Impact of heat stress on sucrose metabolism of watermelon

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Abstract. High temperatures (HT) are a significant threat for crop production, and strategies for maintaining high crop yields and quality under HT stress are crucial agricultural objectives. The changes in sugar metabolism during HT stress were examined in watermelon cv. Crimson Tide leaves. The leaves obtained from plants were subjected to 35, 40, 45, 50, 55 and 60°C. Heat-stress tolerance (HST; LT50), hydrogen peroxide (H2O2), sucrose (Suc), reducing sugars and starch contents and some sucrolytic enzyme activities in leaf samples held at each temperature for 30 minutes were determined. The HST and H2O2 content, rose with increasing temperatures, most noticeably between 50-55°C. As a result, the LT50 value was determined to be 53.84°C. The Suc content increased almost 2-fold between 50-55°C. The reducing sugars and starch content sharply decreased with HT up to 50°C comparing to the control, however both increased almost 2-fold between 50-55°C. The H2O2 may act as a signal molecule at 40-45°C and triggers sucrose metabolism. It was determined that alkaline-INV and SuSy activities were at the maximum level at 40°C. The increase in enzyme activities has been associated with increased energy needs under stress conditions. The findings revealed that sugar metabolism contributes significantly to HST.

1 Introduction

Plants have evolved various mechanisms to combat stress factors as they occur in their environments. After high temperatures (HT), plants have the ability to gain heat stress tolerance (HST) by being exposed to a gradual increase in temperature, resulting in stress acclimation. Plant acclimation to moderately HT has significant effect on induction of tolerance to lethal HT [1]. The term “heat-stress tolerance” (HST; LT50) refers to the temperature generating 50% of the maximal injury based on electrolyte leakage [2].

Long-term exposure to HT can seriously affect a plant's metabolic processes and produce cellular disorder which inhibits growth and development [3]. The estimation of cell membrane stability under heat stress is utilized to express stress tolerance [4, 5, 6]. In plants, extremely reactive and harmful reactive oxygen species [ROS, e.g. superoxide (O2•-), singlet oxygen (‘O2), hydrogen peroxide (H2O2), and hydroxyl radicals (OH•)] are essential for controlling a wide range of biological functions. Because of their toxicity and crucial function in signalling, ROS levels in cells must be strictly controlled [7]. Reactive oxygen species and low-molecular-weight antioxidants work together in the cellular signalling hub to combine environmental data with controls over plant growth, development, and stress tolerance [8].

Osmoprotectants protect the plant by preventing damage to its cellular machinery in response to a stressful environment [9]. They are organic, highly soluble, low-molecular-weight, electrically neutral, and nontoxic chemicals [10]. Betaine and associated molecules, sugars and polyols, and amino acids are the three main groups of osmoprotectants [11, 12]. Membrane integrity strengthening, enzymatic/antioxidant activity balancing, and water modifications are physiological responses associated with the action of osmoprotectants in plants exposed to abiotic stressors [9, 11].

Sugars are also recognized as chemical signalling molecules for a specific receptor, as well as sensor molecules that may detect specific stimuli for normal plant functioning under stress and normal conditions [13]. Sugar plays a protective role by acting on membranes and macromolecules, triggering a signalling cascade system that modulates gene expression in response to both internal and external stimuli under stress circumstances such as drought, salt, and oxidative stress [14].

Watermelon (Citrullus lanatus L.) is an important plant worldwide and it occupies 9.5% of the total vegetable production. Sugar accumulation, the primary factor affecting watermelon fruit quality, is governed by both sugar translocation and metabolism in developing fruits. Watermelon, contains sucrose (Suc), fructose (Fru), and glucose (Glc) as predominant sugars [15]. Sucrose metabolism in plants involves several enzymes, such as sucrose phosphate synthase (SPS; EC 2.4.2.14), sucrose synthase (SuSy; EC 2.4.1.13), and invertase (INV; EC 3.2.1.26) [16]. The irreversible hydrolysis of Suc into Glc and Fru is catalyzed by the INVs [17]. The cytoplasmic enzyme SuSy, also known as UDP-D-Glc: D-Fru 2-a-glucosyltransferase (EC 2.4.2.13), catalyzes the reversible cleavage of Suc with uridine 5-diphosphate (UDP) to produce UDP-Glc and Fru [18].
The aim of this study is to determine the alterations caused by HT in the sugar metabolism and activity of sucrolytic enzymes in watermelon seedlings.

2 Material and method

2.1 Plant material and heat stress treatments

Leaves of watermelon cv. Crimson Tide were used in the current study. Samples were taken from plants growing in a field in Eskisehir, Türkiye, which has ideal watermelon-growing circumstances (longitude: 39°45'38''N, latitude: 30°28'47''E). Samples of completely formed leaves, ideally the third from the apex, were taken for experiments.

The controlled heat stress treatments were performed on the leaf samples as previously described by Arora et al. [19], with slight adjustments. In short, the leaves were collected into pyrex tubes, covered, and placed in a water bath. After a 30-minute acclimation in a 30°C water bath, the water temperature was raised gradually by 5°C every half hour from 35 to 60°C to induce heat stress. The samples obtained at every temperature were divided into two groups: one group was used for determining HST, soluble sugars and starch contents while the other group was immediately fixed in liquid nitrogen (N2) and stored at -80°C until further analysis of sucrolytic enzymes activities.

2.2 Heat stress tolerance (HST)

The HST (LT50) was calculated as the temperature causing half maximal percent (50%) injury based on electrolyte leakage (EL) [20]. Leaf discs with a diameter of 1.5 cm, were taken from leaves then they were rinsed in deionized water and placed in tubes holding 15 mL of distilled water for EL measurements. After shaking the samples at 100 rpm for 4 hours at room temperature, the amount of EL (EC1) was determined with an EC meter (Mettler Toledo, SevenEasy S30, Colombus Ohio, USA). The tubes were autoclaved at 121°C for 15 minutes subsequently total EL (EC2) was measured and EL was calculated using the following equation [20]:

\[
EL \text{ (%) } = \frac{(EC1/EC2) \times 100}{100}
\]

2.3 Hydrogen peroxide (H2O2) content

The H2O2 content of the leaves was calculated using the method of [21]. Shortly liquid N2-frozen leaf tissues weighing 1.0 g were homogenized in 4 mL of perchloric acid (HClO4) containing 1% (v/v) polyvinylpolypyrrolidone (PVPP) and sand. The homogenate was centrifuged at 10 000 g for 20 minutes at 4°C. Following that, the supernatant [adjusted to pH 7.5 with 4 M potassium hydroxide (KOH)] was centrifuged at 1000 g for a minute. A 400 μL aliquot and 1.6 mL dH2O were placed into 2 mL columns of AG 1-X8 Resin 100-200 mesh chloride form, 0.84 cm (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and the eluent was utilized to quantitate H2O2. One mL of reaction mixture [0.1 M sodium phosphate (Na2PO4), 3.3 μmoles 3-dimethylaminobenzoic acid (DMAB), 0.07 μmoles 3-methyl-2-benzothiazolinone hydrazone (MBTH), and 10 μg horseradish peroxidase (Sigma Chemical Co. Ltd.), pH 6.5] was added to 1.0 mL of the tissue extract to start the reaction and the absorbance was recorded at 590 nm. The H2O2 concentrations were estimated by using an H2O2 standard curve.

2.4 Soluble sugars and starch contents

Soluble sugars were extracted by suspending 0.1 g of leaves in 5 mL of 80% (v/v) ethanol for an hour in 85°C water bath and then collecting the ethanolic liquid. This procedure was repeated four times [for 1 h, 30 min, and twice 15 min]. The ethanolic solutions were mixed and evaporated to dryness at 55°C with the help of constant ventilation. One mL of distilled water was used to dissolve the dried sugars. The Suc contents were assessed spectrophotometrically (Perkin Elmer Lambda 25, USA) at 620 nm and using the anthrone reagent technique [22]. Reducing sugars contents were measured colorimetrically at 550 nm with dinitrosalicylic acid [23] using Glc as the standard.

Starch was evaluated by measuring Glc after amyloglucosidase digestion of the ethanol-insoluble residue [24].

2.5 Sucrose metabolizing enzymes

The method of Aloni et al. [25] was used to measure the activity of soluble (cytosolic) acid-INV in leaf tissues. Briefly 0.5 g of tissue samples were homogenized in 5 ml of ice-cold buffer containing 25 mM HEPES (N2-ethanesulphonic acid) pH 7.2, 2 mM DL-Dithiothreitol (DDT), and 3 mM diethyldithiocarbamic acid (DIECA) as an antioxidant. This mixture underwent a 20 000 g centrifugation at 4 °C for 20 minutes. A 100 μL supernatant was incubated in 10 mL of 0.1 N phosphate citrate buffer (pH 5.0) and 20 mM Suc. After 30 minutes of incubation at 37°C, the reaction was stopped by adding 1 mL of the dinitrosalicylic acid reagent. The resultant sugars were identified colorimetrically after 5 minutes of boiling.

The alkaline-INV and SuSy activities were calculated in accordance with Aloni et al. [26]. Following extraction as described for acid-INV, the mixture was dialyzed overnight to separate the internal sugars. The aliquots of 200 μL was incubated in a buffer containing 0.1 M phosphate-citrate buffer (pH 7.0), 200 mM Suc, and 5 mM UDP, the enzyme activity was measured as the rate of Suc breakdown. The dinitrosalicylic acid reaction was used to determine the Fru produced after 30 minutes of incubation at 37 °C. The information was presented using fresh mass. The crude enzyme extracts’ total soluble protein contents were calculated according to Bradford [27].
2.6 Statistical analysis

A randomized block design was used to set up the experiment. All experiments employed five technical replications. Using SPSS software (version 20, Chicago, IL, USA), the data were subjected to analysis of variance (ANOVA), and the means were assessed using the Duncan test at p< 0.05.

3 Results and discussion

3.1 HST Heat stress tolerance (HST)

The EL is a standard method for assessing cell membrane thermostability and an effective marker for heat tolerance in the selection of heat-tolerant plants [4, 5, 6]. Fig. 1 presents the HST (as LT50) of watermelon leaf samples. The rate of injury (%) rose with increasing temperatures, and doubled between 50-55°C (29.90%-56.07%, respectively). Based on the average values, rate of injury (%) was the highest in 60°C (65.72%) than in all other high temperature treatments. Indeed, the average HST of watermelon plant was calculated as 53.84°C. The effect of HT on rate of injury was statistically significant (Table 1). Similarly, rate of injury was increased in strawberry [28], pepper [29], green bean [30] and tomato [6] plants subjected to HT.

3.2 H2O2 content

The ROS are generated in chloroplasts, mitochondria, and peroxysomes under ideal conditions, they can also be produced by heat stress [31], which causes lipid peroxidation and destruction, resulting in membrane damage [32]. The H2O2 content of leaf tissues increased stepwise from control to 60°C, most noticeably between 50-55°C as presented in Figure 2. Based on the average values, H2O2 content was the highest in 60°C (137.11 nmol/gFW) than in all other high temperature treatments. Indeed, the H2O2 content of leaf tissues was calculated as 53.84°C. The effect of HT on rate of injury was statistically significant (Table 1). Similarly, rate of injury was increased in strawberry [28], pepper [29], green bean [30] and tomato [6] plants subjected to HT.

The H2O2, a readily diffusible and relatively long-lived ROS, is a key actor in stress signalling pathways [35]. The results of H2O2 are parallel to rate of injury results in which plants exhibited higher injury rates between 50-55°C.

3.3 Soluble sugars and starch contents

Carbohydrate metabolism is an important response process for plants in response to abiotic stress. Saccharides like Suc, Glc, and Fru can function as critical molecules and substrates in a variety of metabolic reactions [36]. They can also act as a signal in the stress response [37].

The Suc content increased almost 2-fold between 50-55°C as presented in Figure 3. The Suc content was higher in 55°C (25.62 mg/ g FW) than in control (11.69 mg/ g FW) plants. The effect of HT on the Suc content was statistically significant (Table 1).
Table 1. The rate of injury, H₂O₂, Suc, reducing sugars and starch contents, and Acid-INV, Alkaline-INV and SuSy activities of watermelon plants at different temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Rate of Injury</th>
<th>H₂O₂ content</th>
<th>Suc content</th>
<th>Reducing sugars content</th>
<th>Starch content</th>
<th>Acid-INV activity</th>
<th>Alkaline-INV activity</th>
<th>SuSy activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>53.61c</td>
<td>11.69b</td>
<td>16.53a</td>
<td>4.61a</td>
<td>0.63ab</td>
<td>0.63ab</td>
<td>0.66b</td>
</tr>
<tr>
<td>35°C</td>
<td>14.04d</td>
<td>59.94c</td>
<td>11.37b</td>
<td>15.89a</td>
<td>1.50c</td>
<td>0.68a</td>
<td>0.69a</td>
<td>0.59bc</td>
</tr>
<tr>
<td>40°C</td>
<td>15.70d</td>
<td>67.27c</td>
<td>14.15b</td>
<td>15.07a</td>
<td>1.30c</td>
<td>0.54b</td>
<td>0.74a</td>
<td>0.78a</td>
</tr>
<tr>
<td>45°C</td>
<td>17.52d</td>
<td>67.76c</td>
<td>12.61b</td>
<td>10.86b</td>
<td>0.97c</td>
<td>0.55b</td>
<td>0.50bc</td>
<td>0.52cd</td>
</tr>
<tr>
<td>50°C</td>
<td>29.90d</td>
<td>99.27c</td>
<td>14.00b</td>
<td>7.70c</td>
<td>0.87c</td>
<td>0.53b</td>
<td>0.46c</td>
<td>0.49ad</td>
</tr>
<tr>
<td>55°C</td>
<td>56.07d</td>
<td>127.68c</td>
<td>25.62a</td>
<td>11.03b</td>
<td>1.50c</td>
<td>0.22c</td>
<td>0.38c</td>
<td>0.44d</td>
</tr>
<tr>
<td>60°C</td>
<td>65.72a</td>
<td>137.11a</td>
<td>24.01a</td>
<td>12.17b</td>
<td>3.33b</td>
<td>0.15c</td>
<td>0.43c</td>
<td>0.52cd</td>
</tr>
</tbody>
</table>


Data are the means of five replicates. Mean values labeled with different letters in columns were significantly different at p<0.05.

Fig. 4. The reducing sugars content of watermelon leaves in response to heat stress. Vertical bars indicate ±SE of five replicates.

Sugar molecules are now being considered by plant researchers as a new strategy for protecting plants from unfavorable conditions due to their significant antioxidant capacity [13]. Heat stress increased sucrose accumulation in potato leaves [38]. Luo et al. [39] found that trehalose largely removed H₂O₂ and O₂⁻ in wheat under high temperature stress, depending on concentration. After being pre-treated with Glc, the cucumber was subjected to high temperature and the O₂⁻ concentration of the pre-treatment with Glc was increased, and was observed to clearly reduce H₂O₂ content [40].

The starch content sharply decreased with HT up to 50°C comparing to the control, however almost 2-fold between 50-55°C as presented in Figure 5. The reducing sugars content was the highest at control (16.50 mg/g FW) and the lowest at 50°C (7.70 mg/g FW). The effect of HT on the leaf Suc reducing sugars content was statistically significant (Table 1).

High temperature (40°C) caused an increase in carbohydrate concentration [41]. The total non-structural carbohydrate and starch concentrations in Agrostis palustris Huds. plants exposed to high temperatures decreased [42]. Similarly, heat stress decreased starch accumulation in mature leaves [38].

Fig. 5. The starch content of watermelon leaves in response to heat stress. Vertical bars indicate ±SE of five replicates.

3.4 Sucrose metabolizing enzymes

The activity of acid-INV, alkaline-INV and sucrose synthase (SuSy) enzymes reduced as the temperature rose as presented in Figures (6-8). The acid-INV activity has dropped by about half, from 0.53 nmol/mg prot./h to 0.22 nmol/mg prot./h, between 50-55°C (Fig. 6). The highest acid-INV enzyme activity was detected at 50°C (0.68 nmol/mg prot./h) while the lowest activity was detected at 60°C (0.15 nmol/mg prot./h). The effect of HT on the leaf acid-INV activity was statistically significant (Table 1).

The alkaline-INV activity has reached a peak at 40°C (0.74 nmol/mg prot./h) and steadily reduced while temperature increased (Fig. 7). The lowest activity was detected at 55°C (0.38 nmol/mg prot./h). The effect of HT on the leaf alkaline-INV activity was statistically significant (Table 1). Younis et al. [43] found that INV activity decreased in broad bean plants exposed to HT of 40°C.
The highest leaf SuSy activity was detected at 40°C (0.78 nmol/mg prot./h) and the lowest activity was detected at 55°C (0.44 nmol/mg prot./h) as presented in Figure 8. The effect of HT on the leaf SuSy activity was statistically significant (Table 1). Likewise, SuSy activity decreased in maize plants subjected to HT [44].

Considering the central role of INV and SuSy in carbon metabolism and the fact that carbohydrates account for approximately 90% of biomass yield in plants, elucidating the mechanisms underlying the signalling role of sucrose metabolism not only advances basic biology but promises innovative solutions to increase crop yield [45].

4 Conclusion

In conclusion, the LT$_{50}$ value was determined to be 53.84°C. The H$_2$O$_2$ may act as a signal molecule at 40-45°C and triggers Suc metabolism. The amount of Suc increased in parallel with the increase in temperature ‘Crimson Tide’ watermelon variety and that this increase contributed to its tolerance to high temperature stress up to the LT$_{50}$ value. The amount of reducing sugars first decreases with the increase in temperature and has started to increase again since 40°C. The first source of increased energy needs under HT conditions is reducing sugars and starch. This situation was thought to be related to the high hydrolysis of Suc at 40°C. According to the results of enzyme activity analyses, it was determined that alkaline-INN and SuSy activities were at the maximum level at 40°C. The increase in enzyme activities has been associated with increased energy needs under stress conditions. Sugar accumulation is thought to be related to HST.

The findings of this study contributed to a better understanding of the alterations in Suc metabolism of the ‘Crimson Tide’ watermelon variety under heat stress. In the future, this study will assist determining the Suc metabolism related genes involved in osmoprotection and producing HT-tolerant watermelon varieties.

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