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Original Article

Pre-harvest drought stress treatment improves antioxidant activity and sugar accumulation of sugar apple at harvest and during storage

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ABSTRACT

Physico-chemical and quality changes in 72 sugar apple (*Annona squamosa* Linn.) fruits subjected to pre-harvest drought stress were analyzed at harvest and during storage at 10 °C or 15 °C, with 90–95% relative humidity. At harvest, the ascorbic acid, sugar and endogenous abscisic acid concentrations increased while the concentration of the substrate indicating a 50% loss in 2,2-diphenyl-2-picrylhydrazyl scavenging activity (DPPH EC_{50}) decreased in fruit from drought-treated trees compared with fruit from well-watered trees (control). The fresh weight loss of fruit stored at 15 °C was higher than at 10 °C, with no significant effect of drought treatment. In contrast, fruit firmness was reduced by drought treatment compared with the control during storage at both temperatures. Respiration, ethylene production and the endogenous abscisic acid and total sugar concentrations were higher in fruit from the drought-treated trees kept at 15 °C. The total ascorbic acid concentration was higher in fruit from drought-stressed trees kept at 10 °C compared with other treatments. This was concomitant with the DPPH EC_{50} value, which was lowest in fruit from drought-stressed trees stored at 10 °C. These results implied that pre-harvest drought stress treatment activated antioxidant activity and increased sugar concentration in sugar apple fruit. In addition, pre-harvest drought stress hastened fruit ripening. Thus, based on the results, storage of sugar apple fruit at 10 °C is recommended as this induces antioxidant activity which delays chilling injury for 8 d.

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Introduction

The incidence of drought stress during fruit and vegetable production is occurring more frequently with climate change patterns from global warming, which in turn are leading to limited water resources (Whitmore, 2000). Water stress during the production of some agricultural products can influence fruit physiology and morphology, which may affect fruit quality (Toivonen and Hodges, 2011). Abscisic acid (ABA) synthesis is one of the mechanisms in response to water stress in plants; ABA is synthesized and then triggers stomatal closure, thereby reducing water loss via transpiration (Wilkinson and Davies, 2010). Furthermore, the onset of fruit ripening is also accelerated under water deficit conditions, such as in peach (*Prunus persica* L.; Mercier et al., 2009) and apple (*Malus domestica* Borkh.; El-Soda et al., 2014). These effects contribute to the production of ethylene, which is coordinated with fruit-ripening processes in many fruits, such as banana (*Musa* × *paradisiaca* L.) and strawberry (*Fragaria* × *ananassa* D.) according to Barry and Giovannoni (2007). There are many reports of the effect of water stress on fruit quality. Miller et al. (1998) observed that water stress during fruit set decreased the fruit weight but increased the total soluble solids concentration in kiwifruit (*Actinidia deliciosa* cv. Hayward). Terry et al. (2007) also found higher fructose and glucose concentrations in strawberry subjected to water deficit conditions. Moreover, Pérez-Pastor et al. (2007) showed the benefits of deficit irrigation treatments on apricot (*Prunus armeniaca* L.), when they observed a slight increase

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in total soluble solids and firmness at harvest and during cold storage. Under stress conditions, ascorbic acid causes plants to resist stress by reducing the reactive oxygen species constituted by the stress (Ahmed et al., 2014). Thus, the antioxidative system in plants plays an important role in eliminating free radicals from plants under stress conditions. However, the effect of water stress on fruit quality and postharvest change is still complex and variable as water stress affects so many biological processes in plants. Apart from pre-harvest environmental factors, the postharvest control conditions, specifically temperature, greatly affect the visual quality, chemical composition and eating quality of fresh produce. Good management of temperature is the most important and simplest procedure for delaying the deterioration of fresh fruits and vegetables. In addition, the optimum storage temperature can retard softening and color changes of fruits and vegetables, as well as slow down metabolic changes and moisture loss (Nunes, 2008).

Sugar apple (*Annona squamosa* Linn.) is a drought tolerant plant cultivated in subtropical and tropical areas (Egydio-Brandão and Santos, 2016). It is an important commercial crop in Thailand, where it is mainly cultivated in the northeast, which is an arid area (Pratcharoenwanich et al., 2014). Drought stress decreased the stomatal conductance and CO_2 assimilation rate and increased the soluble sugar and free amino acid concentrations in young sugar apple plants (Rodrigues et al., 2010). A more recent previous study found that endogenous ABA and ascorbic acid concentrations in the leaves and fruit of the sugar apple tree increased under drought conditions (Kowitcharoen et al., 2015). In addition, changes in the respiration rate and the sugar and chlorophyll concentrations in sugar apple fruit during development have also been reported (Pal and Kumar, 1995). With regard to postharvest research, the ripening rate of 'Balanagar' sugar apple fruit was delayed when the storage temperature decreased (Vishnu Prasanna et al., 2000). Sugar apple is perishable; therefore an optimum postharvest storage temperature is critical to ensure improved storage life and prevent chilling injury (CI). However, the optimum storage temperature varies in the range 7–20 °C (Vishnu Prasanna et al., 2000). Broughton and Guat (1979) suggested that storage temperatures below 15 °C cause CI in sugar apple. In addition, Chunprasert et al. (2006) reported that sugar apple is susceptible to CI at temperatures lower than 13 °C.

Although several studies have described the physiological and biochemical changes that occur in sugar apple during growth, drought stress and storage, there is little information on the influence of pre-harvest drought treatment on the postharvest quality changes in sugar apple. Therefore, the current study aimed to investigate the effect of pre-harvest drought stress treatment on sugar apple performance at harvest and during storage at low temperatures.

Materials and methods

Plant material and treatments

The plant material consisted of 6-year-old, fruit-bearing sugar apple trees (*Annona squamosa* L., cv. 'Fai') trained using a modified central leader system, grown in an orchard with sandy loam soil at the Pakchong Research Center, Faculty of Agriculture, Kasetsart University (Nakhon Ratchasima, Thailand) located at 14°N, 101°E at an altitude of 317 m above mean sea level. The experiment was carried out in a randomized 2 × 2 factorial design. Two irrigation treatments were applied: well-watered (untreated control), where six sugar apple trees were irrigated during the experiment (30 L/tree/day), and a drought treatment, where six sugar apple trees were not watered for 30 d before harvest. The average amount of rainfall during the experiment period was 1.45 mm/day. Guard trees were grown between the untreated control and drought areas. In total, 72 sugar apple fruits, with uniform color and free from defects, were harvested from the six trees in each treatment (12 fruit/tree), 110 d after full bloom, and were composited in each treatment. Harvested fruits were transported to the Postharvest quality assurance laboratory, King Mongkut's University of Technology Thonburi (Bangkok, Thailand) within 2 h. The fruits were washed with tap water and dried at room temperature. After drying, the fruits in each group were unpacked and randomly redivided into two test groups. In the first group, fruits were kept at 15 °C and 90–95% relative humidity, whereas the second group was kept at 10 °C and 90–95% relative humidity. Fruits from each treatment combination were randomly sampled at 2-day intervals to evaluate the fruit quality and biochemical changes, using three replicates. The collected samples were immediately frozen using liquid nitrogen and kept at −80 °C until analysis, and they were lyophilized before analysis.

Measurement of soil water potential

The soil water potential was measured using a tensiometer (Eastern Agritec; Rayong, Thailand). Three tensiometers were randomly installed at 30 cm soil depth under the trees, and 60 cm distance from the trees in each treatment area. Three trees were used for the soil water potential investigation. The soil water potential (measured in bars) was measured at weekly intervals during the drought stress period.

Analysis of total ascorbic acid concentration

The total ascorbic acid concentration was measured following the method of Roe et al. (1948). A 5 g sample of pulp was homogenized in 20 mL of 5% (weight per volume, w/v) metaphosphoric acid. The homogenate was filtered through filter paper. A 0.4 mL sample of the filtrate was added to 0.2 mL 0.02% (w/v) indophenol solution, and then 2% (w/v) thiourea and 2% (w/v) 2, 4-dinitrophenylhydrazine solution were added, respectively. The mixed solution was incubated for 3 h at 37 °C and then 1 mL 85% (volume per volume, v/v) sulfuric acid was added, and left for 30 min at room temperature. Absorbance was measured at 525 nm using a spectrophotometer (model: UV-1501; Shimadzu; Kyoto, Japan). The same procedure was repeated for a range of ascorbic acid solutions to obtain the standard curve.

Analysis of 2,2-diphenyl-2-picrylhydrazyl-radical scavenging activity

Peel and pulp samples (0.1 g dry weight, DW) were homogenized in 20 mL of 80% ethanol and filtered. Analysis of 2,2-diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging activity was carried out according to the method of Kondo et al. (2004). A test sample of 20 μL was added to 980 μL of 0.1 M DPPH in ethanol, and the combination was mixed and kept for 20 min at room temperature in the dark. The concentration of the antioxidant sample was made from zero to full inhibition at the point where 50% inhibition of reaction in the solution of the sample and DPPH, and the decrease in absorbance at 516 nm was monitored. The data were shown as EC_{50} [half maximum (50%) effective concentration] values.

Analysis of sugar concentration

The sugar concentration was analyzed as reported previously (Kondo et al., 2014). A 1 g dried pulp sample in 10 mL 80% (v/v) ethanol was boiled for 15 min, cooled and then homogenized. The homogenate was filtered and evaporated. The residue was re-

dissolved with 3 mL distilled water and analyzed using high performance liquid chromatography (model L-6200; Hitachi, Tokyo, Japan) with a Shodex ODP2 HP–4E column (Showa Denko; Tokyo, Japan; 4.6 mm internal diameter × 25 cm). The column temperature was set at 30 °C and the mobile phase flow rate was 1 mL/min (75% (v/v) acetonitrile). A refractive index detector was used to identify sugar components.

Measurement of ethylene production and respiration rate

The sugar apple fruit was placed in an air-tight plastic box (700 mL volume) for 2 h at 10 °C or 15 °C. A 1 mL sample of headspace gas was collected and analyzed. The respiration rate (production of carbon dioxide from the fruit) was determined using a gas chromatograph (GC–8A; Shimadzu, Kyoto, Japan) equipped with a 1.8 m packed column of WG–100 at 50 °C and a thermal conductivity detector. The injector temperature was 50 °C, the detector temperature was 80 °C and He gas was used as carrier gas. Ethylene production was analyzed using a gas chromatograph (GC–14B, Shimadzu, Kyoto, Japan) equipped with a 2 m packed column of 80/100-mesh Porapak Q at 80 °C, and a flame ionization detector. The injector temperature was 120 °C, the detector temperature was 120 °C and N_2 gas was used as carrier gas.

Analysis of endogenous abscisic acid concentration

The analysis of the endogenous ABA concentration was performed according to Kondo et al. (2014) with some modifications. A 0.3 g dried sample was homogenized in 20 mL of cold 80% (v/v) methanol with 20 μg ABA–d_6 as an internal standard. The homogenate was centrifuged and then filtered through filter paper, and the residue was washed with 20 mL cold 80% (v/v) methanol, centrifuged, and filtered again. The filtrate was evaporated, then the aqueous residue was adjusted to pH 2.5 with 0.1 M hydrochloric acid and extracted three times with 20 mL 100% (v/v) ethyl acetate. The ethyl acetate phase was evaporated to dryness, re-dissolved three times in 1 mL ethyl acetate and dried. The residue was re-dissolved in 1 mL 4.8 M acetonitrile containing 20 mM acetic acid, filtered through a nitrocellulose filter, purified using high performance liquid chromatography using an ODS Mightysil RP-18 column (250 mm × 4.6 mm internal diameter) with a gradient of 4.8–9.6 M acetonitrile containing 20 mM acetic acid over a period of 30 min, and then held in 9.6 M acetonitrile for 5 min. The fraction containing ABA was collected, evaporated to dryness, re-dissolved three times in 0.5 mL methanol and dried in vacuo. The residue was re-dissolved using 1 mL 10% (v/v) methanol in diethyl ester and then methylated with diazomethane for 10 min. The methyl ester of ABA was quantified and identified using gas chromatography-mass spectrometry-selected ion monitoring (model QP5000; Shimadzu; Kyoto, Japan) with an InertCap 1 MS column (GL Sciences; Tokyo, Japan; 0.25 mm internal diameter × 30 m, 0.25 μm film thickness) and a linear helium flow of 50.2 cm/s. The column temperature was set as follows: 60 °C for 2 min, then increasing from 60 °C to 270 °C at 10 °C/min and finally 270 °C for 35 min. The ions were measured as ABA–d_0 methyl ester/ABA-d_6 methyl ester at *m/z* 190, 260 and 194. The ABA concentration was calculated from the ratio of the peak areas for *m/z* 190 (d_0)/194 (d_6). To identify ABA methyl ester in the samples, fragmentation ion patterns were compared with those of the chemical standard in total monitoring mode.

Measurement of fresh weight loss

Three replications of fruit in each treatment were separated for weight loss investigation. The initial weight of each fruit was measured and recorded before storage in the cold room. Fruit were weighed every 2 d. The fruit weight loss (%) was determined as the difference between the initial and final weights and compared with the initial weight.

Measurement of fruit firmness

Three replications of fruit in each treatment were measured for fruit firmness through the peel. Two measurements were taken on the two opposite sides of the fruit using a Texture Analyzer (model: TA-XTPlus, Stable Micro Systems Ltd.; Surrey, England) equipped with a 5 mm diameter puncture probe. The penetration speed of the probe was fixed at 5 mm/s and the probe penetrated 10 mm into the fruit. The fruit firmness value was expressed in Newtons.

Statistical analysis

The data were presented as mean values ± SE. The SPSS analysis of variance procedure (SPSS Inc.; Chicago, IL, USA) was used to determine the treatment effects, and mean separations were analyzed using Duncan's multiple range test at $p \leq 0.05$. A *t*-test (independent) at the 5% level was used to determine treatment mean differences.

Results and discussion

Soil water potential

The soil water potentials in the drought-treated area were significantly lower than those in the untreated control area, and gradually decreased over the time of treatment (Table 1). In addition, it was found that the value of the soil water potential of the untreated control at 4 wk after treatment was lower than that for 1–3 wk. This may have been due to the higher water demand during fruit development. Furthermore, the vapor pressure deficit (VPD) was higher in the fourth week (data not shown). VPD drives transpiration in the plant which is influenced by relative humidity and temperature (Gates et al., 1998).

Total ascorbic acid concentration and 2,2-diphenyl-2-picrylhydrazyl radical scavenging activity

Ascorbic acid is one of the most important nutritional factors in fruits and vegetables as it is known to have many biological functions in the human body, acting as an antioxidant which could reduce the risk of many diseases such as cardiovascular disease and cancer (Harris, 1996). Moreover, it is also involved in plants undergoing growth and development processes, including their responses to environmental stress (Lee and Kader, 2000). From the results, the highest total ascorbic acid concentrations were found in sugar apple fruit from drought-exposed trees at harvest and at 2 d after storage at 15 °C or 10 °C. On day 6 and day 8 after storage, the

Table 1
Values (mean ± SE) of soil water potential in untreated control and drought-treated areas within the sugar apple orchard.

Weeks after treatment	Soil water potential (bar)	
	Untreated control	Drought
Before treatment	−0.09 ± 0.006 ns	−0.09 ± 0.006
1	−0.10 ± 0.023[a]	−0.37 ± 0.029
2	−0.10 ± 0.023[a]	−0.47 ± 0.040
3	−0.06 ± 0.023[a]	−0.57 ± 0.052
4	−0.31 ± 0.020[a]	−0.62 ± 0.046

[†] ns: non-significant.
[a] Significant at the 5% level using *t*-test.

total ascorbic acid concentration in fruit from drought-exposed trees kept at 10 °C was highest, followed by fruit from untreated control trees kept at 10 °C and the fruit from drought-exposed trees kept at 15 °C (Fig. 1A). The increase in ascorbic acid in sugar apple fruit from the drought-treated trees and also in fruit at lower storage temperature may have been caused by abiotic stress conditions. Normally, the stress conditions can induce ABA biosynthesis which can promote H_2O_2 production during periods of stress; H_2O_2 is classified as a kind of stress signaling that may induce the antioxidant system in plant to maintain or increase the ascorbic acid content (Bayoumi, 2008). Gallie (2013) reported that maintaining a normal level of ascorbic acid in plant cells is a consequence to the tolerance of reactive oxygen species (ROS) from stress conditions without increasing sensitivity to drought conditions.

Radical scavenging activity is an indicator of antioxidant functionality and activity and is related to the presence of bioactive compounds (Heim et al., 2002). EC_{50} refers to the concentration of substrate that indicates a 50% loss in DPPH scavenging activity. A high EC_{50} value indicates low antioxidant activity. Drought stress for 30 d before harvesting significantly decreased the DPPH EC_{50} values in both the peel and pulp of sugar apple at harvest (Fig. 1B,C). Subsequently, the DPPH EC_{50} value significantly decreased in the peel of fruit from drought-exposed trees kept at 10 °C, and this was lower than those in other treatments (Fig. 1B).

The fruit from drought-exposed trees kept at 10 °C delayed an increase in the DPPH EC_{50} value in pulp and had a lower value than in other treatments. The DPPH EC_{50} value in pulp of fruit from drought-exposed trees kept at 15 °C gradually increased throughout the storage period. At the end of storage, the DPPH EC_{50} values in pulp of fruit kept at 10 °C were lower than at 15 °C, with no significant differences between treatments (control and drought treatment) at the same storage temperature (Fig. 1C). Alali et al. (1999) reported that sugar apple peel contains many bioactive compounds and that the peel is an important part of the fruit which is exposed to environment, and functions as protection

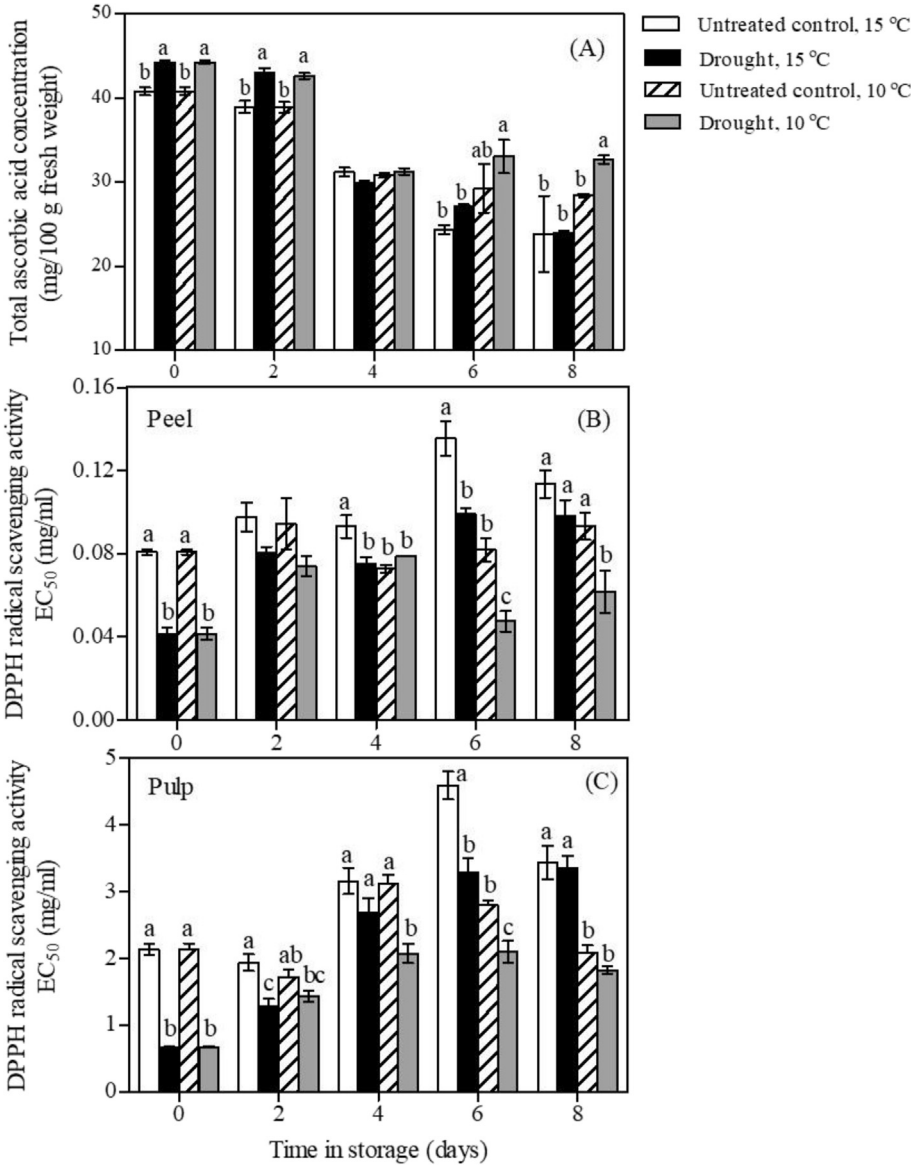


Fig. 1. Total ascorbic acid (A) concentration in sugar apple fruit, and DPPH radical scavenging activity (EC_{50} value) in peels (B) and pulps (C) of sugar apple fruit subjected to drought stress at harvest and during storage at 10 °C or 15 °C. Data are means ± SE of three replicates. Mean separation in each storage period determined using Duncan's multiple range test at 5% test level.

against infection by pathogens and pests. Therefore, to understand sugar apple fruit physiology, the current study involved the measurement of the DPPH scavenging activity in the peel separately from the pulp.

The generation of ROS has been shown to be induced by water deficit (Shao et al., 2008). The enhancement of production and the ability of antioxidants may play an important role in the detoxification of ROS. Samieiani and Ansari (2014) reported that water stress raised the DPPH radical scavenging activity in many groundcover plants. As noted, ascorbic acid has a function as an antioxidant (Noctor and Foyer, 1998). In the current study, the ascorbic acid concentration and DPPH radical scavenging activity were enhanced in fruit from drought-exposed trees at harvest, and 2 d after being held at 15 °C or 10 °C. This suggested that an increase in ascorbic acid concentrations may have a role in the scavenging of free radicals, which accumulate under drought conditions as mentioned earlier. In general, the degradation of ascorbic acid is rapid after harvesting and increases as the storage time and temperature increases (Nunes, 2008). Previous study reported that ascorbic acid concentration in broccoli stored at 1 °C decreased progressively during storage (Serrano et al., 2006). In contrast, the ascorbic acid concentration in citrus fruits (lemon, orange and lime) was higher at 20 °C than at 30 °C (Njoku et al., 2011). These results indicated that the change in the ascorbic acid concentration in a plant is temperature dependent. The current study found that there were higher ascorbic acid concentrations and lower DPPH EC_{50} values in sugar apple fruit stored at lower temperature after 4 d of storage, especially with fruit from drought-exposed trees. Moreover, the DPPH EC_{50} values were lower in the peel than in the pulp (Fig. 1B,C), indicating that sugar apple peel had a higher antioxidant activity than sugar apple pulp. This finding may imply that sugar apple fruit accumulates various antioxidants in the peel to prevent infestation of pests and diseases (Manochai et al., 2014). Although the sugar apple peel is not usually a consumed part of the fruit, as it has high free radical scavenging effects, it is possible that it can be used as a source of antioxidant in the pharmaceutical and food industry. In addition, for greater understanding of the effect of drought stress treatment on the antioxidative system in sugar apple fruit, changes in the enzymatic antioxidant activity should be investigated in future work.

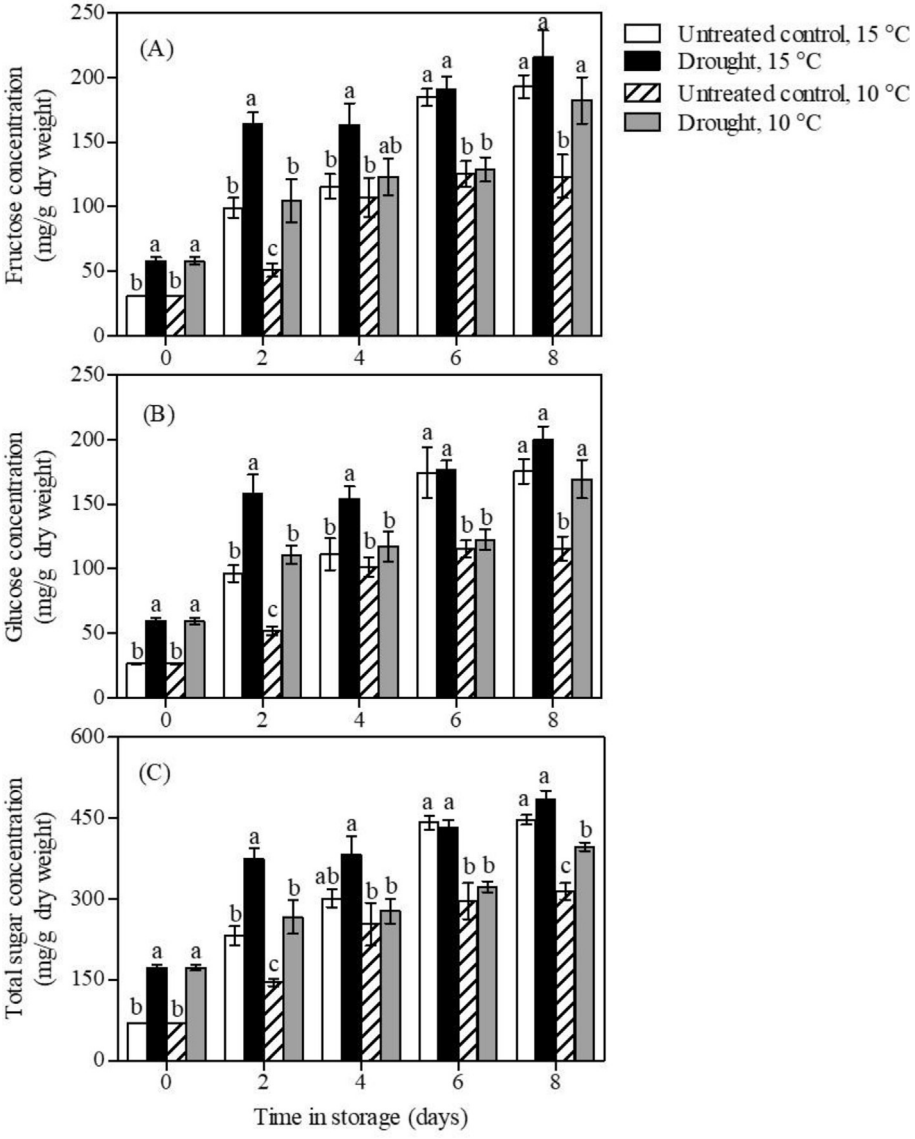


Fig. 2. Fructose (A), glucose (B), and total sugar (C) concentrations in sugar apple fruit subjected to drought stress at harvest and during storage at 10 °C or 15 °C. Data are means ± SE of three replicates. Mean separation in each storage period determined using Duncan's multiple range test at 5% test level.

Sugar concentration

Soluble sugar is one of the major osmotic compounds that accumulate in the fruit of many kinds of fruit trees (Ripoll et al., 2014). The sugar concentration in sugar apple is an important parameter that relates to fruit quality, especially to the taste and has also been related to the response to drought stress (Kowitcharoen et al., 2017). The current study found that the fructose, glucose and total sugar concentrations significantly increased in the fruit from drought-stressed trees at harvest. (Fig. 2A–C). Previous studies also reported that drought stress induced sugar accumulation in Satsuma mandarin (*Citrus unshiu* Marc. 'Okitsu-Wase'; Yakushiji et al., 1998) and peach (Kobashi et al., 2000). This suggested that the increase in sugar concentrations may be associated with plant defense mechanisms against drought stress. Sugar acts as a compatible solute that accumulates in cells, which has a role in osmotic adjustment, preventing turgor loss in tissue (Clifford et al., 1998). Moreover, sugar enhancement by drought may improve the quality of sugar apple fruit, as such fruit was sweeter. During storage, the fructose, glucose and total sugar concentrations in fruit from the drought-exposed trees stored at 15 °C significantly increased and were higher than those for the other treatments during the first 4 d of storage (Fig. 2A–C). Thereafter, the fructose, glucose and total sugar concentrations increased at 15 °C, and corresponded with increasing ethylene production. At the end of storage, there was no difference in the levels of fructose and glucose in the fruit from control trees stored at 15 °C and the fruit from drought-exposed trees stored at 15 °C or 10 °C, but these levels were significantly higher than those in the fruit from control trees stored at 10 °C (Fig. 2A,B). However, the total sugar concentrations were significantly higher in fruit from both control and drought-exposed trees stored at 15 °C compared with fruit stored at 10 °C (Fig. 2C). Vishnu Prasanna et al. (2000) reported an elevated sugar concentration at high temperatures (25 °C and 20 °C) compared with low temperatures (15 °C or 10 °C). Ethylene has been implicated as having a role in the conversion of starch to sugar in fruit (Watkins, 2003). During the ripening process of climacteric fruit, the fruit emit ethylene and the ethylene signal causes the hydrolysis of starch into soluble sugars such as sucrose and glucose, associated with acceleration of amylase in order to increase sweetness (Koning, 1994). This could imply that induction of sugar accumulation in sugar apple fruit during storage was associated with ethylene production. Although high sugar and antioxidant activity were observed in sugar apple fruit from drought-stressed trees, reductions in fruit size and weight were found (data not presented). Cells were smaller in pear fruit (*Pyrus communis* L.) that had experienced water deficit (Lopez et al., 2011).

Ethylene production and respiration rate

At harvest, the drought stress treatment had no significant effect on ethylene production and the respiration rate in sugar apple fruit (Fig. 3A,B). The ethylene production rate in fruit from the drought treatment that was kept at 15 °C sharply increased to a peak on day 4. The changes in ethylene production in fruit from the untreated control kept at 15 °C or 10 °C showed a similar trend, which increased and reached a peak on day 6 and decreased thereafter (Fig. 3A). This finding agreed with a previous report that water deficit causes an increase of endogenous ethylene, which leads to accelerated ripening of fruit such as bananas (Burdon et al., 1994). Storage temperature affected the rate of ethylene production, where a higher rate was observed in fruit held at 15 °C compared with fruit held at 10 °C, indicating that higher temperatures accelerated physiological changes. These results suggested that drought stress may induce fruit ripening by enhancing ethylene production, especially at higher storage temperatures and as a consequence of the accumulation of a higher sugar concentration.

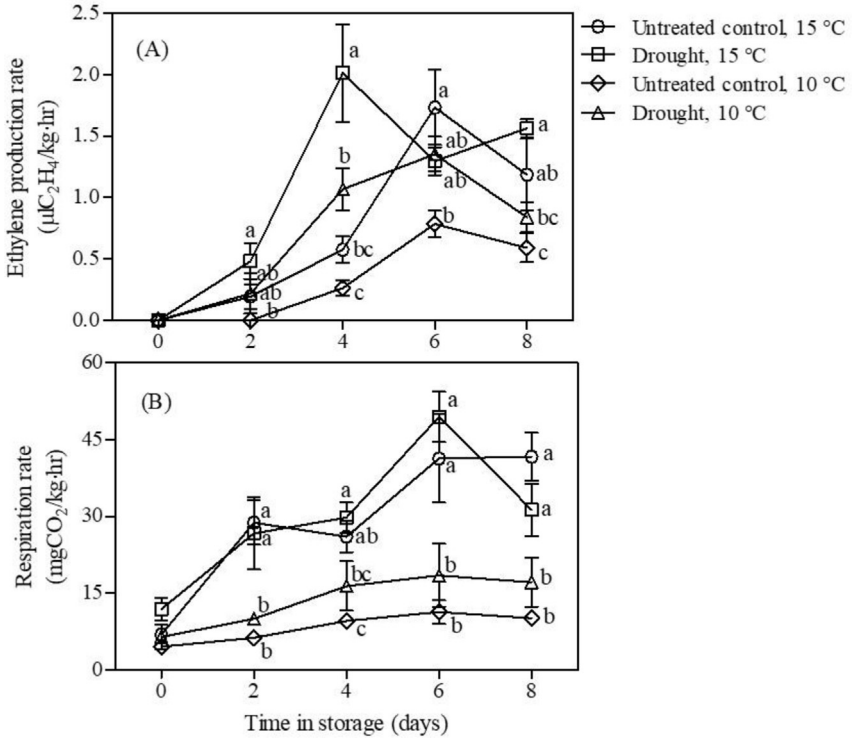


Fig. 3. Ethylene production (A) and respiration rate (B) of sugar apple fruit subjected to drought stress at harvest and during storage at 10 °C or 15 °C. Data are means ± SE of three replicates. Mean separation in each storage period determined using Duncan's multiple range test at 5% test level.

Drought stress had no effect on the respiration rate during storage. In fact, increasing the respiration rate in sugar apple fruit positively correlated with increasing the storage time (Fig. 3B). The rate of increase was significantly higher at 15 °C than at 10 °C. These results suggested that low temperatures may slow down the metabolic activities and consequently delay fruit senescence as reported in many studies (Mworia et al., 2012; Freitas and Mitcham, 2013; Li et al., 2017). Storage at 10 °C effectively reduced the respiration and ethylene production rate resulting in delayed fruit ripening and retardation of sugar accumulation.

Endogenous abscisic acid concentration

ABA is an important plant hormone associated with fruit development, physiology and drought stress tolerance (Ripoll et al., 2014). ABA synthesis is a rapid response to drought stress in plants and is also a health-promoting phytochemical that can be found in fruits and vegetables and is effective against diseases such as type II diabetes, obesity-related inflammation and atherosclerosis-induced hypertension (Guri et al., 2007, 2010). To understand sugar apple fruit physiology, changes in the ABA concentration in fruits subjected to drought stress during storage were analyzed in the peel separately from the pulp. This study found that the endogenous ABA concentrations in the peel and pulp of sugar apple fruit from drought-exposed trees at harvest were significantly higher than those in the control, well-watered trees (Fig. 4A,B). The increasing ABA concentration may be associated with drought stress tolerance systems (Kowitcharoen et al., 2015). This result was consistent with a previous report that endogenous ABA concentrations in peach fruit increased significantly under drought stress (Kobashi et al., 2000). The synthesis of ABA appeared to change over time during the first 4 d of storage; the ABA concentrations in the peel and pulp of fruit from drought-exposed trees stored at either 15 °C or 10 °C remained higher than those of fruit from untreated trees stored at these temperatures (Fig. 4A,B), indicating that drought stress affected the ABA accumulation in the peel and pulp of sugar apple. However, over the following days of storage, temperature also seemed to affect ABA accumulation, where fruit from drought-exposed and untreated trees held at 15 °C had increased ABA concentrations. This result may have been due to drought stress and the higher temperatures inducing fruit senescence, which led to an increase in ABA synthesis. Kondo et al. (2002) reported that *trans*-ABA may increase with mangosteen fruit senescence. The change in the ABA concentrations in the current experiment implied that a pre-harvest drought stress treatment may provide a way to stimulate the synthesis and accumulation of this beneficial phytochemical in sugar apple.

Fresh weight loss and fruit firmness

The percentage of fresh weight loss in sugar apple fruits from the untreated control and drought-exposed trees kept at 15 °C substantially increased with storage time. While a gradual increase in the weight loss was recorded in sugar apple fruit from untreated control and drought-treated trees kept at 10 °C, there was a significant difference in the fresh weight loss between storage temperatures (Fig. 5). A higher weight loss at higher temperature could be related to the acceleration of transpiration, respiration and ripening as previously reported by Lebibet et al. (1995) and Vishnu Prasanna et al. (2000). In the current study, the rate of respiration increased rapidly at 15 °C (Fig. 3B) and may have been the main factor influencing the weight loss that was observed.

Drought stress treatment for 30 d before harvesting the sugar apple fruit had no significant effect on fruit firmness at harvest (Fig. 6). However, the fruit firmness in all treatments decreased as storage progressed and as storage temperature increased. Fruit

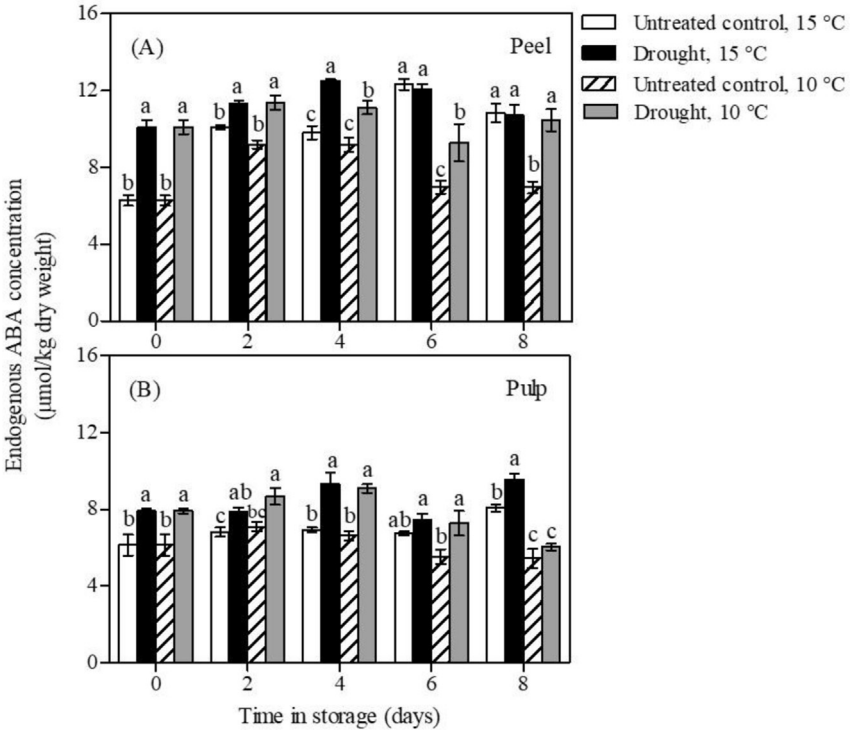


Fig. 4. Endogenous ABA concentration in peel (A) and pulp (B) of sugar apple fruit subjected to drought stress at harvest and during storage at 10 or 15 °C. Data are the means ± SE of three replicates. Mean separation at each storage period determined using Duncan's multiple range test at 5% test level.

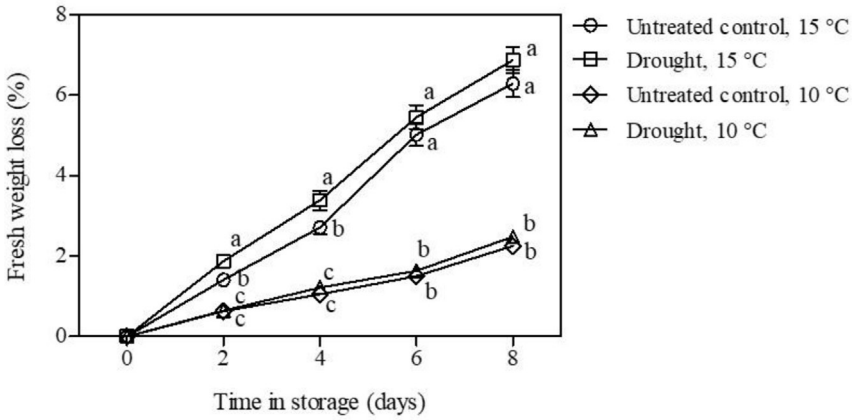


Fig. 5. Fresh weight loss of sugar apple fruit subjected to drought stress at harvest and during storage at 10 or 15 °C. Data are means ± SE of three replicates. Mean separation at each storage period determined using Duncan's multiple range test at 5% test level.

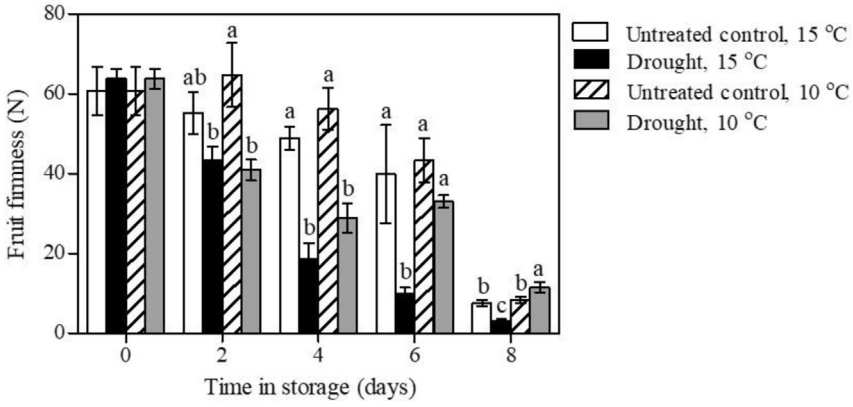


Fig. 6. Fruit firmness of sugar apple fruit subjected to drought stress at harvest and during storage at 10 or 15 °C. Data are means ± SE of three replicates. Mean separation at each storage period determined using Duncan's multiple range test at 5% test level.

from drought-exposed tree stored at 15 °C showed rapid loss of firmness compared with that of other treatments. This could be ascribed to drought stress accelerating fruit softening as mentioned above, and this response may be associated with the induction of ethylene production in response to drought stress. An ethylene-mediated response may up-regulate cell wall modification enzymes and subsequently accelerate softening (Toivonen and Hodges, 2011). In addition, the current study found that the decrease in fruit firmness in all treatments had a very high correlation with fresh weight loss (R^2 = 0.93, data not shown); this change was due to the fresh weight loss causing a reduction in turgor pressure (Harker and Hallett, 1994). These results were in agreement with Shackel et al. (1991) who found a decrease in turgor pressure of tomato (*Lycopersicon esculentum* Mill.) coincided with losses in fruit firmness.

In summary, ascorbic acid, sugar and ABA concentrations and antioxidant activity increased in fruit from drought-exposed trees at harvest. In addition, drought stress also activated antioxidant activity, enhanced sugar accumulation and induced fruit ripening in sugar apple fruit during low temperature storage. The changes in fruit qualities were temperature dependent; at 15 °C the sugar apple ripened faster than at 10 °C. These results suggested that pre-harvest drought stress treatments may enhance the eating quality of sugar apples, especially the increase of antioxidant activity and sugar concentration. However, the induction of fruit ripening by drought stress treatment must be considered for application in proper postharvest technology.

Conflict of interest

There is no conflict of interest.

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