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EFFECT OF WAXING ON POSTHARVEST QUALITY AND STORABILITY OF GINGER (*ZINGIBER OFFICINAL* ROSCOE)

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Abstract

Desiccation remains a major limiting factor affecting the storage life of many horticultural crop species. The process of waxing, which provides a seal to protect against water loss, is currently the main method for maintaining postharvest quality of many crops. However, the effects of waxing on reducing desiccation, thereby improving postharvest quality of ginger remains unclear. This study investigates the effects of waxing on the common physiological and molecular markers of desiccation, including fresh weight loss, antioxidant system, and endogenous hormones of ginger postharvest. Results showed that both 2% and 4% wax treatments significantly inhibited rhizomes desiccation, marked by the reduced fresh weight loss and maintenance of total protein content in ginger rhizomes. Wax application significantly inhibited the rate of increase in the activities of antioxidant enzymes, including SOD, POD, and CAT, as well as lowered the concentration of MDA-each of which are required for desiccation tolerance. In addition, ABA biosynthesis and signaling were repressed by 2% and 4% wax treatments, indicating that regulation of desiccation by ABA was required. These results demonstrate that waxing of ginger rhizomes adequately reduce the rate at which desiccation may occur in horticultural crops. Therefore, the application of wax is an effective method to reduce postharvest loss of ginger rhizome.

Key words: *Zingiber officinale* Roscoe, Postharvest rhizomes desiccation, Antioxidant enzyme activities, Endogenous hormones.

Introduction

Ginger (*Zingiber officinale* Roscoe) is an herbaceous perennial plant, belonging to the family of Zingiberaceae, whose rhizomes are widely consumed as spice of economic, medicinal and cultural importance (Kaushal *et al*., 2017). Ginger is cultivated in numerous countries, including China, India and Nigeria, with an annual production upwards of 100,000 tons on a dry weight basis (Kemper, 1999; Jangam & Thorat, 2010; Kaushal *et al*., 2017). As a traditional herbal medicine, the bioactive components of ginger have shown therapeutic properties as an antioxidant, anti-inflammatory and anticancer agent (Bartley & Jacobs, 2000; Tepe *et al*., 2006).

Desiccation is a major postharvest disorder affecting the storage life of fresh ginger, with surface shriveling of the rhizome resulting form 10% fresh weight loss, shortening the shelf-life and reducing the quality of the rhizome (Kaushal *et al*., 2017; Pun *et al*., 2016). Desiccation is controlled by regulatory mechanisms directing the re-programming of the plant to survive under dehydration (Shinozaki & Yamaguchi-Shinozaki, 2007). Plant hormones, such as abscisic acid (ABA), are crucial for regulating desiccation by inducing stress-responsive gene expression and stomatal closure (Frey *et al*., 2012; Kim *et al*., 2010). Specifically, *RD22* is dehydration response gene induced via ABA treatment and is often a marker of early ABA response to desiccation (Hanana *et al*., 2008). Auxin is also implicated in enhancing response to dehydration stress by altering root architecture (Uga *et al*., 2013). Another indicator of postharvest dehydration, is the rapid accumulation of reactive oxygen species (ROS) that enhances cellular damage (Chai *et al*., 2005; Kranner & Birtić 2005). This ROS accumulation requires the action of antioxidant enzyme systems to provide oxidative protection during desiccation (Kranner & Birtić, 2005; Moharramnejad *et al*., 2019). Overall, several physiological and molecular responses occur during desiccation which can indicate the progress of postharvest water loss.

To maintain postharvest quality, many horticultural crops are treated with a water-soluble surface coating via a process of waxing (Ganai *et al*., 2015; Petracek *et al*., 1998; Segall *et al*., 1974). Waxing is recognized as a simple, pollution-free, and inexpensive strategy for extending the shelf life of fresh produce by modifying the internal atmosphere surrounding the produce (Baldwin, 2001). In addition to providing a seal to protect crops against water loss, wax coating provides a gloss increasing the aesthetic value of the produce (Kaplan, 1986; Ummarat *et al*., 2015). For instance, applying a wax coating to apples, not only imparted a high gloss to hide bruising that occurs during postharvest treatment, but also formed a modified atmosphere around the fruit to preserve firmness and prolong shelf-life (Ganai *et al*., 2015). Similarly, the shelf-life of cut anthuriums could be maintained up to 20 days under ambient conditions after treatment with a wax coating (Mujaffar & Sankat, 2003). With the varying composition of different natural and synthetic waxes, it is critical that the wax treatment applied be tested prior to use and tailored for each commodity (Baldwin, 2001).

Currently, little is known about the potential effects of waxing to maintain the postharvest quality of ginger. This study aimed to uncover the physiological and molecular mechanisms affected by the process of waxing by evaluating the fresh weight loss, protein content, antioxidant enzyme activities, content of endogenous hormones and the expression level of genes involved in desiccation and abscisic acid (ABA) biosynthesis.

Materials and Methods

Ginger rhizomes: Ginger (*Z. officinale* Ros. cv. zhugen) were grown in a greenhouse with temperature: 25 °C, relative air humidity: 60%, and 14 h light with 200 $\mu EM^{-2} S^{-1}$ light intensity / 10 h dark (Jiang *et al*., 2018). Rhizomes were harvested after commercial maturity, and were delivered to the lab within 1 h. Rhizomes without rot and vascular discoloration were used for future analysis (Liu *et al*., 2016).

Waxing treatment: Before wax treatment, 1% (v/v) sodium hypochlorite was used for disinfection of ginger rhizomes. 2% (v/v), 4% (v/v), and 10% (v/v) of commercially-available morpholine fatty acid salt wax (FMC) were used for waxing treatment, using deionized water as control. The wax treatments of the rhizomes in the four groups were soaked in the solution supplied with the different concentrations of wax for 2 min, air-dried and stored under ambient condition (Temperature 18±3°C, RH 70±5%). Phenotypes of rhizomes were assess daily. Three biological and technical replicates were performed.

Effects of waxing on postharvest quality of ginger rhizome: To identify the effects of waxing on postharvest quality of ginger rhizome, fresh weight loss and total protein content were monitored after 7 days of storage. Initial weight of ginger rhizomes were measured before storage (A) and daily under storage (B). Fresh weight loss (%) was calculated as (A-B)/A×100. For total protein content assay, about 0.2 g of pooled tissue samples were prepared as fine powder in liquid nitrogen, and physiological saline solution was added according to proportion 1 (g): 9 (mL). The homogenized solution was centrifuged at 2500 rpm for 10 min (4 °C), and the supernatant was used to assay. Total protein content was analyzed using Total Protein Quantitative Assay kit (NanJing Jian Cheng Bioengineering Institute, China).

Activities of antioxidant enzymes and MDA content assays: Activities of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA) content were performed using Superoxide Dismutase (SOD) Assay Kit, Peroxidase (POD) Assay Kit, Catalase (CAT) Assay Kit, and Malondialdehyde (MDA) Assay kit (NanJing JianCheng Bioengineering Institute, China).

Endogenous ABA and IAA content of ginger rhizomes assays: 0.2 g of pooled tissue samples were crushed in liquid nitrogen, and transferred into 2 mL tubes, and adding extraction solvent (2-propanol/H_2O/HCL [2:1:0.002, v/v/v]) according to the ratio of sample: solvent at 1:10 (mg/ µL). Samples were shaken at 100 rpm for 30 min (4°C). 1 mL dichloromethane was added to each tube, and then shaken at 100 rpm for 30 min (4°C). Each tube was centrifuged at 13,000 g for 5 min (4°C), the solvent from the lower phase was transferred into new 2 mL tube, and then was dried using Organomation Nitrogen Evaporators. Before analysis, PBS (pH 7.4) added into each tube according to proportion 1 (g): 9 (mL). Endogenous ABA or IAA content of ginger rhizomes were performed using ABA Elisa Kit and IAA Elisa Kit (Shanghai Jinhua Science and Technology co. LTD, China).

RNA isolation and gene expression analysis: Total RNA was extracted from ginger rhizomes using an Easy Pure plant RNA Kit (Huayueyang Biotechnology co., Ltd, China). 0.5 µg of total RNA was used for first-stand cDNA synthesis. The final reaction volume was 10 µL using HiScript® II Q Select RT Supermix for qPCR (Vazyme, China) following the manufacture's protocol. Briefly, the reactions were incubated at 25°C for 10 min, 50°C for 30 min, 85°C for 5 min, and then maintained at 4 °C. RT-PCR analysis was performed using 2×Taq Master Mix (CWBIO, China). The PCR cycles were performed as follow: denaturation at 94°C for 5 min; 30 cycles of 94°C for 30 s, 58°C for 30 s, 72°C 30 s; followed by final extension at 72°C for 10 min; and then maintained at 4°C. The primer pairs using for semi-quantitative RT-PCR are given as follow: 5'-GTCCACTTCACTCGACCCAG-3' and 5'-TCGATCGTGTACATCTGCCG-3' for *ZoRD22*, 5'-GACTTCGCTATCACCGAGCA-3' and 5'-AGGTTCCGGTTCACCATTCC-3' for *ZoNECD5*, and 5'-CGCCACACAGGAGTTATGGT-3' and 5'-AGCAGTGGTGGTGAACGAAT-3' for *ZoActin5*. Each treatment group had three biological and technical replicates.

Data analysis

Data was analyzed by one-way ANOVA and Duncan's multiple range tests by SPSS version 16.0 (SPSS Inc., USA). Effects were considered to be significant if the *P* value was less than 0.05.

Results

Effects of waxing on decreasing shriveling of ginger rhizomes: 2% and 4% wax treatments effectively decreased the shriveling of ginger rhizomes compared to control; though no significant difference between the 2% and 4% wax treatments was observed (Fig. 1). While the 10% wax treatment showed no significant effect relative to the control (Fig. 1).

Effects of waxing on fresh weight loss and total protein content of ginger rhizomes: Treatment of ginger rhizomes with 2% and 4% wax treatment significantly inhibited fresh weight loss; however there was no observable difference between these treatments (Fig. 2A). While, 10% wax treatment mildly inhibited fresh weight loss compared to control. On the other hand, ginger rhizomes with a 2% and 4% wax treatment each had a higher total protein content, while the 10% wax treatment showed similar total protein content as the control (Fig. 2B).

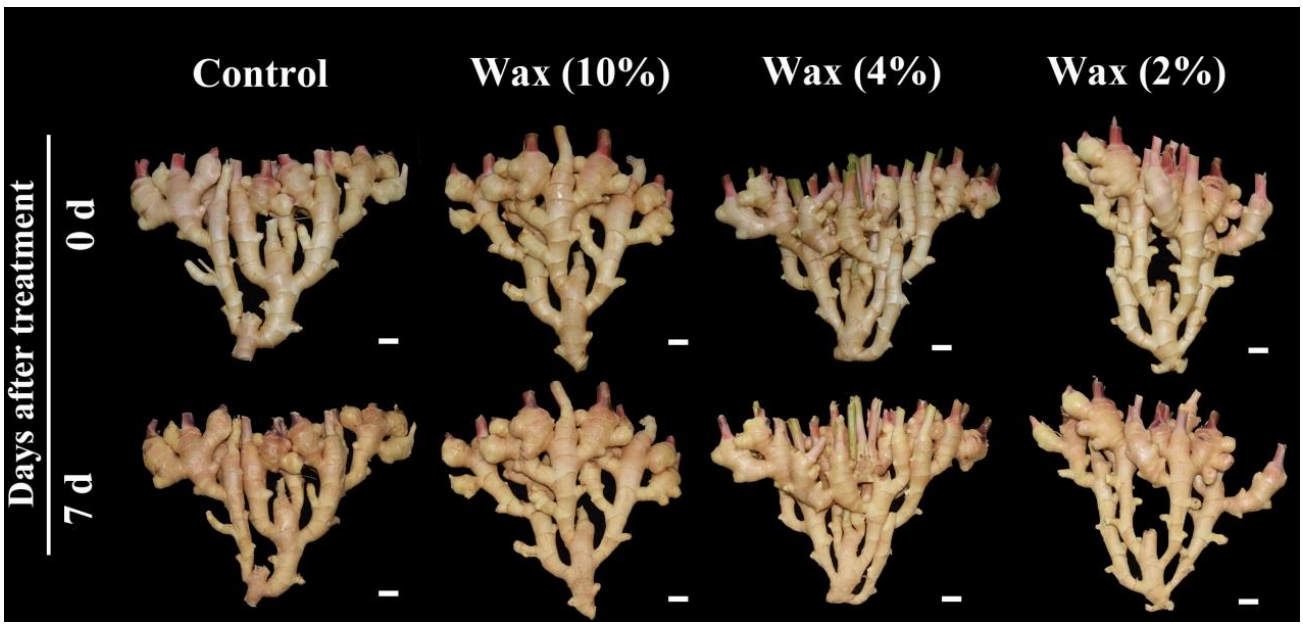

Fig. 1. Effects of waxing on decreasing postharvest shriveling of ginger rhizomes. Ginger rhizomes phenotypes were recorded and photographed daily. Bar = 1 cm.

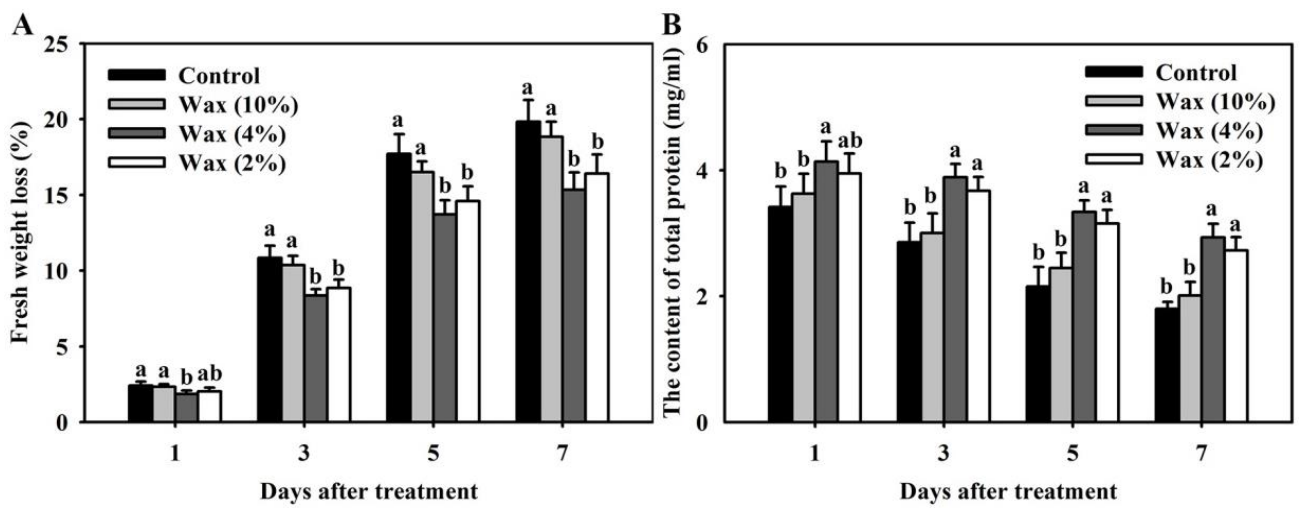

Fig. 2. Effects of waxing on the quality of ginger rhizomes during postharvest storage. (A) fresh weight loss; (B) the content of total protein. Values are the means of data from three experiments with three different biological replicates in each experiment ± standard deviation (n=10). Columns with different letters at each time point indicate significant differences between treatments within 2 days according to Duncan's multiple range test ($t<0.05$).

Effects of waxing on activities of antioxidant enzyme and content of MDA: Corresponding to the pattern of fresh weight loss and total protein content, waxing at 2% and 4% concentration also markedly inhibited the activity of POD, SOD, and CAT in ginger rhizomes (Fig. 3A-C). However, in the days following the respective wax treatment, the three antioxidant enzyme activities also gradually increased, though at a slower rate than the control (Fig. 3A-C), Similar results were observed when assessing MDA content in ginger rhizomes, with increasing wax concentration leading to a decrease in overall MDA content (Fig. 3D). Though following initial wax treatment, the MDA content also steadily increased; though slower than that of the control (Fig. 3D). Comparably to its effect on fresh weight loss and total protein content, the 10% wax treatment showed no significant effect on regulation of antioxidant enzyme activities or MDA content.

Effects of waxing on endogenous ABA or IAA in ginger rhizomes: 2% and 4% wax treatments markedly inhibited endogenous ABA biosynthesis (Fig. 4A). Endogenous ABA content progressively accumulated in both the 2% and 4% wax treatments in the days following post-treatment, which is consistent with the increased water loss associated with postharvest (Fig. 4A). No significant difference was observed between the 2% and 4% treatments (Fig. 4A). The 10% wax treatment had a minimal effect on the endogenous ABA content (Fig. 4A). No significant difference in IAA content was measured in each wax treatment compared to the control, across the days post-treatment (Fig. 4B).

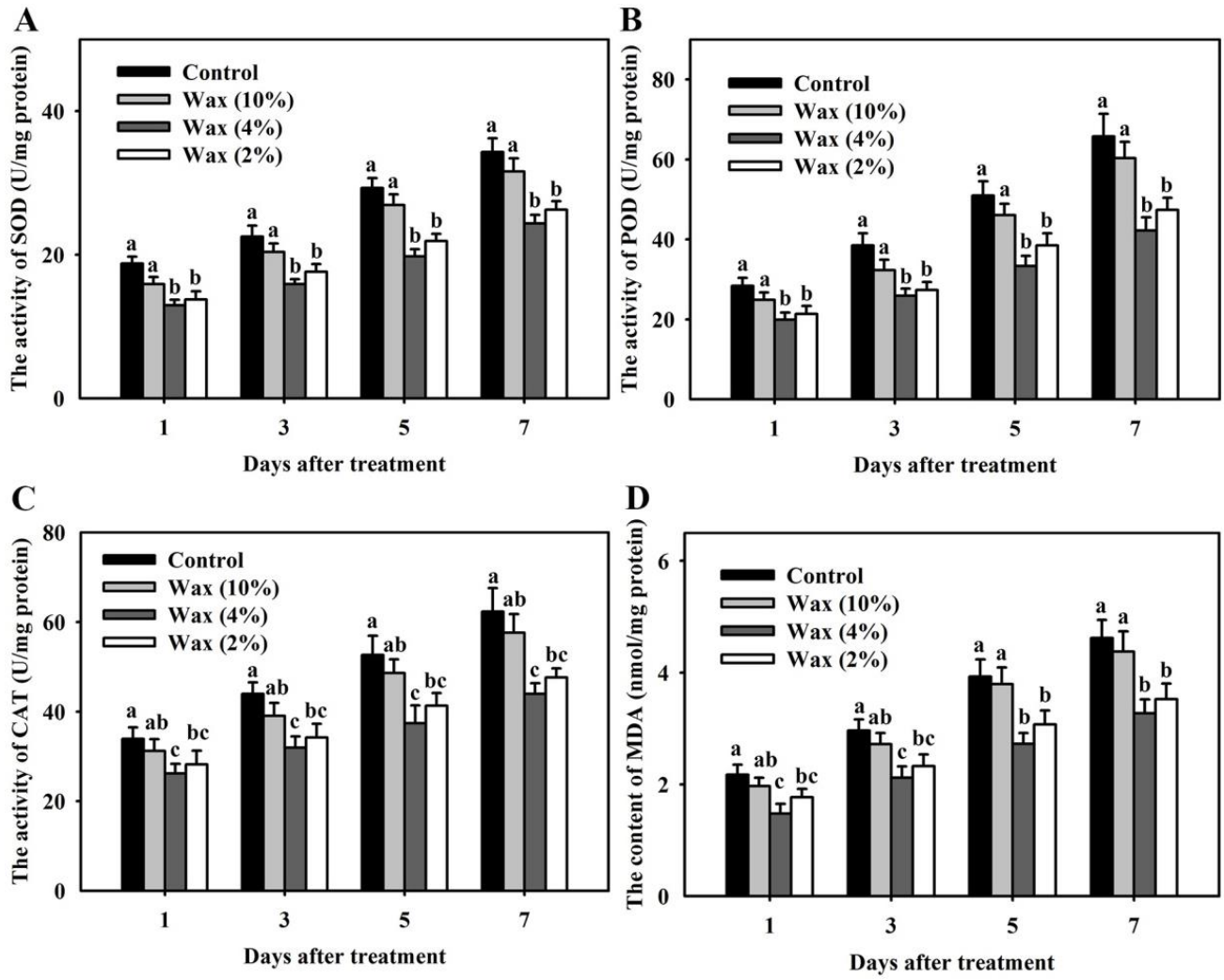

Fig. 3. The activities of SOD (A), POD (B), CAT (C); and content of MDA (D) in ginger rhizomes during postharvest storage. Values are the means of data from three experiments with three different biological replicates in each experiment ± standard deviation (n=10). Columns with different letters at each time point indicate significant differences between treatments within 2 days according to Duncan's multiple range test (t<0.05).

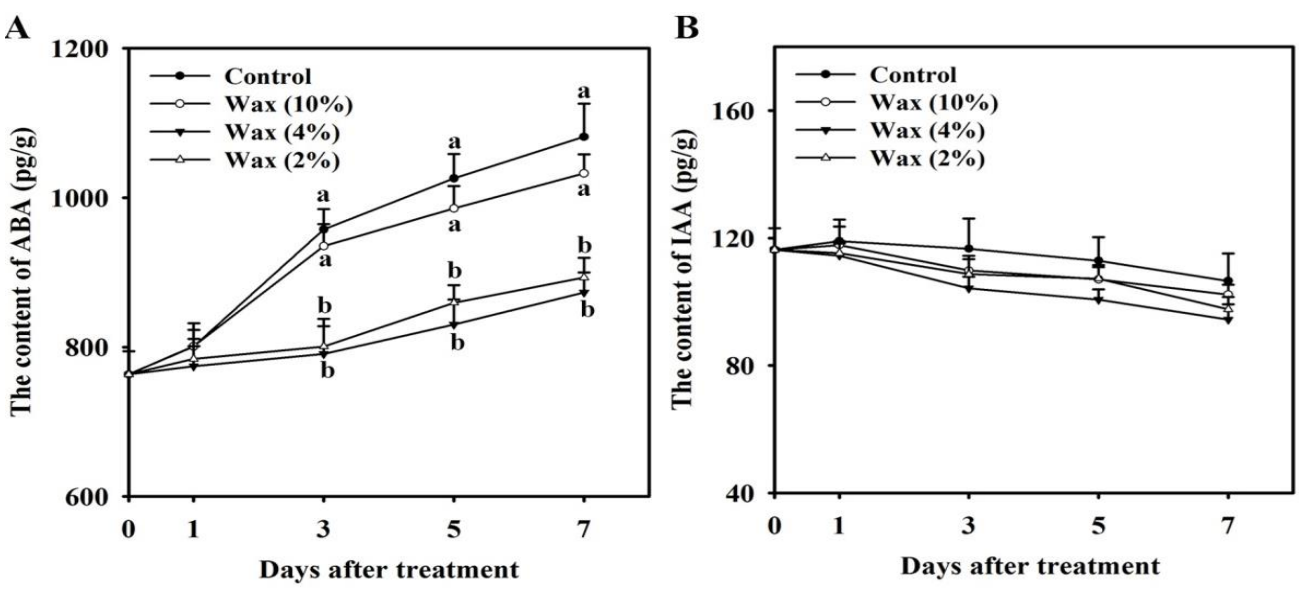

Fig. 4. The content of ABA (A) and IAA (B) in ginger rhizomes during postharvest storage. Values are the means of data from three experiments with three different biological replicates in each experiment ± standard deviation (n=10). Columns with different letters at each time point indicate significant differences between treatments within 2 days according to Duncan's multiple range test (t<0.05).

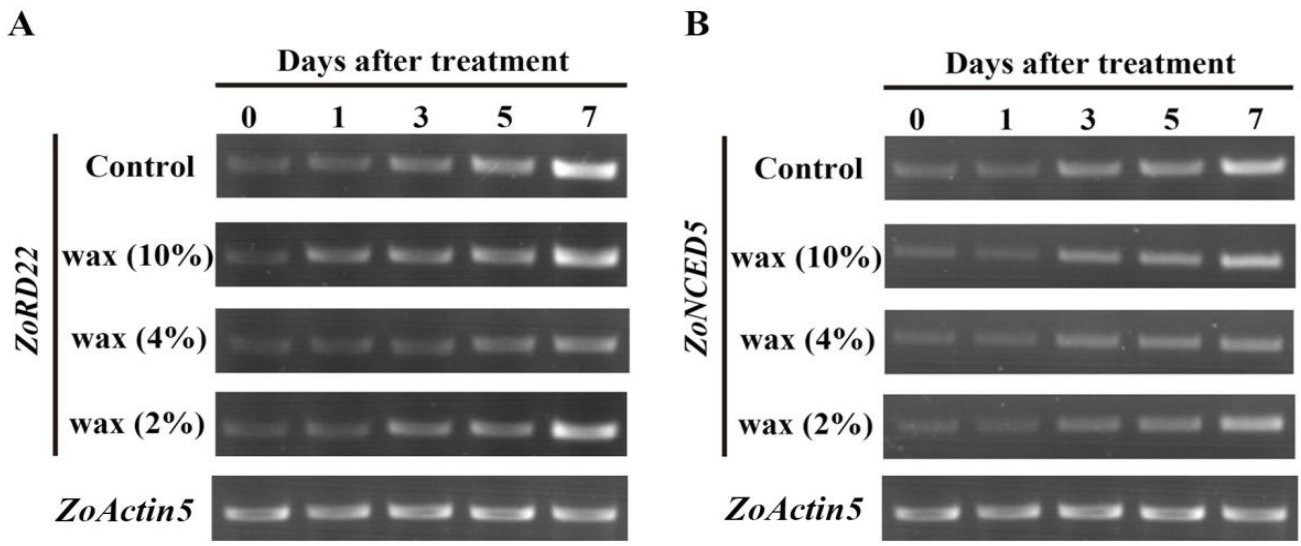

Fig. 5. The expression of *ZoRD22* (A) and *ZoNCED5* (B) in ginger rhizomes during postharvest storage. Total RNA was prepared from three individual samples at each time point and RT-PCR was repeated at least three times for all samples. *ZoActin5* was used as an internal control to normalize the amount of total RNA.

Effects of waxing on expression of *ZoRD22* and *ZoNCED5*: With decreased ABA content in wax treated ginger rhizomes, to test whether ABA biosynthesis and response were also inhibited the expression level of *ZoNCED5* and *ZoRD22* was measured using RT-PCR. *ZoRD22* and *ZoNCED5* transcripts gradually accumulated, consistent with the dehydration associated with postharvest (Fig. 5). Expression of *ZoRD22* and *ZoNCED5* transcripts in ginger rhizomes treated with 2% and 4% wax were markedly inhibited, though no notable difference between 10% wax treatment and control was observed (Fig. 5).

Discussion

FMC, a commercially-available morpholine fatty acid salt wax, and its derivatives can effectively maintain the quality of horticultural products by prolonging their shelf life (Petracek *et al*., 1998; Mujaffar & Sankat, 2003). Although waxing extends shelf life, a loss of flavor quality was reported in some varieties of mandarin (*Citrus reticulata* Blanco) due to an enhance development of anaerobic conditions following wax treatment (Ummarat *et al*., 2015). Therefore, it is vital to identify the effect of waxing on the postharvest quality of ginger prior to commercial consumption.

This study shows that waxing was effective to inhibit the physiological and molecular mechanisms associated with desiccation in ginger rhizomes. The regulatory effect was observed when ginger rhizomes were treated with wax concentration at 2%, with the effects increasing under 4% wax treatment. Though, 10% wax treatment showed no significant effect on controlling water loss of ginger rhizomes (Fig. 1, 2A). This indicates that a deleterious effect may occur under high concentration wax treatment of ginger rhizomes, such that it is necessary to determine the concentration of wax treatment that effectively mitigates the desiccation of ginger rhizomes. Similar effects of waxing were found in other horticulture crops. For instance, using low amounts of shellac, another type of wax coating, significantly improved the quality and sensory of 'Galia-type' melon (Fallik *et al*., 2005).

During drought stress, which causes similar changes at the molecular level as desiccation, protein residues may be modified by chemical processes such as domination, isomerization, or oxidation, which results in protein degradation (Ingram & Bartels, 1996). Following harvest, we found that the total protein content in ginger rhizomes steadily decreased; however treatments with 2% and 4% wax significantly decreased the rate at which protein degradation was occurring in ginger rhizomes. Therefore, particular concentrations of wax treatment can slow down the rate of water loss in ginger rhizomes.

Free radicals accumulate when plants suffer a wide range of stresses, including dehydration, as they cause molecular and cellular damage (França *et al*., 2007; Smirnoff, 1993). Desiccation tolerance requires the action of antioxidant enzymes to scavenge for active oxygen species (AOS), to prevent the deleterious effects associated with free radicals (Niedzwiedz-Siegien *et al*., 2004). Increase in SOD activity was detected across multiple plant species, including *Lotus corniculatus*, barley, and wheat, under water stress (Acar *et al*., 2001; Borsani *et al*., 2001; Sgherri *et al*., 2000). Different stages of development result in different antioxidant enzyme activities, such as the decreased SOD activity exhibited in immature bean seeds (Bailly *et al*., 2001). In addition, the POD activity was also significantly enhanced in wheat seedling under dehydration condition (Niedzwiedz-Siegien *et al*., 2004). Similarly, the regulation of CAT activity during dehydration stress is different across developmental stages, and is species specific (Bailly *et al*., 2001; Fu & Huang, 2001). In this study, the induction of SOD, POD, and CAT activity were repressed by 2% and 4% wax treatments, indicating that the wax treatment did delay desiccation as antioxidant enzymes were not needed to prevent the damage associated with desiccation. As an indicator of lipid peroxidation, MDA accumulated in plants under episodic drought (Leprince *et al*., 1994; Price & Hendry, 1991; Seel *et al*., 1991). We

found the content of MDA sharply accumulated during postharvest water loss, however 2% and 4% wax treatment inhibited the rate of accumulation. Based on the markers associated with desiccation, low concentrations of wax treatment substantially delayed the onset of desiccation in ginger rhizomes.

Plants have evolved highly complex molecular systems to response to dehydration stress, including hormone signaling pathways and metabolic regulation. Among them, ABA-dependent gene expression plays a vital role (Bray, 2002; Urano *et al*., 2017). In our study, we found that wax treated ginger rhizomes had significantly decreased ABA content and ABA-induced gene expression, accompanied with lower of transcript accumulation of *ZoNCED5* or *ZoRD22* (Figs. 4A & 5). In addition to ABA, several other plant hormones also regulate dehydration stress (Claeys & Inze, 2013). For instance, auxin enhances water absorption of plant from deep layers through modification the root architecture (Ruf *et al*., 2011). However, IAA content did not significantly changed in wax treated ginger rhizomes under postharvest dehydration.

Conclusion

This study demonstrates that low concentration (2% and 4%) of wax treatment significantly maintained the postharvest quality of ginger rhizomes by reducing desiccation responses, such as water loss, protein content, antioxidant enzyme activities, ABA biosynthesis and signaling. However, other potential effects of waxing on ginger rhizomes remain to be assessed.

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