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The Relationship Between Yield and the Antioxidant Defense System in Tomatoes Grown Under Heat Stress

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Four putative heat-tolerant tomato (*Lycopersicum esculentum*) cultivars (Tamasabro, Heat Wave, LHT-24, and Solar Set) and one putative heat-sensitive tomato cultivar (Floradade) were grown in the field under non-stress (average daily temperature of 26°C) and heat-stress (average daily temperature of 34°C) conditions. At anthesis, approximately five weeks after being transplanted to the field, leaf samples were collected for antioxidant analyses. Yield was determined by harvesting ripe fruit seven weeks after the collection of leaf samples. Heat stress resulted in a 79.1% decrease in yield for the heat-sensitive Floradade, while the fruit yield in the heat-tolerant cultivars Heat Wave, LHT-24, Solar Set, and Tamasabro was reduced 51.5%, 22.1%, 43.8%, and 34.8% respectively. When grown under heat stress, antioxidant activities were also greater in the heat-tolerant cultivars. Superoxide dismutase (SOD) activity increased up to 9-fold in the heat-tolerant cultivars but decreased 83.1% in the heat-sensitive Floradade. Catalase, peroxidase, and ascorbate peroxidase activity increased significantly in all cultivars. Only Heat Wave showed a significant increase in glutathione reductase in response to heat stress but all heat-tolerant cultivars exhibited significantly lower oxidized ascorbate/reduced ascorbate ratios, greater reduced glutathione/oxidized glutathione ratios, and greater α-tocopherol concentrations compared to the heat-sensitive cultivar Floridade. These data indicate that the more heat-tolerant cultivars had an enhanced capacity for scavenging active oxygen species and a more active ascorbate-glutathione cycle and suggest a strong correlation between the ability to up-regulate the antioxidant defense system and the ability of tomatoes to produce greater yields when grown under heat stress.

Keywords: Heat stress, oxidative stress, tomatoes, antioxidant enzymes, antioxidant scavengers

INTRODUCTION

In plants, it is well documented that levels of cytotoxic activated oxygen species such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\cdot}OH$) increase during periods of environmental stress.[1,2,3,4,5,6,7,8,9,10] This phenomenon is particularly critical in plants because photosynthetic tissues are oxygenic so internal oxygen concentrations are maintained at high levels. Leakage from the electron transport chain

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within the chloroplast is a major source of electrons which may react with molecular oxygen to form the reactive species. The extent of this leakage is illustrated by Asada and Takahashi's[11] estimate that approximately 1% of the total oxygen consumed by plants becomes reactive. Hence, the process of photosynthesis can be viewed as having serious repercussions since large amounts of oxygen are produced in the immediate vicinity of a powerful oxidation-reduction system that can immediately reduce oxygen to the superoxide free radical.[12]

A number of antioxidants serve as the first line of defense against the potentially cytotoxic active oxygen metabolites. Superoxide dismutase (SOD) is a major scavenger of $O_2^{\cdot-}$, and its enzymatic action results in the formation of H_2O_2. Catalase, ascorbate peroxidase[13] (AP) and a variety of non-specific peroxidases[14] catalyze the breakdown of H_2O_2. In an ascorbate-glutathione cycle, the enzymatic action of AP produces the monodehydroascorbate radical (MDAR) which can dismutate spontaneously or be enzymatically reduced to dehydroascorbate (DAsA) by NADPH -dependent MDAR reductase.[15] DAsA can then be reduced to ascorbate (AsA) by reduced glutathione (GSH) either non-enzymatically or enzymatically in a reaction mediated by DAsA reductase.[8] The resulting oxidized glutathione (GSSG) is then converted back to the reduced form by NADPH-dependent glutathione reductase (GR).[16] Under normal conditions, reactive oxygen species are efficiently decomposed via these antioxidant systems. However, under abnormal environmental conditions the production of active oxygen metabolites may exceed the antioxidant capacity. Many environmental perturbations have been linked to the excess production of these activated oxygen species, but plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to active oxygen-mediated damage.[1,3,4,5,6,17,18,19,20]

The devastating effect of high temperature on crop plants is well documented. Under heat stress, C_3 plants close stomates in an attempt to conserve water. Stomatal closure reduces carbon dioxide levels within the plant cells resulting in increased photorespiration.[21] It has been frequently reported that changes in activities of ribulose 1,5-bisphosphate carboxyoygenase (Rubisco), other Calvin cycle enzymes, or electron transport rates occur with elevated temperatures.[22,23,24] Additional studies employing simultaneous measurements of chlorophyll *a* fluorescence and CO_2 exchange indicate that the ratio of electron transport to CO_2 increases with rising temperature.[25] Other studies have shown that high temperatures disrupt membrane structure and associated functions.[21,26,27] This high temperature-associated membrane damage leads to the loss of plasma membrane integrity and subsequently causes leakage of solutes and other intracellular components.[28,29,30] In addition, it has been shown that high temperatures cause injury to chloroplast envelope membranes and a loss of water from the chloroplasts in maple leaves.[31] It has also been established that elevated temperatures can completely arrest the photosynthetic apparatus. The first step in the inactivation of the photosynthetic apparatus by elevated temperatures is with the blockage of photosystem II's reaction centers. This leads to a dissociation of antennae pigment protein complexes from the central core of the light harvesting apparatus of photosystem II.[32,33,34] The dissociation of the antennae pigment protein complexes is thought to be related to the phase separation of non-bilayer-forming lipids in the thylakoid membranes.[34] These latter findings could have been due to increased lipid peroxidation resulting from the presence of excessive reactive oxygen metabolites.

Rabinowitch and Fridovitch[2] have shown that the susceptibility of tomatoes to sun scald resulting from a combination of heat and high light intensity is correlated with SOD activity, but to date, the relationship between the antioxidant defense system and reduction in yield due to heat stress has not been thoroughly examined in tomatoes (*Lycopersicum esculentum*). As with most

crops, there is variation in the capacity to tolerate heat stress among various tomato cultivars, and the purpose of this research was to examine the relationship between heat stress, yield, and antioxidant levels in putative heat-tolerant and heat-sensitive cultivars of tomatoes.

MATERIALS AND METHODS

Growth And Harvest of Tomatoes

Both a spring (unstressed) and a summer (heat-stressed) crop of the tomato cultivars Tamasabro, Heatwave, LHT-24, Solar Set, and Floradade were grown in the field at the Louisiana State University Agricultural Center's Red River Research Station in Bossier City, Louisiana. Heatwave, LHT-24, and Solar Set are putative heat-tolerant cultivars. Floradade is a putative heat-sensitive cultivar. The heat tolerance of Tamasabro, a supposedly heat-tolerant cultivar developed in Japan, has not previously been tested in the United States. For each crop, six-week-old seedlings of each cultivar were transplanted in 5 × 75-ft plots with an in-row spacing of 18 inches. Two weeks prior to transplanting, preplant fertilizer consisting of 80N-105P-199K lb/acre was broadcast and incorporated into the soil of each plot. A black polyethylene mulch was applied to the beds in tests conducted under optimal temperature (spring), and white on-black polyethylene mulch was applied to the tests conducted under above-optimal temperature (summer). Plants were staked and tied using the weave and tie system. Drip irrigation was applied as needed based on tensiometer readings of –10 to 15 centibars.[35] The spring crop of cultivars was transplanted to the field on 4-6-94 and the tomatoes were harvested on 7-5-94 while being exposed to average daily temperature of 26°C. The summer crop of cultivars were transplanted to the field on 6-16-94 and harvested on 8-30-94. This crop experienced an average daily high temperature of 34°C throughout its growth period. At anthesis, approximately five weeks after transplanting, four samples per cultivar from each crop were harvested and placed in labeled bags and immediately frozen at –70°C for later antioxidant analyses. For each crop, ripe fruit was harvested for seven weeks and fruit yield for each cultivar was calculated on the basis of total weight of all fruit sizes.

Antioxidant Analyses

Samples were prepared for catalase, peroxidase, and GR analyses by a modification of the method outlined by Foster and Hess.[36] One g of frozen leaf tissue was homogenized in 3 mL of an ice-cold solution containing 100 mM Tris (pH 7.0), 10 mM D-isoascorbic acid, 2% (w/v) PVP-10, 0.1 mM EDTA, 0.2% (v/v) Triton X-100 and 1 drop of antifoam A emulsion. The homogenate was then centrifuged at 4°C for 20 min at 15,000g. One mL of the supernatant was centrifuge-desalted through a 10 mL bed of Sephadex G50-300.[37] A portion of the eluent was analyzed immediately for catalase activity, and the remainder was stored at –70°C for subsequent analysis of GR and peroxidase activities. AP was extracted by homogenizing 1 g of the frozen leaf tissue in 3 mL of an ice-cold solution containing 50 mM Pipes buffer (pH 6.8), 6 mM L-cysteine hydrochloride, 10 mM D-isoascorbate, 1 mM EDTA, 0.3% (v/v) Triton X-100, 1% (w/v) PVP-10 and 1 drop of antifoam A emulsion.[38] The homogenate was centrifuged at 4°C for 20 min at 15,000g, and 1 mL of the supernatant was centrifuge-desalted following the procedure outlined by Anderson *et al.*[38] Ascorbate was extracted by homogenizing 1 g of frozen leaf tissue in 1 mL of 6% (w/v) m-phosphoric acid (pH 2.8) containing 1 mM EDTA.[38] After centrifugation at 20,000g for 15 min, the supernatant was removed, filtered through a 45 µm ultrafilter, and stored at –70°C prior to analysis. Samples were prepared for glutathione, cysteine, and cystine analyses by homogenizing 1 g of frozen tissue in 1 mL of 5% (w/v) TCA. The homogenate was centrifuged for

15 min at 20,000g, and after the supernatant was removed and filtered through a 45 m. ultrafilter, the sample was stored at –70°C for subsequent glutathione analysis. Samples were prepared for α-tocopherol analysis by homogenizing 10 g of frozen leaf material in 30 ml of absolute ethanol and 10 ml of 250 grams per liter of Na-ascorbate. After centrifugation for 10 minutes at 2,500g, the supernatant was removed, partitioned by adding 10 ml of petroleum ether and recentrifuged for 10 minutes at 1500 × g. The upper petroleum ether phase was removed, evaporated to dryness, and the residue dissolved in 0.7 ml of methanol.

Catalase activity was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 1.9 mL H_2O, 1 mL 0.059 M H_2O_2 in 50 mM KPO_4 buffer (pH 7.0), and 0.1 mL extract.[39] Total SOD activity was measured by determining the amount of enzyme required to produce 50% inhibition of the reduction of cytochrome C by superoxide generated by xanthine oxidase in a reaction mixture consisting of 900 μL SOD cocktail which contained 0.1 mM EDTA, 0.01 mM ferricytochrome C, and 0.05 mM xanthine in 50 mM KPO_4 buffer (pH 7.8); 50 μL catalase at 0.05 units/mL; 20 to 50 μL extract; and 50 μL xanthine oxidase at 0.05 units/mL.[40] GR activity was determined by monitoring the glutathione-dependent oxidation of NADPH at 340 nm in a reaction mixture containing 950 μL of 0.15 mM NADPH, 0.5 mM GSSG, and 3 mM $MgCl_2$ in 50 mM Tris (pH 7.5) and 50 μL extract.[41] Peroxidase activity was measured by monitoring at 675 nm the H_2O_2-dependent oxidation of reduced 2,3',6-trichloroindophenol in a reaction mixture containing 950 μL of 120 mM H_2O_2, 17 mM $Na_2S_2O_3$, and 0.3 mM 2,3',6-trichloroindophenol in 40 mM $NaPO_4$ (pH 6.0), and 50 μL of extract[42] AP activity was assayed by monitoring the ascorbic acid-dependent reduction of HPLC method of Reed *et al.*[45] S-carboxymethylated thiol derivatives were prepared by adding 100 μL of 40 mM iodoacetic acid to 500 μL of acid extract and adjusting the pH to 7.0 with 1M K_2CO_3. After 1 hour, 500 μL of 1.5% (v/v) 1-fluoro-2,4 dinitrobenzene (FDNB) in absolute ethanol was added to the sample, the pH was adjusted to greater than 10 with 1M K_2CO_3, and the reaction mixture was allowed to sit in the dark at room temperature for 4 h to form the S-carboxymethyl, FDNB derivatives. The derivatized samples were wrapped in foil and stored at 4°C for subsequent separation and analysis. Prior to injection, precipitates were removed by centrifugation. Separation was accomplished using a 4.1 × 250 Versa-Pack amine column (AllTeck #28142) protected by a Bond-Pak amine precolumn insert (Waters #26260) and a gradient solvent system delivered at a flow rate of 1 mL/min. At the beginning of each run, the solvent system consisted of 75% solvent A (80% v/v methanol in water) and 25% solvent B (4 M sodium acetate containing 64% v/v methanol in water at pH 4.5). After 10 min, the gradient was changed linearly until it reached a ratio of 5% solvent A and 95% solvent B 50 min into the run. The gradient was then immediately shifted back to 75% solvent A and 25% solvent B and allowed to equilibrate for 10 min before the next injection. GSH, GSSG, cysteine, and cystine contents were determined by measuring the absorbance of the s-carboxyl methyl, DNP derivatives at 365 nm. Peaks were calculated using the Waters Millineum software and a standard curve based on derivatives prepared from GSH, GSSG, cysteine hydrochloride, and cystine at concentrations ranging from 20 to 200 μg/mL. Total glutathione values were determined from the sum of the GSH and GSSG values. The glutathione values are expressed as μg/g fresh weight. α-Tocopherol was determined by the HPLC method of Spychalla and Desborough.[6] α-Tocopherol separation was accomplished by employing a 3.9 × 150 mm Nova-Pak C_{18} octadecyl reverse-phase column (Waters #86344) protected by a Nova-Pak C_{18} precolumn insert (Waters #15220), and α-tocopherol content was measured at 295 nm. The solvent system consisted of 95% (v/v) methanol in water delivered at 2 ml/minute, and peak

areas were calculated using the Waters Millenium software. A standard curve was prepared with α-tocopherol at concentrations ranging from 25 to 400 μg/ml in 100% methanol. Sample volume was 200 μl. The α-tocopherol data is expressed in μg/g fresh weight.

Statistical Analyses

A one way analysis of variance was used to analyze statistical differences among cultivars within a treatment (*i.e.* the Spring crop under no heat stress or the Summer crop under heat stress), and the significant differences between a cultivar grown under no stress and the same cultivar grown under heat stress were determined by Student's *t* test. Data are presented as means ± SE (n = 4). Significance is expressed at the 95% confidence level.

RESULTS

In the unstressed spring crop, the putative heat-sensitive cultivar, Floradade, produced the third highest yield with two of the putative heat-tolerant cultivars (Heat Wave and Solar Set) producing slightly more Kg of fruit per plant and two (LHT-24 and Tamasabro) producing slightly less (Fig. 1). In the heat-stressed summer crop, all four heat-tolerant cultivars produced greater yields than Floradade. Although heat stress reduced total fruit yield in all cultivars, reduction in yield among the heat-tolerant cultivars was less severe than in the heat-sensitive cultivars (Fig. 1). Total fruit yield in the heat-tolerant cultivars Heat Wave, LHT-24, Solar Set and Tamasabro was reduced 54.4%, 22.1%, 43.8%, 34.8% respectively. In contrast, yield in the heat-sensitive cultivar Floradade was reduced 79.1%.

The antioxidant enzyme activities of the different cultivars grown in the spring and summer are presented in Figure 2. In the unstressed spring crop, there were significant differences in constitutive SOD activities among the cultivars. SOD activity was significantly greater in LHT-24 and Solar Set than in Heat Wave, while the SOD activities in Tamasabro and Floradade fell in between the two extremes. Heat stress resulted in a significant 9-fold increase in SOD activity in Heat Wave and a 83.1% decrease in activity in Floradade. SOD activity did not differ significantly in the other cultivars when grown under heat-stress conditions. In the summer crop, Heat Wave had more than twice the SOD activity of any other cultivar, and of particular note is the fact that Floradade exhibited the lowest SOD activity.

Examination of the enzymes that break down the H_2O_2 generated by SOD revealed that there

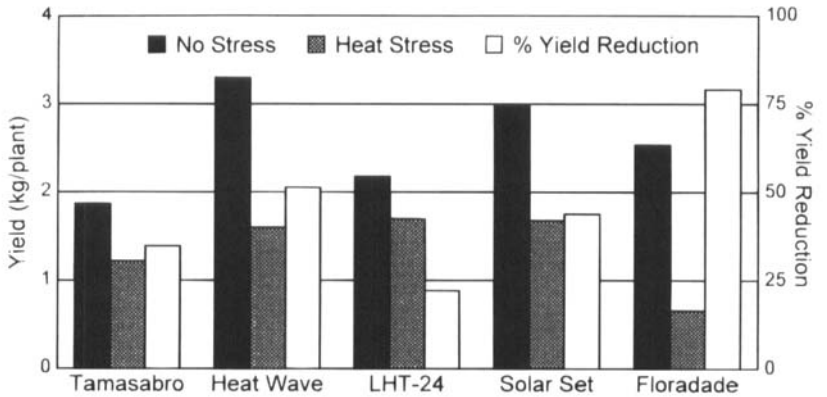

FIGURE 1 Yield (kg/plant) and yield reduction of Tamasabro, Heat Wave, LHT-24, Solar Set and Floradade grown in the field under no stress and heat-stress conditions.

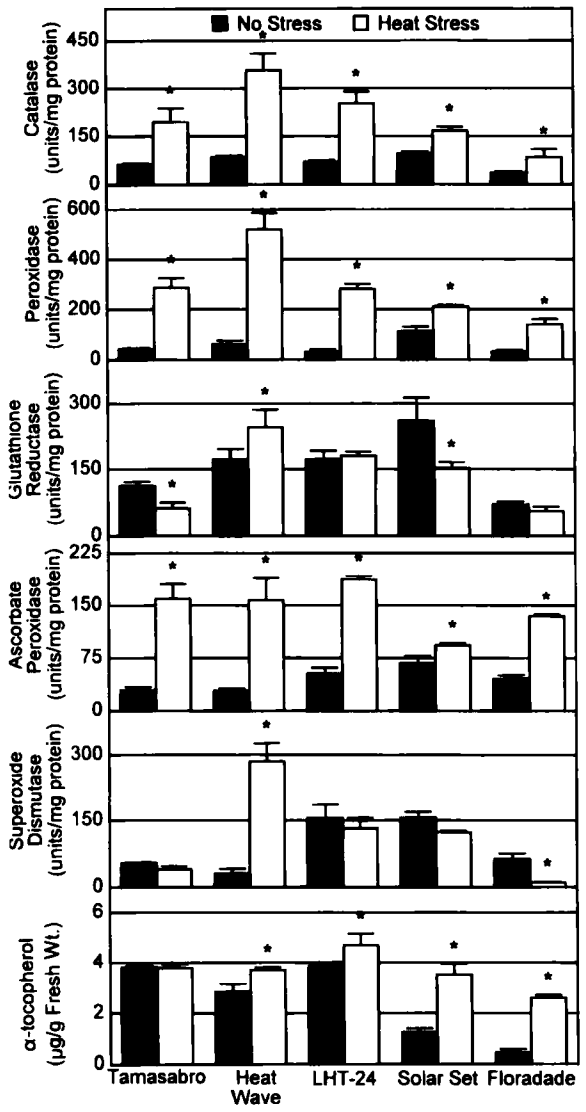

FIGURE 2 Catalase, peroxidase, glutathione reducatase, ascorbate peroxidase, and superoxide dismutase activities ± SE and α-tocopherol concentrations ± SE in Tamasabro, Heat Wave, LHT-24, Solar Set, and Floradade grown under no stress and heat stress. (* denotes significant differences between cultivars grown under heat stress conditions as compared to those same cultivars grown under no stress).

were no significant differences in catalase or peroxidase activity among the cultivars grown during the spring (Fig. 2). When grown under heat stress during the summer, catalase activity increased significantly in all cultivars. This increase ranged from 175% in Solar Set to 419% in Heat Wave. Catalase activity was significantly greater, (183%, 213%, and 423% respectively) in Heat Wave than in Tamasabro, Solar Set, or Floradade, and significantly greater in LHT-24 than in Floradade and Solar Set. Floradade exhibited significantly lower catalase activity than all other cultivars grown under heat-stress conditions. Peroxidase activity in the different cultivars resembled catalase activity in that there was no significant differences among cultivars grown under nonstress conditions (Fig. 2). Under heat stress, peroxidase activity increased 2- to 9-fold in all cultivars. Compared to all other cultivars, peroxidase activity was significantly greater in Heat Wave and significantly lower in Floradade.

Constitutive levels of AP in Solar Set were significantly greater than all cultivars grown in the spring except LHT-24 (Fig. 2). When grown under heat stress, AP activity increased significantly in all cultivars. This increase ranged from 38% in Solar Set to 453% in Heat Wave. Significant differences in GR activity were observed among unstressed cultivars grown during the spring (Fig. 2). GR activity in Solar Set and Floradade were significantly greater and lower, respectively, than all other cultivars. In response to heat stress, GR activity decreased significantly in Tamasabro and Solar Set, but increased significantly in Heatwave. LHT-24 and Floradade showed no significant change. There were also significant differences in GR activity among the cultivars grown during the summer. Under heat stress, GR activity was significantly greater in Heat Wave than all other cultivars, while Tamasabro and Floradade were significantly lower.

In the spring crop there were also significant differences in α-tocopherol concentrations among cultivars (Fig. 2). The constitutive levels of α-tocopherol in Tamasabro and LHT-24 were significantly greater than the other cultivars while levels in Floradade were significantly lower than the other cultivars. Under heat stress all cultivars

except Tamasabro exhibited significant increases in α-tocopherol.

Total ascorbate, reduced ascorbate (AsA), and oxidized ascorbate (DAsA) concentrations are shown in Figure 3. In the spring crop, constitutive total ascorbate, AsA, and DAsA concentrations did not differ significantly among cultivars. However, in response to heat stress, total ascorbate concentrations decreased significantly in all five cultivars and levels in Tamasabro fell significantly below the other cultivars. AsA concentrations increased significantly (121%–249%) in all cultivars in response to heat stress. In the summer crop, Solar Set exhibited significantly greater AsA concentrations than Tamasabro, LHT-24, and Floradade. A significant decrease in DAsA concentrations accompanied the increase in AsA in all five cultivars in response to heat stress. In the heat stressed plants, the DAsA concentration in Floradade was significantly greater than all other cultivars. LHT-24 also exhibited a DAsA content that was signficantly greater than Heat Wave and Tamasabro. The DAsA/AsA ratios were not significantly different among the cultivars grown during the spring, however, there was a significant decrease in the DAsA/AsA ratios of all five cultivars in response to heat stress. Under heat stress, Floradade showed a significantly greater DAsA/AsA ratio than all other cultivars. Hence, this cultivar had a greater

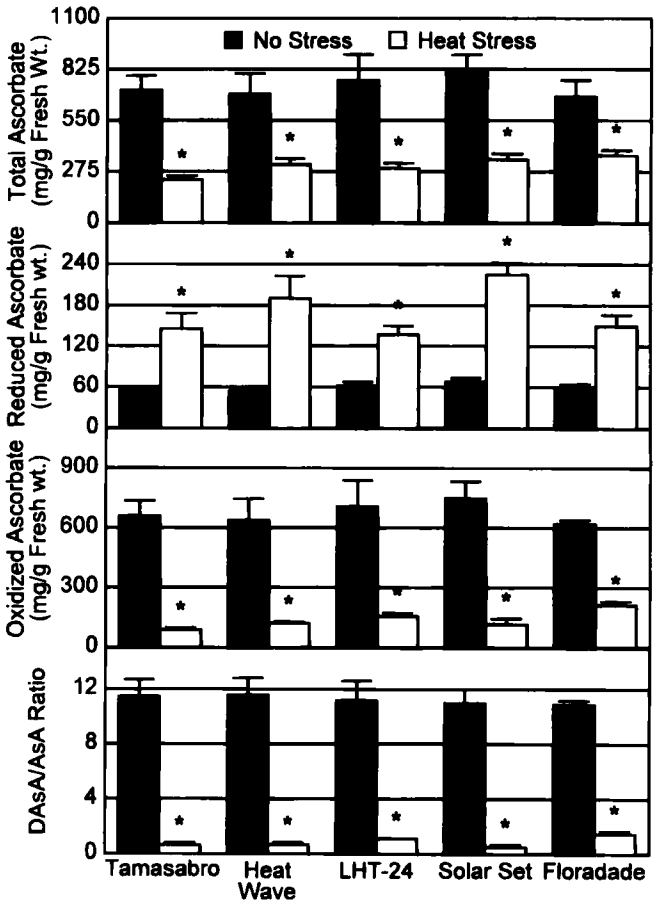

FIGURE 3 Total ascorbate, reduced ascorbate (AsA), oxidized ascorbate (DAsA), and the ratio of oxidized to reduced ascorbate ± SE for Tamasabro, Heat Wave, LHT-24, Solar Set and Floradade grown in the field under no stress and heat-stress conditions. (* denotes significant differences between cultivars grown under heat stress conditions as compared to those same cultivars grown under no stress).

percentage of available ascorbate present in the reduced form.

In the spring crop, there were significant differences in the concentrations of total glutathione among cultivars (Fig. 4). Levels in Solar Set were significantly greater than all cultivars except Tamasabro, while levels in Heat Wave were significantly lower than all other cultivars. Heat Wave, Solar Set, and Floradade showed significant increases in total glutathione when grown under heat-stress conditions, but there were no significant differences in total glutathione concentrations among cultivars in the summer crop. Significant 3- to 12-fold increases in GSH concentrations were observed in all five cultivars when grown under heat stress; however, there were no significant differences in GSH concentration among cultivars grown during the summer (Fig. 4). Only Tamasabro showed no significant increase in GSSG levels when grown in the summer (Figure 4). Under heat stress, Solar Set and Floradade had significantly greater GSSG concentrations than Tamasabro, LHT-24, and Heat Wave. In the nonstressed plants grown during the spring, the GSH/GSSG ratio was significantly lower in Heat Wave than in the other cultivars which exhibited very similar GSH/GSSG ratios (Fig. 4). Significant increases ranging from 25%

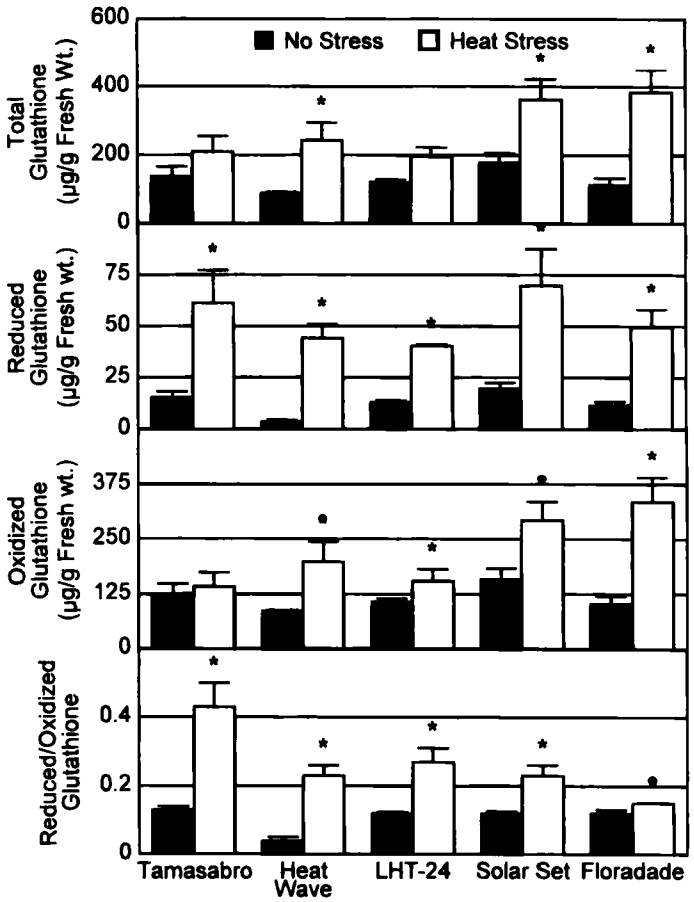

FIGURE 4 Total glutathione, reduced glutathione (GSH), oxidized glutathione (GSSG), and the ratio of oxidized to reduced glutathione ± SE for Tamasabro, Heat Wave, LHT-24, Solar Set and Floradade grown in the field under no stress and heat-stress conditions. (* denotes significant differences between cultivars grown under heat stress conditions as compared to those same cultivars grown under no stress).

for Floradade to 475% for Heat Wave were observed in the GSH/GSSG ratios for all cultivars grown under heat stress (Fig. 4). In the summer crop, Tamasabro exhibited the greatest GSH/GSSG ratio, Floradade exhibited the lowest, and the other cultivars ranged between the two extremes. Thus, the heat tolerant cultivars all had a significantly greater proportion of the total glutathione available as GSH than did the more heat-sensitive Floradade.

During the spring, levels of cystine and cysteine were significantly greater in Tamasabro than Heat Wave and LHT-24 (Fig. 5). Under these nonstress conditions, LHT-24 and Solar Set exhibited significantly greater cysteine/cystine ratios compared to Floradade. In the summer crop, both cystine and cysteine increased significantly in all cultivars, but the cysteine/cystine ratios remained relatively constant with no significant change in LHT-24 or Solar Set. Under heat stress, there were some significant differences in the concentrations of these compounds among the different cultivars. Solar Set and Floradade had significantly greater concentrations of cystine than Tamasabro, Heat Wave, and LHT-24. The cysteine concentration was significantly lower in Tamasabro than in either Solar Set or Floradade. Tamasabro also exhibited a slight, but significantly lower, cysteine/cystine ratio compared to all cultivars.

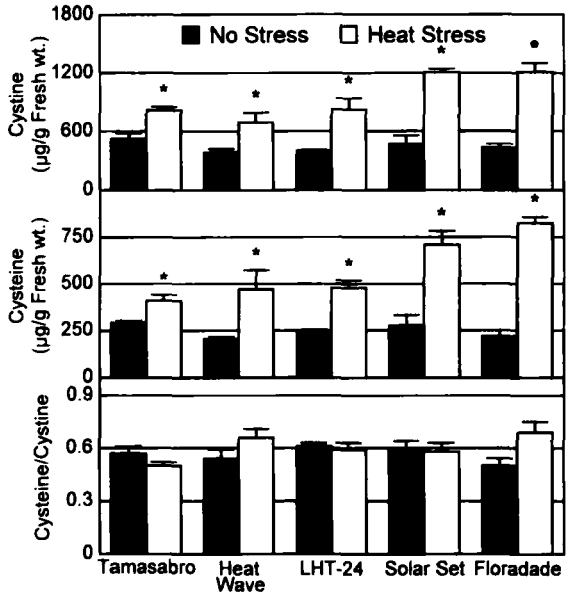

FIGURE 5 Cystine, cysteine, and the ratio of cysteine to cystine ± SE for Tamasabro, Heat Wave, LHT-24, Solar Set and Floradade grown in the field under no stress and heat-stress conditions. (* denotes significant differences between cultivars grown under heat stress conditions as compared to those same cultivars grown under no stress).

DISCUSSION

Since yields were reduced in all cultivars when grown in the summer as compared to being grown in the spring, it can be concluded that all five cultivars were susceptible to heat stress. When grown under conditions of heat stress, Heat Wave, LHT-24 and Solar Set similarly produced the highest yields. Floradade produced the lowest and Tamasabro produced yields between the high and the low. In addition, heat stress reduced the yields of the heat-tolerant cultivars substantially less than that of the heat-sensitive Floradade. Hence, it can also be concluded that Heatwave, LHT-24, Solar set, and Tamasabro were more heat tolerant than Floradade.

The degree of heat tolerance observed among the cultivars appeared to be correlated with antioxidant status. The lowest-yielding cultivar, Floradade, had the lowest antioxidant profile, while the highest-yielding cultivars, Heat Wave, LHT-24, and Solar Set exhibited considerably greater antioxidant profiles. In the summer crop, a heat-induced increase in SOD activity in Heat Wave and a heat-induced decrease in SOD activity in Floradade resulted in the three highest-yielding cultivars exhibiting the highest SOD activities and the lowest-yielding cultivar exhibiting the lowest SOD activity. The intermediate-yielding cultivar, Tamasabro, also exhibited an intermediate SOD activity. There were no significant differences in catalase or peroxidase activity among cultivars grown during the spring suggesting that all cultivars had similar capacities for the breakdown of

hydrogen peroxide when grown under non-stressed conditions. During the summer, however, catalase and peroxidase activity increased significantly in all cultivars. When grown under heat stress, the greater-yielding cultivars had the greatest catalase and peroxidase activities, and Floradade, the lowest yielding cultivar, exhibited the lowest activities. An elevation in SOD activity occurring without an accompanying increase in the enzymes needed to scavenge H_2O_2 can lead to increased cytotoxicity by the extremely destructive hydroxyl radical generated from H_2O_2 in a metal-catalyzed Haber-Weiss reaction.[46] The greater SOD activities observed among the heat-tolerant cultivars indicate there was an increased capacity to scavenge the superoxide free radical, and generate hydrogen peroxide. Although all cultivars exhibited significant heat-induced enhancement of catalase and peroxidase activity, activity of both enzymes was significantly greater in the relatively heat-tolerant cultivars compared to the heat-sensitive Floradade, indicating that the heat-tolerant cultivars had a greater capacity for the decomposition of H_2O_2 generated by SOD. Increases in SOD, catalase, and peroxidase have been correlated with resistance to other environmental stresses. SOD activity has also been shown to be elevated in paraquat-resistant tobacco[47] and maize,[48] chilling-resistant spinach,[49] and salt-tolerant cotton callus tissue.[19] Catalase activity has also been shown to be significantly greater in paraquat-tolerant lines of perennial ryegrass than in paraquat-sensitive lines[3] and in salt-tolerant cotton cultivars as compared to salt-sensitive cultivars.[18] Increased peroxidase activity has also been reported in paraquat-tolerant lines of ryegrass[3]and salt-tolerant cultivars of cotton.[18]

In the spring crop, there were no significant differences in total ascorbate, DAsA, or AsA concentrations, or the DAsA/AsA ratios among cultivars. However, under heat stress, AsA concentrations increased significantly in all cultivars, while DAsA concentrations and the DAsA/AsA ratios decreased significantly. Total ascorbate concentrations also decreased significantly in all cultivars due to the fact that DAsA concentrations decreased more than the AsA concentrations increased. These changes indicated a decrease in ascorbate oxidation and an increase in ascorbate reduction. Ascorbate peroxidase also increased significantly in all cultivars in response to heat stress. Ascorbate oxidation can be explained by two reactions other than that catalyzed by ascorbate-specific peroxidase. Univalent oxidation of ascorbate by the superoxide radical or the α-chromoxyl radical of oxidized α-tocopherol can result in the production of monodehydroascorbate[16] which is then converted, both nonenzymatically and enzymatically, to AsA and DasA. Ascorbate oxidation and α-tocopherol concentrations are closely related,[16] and α-tocopherol concentrations in both the spring and summer crop were significantly greater in the more heat-tolerant cultivars than in the heat-sensitive Floradade. These results indicate that the more heat-tolerant cultivars have an enhanced free radical scavenging capacity. The lower DAsA/AsA ratios in the heat-tolerant cultivars indicate that these cultivars had a higher proportion of their total ascorbate present in the reduced form suggesting that under heat-stress conditions, the heat-tolerant cultivars had greater ascorbate turnover rates than the heat-sensitive cultivar. This observation may be due to any one or all of three different mechanisms. First, it has been proposed that MDAR reductase plays a crucial role in maintaining ascorbate in the reduced state,[50] and studies have shown that this enzyme's activity increases 80% to 90% in Mn^{2+}-deficient spruce needles.[8] A second explanation is that DAsA reductase is responsible for the enzymatic reduction of DAsA to AsA. DAsA reductase activity has been shown to be as much as 100% greater in an internal rust spot (IRS)-resistant strain of potato than in an IRS-sensitive clone,[5] and Cakmak and Marschner[7] demonstrated significantly greater activities of this enzyme in Mg^{2+}-deficient bean leaves. The third explanation provided by Foyer *et al.*[16] suggests that the glutathione pool is involved in recycling of the ascorbate pool. Neither MDAR reductase nor DAsA reductase were assayed in the present

study, but analysis of the glutathione pool lends support to the hypothesis that the glutathione pool is involved in recycling of the ascorbate pool.

Total glutathione concentrations tended to increase in all plants grown during the summer primarily due to significant increases in GSH. Although the concentration of GSSG increased significantly in all cultivars under heat-stress conditions, the GSH/GSSG ratios were significantly greater in the higher-yielding cultivars than in the heat-sensitive Floradade. The low GSH/GSSG ratio in Floradade indicates that a much smaller percentage of the glutathione pool was in the reduced state. Variances in GSH/GSSG ratios reflect differences in glutathione turnover rates that are patially controlled by GR activity. With the exception of Heat Wave, heat stress did not appear to induce a significant increase in glutathione reductase activity, but the activity of this enzyme was expressed constitutively at greater levels in the higher-yielding cultivars than in Floradade. The greater GR activities and GSH/GSSG ratios observed in the higher-yielding cultivars under heat stress indicate that these heat-tolerant cultivars had an increased glutathione turnover rate and a more active ascorbate-glutathione cycle than the less heat-tolerant Floradade. The greater GR activities resulted in a larger pool of GSH which the heat-tolerant cultivars could use to increase AsA concentrations and produce more favorable DAsA/AsA ratios than the one observed in the heat-sensitive Floradade. Cysteine serves as a precursor for GSH, and under heat stress both cystine and cysteine pools increased significantly in all cultivars. These data suggest that during heat-induced oxidative stress, AsA concentrations increase at the upper end of the ascorbate-glutathione cycle and GSH concentrations increase at the lower end in order to keep the GSH/GSSG ratio favorable towards ascorbate reduction. In addition, cystine and cysteine concentrations increase in order to provide the precursor necessary to maintain greater levels of GSH. An up-regulation of the ascorbate-glutathione cycle has also been observed under other environmental stress conditions. Ascorbate peroxidase activity has also been reported to increase in Mg^{2+}-deficient bean leaves,[7] chilling-resistant spinach,[49] winter needles of eastern pine,[38] and salt-tolerant cotton callus tissue.[19] GSH concentrations in chilling-sensitive cucumbers have been shown to be much lower than the GSH concentration in chilling-resistant peas,[4] and other studies have also revealed that the GSH/GSSG ratio was significantly higher in an IRS-resistant strain of potato than in an IRS-sensitive strain[5] and in salt-tolerant cultivars of cotton as compared to salt-sensitive cultivars.[18] GR activity has been reported to increase in cotton subjected to elevated atmospheric oxygen treatments[36] and salt stress,[18] an IRS-resistant strain of potato,[5] Mg^{2+}-deficient bean leaves,[7] and white pine needles during the winter months.[38]

It is not known whether the heat-induced increases in antioxidant enzyme activities observed in this study were due to an increased synthesis of the enzymes or an increased activation of constitutive enzyme pools. Other studies have shown the first explanation to be likely. An increase in the transcription of genes involved in the synthesis of various stress metabolites have been reported,[51,52,53] and Yamaguchi-Shinozaki and Shinozaki[54] have identified a *cis*-acting element responsible for induction of an *Arabidopsis* involved in responsiveness to drought, low-temperature, and high-salt stress. It has also been shown that cytoplastic and chloroplastic Cu,Zn SOD transcripts increase when tomatoes are mechanically wounded or treated with sublethal doses of paraquat,[55,56] and Scandalios[57] has shown that the catalase CAT1 mRNA levels increase substantially in the presence of abscisic acid. When compared to non-hardened spinach leaves, cold hardened leaves had increased GR activity plus additional isoforms of the enzyme.[58] Hence, it may well be that in tomatoes, acclimation to elevated temperatures and the capacity to produce higher yields under high-temperature stress is due, at least in part, to the up-regulation of the genes encoding these antioxidant enzymes. Heat Wave, LHT 24, and Solar Set had the highest

antioxidant profiles, while Floradade had the lowest. Tamasabro also had an antioxidant profile similar to that of the higher-yielding cultivars, yet its yield was somewhat lower. This may be due to the fact that Tamasabro was lower than the higher-yielding cultivars in both SOD and GR. These two enzymes may represent limiting factors in the development to heat stress, and thus resulted in lower yield in this cultivar as compared to the higher-yielding cultivars. On the other hand, Tamasabro was developed to withstand temperatures in excess of 32°C in Japan, and, other environmental factors in addition to heat stress may have affected the yield of this cultivar when grown in Louisiana. Nevertheless, all four putative heat-tolerant cultivars produced greater yields than Floradade when grown under heat-stress conditions, and the four putative heat-tolerant cultivars had the greatest antioxidant profiles. It should be noted again, however, that all five cultivars produced lower yields when grown under heat stress, and no one cultivar was high in every one of the antioxidant parameters measured. For example, Heat Wave had only moderate AP activity and a low GSH/GSSG ratio; LHT-24 had only moderate SOD, catalase, peroxidase, and GR activities; Solar Set exhibited moderate SOD and GR activity and low catalase, peroxidase, and AP. Hence, it may be possible to further increase yields under heat-stress conditions by genetically improving the antioxidant status in even the higher-yielding cultivars.

It has been shown that heat-stress induces stomatal closure results in lower internal carbon dioxide concentrations.[21] The lower carbon dioxide levels would cause a decrease in carbon dioxide fixation rates and reduce the sink for NADPH produced by the light reaction of photosynthesis while electron transport and oxygen evolution would continue at a steady rate. Since Rubisco is both a carboxylase and an oxygenase, the higher molecular oxygen/carbon dioxide ratio would favor photorespiration.[59] In photorespiration, C_3 plants such as tomato enter into a complex process in which glycolate is transported into an adjacent peroxisome and then oxidized to glyoxylate in a reaction catalyzed by glycolate oxidase. This reaction is responsible for producing substantial amounts of H_2O_2. While photorespiration can also serve as a sink for NADPH, the photorespiration rate would eventually decline due to the inhibition of glycine oxidation caused by the heat induced leakage of NAD^+ from the mitochondria.[60] Since NADPH is utilized downstream from the glycine oxidation step, there would be a further reduction in the size of the sink for NADPH and would inevitably result in the transfer of electrons from ferredoxin to molecular oxygen[12] and the production of the superoxide free radical. The elevation of both H_2O_2 and superoxide concentrations would cause lipid peroxidation which would ultimately result in injury to the chloroplast and other organelle membranes, dissociation of antennae pigment protein complexes, metabolite leakage, and perhaps the induction of antioxidant enzyme encoding genes.

Recently it has been suggested that ABA may confer a degree of tolerance to environmental stress,[61] and an increase in ABA in vegetative tissues is often associated with increases in stress-induced gene expression.[62] ABA has been shown to positively enhance the catalase *Cat1* transcript in maize,[63] and Galvez *et al.*[64] have shown that ABA is the likely inducer for the increased transcription of eleven mRNAs associated with the synthesis of early salt-stress induced proteins in *Lophopyrum elongatum*. Hence, the increase in antioxidant enzyme activity observed in the heat-tolerant cultivars may have been due to enhanced gene induction by elevated levels of ABA. Elevated temperatures could possibly be responsible for elevating ABA concentrations which, in turn, could be responsible for the enhanced induction of antioxidant genes; however, increases in ABA levels appears to be a general response to a number of environmental stress conditions. There are cases in which genes regulated by exogenous ABA are not markedly induced by stress[62] and cases

where genes that induced by stress are not responsive to exogenous ABA.[54] There is considerable evidence that $O_2^{\cdot-}$ [65] and perhaps H_2O_2[57] serve as signal induction molecules for stress-induced cellular responses. Superoxide is generated under most types of environmental stress. It may well be that heat stress results in electron leakage and production of $O_2^{\cdot-}$ which serves as the signal molecule for the induction of stress metabolites including ABA. The elevated ABA levels could then result in the enhanced induction of antioxidant enzyme encoding genes.

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