

Evaluation of tomato genotypes for high temperature tolerance using certain reproductive and fruit traits by factor analysis

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Abstract

High atmosphere temperature is the most significant environmental factor and its negative impact on plant growth and productivity causes large losses in agricultural production. Fourteen tomato genotypes (G1;U64-16, G2;U4-10, G3;U2-29, G4;U117-2 G5; CLN1621L, G6; BL1176, G7; CLN2418A, G8; BL1175, G9; BL1173, G10; CLN2001A, G11; CLN2413R, G12; CL5915-93D4-1-0-3, G13; BL1174, and G14; CLN2498E) were evaluated at three temperature conditions. Three field experiment was carried out at optimum (OT, 28/21°C day/night), moderate high temperature (MHT, 32/22°C day/night) and high temperature (HT, 37/27°C day/night) conditions. Fruit set rate (Fr.S), number of produced pollen grains per flower (P.Pr), number of released pollen per flower (P.R), percentage of viable pollen (P.Via), in vitro pollen germination (P.Ger), number of seed per fruit (S./Fr), aborted fruit rate (A.Fr), fruit weight (Fr.We), fruit length (Fr.Len), fruit diameter (Fr.Dia) and seed germination (S.Ger) were scored. The temperature damage threshold was determined for the mentioned properties. The temperature slightly over the OT reduced the pollen characteristics, Fr.S and S./Fr. The results revealed that the P.R, P.Pr and P.Ger were the most important factors to determine the fruit set under for the temperatures above the optimum and could be used in breeding programs aiming to obtain better fruit set under HT. The P.Pr and P.R were readily affected by the increase in temperature compared to P.Ger and P.Via. The damage threshold temperature was 43.9 °C for P.Ger and 45.9 °C for P.Via.

Key words: Heat stress, pollen, damage threshold, biplot, climate change

Introduction

The climate changing is continues to grow and the Earth is heating up because of global warming. The global average temperature increased by 1.04 °C between 2014 and 2018. By 2030, this increase will reach 1.5 °C. The temperature rises higher than the global average is already happening in many regions and seasons (IPCC, 2018). High temperatures (HT) have become an increasing agricultural problem in many parts of the World. As the global climate changes, tomatoes will be exposed to more frequent and severe temperature stress.

Tomato has adapted to a wide range of climatic conditions from temperate to hot and humid tropics. The optimum

temperature for tomato varieties growth, fruit set, and good yield is between 21 °C - 29.5 °C daytime and between 18.5 °C – 21 °C night temperature (Jones, 2008, Camejo et al., 2005). Heat tolerance screening is performed in a variety of environments, including fully controlled phytotron, growth chambers, greenhouse, and in the open field. However, findings from screening under controlled environments cannot always be transferred to the field due to uncontrolled factors that respond to the plant's heat stress (Ayenan, 2019).

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The response and sensitivity of plants to HT varies between genotypes as well as in plant development stages (Wahid et al., 2007). Generative organs of tomato are more sensitive to HT than vegetative organs (Abdul-Baki, 1991, Peet et al., 1997, Sato et al., 2006). For this reason, it is important to consider the generative properties in HT tolerant genotype selection. Since there is a large genetic diversity in tomato, varieties with improved heat tolerance can be improved. This study aimed to evaluate the effects of HT on certain pollen properties and fruit set by using factor analysis and biplot graph and determination of critical temperature thresholds of significance to tomatoes.

Materials and Methods

The experiments carried on field conditions (in the experimental field of GAP Training Extension and Research Center located in Sanliurfa, TURKEY; latitude of 37° 08' N, longitude 38° 46' E, 464 m above sea level). The site was in a continental semi-arid temperate zone.

Plant Materials and Experimental Conditions

Four local tomato (*S. lycopersicon*) genotypes (G1; U-64-16, G2; U-4-10, G3; U-2-29 and G4; U-117-2) selected from the Sanliurfa province, Southeastern Anatolia Region of Turkey and 10 Asian Vegetable Research and Development Center's (AVRDC) tomato lines (G5; CLN1621L, G6; BL1176, G7; CLN2418A, G8; BL1175, G9; BL1173, G10; CLN2001A, G11; CLN2413R, G12; CL5915-93D4-1-0-3, G13; BL1174, and G14; CLN2498E) that had been previously reported as being heat-tolerant, were used as plant materials.

Three experiments were set through the growing season to provide optimum temperature (OT; stress free), moderate high temperature (MHT) and high temperature (HT) regimes. The growing period from transplanting to end were May 4 – June 8 (OT), May 16- June 22 (MHT) and June 17- August 2 (HT) for OT, MHT and HT stress treatments, respectively. Sampling dates were 23 May- 8 June / 7 June-22 June / and 17 July- 2 August, respectively. Air temperature in the experimental site was measured by a mini data logger (Hobo H8, Onset Computer Corp., MA, USA). The average of day/night temperatures was calculated as the mean readings of each 1 hour during growing period. The average temperatures recorded during flowering and fruit development were; OT: 28 °C day/21 °C night; MHT: 32 °C day/22 °C night; HT: 37 °C day/27 °C night.

Fruit set ratio (Fr.S), number of pollen grains produced per flower (P.Pr), number of pollens released per flower (P.R), percentage of viable pollen (P.Via), in vitro germination of pollen (P.Ger), number of seed per fruit (S./Fr), aborted fruit ratio (A.Fr), fruit weight (Fr.We), fruit length (Fr.Len), fruit diameter (Fr.Dia) and seed germination (S.Ger) were scored. For fruit set ratio, in each repetition the ratio of fruits to the total number of flowers in the first two clusters marked in three plants was calculated as a percentage.

The number of pollen produced per flower was assessed according to Eti (1991). Ten flower buds from each genotypes were randomly collected a day before anthesis. The flower buds were incubated in a glass bottle at the room temperature for 24 hours until opening. Pollen were homogenized with distilled water. Total pollen grains were counted by a hemocytometer slide by a light microscope and then the value was adjusted per flower. Four replications were employed in pollen counting (two random microscopic fields containing

more than 100 pollen grains per slide, and two slides per genotypes).

For counting of number of released pollen per flower the anthers of newly opened 5 flowers from each repetition were placed in a 2 ml glass tube and vibrated by tooth brush for 10 seconds. The released pollen grains were homogenized with 1 ml of distilled water and counted with the help of a hemocytometer.

In vitro viability of pollen (produced under three temperature conditions) were scored by 1% Triphenyl Tetrazolium Chlorid (TTC) test according to Eti (1991).

In vitro germination of pollen (produced under three temperature conditions) was evaluated by incubation of pollen grain at temperatures 25 °C for 12 hours on germination medium (15% (w/v) sucrose, 50 ppm boric acid, 100 ppm calcium nitrate and 1% (w/v) agar).

The aborted flower was scored in the marked first two clusters of three plants from each plot. Yellowed flowers, having little separation in the breaking layer or tags left in the flowerless pedicel was evaluated as an aborted flower.

The seeds extracted from the fruits obtained at each temperature period were germinated between the papers in the incubator at 25 °C temperature. Fifty seeds were used from each genotype with 4 repetitions and the seed germination (S.Ger) rate was calculated.

The temperature damage threshold was estimated by regression analysis.

Statistical analysis

Statistical analyzes were performed by Minitab 17 Statistical Software (Anderson, 1984). In this study, plant characters of 14 tomato genotypes under 3 different temperature conditions were evaluated by factor analysis and biplot graph. Regression analysis was used to predict the temperature threshold for each character

Results and Discussion

Fruit set and fruit set components of 14 tomato genotypes were evaluated to assess the performance, stability and adaptability of the genotypes to different temperature conditions. The Fr.S was considered a target trait and all other traits were considered as related traits of the target trait. Relationships between properties observed in OT, MHT and HT conditions were interpreted using factor analysis.

Factor analysis revealed that, PC1 44.12% and PC2 18.92% constituted 63.04% of the total variation in OT conditions. Fr.S was found to be highly negatively correlated with Fr.Len, Fr.We, Fr.Dia and S./Fr (Table 1). An independent correlation was found between Fr.S and S.Ger while a positive correlation was recorded between Fr.S and P.Via. The highest Fr.S percentage was recorded in G14, G6, G5 and G8 genotypes, which had the smallest fruit size (Fr.Len, Fr.We, Fr.Dia) and the lowest stability. The most stable properties under OT conditions were determined as Fr.Len, Fr.We, Fr.Dia, S./Fr, and P.R (Figure 1). Although G9 and G13 had the highest performance in terms of P.Pr and P.Ger, they did not have the same performance in terms of Fr.S.

Fr.S and S.Ger was not likely to be correlated. Generally, considering all the traits examined G1, G2, G3, G4, G7 and G10 have been better performing genotypes than others. However, while G7 and G10 were unstable, G1, G2, G3 and G4 were defined as the most stable genotypes.

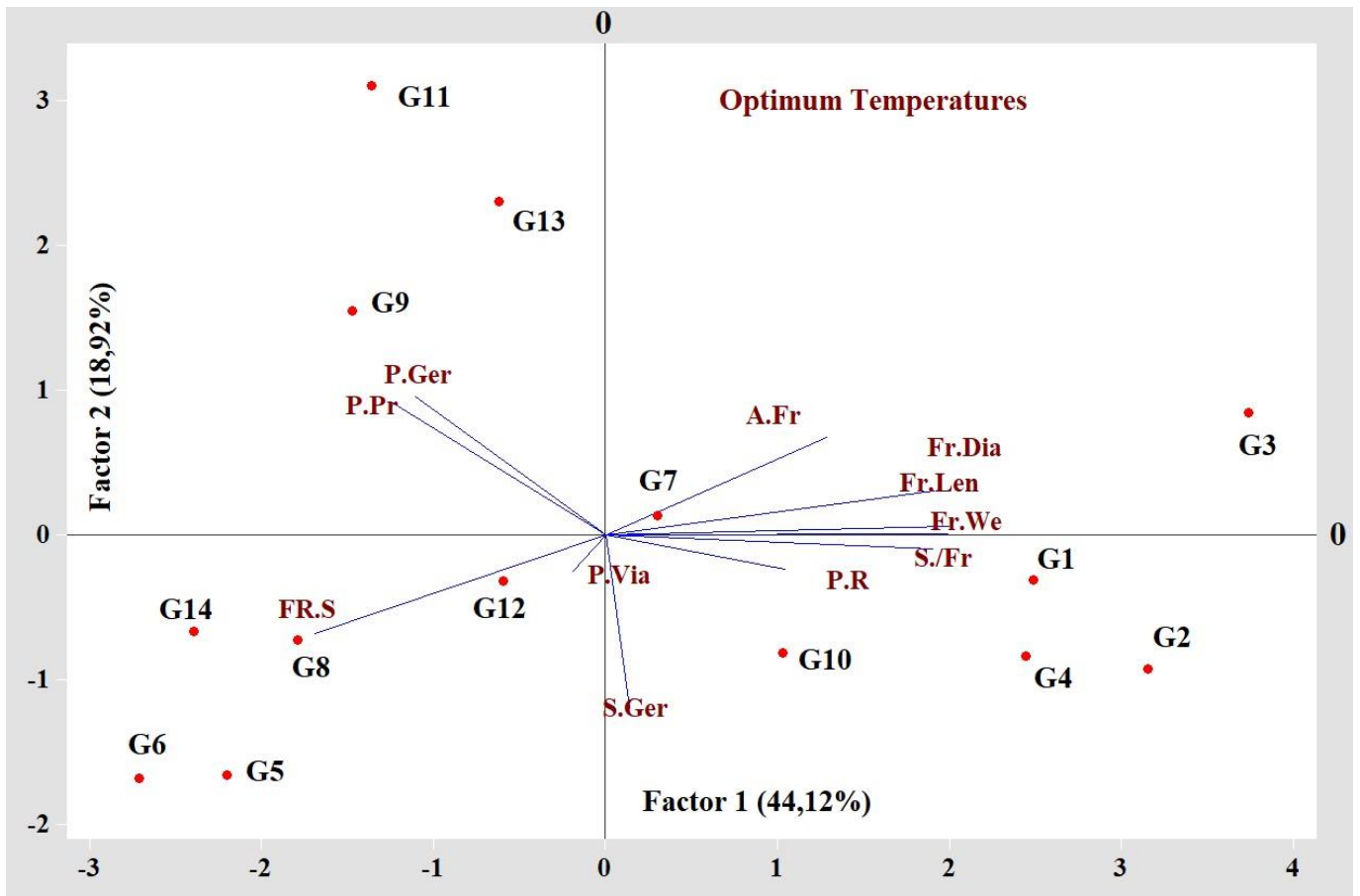


Fig 1. At optimum temperature factor analysis and biplot showing genotypes performance and traits correlated with fruit set.

PC1 39.78%, and PC2 20.67% constituted 60.45% of the total variation in MHT conditions (Table 2). Under MHT conditions, there was a significant negative correlation between Fr.S and Fr.Len, Fr.We, Fr.Dia, S./Fr, S.Ger, P.R and P.Pr. A positive correlation was found between Fr.S and

P.Via. Local genotypes (G1, G2, G3 and G4) that showed the highest performance in OT conditions were found to be the best genotypes in MHT conditions. Fr.Len, Fr.We, Fr.Dia, S./Fr, S.Ger and P.R were the most stable properties (Fig 2).

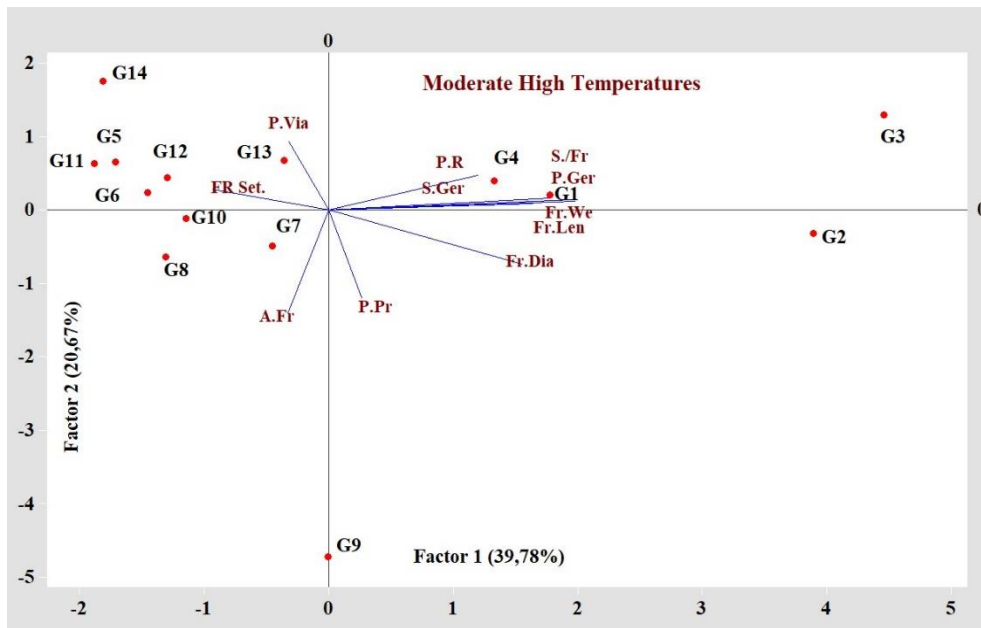


Fig. 2. At moderate high-temperature factor analysis and biplot showing genotypes performance and traits correlated with fruit set.

In HT condition PC1 47.80 %, and PC2 19.53 % constituted 67.33 % of the total variation. In HT case, the highest positive correlation was found between Fr.S and P.Pr, P.R. An independent correlation was found between Fr.S and P.Via, P.Ger.

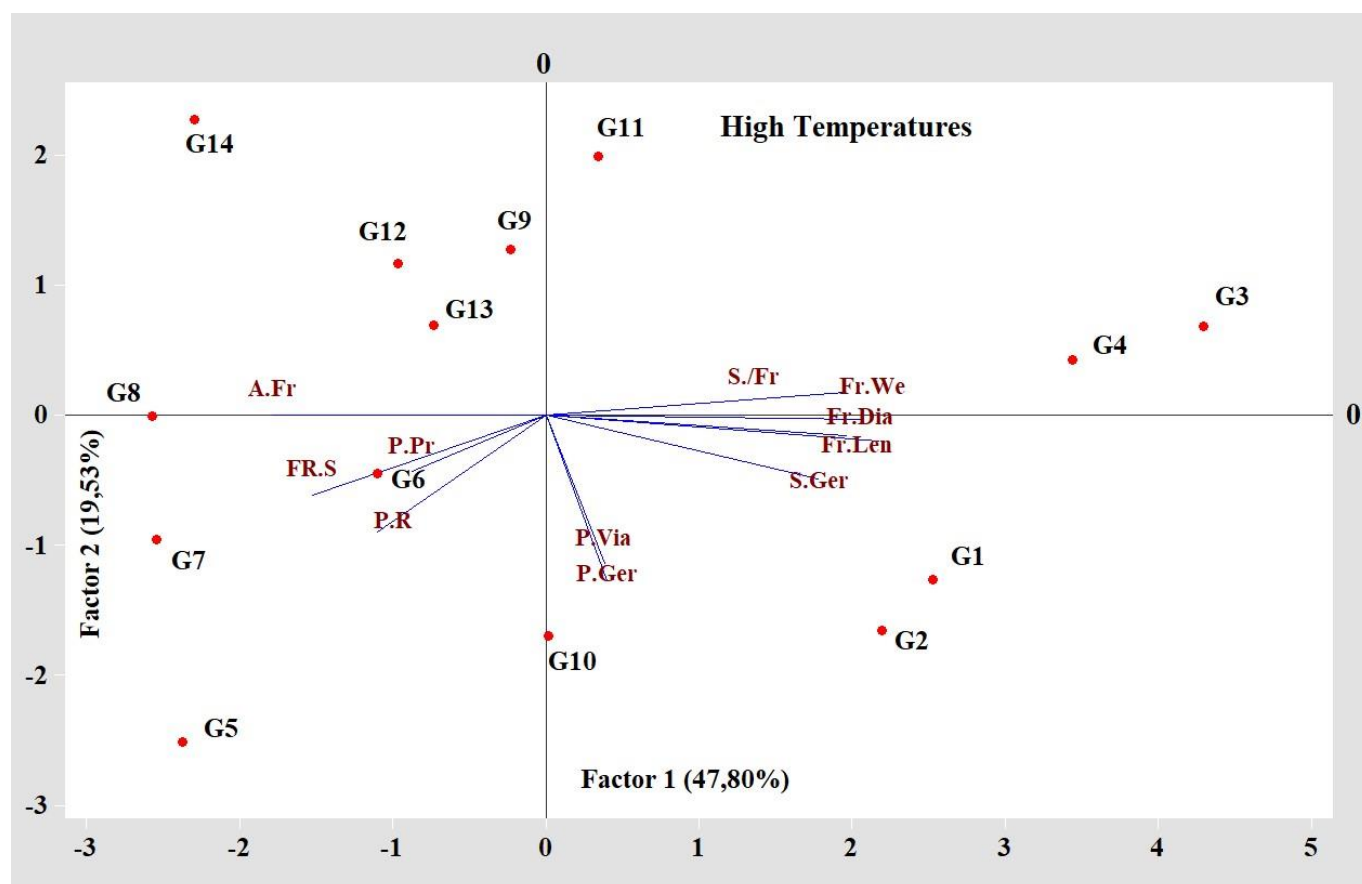


Fig. 3. At high-temperature factor analysis and biplot graph showing genotypes performance and traits correlated with fruit set.

In HT stress conditions, considering all the traits examined the performance of 4 local genotypes was found to be higher than other genotypes (Fig 3). In this case, the fact that local genotypes have high performance in general under three different temperature levels shows that these genotypes are stable. S./Fr, Fr.We, Fr.Len, and Fr.Dia have been highly associated with Fr.S in all three temperature conditions and showing significant reduction in HT stress condition.

The Fr.S values recorded in G5, G6, G8, and G14 in OT conditions, G5, G6, G11, and G12 in MHT and G5, G6, and G7 in HT conditions were found higher than other genotypes. Genotypes that have high Fr.S performance at three temperature conditions were the lowest in terms of Fr.We, Fr.Len and Fr.Dia (have small fruits). These genotypes were found to be the unstable and highly variable. In addition, Fr.S was determined as the trait with the lowest stability.

Genotypes (G5, G6, G7, G8, G9, G10, G11, G12, G13, and G14) that performed poorly in MHT and HT conditions also performed poorly in OT conditions. Although G10 and G7 perform well in OT conditions, it was found that this performance could not be maintained with the increase in temperature.

High stability is important if the overall average performance is also high when selection make between genotypes. According to Figure 1, 2 and 3, it was determined that G1, G2, G3 and G4 were quite stable in terms of Fr.We, Fr.Len, Fr.Dia and S./Fr and their performances were consistent in terms the mentioned properties.

Based on the results obtained from principal component analysis, the Fr.We, Fr.Len and Fr.Dia were stable in three temperature conditions. The Fr.S related traits except that Fr We, Fr Len and Fr.Dia differed significantly at the different temperature conditions.

When the results obtained in MHT and HT conditions were compared with OT conditions, it was determined that G1, G2, G3 and G4 did not show significant differences and were stable compared to environmental conditions. It was determined that the genotypes (such as G8, G14, G5, and G6) with the highest Fr.S under OT conditions differ significantly in MHT and HT conditions and were not stable. The most stable genotype stated by the Fr.S percentage under all three temperature conditions was G6. In contrast, G1, G2, G3 and G4 had the highest fruit size and stability, and the lowest Fr.S compared to OT conditions.

The P.Pr decreased 40.10% in MHT and 90.19% in HT conditions, while P.R decreased 39.48% in MHT and 92.40% in HT conditions. Similarly Fr.S rates decreased by 13.56% in MH and 73.29% in HT conditions compared to OT. In contrast A.Fr under HT conditions increased by 45.21%. The S./Fr loss due to HT was 52.21 % (Table 4, Fig. 4).

P.Pr and P.R ability under high temperature were revealed to be the most important factors determining the Fr.S ability and could be used in breeding programs aiming for better fruit set under HT. Germination rates of seeds obtained from fruits grown at MHT and HT conditions also decreased. When compared to optimum conditions, the germination rate of seeds obtained under MHT and HT conditions decreased by 13.59 %

and 44 %, respectively. It was determined that in HT conditions Fr.S was directly related to P.Pr and P.R. In addition to both P.Pr and P.R reduction in HT conditions, P.Via significantly decreased, therefore Fr.S and S./Fr

decreased. The S./Fr under HT conditions and accordingly the fruit size decreased. Exposure to higher than the optimal temperature was negatively affecting pollen traits, leading to low seed set.

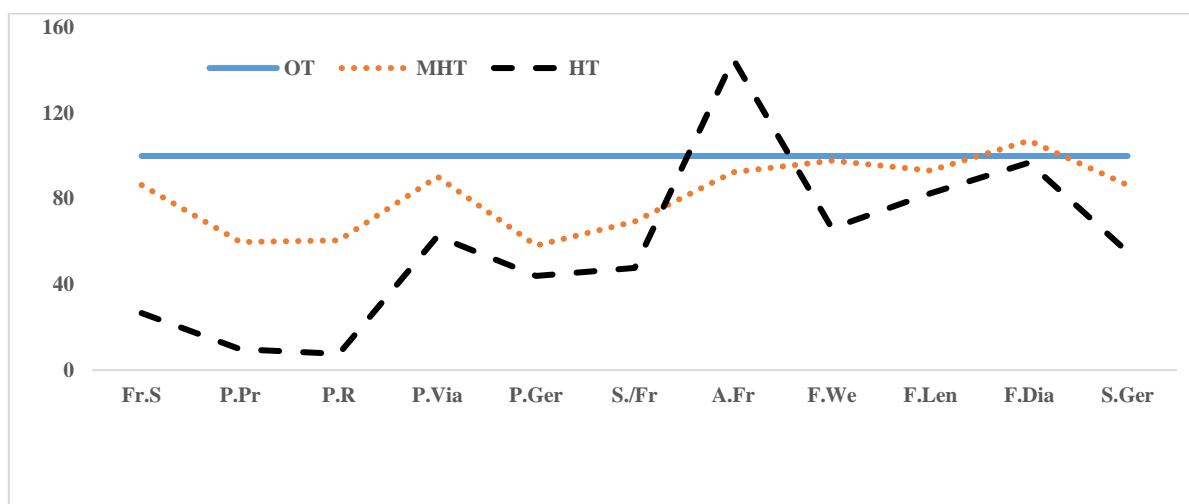


Fig. 4. Increase or decrease rates of the properties examined in the experiment against the optimum temperature

Pollen characteristics under high temperature were revealed to be the most important factors determining the fruit set ability and could be used in breeding programs aiming for better fruit set under high temperatures. High-temperature conditions caused deficient fruit set in tomatoes. It has been reported that the decrease in pollen viability under high temperatures is associated with a significant decrease in tomato fruit set (Peet et al., 1998, Sato et al., 2006, Pressman et al., 2002, 2006, Soyly and Comlekcioglu, 2009, Xu et al., 2017).

HT condition resulted in a significant decrease in the performance of all reproductive traits. Important variation was found among genotypes under the three ambient temperature conditions. Rating of screened local genotypes by biplot analysis can provide genetic diversity useful for plant breeding for heat stress tolerance. Many researchers have used biplot analysis in various plant species for the evaluation and selection of genotypes and cultivars tested in tomato (Joshi et al., 2011, Naranjo et al., 2016, Sharma et al., 2020).

The P.Pr and P.R and fruit set ability of G5 and G6 genotypes under HT conditions were higher compared to the other genotypes examined. Under HT condition, only local genotypes produced fruits with seeds. The genotypes of AVRDC that had been previously reported as being heat-tolerant produced fruits almost no seeds. It is understood that AVRDC lines tend to form parthenocarpic fruit under stress conditions. Despite the high performances of AVRDC genotypes in terms of P.Pr, PR and Fr.S under HT conditions, the low rate of S./Fr may be due to the negative effect of HT to stigma position according to anther, and closely related to pollen hydration.

HT cause to bud drop, abnormal flower development, poor pollen production, dehiscence, and viability, ovule abortion and poor viability, reduced carbohydrate availability, and other reproductive abnormalities that results with failure in fruit set in tomato (Hazra et al 2007, Bhardwaj, 2012). Thamburaj and Singh (2011) reported that at temperatures above 25 °C pollination and fruit set are negatively affected in tomatoes, on the other hand Bhardwaj (2012), recorded that it

caused significant losses in tomato yield due to reduced fruit set, smaller and lower quality fruits.

In the screening of genotypes for HT tolerance in tomatoes, a large number of properties are studied, which are based on the capacity of the fruit set, which is affected by many factors at high temperatures (Golam et al., 2012, Xu et al., 2017, Driedonks et al., 2018). Morphological, cytological and physiological characteristics of plants are examined in high temperature tolerant tomato breeding.

The critical threshold in agriculture is defined as the crisis point at which the production of a crop becomes not feasible due to identifiable change in a production factor (Kenny et al., 2000) or as dangerous levels of change (Arnell, 2000).

Critical temperature values (temperature damage threshold) for the properties examined in this study were calculated and presented in Table 5.

P.Pr and P.R were quickly affected by temperature increase compared to P.Ger and P.Via. It was determined that when the ambient temperature was above 37.8 °C, 38.2 °C and 38.8 °C, P.R, P.Pr, and Fr.S were negatively affected, respectively. However, P.Ger and P.Via were not affected quickly, it was found that the damage threshold temperature was 43.9 °C for P.Ger and 45.9 °C for P.Via and S./Fr. It means that in a field with an average atmospheric temperature of 45.9 °C, the viability of the pollen is completely absent and the seeded fruit is not formed.

Although the damage threshold for P.Via and P.Ger were greater than P.Pr and P.R, 38.8 °C was critical for the Fr.S due to the absence of P.Pr and P.R. It means there was completely absence Fr.S above the temperature of 38.8 °C.

It was considered that pollen is more heat stress sensitive than both vegetative tissues and the female gametophyte. But this sensitivity to heat stress is not uniform during pollen development and function (Raja et al., 2019). P.Pr and PR were more sensitive than P.Via.

Due to climate change, global warming and population pressure, it is necessary to be prepared for food safety. New cultivars will need to adjust further in this evolving global environment. We should achieve the required production,

under scarce land and water resources and the negative impacts of climate change.

High temperature stress has become an increasing agricultural problem in tomato production worldwide because it decreases fruit set due to its negative effects on pollen development and fertility (Phama et al., 2020).

In order to adapt to current and future HT stress, there is a serious need for the development of heat resistant varieties and an effective change in agricultural practices. The main purpose of plant growers is to develop high-yielding varieties resistant to biotic and abiotic stress factors.

The results obtained for G1, G2, G3 and G4 genotypes under MHT and HT conditions and OT conditions were similar; therefore these genotypes were recognized as stable and could be valuable sources of heat-tolerant germplasm for tomato breeding studies. Local genotypes (G1, G2, G3 and G4) had both high average performance and high stability under all three temperature conditions. However, the most stable of these genotypes does not mean they are the best or most tolerant to HT. High P.Pr, P.R and P.Ger traits can be combined into one genotype for higher Fr.S ratio in HT conditions.

Flowering in tomatoes continues over a long period of the growing season. Therefore, the HT that will occur during the production season or at any time during this period will have negative effects.

Considering that a large number of genotypes are screened for HT tolerance, these studies take a long time and require high cost and labor. Determine the appropriate feature to score the HT tolerance is very important. Morphological or cytological markers provide important advantages to shorten the time in breeding studies. Fruit set ratio is a complex trait in germplasm screenings and is affected by many reproductive traits. For this reason, it is important to evaluate the characteristics that determine the fruit setting ability and to determine the genotypes that show better performance in general. Investigating of various factors that affect the fruit set separately and then evaluating them together can be an effective strategy in germplasm screening.

Conclusions

As a conclusion of this experiment, biplot analysis has been determined to be important and effective in evaluating genotypes in terms of many characteristics in different environmental conditions, determining genotypes that exhibit high performance in terms of desired characteristics and making an effective selection. Genotype and environmental interaction need to be considered in the development of stable varieties for a particular location. Local genotypes (G1, G2, G3 and G4) showed both high average performance and high stability under all three temperature conditions. However, the most stable of these genotypes does not mean they are the best or most tolerant to HT. High P.Pr, P.R and P.Ger traits can be combined into one genotype for higher Fr.S ratio in HT conditions. The data obtained in this study highlighted the importance of pollen viability for tomato fruit set ability as much as P.Pr and P.R under HT growth conditions. The P.Via could be considered a potential reliable indicator for HT stress.

Conflict of Interest

There is no conflict of this manuscript.

Authors' contribution

N. C.: Advisor of this master thesis, planned of overall research, wrote this article, M.K.S.: Conducted the field experiments, collected the data, collected and prepared of samples and laboratory analysis and M. O.: Performed the statistical analyses.

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