Review

Role of alternative oxidase in postharvest stress of fruit and vegetables: Chilling injury

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Chilling injury (CI) imposes a limitation for extending storage of fruit and vegetables. Chilling injury can result from oxidative stress caused by reactive oxygen species (ROS). Alternative oxidase (AOX) as a ROS avoidance genes play pivotal role in defense mechanism against chilling injury derived oxidative stress. Postharvest treatment of fruit and vegetables through methyl salicylate, methyl jasmonate and storage in atmosphere with high O_2 could induce AOX gene expression and led to chilling tolerance. In this review, the main topic is the role of postharvest treatments in tolerance to chilling injury via induction of AOX gene expression and AOX physiological roles in fruit ripening.

Key words: Chilling injury, alternative oxidase, methyl salicylate, methyl jasmonate, reactive oxygen species, fruit ripening.

INTRODUCTION

Low temperature storage is a postharvest technology used widely to extend the postharvest life of fruit and vegetables and allows the preservation of fruit and vegetables quality after harvest, because low temperatures decrease the speed of cell metabolism and delay plant senescence and fruit ripening (Sevillano et al., 2009). Tropical and subtropical fruit and vegetables are sensitive to low temperatures and suffer chilling injury (CI). The incidence of chilling injury as a physiological disorder limits the application of low temperature storage. Therefore, the impact of chilling injury on the agro-food industry has serious economic consequences (Sevillano et al., 2009). Chilling injury of fruit and vegetables negatively affect their quality and therefore their marketing (Lafuente et al., 2005; Lafuente and Zacarias, 2006; Mulas and Schirra, 2007). To increase the tolerance of fruit and vegetables to chilling injury, postharvest treatments such as signaling molecules: Methyl jasmonate (MeJA) or methyl salicylate (MeSA) (Fung et al., 2004, 2006) or use of storage with high

(Zheng et al., 2008) had been developed. Further, the induction of the AOX gene expression by these pretreatments had been shown. Also, there is a direct correlation between the induction of AOX gene expression and the increase tolerance to chilling injury. In the present review, the literature in which the role of AOX had been studied in relation to the use of treatments for harvested fruit and vegetables was analyzed. Besides, the role of AOX in the fruit ripening and their biochemical mode of action were also discussed.

CHILLING INJURY IN HORTICULTURAL CROPS

Tropical and subtropical fruit and vegetables are sensitive to low temperatures and suffer from chilling injury. The occurrence of chilling injury limits the use of cold storage. Therefore, the impact of chilling injury on the horticultural industry has serious economic consequences. Understanding the physiological mechanisms responsible

the activation and development of this physiological disorder would allow the design of strategies to avoid or delay CI appearance (Sevillano et al., 2009). Cell membranes are primary sites for development of CI (Rui et al., 2010). Phase transitions from a flexible liquid crystalline to a solid gel structure occur in membrane of chilled tissue (Lyons, 1973). The fatty acid composition of membrane lipids can determine the existence of a temperature induced phase transition (Martin et al., 1976). In order to explain the impacts of CI on cell membranes and its manifestations at the molecular level when CI occurs, Lyons (1973) proposed the so-called 'membrane theory'. The membrane theory of CI suggests that the immediate effect of low temperatures is a global increase in the microviscosity of the membrane matrix due to a diminution of random rotation or folding movements of the aliphatic chains of fatty acids and to a reduction in the degree of desaturation of these acids. Below a critical value of temperature called the transition temperature, this event leads to a reorganization of the membrane lipids in a rigid structure called solid gel. A higher proportion of unsaturated fatty acids provide higher tolerance to low temperature, as has been reported, for example, in banana, pomegranate and loquat fruit (Mirdehghan et al., 2007; Promyou et al., 2008; Cao et al., 2009). Maintenance of membrane integrity at low temperature has been reported to be important in the resistance to chilling temperature (Wonsheree et al., 2009). As indicators of membrane damage, electrolyte leakage and malondialdehyde (MDA) content are generally considered to be indirect measurements of membrane integrity and can reflect the occurrence of CI and loss of membrane integrity (Shewfelt and Purvis, 1995). Lipolytic cascade in membrane lipids deterioration during senescence and CI was achieved by the concerted activities of a variety of membranous lipolytic enzymes such as phospholipase D (PLD) and lipoxygenase (LOX) (Pinhero et al., 1998). LOX and PLD catalyze peroxidation of polyunsaturated fatty acids and are believed to be major contributors to membrane damage and thus CI in plant (Pinhero et al., 1998; Wang, 2001). Mao et al. (2007) showed that the development of CI in cucumber fruit was accompanied by increase in PLD and LOX activities when exposed to chilling stress, and that the enhanced tolerance to CI by heat treatment was related to the reduction in activities of both enzymes. Rui et al. (2010) reported that the heat treatment increased LOX and PLD activity in response to chilling stress in loquat fruit and the reduction of internal browning (IB), main symptom of chilling injury in loquat fruit by heat treatment was associated with the reduction of PLD and LOX activities. This result suggested that these two enzymes might be associated with the initiation of CI by being involved in membrane deterioration and signalling pathway in response to chilling stress. Changes in membrane structure and composition are considered as the primary event of CI and lead to a loss of permeability control and

metabolic dysfunctioning (Lyons, 1973). It was reported that membrane lipids from chilling resistant plant species showed higher content of unsaturated fatty acids than did sensitive species (Wonsheree et al., 2009). Heat treatment induced acclimatization of pomegranate and banana fruit to low temperature and thus reduced CI by maintaining higher unsaturated/saturated fatty acid ratio (Mirdehghan et al., 2007; Promyou et al., 2008). Cao et al. (2009) showed that the decrease of lipid unsaturation was involved in the induction of CI in loquat fruit. The higher unsaturated/ saturated fatty acid ratio contributed to the reduced CI in methyl jasmonate treated fruit. Rui et al. (2010) suggested that the loss of membrane integrity, decrease in membrane lipid unsaturation and increases in PLD and LOX activities may be involved in the development of IB and CI in loquat fruit. The reduction of IB in chilled loquat fruit by heat treatment might be due to maintenance of membrane integrity, higher unsaturated/saturated fatty acid ratio and reduced PLD and LOX activities.

Apart from the direct effect of low temperatures on the molecular organization of membrane lipids, the loss of integrity of the membrane itself is boosted by oxidative processes, since low-temperature stress increases the levels of reactive oxygen species (ROS) (Sevillano et al., 2009). Defense against oxidative stress consists of two lines: The first line of defense is termed ROS scavenging genes which includes superoxide dismutase (SOD), catalase (CAT), the ascorbate/glutathione (AsA-GSH) cycle, the glutathione peroxidase and thioredoxin system (Møller, 2001). SOD catalyses the dismutation of $O_2^{\bullet-}$ to H_2O_2 and CAT scavenge H_2O_2 to form oxygen and water. The AsA-GSH cycle is also an important mechanism in the removal of ROS in plants. Its activation seems to produce a positive effect by inhibiting the development of CI. Sato et al. (2001) showed that the induction of APX activity by means of a heat treatment is a key element in the protection of rice against a later exposure to low temperatures. The GSH content and GR activity are also significant in plants showing tolerance to low temperatures. Cold-tolerant genotypes of tomato (Walker aand Mckersie, 1993) accumulate more GSH, and during cooling, their GR activity is higher than that of sensitive genotypes. Moreover, the rise in GSH content and GR activity in cold-sensitive genotypes of corn causes a diminution of CI (Kocsy et al., 2001).

The second line of defense is termed ROS avoidance genes which include alternative oxidase (AOX). The plant AOX pathway branches from the main respiratory electron transport chain, bypasses the final steps of the cytochrome respiratory pathway and catalyses the oxidation of ubiquinol. It was suggested that by maintaining the flow of mitochondrial electrons, AOX maintained activation of NAD(P)H dehydrogenase and proton-pumping NADH dehydrogenase (Møller, 2001) and helps in generation of sufficient ATP required for the rapid adaptation and the maintenance of plant growth rate

homeostasis (Moore et al., 2002; Hansen et al., 2002). In this process, AOX is involved in the reduction of ROS by preventing electrons from reducing O_2 to $O_2^{\cdot-}$ and thus reduces the level of O_2 in the mitochondria (Møller, 2001; Wagner and KRAB, 1995; Mittler, 2002). The concept of AOX acting as an antioxidant enzyme has been shown in isolated bell pepper mitochondria (Purvis, 1995) and in intact tobacco cells (Maxwell et al., 1999). It was proposed that the alternative respiratory pathway mediated chilling injury by keeping the production of ROS in balance with the levels of antioxidants and active oxygen scavenging enzyme systems (Purvis, 1995).

AOX FUNCTIONS IN FRUIT AND VEGETABLES

Two main hypotheses for roles of AOX activity in plants suggested that: (i) AOX activity allows continued TCA cycle carbon flow to occur under conditions when the cytochrome pathway is inhibited or restricted by the availability of ADP (Bahr and Bonner, 1973; Vanlerberghe and Mcintosh, 1994). When mitochondrial electron transport via the cytochrome pathway is inhibited or restricted, it results in the inhibition of TCA cycle and glycolysis. Reducing power (NAD (P) H) accumulates, biosynthesis stops and aerobic fermentation takes place which may lead to tissue damage by ethanol production (Vanlerberghe et al., 1995). An alternative electron transport pathway to oxygen could prevent redirection of carbon metabolism to the fermentative pathways and allow activity of TCA cycle to continue. Palmer (1976) suggested that this was probably the primary function of the AOX. Such a function was later modified by Lambers (1985), who suggested that the alternative pathway functions as an "energy overflow". It was proposed that the alternative pathway became engaged when the cytochrome pathway was saturated (Bahr and Bonner, 1973) such as the one that occur in the presence of excess sugars. (ii) The AOX has a role in the defence mechanism against oxidative stress such as chilling injury (Sevillano et al., 2009). Mittler (2002) proposed that the AOX reduced ROS production by means of two mechanisms: preventing the reduction of O_2 to superoxide radical and reducing the amount of O_2, the substrate for the formation of ROS in cell organelles.

There are two energy dissipating systems in plants: The redox energy dissipating pathway (AOX) and the proton electrochemical gradient (H^+) energy dissipating pathway or plant uncoupling mitochondrial protein (PUMP) which leads to the same final effect such as a decrease in ATP synthesis and an increase in heat production (Sluse and Jarmuszkiewicz, 2000). Linoleic acid, an abundant free fatty acid (FFA) in plants which activates PUMP, strongly inhibits cyanide resistant respiration mediated by AOX. Sluse and Jarmuszkiewicz (2000) reported that the AOX and PUMP acts sequentially during postharvest ripening of tomato fruit,

AOX activity decreases in early post-growing stages and PUMP activity is decreased in late ripening stages. Thermogenesis occurs during fruit ripening (Cruz-Hernandez and Gomez-Lim, 1995; Kumar et al., 1990; Kumar and Sinha, 1992) and FFA concentration increases during the post-growing stage (Rouet-Mayer et al., 1995). Sluse and Jarmuszkiewicz (2000) indicated a clear regulation of AOX activity through a decrease in protein expression during tomato fruit ripening, and a decrease in PUMP protein expression only after the yellow stage that parallels PUMP activity. Sluse and Jarmuszkiewicz (2000) suggested that AOX and PUMP acts sequentially. AOX would be active mainly during the growing period, thereby providing a safety balance between redox potential, phosphate potential and biosynthesis demand, whereas PUMP would start working in the post-growing stage when the FFA concentration increases, thereby providing a mechanism for heat generation via a decrease in the efficiency of oxidative phosphorylation in parallel with the termination of biosynthetic processes.

Considine et al. (2001) reported that the expression of PUMP genes is strongly enhanced during fruit ripening, suggesting a developmental stage specific regulation of PUMP. The increase in mRNA levels after the climacteric ripening phase directly correlates with the increase of mango PUMP content. In tomato, the studies on fruit ripening show that PUMP expression adapts to physiological conditions. Holtzapffel et al. (2002) showed an increase in transcript and protein levels in the later stages of on-vine fruit ripening and Almeida et al. (1999) reported that the activity of PUMP decreases during the later stages of postharvest fruit ripening. Almeida et al. (1999) also reported a higher amount of AOX in tomato fruit in postharvest ripening. However, FA concentrations also increase during the post growing stage. The PUMP and AOX pathways could therefore operate with different efficiencies under distinct physiological conditions. AOX would be active mainly during high biosynthetic activities in early stages of tomato fruit ripening, whereas PUMP would be functionally silent. With increasing FFA concentration in post growth stages, as in fruit ripening but perhaps also in senescence and flowering, PUMP activity could reach maximum velocity while AOX activity is switched off (Jarmuszkiewicz et al., 2001).

POSTHARVEST TREATMENTS, AOX GENE EXPRESSION AND CHILLING TOLERANCE IN FRUIT AND VEGETABLES

Although chilling injury can be prevented by maintaining the commodity at temperatures above the critical threshold, these temperatures can significantly reduce the product's shelf life (Troncoso-Rojas and Tiznado-Hernandez, 2006). Fruit and vegetables that are susceptible to chilling injury have a short storage life in

low temperature storage. Low temperature storage cannot be used to slow losses and pathogen growth in this commodity, which has serious economic consequence on the agro-industry.

Valero and Serrano (2010) reported that the potential symptoms of chilling injury in fruit and vegetables are surface lesions, pitting, sunken and discoloration area, water soaking of tissue, water loss, desiccation, shriveling, internal browning, mealiness and browning in the flesh, tissue breakdown, failure of fruit to ripen or uneven or slow ripening, accelerated senescence and ethylene production, shortened storage or shelf life, loss of flavor, loss of growth or sprouting capability, wilting and increased decay due to leakage of cell metabolites.

Due to consumer concerns about the presence of chemicals in fruit and vegetables, the use of environmentally friendly technologies such as salicylic and jasmonic acids and their methyl esters to avoid the development of chilling injury have been considered in many studies. The exposure of fruit and vegetables to these signaling molecules is used as a strategy to protect the commodity from the subsequent stress represented by the cold storage. From a biochemical point of view, evidence that links this protective effect with the expression of AOX has been found. The present review discusses the application of postharvest treatments and the link between treatment application and AOX gene expression.

SIGNALING MOLECULES: SALICYLATES AND JASMONATES

The increasing demand for consumption of fresh fruit and vegetables, along with more restriction on the use of synthetic chemicals to preserve produce quality and reduce CI, has encouraged scientific research to develop new technologies based on natural product such as salicylic acid and methyl salicylate (MeSA), and jasmonic acid and methyl jasmonate (MeJA).

SA and MeSA are endogenous signal molecules, playing pivotal roles in regulating stress responses and plant development. Recently, SA has received a particular attention because it is a key signal molecule for expression of multiple modes of plant stress resistance. Although, the focus has been mainly on the roles of SA on biotic stresses, several studies support major roles of salicylates in modulating plant response to several abiotic stresses, such as UV light, drought, salinity, chilling stress and heat shock. Salicylates delay the ripening of fruits, probably through inhibition of ethylene biosynthesis or action, and maintain postharvest quality (Asghari and Aghdam, 2010). Although, there are many methods to reduce CI in various fruit and vegetables, SA and MeSA treatments are inexpensive, easy to apply and can be used on various horticultural crops (Asghari and Aghdam, 2010). SA has been shown to induce expression of AOX

and increase the antioxidant capacity of the cells. Increases in AOX transcript levels using SA and MeSA before cold treatment reduces the incidence of CI in freshly green bell peppers (Fung et al., 2004). Lipid peroxidation is closely tied to CI in plant cells and this leads to MDA accumulation. MDA accumulation is prevented by SA treatment (Asghari and Aghdam, 2010). SA as a signal triggers the induction of cyanide resistance respiration in plant cells by affecting the AOX enzyme activity (Raskin et al., 1989). In fruit and vegetables, SA affects AOX activity leading to decrease in the harmful effects of different post-harvest oxidative stresses such as chilling injury, prevents fermentation and maintains low respiration rates and decreases fruit ripening and senescence rates. Respiration of harvested crops is highly dependent on ethylene production and activity and any factor increasing the production and activity of ethylene leads to increases in respiration and consequently increases the senescence rate. Effect of SA in decreasing the respiration rate is mainly due to its negative effects on ACC, ACO, PG, PME, cellulase and antioxidant enzymes leading to decrease in ethylene production and action (Asghari and Aghdam, 2010).

MeJA treatment also regulates diverse processes such as skin color development by promoting β-carotene synthesis and chlorophyll degradation, CI and ion leakage. MeJA treatment reduces the development of CI symptoms in a wide range of fruit and vegetables, including mango (González-Aguilar et al., 2000), guava (González-Aguilar et al., 2004), tomato (Ding et al., 2001) and loquat (Cao et al., 2010). Fruit and vegetables treated with MeJA have higher sugar, organic acid and vitamin C levels. MeJA stimulate ethylene biosynthesis by increasing ACS and ACO activity, which in turn enhances fruit ripening (González-Aguilar et al., 2006). MeJA treatment inhibits the gray mould rot caused by *Botrytis cinerea* and reduces decay caused by green mould *Penicillium digitatum* (Gonzalez-Aguilar et al., 2006). Recently, it was reported that MeJA induced the expression of pathogen related proteins (PRs) and AOX genes, increase the transcript accumulation of heat shock proteins (HSPs), and enhanced antioxidant system activity. These findings help to explain the mode of action of MeJA in increasing chilling tolerance in fruit and vegetables (Gonzalez-Aguilar et al., 2006).

Fung et al. (2004) reported that the treatment of pepper fruit with MeSA or MeJA vapors increased preferentially the transcription levels of AOX. These authors showed that the overnight treatment with MeSA or MeJA vapors increased transcript levels of AOX (1.5 kb) even at room temperature of 25°C, whereas no change was observed with untreated control. In addition to the expected 1.5 kb AOX transcript, RNA gel blot analysis by Fung et al. (2004) revealed an extra 3.5 kb transcript which was induced only at 0°C. At 0°C, both AOX transcripts reached maximal levels firstly in MeSA treated fruit, secondly in MeJA treated fruit and lastly in controls.

Fung et al. (2004) showed that the MeSA and MeJA vapors increased resistance against chilling injury in freshly harvested green bell pepper. They also showed that the increase in AOX transcript levels by MeJA or MeSA before cold treatment was correlated with reduced incidence of chilling injury in green bell pepper. AOX transcript, protein levels and activity were shown to respond to multiple developmental and environmental triggers (Considine et al., 2002). For example, AOX transcript and protein can be increased by low temperature (Ito et al., 1997; Djajanegara et al., 1999). Mutated AOX gene was linked to quantitative trait loci (QTL) for low temperature tolerance in a rice cultivar (Ribas-Carbo et al., 2000).

Fung et al. (2004) reported that the expression of AOX family 1 gene(s) in pepper was tightly associated with low temperature stress, suggesting that family 1 gene play a role in stress-induced conditions in plants. Within 6 h of rewarming, AOX transcripts (3.5 and 1.6 kb) in pepper fruit are depleted suggesting the presence of a high turnover rate for AOX transcripts. Low levels of CaAOX1 transcripts are detected in fruit stored at 20°C and freshly harvested. This suggests that the CaAOX1 transcript is constitutively expressed throughout fruit development and corresponds to basal levels necessary for alternative respiration (Fung et al., 2004).

Fung et al. (2004) showed that the CaAOX1 transcripts (1.5 kb) reach high levels within 2 days at 5°C but fruit at 0°C takes up to 7 days to attain the same level. Purvis (2001) reported that the higher AOX protein levels were found in mitochondria from pepper fruit stored at 4°C than at 1°C. Fung et al. (2004) suggested that the delay of CaAOX1 transcript accumulation at 0°C can be overcome by application of MeJA or MeSA vapors. Both MeSA and MeJA reduce short-term external surface pitting and long-term internal seed blackening symptoms in pepper fruit. Though, MeSA induced higher and earlier expression of CaAOX1 transcripts, MeJA (10^{-4} M) was more effective than MeSA (10^{-4} M) at alleviating chilling injury. It is possible that the beneficial effects for chilling resistance from enhancement of alternative oxidase might be compromised by the death-inducing properties of MeSA (Alvarez, 2000).

Fatty acid desaturase (18:1 FAD) gene for the production of polyunsaturated membrane lipid (Miquel et al. 1993), the catalase and alternative oxidase (AOX) genes in the oxidative stress defense mechanism (Kerdnaimongkol and Woodson, 1999; Abe et al., 2002), cold regulated (COR) genes such as dehydrins (Thomashow, 1998) and CBF1 genes, a member of AP2/EREBP transcription factor family (ZHAO et al., 2009) are mechanisms correlated with the acquisition of chilling tolerance.

AOX action maintains the flow of mitochondrial electrons and avoids production of ROS (Purvis, 1997; Maxwell et al., 1999; Møller, 2001) which was thought to be the main factor which resulted in CI (Purvis et al.,

1995). MeSA and MeJA induced CaAOX1 gene expression is correlated with resistance to CI in peppers (Fung et al., 2004). One unexplained phenomenon of CaAOX1 expression was the detection of two CaAOX transcripts (1.5 and 3.5 kb) at low temperature. A longer transcript was also detected in rice and suggested to be the unprocessed transcript of AOX (Ito et al., 1997). Family 1 AOX genes typically occur as multigene families in plants (Ito et al., 1997). For example, closely related LeAOX1a and 1b gene transcripts are expressed in chilled tomatoes (Holtzapffel et al., 2003). By using a yeast expression system, the 1b protein was shown to be functional and had altered regulatory properties in comparison to 1a. The 1b protein was suggested to be a less regulated form of AOX that remains active under stress conditions (Holtzapffel et al., 2003). Overnight exposure to MeSA or MeJA was effective at increasing resistance to CI in tomatoes at the mature green maturity stage (Ding et al., 2002) and in peppers (Fung et al., 2004).

Fung et al. (2006) treated pink tomato fruit overnight with MeJA or MeSA at a concentration of 10^{-4} M before cold storage at 0°C. CI index was recorded based on overall fruit appearance by taking into account the degree of shrivel, pitting, decay and fruit color. These authors reported that the significant differences in CI index were found among treated and untreated pink tomato fruit only after 3 weeks of storage and only with MeSA treatment. For MeJA treated fruit, no differences were observed in untreated fruit except for less decay. For MeSA treated fruit, CI index was significantly lower in terms of the degree of shrivel and decay. Red color development was inhibited in both untreated and MeJA treated fruit and was characterized by the development of pale orange and yellow pigmentation. In summary, MeSA (but not MeJA) efficiently delayed CI in tomatoes treated at the pink maturity stage (Fung et al., 2006).

RNA gel blot analysis carried out by Fung et al. (2006) indicated that LeAOX1a mRNA abundance increased during the 24 h exposure to exogenous MeSA and also during the first day at 0°C in both MeJA and MeSA treated fruit. In addition to the 1.6 kb LeAOX1a transcript, a 3 kb transcript was detected when fruits were transferred at 1°C. Both 1.6 and 3 kb LeAOX1a transcript levels remained high in treated and untreated fruit throughout the first 5 days of 0°C storage. The expression profile of LeAOX Family 1 transcripts in fruit appeared to be determined almost entirely by LeAOX1a expression. The transcript levels of the LeAOX Family 2 gene appeared to be increased by MeSA and MeJA during days 1 and 2, and thereafter remained unchanged during cold storage. A 3 kb transcript was also detected for the LeAOX Family 2 gene during 0°C storage.

Fung et al. (2006) concluded that the CI index and degree of severity (shrivel and decay) correlates with the expression pattern of the LeAOX1a and LeAOX2 genes in MeSA treated fruit before and during the first day of

cold storage. The three closely related LeAOX Family 1 genes are expressed at different levels in fruit and LeAOX1a is responsible for the vast majority of LeAOX Family 1 expression in fruit during cold storage and accumulation of its mRNA correlates with chilling resistance. MeJA and MeSA treatments induced chilling resistance in mature green tomatoes (Ding et al., 2002) and green bell peppers (Fung et al., 2004). However, MeJA is not as effective as MeSA in alleviating CI of pink maturity stage tomatoes. Fung et al. (2006) results confirms previous work demonstrating that tomato fruit at different maturity stages respond differently to plant growth regulator treatment (Ding and Wang, 2003). Also, chilling tolerance of maize, cucumber and rice seedling leaves and roots was shown to be differentially affected by salicylic acid (Kang and Saltveit, 2002). The delayed induction of LeAOX1a and LeAOX2 transcript levels in MeJA treated fruit may provide one explanation for the lower degree of shrivel found among chilling resistant MeSA treated tomato fruit but not in MeJA or control fruit. Among the AOX gene family members, LeAOX1 transcripts accumulate to the highest levels during cold storage and LeAOX1a mRNA abundance is higher in comparison to that of the 1b and 1c genes in fruit throughout the course of the treatment.

HIGH OXYGEN STORAGE

Storage of horticultural crops under superatmospheric O_2 concentration may stimulate, have no effect, or reduce ethylene production and rates of respiration, depending on the commodity, maturity and ripeness stage, O_2 concentration, storage time and temperature, and concentrations of CO_2 and ethylene present in the atmosphere. Ripening of mature-green climacteric fruits was slightly enhanced by exposure to 30 to 80 kPa O_2, but levels above 80 kPa retarded their ripening and caused O_2 toxicity disorders on fruits. High O_2 concentrations enhance the effects of ethylene on fresh fruits and vegetables, including ripening, senescence and ethylene-induced physiological disorders such as bitterness of carrots and russet spotting on lettuce. While superatmospheric O_2 concentrations inhibit the growth of bacteria and fungi, they are much more effective if combined with elevated (15 to 20 kPa) CO_2, which is a fungistatic gas (Kader and Ben-Yehoshua, 2000). AOX gene expression is enhanced by elevated O_2 atmospheres (Zheng et al., 2008). Zheng et al. (2008) reported that the transcript levels of antioxidative genes including SOD, APX and CAT which were relatively constant during storage at 5°C with high oxygen treatment in freshly harvested zucchini squash. However, the expressions of AOX were induced slightly in squash treated with 60 and 100% oxygen for 3 days when compared with the control squash. These increases in AOX transcript levels were correlated with the increased

chilling resistance in the treated squash. The ROS scavenging enzyme activities including SOD, APX, CAT and POD in treated zucchini squash were also higher than those in the control for the first 3 days at 5°C. Transcript levels of AOX increased substantially between 3 and 6 days in all treatments, suggesting the involvement of alternative respiratory pathway during chilling stress. All the enzyme activities in 100% oxygen treated squash started to decline after 6 or 9 days of cold storage to a level comparable or lower than those of the control. These declines were correlated to the loss of chilling resistance in the 100% oxygen treated tissue as indicated in the chilling injury index. However, squash treated with 60% oxygen maintained elevated levels of all enzyme activities except POD and sustained the least chilling injury throughout the 15 days of storage at 5°C. The oxygen radical absorbance capacity (ORAC) values and total phenol content remained high in squash treated with 60 and 100% oxygen for the first 9 days, then their levels in the 100% oxygen treated samples declined sharply while those in the 60% oxygen treated samples maintained elevated, indicating that both ORAC activity and phenolic content may also contribute to the resistance of tissue against chilling injury. The 100% oxygen treated squash showed the lowest respiration rate and 60% oxygen treated samples had the lowest ethylene production. Zheng et al. (2008) results may be an indication of the low chilling injury in the high oxygen treated squash. Zheng et al. (2008) results showed that the ORAC and total phenolic levels in zucchini skin peels were both induced by cold storage and further enhanced by 60% oxygen storage. The enhanced antioxidative enzyme activities and the overall antioxidative capacity (ORAC and phenolic levels) seem to correlate with the reduced chilling injury.

In peppers, treatment with MeJA and MeSA reduced chilling injury and induced preferentially the transcript level of AOX gene, while no change in transcript levels of ROS scavenging genes were detected during the early cold storage at 0°C (Fung et al., 2004). Zheng et al. (2008) showed that the elevated level of oxygen was effective in reducing chilling injury in zucchini. Similarly, Zheng et al. (2008) observed slight induction of only AOX gene transcript during early chilling period at day 3 when no difference in chilling injury symptom was visible among treated and control squash. The detectable differences in AOX transcript levels among treated and control zucchini and peppers (Fung et al., 2004) suggested possible correlation between AOX gene expression level and chilling injury. Zheng et al. (2008) results are consistent with the idea that global reprogramming of metabolism occurs during low temperature stress (Sung et al., 2003; Graya and Heath, 2005; Hannah et al., 2005). Zheng et al. (2008) observed that cold storage resulted in inhibition of primary metabolic pathways of respiration and induction of AOX transcript of alternative respiratory chain. This is also

accompanied by the antioxidant system as demonstrated by their enzyme activities and by the ORAC assay that presumably function to provide immediate relieve to the adverse redox status. On the other hand, the plant system quickly remobilize its primary metabolites into various secondary metabolite biosynthetic pathway (Kaplan et al., 2004; Renaut et al., 2005) as also shown by Zheng et al. (2008) data from the total phenolic compound that increased by 30% during cold storage.

CONCLUSION

AOX is a best candid gene for manipulation of resistance to chilling injury in fruit and vegetables. Postharvest treatments increased the AOX gene expression and can be used in postharvest technology as an important method for reducing chilling injury of fruit and vegetables. The applied technologies for the reduced CI impacts via the increase of AOX gene expression are based mainly on the application of signalling molecules such as SA, MeSA and JA, and MeJA or storage with high O_2. Biotechnology has also offered way to reduce the impact of CI in sensitive fruit and vegetables with the generation of genetically modified plants by the overexpression of AOX genes that act as an antioxidant avoidance gene and controls the ROS production in fruit and vegetables under chilling temperature.

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