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Expression of genes for the biosynthesis of compatible solutes during pollen development under heat stress in tomato (*Solanum lycopersicum*)

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ABSTRACT

Accumulation of compatible solutes is considered a key adaptation mechanism in many plants in response to abiotic stress. The expression of four genes, involved in sucrose metabolism (SPS and SuSy), biosynthesis of galactinol (GoLS1) and proline accumulation (P5CS) was compared: at meiosis (MM), vacuolated and mature stages of pollen development in heat tolerant and heat sensitive tomato genotypes. The results showed differences in gene expression across tomato genotypes and stages of pollen development. Three genes (P5CS, SPS and SuSy) were up regulated in heat tolerant genotype CLN1621L at the mature stage and one gene (P5CS) in genotype CLN5915-93D at the MM stage. Two genes (SPS and GoLS1) were down regulated in heat sensitive genotype CA4 and one gene (GoLS1) in genotype CLN2498E at the MM stage. Additionally, the continuous exposure of tomato genotypes to temperatures of 35 °C/28 °C day/night completely impaired flower development in genotypes CA4 and CLN2498E but not in genotypes CLN1621L and CLN5915-93D. Tomato genotypes CLN1621L and CLN5915-93D produced fully developed flowers containing mixture of non viable pollens and very few viable pollens grains. Membrane permeability was affected at all stages of development under heat stress with heat tolerant genotypes CL5915-93D4, CLN2498E and CLN1621L showing varying degrees of heat acclimation. Significant increases in total chlorophyll were seen in all genotypes in response to heat stress. The expression of compatible solute genes at MM is more critical than at mature stage for the development of viable pollen grain.

Introduction

Heat stress is one of the most limiting environmental factors for agriculture, accounting for significant crop losses worldwide. Agricultural production and yield are predicted to be affected by increasing temperatures resulting from global warming (Ainsworth and Ort, 2010), with the rate of increase in the production of major crops decreasing (Fischer and Edmeades, 2010).

Temperatures in the tropics and subtropics are predicted to exceed current maximums by the end of the 21st century (Battisti and Naylor, 2009) and heat wave events are set to become more frequent (Tebaldi et al., 2006). Substantial crop losses due to elevated temperatures have been predicted in both temperate and subtropical areas and there is an urgent need to mitigate against these losses through agricultural policy and crop adaptation (Teixeria et al., 2013).

Sexual reproduction is highly sensitive to heat stress, affecting flower development and number, and pollen production and viability. This in turn results in reduced seed set and yield (Prasad et al., 2006; Das et al., 2014). It has been recognized as the most heat stress susceptible phase in cereals (Monterroso and Wien, 1990; Barnaba et al., 2008) and vegetables (Erickson and Markhart, 2002), with male reproductive stages more sensitive to heat stress than female or vegetative stages of growth (Sakata and Higashitani, 2008).

Tomato (*Solanum lycopersicum*) is among the most popular and the second most consumed vegetable worldwide after potatoes (FAO, 2005). It grows under diverse environmental conditions worldwide with optimal temperatures of 25–30 °C day and 20 °C

Abbreviations: FDA, fluorescein diacetate; GoLS1, galactinol synthase; KGMN, Kigamboni; LSD, least significant difference; MP, mature pollen; MM, meiosis stage; P5CS, pyrroline 5 carboxylate synthase; RCBD, randomized complete block design; REST, Relative Expression Software Tool; RI, relative injury; SPS, sucrose phosphate synthase; SuSy, sucrose synthase; UDSM, The University of Dar-es-Salaam; VM, vacuolated stage.

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night (Camejo et al., 2005). Tomato production in tropical and subtropical areas is constrained by above optimal (<27 °C) temperatures that cause heat stress in tomatoes. Exposure to heat stress during the reproductive stage is the most critical cause of yield reduction as it affects the pollen developmental process (Peet et al., 1998; Firon et al., 2006) leading to poor pollen formation and loss of pollen viability (Sato et al., 2002). The major effect of heat stress on the pollen development process is the disruption of carbohydrate metabolism and proline translocation (Sato et al., 2006) resulting into a decrease in starch and sugar concentration in mature pollen (MP) grains with a subsequent loss of pollen viability (Pressman et al., 2002). This in turn results in a failure of fruit set and associated crop losses at high temperatures (Abdul-Baki and Stommel, 1995).

The major difference between heat tolerant and heat sensitive tomato genotypes growing under heat stress conditions is the ability to accumulate starch and soluble sugars by the former during pollen development (Firon et al., 2006). As a consequence heat tolerant tomatoes produce viable pollen and high fruit set under heat stress (Firon et al., 2006). However it is not well understood how plants manage to maintain the appropriate levels of starch and soluble sugars under heat stress. Different genotypes adjust their metabolic pathways during heat stress to achieve a new state of homeostasis (Suzuki and Mittler, 2006). It is not clear whether heat tolerant varieties accumulate starch and soluble sugars through increased biosynthesis, decreased degradation (Demnitz-King et al., 1997), or by increased uptake (Aloni et al., 1996) of compatible solutes causing their accumulations in cells and tissues.

In this study we investigated the gene expression profiles of four compatible solute genes; sucrose phosphate synthase (SPS), sucrose synthase (SuSy), galactinol synthase (GoLS1) and pyrroline 5 carboxylate synthase (P5CS) at three stages of pollen development in heat tolerant and heat sensitive tomato genotypes subjected to heat stress. The selected genes encode key enzymes responsible for sucrose metabolism, galactinol and proline biosynthesis respectively and were selected based on their reported involvement in heat stress tolerance. The SuSy gene is involved in controlling sucrose import capacity in young tomato fruit, which in turn affects fruit set (D'Aoust et al., 1999), whilst elevated levels of SPS in leaves have been shown to improve heat stress tolerance by enhancing photosynthetic capacity (Hong et al., 2009). The GoLS1 gene encodes a key enzyme involved in the synthesis of raffinose family oligosaccharides, galactinol, which accumulates in plants subjected to heat stress (Panikulangara et al., 2004). P5CS encodes for proline, a compatible solute known to be induced by heat stress as a protective mechanism (Verbruggen and Hermans, 2008).

During the process of anther development some stages have been reported to be more sensitive to heat stress than others (Giorno et al., 2013), including pollen meiosis (MM) in *Arabidopsis thaliana* (Kim et al., 2001), and pollen tapetum differentiation in barley (Sakata and Higashitani, 2008). In the present study, the stages of anther development selected correspond to the pollen MM stage, vacuolated stage, and MP stage which cover early, intermediate and late pollen development, in order to identify which stages of anther development are most sensitive to heat stress in tomato.

We hypothesized that the expression of compatible solute genes differ in tomato genotypes at key stages of pollen development and in addition increased gene expression corresponds with the production of viable pollen grains and high fruit set under heat stress. A better understanding of the effect of heat stress on gene expression at individual stages of pollen development could potentially reveal metabolic adjustments in heat tolerant genotypes. Understanding the metabolic changes that occur in heat tolerant varieties/lines during heat stress is essential for the production of tomato crops adapted to variable temperature increases.

Materials and methods

Source of germplasm

Tomato (*Solanum lycopersicum* L.) seeds were obtained from the Genetic Resources and Seed Unit of AVRDC—The World Vegetable Center, Taiwan. Four cultivars were used: CLN 1621L and CL5915 generated from crosses between a heat tolerant *S. lycopersicum* and a heat sensitive Gemini-virus resistant variety *S. lycopersicum* either CA4 or FLA 456; moderately tolerant genotype CLN 2498E generated by crossing *S. lycopersicum* to *Solanum pimpinellifolium*, and a heat sensitive genotype CA4.

Experimental design

Growth chamber experiments were carried out at AVRDC headquarters, Taiwan (latitude 23°N, longitude 120°E). Seeds were germinated in 5 cm pots and the seedlings transplanted to 1 L pots containing 1 kg of potting mixture and placed in controlled temperature growth chambers 21 d after germination. One growth chamber was set at optimum temperature condition 24 °C/18 °C, day/night as the control. A second growth chamber was set at 35 °C/28 °C, day/night for the heat treatments. Each growth chamber contained four accessions with three replications in a complete random design. The plants were monitored and scored for the number of flowers and trusses, number of pollen and pollen viability, fruit set, cell membrane thermostability, and leaf chlorophyll content.

Field trials were carried out in Tanzania at three different locations across the hot and cool seasons of 2010/2011. Seedlings were transplanted at 21 d after emergence and each genotype was planted in a plot of 6 plants in two rows of 3 plants spaced at 60 × 60 cm in a randomized complete block design (RCBD), with three replications at each location. Two sites Ruvu, Kibaha District (latitude 6.8083°S, longitude 38.6583°E), Kigamboni (KGMN) (latitude 6.8167°S, longitude 39.3167°E) maintained average monthly temperatures of above 33 °C day and 27 °C night with a daily range of 28–43 °C/25–38 °C day/night throughout the hot season, therefore qualifying for screening the genotypes for heat stress tolerance (hot season). Cool season experiments were carried out at The University of Dar-es-Salaam (UDSM) (latitude 6.7806°S, longitude 39.2033°E) with average monthly temperatures of 29 °C day and 19 °C night with a daily range of 29–30 °C/18–19 °C day/night throughout the cool season. Plants were scored for number of flowers, number of fruits, fruit set percentages, plant height, number of d to maturity and membrane thermostability according to standardized protocol developed by AVRDC. Morphological and physiological analysis was carried out at the seedling, vegetative and flowering developmental stages. ANOVA for RCBD was used to test the differences of the target traits in the four genotypes. The difference across seasons was analyzed using two sample T test and the mean comparison for all pairs of target traits were tested by least significant difference (LSD) for all pair wise comparison using statistix 8.1 1985–2005 analytical software.

Cell membrane thermostability

Cell membrane thermostability tests were carried out according to Camejo et al. (2005) with a modification on temperature treatments of the leaf disks as described below. Leaf disks of 2 cm^2 in diameter were collected at seedling, vegetative and flowering stages of tomato growth. At each stage 12 leaf disks were sampled per plant from newly formed leaves, and the electrical conductivity