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Post-harvest application of boric acid on grapes to improve the shelf life and maintain the quality

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Abstract: Boric acid (BA) is commercially acceptable and economically feasible material to enhance the shelf life of pears, oranges and other horticultural plants. Here, we investigated the effect of BA on the shelf life and post-harvest quality of table grapes (cv. 'Kyoho'). The grapes were immersed in a BA solution with different concentrations [0 (as the control), 10, 30, 50 mM] for 10 min and stored at 25 ± 1 °C for 10 days. Compared to the control, the BA treatments maintained higher berry firmness by inhibiting the activity of polygalacturonase (PG) and cellulase. In addition, the BA-treated grapes maintained higher antioxidant enzyme activities, such as catalase (CAT) and superoxide dismutase (SOD), and lower metabolic toxic products, like the superoxide anion (O_2^-) production rate, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content than the control. The experimental results showed that the post-harvest application of BA effectively delays the senescence of grapes compared with the control, and the 10 mM BA treatment had the most obvious effect.

Keywords: Kyoho; boric acid; postharvest; antioxidant enzyme

The grape (*Vitis vinifera* L.) is one of the most widely consumed fruits all over the world. At present, China has become one of the world's biggest grape producers. Over 84% of the total land used for grape production in China is cultivated for the table grapes (Sun et al. 2020). However, being a non-climacteric fruit, grapes show a reduction in shelf life due to a rapid loss of weight and firmness after harvest. Moreover, because of the thin pericarp and succulent flesh, table grapes are vulnerable to mechanical damage and fungal infections, which leads to rotting, decay and dehydration during storage (Meng et al. 2010). Senescence and post-harvest diseases have recently limited the development of the table grape market. Therefore, it is necessary to explore more storage strategies for table grapes.

In this context, different methods, such as preharvest and post-harvest applications of kombucha (Zhou et al. 2019), short-term high CO_2 (Vazquez-Hernandez et al. 2018), an aloe vera gel (Ehtesham

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Nia et al. 2021), a calcium-based preparation aerosol (Cherviak et al. 2021), edible coatings (1.5% chitosan and 1.0% poly-ε-lysine) (Chen et al. 2019) were used to maintain the firmness and prolong the shelf life of fresh table grapes. In addition, chemical fungicides are widely used in vineyards to control the post-harvest diseases of grapes (Ehtesham Nia et al. 2021). However, a large amount of chemical spraying will lead to adverse effects for the environment and on consumer health. Hence, there is an urgent need to explore more environmentally friendly and cost-effective methods to solve these problems.

Boron is accepted as an essential nutrient for all vascular plants, animals and humans. Boron regulates the metabolic activities by interacting with magnesium, calcium and vitamin D, which are all necessary for bone metabolism (Devirian, Volpe 2003). In addition, boron has disinfectant and bactericidal properties which inhibit the fruit decay after harvest and play a crucial role in maintaining the rigidity of the fruit cytoderm and phenolic concentration (Kaur et al. 2019). It was reported that boron has an obvious effect on the prevention and control of grey mould of table grapes caused by *B. cinerea* (Qin et al. 2010). Additionally, many studies have revealed that boric acid (BA) has chemical properties inhibiting the initial increase of ethylene production (Ahmadnia et al. 2013) and suppresses the activity of 1-Aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase (Moon et al. 2020). It has also been reported that BA enhances the storage life of tomatoes (Wang, Morris 1992), retains the storability and quality of pear fruits (Kaur et al. 2019), extends the shelf life and quality maintenance of guavas (Singh et al. 2017) and improves the post-harvest quality of cut carnations (Ahmadnia et al. 2013). However, little information is available on the effect of BA applications on the post-harvest quality of table grapes during storage. Accordingly, the objective of the present study was to evaluate the potential of a post-harvest treatment of BA to extend the shelf life of table grapes during storage. It was hypothesised that different concentrations of BA would enhance the storability and quality of the table grape ('Kyoho'). To evaluate the sensory attributes, the weight loss rate and firmness of the post-harvest grapes with the addition of BA were determined. To evaluate the reactive oxygen species (ROS) metabolic indicators causing the post-harvest senescence, the O_2 production rate, H_2O_2 content, superoxide dismutase (SOD) and catalase (CAT) activity, and malondialdehyde (MDA) content of the table grapes were also determined. The total soluble solid (TSS), ascorbic acid (AsA) content as well as the polygalacturonase (PG) and cellulase activity were measured to evaluate the nutritional value and quality-related chemical parameters.

MATERIAL AND METHODS

Plant materials and experimental treatments. Table grapes ('Kyoho') that were collected from a grape vineyard were employed in this study. The grapes were selected based on the uniformity in shape and appearance, and due to the absence of any visible defects. All the grapes were harvested after ripening and analysed at the Engineering Technology Research Center of Quality Regulation and Controlling of Horticultural Plants. The grapes were divided into four sets and immersed in 0, 10, 30 and 50 mM BA for 10 minutes. The concentration gradient and treatment time were set according to previous reports (Singh et al. 2017; Kaur et al. 2019). Then the grapes were wrapped in a preservative film and stored in a tissue culture room at 25 ± 1 °C for 10 days. Grape berries were taken every two days for a total of 6 times, i.e., sampling at 0, 2, 4, 6, 8, 10 days after the treatment. The sampled grape berries were wrapped in aluminium foil and immediately frozen in liquid nitrogen and then stored at –40 °C for the subsequent analysis of the physiological indicators. Each treatment was carried out in three replicates, each of which contained five clusters of grapes.

Determination of the weight loss rate, firmness, TSS content. The weight of the BA-treated 'Kyoho' grapes was measured at the 0^{th} storage day and 10^{th} storage day using an analytical balance. The weight loss rate was calculated by the following formula:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

The firmness of the berries was measured using a durometer (FT–327, Wuxi, China) and the TSS concentration was measured with a saccharimeter (ATC–32, Shanghai, China), as described previously (Guo et al. 2019).

Determination of the ascorbic acid (AsA) content. The AsA was measured according to the method described previously (Ge et al. 2015). Fro-

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zen tissue (0.5 g) was homogenised with 4.0 mL of pre-chilled 5% metaphosphoric acid and centrifuged at 12,000 × g for 10 min at 4 °C. The supernatant was used to measure the AsA content. The mixture solution was measured at 525 nm and expressed as mg AsA/g fresh weight (FW). A standard curve with ascorbic acid was used to calculate the AsA content.

Determination of the superoxide anion (O_2^-) production rate and H_2O_2 content. The production rate was measured according to the methods in (Ge et al. 2015). The absorbance of the extracting solution was recorded at 530 nm. A standard curve with sodium nitrite was used to calculate the production rate following the reaction equation of with hydroxylamine. The production rate of was expressed as nmol/min/g FW.

The H_2O_2 content of the grape berries was measured spectrophotometrically after reaction with potassium iodide (Chakrabarty, Datta 2007). The reaction mixture was measured at 390 nm, a 10% Trichloroacetic acid (TCA) solution was used as the control. The H_2O_2 content was calculated using a standard curve with known H_2O_2 concentrations.

Determination of the SOD activity and CAT activity. The SOD and CAT were extracted and assayed according to the methods described previously with some modifications (Sun et al. 2011). Frozen grape berry tissue (0.5 g) was extracted using 2.0 mL of a 0.05 M sodium phosphate buffer (pH 7.8) containing 0.1% (w/v) polyvinyl pyrrolidone for 5 minutes. The extract solution was centrifuged for 20 minutes at 12.000 × g at 4 °C. The supernatant was collected for the determination of the SOD and CAT activities.

The SOD activity was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). A total of 0.5 mL of the enzyme solution was added into 3.0 mL of an assay reagent consisting of 130 mM methionine, 30 μM Ethylenediaminetetraacetic acid (EDTA), 750 μM NBT, 20 mM riboflavin in a 0.05 M sodium phosphate buffer (pH 7.8). The reaction solutions were incubated for 20 minutes under 4 000 lux illumination. The absorbance of the sample was spectrophotometrically measured at 560 nm and a 0.05 M sodium phosphate buffer (pH 7.8) was used as the control. The SOD activity was expressed as U/g FW, where 1 U is the amount of enzyme that caused the 50% inhibition of the NBT reduction.

The assay mixture for determining the CAT activity consisted of 0.3 mL of 0.1 M H_2O_2 prepared by the 0.05 M sodium phosphate buffer (pH 7.8) and 0.5 mL of the enzyme solution. The decrease in absorbance at 240 nm was recorded for 2 minutes at 25 °C and the CAT activity was expressed as U/g FW/min, where 1 U was defined as the amount of enzyme that caused a change of 0.01 in absorbance per minute.

Determination of the malondialdehyde (MDA) content. The method described previously with some modifications (Ehtesham Nia et al. 2021) was employed to measure the MDA content. Frozen grape berry tissue (0.5 g) was homogenised for 5 minutes in 5.0 mL of 10% (w/v) trichloroacetic acid. The homogenate was centrifuged for 15 minutes at 12 000 × g. Three millilitres of the supernatant was added to 3.0 mL of 0.67% (w/v) trichloroacetic acid. The mixture solution was heated for 20 minutes at 100 °C, quickly cooled in an ice-bath for 10 minutes and then centrifuged for 15 minutes with 12 000 × g at 4 °C. The absorbances were measured at 532, 450 and 600 nm. The MDA concentration was calculated as follows:

$$\text{MDA content (mmol/g FW)} = [6.45 (OD_{532} - OD_{600}) - 0.56OD_{450}] \times 5 \text{ mL}/0.5 \text{ g}$$

Determination of the polygalacturonase and cellulase activity. The PG and cellulase activity were measured using the methods described in (Abu-Sarra, Abu-Goukh 2015). The reaction mixture contained 0.5 ml of a crude enzyme, 2.0 ml of 0.5% pectin was incubated at 37 °C for 30 minutes. After the constant temperature reaction, 3.5-dinitrosalicylic acid (DNS) was added, and the mixed solution was boiled for 5 minutes. The absorbance was measured at 540 nm. One pectinase activity unit was 1.0 mg of galacturonic acid produced by pectin decomposition at 37 °C per gram of fresh sample per minute.

The cellulase activity was determined by the same procedure as the PG assay, but the reaction temperature and time was 40 °C, 60 minutes, and the substrate was 1% carboxymethyl cellulose. The cellulase activity unit was 1.0 mg of glucose produced by the decomposition of carboxymethyl cellulose at 40 °C per gram of fresh sample per minute.

Statistical analysis. The data presented as mean ± standard deviation (SD) from three replicates were tested using the SPSS 21.0 software (IBM, Chicago, IL, USA). Significant differences among the mean values were determined using Duncan's multiple range test, and differences at $P < 0.05$ were consid-

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ered significant. The figures were produced using GraphPad Prism 9.0.

RESULTS

Effects of the BA treatment on the weight loss and firmness of the 'Kyoho' berries. In the 10 mM BA treatment group, the weight loss rate was significantly lower than the control, while there was no significant difference among the other groups (Figure 1A). The results showed that that the three treatments of BA are not all beneficial to the water loss index, and only 10 mM BA was effective in preventing the weight loss of the berries.

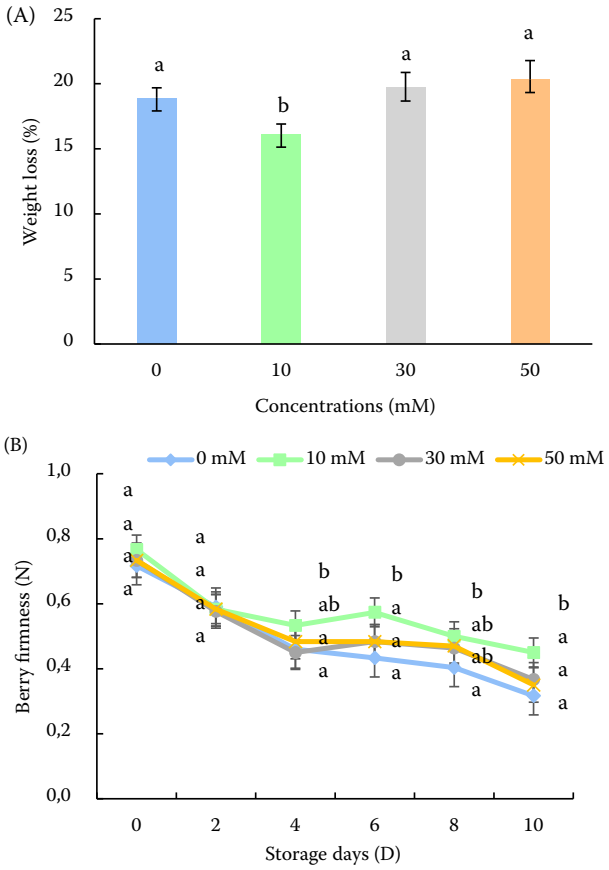

Figure 1. Effects of the boric acid (BA) treatment on the weight loss rate (A) and firmness (B) of 'Kyoho' grape berries

The concentrations of the BA treatment were 0 (as the control), 10, 30 and 50 mM. The vertical bars indicate the mean ± standard deviation (SD), n – 3 replicates

The bars followed by the same letter are not significantly different at $P < 0.05$. Significant differences among the means were determined using Duncan's multiple range test

The firmness of grape berries gradually decreased during storage (Figure 1B), and it was higher in the 10 mM BA treatment group than that of berries in the other BA treatment and the control groups. This suggests that the 10 mM BA treatment could prevent a reduction in the grape quality and firmness during storage to a certain extent (Figure 1B).

Effects of the BA treatment on the TSS content and AsA content. The variation trend of the TSS after the BA treatment with the different concentrations was consistent with that of the control (Figure 2A). The TSS content in the 10 and 30 mM treatments were significantly different on the second and fourth days of storage, but there was no significant difference at the other times (Figure 2A).

The AsA content of the 10 mM BA treatment grape berries were significantly higher than the control over all the storage days (Figure 2B). In addition, the AsA content of the 30 mM BA treatment was also significantly higher than the control except on the 8th storage day (Figure 2B).

Effects of the BA treatment on the MDA content, superoxide anion (O_2^-) production rate and H_2O_2 content. Changes in the MDA content of the grapes are shown in Figure 3A. The MDA content of the berries increased during the first 4 days of storage, reaching the peak on day 4, and then declined from day 4 to day 10. At the peak levels, the MDA content was the lowest in the10 mM BA treatment group (Figure 3A). Over all the storage times other than at the 10th storage day, the MDA content in the 10 mM BA treatment was significantly lower than the control. The data show that the BA inhibited the production of MDA, and it effectively slowed the senescence rate of the grape berries at aconcentration of 10 mM (Figure 3A).

The superoxide anion (O_2^-) production rate of the grape berries showed a similar profile in all the treatment groups, and the BA treatment groups were lower than the control group on the whole, especially in 10 mM BA treatment group (Figure 3B). Additionally, the 10 mM BA treatment had the lowest hydrogen peroxide content in the grape berries among all the treatments (Figure 3C).

Effects of the BA treatment on the SOD and CAT activities. The patterns of the SOD activity in the different treatments are shown in Figure 4A and the SOD activity gradually increased during storage (Figure 4A). The SOD activity of the berries in the 10 mM BA treatment was higher than that of the control group on the whole (Figure 4A).

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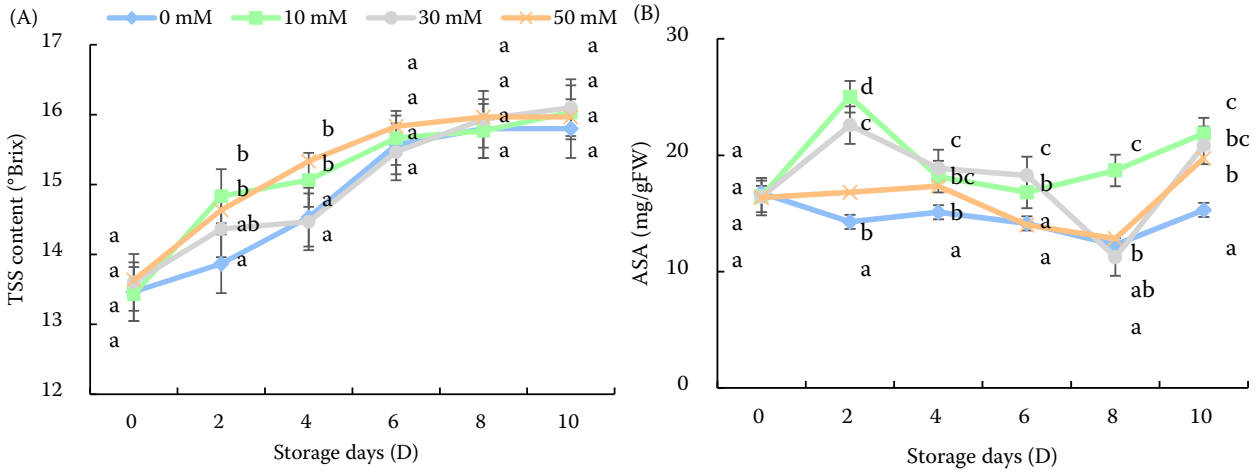

Figure 2. Effects of the boric acid (BA) treatment on the TSS (A) and AsA content (B) of 'Kyoho' grape berries

The concentrations of the BA treatment were 0 (as the control), 10, 30 and 50 mM

The vertical bars indicate the mean ± standard deviation (SD), n – 3 replicates

The bars followed by the same letter are not significantly different at $P < 0.05$. Significant differences among the means were determined using Duncan's multiple range test

The CAT activity profile was similar among the different BA treatments and the control, with a gradual increase from day 0 to day two and from day four to day six, followed by a decrease from day to to day four and from day six to day 10 (Figure 4B). The CAT activity was significantly higher in the 10 mM BA treatment group than in the control from day 0 to day eight. During the storage, the highest

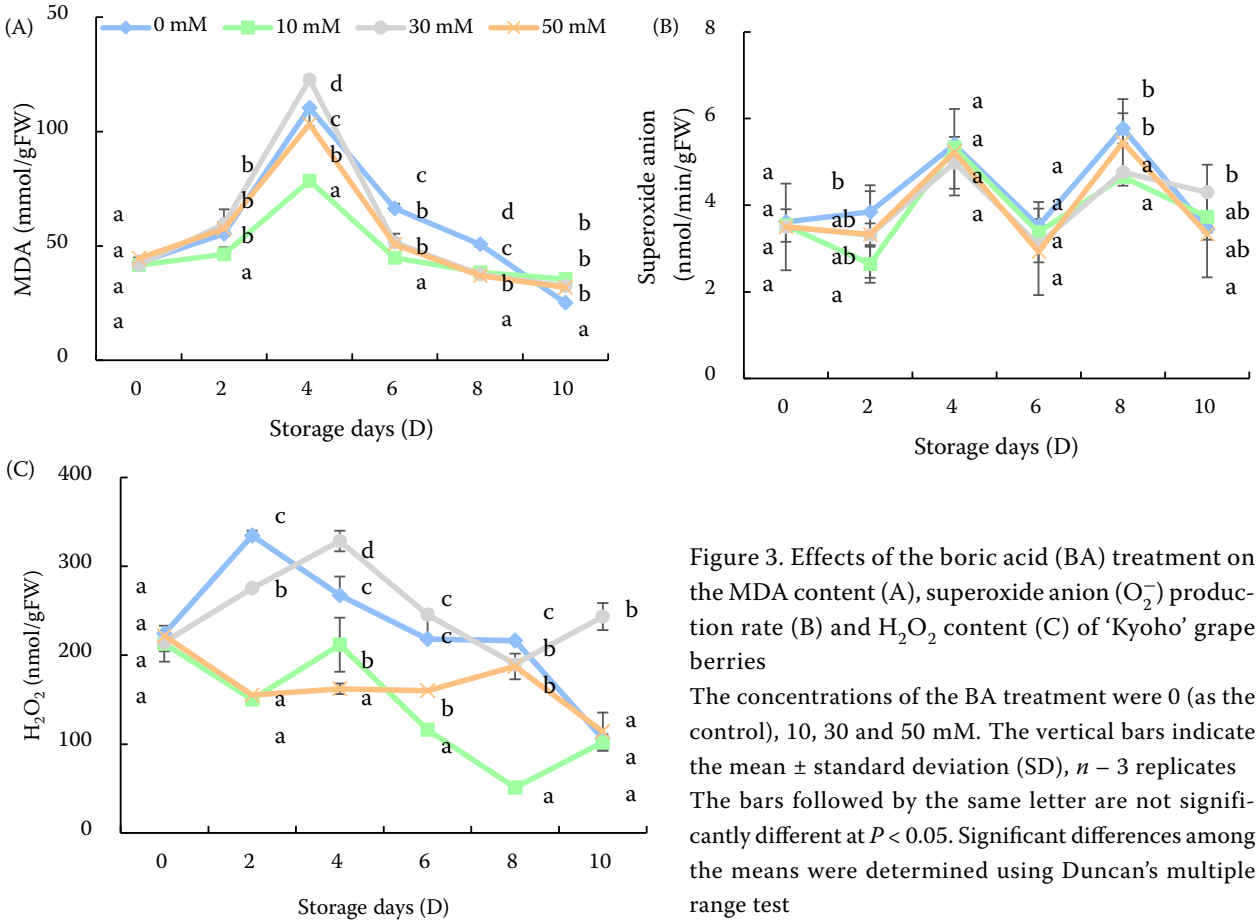

Figure 3. Effects of the boric acid (BA) treatment on the MDA content (A), superoxide anion (O_2^-) production rate (B) and H_2O_2 content (C) of 'Kyoho' grape berries

The concentrations of the BA treatment were 0 (as the control), 10, 30 and 50 mM. The vertical bars indicate the mean ± standard deviation (SD), n – 3 replicates

The bars followed by the same letter are not significantly different at $P < 0.05$. Significant differences among the means were determined using Duncan's multiple range test

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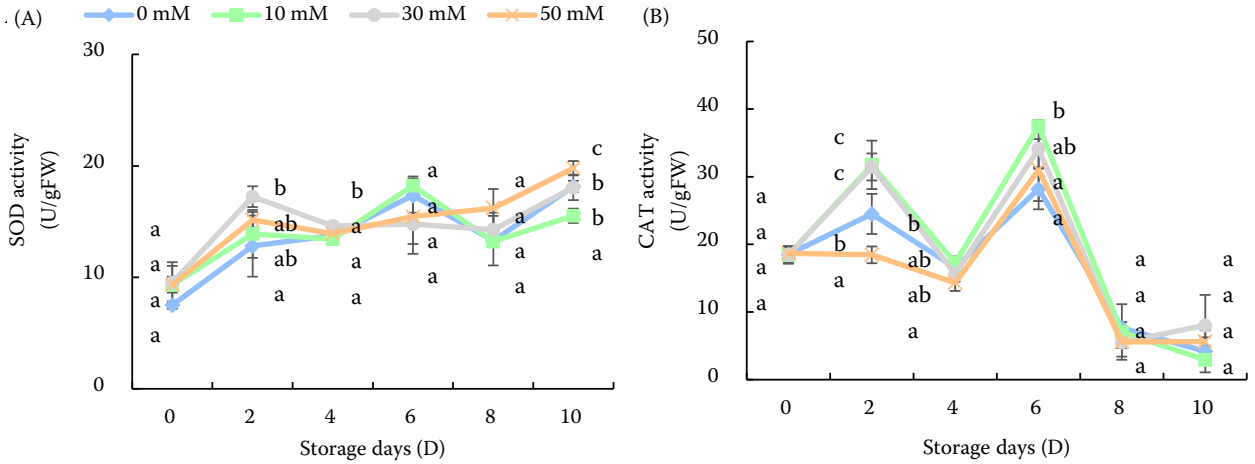

Figure 4. Effects of the boric acid (BA) treatment on the SOD (A) and CAT (B) activities in 'Kyoho' grape berries
The concentrations of the BA treatment were 0 (as the control), 10, 30 and 50 mM
The vertical bars indicate the mean ± standard deviation (SD), n – 3 replicates
The bars followed by the same letter are not significantly different at $P < 0.05$. Significant differences among the means were determined using Duncan's multiple range test

CAT activity was detected on day six in the 10 mM BA treated berries (Figure 4B).

Effects of the BA treatment on the PG and cellulase activities. The PG and cellulase activities in the 10 mM treatment group were lower than that in the control group (Figure 5). The PG activity increased gradually and then stabilised at a certain range (Figure 5A), moreover, the cellulase activ-

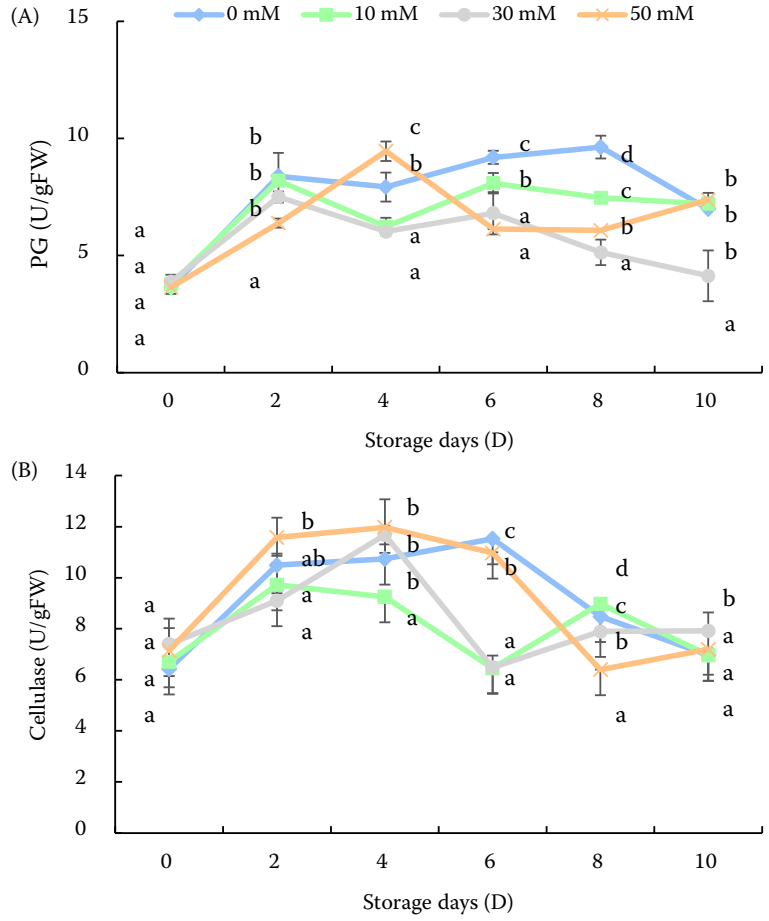

Figure 5. Effects of the boric acid (BA) treatment on the PG (A) and cellulase (B) activities in 'Kyoho' grape berries
The concentrations of the BA treatment were 0 (as the control), 10, 30 and 50 mM
The vertical bars indicate the mean ± standard deviation (SD)
n – 3 replicates
The bars followed by the same letter are not significantly different at $P < 0.05$ Significant differences among the means were determined using Duncan's multiple range test

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ity in the grape berries firstly increased, then decreased slightly, and significant differences were observed among the various BA treatment groups (Figure 5B).

DISCUSSION

It is essential to maintain the post-harvest quality of grapes during storage, which are mostly consumed in a fresh state (Jung et al. 2018). In order to improve the shelf life of table grapes, it is very important to delay the ageing process of grapes. Senescence is a complex genetic programming process that is used to describe a series of events that culminate in cell death at the last development period, including structural deterioration and macromolecule degradation (Noodén et al. 1997). In table grapes, senescence is closely related to the reactive oxygen species accumulation (Zhang et al. 2019). The excessive production of ROS can damage the cells and accelerate the senescence of grapes. Plant cells have developed two main scavenging mechanisms of ROS under oxidative stress which can be categorised as an enzymatic system and a non-enzymatic system (Shao et al. 2008). In this study, the BA treated grapes had a lower H_2O_2 content and superoxide anion (O_2^-) production rate than of the control except for the individual storage days in the 30 mM treatment group (Figure 3), indicating that the BA treatment may control the excessive production of ROS to a certain extent during storage of table grapes. Meanwhile, the BA treated grapes maintained a higher CAT and SOD activity than the control and the highest CAT activity was detected on the 6th day of storage in the 10 mM BA treated berries (Figure 4).

Membrane deterioration and degradation is an early and essential characteristic of the signal transduction pathways that occur in plant senescence (Bhattacharjee 2005). In the meantime, membrane lipid peroxidation is the main cause of plant cell senescence, leading to the loss of the membrane integrity, physical structure and fluidity, thus affecting the protein function. (Shewfelt, Del Rosario 2000). The loss of membrane integrity is associated with the senescence of grapes and accompanied by the disorder of ROS, especially the high levels of H_2O_2 and MDA. Malondialdehyde, as a toxic by-product of ROS metabolism and the end product of lipid peroxidation, is used to reflect the degree of lipid peroxidation of the cell membrane (Hodges et al. 1999). The MDA content of the 10 and 50 mM BA treatment groups were lower than that of the control, and the MDA content of the 10 mM BA treatment group was the lowest (Figure 3A). The measurement results of the hydrogen peroxide content (Figure 3C) in the samples were similar to the MDA content, and the 10 mM BA treatment had the lowest hydrogen peroxide content in the grape berries among all the treatments. The results showed that the BA treatment significantly reduced the over-production of MDA and H_2O_2 during the storage period and inhibited the lipid peroxidation. This is possibly related to the previous discovery that boron helps maintain the plasma membrane integrity by stimulating the activity of ATPase (Ferreira et al. 2021).

In previous reports, it has been demonstrated that boron could control disease in grapevines caused by fungus and grey mould on table grapes caused by *B. cinerea* (Qin et al. 2010). Additionally, BA is a commercially acceptable and an economically feasible and an environmentally safe management strategy to enhance the shelf life of many horticultural plants. In the present experiment, there was a reduction in the fruit firmness with a storage period in the BA treated as well as the control fruit, and the 10 mM BA treatment group maintained the highest firmness of the grape berries (Figure 1B). This is likely due to the function of boron in the synthesis of the cell wall composition and the regulation of the cell wall stability. The role of borates as an antifungal complex in the control of post-harvest diseases in various fruits has also been demonstrated (Shi et al. 2011). In addition, this could be related to the fact that BA keeps the cell wall rigid by forming links to the carboxyl groups of pectin compounds in the cell wall (O'Neill et al. 2004). These complexes resist cell wall deterioration enzymes, including polygalacturonase, cellulase, and inhibit the rate of softening during storage. Meanwhile, the BA treated grapes maintained a significantly low PG activity and cellulase activity (Figure 5), which often cause fruit softening and degradation of the cell wall components due to the depolymerisation of the celluloses, hemicelluloses and pectin substances which decrease the thickness and rigidity following the degradation of the cellulose fibre (Ge et al. 2019). It is presumably because this element improves the car-

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bohydrate metabolism and translocation, whose effect is to provide a substrate for cell respiration and cell wall synthesis. It is also reported to play a role in processes such as the cell capture and transport, cell wall formation, cell membrane function and antioxidant defence system (Riaz et al. 2021).

Our results indicated that the post-harvest application of BA delayed the senescence process of grapes. In general, the 10 mM BA treatment had the best fresh-keeping effect. First, the BA treated grapes maintained higher antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD); second, they maintained lower metabolic toxic products like the superoxide anion (O_2^-) production rate, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content than the control. Consequently, the senescence of grape berries during storage is moderated by the BA treatment.

CONCLUSIONS

Based on the results of the effect of BA on the storage performance of grape berries, it can be concluded that BA alleviated the post-harvest senescence of the grapes, and the 10 mM BA treatment was found to be the most effective in improving the quality of grapes after harvest by maintaining higher berry firmness (by inhibiting the activity of PG and cellulase), AsA content and moisture content. Moreover, it also resulted in a lower MDA content, superoxide anion (O_2^-) production rate and H_2O_2 content during storage. Overall, the post-harvest application of 10 mM BA effectively delays the senescence of grapes by regulating the activity of the cell wall degrading enzymes and the level of ROS metabolism. The results obtained in this study can be used as the basis for more detailed mechanism studies, helping researchers and producers to promote the development and application of post-harvest preservation agents.

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