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Influence of Grafting on Growth, Development and some Physiological Parameters of Tomatoes under Controlled Heat Stress Conditions

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Summary

This study investigated the effect of grafting on the vegetative growth and reproductive development of tomato plants under controlled prolonged heat stress conditions. The heat tolerant tomato cultivar (cv.) 'Summerset' and the eggplant cv. 'Black Beauty' were selected as rootstocks and the heat sensitive tomato cv. 'UC 82-B' as scion. Grafted (tomato/tomato (T/T), and tomato/eggplant (T/E)) and non-grafted plants were grown under high (37/27 °C day/night) temperature and control (26/20 °C) temperature conditions in growth chambers. The experiments were conducted twice with the same conditions. This study indicated that grafted plants developed better under heat stress conditions than the non-grafted ones. T/E plants, for instance, showed significantly higher values of chlorophyll fluorescence at late fruiting stage, greater leaf area, leaf fresh and dry weight, number of pollen grains per flower and lower values of electrolyte leakage than non-grafted 'UC 82-B' at 37/27 °C. Also T/T plants had a better vegetative growth than 'UC 82-B'. However, there was no positive effect on reproductive traits including yield due to grafting. Further combinations with good productive scions and heat stress tolerant rootstocks need to be tested.

Key words. cell membrane thermostability – chlorophyll fluorescence – electrolyte leakage – fruit set and thermotolerance – pollen production

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops in the world. In semi-arid regions, low humidity and high summer temperatures caused by high levels of radiation, limit the production of tomato into the cooler part of the year, and leads to the seasonality of the crop production (Abdalla and Verkerk 1968; Abdelmageed and Gruda 2007).

High day and/or night temperatures interfere with tomato fruit set (Berry and Uddin 1988). Overcoming heat stress problems will have a positive impact on tomato production. Several breeding programs have been made to improve the heat tolerance of tomato, but with limited commercial success. Thus, cultural methods are needed to enhance tomato fruit set (Abdelmageed et al. 2004).

One possibility to overcome this problem would be to graft a heat sensitive cv. onto rootstocks that are more efficient and capable of absorbing water and nutrients as well as able to tolerate high soil temperatures. Thus, heat tolerant genotypes could be used to increase yield and extend the tomato fruit production season.

The grafting technique can be used to reduce diseases caused by soil pathogens (Lee et al. 1998), improve low temperature tolerance (Ahn et al. 1999), enhance salt tolerance (Fernandez-Garcia et al. 2004), induce flood tolerance (Liao and Lin 1996), increase drought tolerance (Iacono et al. 1998), and enhance nutrient and water uptake (Fernandez-Garcia et al. 2002).

There have been a few studies on the effect of grafting on heat stress in tomato plants. According to Rivero et al. (2003a, b) and Abdelmageed et al. (2004) grafting heat sensitive tomato cultivars onto more resistant rootstock cultivars improves plant adaptation to heat stress conditions. On the other hand, eggplants are adapted to hot arid conditions, have a better soil temperature tolerance than tomatoes and posses a highly efficient root system with respect to water uptake (Abdelhafeez et al. 1975).

The objective of this study is to examine if there is any positive effect of grafting on the vegetative growth and reproductive development of tomato plants under heat stress conditions.

Materials and Methods

The heat tolerant tomato cv. 'Summerset' and the eggplant cv. 'Black Beauty' were selected as rootstock and the heat sensitive cv. 'UC 82-B' as scion. Self-grafted treatments were excluded. According to Khah et al. (2006), self-grafted tomatoes had a lower yield under both cultivation conditions (greenhouse and open field). In addi-

tion, LEONARDI and GIUFFRIDA (2006) found that self-grafted plants were less vigorous and produced less dry epigeous biomass than plants grafted on interspecific rootstock. Seeds were sown in flat trays filled with a standard peat mixture substrate for germination from Stender AG Company, Germany. According to the producer the substrate contains 50–300 mg L^{-1} N, 80–300 mg L^{-1} P_2O_4 and 80–400 mg L^{-1} K_2O fertilizer and had a pH of 5.0–6.5. The eggplant 'Black Beauty' was sown one week earlier than the two tomato cultivars. Fifteen days after sowing (DAS), the seedlings were transplanted into 9 cm pots filled with a standard peat mixture substrate from the same company. The plants were watered daily. Twice a week 40 ml of 0.2 % soluble fertilizer (12N-4P-6K) were applied to each pot. The transplants were grown in the greenhouse of the Department of Vegetable Crops, Institute for Horticultural Sciences, Humboldt University of Berlin (Latitude 52 ° 30 ` N, Longitude 13 ° 25 ` E).

Twenty two DAS, tomato cv. 'UC 82-B' was grafted onto the eggplant rootstock 'Black Beauty' (T/E) and tomato 'Summerset' (T/T), using the pin grafting method described by LEE et al. (1998). Specifically, grafting ceramic pins were used to hold the rootstock and scion together tightly. Grafted plants were covered by a transparent plastic cover for 7 d, in order to maintain a high relative humidity and to avoid plant dehydration by water loss.

Grafted and non-grafted tomato plants at 29 DAS were transferred into 14 cm diameter pots filled with same substrate. Thirty five DAS the transplants were divided into two sets, one set was transferred to a growth chamber maintained at normal temperatures, 26/20 °C (day/night) for 13/11 h. The other set was transferred to a second growth chamber maintained at high temperatures, 37/27 °C (day/night) for 13/11 h. During the day 550 µmol m^{-2} s^{-1} irradiance of overhead lighting from a combination of fluorescent and incandescent lamps, was provided for each set.

Five plants of each plant type were placed randomly in each growth chamber. Plants were rotated within the growth chamber every week to avoid any potential positional effects. The experiments were conducted twice with the same conditions.

Data collected

Leaf area (cm^2), leaf fresh weight, leaf dry weight, stem fresh weight and dry weight (g $plant^{-1}$), number of fruits per plant, fruit fresh weight (g $plant^{-1}$), number of pollen grains per flower, and percent of fruit set were recorded according to ABDELMAGEED and GRUDA (2007). In addition, electrolyte leakage and chlorophyll fluorescence were measured.

Electrolyte leakage test procedure was determined by a conductivity method similar to that described by CHEN et al. (1982) with some modifications. Young fully expanded leaves from 3-month-old plants were excised and washed with tap water. After drying with filter paper, 2 g fresh weight of leaves were cut into pieces (about 2 cm^2) using a surgical blade. After that, the materials were placed in vial flasks with 60 ml deionized water and maintained for 20 h followed by reading of conductivity of the solution with the conductivity meter (Digital Conductivity Meter, GMH Model 3410, Greisinger Electronic, Germany). The flasks were shaken on a rotary shaker at 26 °C for 2 h and the electrolyte leakage diffused from the leaves tissues were measured again. Four replicates for each time point were performed. The percentage of electrolytes originally diffused out was calculated as follows:

$$\% \text{ electrolyte} = C_1 / C_2 \times 100 \quad (1)$$

where C_1 and C_2 are the conductivities of the solution before and after shaking respectively.

Chlorophyll fluorescence of fully expanded leaves was measured in vivo using a portable pulse amplitude modulated fluorometer (PAM 2000, Heinz Walz GmbH, Effeltrich, Germany) equipped with the leaf clip 2030-B, at vegetative, flowering, and late fruit set stage according to the protocol described by GENTY et al. (1989). The measurements were replicated five times for each leaf, for five leaves from each plant and on five plants for each treatment. The plants were adapted to the dark for 20 min in the growth chamber at room temperature. The leaf was exposed to a weak modulated beam light at a frequency of 600 Hz to determine the minimal fluorescence yield (F_0) when all PSII reactions centers were fully oxidized. The leaf sample was then illuminated with a saturating pulse beam at a frequency of 20 KHz to estimate the maximal fluorescence yield (F_m) when all PSII reactions centers were reduced. The variable fluorescence yield (F_v) and optimal quantum yield (F_v/F_m) was measured according to GENTY et al. (1989).

Measurements of the quantum efficiency of electron transfer at PSII (Φ_{PSII}) was calculated according to GENTY et al. (1989) and MAXWELL and JOHNSON (2000).

Statistical analysis

Collected data was analyzed using the SPSS statistical package software version 10.0. (Chicago, USA). Analysis of variance (ANOVA) with mean separation by Duncan's multiple range test was used for both temperature regimes separately to determine the significance of variation among the different plant types. The experiments were conducted twice. The same conclusions were drawn from each experiment. Thus, a combined analysis of variance was performed and the data is presented as mean values of 10 replicates across the two experiments.

Results and Discussion

Cell membrane thermostability (CMT)

In this study, electrolyte leakage was selected to compare increase in the membrane damage of the different plant types when subjected to high temperatures. Heat stress increased permeability of the plasmalemma, reducing its ability to retain solutes and water. The leakage of electrolytes through the plasmalemma results in reduced photosynthetic and mitochondrial activity of plant cells (SHANAHAN et al. 1990; RISTIC et al. 1996). A trend for a higher electrolyte leakage was obtained at 37/27 °C (day/night) temperatures in the present study (Fig. 1). Thus, the result here indicates that plant tissues were injured at high temperatures, the membrane permeability was increased and their ability to retain solutes and water was reduced (CHEN et al. 1982; SHEN and LI 1982; CAMEJO et al. 2005).

Moreover, there was a difference in cell membrane thermostability (relative injury) among the leaves of various plant types. Furthermore, there was a significant differ-

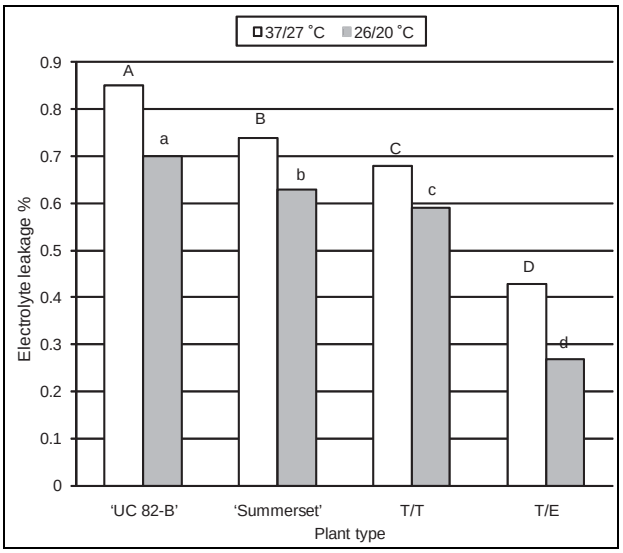


Fig. 1. Influence of grafting on electrolyte leakage of tomatoes grown under two controlled temperature regimes (°C). Bars bearing similar letter(s) within each temperature regime among the different plant types are not significantly different at P≤0.05, according to Duncan's multiple range test for a given temperature regime. T/T = 'UC 82-B'/'Summerset', T/E = 'UC 82-B'/'Black Beauty'. n = 10 plants.

ence between the grafted and non-grafted plants; grafted plants showed lower electrolyte leakage than in non-grafted ones, irrespective of the growth temperature. The highest electrolyte leakage was measured in 'UC 82-B' (Fig. 1).

Thus, the result here indicates a possible association between the damage to cell membrane as reflected by an increased leakage of electrolytes and heat tolerance of the different plant types of tomato. This agrees with preliminary results (Abdelmageed et al. 2004).

Chlorophyll fluorescence of intact leaves

Under heat stress conditions, the optimal quantum yield (F_v/F_m) was reduced in all plant types, irrespective of the growth stage. The reduction in optimal quantum yield (photochemical efficiency) by high temperature regimes is likely to be attributed to inhibition of the water splitting apparatus as reported by Weis (1982) and Weis and Berry (1987). T/E had higher optimal quantum yield (F_v/F_m) than 'UC 82-B' at high temperature regime at the late fruiting stage (Table 1), indicated that T/E was more tolerant to heat stress than 'UC 82-B' at this growth stage. Furthermore, optimal quantum yield (F_v/F_m) for 'UC 82-B' and T/T were significantly different at the flowering stage at the high temperature regime and at the late fruiting stage at the normal temperature (Table 1).

Berry and Bjorkman (1980), and Willits and Peet (2001) reported a decline in the functioning of primary photochemical reactions, primarily involving inhibition of PSII, located in the thylakoid membrane system. A similar response also observed in our study for the quantum efficiency at PSII (Φ_{PSII}) of electron transfer at the high temperature regime (Table 1). Moreover, T/E and 'Summerset' had a tendency for higher quantum efficiency of electron transfer at PSII (Φ_{PSII}) at the high temperature regime during the late fruiting stage.

The reduction of both the optimal quantum yield (F_v/F_m) and the quantum efficiency of electron transfer at PSII (Φ_{PSII}) at the high temperature regime indicated that an important portion of the PSII reaction centre caused damage to the thylakoid membrane. This result is in agreement with those of Karim et al. (1997) and Camejo et al. (2005). According to Genty et al. (1989), the quantum yield of electron transfer at PSII (Φ_{PSII}) is the product of the efficiency of the open PSII reaction centres (F'_v/F'_m) and the photochemical quenching factor ($_qp$). Therefore, the decrease in net photosynthetic O_2 evolution and Φ_{PSII} in leaves grown at a high temperature regime is due primarily to the decrease in $_qp$, in our study (data not shown).

Vegetative growth

High temperature conditions (37/27 °C) markedly reduced the vegetative growth of the different plant types. All plant types showed a trend for lower fresh and dry weights of leaves and stems, and reduced leaf area at the

Table 1. Influence of grafting on optimal quantum yield (F_v/F_m) and quantum efficiency of electron transfer at PSII (Φ_{PSII}) at different growth stages of tomatoes grown under two controlled temperature regimes (°C).

	Optimal quantum yield (F_v/F_m)						Quantum efficiency of electron transfer at PSII (Φ_{PSII})					
Growth stage	Vegetative		Flowering		Late fruiting		Vegetative		Flowering		Late fruiting	
Temperature (day/night) (°C)	37/27	26/20	37/27	26/20	37/27	26/20	37/27	26/20	37/27	26/20	37/27	26/20
'UC 82-B'	0.68 A	0.71 a	0.68 A	0.70 ab	0.66 B	0.69 b	0.68 A	0.72 a	0.68AB	0.70 ab	0.67AB	0.69 b
'Summerset'	0.64 B	0.72 a	0.68 A	0.69 b	0.68AB	0.67 c	0.64 B	0.72 a	0.67AB	0.69 b	0.68 A	0.68 b
T/T	0.68 A	0.73 a	0.64 B	0.68 b	0.65 B	0.71 a	0.69 A	0.73 a	0.65 B	0.69 b	0.64 B	0.72 a
T/E	0.69 A	0.72 a	0.68 A	0.71 a	0.70 A	0.71 a	0.69 A	0.72 a	0.68 A	0.71 a	0.70 A	0.71 a
Mean	0.67	0.72	0.67	0.70	0.67	0.70	0.67	0.72	0.67	0.70	0.67	0.70

Means followed by the same letter(s) within each column are not significantly different at P≤0.05, according to Duncan's multiple range test for a given temperature regime. T/T = 'UC 82-B'/'Summerset', T/E = 'UC 82-B'/'Black Beauty'n = 10 plants

Table 2. Influence of grafting on vegetative growth parameters of tomatoes grown under two controlled temperature regimes (°C).

Parameters	Leaf area (cm^2)		Leaf fresh weight (g)		Leaf dry weight (g)		Stem fresh weight (g)		Stem dry weight (g)	
Temperature (day/night) (°C)	37/27	26/20	37/27	26/20	37/27	26/20	37/27	26/20	37/27	26/20
'UC 82-B'	1060.4 C	1824.3 c	37.9 D	71.9 c	6.09 D	8.44 b	27.33 B	25.54 c	3.26 B	2.86 d
'Summerset'	1064.9 C	4060.6 a	57.5 C	170.4 b	9.15 C	22.73 a	25.04 B	54.12 b	3.15 B	6.72 c
T/T	2915.9 A	3687.7 ab	144.6 A	193.1 a	18.30 A	23.16 a	44.71 A	59.90 a	5.32 A	10.12 a
T/E	1508.0 B	3387.2 b	92.2 B	158.6 b	11.19 B	22.02 a	15.65 C	50.88 b	3.07 B	8.71 b
Mean	1637.3	3239.9	83.1	148.5	11.18	19.09	28.18	47.61	3.70	7.10

Means followed by the same letter(s) within each column are not significantly different at P≤0.05, according to Duncan's multiple range test for a given temperature regime. T/T = 'UC 82-B'/'Summerset', T/E = 'UC 82-B'/'Black Beauty'. n = 10 plants.

Table 3. Influence of grafting on generative development of tomatoes grown under two controlled temperature regimes (°C).

Parameters	No. of pollen grains $flower^{-1}$		Fruit fresh weight (g)		No. of fruits $plant^{-1}$		Fruit set percentage	
Temperature (day/night) (°C)	37/27	26/20	37/27	26/20	37/27	26/20	37/27	26/20
'UC 82-B'	45.0 C	71.0 b	0.00 B	276.40 a	0.0 B	10.2 b	0.0 B	21.5 bc
'Summerset'	66.8 A	88.0 a	13.13 A	284.00 a	3.2 A	10.6 b	25.7 A	26.9 b
T/T	47.0 C	73.0 b	3.85 B	276.00 a	0.8 B	10.4 b	5.0 B	17.1 c
T/E	58.0 B	75.0 b	3.83 B	272.00 a	0.8 B	12.8 a	4.4 B	53.8 a
Mean	54.2	76.9	5.20	277.10	1.2	11.0	8.8	29.8

Means followed by the same letter(s) within each column are not significantly different at P≤0.05, according to Duncan's multiple range test for a given temperature regime. T/T = 'UC 82-B'/'Summerset', T/E = 'UC 82-B'/'Black Beauty'. n = 10 plants.

high temperature regime in comparison to the control (Table 2). This is in agreement with the results obtained by Abdelhafeez et al. (1975). However, the leaf area and the fresh and dry weights of the leaves for both T/E and T/T were significantly higher than that from 'UC 82-B' at the high temperature regime. This might be attributed to the ability of these plant types to produce more assimilates than the heat sensitive cv. 'UC 82-B'. Furthermore, it is interesting that grafted T/T plants showed better vegetative parameters compared to the heat tolerant cv. 'Summerset', which supported the results obtained by Rivero et al. (2003a, b). This result indicated that greater dry matter partitioning might contribute to improvement of crop productivity by increasing total carbohydrate production (Nkansah and Ito 1994).

Reproductive development

At the high temperature regime, the number of pollen grains produced and released be all plant types was markedly reduced (Table 3). Similar results were obtained by Sato et al. (2000) and Abdelmageed et al. (2004). However, T/E plants produced a significantly higher number of pollen grains when compared to 'UC 82-B' at the high temperature regime. Also T/T had a tendency for a higher number of pollen grains compared to 'UC 82-B' (Table 3).

High temperatures sharply reduced the number of fruits, fruit fresh weights per plant, and fruit set percentage. This is consistent with the results obtained by Sato et al. (2004). The differences between T/T or T/E and 'UC 82-B' were not significant. However, the grafted plant types showed a better trend than the non-grafted ones. This might be due to a disruption of male reproductive development in 'UC 82-B'. Contrary to results from vegetative growth, the heat tolerant cv. 'Summerset' showed better generative parameters than grafted plants (Table 3). These results were observed also in the preliminary work (Abdelmageed et al. 2004) and this might be due to the higher growth potential of this cultivar under heat stress conditions.

In summary, this study indicated that the grafting of heat sensitive tomato plant onto a heat tolerant tomato or eggplant rootstock improves vegetative growth, but not yield under heat stress conditions. The results of this study are in agreement with the results of Rivero et al. (2003a, b). They found that the use of grafted plants under excessively high temperatures implies an advantage

for the plant under such conditions through an intrinsic mechanism of resistance to high temperatures that is derived from a resistant rootstock. Therefore promoting better growth under situations of thermal stress is directly reflected in greater biomass production. However, there was no positive effect on reproductive traits including yield. In order to improve the generative development of tomato growth under heat stress conditions, further combinations with good productive scions and heat stress tolerant rootstocks need to be tested in laboratory and field conditions.

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