CHAPTER 1

Introduction

Principle and rationale

The oxidation-reduction (redox) status is an important regulator for various metabolic functions. The cellular redox state can be visualized in terms of the redox state or redox status of the individual redox-active molecules in the cell (Potters *et al*., 2010).

Cellular respiration, one of the major redox systems, supplies energy for various cellular functions such as growth and survival. Electron transport chain (ETC) utilizes the potential energy stored in reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$) to produce adenosine triphosphate (ATP). Inner mitochondrial membrane enzymes such as NADH dehydrogenase, succinate dehydrogenase (SDH), cytochrome *c* oxidase (CCO) transfer electrons from NADH and producing proton gradient. ATP synthase uses energy from the proton-motive flow to produce ATP (Soole and Menz, 2013; Schertl and Braun, 2014). Redox status, in respiration, can be envisioned as the ratio of oxidized nicotinamide adenine dinucleotide (NAD^+) to NADH (NAD^+/NADH) and ubiquinone (Q) to ubiquinol (QH_2) (Q/QH_2). In a plant cell, both redox and energy status as well as respiratory enzyme activities have been shown to be affected by internal and external factors (Yang *et al*., 2009; Jin *et al*., 2013; 2014; Ostaszewska *et al*., 2014).

Free radical scavenging system is also one of the major cellular redox systems. It minimizes accumulation of reactive oxygen species (ROS) and repairs oxidative damage. Ascorbate-glutathione (ASA-GSH) cycle or Halliwell-Asada cycle, is one such system. Four antioxidative enzymes, ascorbate peroxidase (APX), monodehydro-

ascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) and others such as ascorbate (ASA) and reduced glutathione (GSH), work in concert with dissipate hydrogen peroxide (H_2O_2) or other ROS (Noctor and Foyer, 1998). In ASA-GSH cycle, there is a complex relationship between ASA, GSH and reduced nicotinamide adenine dinucleotide phosphate (NADPH) since NADPH is the ultimate reducing equivalent of ASA-GSH metabolism. It is regenerated from its oxidized form (oxidized nicotinamide adenine dinucleotide phosphate, $NADP^+$) by a group of NADPH-generating dehydrogenases located in cytosol such as glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) (Corpas and Barroso, 2014; Manai *et al*., 2014). Redox status, in ASA-GSH cycle, can be envisioned as the ratio of ASA to dehydroascorbate (DHA) (ASA/DHA), GSH to oxidized glutathione (GSSG) (GSH/GSSG) and NADPH to $NADP^+$ (NADPH/$NADP^+$). In a plant cell, the redox status as well as antioxidative enzyme activities have also been shown to be affected by both internal and external factors (Potters *et al*., 2010).

Considerable evidence suggested that the redox status is a key factor controlling senescence in some horticultural crops. Wang *et al*. (2013) reported that alteration in the redox potential and, consequently, the reduction in energy production during various stress conditions leads to senescence of fruits. Stress-induced reduction in energy status in many types of fruits in storage has been demonstrated. Reduction in ATP content and energy charge (EC) in pear fruit coincided with amplification of flesh browning when the fruit was stored at 0°C (Saquet *et al*., 2003). During low temperature storage, it was found that ATP content, EC and SDH as well as CCO activities in pear fruit decreased significantly while chilling injury was significantly increased (Jin *et al*., 2013; 2014). In other fruits such as mango (Li *et al*., 2014), longan (Chen *et al*., 2014), litchi (Yang *et al*., 2009; Wang *et al*., 2013) and banana (Wang *et al*., 2015b), the energy status was similarly found to be greatly reduced in conjunction with an increase in injury or other storage damages. In addition to the energy status, stress conditions also caused changes in the cellular redox status. For example, an increase in QH_2/total ubiquinone (Qt) ratio of litchi fruit coincided with a decrease in ATP, while browning symptom increased under oxidative stress (Yang *et al*., 2009). The NAD^+/NADH ratio of bean seedlings and *Arabidopsis* was found to be significantly decreased in conjunction with a decrease in

ATP, while growth was inhibited after sulphur-deficient stress (Juszczuk and Ostaszewska, 2011; Ostaszewska *et al*., 2014).

Moreover, in free radical scavenging, it was also suggested that the role of ASA-GSH cycle against oxidative stress is controlled by ASA/DHA and GSH/GSSG ratios, and is related to senescence in some horticultural crops (Potters *et al*., 2010). Alteration of cellular redox status causes a reduction in ASA-GSH cycle capacity which ultimately brings about plant senescence. For example, the decrease in ASA/DHA and GSH/GSSG ratios leads to significant decrease in the activities of APX, DHAR and GR of broccoli while senescence occurs during storage (Mori *et al*., 2009). In loquat fruit, decreased ASA/DHA and GSH/GSSG ratios also relates to significantly decreased activities of APX, MDHAR, DHAR and GR while flesh browning is found to increase during cold storage (Cai *et al*., 2011). Similarly, ASA/total ascorbate and GSH/total glutathione ratios of tomato fruit decreases while disease symptom increases when the fruit is 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 fruit (Ambuko *et al*., 2013) and plum fruit (Singh and Singh, 2013) also decrease while chilling injury increases in low temperature storage. The activities of APX, MDHAR, DHAR and GR of plum fruit are also found to be concurrently decreased (Singh and Singh, 2013). Moreover, the redox status of NADPH (NADPH/$NADP^+$ ratio) and activities of G6PDH and 6PGDH are also affected by stresses. For example, the decline in NADPH redox balance in bean seedling was found during sulphur-deficient stress while the decrease in G6PDH has been reported in rice under salt stress (Juszczuk and Ostaszewska, 2011; Zhang *et al*., 2013). Similarly, salt stress was shown to induce the decrease in NADPH/$NADP^+$ ratio, G6PDH and 6PGDH activities in the root which coincided with a decrease in the growth of tomato seedlings (Manai *et al*., 2014).

These findings suggest that aging and senescence are closely related to cellular energy supply and free radical scavenging system. Maintaining the energy efficiency and improving the capacity of free radical scavenging system in the cell could, therefore, delay aging and inhibit the occurrence of deterioration to a certain extent.

In order to delay or reduce senescence via alteration of energy and redox status, various chemicals and physical methods have been employed. Prior treatment with chemical such as methyl jasmonate (MJ), oxalic acid (OA) (Jin *et al*., 2013; 2014; Li *et al*., 2014), nitric oxide (NO) (Wang *et al*., 2015b) and pure oxygen (Su *et al*., 2005) had been shown to increase ATP content, EC and some respiratory enzyme activities and lessen damage during storage of many fruits such as pear, mango, banana and longan. Peach fruit exposed to increased atmospheric pressure exhibited similar outcome during storage (Wang *et al*., 2015a). Several lines of evidence indicate that some chemicals significantly improved redox balance between free radical producing system and antioxidant system and reduced oxidative stress. Mori *et al*. (2009) reported that delayed senescence in broccoli by ethanol vapor associated with increasing ASA/DHA and GSH/GSSG ratios and activities of APX, MDHAR, DHAR and GR. Cai *et al*. (2011) found that application of MJ on loquat fruit before storage increased ASA/DHA and GSH/GSSG ratios and activities of APX, MDHAR, DHAR and GR while chilling injury decreased. Zhu and Tian (2012) also reported that tomato treated with MJ before inoculation with *Botrytis cinerea*, ASA/total ascobate and GSH/total glutathione ratios significantly increased while disease symptom decreased.

Chlorine dioxide (ClO_2) is a new chemical found to reduce and delay aging and senescence including browning and disease in many types of fruits such as apple (Fu *et al*., 2007), litchi (Wu *et al*., 2011), tomato, cantaloupe and strawberry (Trinetta *et al*., 2013). Applications of gaseous ClO_2 on longan fruit before storage reduced pericarp browning significantly by reducing the oxidation of phenolic compound, delaying the occurrence of disease and maintaining higher fruit quality (Saengnil *et al*., 2014). Similarly, ClO_2 treatment of longan fruit reduced pericarp browning apparently by enhancing the antioxidant defense system (Chomkitichai *et al*., 2014a; 2014b). However, the underlying reasons were not known. This study explores the involvement of ClO_2 in improving cellular redox homeostasis and enhancing energy production and free radical scavenging during longan fruit senescence.

Objectives

1. To study the associations of redox status in energy production and free radical scavenging with senescence of ‘Daw’ longan pericarp during storage.

2. To study the effects of gaseous ClO_2 on redox status in energy production and free radical scavenging in relation with senescence of ‘Daw’ longan pericarp during storage.

Usefulness of research

1. The knowledge about the associations of cellular redox homeostasis involved in energy production and free radical scavenging with senescence of ‘Daw’ longan during storage will be elucidated.

2. The mechanisms of gaseous ClO_2 on the reduction of senescence of ‘Daw’ longan will be evaluated through biochemical responses involved in cellular redox homeostasis.

3. The outcome of this research can be applied to extend storage life or prolong senescence of longan and other fruits.

Scope of research

Effects of gaseous ClO_2 fumigation on redox status involved in energy production and free radical scavenging of ‘Daw’ longan during storage.

Duration of research

Three years from August 2013 to August 2016