CHAPTER 2

Literature Review

Oxidation-reduction (redox) reaction and cellular redox status in plant cells

Oxidation-reduction reactions, also called redox reactions, involve transferring of an electron from an electron donor (a reducer), to an electron receptor (an oxidizer). Upon transferring of the electron, the oxidative state of both reacting species changes. The oxidizing partner receives an electron lowering its oxidation number. In contrast, the oxidation number increases as the reducer gives up its electron. The pair of the oxidizing and reducing agent is called a redox pair. A redox couple is a reducing species and its corresponding oxidized form, for example; Fe^{2+}/Fe^{3+} (Hopkins and Huner, 2008).

A redox reaction can be simply illustrated by the following equations. Compound A in its reduced form (A_{red}) becomes oxidized (A_{ox}) when it gives up an electron (e^-) (Equation 2.1). Oxidized compound B (B_{ox}) is reduced (B_{red}) when it accepts an electron (Equation 2.2). When these two reactions are coupled, the net effect is the transfer of an electron from compound A to compound B (Equation 2.3) (Hopkins and Huner, 2008).

$$A_{red} \rightarrow A_{ox} + e^- \quad (2.1)$$

$$B_{ox} + e^- \rightarrow B_{red} \quad (2.2)$$

$$A_{red} + B_{ox} \rightarrow A_{ox} + B_{red} \quad (2.3)$$

The redox reaction is essential in cellular metabolism and represents a major fraction of all catabolic and anabolic reactions. The reactions can be found throughout

the cell, from the cell wall to deep within the nucleus of the cell (Dietz and Pfannschmidt, 2011). As a specific biological example, consider the reduction of 3-phosphoglyceric acid (PGA) to glyceraldehyde-3-phosphate (G3P) by NADPH (Hopkins and Huner, 2008):

$$PGA + NADPH + H^+ \rightarrow G3P + NADP^+ \quad (2.4)$$

The reactions may be separated into two half-reactions, an electron donating and receiving reaction. Equation 2.5 represents the reducing half by which two electrons are released from the reducer, NADPH. The oxidizing reaction, Equation 2.6, portrays the oxidizer, PGA, accepting two electrons transforming into G3P (Hopkins and Huner, 2008):

$$NADPH \rightarrow NADP^+ + H^+ + 2e^- \quad (2.5)$$

$$PGA + 2e^- + H^+ \rightarrow G3P \quad (2.6)$$

The oxidation of NADPH to $NADP^+$ is coupled with the reduction of PGA to G3P. A reduced/oxidized pair such as PGA/NADPH is known as a redox couple. It should be noted that a redox reaction often involves a transfer of protons. The positively charged protons balance the negative charges of the acquired electrons maintaining electroneutrality. The involvement of protons indicates that the redox reaction is pH sensitive (Hopkins and Huner, 2008).

The oxidation-reduction status (the redox status) is an important regulator of various metabolic functions of cells (Kocsy *et al*., 2013). The cellular redox state is made tangible in terms of the redox state or the redox status of the individual redox-active molecules in a cell. For each redox-active molecule, its redox status can be defined as a proportion of reduced molecules in relation to the total population, or, alternatively, as a ratio between reduced and oxidized forms within a pool as well as the sum of reducing and oxidizing redox-active molecules (Potters *et al*., 2010).

This redox state maintains general cell homeostasis, and in particular, the ability of the cell to deal with redox events. As such, redox state is not just the control point in plant stress response, but rather plays a far more fundamental role in every living cell.

Indeed, redox control during plant growth, development and senescence is involved with regulation of non-stress-related processes such as expression of large numbers of genes, energy production in mitochondria and chloroplasts, oxidation and reduction reactions, as well as many reactions in primary and secondary metabolism (Figure 2.1) (Potters *et al*., 2010; Handy and Loscalzo, 2012; Kocsy *et al*., 2013). Mitochondrial energy production and free radical scavenging activity are the major redox systems controlling cellular redox balance as well as interacting with various transducers and effectors in responses specific to oxidative stress (Noctor *et al*., 2007; Potters *et al*., 2010). The ratios of oxidized to reduced forms of nicotinamide adenine dinucleotide and ubiquinone (NAD^+/NADH and Q/QH_2) were identified as potential redox status in energy production whereas the ratios of reduced to oxidized forms of ascorbate, glutathione and nicotinamide adenine dinucleotide phosphate (ASA/DHA, GSH/GSSG and NADPH/$NADP^+$) have been identified as potential redox status in free radical scavenging (Yang *et al*., 2009; Potters *et al*., 2010; Ostaszewska *et al*., 2014).

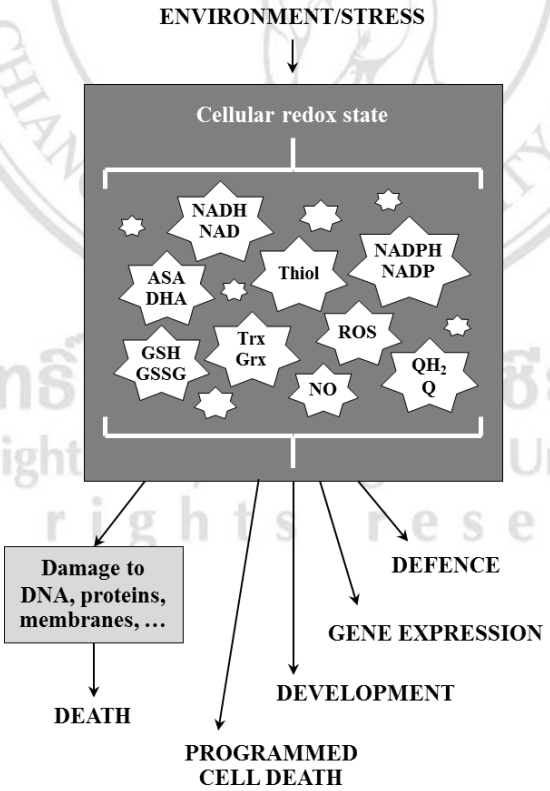

Figure 2.1 Overview of cellular redox state in different responses (modified from Noctor *et al*., 2007; Potters *et al*., 2010; Kocsy *et al*., 2013)

The levels of many redox-active metabolites vary depending on developmental stage, growth conditions, sub-cellular location and stress exposure. The relationships between metabolite concentration and stress are complex. Consequently, the concept of a cellular redox state, which regulates stress responses, is inherently associated with the kinetics of stress exposure especially the balance between damage and acclimation responses. Stress also alters the redox state of metabolites and this may either constitute damage or rather be an active acclimation response (Potters *et al*., 2010).

Plant senescence

Senescence is the final stage in the development of plant organs, during which a series of irreversible events leads to breakdown and death of the plant cells (Sacher, 1973). Plants have both stress-induced and age-related developmental aging. Current physiological understanding of the senescence condition and its positive roles in plant growth, differentiation, adaptation, survival and reproduction, supports a definition that acknowledges senescence to be a phase of development that: (1) is a trans differentiation episode following the completion of growth; (2) may or may not be succeeded by death and (3) is absolutely dependent upon cell viability and expression of specific genes. It is marked by general decline in metabolic activities, accompanied by a decline in energy synthesis and chloroplast activities, change in DNA and RNA response and an increase in permeability of cytoplasmic membranes in the cells (Thomas, 2013).

After harvest, horticultural crops, fresh fruits, vegetables, and ornamentals immediately initiate and actively undergo senescence. Many biochemical processes of senescence irreversibly change the tissue composition of the crop until it becomes unmarketable. The period during which consumption is considered acceptable is called postharvest shelf life. The biological deterioration composes of ethylene production, compositional changes, growth and development, physiological breakdown and pathological breakdown whereas the influencing environmental factors are temperature, relative humidity, atmospheric composition, ethylene, light and chemicals (Sacher, 1973; Paliyath *et al*., 2008). Since mitochondrial energy production and free radical scavenging are the major redox system in plant cell, it is interesting to know that the

biological and environmental factors can affects the changes in redox reaction, redox active molecules and redox status in relation to energy production and free radical scavenging, which involved plant senescence.

Role of energy and energy production in plant cell

ATP is cellular energy source dictating cell functions and viability. ATP hydrolysis generates energy needed for cellular functions such as macromolecular biosynthesis, membrane transport and signal transduction (Mishra *et al*., 2006; Tanaka *et al*., 2010). Extracellular ATP has been shown to be signal indicator of plant stress response (Tanaka *et al*., 2010) whereas intracellular ATP is a substrate for kinases in the phosphorylation of several target molecules during the signal transduction (Mishra *et al*., 2006). In plant cell, the levels of cellular energy can be defined in term of "energy status". The energy status is determined by ATP content, energy charge (EC = [ATP + 1/2 adenosine diphosphate (ADP)]/[ATP + ADP + adenosine monophosphate (AMP)]) and the ratio of ATP to ADP (Yang *et al*., 2009).

As shown in Figure 2.2, ATP is synthesized in an energetically unfavorable phosphorylation reaction in which a phosphate group is added to ADP by ATP synthase. When required, ATP gives up this energy packet in an energetically favorable hydrolysis to ADP and inorganic phosphate (Pi). The regenerated ADP is, then, available for phosphorylation by the synthase (Alberts *et al*., 2010).

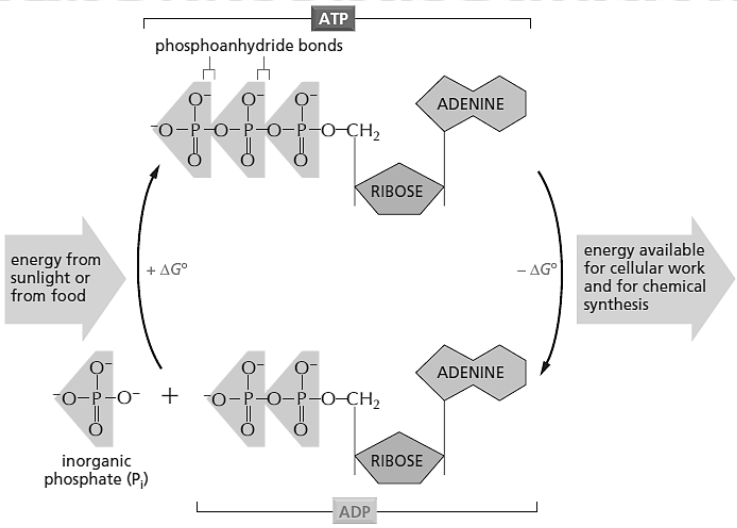

Figure 2.2 The interconversion of ATP and ADP (Alberts *et al*., 2010)

In plant cell, ATP is synthesized during photosynthesis in addition to regular cellular respiration. In respiration, the majority of ATP is synthesized via oxidative phosphorylation at the inner mitochondrial membrane while a small amount is produced during glycolysis in the cytoplasm (Soole and Menz, 2013; Zhou *et al*., 2014). The oxidative process by which ATP is produced can generally be depicted as followed. Electrons are sequentially transferred from NADH or $FADH_2$ to the final electron acceptor, molecular oxygen (O_2). After receiving electrons from NADH, NADH dehydrogenase (Complex I) which donates them to Q, a mobile lipophilic electron carrier, reducing it to QH_2. Electrons from $FADH_2$ are channeled through Complex II or SDH which also donates the electrons to Q. QH_2 is oxidized by Complex III via the Q-cycle and reduces the mobile peripheral protein cytochrome *c*. By interacting with Complex IV or CCO, the electrons carried by cytochrome *c* are donated to the terminal electron acceptor, O_2. During these electron movements, protons are pumped from the mitochondrial matrix to the intermembrane space at Complexes I, III, and IV, generating a proton gradient or the proton-motive force (pmf or $\Delta\mu_{H+}$). The gradient created is utilized by the inner mitochondrial membrane-bound to F_0F_1-ATPase to synthesize ATP. ADP and Pi are bound to the F_1 subunit located in the mitochondrial matrix resulting in a tightly bound ATP. Protons passing through the F_0 subunit cause structural changes to the F_1 subunit, releasing ATP (Figures 2.3 and 2.6) (Vedel *et al*., 1999; Hopkins and Huner, 2008; Taiz and Zeiger, 2010; Soole and Menz, 2013; Schertl and Braun, 2014).

The ATP producing steps described above is shared in essentially the same form by virtually all aerobic organisms: plants, animals, and microorganisms. Plant mitochondria contain, in addition, several other redox enzymes, at least two of which are unique to plants. These enzymes have been discovered largely by their insensitivity to certain classic inhibitors of electron transport. Unlike animal mitochondria, plant mitochondria contain dehydrogenases capable of oxidizing cytosolic and intermembrane NADH and NADPH. Electrons are subsequently donated to the ubiquinone pool. However, because these membranes bound dehydrogenases do not traverse the inner mitochondrial membrane, no protons are translocated as does Complex I. Consequently, only two ATP can be formed from the transfer of each pair of electrons to oxygen

(Figure 2.4). Thus, the inner membrane of plant mitochondria contains four distinct NAD(P)H dehydrogenases: (1) an internal NADH dehydrogenase (2) a rotenone-insensitive NADPH dehydrogenase (3) an external NADH dehydrogenase and (4) an external NADPH dehydrogenase (Figures 2.4 and 2.6) (Hopkins and Huner, 2008; Taiz and Zeiger, 2010).

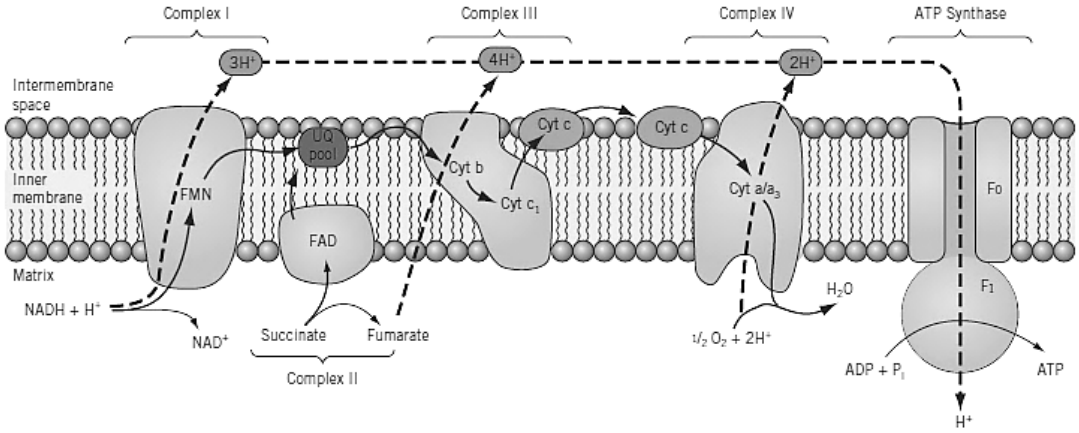

Figure 2.3 Schematic representation of the electron transport chain (ETC) and proton pumping sites in the inner membrane of a plant mitochondria. Solid arrow indicates the path of electrons from NADH or succinate to molecular oxygen. Energy conserved in the proton gradient is used to drive ATP synthesis through the F_0-F_1-ATPase coupling factor elsewhere in the membrane (Hopkins and Huner, 2008)

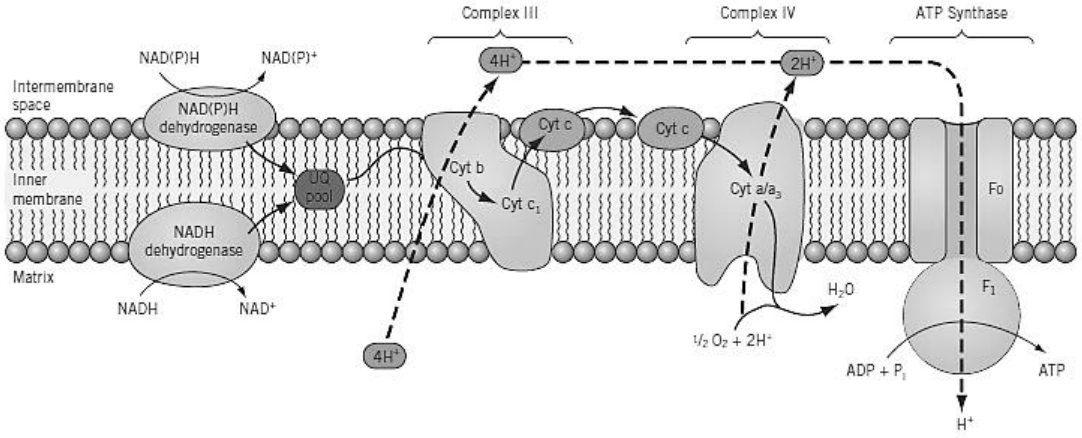

Figure 2.4 Alternative electron transport pathways in plant mitochondria. Electrons entering the chain through the alternative dehydrogenases will pass through two phosphorylating sites rather than three (Hopkins and Huner, 2008)

A feature of plant mitochondrial ETC is the presence of two terminal oxidases. In addition to cytochrome *c* oxidase pathway (COX), an alternative oxidase (AOX) is present that directly couples the oxidation of QH_2 with the reduction of O_2 to H_2O. AOX introduces a branch in the ETC such that electrons in QH_2 are partitioned between the cytochrome pathway (Complex III, cytochrome *c*, Complex IV) and AOX. Notably, AOX dramatically reduces the energy (ATP) yield of respiration since it is not proton pumping and since electrons flowing to AOX bypass the proton pumping Complexes III and IV. Electron flow to AOX still yield ATP, but at a reduced level. However, if electron flow to AOX is being supported by an alternative dehydrogenase or by Complex II (that, unlike Complex I, are not proton-pumping), then electron flow will be completely uncoupled from ATP turnover (Figures 2.5 and 2.6) (Juszczuk and Rychter, 2003; Hopkins and Huner, 2008; Taiz and Zeiger, 2010; Vanlerberghe, 2013).

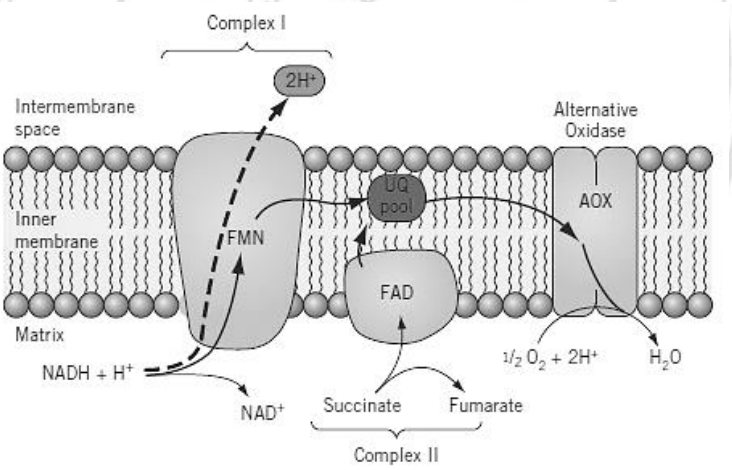

Figure 2.5 Alternative respiratory pathway. Electrons intercepted by the alternative oxidase pass through one or no phosphorylating sites (Hopkins and Huner, 2008)

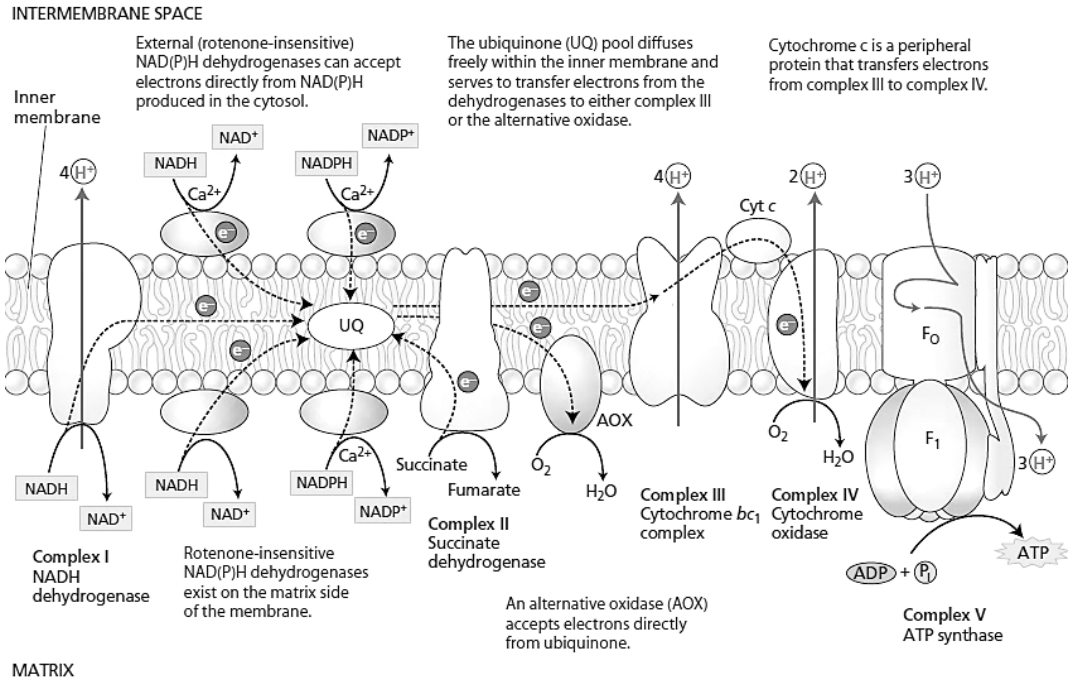

Figure 2.6 Organization of the electron transport chain and ATP synthesis in the inner membrane of plant mitochondria (Taiz and Zeiger, 2010)

It is generally believed that biotic and abiotic stresses cause plant cells to consume high amount of energy in order to survive. Reduction in energy production during various stresses, thus, leads to senescence of fruits. Many studies have reported that stress causes a reduction in the energy status and respiratory enzyme activity of fruits during storage. For example, reduction in ATP content and EC in pear fruit coincided with amplification of flesh browning when the fruit was stored at 0°C (Saquet *et al*., 2003). Similarly, ATP content and EC in litchi fruit also significantly decreased while membrane permeability and pericarp browning significantly increased when fruit were stored at 25°C (Yang *et al*., 2009; Wang *et al*., 2013). Moreover, ATP content, EC and SDH and CCO activities in peach fruit significantly decreased while membrane damage and chilling injury significantly increased when fruit was stored at 0°C (Jin *et al*., 2013; Jin *et al*., 2014). ATP content and EC in mango fruit significantly decreased while chilling injury significantly increased when fruit was stored at 10°C (Li *et al*., 2014). ATP content and EC in longan fruit also significantly decreased while membrane permeability, fruit disease and pericarp browning significantly increased

when fruit was induced by pathogen or stored at 0°C (Chen *et al*., 2014). Furthermore, ATP content and EC in peach fruit significantly decreased while membrane damage, fruit disease and ripening process significantly increased when fruit was stored at 25°C (Wang *et al*., 2015a). ATP content, EC and SDH activity in banana fruit significantly decreased while membrane permeability and chilling injury significantly increased when fruit was stored at 7°C (Wang *et al*., 2015b).

Considerable evidence pointed to the fact that energy seems to be important on aging and senescence of fruits and vegetables. Exogenously added ATP could also tip the cellular energy balance in favor of diminishing and controlling aging and senescence of fruits and vegetables. Yi *et al*. (2008) reported that application of 1 mM ATP on litchi fruits before inoculation with *Peronophythora litchi* and stored at 25°C for 6 days significantly reduced pericarp browning and fruit disease symptom by increasing ATP content and EC and decreasing oxidative membrane damage. Litchi fruits immersed in 1 mM ATP solution before storage at 25°C for 5 days showed significant reduction in pericarp browning by increasing ATP content and EC and decreasing oxidative membrane damage (Yang *et al*., 2009). Moreover, 1 mM ATP-treated litchi fruit stored at 28°C for 6 days was found to have enhanced enzymatic antioxidant and antioxidant capacity while pericarp browning decreased (Yi *et al*., 2010). Similarly, exogenous application of 0.8 or 1 mM ATP to longan fruits before storage at 25°C for 6 days significantly reduced pericarp browning (Yao *et al*., 2014; Chen *et al*., 2015). Hence, increase in cellular energy by ATP supplement could be utilized to delay postharvest deterioration of fruits and vegetables.

Redox status in energy production

Plant mitochondria, apart from ATP biosynthesis, coordinate redox homeostasis of the cell in response to developmental changes as well as variable and/or stressful environmental conditions (Rhoads and Subbaiah, 2007; Sweetlove *et al*., 2007). Redox status, in respiration, can be envisioned as the ratio of NAD^+ to NADH (NAD^+/NADH) and Q to QH_2 (Q/QH_2). In a plant cell, the redox and the energy status as well as respiratory enzyme activities have been shown to be affected by both internal and external factors. Any changes in respiratory redox balance disturb respiratory enzyme

activities and ATP biosynthesis (Yang *et al*., 2009; Jin *et al*., 2013; Jin *et al*., 2014; Ostaszewska *et al*., 2014).

Considerable evidence suggested that stress conditions caused changes in the cellular redox status in energy production and, consequently, affect senescence. For example, a decrease in Q/QH_2 ratio of litchi fruits coincided with a decrease in ATP content and EC under oxidative stress, resulting in oxidative damage of membrane and pericarp browning during storage at 25°C (Yang *et al*., 2009). NAD^+/NADH ratio of bean seedlings decreased significantly in conjunction with the decrease in ATP content, ATP/ADP ratio and COX capacity after sulphur-deficient stress, resulting in a delay of seedling growth (Juszczuk and Ostaszewska, 2011). Moreover, NAD^+/NADH ratio of *Arabidopsis* also decreased significantly and coincided with a decrease in ATP content, ATP/ADP ratio and COX capacity after long-term sulphur-deficient stress, resulting in a delay of growth (Ostaszewska *et al*., 2014). This evidence suggests that senescence is closely related to the redox status and cellular energy supply. Maintaining healthy redox status and energy efficiency in the cell could, therefore, delay or inhibit cellular deterioration to a certain extent.

Role of cellular energy and redox status in preventing senescence in plants

In order to delay or reduce senescence via alteration of energy and redox status, various chemicals and physical methods have been employed. For example, application of 100% oxygen on longan fruits during storage at 28°C for 6 days significantly increased ATP content and EC while oxidative membrane damage and pericarp browning decreased (Su *et al*., 2005). Application of 1 mM ATP solution for 3 minutes on litchi fruit before storage at 25°C significantly increased ATP content and EC relating to ubiquinone redox balance while oxidative membrane damage and pericarp browning decreased (Yang *et al*., 2009). Similarly, application of 1 µM MJ vapor for 24 hours or 5 mM OA solution for 10 minutes on peach fruits prior to 0°C storage significantly increased ATP content, EC and SDH and CCO activities while oxidative membrane damage and chilling injury decreased (Jin *et al*., 2013; Jin *et al*., 2014). Li *et al*. (2014) reported that application of 5 mM OA solution for 10 minutes on mango before storage at 10°C for 49 days significantly increased ATP content and EC while

chilling injury decreased. Wang *et al*. (2015a) reported that decreased atmospheric pressure on peach fruits to 10-20 KPa before cold storage significantly increased ATP content and EC while oxidative membrane damage decreased and senescence was delayed. Furthermore, application of 0.05 mM NO for 5 minutes on banana fruit before storage at 7°C for 20 days significantly increased ATP content, EC and SDH and CCO activities while oxidative membrane damage and chilling injury decreased (Wang *et al*., 2015b).

Oxidative stress and antioxidative ascorbate-glutathione cycle

Even under optimal conditions, ROS are mainly produced at a low level in organelles such as chloroplasts, mitochondria, peroxisomes and glyoxysomes (Ahmad *et al*., 2010). However, during biotic and abiotic stress, ROS production is dramatically elevated. ROS may be produced in response to abiotic and biotic stresses, and associated with a number of physiological responses in plants. When the generation of ROS exceeds the capacity of the plant to maintain cellular redox homeostasis, or when the production of ROS exceeds the capacity of the plant to scavenge them, the oxidative stress occurs (Hodges *et al*., 2004; Bhattacharjee, 2013). Oxidative stress can be induced at various subcellular sites and initiated by an increased production of ROS as a result of numerous processes. The three sites related to ROS generation are the apoplastic region (cell wall, apoplastic space and the external surface of the plasma membrane), the cytoplasm and cellular organelles (chloroplasts, mitochondria and peroxisomes/glyoxysomes) (Toivonen, 2004). ROS include such compounds as superoxide radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), H_2O_2 and the hydroxyl radical ($OH^{\bullet}$). They are partially reduced forms of O_2, resulting from either the excitation of O_2 to form $^1O_2$ or the transfer of one, two or three electrons to O_2 to form $O_2^{\bullet-}$, H_2O_2 or $OH^{\bullet}$, respectively (Mittler, 2002; Hodges *et al*., 2004). H_2O_2 has important useful functions in several cellular processes and its production increases in stressed plant tissues (Purvis, 2004).

The toxicity of ROS is due to their ability to initiate radical cascade reactions that lead to the production of $OH^{\bullet}$ and other destructive species which can cause protein damage, lipid peroxidation, DNA damage and finally cell death (Hossain *et al*., 2012).

ROS accumulation may cause oxidative damage to lipids, forming toxic products such as malondialdehyde (MDA), a secondary end product of polyunsaturated fatty acid oxidation. Thus, MDA is usually an indicator of the degree of plant oxidative stress and the structural integrity of the membranes of plants subjected to the abiotic stress (Hodges *et al*., 1999; Sharma *et al*., 2012). Therefore, oxidative stress, resulting from the deleterious effects of ROS, is an important phenomenon in many biological systems. These oxidative stresses can be caused at various subcellular sites and initiated by the accumulation of ROS (Toivonen, 2004; Sharma *et al*., 2012).

Scavenging of ROS is important for the maintenance of normal plant growth. Plants have evolved an efficient antioxidant defense system that would prevent the accumulation of ROS and repair oxidative damage. This system involves both lipid-soluble antioxidants (α-tocopherol and carotene), water-soluble reductants (ASA and GSH), and enzymes such as catalase (CAT), APX, superoxide dismutase (SOD) and GR (Mittler, 2002; Imahori *et al*., 2008; Ahmad *et al*., 2010; Sharma *et al*., 2012).

The ASA-GSH cycle, also known as the Halliwell-Asada cycle, is one antioxidant defense system, involves a series of reactions with four antioxidant enzymes that act in concert in dissipation of H_2O_2 and other ROS (Noctor and Foyer, 1998). When ROS such as H_2O_2 is generated, it is scavenged via the oxidation of ASA forming monodehydroascorbate (MDHA). This reaction is catalyzed by APX (Figure 2.7 and Equation 1 in Table 2.1). The recycling of MDHA to produce ASA can occur in the following two pathways. Firstly, MDHAR directly converts MDHA to ASA (Figure 2.7 and Equation 2 in Table 2.1). Secondly, DHAR catalyzed the reaction between GSH and DHA, from non-enzymatic disproportionation of MDHA, turning it to ASA. (Figure 2.7 and Equation 3 in Table 2.1). Regeneration of GSH from GSSG by GR requires electrons from NADPH (Figure 2.7 and Equation 4 in Table 2.1) (Foyer and Noctor, 2011).

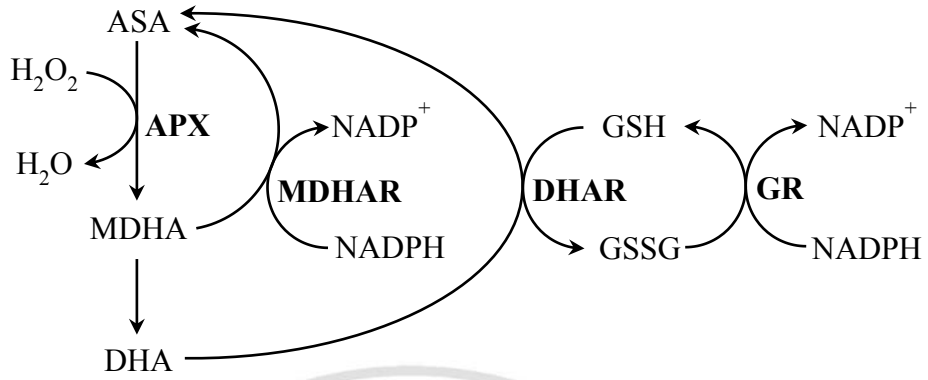

Figure 2.7 H_2O_2 scavenging by ASA-GSH cycle (modified from Mittler, 2002 and Foyer and Noctor, 2011)

Table 2.1 Reaction of four antioxidant enzymes in ASA-GSH cycle (modified from Sharma *et al.*, 2012)

ASA-GSH cycle enzymes	Reactions catalyzed
Ascorbate peroxidase (APX)	$H_2O_2 + ASA \rightarrow 2H_2O + MDHA$……..….(1)
Monodehydroascorbate reductase (MDHAR)	$MDHA + NADPH \rightarrow ASA + NADP^+$……(2)
Dehydroascorbate reductase (DHAR)	$DHA + 2GSH \rightarrow ASA + GSSG$……..…..(3)
Glutathione reductase (GR)	$GSSG + NADPH \rightarrow 2GSH + NADP^+$...…..(4)

NADPH is an important cofactor required for cell growth, proliferation and detoxification. Thus, it is one of the main end-products of several metabolic pathways and is also an indispensable substrate for reductive biosynthetic reactions in plants. NADPH is ultimate primary source of reducing equivalents for the ASA-GSH cycle which is used by the GR enzyme to maintain the level of GSH or by the MDHAR enzyme to maintain the level of ASA (Figure 2.7 and Table 2.1) (Corpas and Barroso, 2014). In plant cells, NADPH is regenerated from its oxidized form by a group of NADPH-generating dehydrogenases in the pentose phosphate pathway located in cytosol such as G6PDH and 6PGDH (Figure 2.8) (Valderrama *et al*., 2006; Corpas and Barroso, 2014; Manai *et al*., 2014).

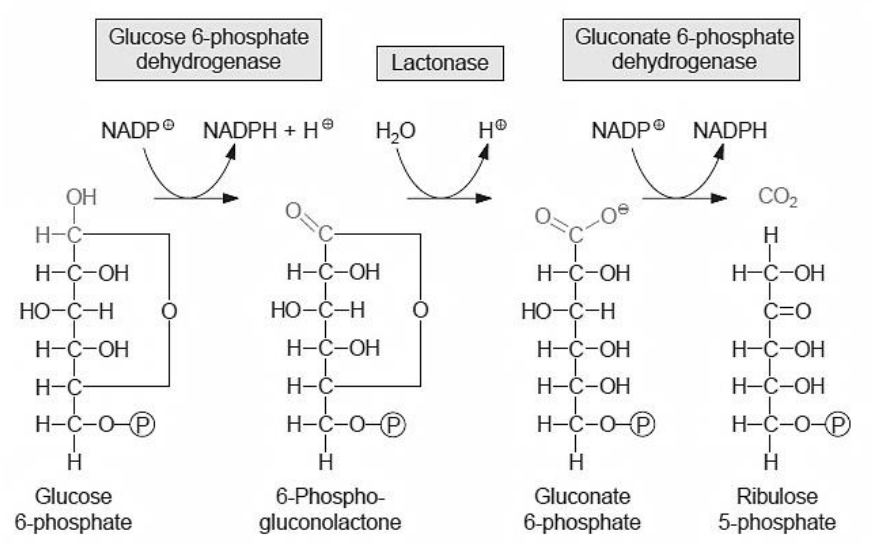

Figure 2.8 Regeneration of NADPH by some NADPH-generating dehydrogenases in the pentose phosphate pathway (Heldt and Piechulla, 2011)

Redox status in antioxidative ASA-GSH cycle

Redox status, in ASA-GSH cycle, can be envisioned as the ratio of ASA to DHA (ASA/DHA) and GSH to GSSG (GSG/GSSG). ASA/DHA and GSH/GSSG ratios are linked through the ASA-GSH cycle with APX, DHAR, MDHAR and GR (Fotopoulos *et al*., 2010). In a plant cell, the redox status and ASA-GSH cycle enzyme activities have been shown to be affected by internal and external factors. A low ratios of ASA/DHA and/or GSH/GSSG by decreased ASA and GSH or increased DHA and GSSG, may reduce the capacity of ASA-GSH cycle to scavenge ROS, leading to oxidative membrane damage and cell senescence (Foyer and Noctor, 2011).

It was suggested that ASA-GSH redox status including ASA/DHA and GSH/GSSG ratios is a key factor controlling ASA-GSH cycle, relating to senescence in some horticultural crops (Potters *et al*., 2010). Many studies have found that alteration of redox status causes a reduction in ASA-GSH cycle capacity leading to plant senescence. For example, a decrease in ASA/DHA and GSH/GSSG ratios related to a significant decrease in activities of APX, DHAR and GR of broccoli while senescence occurred during storage at 20°C for 5 days (Mori *et al*., 2009). In loquat fruit, decreased ASA/DHA and GSH/GSSG ratios also related to a significant decrease in activities of

APX, MDHAR, DHAR and GR while flesh browning increased significantly during cold storage (Cai *et al*., 2011). Similarly, ASA/total ascobate and GSH/total glutathione ratios of tomato fruit decreased significantly while disease symptom increased when the fruit was inoculated with *Botrytis cinerea* and stored at 25°C for 6 days (Zhu and Tian, 2012). In addition, ASA/DHA and GSH/GSSG ratios in banana (Ambuko *et al*., 2013) and plum (Singh and Singh, 2013) also decreased significantly while chilling injury increased significantly in low temperature storage. The activities of APX, MDHAR, DHAR and GR of plum were also found to decrease concurrently (Singh and Singh, 2013).

For the function of ASA-GSH cycle, there is a close interplay among ASA, GSH and NADPH. The redox status of NADPH (NADPH/$NADP^+$ ratio) and activities of G6PDH and 6PGDH also affected by stresses. For example, the decline in NADPH redox balance coincided with a decrease in growth has been reported in bean seedling during sulphur-deficient stress (Juszczuk and Ostaszewska, 2011) while the decrease in G6PDH has also been reported in rice under salt stress (Zhang *et al*., 2013). Similarly, NADPH/$NADP^+$ ratio also decreased and coincided with a decrease in the growth of *Arabidopsis* after long-term sulphur-deficient stress (Ostaszewska *et al*., 2014). Moreover, salt stress was shown to induce the decrease in GSH/GSSG and NADPH/$NADP^+$ ratios, G6PDH and 6PGDH activities in the root due to the cellular oxidative damage which coincided with a decrease in growth of tomato seedlings (Manai *et al*., 2014). Drought stress was shown to decrease in G6PDH and increase in 6PGDH activity in the root due to the cellular oxidative damage of lotus (Signorelli *et al*., 2013). Furthermore, expression of G6PDH genes is a key antioxidant system against salt-induced oxidative stress in olive (Valderrama *et al*., 2006).

Role of free radical scavenging and redox status in preventing senescence in plants

Studies conducted in a number of plant species under biotic and abiotic stress conditions have elucidated the fact that a high ratio of ASA/DHA and/or GSH/GSSG sustained by increased ASA and GSH or diminution of DHA and GSSG, may be the key element for efficient protection against biotic and abiotic stress-induced oxidative stress (Fotopoulos *et al*., 2010; Foyer and Noctor, 2011).

In order to delay or reduce senescence by mean of maintaining the redox status balance, various chemical methods have been employed. In harvested horticultural crops, Mori *et al*. (2009) reported that delayed senescence by an application of 40 pM ethanol vapor on broccoli during storage at 20°C associated significantly with increasing ASA/DHA and GSH/GSSG ratios and activities of APX, MDHAR, DHAR and GR. Moreover, application of 100 µM NO solution for 24 hours on *Phalaenopsis* flower significantly increased ASA/total ascobate ratio and activities of APX, MDHAR, DHAR and GR while oxidative damage decreased which coincided with an increase in vase life (Tewari *et al*., 2009). Application of 10 µM MJ vapor for 24 hours on loquat fruit before storage at 1°C for 35 days significantly increased ASA/DHA and GSH/GSSG ratios and activities of APX, MDHAR, DHAR and GR while chilling injury decreased (Cai *et al*., 2011). Similarly, tomato treated with 10 mM MJ solution for 10 minutes before inoculating with *Botrytis cinerea*, ASA/total ascobate and GSH/total glutathione ratios increased significantly while disease symptom decreased (Zhu and Tian, 2012).

Moreover, in other plants, application of 250 µM NO on Chickpea before exposure to cadmium (Cd) increased ASA/DHA and GSH/GSSG ratios and activities of APX, MDHAR, DHAR and GR significantly while Cd stress decreased which coincided with an increase in growth (Kumari *et al*., 2010). Moreover, Ramakrishna and Rao (2013) reported that application of 0.5-2 µM 28-homobrassinolide on radish seedlings before exposure to zinc increased GSH/GSSG ratio and activity of GR significantly while zinc stress decreased which coincided with an increase in growth. In addition, application of 1 mM putrescine on *Salvinia natans* that exposed by H_2O_2 increased GSH/GSSG ratio and activity of GR significantly while oxidative damage decreased which coincided with an increase in growth (Mandal *et al*., 2014). Application of 0.6 mM hydrogen sulfide (H_2S) on maize leaves before exposure to salt stress increased ASA/DHA and GSH/GSSG ratios and activities of APX, MDHAR, DHAR and GR significantly while salt stress damage decreased which coincided with an increase in growth (Shan *et al*., 2014). Furthermore, application of 10 mM trehalose on rice before exposure to copper stress increased ASA/DHA and GSH/GSSG ratios

and activities of APX, MDHAR, DHAR and GR significantly while oxidative damage decreased which coincided with an increase in growth (Mostofa *et al*., 2015).

Longan

Longan (*Dimocarpus longan* Lour.) is an evergreen tree in the Sapindaceae family. Longan is grown commercially in many countries, including China, Thailand, India and Vietnam. Longan fruit are non-climacteric and will not continue to ripen once removed from the tree. Consequently, fruit must be harvested when the skin becomes yellow-brown and their flesh reaches optimal eating quality (15.5-16.0 °Brix) (Jiang *et al*., 2002). 'Daw' longan is an important economic variety in Thailand, with white, juicy and sweet edible flesh (Chanrittisen and Chomsri, 2010). However, longan fruit undergo rapid pericarp browning and develop disease after just a few days at room temperature, resulting in a short storage life and reducing market value, since pericarp appearance is a key factor determining consumer selection (Jiang *et al*., 2002; Khunpon *et al*., 2011; Saengnil *et al*., 2014).

Pericarp browning is caused by two key enzymes, polyphenol oxidase (PPO) and peroxidase (POD), oxidizing phenols to quinones, which are then polymerized to form brown pigments (Jiang *et al*., 2002; Queiroz *et al*., 2008). Moreover, longan fruit are susceptible to postharvest pathogens. High sugar and moisture induce microorganisms to rot the fruit rapidly, which also causes severe browning (Apai, 2010). Consequently, prevention or inhibition of enzymatic browning and fruit decay is important for improving fruit marketing (Duan *et al*., 2007; Ciou *et al*., 2011).

Effective postharvest treatments for preventing longan fruit senescence include heat treatment, irradiation, coating, controlled atmosphere, plant extract, plant growth regulator and chemical treatment (Jiang *et al*., 2002). Previous studies provided an alternative chemical treatment using chlorine dioxide (ClO_2) fumigation for reducing pericarp browning and preventing fruit decay of longan fruit (Saengnil *et al*., 2014; Chumyam *et al*., 2015).

Chlorine dioxide and its role in senescence

ClO_2 is a strong oxidizing and sanitizing agent. It has a broad and high biocidal activity and has been used to control microorganisms, including foodborne pathogens (Du *et al*., 2009). It is a powerful oxidant that reacts with many organic compounds but produces much less toxic chlorinated byproducts than chlorine and hypochlorite (ClO^-). It is a greenish yellow to orange gas at room temperature with a characteristic pungent chlorine-like odor. It can be formed by the reaction of sodium chlorite ($NaClO_2$) and gaseous chlorine (Cl_2(g)), hypochlorous acid (HOCl) or hydrochloric acid (HCl). The reactions (World Health Organization, 2002) are:

$$2NaClO_2 + Cl_2\,(g) \rightarrow 2ClO_2\,(g) + 2NaCl$$

$$2NaClO_2 + HOCl \rightarrow 2ClO_2\,(g) + NaCl + NaOH$$

$$5NaClO_2 + 4HCl \rightarrow 2ClO_2\,(g) + 5NaCl + 2H_2O$$

ClO_2 is a new chemical found to reduce and delay aging and senescence including browning and disease in many types of fruits and vegetables (Gómez-López *et al*., 2009). Fu *et al*. (2007) found that 50 mg L^{-1} of ClO_2 solution was most efficient in reducing browning and inhibiting PPO activity of apple juices during storage at 15°C for 24 hours, whereas 100 mg L^{-1} of ClO_2 was most efficient in reducing browning and inhibit PPO activity of fresh-cut lotus root during storage at 4°C for 10 days (Du *et al*., 2009). Chen *et al*. (2010) also found that 100 mg L^{-1} of ClO_2 was most efficient in inhibiting PPO and POD activities of fresh-cut asparagus lettuce during storage at 4°C for 10 days. Moreover, 120 mg L^{-1} solution of ClO_2 reduced pericarp browning and inhibited PPO and POD activities of lychee fruit during storage at 20°C for 7 days. It also reduced MDA content during storage (Wu *et al*., 2011).

ClO_2 in both solution and gaseous form has been used to reduce microorganism in horticultural crops (Gómez-López *et al*., 2009). For example, application of 60-80 mg L^{-1} ClO_2 solution for 15 minutes maintained the quality of mulberry by reducing bacteria, yeast and mold during storage at -1°C for 14 days (Chen *et al*., 2011).

Similarly, the quality of fresh-cut Red Chard submerged in 3 mg L^{-1} ClO_2 solution for 1 minute was maintained as a result of *Escherichia coli* and *Salmonella* reduction during storage (Tomás-Callejas *et al*., 2012). Application of 10 mg L^{-1} ClO_2 solution for 5 minutes maintained the quality of star fruit, guava and pineapple by reducing *Escherichia coli* and *Staphylococcus aureus* (Har and Perera, 2013). In addition, fumigation with 1.33, 1.74 and 1.29 mg L^{-1} ClO_2 gas maintained the quality of carrot, lettuce and cabbage by reducing bacteria and yeast during storage at 7°C for 9 days (Gómez-López *et al*., 2007; 2008). Similarly, fumigation with 2-10 mg L^{-1} ClO_2 gas for 10-180 seconds maintained the quality of tomato by reducing *Salmonella enterica* during storage at 25°C for 28 days (Trinetta *et al*., 2010). It has been shown that the quality of tomato, cantaloupe and strawberry was maintained after exposing to 10 mg L^{-1} ClO_2 gas for 10-180 seconds. *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes* were lowered during storage at 25°C for 28 days (Trinetta *et al*., 2013).

It has been reported that gaseous ClO_2 can reduce and delay senescence by reducing pericarp browning and maintaining the quality of 'Daw' longan fruit. Fumigation of longan with 10 mg L^{-1} ClO_2 for 10 minutes was the most effective treatment to reduce pericarp browning and maintain fruit the quality for 5 days. Activities of PPO and POD during storage were found to be lower after ClO_2 treatment resulting in the reduction of pericarp browning of longan fruit cv. Daw (Saengnil *et al*., 2014). Moreover, ClO_2 treatment lessened longan fruit decay by decreasing longan pathogenic fungi such as *Fusarium*, *Cladosporium* and *Lasiodiplodia* (Chumyam *et al*., 2015).

The induction of antioxidant defense system to reduce oxidative damage from ROS is thought to be a possible mechanism of ClO_2 effect (Chomkitichai *et al*., 2014a; 2014b). However, no studies have been conducted on the changes in cellular redox balance and relationship between redox status, energy production and antioxidative ASA-GSH cycle of 'Daw' longan fruit during senescence. Therefore, the present study investigated changes in redox status related to enzyme activities in energy production and antioxidative ASA-GSH cycle during storage of harvested longan fruits cv. Daw and the effects of ClO_2 on redox status associated with enzyme activities in energy production and antioxidative ASA-GSH cycle for alleviating senescence.