CHAPTER 2

Literature Review

Thai mango export and problems

Mango is an exotic economic fruit of Thailand, which is widely grown in many regions. Mango fruits are domestically consumed and exported to international markets. The quantity and value of exported mango have increased during the past 7 years. In 2013, thirty three thousand tons of fresh mango fruits were exported to international markets giving an income to Thailand of 853.48 million baht (Thai Customs Department, 2014).

In 2013, fresh mangoes were mostly exported to Asian countries such as Vietnam, Korea, Japan, Malaysia and China (92.67%) and the minority was exported to some countries in Europe, America and other countries (7.33%) (Thai Customs Department, 2014). Among the mango cultivars, Nam Dok Mai No. 4 is one of the most popular cultivar in the international market because it has lusciously fragrant, sweet juicy mesocarp and golden yellow exocarp color that attract consumers, especially in Japan (Office of Agricultural Economics, 2009).

Increasing popularity and demand for high quality Thai mangoes in the international market over the past two decades has created a shift in transportation linkages away from air and towards marine shipment (Evans, 2008). Exportation of Thai mangoes via marine shipment could be done at lower cost and higher volume than air shipment but transit time may be more than 2 weeks. Mango fruit ripes and deteriorates quickly and has a short postharvest life at ambient temperature (Pantastico *et al.*, 1984; Pesis *et al.*, 2000). It is limited by physiological deterioration related to over-ripening and by pathogen development (Johnson and Coates, 1993), causing a commercial problem in distant markets such as Europe and America. Extending mango postharvest life will enhance the export quantity and distribution of Thai mango in the international market.

Keeping mango at low temperature is considered to be the most effective method for maintaining the quality as it retards respiration, ethylene production, ripening and senescence. It has been suggested that a temperature of 13 °C with 90-95% RH is optimum for mango storage for 2-3 weeks (Camelo, 2004; Department of Agriculture, 2011). Shipping time to Europe and America is longer. Therefore, low temperature storage below 13 °C has been developed to extend mango storage life. There are several studies on how to extend postharvest life of mango fruit below 13 °C such as 2 °C (Zhao *et al.*, 2006), 4 °C (Ketsa *et al.*, 2000; Phakawatmongkol *et al.*, 2004; Chidtragool *et al.*, 2011; Chongchatuporn *et al.*, 2013), 5 °C (González-Aguilar *et al.*, 2001; Ding *et al.*, 2007), 7 °C (González-Aguilar *et al.*, 2000) and 8 °C (Phakawatmongkol *et al.*, 2004). Unfortunately, mango fruits are susceptible to chilling injury (CI), which leads to a storage defect when fruits are stored at 2-8 °C for a long period of time (Ketsa *et al.*, 2000; González-Aguilar *et al.*, 2000, 2001; Phakawatmongkol *et al.*, 2004; Zhao *et al.*, 2006; Ding *et al.*, 2007; Chidtragool *et al.*, 2011; Chongchatuporn *et al.*, 2013). It is important to find methods to protect or reduce CI of mango fruit during storage at low temperature with less quality loss and increased storage and shelf life. It must be also practical, easy, cheap and safe to apply.

Chilling injury (CI) in plant

CI is an environmental stress and physiological disorder that occurs in many fruits and vegetables under storage or growth at low temperature (Sharma *et al.*, 2012). It may occur in the field, in transit or distribution and in retail or home refrigerators. Most crops of tropical and subtropical origins are sensitive to CI. Some crops in temperate zone are also susceptible. These crops are injured by low, but nonfreezing, temperatures. The critical temperature for CI varies with the commodity, but it generally occurs when the product is stored at temperatures below 10-13 °C. At this temperature, the tissues are weaken because they are unable to carry on normal metabolic processes. Various physiological and biochemical alterations occur in the sensitive species in response to low-temperature exposure. These alterations lead to the development of a variety of CI symptoms (Wang, 1994).

Some common CI symptoms in fruits are shown in Table 2.1. They include pitting, discoloration, water-soaked appearance, internal breakdown, failure to ripen,

loss of flavor and aroma and decay. The onset of symptom expression may take hours to months, depending on temperature, time and sensitivity of plant. The symptoms are also often not apparent while the fruits are at a low temperature, but develop later, when the fruits are brought to warmer temperatures for ripening or are displayed for sale (Wang, 1994).

Table 2.1 Chilling injury symptoms on some fruits.

Fruits	CI temperatures	CI symptoms	References
cucumber	2 °C	surface pitting and dark watery patches	Yang *et al.*, 2011
guava	5 °C	skin browning, abnormal ripening and decay	González-Aguilar *et al.*, 2004
lemon	1.5 °C	surface pitting, browning	Safizadeh *et al.*, 2007
loquat	1 °C	stuck peel, firm, juiceless flesh, and internal browning	Cao *et al.*, 2009
mume	1, 6 °C	surface pitting	Imahori *et al.*, 2008
pear	2 °C	surface pitting, browning and dehydration	Al-Qurashi and Awad, 2012
peach	0 °C	internal browning and flesh mealiness	Jin *et al.*, 2009b
pepper	1, 5 °C	surface pitting	Lim *et al.*, 2009
pineapple	10 °C	surface browning of the pulp adjacent to the core	Nilprapruck *et al.*, 2008
pomegranate	2 °C	pitting, browning and dehydration	Sayyari *et al.*, 2009
tomato	3 °C	surface pitting, water soaking, abnormal ripening and decay	Côté *et al.*, 1993
zucchini squash	5 °C	surface pitting	Wang, 1995

Mango fruits are considered to be a climacteric and ripen rapidly after harvest. Storage, handling and transport of mango fruits are limited by susceptibility to diseases, sensitivity to storage temperatures below 10-13 °C and perishability due to ripening and softening (Acosta *et al.*, 2000). The combination of storage temperature and duration of storage are important factors that lead to chilling and induce physiological and metabolic dysfunctions in plant cells. These dysfunctions lead to various visible disorders that are commonly used to assess the degree of CI (Walker *et al.*, 1990).

In mango fruit, the most common visual symptoms of CI are dark, discolorations of the exocarp, beginning around lenticels and spreading outwards to produce a more or less circular lesion, pitting on the fruit exocarp, development of off-flavor, discoloration of the mesocarp and overall poor fruit quality (Nair *et al.*, 2003). The symptoms depend on the mango cultivar, storage temperature and duration of exposure to low temperature. For example, Lederman *et al.* (1997) reported that storage temperature at 0, 2 and 5 °C induced CI symptoms manifested as surface pitting and water soaking in Keitt mango fruits which appeared on Week 2 during storage at 0 and 2 °C, while at 5 °C, CI symptoms appeared only 3 days after the fruit had been transferred to shelf conditions (Lederman *et al.*, 1997). Ding *et al.* (2007) and Wang *et al.* (2008) reported that surface pitting and sunken lesions were found in mango cv. Zill and Tainong after exposure to low temperature at 5 °C for 20 days and 4 °C for 7 days, respectively. González-Aguilar *et al.* (2000) reported that mango fruit cv. Tommy Atkins showed CI symptoms as surface pitting that appeared on Day 7 after exposure to 7 °C. González-Aguilar *et al.* (2001) reported that mango fruit cv. Kent also showed surface pitting that appeared on Day 14 after exposure to 5 and 10 °C. CI symptoms in stored mango at 5 °C were higher than those stored at 10 °C (González-Aguilar *et al.*, 2001). The lower temperature at 2 °C also induced CI symptoms as water soaking in mango fruit cv. Wacheng during storage at 2 °C for 12 days (Zhao *et al.*, 2006).

In Thai mango cultivars, low temperature storage at 4, 8 and 12 °C induced CI symptoms in cvs. Kaew, Rad, Okrong, Tongdum, Nam Dok Mai and Nungklangwun. CI symptoms in these cultivars manifested as exocarp, mesocarp and endocarp browning. Nam Dok Mai was most sensitive to CI, based on exocarp browning during storage at 4, 8 and 12 °C (Phakawatmongkol *et al.*, 2004). In Nam

Dok Mai Si Thong, CI symptoms were surface pitting and exocarp browning which occurred on Days 20 and 30 after storage at 5 and 8 °C respectively (Pattanapo *et al.*, 2010). Chidtragool *et al.* (2011) reported that Nam Dok Mai mango fruit showed CI symptoms as exocarp browning during storage at 4 °C on Day 6 of storage, while CI symptoms were not found in cv. Choke Anan. Chongchatuporn *et al.* (2013) also found that exocarp browning occurred in Nam Dok Mai on Day 5 of storage at 4 °C, while this symptom was found in cv. Choke Anan on Day 10. These results indicate that Nam Dok Mai is most sensitive to CI, based on exocarp browning during storage below 13 °C (Phakawatmongkol *et al.*, 2004; Chidtragool *et al.*, 2011; Chongchatuporn *et al.*, 2013).

CI symptoms usually develop more strongly once the mango fruits are returned to non-chilling temperatures (Lederman *et al.*, 1997; González-Aguilar *et al.*, 2000, 2001; Ding *et al.*, 2007; Wang *et al.*, 2008; Phakawatmongkol *et al.*, 2004; Chidtragool *et al.*, 2011; Chongchatuporn *et al.*, 2013).

Besides visual injury symptoms, CI in mango fruits stored at 4 °C accelerated softening of the fruit after they were transferred to 20 °C for ripening (Kane *et al.*, 1982). González-Aguilar *et al.* (2001) reported CI induced high decay and low overall quality in Kent mangoes after storage at 5 °C for 14 days. The inhibition in β-carotene synthesis and chlorophyll degradation were found in CI fruit after 14 days in fruits stored at 5 °C. These fruits lacked yellow and red color development in the exocarp after transfer to room temperature. There is a significant decrease in total soluble solids (TSS) and organic acid, especially citric acid that retarded or inhibits fruit ripening (González-Aguilar *et al.*, 2001). Wang *et al.* (2008) reported that low sensory quality, indicated by the appearance, taste, texture and odor of ripe fruit was found in mango cv. Tainong after storage at 4 °C for 7 days.

Mechanisms involved in chilling injury

The mechanisms involved in the CI of plant tissues are poorly understood. Many hypotheses about development of CI in plants have been made (Lyons, 1973; Parkin *et al.*, 1989; Kaniuga, 2008; Sevillano *et al.*, 2009; Aghdam and Bodbodak, 2013). Lyons (1973) presented a schematic pathway of the events leading to CI in sensitive

plant tissues resulting from the phase transition in cellular membranes (Figure 2.1). As the temperature is lowered in chilling-sensitive species, the membrane lipid bilayers change from their normal flexible liquid-crystalline to solid gel. This results in a decrease in membrane flexibility that causes cracks or channels, leading to increased permeability. This immediate effect on permeability leads to an upset in ion balance as well as ion leakage that results from chilling in some tissues. The phase transition and solidification of membranes also disrupt normal functioning of membrane bound enzymes. The reaction rate of these enzymes is inhibited by increased activation energy leading to imbalances in metabolism and accumulation of toxic metabolites in plants. CI and death of cells and tissues can occur in plants exposed to low temperature for a long time.

Another possibility is associated with an increase in oxidative stress during low temperature storage (Hodges *et al*., 2004; Kaniuga, 2008; Sevillano *et al*., 2009; Aghdam and Bodbodak, 2013). Shewfelt and del Rosario (2000) suggested that low temperature storage induced oxidative stress from excess free radicals accumulation (Figure 2.2). The cause of free radicals accumulation is loss of equilibrium between the production of free radicals and the scavenging of free radicals (antioxidant defense system). Free radicals are highly reactive with biomolecules such as lipids, proteins and DNA in plant cell that lead to lipid peroxidation, protein and DNA damage in plant cells during low temperature conditions (Mittler, 2002; Gill and Tuteja, 2010). An increase in lipid peroxidation of membranes leads to membrane breakdown resulting in metabolic imbalances within the cell manifested at the tissue level as visible CI symptoms (Shewfelt and del Rosario, 2000).

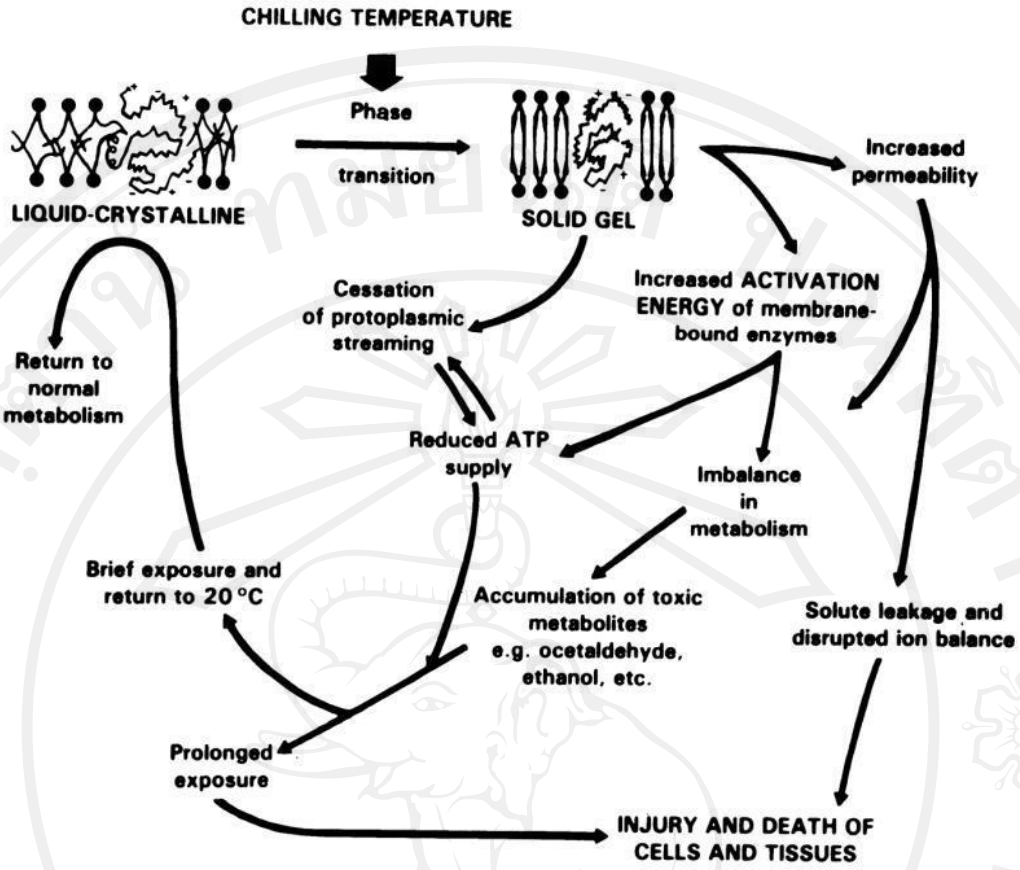

Figure 2.1 Schematic pathway of the events leading to chilling injury in sensitive plant tissues (Lyons, 1973).

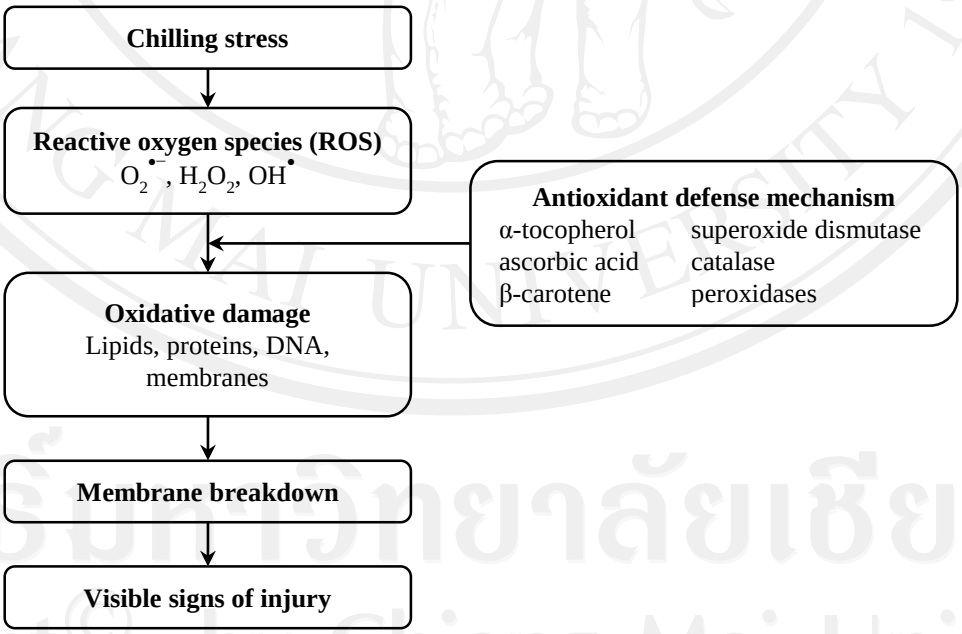

Figure 2.2 Conceptual model to explain CI development (modified from Shewfelt and del Rosario, 2000).

Free radicals and their role during low temperature stress

Free radicals are atoms or groups of atoms with at least one unpaired electron in their orbitals and is therefore unstable (Pala and Tabakçioğlu, 2007). Reactive oxygen species (ROS) are a group of free radicals, reactive molecules and ions that are derived from O_2. It has been estimated that about 1% of O_2 consumed by plants is diverted to produce ROS in various subcellular loci such as chloroplasts, mitochondria and peroxisomes (Sharma *et al.*, 2012). ROS are well recognized for having a dual role as deleterious and beneficial species depending on their concentration in plants. At high concentrations ROS causes damage to biomolecules, whereas at low/moderate concentrations it acts as second messenger in intracellular signaling cascades that mediate several responses in plant cells (Yuan and Lin, 2008, Sharma *et al.*, 2012). ROS such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$) and lipid peroxide radical ($LOO^{\bullet}$) are the most prevalent free radicals in plants (Dat *et al.*, 2000; Arora *et al.*, 2002; Bloknina *et al.*, 2003; Apel and Hirt, 2004; Møller *et al.*, 2007).

Plant cells continuously generate free radicals naturally as by-products of aerobic metabolism, from the electron transport chains of photosynthesis and respiration (Dat *et al.*, 2000; Bloknina *et al.*, 2003; Apel and Hirt, 2004; Gill and Tuteja, 2010). Leakage of electrons to oxygen (O_2), from the electron transport chains, releases and forms $O_2^{\bullet-}$ (Apel and Hirt, 2004; Gill and Tuteja, 2010). $O_2^{\bullet-}$ can be further reduced by superoxide dismutase (SOD) dismutation to H_2O_2. About 1-5% of mitochondrial O_2 consumption leads to H_2O_2 production, which is a stable ROS with long half-life (Gill and Tuteja, 2010). This H_2O_2 reacts with reduced Fe^{2+} and Cu^{+} to produce highly toxic $OH^{\bullet}$. $OH^{\bullet}$ is also produced from $O_2^{\bullet-}$ and H_2O_2 by the Fenton and Haber-Weiss reactions (Dat *et al.*, 2000; Arora *et al.*, 2002; Gill and Tuteja, 2010; Sharma *et al.*, 2012). These ROS are capable to initiate lipid peroxidation and lead to $L^{\bullet}$ and $LOO^{\bullet}$ (Sharma *et al.*, 2012).

1. Superoxide radical ($O_2^{\bullet-}$)

ROS appears continuously during photosynthesis in chloroplasts by partial reduction of O_2 molecules or energy transferred to them. The major site of $O_2^{\bullet-}$

production is the thylakoid membrane-bound primary electron acceptor of photosystem I (Gill and Tuteja, 2010). The production of ROS is an inevitable consequence of aerobic respiration in mitochondria (Purvis and Shewfelt, 1993; Møller, 2001). When the terminal oxidases, cytochrome c oxidase and the alternative oxidase react with O_2, four electrons are transferred and H_2O is released. Occasionally O_2 can react with other electron transport chain (ETC) components such as nicotinamide adenine dinucleotide (NADH) dehydrogenase, succinate dehydrogenase and ubiquinol-cytochrome C reductase. Here, only one electron is transferred, and the result is $O_2^{\bullet-}$ (Purvis and Shewfelt, 1993; Turrens, 2003).

$O_2^{\bullet-}$ is a moderately reactive ROS with approximately 2-4 μs of half-life. It is usually the first ROS to be generated (Møller *et al.*, 2007; Gill and Tuteja, 2010). The generation of $O_2^{\bullet-}$ may trigger the formation of more reactive ROS like $OH^{\bullet}$ and more possibly $^1O_2$, each of which may cause peroxidation to membrane lipids and cellular weakening. $O_2^{\bullet-}$ can donate an electron to iron (Fe^{3+}) to yield a reduced form of iron (Fe^{2+}) which can then reduce H_2O_2, produced as a result of SOD led dismutation of $O_2^{\bullet-}$, to $OH^{\bullet}$. The reactions through which $O_2^{\bullet-}$, H_2O_2 and iron rapidly generate $OH^{\bullet}$ is called the Haber-Weiss reaction, whereas the final step which involves the oxidation of Fe^{2+} by H_2O_2 is referred to as the Fenton's reaction (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

$$O_2^{\bullet-} + Fe^{3+} \longrightarrow {}^1O_2 + Fe^{2+}$$

$$2O_2^{\bullet-} + 2H^{+} \xrightarrow{SOD} O_2 + H_2O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{-} + OH^{\bullet} \text{ (Fenton reaction)}$$

Exposure to low temperature stress increases $O_2^{\bullet-}$ content in many plants. It was found that $O_2^{\bullet-}$ content in the mesocarp of mango fruit cv. Zill decreased during the first 10 days and then increased continuously after Days 10-30 during storage at 5 °C, correlated with an increase in CI symptoms expressed as exocarp pitting on Days 20 and 30 of storage (Ding *et al.*, 2007). In other fruits, Cai *et al.* (2006) reported that the $O_2^{\bullet-}$ content in the flesh of loquat fruit cv. Luoyangqing gradually increased throughout cold storage at 0 and 5 °C with increased in CI symptoms expressed as peel and flesh

browning during storage. Cao *et al.* (2009) also found that $O_2^{\bullet-}$ content increased in flesh of loquat fruit cv. Fuyang during storage at 1 °C for 35 days and correlated with increasing in CI symptoms such as browning discoloration near the core, firm and juiceless flesh. In addition, Yang *et al.* (2011) found that $O_2^{\bullet-}$ content increased in flesh tissue of cucumber throughout storage at 2 °C for 15 days and correlated with increased CI symptom. Yang *et al.*, 2012 found that $O_2^{\bullet-}$ content increased in flesh tissue of kiwifruit cv. Hongyang during storage at 0 °C for 80 days and correlated with high CI symptoms on Day 80. In kiwifruit cv. Hayward, it was also found that cold storage at 0 °C for 120 days increased $O_2^{\bullet-}$ accumulation in flesh tissue and correlated with CI symptoms which appeared on Day 60 onwards (Yang *et al.*, 2013).

2. Hydrogen peroxide (H_2O_2)

H_2O_2 can be produced from $O_2^{\bullet-}$ that accepts one electron and two protons or by superoxide dismutase (SOD) catalyzed reaction to H_2O_2 (Sharma *et al.*, 2012). H_2O_2 is moderately reactive and has a relatively long half-life (1 ms). Other ROS such as $O_2^{\bullet-}$, $OH^{\bullet}$ and $^1O_2$, have a much shorter half-life (2-4 μs) (Møller *et al.*, 2007; Gill and Tuteja, 2010). Excess H_2O_2 in plant cells leads to the occurrence of oxidative stress. H_2O_2 may inactivate enzymes by oxidizing their thiol groups (Gill and Tuteja, 2010).

H_2O_2 plays a dual role in plants. At low concentrations, H_2O_2 acts as a signal molecule involved in acclimatory signaling triggering tolerance to various biotic and abiotic stresses. At high concentrations, H_2O_2 leads to programmed cell death (PCD) (Mittler, 2002; Gill and Tuteja, 2010). H_2O_2 has also been shown to act as a key regulator in a broad range of physiological processes, such as senescence, photorespiration and photosynthesis, stomatal movement, growth and development. H_2O_2 is a second messenger for signals generated by means of ROS because of its relatively long life and high permeability across membranes (Møller *et al.*, 2007; Gill and Tuteja, 2010).

Low temperature storage induces H_2O_2 accumulation in many fruits. For example, H_2O_2 accumulation increased in the mesocarp of mango fruit cv. Zill during the first 20 days of storage at 5 °C with CI symptoms first occurring on Day 20 (Ding *et al.*, 2007). In cucumber, an increase in H_2O_2 content was found in flesh tissue during

storage at 2 °C for 15 days along with an increase in CI (Yang *et al.*, 2011). In loquat fruit, H_2O_2 content increased in the flesh along with an increase in CI symptoms during storage at 1 °C for 35 days (Cao *et al.*, 2009). In peach, low temperature storage at 0 °C for 35 days induced an increase in H_2O_2 content in flesh tissue throughout storage time and correlated with increased CI symptoms expressed as internal browning and flesh mealiness (Jin *et al.*, 2009a and 2013). In kiwifruit cv. Hongyang, storage at 0 °C for 80 days induced H_2O_2 accumulation in flesh tissues along with high CI symptom appeared on Day 80 (Yang *et al.*, 2012). Yang *et al.* (2013) also reported that H_2O_2 content gradually increased in flesh tissue of kiwifruit cv. Hayward during storage at 0 °C for 120 days and CI symptoms appeared on Day 60 and afterwards.

Accumulation of H_2O_2 content was also found in many plant seedlings during growth under low temperature stress and correlated with CI symptoms development. An increase in H_2O_2 content in leaf tissue was found in banana seedlings during growth under low temperature stress at 5 °C for 3 days along with CI symptoms in leaf expressed as necrosis and wilting (Kang *et al.*, 2003). An increase in H_2O_2 content was also found in leaves of cucumber seedlings grown at 10 °C for 6 days (Lei *et al.*, 2010). In eggplant seedlings, H_2O_2 content gradually increased in leaves growing under low temperature stress at 4 °C for 9 days and with CI symptom in the leaves (Chen *et al.*, 2011).

3. Hydroxyl radical ($OH^{\bullet}$)

Hydroxyl radicals are among the most highly reactive ROS known (Møller *et al.*, 2007; Gill and Tuteja, 2010; Sharma *et al.*, 2012). In the presence of suitable transitional metals, especially Fe, $OH^{\bullet}$ can also be produced from $O_2^{\bullet-}$ and H_2O_2 at neutral pH and ambient temperatures by the catalyzed-iron, $O_2^{\bullet-}$ driven Fenton reaction (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

$$H_2O_2 + O_2^{\bullet-} \xrightarrow{Fe^{2+}, Fe^{3+}} OH^- + O_2 + OH^{\bullet}$$

These $OH^{\bullet}$ are thought to be largely responsible for mediating oxygen toxicity *in vivo* (Gill and Tuteja, 2010; Sharma *et al.*, 2012). $OH^{\bullet}$ can potentially react with all

biological molecules viz. DNA, proteins, lipids and almost any constituent of cells. Due to the absence of any enzymatic mechanism for the elimination of this highly reactive ROS, excess production of $OH^{\bullet}$ ultimately leads to cell death (Gill and Tuteja, 2010). Low temperature storage induced $OH^{\bullet}$ accumulation in eight wild almond species (Sofo *et al.*, 2014). The content of $OH^{\bullet}$ increased in the leaves of almond after exposure to low temperature at 4 °C for 3 days (Sofo *et al.*, 2014).

4. Singlet oxygen ($^1O_2$)

$^1O_2$ is the first excited electronic state of O_2 and is an unusual ROS because it is not related to electron transfer to O_2. Insufficient energy dissipation during photosynthesis can lead to formation of chlorophyll (Chl) triplet state which can react with $^3O_2$ to give up the very reactive $^1O_2$. It has been found that the formation of $^1O_2$ during photosynthesis has a powerful damaging effect on photosystem I and photosystem II as well as on the whole photosynthetic machinery. Various abiotic stresses such as salinity, drought etc. lead to closing of stomata and resulting low intercellular CO_2 concentration in chloroplast favors the formation of $^1O_2$ (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

The half-life of $^1O_2$ in a cell has been measured to be approximately 1 μs. A fraction of $^1O_2$ may be able to diffuse over considerable distances of 30 nm (Møller *et al.*, 2007). $^1O_2$, an oxidizing agent for a wide range of biological molecules, can react with proteins such as tryptophan, tyrosine, methionine, histidine and cysteine; nucleic acids mainly guanine and lipids is thought to be the most important species responsible for light induced loss of PS II activity which may trigger cell death (Møller *et al.*, 2007). There is no report on the change of $^1O_2$ in plant during low temperature stress.

Oxidative membrane damage during low temperature stress

Low temperature stress response has been related to oxidative damage and the appearance of CI symptoms. Over-production of ROS is a main cause of oxidative membrane damage. It occurs when ROS levels exceed the capacity of the defense mechanisms to neutralize them causing “oxidative stress” (Gill and Tuteja, 2010; Sharma *et al.*, 2012), which then leads to oxidation of lipids and proteins, nucleic acid

damage, enzyme inhibition and membrane damage in both cellular and organelle membranes. Consequently, it activates the PCD pathway, which decreases productivity and causes cell injury and ultimate death (Bloknina *et al.*, 2003; Møller *et al.*, 2007; Gill and Tuteja, 2010; Sharma *et al.*, 2012). Membrane lipid peroxidation is catalyzed by lipoxygenase (LOX) and leads to the production of conjugated diene and malondialdehyde (MDA) as biomarkers (Gill and Tuteja, 2010; Sharma *et al.*, 2012). Lipid peroxidation alters membrane properties and causes cell defects such as ion leakage and cellular decompartmentation (Bloknina *et al.*, 2003; Duan *et al.*, 2007; Yang *et al.*, 2009). LOX activity, MDA content and electrolyte leakage (EL) are parameters to measure the level of oxidative membrane damage under various stresses.

1. Lipoxygenase (LOX) activity

LOX catalyzes peroxidation of polyunsaturated fatty acids (PUFA) and is believed to be a major contributor to chilling-induced membrane damage in plant tissue (Pinhero *et al.*, 1998). LOX catalyzes the specific dioxygenation of PUFA using O_2 as the second substrate. This enzyme produces both reactive fatty acid hydroperoxides and activated $O_2^{\bullet -}$, which may propagate non-enzymatic oxygenation of membrane lipids (Lynch and Thompson, 1984). Fatty acid hydroperoxide, the initial product of the LOX reaction, is deleterious to membranes and proteins (Bhattacharjee, 2012).

Low temperature induces an increase in LOX activity that correlates with CI development. Increase in LOX activities were observed in chilled mango fruit cv. Zill (Ding *et al.*, 2007). LOX activity in the mesocarp of mango fruit was 200% higher than the original level after storage at 5 °C for 30 days. The increase in LOX activity associated with CI symptoms development in mango fruits that occurred on Day 20 of storage at 5 °C (Ding *et al.*, 2007).

LOX activity has been shown to increase in ROS production associated with CI development. LOX activity in the flesh of loquat fruit increased throughout storage at 1 °C for 35 days and correlated with increased CI symptoms. LOX activity in loquat flesh was 87% higher than original level after 35 days of storage (Cao *et al.*, 2009). Increase in LOX activity was also found in anthurium flowers during storage at 4 °C for 20 days and correlated with increased CI symptoms in these flowers. LOX activity in

anthurium was 50% higher than the original level after 20 days of storage (Promyou *et al.*, 2012).

2. Malondialdehyde (MDA) content

MDA is the end product of peroxidation of membrane fatty acids and the level of this compound is used as a marker of oxidative stress since an increase in this compound indicates damage of cell membrane integrity (Hodges *et al.*, 1999). Low temperature induced an increase in MDA content in chilled mango fruit cv. Zill. MDA content in the mesocarp was 10% higher than the original level after storage at 5 °C for 30 days. The increase in MDA content was associated with CI symptom development in mango fruits occurred on Day 20 of storage (Ding *et al.*, 2007).

CI associates with oxidative damage that enhances ROS production and accumulation resulting in membrane lipid peroxidation and increased in MDA content. In lemon, MDA content increased in the fruit after being exposed to low temperature at -0.5 °C for 42 days and was associated with increased CI symptoms. It was 88% higher than the original level after 42 days of storage (Siboza and Bertling, 2013). In cucumber, MDA content increased in the fruit after exposure to low temperature at 2 °C for 15 days and was associated with increased CI symptoms. It was 185% higher than the original level after 15 days of storage (Yang *et al.*, 2011). MDA content increased in green bell pepper fruit during storage at 3 °C for 18 days. It was 133% higher than the original level after 18 days of storage (Wang *et al.*, 2012).

The relationship between MDA content and the development of CI was also found in other parts of plants. For example, Lei *et al.* (2010) reported that MDA content in cucumber seedlings increased under low temperature stress associated with increased CI symptoms. It was 100% higher than the original level after 6 days of stress. Low temperature storage at 4 °C induced the increase in MDA content in anthurium flowers and was associated with an increase in CI symptoms. It was 71% higher than the original level after 20 days of storage (Promyou *et al.*, 2012). MDA content increased in bamboo shoot during storage at 1 °C for 50 days and correlated with CI development. It was 52% higher than the original level after 50 days of storage (Luo *et al.*, 2012).

3. Electrolyte leakage (EL)

EL is an effective parameter to assess membrane permeability and is used as an indicator of membrane damage (Marangoni *et al.*, 1996). EL measures intracellular water, ions and metabolites that leak from cell membranes indicating membrane damage (Marangoni *et al.*, 1996).

Increased membrane permeability and increased rates of ion leakage are associated with CI during low temperature stress. An increase in EL was observed in chilled mango cv. Tommy Atkins. EL in the exocarp of mango fruit was 84% higher than the original level after storage at 7 °C for 21 days. The increase in EL was associated with CI symptom development on Day 7 of storage (González-Aguilar *et al.*, 2000).

The relationship between EL and development of CI was also found in other fruits. In cucumber, EL increased in the fruit after exposure to low temperature at 2 °C for 15 days and was associated with increased CI symptoms (Yang *et al.*, 2011). EL in cucumber was 440% higher than the original level after 15 days of storage (Yang *et al.*, 2011). EL increased in green bell pepper during storage at 3 °C for 18 days and correlated with CI development. It was 300% higher than the original level after 18 days of storage (Wang *et al.*, 2012). Meng *et al.* (2009) reported that EL increased in peach during storage at 5 °C for 21 days associated with increased CI symptoms. It was 800% higher than the original level after 21 days of storage. Nilprapruck *et al.* (2008) reported that EL increased in pineapple fruit during storage at 10 °C for 28 days with increased in CI symptoms. It was 42% higher than the original level after 28 days of storage. CI increased in guava fruit during storage at 5 °C for 15 days and with increased of CI symptoms. The EL of red and white guava fruits were 108 and 140% respectively and higher than the original level after 15 days of storage (González-Aguilar *et al.*, 2004).

Correlation of increasing EL and the development of CI was also found in other plant parts. Lei *et al.* (2010) reported that EL increased in cucumber seedlings under low temperature stress with increased CI symptoms. It was 125% higher than the original level after 6 days of stress conditions. Promyou *et al.* (2012) reported that low temperature storage at 4 °C induced the increase in EL in anthurium flowers with

increased in CI symptoms. It was 400% higher than the original level after 20 days of storage. EL increased in bamboo shoot during storage at 1 °C for 50 days and correlated with CI development. It was 200% higher than the original level after 50 days of storage (Luo *et al.*, 2012).

Antioxidant defense system during low temperature stress

Plants possess a complex antioxidant defense system comprising of non-enzymatic and enzymatic components to scavenge ROS (Smirnoff, 2005). In plant cells, specific ROS producing and scavenging systems are found in different organelles such as chloroplasts, mitochondria, and peroxisomes. ROS scavenging pathways from different cellular compartments are coordinated (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

Under normal conditions, potentially toxic oxygen metabolites are generated at a low level and there is an appropriate balance between production and quenching of ROS. This may be perturbed by a number of adverse environmental factors, giving rise to rapid increase in intracellular ROS levels, which can induce oxidative damage to lipids, proteins, and nucleic acids. In order to avoid oxidative damage, higher plants raise the level of their endogenous antioxidant defenses (Gill and Tuteja, 2010; Sharma *et al.*, 2012). Various components of the antioxidant defense system are involved in ROS scavenging have been manipulated, overexpressed or downregulated to add to the present knowledge and understanding the role of the antioxidant systems.

Enhancement of the antioxidant defense system responds to various environmental stresses such as drought, salinity, chilling, metal toxicity, UV-B radiation and pathogen attack (Sharma *et al.*, 2012). Under stress condition, ROS drastically increased in plants disturbing the normal balance of $O_2^{\bullet -}$, $OH^{\bullet}$ and H_2O_2 in the intracellular environment. To detoxify ROS, a highly efficient antioxidant defense system is induced in cells. The antioxidant defense system includes enzymatic and non-enzymatic antioxidants increased to scavenge excess ROS under stress conditions (Gill and Tuteja, 2010; Sharma *et al.*, 2012). Increased antioxidant defense system activity in plants is subjected to low temperature stress (Sevillano *et al.*, 2009; Gill and Tuteja, 2010; Sharma *et al.*, 2012; Aghdam and Bodbodak, 2013).

Enzymatic antioxidants

The enzymatic components of the antioxidant defense system are comprised of several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), enzymes of ascorbate-glutathione (AsA-GSH) cycle ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Mittler, 2002; Foyer and Noctor, 2005; Gill and Tuteja, 2010; Sharma *et al.*, 2012). These enzymes operate in different subcellular compartments and respond in concert when cells are exposed to oxidative stress (Gill and Tuteja, 2010; Sharma *et al.*, 2012). The potential role of antioxidant enzymes in protecting plant from low temperature stress can be explained as follows.

1. Superoxide dismutase (SOD)

Metalloenzyme SOD is the most effective intracellular enzymatic antioxidant which is ubiquitous in all aerobic organisms and is in all subcellular compartments prone to ROS mediated oxidative stress. It is well established that various environmental stresses often lead to an increased generation of ROS where SOD has been proposed to be important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of ROS. The SODs remove $O_2^{\bullet-}$ by catalyzing its dismutation, one $O_2^{\bullet-}$ being reduced to H_2O_2 and another oxidized to O_2 (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} 2H_2O_2 + O_2$$

It removes $O_2^{\bullet-}$ and hence decreases the risk of $OH^{\bullet}$ formation via the metal catalyzed Haber-Weiss-type reaction. This reaction has a 10,000-fold faster rate than spontaneous dismutation. SODs are classified by their metal cofactors into three known types: copper/zinc (Cu/Zn-SOD), manganese (Mn-SOD) and iron (Fe-SOD), which are localized in different cellular compartments. The subcellular distribution of these isozymes is also distinctive. The Mn-SOD is found in the mitochondria of eukaryotic cells and in peroxisomes; some Cu/Zn-SOD isozymes are found in the cytosolic

fractions, and also in chloroplasts of higher plants. The Fe-SOD isozymes, often not detected in plants, are usually associated with chloroplasts when present (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

Changes in SOD activity under low temperature stress have been associated with CI development. Ding *et al.* (2007) reported that SOD activity increased in the mesocarp of mango fruit cv. Zill during storage at 5 °C. Enzyme activity decreased after Day 20 of storage, with CI symptoms occurred on Day 20. Chongchatuporn *et al.* (2013) also reported that rapid decrease in SOD activity was related to CI symptoms in mango fruit cv. Nam Dok Mai during storage at 4 °C. There were no CI symptoms associated with high SOD activity at 12 °C. Zhao *et al.* (2006) reported that SOD activity was slightly decreased in mango fruit cv. Wacheng during storage at 2 °C for 12 days with slight CI symptoms appearing in the fruit on Day 12.

The relationship between SOD activity and CI symptoms were also found in other fruits and flowers. Yang *et al.* (2011) reported that SOD activity increased in cucumber during storage at 2 °C. Enzyme activity decreased after Day 6 of storage, with CI symptoms occurring on Day 6. Yang *et al.* (2013) found that SOD activity increased in kiwifruit after being exposed to low temperature at 0 °C, but the activity of this enzyme decreased 30 days after storage leading to CI symptoms afterwards. SOD activity rapidly increased in anthurium flowers at the first 4 days after being exposed to low temperature at 4 °C and then decreased afterward leading to CI symptoms on Day 8 of storage (Promyou *et al.*, 2012). The opposite result was found by Cao *et al.* (2009) where the activity of SOD increased along with an increase in CI symptoms in loquat fruit during storage at 1 °C for 35 days.

2. Catalase (CAT)

CATs are tetrameric hemes containing enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2 (Mittler, 2002).

$$2H_2O_2 \xrightarrow{CAT} O_2 + 2H_2O$$

It is indispensable for ROS detoxification during stressed conditions. CAT has one of the highest turnover rates for all enzymes and one molecule of CAT can convert about 6 million molecules of H_2O_2 to H_2O and O_2 per minute. It is important in the removal of H_2O_2 generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration and purine catabolism (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

There is a correlation between CAT activity and CI caused by low temperature. The activity of CAT rapidly increased in the mesocarp of mango cv. Zill at the first 10 days during storage at 5 °C and then decreased afterwards with associated CI symptoms occurring on Day 20 (Ding *et al.*, 2007). CAT activity in the exocarp of mango cv. Nam Dok Mai increased slightly during the first 5 days of storage at 4 °C and then rapidly decreased afterwards resulting in CI symptoms on Day 5 of storage (Chongchatuporn *et al.*, 2013). CAT activity in mango cv. Wacheng decreased during storage at 2 °C for 12 days with associated CI symptoms appearing on Day 12 (Zhao *et al.*, 2006).

The relationship between CAT activity and CI symptoms was also found in other fruits and flowers. Yang *et al.* (2011) reported that CAT activity increased in cucumber during storage at 2 °C. Enzyme activity decreased after Day 9 of storage, while CI symptoms occurred on Day 6. Wang *et al.* (2012) reported that CAT activity increased in green bell pepper during storage at 3 °C for 6 days. Enzyme activity decreased after Day 6 of storage and CI symptoms occurred on Day 6. Yang *et al.* (2013) found that CAT activity increased in kiwifruit for the first 40 days after being exposed to low temperature at 0 °C and then decreased afterwards, and developing to CI symptoms. CAT activity of anthurium flowers rapidly increased and reached its peak on Day 8 after being exposed to low temperature at 4 °C and then decreased afterward with CI symptoms occurring on Day 8 of storage (Promyou *et al.*, 2012). Cao *et al.* (2009) reported that the activity of CAT gradually decreased, along with an increase in CI symptoms in loquat fruit during storage at 1 °C for 35 days.

3. Ascorbate peroxidase (APX)

APX is a central component of the AsA-GSH cycle and has an essential role in the control of intracellular ROS levels. APX uses two molecules of AsA to reduce H_2O_2 to water with a concomitant generation of two molecules of MDHA (Asada, 1992; Gill and Tuteja, 2010; Sharma *et al.*, 2012).

$$AsA + H_2O_2 \xrightarrow{APX} MDHA + 2H_2O$$

The APX family consists of at least five different isoforms including mitochondrial, thylakoid, glyoxisome membrane, chloroplast stromal soluble and cytosolic forms (Noctor and Foyer, 1998). APX has a higher affinity for H_2O_2 (µM range) than CAT and peroxidase (POD) (mM range) and may have a more crucial role in the management of ROS during stress (Gill and Tuteja, 2010).

The relationship between APX activity and CI symptoms in mango during low temperature storage has been reported. The activity of APX rapidly increased in the mesocarp of mango cv. Zill during the first 10 days of storage at 5 °C and then decreased afterwards with CI symptoms on Day 20 (Ding *et al.*, 2007). APX activity in mango cv. Wacheng increased during the first 6 days during storage at 2 °C for 12 days and then decreased afterwards with CI symptoms appearing in the fruit on Day 12 (Zhao *et al.*, 2006). Chongchatuporn *et al.* (2013) reported that APX activity in the exocarp of mango cv. Nam Dok Mai increased throughout storage time at 4 °C, while the CI symptoms occurred on Day 5 of storage.

The relationship between APX activity and CI symptoms were also found in other fruits. Yang *et al.* (2011) reported that APX activity increased in cucumber during storage at 2 °C for 6 days. Enzyme activity decreased after Day 6 of storage, with CI symptoms occurring on Day 6. Wang *et al.* (2012) reported that APX activity increased in green bell pepper during storage at 3 °C for 6 days. Enzyme activity decreased after Day 6 of storage with CI symptoms occurring on Day 6. Yang *et al.* (2013) found that APX activity rapidly increased in kiwifruit for the first 10 days after exposure to low temperature at 0 °C and then decreased with CI symptoms. Cao *et al.* (2009) and Cai

et al. (2011) reported that the activity of APX gradually decreased along with an increase in CI symptoms in loquat fruit during storage at 1 °C for 35 days.

4. Monodehydroascorbate reductase (MDHAR)

MDHAR is a flavin adenine dinucleotide (FAD) enzyme that catalyzes the regeneration of AsA from the mono-dehydroascorbate (MDHA) radical using nicotinamide adenine dinucleotide phosphate (NAD(P)H) as the electron donor. Its activity is widespread in plants. The isoenzymes of MDHAR have been reported to be present in several cellular compartments such as chloroplasts, cytosols, mitochondria and peroxisomes (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

Change in MDHAR activity in plants may be attributed to CI development during low temperature storage. Cai *et al.* (2011) reported that MDHAR activity decreased in loquat fruit after 35 days of storage at 1 °C with CI symptoms occurring on Day 35. Chen *et al.* (2011) reported that MDHAR activity increased in the leaves of eggplant seedlings under chilling stress at 4 °C for 9 days with CI symptoms appearing on the leaves.

5. Dehydroascorbate reductase (DHAR)

DHAR catalyzes the reduction of DHA to AsA using GSH as the reducing substrate and has an important role in maintaining AsA in its reduced form. Despite the possibility of enzymic and nonenzymic regeneration of AsA directly from MDHA, some DHA is always produced when AsA is oxidized in leaves and other tissues. DHA, a very short-lived chemical, can either be hydrolyzed irreversibly to 2,3-diketogulonic acid or recycled to AsA by DHAR (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

DHAR activity changed in plants exposed to low temperatures, associated with CI development. Cai *et al.* (2011) reported that DHAR activity decreased in loquat fruit after 35 days of storage at 1 °C with CI symptoms appearing on Day 35. Chen *et al.* (2011) reported that DHAR activity increased in leaves of eggplant seedlings under chilling stress at 4 °C for 9 days with CI symptoms appearing on the leaves.

6. Glutathione reductase (GR)

GR is a flavo-protein oxidoreductase, found in both prokaryotes and eukaryotes. It is a potential enzyme of the ASH-GSH cycle and has an essential role in the defense system against ROS by sustaining the reduced status of GSH. It is localized predominantly in chloroplasts, but small amounts have also been found in mitochondria and cytosols. GR catalyzes the reduction of GSH, a molecule involved in many metabolic regulatory and antioxidative processes in plants where GR catalyses the NAD(P)H dependent reaction of disulfide bond of glutathione disulfide (GSSG) and is important for maintaining the GSH pool. GSSG consists of two GSH linked by a disulfide bridge which can be converted back to GSH by GR. GR is involved in defense against oxidative stress (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

Development of CI in mango is caused by GR activity during low temperature storage. The activity of GR rapidly increased in the mesocarp of mango cv. Zill for the first 10 days during storage at 5 °C and then decreased afterwards with CI symptoms occurring on Day 20 (Ding *et al.*, 2007). GR activity in mango fruit cv. Wacheng decreased throughout storage time at 2 °C for 12 days with CI symptoms appearing on Day 12 (Zhao *et al.*, 2006).

The relationship between GR activity and CI symptoms was also found in other fruits. Cai *et al.* (2011) reported that GR activity decreased in loquat fruit after 35 days of storage at 1 °C with CI symptoms appearing on Day 35. Wang *et al.* (2012) reported that GR activity decreased in green bell pepper throughout storage time at 3 °C for 18 days, along with an increase in CI symptoms that first appeared on Day 6.

Non-enzymatic antioxidants

Non-enzymatic components of the antioxidant defense system include ascorbic acid (AsA), glutathione (GSH), tocopherol, carotenoids, and phenolic compounds. They interact with numerous cellular components in addition to having crucial roles in defense response to oxidative stress in plants.

1. Ascorbic acid (AsA)

AsA is the most abundant, powerful and water soluble antioxidant preventing or minimizing oxidative damage caused by ROS in plants (Valpuesta and Botella, 2004; Gallie, 2013). It occurs in all plant tissues, usually being higher in photosynthetic cells and meristems. In plants, mitochondria have a central role in the metabolism of AsA (Valpuesta and Botella, 2004). Plant mitochondria not only synthesize AsA by L-galactono-γ-lactone dehydrogenase, but also take part in the regeneration of AsA from its oxidized forms (Gill and Tuteja, 2010). More than 90% of AsA is localized in cytoplasm, but unlike other soluble antioxidants, a substantial portion is transported to the apoplast, where it is present in millimolar concentration. Apoplastic AsA is believed to represent the first line of defense against potentially damaging external oxidants (Sharma *et al.*, 2012).

AsA protects critical macromolecules from oxidative damage. Under normal physiological conditions, it mostly exists in a reduced state in chloroplasts where it also acts as a cofactor of violaxanthin de-epoxidase, thus, sustaining dissipation of excess excitation energy. It provides membrane protection by directly reacting with $O_2^{\bullet -}$, H_2O_2 and regenerating α-tocopherol from tocopheroxyl radical and preserves the activities of the enzymes that contain prosthetic transition metal ions (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

AsA has a key role in removal of H_2O_2 via the AsA-GSH cycle. Oxidation of AsA occurs in two sequential steps, first producing MDHA and subsequently DHA. In the AsA-GSH cycle, two molecules of AsA are utilized by APX to reduce H_2O_2 to water with concomitant generation of MDHA. MDHA is a radical with a short life time and can spontaneously dismutate into DHA and AsA or is reduced to AsA by NAD(P)H dependent enzyme MDHAR. DHA is also highly unstable at pH values greater than 6.0 and is decomposed to tartarate and oxalate (Gallie, 2013). To prevent this, DHA is rapidly reduced to AsA by the enzyme DHAR using reducing equivalents from GSH (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

The change in AsA content is associated with CI development in mango fruits during low temperature storage. The AsA content rapidly increased in the mesocarp of

mango cv. Zill during the first 10 days of storage at 5 °C and then decreased afterwards with CI symptoms occurring on Day 20 (Ding *et al.*, 2007). This disagrees with Zhao *et al.* (2006) who reported that AsA content in mango cv. Wacheng did not change during storage at 2 °C for 12 days, but CI symptoms occurred on Day 12 indicating that AsA is not associated with CI development in Wacheng mango. Chongchatuporn *et al.* (2013) also reported that AsA content was not associated with CI development in Nam Dok Mai mango during storage at 4 °C.

The relationship between AsA content and CI symptoms were also found in other fruits. Cai *et al.* (2011) reported that AsA content decreased in loquat fruit after 35 days of storage at 1 °C with CI symptoms appearing on Day 35. Wang *et al.* (2012) reported that AsA content decreased in green bell pepper throughout storage time at 3 °C for 18 days, along with an increase in CI symptoms that first appeared on Day 6. Sayyari *et al.* (2011b) reported that AsA content decreased in pomegranate fruit throughout storage time at 2 °C for 84 days, along with increased CI symptoms on Day 14 of storage. Cao *et al.* (2009) reported that AsA content decreased in cucumber throughout storage time at 1 °C for 18 days, along with increased CI symptoms on Day 6 of storage. Jin *et al.* (2009a) reported that AsA content decreased in peach throughout storage time at 0 °C for 5 weeks along with an increase in CI symptoms during storage. AsA content in leaves of eggplant seedlings increased during chilling stress at 4 °C for 9 days with CI symptoms appearing in the leaves (Chen *et al.*, 2011).

2. Glutathione (GSH)

Tripeptide glutathione (γ-glutamyl-cysteinyl-glycine, GSH) is one of the crucial metabolites in plants which is considered as the most important intracellular defense against ROS induced oxidative damage. It occurs abundantly in reduced form in plant tissues and is localized in all cell compartments viz. cytosols, endoplasmic reticulae, vacuoles, mitochondria, chloroplasts, peroxisomes and apoplasts (Gill and Tuteja, 2010).

GSH plays a central role in several physiological processes, including regulation of sulfate transport, signal transduction, conjugation of metabolites, detoxification of xenobiotics and the expression of stress-responsive genes. GSH also has an important

role in several growth and development related events in plants, including cell differentiation, cell death and senescence, pathogen resistance and enzymatic regulation (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

GSH is necessary to maintain the normal reduced state of cells so as to counteract the inhibitory effects of ROS-induced oxidative stress. It is a potential scavenger of $^1O_2$, H_2O_2 and a most dangerous ROS like $OH^{\bullet}$. GSH has a key role in the antioxidant defense system by regenerating another potential water soluble antioxidant like ASA, via the AsA-GSH cycle. When the intensity of stress increases, GSH concentrations usually decline and redox state becomes more oxidized, leading to deterioration of the system (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

The change in GSH content is associated with CI development in mango fruits during low temperature storage. The GSH content rapidly increased in the mesocarp of mango cv. Zill during the first 20 days of storage at 5 °C and then decreased afterwards with CI symptoms occurring on Day 20 (Ding *et al.*, 2007). Zhao *et al.* (2006) reported that AsA content in mango cv. Wacheng increased throughout storage time at 2 °C for 12 days with CI symptoms occurring on Day 12.

The relationship between GSH content and CI symptoms were also found in loquat fruits. GSH content decreased in loquat fruit after 35 days of storage at 1 °C with CI symptoms appearing on Day 35 (Cai *et al.*, 2011). GSH content in leaves of eggplant seedlings increased during chilling stress at 4 °C for 9 days with CI symptoms appearing in the leaves (Chen *et al.*, 2011).

3. Phenolic compounds

Phenolics are diverse secondary metabolites (flavonoids, tannins, hydroxycinnamate esters and lignin) which possess antioxidant properties. They are abundantly found in plant tissues. Polyphenols contain an aromatic ring with OH or OCH_3 substituents which together contribute to their biological activity, including antioxidant action. They have been shown to outperform well-known antioxidants, AsA and α-tocopherol, in *in vitro* antioxidant assays because of their strong capacity to donate electrons or hydrogen atoms (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

Polyphenols can chelate transition metal ions, directly scavenge ROS, and inhibit lipid peroxidation by trapping the lipid alkoxyl radical. They also modify lipid packing order and decrease fluidity of the membranes. These changes can hinder diffusion of free radicals and restrict peroxidative reactions. Flavonoids and phenylpropanoids are oxidized by POD and act in the H_2O_2-scavenging, phenolic/AsA/POD system. There is some evidence of induction of phenolic metabolism in plants as a response to multiple stresses (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

The change in content of phenolic compounds is associated with CI development in mango fruits during low temperature storage. It decreased in mango cv. Wacheng throughout storage time at 2 °C for 12 days with CI symptoms occurring on Day 12 (Zhao *et al.*, 2006). Chidtragool *et al.* (2011) reported that total phenolic content in exocarp of mango cv. Nam Dok Mai decreased throughout storage time at 4 °C for 30 days, along with increased CI symptoms in the exocarp after Day 6 of storage.

The relationship between total phenolic content and CI symptoms were also found in peach. Total phenolic content decreased in peach throughout storage time at 0 °C for 5 weeks and with CI symptoms appeared on Week 5 (Jin *et al.*, 2009a). Sayyari *et al.* (2011b) reported that total phenolic content in pomegranate decreased throughout storage time at 2 °C for 84 days along with an increase in CI symptoms.

4. α-Tocopherols (Vitamin E)

Tocopherols, lipid soluble antioxidants, are considered potential scavengers of ROS and lipid radicals (Gill and Tutaja, 2010) and major antioxidant in biomembranes, where they have both antioxidant and non-antioxidant functions (Sharma *et al.*, 2012).

Tocopherols are considered general antioxidants for protection of membrane stability, including quenching or scavenging ROS like $^1O_2$. Tocopherols are localized in plants in the thylakoid membrane of chloroplasts. Out of four isomers of tocopherols (α-, β-, γ-, δ-) found in plants, α-tocopherol has the highest antioxidative activity due to the presence of three methyl groups in its molecular structure. It is synthesized from

γ-tocopherol in the chloroplasts by γ-tocopherolmethyltransferase (γ-TMT; VTE4) (Gill and Tutaja, 2010).

Tocopherols have been shown to prevent the chain propagation step in lipid autooxidation which makes it an effective free radicals trap. It has been estimated that one molecule of α-tocopherol can scavenge up to 120 $^1O_2$ molecules by resonance energy transfer (Gill and Tutaja, 2010).

5. Carotenoids

Carotenoids also belong to the group of lipophilic antioxidants and are able to detoxify various forms of ROS. In plants, carotenoids absorb light in the region between 400 and 550 nm of the visible spectrum and pass the captured energy to the chlorophyll (Chl) (Gill and Tutaja, 2010).

As antioxidants, they scavenge $^1O_2$ to inhibit oxidative damage and quench triplet sensitizer ($^3Chl^*$) and excited chlorophyll (Chl^*) molecules to prevent the formation of $^1O_2$ to protect the photosynthetic apparatus. Carotenoids also serve as precursors to signaling molecules that influence plant development and biotic/abiotic stress responses. The ability of carotenoids to scavenge, prevent or minimize the production of triplet chlorophyll may be accounted for by their chemical specificity (Gill and Tutaja, 2010; Sharma *et al.*, 2012).

Total antioxidant capacity (TAC)

TAC is a useful mean to evaluate the effectiveness of the antioxidant defense systems of plants. TAC reflects the presence and the activity of antioxidant components. The most popular methods of evaluating TAC levels are DPPH and ABTS radical scavenging activity methods.

1. DPPH radical scavenging activity

DPPH$^\bullet$ (1,1-diphenyl-2-picrylhydrazyl) is one of the few stable organic nitrogen radicals. The DPPH assay is based mainly on the electron transfer reaction, while hydrogen-atom abstraction is a marginal reaction pathway. The interactions between antioxidants and DPPH$^\bullet$ are also determined by the antioxidant structural conformation.

Some compounds react very rapidly with DPPH$^{\bullet}$ and they reduce the number of DPPH$^{\bullet}$ molecules corresponding to the number of available hydroxyl groups. DPPH$^{\bullet}$ can be used to determine natural antioxidants both hydrophilic and hydrophobic. The DPPH test does not support the determination of TAC compounds whose spectra overlap with DPPH$^{\bullet}$ spectra, in particular carotenoids (Prior *et al.*, 2005).

A DPPH$^{\bullet}$ solution in methanol has an intensive deep purple color with a strong VIS absorption at 517 nm. When it reacts with an antioxidant, the DPPH$^{\bullet}$ radical is converted into DPPH and its color changes from purple to yellow. The antioxidant effect may be easily evaluated by observing the decrease in VIS absorption at 517 nm (Mun'im *et al.*, 2003).

2. ABTS radical scavenging activity

ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) is converted into its radical cation (ABTS$^{\bullet+}$) by addition of sodium persulphate. ABTS$^{\bullet+}$ is reactive towards most antioxidants and it is soluble in both aqueous and organic solvents. The ABTS$^{\bullet+}$ method is useful in determining the antioxidant activity of both lipophilic (e.g. α-tocopherol or β-carotene) and hydrophilic antioxidants in various matrices (body fluids, food extracts, etc.) (Martysiak-Żurowska and Wenta, 2012). ABTS$^{\bullet+}$ reacts rapidly with antioxidants and they can be applied over a wide pH range. Selected substances, including most phenolic compounds, reduce ABTS$^{\bullet+}$ if its redox potential is lower than that of ABTS (0.68 V) (Vajragupta *et al.*, 2006).

ABTS is converted into its radical cation (ABTS$^{\bullet+}$) by addition of sodium persulphate. This blue-green radical cation absorbs light at 734 nm. During this reaction, the blue-green ABTS radical cation is converted back into its colorless neutral form. The reaction may be monitored spectrophotometrically (Huang *et al.*, 2005).

Previous studies showed that TAC decreased during low temperature storage in many fruits associated with CI symptom development. Siboza and Bertling (2013) reported that TAC by DPPH assay gradually decreased in lemon throughout storage at -0.5 °C for 42 days, along with an increase in CI symptoms that first appeared on Day 21. Yang *et al.* (2011) reported that TAC by DPPH assay decreased in cucumber

throughout storage at 2 °C for 15 days along with an increase in CI symptoms that first appeared on Day 6. Sayyari *et al.* (2011a, b) reported that TAC by ABTS assay increased in pomegranate during the first 14-28 days after exposure to low temperature at 2 °C. TAC decreased 14-28 days after storage and correlated with CI symptoms which occurred on Day 14. TAC by ferric reducing/antioxidant power (FRAP) assay in anthurium flowers decreased throughout storage at 4 °C for 20 days along with an increase in CI symptoms that first appeared on Day 8 (Promyou *et al.*, 2012).

From previous studies on free radicals content, membrane damage and antioxidant defense system during storage at low temperature, it was found that low temperature induced an increase in ROS accumulation along with an increase in oxidative membrane damage and increase in antioxidant defense system activity in both enzymatic and non-enzymatic antioxidant that have important role for scavenging ROS during low temperature storage. Antioxidant enzyme activity and antioxidant contents also decreased after a long storage, leading to high ROS accumulation. The excess ROS is highly reactive with biomolecules especially induced lipid peroxidation of membrane lipids, leading to membrane damage and resultant CI symptoms in plants.

Postharvest treatments for reducing chilling injury

An obvious way to avoid chilling damage is to avoid chilling temperatures. If chilling can not be avoided, treatments should be developed either to increase the tolerance of the tissue before chilling or to reduce the development of injury symptoms. Various methods such as modified atmosphere packaging (Kumpoun and Uthaibutra, 2010), cold-shock treatment (Zhao *et al*., 2006) and an application of plant growth regulators (PGRs) (Ding *et al.*, 2007) have been applied to mango fruits to alleviate CI. Amongst those methods, PGRs treatments are very effective, cheap, easy to set up and applicable to various fruit products (Cao *et al.*, 2009; Jin *et al.*, 2009a; Sayyari *et al.*, 2009).

Among the various chemical treatments developed, salicylic acid (SA) and methyl jasmonate (MJ) have been efficient in reducing the incidence of CI symptoms in mango. Treatment efficiency varies with the SA and MJ concentrations and storage regime. SA and its derivatives can be used to reduce CI symptoms in mango cv. Zill

(Ding *et al.*, 2007), pomegranate (Sayyari *et al.*, 2009, 2011a, b), plum (Luo *et al.*, 2011), pear (Al-Qurashi and Awad, 2012), tomato (Aghdam *et al.*, 2012) and lemon (Siboza and Bertling, 2013). MJ and its derivatives can be used to reduce CI symptoms in mango cvs. Tommy Atkins and Kent (González-Aguilar *et al.*, 2000, 2001), guava (González-Aguilar *et al.*, 2004), loquat (Cai *et al.*, 2011), peach (Jin *et al.*, 2013), pomegranate (Sayyari *et al.*, 2011a) and lemon (Siboza and Bertling, 2013). Moreover, it was found that 2,4-dichlorophenoxyacetic acid (2,4-D) can reduce CI symptoms in mango cv. Tainong (Wang *et al.*, 2008) and pear (Al-Qurashi, 2012). Brassinolide can be used to reduce CI symptoms in green bell pepper (Wang *et al.*, 2012).

Reduction of chilling injury by salicylic acid (SA) and methyl jasmonate (MJ)

SA belongs to an extremely diverse group of plant phenolic compounds (Hayat *et al.*, 2010). SA possesses an aromatic ring with a hydroxyl group or its functional derivatives (Figure 2.3a). Free SA is a crystalline powder that melts at 157-159 °C. It is moderately soluble in water, but highly soluble in polar organic solvents. The esterification of the phenolic hydroxyl group of SA with the acetyl group from acetic anhydride or acetyl chloride results in acetylsalicylic acid (ASA). Methyl salicylate (MeSA) can be produced by esterifying SA with methanol. SA is well known naturally occurring signaling molecule that has a key role in establishing and signaling a defense response against various pathogenic infections (Hayat *et al.*, 2010) and response against abiotic stress such as water, salinity and cold stress (Hayat *et al.*, 2010; Miura and Tada, 2014).

MJ is a volatile organic compound in plants (Figure 2.3b). It is derived from jasmonic acid (JA) and the reaction is catalyzed by JA carboxyl methyltransferase (Cheong and Choi, 2003). MJ is a colorless liquid that melts below 25 °C. MJ and JA, collectively referred to as jasmonates, are important cellular regulators involved in plant development processes such as germination, root growth, fertility and fruit ripening and senescence. MJ activates plant defense mechanisms in response to biotic stress such as pathogens, insects and wounding and abiotic stress such as drought, low temperature and salinity (Creelman and Mullet, 1997; Cheong and Choi, 2003).

SA, MJ and its derivative treatments at non-toxic concentrations alleviate postharvest CI in fruits, vegetables and flowers. CI of mango cv. Zill, pomegranate, plum, pear, tomato and lemon fruits and anthurium flowers were reduced by SA treatment (Ding *et al.*, 2007; Sayyari *et al.*, 2009, 2011a, b; Luo *et al.*, 2011; Al-Qurashi and Awad, 2012; Aghdam *et al.*, 2012; Promyou *et al.*, 2012; Siboza and Bertling, 2013). CI of mango cvs. Tommy Atkins and Kent, guava, loquat, peach, pomegranate and lemon fruits were also reduced by MJ treatment (González-Aguilar *et al.*, 2000, 2001; González-Aguilar *et al.*, 2004; Cai *et al.*, 2011; Jin *et al.*, 2013; Sayyari *et al.*, 2011a; Siboza and Bertling, 2013).

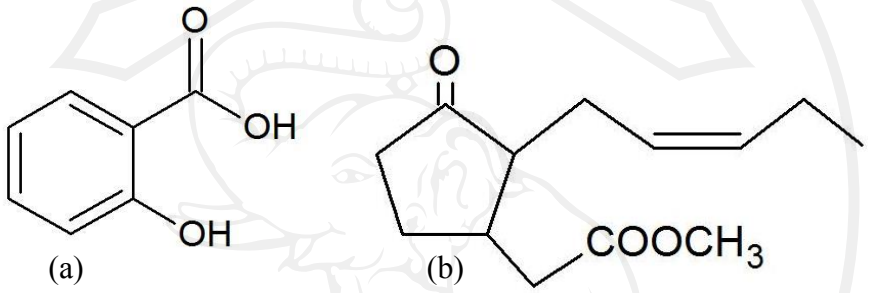

Figure 2.3 Chemical structures of SA (a) and MJ (b)
(Hayat and Ahmad, 2007; Cheong and Choi, 2003).

SA and MJ on chilling injury symptoms during low temperature stress

SA or MJ treatments prior to low temperature storage can be used to reduce deterioration and development of CI symptoms in many fruits. The optimum concentrations of SA and MJ for reducing CI symptoms depend on the fruit species and cultivar (González-Aguilar *et al.*, 2000, 2001; Han *et al.*, 2006; Ding *et al.*, 2007; Sayyari *et al.*, 2009).

SA can be used for reducing CI symptoms in mango. Ding *et al.* (2007) reported that mango cv. Zill dipped in SA at a concentration of 2 mM for 10 minutes before storage at 5 °C significantly reduced CI during storage for 30 days and 5 days of shelf life at 25 °C. CI symptoms in SA treated fruits were 16 and 20% lower than the control at Day 30 of cold storage and shelf life, respectively. Exposing fruits to 0.1 mM MeSA for 12 hours reduced CI symptoms in mango cv. Zill during storage at 5 °C for 35 days

(Han *et al.*, 2006). The CI severity of MeSA treated fruit was 32% lower than control fruits at the end of storage.

SA has been applied to reduce CI symptoms in other fruits. SA immersion at 1 mM for 5 minutes was effective in alleviating CI of peach cv. Beijing 24 during storage at 0 °C for 28 days and subsequent shelf life period at 20 °C for 3 days (Wang *et al.*, 2006). CI index of 1 mM SA treated fruits was 56% lower than the control fruits during shelf life while SA concentrations at 0.35 and 0.70 mM did not reduce CI. In pomegranate cv. Malas Saveh, dipping fruits in 0.7, 1.4 and 2 mM SA solutions for 10 minutes reduced CI symptoms during storage at 2 °C for 3 months (Sayyari *et al.*, 2009). The effectiveness of CI reduction increased with increasing SA concentrations. SA concentration at 2 mM was most effective in reducing CI in pomegranate and CI of 2 mM SA treated fruits was 58% lower than the control fruits at the end of storage. Dipping plums in 1.5 mM SA solution for 10 minutes reduced CI symptoms during storage at 1 °C for 60 days (Luo *et al.*, 2011). The CI index of SA treated fruit was 38% lower than the control fruits for the shelf life period (20 °C for 3 days) after Day 60 of cold storage. In tomato, dipping in 1 and 2 mM SA solution for 5 minutes could effectively reduce CI during storage at 1 °C for 3 weeks and 2 mM was the most effective concentration that reduced CI by 90% for the shelf life period (20 °C for 3 days) after Week 3 (Aghdam *et al.*, 2012). Dipping lemons in 2 mM SA solution for 30 seconds delayed and reduced CI symptoms during storage at -0.5 °C for 42 days (Siboza and Bertling, 2013). CI in the control fruits occurred on Day 21 while SA treated fruits occurred on Day 42. CI of SA treated fruits was 52% lower than the control fruits for the shelf life period (25 °C for 7 days) after Day 42.

ASA is a close analogue of SA and when applied exogenously, it is converted to SA spontaneously, having similar effects to SA in plant defense processes (Hayat and Ahmad, 2007). Dipping loquat fruits in 1 mM ASA solution for 5 minutes reduced CI symptoms during storage at 0 °C for 39 days (Cai *et al.*, 2006). CI symptoms manifested as internal and external browning were 47 and 40% respectively lower than the control fruits at Day 39 of storage. Dipping pomegranate cv. Mollar de Elche in 0.1, 0.5 and 1 mM ASA solution for 10 minutes effectively reduced CI during storage at 2 °C for 84 days. A concentration of 1 mM was the most effective concentration that

reduced CI by 57% of the shelf life period (20 °C for 4 days) after Day 84 (Sayyari *et al.*, 2011b).

MeSA is also a volatile plant compound synthesized from salicylic acid having a role in the plant defense mechanism as well as during plant growth and development which could be converted back to salicylic acid (Hayat and Ahmad, 2007). MeSA vaporized before storage at low temperature and reduced CI of tomato fruits (Ding *et al.*, 2001, 2002; Fung *et al.*, 2006). MeSA vaporized fruits with 0.01, 0.1 and 0.5 mM for 16 hours reduced CI in tomato fruits during storage at 5 °C for 28 days. The most effective concentration was 0.01 mM which reduced CI by 60% of the shelf life period (20 °C for 1 days) after Day 28 (Ding *et al.*, 2001, 2002). The effectiveness on CI reduction is reduced when MeSA concentration increases (Ding *et al.*, 2002). Fung *et al.* (2006) reported that fumigating fruit with 0.1 mM for 24 hours also reduced CI in tomato fruit during storage at 0 °C for 21 days. The CI of MeSA treated fruit was 27% lower than control fruit of the shelf life period (25 °C for 3 days) after Day 21. In sweet pepper, MeSA vaporization with 0.1 mM of fruit for 24 hours reduced CI during storage at 0 °C for 13 days (Fung *et al.*, 2004). A CI symptom of MeSA treated fruit was 18% lower than control fruit at the end of storage. MeSA at 0.01 and 0.1 mM also reduced CI symptoms in pomegranate fruits during storage at 2 °C for 84 days (Sayyari *et al.*, 2011a). CI of 0.01 and 0.1 mM MeSA treated fruits were 63 and 58% lower of the shelf life period (20 °C for 4 days) after Day 84, without significant differences among concentrations used (Sayyari *et al.*, 2011a).

MJ can be used for reducing CI symptoms in mango fruits. González-Aguilar *et al.* (2000) reported that application of MJ vapor at 0.1 mM for 24 hours to mango cv. Tommy Atkins reduced CI during storage at 7 °C for 21 days and subsequent storage at 20 °C for 5 days. CI symptoms in MJ treated fruits were 53 and 46% lower than the control fruits at Day 21 of cold storage and shelf life, respectively. They also found that exposure of mango cv. Kent to MJ vapor at 0.01 and 0.1 mM for 20 hours and then kept at 5 °C effectively reduced CI during storage for 14 days and subsequent storage at 20 °C for 7 days. The results showed that MJ at 0.1 mM was more effective in reducing CI than MJ at 0.01 mM. CI symptoms in 0.1 mM MJ treated fruits were 86 and 85% lower at Day 14 of cold storage and shelf life, respectively. Moreover,

0.1 mM MJ was most effective in reducing CI in these fruits during storage at 10 °C for 14 days and subsequent shelf life (González-Aguilar *et al.*, 2001).

MJ has been applied to reduce CI symptoms in other fruits. Treating guava with 0.01 and 0.1 mM MJ for 8 hours was effective in reducing CI when stored at 5 °C for 15 days (González-Aguilar *et al.*, 2004). CI symptom of MJ treated fruits was 43% lower at shelf life period (25 °C for 2 days) after Day 15. Dipping pineapple fruit in 0.01, 0.1 and 1 mM MJ for 5 minutes reduced CI during storage at 10 °C for 28 days, without significant differences between MJ concentrations (Nilprapruck *et al.*, 2008). CI of MJ treated fruits was 23-27% lower at the end of storage. MJ at 0.01 and 0.1 mM also reduced CI symptoms in pomegranate during storage at 2 °C for 84 days. CI of 0.01 and 0.1 mM MeSA treated fruit were 68 and 54% lower of the shelf life period (20 °C for 4 days) after Day 84, without significant differences among concentrations used (Sayyari *et al.*, 2011a). Tomato treated with 0.01, 0.1 and 0.5 mM MJ for 16 hours reduced CI during storage at 5 °C for 28 days and 0.01 mM was the most effective concentration that reduced CI by 47% of the shelf life period (20 °C for 1 day) after Day 28 (Ding *et al.*, 2001, 2002). The effectiveness of CI reduction reduced when MeSA concentration increased (Ding *et al.*, 2002). Treatment with MJ at 0.01 mM also reduced CI in peach during storage at 1 °C for 35 days (Cai *et al.*, 2011). The CI of MJ treated fruit was 80% lower at the end of storage. In peach, treatment with 0.001 mM for 24 hours reduced CI symptoms as internal browning and flesh mealiness by 75 and 60% respectively during storage at 0 °C for 5 weeks (Jin *et al.*, 2009b).

SA and MJ on free radicals production during low temperature stress

SA or MJ treatments before low temperature storage reduced free radicals such as $O_2^{\bullet-}$ and H_2O_2 production and accumulation in plants. The reduction is associated with increasing CI tolerance in many plants. Dipping mango cv. Zill in 2 mM SA for 10 minutes reduced $O_2^{\bullet-}$ accumulation but promoted H_2O_2 accumulation in the mesocarp during storage at 5 °C for 30 days (Ding *et al.*, 2007). This suggests that the effect of SA on mango CI is probably attributed to less $O_2^{\bullet-}$ accumulation and more H_2O_2 accumulation. Dipping lemon fruits in 2 mM SA solution for 30 seconds before exposure to low temperature at -0.5 °C reduced the production of ROS by 42% at Day

42 and also reduced CI symptoms in SA treated fruit (Siboza and Bertling, 2013). Loquat fruit, dipped in 1 mM ASA solution for 5 minutes reduced $O_2^{\bullet-}$ accumulation during storage at 0 °C for 39 days by 15% and subsequent shelf life at 20 °C for 5 days by 21% (Cai *et al.*, 2006). The reduction in $O_2^{\bullet-}$ accumulation in ASA treated fruit is associated with reducing CI symptoms of loquat fruits.

SA also reduces ROS accumulation in some seedlings associated with induced CI tolerance during growth under low temperature stress. Spraying 0.5 mM SA solution on leaf blades and irrigation of 0.5 mM SA solution to roots of banana seedlings before exposing to chilling stress at 5 °C reduced H_2O_2 content by 35% on day 3 at 5 °C (Kang *et al.*, 2003). A similar result was found in cucumber seedlings sprayed with 0.02 mM SA solution on the leaves before exposure to low temperature at 10 °C that H_2O_2 accumulation was reduced by 28% at Day 6 under low temperature stress (Lei *et al.*, 2010). In eggplant, lower accumulation of H_2O_2 content was found in the seedlings treated by spraying with 0.02 M SA solution before exposure to low temperature at 4 °C for 9 days (Chen *et al.*, 2011). The H_2O_2 content in SA treated seedlings was 34% lower at Day 9 under stress condition. Reduction in H_2O_2 contents by SA treatment is closely related to CI tolerance in banana, cucumber and eggplant seedlings under low temperature stress (Kang *et al.*, 2003; Lei *et al.*, 2010; Chen *et al.*, 2011).

Siboza and Bertling (2013) found that dipping lemon fruits in 0.01 mM MJ solution for 30 seconds before exposure to low temperature at -0.5 °C also reduced the production of ROS by 42% at Day 42 of cold storage and is related to reducing CI symptoms in MJ treated fruit. In peach, MJ vapor of the fruit with 0.001 mM for 24 hours before storage at 0 °C for 5 weeks reduced $O_2^{\bullet-}$ and H_2O_2 accumulation by 18 and 37% respectively at the end of storage (Jin *et al.*, 2013). The reduction in $O_2^{\bullet-}$ and H_2O_2 contents by MJ treatment is closely related to CI tolerance in peach (Jin *et al.*, 2013). Similar results were also found in loquat that MJ vapor with 0.01 mM for 24 hours reduced $O_2^{\bullet-}$ and H_2O_2 accumulation by 20 and 23% respectively with reduced CI symptoms in MJ treated fruit during storage at 1 °C for 35 days (Cao *et al.*, 2009).

All of these results show that treatments with SA and MJ reduced ROS production and accumulation during low temperature storage and induced CI tolerance

in SA and MJ treated fruits. The mechanisms of SA and MJ in reducing ROS contents may be associated with enhancing the antioxidant defense system in the treated fruits.

SA and MJ on oxidative membrane damage during low temperature stress

SA or MJ treatments before low temperature storage reduce membrane damage in plants by lower LOX activity, MDA content or EL than untreated fruits. The reduction in membrane damage by SA and MJ treatments is associated with increasing CI tolerance in many plants (González-Aguilar *et al.*, 2000, 2004; Ding *et al.*, 2007; Siboza and Bertling, 2013).

Studies on mango cv. Zill showed that dipping the fruits in 2 mM SA solution for 10 minutes reduced LOX activity and MDA content during storage at 5 °C for 30 days and reduced CI symptoms in SA treated fruit (Ding *et al.*, 2007). LOX activity and MDA content in SA treated fruits were 48 and 15% lower at Day 30 of cold storage (Ding *et al.*, 2007).

Pomegranate cv. Malas Saveh, dipped in 2 mM SA solution for 10 minutes showed, EL to be 16% lower during storage at 2 °C for 3 months (Sayyari *et al.*, 2009). A positive relationship between CI and EL ($R^2 = 0.696$) was also found in pomegranate which indicated that SA might partially maintain membrane integrity associated with reduced CI symptoms by SA (Sayyari *et al.*, 2009). Sayyari *et al.* (2011b) found that dipping the fruits in 1 mM ASA for 10 minutes before storage at 2 °C reduced EL associated with reducing CI symptoms in pomegranate cv. Mollar de Elche during 3 months of storage. The EL of ASA treated fruit was 14% lower at Day 84 plus shelf life. In loquat, dipping the fruits in 1 mM ASA for 5 minutes also reduced EL and reduced CI symptoms during storage at 0 °C for 39 days (Cai *et al.*, 2006). The EL of ASA treated fruit was 14% lower at Day 39 of cold storage. Dipping lemon in 2 mM SA solution for 30 seconds before storage at -0.5 °C was effective in reducing MDA content by 29% at Day 42 and reduced CI symptoms in SA treated fruits (Siboza and Bertling, 2013).

SA and its derivative also reduced membrane damage in flowers, shoots and seedlings under low temperature conditions. In cucumber seedlings, a single spray with

0.02 mM SA reduced MDA content and EL of seedlings under low temperature stress (Lei *et al.*, 2010). The MDA content and EL of SA treated seedlings were 24 and 30% respectively lower than the control seedlings at Day 6 under low temperature stress. In anthurium flowers, dipping scape in 2 mM SA solution for 15 minutes reduced LOX activity, MDA content and EL of flowers during storage at 4 °C for 20 days and reduced CI symptoms in SA treated flowers (Promyou *et al.*, 2012). The LOX activity, MDA content and EL of SA treated flowers were 41, 29 and 44% respectively lower at Day 20 of low temperature storage. Dipping bamboo shoots in 1 mM SA solution for 15 minutes reduced the increase of MDA content and EL in the SA treated shoots during storage at 1 °C for 50 days and increased CI tolerance in SA treated shoots (Luo *et al.*, 2012). The MDA content and EL of SA treated shoots were 7 and 22% respectively lower than the control shoots at the end of storage.

Similar results were also found in MJ treated fruits. In mango cv. Tommy Atkins, treatment with MJ vapor at 0.1 mM for 24 hours reduced EL in MJ treated fruits by 12% and increased CI tolerance during storage at 7 °C for 21 days (González-Aguilar *et al.*, 2000).

In loquat, MJ vapor at 0.01 mM for 24 hours reduced LOX activity in MJ treated fruits by 13% during storage at 1 °C for 35 days (Cao *et al.*, 2009). In peach, MJ vapor of fruit with 0.001 mM for 24 hours reduced MDA content and EL in MJ treated fruits by 16 and 18% respectively during storage at 0 °C for 5 weeks and was closely related to delayed CI symptom development in MJ treated fruit (Jin *et al.*, 2013). In guava, the reduction in EL was found in the fruits treated with 0.01 and 0.1 mM MJ vapor for 8 hours before storage at 5 °C for 15 days and correlated with reduced CI symptoms by MJ (González-Aguilar *et al.*, 2004). In pomegranate, treatments with 0.01 and 0.1 mM MJ vapor for 16 hours also reduced EL in MJ treated fruit by 11 and 13% respectively and reduced CI symptoms in MJ treated fruits during storage at 2 °C for 3 months (Sayyari *et al.*, 2011a). In lemon, dipping the fruit in 0.01 mM MJ solution for 30 seconds before storage at -0.5 °C was effective in reducing MDA content by 43% at Day 42 and reduced CI symptoms in MJ treated fruit (Siboza and Bertling, 2013).

All of these results show that treatment with SA and MJ reduced oxidative damage of membranes during low temperature storage which induced CI tolerance in

SA and MJ treated fruits. The mechanism of SA and MJ that reduced membrane damage may be associated with the reduction in ROS production and accumulation during low temperature storage.

SA and MJ on antioxidant defense system during low temperature stress

The mechanisms of SA and MJ in reducing CI of the fruits involve enhancing of their antioxidant defense system. Many reports showed that treatment with SA and MJ before exposure to chilling stress increased both enzymatic and non-enzymatic antioxidant components which increased their ability to reduce and scavenge ROS and reduce membrane damage during low temperature storage (Wang *et al.*, 2006; Ding *et al.*, 2007; Sayyari *et al.*, 2011a, b; Siboza and Bertling, 2013).

In mango cv. Zill, dipping the fruits in 2 mM SA solution for 10 minutes before storage at 5 °C had significantly higher AsA and GSH contents and activities of SOD, CAT, POD, APX and GR than those in the control fruits (Ding *et al.*, 2007). It also showed that SA was most effective in increasing GSH content by 450% at Day 30 of cold storage (Ding *et al.*, 2007).

Dipping peach cv. Beijing 24 in 1 mM SA solution for 5 minutes before storage at 0 °C had significantly higher APX and GR activities and higher reduced-to-oxidized ascorbate ratio (AsA/DHAsA) than those in the controls during cold storage for 28 days and subsequent shelf life at 20 °C for 3 days (Wang *et al.*, 2006). The activities of APX, GR and AsA/DHAsA ratio in SA treated fruits were 33, 50 and 66% respectively compared with control fruits at Day 28 of cold storage. Dipping pomegranate cv. Mollar de Elche in 1 mM ASA solution for 10 minutes before storage at 2 °C had significantly higher contents of ascorbic acid, total phenolics and total anthocyanins and TAC than those in the control fruits during storage for 84 days (Sayyari *et al.*, 2011b). The contents of ascorbic acid, total phenolics and total anthocyanins and TAC were 140, 19, 18 and 70% respectively at Day 84. ASA is more effective in enhancing ascorbic acid than total phenolics and total anthocyanin (Sayyari *et al.*, 2011b). MeSA vapor of pomegranate cv. Mollar de Elche with 0.01 or 0.1 mM for 16 hours before storage at 2 °C also increased the contents of total phenolics and anthocyanins and TAC in MeSA treated fruits during storage for 84 days (Sayyari *et al.*, 2011a, b). In lemon, dipping the

fruit in 2 mM SA solution for 30 seconds before storage at -0.5 °C had higher TAC throughout 42 days of cold storage (Siboza and Bertling, 2013). The TAC by DPPH method of SA treated fruit was 100% higher than the control fruit at the end of storage.

SA treatment also increases the antioxidant defense system in other parts of plants. Dipping the stems of anthurium flowers in 2 mM SA solution for 15 minutes before storage at 4 °C had significantly higher CAT activity during storage for 20 days (Promyou *et al.*, 2012). CAT activity of SA treated flowers was 150% higher at the end of storage while SA did not affect TAC in these flowers. In banana seedlings, spraying on leaves and irrigating roots with 0.5 mM SA solution had higher SOD, CAT and APX activities during exposure to low temperature at 5 °C for 3 days (Kang *et al.*, 2003). The activities of SOD, CAT and APX in SA treated seedlings were 58, 68 and 160% respectively at Day 3 under low temperature stress. In eggplant seedlings, spraying the leaves with 0.02 M SA solution had higher AsA and GSH contents and APX, DHAR and MDHAR activities during growth at 4 °C for 9 days (Chen *et al.*, 2011). The contents of AsA and GSH in SA treated seedlings were 70 and 133% respectively while activities of MDHAR, DHAR and APX were 40, 50 and 122% respectively higher than the control seedlings at Day 9 under low temperature stress.

Similar results were also found in MJ treated fruits. In peach cv. Baifeng, vaporizing of the fruits with 0.001 mM MJ for 24 hours before storage at 0 °C increased AsA and total phenolics contents and SOD activity by 36, 60 and 45% respectively at Week 5 of storage (Jin *et al.*, 2009b). In loquat cv. Fuyang, fruits treated with 0.01 mM MJ vapor for 24 hours before storage at 1 °C had higher SOD, APX and CAT activities than those in the control fruits by 47, 113 and 118% respectively at Day 35 of cold storage (Cao *et al.*, 2009). Loquat cv. Jiefangzhong treated with 0.01 mM MJ vapor for 24 hours before storage at 1 °C had higher contents of AsA and GSH and higher activities of MDHAR, DHAR, GR, APX, GPX and GST at Day 35 (Cai *et al.*, 2011). The contents of AsA and GSH in MJ treated peach fruits were 70 and 100% higher than the control fruits, while the activities of APX, DHAR, GST, GPX, MDHAR and GR in SA treated fruits were 23, 42, 48, 52, 114 and 173% higher at the end of storage. In pomegranate cv. Mollar de Elche, MJ vapor of 0.01 or 0.1 mM for 16 hours before storage at 2 °C had significantly higher total phenolics and total anthocyanins contents

and higher total antioxidant activity during storage for 84 days (Sayyari *et al.*, 2011a). Dipping lemon fruits in 0.01 mM MJ solution for 30 seconds before storage at -0.5 °C had higher TAC than the control fruits throughout 42 days of cold storage (Siboza and Bertling, 2013). The TAC by DPPH method of MJ treated fruits was 400% higher than control fruits at the end of storage.

SA and MJ also induce some antioxidant gene expression in some fruits during storage at low temperature. Sweet pepper treated with 0.1 mM MeSA or 0.1 mM MJ for 24 hours before storage at 0 °C had higher transcription levels of SOD, CAT and APX genes at Day 3 of storage time (Fung *et al.*, 2004). Tomato fruits treated with MJ vapor of 0.01 mM for 16 hours before storage at 5 °C maintained high transcription levels of the CAT gene throughout storage time for 28 days. Tomato fruits treated with 0.01 mM MeSA for 16 hours before storage at 5 °C reduced transcription levels of the CAT gene for the first 3 days of storage and then increased high transcription level after Day 3 and afterward. Conversely, the CAT transcripts were maintained at high levels only in the first 3 days and then gradually decreased thereafter in control fruit (Ding *et al.*, 2002). Spraying leaves of eggplant seedlings with 0.02 M SA solution also enhanced expression of stress-responsive genes under chilling stress. The highest transcript levels of GST1, GST2, GPX1, GPX2, GSH, MDHAR, GR and DHAR upon pretreatment with SA were 6.21, 6.24, 7.76, 5.99, 6.54, 6.44, 2.86 and 2.15 folds respectively as compared to control seedlings (Chen *et al.*, 2011). SA also enhanced the expressions of CAT and GPX genes in sweet cherry fruit by dipping in 2 mM SA solution before storage at 20 °C for 4 days under a pathogen stress condition (Xu and Tian, 2008). The increase in expression levels of CAT and GPX genes is associated with increases of CAT and GPX activity in sweet cherry, resulting in pathogen resistance in SA treated sweet cherry fruit (Xu and Tian, 2008).

The increase in antioxidant enzyme activities, antioxidant contents or some antioxidant gene expression in the plants mentioned above correlate with reduced CI symptoms or induced CI tolerance in these plants by increasing the ability to reduce free radicals accumulation and oxidative membrane damage during low temperature storage.

SA and MJ on fruit quality during low temperature stress

SA and MJ treatments not only reduced CI symptoms, but also maintained or improved the fruit quality in mango fruits. González-Aguilar *et al.* (2000) found that treatment with 0.1 mM MJ for 24 hours before storage at 7 °C for 21 days decreased weight loss and maintained fruit firmness of Tommy Atkins mango fruit during storage. MJ treatment also increased TSS of ripe mango fruits and delayed the exocarp color change of mango fruit during storage at 7 °C (González-Aguilar *et al.*, 2000). González-Aguilar *et al.* (2001) reported that treatment with 0.01 and 0.1 mM MJ for 20 hours before storage at 5 °C reduced CI and improved quality of Kent mango fruit. Treatment with 0.01 mM MJ reduced fruit decay but accelerated ripeness and overall quality of Kent mango fruits (González-Aguilar *et al.*, 2001). Treatment with 0.01 mM MJ before cold storage improved yellowness of exocarp color and increased TSS, organic acid and sugar contents of Kent ripe mango fruits (González-Aguilar *et al.*, 2001). MJ has been reported to improve peel color by promoting β-carotene synthesis and chlorophyll degradation (Pérez *et al.*, 1993) and stimulates anthocyanin accumulation (Sayyari *et al.*, 2011a). Ding *et al.* (2007) reported that dipping the fruits in 2 mM SA for 10 minutes before storage at 5 °C for 30 days increased TSS of ripe Zill mango fruits on Days 20 and 30 of storage.

SA and MJ treatments also improved the quality of other fruits. Meng *et al.* (2009) reported that treatment with 0.1 mM MJ for 12 hours before storage at 5 °C for 21 days retarded decreases firmness of peach cv. Jiubao during cold storage. MJ maintained fruit firmness by decreasing the activity of endo-polygalacturonase and pectin methyl esterase that catalyzed cell wall degradation leading to fruit softening (Meng *et al.*, 2009). Sayyari *et al.* (2011a) reported that treatment with 0.01 and 0.1 mM MJ and MeSA for 16 hours before storage at 2 °C for 84 days also retarded decreases in firmness of pomegranate after cold storage. Tareen *et al.* (2012) found that dipping the fruits in 0.5-2 mM SA solution for 5 minutes before storage at 0 °C for 5 weeks improved the quality of peach cv. Flordaking during cold storage. SA treatment delayed the skin color changes manifested as higher lightness and hue angle values than the control fruits. SA treatments also reduced fruit decay and weight loss of peach (Tareen *et al.*, 2012). SA has been reported to reduce fruit weight losses by closing stoma in

Vicia faba (bean) and ‘Ponkan’ mandarin fruit (Manthe *et al.*, 1992; Zheng and Zhang, 2004). SA treatments retarded decreases in fruit firmness but enhanced TSS of peach (Tareen *et al.*, 2012). MeSA and MJ increased resistance to pathogens, thereby decreasing the incidence of decay of tomato fruit during storage at 5 °C for 28 days by treatment with 0.01 mM MeSA and MJ for 16 hours (Ding *et al.*, 2001, 2002). MeSA and MJ treatments induce the synthesis of some stress proteins, such as intracellular and extracellular β-1,3-glucanase and intracellular chitinase, which leads to increased resistance to pathogens, thereby decreasing the incidence of decay in tomato fruits (Ding *et al.*, 2002).