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Pollen Viability and Fruit Set of Tomato Introgression Lines (*Solanum esculentum X L. Chmielewskii*) at Moderately High Temperature Regimes

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Abstract: The rise of global temperature nowadays becoming the most important problem in all crop production in general and in tomato production in particular. Several studies have shown that high temperature affected fruit set and subsequent yield of tomato crop. To overcome this, breeding for high temperature tolerance is better option. This study was initiated to identify moderately high temperature tolerant tomato genotypes and to elucidate physiological and possibly genetic background of such possible tolerance. The study was conducted in multi-span Venlo-type greenhouse compartment with 58 tomato genotypes. Two moderately high temperature regimes, 28/22°C and 25/19°C (day/night) were compared one after the other on the same plants. Significant interaction effects between genotype and temperature was observed for pollen germination and fruit set percentage with highly significant interaction effect was observed at temperature regime of 28/22°C. Fruit set percentage was affected at moderately high temperature regimes in genotypic dependent way. Genotype 1, 5, 7, 12, 14, 16, 17, 44 and 56 were performed well in their fruit set at both temperature regimes whereas fruit set on genotype 6, 23, 46, 48, 51, 60 and 61 was severely affected. Genotypes which have had higher fruit set percentage also showed higher pollen germination percentage. Average number of flower per truss was also affected by the main effect of genotype and temperature. Higher numbers of flowers were produced at 28/22°C than at 25/19°C temperature regime. The genotypes also responded differently to moderately high temperature regimes in their number of total fruit per plant and number of ripen fruit per plant. Therefore, it is important to take into account the links between the phenotype and introgression position for further research to find out genes responsible for the enhanced high temperature tolerance.

Key words: Tomato · Pollen Germination · Fruit Set · Genotypes and Temperature

INTRODUCTION

Tomato (*Solanum esculentum* Mill.) is one of the most important widely grown and consumed vegetable crops next to potato in the world. It is also a model plant species, due to its diploid, relatively compact and recently sequenced genome and its large genetic and genomic resources [1]. Nowadays, there is a growing demand for tomato production and consumption is constantly growing. The worldwide production of tomato is estimated to 60 million tons on a cultivated area of almost 4.8 million hectares in 2011 [2]. The crop plays a vital role in both fresh fruit market and in processed food industries (e.g. tomato soup, tomato sauces). It is also considered as protective food because of its particular nutritive value, as it provides important nutrients such as lycopene, beta-carotene, flavonoids, vitamin C and E [3].

Despite its high genetic productivity potential and quality, tomato fruit set and yield are considerably affected by adverse environmental conditions (temperature, light and humidity) and poor nutrition. Now days, the increasing of temperature due to global warming is a threat of crop production and productivity [4]. Besides this high temperature has been a limiting factor in summer tomato production by increasing production cost (cooling the greenhouse by low temperature). High temperature is known to limit vegetative and reproductive processes of tomato and

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ultimately reduces yield [5]. In addition many physiological processes in tomato plant are disturbed as the result of high temperature stress. According to EI Ahmadi and Stevens [6] temperature above the optimum value influences fruit set, number of flower and number of seed per fruit. The optimum temperature for fruit set of tomato is between 21-24°C depending on developmental stage [7].

Reproductive processes of tomato plant are more sensitive to high temperature than vegetative growth although the sensitivity to the stress appears to differ from cultivar to cultivar [8]. It has been suggested that 9-10 days before an thesis is the most sensitive stage in reproductive processes of tomato under high temperature [9]. Moreover, the effect of high temperature is more pronounced on male reproductive gametes than female gametes [10]. Poor fruit set in tomato under moderately high temperature is mainly due to poor pollen release and pollen germination [11]. These authors also suggested that the responses of the cultivars are different to moderately high temperature in their fruit set.

Therefore, it is paramount important to find better strategies to cope with high temperature to increase fruit set and thereby productivity. Breeding for high temperature tolerance is a better option as so far breeding goals focus on characteristics that ensure high yields and quality with minimum production costs [12]. Currently, tomato cultivars have limited genetic variations in temperature response. However, wild tomato species like *L. chmielewskii* is a potential source to improve cultivated tomato for high temperature limitation [13]. Since this wild tomato species have large genetic variations which can be utilized for improving specific trait or as a source of variation for several traits of agronomic interest [14]. In addition *L. chmielewskii* can easy be crossed with cultivated tomatoes to generate fertile offspring and also believed that used as sources of tolerance to salinity and drought stresses [15].

Identification of tolerant tomato genotypes for high temperature can be accomplished by evaluating genotypes for traits sensitive to high temperature and at the same time directly related to yield like flowering, pollen germination and fruit set. In view of this, EU-SOL is one of a projects involved in improving tomato cultivars for better yield and quality. Within EU-SOL project backcross inbred lines (BILs) genetic library of *Solanum esculentum* (Moneyberg) *x L.chmielewskii* (La 1840) has been already developed. These inbred lines contain a single homozygous segment of the donor genome. All introgression lines together provide the complete genome coverage of the donor genome. However, this BILs genetic library is still not exploited for the possible difference in response to high temperature stress. Therefore, evaluating these BILs are necessary to make usable for further breeding program. The aim of this study was to identify moderately high temperature tolerant tomato genotypes developed from *Solanum esculentum* (Moneyberg) *x L.chmielewskii* (La 1840) and to elucidate physiological and possibly genetic background of such possible tolerance. Therefore, the response of these genotypes to moderately high temperature regimes were investigated in this research based on their pollen germination and subsequent fruit set.

MATERIALS AND METHODS

Planting Materials: Fifty eight tomato introgression lines (Genotypes) were used which developed by crossing between (Moneyberg) and *L.chemielewskii* (La 1840) including the genotype Moneyberg (1). A BC1 population derived from cultivated tomato 'Moneyberg' and a 'Wild' relative (*L. chmielewski*) is fingerprinted by using AFLP markers. Based on the BC1 population a genetic map is constructed in order to demonstrate graphical genotypes of each of the BC1 individuals and 10BC1 individuals were selected. The selected BC1 individuals were used for further backcrossing to generate the BC2 and selection was continued till BC3 populations. From BC3 population, 53 BC3 plants were selected and these have been selected in order to generate homozygous segments. Thus, the introgression lines consist of homozygous lines and differ for many traits. Most of these traits are not observable in the original parents. Each introgression lines contain specific characteristics for instance, fruit color (red, orange, yellow and pink), taste and flavor. List of these genotypes with relevant details are presented in Appendix 1.

Experimental Set -Up: The experiment was conducted in a multi-span Venlo-type greenhouse compartment (Unifarm, Wageningen University, the Netherlands, latitude 52°N). Tomato seeds were sown on 5 June 2008 in glasshouse of Unifarm and seedling were transplanted on 16 July 2008 to another greenhouse compartment on rock-wool slab in a total of 14 rows. Each row has 12 slabs and three plants per genotypes were planted per slab resulting to 36 plants per row. The total plant population was 3.8 plants per m^2, which is normal, stem density in summer in the Netherlands.

The experiment was arranged in Randomized Complete Block Design (RCBD) with three replications. Three plants per genotype were planted per block and a total of six plants per genotypes. Five plants per genotypes were considered for measurements. All axillary shoots were removed weekly and plants were trained according to the high wire system [16]. The older leaves below the lowest ripening truss were removed weekly. Standard nutrient solutions were applied six minutes per day (133ml per minute per plant) by drip irrigation system until drainage was observed during each irrigation.

Temperature Treatments: Two sets of moderately high temperature treatments, 28/22°C and 25/19°C (day/night) were used one after the other on the same plants. At the beginning of the experiment, the temperature was set to 28/22°C (Day/night) and maintained for six weeks from 4th of August to 17th of September. After 17th of September the temperature was lowered to 25/19°C (Day/night) and kept for six weeks till 30th of October, 2008.

Measurements: Different parameters were considered in this research activity and were collected accordingly. First fruit set percentage was calculated for all genotypes at 28/22°C and the genotypes were divided into three groups based on their fruit set percentage at this temperature regime: higher group (Greater than 60 % fruit set) and medium group (Between 50-60 % fruit set) and lower group (less than 50 % fruit set). Ten genotypes from each category were selected to determine pollen germination, fruit fresh weight, fruit dry weight, average fresh weight per fruit and average dry weight per fruit. Therefore, in total 30 genotypes were considered for measurements. Since doing pollen germination and destructive measurements for all genotypes are time taking and labor intensive.

***In vitro* Pollen Germination:** Pollen viability was determined by in vitro germination of pollen grain in a medium containing 20 % of sucrose and 100ppm boric acid, by hanging drop method (Slide turn down). Pollen grain used for in vitro germination were taken from 6-7 trusses for 28/22°C and 8-10 trusses for 25/19°C depending on availability of flower at anthesis for each genotype. Pollen was collected from four plants per genotype at anthesis for both sets of moderately high temperature regimes (28/22°C and 25/19°C) by using black paper. After collection, pollen was incubated under 100% RH to avoid desiccation immediately after collection. For the germination assay 0.5ml of germination solution was added to 2ml microtube. Then the tube containing solution was incubated for 15 minutes over water bath at 25°C and RH (100%). Here after pollen sample and incubated germination solution were mixed and homogenized. A drop of these mixture was placed on a slide and slide turns down immediately (Hanging drop assay) and incubated over water bath at 25°C for germination. After five hours, pollen was observed under light microscope for their pollen tube growth. Number of germinated and total pollen counted for calculation ranged between 2- 45 and 7- 80, respectively.

$$\text{Percentage pollen germination} = \frac{\text{Number of germinated pollen}}{\text{Total number of pollen}} \times 100$$

Statistical Analysis: Data were analyzed using genstat statistical software (11th ed.) with two- way analysis of variance (ANOVA), considering genotype and temperature as factors for fruit set, aborted fruit, aborted flower and pollen germination percentage. For number of fruit, number of flower, fruit fresh weight, fruit dry weight and average dry weight of ripen fruit, one- way ANOVA was carried out. Linear regression analysis was also conducted for fruit set and pollen germination. LSD derived from ANOVA was used to carry out mean separation.

RESULTS

Interaction Effect of Genotype and Temperature on Fruit Set Percentage: A significant interaction between genotype and temperature was observed for percentage of fruit set ($P<0.001$, Fig. 1). Genotype 16, 23, 43, 46, 51, 54 and 58 showed significantly higher fruit set percentage at 25°C when compared to percentage fruit set at 28°C whereas genotypes 1, 12 17, 29 and 56 showed no significance difference of temperature effect on percentage of fruit set. Furthermore, the largest reduction in fruits set caused by moderately high temperature of 28/22°C for genotype, 23, 46 and 51.

Genotype 23 had the lowest percentage of fruit set at temperature regime of 28/22°C and genotype 46 was the lowest at 25/19°C while genotype 56 showed the highest fruit set percentage followed by genotype Moneyberg at both temperature regimes (Fig. 1). Fruit set percentage increased by 45% and 32% for genotype 23 and 51, respectively, when the temperature was lowered to 25°C whereas the increment for the genotypes 56 and 1 was only 6% and 7%, respectively.

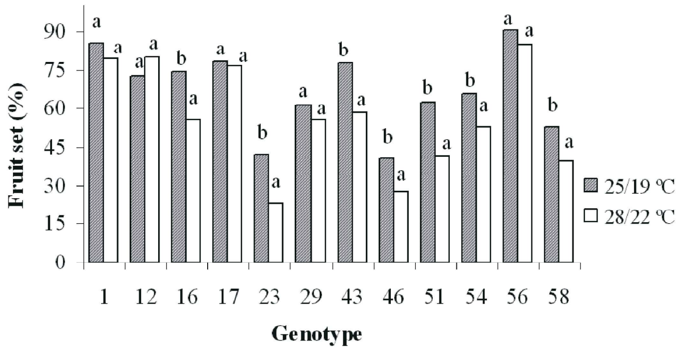

Fig. 1: Effect of genotype temperature interaction on percentage of fruit set at moderately high temperature regimes for truss number 3-5 (28/22°C) and 8-10 (25/19°C) for the selected four genotypes per group. Values are averages of four plants. Different letters indicate significant differences within genotype according to LSD–test; α = 0.05, LSD=9.65

Table 1: Genotype by temperature interaction effect on percentage of aborted fruit and flower at moderately high temperature regimes for truss number 3-5 (28/22°C) and 8-10 (25/19°C) for the selected four genotypes per group. Values are averages of four plants

	Aborted fruit (%)		Aborted flower (%)	
	Temperature (°C)		Temperature (°C)	
Genotype	28/22	25/19	28/22	25/19
1	12	14	8	1
12	11	24	9	4
16	26	18	18	8
17	17	13	6	8
23	19*	23	58*	36
29	43	31	7	8
43	8	12	33*	11
46	64*	30	8*	30
51	23	17	37*	20
54	23	16	24*	18
56	15	9	0	0
58	21	20	39*	27

F-Prob.[a] < 0.001 <0.001

LSD[b] 13 12

[a] F-Probability (Significant levels presented in bold). [b] Least significant difference, * indicate significance difference within genotype according to LSD–tes ; α = 0.05

Genotype temperature interaction effect was observed on percentage of aborted fruit and flowers (P<0.001, Table 1). Significantly higher percentage of aborted fruits was observed for genotypes 16, 29, 46, 51, 54 and 58 at 28/22°C. The largest flower abortion occurred in genotype 23 which counted to 57 % and 35.5% at 28°C and 25°C respectively (Table 1). Surprisingly, no flower abortion was observed for genotype 56 at both temperature regimes.

Effect of Genotype and Temperature on Average Number of Flower per Truss: For number of flower production the two- way interaction between genotype and temperature was not significant (P=0.193) but the main effects of genotype and temperature were highly significant (P<0.001, Fig. 2 and Fig. 3). Genotype 29 produced higher number of flower per truss which is followed by genotype 12 and 58 but genotype 51 had lower number of flower per truss. There was higher flower number per truss at 28/22°C compared to 25/19°C.

Interaction Effect of Genotype and Temperature on Pollen Germination: Genotypes were divided into three groups (Higher, medium and lower groups) based on their fruit set percentage at 28/22°C. Ten genotypes from each group (Total 30 genotypes) were selected for pollen germination test. Two genotypes from each groups and Moneyberg were selected for comparison and presented in Fig. 4. Genotypes 17 and 56 from higher group, 43 and 54 from medium group, 51 and 58 from lower group were selected for comparison. Genotype and temperature interaction effect was higher at 28/22°C than at 25/19°C on pollen germination percentage. Genotype 56 had the highest pollen germination percentage whereas genotype 51 had the lowest. Genotype 51 also showed the greatest interaction effect on its percentage of pollen germination than the other.

Pollen germination perecentage was increased by 31 % and 30 % for genotype 58 and 51 respectively, while the temperature decreased from 28/22°C to 25/19°C. But genotype 56 increased only by 2 % followed by genotype 17 (3%). A positive significant linear relationship was observed between fruit set and pollen germination percentage at 28/22°C (Fig. 4a) and at 25/19°C (Fig. 4b).

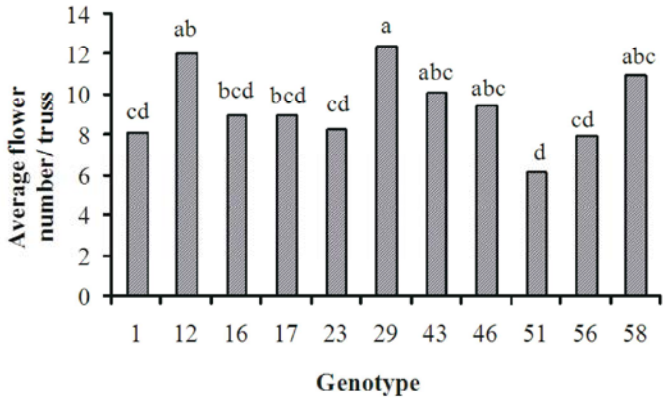

Fig. 2: Effect of genotype on average number of flower per truss at 28/22°C (Observation on truss number 3, 4 and 5) and 25/19°C (Observation on truss number 8, 9 and 10) for the selected four genotypes per group. Values are averages of four plants. Different letters indicate significant difference between genotype according to LSD–test; α = 0.05. Average values over two temperature regimes

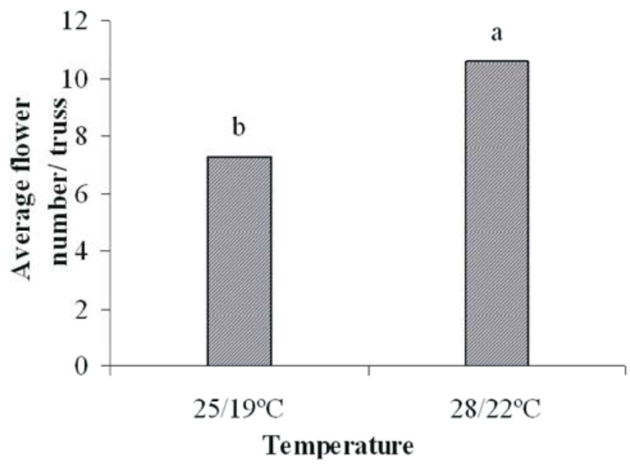

Fig. 3: Effect of temperature on average number of flower per truss at 28/22°C (Observation on truss number 3, 4 and 5) and 25/19°C (Observation on truss number 8, 9 and 10). Average values over genotypes. Values are averages of four plants. Different letters indicate significant difference between temperatures according to LSD–test; α= 0.005.

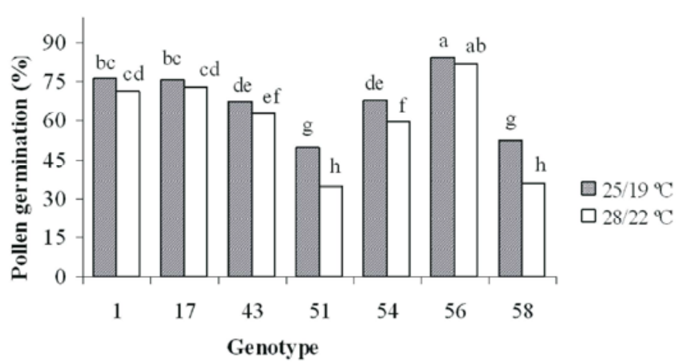

Fig. 4: Interaction effect of genotype and temperature on pollen germination percentage at 28/22°C (Observation on truss number 6 and 7) and 25/19°C (Observation on truss number 8 and 9) for two genotypes per group and Moneyberg. Values are averages of four plants. Different letters indicate significant difference within and between genotypes according to LSD–test; α = 0.05, LSD=6.914

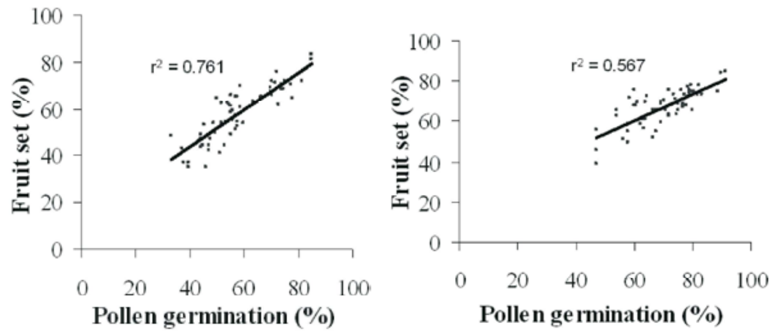

Fig. 8: Relationship between fruit set and pollen germination percentage at 28/22°C (a) and at 25/19°C (b). This included the data from 30 selected genotypes. r^2= adjusted R square

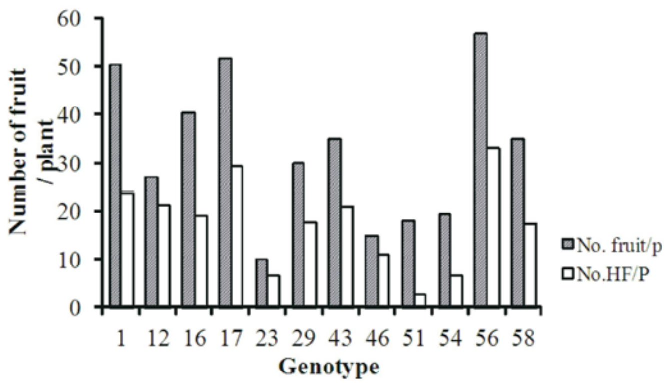

Fig. 5: Genotypic difference in total fruit number (Ripen and green) per plant (No. fruit/p) and number of ripen fruit per plant (No. HF/p) at (28/22°C and 25/19°C) for the selected four genotypes per group. Values are averages of four plants

Genotypic Difference in Number of Total Fruit and Ripen Fruit per Plant: The number of total fruit (ripe and green) and ripen fruit per plant at moderately high temperature showed significant differences between genotypes ($P<0.001$, Fig. 5). Genotype 56 and 23 gave the highest (56.5) and the lowest (9.75) total fruit number per plant, respectively. Genotype 17 and 1 had also higher total fruit number per plant. Moreover, number of ripen fruit per plant was higher for genotype 56 whereas genotypes 51 had the lowest. Genotypes 58 had higher number of total fruit and ripen fruit compared to genotype 54.

DISCUSSION

Fruit set is a trait that strongly affected under high temperature stress and it was used as key factor to select tolerant tomato genotypes at moderately high temperature. The result showed that fruit set percentage of the genotypes was affected by moderately high temperature regimes and the response varied among the genotypes. Several studies have also shown that fruit set in tomato reduced at moderately high temperature stress [11, 17]. The result of Sato *et al*. [11] showed that cultivars difference was observed in their fruit set percentage under high temperature stress.

For most of the genotypes the percentage of fruit set was markedly reduced at 28/22 °C. For example, the fruit set percentage of genotypes 23 and 51 was increased by 45 % and 32 % when temperature was reduced from 28/22°C to 25/19 °C, respectively. In the present study eight genotypes (1, 5, 7, 12, 14, 16 17, 44, 56) performed well at both temperature regimes, especially, genotype 56 showed the highest fruit set percentage 85 % and 91 % at 28/22 °C and 25/19 °C, respectively compared to the other genotypes. These genotypes have an introgression on different chromosomes (Genotype 5 on chromosome 3, genotype 7 on chromosome 6, genotype 12 on chromosome 1, genotype 14 on chromosome 2, genotype 17 on chromosome 7, genotype 44 on chromosome 9 and genotype 56 on chromosome 10+11) (Appendix 1). The higher fruit set percentage for the genotype 5, 7, 12, 14, 17 and 44 at moderately high temperature stress might not related to specific gene found on the chromosome 3, 6, 1, 2, 7, and 9 respectively. Because there are other genotypes with lower fruit has introgression on the same chromosome with genotypes 5, 7, 12, 14, 17 and 44. For instance, genotype 42 introgression on chromosome 6 and genotype 58 introgression on chromosome 1. The introgressions on different chromosomes of these genotypes make it difficult to draw conclusions about specific introgression on chromosomes linked to the tolerance to high temperature. However, genotype 56 have an introgression on chromosomes 10 +11 and no any other genotypes have the same introgression on chromosome. This indicated that the better performance of the genotype at moderately high temperature stress is linked to specific introgression on chromosome 10+11.

Few genotypes on the other hand showed decreased percentage of fruit set as temperature decreased from 28/22 °C to 25/19 °C. The reason for the reduction in the percentage of fruit set for some genotypes at 25/19 °C temperature regime was most likely attributed to the lower capacity of the genotypes to flower and set fruit at their later stage.

Pollen germination percentage of the genotypes was found to be affected at moderately high temperature regimes. Higher reduction in pollen germination percentage was observed at 28/22°C for genotype 51 (35%) and 58 (36%) than at 25/19°C (Fig.3). The reduction of fruit set percentage of tomato cultivars at moderately high temperature could be explained by poor pollen germination [11]. Genotype 56 had the highest percentage of pollen germination at both temperature regimes as showed for fruit percentage. The difference in the pollen germination percentage of the genotypes was mainly attributed to difference in ability of their fruit set percentage. The study carried out by Firon *et al.* [18] also showed that poor pollen viability and germination lead to reduction of percentage of fruit set in tomato at moderately high temperature stress but of course varied among cultivars.

The present study also indicated that there is a strong relationship between fruit set and pollen germination percentage at moderately high temperature regimes (Fig.4a, b). Thus, the study showed that more than 55 % of the difference in fruit set percentage between genotypes was explained by difference in pollen germination. The other factors might be due to differences in pollen release, pollen tube growth, abnormal development of female reproductive tissues and lack proper ovule fertilization. As explained by Sato *et al*. [11] and Peet *et al*. [19] under high temperature tomato pollen release was affected and in turn contributes to the reduction of fruit set percentages.

The effect of high temperature on tomato pollen germination also associated with reduction of total soluble sugars in pollen grains. The reason for such reduction in sugar content of tomato pollen grains under high temperature is still not clear, but it has been suggested that higher temperature could disrupt sucrose hydrolysis in developing pollen [4]. This leads to loss of pollen quality and subsequent fruit set as pollen grain needs enough sugar to be viable [20]. Wallwork *et al*. [21] indicated that enzymes involved in starch biosynthesis can easily be affected at higher temperatures resulting to lower sugar contents.

Average number of flower production per truss was significantly affected by moderately high temperature regimes. In the present study, higher flower production per truss was observed at 28/22 °C (11) compared to 25/19°C (7) (Fig. 1). This is due to the fact that high temperature increases flower initiation. The decrease in flower number at 25/19 °C temperature regime was also caused by low light condition. Peet *et al*. [22] also found higher flower number per truss at 32 °C (11) than at 30°C (7.37) and 28 °C (7). In contrast, no significant difference was observed between tomato cultivar in their number of flowers grown under the temperature regimes of 28°C and 32°C [11]. The difference in the result of these two experiments might be due to the difference in the experimental conditions. Sato *et al*. [4] compared at two higher temperatures (28 °C and 32 °C) separately in their experiment.

Genotypes showed significant differences in the number of flowers production per truss (Fig. 2). Genotype 12, 29 and 58 produced higher of number flowers per truss whereas genotype 51 produced lower number of flowers per truss. The difference observed on flower development was genotypic dependent in addition to temperature effect [23]. To select genotypes that withstand higher temperatures, it is inevitable to consider other related traits like pollen germination capacity and fruit set. For instance, at 28/22°C, two genotypes, 23 and 56 produced comparable number of flowers per truss. However, in genotype 23, only 22 % of the flowers were developed in to fruit which was the lowest of all genotypes in fruit set percentage. For genotype 56, about 84 % of flowers were developed to fruit.

The pattern of allocation flowers to the different categories of flower fate were varied greatly among genotypes in response to temperature. The fate can be described in terms of flower and fruit abortion. The highest percentage of fruit abortion was seen in genotype 46 (62%) where as the lowest was observed in genotype

43 (8%). The highest percentage of flower abortion was observed in genotype 23 (58%) at 28/22°C whereas genotype 56 had the lowest number of flower abortion at both temperature regimes indicating that this genotype is the most tolerant. In this experiment, genotype 46 and 23 were found to be the most sensitive genotypes to higher temperature. Though it depends on the genotypes, higher temperature not only accelerates flower production but also increases flower and fruit abortions leading to lower fruit set percentage. Recently, Yebirzaf *et al*. [24-26], reported different tomato genotypes responded differently to growing condition (e.g. greenhouse and open field) in their growth, yield and quality of the fruit.

CONCLUSIONS

Pollen germination and subsequent fruit set of tomato genotypes were decreased at moderately high temperature regimes and the reduction was genotypic dependent. From all tested genotypes, genotypes 5, 7, 12, 14, 16, 17, 44, 56 and Moneberg were found to be the most tolerant to moderately high temperature, whereas genotype 6, 23, 46, 48, 51, 60 and 61 were the most sensitive genotypes based on their pollen germination and fruit set ability. A positive correlation between fruit set and pollen germination percentage of the genotypes has been observed at moderately high temperature regimes. Pollen germination is a major limiting factor for tomato fruit set at moderately high temperature stress. Thus, genotype 56, introgression on chromosome 10+11 was the best of all genotypes in most important parameters (Pollen germination, fruit set and number of ripen fruit). Thus, it is necessary to be taken into account for further breeding at molecular level. Considering specific introgression on chromosome 10+11 helps to find responsible genes for high temperature tolerance for this genotype.

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Supplementary Data

Appendix 1: Gene Code, chromosome number with main introgression and fruit color of each of the 58 genotypes used in this experiment

Genotype	Gene code	Main introgression on chromosome	Fruit color
1	Moneyberg	Parent	red
2	La1840	wild type	n.a
3	TKM6U 0025	6	orange
4	TKM6U 0051	6	red
5	TKM6U 0062	3	yellow
6	TKM6U 0070	6	red
7	TKM6U 0080	6	red
9	TKM6U 0092	9	red
10	TKM6U 0099	8	red
11	TKM6U 0112	11	red
12	TKM6U 0171	1	red
13	TKM6U 0637	10	red
14	TKM6U 0648	2	red
15	TKM6U 0659	8	red
16	TKM6U 0669	2	red
17	TKM6U 0674	7	red
18	TKM6U 0810	12	orange
19	TKM6U 0945	6	orange
20	TKM6U 1055	2+3	red
21	TKM6U 1190	4	red
22	TKM6U 0016	1	red
23	TKM6U 0439	1+5	pink
24	TKM6U 0455	11	red
25	TKM6U 0475	3	red
26	TKM6U 0486	4	red
27	TKM6U 0540	10	red
28	TKM6U 0571	4	red

Appendix 1: Continue

Genotype	Gene code	Main introgression on chromosome	Fruit color
29	TKM6U 0580	4	red
30	TKM6U 0595	8	red
31	TKM6U 0618	12	red
32	TKM6U 0623	7	red
33	TKM6U 0193	2	
Red+orange+yellow+pink			
34	TKM6U 0190	12	red
35	TKM6U 0206	2	red
36	TKM6U 0217	3	red
38	TKM6U 0277	5	red
39	TKM6U 0005	8	red
40	TKM6U 0016	1+2	orange
41	TKM6U 0083	5	red
43	TKM6U 0009	8	red
44	TKM6U 0030	9	red
45	TKM6U 0057	5	red
46	TKM6U 0103	12	orange
47	TKM6U 0142	4	red
48	TKM6U 0168	9	red
49	TKM6U 0225	8	red
50	TKM6U 0234	6	orange
51	TKM6U 0283	12	red
52	TKM6U0306	3	red
53	TKM6U 0308	12	red
54	TKM6U 0338	9	red
55	TKM6U 0372	9	red
56	TKM6U 0398	10+11	red
57	TKM6U 0422	7	red
58	TKM6U 0438	1	pink
59	06SG259	n.a [b]	orange
60	06SG260	n.a	pink
61	06SG261	n.a	n.a

[b] Not available.