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Araştırma Makalesi/*Article*

Determination of High Temperature Tolerance via Screening of Flower and Fruit Formation in Tomato

Nuray COMLEKCIOGLU [1*] Mustafa KEMAL SOYLU[2]

[1]Department of Horticulture, Faculty of Agriculture, University of Harran , Sanliurfa, Turkey
[2]Southeastern Anatolia Project Training Extension and Research Center, Sanliurfa, Turkey
*Corresponding author E-mail: nuray@harran.edu.tr; nuraycom01@hotmail.com

Abstract : The effects of high temperature stress on flower and fruit development were examined in fourteen tomato genotypes. Four genotypes were selected from landraces in Sanliurfa province (U-4-10, U-64-16, U-2-29, and U-117-2) and the other genotypes were provided by the Asian Vegetable Research and Development Center (AVRDC). The field experiment was designed to yield three temperature regimes; optimum (OT, 28/21°C day/night), moderate high (MHT, 32/22°C day/night) and high (HT, 37/27°C day/night) by transplanting the seedlings at different time intervals. The parameters of seeded fruit (SF), parthenocarpic (seedless), fruit (PF), undeveloped flower (UF), and aborted flower (AF) were used to evaluate flower and fruit development. The HT regime resulted in dramatic decreases in seeded fruit and increases in parthenocarpic fruit, undeveloped flower and flower abortion. The results showed that four domestic tomato genotypes (U-4-10, U-64-16, U-2-29, and U-117-2) could be valuable source of heat-tolerant germplasm for tomato breeding programs. Percentage of SF, PF production and rate of AF may be reliable criteria for determination of heat tolerance.

Key words: High Temperature, Flower development, Tomato, Parthenocarpy

Domateste Yüksek Sıcaklığa Toleransın Çiçek ve Meyve Oluşumu ile Belirlenmesi

Özet: Yüksek sıcaklık stresinin ondört domates genotipinde çiçek ve meyve gelişimine etkileri incelenmiştir. Dört genotip Şanlıurfa yerli domatesinden seçilmiş (U-4-10, U-64-16, U-2-29 ve U-117-2) ve diğer genotipler Asya Sebze Araştırma ve Geliştirme Merkezi (Asian Vegetable Researc and Development Center; AVRDC)'nden sağlanmıştır. Üç arazi denemesi üç sıcaklık rejimi sağlamak üzere planlanmıştır; optimum (OT, 28/21°C gündüz/gece), orta yüksek (MHT, 32/22°C gündüz/gece) ve yüksek (HT, 37/27°C gündüz/gece). Çiçek ve meyve gelişiminin değerlendirilmesinde tohumlu meyve (SF), partenokarpik (tohumsuz) meyve (PF), gelişmemiş çiçek (UF) ve aborsiyona uğramış çiçek (AF) kriterleri kullanılmıştır. Yüksek sıcaklık rejimi bütün genotiplerde tohumlu meyve oranında dramatik düşüşlere, partenokarpik meyve, gelişmemiş çiçek ve aborsiyona uğramış çiçek oranında da artışlara yol açmıştır. Sonuçlar dört yerli domates genotipinin (U-4-10, U-64-16, U-2-29, U-117-2) domates ıslahında sıcağa tolerans için değerli bir gen kaynağı olabileceğini göstermiştir. SF yüzdesi, PF üretimi ve AF oranı sıcağa toleransın belirlenmesinde güvenilir kriterler olabilir.

Anahtar kelimeler: Yüksek Sıcaklık, Çiçek gelişimi, Domates, Partenokarpi

Introduction

Plant growth and productivity are significantly depended on environmental factors and world agriculture will be affected by climate changes (Kandlikar and Risbey 2000). Effects of global warming on agricultural productivity indicate that high temperature (HT) will have detrimental effects in many developing countries (Mendelsohn and Dinar 1999). In tropical and subtropical regions heat stress may become a major limiting factor for crop production. High temperature (HT) stress has been reported as one of the most important causes of change in plant morphology, physiology and biochemical aspects, which reduces plant growth and development in many crops, including tomato. In tomato, physiological parameters such as seed germination, seedling and vegetative growth, flowering, fruit set, and fruit ripening are adversely affected at the temperature above 35 °C (Thomas and Prasad 2003; Wahid et al. 2007). The researchers reported that reproductive development was affected by high temperature stress more than vegetative development (Sato et al. 2002; Abdelmageed et al. 2003). HT limited flower bud initiation and development and resulted in abortion of the flowers and reduction in yield of many crops

(Peet et al. 1998; Sato et al. 2000; Cross et al. 2003; Young et al. 2004). HT tolerant tomato genotypes provide valuable tool for improving new cultivars. The selection of crops or species tolerant to HT stress would be the best and the easiest strategy for increasing fruit set at HT in tomato (Warner and Erwin 2005). Local populations are the valuable source of heat-tolerant genes for tomato genetic improvement. Plant breeders have been interested in developing new cultivars with higher yielding and resistance to pests and diseases, tolerance of drought, salinity and other abiotic stresses. This caused to narrow genetic base of landraces. Diversity within cultivated plants has been replaced by genetic uniformity of new cultivars. Plant breeders need the genetic diversity of genes found in wild and landraces to be able to develop new cultivars of crop plants in the future; therefore evaluation and conservation of the landraces is important (Ford-Lloyd 2003). Several methods have been used to evaluate variation in heat tolerance of genotypes. It has been reported that in evaluating individual flowers of either crop plants or landraces for tolerance to high temperature it is critical to observe whether the fruit set is seeded or seedless (Sato et al. 2002). Evaluating genotypes through the fate of flower development for heat tolerance is an important technique because these processes are directly related to yield.

The objective of this study was to investigate the flower and fruit formation (as seeded fruit, parthenocarpic fruit, undeveloped flowers, or aborted flowers) of fourteen tomato genotypes under different temperature regimes in order to find out the most important selection criteria for a better fruit set at high temperatures.

Materials and Methods

Plant Materials and Experimental Conditions

Field trials were carried out in the experimental farm of SAP (Southeastern Anatolia Project) Training Extension and Research Center located in Sanliurfa, TURKEY in 2005. Four inbred lines which selected from Sanliurfa province (U-4-10, U- 64-16, U-2-29 and U-117-2) and 10 heat tolerant tomato genotypes provided from AVRDC (CLN1621L, BL1176, CLN2418A, BL1175, BL1173, CLN2001A, CLN2413R, CL5915-93D4-1-0-3, BL1174, and CLN2498E) were used as plant materials. The field experiments were designed to yield different three temperature regimes as optimum (OT), moderate high (MHT) and high (HT) by transplanting the seedlings at different time intervals. Seeds were sown in multi-pot trays (70 pots per tray) filled with peat (on 25 March, 6 April, and 6 May of 2005 for OT, MHT and HT regimes respectively). The seedlings were grown in greenhouse (maintained at 22-26/18-20 ^{O}C, day/night by electric heating with thermostat) until date of the transplantation to the field. Seedling were directly transplanted to the field at different three date (4 May, 16 May and 17 June 2005) and plants were naturally grown in the field to establish different three temperature regimes at flowering and fruit set periods. The growing periods from transplanting to the end were May 4 – June 8 (OT), May 16- June 22 (MHT) and June 17- August 2 (HT) for optimum, moderate high and high temperature stress treatments, respectively. Plants were spaced with 0.80 cm in a row and 140 cm between rows. The irrigation water was applied by drip irrigation system. The soil of the experimental site was red sandy loam in texture, medium in organic carbon (0.65%). All treatment plots received the same amount of fertilizer (250 kg ha^{-1} N; 80 kg ha^{-1} P_2O, 300 kg ha^{-1} K_2O_5 and 40 CaO kg ha^{-1}) with automatic fertigation system. A mini data logger (Hobo H8, Onset Computer Corp., MA, USA) was used to monitor air temperature and humidity in the experimental site. The experiments were setup according to completely randomized block design with three replications. Each plot consisted of 15 plants. Square root transformation was performed for analysis of variance. Data were analyzed using Jump Statistical Software (SAS Institute, Cary, NC, USA).

Flower and Fruit Formation

First and second flower cluster of plants were tagged and allowed to develop until fruits reached to the full maturity. Fate of flowers was classified into four classes, as seeded fruit, parthenocarpic fruit, undeveloped flower/fruit and aborted flower. According to Sato et al. (2004), fruits cross cut and fruits containing visible seeds were classified as seeded fruits (SF), and fruits with no visible seeds as were evaluated as parthenocarpic (PF). Fruits and flowers still attached to the peduncle at harvest time but too small to examine of seed content were recorded as undeveloped flowers/fruits (UF), and peduncles without fruits or flowers as aborted flowers (AF). Percentages were calculated for each genotype and seed number was counted in per fruit.

Results and Discussion

Seeded Fruit

The field average temperatures were 28/21°C (day/night) for the first growing season (OT), 32/22°C (day/night) for the second (MHT) and 37/27°C (day/night) for the third season (HT).The percentages of SF of genotypes under three temperature regimes were shown in Table 1. The production of SF was significantly ($P < 0.01$) reduced with HT and the amount of reduction varied among genotypes. BL1176, CL5915-93D4-1-0-3 and CLN1621L had the highest percentage (89.11%, 86.86% and 83.72%, respectively) of SF set at OT; on the other hand, SF set reduced dramatically to 23.81%, 26.42% and 28.62 %, respectively, under HT conditions. Reduction rate of SF set under HT was about 77 % when compared with OT. However, the SF set was not significantly limited under MHT condition except U-117-2 and CLN2498E genotypes. In the present study, a strong negative correlation has been observed between fruit set and HT ($r = - 0.781$). In our previous, study positive correlation has been observed between number of produced pollen grain and fruit set ($r = 0.61$) and strong negative correlations between number of produced pollen grain and HT ($r= - 0.74$) (Soylu and Comlekcioglu 2009). Bertin, (1995) reported that, fruit set in tomato plants failed under HT conditions due to competition for assimilates**.** Poor fruit set at HT has also been related with low levels of carbohydrates available to support ovule growth and growth regulators (Peet et al. 1997; Kinet and Peet, 1997). The results of Sato et al. (2000) showed that HT (32/26 ºC day/night) limited the fruit set in tested five tomato cultivars and only one could set fruit suggesting that poor fruit set at HT was due to the effect of temperature on pollen grain release and germination**.** Sato et al. (2004) found that under HT (32/28 ◦C day/night) SF set was less than half compared to control (26/22 ◦C day/night) temperature conditions. Peet at al. (1997) noted that fruit set, total number and weight of fruit per plant decreased as daily mean temperature increased from 25 °C to 26 °C and from 28 °C to 29 °C, respectively.

Table 1. Percentage of seeded fruit set at three temperature regimes

Genotypes	OT (28/21 ^{O}C)	MHT (32/22 ^{O}C)	HT (37/27 ^{O}C)	Genotype Mean
U-64-16	60.68 g-ı	67.40 e-g	13.32 qr	42.90 c
U-4-10	64.16 f-h	61.00 g-ı	16.32 q	43.82 c
U-2-29	39.94 ı	33.52 mn	6.25 s	23.72 f
U-117-2	40.83 ı	10.43 r	5.34 s	15.84 g
CLN1621L	83.72 ab	79.21bc	28.62 no	60.84 a
CL5915-93D4-1-0-3	86.86 ab	70.39d-f	26.42 o	58.06a
CLN2418A	54.46 ı-k	70.39 d-f	21.53 p	46.24 c
CLN2001A	78.68 b-d	64.48 f-h	20.98 p	51.27 b
CLN2498E	67.57 e-g	38.81 lm	2.59 t	28.73 e
CLN2413R	60.68 g-ı	54.91 ı-k	24.60 op	45.16 c
BL1173	53.29 jk	58.06 h-j	4.33 st	32.15 d
BL1174	68.23 e-g	48.72 k	20.52 p	43.43 c
BL1175	58.83 h-j	52.56 jk	27.67 o	45.29 c
BL1176	89.11 a	74.13 -c-e	23.81op	58.37 a
Temperature mean	63.84 a	54.02 b	15.76 c	42.56
CV (%)	4.95			
LSD(Temperature, A)	0.162**			
LSD (Genotype, B)	0.284**			
LSD (A*B)	0.493**			

**Significant at P<0.01, OT ; optimum temperature (28/21 ^{O}C), MHT; moderate high temperature (32/22 ^{O}C), HT; high temperature (37/27 ^{O}C)

Seed Number per Fruit

Elevated temperature decreased seed number per fruit in all tested genotypes except U-117-2 (Table 2). The degree of sensitivity to elevated temperature differed among genotypes. The smallest effects of HT were determined on genotypes U-117-2 and U-2-29, but no detrimental effects of MHT were observed. In the case of HT condition, seed per fruit resulted less than half (more than 50% reduction) when compared with OT. In general the local genotypes were able to set seed more than those of AVRDC genotypes at all temperature regimes (Table 2).

It has been reported that heat stress conditions in heat-sensitive cultivars caused a reduction in the fruit set and markedly reduced the number of seed per fruit because of reduced number of pollen grain, viability and germination. In the heat-tolerant cultivars, however, the number of fruits and the number of seed per fruit were less affected by high temperatures (Sasaki et al. 2005; Firon et al. 2006). Peet et al. (1998) noted a similar response to HT, at growth temperatures of 29 °C seed number per fruit was only 16,4%, compared with those at 25°C. In our previous study strong positive correlation has been observed between seed number per fruit and fruit diameter (r = 0.71)(Soylu and Comlekcioglu 2009).

Table 2. Seed number per fruit at three temperature regimes

Genotypes	OT (28/21 ^{O}C)	MHT (32/22 ^{O}C)	HT (37/27 ^{O}C)	Genotype Mean
U-64-16	132.11 b	100.33 de	42.55 l-p	91.67 b
U-4-10	173.67 a	123.45 bc	62.08 g-k	119.73 a
U-2-29	123.39 bc	126.78 b	114.33 b-d	121.50 a
U-117-2	117.78 b-d	51.00 j-m	125.44 b	98.07 b
CLN1621L	68.22 f-j	61.56 g-l	29.56 o-q	53.11 fg
CL5915-93D4-1-0-3	73.44 f-h	55.00 h-l	34.33 m-q	54.26 e-g
CLN2418A	119.55 bc	83.44 ef	16.89 q	73.29 c
CLN2001A	80.05 fg	68.00 f-j	28.55 o-q	58.87 d-f
CLN2498E	63.45 g-k	46.28 k-p	32.33 m-q	47.35 g
CLN2413R	119.89 bc	44.89 k-p	28.56 o-q	64.44 c-e
BL1173	105.78 cd	47.33 k-o	48.83 k-n	67.31 cd
BL1174	85.45 ef	54.89 h-l	30.67 n-q	57.00 d-g
BL1175	73.22 f-h	72.94 f-ı	50.83 j-m	65.67 cd
BL1176	54.00 ı-l	27.89 pq	19.28 q	33.72 h
Temperature mean	99.29 a	68.84 b	47.45 c	
CV (%)	16.28			
LSD(Temperature, A)	5.99**			
LSD (Genotype, B)	10.98**			
LSD (A*B)	19.02**			

**Significant at P<0.01, OT ; optimum temperature (28/21 ^{O}C), MHT; moderate high temperature (32/22 ^{O}C), HT; high temperature (37/27 ^{O}C)

Parthenocarpic Fruit

There was also poor PF set in most of genotypes at HT in the present experiments. No PF has been observed under OT. At MHT conditions only BL1176 produced 3.09 % PF. U-64-16, U-117-2, CLN2498E and BL1173 were not able to produce PF at any of temperature regimes. BL1175 produced the highest percentage of PF (23.35 %) and CLN1621L had the lowest (4.04%) under HT (Table 3). Similar to our results, Sato et al (2001) reported that HT stress increased the frequency of PF. Positive correlation has been found between PF rate and HT (r = 0.57). Parthenocarpy in tomato fruit can be induced environmentally or can arise genetically (Pascual et al. 2007). Several researches reported that under HT stress most of flowers developed into PF due to poor flower fertilization and high percentage of flower abortion (Barringer et al. 1981; Sato et al. 2001, 2004). Sato et al. (2001) noted that it is critical to observe whether the fruit set is parthenocarpic or seeded in evaluating the genotypes for tolerance to HT. In this study it was observed that HT had no effect on flowering, but induced PF set and flower abortion.

Table 3. Percentage of parthenocarpic fruit at three temperature regimes

Genotypes	OT (28/21 ^{O}C)	MHT (32/22 ^{O}C)	HT (37/27 ^{O}C)	Genotype Mean
U-64-16	0.00 ı	0.00 ı	0.00 ı	0.00 f
U-4-10	0.00 ı	0.00 ı	4.92 fg	1.64 de
U-2-29	0.00 ı	0.00 ı	6.28 ef	2.09 c-e
U-117-2	0.00 ı	0.00 ı	0.00 ı	0.00 f
CLN1621L	0.00 ı	0.00 ı	4.40 g	1.47 e
CL5915-93D4-1-0-3	0.00 ı	0.00 ı	8.47 cd	2.82 bc
CLN2418A	0.00 ı	0.00 ı	7.33 de	2.44 b-d
CLN2001A	0.00 ı	0.00 ı	7.78 c-e	2.59 bc
CLN2498E	0.00 ı	0.00 ı	0.00 ı	0.00 f
CLN2413R	0.00 ı	0.00 ı	7.97 c-e	2.66 bc
BL1173	0.00 ı	0.00 ı	0.00 ı	0.00 f
BL1174	0.00 ı	0.00 ı	10.30 b	3.43 b
BL1175	0.00 ı	0.00 ı	23.35 a	7.78 a
BL1176	0.00 ı	3.09 h	9.37 bc	4.16 a
Temperature mean	0.00 c	0.22 b	6.44 a	
CV (%)	25.26			
LSD(Temperature, A)	0.054**			
LSD (Genotype, B)	0.173**			
LSD (A*B)	0.300**			

**Significant at P<0.01, OT ; optimum temperature (28/21 ^{O}C), MHT; moderate high temperature (32/22 ^{O}C), HT; high temperature (37/27 ^{O}C)

Undeveloped Flower/Fruit

Undeveloped flower/fruit rate significantly varied among genotypes with changed environmental conditions. The highest rate of UF was 72.08% and 62.90% in genotype CLN2001A and CLN2498E, respectively, under HT conditions. CLN1621L, CL5915-93D4-1-0-3 and CLN2001A under MHT and HT, CLN2413R at MHT, and CLN2418A, CLN2498E and BL1175 under HT had higher UF rate than those at OT. Decreases in UF rate induced flower abortion in these genotypes. The rest genotypes had higher UF rates when compared with MHT and HT (Table 4). This can be related to the competition for available assimilates between rapidly growing older fruits and young fruits at OT (Bertin 1995; Marcelis et al. 2004).

Table 4. Percentage of undeveloped Flower/Fruit at three temperature regimes

Genotypes	OT (28/21 ^{O}C)	MHT (32/22 ^{O}C)	HT (37/27 ^{O}C)	Genotype Mean
U-64-16	25.30 f-j	18.58 k-n	10.18 q-s	17.47 gh
U-4-10	23.62 g-k	21.16 ı-m	13.03 o-s	18.92 fg
U-2-29	32.26 d-f	15.68 m-p	12.46 o-s	19.27fg
U-117-2	29.59 e-h	13.62 n-r	5.34 t	14.52 h
CLN1621L	13.62 n-r	17.14 l-o	63.04 ab	27.67 cd
CL5915-93D4-1-0-3	12.82 o-s	23.04 h-l	61.15 ab	29.16 c
CLN2418A	39.31 d	26.32 e-ı	57.00 bc	39.94 b
CLN2001A	19.01 j-n	30.69 e-g	72.08 a	37.58 b
CLN2498E	25.91 e-ı	50.55 c	67.90 a	46.38 a
CLN2413R	32.95 de	25.60 f-ı	9.67 rs	21.53 ef
BL1173	26.21 e-ı	16.89 m-o	11.02 p-s	17.47 gh
BL1174	27.14 e-ı	24.50 g-k	20.88 ı-m	24.11 de
BL1175	33.06 de	24.80 g-k	54.46 bc	36.48 b
BL1176	8.58 st	14.36 n-q	49.14c	20.98 ef
Temperature mean	24.11 b	22.37 c	31.14 a	26.53
CV (%)	8.24			
LSD(Temperature, A)	0.111**			
LSD (Genotype, B)	0.392**			
LSD (A*B)	0.679**			

**Significant at P<0.01, OT ; optimum temperature (28/21 ^{O}C), MHT; moderate high temperature (32/22 ^{O}C), HT; high temperature (37/27 ^{O}C)

The oldest flower on the cluster (developed either seeded or parthenocarpically at OT) had inhibitory effects on fruit set of later open flower and caused to remain undeveloped or dropped. UF is not recommended as a reliable criterion for heat tolerance screening in tomato since can be related to many factors. Flower abortion may refer to loss of pollen production, altered pollination or fertilization and subsequent seed development.

Aborted Flower

Percentage of AF was presented in Table 5. The highest rate of AF was 89.16% in U-1117-2. In general, local genotypes had higher AF rate than the genotypes delivered from AVRDC. The positive correlation calculated between elevated temperature and the AF (r=0.57). Floral anomalies were observed at HT in all genotypes. Most of flower buds had underdeveloped inner whorls restricted within the calyx. Heat stress due to increased temperature caused floral abortion in tomato. Similar finding reported that plants produced AF at stress conditions (Sun et al. 2004; Young et al. 2004). Under OT condition 63.84% flowers set SF, 0.00% developed into PF (no PF under OT), 24.11% stayed UF and 10. 06% of flowers aborted as the mean of all tested genotypes. While 9.30% flowers set SF, 6.44% developed into PF, 31.14% stayed UF and 46.26% of flowers aborted under HT (Table 1-3-4 and 5). The plants regulated the fate of flowers in response to changing environmental conditions. This study was conducted in semiarid conditions and with elevation of temperature, relative humidity decreased significantly. HT and low humidity occurred in the field simultaneously and combination of these two stresses had significant detrimental effects on fruit set in tomato. In the present experiment, HT and dry periods with the humidity from 20% to 30% caused dehydration of the stigma and unfavourable surface for pollen reception (pollen did not stick to the dry stigma), germination and degeneration in unfertilized ovules. Flower drop was a result of lack of fertilization, flower anomaly and low stigma receptivity. Our results showed that four local tomato genotypes (U-4-10, U-64-16, U-2-29, and U-117-2) are the valuable source of heat-tolerant genes for tomato breeding when compared with genotypes provided by AVRDC. It has been concluded that percentage of fruit set, parthenocarpic fruit production and rate of flower abortion may be a reliable criterion for selection of heat tolerance cultivars and a successful breeding program for enhanced fruit set at high temperatures should include these features. Percentage of undeveloped flowers is not recommended as a criterion for determination for heat tolerance.

Table 5. Percentage of aborted flower at three temperature regimes

Genotypes	OT (28/21 ^{O}C)	MHT (32/22 ^{O}C)	HT (37/27 ^{O}C)	Genotype Mean
U-64-16	13.91 lm	13.73 lm	76.44 bc	34.69 bc
U-4-10	12.16 mn	17.73 kl	70.48 cd	33.45 d
U-2-29	27.70 g-h	50.64 f	81.16 a-c	53.17 b
U-117-2	29.32 g	75.89 b-d	89.16 a	64.79 a
CLN1621L	0.00 vx	6.50 t-w	12.28 n-q	6.26 h
CL5915-93D4-1-0-3	2.10 y	4.78 q-s	6.86 mn	4.58 h
CLN2418A	6.12 q-u	3.11 u-x	21.35 ı-k	10.19 g
CLN2001A	2.59 vx	3.50 r-v	8.28 p-s	4.79 h
CLN2498E	6.34 q-t	10.53 n-p	29.49 g	15.45 f
CLN2413R	6.07 q-t	19.18 jk	65.57 de	30.27 e
BL1173	20.43 jk	25.00 g-j	83.51 ab	42.98 c
BL1174	4.48 s-w	26.65 g-ı	58.38 ef	29.84 e
BL1175	7.75 o-r	22.51 h-k	17.88 kl	16.05 f
BL1176	1.92 x	11.22 m-o	26.85 g-ı	13.33 g
Temperature mean	10.06 c	20.78 b	46.26 a	
CV (%)	8.90			
LSD(Temperature, A)	0.455**			
LSD (Genotype, B)	0.368**			
LSD (A*B)	0.637**			

**Significant at P<0.01, OT ; optimum temperature (28/21 ^{O}C), MHT; moderate high temperature (32/22 ^{O}C), HT; high temperature (37/27 ^{O}C)

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