

Reduction in pollen germination per cent at 35 °C and 40 °C compared to room temperature is presented in Table 10. Vellayani Vijay showed 24.23% and 44.84% reduction in pollen germination at 35 °C and 40 °C respectively compared to room temperature condition. EC 528368, EC 620494 and EC 620428 recorded lower reduction in pollen germination under 35 °C as well as 40 °C compared to Vellayani Vijay. EC 549819 (82.48%) showed the highest reduction in pollen germination at 35 °C, and Manulakshmi and AVTO 1314 exhibited 100 per cent reduction at 40 °C compared to room temperature condition (Table 10). AVTO 0922 (6.92%) and EC 620428 (20.10%) recorded the lowest reduction in pollen germination when incubated under 35 °C and 40 °C respectively, compared to the incubation under room temperature condition.

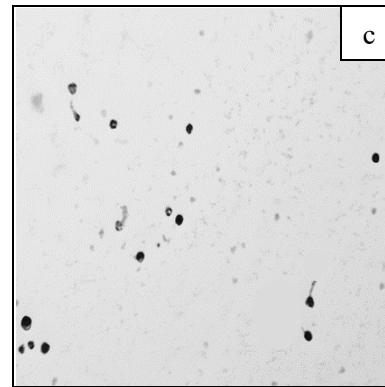
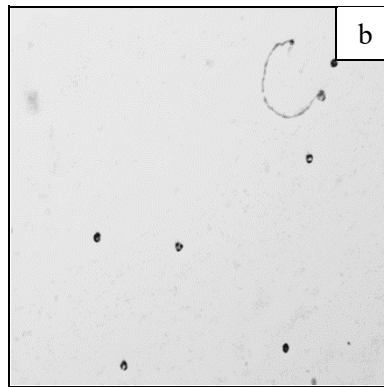
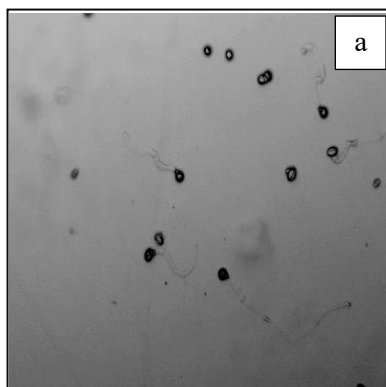
While comparing the pollen characters *viz.*, pollen viability, and pollen germination at room temperature, 35°C and 40°C, (Fig. 4a) a decreasing trend was observed. Pollen viability was higher than pollen germination under room temperature

followed by pollen germination under 35 °C and 40 °C sequentially. EC 636872 and Manulakshmi showed lower germination per cent at all temperatures in spite of high pollen viability. Although pollen viability was highest for EC 523851, followed by EC 567305, EC 538153, EC 528360, EC 620486 and AVTO 0922, pollen germination at room temperature was highest for AVTO 0301 and AVTO 1706. AVTO 0922, AVTO 0301, AVTO 1725, AVTO 1706 and Vellayani Vijay showed higher pollen germination at 35 °C. Pollen of AVTO 1314, and Manuprabha showed very low germination at 40 °C, while, EC 620494, EC 620428, EC 620486, EC 528368, AVTO 1706, Akshaya and Vellayani Vijay had high pollen germination at 40 °C.

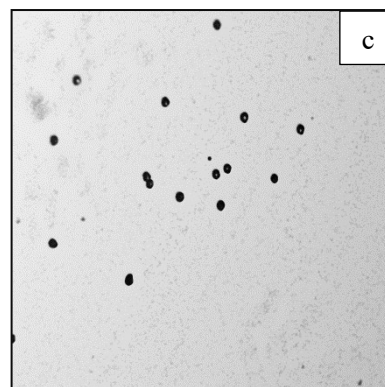
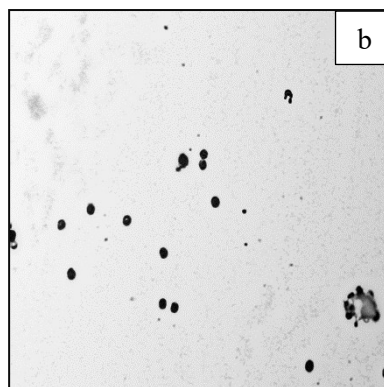
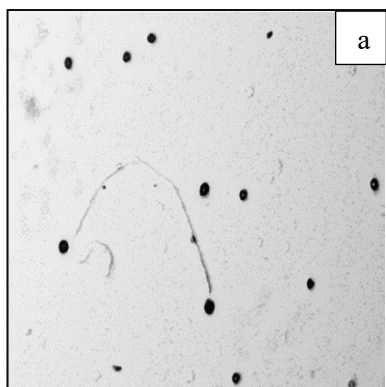
According to Sato *et al.* (2000), the most crucial elements influencing the fruit set in tomato, apart from pollen release, are variations in pollen germination under heat stress. Zhou *et al.* (2015) reported that heat stress at 36 °C and 40 °C significantly reduced *in vitro* pollen germination in tomato. At 36 °C, the heat-tolerant group had significantly higher pollen germination than the heat-sensitive group. Current study also demonstrated a significant reduction in pollen germination under 35 °C and 40 °C and genotypic variation in pollen germination under 35 °C than 40 °C. A significant decrease in pollen germination (15.29–67.59%) was reported at an average day/night temperature of 38 °C/22.2 °C (Srivastava *et al.*, 2012). Per cent reduction in pollen germination under 35 °C ranged from 6.92 to 82.48 per cent in our study, which was similar to the findings of Srivastava *et al.* (2012). Laxman *et al.* (2018) reported that, pollen germination of tomato ranged between 12.7 and 37.2 per cent under moderate elevated temperature condition (~2 °C above the optimum temperature of 30 °C) which was in agreement with our study, which recorded pollen germination between 2.6 and 32.48 per cent at 35 °C. The rate of pollen germination and pollen tube growth were decreased in tomato at temperatures higher than 30 °C and 35 °C (Lee *et al.*, 2022). Reduction in pollen germination at higher temperature was evident in our study also.

Table 10. Percentage reduction in pollen germination at different temperature conditions compared to room temperature

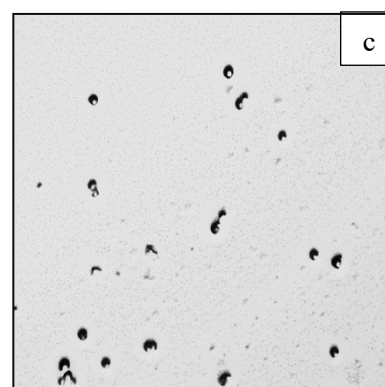
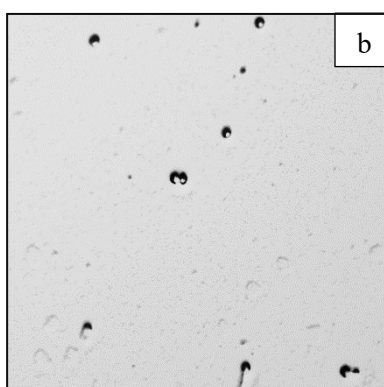
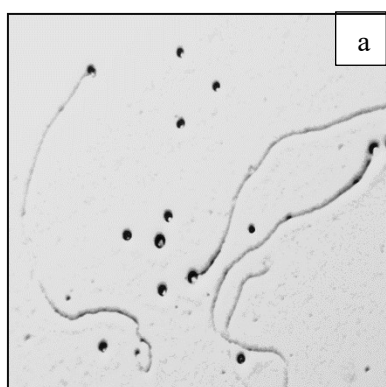
Sl. No.	Accessions	Per cent reduction at 35 °C	Per cent reduction at 40 °C
1	Akshaya	43.30	46.12
2	Anagha	70.77	71.67
3	Arka Abhed	48.74	84.64
4	Arka Rakshak	31.87	90.69
5	Arka Samrat	17.73	79.47
6	Arka Vishesh	69.78	84.54
7	AVTO 0301	43.02	89.94
8	AVTO 0922	6.92	90.43
9	AVTO 1314	41.88	100.00
10	AVTO 1702	43.29	90.70
11	AVTO 1706	17.88	77.37
12	AVTO 1707	67.18	85.82
13	AVTO 1725	11.12	87.44
14	AVTO 1726	44.80	90.46
15	EC 315489	45.99	77.70
16	EC 523851	24.46	86.11
17	EC 528360	54.73	92.75
18	EC 528368	13.66	37.34
19	EC 538153	78.44	95.53
20	EC 549819	82.48	95.26
21	EC 567305	46.36	90.12
22	EC 620428	11.59	20.10
23	EC 620486	61.16	61.71
24	EC 620488	76.61	92.87
25	EC 620494	9.21	40.49
26	EC 631354	63.09	91.01
27	EC 636872	29.35	34.51
28	Manulakshmi	31.81	100.00
29	Manuprabha	44.66	98.85
30	Vellayani Vijay	24.23	44.84



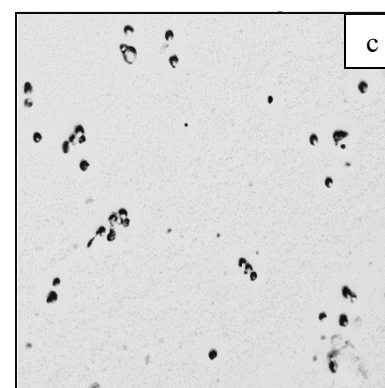
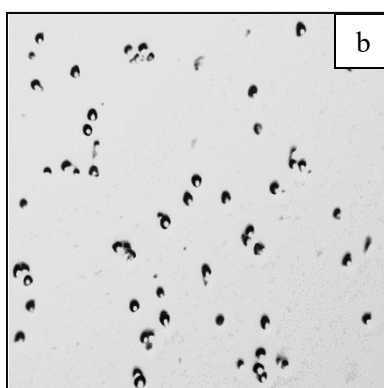
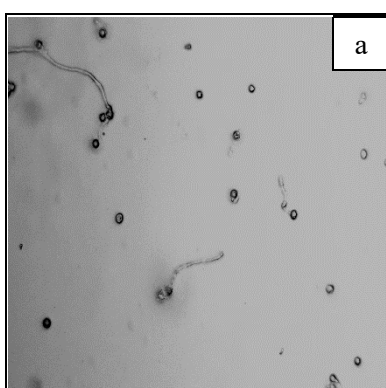
EC 620494



EC 620428



EC 528368



Vellayani Vijay

Plate 3. Pollen germination of genotypes under lab screening

Note: a) Pollen germination at room temperature b) Pollen germination at 35 °C c) Pollen germination at 40 °C

Note: Magnification 40X

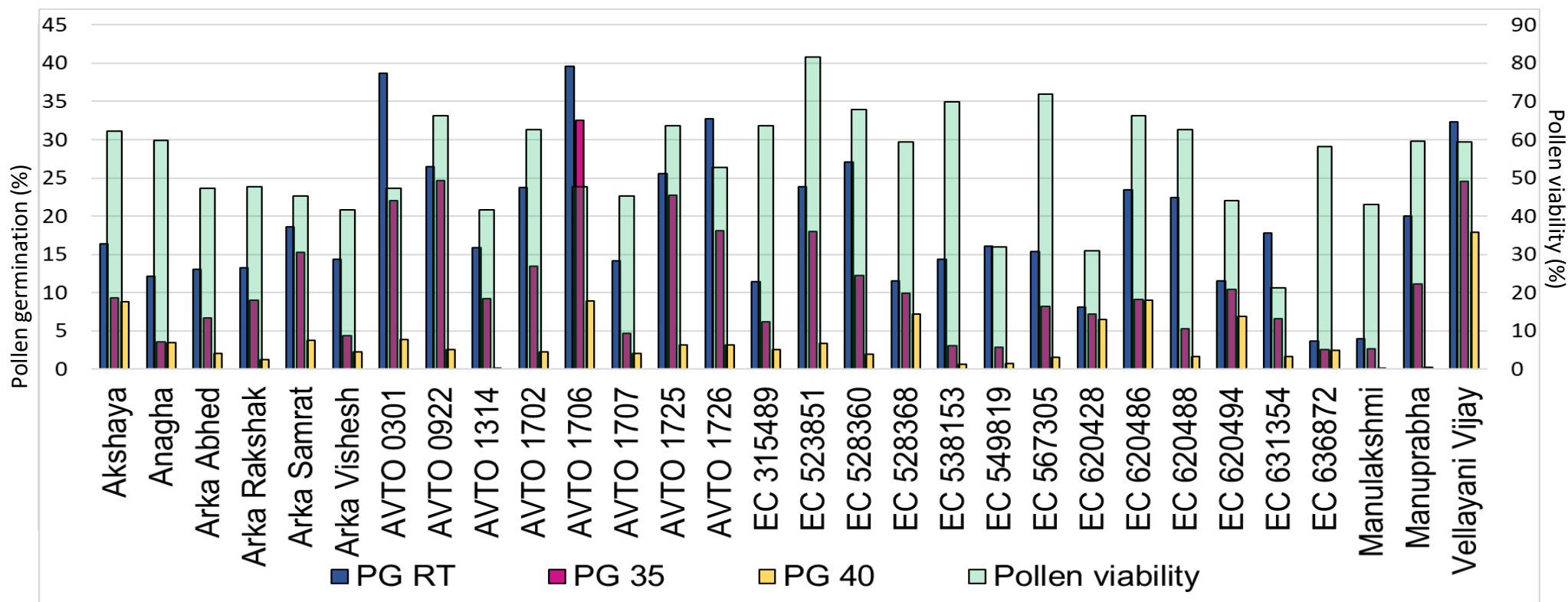


Fig 4a. Performance of genotypes for pollen viability and pollen germination at room temperature (PG RT), 35 °C (PG 35) and 40 °C (PG 40).

4.1.4 Style length

AVTO 1707 (0.82 cm) and EC 620488 (0.35 cm) exhibited highest and lowest style length compared to other genotypes (Table 11). EC 523851, EC 620494, EC 636872, Arka Abhed, AVTO 1726, Manulakshmi, EC 631354, EC 528368, Vellayani Vijay, AVTO 1702, AVTO 1725, Arka Samrat, AVTO 0922, EC 549819 and EC 567305 exhibited lower style length than the overall mean (0.59 cm) and was on par with Vellayani Vijay (0.52 cm). EC 620488 (0.35 cm) exhibited significantly lower style length than Vellayani Vijay (0.52 cm).

Pistil length observed by Pan *et al.* (2019) ranged between 0.57 cm to 0.6 cm under normal conditions (day/night temperature 25/20 °C). In the present study, style length ranged between 0.35 and 0.82 cm and the overall mean was 0.59 cm which indicates larger variation for the trait compared to those observed by Pan *et al.* (2019). Style length varies among different tomato varieties. Under controlled conditions (29/19 °C), studies reported higher style protrusion in wild tomato accessions than cultivated types (Driedonks *et al.*, 2018). This shows that wild tomatoes may have longer styles, which might be beneficial for reproductive success and adaptation in their native habitat.

4.1.5 Style protrusion

Akshaya and Anagha were on par with Vellayani Vijay (45.0%) in style protrusion under open condition (31.75/23.10 °C, Max/Min). EC 549819 and EC 528360 had significantly higher style protrusion and were inferior to Vellayani Vijay for the trait. All other genotypes were superior to Vellayani Vijay (Table 11). EC 528360 (76.33%) recorded the highest style protrusion. EC 620494 and EC 523851 did not display any style protrusion.

Pan *et al.* (2019) conducted experiments using seven varieties of tomato and observed that there was no stigma protrusion under normal condition (25/20 °C) compared to moderate elevated temperature (33/28 °C), which exhibited 23.5 per cent stigma exertion. In our study, the temperature was 31.75/23.1 °C, which was similar to the moderate elevated temperature mentioned by Pan *et al.* (2019). Srivastava *et al.* (2012) reported a significant increase in stigma exertion (7.82-50.35%) at an average day/night temperature of 38 °C/22.2 °C.

Table 11. Performance of genotypes for reproductive traits

Sl. No.	Accessions	Pollen viability (%)	Pollen germination (%)			Style length(cm)	Style protrusion (%)
			Room temperature	35 °C	40 °C		
1	Akshaya	62.28(126.19 ^{abcde})	16.35 (29.73 ^{cdef})	9.28 (16.75 ^{def})	8.81 (15.90 ^b)	0.61 ^{efghi}	45.67(85.64 ^{cd})
2	Anagha	59.83 (116.34 ^{bcde})	12.19 (21.99 ^{cdef})	3.57 (6.42 ^f)	3.45 (6.22 ^{bcdef})	0.63 ^{defgh}	43.33(80.70 ^{de})
3	Arka Abhed	47.33 (88.78 ^{cdefg})	13.10 (23.66 ^{cdef})	6.71 (12.29 ^{ef})	2.01 (3.78 ^{cdef})	0.54 ^{hijkl}	15.33(27.71 ^{lm})
4	Arka Rakshak	47.67 (89.49 ^{cdefg})	13.21 (23.89 ^{cdef})	9.00 (16.25 ^{ef})	1.23 (2.39 ^{def})	0.65 ^{cdefg}	23.67(43.01 ^{hijk})
5	Arka Samrat	45.22 (84.47 ^{defg})	18.56(33.68 ^{bcdef})	15.28(27.65 ^{bcdef})	3.81 (6.86 ^{bcdef})	0.49 ^{ijkl}	20.00 (36.25 ^{ijkl})
6	Arka Vishesh	41.66 (77.37 ^{efg})	14.37 (25.96 ^{cdef})	4.34 (7.81 ^f)	2.22 (4.00 ^{cdef})	0.59 ^{efghij}	24.33(44.47 ^{ghijk})
7	AVTO 0301	47.33 (88.78 ^{cdefg})	38.67 (72.69 ^a)	22.03(40.02 ^{abcd})	3.89 (7.01 ^{bcdef})	0.72 ^{abcd}	20.67 (37.47 ^{ijkl})
8	AVTO 0922	66.22(130.34 ^{abcde})	26.44(48.24 ^{abcd})	24.61 (44.78 ^{ab})	2.53 (4.55 ^{cdef})	0.48 ^{kl}	7.67 (13.84 ^{no})
9	AVTO 1314	41.66 (77.37 ^{efg})	15.83 (28.73 ^{cdef})	9.20 (16.59 ^{def})	0.00 (0.50 ^f)	0.74 ^{abc}	23.33 (42.40 ^{hijk})
10	AVTO 1702	62.56 (121.67 ^{bcde})	23.78(43.31 ^{bcde})	13.48(24.35 ^{bcdef})	2.21 (3.98 ^{cdef})	0.50 ^{ijkl}	11.67 (21.05 ^{mn})
11	AVTO 1706	47.67 (89.49 ^{cdefg})	39.56 (73.21 ^a)	32.48 (62.39 ^a)	8.95 (16.15 ^b)	0.77 ^{ab}	19.33 (35.03 ^{kl})
12	AVTO 1707	45.22 (84.47 ^{defg})	14.17 (25.64 ^{cdef})	4.65 (8.38 ^f)	2.01 (3.61 ^{cdef})	0.82 ^a	27.33 (49.84 ^{ghij})
13	AVTO 1725	63.67(124.28 ^{abcde})	25.55(46.88 ^{abcd})	22.71 (41.26 ^{abc})	3.21 (5.78 ^{bcdef})	0.50 ^{ijkl}	23.00 (41.78 ^{hijk})
14	AVTO 1726	52.67(99.91 ^{cdef})	32.72 (60.40 ^{ab})	18.06(32.71 ^{bcde})	3.12 (5.78 ^{bcdef})	0.54 ^{hijkl}	19.67 (35.64 ^{kl})
15	EC 315489	63.67(124.28 ^{abcde})	11.48 (20.73 ^{def})	6.20 (11.35 ^{ef})	2.56 (4.77 ^{cdef})	0.67 ^{bcde}	30.00 (54.88 ^{gh})
16	EC 523851	81.64(176.62 ^a)	23.83 (43.50 ^{bcd})	18.00(32.61 ^{bcde})	3.31 (5.96 ^{bcdef})	0.55 ^{fghijk}	0.00 (0.01 ^p)
17	EC 528360	67.79(136.11 ^{abcd})	27.04 (49.51 ^{abc})	12.24(22.09 ^{bcdef})	1.96 (3.86 ^{cdef})	0.65 ^{cdef}	76.33 (156.50 ^a)
18	EC 528368	59.42(120.11 ^{bcde})	11.49 (20.75 ^{def})	9.92 (17.90 ^{cdef})	7.20 (13.15 ^{bc})	0.52 ^{ijkl}	31.00 (56.79 ^{fg})
19	EC 538153	69.85(141.29 ^{abc})	14.33 (25.89 ^{cdef})	3.09 (5.57 ^f)	0.64 (1.49 ^{ef})	0.72 ^{abcd}	25.67(46.74 ^{ghijk})
20	EC 549819	32.04(58.94 ^{fg})	16.04 (29.00 ^{cdef})	2.81 (5.22 ^f)	0.76 (1.70 ^{ef})	0.47 ^{kl}	58.67 (112.89 ^b)
21	EC 567305	71.82(154.37 ^{ab})	15.38 (27.81 ^{cdef})	8.25 (14.90 ^{ef})	1.52 (3.06 ^{cdef})	0.44 ^{lm}	2.34 (4.21 ^{op})
22	EC 620428	30.94(57.05 ^{fg})	8.11 (14.61 ^{ef})	7.17 (12.93 ^{ef})	6.48(11.88 ^{bcde})	0.59 ^{efghij}	37.33 (68.91 ^{ef})
23	EC 620486	66.22(130.34 ^{abcde})	23.49(42.82 ^{bcde})	9.12 (16.47 ^{def})	8.99 (16.22 ^b)	0.65 ^{cdef}	52.33 (99.17 ^c)
24	EC 620488	62.52(129.19 ^{abcde})	22.45(40.83 ^{bcde})	5.25 (9.46 ^{ef})	1.60 (3.05 ^{cdef})	0.35 ^m	22.00 (39.93 ^{ijkl})
25	EC 620494	44.06(84.89 ^{defg})	11.51 (20.85 ^{cdef})	10.45(19.05 ^{cdef})	6.85(12.53 ^{bcd})	0.55 ^{fghijk}	0.00 (0.01 ^p)
26	EC 631354	21.16(38.38 ^g)	17.80(32.41 ^{bcdef})	6.58 (11.85 ^{ef})	1.60 (3.05 ^{cdef})	0.53 ^{hijkl}	29.00 (52.96 ^{ghi})
27	EC 636872	58.08(114.64 ^{bcde})	3.69 (6.64 ^f)	2.60 (4.85 ^f)	2.41 (4.51 ^{cdef})	0.55 ^{ghijkl}	8.00 (14.44 ^{mno})
28	Manulakshmi	43.09 (80.32 ^{efg})	3.93 (7.08 ^f)	2.68 (4.83 ^f)	0.00 (0.50 ^f)	0.53 ^{hijkl}	29.33 (53.62 ^{gh})
29	Manuprabha	59.67(116.53 ^{bcde})	20.02(36.29 ^{bcde})	11.08 (20.00 ^{cdef})	0.23 (0.75 ^f)	0.74 ^{abc}	21.33(38.72 ^{kl})
30	Vellayani Vijay	59.47(117.95 ^{bcde})	32.37 (60.50 ^{ab})	24.52 (44.80 ^{ab})	17.85(32.32 ^a)	0.52 ^{ijkl}	45.00 (84.11 ^d)
	CD	22.78 (53.75)	15.28 (28.74)	12.29 (23.65)	5.94 (10.56)	0.11	7.17 (13.68)
	SE(d)	11.39 (26.87)	7.64 (14.37)	6.14 (11.83)	2.97 (5.28)	0.05	3.59 (6.84)

Note: Transformed values given in parenthesis.

^aMean followed by a common letter are not significantly different at the 5% level.

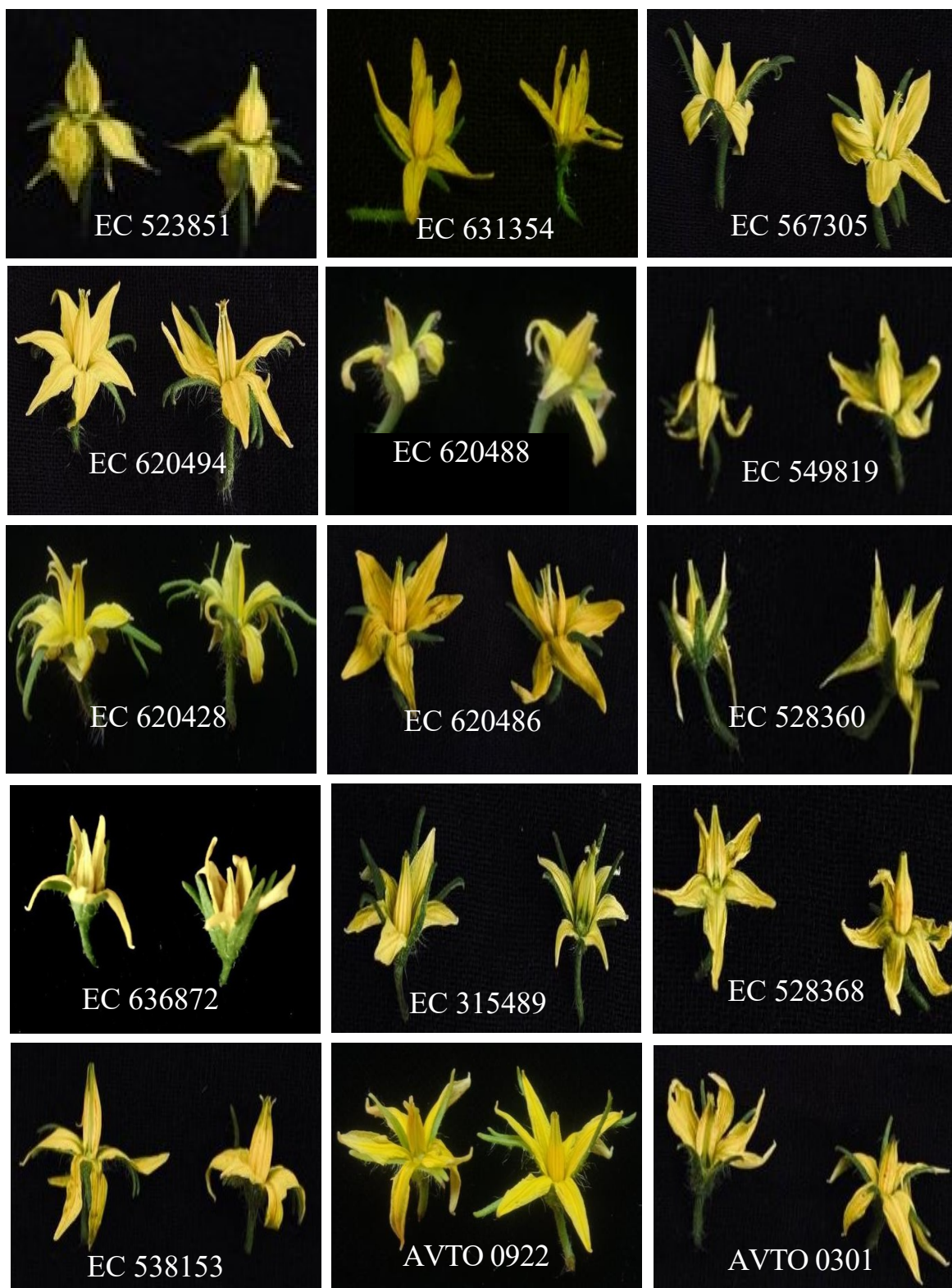


Plate 4a. Style protrusion of genotypes under laboratory screening

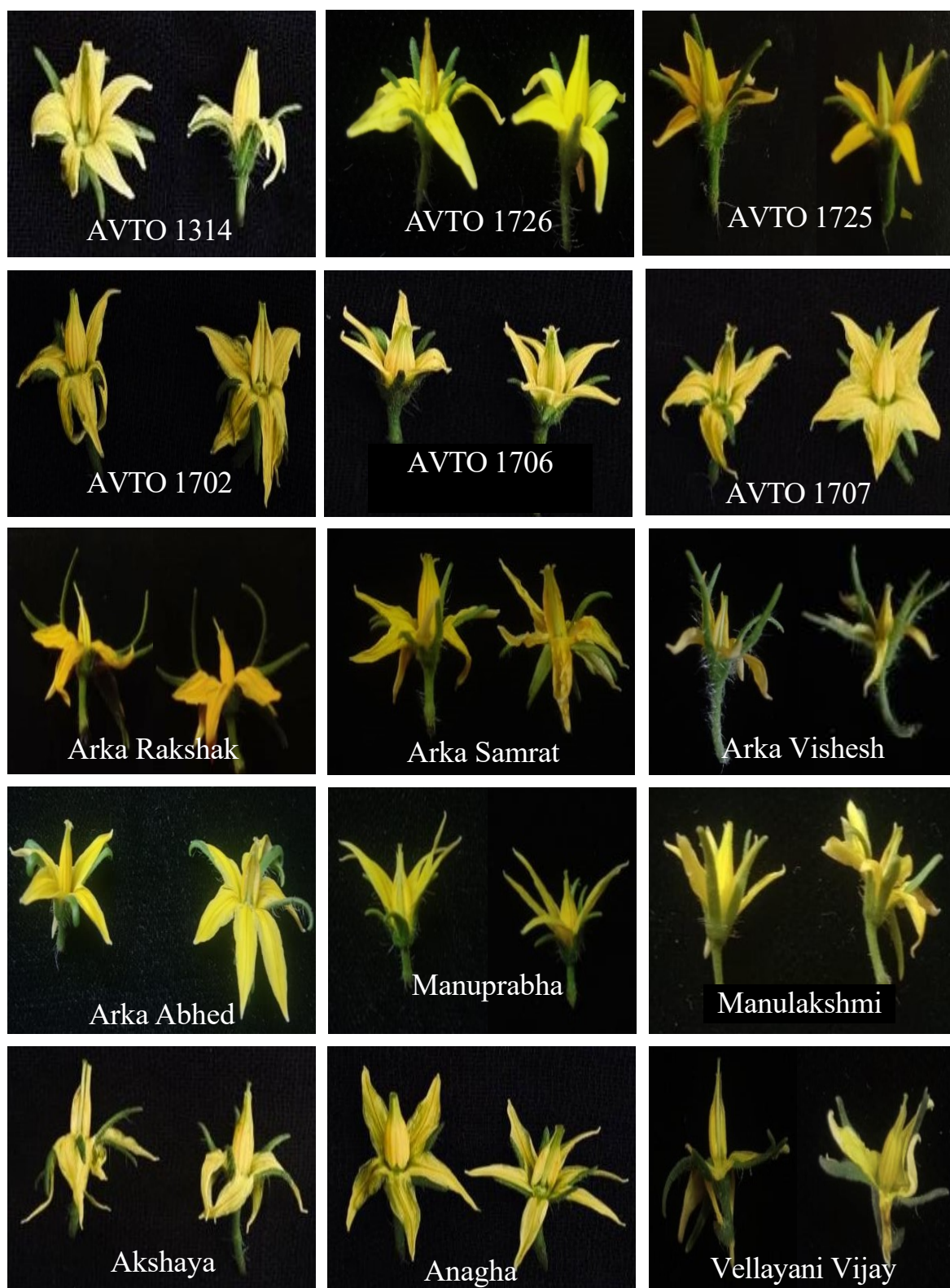


Plate 4b. Style protrusion of genotypes under laboratory

4.1.6 Identification of superior genotypes

Pollen germination and pollen viability are the male reproductive characters influenced by temperature. High pollen viability and germination per cent under heat stress condition are considered as characters of tolerant genotypes. Scatter plot helped to identify genotypes with high pollen viability and pollen germination, which lies in the first quadrant of the scatter plot *ie.*, EC 523851, EC 528360, EC 620486, EC 620488, AVTO 0922, AVTO 1702, Manuprabha and Vellayani Vijay were superior with respect to pollen viability and pollen germination at room temperature condition (Fig. 4b). EC 523851, EC 528360, AVTO 0922, AVTO 1702, AVTO 1725 and Vellayani Vijay were superior with respect to pollen viability and pollen germination at 35 °C (Fig. 4c). When pollen viability and pollen germination at 40 °C were considered EC 528368, EC 620486, Akshaya and Vellayani Vijay were superior (Fig. 4d).

Genotypes with high pollen viability and low style protrusion are also considered superior, which falls in the second quadrant of the scatter plot (Fig. 4e). EC 315489, EC 523851, EC 528368, EC 538153, EC 567305, EC 620486, EC 620488, EC 636872, AVTO 0922, AVTO 1725 and Vellayani Vijay were superior with high pollen viability and low style protrusion.

Pollen germination and style protrusion are the two main male and female traits respectively, that will influence yield of tomato under heat stress. Hence, genotypes were classified based on pollen germination at 40 °C and style protrusion into four groups *viz.*,

Group 1: Genotypes with >2.5% pollen germination and <20% style protrusion.

Group 2: Genotypes with <2.5% pollen germination and <20% style protrusion.

Group 3: Genotypes with >2.5% pollen germination and >20% style protrusion.

Group 4: Genotypes with <2.5% pollen germination and >20% style protrusion.

The combination of these traits with other characteristics, such as electrolyte leakage and the percentage decrease in pollen germination at 35 °C and 40 °C, was examined. Genotypes were selected from all the groups for evaluation of contrasting genotypes under polyhouse condition.

Group 1 represented the best combination with high pollen germination and low style protrusion and consisted of Arka Samrat, EC 620494, EC 523851, AVTO 0922, AVTO 1706 and AVTO 1726. Pollen germination in EC 620494 was higher at 40 °C with no style protrusion, and the percentage reduction in pollen germination at 35 °C and 40 °C was low. Although style protrusion was low, and pollen germination at 40 °C was high in AVTO 1726, the percentage reduction in pollen germination was high at 35 °C and 40 °C. Hence, EC 620494 and AVTO 1726 were selected from group 1 for polyhouse evaluation.

Group 2 included EC 567305, EC 636872, AVTO 1702 and Arka Abhed. AVTO 1702 had low style protrusion, low pollen germination at 40 °C, high membrane stability and high reduction in pollen germination. Pollen germination at 40 °C, style protrusion, membrane stability and pollen germination reduction were all lower in EC 636872. Significantly higher stigma protrusion, increased pollen germination at 40 °C, and a high per cent decrease in pollen germination at 35 °C and 40 °C were noted in EC 620486. AVTO 1702, EC 636872 and EC 620486 were selected from group 2 for further evaluation.

Group 3 included Akshaya, EC 620486, EC 620428, EC 528368, EC 315489, AVTO 0301, AVTO 1725, Vellayani Vijay and Anagha. AVTO 1725 and EC 315489 had high style protrusion and pollen germination at 40 °C, low per cent reduction on pollen germination at 35 °C and high at 40 °C. EC 620428 had high style protrusion and pollen germination at 40 °C, and lowest per cent reduction of pollen germination at 35 °C and 40 °C. Thus, from group 3, AVTO 1725, EC 315489 and EC 620428 were chosen for polyhouse evaluation.

EC 620488, EC 631354, EC 528360, EC 538153, EC 549819, AVTO 1314, AVTO 1707, Arka Rakshak, Arka Vishesh, Manulakshmi, and Manuprabha belonged to Group 4. Manulakshmi exhibited a 100 per cent reduction in pollen germination at 40 °C, a significantly low membrane stability, a low pollen germination rate at 40 °C, and high style protrusion. Significantly high stigma protrusion, reduced pollen germination at 40 °C, and maximum reduction in pollen germination at 35 °C and 40 °C were observed in EC 549819. Manulakshmi and EC 549819 were the genotypes selected from group 4 for polyhouse evaluation.

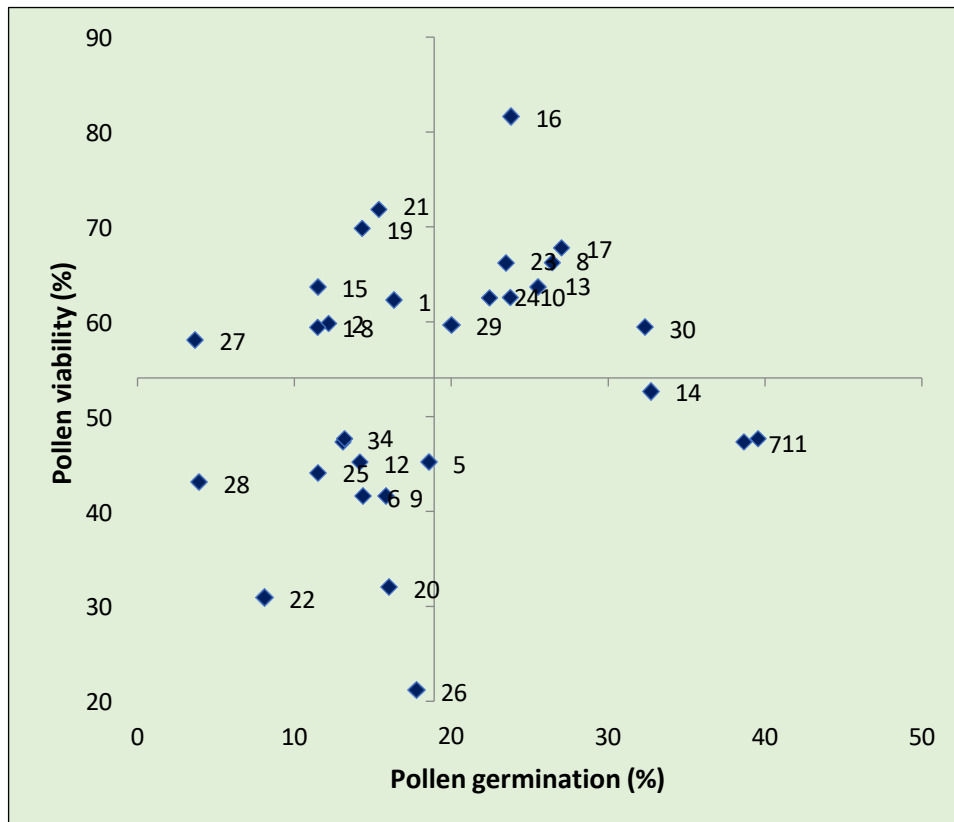


Fig. 4b Pollen viability vs pollen germination at room temperature

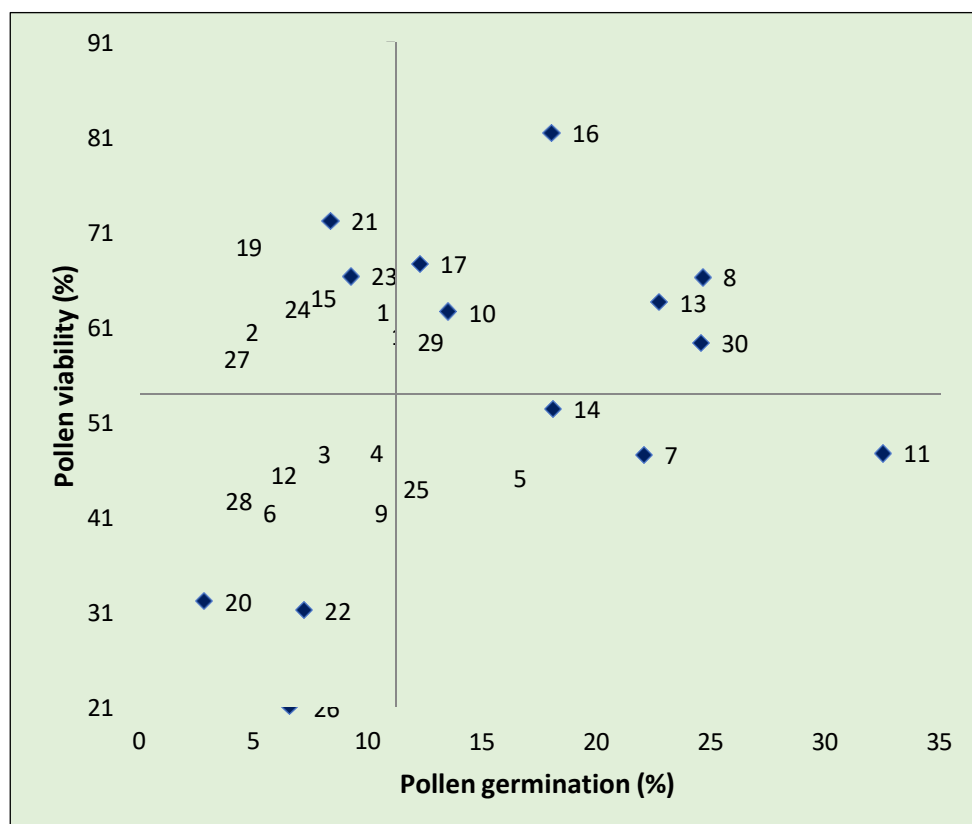


Fig. 4c Pollen viability vs pollen germination (35 °C)

- 1 Akshaya
- 2 Anagha
- 3 Arka Abhed
- 4 Arka Rakshak
- 5 Arka Samrat
- 6 Arka Vishesh
- 7 AVTO 0301
- 8 AVTO 0922
- 9 AVTO 1314
- 10 AVTO 1702
- 11 AVTO 1706
- 12 AVTO 1707
- 13 AVTO 1725
- 14 AVTO 1726
- 15 EC 315489
- 16 EC 523851
- 17 EC 528360
- 18 EC 528368
- 19 EC 538153
- 20 EC 549819
- 21 EC 567305
- 22 EC 620428
- 23 EC 620486
- 24 EC 620488
- 25 EC 620494
- 26 EC 631354
- 27 EC 636872
- 28 Manulakshmi
- 29 Manuprabha
- 30 Vellayani Vijay

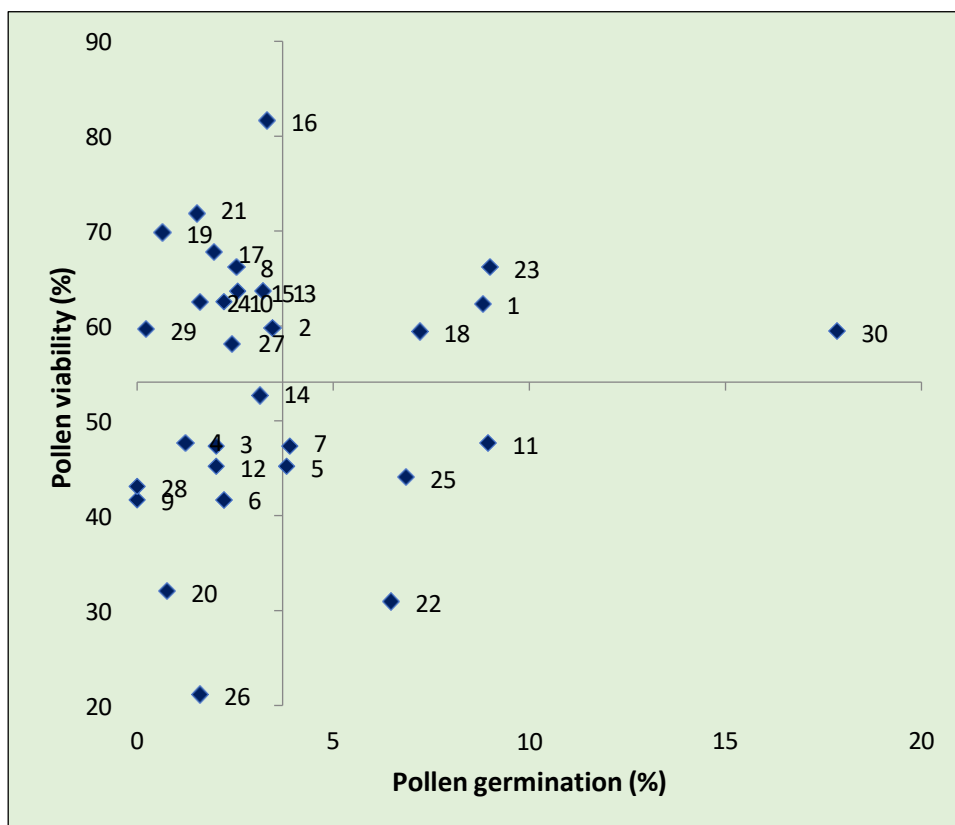


Fig. 4d Pollen viability vs pollen germination (40 °C)

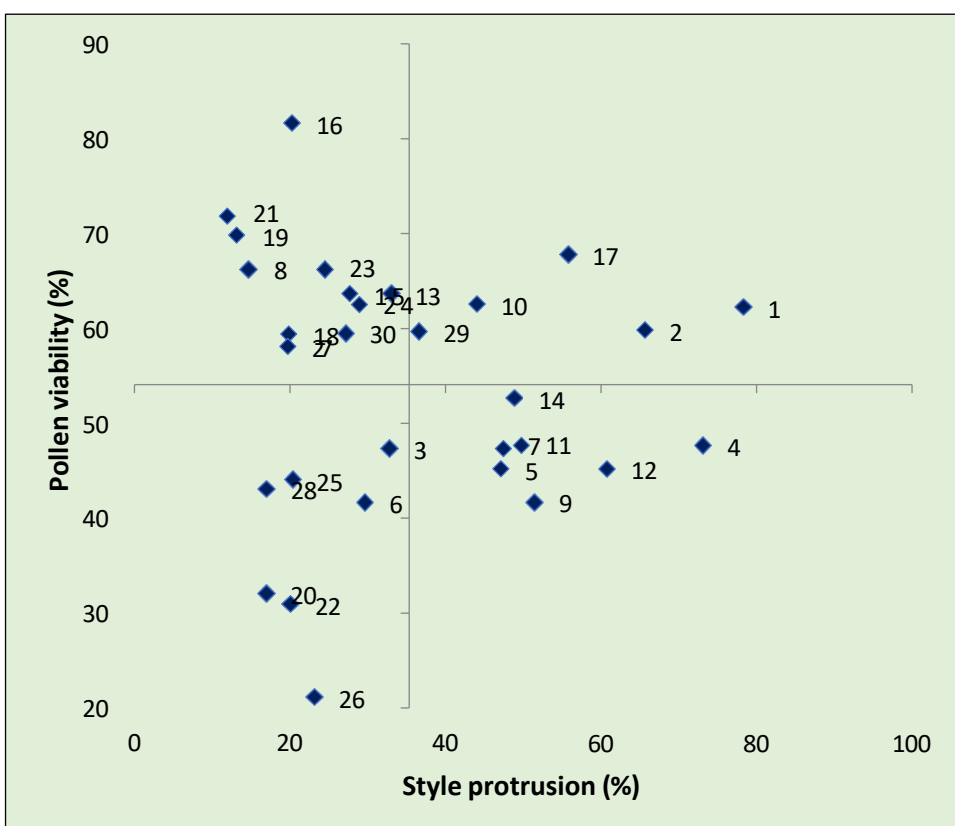


Fig. 4e Pollen viability vs style protrusion

- 1 Akshaya
- 2 Anagha
- 3 Arka Abhed
- 4 Arka Rakshak
- 5 Arka Samrat
- 6 Arka Vishesh
- 7 AVTO 0301
- 8 AVTO 0922
- 9 AVTO 1314
- 10 AVTO 1702
- 11 AVTO 1706
- 12 AVTO 1707
- 13 AVTO 1725
- 14 AVTO 1726
- 15 EC 315489
- 16 EC 523851
- 17 EC 528360
- 18 EC 528368
- 19 EC 538153
- 20 EC 549819
- 21 EC 567305
- 22 EC 620428
- 23 EC 620486
- 24 EC 620488
- 25 EC 620494
- 26 EC 631354
- 27 EC 636872
- 28 Manulakshmi
- 29 Manuprabha
- 30 Vellayani Vijay



EC 523851



EC 631354



EC 567305



EC 620494



EC 620488



EC 549819



EC 620428



EC 620486



EC 528360

Plate 5a. Genotypes used for laboratory screening



EC 636872



EC 315489



EC 528368



EC 538153



AVTO 0922



AVTO 0301



AVTO 1314



AVTO 1726



AVTO 1725

Plate 5b. Genotypes used for laboratory screening



AVTO 1702



AVTO 1706



AVTO 1707



Arka Abhed



Arka Rakshak



Arka Vishesh



Arka Samrat



Manuprabha



Manulakshmi

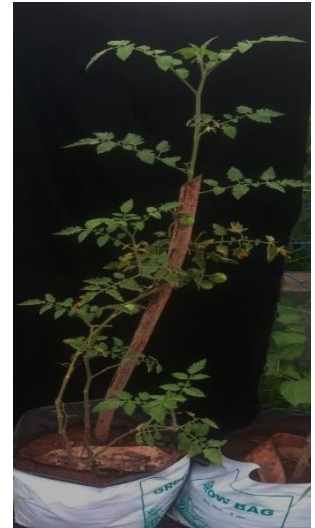
Plate 5c. Genotypes used for laboratory screening



Akshaya



Anagha



Vellayani Vijay

Plate 5d. Genotypes used for laboratory screening

AVTO 1706, EC 620486 and EC 538153 exhibited intermediate traits from different groups. Vellayani Vijay was used as the check variety during evaluation. Therefore, the following genotypes were chosen for polyhouse evaluation: AVTO 1702, AVTO 1706, AVTO 1725, AVTO 1726, EC 315489, EC 538153, EC 549819, EC 620428, EC 620486, EC 620488, EC 620494, EC 636872, Manulakshmi and Vellayani Vijay.

4.2 Evaluation under polyhouse condition

AVTO 1702, AVTO 1706, AVTO 1725, AVTO 1726, EC 315489, EC 538153, EC 549819, EC 620428, EC 620486, EC 620488, EC 620494, EC 636872, Manulakshmi and Vellayani Vijay were selected for polyhouse screening. Observations were statistically analyzed using two factor factorial design, with genotypes and growing conditions (open and polyhouse) taken as two factors.

4.2.1 Electrolyte leakage and membrane stability

While considering the interaction effect of membrane stability and electrolyte leakage under open and polyhouse conditions, there was no significant difference between genotypes (Table 12).

When the effect of electrolyte leakage on genotypes alone was considered, AVTO 1725 (19.16%) was superior than all other genotypes. AVTO 1702, AVTO 1706, Manulakshmi, EC 620428 and EC 538153 were on par with AVTO 1725 (Table 13). EC 636872 (40.40%) and AVTO 1725 (19.16%) recorded the highest and lowest electrolyte leakage.

Heat stress treatment of 42/26 °C (day/night temperature) was imposed on tomato genotypes under heat chamber and Alsamir *et al.* (2017) recorded an average electrolyte leakage of 29.79 ± 0.96 μ mhos/cm. According to Akhoundnejad and Dasgan (2018), the overall average value of membrane damage on leaf cells of tomato genotypes grown during the summer months was 20.54 per cent. In contrast, the current study recorded an average electrolyte leakage of 30.09 per cent at 34/25 °C (Max/Min temperature). When compared to the control, a 44.7 per cent increase in membrane damage on leaf cells of tomato genotypes under high temperature stress has been reported (Akhoundnejad and Dasgan, 2018). Increase as well as reduction in electrolyte leakage was observed under polyhouse (34/25 °C) compared to open condition (30/23 °C) (Table 16). Average increase and decrease in electrolyte leakage

recorded was 31.84 and 12.45 per cent which was lower compared to the study conducted by Akhoundnejad and Dasgan (2018).

When the effect of membrane stability of genotypes alone was considered, AVTO 1725 (80.84%) was superior than all other genotypes. AVTO 1702, AVTO 1706, Manulakshmi, EC 620428 and EC 538153 were on par with AVTO 1725. EC 636872 and EC 549819 were statistically inferior than Vellayani Vijay (Table 14). AVTO 1725 (80.34%) recorded the highest and EC 636872 (59.60%) recorded the lowest membrane thermostability index. Lokesh *et al.* (2019) evaluated six tomato genotypes under polytunnel, where temperature reached 40 ± 2 °C, where they obtained membrane stability index ranging between 50 and 70 per cent after nine days exposure. We obtained an average 65.44 per cent membrane stability, which lies within the limits of results reported by Lokesh *et al.* (2019).

Table 12. Interaction effect of genotype and growth condition on physiological traits

Sl. No.	Genotypes	Membrane thermostability (%)		Electrolyte leakage (%)	
		Open	Polyhouse	Open	Polyhouse
1	AVTO 1702	72.81	76.63	27.20	23.37
2	AVTO 1706	77.99	69.76	22.01	30.24
3	AVTO 1725	81.55	80.13	18.45	19.87
4	AVTO 1726	70.47	70.92	29.53	29.08
5	EC 315489	73.57	68.12	26.43	31.88
6	EC 538153	73.65	72.04	26.35	27.95
7	EC 549819	66.78	66.52	33.22	43.48
8	EC 620428	78.24	67.75	21.76	32.25
9	EC 620486	68.64	58.22	31.36	41.78
10	EC 620488	74.05	66.33	25.96	33.67
11	EC 620494	58.89	72.54	41.41	27.46
12	EC 636872	72.18	47.02	27.82	52.97
13	Manulakshmi	74.68	74.82	25.31	25.18
14	Vellayani Vijay	68.85	64.42	31.16	35.57

The genotypes showed an increase as well as reduction in electrolyte leakage and membrane thermostability index under polyhouse compared to open condition (Table 13). AVTO 1702, AVTO 1726, EC 620494 and Manulakshmi showed increase

in membrane thermostability index and reduction in electrolyte leakage. While, all other genotypes exhibited higher increase and decrease in electrolyte leakage and membrane stability respectively (Table 13).

Tomato genotypes when subjected to two pre-conditioned treatments (30/23 °C and 35/28 °C) for four days prior to heat stress exposure, preconditioned plants had better osmotic adjustment than control plants (Morales *et al.*, 2003). Since the polyhouse study was conducted in May- June (end of summer season) when the temperature was high, a similar pre-conditioning might have taken place in the plants. This might be the reason for an increase in membrane stability index observed in the current study.

Table 13. Response of genotypes to high temperature condition under polyhouse for physiological traits

Sl. No.	Genotypes	Membrane thermostability (%)	Electrolyte leakage (%)
1	AVTO 1702	74.72 ^{ab}	25.28 ^{de}
2	AVTO 1706	73.87 ^{ab}	26.13 ^{de}
3	AVTO 1725	80.34 ^a	19.16 ^e
4	AVTO 1726	70.69 ^{bcd}	29.31 ^{bcd}
5	EC 315489	70.85 ^{bcd}	29.15 ^{bcd}
6	EC 538153	72.84 ^{abc}	27.16 ^{cde}
7	EC 549819	40.82 ^g	38.35 ^{ab}
8	EC 620428	72.99 ^{abc}	27.00 ^{cde}
9	EC 620486	53.53 ^{def}	36.57 ^{abc}
10	EC 620488	70.18 ^{bcd}	29.81 ^{bcd}
11	EC 620494	49.00 ^{efg}	34.28 ^{abcd}
12	EC 636872	44.96 ^{fg}	40.40 ^a
13	Manulakshmi	74.75 ^{ab}	25.25 ^{de}
14	Vellayani Vijay	66.63 ^{bcd}	33.37 ^{abcd}
	CD	9.98	9.98
	SE(d)	4.98	4.98

4.2.2 Pollen viability

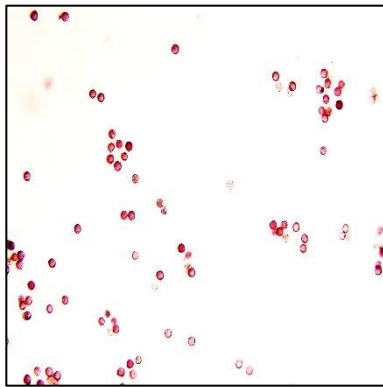
Highest and lowest pollen viability was recorded for EC 549819 (18.18%) and EC 620486 (3.29%) respectively under open condition (Table 15). AVTO 1725, EC 315489, EC 620486, EC 620494, EC 636872 and EC 620428 which exhibited less than 10 per cent pollen viability were considered as inferior genotypes with respect to pollen viability.

A reduction in pollen viability under polyhouse compared to the open condition was shown by all the genotypes. Vellayani Vijay showed 26 per cent reduction in pollen viability under polyhouse condition compared to open condition (Table 16). EC 549819, EC 620488 and AVTO 1706 had lower pollen viability and higher per cent reduction in pollen viability than Vellayani Vijay under poly house condition (Table 15 and 16).

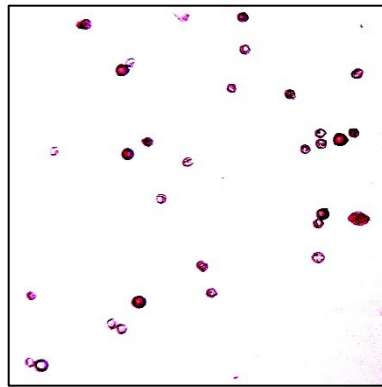
Under heat stress conditions (35/25 °C day/night, 16/8 h light/dark), Pham *et al.* (2020) observed reduced pollen viability by 50 per cent in tomato genotypes HT7 and WT. Tomatoes exposed to temperatures between 43 and 45 °C for three to seven days prior to anthesis had a 20 per cent decrease in pollen viability (Krishna *et al.*, 2019). Reduction in pollen viability observed in the current study under polyhouse (34/25 °C), compared to open condition (30/23 °C), ranged between 6.90 to 85.17 per cent. Average reduction in pollen viability recorded was 43.80 per cent which was comparable with the study conducted by Pham *et al.* (2020) and higher than that reported by Krishna *et al.* (2019).

4.2.3 Pollen germination

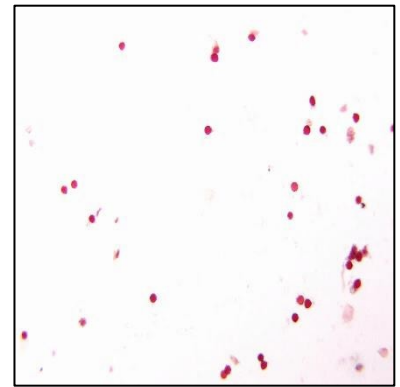
Vellayani Vijay was superior to all other genotypes for pollen germination under open and polyhouse conditions, except EC 549819 which had higher pollen germination than Vellayani Vijay under polyhouse condition (Table 15). Vellayani Vijay (15.97%) and EC 636872 (3.59%) exhibited highest and lowest pollen germination under polyhouse condition (Table 15). Pollen germination under open and polyhouse conditions were on par for EC 549819, EC 620428 and EC 636872 indicating that they performed equally well under both conditions and thus can be considered heat tolerant (Table 14). Reduction in pollen germination was observed for all the genotypes under polyhouse compared to open condition (Table 16). EC 549819 exhibited the lowest reduction in pollen germination.



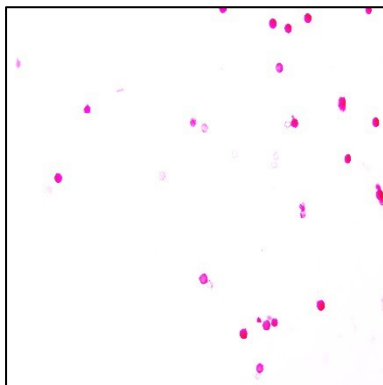
EC 549819



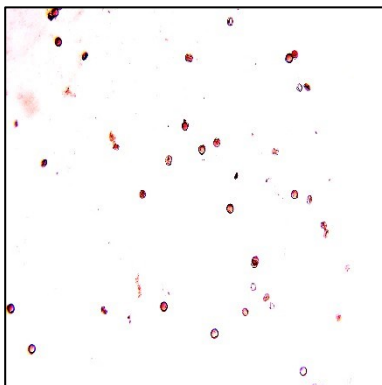
EC 620488



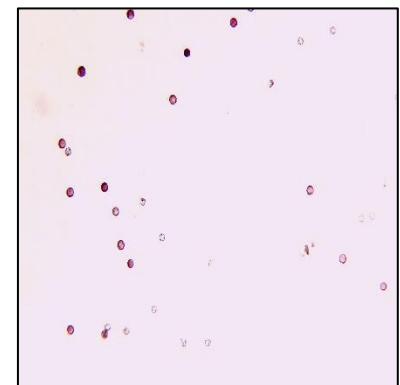
AVTO 1706



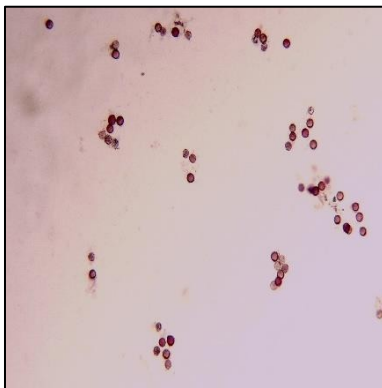
EC 620486



EC 620428



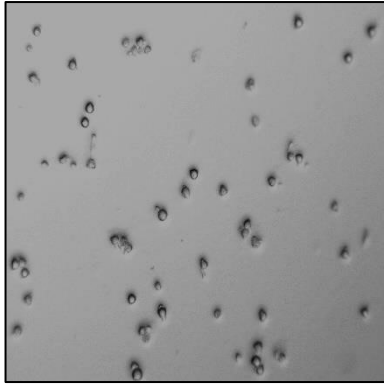
EC 636872



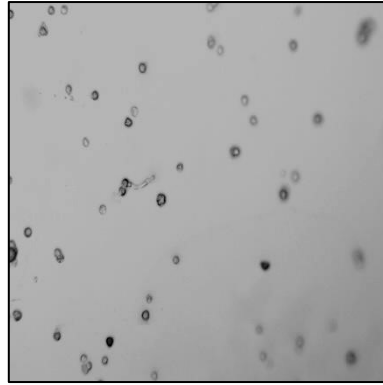
Vellayani Vijay

Plate 6. Pollen viability of genotypes inside polyhouse

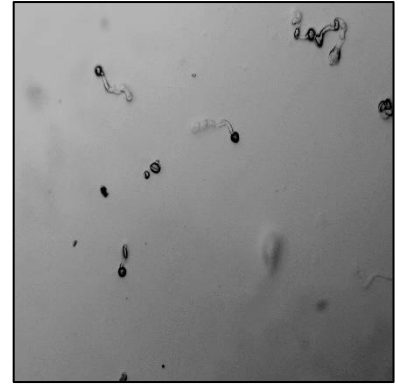
Note: Magnification 40X



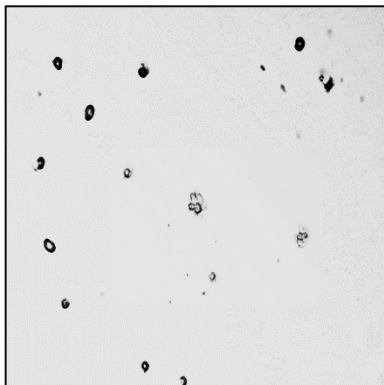
Vellayani Vijay



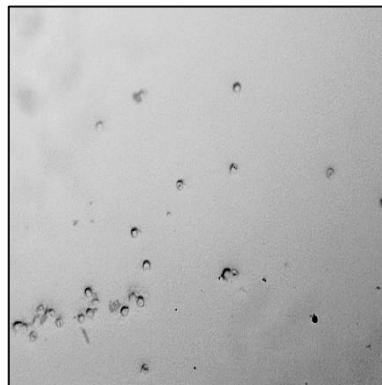
EC 549819



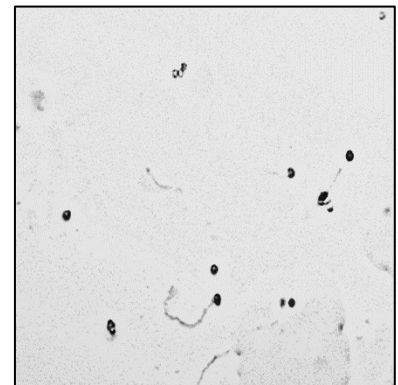
EC 620486



EC 620488



EC 636872



AVTO 1725

Plate 7. Pollen germination of genotypes inside polyhouse

Note: Magnification 40X

AVTO 1725, EC 315489, EC 620486, EC 620488, EC 636872 and Manulakshmi showed high reduction in pollen germination under polyhouse condition and hence can be considered to possess low thermotolerance for the trait (Table 16).

Firon *et al.* (2012) and Jegadeesan *et al.* (2018) reported that pollen incubated under 50 °C for 2 hours resulted in a significant reduction in germination rate. Pollen developed under polyhouse condition (48/23 °C, Max/Min temperature) exhibited an average reduction in pollen germination of 69.22 per cent in this study. All genotypes recorded reduction in pollen germination under polyhouse compared to open condition (30/23 °C).

4.2.4 Style length

Style length under open and polyhouse conditions were comparable for EC 549819, EC 620486, EC 620488, EC 620494, EC 636872, AVTO 1702 and AVTO 1725 indicating thermotolerance of these genotypes, for the trait (Table 14). EC 620488 had the lowest style length than all other genotypes in this study. EC 620494, EC 636872 and Vellayani Vijay was on par with EC 620488 for style length under polyhouse condition. AVTO 1702, AVTO 1706, EC 620486, EC 538153, EC 549819, EC 620428, AVTO 1726, EC 315489 and Manulakshmi had significantly higher style length than Vellayani Vijay. AVTO 1706 (0.65 cm) and EC 620488 (0.40 cm) recorded highest and lowest style length respectively (Table 15).

EC 549819, EC 620488, EC 620494, EC 636872 and Vellayani Vijay showed a reduction in style length under poly house compared to open condition, while all other genotypes exhibited an increase in style length (Table 16). EC 315489 had the highest increase in style length followed by AVTO 1706.

Saeed *et al.* (2007) found that varying tomato genotypes had longer styles by 25–55 per cent at higher temperatures, whereas in the current study we obtained 6.38 – 51.11 per cent longer styles. An increase in style length (7.34 mm) during high temperature condition (>32 °C/21 °C) was also reported by Akhtar *et al.* (2012). In contrast to these studies, we also observed reduction in style length, ranging between 2.08 – 26.53 per cent.

4.2.5 Style protrusion

Manulakshmi, was the superior genotype (with lowest style protrusion) among the genotypes evaluated (Table 15). EC 315489, EC 549819, EC 538153, EC 620428, EC 620486, EC 620488, EC 620494, EC 636872 and Vellayani Vijay were on par with Manulakshmi. AVTO 1706 was significantly inferior than Vellayani Vijay. Highest and lowest style protrusion under polyhouse condition was observed in AVTO 1706 (54.15%) and Manulakshmi (15.58%) respectively.

Vellayani Vijay exhibited a decreasing trend for style protrusion under polyhouse compared to open condition. All other genotypes showed a percent increase in style protrusion under polyhouse condition (Table 16). EC 549819 and EC 538153 showed the highest increase in style protrusion, while EC 315489 showed no change.

High temperature stress during anthesis induced stigma exsertion in various tomato cultivars, as previously reported (Dane *et al.*, 1991; Lohar and Peat, 1998; Srivastava *et al.*, 2012; Pan *et al.*, 2019; Saeed *et al.*, 2007). In contrast to these studies, increase as well as decrease in style protrusion was observed in the current study. Pan *et al.* (2019) subjected tomato genotypes to two elevated temperature conditions, namely moderate elevated temperature ($33\text{ }^{\circ}\text{C}/28 \pm 1\text{ }^{\circ}\text{C}$) and high temperature ($35 \pm 1\text{ }^{\circ}\text{C}/30 \pm 1\text{ }^{\circ}\text{C}$), for 12 days. Under both conditions, the stigmas protruded visibly from the anther cone. In comparison to moderately elevated temperatures (23.5%), the proportion of protruding stigmas was significantly higher at high temperatures (65.2%). Increase in style protrusion ranged between 3.26 – 79.91 per cent and reduction of 11.58 per cent was observed in the present study.

EC 549819, EC 620488, EC 620494 and EC 636872 showed decreasing style length but an increasing trend for style protrusion. This can be attributed to the accompanying decrease in stamen length under high temperature. Contrary to the stigma exsertion seen in wild tomato species, which is brought on by excessive pistil elongation, Pan *et al.* (2019) reported the occurrence of high temperature induced stigma exsertion, which is caused by more severely shortened stamens than pistils.

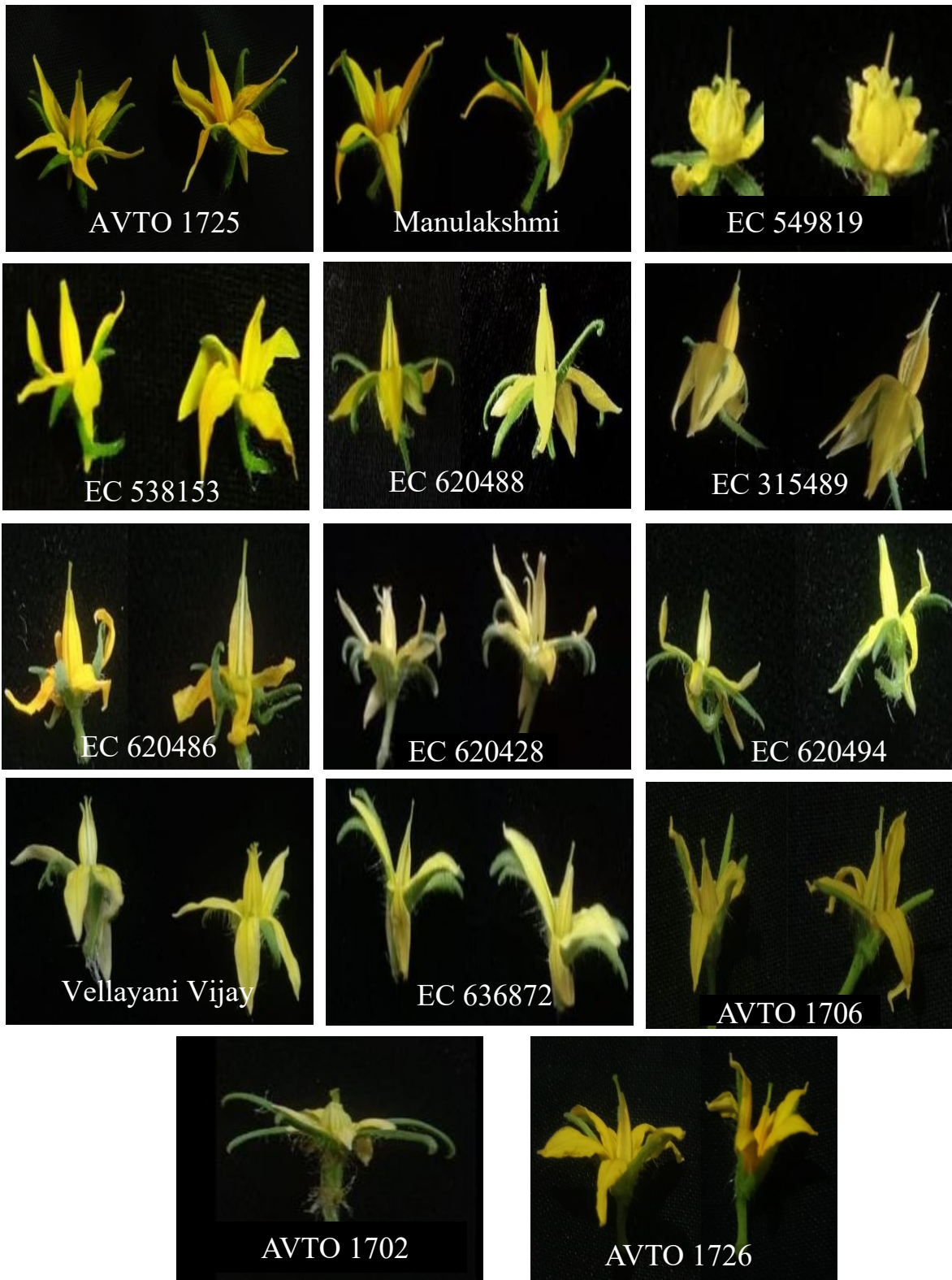


Plate 8. Style protrusion exhibited by genotypes inside polyhouse

4.2.6 Better performing genotypes under polyhouse evaluation

Membrane thermostability and floral characters (pollen viability and stigma exsertion) are critical parameters that determine the thermotolerance of a genotype and based on our observations, superior genotypes were identified for these traits. AVTO 1702 and Manulakshmi showed superiority along with low reduction in electrolyte leakage under polyhouse condition compared to open condition. Under poly house condition, AVTO 1702, AVTO 1726, EC 538153, Manulakshmi and Vellayani Vijay had high pollen viability. Vellayani Vijay and EC 549819 had high pollen germination under poly house condition. EC 549819 also showed low per cent reduction for pollen germination in polyhouse compared to open condition. Genotypes with smaller style and reduction in style length under poly house were Vellayani Vijay, EC 620488, EC 620494 and EC 636872. EC 315489 showed no significant reduction in style protrusion under poly house compared to open condition and Vellayani Vijay exhibited some per cent reduction in style protrusion.

Based on the screening of 14 selected genotypes under polyhouse condition, Manulakshmi, AVTO 1702, EC 549819, EC 620428, EC 636872, EC 315489 and Vellayani Vijay can be considered as superior genotypes for thermotolerance traits

Table 14. Interaction effect of genotype and growth condition on reproductive traits

Sl. No.	Genotypes	Pollen viability (%)		Pollen germination (%)		Style length (cm)		Style protrusion (%)	
		Open	Polyhouse	Open	Polyhouse	Open	Polyhouse	Open	Polyhouse
1	AVTO 1702	16.55 (29.93)	11.67 (21.07)	11.86 (21.40 ^{cd})	2.69 (4.84 ^{ijkl})	0.47 ^{hijkl}	0.50 ^{fghij}	31.47 (57.63)	51.39 (98.77)
2	AVTO 1706	16.91 (30.58)	14.00 (25.28)	8.94 (16.12 ^{defg})	3.36 (6.05 ^{ijkl})	0.57 ^{cdef}	0.74 ^a	52.47 (99.58)	55.83 (107.12)
3	AVTO 1725	33.71 (63.65)	5.00 (9.00)	17.68 (32.00 ^b)	1.86 (3.35 ^{ijkl})	0.44 ^{ijklm}	0.49 ^{ghijk}	30.37 (55.60)	49.72 (101.80)
4	AVTO 1726	10.49 (18.93)	7.26 (13.10)	10.33 (18.63 ^{cdef})	5.19 (9.36 ^{ghijkl})	0.52 ^{efghi}	0.59 ^{cd}	26.61 (48.53)	36.23 (66.82)
5	EC 315489	20.92 (37.98)	9.67 (17.84)	8.62 (15.54 ^{defg})	1.47 (2.64 ^{kl})	0.45 ^{ijklm}	0.68 ^{ab}	30.00 (54.89)	30.00 (55.58)
6	EC 538153	20.46 (37.15)	12.00 (21.7)	10.87 (19.60 ^{cde})	2.66 (4.79 ^{ijkl})	0.56 ^{defg}	0.67 ^{ab}	12.22 (22.05)	21.11 (38.30)
7	EC 549819	18.83 (34.18)	17.53 (31.73)	8.93 (16.10 ^{defg})	8.92 (16.09 ^{defg})	0.48 ^{hijk}	0.47 ^{hijk}	15.38 (27.80)	27.67 (38.06)
8	EC 620428	13.79 (24.91)	5.78 (10.40)	6.63 (11.95 ^{efghij})	2.72 (4.89 ^{ijkl})	0.54 ^{defgh}	0.64 ^{bc}	19.75 (35.78)	27.67 (50.49)
9	EC 620486	4.96 (8.93)	1.63 (2.94)	14.15 (25.58 ^{bc})	1.68 (3.02 ^{ijkl})	0.61 ^{bcd}	0.68 ^{ab}	26.93 (49.12)	32.94 (61.13)
10	EC 620488	6.97 (12.56)	5.88 (10.60)	8.59 (15.50 ^{defg})	0.45 (0.81 ^l)	0.40 ^{lmn}	0.39 ^{mn}	30.32 (55.47)	38.89 (72.19)
11	EC 620494	11.85 (21.38)	3.84 (6.92)	10.63 (19.17 ^{cdef})	3.54 (6.38 ^{hijkl})	0.47 ^{hijkl}	0.40 ^{lmn}	21.83 (39.62)	24.44 (45.05)
12	EC 636872	14.83 (26.79)	3.72 (7.05)	5.67 (10.21 ^{fghijk})	1.50 (2.70 ^{kl})	0.47 ^{hijkl}	0.43 ^{klmn}	15.77 (28.57)	18.33 (33.39)
13	Manulakshmi	17.01 (30.81)	10.35 (18.69)	8.43 (15.19 ^{defgh})	1.90 (3.42 ^{ijkl})	0.50 ^{fghij}	0.58 ^{cde}	15.33 (27.71)	15.83 (28.77)
14	Vellayani Vijay	18.45 (33.40)	13.65 (24.66)	24.94 (45.48 ^a)	7.00 (12.61 ^{defghi})	0.49 ^{ghijk}	0.36 ⁿ	32.99 (60.84)	29.17 (53.58)

Note: Transformed values given in parenthesis.

^aMean followed by a common letter are not significantly different at the 5% level.

Table 15. Response of genotypes to high temperature condition under polyhouse for reproductive traits

Sl. No.	Genotypes	Pollen viability (%)	Pollen germination (%)	Style length (cm)	Style protrusion (%)
1	AVTO 1702	9.61 (17.34)	7.28 (13.12 ^{bcd})	0.49 ^d	41.43 (78.20 ^{ab})
2	AVTO 1706	10.45 (18.86)	6.15 (11.09 ^{cde})	0.65 ^a	54.15 (103.35 ^a)
3	AVTO 1725	14.35 (27.22)	9.77 (17.67 ^b)	0.47 ^{de}	40.05 (78.70 ^{ab})
4	AVTO 1726	7.21 (12.99)	7.76 (13.99 ^{bcd})	0.56 ^c	31.42 (57.67 ^{bcd})
5	EC 315489	8.63 (15.58)	5.17 (9.31 ^{de})	0.56 ^c	30.00 (55.23 ^{bcd})
6	EC 538153	9.56 (17.27)	6.77 (12.20 ^{bcd})	0.62 ^{ab}	16.67 (30.18 ^{de})
7	EC 549819	18.18 (32.95)	8.93 (16.09 ^{bc})	0.48 ^d	17.97 (32.93 ^{de})
8	EC 620428	6.45 (11.62)	4.67 (8.42 ^{de})	0.59 ^{bc}	23.71 (43.14 ^{cde})
9	EC 620486	3.29 (5.93)	7.91 (14.29 ^{bcd})	0.64 ^a	29.93 (55.13 ^{bcd})
10	EC 620488	6.43 (11.58)	4.52 (8.16 ^{de})	0.40 ^f	34.60 (63.83 ^{bc})
11	EC 620494	7.84 (14.15)	7.09 (12.78 ^{bcd})	0.44 ^{def}	23.14 (42.34 ^{cde})
12	EC 636872	9.27 (16.75)	3.59 (6.46 ^e)	0.45 ^{de}	17.05 (30.98 ^{de})
13	Manulakshmi	7.01 (12.66)	5.17 (9.31 ^{de})	0.54 ^c	15.58 (28.25 ^e)
14	Vellayani Vijay	11.05 (19.94)	15.97 (29.04 ^a)	0.42 ^{ef}	31.08 (57.21 ^{bcd})
	CD	8.85 (15.90)	3.49 (6.36)	0.05	14.20 (28.99)
	SE(d)	4.17 (7.96)	1.74 (3.18)	0.03	7.08 (14.47)
	SE(m)	2.95 (5.63)	1.23 (2.24)	0.02	5.01 (10.24)

Note: Transformed values given in parenthesis.

^aMean followed by a common letter are not significantly different at the 5% level.

Table 16. Per cent change in thermotolerance traits under polyhouse compared to open condition

Sl. No.	Genotypes	Pollen viability	Pollen germination	Style length	Style protrusion	Membrane thermostability	Electrolyte leakage
1	AVTO 1702	-29.52%	-77.32%	6.38%	63.30%	5.25%	-14.08%
2	AVTO 1706	-17.19%	-62.42%	29.82%	6.40%	-10.55%	37.39%
3	AVTO 1725	-85.17%	-89.48%	11.36%	63.71%	-1.74%	7.70%
4	AVTO 1726	-30.74%	-49.76%	13.46%	36.15%	0.64%	-1.52%
5	EC 315489	-53.79%	-82.95%	51.11%	0.00%	-7.41%	20.62%
6	EC 538153	-41.35%	-75.53%	19.64%	72.75%	-2.19%	6.07%
7	EC 549819	-6.90%	-0.11%	-2.08%	79.91%	-0.39%	30.89%
8	EC 620428	-58.10%	-58.97%	18.52%	28.62%	-13.41%	48.21%
9	EC 620486	-67.08%	-88.13%	11.48%	22.32%	-15.18%	33.23%
10	EC 620488	-15.67%	-94.76%	-2.50%	28.27%	-10.43%	29.70%
11	EC 620494	-67.56%	-66.70%	-14.89%	11.96%	23.18%	-33.69%
12	EC 636872	-74.93%	-73.54%	-8.51%	16.23%	-34.86%	90.40%
13	Manulakshmi	-39.15%	-77.46%	16.00%	3.26%	0.19%	-0.51%
14	Vellayani Vijay	-26.00%	-71.93%	-26.53%	-11.58%	-6.43%	14.15%

Note: Negative values indicate per cent reduction.

Positive values indicate per cent increase.



AVTO 1702



AVTO 1706



AVTO 1725



AVTO 1726



EC 315489



EC 538153



EC 549819



EC 620428



EC 620486

Plate 9a. Genotypes grown in polyhouse condition



EC 620488



EC 620494



EC 636872



Manulakshmi



Vellayani Vijay

Plate 9b. Genotypes grown in polyhouse condition

4.3 Field screening for thermotolerance

During the March–May season (summer), all the 30 genotypes were grown under natural field conditions, and various growth and yield parameters were recorded.

4.3.1 Pollen viability

Pollen viability of EC 528360 (55.5%) was significantly different from every other genotype and also superior than Vellayani Vijay. All other genotypes were on par with Vellayani Vijay (8.25%) (Table 17). EC 528360 (55.50%) and AVTO 1314 (1.85%) recorded highest and lowest pollen viability. EC 528360 and Vellayani Vijay showed 18.13 and 86.13 per cent reduction in pollen viability respectively, compared to favourable season (October) (Table 18). EC 528360 and EC 549819 showed less than 50 per cent reduction in pollen viability, whereas, AVTO 1314 exhibited more than 90 per cent reduction in summer season compared to favourable season.

Plotting pollen viability observed for summer (May) and favourable season in a scatter plot revealed that EC 636872, EC 523851, EC 528360, EC 528368, Anagha and Akshaya performed well in both seasons (Fig. 5a). EC 528360 can be considered as the superior genotype with respect to pollen viability, as it is superior to Vellayani Vijay, and performed well under both seasons, as well as showed lower reduction in summer compared to favourable season.

Srivastava *et al.* (2012) observed a reduction in tomato pollen viability ranging from 8.99 to 66.59 per cent under 39/24 °C. Percentage reduction in pollen viability of tomato from 36.61 to 87.90 per cent under high temperature conditions (>32 °C/21 °C) was reported by Akhtar *et al.* (2012). Pollen viability under 42.8/29.1 °C (average Max/Min temperature) showed reduction ranging between 18.13 to 95.56 per cent in the current study, which was higher than the studies mentioned above. This decrease can be attributed to high temperature condition that prevailed during the period under study. At high temperature, plants transpire more, and hence yield reduction is caused by the impaired pollen, anther development and reduced pollen viability in tomato (Vijayakumar *et al.*, 2021).

4.3.2 Style length

EC 636872, EC 631354, EC 620494, EC 620488, EC 567305, EC 549819, EC 528368, EC 523851, EC 315489, AVTO 1725, AVTO 1702, AVTO 0922, Arka Samrat and Arka Abhed were on par with Vellayani Vijay (0.41 cm) (Table 17). All other genotypes were inferior than Vellayani Vijay. Highest style length was recorded for AVTO 1314 (0.66 cm) and lowest by EC 567305 and EC 620488 (0.39 cm) respectively. Arka Vishesh, Akshaya, and EC 620488 showed 4.24, 4.10, and 11.43 per cent increase in style length respectively compared to that recorded in the favourable season. All other genotypes showed a percentage decrease in style length ranging from 0.46 to 33.58 per cent (Table 18).

Scatter plot revealed that the genotypes displayed a similar performance under favourable and summer seasons (Fig. 5b). Arka Abhed, Arka Samrat, AVTO 0922, AVTO 1702, AVTO 1725, AVTO 1726, EC 523851, EC 528368, EC 549819, EC 567305, EC 620488, EC 620494, EC 631354 and EC 636872 had lower style length during both seasons, than the overall mean (Fig. 5b).

Saeed *et al.* (2007) found that varied tomato genotypes had longer styles by 25–55 per cent at higher temperatures. An increase in style length of tomato (7.34 mm) during high temperature condition (>32 °C/21 °C) was also reported by Akhtar *et al.* (2012). In contrast to these studies, the current study observed a reduction in style length as well. Increase and decrease in style length that ranged between 4.10 – 11.43 per cent and 0.48 – 33.58 per cent respectively, were observed. This reduction in style length can be attributed to overall reduction in flower size during summer season.

4.3.3 Style protrusion

Genotypes AVTO 0922, EC 538153, EC 549819, EC 567305 and Manulakshmi had significantly lower style protrusion, and hence were superior to Vellayani Vijay (Table 17). Arka Abhed, Arka Vishesh, AVTO 1725, EC 315489, EC 523851, EC 528368, EC 620428, EC 620486, EC 620488, EC 620494, EC 631354 and EC 636872 were on par with Vellayani Vijay (27.16%) for the trait. Akshaya (78.32%) and EC 567305 (11.94%) recorded the highest and lowest style protrusion, respectively. All improved lines and hybrids (genotypes other than EC accessions), except Manulakshmi and Vellayani Vijay showed increase in style

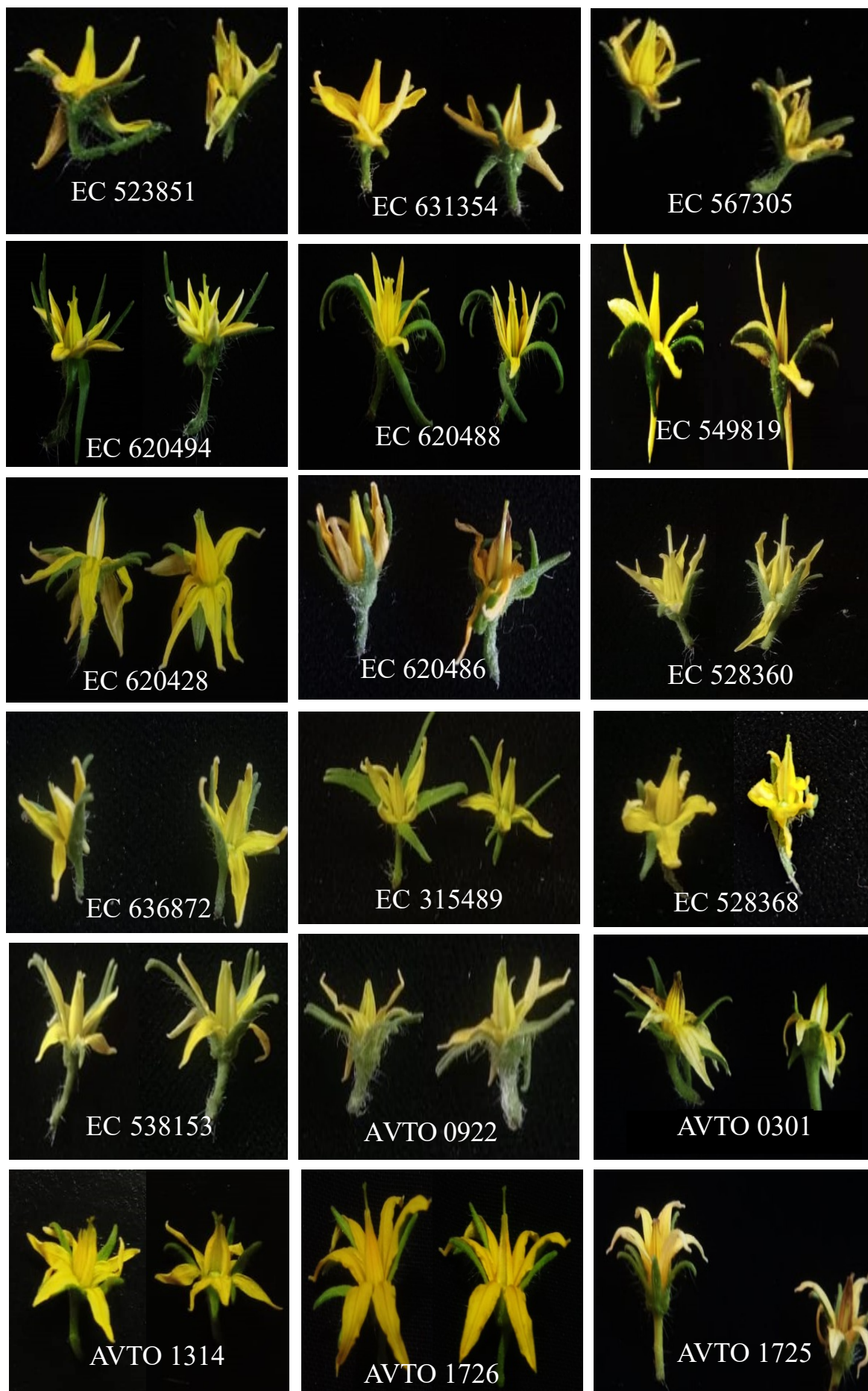


Plate 10a. Protrusion of genotypes in summer (May)

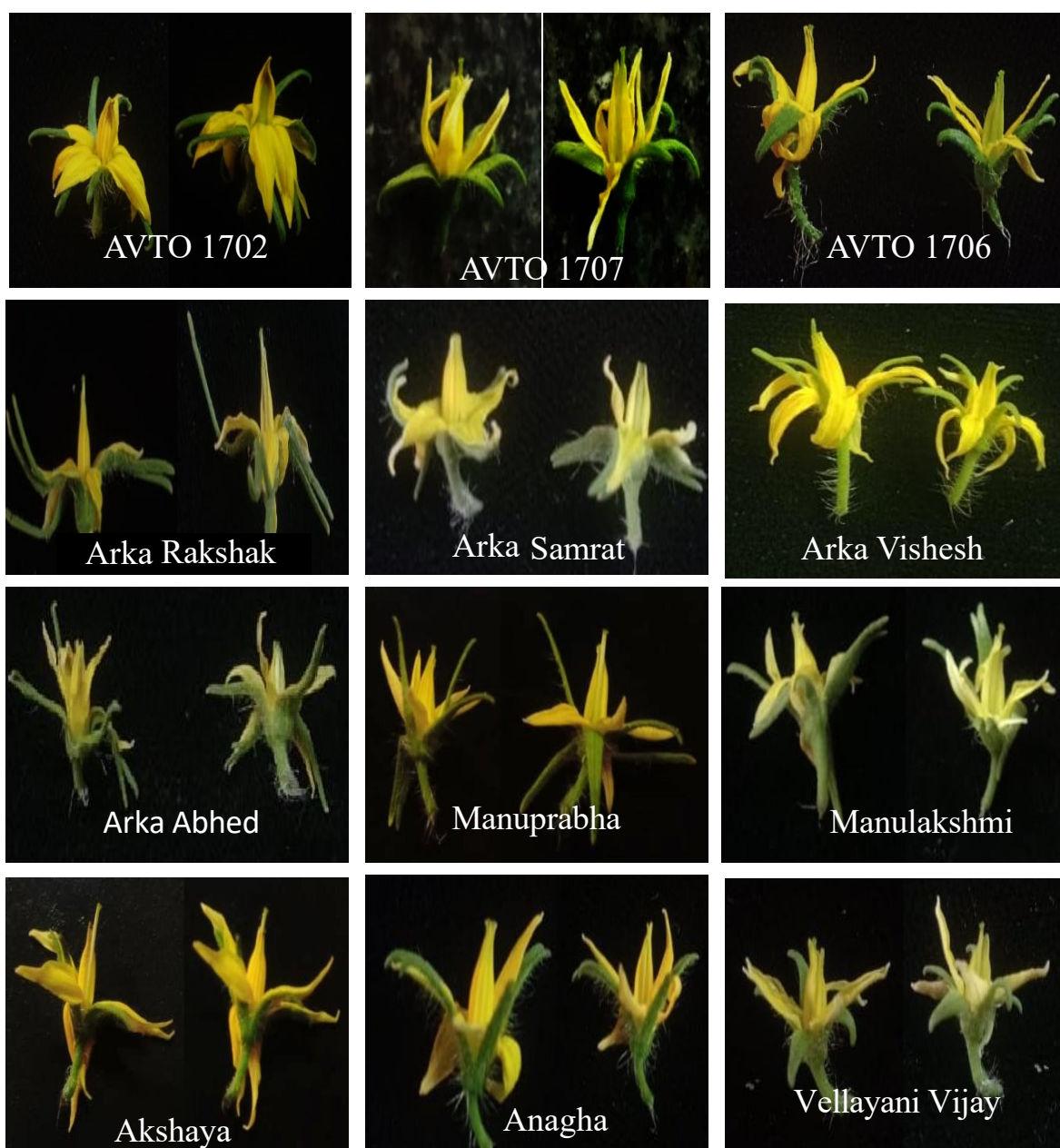


Plate 10b. Protrusion of genotypes in summer (May)

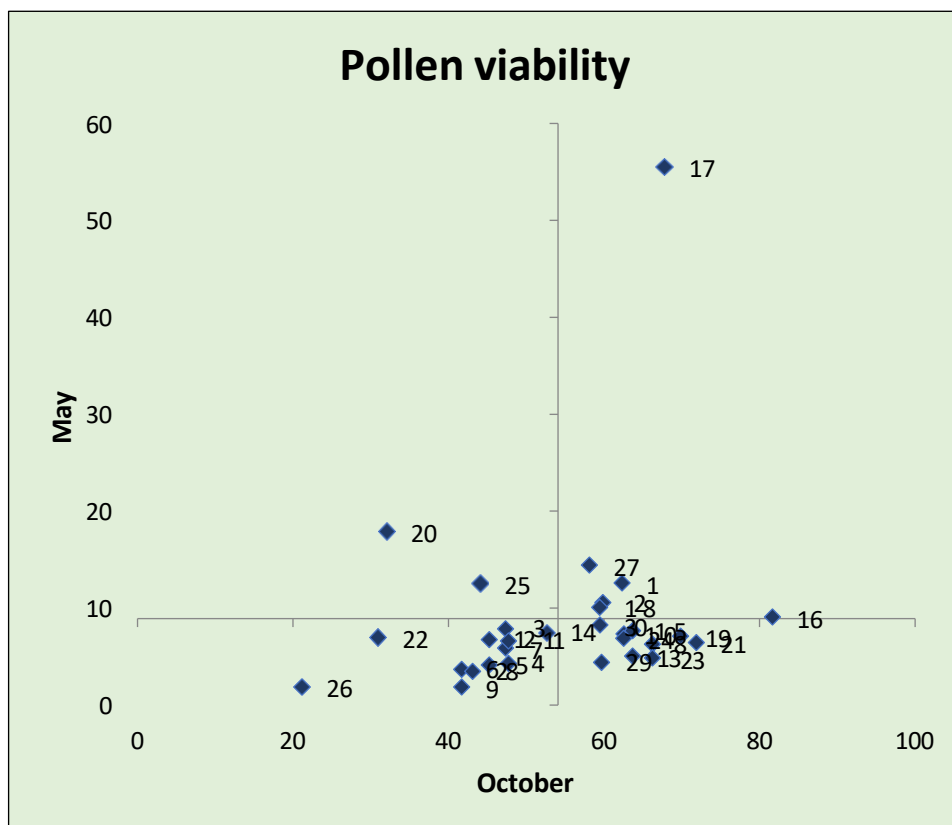


Fig 5a. Comparison between pollen viability under favourable (October) and high temperature (May) conditions

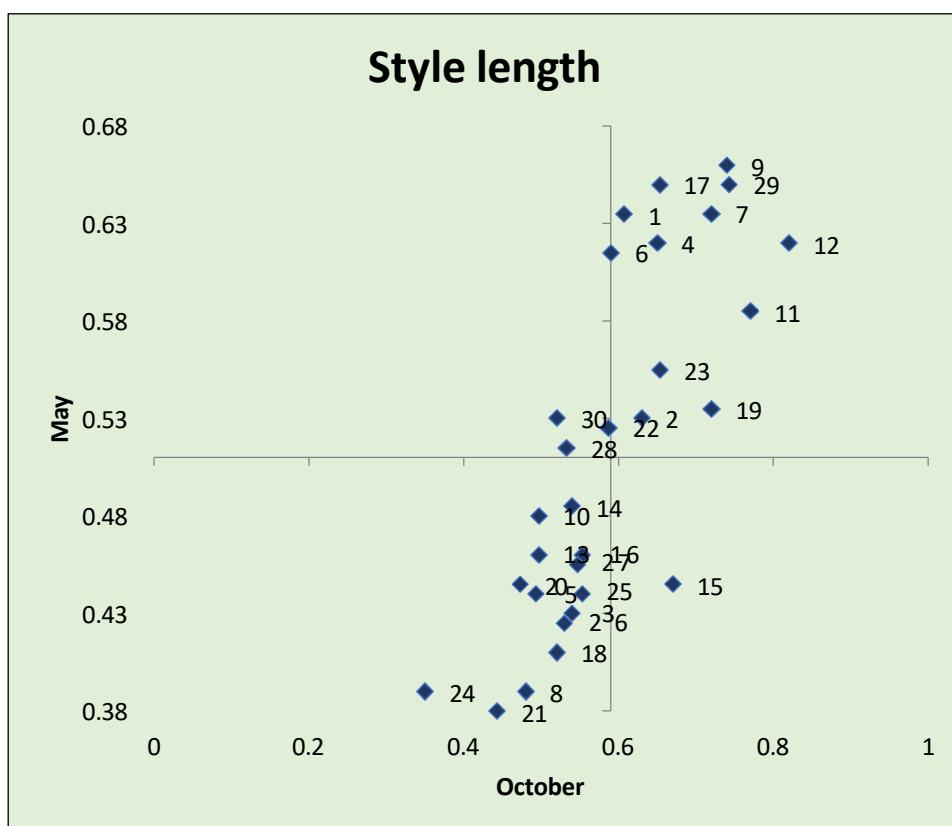


Fig 5b. Comparison between style length under favourable (October) and high temperature (May) conditions

- 1 Akshaya
- 2 Anagha
- 3 Arka Abhed
- 4 Arka Rakshak
- 5 Arka Samrat
- 6 Arka Vishesh
- 7 AVTO 0301
- 8 AVTO 0922
- 9 AVTO 1314
- 10 AVTO 1702
- 11 AVTO 1706
- 12 AVTO 1707
- 13 AVTO 1725
- 14 AVTO 1726
- 15 EC 315489
- 16 EC 523851
- 17 EC 528360
- 18 EC 528368
- 19 EC 538153
- 20 EC 549819
- 21 EC 567305
- 22 EC 620428
- 23 EC 620486
- 24 EC 620488
- 25 EC 620494
- 26 EC 631354
- 27 EC 636872
- 28 Manulakshmi
- 29 Manuprabha
- 30 Vellayani
Vijay

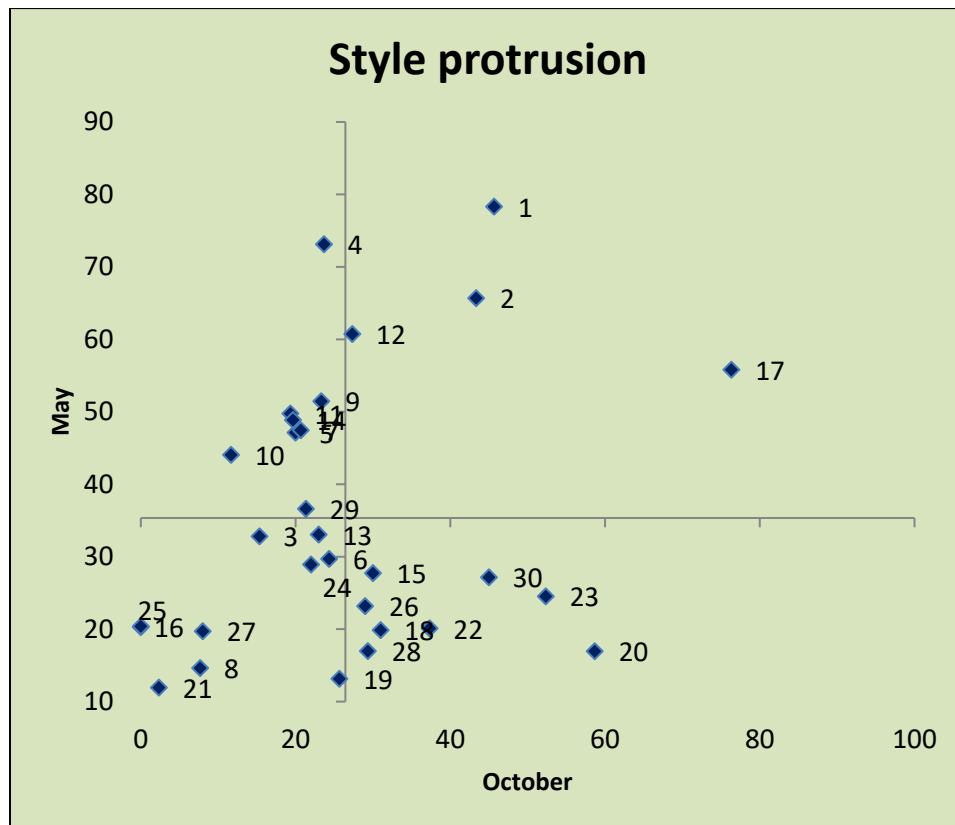


Fig 5c. Comparison between style protrusion under favourable (October) and high temperature (May) conditions

1 Akshaya	16 EC 523851
2 Anagha	17 EC 528360
3 Arka Abhed	18 EC 528368
4 Arka Rakshak	19 EC 538153
5 Arka Samrat	20 EC 549819
6 Arka Vishesh	21 EC 567305
7 AVTO 0301	22 EC 620428
8 AVTO 0922	23 EC 620486
9 AVTO 1314	24 EC 620488
10 AVTO 1702	25 EC 620494
11 AVTO 1706	26 EC 631354
12 AVTO 1707	27 EC 636872
13 AVTO 1725	28 Manulakshmi
14 AVTO 1726	29 Manuprabha
15 EC 315489	30 Vellayani Vijay

protrusion (18.06 to 73.52%). Arka Abhed, Arka Vishesh, AVTO 0922, AVTO 1725, EC 523851, EC 538153, EC 567305, EC 620488, EC 620494 and EC 636872 displayed low style protrusion under favourable (October) as well as summer seasons (Fig. 5c). Most of the EC accessions evaluated in this study demonstrated a decrease in style protrusion in summer. However, EC 523851, EC 567305, EC 620494 and EC 636872 showed a high increase in style protrusion in summer compared to October, indicating the low tolerance of these genotypes (Table 18).

As seen in the scatter plot, Akshaya, Anagha, EC 528360 and AVTO 1707 displayed high style protrusion in October and summer season and had significantly higher style protrusion than Vellayani Vijay in summer season (Fig. 5c).

Stigma exertion in tomato is reported to exhibit a significant increase in the range 7.82 to 50.35 per cent under an average temperature of 38/22.2 °C (Srivastava *et al.*, 2012). In contrast to these studies, we observed both increase and decrease in style protrusion. Majority of the genotypes showed an increase ranging between 18.06 – 99.99 per cent. However, some genotypes recorded reduction in style length ranging from 8.21 to 95.26%. Pan *et al.* (2019) observed that stigma exertion of tomato occurred as a result of severely shortened stamens than pistils. This might be responsible for increased style protrusion despite a decrease in style length in some of the genotypes evaluated in our study.

4.3.4 Fruit set

Genotypes which showed higher fruit set than overall mean (34.67%) are Akshaya, Anagha, Arka Rakshak, Arka Vishesh, AVTO 1314, AVTO 1706, AVTO 1707, AVTO 1725, EC 620428, EC 636872 and Vellayani Vijay. Akshaya (68.24%), Arka Rakshak (57.30%), AVTO 1314 (75.62%), and EC 636872 (78.45%) were significantly superior to Vellayani Vijay and significantly different from other genotypes (Table 17). Arka Vishesh, AVTO 1707, AVTO 1725, EC 620428 and Manuprabha were on par with Vellayani Vijay (42.88%). Highest and lowest fruit set were shown by EC 636872 (78.46%) and EC 315489 (14.44%) respectively.

Zhou *et al.* (2015) exposed tomato inflorescences to high temperatures (36/28 °C), and observed that plants in the heat-tolerant group had a higher average fruit set rate (>60%) and the plants in the heat-sensitive group had a lower average fruit set rate (<20%). Srivastava *et al.* (2012) conducted experiments in tomato during autumn – winter (26/18 °C) and spring – summer season (36/28 °C), and observed a reduction in fruit set/truss in the range 21.70 to 56.15 per cent during summer season. Fruit set is hampered at day/night temperatures above 32 °C/21 °C due to inefficient physiological processes during the flowering and fruit development stages (Muthuvel *et al.*, 2000). According to Akhtar *et al.* (2012), some genotypes set no fruits during the fruit retention stage, while others showed fruit drop with increased fruit maturity due to lack of fertilization and pedicel abscission. In accordance with the above studies, current study also showed a low fruit set per cent at high temperature. In the current study, where temperature prevailed was 42.8/29.1 °C (Max/Min temperature), fruit set ranged between 14.44 – 78.46 per cent which was comparable with the above studies.

4.3.5 Deformed fruits per cent

Deformed fruits per cent, which gives an indication of the flowers which failed to reach the harvestable fruit stage, was more than 80 per cent for all the genotypes evaluated. EC 538153, EC 528360, EC 523851, Arka Rakshak, AVTO 1314, AVTO 1707, Arka Samrat, Arka Abhed, AVTO 0922, EC 636872, Manuprabha and Arka Vishesh showed lesser per cent of deformed fruits than the overall mean (92.15%). There was no significant difference between genotypes for the trait deformed fruits per cent (Table 19). EC 528368 (94.99%) and Arka Vishesh (84.74%) exhibited highest and lowest deformed fruits per cent (Table 19).

When tomato plants face prolonged heat stress with an average temperature of 34 °C/19 °C, a substantial proportion (34%) of flowers drop prematurely (Vijayakumar and Beena, 2020). Also, a significant number of flowers fail to develop into fruits due to the adverse impact of high temperatures. Srivastava *et al.* (2012) observed an increase in tomato flower drop in the range 22.55 to 42.56 per cent under summer season (36/28 °C).

Table 17. Performance of genotypes for reproductive traits under open field condition in summer season

Sl. No.	Accessions	Pollen viability (%)	Style length (cm)	Style protrusion (%)	Fruit set (%)
1	Akshaya	12.59 (22.72 ^{bcd})	0.64 ^{ab}	78.32 (162.11 ^a)	68.24 (135.25 ^b)
2	Anagha	10.58 (19.08 ^{bcd})	0.53 ^{defg}	65.69 (129.56 ^b)	35.01 (64.47 ^{gh})
3	Arka Abhed	7.87 (14.18 ^{bcd})	0.44 ^{klm}	32.82 (60.22 ^{gh})	25.86 (47.09 ^{ijklm})
4	Arka Rakshak	4.30 (7.73 ^{cd})	0.62 ^{abc}	73.13 (147.78 ^a)	57.30 (109.85 ^c)
5	Arka Samrat	4.14 (7.46 ^{cd})	0.44 ^{klm}	47.13 (88.34 ^e)	28.02 (51.13 ^{hijk})
6	Arka Vishesh	3.68 (6.62 ^{cd})	0.62 ^{abc}	29.70 (54.28 ^{gh})	47.53 (89.15 ^d)
7	AVTO 0301	5.87 (10.57 ^{cd})	0.64 ^{ab}	47.47 (89.05 ^e)	21.75 (39.47 ^{klmnop})
8	AVTO 0922	6.27 (11.29 ^{cd})	0.40 ^{lm}	14.64 (26.44 ^{lm})	22.15 (40.21 ^{klmno})
9	AVTO 1314	1.85 (3.33 ^d)	0.66 ^a	51.46 (97.30 ^d)	75.63 (154.40 ^a)
10	AVTO 1702	7.33 (13.21 ^{bcd})	0.46 ^{ijkl}	44.06 (82.22 ^{ef})	24.64 (44.82 ^{ijklmn})
11	AVTO 1706	6.61 (11.91 ^{cd})	0.59 ^{bcd}	49.76 (93.80 ^{de})	35.78 (65.87 ^{fg})
12	AVTO 1707	6.74 (12.15 ^{cd})	0.62 ^{abc}	60.74 (117.53 ^{bc})	47.84 (89.79 ^d)
13	AVTO 1725	5.07 (9.13 ^{cd})	0.47 ^{ghijk}	33.06 (60.68 ^{gh})	38.70 (71.54 ^{ef})
14	AVTO 1726	7.41 (13.35 ^{bcd})	0.49 ^{ghij}	48.87 (91.96 ^{de})	16.64 (30.09 ^{op})
15	EC 315489	7.67 (13.81 ^{bcd})	0.45 ^{klm}	27.73 (50.58 ^{ghij})	14.44 (26.09 ^p)
16	EC 523851	9.08 (16.30 ^{bcd})	0.46 ^{mijkl}	20.28 (36.76 ^{ijklm})	29.83 (54.53 ^{ghij})
17	EC 528360	55.50 (108.23 ^a)	0.65 ^{ab}	55.80 (106.95 ^{cd})	29.84 (54.61 ^{ghij})
18	EC 528368	10.06 (18.15 ^{bcd})	0.42 ^{klm}	19.86 (35.99 ^{klm})	27.40 (49.96 ^{ijkl})
19	EC 538153	7.11 (12.81 ^{bcd})	0.54 ^{def}	13.15 (23.73 ^m)	27.67 (50.47 ^{ijk})
20	EC 549819	17.89 (32.39 ^b)	0.45 ^{ijklm}	16.95 (30.65 ^{klm})	24.40 (44.39 ^{ijklmn})
21	EC 567305	6.43 (11.58 ^{cd})	0.39 ^m	11.94 (21.55 ^m)	22.56 (40.95 ^{klmnop})
22	EC 620428	6.99 (12.59 ^{cd})	0.53 ^{deigh}	20.10 (36.43 ^{klm})	49.41 (93.13 ^a)
23	EC 620486	4.87 (8.76 ^{cd})	0.56 ^{cde}	24.54 (44.63 ^{hijk})	20.10 (36.44 ^{lmnop})
24	EC 620488	6.86 (12.35 ^{cd})	0.39 ^m	28.93 (52.84 ^{ghij})	18.33 (33.19 ^{nop})
25	EC 620494	12.53 (22.61 ^{bcd})	0.45 ^{klm}	20.39 (36.97 ^{ijklm})	30.29 (55.42 ^{gh})
26	EC 631354	1.87 (3.37 ^d)	0.43 ^{klm}	23.18 (42.10 ^{ijkl})	24.10 (43.82 ^{ijklmno})
27	EC 636872	14.43 (26.06 ^{bc})	0.46 ^{hijkl}	19.71 (35.74 ^{klm})	78.46 (162.47 ^a)
28	Manulakshmi	3.46 (6.22 ^d)	0.52 ^{efghi}	16.96 (30.68 ^{klm})	19.33 (35.03 ^{mnop})
29	Manuprabha	4.41 (7.94 ^{cd})	0.65 ^{ab}	36.61 (67.53 ^{fg})	35.93 (66.17 ^{efg})
30	Vellayani Vijay	8.25 (14.87 ^{bcd})	0.41 ^{klm}	27.16 (49.60 ^{nj})	42.88 (79.85 ^{de})
	CD	9.20 (19.67)	0.07	8.41 (17.65)	7.05 (13.83)
	SE(d)	4.50 (9.63)	0.03	4.12 (8.64)	3.45 (6.77)

Note: Transformed values given in parenthesis.

^aMean followed by a common letter are not significantly different at the 5% level.

Table 18. Per cent change in reproductive traits in summer season compared to favourable season (October-December)

Sl. No.	Accessions	Pollen viability	Style length	Style protrusion
1	Akshaya	-79.79%	4.10%	41.69%
2	Anagha	-82.32%	-15.87%	34.03%
3	Arka Abhed	-83.38%	-20.37%	53.27%
4	Arka Rakshak	-90.99%	-4.62%	67.63%
5	Arka Samrat	-90.85%	-10.75%	57.56%
6	Arka Vishesh	-91.18%	4.24%	18.06%
7	AVTO 0301	-87.61%	-11.81%	56.46%
8	AVTO 0922	-90.54%	-18.75%	47.61%
9	AVTO 1314	-95.56%	-10.81%	54.66%
10	AVTO 1702	-88.28%	-3.42%	73.52%
11	AVTO 1706	-86.13%	-24.03%	61.15%
12	AVTO 1707	-85.10%	-24.39%	55.00%
13	AVTO 1725	-92.04%	-7.44%	30.43%
14	AVTO 1726	-85.93%	-10.19%	59.75%
15	EC 315489	-87.96%	-33.58%	-8.21%
16	EC 523851	-88.88%	-16.82%	99.99%
17	EC 528360	-18.13%	-0.46%	-36.81%
18	EC 528368	-83.07%	-21.15%	-56.09%
19	EC 538153	-89.82%	-25.69%	-95.26%
20	EC 549819	-44.17%	-5.92%	-71.12%
21	EC 567305	-91.05%	-14.22%	80.44%
22	EC 620428	-77.42%	-10.56%	-85.74%
23	EC 620486	-92.65%	-15.01%	-53.12%
24	EC 620488	-89.04%	11.43%	23.95%
25	EC 620494	-71.57%	-20.43%	99.99%
26	EC 631354	-91.16%	-19.81%	-25.13%
27	EC 636872	-75.16%	-16.82%	59.41%
28	Manulakshmi	-91.98%	-3.38%	-72.95%
29	Manuprabha	-92.62%	-12.52%	41.73%
30	Vellayani Vijay	-86.13%	-1.20%	-65.72%

In the current study conducted under 42.8/29.1 °C (Max/Min temperature) condition deformed fruits per cent recorded was between 84.74 – 95.57 per cent, which is higher than that reported by the above studies. This increase can be attributed to higher temperature condition that prevailed during the study.

4.3.6 Plant height

Anagha, Arka Abhed, EC 315489, EC 523851, EC 528360, EC 538153, EC 567305, EC 620486, EC 620488 and EC 636872 were on par with Vellayani Vijay (35.70%) (Table 19). Arka Rakshak, Arka Samrat, Arka Vishesh, EC 631354, Manulakshmi, EC 549819, EC 528368, AVTO 1725, AVTO 1314, AVTO 0301, AVTO 1706, AVTO 1726, AVTO 0922 and AVTO 1707 showed higher plant height than the overall mean (47.78 cm), which means that these genotypes showed better vegetative growth even under heat stress condition. Arka Rakshak (68.04 cm) and EC 567305 (29.47 cm) were the genotypes with highest and lowest plant height respectively (Table 19).

Solankey *et al.* (2017) observed plant height ranging from 12.60 to 114.33 cm during heat stress condition (>35 °C). Chippy *et al.* (2021) reported plant height ranging between 72.62 to 118.44 cm (mean - 93.52 cm) while screening 10 genotypes. In current study, plant height ranged from 29.47cm to 68.04cm which was similar to the above studies. Bhattarai *et al.* (2021) observed a significant reduction in plant height (24.3%) under heat stress (34/24 °C) compared to control condition (26/20 °C). Vijayakumar *et al.* (2021) observed a percentage increase in the range of 14 to 70 per cent in tomato cultivars under heat stress condition under polyhouse (36±2 °C) which they attributed to the shade in polyhouse as well.

4.3.7 Days to 50 per cent flowering

Reducing the time taken to reach 50 per cent flowering in tomatoes under high temperature condition plays a crucial role in plant adaptation. Early flowering was shown by EC 528368 (27 days after transplanting (DAT)) and late flowering by AVTO 0301 (55 DAT). Akshaya, Anagha, Arka Samrat, Arka Vishesh, AVTO 1702, AVTO 1726, EC 315489, EC 523851, EC 528360, EC 528368, EC 549819, EC 620428 and EC 636872 showed early flowering and were significantly superior to Vellayani Vijay. All other genotypes were on par with Vellayani Vijay (50.50 DAT) with respect to the trait days to 50 per cent flowering (Table 19). AVTO 0301 and AVTO 1706 flowered later (55 DAT) compared to all other genotypes, while EC 528368 flowered earlier (26.50 DAT) than all other genotypes (Table 19).

Solankey *et al.* (2017) also reported earlier days to first flowering under high temperature (38.9 °C). According to Chipsey *et al.* (2021) days to 50 per cent flowering ranged between 43.22 to 54.22 days (mean – 47.76 days). In the present study, days to 50 per cent flowering ranged between 26.50 to 55 days after transplanting (mean – 45.58 days) which is comparable to the earlier studies.

4.3.8 Number of fruits/plant

Vellayani Vijay, Anagha, Akshaya, Manuprabha, EC 636872, EC 549819, AVTO 1707 and AVTO 1702 showed higher number of fruits per plant than overall mean (1.48). Vellayani Vijay (2.94) and EC 636872 (2.72) were on par with each other and significantly different from every other genotype (Table 19). Vellayani Vijay (2.94) and EC 538153 (1.09) were the genotypes showing highest and lowest number of fruits per plant respectively.

Alsamir *et al.* (2017) reported 88.8 per cent reduction in the number of fruits in tomato due to high temperature stress. When air temperature increased from 25 °C to 29 °C, the number of fruits per plant in tomato was found to be reduced in earlier studies as well (Harel *et al.*, 2014).

4.3.9 Average fruit weight

Arka Rakshak (34.42 g) recorded the highest and EC 549819 (2.91 g) recorded the lowest average fruit weight among the genotypes evaluated (Table 19). Arka Rakshak, AVTO 1706, Anagha, Arka Samrat, AVTO 1314, AVTO 0301, EC 620428, EC 631354, AVTO 1726, Akshaya, AVTO 1707, Arka Abhed, Manuprabha, Arka Vishesh, AVTO 1725, AVTO 1702, Manulakshmi and AVTO 0922 showed higher average fruit weight than the overall mean (16.65 g). Arka Rakshak (34.42 g) was significantly different from every other genotype (Table 19). Vellayani Vijay showed low average fruit weight (9.51 g), even though the number of fruits per plant was significantly higher. EC 528360 and EC 620488 were on par with Vellayani Vijay (2.94 g). EC 528368, EC 549819, EC 567305 and EC 636872 were inferior to Vellayani Vijay and all other genotypes were superior to Vellayani Vijay.

Average fruit weight reduced from 52.87 g to 27.63 g during high temperature stress condition (>32 °C/21 °C) in tomato (Akhtar *et al.*, 2012). According to Solankey *et al.* (2017) average fruit weight reduced under high

temperatures (38.9 °C). Rajametov *et al.* (2021) reported reduction in fruit weight by 31.9 per cent in heat susceptible tomato cultivars, whereas, heat tolerant cultivars showed 3.6 per cent increase in fruit weight.

4.3.10 Fruit yield/plant

Arka Rakshak (37.11 g) recorded highest fruit yield per plant and lowest was recorded by EC 549819 during summer field screening (3.56 g) (Table 19). Arka Rakshak, Akshaya, AVTO 1707, Anagha, AVTO 0301, Manuprabha, AVTO 1314, Vellayani Vijay, Arka Samrat, Arka Vishesh, AVTO 1706, EC 631354, Manulakshmi and AVTO 1726 showed higher fruit yield per plant than the overall mean (17.19 g). Arka Rakshak (37.11 g/plant) had significantly higher yield compared to other genotypes (Table 19). Akshaya, Anagha, Arka Abhed, Arka Samrat, Arka Vishesh, AVTO 0301, AVTO 1314, AVTO 1706, AVTO 1707, AVTO 1725, AVTO 1726, EC 620428, EC 631354, Manulakshmi and Manuprabha were on par with Vellayani Vijay (24.34 g/plant) (Table 19).

Yield recorded in the present study under summer season was very low. Yield reduction ranging from 69 to 99 per cent has been reported in tomato crop under heat stress by Vijayakumar *et al.* (2021). According to Singh *et al.* (2005) reduced allocation of assimilates under high temperature stress compared to control temperature condition led to decreased yield. Islam (2011) pointed out that reduced supply of photosynthates and poor production of growth regulators in sink tissues are the reasons for reduced yield related traits, which was supported by Hasanuzzaman *et al.* (2013).

Table 19. Performance of genotypes for growth and yield related traits in summer season under open field condition

Sl. No.	Accessions	Plant height (cm)	Deformed fruits (%)	Days to 50% flowering	Number of fruits/plant	Fruit yield/ plant (g)	Average fruit weight (g)
1	Akshaya	46.95 ^{efghi}	92.79 (214.04)	39.50 ^{jk}	1.75 ^{de}	27.00 ^b	21.43 ^{cd}
2	Anagha	41.15 ^{hijk}	93.06 (215.61)	35.00 ^{kl}	1.88 ^{cd}	26.53 ^{bc}	16.38 ^{gh}
3	Arka Abhed	38.83 ^{ijk}	89.25 (199.43)	52.50 ^{abc}	1.14 ^{gh}	16.17 ^{efghijk}	20.17 ^{cde}
4	Arka Rakshak	68.04 ^a	91.08 (206.29)	49.00 ^{bcdef}	1.44 ^{defgh}	37.11 ^a	34.42 ^a
5	Arka Samrat	66.87 ^{ab}	89.73 (203.03)	42.50 ^{ghij}	1.29 ^{efgh}	22.57 ^{bcdef}	25.22 ^b
6	Arka Vishesh	66.20 ^{ab}	84.74 (182.46)	45.50 ^{efgh}	1.42 ^{defgh}	21.64 ^{bcdefg}	18.61 ^{ef}
7	AVTO 0301	50.20 ^{cdef}	93.63 (218.57)	55.00 ^a	1.42 ^{defgh}	25.74 ^{bc}	22.21 ^c
8	AVTO 0922	49.15 ^{defgh}	89.24 (199.38)	51.50 ^{abcd}	1.13 ^{gh}	10.99 ^{ijklmn}	18.23 ^{efg}
9	AVTO 1314	53.70 ^{cde}	90.55 (203.85)	50.00 ^{bcde}	1.34 ^{efgh}	24.34 ^{bcde}	25.50 ^b
10	AVTO 1702	44.30 ^{fghi}	94.07 (220.63)	42.00 ^{hij}	1.55 ^{defgh}	15.16 ^{fghijk}	17.59 ^{fg}
11	AVTO 1706	49.80 ^{defg}	94.06 (220.94)	55.00 ^a	1.39 ^{defgh}	20.33 ^{bcdefgh}	18.22 ^{efg}
12	AVTO 1707	48.67 ^{defgh}	89.75 (200.56)	49.00 ^{bcdef}	2.26 ^{bc}	26.53 ^{bc}	13.57 ⁱ
13	AVTO 1725	54.54 ^{cde}	93.39 (217.08)	48.00 ^{cdef}	1.18 ^{fgh}	16.26 ^{defghijk}	18.88 ^{ef}
14	AVTO 1726	49.67 ^{defg}	94.62 (223.81)	45.50 ^{efgh}	1.24 ^{fgh}	18.23 ^{cdefghijk}	21.07 ^{cd}
15	EC 315489	33.22 ^{kl}	94.53 (222.96)	34.00 ^l	1.14 ^{gh}	9.80 ^{klmn}	12.83 ⁱ
16	EC 523851	39.20 ^{ijk}	91.35 (208.49)	44.50 ^{fghi}	1.33 ^{efgh}	13.31 ^{ghijkl}	12.77 ⁱ
17	EC 528360	43.12 ^{fghij}	91.72 (209.07)	40.50 ^{ij}	1.42 ^{defgh}	12.54 ^{hijklm}	10.63 ^j
18	EC 528368	54.60 ^{cde}	94.99 (227.04)	26.50 ^m	1.45 ^{defgh}	6.52 ^{lmn}	4.61 ^k
19	EC 538153	41.45 ^{ghijk}	92.03 (211.45)	52.50 ^{abc}	1.09 ^h	11.93 ^{hijklmn}	14.53 ^{hi}
20	EC 549819	54.74 ^{cde}	94.77 (224.44)	29.00 ^m	1.61 ^{deg}	3.56 ⁿ	2.91 ^k
21	EC 567305	29.47 ^l	94.61 (223.41)	47.00 ^{defg}	1.17 ^{fgh}	4.05 ^{mn}	4.17 ^k
22	EC 620428	44.22 ^{fghi}	95.57 (229.18)	45.50 ^{efgh}	1.30 ^{efgh}	16.77 ^{defghijk}	21.92 ^c
23	EC 620486	34.07 ^{kl}	93.67 (218.43)	50.00 ^{bcde}	1.24 ^{fgh}	11.26 ^{ijklmn}	12.70 ^{ghi}
24	EC 620488	42.94 ^{fghij}	92.97 (216.30)	53.00 ^{ab}	1.11 ^{gh}	5.70 ^{lmn}	9.02 ^j
25	EC 620494	47.29 ^{efghi}	92.67 (214.12)	50.00 ^{bcde}	1.30 ^{efgh}	14.01 ^{fghijkl}	14.15 ⁱ
26	EC 631354	58.50 ^{bc}	94.10 (220.58)	50.00 ^{bcde}	1.27 ^{efgh}	19.81 ^{bcdefghi}	21.03 ^{cd}
27	EC 636872	43.02 ^{fghij}	88.43 (195.33)	35.00 ^{kl}	2.72 ^{ab}	9.91 ^{klmn}	3.72 ^k
28	Manulakshmi	56.00 ^{cd}	93.71 (218.57)	48.50 ^{bcdef}	1.29 ^{efgh}	18.75 ^{bcdefghij}	17.54 ^{fg}
29	Manuprabha	47.70 ^{defgh}	87.09 (190.65)	51.00 ^{abcd}	1.66 ^{def}	24.82 ^{bcd}	19.71 ^{de}
30	Vellayani Vijay	35.70 ^{ijkl}	92.33 (211.88)	50.50 ^{abcd}	2.94 ^a	24.34 ^{bcde}	9.51 ^j
	CD	8.50	NS	4.93	0.50	8.63	2.07
	SE(d)	4.16	2.73 (12.21)	2.41	0.25	4.22	1.01

Note: Transformed values given in parenthesis.

^aMean followed by a common letter are not significantly different at the 5% levels.

4.4 Better performing genotypes based on overall performance

Thermotolerance of tomato is determined by its membrane thermostability, floral characters (pollen viability and stigma exertion), fruit set and fruit yield per plant. Based on the observations recorded, superior genotypes were identified for the important traits.

Superior genotypes with respect to electrolyte leakage were Arka Samrat, Arka Vishesh, EC 538153, AVTO 1314 and Manuprabha under laboratory screening, and AVTO 1702, AVTO 1706, AVTO 1725, EC 620428, EC 538153 and Manulakshmi under polyhouse screening. Performance of EC 549819, EC 620428 and EC 636872 was similar under polyhouse as well as open condition.

EC 636872, EC 523851, EC 528360, EC 528368, Anagha and Akshaya performed well for pollen viability under laboratory study as well as field screening. EC 528360 showed superior pollen viability and lower reduction in pollen viability under field screening as well.

Genotypes AVTO 0922, EC 538153, EC 549819, EC 567305 and Manulakshmi had significantly lower style protrusion, and hence were superior to Vellayani Vijay. Arka Abhed, Arka Vishesh, AVTO 0922, AVTO 1725, EC 523851, EC 538153, EC 567305, EC 620488, EC 620494, and EC 636872 displayed low style protrusion under favourable (October) as well as summer season indicating their thermotolerance.

High temperature stress leads to early flowering in tomato. Genotypes which showed early flowering and were significantly superior to Vellayani Vijay were, Akshaya, Anagha, Arka Samrat, Arka Vishesh, AVTO 1702, AVTO 1726, EC 315489, EC 523851, EC 528360, EC 528368, EC 549819, EC 620428, and EC 636872.

Akshaya, Arka Rakshak, AVTO 1314, and EC 636872 were significantly superior to Vellayani Vijay for fruit set in summer. EC 538153, EC 528360, EC 523851, Arka Rakshak, AVTO 1314, AVTO 1707, Arka Samrat, Arka Abhed, AVTO 0922, EC 636872, Manuprabha and Arka Vishesh showed lower deformed fruits per cent.

In summer, Akshaya, Anagha, Arka Abhed, Arka Samrat, Arka Vishesh, AVTO 0301, AVTO 1314, AVTO 1706, AVTO 1707, AVTO 1725, AVTO 1726,

EC 620428, EC 631354, Manulakshmi and Manuprabha were on par with Vellayani Vijay for fruit yield per plant. Arka Rakshak was the significantly superior genotype. Akshaya, Arka Rakshak and AVTO 1314 were the genotypes exhibiting higher fruit set, lower deformed fruits per cent and higher yield per plant.

EC 636872 and EC 523851 showed superiority in both (October and May) the seasons for thermotolerance traits which was depicted in the scatter plot diagram (Fig. 4f, g, h) and hence can be considered as thermotolerant.

Based on laboratory, polyhouse and field screening of genotypes for thermotolerant traits, EC 549819, EC 636872, EC 523851 and Vellayani Vijay was considered as superior genotypes. Even though EC 549819 was superior for thermotolerant traits it exhibited inferior performance in yield traits. EC 636872 was significantly superior for fruit set and plant height. EC 523851 was superior for plant height and average fruit weight. EC 620486, EC 538153 and EC 528360 were superior for plant height and average fruit weight even though they are inferior for thermotolerant traits. AVTO 1314 and Akshaya exhibited superiority for fruit set, plant height, fruit yield per plant and average fruit weight. Anagha showed superiority for plant height, fruit yield per plant and average fruit weight. AVTO 1707 was superior for fruit set, plant height, fruit yield per plant and average fruit weight.

Even though Akshaya, Arka Rakshak, and AVTO 1314 failed to perform well for thermotolerance traits *per se*, they exhibited superior yield traits even in summer season. EC 636872 and EC 523851 showed superiority for thermotolerance traits under laboratory screening. Performance of EC 549819, EC 620428, and EC 636872 was similar under polyhouse and open condition with high pollen germination, coupled with low style protrusion. Hence, Akshaya, Arka Rakshak and AVTO 1314 can be used for yield improvement; and EC 636872, EC 523851, EC 549819, EC 620428 and EC 636872 can be used for improving specific thermotolerance traits in tomato breeding programmes.



EC 523851



EC 631354



EC 567305



EC 620494



EC 620488



EC 549819



EC 620428



EC 620486



EC 528360

Plate 11a. Genotypes under field evaluation



EC 636872



EC 315489



EC 528368



EC 538153



AVTO 0922



AVTO 0301



AVTO 1314



AVTO 1726



AVTO 1725

Plate 11b. Genotypes under field evaluation



AVTO 1702



AVTO 1706



AVTO 1707



Arka Rakshak



Arka Samrat



Arka Vishesh



Arka Abhed



Manuprabha



Manulakshmi

Plate 11c. Genotypes under field evaluation

4.4 Genetic parameters

Phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2) and genetic advance (GA) for ten characters were assessed and presented in Table 20.

The GCV values ranged from 1.91 to 100.55 per cent, and the PCV values ranged from 3.48 to 112.07 per cent. Deformed fruits per cent showed low GCV and PCV. Days to 50 per cent flowering and style length showed moderate GCV and PCV. High GCV and PCV was shown by pollen viability, style protrusion, number of fruits per plant, fruit set, average fruit weight and fruit yield per plant. Plant height showed high PCV and moderate GCV. All the characters except deformed fruits per cent, exhibited high heritability coupled with high GA as per cent of mean (GAM). Thus, these characters show additive gene effects and can be relied upon for selection. PCV is slightly higher than GCV for all traits indicating that environment plays an important role in trait expression.

High GCV and PCV was observed for the number of fruits per plant and fruit yield per plant by Kadyan *et al.* (2023) in tomato. They also reported moderate PCV and GCV for average fruit weight and lower PCV and GCV for plant height and days to 50 per cent flowering.

Varun *et al.* (2023) evaluated 76 genotypes under high temperature stress in open condition. They reported high GCV and PCV for fruit yield per plant, number of fruits per plant, and average fruit weight. Number of fruits per plant, plant height and pollen viability showed high heritability and fruit yield per plant, number of fruits per plant, average fruit weight showed high heritability coupled with high GA. Ara *et al.* (2009) reported high PCV for fruit yield per plant and number of fruits per plant. According to Narayan *et al.* (2020) number of fruits per plant and fruit yield per plant exhibited high GCV and heritability.

Yield per plant, average fruit weight and number of fruits per plant showed high GA, while pollen viability and plant height exhibited moderate GA (Varun *et al.*, 2023). High levels of GCV, PCV, H^2 and GA were reported for fruit yield per plant, number of fruits per plant and plant height by Meena *et al.* (2018) also. Plant height, number of fruits per plant and yield per plant showed high heritability and genetic advance (Sherpa *et al.*, 2014).

Table 20. Genetic parameters of morphological traits observed in the study

	V_g	V_p	V_e	PCV (%)	GCV (%)	H² (%)	GAM (%)
Pollen viability	80.46	99.95	19.49	112.07	100.55	89.72	185.83
Style length	0.01	0.01	0.00	17.97	16.88	93.93	32.66
Style protrusion	338.10	355.65	17.55	53.37	52.04	97.51	104.52
Plant height	84.41	101.86	17.45	21.13	19.23	91.01	36.06
Days to 50% flowering	52.82	58.77	5.95	16.82	15.94	94.77	31.14
No of fruits/plant	51.94	67.08	15.14	46.62	41.03	88.01	74.37
Fruit set	283.61	295.92	12.31	49.62	48.58	97.90	97.97
Deformed fruits	3.10	10.25	7.16	3.48	1.91	54.89	2.16
Average fruit weight	45.96	56.17	10.21	45.02	40.72	90.45	75.88
Fruit yield/plant	54.24	72.40	18.16	49.51	42.85	86.55	76.41

4.5 Correlation analysis

The association between ten characters was worked out in all possible combinations and is presented in Table 21.

Pollen viability was positively correlated with style length, style protrusion and the number of fruits per plant though not significant. Akhtar *et al.* (2012) also reported a significant positive correlation between pollen viability and number of fruits per plant, fruit weight and fruit yield per plant.

Style length showed significant positive correlation with style protrusion, fruit set, average fruit weight, and fruit yield per plant. Style protrusion showed positive significant correlation with style length, fruit set, average fruit weight, and fruit yield per plant. Plant height showed significant positive correlation with fruit yield per plant and average fruit weight. Plant height and number of fruits per plant showed positive non-significant correlation in earlier studies also (Kadyan *et al.* 2023).

Days to 50 per cent flowering was positively correlated with fruit yield per plant, and showed a significant positive correlation with average fruit weight. A cultivar's ability to produce fruits earlier due to early anthesis at high temperatures can be viewed as a positive adaptation to heat stress. Days to flowering and fruit yield per plant were found to be strongly correlated (Kugblenu *et al.*, 2013).

Number of fruits per plant showed significant positive correlation with fruit set, and positive correlation with pollen viability, style length, style protrusion and fruit yield per plant. Fruit set showed significant positive correlation with number of fruits per plant and fruit yield per plant; and significant negative correlation with deformed fruits per cent. Fruit set showed significant positive correlation with style length and style protrusion as well. Cross-pollination can result in increased fruit set in wild tomatoes, which is frequently associated with longer style lengths. A longer style in wild tomato species can facilitate cross-pollination by native insects, which is essential for fruit production, especially outside their native region, where there are fewer insect pollinators (Seed Savers, 2016).

Deformed fruits per cent exhibited significant negative correlation with fruit set. Deformed fruits per cent showed negative correlation with style length and style protrusion although not significant. Negative correlation between number of

flowers shed and fruit yield per plant was reported by Saeed *et al.* (2007) similar to the current study.

Average fruit weight was significantly positively correlated with days to 50 per cent flowering, fruit yield per plant, plant height, fruit set, style length, and style protrusion. Lin *et al.* (2006) found a positive correlation between fruit weight and yield, whereas, Kugblenu *et al.* (2013) found no correlation between these two traits. Akhtar *et al.* (2012) also reported that the number of fruits per plant and fruit weight were negatively correlated.

Fruit yield per plant showed significant positive correlation with fruit set, average fruit weight, plant height, style length and style protrusion. Fruit yield per plant was positively correlated with days to 50 per cent flowering and number of fruits per plant. Similar to the present study, fruit yield per plant showed positive correlation with number of fruits per plant and fruit weight in the studies conducted by Narayan *et al.* (2020). Fruit yield per plant was reported to show a strong significant positive correlation with average fruit weight (Kadyan *et al.* 2023), as observed in the present study. In contrast, Saeed *et al.* (2007) reported that stigma tube elongation and yield are negatively correlated.

Table 21. Correlation between morphological characters observed during field screening in summer season

	Pollen viability	Style length	Style protrusion	Plant height	Days to 50% flowering	No of fruits/plant	Fruit set	Deformed fruits	Average fruit weight	Fruit yield/plant
Pollen viability	1.00	0.22	0.16	-0.19	-0.35	0.11	-0.01	0.04	-0.34	-0.25
Style length		1.00	0.56**	0.12	0.25	0.19	0.48**	-0.33	0.39*	0.56**
Style protrusion			1.00	0.27	0.01	0.15	0.38*	-0.10	0.64**	0.74**
Plant height				1.00	-0.01	-0.11	0.22	-0.29	0.47**	0.45*
Days to 50% flowering					1.00	-0.19	-0.08	-0.20	0.42*	0.33
No of fruits/plant						1.00	0.52**	-0.26	-0.20	0.27
Fruit set							1.00	-0.401*	0.21	0.45*
Deformed fruits								1.00	-0.17	-0.31
Average fruit weight									1.00	0.82**
Fruit yield/plant										1.00

** Correlation is significant at 0.01

* Correlation is significant at 0.05 level

4.7 Molecular marker analysis

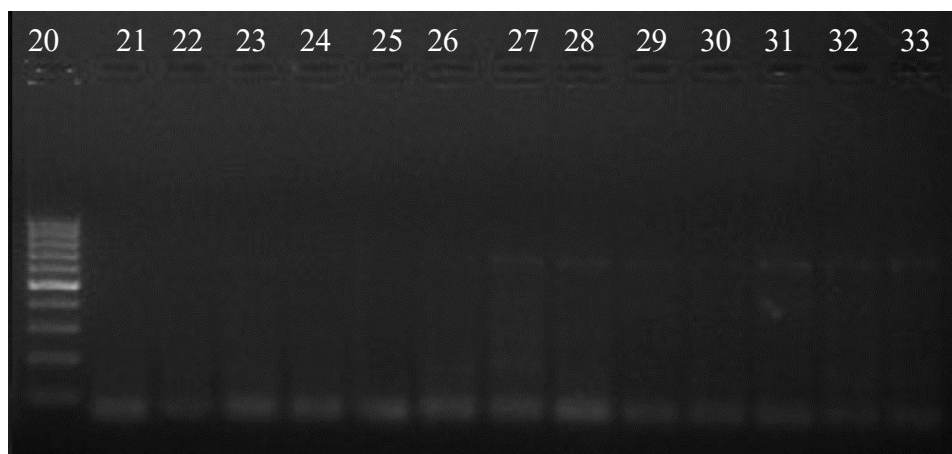
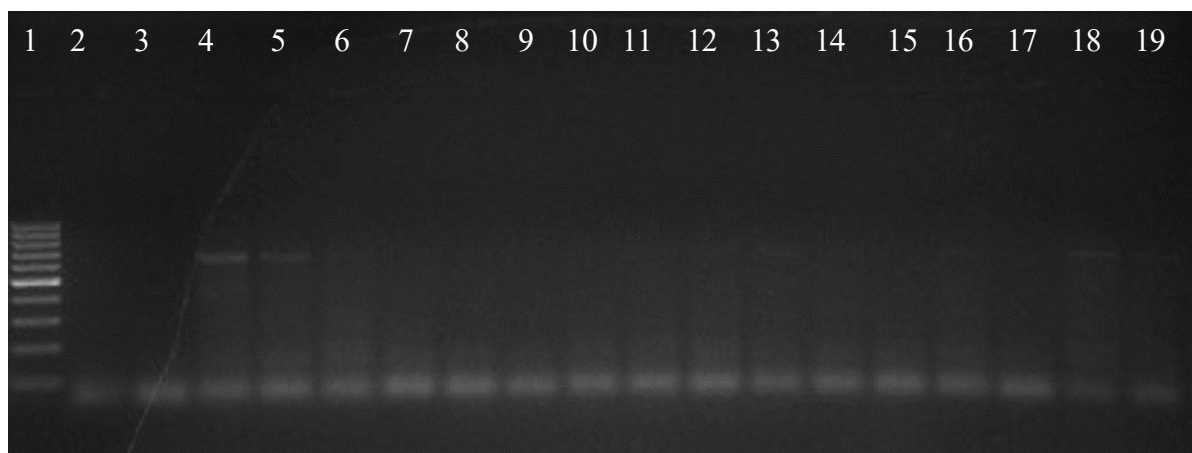
Genomic DNA of thirty genotypes (Table 22) were isolated using CTAB method and were analysed for the presence of two reported markers. Molecular marker screening carried out using SSR and RAPD primers *viz.*, SSR 63 and A16 respectively, showed amplification. The DNA used was of good quality and quantity.

While PCR products of SSR 63 primer were analysed, amplicon of size between 100 and 200 bp was observed for twelve out of thirty genotypes (Plate. 12). Vellayani Vijay, EC 528360, AVTO 1725, Akshaya, Anagha, AVTO 1314, EC 620494, EC 538153, AVTO 0301, AVTO 1707, Arka Rakshak, Arka Vishesh, EC 315489, AVTO 1702, EC 631354, and Manulakshmi showed amplification between 100 and 200 bp. In contrast, Shaji *et al.* (2020) used twenty five primers to screen twenty two tomato genotypes and obtained polymorphic band with size ~ 250bp for SSR 63 primer. Hence, the results obtained in the present study were not conclusive.

PCR products of A16 primer showed amplification between 100 and 200 bp for certain genotypes. Genotypes AVTO 1725, Akshaya, Anagha, AVTO 1314, EC 620494, EC 538153, AVTO 0301, AVTO 1707, Arka Rakshak, EC 620488, EC 620428, EC 528368, AVTO 1726, EC 631354, Manulakshmi, Manuprabha and EC 549819 showed amplification between 100 and 200 bp (Plate.13). While examining heat tolerance in genotypes using ten RAPD and six ISSR primers, Kamel *et al.* (2010) reported that RAPD marker A16 as a reliable marker for heat tolerance having polymorphic band at 100 bp. A similar polymorphism could not be observed in the present study.

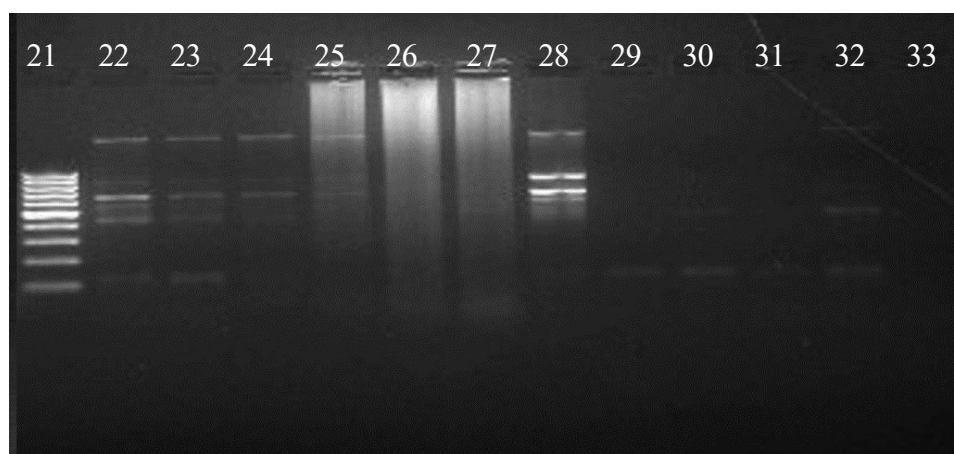
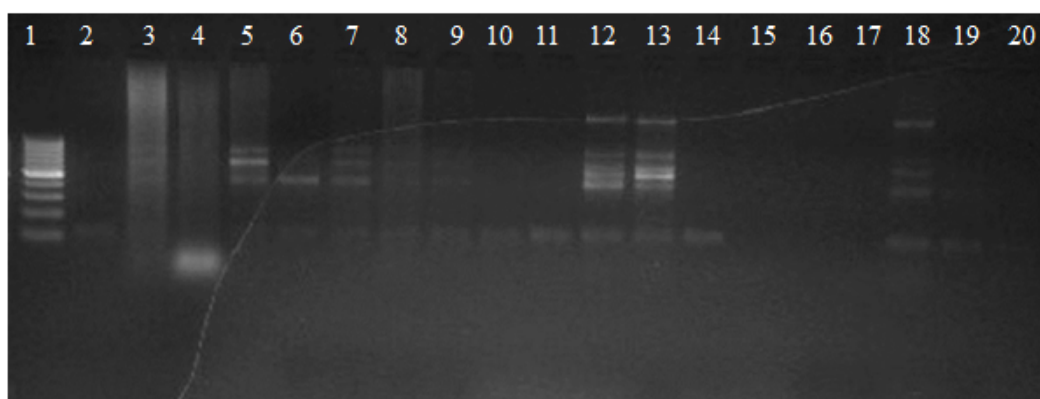
Table 22: Quantity and quality of isolated DNA from genotypes used for the study

Sl. No.	Accessions	A ₂₆₀ /A ₂₈₀	Quantity (µg/mL)
1	AVTO 0301	2.09	2394.1
2	AVTO 0922	2.15	4219.6
3	AVTO 1314	2.02	1865.1
4	AVTO 1702	2.17	2892.6
5	AVTO 1706	1.99	476.10
6	AVTO 1707	2.17	2988.9
7	AVTO 1725	2.15	2468.0
8	AVTO 1726	2.16	3128.3
9	EC 315489	2.18	3538.9
10	EC 523851	2.14	1429.6
11	EC 528360	2.14	3092.8
12	EC 528368	2.17	850.85
13	EC 538153	2.16	1895.3
14	EC 549819	2.18	1764.9
15	EC 567305	2.13	1317.9
16	EC 620428	2.19	1746.6
17	EC 620486	2.18	3324.7
18	EC 620488	2.15	1704.2
19	EC 620494	2.18	2954.4
20	EC 631354	2.16	2022.6
21	EC 636872	2.16	1643.1
22	Arka Abhed	2.03	2751.3
23	Arka Rakshak	2.12	4197.7
24	Arka Samrat	2.02	4642.5
25	Arka Vishesh	2.18	2904.1
26	Akshaya	2.04	1338.3
27	Anagha	2.20	3107.0
28	Manuprabha	2.11	1495.8
29	Manulakshmi	2.16	1641.4
30	Vellayani Vijay	2.14	1888.7



- | | |
|---------------------|------------------|
| 1. 100bp ladder | 17. AVTO 0922 |
| 2. Negative control | 18. EC 620488 |
| 3. EC 620486 | 19. EC 620428 |
| 4. Vellayani Vijay | 20. 100bp ladder |
| 5. EC 528360 | 21. AVTO 1726 |
| 6. AVTO 1725 | 22. AVTO 1706 |
| 7. Akshaya | 23. EC 523851 |
| 8. Anagha | 24. EC 567305 |
| 9. AVTO 1314 | 25. EC 636872 |
| 10. EC 620494 | 26. AVTO 1702 |
| 11. EC 538153 | 27. EC 631354 |
| 12. AVTO 0301 | 28. Manulakshmi |
| 13. AVTO 1707 | 29. Manuprabha |
| 14. Arka Rakshak | 30. EC 549819 |
| 15. Arka Vishesh | 31. EC 528368 |
| 16. EC 315489 | 32. Arka Abhed |
| | 33. Arka Samrat |

Plate 12. Amplification of the marker SSR 63



- | | |
|--------------------|----------------------|
| 1. 100bp ladder | 18. EC 620488 |
| 2. Vellayani Vijay | 19. EC 620428 |
| 3. EC 620486 | 20. Arka Samrat |
| 4. Arka Abhed | 21. 100 bp ladder |
| 5. EC 528360 | 22. EC 528368 |
| 6. AVTO 1725 | 23. AVTO 1726 |
| 7. Akshaya | 24. AVTO 1706 |
| 8. Anagha | 25. EC 523851 |
| 9. AVTO 1314 | 26. EC 567305 |
| 10. EC 620494 | 27. EC 636872 |
| 11. EC 538153 | 28. AVTO 1702 |
| 12. AVTO 0301 | 29. EC 631354 |
| 13. AVTO 1707 | 30. Manulakshmi |
| 14. Arka Rakshak | 31. Manuprabha |
| 15. Arka Vishesh | 32. EC 549819 |
| 16. EC 315489 | 33. Negative control |
| 17. AVTO 0922 | |

Plate 13. Amplification of RAPD marker - A16

SUMMARY

5. SUMMARY

The study on “Screening of tomato (*Solanum lycopersicum* L.) genotypes for high temperature tolerance” was conducted to screen 30 tomato genotypes for thermotolerance and yield traits, and further screening them using reported molecular markers. Screening was done based on laboratory, polyhouse and field evaluation.

Under lab screening, electrolyte leakage, pollen viability, pollen germination, style length and style protrusion were evaluated. Major findings of lab screening are listed below.

- ✓ Arka Samrat, EC 538153, AVTO 1314 and Manuprabha were significantly superior genotypes than Vellayani Vijay with respect to electrolyte leakage and membrane thermostability.
- ✓ EC 523851 recorded the highest pollen viability and was significantly superior than Vellayani Vijay, followed by AVTO 0922, EC 620486, EC 567305, EC 538153, and EC 528360.
- ✓ A declining trend was evident when we compared the pollen characteristics, such as pollen viability, and pollen germination at room temperature, 35 °C, and 40 °C.
- ✓ Despite having high pollen viability, EC 636872 and Manulakshmi exhibited lower germination percentage at all temperatures.
- ✓ EC 528368, EC 620486, Akshaya and Vellayani Vijay were superior when pollen viability and pollen germination at 40 °C were considered together. EC 528368, EC 620494 and EC 620428 recorded lower reduction in pollen germination under 35 °C as well as 40 °C compared to Vellayani Vijay.
- ✓ Considering the combination of pollen viability and low style protrusion, EC 315489, EC 523851, EC 528368, EC 538153, EC 567305, EC 620486, EC 620488, EC 636872, AVTO 0922, AVTO 1725, and Vellayani Vijay were superior.

Thermotolerant traits, viz., electrolyte leakage, pollen viability, pollen germination, style length, and style protrusion for selected genotypes, were also evaluated in the polyhouse. Major findings of polyhouse screening are listed below.

- ✓ AVTO 1725, AVTO 1702, AVTO 1706, EC 620428, EC 538153 and Manulakshmi were the superior genotypes with respect to electrolyte leakage.
- ✓ EC 549819, EC 620488 and AVTO 1706 had low pollen viability and high per cent reduction under polyhouse compared to open condition. All other genotypes were superior for pollen viability. All genotypes displayed a decrease in pollen viability and pollen germination under polyhouse compared to the open condition.
- ✓ EC 549819, EC 620428, and EC 636872 showed comparable values for pollen germination under open and polyhouse conditions. Vellayani Vijay was superior than all other genotypes for pollen germination under open and polyhouse condition.
- ✓ Reduction in style length under polyhouse compared to open condition was observed in EC 549819, EC 620488, EC 620494, EC 636872 and Vellayani Vijay, EC 315489 and AVTO 1706 had the highest increase in style length.
- ✓ Except Vellayani Vijay all other genotypes displayed an increase in style protrusion under polyhouse than open condition. EC 549819 and EC 538153 showed highest increase in style protrusion and EC 315489 had no change in style protrusion while comparing open and polyhouse conditions.
- ✓ EC 549819, EC 620428, and EC 636872 were identified as superior genotypes from polyhouse screening considering style protrusion and pollen germination.

Field screening included screening based on thermotolerant as well as growth and yield traits in summer season. Major findings of field screening are listed below.

- ✓ EC 528360 had significantly higher pollen viability than Vellayani Vijay and displayed a lower difference between seasons.
- ✓ Compared to the favourable season, Arka Vishesh, Akshaya, and EC 620488 displayed a rise in style length of 4.24 per cent, 4.10 per cent, and 11.43 per cent, respectively.

- ✓ Style protrusion increased in all improved lines and hybrids, other than EC accessions. Low style protrusion was seen in Arka Abhed, Arka Vishesh, AVTO 0922, AVTO 1725, EC 523851, EC 538153, EC 567305, EC 620488, EC 620494, and EC 636872 in both favourable (October) and summer seasons, indicating their thermotolerance.
- ✓ Akshaya, Arka Rakshak, AVTO 1314, and EC 636872 had significantly higher fruit set per cent than Vellayani Vijay.
- ✓ Genotypes evaluated did not differ significantly for deformed fruits per cent and was higher than 80 per cent for every genotype examined.
- ✓ Anagha, Arka Abhed, EC 315489, EC 523851, EC 528360, EC 538153, EC 567305, EC 620486, EC 620488, and EC 636872 were statistically comparable to Vellayani Vijay for plant height.
- ✓ Genotypes that flowered earlier than Vellayani Vijay were Akshaya, Anagha, Arka Samrat, Arka Vishesh, AVTO 1702, AVTO 1726, EC 315489, EC 523851, EC 528360, EC 528368, EC 549819, EC 620428, and EC 636872.
- ✓ Vellayani Vijay and EC 636872 were on par with each other and significantly different from every other genotype for number of fruits per plant.
- ✓ Considering average fruit weight, Arka Rakshak (4.42 g) differed significantly from all other genotypes. Vellayani Vijay had a low average fruit weight, in spite of having a significantly higher number of fruits per plant. Arka Rakshak had a significantly higher yield than all other genotypes. Akshaya, Anagha, Arka Abhed, Arka Samrat, Arka Vishesh, AVTO 0301, AVTO 1314, AVTO 1706, AVTO 1707, AVTO 1725, AVTO 1726, EC 620428, EC 631354, Manulakshmi, and Manuprabha were on par with Vellayani Vijay.

Pollen viability, style protrusion, number of fruits per plant, fruit set, average fruit weight, and fruit yield per plant showed high GCV and PCV. Except for the percentage of deformed fruits, all other characters had a high heritability and GAM. Thus, these characters exhibit additive gene effects and can be used for selection.

Correlation analysis revealed

- ✓ A significant positive correlation of style length with style protrusion, fruit set, average fruit weight, and fruit yield per plant was observed.

- ✓ Fruit set exhibited a positive correlation with the number of fruits per plant, pollen viability, style length, style protrusion, and fruit yield per plant.
- ✓ Fruit yield per plant showed a positive correlation with fruit set, average fruit weight, plant height, style length, and style protrusion.

AVTO 0922, EC 523851, EC 528368, EC 549819, EC 620494, EC 636872 and Vellayani Vijay were identified as genotypes exhibiting heat tolerant traits considering evaluations under laboratory, polyhouse and open field conditions.

Akshaya, Anagha, Arka Rakshak, AVTO 0301, AVTO 1314, AVTO 1707, Manuprabha and Vellayani Vijay exhibited higher yield, and except AVTO 0301 all of them had high fruit set per cent in summer.

Hence, thermotolerant genotypes, and genotypes having high yield in summer identified in the study can be used as donors for specific reproductive traits in crop improvement for thermotolerance in tomato. Vellayani Vijay had heat tolerance traits as well as superior yield traits indicating its suitability for incorporation in crop improvement programmes.

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6. REFERENCES

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**SCREENING OF TOMATO (*Solanum lycopersicum* L.) GENOTYPES FOR HIGH
TEMPERATURE TOLERANCE**

by
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(2021-11-076)

ABSTRACT

**Submitted in partial fulfilment of the
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**Faculty of Agriculture
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Abstract

Tomato (*Solanum lycopersicum* L., 2n=24) cultivation is prevalent in both tropical and sub-tropical climates. For optimum yield and quality, the crop needs a climate that is dry and fairly cold, and are sensitive to hot and humid climate. In the current context of global warming, high temperature is considered as a major threat to agriculture with profound consequences on yield and quality. Plant morphology, physiology, biochemistry, and molecular pathways are disrupted by heat stress. The optimum temperature range for fruiting in tomato is narrow viz., 15 to 21 °C at night and 30 to 35 °C during the day. Depending on the stage of growth, a daily mean temperature between 21 and 24 °C is ideal for tomato.

The current study, ‘Screening of tomato (*Solanum lycopersicum* L.) genotypes for high temperature tolerance’ was conducted during 2021–2023 at the Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara. Thirteen NBPGR accessions, four hybrids from IIHR, Bengaluru, eight improved lines from the World Vegetable Centre, Taiwan, and five KAU varieties made up the 30 tomato genotypes used in the study.

Genotypes were subjected to laboratory screening for thermotolerant traits, and selected genotypes were taken for polyhouse screening. Field screening was done during summer for evaluating the performance of all the genotypes for thermotolerance and yield traits. All experiments were laid out in completely randomized design with three replications for laboratory and polyhouse screening, and two replications for field screening. Physiological traits (electrolyte leakage and membrane stability) and morphological traits (pollen viability, pollen germination, style length and style protrusion) were evaluated under laboratory and polyhouse screening. Plant height and yield characters (days to 50 per cent flowering, fruit set per cent, deformed fruits per cent, number of fruits per plant, average fruit weight and fruit yield per plant) were recorded under field screening along with morphological traits.

Arka Samrat, EC 538153, AVTO 1314, and Manuprabha were superior genotypes for electrolyte leakage and membrane thermostability under laboratory screening. EC 528368, EC 620486, Akshaya, and Vellayani Vijay outperformed other varieties in terms of pollen viability and germination at 40 °C. Superior genotypes

based on pollen viability and style protrusion were EC 315489, EC 523851, EC 528368, EC 538153, EC 567305, EC 620486, EC 620488, EC 636872, AVTO 0922, AVTO 1725 and Vellayani Vijay. Based on style protrusion and pollen germination at 40 °C, and their combination with other traits, 14 genotypes (AVTO 1702, AVTO 1706, AVTO 1725, AVTO 1726, EC 315489, EC 538153, EC 549819, EC 620428, EC 620486, EC 620488, EC 620494, EC 636872, Manulakshmi, and Vellayani Vijay) were selected for polyhouse evaluation.

Polyhouse screening was done inside a non-ventilated structure to ensure temperature build up, and an increase of 4-5°C was found inside the structure compared to ambient condition. All genotypes showed a decrease in pollen viability and germination under polyhouse compared to the open condition. All the genotypes, except Vellayani Vijay showed an increase in style protrusion. Superior genotypes with respect to electrolyte leakage were AVTO 1725, AVTO 1702, AVTO 1706, EC 620428, EC 538153, and Manulakshmi. EC 549819, EC 620428, and EC 636872 were considered as superior genotypes based on style protrusion and pollen germination, as they are important thermotolerant traits.

Field screening was carried out to assess the yield traits along with thermotolerant traits in summer season. EC 528360 was found to be superior in pollen viability and style length. Style protrusion increased in all breeding lines and hybrids, except EC accessions. Genotypes like Akshaya, Arka Rakshak, AVTO 1314, and EC 636872 were significantly superior for fruit set percentage compared to Vellayani Vijay. Deformed fruits percentage showed no significant variation between genotypes, and was more than 80 per cent for every genotype examined. Vellayani Vijay and EC 636872 exhibited higher fruit number per plant. Arka Rakshak had a significantly higher average fruit weight than all other genotypes.

Pollen viability, style protrusion, number of fruits per plant, fruit set, average fruit weight, and fruit yield per plant all demonstrated high GCV and PCV. The GAM and high heritability of the remaining characters suggested that they had additive gene effects and can be used for selection. Correlation analysis of observations recorded for field screening showed that style length and style protrusion had a significant positive correlation with fruit set per cent, average fruit weight and fruit yield per plant.

Molecular markers did not reveal polymorphism in the present study and needs further investigation to identify reliable markers.

AVTO 0922, EC 523851, EC 528368, EC 549819, EC 620494, EC 636872, and Vellayani Vijay were identified as heat-tolerant genotypes based on laboratory, polyhouse, and open field evaluations. Akshaya, Anagha, Arka Rakshak, AVTO 0301, AVTO 1314, AVTO 1707, Manuprabha, and Vellayani Vijay had higher yields and, with the exception of AVTO 0301, all had high fruit set percentage in the summer season. In general, thermotolerant genotypes performed moderate to poor in terms of yield traits. Hence, the study demonstrated that specific donors for thermotolerance traits and yield traits needs to be included in crop improvement programmes for heat tolerance in tomato. Vellayani Vijay had heat tolerance traits as well as superior yield traits indicating its suitability for incorporation in crop improvement programmes.