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Effect of high temperature and excessive light on glutathione content in apple peel

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Abstract The present experiment was conducted to examine the changing patterns of glutathione (GSH) contents in apple peel stressed by high temperature and excessive solar radiation. By comparing the parameters of temperature and light conditions where fruits grow with the GSH contents in them, the mutual relationship was disclosed. Meanwhile, the changes of antioxidant capacity of fruits stressed at different levels were studied under artificially controlled conditions in a laboratory. Also, the effect of applying various types of exogenous substances was evaluated on endogenous GSH contents. The results indicated that within a certain range, a positive correlation was found between the stressed extents to which fruits were subject by high temperature and excessive light, and GSH contents in fruit peel. Moreover, fruits on southwest (SW) exposure contained a very significantly higher amount of GSH than those on other exposures. In laboratory experiments, it was proved that fruit GSH contents increased with temperatures within a certain range but they declined beyond a given limit. The temperature rising modes had a great influence on fruit GSH contents, and gradual temperature increase was favorable to an increase of antioxidant capability in fruit peel, thus providing a theoretical basis for exerting appropriate acclimation on fruits. It was also documented that application of four exogenous formulations could significantly increase the endogenous GSH contents, among which AsA (ascorbic acid) + BA (benzoic acid) and SA (salicylic acid) treatments exhibited better results, 33.97% and 31.81% higher than the control, respectively.

Keywords apple, fruit, high temperature, excessive light, stress, glutathione (GSH)

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1 Introduction

Apple fruit sunburn is a common disorder that is usually regarded as a result of a bio-oxidation process in fruit peel stressed by both high temperature and excessive solar radiation (Schrader et al., 2003; Zhang et al., 2004). Glutathione (GSH) is an important endogenous antioxidant and plays a key role in the balance of oxidative and reductive system in a plant body. It has been reported that GSH could scavenge active oxygen radicals, prevent peroxidation of membrane lipid and maintain the stability of membrane system (Chen and Liu, 1998; Chen and Liu, 2000; Wang and Li, 2002). Some researchers have preliminarily demonstrated on peppers, tomatoes, litchis and figs that drought, low temperature, excessive light, heavy metals, salts and water stresses could cause an abrupt increase in GSH contents in leaves or fruits, which is positively correlated with antioxidant capacity (Yao et al., 2000; Ding et al., 2006; Lin et al., 2006). Moreover, environmental induction or exogenous GSH application has shown the potential to improve the antioxidant capacity of plants to environmental stresses (Smith et al., 1989; Hua et al., 2003; Liu et al., 2004; Zhu et al., 2005; Wang et al., 2006). However, no report has been found so far on the relationship between the degrees of high temperature and excessive light stresses and the changes of GSH contents in apple fruits. The present experiment aims at examining the changing patterns of endogenous GSH of fruits stressed by high temperature and excessive light, as well as the effect of exogenous agent application on fruit antioxidant capacity, so that further studies could be undertaken to develop effective ways to prevent heat-related damage.

2 Materials and methods

2.1 Materials

The experiment was carried out in 2005 and 2006 at the Beizhang orchard, situated in an agricultural suburb of Baoding, as well as in the key-discipline laboratory of pomology,

Ministry of the People's Republic of China located at Agricultural University of Hebei. Ten-year-old apple trees (*Malus domestica* Borkh. cv. Red Fuji) on crab apple rootstocks (*M. micromalus* Mill.) were employed with a planting density of 3 m × 5 m. Those trees were managed quite well and pruned to a free spindle system with ideal reception of sunlight. Sample trees were chosen carefully from the orchard, which had a good light reception, consistent growth, medium vigor and evenly loaded crops.

2.2 Methods

2.2.1 Comparison of ecological conditions among different exposures on the canopy

On three sample trees, the four exposures of southeast (SE), northeast (NE), southwest (SW) and northwest (NW) were divided on the canopy and three uniform-growing fruits were selected on each of them from the exterior canopy, 1.5 m above the ground. A single tree served as a plot and randomized-block arrangement was adopted with three replicates. After three successive clear days around July or August, the fruit surface temperatures on the exposed side were measured with a digital temperature probe (Model JM222) at 1 h intervals, beginning from 8 a.m. to 3 p.m.. Meanwhile, the intensity of solar radiation near the fruit surface was obtained with a digital luminometer (Model PES1332A) and the ambient temperatures and solar radiation were recorded simultaneously.

2.2.2 Comparison of GSH contents in fruit peel among different exposures

After three successive sunny days around mid-July, the fruits from different exposures on a tree canopy were collected at 3 p.m. on the fourth day and peeled on the exposed side immediately in a laboratory. The skin contained both epidermal layer and adjacent flesh tissue, about 0.5–1 mm in thickness. Fruit peel was put into an extra-cold refrigerator (−72°C) for storage. Every treatment included three apples with three replicates. The field trial was designed in a randomized-block way and Duncan's new multiple range test was performed for statistical analysis (same as below).

2.2.3 Comparison of GSH contents in fruit peel stressed by different temperatures

In mid-July, uniform attached fruits were covered with clear plastic wrap and put into a water incubator. Fruits were treated for 20 min at 25°C, 35°C, 48°C and 55°C, respectively. Pretreatment was required before time was being counted when fruit surface temperature reached the designed target. During treatment, a multiple-point temperature probe (Model TH-74) was used for monitoring fruit temperatures. A complete-randomized design was adopted with three fruits for each treatment, and three replicates were followed. After

a one-hour stay in the laboratory, the peel of fruits was taken off and put into an extra-cold refrigerator (−72°C) for storage.

2.2.4 Comparison of GSH contents in fruit peel influenced by fluctuating temperatures

In mid-August, uniform detached fruits were covered with clear plastic wrap and put into a water incubator. Three treatments were applied: (1) gradual temperature rising (T1): fruit temperatures increased from 25°C to 48°C via four stops (25°C→30°C→40°C→48°C); (2) direct temperature rising (T2): fruit temperatures increased from 25°C to 48°C via two stops (25°C→48°C); (3) fluctuating temperature rising (T3): fruit temperatures increased from 25°C to 48°C via four stops (25°C→45°C→25°C→48°C). Treatments of both T1 and T3 lasted for 24 h. During treatment, fruits stayed at that temperature for 4 h whenever fruit temperatures reached stop temperatures of 25°C, 30°C, 40°C or 45°C, and as soon as fruit temperature reached 48°C, they were allowed to stay at that temperature for 20 min. The surplus time was distributed evenly among the process of heating. After treatment, fruits were peeled and the skin was put into a refrigerator. Complete-randomized design was adopted with three replicates (three fruits serving as a plot).

2.2.5 Effects of light intensity on GSH contents in fruit peel

In mid-July, fruits on SW canopy were covered with black cloth (<5% of natural light) or shading net (about 70% of natural light), leaving natural exposed ones as the control (natural light). The light intensity was measured near the fruit surface from 12 a.m. to 3 p.m. A complete-randomized design was adopted with three fruits for each treatment, and three replicates were followed. After a one-hour stay in the laboratory, the exposed part of fruits was peeled and put into an extra-cold refrigerator (−72°C) for storage.

2.2.6 Effects of exogenous formulations on GSH contents in fruit peel

Fifteen trees were chosen and the field trial was arranged in a randomized-block design with three replicates (a single tree serving as a plot). Uniform fruits on SW exposure of tree canopy were sprayed at approximately 8 a.m. in a clear, calm and sunny day of mid-August with the following formulations: (1) AsA 15 mmol/L + CA (catalase) 7.5 mmol/L; (2) AsA 15 mmol/L + 6-BA 90 μmol/L; (3) CaCl₂ 20 mmol/L; (4) SA 0.2 mmol/L and (5) CK. Formulations were prepared by dissolving the agent with ethanol and then adding Tween-80 into the solution for emulsification. Control formulation contained the same amount of ethanol and Tween-80 in water. Fruits were picked up at 3 p.m. the following day. Three fruits were harvested from each plot. In a laboratory, the peel of fruits was removed and put into an extra-cold refrigerator (−72°C) for storage.

2.3 Assay of GSH contents

The method described by Chen and Wang (2002) was followed. 0.5 g of peel tissue was ground and extracted with 5 mL trichloroacetic acid. Then the syrup was centrifuged at 15000 r/min for 20 min. The supernatant liquid was prepared for the quantitative assay. 0.25 mL of sample solution was mixed with 2.6 mL NaH_2PO_4 (150 mmol/L, pH 7.7), and 0.18 mL (5, 5'-dithiobis (2-nitrobenzoic acid)) (DTNB) agent (75.3 mg DTNB dissolved in 30 mL 100 mmol/L PBS, pH 6.8), with phosphoric acid buffer solution (PBS) as the blank control instead of DTNB. The reaction was conducted for 5 min at 30°C with the solution evenly shaken. The GSH concentrations were determined by measuring the optic density (OD) values at 412 nm. Its concentrations in samples were calculated based on an equation or the standard curve made with GSH.

3 Results

3.1 Comparison of fruit surface temperature and light reception on different exposures of a canopy

As far as different exposures were concerned, a great variation existed in fruit surface temperatures by time course on a sunny day (Fig. 1). From 10 a.m. to 3 p.m., the fruits with highest temperatures on a tree canopy shifted from one exposure to another with the continuous changes of sunlight incident angles. As shown in Fig. 1, fruit surface temperatures increased gradually from 10 a.m. to 3 p.m. with air temperatures. The maximum temperatures of fruits on SE, NE and interior canopy (IC) were reached at 2 p.m. and a decline occurred thereafter. However, the fruit surface temperature on SW exposure achieved the maximal value at 3 p.m. (42.5°C). It also indicated that the SW fruits had a much larger differential of daily temperature range than those on other exposures, and remained to be the maximal temperature from 12 a.m. to 3 p.m.. In terms of the absolute values, the daily maximum fruit temperatures on different exposures ranked in such a descending order: SW > SE > NW > NE > IC. Thus, it was concluded that there was a great difference in fruit surface temperatures on different parts of a canopy because of the constant changes in light reception.

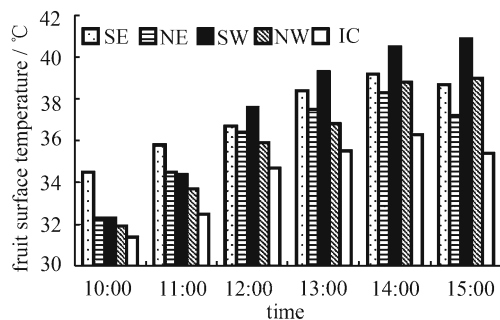


Fig. 1 Comparison of fruit surface temperatures among different exposures by time course

A great difference also existed in light intensity received by fruits on different exposures on a tree canopy (Fig. 2). From 10 to 11 a.m., fruits on SE exposure obtained the maximal solar radiation, reaching 410 W/m^2 at 11 a.m. and the light intensities on both SE and SW fruits remained approximately the same at 12 a.m., being 515 and 519 W/m^2 , respectively. However, light intensities on SE and NE fruits decreased and those on SW and NW exposures increased from 12 a.m. to 3 p.m. as a result of the changes of incident sunlight. As the fruits in the interior canopy mainly received scattered light, no significant changes happened in light intensity during the daytime, always maintaining a lower level. On a clear day, the SW fruits received the maximum solar radiation and temperatures were much higher than those on other exposures. Meanwhile, the scope of temperature changes was much higher than that on other exposures. For these reasons, it was concluded that the high temperature and excessive light stresses were most likely to emerge on SW exposure of a tree canopy.

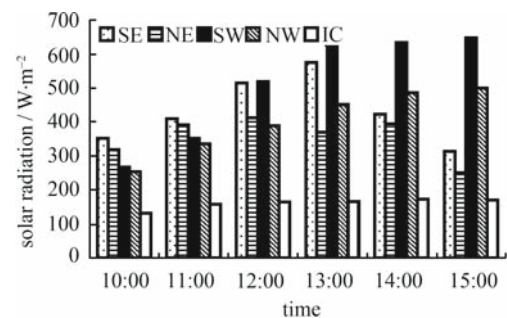


Fig. 2 Comparison of solar radiation received by fruits among different exposures by time course

3.2 Comparison of GSH contents in fruit peel among different exposures of a tree canopy

Under natural conditions, there was a great variation in GSH contents among fruits on different exposures (Fig. 3). The GSH contents of fruits on both SW and SE exposures were very significantly higher than those on the additional three exposures, being 36.37% and 25.18% above their average values, respectively. The results indicated that fruit antioxidant capacity increased gradually as a consequence of successive induction by high temperature and excessive light, while GSH contents rose significantly in peel tissues. Moreover, within a certain range, the more the fruits were stressed, the higher the GSH contents in fruit peel. For this reason, fruits on the exposures generally had a higher resistance to heat stress than those on low-temperature-and-light-stressed exposures. This explained satisfactorily why the interior fruits were prone to getting sunburned once they received accidental sunlight as a result of limbs being bent under a heavy fruit load in summer, as well as why shaded fruits become injured easily in the field when carelessly exposed in full sun after harvest.

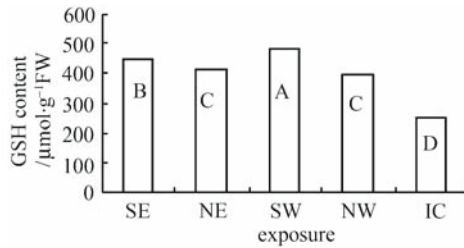


Fig. 3 Comparison of GSH contents in fruit peel on different canopy exposures

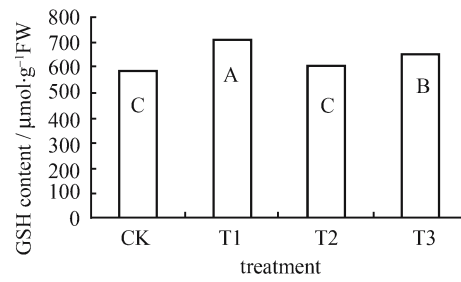


Fig. 5 Changes of GSH contents in fruit peel among different temperature-rising modes

3.3 Effect of high temperature stress on GSH contents in fruit peel

As shown in Fig. 4, as the heating temperatures increased and the stressed intensity heightened, GSH contents grew markedly within a certain range, reaching a significant level of 1%. However, when the treatment reached a temperature of 55°C when sunburn symptoms appeared, GSH contents declined sharply. The results suggested that the endogenous GSH could be regarded as an immediate response to heat shock, which prevented the peel tissue from being injured to some extent, but GSH contents may decrease beyond a temperature limit because damage may also happen to the related enzymes or oxidative and reductive chains in cells.

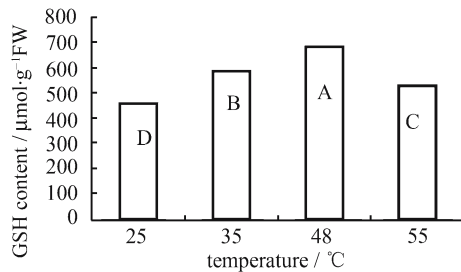


Fig. 4 Changes of GSH contents in fruit peel under different high-temperature stresses

3.4 Effect of fluctuating temperatures on GSH contents in fruit peel

Fluctuating temperatures could affect GSH contents in fruit peel substantially (Fig. 5). Three types of temperature rising modes could consistently raise the GSH contents in fruit peel, with T₁ and T₃ increasing by 26.25% and 14.49%, respectively, over the control. Gradual temperature rising (25°C→30°C→40°C→48°C) had the highest GSH contents because it was favorable for acclimation of fruits to high temperatures, and fluctuating temperature rising (25°C→45°C→25°C→48°C) could also induce endogenous GSH contents to increase significantly than the control even though fruit temperatures underwent an up-and-down pattern. However, direct temperature rising (25°C→48°C) resulted in an insignificant increase of GSH level partly because fruits were hardly adapted to a higher temperature stress within a short period of

time even though fruit temperatures increased eventually from low to high. Therefore, it was suggested that within a certain range, gradual temperature rising favored the acclimation of fruits to high temperatures, whereas the contrary was true if the temperature rising process proceeded too quickly or the temperature differential was too large.

3.5 Effect of light intensity on GSH contents in fruit peel

Figure 6 shows the GSH contents in fruits receiving different intensities of sunlight. As light intensities rose, fruit GSH contents increased gradually with the control and net shading treatments being 63.73% and 28.66% higher than black cloth treatment, respectively. The results indicated that higher light intensities could induce fruits to synthesize GSH. Thus, it is implicated that fruit antioxidant capacity may be improved by applying appropriate light induction, which might be of great significance in lessening the light-related loss caused by improper bagging practices.

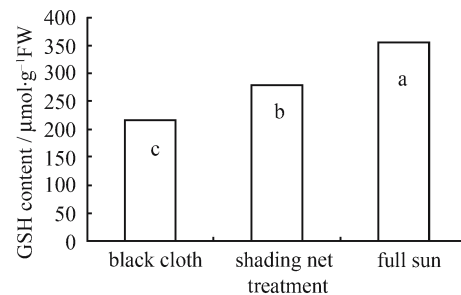


Fig. 6 Comparison of GSH contents in fruit peel exposed under different light intensity

3.6 Effect of exogenous formulations on GSH contents in fruit peel

As shown in Fig. 7, four types of formulations applied could consistently increase the endogenous GSH contents in fruit peel, among which AsA + BA and SA sprays showed to be more effective, 33.97% and 31.81% higher than the control, respectively. The results suggested that application of exogenous formulations could raise the contents of endogenous antioxidants, thus enhancing antioxidant capacity of fruits to high temperature and excessive light stresses.

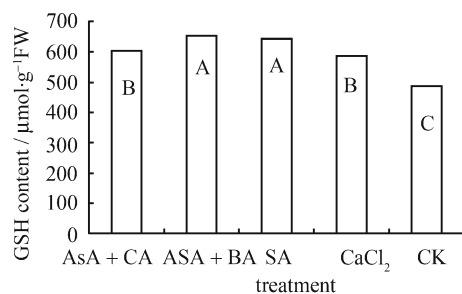


Fig. 7 Comparison of GSH contents in fruit peel among different treatments

4 Discussion

4.1 Role of GSH in fruit response to high temperature and excessive light stresses

Glutathione is a group of small-molecular antioxidants existing widely in plants. It has been reported that the GSH pool size and its status of oxidation or reduction in plants are closely associated with the tolerance of plants to environmental stresses (Chen, 1993; Chen et al., 2004). Generally, the endogenous GSH could respond immediately to low temperatures and water stress (Kuroda et al., 1992; Wang et al., 1995; Chen and Liu, 1999). Furthermore, our results also suggested that high temperature and excessive light could also heighten GSH contents in fruits within a certain range, indicating that the endogenous GSH contents could respond immediately to those stresses in a similar way, which was considered to alter the fruit antioxidant capacity. Since one of the important pathways to scavenge active oxygen species in plants is accomplished by ascorbate-glutathione cycles (Grodén and Beck, 1979; Foryer et al., 1989; Foyer, 1998), it is presumed that the role of GSH in an immediate response to heat shock was probably ascribed to its particular function in the cycles. Therefore, further studies should be done on the changing characteristics of GSH in the cycles, the exact relationship between endogenous GSH pool size and fruit antioxidant capacity as well as the efficacy of exogenous formulations on the changes of endogenous GSH pool size under different levels of high temperature and excessive light stresses.

4.2 Prospects of GSH application in preventing apple fruit sunburn

It has been found on maize and mango crops that heat induction could improve their antioxidant capacity by increasing GSH contents in leaves or fruits (Dai et al., 1999; Li and Gong, 2003). Thus, it is assumed that wherever sunburn is a problem, heat-related damage of fruits might be reduced by improving temperature and light environments around fruits, such as applying judicious pruning systems, adopting proper growing practices and so on. In addition, it has been proven

that the application of GSH or Ca could raise the amount of endogenous GSH and antioxidant capacity in plant organs (Guan et al., 1998; Zhang et al., 2001), and in our experiment, spraying with AsA + BA and SA also led to a significant rise in GSH contents. Those results undoubtedly provide a new approach to pursuing effective practices to highlight fruit antioxidant capacity via external regulations.

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