ROLE OF POLLEN STARCH AND SOLUBLE SUGAR CONTENT ON FRUIT SET IN TOMATO UNDER HEAT STRESS

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SUMMARY

The effects of heat stress on pollen starch and soluble sugar content in relation to fruit set were examined in 4 tomato genotypes under controlled condition at 2 temperature regimes, including optimal and high temperature. The optimal temperature conditions (OT, 28/21 °C) resulted in starch accumulation in the pollen grains, where it reached a maximum value before anthesis and diminished towards anthesis. Total soluble sugar concentration gradually increased in the pollen grains reaching a maximum at anthesis. Continuous exposure of the plants to high temperatures (HT, 37/27 °C) prevented the transient increase in starch concentration and led to decrease in the concentrations of soluble sugars in the pollen grains at anthesis. It also markedly reduced the percent pollen viability, pollen germination, and fruit set, as compared to optimal temperature (OT, 28/21°C). These results suggest that a major effect of heat stress on pollen development is a decrease in starch concentration before anthesis, which results in a decreased soluble sugar concentration in the mature pollen grains at anthesis. These events possibly contribute to the decreased pollen viability, pollen germination, and ability to fruit set in tomato. The results strongly indicated that 2 tomato genotypes (Pusa Sadabahar and NDTV-R-60) could be a valuable source of heat-tolerant germplasm for tomato breeding programs.

Key words: Heat stress, starch, soluble sugar, pollen viability, pollen germination, fruit set

Key findings: The results of present investigation emphasize the use of starch and soluble sugar content in pollen grains as the most important biochemical parameter for screening of tomato genotypes for improved fruit set in heat stress conditions. It was concluded that Pusa Sadabahar and NDTV-R-60 tomato genotypes tolerant to the heat stress can be a source of heat tolerance and considerable practical value for studying the mechanism of heat stress tolerance and for providing genetic resources for the development of heat tolerant cultivars.

INTRODUCTION

In tropical and sub-tropical regions, heat stress may become a major limiting factor for crop production. In agricultural crops, rising temperatures may lead to changed geographical distribution and growing season. Thus, climate change can have dramatic effects on agricultural production. The ability to adjust to the effects of climate change will be a key adaptive measure in the agricultural sector.
High temperature stress has several major effects on reproductive tissues that contribute to poor seed set and yield. Reproductive development has been affected more adversely at high temperature stress than the vegetative development (Sato et al., 2002; Abdelmajeed et al., 2003). Successful fertilization is a key factor for many agricultural crop species. In tomato, reproductive phase is a good candidate to be affected by climate change. The intensity of heat stress during gamete development affects plant reproduction with immediate and long-term effects. Among the different aspects related to pollen fertility and functioning, the relevance of the carbohydrates metabolism has been demonstrated by a number of studies.

Environmental stresses were shown, in a number of experimental systems, to affect sugar metabolism in the anther and in developing pollen. Normal starch accumulation during pollen development was inhibited in the anthers of water stressed rice plants causing male sterility (Sheoran and Saini, 1996). Similar metabolic changes were observed in water-stressed wheat (Dorion et al., 1996). A significant reduction was found in the number of pollen grains per flower and a reduction in their viability and germination in tomatoes grown under chronic high-temperature conditions (Pressman et al., 2002).

The initial period of pollen tube growth is considered autotrophic, when the pollen tube would use the reserves stored in the pollen grain (Shivanna, 2003). A range of cytosolic and plastidial carbohydrates were found among pollen reserves, from polysaccharides to small soluble molecules (Pacini, 1996). Starch and sucrose are important pollen reserves, whose presence and amount can vary among species (Baker and Baker, 1979; Singh et al., 1978; Speranza et al., 1997). Especially in perennials, the male gametophytic function can vary naturally along the plant life (Bellani et al., 1985a and Bellani et al., 1985b). However, probably in view of the ease in handling, most of the work has centered on the male functional gametophytic development. Thus, high temperatures affects the quantity and morphology of pollen, anther dehiscence and pollen wall architecture, as well as the chemical composition and metabolism of pollen (Aloni et al., 2001; Prasad et al., 2002 and Koti et al., 2005). Biosynthesis of starch and soluble sugar in pollen grains during the final phases of pollen maturation is critical in determining pollen quality not only because starch is a reserve source of energy for pollen germination but it may also serves as a checkpoint of pollen maturity. In dicots, such as tomato, starch accumulation peaks at 3 days before anthesis. The pollen grains of tomato plants (Solanum lycopersicum L.) can be considered starchless at maturity, because most of the starch is converted to soluble sugar at anthesis (Carrizo et al., 2010) as in several other tomato cultivars (Pressman et al., 2002 and Firon et al., 2006) but their content of soluble carbohydrates is peculiar. In fact, reducing sugars predominate, whereas sucrose can be absent or in a very low amount; the regular presence of maltosaccharides is also interesting (Carrizo et al., 2009 and Carrizo et al., 2010). The non-viability and germination of pollen grains were also associated with a marked reduction in starch and soluble sugar content in the developing pollen grains before anthesis and at the final phase of pollen maturity.

High temperatures during reproductive development have been reported to limit the flower bud initiation with significant increment in flower drop (Hanna and Hernandez, 1982) and significant decrease in fruit set (Berry and Rafique, 1988), leading to a sharp decrease in tomato fruit yield. The importance of carbohydrates in pollen grain development, in particular the role assigned to starch content before anthesis and soluble sugar content at anthesis, can be examined by studying pollen viability, pollen germination, and fruit set under high temperature conditions in tomato genotypes. The aims of the present work were to determine the starch content in pollen grains before anthesis and soluble sugar content in pollen grains at anthesis, and to analyze the possible relationships between starch and soluble sugar content in pollen grains, along with the ability to fruit set in tomato genotypes. The studies of the carbohydrate metabolism in pollen grains have been generally done in standardized conditions, and eventual natural variations have been overlooked. Regarding soluble carbohydrates, even if they can be
variable among species (Aloni et al., 2001 and Castro and Clement, 2007) the soluble carbohydrates content in the pollen grains of a species is constant, and it has not yet been investigated if it can fluctuate commensurate with the high temperature stress. To address this question, pollen samples were taken along 2 temperature regimes in 4 tomato genotypes (heat tolerant and heat susceptible) to describe its starch and soluble sugar content in pollen grains and to define any degree of variation.

**MATERIALS AND METHODS**

**Plant material, growth conditions and heat stress application**

The observations on effects of heat stress were made in the 4 tomato (Solanum lycopersicum L.) genotypes. The experiment was conducted in 2 consecutive years, 2010 and 2011 in Plant Abiotic Stress Laboratory, Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. Seeds of 4 contrasting tomato genotypes, including Pusa Sadabahar, NDTV-60, H-86 and Floradade (relatively heat tolerant and heat susceptible), respectively, were obtained from the Department of Genetics and Plant Breeding. Three-week old seedlings (2 seedlings per pot) were transplanted in small plastic pots (15 cm top diameter) filled with a mixture of sand, vermiculate, FYM (2:1:1), while upon establishment were thinned to 1 per pot. Proper care was taken by watering them regularly and spraying with 1 g composite fertilizer per liter of water (containing N, P, K in the ratio of 18:18:18) was applied to each plant at fortnight interval. The pots were arranged in completely randomized design in 2 sets and each set consisted of 12 pots kept in a plant growth chamber under 2 temperature regimes, designated as optimum temperature (OT, 28/21 °C day/night) and high temperature (HT, 37/27 °C day/night) to be imposed 15 days after transplanting to the plant growth chambers (3m x 3m) under a 14/10 hours day/night cycle and 85% humidity.

**Isolation of pollen grains for starch and sugar analysis**

The sampling was done during the peak of flowering, each sample contained pollen from 1 to 2 days, obtained from several flowers (10–50). The quantification of starch and soluble sugar in pollen grains will be referred to the pollen fresh weight before anthesis and at anthesis. Anthers collected from at least 10 flowers were dissected according to (Aouli et al., 2001). Pollen grains were obtained by slicing the anthers transversely and vortexing them in cold, sucrose-free germination solution. The solution was then filtered through cheesecloth to remove the anther walls, and pollen grains were separated by centrifugation for 10 minutes at 8000 g. The released pollen grains were immediately suspended in 80% ethanol at 75 °C for 30 min. The carbohydrate concentrations in pollen samples were analyzed according to (Hubbard et al., 1990 and Stoop and Pharr, 1994). Freeze-dried or ethanol-suspended samples were extracted 3 times in hot 80% (v/v) ethanol. The supernatant was dried in vacuo at 40 °C and resolubilized in water. Soluble sugars were determined by HPLC with a Fast Carbohydrate column (Bio-Rad, Hercules, CA, USA) operated at 85 °C with deionized, degassed water as eluent and detection by a refractometer. The insoluble residue that remained after ethanolic extraction was resuspended in 2 ml of 30 mM HCl and boiled for 30 minutes. After cooling, the pH was adjusted to 4.5 with KOH. The gelatinized starch was digested for 60 minutes at 50 °C with ∼36 units of amylglucosidase from Aspergillus oryzae (Hubbard et al., 1990). The reaction mixture was incubated at 25 °C for 30 minutes and absorbance at 340 nm was measured.

**Determination of pollen viability, pollen germination and fruit set**

Pollen viability, pollen germination, and fruit set were assessed under controlled conditions. Percentage of pollen viability was tested a day before anthesis. Ten flower buds were collected from 3 plants per genotype. PolLens were removed from the anthers by using a needle. The pollen grains were inoculated on glass slide for
determining the number of viable pollen grains through triphenyl tetrazolium chloride (TTC) test as per (Eti, 1991). Mixed pollens were used each time, counting stained pollen over 300 (3 x 100). The percentage of viable pollens was more or less constant along each temperature regime; therefore average values have been shown. Pollen germination was tested following the Bedinger’s liquid tomato pollen germination media protocol (available online at http://www.irbtomato.org).

Flowers were collected from 3 plants per genotype at anthesis. Pollen grains, collected from the anthers using a needle from 10 randomly selected flowers, were put on a slide and mixed using a nylon hairbrush. After mixing, pollen grains were immediately transferred within 30 minutes of picking the flowers on to the growth medium tube. Investigation on pollen germination and pollen tube growth was conducted by placing the pollens on a slide with the germinating media. A pollen grain was considered to have germinated when the length of the germinated pollen tube was equal to or longer than the diameter of the pollen as suggested by (Luza et al., 1987). Counts on pollen germination were made randomly in 3 replications under a microscope and average results were expressed in percentage. Fruit set was also expressed in percentage by counting the total number of flowers as well as total number of fruits per plant. Experimental data were subjected to analysis of variance (ANOVA) for factorial complete randomized block design with genotypes and temperatures as factors were carried out for each parameter measured. Significance was tested by variance ratio (~F value) at 5% and least significant difference (LSD) was worked out for comparing means for all parameters.

RESULTS

High temperature (HT) conditions (37/27 °C) markedly reduced the starch content in pollen grains before anthesis in all tomato genotypes data were presented (Table 1) for optimum and high temperature regimes for all traits. The reduction in starch content was found maximum in heat sensitive tomato genotype Floradade (68.4%) followed by H-86 (65.4%) and minimum was found in heat tolerant tomato genotype Pusa Sadabahar (3.7%), and NDTV R-60 (3.8%) as compared to optimum temperature (OT) (27/21 °C). High temperature (HT) (37/27 °C) also significantly reduced the soluble sugar content in pollen grains of heat sensitive genotypes H-86 (48.6%) and Floradade (47.1%) at anthesis but little increments were obtained in both heat tolerant genotypes Pusa Sadabahar (-4.2%), and NDTV R-60 (-4.2%). The highest reduction was noticed in H-86 (48.6%) and Floradade (47.1%), respectively and minimum reduction was observed in Pusa Sadabahar (-4.2 %) and NDTV R-60 (-4.2%) in comparison to optimum temperature (OT) (27/21 °C). The highest reduction of pollen viability and pollen germination was found in heat sensitive genotype Floradade (87.8 and 85.1%) and H-86 (75.3 and 31.1%) respectively (Table 2). The small reduction in pollen viability and pollen germination values was observed in genotype Pusa Sadabahar (23.7 and 21.0%) and NDTV R-60 (52.2 and 51.9%), respectively, being the less affected to high temperature, appeared more heat tolerant. Heat stress also markedly reduced the number of fruit set in all 4 genotypes in comparison to optimum temperature. The highest reduction of fruit set was found in Floradade (76.1%) and H-86 (82.0%). The lowest reduction was observed in Pusa Sadabahar (27.9%) and NDTV R-60 (35.7%). However, genotype Pusa Sadabahar set more fruits (52.7%) at high temperature. Statistically significant differences in number of fruit set were observed among all genotypes in optimum temperature and high temperature conditions.

DISCUSSION

The effects of heat stress on reproductive development and fruit set were investigated in heat-tolerant and heat sensitive tomato genotypes that were grown under controlled environmental conditions with 2 temperature regimes, optimum temperature and high temperature. It was found that heat stress had statistically significant effects on pollen
development, fruit set and all examined genotypes showed significant variation. Our findings showed that high temperature strongly disturbed the carbohydrate metabolism in pollen grains of tomato genotypes. The elevated temperature caused reduction in starch content in pollen grains at before anthesis in all evaluated genotypes. Higher reduction in starch content was observed in sensitive genotypes as compared to tolerant genotypes prior to anthesis, developing pollen grains and anthers accumulate starch temporarily, but a moderate temperature increase reduces starch concentration in developing pollen grains, and pollen viability decreases (Sheoran and Saini, 1996). Heat stress may limit starch accumulation in pollen grains, either by reducing the availability of assimilates or by inhibiting the activities of enzymes in relation to biosynthesis of starch. The assimilate-transport of carbon to the trusses and apex, assimilate-import by the flower buds, and conversion of carbon to starch were inhibited in heat-sensitive and heat-tolerant cultivars, and that the inhibition was stronger in the heat-sensitive one (Dinar and Rudich, 1985a; Dinar and Rudich, 1985b and Lalonde et al., 1997). Reduced starch deposition in water-stressed wheat pollen could be the result of a lower expression of ADP glucose pyrophosphorylase (Lalonde et al., 1997). Under high temperature, the strong reason for decreased starch content in pollen grains at before anthesis is still not known. Soluble sugar content in pollen grains was also influenced by heat stress at anthesis stage. A smaller increment in soluble sugar content was found in both heat-tolerant

Table 1. Starch and soluble sugar content in pollen grains before anthesis and at anthesis respectively, of tomato genotypes (Pooled data of 2010 and 2011).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Starch content (mg g⁻¹ fr. wt.)</th>
<th>Soluble sugar content (mg g⁻¹ fr. wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OT</td>
<td>HT</td>
</tr>
<tr>
<td>PUSA SADABAHAR</td>
<td>275.3</td>
<td>265.2</td>
</tr>
<tr>
<td>NDTVR-60</td>
<td>283.1</td>
<td>272.3</td>
</tr>
<tr>
<td>H-86</td>
<td>285.2</td>
<td>98.7</td>
</tr>
<tr>
<td>FLORADADE</td>
<td>280.8</td>
<td>88.8</td>
</tr>
</tbody>
</table>

SEM + LSD (P = 0.05)

G 2.5 7.5
T 1.8 5.3
G x T 3.5 10.6

OT = Optimum temperature, HT = High temperature

Table 2. Percent pollen viability, pollen germination and fruit set in tomato genotypes (Pooled data of 2010 and 2011).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Pollen viability %</th>
<th>Pollen germination %</th>
<th>Fruit set %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OT</td>
<td>HT</td>
<td>% Reduction</td>
</tr>
<tr>
<td>PUSA SADABAHAR</td>
<td>88.4</td>
<td>67.4</td>
<td>23.7</td>
</tr>
<tr>
<td>NDTVR-60</td>
<td>81.8</td>
<td>39.1</td>
<td>52.2</td>
</tr>
<tr>
<td>H-86</td>
<td>90.0</td>
<td>22.2</td>
<td>75.3</td>
</tr>
<tr>
<td>FLORADADE</td>
<td>93.5</td>
<td>11.4</td>
<td>87.8</td>
</tr>
</tbody>
</table>

SEM + LSD (P = 0.05)

G 0.8 2.3
T 0.5 1.6
G x T 1.1 3.3

OT = Optimum temperature, HT = High temperature
genotypes but higher reduction was found in heat-sensitive genotypes. It was reported that during germination, pollen grains depend on simple sugar as metabolic substrates for their germination (Stanley, 1971). It was also demonstrated that the adverse effects of heat stress on quality of tomato pollen grains were associated with impairment of starch accumulation in the pollen grains and a consequent decrease in soluble sugar content in the mature pollen (Pressman et al., 2002). The ability of pollen grains of heat-tolerant genotypes to accumulate more starch before anthesis and soluble sugar at anthesis provides the pollen grains with an instant energy source for their germination. The data obtained in present study indicate that starch and soluble sugar content in pollen grains influence the viability of pollen grains, pollen germination and finally caused a mark reduction in fruit set in both heat-tolerant and heat-sensitive tomato genotypes. Differences between cultivars in pollen viability and pollen germination under heat stress have been shown to be the most important factors in determining their ability to set fruit (Srivastava et al., 2012). Therefore, it was concluded that at least these 2 important features make the pollen grains of heat-tolerant genotypes better able to function under heat stress conditions. In order to breed more heat-tolerant tomatoes it would be useful to single out the factors that mostly limit the ability to set fruit (Sato et al., 2000). The results of present investigation emphasize the use of starch and soluble sugar content in pollen grains as the most important biochemical parameter for screening of tomato genotypes for improved fruit set in heat stress conditions and Pusa Sadabahar and NDTVR-60 tomato genotypes can be a source of heat tolerance in further breeding programs.

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REFERENCES


