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Genetic Variation for Tolerance to High Temperatures in Tomato Using Critical Sterility Temperature

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) reproduction is influenced by temperature, prominently impacting yield. The ideal daytime range is between 25°C and 30°C and the nighttime temperature is around 20°C for tomato and deviations of a few degrees from these temperatures can have negative effects. The study assessed the effect of temperature on tomato pollen germination and aimed to ascertain the critical sterility temperature for pollen germination. In an open field, pollen from 10 genotypes (Anagha, Manuprabha, Vellayani Vijay, Akshaya, Nandi, IIHR26372, Pusa

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Rohini, Arka Saurabh, Arka Rakshak, and Arka Vikas) underwent *In-vitro* temperature exposure ranging from 32 to 40°C at 2°C intervals. Variations in *In-vitro* pollen germination percentage were observed across the genotypes, notably revealing a sharp decline after 36°C. This decline indicated a pivotal shift in pollen germination and viability. Genotypes, Anagha and Arka Saurabh, were identified as temperature tolerant and susceptible, respectively. The findings present an opportunity to incorporate temperature-dependent pollen response functions and identified genotypes into tomato breeding programs, offering significant implications for enhancing yield under varying temperature conditions.

Keywords: Temperature; pollen germination; tomato; critical temperature; pollen selection.

1. INTRODUCTION

The tomato (*Solanum lycopersicum* L.) is a globally significant horticultural crop with substantial economic value, but it faces increasing challenges from rising temperatures due to global climate change. When temperatures exceed 25 °C, there is a notable decrease in fruit quantity, weight, and seed count per fruit [1]. Exposure to extreme heat (45 °C) for short periods can induce programmed cell death (PCD), release of cytochrome c, and production of caspase-like enzymes [2]. The plant's reproductive phase is typically more susceptible to high temperatures than its vegetative phase [3,4]. Elevated temperatures during flowering significantly affect pollen viability and germination, leading to reduced fruit set and yield [1]. Various crops, including bell pepper, corn, tomato, cowpea, and soybean, exhibit sensitivity to above-optimal temperatures during fruit set [5,6,7,8,9]. Numerous studies have focused on heat stress effects on pollen development [10,11,12,13]. Research by [11] revealed that heat stress causes male sterility in tomatoes, but male sterile plants grown at 29 °C could produce fruit when pollinated by pollen developed at 25°C. In tomatoes, pollen germination and tube development are impaired at temperatures above 30°C [14]. Research indicates that the ideal temperature for pollen germination *In vitro* ranges from 15 to 22 °C, while 25 °C is optimal for in vivo germination [15,16]. Pollen viability and germination success can be negatively affected by temperatures exceeding 30°C. The male reproductive organs (stamens) are particularly susceptible to heat stress, which can lead to a decrease in the quantity of pollen grains produced and released by the anther, as well as reduced pollen viability and germination rates [17,18]. Pollen germination and viability are especially vulnerable to high temperatures, making them key indicators of heat tolerance.

To assess high-temperature tolerance in tomato, researchers employ various physiological and biochemical tests, with the pollen germination percentage assay playing a crucial role. These tests provide direct insights into how well tomato plants reproduce under heat stress conditions [11]. By examining pollen responses to elevated temperatures, plant breeders can identify and choose varieties with enhanced tolerance characteristics, thus improving breeding programs aimed at developing heat-resistant tomato cultivars [19]. This approach offers a rapid and efficient screening method to aid in the selection of robust tomato varieties capable of thriving in warmer environments. This study seeks to examine the methodology for evaluating tomato varieties' high-temperature tolerance using pollen germination assays.

2. MATERIALS AND METHODS

2.1 Plant Growth

The study was carried out at the Plant Physiology Department of the Agriculture College in Vellayani, situated at 8°5'N latitude, 76°9'E longitude, and 29 meters above sea level. The research involved ten tomato varieties: Anagha, Manuprabha, Vellayani Vijay, Akshaya, Nandi, IIHR26372, Pusa Rohini, Arka Saurabh, Arka Rakshak, and Arka Vikas. These varieties were grown in pots containing an equal mixture of farmyard manure (FYM), sand, and soil with 4 replications of each. The pots were positioned in an open field, and the plants received nutrients and pest control treatments as recommended by Kerala Agricultural University.

2.2 Temperature Treatments

Mature pollen grains were harvested from the chosen genotypes between 8:00 and 10:00 AM. The collected pollen was then exposed to various temperatures (ranging from 32°C to 40°C, in 2°C increments) for two hours. This incubation took place in a BOD incubator (Rotary shaker cum BOD, Rotek, ROSI-1) using a standardized pollen germination medium in petri plates of 3 replications.

2.3 Pollen Germination Measurements

After incubation, pollen germination was assessed using a compound microscope (Leica DC 7.5 V, 10X). The germination percentage was calculated by determining the ratio of germinated pollen to the total pollen count per field of view, expressed as a percentage. The replicated data on maximum pollen germination underwent analysis using two-way ANOVA through GRAPES statistics [20]. The critical temperature, where only 20-30% of the pollen grains germinated, was also determined for each variety.

3. RESULTS AND DISCUSSION

3.1 Pollen Germination

Pollen grains started germinating in about 30 minutes on contact with the *in vitro* pollen germination medium. The effect of five constant temperature regimes from 32 to 40 ◦C at 2 ◦C intervals on the *in vitro* pollen germination of ten tomato genotypes after 2hrs incubation was observed and expressed as the percentage of germinated pollen grains (Table 1). The pollen germination percentage decreased significantly with increase in temperature. Fig. 1 shows the variation for pollen germination in response to temperature of two genotypes for clarity. Maximum germination percentage ranged from 24.57 % (Manuprabha) to 46.63% (Anagha), with a mean of 33.01 % were observed at 32ºC. After 36⁰C, the mean pollen germination percentage decreased below 20%. The pollen germination observed for the tomato genotypes at a temperature of 40◦C was very low; and the germination rates were below 1% in three genotypes (Arka saurabh, Arka Vikas and Pusa Rohini). Differences among pollen germination means were found statistically significant and the highest germination percentages were observed in Anagha (24.63 %) followed by Vellayani Vijay (22.79 %) and IIHR 26372 (21.5 %) and the three genotypes which showed least mean pollen germination were Pusa rohini (15.13%), Manuprabha (15.37%) and Arka Saurabh (16.04%) (Fig 1). Fig. 2 shows the variation in pollen germination in response to temperature of two tomato genotypes for clarity.

3.2 Critical Sterility Temperature

Critical temperature was assessed as the temperature where 20-30% of the pollen only germinates. Critical temperatures for pollen germination differed among genotypes. There was considerable variability in the critical temperatures for pollen germination among the genotypes. The genotypes Arka Saurabh, Arka Vikas, Pusa Rohini, Manuprabha and Akshay showed less than 20 % germination percentage after 34º C while Vellayani vijay, Anagha, Arka Rakshak, IIHR 26372 and Nandi has showed pollen germination percentage less than 20 only after 36ºC. The mean pollen germination % at each temperature were taken and was observed that germination percentage was 21.04 % at 36ºC and decreased drastically to 9.29 % at 38⁰C. So 36⁰C was identified as the critical temperature for pollen selection.

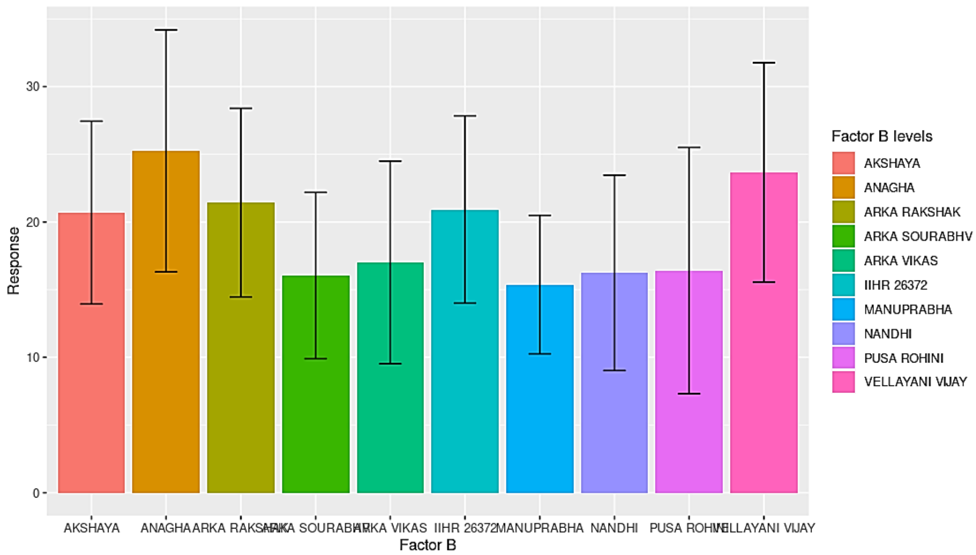

Fig. 1. Pollen germination of tomato genotypes

Table 1. Pollen germination (%) after 2 hrs incubation at different temperatures

Treatment	Varieties										Mean
	Akshaya	**Vellayani Vijay**	**Manu prabha**	**Pusa Rohini**	**Anagha**	**Arka Rakshak**	**Arka sourabhv**	**Arka vikas**	**IIHR 26372**	**Nandhi**	
32^0C	31.33	42.33	24.57	33.6	46.63	32.64	26.15	25.10	34.96	32.9	33.01
34^0C	29.89	33.9	21.01	22.11	34.96	26.13	26.26	32.01	29.79	30.93	28.70
36^0C	19.97	21.27	17.94	19.69	22.74	25.88	16.34	19.033	25.63	21.89	21.04
38^0C	14.24	12.18	10.33	0.147	12.667	10.63	11.29	8.9	12.453	.117	9.29
40^0C	5.74	4.26	2.99	0.121	6.117	9.836	0.172	0.02	4.663	4.09	3.80
Mean	20.2	22.79	15.37	15.13	24.63	21.02	16.04	17.01	21.5	18.01	
	Temperature (T)	Variety (V)	TXV								
SE m±	1.205	1.703	3.80								
CD (0.05)	3.38	4.781	5.72								

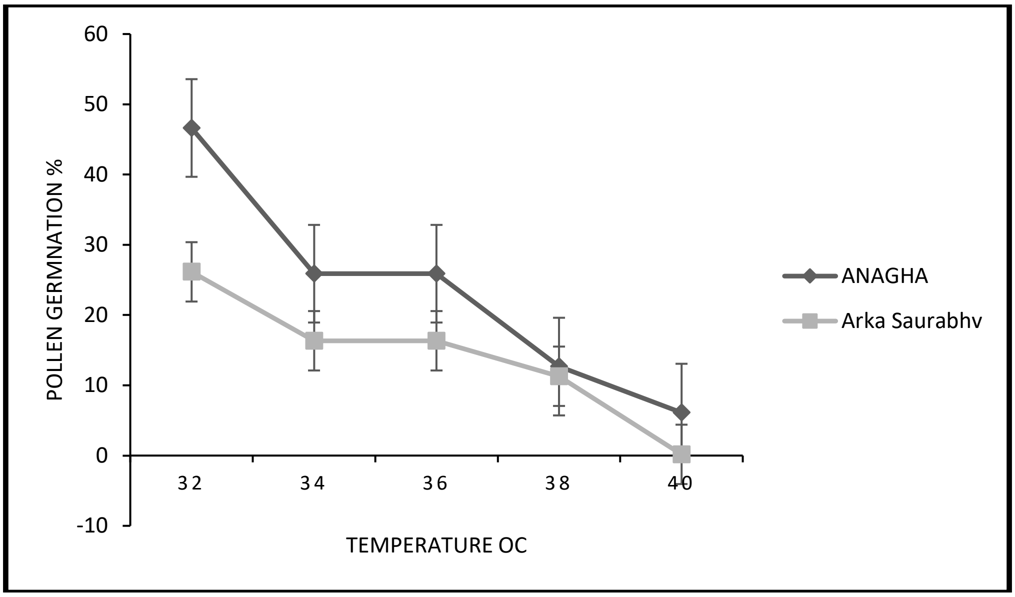

Fig. 2. ***In vitro* pollen germination responses to temperature of two tomato genotypes (Anagha and Arka Saurabh)**

3.3 Discussion

Temperature plays a crucial role in influencing plant growth, pollen production, and reproductive success. Most plant species thrive within a specific temperature range for pollen germination, with tomato preferring 20-30°C [11]. Studies have demonstrated that temperatures outside this range significantly diminish germination rates and pollen tube length [21]. This research corroborates previous findings, observing a marked decrease in pollen germination under elevated temperature conditions. For instance, [12] reported a considerable decline in tomato pollen viability and germination when daytime temperatures exceeded 32°C. Additionally, [7] emphasized that extended exposure to temperatures above 30°C during flowering resulted in reduced pollen germination and fruit set, highlighting tomato pollen's vulnerability to heat stress. This study revealed notable variations in pollen germination among different cultivars, suggesting that pollen can be utilized as a means to select genotypes based on their temperature tolerance. Typically, genotypes that exhibit high-temperature tolerance display superior pollen germination and viability compared to their sensitive counterparts [22]. Employing pollen germination as a screening method at critical temperatures can offer valuable insights into genotypes' heat tolerance levels. Research has indicated a 13-fold reduction in germinated pollen as temperatures progressively increased from the optimal level [11]. Marine et al. [23] categorized different tomato genotypes into tolerant and susceptible based on pollen viability. A comparable investigation on soybeans employed in vivo pollen germination to evaluate genotypic heat tolerance, grouping varieties into tolerant, intermediate, and susceptible categories based on pollen germination at different heat levels [24]. Similar research on upland cotton identified 35 °C as a critical temperature for pollen viability, with genotypes showing less reduction in pollen germination at this temperature considered more heat-resistant [25].

While some studies have pinpointed specific critical temperatures for pollen germination, others have used temperature ranges to establish minimum, optimum, and maximum cardinal temperatures for pollen germination and tube growth. These values can be used to calculate a cumulative temperature response index (CTRI) for screening genotypes [26]. This approach has been applied to various plants, including coconut and capsicum, to identify heat-stress tolerant genotypes [27,26]. The use of pollen germination at critical temperatures as a screening tool provides valuable insights into genotypes' tolerance levels to elevated temperatures.

4. CONCLUSION

Significant genetic variations were noted in tomato genotypes regarding pollen germination and critical sterility temperature. 36ºc was identified as critical temperature for pollen selection where 20% pollen germination was observed. Beyond critical temperature, pollen germination percentage decreased below 20 % except in Manuprabha, Arka sourabhv, and Arka vikas in which the percentage germination reduced below 20 after 34^0 c itself. Anagha, Vellayani vijay and IIHR 26372 which showed higher germination at the critica sterility temperature was selected as tolerant and Arka Saurabhv, Manuprabha and Pusa Rohini with least germination percentage as susceptible. The sensitivity of pollen germination rate and tube growth makes them valuable indicators of thermal tolerance, reflecting the ability of pollen to function in elevated temperatures. Assessing the performance of different tomato varieties in controlled high-temperature environments can provide valuable insights into their thermal tolerance mechanisms. However, further studies are required to confirm heat tolerance with respect to various attributes contributing to tolerance mechanisms.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

We hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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