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Germplasm diversity for resource protection in crop production

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Abstract

Huge problems in greenhouse tomato production in the tropics are caused by high temperatures. Greenhouses are used to protect plants from destruction due to heavy rainfalls, storms, and insect infestations. For worthwhile tomato production, it is absolutely necessary to decrease the air temperature inside the greenhouse. But the use of more or increasingly powerful cooling systems might be as well uneconomical as resource encumbering. Genetic diversity of heat tolerance of the tomato (*Solanum lycopersicum* L.) has already been described in the literature.
Two strategies were used to evaluate and combine the potentials of resource protection: Reducing the stress factor and finding genotypes adapted to the stressor. The experiments were conducted during different seasons at the experimental facilities of the project 'Protected cultivation- an approach to sustainable vegetable production', situated on the campus of the Asian Institute of Technology (AIT), Klong Luang, Pathum Thani, Central Thailand, and on the campus of the Leibniz University in Hannover, Germany. For the first strategy, we assessed the response of miscellaneously heat tolerant genotypes to different microclimatic conditions aroused by different greenhouse cover materials and ground mulches. We analyzed the plant's response to UV absorbing greenhouse cover films and different colored mulch foils and evaluated the influence of UV and NIR radiation. Several traits associated with heat tolerance were recorded. For the second strategy the most heat tolerant genotype and a heat sensitive variety were selected and used as parents for developing a segregating F_2 population. We evaluated the same traits under high temperature treatment as in the prior experiments. Currently, AFLPs are being used to construct linkage groups. We will be able to map some of the genes responsible for heat tolerance and it is envisaged creating molecular markers as useful tools for plant breeders. These results could contribute to the breeding of new varieties with better adaptation to heat stress, and reduce the energy consumption for cooling systems.

Introduction

Major constraints for sustainable vegetable production in the tropics are damages of plants and fruits by unfavorable weather conditions (Midmore et al. 1997). Especially the temperatures in the tropical lowlands exceed the optima for most vegetable crops almost throughout the year. These conditions do not only reduce the yield but avail the occurrence of plant diseases, insect pest infestations, and associated virus infections, amongst others. A self-evident solution to protect crops from such detrimental environmental effects is the utilization of greenhouses (GHs). Most GHs in tropical and subtropical regions consist of plastic roofs with open or net covered

sidewalls and ventilation openings (Möller et al. 2004) to minimize insect immigration and reduce the frequency of pesticide application. The covered ventilation openings lead to a reduction of the air exchange rate (Harmanto et al. 2006; Teitel 2001) influencing the microclimatic conditions inside the GHs leading to an additional increase of the temperature. The effects of high temperatures on plants range from hastening of fruit ripening (Adams et al. 2002) to losses of almost entire yields (Kleinhenz et al. 2006). Reduced flower production and flower abscission (Abdul-Baki 1991; Dane et al. 1991), and increased incidences of physiological disorders, e.g. fruit cracking (Peet 1992) and blossom-end rot (Ho et al. 1993) are just some of the observed characters. Even though tomatoes are able to produce parthenocarpic fruits, these fruits are not usable for marketing. Parthenocarpic fruits are cavernous, misshaped and unappealing to consumers. In the literature the results for phenotypic traits in the tomato crop were sometimes rather ambiguous. A reduction of flower formation under heat stress was reported (El-Ahmadi et al. 1979; Lohar et al. 1998) and no reduction or an increased number of flowers under different high temperature treatments (Peet et al. 1997). Differing results were also found for the number of pollen released. Both, a decrease of the pollen number released under heat stress (Abdalla et al. 1967) and no differences in different temperature treatments were found (Sato et al. 2006). This ambiguity indicates the genetic variance within the tomato species. Therefore, we tested several varieties referred to be heat tolerant by their breeders under heat stress conditions (herein further referred to as 'variety trial'). Additionally, we tested the plant response of different greenhouse set-ups (herein further referred to as 'set-up trial'). Easy to implement methods were studied as a possibility for adapting the inside GH-microclimate at suitable values for fruit production. Climatic data such as inside air temperature, outside air temperature, relative humidity, and global radiation were collected to give suggestions for further greenhouse constructions. Different parameters important for and related with fruit set, e.g. number of flowers and fruits, were evaluated in our experiments. In literature the male reproduction system seems to be the most sensitive part in flower and fruit formation. Therefore, the traits pollen amount and pollen viability were specified as main factors to be evaluated. From these results we chose the genotype seeming to be the most heat tolerant and created an F_2 mapping population. In this population we investigated the same traits as described before (herein further referred to as 'F_2 experiment'). At present molecular marker data are collected to find markers linked to these traits. The combination of these experimental results might finally offer possibilities to reduce the energy consumption for the tomato production under tropical climate conditions.

Materials and Methods

1.1. Experimental sites and greenhouse set-ups

The experiments were conducted in five GHs at the experimental facilities of 'Protected cultivation project', situated on the campus of the Asian Institute of Technology (AIT), Klong Luang, Pathum Thani, Central Thailand and in greenhouse facilities of the Department of Horticulture of the Leibniz University of Hannover, Germany. The greenhouse cabin (GC) used in Hannover was 8 × 12 × 4 m. In the AIT, GH dimensions were 10 × 20 × 6.4 m (GHbig) and 6.0 × 3.0 × 3.2 m (GHsmall) (L × W × H). The sidewalls of the GHsmall were clad with 40-mesh net ('Econet M', Ab Ludvig Svensson, Kinna, Sweden). A polyethylene film was used as roof cover. The sidewalls and roof ventilation opening of the GHbig were covered with a 50-mesh insect-proof net (BioNet). A UV-absorbing polyethylene film was used to cover the greenhouse roof, gable sides and the portion of the sidewalls near the ground. The roof plastics of two of the GHsmall were additionally coated with a near infra red (NIR) reflecting pigment (Reduheat, Baarle Nasau, Mardenkro, The Netherlands). The pigment paint (mixing ratio 1:2.5 pigment to water) was applied to the roofs one week before the experiments commenced. The GH floors were covered with a bi-colored (black-white) plastic mulch (Silo plus™, FVG, Dernbach,

Germany). Depending on which surface of the mulch was on top, the following four combinations of GHsmall were realized: 1. White mulch and NIR transmissive roof (herein after referred to as 'WTrans'); 2. White mulch and NIR-reflecting roof ('WRef'); 3. Black mulch and NIR-reflecting roof ('BRef'); 4. Black mulch and NIR transmissive roof ('BTrans'). The white surface of the PE mulch was used in all GHbig.

1.2. Crop management

For the 'variety trial' 5 plants of 16 varieties (80 plants in total) were transplanted to a GHbig. 280 additional plants, resulting in a plant density of approx. 1.8 plants m^{-2}, were transplanted. For the 'set-up trial' 36 plants of the tomato cultivar 'FMTT260' (AVRDC-The World Vegetable Center, Shanhua, Taiwan), an indeterminate F_1-hybrid considered to be heat tolerant, were transplanted to each experimental GHsmall in three rows resulting in a planting density of 2 plants m^{-2}. For the 'F_2 experiment' 174 F_2 plants plus two plants of the F_1 generation and both parents, respectively, were transplanted into the GC, resulting in a plant density of approx. 1.8 plants m^{-2}. The temperature regime in the GC was 32/ 28 °C day/ night temperature. In all greenhouses insect pest control took place once a week, spraying of fungicides according to necessity before flowering. Pruning was done twice a week. After the first harvest, senescent leaves were removed regularly up to the first fruit carrying truss and plants were laid down according to necessity. Fertigation was done automatically in different intervals according to physical plant age.

1.3. Data collection

Climate data inside and outside the GHs were monitored continuously with the aid of aspirated psychrometers (sensors: sheathed type K [NiCr-Ni] thermocouples, ∅: 0.5 mm) (BGT, Hannover, Germany). Data were measured and transferred to a purpose-build data-logging system (BGT, Leibniz University Hannover) every 15 s and stored as mean value for 5 minutes. All sensors were calibrated prior to the start of the experiment. The collection of all plant related data was restricted to the plants of the central rows in order to avoid side effects. Pollen viability was evaluated via FDA (Fluorescein diacetat [3',6'-Bis(acetyloxy)-spiro isobenxofuran-1(3H),9'-9H xanthen-3-one 3,6-Diacetoxyfluoran]) staining in conection with fluorescence microscopy or MTT (Methylthiazolyldiphenyl-tetrazolium bromide [3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide]) staining and the amount of pollen released evaluated by a 'Fuchs-Rosenthal' counting chamber via transmitted-light microscopy (AxiovisonA40, ZEISS, Göttingen, Germany). The phenotypic data were rated visually. All plant growth data were recorded weekly and the pollen data were evaluated twice weekly. The harvest in the 'set-up trial' was done twice a week.

1.4. . Statistical Analysis

Statistical design for the 'variety trial' and the 'F_2 experiment' was a Randomized Complete Block Design (RCBD) and for the 'set-up trial' was a two factorial (2-2) scheme consisting of the two factors 'roof cover' and 'mulch color'. Data were analyzed with SAS Version 9.1 (SAS, 2005, SAS Institute Inc., Cary N.C., USA). SAS's 'GLM'-procedure was used to conduct the Analysis of variance (ANOVA). In general a significance level of 0.05 was underlying for the tests except for results mentioned explicitly.

Results

Though all of the tested varieties are referred as to be heat tolerant they showed highly significant differences for several traits investigated. The highest amount of pollen released and the highest percentage of viable pollen was found in the lines bred by the AVRDC (Figure 1, Figure 2). From the 16 tested varieties only 10 produced enough pollen for statistical analysis under the extreme

temperatures in our experiments. Basing on these results the line CLN1621 was chosen as the heat tolerant parent for the creation of the mapping population.

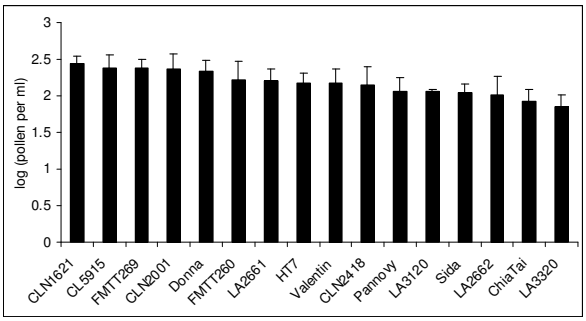

Figure 1: 'Variety trial': the sum of pollen released in 16 tested varieties. The results are in logarithmic scale.

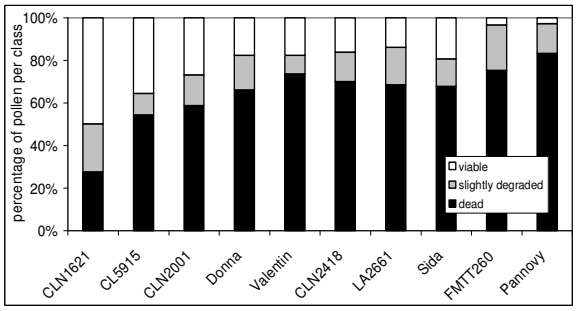

Figure 2: 'Variety trial': the percentage of viable pollen. The data were separated in three different classes: viable (white columns), slightly degenerated (labeled by white to black columns), and dead pollen (black columns). Only 10 of 16 varieties produced enough pollen for statistical analysis.

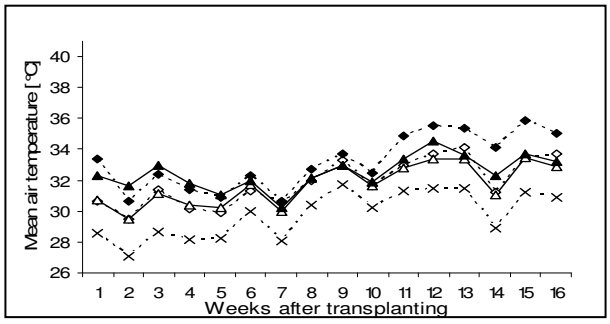

Figure 3: 'Variety trial': the weekly mean temperatures of the average air temperatures inside the greenhouses at daytime during the dry season 2005/06 in Central Thailand. White and black mulched greenhouses are indicated by filled symbols and open symbols, respectively. Transmissive and reflecting roof covers are pictured by rhombic and triangle symbols, respectively.

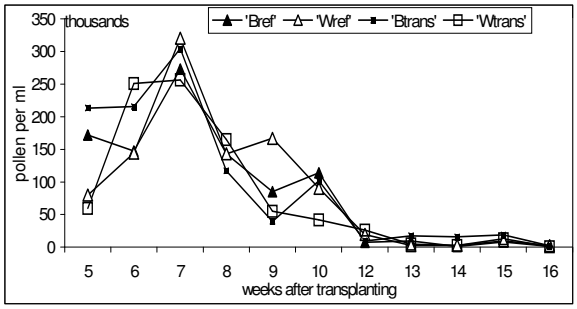

Figure 4: 'Set-up trial': the number of pollen released in four different built houses. The 'WTrans' is indicated by the unfilled black square, 'WRef' by the unfilled triangle, 'BRef' by the filled triangle, and 'BTrans' by the filled square.

In the 'set-up trial' results differed significantly for the pollen traits concerning the time span 13 weeks after transplanting (WAT13) to WAT16. Within this time the daily mean temperatures exceeded 32 °C constantly (Figure 3). Pollen viability was higher in greenhouses with black ground cover while the pollen release was higher in houses with NIR transmissive roofs (data not shown). Additionally a clearly visible decrease over the experimental time in all greenhouses was observed (Figure 4). With increasing plant age and heat exposure the harvested yield dropped to a mean of less then 150 g per WAT (data not shown) and the total number and percentage of parthenocarpic fruits increased (Figure 5). The plant height and the height of the first fruit carrying truss were significantly reduced in the houses with the white mulch color (Figure 6, Figure 7). The time for the first flowers to open was reduced in the houses with white mulch color (data not shown).

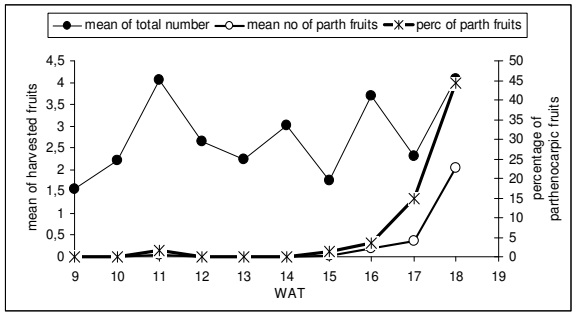

Figure 5: 'Set-up trial': the mean number of fruits (labeled by filled circles) and the mean number of parthenocarpic fruits (labeled by unfilled circles, both on left y-axis) and the percentage of the parthenocarpic fruits (labeled by crosses, right y-axis).

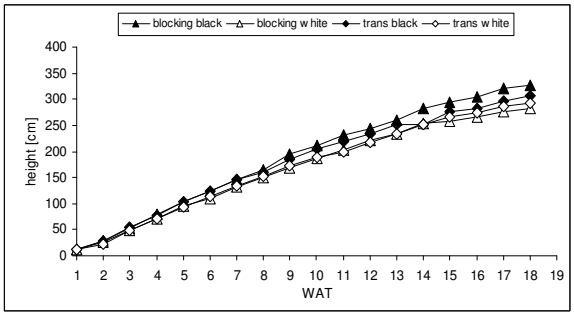

Figure 6: 'Set-up trial': the plant height in cm. The 'WTrans' is indicated by the unfilled black square, 'WRef' by the unfilled triangle, 'BRef' by the filled triangle, and 'BTrans' by the filled square.

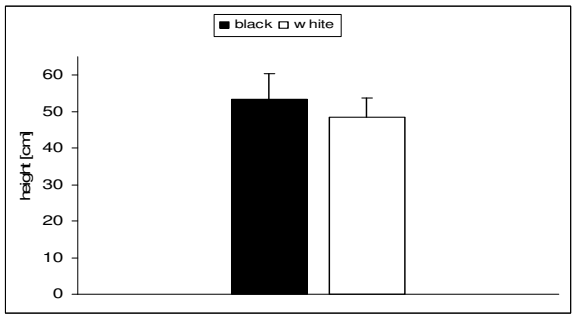

Figure 7: 'Set-up trial': the height of the first inflorescence. The houses with white ground mulch are indicated by the white column and the houses with the black mulch with the black column.

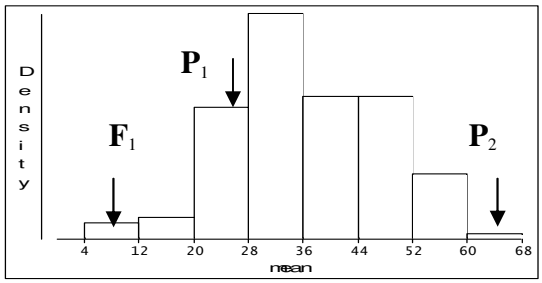

Figure 8: 'F_2 experiment': the distribution of the trait 'pollen viability'. The parents are indicated by arrows. P_1 and P_2 are the heat sensitive and the heat tolerant parent, respectively. F_1 indicates the first offspring of the cross.

In the 'F_2 experiment' the phenotypic data off all 174 genotypes showed high variability for all evaluated traits. The pollen viability ranged from around 4 % to 65 %. The parents were found on the ends of the distribution while the F_1 showed even worse results compared to the heat sensitive parent (Figure 8). Similar results were found for the pollen amount released, the numbers of flowers and fruits, the fruit set and the percentage of parthenocarpic fruits.

Discussion

The climatic conditions, especially the temperatures, in tropical and subtropical regions are uncomfortable for tomato greenhouse production almost all over the year. In our experiments we could proof the genetic variability in heat tolerant tomato lines bred under different climate conditions. Varieties from Taiwan showed the best adaptation to the Thailand environment in consequence of similar conditions during the selecting process. This indicates the influence of other climatic factors on the investigated traits, relative air humidity or global radiation, e.g. Therefore, a strict selection of the grown variety for the regnant climatic conditions is required. A possibility for decreasing the inside temperatures in greenhouses is the use of more or increasingly powerful cooling systems but this might be as well uneconomical as resource encumbering. Different greenhouse set-ups can be a useful tool to help adapting the greenhouse inside climate conditions to suitable values for tomato production. In greenhouses with white mulch color the temperatures were lower compared to the black covered houses in a large part of the experiment. The white ground cover led to a reduced plant height and a reduced height of the

first fruit cluster, and an earlier bloom. At the same time the numbers of fruits did not differ. This results in less work to lay down plants and in an extension of the cultivation time. A steady pollination is required for an economically relevant yield. In the beginning of the 'set-up trial' the temperatures were not high enough to produce heat stress disorders in the pollen. In the ongoing experiment the temperatures were devastating for pollen production and release and their viability. Significant influences could not be found. The pollen release was decreased to a very low level and it was difficult to receive enough pollen for the staining procedure. Overall, the pollen viability was down to a level that can not generate a proper fertilization anymore. The influence of the NIR reflecting pigment on the pollen release can not be explained reasonably. But for different species it was observed that the light spectrum influences the opening process of the anthers.

However, the application of white ground cover can be recommended as it leads to lower temperatures inside the greenhouses and positive effects on several traits related to plant growth. The influence of the shading paint on other relevant traits has to be evaluated further on.

The F_2 population showed high variability for all evaluated traits related to fruit set as expected. The low results of the F_1 offspring might be explained by heterosis of the heat sensitive parent because it concerned to be a F_1 hybrid or by demasking of recessive genes. Presently, we are analyzing molecular markers for creating a linkage map and for mapping QTLs. The results will allow to create primers as useful tools for plant breeders to select their breeding material further on. The combination of the adaptation of the greenhouse environment and the crop itself are valuable sources for energy conservation.

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