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Influences of Brassinosteroide and Hot Water on Postharvest Enzyme Activity and Lipid Peroxidaion of Lime (*Citrus aurantifolia* L.) Fruit During Storage at Cold Temperature

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Abstract
Storage of Lime (*Citrus aurantifolia* L.) fruits, originally a tropical fruits, in low temperature confronts with several difficulties due to the risk of chilling injury (CI). To develop an effective method aiming to reduce CI, the effects of treatments containing 0, 0.5 and 1 Mg/lit brassinosteroids (BRs) and hot water (HW) including 20°C as control, 45 and 55°C for 30 minute on CI was studied. Moreover lipid peroxidaion, hydrogen peroxide, catalase (CAT) and peroxidase (POD) activities were investigated in lime fruits stored at 1°C for 21 days. The CI, lipid peroxidaion, hydrogen peroxide were significantly reduced by BRs treatment particularly at 1Mg/lit and 45°CHW treatment. Furthermore fruits treated with 1Mg/lit BRs and 45 °C HW treatment exhibited significantly higher CAT and POD activities in comparison with the control fruits. These results suggest that BRs and HW treatment protect lime fruits from CI by enhancing antioxidant enzymes such as POD and CAT activities, and reduce lipid peroxidaion, hydrogen peroxide contents, and maintaining membrane integrity.

Keywords: Brassinostroide, Chilling injury, Enzyme activity, HW, Storage

Abbreviations: BRs, Brassinosteroids, CI, Chilling injury, Catalase, CAT, Peroxidase, POD, HW, Hot water; Mg/lit, Milligram per Liter

Introduction

Citrus aurantifolia is an important medicinal and food plant widely cultivated in many parts of the world (Enejoh et al., 2015). In order to extend its commercial life, it is usually harvested at mature stage and stored at low temperatures. Storage of limes as an originally subtropical fruits at low temperature is limited by the risk of chilling injury (CI). Postharvest longevity of some fruits is also limited by physiological disorders especially CI. Pre-storage heat treatments have been shown to effectively reduce the CI susceptibility in Citrus (Porat et al., 2000), persimmon (Lay-Yee et al., 1997), peach (Candir et al., 2008) and plum (Abu-Kpawoh et al., 2002). Heat treatments are physical methods for controlling insects, preventing pathogenic infection, improving resistance against CI, delaying maturity and extending shelf life after harvest (Civello et

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al., 1997; Wang, 1998). Cowley et al. (1992) reported that high temperature applications on persimmon fruits reduce sensitivity against damages caused by cold. Woolf et al. (1997) examined the effects of hot air application (0.5 and 10 h at 34 - 50°C) on CI during storage in persimmon fruit and reported that the application of47°C air for 0.5 - 3 h minimized CI and peel browning. Lay-Yee et al. (1997) applied 47 - 54°C water for 2.5 - 120 min and 20°C air for 60-120 min on Fuyu cultivar and determined that the 50°C water for 30 and 45 min, 52 °C water for 20 and 30 min and 54°C water for 20 min are applicable for insect disinfestations without causing browning in fruit's flesh and peel (Lay-Yee et al. 1997).

Brassinosteroids (BRs) are a group of plant steroids that play vital role in plant growth and development and tolerance to biotic and abiotic stresses, such as cold stress (Yu et al., 2002; Fariduddin et al., 2011), salt injury (Ozdemir et al., 2004), pathogen infection (Nakashita et al., 2003), oxidative damage (Cao et al., 2005) and heat stress (Ogweno et al., 2008). Zhu et al. (2010) reported that the application of 5Mm BRs, reduced postharvest decay caused by Penicillium expansum in jujube fruit and delayed fruit senescence by inhibiting ethylene production and respiration rate. The potential of BRs to enhance chilling tolerance of plants has also been investigated. BRs have been shown to have protective effects on eggplant, cucumber, maize and rice seedlings under chilling stress (Mandava, 1988; He et al., 1991; Hotta et al., 1998). Wang and Zeng (1993) reported that the treatment of rice seedlings with BRs led to an increase in chilling tolerance through inducing effects on membrane stability. Liu et al. (2009) reported that BRs treatment promoted the activities of antioxidant enzymes and increased the contents of ascorbic acid and reduced glutathione under chilling stress, thus scavenging excess reactive oxygen species (ROS) accumulation and decreasing the content of lipid peroxidation and hydrogen peroxide. These findings indicate that BRs are capable of alleviating oxidative injury and improving the resistance of cells against chilling stress. The antioxidant enzymes activation could be considered for potential applications for enhancing defense mechanism against chilling, as it resulted in lower cold-induced peel damage in mandarin fruit (Lafuente et al., 2004). POD and CAT are key enzymes in the metabolism of free radical that has been reported to protect plants against stress conditions. However, to our knowledge, the effects of BRs application on postharvest CI in fruits is still under debate (Li et al., 2012).

In this study we aimed to find the effect of BRs and HW applications on protection against the CI and quality losses during storage and to determine antioxidant activity of enzymes following chemical treatments of lime (Citrus aurantifolia L.) fruit.

Materials and methods

Plant materials and treatments

Lime (*Citrus aurantifolia* L.) fruits were harvested at full maturity stage from a commercial orchard at Jiroft (Kerman Province, Iran) on 20March, 2012 and transported to laboratory of shahid bahonar university of kerman. Fruit with unique size and color, and without mechanical and pathogen damages were selected and treated with 0 (control), 0.5 and 1Mg/lit brassinosteroids for 5min and 20°C (control), 45°C and 55°C HW for 20 min and were stored at 5±1°C, 85-90 % relative humidity for 21 days. Treated fruits used for determination of visible CI symptoms, POD and CAT activities,lipid peroxidation and hydrogen peroxide content.

CI

The symptoms of CI include surface pitting and browning. The decay and CI index was determined according to the method

described by Obenland et al. (2009). Grade levels were classified as: grade 0, the unaffected orange fruits; grade 1, CI symptoms on the fruit with decay of less than 25% ; grade 2, CI symptoms on the fruit with decay of 25–50%; and grade 3, more than 50% decay and CI symptoms on fruits. The decay and CI index is calculated using the following formula: decay and CI index (%) = (decay and CI Grade ×number of fruit at this level) / (highest level × total fruit number) × 100.

Estimation lipid peroxidation

Lipid peroxidation was estimated by determining the malondialdehde (MDA) content in the lime fruit according to method of Heath and Packer (1968); A hundred milligram of fruit samples was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000-g for 5min at 4°C. Aliquot of 0.3 ml supernatant was mixed with 1.2 ml f 0.5% thiobarbituric acid (TBA) prepared in TCA 20%, and incubated at 95°C for 30min. After incubating the reaction in an ice bath for 5 min, samples were centrifuged at 10000-g for 10 min at 25°C. The supernatant absorbance at 532 nm was then measured using a Beckman UV-DU 520 spectrophotometer (USA). After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the155 $mM^{-1}cm^{-1}$ extinction coefficient.

Hydrogen peroxide assay

The assay for H_2O_2 content was carried out by the procedure described by Velikova et al. (2000). Fresh tissues (2g) were homogenized with 10 ml of acetone at 0°C. After centrifugation for 15 min at 6000g at 4°C, the supernatant phase was collected and mixed with 0.1 ml of 5% titanium sulphate and 0.2 ml ammonia, and then centrifuged for 10 min at 6000-g and 4°C. The pellets were dissolved in 3 ml of 10% (v/v) H_2SO_4 and centrifuged for 10 min at 5000g. Absorbance of the supernatant phase was measured at 410 nm. H_2O_2 content was calculated using H_2O_2 as a standard and recorded as µmol/g on fresh weight basis (Velikova et al., 2000).

Assays of enzyme activity

POD activity was assayed according to Kochba et al. (1977), The POD reaction solution (3ml) contains 20 mmol/l phosphate buffer (pH 6.0), 20 mmol/l guaiacol, 40 mmol/l H_2O_2, and 40µl enzyme extract. One unit of activity was defined as the amount of enzyme required to increase 1 absorbance unit in the optical density at 470nm Min-1. Protein content in the enzyme extract was estimated using the Bradford (1976) method. Specific activity of the enzyme was expressed as units per mg protein.

CAT activity

The activity of CAT was assayed according to Beers and Sizer (1952). Fresh samples (200 mg) were homogenized in 5 ml of 50 mM Tris-NaOH buffer (pH 8.0) containing 0.5 mM EDTA, 2% (w/v) PVP and 0.5% (v/v) Triton X-100. The homogenate was centrifuged at 22000-g for 10 min at 4 8°C and after dialysis supernatant was used for enzyme assay. Assay mixture in a total volume of 1.5 ml contained 1000 ml of 100 mM KH_2PO_4 buffer (pH 7.0), 400 ml of 200 mM H_2O_2 and 100 ml enzyme. The decomposition of H_2O_2 was followed at 240 nm (extinction coefficient of 0.036 mM-1 cm-1) by decrease in absorbance. Specific activity of the enzyme was expressed as units per mg protein.

Statistical analysis

The experimental design was a completely randomized design with three replications. Data were analyzed by analysis of variance (ANOVA) and the means were compared ($p \leq 0.05$) by Dancan's multiple rang test (DMRT). All analyses were performed by using SAS version.

Results and discussion

Slight CI symptoms appeared after 7 days in control fruits, and continued to progress

over time. CI is a major factor that reduces quality and limits lime fruit storage longevity. In this study, the plant hormone BRs was applied and significantly reduced postharvest CI in lime fruits (Fig. 1). Our results were consistent with previous reports that BRs could protect seedlings of eggplant, cucumber, maize and rice against chilling stress (Mandava, 1988; He et al., 1991; Hotta et al., 1998). Lipid peroxidation (Fig. 2) and hydrogen peroxide content (Fig. 3) were significantly reduced in treated fruits with BRs (1Mg/lit) and 45°CHW . As shown in Fig. 2 and 3, the lipid peroxidaion, hydrogen peroxide content of control increased significantly during storage time ($P < 0.05$). The POD (POD) (Fig. 4) and CAT (CAT) activities (Fig. 5) in treated fruits with BR and HW increased with chilling stress time but showed an increasing pattern in control fruits during storage time. The POD and CAT activities were higher in BRs and HW treated fruits than in control fruits at day 21 ($P < 0.01$). A correlation was observed between CI index and lipid peroxidation (Fig. 6) and hydrogen peroxide content (Fig. 7). Moreover, the CI index, lipid peroxidation and hydrogen peroxide content were significantly lower in BRs and HW treated fruit than those of control fruit.

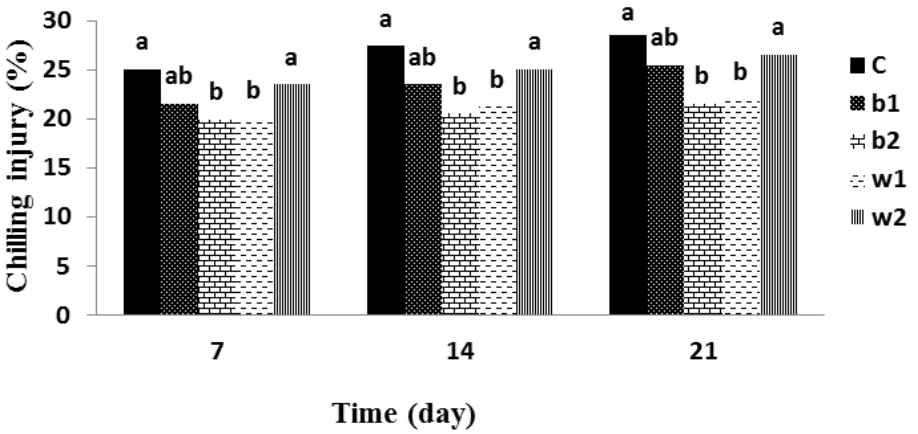

Fig. 1. Effect of BRsBRs and HW treatment on CICI (%) of lime fruit. Means in each column having the same letters are not significantly at 5% level of probability using Duncan' test. C: Control, b1: 0.5 Mg/lit BRs, b2: 1 Mg/lit BRs, w1: 45°C HW and w2: 55°C HW.

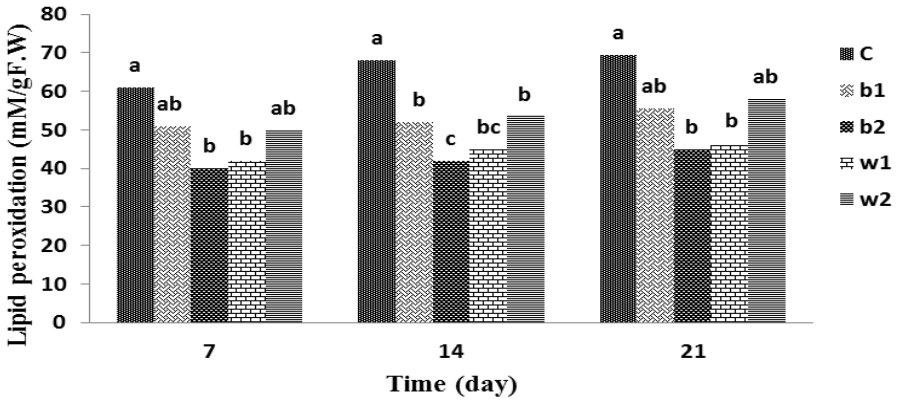

Fig. 2. Effect of BRsBRs and HW treatment on lipid peroxidation (mM/gF.W) of lime fruit. Means in each column having the same letters are not significantly at 5% level of probability using Duncan' test. C: Control, b1: 0.5 Mg/lit BRs, b2: 1 Mg/lit BRs, w1: 45°C HW and w2: 55°C HW.

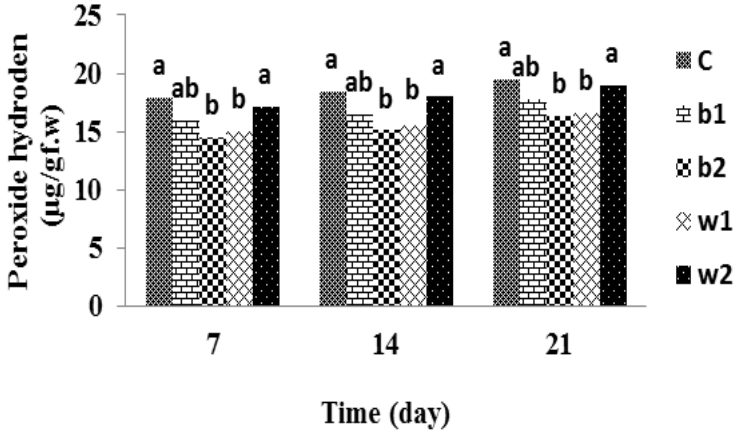

Fig. 3. Effect of BRsBRs and HW treatment on hydrogen peroxide (µg/gf.w) of lime fruit. Means in each column having the same letters are not significantly at 5% level of probability using Duncan' test. C: Control, b1: 0.5 Mg/lit BRs, b2: 1 Mg/lit BRs, w1: 45°C HW and w2: 55°C HW.

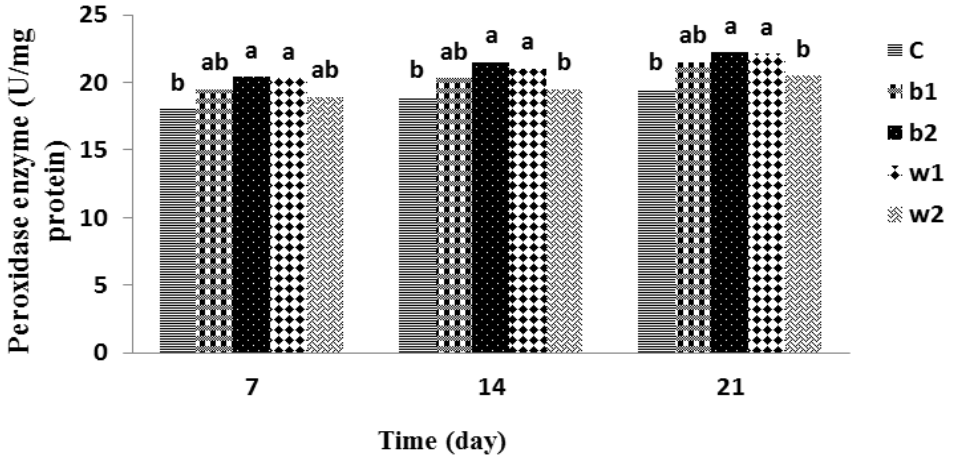

Fig. 4. Effect of BRs and HW treatment on POD activity (Unit/mg protein) of lime fruit. Means in each column with the same letters are not significantly at 5% level of probability using Duncan' test. C: Control, b1: 0.5 Mg/lit BRs, b2: 1 Mg/lit BRs, w1: 45°C HW, and w2: 55°C HW.

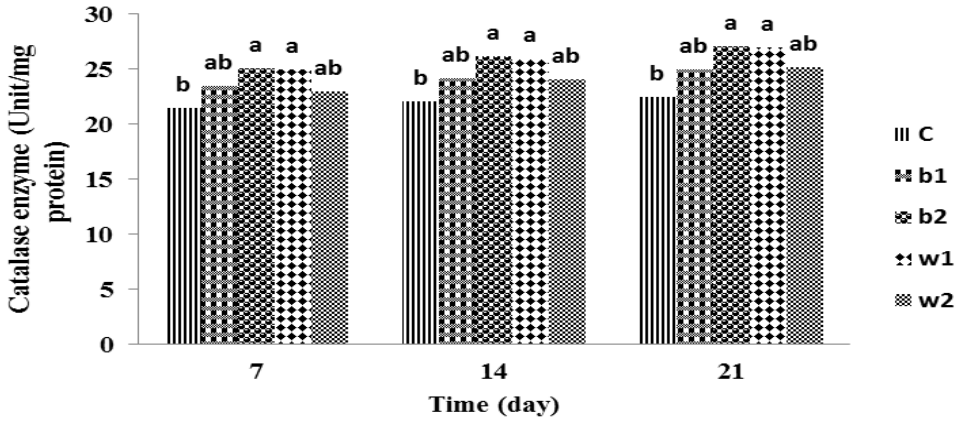

Fig. 5. Effect of BRs and HW treatment on CAT activity (Unit/mg protein) of lime fruit. Means in each column with the same letters are not significantly at 5% level of probability using Duncan' test. C: Control, b1: 0.5 Mg/lit BRs, b2: 1 Mg/lit BRs, w1: 45°C HW, and w2: 55°C HW.

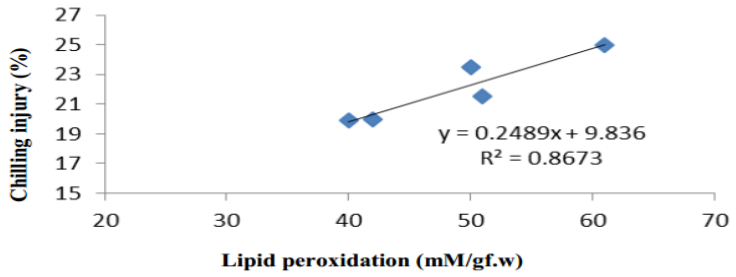

Fig. 6. Correlation between CI and lipid peroxidation of the lime fruit during storage under low temperature.

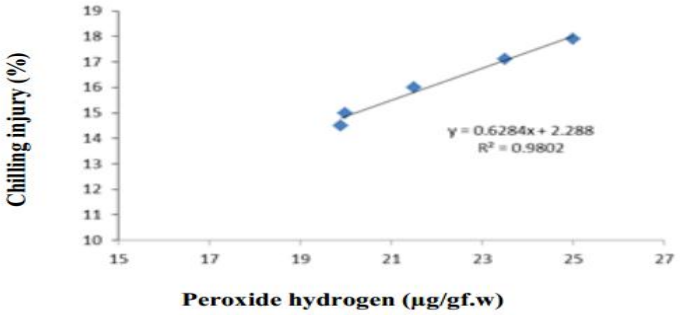

Fig. 7. Correlation between CI and hydrogen peroxide of the lime fruit during storage under low temperature.

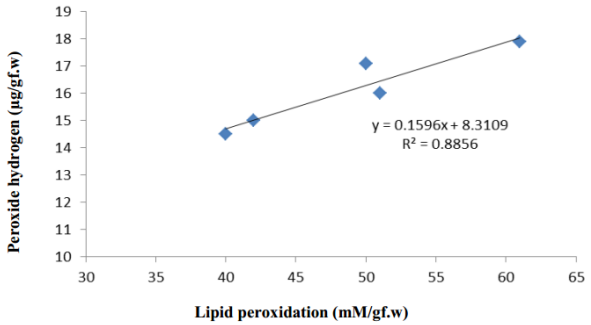

Fig. 8. Correlation between lipid peroxidation and hydrogen peroxide of the lime fruit during storage under low temperature.

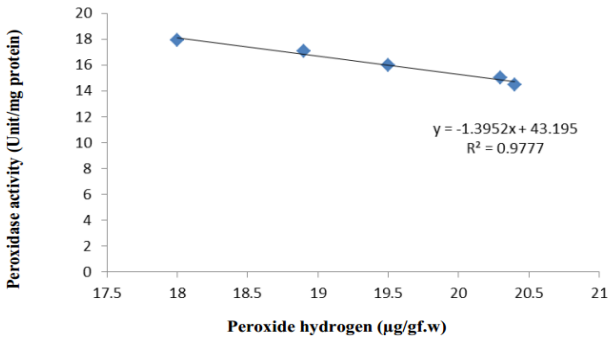

Fig. 9. Correlation between POD activity and hydrogen peroxide of the lime fruit during storage under low temperature.

Treatment with BRs and HW decreased the accumulation of MDA, the lipid peroxidation product, which is considered as an indicator of the loss of structural integrity in membranes and associated with cold stress. ROS accumulation cause oxidative damage to plant cell membranes and forms toxic products such as MDA as a secondary end-product of poly-unsaturated fatty acid oxidation. Thus, MDA is usually considered to be an indicator of plant oxidative stress (Hodges et al., 1999) and of the structural integrity of the membranes in plants subjected to low temperatures. It was reported that treatments with BRs could decrease the lipid peroxidation level induced by abiotic stress, such as oxygen deficiency (Ershova and Khripach, 1996), heat shock (Ogweno et al., 2008) and drought stress (Robinson and Bunce, 2000). The results showed that drought stress increased the H_2O_2 and lipid peroxidation level in fruits; however, this effect was significantly alleviated by application of BRs and HW. BRs maintain the membrane structure stability under stress conditions (Bajguz and Hayat, 2009). Therefore, bean plants treated with BRs, both in the presence and absence of cadmium stress, had a higher membrane stability index and decreased peroxidation of membrane lipids (Rady, 2011). However inhibited lipid peroxidation under chilling stress, which indicate that BRs strongly capable of protecting plants from oxidative damage, enhancing chilling tolerance, protecting membrane integrity and stabilizing antioxidant enzymes (Sharp et al., 1990 ; Bandurska, 1993 ; Bohnert and Jensen, 1996). Moreover, increasing BRs level in response to chilling stress plays a pivotal role in antioxidative stress as a hydroxyl radical scavenger (Yadegari et al., 2007). ,Correlations between the accumulation of antioxidant enzymes and improved cold tolerance have been found mostly in chilling sensitive plants (Zhao et al., 2009). Yusuf et al. (2011) reported that the treatment of Vigna radiata plants with BRs enhanced the activities of antioxidant enzymes both in the absence and presence of boron stress. He suggested that the acceleration of the activities of antioxidant enzymes resulted in an increase in the capacity of tolerance to boron stress. Accordingly, Rady (2011); showed that the treatment of bean plants with BRs enhanced the activities of antioxidant enzymes both in the presence and absence of cadmium stress. POD and CAT activity increased when plants exposed to stresses such as low temperature, pathogen infections, UV radiation, poisonous gases and heavy metals (Grover and Sinha, 1985). POD can scavenge the free radicals under chilling conditions. According to the Fig. 4 and 5, during storage time, antioxidant enzymes activities, upon BRs and HW treatments were significantly higher ($P < 0.05$) when compared to controls. BRs treatment hypothetically induces a defense mechanism which protect the plant tissue during environmental stresses, such as cold (Bajguz and Hayat, 2009). Zhu et al. (2010) reported that the brassinosteroids at a concentration of 5Mm, effectively inhibited development of blue mould rot in jujube fruit via enhancing the activities of POD, CAT and superoxide dismutase (SOD). It is generally accepted that POD and CAT activity increases in fruits under chilling temperature, the enhancement being proposed as a mechanism to alleviate CI symptoms (Lafuente et al., 2003), since pre-treatments such as heat reduces CI, (Chen et al., 2008) and leads to higher increases in POD activity in banana fruit. The increase in POD and CAT activity of BRs and HW treated fruits during storage time along with the amelioration of CI in this study confirms this finding. In conclusion, the present study has proven the beneficial effects of BRs on reducing CI in lime fruits during low temperature storage. Our results suggest that the development of CI in lime fruits increase POD and CAT activities and reduce lipid

peroxidation content, which ultimately results in reduced membrane integrity loses. Consequently, applications of BRs and HW are suggested for reducing CI in lime fruits during low temperature storage

References

1. Abu-Kpawoh J.C, Xi Y.F, Zhang Y.Z, Jin Y.F. 2002. Polyamine accumulation following hot-water dips influences chilling injury and decay in 'Friar' plum fruit. Journal Food Science 67, 2649–2653.
2. Beers R.F, Sizer I.W. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. The Journal of Biological Chemistry 195, 133-140.
3. Bradford M.M. 1976. A rapid and sensitive method for the quantittion of microgram quantities of protein utilizing the principle dye binding. Annual Review of Biochemistry 72, 248-252.
4. Bajguz A, Hayat S. 2009. Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiology and Biochemistry 47, 1–8.
5. Bandurska H. 1993. In vitro and in vivo effect of proline on nitrate reductase activity under osmotic stress in barley. Acta Physiologiae Plantarum 15, 83–88.
6. Bohnert H.J, Jensen R.G. 1996. Strategies for engineering water-stress tolerance in plants. Trends in Biotechnology 14, 89–97.
7. Chen J.Y, He L.H, Jiang Y.M, Wang Y, Joyce D.C, Ji Z.L, Lu W.L. 2008. Role of phenylalanine ammonia-lyase in heat pretreatment-induced chilling tolerance in banana fruit. Physiologia Plantarum 132, 318–328.
8. 8.Cao S, Xu Q, Cao Y, Qian K, An K, Zhu Y, Binzeng H, Zhao H, Kuai B. 2005. Loss of function mutations in DET2 gene lead to an enhanced resistance to oxidative stress in Arabidopsis. Physiologia Plantarum 123, 57–66.
9. Civello P.M, Martinez G.A, Chaves A.R, Anon M.C. 1997. Heat treatment delay ripening and postharvest decay of strawberry fruit. Journal of Agricultural and Food Chemistry 45, 4589-4594.
10. Cowley J.M, Chad field K.D, Baker R.T. 1992. Evaluation of dry heat as a postharvest disinfestation treatment for persimmons. Journal of Crop and Horticultural Science 20, 209-215.
11. Candir E.E, Temizyurek F, Ozdemir A.E. 2008. The effects of hot water dip treatments on the cold storage of Big Top nectarines. Journal of Applied Botany and Food Quality 82, 136-140.
12. Ershova A, Khripach V. 1996. Effect of epibrassinolide on lipid peroxidation in Pisum sativum at normal aeration and under oxygen deficiency. Journal of Plant Physiology 43, 750–752.
13. Enejoh O.S, Ogunyemi I.O, Bala M.S., Oruene I.S, Suleiman M.M, Ambali S.F. 2015. Ethnomedical Importance of *Citrus Aurantifolia* (Christm) Swingle. The Pharma Innovation Journal 4(8), 1-6.
14. Fariduddin Q, Yusuf M, Chalkoo S, Hayat S, Ahmad A. 2011. Homobrassinolide improves growth and photosynthesis in *Cucumis sativus* L. through an enhanced antioxidant system in the presence of chilling stress. Photosynthetica 49, 55–64.
15. Grover A, Sinha S.K. 1985. Senescence of detached leaves in pigeon pea and chick pea: Regulation by developing pods. Physiologia Plantarum 65, 503-507
16. He R.Y, Wang G.J, Wang X.S. 1991. Effects of brassinolide on growth and chill-ing resistance of maize seedlings. Brassinosteroids Chemistry, Bioactivity and Application. In: Cultler G.H, Yokota T, Adam G. American Chemical Society, Washington. 220–230.
17. Hodges D.M, Delong J.M, Forney C.F, Prange R.K. 1999. Improving the thiobar-bituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611.
18. Hotta Y, Tanaka T, Luo B.S, Takeuchi Y, Konnai M. 1998. Improvement of cold resistance in rice seedlings by 5-aminolevulinic acid. Journal of Pest Science 23, 29–33.
19. Heath R.L, Packer L. 1968. Photoperoxidation in isolated chloroplast I. kinetics and stoichiometry of fatty acids peroxidation. Archives of Biochemistry and Biophysics 125, 189-198.
20. Kochba J, Lavee S, Spiegel-Roy P. 1977. Differevces in peroxidase activity and isoenzymes in embryogenic and non-embryogenic shamouti orange ovular callus lines. Plant and Cell Physiology 18, 463-497.
21. Lay-Yee M, Ball S, Forbes S.K, Woolf A.B. 1997. Hot water treatment for insect disinfestation and reduction of chilling sensitivity of 'Fuyu' persimmon. Postharvest Biology and Technology 10, 81-88.

22. Lafuente M.T, Zacarias L, Martinez-Tellez M.A, Sanchez-Ballesta M.T, Granell A. 2003. Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus. Postharvest Biology and Technology 29, 308–317.

23. Lafuente M.T, Sala J.M, Zacarias L. 2004. Active oxygen detoxifying enzymes and phenylalanine ammonia-lyase in the ethylene-induced chilling tolerance in citrus fruit. Journal of Agricultural and Food Chemistry 52, 3606–3611.

24. Li B.Q, Zhang C.F, Cao B.H, Qin G.Z, Wang W.H, Tian S.P. 2012. Brassinolide enhances cold stress tolerance of fruit by regulating plasma membrane proteins and lipids. Amino Acids 43, 2469-80.

25. Liu Y.J, Zhao Z.G, Si J, Di C.X, Han J, An L.Z. 2009. Brassinosteroids alleviate chilling-induced oxidative damage by enhancing antioxidant defense system in suspension cultured cells of Chorispora bungeana. Plant Growth Regulation 59, 207–214.

26. Mandava N.B. 1988. Plant growth-promoting brassinosteroids. Annual review of plant physiology and plant molecular biology 39, 23–52.

27. Nakashita H, Yasuda M, Nitta T, Asami T, Fujikoa S, Arai Y, Sekimata K, Takat-suto S, Yamaguchi I, Yoshida S. 2003. Brassinosteroids functions in a broad range of disease resistance in tobacco and rice. The Plant Journal 33, 887–898.

28. Obenland D, Collin S, Sievert J, Fjeld K, Doctor J, Arpaia M.L. 2008. Commercial packing and storage of navel oranges alters aroma volatiles and reduces flavor quality. Postharvest Biology and Technology 47, 159–167.

29. Ozdemir A.E, Dundar, O. 1999. Effects of hot water dip applications on postharvest of some oranges (in Turkish). National Horticule Congress, Ankara. 126-131.

30. Ozdemir F, Bor M, Demiral T, Turkan I. 2004. Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxida-tive system of rice (*Oryza sativa* L.) under salinity stress. Plant Growth Regulation 42, 203–211.

31. Ogweno J.O, Song X.S, Shi K, Hu W.H, Mao W.H, Zhou Y.H, Yu J.Q, Nogues S. 2008. Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. Plant Growth Regulation 27, 49–57.

32. Porat R, Pavoncello D, Peretz Y, Weiss B, Cohen L, Ben-Yehoshua S, Fallik E, Droby S, Lurie S. 2000. Induction of resistance against Penicillium digitatum and chilling injury in 'Star Ruby' grapefruit by a short hot water brushing treatment. The Journal of Horticultural Science and Biotechnology 75, 428- 432.

33. Rady M.M. 2011. Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. Scientia Horticulturae 129, 232–237.

34. Robinson J.M, Bunce J.A. 2000. Influence of drought-induced water stress on soy-bean and spinach leaf ascorbate-dehydroascorbate level and redox status. International Journal of Plant Sciences 161, 271–279.

35. Sharp R.E, Hsiao T.C, Silk W.K. 1990. Growth of maize primary root at low water potential's. Plant Physiology 93, 1337–1346.

36. Velikova V, Yordanov I, Edreva A. 2000. Oxidative stress and some antioxidant system in acid rain treated bean plants: Protective role of exogenous polyamines. Plant Science 151, 59-66.

37. Yadegari L.Z, Heidari R, Carapetian J. 2007. The influence of cold acclimation on proline, malondialdehyde (MDA), total protein and pigments contents in soybean (*Glycine max*) seedlings. The Journal of Biological Sciences 7, 1436–1441.

38. Yu J.Q, Zhou Y.H, Ye S.F, Huang L.F. 2002. 24-Epibrassinolide and abscisic acid protect cucumber seedlings from chilling injury. The Journal of Horticultural Science and Biotechnology 77, 470–473.

39. Yusuf M, Fariduddin Q, Ahmad A. 2011. 28-Homobrassinolide mitigates boron induced toxicity through enhanced antioxidant system in *Vigna radiata* plants. Chemosphere 85, 1574-1584.

40. Zhu Z, Zhang Z, Qin G, Tian S. 2010. Effects of brassinosteroids on postharvest disease and senescence of jujube fruit in storage. Postharvest Biology and Technology 56, 50–55.

41. Wang C.Y. 1998. Heat treatment affects postharvest quality of kale and collard but not of brussels sprouts. HortScience 33, 881-883.

42. Woolf A.B, Ball S, Spooner K.J, Lay-Yee M, Ferguson I.B, Watkins C.B, Gunson A, Forbes S.K. 1997. Reduction of CI in the sweet persimmon 'Fuyu' during storage by dry air heat treatments. Postharvest Biology and Technology 11, 155-164.