Title of thesis: GENERIC DIFFERENCES IN ANTIOXIDANT CONCENTRATION IN THE FRUIT TISSUES OF FOUR MAJOR CULTIVARS OF APPLES

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Degree: Masters of Science, 2004

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Abstract

Apples have been cultivated for over two millennia and over 10,000 named cultivars exist. Antioxidants protect against oxidative stress and provide resistance against superficial scald in apple (*Malus domestica* Borkh). In the present study four commercially important apple cultivars (Red Delicious, Gala, Fuji and Granny Smith) and source leaves of Gala apple tree were studied. Ascorbate concentration, SOD activity, and protein concentration was determined for Gala leaves and all the four apple (fruit peel and fleshy cortex tissue) cultivars. Gala leaves had the highest ascorbate concentration and SOD activity. Granny Smith fruit peel tissue had the highest and Red Delicious fruit peel tissue had the lowest ascorbate concentration. Granny Smith fruit peel tissue had the highest SOD activity. These results demonstrate that ascorbate concentration and SOD activity in apple tissue may depend on their respective breeding parents, scald susceptibility and the amount of ethylene they produce.

GENERIC DIFFERENCES IN ANTIOXIDANT CONCENTRATION IN THE FRUIT TISSUES OF FOUR MAJOR CULTIVARS OF APPLES

by

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Thesis submitted to the Faculty of Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Masters of Science
2004

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ACKNOWLEDGEMENT

I am very grateful to my major professor, Dr. Bruno Quebedeaux, for his guidance, kindness, assistance, and patience throughout my graduate studies at the University of Maryland.

I wish to express my gratitude to the other members of my committee, Dr. Shyam K. Dube, Dr. George A. Bean for their precious advice and criticism.

I would also like to express my gratitude to Drs. Neelam Pathak, Asif Mehar and Gerald Deitzer for their invaluable help in my research. Special thanks are also given to Laurie Hellman and the greenhouse staff for allowing me to freely use greenhouse facilities and for the routine management of my apple trees.

I would like to thank the Department of Nutrition and Food Science for the financial support throughout my graduate studies.

Finally, I would like to thank my fiancé Mansi Shah, my parents and my brother for being supportive and helpful.

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ABBREVIATIONS

Asc	Ascorbate
AA	ascorbic acid
AOS	active oxygen species
APX	ascorbate peroxidase
CA	controlled atmosphere
CAT	Catalase
CuSOD	copper superoxide dismutase
DAFB	days after full bloom
DHA	Dehydroascorbate
DHAR	dehydroascorbate reductase
DNA	deoxyribonucleic acid
DPA	Diphenylalanine
EDTA	ethylenediaminetetraacetic acid
FAO	food and agriculture organization
FAS	federation of american scientists
FeSOD	iron superoxide dismutase
FW	fresh weight
Gal	Galactose
Glc	Glucose
GPX	glutathione peroxidase
GR	glutathione reductase
GSH	Glutathione
GSSG	glutathione disulphide
GST	glutathione s-transferases
KCN	potassium cyanide
LSD	least significant difference
MDHA	Monodehydroascorbate
MDHAR	monodehydroascorbate reductase
MnSOD	manganese superoxide dismutase
MT	metric ton
NADH	β-nicotinamide adenine dinucleotide, reduced
NADPH	β-nicotinamide adenine dinucleotide phosphate, reduced
NBT	nitroblue tetrazolium
PVPP	poly(vinylpolypyrrolidone)
SOD	superoxide dismutase
TE	tris-EDTA (ethylenediaminetetraacetic acid)
ULO	ultra low oxygen
ZnSOD	zinc superoxide dismutase

CHAPTER I

INTRODUCTION

Apples have been cultivated and vegetatively propagated for over two millennia. Over 10,000 named cultivars exist and breeders worldwide create more new selections annually, but only a few dozen types are widely produced in commerce today (Janick et al. 1996). The common domesticated apple is putatively an interspecific hybrid complex, usually designated *Malus domestica* Borkh. (Korban and Skirvin 1984) or *M. domestica* Borkh. (Phipps et al. 1990). *M. domestica* is now cultivated widely in temperate latitudes or at high elevations in the tropics on all continents except Antarctica. The fruits are eaten fresh, dried or canned or processed into juice, preserves or alcoholic beverages. Besides *M. domestica*, fruits of several other species are consumed fresh or processed or are used for medicinal purposes (Luby 2003).

Fruit ripening has been described as an oxidative phenomenon (Blackman and Parija 1928; Brennan and Frenkel 1977) which requires a turnover of active oxygen species, such as H_2O_2 and superoxide anion (Hamilton 1974). For this to be the case there must be a balance between the production of active oxygen species and their removal by antioxidant systems. It is likely, therefore, that the antioxidant system will play a crucial role in the ripening process (Foyer and Halliwell 1976).

Anaerobic Life has led to the evolution of biochemical adaptations in plants that exploit the reactivity of active oxygen species (Noctor and Foyer 1998). The term active oxygen species is generic, embracing not only free radicals such as superoxide and hydroxyl

radicals but also H_2O_2 and singlet oxygen. It is generally assumed that the hydroxyl radical and singlet oxygen are so reactive that their production must be minimized (Jakob and Heber 1996). The term antioxidant can be considered to describe any compound capable of quenching active oxygen species without itself undergoing conversion to a destructive radical (Nishikimi and Yagi 1996). Antioxidant enzymes are considered as those that either catalyze such reactions or are involved in the direct processing of active oxygen species. Hence, antioxidants and antioxidant enzymes function to interrupt the cascades of uncontrolled oxidation. (Halliwell et al. 1995).

Ascorbate is one of the most powerful antioxidants (Foyer and Halliwell 1976). It is involved in removing active oxygen species and regenerating α-tocopherol (an important lipid-phase antioxidant, Asada 1996). It is also utilized in cell metabolism and may be involved in the control of growth (Navas and Gomez-Diaz 1995), cell division (Liso et al. 1984; Kerk and Feldman 1995), cell wall expansion (Takahama and Oniki 1994).

Efficient destruction of O_2 and H_2O_2 requires the action of several antioxidant enzymes acting in synchrony. Superoxide produced in the different compartments of plant cells is rapidly converted to H_2O_2 by the action of superoxide dismutase (Bowler et al. 1992). In plant cells, the most important reducing substrate for H_2O_2 detoxification is ascorbate (Mehlhorn et al. 1996). Therefore, determination of ascorbate is of interest for two major reasons. First, itis a key component of the plant antioxidant system and it has a number of other proposed physiological roles. Second, plants provide the major source of dietary vitamin C for humans. Functions of ascorbate in plants include its role as an antioxidant (Noctor and Foyer 1998; Smirnoff and Wheeler 2000).

Superoxide dismutase has been identified as an essential component in an organism's defense mechanism and has consequently been the subject of much research. In plants, the role of superoxide dismutase during environmental adversity has received much attention since reactive oxygen species have been found to be produced during many stress conditions.

The present study is done on four commercial apple cultivars; Granny Smith, Red Delicious, Gala and Fuji. The main objectives of this study are:

- To determine generic differences between the four apple cultivars, in terms of their antioxidant concentrations.
- To encourage the exploitation of cultivars chosen for their high antioxidant levels that could be of interest to fruit processing industries, fruit growers; e.g. apart from its vitamin properties, ascorbate is a powerful and natural oxygen scavenger (antioxidant) and that prevents fruit browning and contributes towards better fruit product utilization and preservation.
- To promote the use of cultivars chosen for their high antioxidant levels that could be used for breeding parents or developing genetic hybrids for developing new cultivars.

CHAPTER II

LITERATURE REVIEW

2.1 The Origin and Spread of the Domesticated Apple

The common domesticated apple is putatively an interspecific hybrid complex, usually designated *Malus domestica* Borkh. (Korban and Skirvin 1984) or *M. domestica* Borkh. (Phipps et al. 1990). The anatomy and structure of the mature apple fruit is shown in figure 1. The cultivated apple, *M. domestica*, belongs to the Pomoideae subfamily of the Rosaceae, along with pear (*Pyrus communis*), prune (*Prunus domestica*), cherry (*Prunus avium*). Linnaeus first named the cultivated apple Pyrus malus, but this was superceded by M. communis and M. pumila in the 1800's. Some authors still use these names today for the cultivated apple (along with M. sylvestris). In 1984, Korban and Skirvin proposed *M. domestica* for the cultivated apple, since it does not exist in the wild and therefore must be an interspecific hybrid (Luby 2003).

Malus domestica Borkh is now cultivated widely in temperate latitudes or at high elevations in the tropics on all continents except Antarctica. The fruits are eaten fresh, dried or canned or processed into juice, preserves or alcoholic beverages. Besides *M. domestica*, fruits of several other species are consumed fresh or processed or are used for medicinal purposes (Luby 2003). Table 1 shows dietary value of apple tissue per one hundred grams of edible portion.

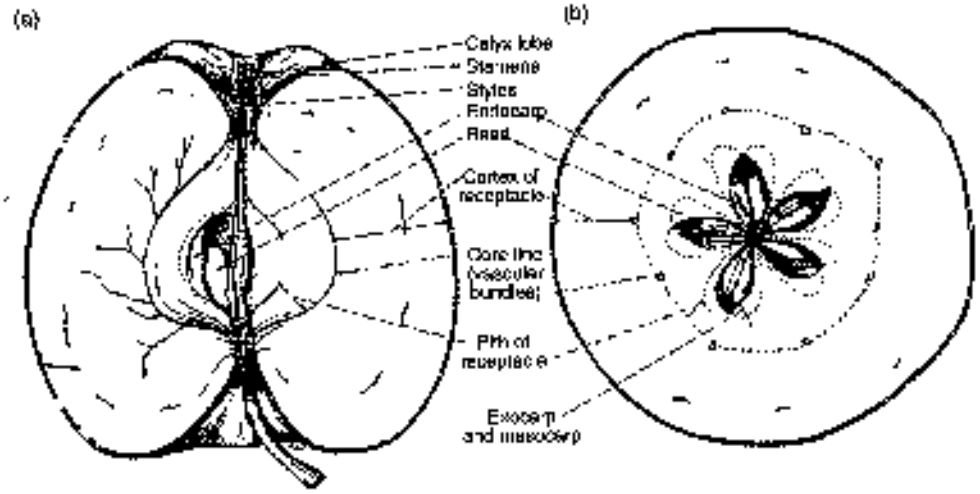

Fig 1: Structure and anatomy of mature apple fruit. (a) vertical selection; (b) equatorial selection (Robbins, 1933)

Table 1: Dietary value, per 100 gram edible portion of apple tissue

water (%)	85
calories	56
protein (%)	0.2
fat (%)	0.6
carbohydrates (%)	14
crude fiber (%)	1

	% of US RDA*
vitamin A	1.8
thiamin, B1	2.1
riboflavin, B2	1.2
niacin	0.6
vitamin C	16.0
calcium	0.9
phosphorus	1.2
iron	3.0
sodium	---
potassium	2.3

* Percent of recommended daily allowance set by FDA, assuming a 154 lb male adult, 2700 calories per day

Source: USDA FAS Agriculture report and U.S. National Agriculture Statistics Service, 2003.

The cultivated apple is believed to have originated in central Asia. Its chief ancestor is probably *Malus sieversii,* from the Heavenly Mountains (Tien Shan) on the border of western China, the former USSR and central Asia (Janick et al. 1996). Apples have been cultivated and vegetatively propagated for over two millennia. Over 10,000 named cultivars exist and breeders worldwide create more new selections annually, but only a few dozen types are widely produced in commerce today (Janick et al. 1996). The high cost of modern production requires a cultivar to have prolific, consistent yields of uniform, commercial quality fruit, be amenable to handling, storage and shipping and generate high consumer demand. Resistance to diseases, pests and storage disorders is also important. In the past, most small farms produced their own apples for fresh or preserved use and local markets. Improvements in storage technology eliminated the need for a succession of short-storing apples from early summer to late winter (Juniper 1998).

During the late 19th and 20th centuries, *M. domestica* cultivars found or bred in Europe, Russia, North America, New Zealand, Japan and Australia were introduced throughout the world and form the basis for most current commercial apple production (Way et al. 1990; Janick et al. 1996).

The center of diversity of the genus Malus is eastern Turkey, and the southwestern Russia region of Asia Minor. The apple and other members of the Pomoideae subfamily were first cultivated there, and then spread throughout Europe. Apples were probably improved through selection over a period of thousands of years. Alexander the Great found dwarfed apples in Asia Minor in 300 BC and brought them to Greece. Apples were

brought to North America with Columbus in the 1490's, and moved to the western US with pioneers, John Chapman (alias Johnny Apple seed) and missionaries during the 1800's. In the mid 1900s, irrigation projects in Washington State began and allowed the development of the multi-billion dollar fruit industry, of which the apple is the leading species. Today, apple production is growing most rapidly in northern California, and remaining steady or declining in the eastern US (Luby 2003).

There are some non-food uses of apples like medicinal, anti-diarrheal, antibiotic activity etc. Who has not heard: An apple a day keeps the doctor away. Apples are a good source of fiber. Apple cider vinegar is good for dieter since it helps clean out you system and helps curve sweets cravings. Apple relives constipation and pectin in apples can lower cholesterol. Apple is also used as laxative and diuretic. Apple is rich in antioxidants and we need antioxidants to protect ourselves against O_2 toxicity. Antioxidants can prevent against tissue damage, aging of skin and cancer (Halliwell 1996).

2.2 Production of apples

Apples grow readily throughout temperate climatic zones. However, commercial apple production is increasingly concentrated in countries and in growing districts that have a strong comparative advantage in apple production and marketing. Falling trade barriers have meant that it has become increasingly difficult for less efficient producers to find shelter from more efficient external competitors. The development of more heat-tolerant cultivars, the increasing popularity of varieties that require a long growing season (such as Granny Smith and Fuji) and advances in irrigation techniques have permitted apple

production to expand successfully into warmer climates. Leading production countries include china and the USA (O'Rourke1994).

2.3 Apple production statistics

A. World - 56,971,000 MT (metric tons)

1. China -	19,490	6. France -	1,954
2. USA -	4,791	7. Iran -	1,944
3. Turkey -	2,500	8. Poland -	1,600
4. Italy -	2,115	9. Argentina -	1,430
5. Germany -	2,026	10. India -	1,320

B. United States - 1998 = 6,019,000 MT. Value $1.22 billion. 5-year average price per lb = $0.18 - 0.25

Leading States:

1. Washington -	50%
2. New York -	10%
3. California -	9%
4. Michigan -	8%
5. Pennsylvania -	4%

Exports (1996-97): 11%: Leading importers include Taiwan, Canada, Mexico, United Kingdom, Hong Kong, Thailand, Singapore, and Saudi Arabia.

Source: FAS agriculture Attache reports and the U.S. National Agriculture Statistical Service (1998).

2.4 World apple production trends

Apple production has been on a term growth trend since the Second World War The growth rate slowed in 1980s, but leaped ahead in the 1990s, to just one factor, the phenomenal expansion of production in China. At the beginning of the 1990s Chinese apple production was about 4 million ton. By the end of the decade it had grown more than five- fold. China has provided all the increase in acreage of apples harvested around the world since the mid-1980s (figure 2). In the 1990s, apple acreage harvested in the rest of the world has been declining (O'Rourke 2000).

In Figure 3, Food and Agriculture Organization data indicate that China's share of world apple production has gone from 10.7% in 1990 to 36.7% in the year 2000. World production trends for year 2003 are given in figure 4.

2.5 Major cultivars

International information flow has also had a profound effect on the popularity of different apple cultivars. In most countries, growers continue to produce many cultivars that have been traditional in their region. However, newer cultivars have gradually been introduced for varied production and marketing reasons. For example, Red Delicious and

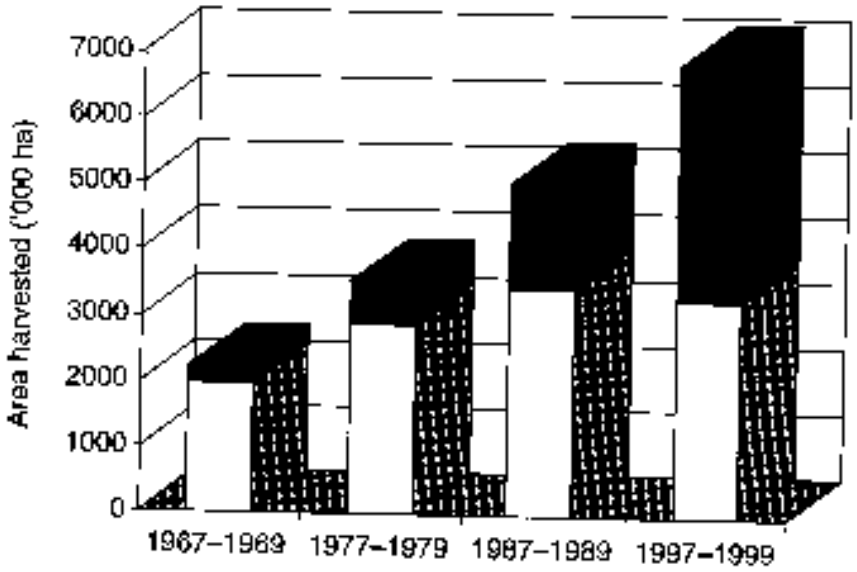

Figure 2: Apple area harvested in China ■ **verses total world** □ **, 1967-1999 (FAO, 2000)**

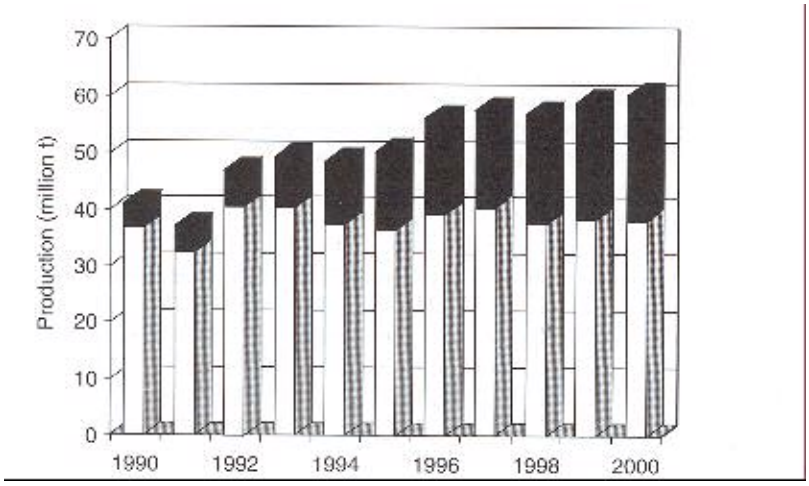

Figure 3: Apple production harvested in China■ verses total world□ , 1999-2000 (FAO, 2000)

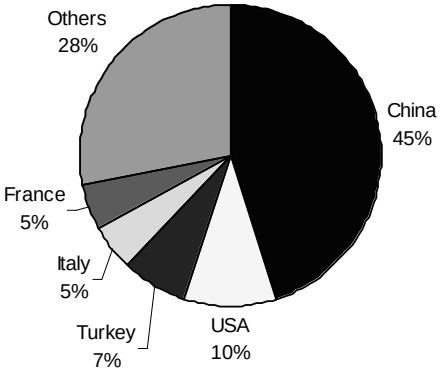

Figure 4: Key apple producer's share of world apple production

Source: USDA FAS Agriculture report, U.S. National Agriculture Statistics Service, 2003.

Golden Delicious became popular in the USA in the 1950s and 1960s, partly because they provided attractive display opportunities for the booming supermarket retail business. Granny Smith entered the market initially as an off-season cultivar. Production expanded in the northern hemisphere in the 1970s and 1980s as the demand grew for a tarter apple. The arrival of Jonagold, Gala, Fuji and Braebum in the 1980s and 1990s coincided with the growth of hypermarkets and a large expansion of the retail shelf space devoted to produce.

Official data on cultivars are either very limited or not available at all for many countries. Estimates of cultivar trends for 34 major producing countries (not including China) were developed by O'Rourke (2001). These suggest that the volume of production of Red Delicious, Golden Delicious and Granny Smith apples will be stable in the next few years, but that there will be significant gains in volume of Gala, Braebum, Pink Lady and, to a lesser extent, Fuji (Table 2). If China is included, Fuji production will continue to leap ahead because China is estimated to have 45% of its production in this one cultivar.

2.6 Chilling requirements

The apple (*M. domestica*) is a fruit tree in the general category of temperate plants, which are characterized as requiring an annual cold period to satisfy their 'chilling requirement'. If the chilling requirement is not satisfied, the buds will not open; if the chilling requirement is partially met, the buds will open sporadically and both the bloom and harvest periods will be abnormally extended. Most apple cultivars require between

Table 2: Production trends (in ‘000 tons) of major apple exclusive of China (O’Rourke, 2001)

Cultivar	2000 Estimated	2005 Projected	2010 Projected
Red Delicious	5334	5422	5423
Granny Smith	1719	1805	1828
Fuji	1433	1671	1857
Gala	1688	2188	2594
Braeburn	429	574	710
Golden delicious	4982	5222	5212
Pink Lady	61	129	194

1200 and 1500 h of chilling in the range of 4-7°C (generally 45° F and below) (Linden et al. 1996).

Chilling is achieved, between roughly November 1st and February 15th in northern latitudes, with the most benefit derived from chilling hours occurring in December and January. These hours are cumulative and need not be continuous. Temperatures below 0°C or above about 10°C do not provide chilling and may actually negate previously accumulated chilling hours. The number of chilling hours required for each fruit variety is different to some extent (Regnold et al. 2001; Gardea et al. 2000).

There are some "low-chill" varieties successfully developed, these varieties require 400 chilling hours or less. Some apple cultivars (e.g. Anna, Dorsett Golden) require as little as 250-300 Hrs of chilling and can be grown in the sub-tropics, whereas others (e.g. McIntosh, Golden Delicious) require much more chilling (1000-1600 Hrs) (Palmer 2003).

2.7 Controlled atmosphere storage

The apple is one of the few fruits that can tolerate long-term storage without significant loss of quality (Awad and Jager 2003). Some other fruits kept in controlled atmosphere are pear, cherry and tomato. Controlled atmosphere storage has allowed the Washington industry to grow to its current level. Firmer, less ripe fruit are placed in long-term controlled atmosphere (150-200 days), while more mature fruit are sold directly or placed in short-term controlled atmosphere (Lau 1998).

The apple is the predominant horticulture commodity stored under controlled atmosphere conditions (Leja et al. 2003; Waltkins 2003). The objective of controlled atmosphere storage is to lower oxygen and increase carbon dioxide concentrations to levels that will maintain fruit quality by decreasing respiratory metabolism and reducing ethylene production and action, but not to levels that induce fermentation or other damaging events (Bishop 1996; Watkins 2003). Carbon dioxide and oxygen concentrations are typically between 2-3% (Fernandez-Trujillo et al. 2001; Leja et al. 2003; Waltkins 2003).

Controlled atmosphere storage was originally adopted as a tool to permit companies to market selected cultivars of apples for an additional month or two. As the technology has evolved, controlled atmosphere has enabled more and more cultivars to be held for longer periods. It has been an excellent tool for managing the flow of product to market. However, the advantages of controlled atmosphere in lengthening the sales period have been offset by the increasing availability of new-crop apples from the opposite hemisphere within 6 months of harvest. In recent years, controlled atmosphere storage has been used increasingly to ensure better firmness or to control fruit disorders that affect apples in regular cold storage. Controlled atmosphere has become a highly complex tool for ensuring that apples meet customer quality standards at any time during the marketing year. As controlled atmosphere has become more pervasive, buyers have been less willing to pay a premium for these quality improvements. Controlled atmosphere capacity continues to grow in Europe, North America and the southern hemisphere because larger operators want the flexibility in marketing that controlled atmosphere provides (Waltkins 2003). The volume of apples still in controlled

atmosphere storage in the northern hemisphere during later months of the season continues to rise. This has tended to reduce the price advantage formerly earned by late-season controlled atmosphere fruit. That fruit is now beginning to crowd the market and depress the price of new, off-season apples from the southern hemisphere. Essentially, the seasonal niches that controlled atmosphere storage once-made possible have shrunk (Waltkins 2003).

2.8 Superficial scald

Scald is a physiological disorder of apples and pears that causes important loss of market value after storage (Soria et al. 1999; Zanella 2003). In scald some dark brown spots appear on the peel tissue of the apple fruit. It is caused by oxidation of α-farnesene, which leads to scald formation. It was reported earlier that antioxidants inhibit oxidation of α-farnesene and thus may block the sequence of events leading to scald formation (Barden and Bramlage 1994).

2.9 Breeding Parents

All over the world, the great commercial cultivars are included on a large scale in breeding programs to improve fruit quality. Golden Delicious and its derivatives, as Gala, are the parents that are most often used in the crosses. Jonathan, Red Delicious, Granny Smith, Idared and more recently Fuji and Braeburn are also frequently used.

2.10 Cultivar Descriptions

2.10.1 Red Delicious

Red Delicious (also know as Delicious) arose unwanted in the field of Jesse Hiatt of Peru, Iowa, USA, in about 1872 (Maas 1970; Fear and Domoto 1998). Mr Hiatt cut the tree down twice before allowing it to persist, because it was not growing in the row. The parentage is unknown; a nearby Yellow Bellflower tree may have been the seed parent (Maas 1970; Khanizadeh and Cousineau 1998), or Delicious may have arisen as a sprout from a seed or a seedling rootstock. Mr Hiatt was impressed by the fruit and named the cultivar Hawkeye. It was eventually purchased by Stark Brothers Nursery, who renamed it Delicious and introduced it commercially in 1895. In its various forms, Delicious has become the world's most important and best-studied cultivar. It has long been the backbone of the US industry. With its high color and distinctive appearance, Delicious is frequently used as a generic apple in advertisements and artwork. Delicious is an important cultivar in the USA (especially Washington), the European Union, Australia, China and many other countries. Synonyms: Hawkeye, Red Delicious, Stark Delicious.

2.10.1.1 Tree Structure

Standard trees are moderately vigorous, upright-spreading, spurring fairly freely. It is medium in precocity, productivity and regularity of bearing. These trees are widely adaptable, but perform best in areas with warm summers, high light intensity and

adequate water-supply and bears primarily on spurs. These trees are not prone to pre-harvest drop.

2.10.1.2 Fruit Quality

Maturity indices include days after full bloom, ground color change from green towards yellow, flesh color change from green to cream, starch index and soluble solids. Size medium to large; adequate thinning important for fruit size and return bloom. Fruit shape is oblong conic to truncate conic, sometimes waisted below apex; prominently ribbed and irregular with very pronounced crowning at apex. Stem cavity is wide and fairly deep, usually russet-free. Skin of Delicious is very tough, resistant to bruising, russet-free, dry, smooth and glossy. Ground color greenish yellow, over color varies with strain; original said to be strawberry red with darker stripes (Maas 1970); newer strains can be close to 100% dark crimson. Flesh color is cream, sometimes tinged with green, very firm, fine-grained, juicy, and mealy if overripe. The flavor is sweet, low in acid, aromatic, distinctive (Waltkins 2003).

2.10.1.3 Commercial Uses

It is very good for dessert, fair to poor for culinary uses, fair for sauce, good for juice, fair to poor for drying, good for minimally processed slices (Smock and Neubert 1950; Kim et al. 1993; Root 1996; Lisowa et al. 1997). Delicious cultivars are exceptionally good for handling and shipping.

2.10.1.4 Storage and postharvest

Delicious can be stored 3-4 months in air (Smock and Neubert 1950). Optimal controlled-atmosphere storage recommendations vary with region, ranging from 0.7 to 2.5% O_2, 0 to 4.5% CO_2, -0.5 to 1.1°C, for storage of 6-11 months (Kupferman 1997). Delicious is not chilling-sensitive or sensitive to low O_2. It is prone to scald, particularly if picked too early (125-135 DAFB). Spur types should be picked 7-10 days later than standard strains for comparable scald control (Fisher and Ketchie 1989). The lobes are sensitive to heat injury and can develop symptoms resembling scald (Meheriuk and McPhee 1984).

Later harvested fruit can also become mealy in storage. 'Delicious' can get bitter pit, but it is preventable by proper fertilization practices and crop-load management. It is susceptible to moldy core.

2.10.2 Fuji

Fuji is the offspring of a Ralls Janet x Delicious cross made in 1939 (Smith 1971; Kikuchi et al. 1997). It was named Fuji in 1962 by the Horticulture Research Station in Morioka, Japan. The name commemorates Fujisaki in Aomori, Japan, where the cross was made (Yoshida et al. 1998). Fuji is the most important apple in Japan and China and is a major cultivar in Korea, Brazil, Argentina, Chile and Australia. It has been planted extensively in both hemispheres in the past decade. About 80% of current Fuji acreage is

located in China (Avermaete 1999). Fuji has a long storage and shelf life, perhaps because of its low ethylene production and low respiration rate (Yoshida et al. 1998).

2.10.2.1 Tree Structure

Fuji tree is vigorous, spreading and, productive but somewhat slow to spur in initial years (Tustin, 1994) and produces some blind wood. It is difficult to thin chemically, strong tendency to bear biennially. No preharvest drop. Trees are winter-hardy but with a lower chilling requirement than McIntosh or Delicious (Ghariani and Stebbins 1994); estimations of chilling requirement vary from 600-800 h (Barahona et al. 1992) to 1050 units (Ghariani and Stebbins 1994). Fuji is more sensitive to bloom frost than Delicious or Jonagold (Shibata and Mizuno 1988).

2.10.2.2 Fruit Quality

Fuji is harvested 140-180 days after full bloom, early to mid- November in Japan. It may require several picks, depending on color management. Ground color, over color and starch disappearance are used as maturity indices, but not firmness or soluble solids (Argenta et al. 1995; Britz 1998; Yoshida et al. 1998). Its ground color pale yellow-green with red blush and darker stripes (Smith 1971). Color is frequently poor on standard strain without special management. Elaborate practices to boost color development have been developed in Japan, including bagging (Arakawa 1998), leaf removal, reflective mulches and fruit turning. Its Flesh is cream, above average in crispness, firmness, juiciness, fine-grained. Its flavor is sweet and very mild, high in sugar and low in acidity. It is low in dietary fiber compared with other cultivars (Gheyas et al. 1997).

2.10.2.3 Commercial Uses

It is excellent for dessert, good for processing quality, except cider (Yiem et al. 1980).

2.10.2.4 Storage and postharvest

Fuji is highly prone to water-core, prone to stem-end cracking, bitter pit, cork spot and external brown staining. Compared with other cultivars, Fuji has a very slow rate of firmness loss and a long shelf life (Yoshida et al. 1998). For short-term storage, controlled atmosphere is no better than regular atmosphere storage for firmness retention, but controlled atmosphere improves retention of acidity (Drake 1993). Fuji is susceptible to internal browning in long-term controlled atmosphere (~ 6 months); the problem is worse with mature fruit. Short-term (3 days) exposure of fruit samples at harvest to 20 kPa CO_2 may be useful as a predictor of susceptibility to internal browning (Volz et al. 1998). Fuji is also susceptible to core browning, a disorder whose incidence rises with later picking and preharvest calcium treatments (Yoshida et al. 1998). Early-picked Fuji may develop scald, but it is preventable with Diphenylalanine, ultra-low oxygen or hypobaric storage (Kupferman 1997; Yoshida et al. 1998). Optimum controlled atmosphere conditions vary with region, ranging from 0.7 to 2.5% O_2 and 0.5 to 2.0% CO_2 at 0-1°C, for 7-11 months of storage (Kupferman 1997).

2.10.3 Granny Smith

Granny Smith is a chance seedling discovered on the farm of Maria Ann and Thomas Smith of Ryde, New South Wales, Australia. The original tree was fruiting by 1868. Granny Smith is believed to be an open-pollinated seedling of French Crab, but it also resembles some of the American Greening cultivars and Cleopatra (Warrington 1998). Although there have been significant plantings of Granny Smith since the 1920s in Australia, it has only become an important apple in world trade since 1950. Granny Smith is chiefly a southern hemisphere apple. It has been grown extensively in Australia, Argentina, Chile, New Zealand and South Africa. Unmet consumer demand stimulated significant planting in western North America and southern Europe in the 1970s. Today Granny Smith accounts for a third of southern hemisphere exports (Avermaete 1999).

2.10.3.1 Tree Structure

Granny Smith is moderately vigorous, upright-spreading, spurs fairly freely. (Bultitude 1983; Khanizadeh and Cousineau 1998). It is precocious, bearing heavy annual crops. Its production of 120-130 ton per hectare is routine in New Zealand, but yield is frequently lower elsewhere (Warrington 1998). Cold-hardiness is moderate and, adequate for central Washington State but not Quebec or Minnesota (Khanizadeh and Cousineau 1998; Luby et al. 1999). It blooms mid to late season. Chilling requirement is reported as 400-600 h in Algeria (less than Delicious or Golden Delicious), 600-800 h in Ecuador, 1040 units in the USA and, is similar to Fuji or Jonagold (Semadi 1988; Barahona et al. 1992; Ghariani and Stebbins 1994).

2.10.3.2 Fruit Quality

Granny Smith is harvested 170-210 days after full bloom in a single pick (Janick et al. 1996; Khanizadeh and Cousineau 1998; Warrington 1998). Its Ground color is bright green, becoming greenish yellow. Sometimes it has a slight pink blush present but no stripes. Its flesh is greenish white, very firm, rather coarse, juicy, sub-acid, tart- sweet, refreshing, but is lacking in flavor (Warrington 1998).

2.10.3.3 Commercial Uses

Granny Smith is a good dual-purpose dessert and culinary apple. The fruit is especially high in pectin (Blagov1998).

2.10.3.4 Storage and postharvest

Granny Smith is a long-keeping apple, possibly because of its low ethylene production (Warrington 1998). It is very susceptible to superficial scald, particularly if picked too early. Premium postharvest quality is attained when the fruit is picked with a medium green ground color and when all starch has disappeared from the core area (Warrington 1998). Optimal controlled atmosphere conditions range from 0.8 to 2.5% O_2, 0 to 5.0% CO_2, -0.5 to 2°C, for 7-11 months of storage (Kupferman 1997). Incidences of core flush and scald are higher in warm climates. Granny Smith may develop bitter pit and water-core if picked too late (Khanizadeh and Cousineau 1998).

2.10.4 Gala

Gala is the product of two generations of controlled crossing by amateur breeder, I.H. Kidd of Wairarapa, New Zealand. Gala is the offspring of a cross of Kidd's Orange Red x Golden Delicious made about 1934. Kidd's Orange Red was itself a cross of Delicious x Cox's Orange Pippin (Noiton and Alspach 1996). The apple was named Gala in 1962, and released for commercial planting in 1965. However, it did not become really popular until the mid-1970s when several red color sports appeared (Tustin 1990). Gala is an important cultivar in New Zealand, Brazil, Argentina, Chile, Australia, China, the USA and Europe (especially France), and has been planted extensively in both hemispheres in the past decade. Synonym: Kidd's D.8.

2.10.4.1 Tree Structure

Gala tree is highly precocious, bears prolific annual crops. Its wood is brittle and prone to breakage. It is easy to train with modern methods, widely adapted. Fruit size and color is very responsive to light exposure. Cold-hardiness is moderate and, similar to Golden Delicious (Luby et al. 1999). Estimates of chilling requirement vary from 600-800 hours (Barahona et al. 1992).

2.10.4.2 Fruit Quality

Gala is harvested 120-140 days after full bloom, 2-4 weeks before Delicious. The fruit adhere well to the tree and can be hard to pick; preharvest drop is minimal. Ripening is uneven and, requires at least three picks, based on ground color change from green to creamy yellow. Fruit size is small to medium and usually uniform. The skin is russet-free, smooth and glossy, becoming greasy and, not susceptible to bruising. Occasionally some scarf skin appears at the base. It develops stem-end cracking when fully to over mature. Its ground color is creamy yellow to golden yellow and is, partly to fully flushed and flecked with bright orange-red and strewn with deeper red stripes. Its flesh is pale yellow, juicy, firm, crisp and, fine-grained. Flavor is sweet, low in acid, refreshing, aromatic with excellent quality (Hampson and Kemp 2003).

2.10.4.3 Commercial Uses

Gala is mainly used as dessert. It is suitable for drying (Hampson and Kemp 2003).

2.10.4.4 Storage and postharvest

Gala has no significant storage disorders but only a medium-term storage life (Tustin 1990). Controlled atmosphere recommendations vary with region and strain; optimum conditions range from 1 to 3% O_2, < 0.5 to 2% CO_2, 0 to 3°C for storage periods of 4-9 months (Kupferman 1997).

2.11 Cross Pollination

In crops whose marketable organs are fruits and or seeds, such as apple, flowering, fruit set and development are critical for economic return. Some apple cultivars tend to set too many fruits resulting in small size and inhibition of subsequent flowering. Hence, biennial bearing unless the crop is reduced by early thinning. Pollination and fertilization are essential for fruit set. Although some cultivars are self-fruitful, cross-pollination is required in most and is usually advantageous even in self-fruitful ones. Bees are the primary pollinators, and climatic conditions during bloom are critical for fruit set. When fruit set is excessive, the fruits must be thinned mechanically or chemically to encourage fruit growth and flower-bud formation. Cultivars differ in the time required for maturation, some ripening in mid summer and some in late autumn. Preharvest drop can be a problem in some years and with some cultivars. Chemicals can be used either to hasten ripening or to delay it, allowing growers to harvest earlier or later than would otherwise be the case (Luby 2003).

2.12 Fruit ripening

Fruit ripening has been described as an oxidative phenomenon (Blackman and Parija 1928; Brennan and Frenkel 1977) which requires a turnover of active oxygen species, such as H_2O_2 and superoxide anion (Hamilton 1974, Jimenez et al. 2002). For this to be the case, there must be a balance between the production of active oxygen species and their removal by antioxidant systems. It is likely, therefore, that the antioxidant system

will play a crucial role in the ripening process. This system includes catalase, superoxide dismutase, some peroxidases and the enzymes involved in the ascorbate-glutathione cycle: ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase (Scandalios et al. 1980; Dalton 1995; Asada 1996). These enzymatic components, together with the low-molecular-weight antioxidants ascorbate and glutathione, ultimately scavenge H_2O_2 at the expense of NADPH or NADH (Foyer and Halliwell 1976; Jimenez et al. 2002).

Ascorbate is one of the most powerful antioxidants (Foyer and Halliwell 1976). It is involved in removing active oxygen species and regenerating α-tocopherol (an important lipid-phase antioxidant, Asada 1996). It is also utilized in cell metabolism and may be involved in the control of growth (Navas and Gomez-Diaz 1995), cell division (Liso et al. 1984; Kerk and Feldman 1995), cell wall expansion (Takahama and Oniki 1994) and organogenesis (Jimenez et al. 2002).

2.13 Keeping active oxygen under control

Anaerobic life has led to the evolution of biochemical adaptations that exploit the reactivity of active oxygen species (Noctor and Foyer 1998). The term active oxygen species is generic, embracing not only free radicals such as superoxide and hydroxyl radicals but also H_2O_2 and singlet oxygen. It is generally assumed that the hydroxyl radical and singlet oxygen are so reactive that their production must be minimized (Jakob and Heber 1996), O_2 and H_2O_2 are synthesized at very high rates even under optimal conditions. They are involved in virtually all major areas of aerobic biochemistry (e.g.

respiratory and photosynthetic electron transport; oxidation of glycolate, xanthine, and glucose) and are produced in copious quantities by several enzyme systems (e.g. plasmalemma-bound NADPH dependent superoxide synthase and superoxide dismutase) (Bowler et al. 1992). The chief toxicity of O_2 and H_2O_2 is thought to reside in their ability to initiate cascade reactions that result in the production of the hydroxyl radical and other destructive species such as lipid peroxides. These dangerous cascades are prevented by efficient operation of the cell's antioxidant defenses. In some circumstances, however, the destructive power and signaling potential of active oxygen speciesare utilized as an effective means of defense (Chen et al. 1993, Foyer et al. 1997). The term antioxidant can be considered to describe any compound capable of quenching active oxygen species without itself undergoing conversion to a destructive radical (Nishikimi and Yagi 1996). Antioxidant enzymes are considered as those that either catalyze such reactions or are involved in the direct processing of active oxygen species. Hence, antioxidants and antioxidant enzymes function to interrupt the cascades of uncontrolled oxidation. The comparatively few classes of antioxidant enzymes have been well characterized are listed in Table 3. Of the numerous enzymes and metabolites potentially covered by the above definitions, many remain uncharacterized (Halliwell et al. 1995). These enzymes catalyze redox reactions, many of which rely on electrons supplied by reductants of low molecular weight.

Among these low-molecular-weight antioxidants, ascorbate and glutathione are of paramount importance. They fulfill multiple roles in defense reactions and are major assimilate sinks, present in many tissues at millimolar concentrations (Noctor et al. 1997).

Table 3: Major antioxidant enzymes in plants (Noctor and Foyer 1998)

Enzyme	Abbreviation in text	EC number
superoxide dismutase	SOD	1.15.1.1
ascorbate peroxidase	APX	1.11.1.11
monodehydroascorbate reductase	MDHAR	1.6.5.4
dehydroascorbate reductase	DHAR	1.8.5.1
glutathione reductase	GR	1.6.4.2
Catalase	CAT	1.11.1.6
glutathione peroxidase	GPX	1.11.1.9
guaiacol-type peroxidases	—	1.11.1.7
glutathione s-transferases	GST	2.5.1.18

In spite of this, and despite the repeated observation of increased levels of ascorbate and glutathione in stress conditions, very little consideration has been given to the regulation of the pathways that influence their cellular concentration

2.14 The antioxidant enzymes

Efficient destruction of O_2 and H_2O_2 requires the action of several antioxidant enzymes acting in synchrony. Superoxide produced in the different compartments of plant cells is rapidly converted to H_2O_2 by the action of superoxide dismutase (Bowler et al. 1992). In organelles such as the chloroplast, which contain high concentrations of ascorbate, direct reduction of O_2 by ascorbate is also rapid (Buettner and Jurkiewicz 1996). As has frequently been pointed out, dismutation of O_2 simply serves to convert one destructive active oxygen species to another. H_2O_2 is a strong oxidant that is rapidly oxidized; it cannot be allowed to accumulate to excess in organelles such as the chloroplast, where photosynthesis occurs.

In plant cells, the most important reducing substrate for H_2O_2 detoxification is ascorbate (Mehlhorn et al. 1996). Ascorbate peroxidase uses two molecules of ascorbate to reduce H_2O_2 to water, with the concomitant generation of two molecules of monodehydroascorbate. Monodehydroascorbate is a radical with a short lifetime that, if not rapidly reduced, and is disproportionates to ascorbate and dehydroascorbate. Despite the possibility of enzymic and non enzymic regeneration of ascorbate directly from monodehydroascorbate, rapid disproportionation of the monodehydroascorbate radical means that some dehydroascorbate is always produced when ascorbate is oxidized in

leaves and other tissues. Dehydroascorbate is reduced to ascorbate by the action of dehydroascorbate reductase, using glutathione as the reducing substrate (Foyer and Halliwell 1976). This reaction generates glutathione disulphide, which is in turn re-reduced to glutathione by NADPH, a reaction catalyzed by glutathione reductase. The removal of H_2O_2 through this series of reactions is known as the ascorbate-glutathione cycle (Figure 5). Ascorbate and glutathione are not consumed in this pathway but participate in a cyclic transfer of reducing equivalents, involving four enzymes, which permits the reduction of H_2O_2 to H_2O using electrons derived from NAD(P)H (Figure 5).

2.15 Antioxidants and the Regulation of Photosynthesis

The production of O_2 and other active oxygen species is frequently considered to be a deleterious event since oxidant accumulation invariably leads to oxidative stress. During photosynthesis, however, active oxygen species are produced and destroyed in a concerted manner that contributes to the regulation of electron transport (Asada 1992; Asada 1997; Foyer et al. 1997).

Recent years have witnessed a plethora of reports correlating increases in one or more of the antioxidant enzymes with either stress conditions or ameliorated stress resistance. Abiotic conditions that have been studied include, among others, low temperature, high salinity, herbicide challenge, drought, woundingultraviolet irradiation, SO_2 fumigation, and ozone exposure (Noctor and Foyer 1998). In work where several enzymes have been studied under the same stress conditions, differential responses have frequently been

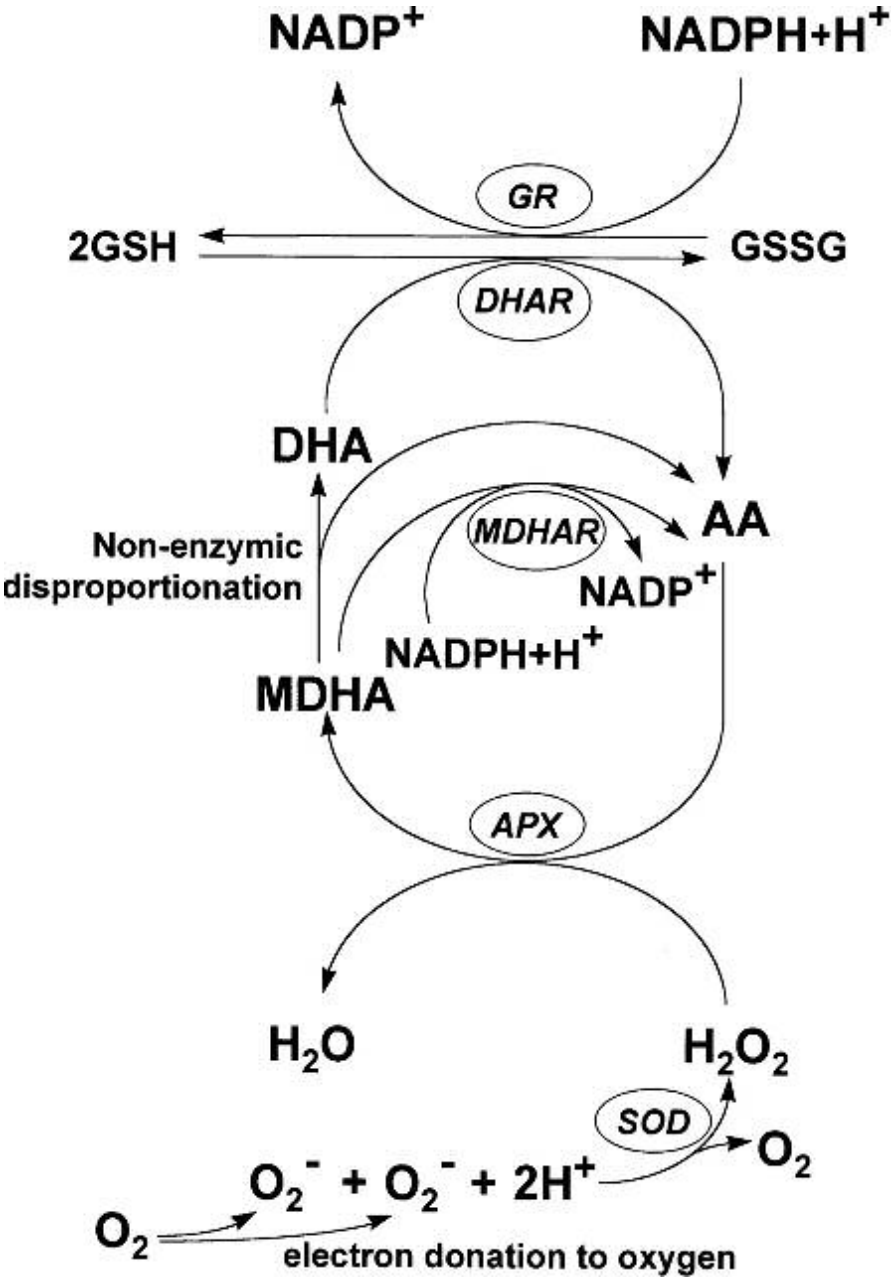

Figure 5: The ascorbate-glutathione cycle (Noctor and Foyer 1998)

observed (Noctor and Foyer 1998). The degrees to which the activities of individual antioxidant enzymes are increased as a result of stress imposition is extremely variable and, in many cases, relatively minor.

2.16 Ascorbate

2.16.1 Introduction

Understanding ascorbate biosynthesis is of interest for two major reasons. First, it is a key component of the plant antioxidant system and it has a number of other proposed physiological roles. Second, plants provide the major source of dietary vitamin C for humans (Planchon et. al. 2004). Functions of ascorbate in plants include its role as an antioxidant (Noctor and Foyer 1998; Smirnoff and Wheeler 2000). Ascorbate and cell wall localized ascorbate oxidase are suggested to be involved in cell division and growth (Smirnoff 2000). As the functions of ascorbate and the reactions concerned with regenerating it from its two oxidized forms, monodehydroascorbate and dehydroascorbate, have been extensively reviewed and studied (Smirnoff 2000).

2.16.2 Historical perspective

Isolation of vitamin C from plant and mammalian sources, and crystallization of the physiologically active compound, provided evidence to show that both vitamin C and the crystalline compound were in reality the same substance which were accomplished in 1932 (Smirnoff et al. 2001). Scarcely a year later, the structure of vitamin C, now assigned the trivial term ascorbic acid, was established as L-threo-hex-2-enono-1,4-lactone and its synthesis was achieved. Industrial production of ascorbic acid, which

quickly followed, provided access to an unlimited supply of this vitamin and opened new avenues for exploration of its physiological, biochemical, and biomedical properties (Smirnoff et al. 2001).

In 1954, Isherwood et al., proposed a scheme analogous to that from the rat study for ascorbic acid biosynthesis in plants. They based their scheme on the finding that cress seedlings (Lepidium sativum L.) readily oxidized L-galactono-1,4-lactone (GalL) to AA and they invoked a D-galacto/L-galacto transition as the basic difference between the animal and plant pathways:

$$D-Galactose \rightarrow D-Galactouronate \rightarrow L-Galactonate \rightarrow L-GalL \rightarrow AA$$

Although it was assumed that the initial step involved oxidation of D-galactose, this premise was not tested and these investigators did not undertake radiotracer studies. Subsequently, Loewus et al. 1956 did undertake such studies and found that conversion proceeded directly, i.e. without inversion of the carbon chain of D-Glc.

All plants, and all animals except primates and guinea pigs, can synthesize ascorbic acid. In plants, ascorbate can accumulate to millimolar concentrations in both photosynthetic and nonphotosynthetic tissues (Noctor and Foyer 1998). Leaves often contain more ascorbate than chlorophyll, with the ascorbate pool representing over 10% of the soluble carbohydrate. Of the many functions ascribed to ascorbic acid, relatively few are well characterized. It is clear, however, that ascorbate is a major primary antioxidant (Noctor and Foyer 1998), reacting directly with hydroxyl radicals, superoxide, and singlet oxygen (Buettner and Jurkiewicz 1996). In addition to its importance in photoprotection and the regulation of photosynthesis (Forti and Elli 1995), ascorbate plays an important role in

preserving the activities of enzymes that contain prosthetic transition metal ions (Padh 1990). Ascorbate is also a powerful secondary antioxidant, reducing the oxidized form of α-tocopherol, an important antioxidant in nonaqueous phases (Padh 1990).

2.16.3 Biosynthesis of Ascorbate

Since ascorbate is an essential metabolite implicated in vital cell functions, it is surprising that the pathway of ascorbate synthesis in plants remains to be established (Nishikimi and Yagi 1996; Smirnoff 1996). In animals, ascorbate deficiency leads to “scurvy.” In plants, however, the effects of ascorbate depletion are less easily discernible. The hypothesis that ascorbate is synthesized from glucose is widely accepted, but measured rates of conversion of labeled glucose into ascorbate are very low (Noctor and Foyer 1998).

2.17 Superoxide Dismutase

2.17.1 Introduction

Oxidative stress, resulting from the deleterious effects of reduced oxygen species, is an important phenomenon in many biological systems. Superoxide dismutases have been identified as an essential component in an organism's defense mechanism and have consequently been the subject of much research. In plants, the role of superoxide dismutase during environmental adversity has received much attention since reactive oxygen species have been found to be produced during many stress conditions (Gong et. al. 2001).

2.17.2 An over view of the defense mechanism against oxidative stress

The phenomenon of oxidative stress arises from the deleterious reactions of oxygen, which are an unfortunate consequence of life for any aerobic organism. These reactions are mediated by reduced oxygen species such as superoxide radicals and hydrogen peroxide (Kondo et al. 2002). By themselves they are relatively unreactive, but they can form species damaging to essential cellular components. In the presence of metal ions (such as iron), superoxide and hydrogen peroxide can react in a Haber-Weiss reaction to form hydroxyl radicals:

$$H_2O_2 + O_2^- \xrightarrow{Fe^{2+}+Fe^{3+}} OH^- + O_2 + OH^{\cdot} \qquad \text{(Haber-Weiss reaction)}$$

Hydroxyl radicals (and their derivatives) are among the most reactive species known to chemistry (Cadenas 1989), and are able to react indiscriminately to cause lipid peroxidation, the denaturation of proteins, and the mutation of DNA. Lipid peroxidation is commonly used as an indicator of oxidative stress, although it can be caused by other reactive species (Bowler et al. 1992; Du and Bramlage 1994). In addition, singlet oxygen, which is formed when excitation energy is transferred to oxygen, also produces deleterious effects (Cadenas 1989).

Superoxide radicals, hydrogen peroxide, and singlet oxygen are formed from many cellular reactions (Bowler et al. 1992). In general, superoxide can arise when electrons are misdirected and donated to oxygen. Mitochondrial electron transport, for example, is

a well- documented source of superoxide radicals, as is the electron transport chain of the photosynthetic apparatus within the chloroplast. An additional problem for the chloroplast is the transfer of excitation energy from chlorophyll to oxygen, which can generate singlet oxygen (Bowler et al. 1992).

Protective mechanisms have evolved that keep these deleterious reactions to a minimum. Since hydroxyl radicals are far too reactive to be controlled easily, aerobic organisms eliminate the less-reactive forms as efficiently as possible and prevent their coming into contact with each other. This defense involves both enzymic and non-enzymic mechanisms. Superoxide dismutases (EC 1.15.1.1), originally discovered by McCord & Fridovich in 1969, react with superoxide radicals at almost diffusion-limited rates to produce hydrogen peroxide:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \qquad \text{(SOD)}$$

This enzyme is unique in that its activity determines the concentrations of O_2^- and H_2O_2, the two Haber-Weiss reaction substrates, and it is therefore likely to be central in the defense mechanism. Its importance has been established by the demonstration that SOD-deficient mutants of *Escherichia coli* and yeast are hypersensitive to oxygen. It is present in all aerobic organisms and in subcellular compartments where oxidative stress is likely to arise (Bowler et al. 1992).

The three known types of SOD are classified by their metal cofactor: the copper/zinc (Cu/ZnSOD), manganese (MnSOD), and iron (FeSOD) forms. Experimentally, these three different types can be identified by their differential sensitivities to KCN and H_2O_2. Cu/ZnSOD is characterized as being sensitive to both H_2O_2 and KCN, FeSOD is sensitive to H_2O_2 only, and MnSOD is resistant to both inhibitors. The FeSOD and MnSOD enzymes are structurally similar; indeed, the apoenzymes have been found to function with either metal present at the active site. The Cu/ZnSOD, however, is structurally unrelated. All prokaryotic organisms so far studied contain MnSOD and/or FeSOD; Cu/ZnSOD is absent except in a few cases. Eukaryotic algae (except those with phragmoplastic cell division) and protozoa possess MnSOD and FeSOD but not Cu/ZnSOD. Cu/ZnSOD has been found in all higher eukaryotes within animal kingdom, as has MnSOD; while Cu/ZnSOD is cytosolic, MnSOD is found in mitochondria (Bowler et al. 1992). The phylogenic distribution of SOD thus indicates that MnSOD and FeSOD are ancient; while they probably evolved before eukaryotes and prokaryotes diverged, Cu/ZnSOD has evolved independently at some point near the beginning of eukaryotic lineage. Hence the enzyme has evolved twice (Bowler et al. 1992).

Hydrogen peroxide is disposed by catalases (E.C.1.11.1.6) and peroxidases (EC 1.11.1.7). In plants, catalase is found predominantly in peroxisomes (and also in glyoxysomes) where it functions chiefly to remove the H_2O_2 formed during photo-respiration (or during β-Oxidation of fatty acids in glyoxsomes) (Bowler et al. 1992). In spite of its restricted location it may play a significant role in defense against oxidative

stress since H_2O_2 can readily diffuse across membranes. Many different peroxidases occur in plants; unlike catalase, and these require a substrate (R) for catalysis:

$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad \text{(CAT)}$$

$$H_2O_2 + RH_2 \rightarrow 2H_2O + R \qquad \text{(POD)}$$

Some of these enzymes have broad substrate specificity while others can only function with one. The peroxidases with broad specificity are often found in cell wall where they utilize H_2O_2 to generate phenoxy compounds that then polymerize to produce components such as lignin (Greppin et al. 1986). In addition to their role in the biosynthesis of cellular components, reactive oxygen species are thought to act as secondary messengers in cells.

Of more importance in the context of oxidative stress is a chloroplast-localized, ascorbate-specific peroxidase activity found mainly in the chloroplast. Together with glutathione reductase and dehydroascorbate reductase it is thought to remove H_2O_2 through a mechanism termed the Halliwell-Asada pathway, named after its discoverers (Foyer and Halliwell 1976; Nakano and Asada 1980) (Figure 6). Since the action of superoxide sismutase results in the formation of H_2O_2, it is also intimately linked with this pathway. Glutathione reductase, the other key component, has a regulatory function because of the dependence of its activity on the availability of NADPH (Smith et al. 1989). It has been found not only in the chloroplast but also in the mitochondria and the

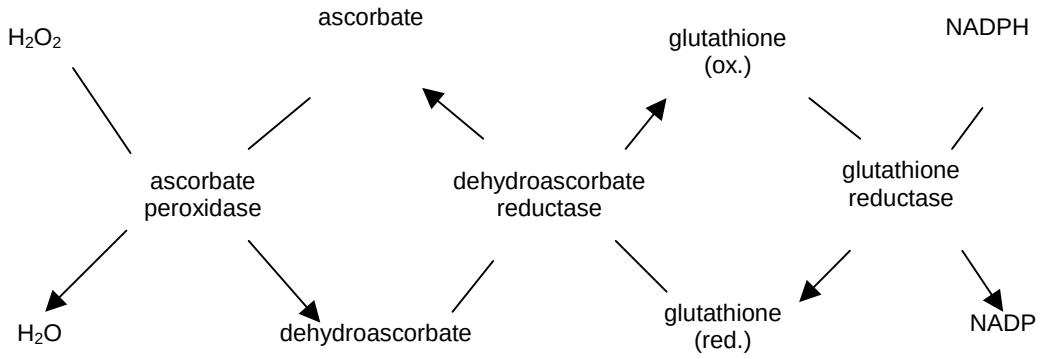

Figure 6: Halliwell-Asada Pathway (Bowler et al. 1992)

cytoplasm (Edwards et al. 1990), where it may also cooperate with superoxide dismutase to remove superoxide radicals. Besides dehydroascorbate, ascorbate peroxidase activity also generates monodehydroascorbate. The ascorbate radical is converted back to

ascorbate by a monodehydroascorbate reductase that can use both NADH and NADPH as, reductants (Bowler et al. 1992). The importance of this system relative to the Halliwell-Asada pathway remains to be evaluated.

In addition, plant cells contain relatively high levels of ascorbate, glutathione, and α-tocopherol, which are efficient oxyradical scavengers. The lipophyllic α-tocopherol is present in large amounts in thylakoid membranes where it blocks the chain-propagating reactions of lipid peroxidation. Carotenoids are another essential component of thylakoid membranes because they can quench singlet oxygen extremely rapidly (Knox and Dodge 1985). Thus superoxide dismutase is intertwined with other enzymes and antioxidants in what is likely a highly optimized balance that reduces the risk of hydroxyl radical formation. Discussion of the role of superoxide dismutase therefore necessitates consideration of the oxidant stress defense system as a whole.

2.17.3 Response of superoxide dismutase to Environmental Stresses

Chloroplastic superoxide dismutase is generally the most abundant superoxide dismutase in green leaves, while in germinating seedlings and in etiolated material the cytoplasmic and mitochondrial superoxide dismutases are prevalent (Foster and Edwards 1980;

Jackson et al. 1978; Tsang et al. 1991). This distribution presumably reflects changes occurring in the subcellular sites of oxyradical formation i.e. during the greening process photosynthetic reactions become more dominant in cell metabolism, necessitating an increase in chloroplastic superoxide dismutase. During subsequent growth to maturity, superoxide dismutase activities appear to change a little. However it has been observed that expression of the different enzymes is to some extent determined by the availability of their metal cofactors (Del-Rio et al. 1991).

As the plant senesces, activity of all superoxide dismutase enzymes, together with that of oxygen-detoxifying enzymes such as catalase and gluththione reductase, decreases (Dhindsa and Matowe 1981; Tanaka and Sugahara 1980). Processes that enhance the formation of oxyradicals and initiate lipid breakdown, such as lipoxygenases enzymes, are stimulated in senescing plant tissue. The addition of hydrogen peroxide or hydrogen peroxide generating compounds to excised rice leaves promotes senescence (Parida et al. 1978). These observations are consistent with the proposal that free radicals play an important role in aging and senescence processes (Bowler et al. 1992).

Regulation of superoxide dismutase genes also appears to be very sensitive to environmental stress, presumably as a consequence of increased oxygen radical formation. This section documents the evidence for the generation of oxidative stress during different types of environmental adversity and summarizes the corresponding regulation of superoxide dismutase enzyme activities which have been observed.

2.17.4 Photoinhibition

The production of hydrogen peroxide by illuminated chloroplasts was first demonstrated by Mehler in 1951. It has subsequently been shown that almost all of this hydrogen peroxide is derived from superoxide formed by the univalent transfer of electrons to oxygen from the electron acceptor of photosystem I, and more particularly ferredoxin (Bowler et al. 1992).

In general, superoxide radicals are more likely to be formed during periods of high photosynthetic activity; the disturbance of normal photosynthetic reactions increases this likelihood even further.

Some documented results indicate that the chloroplastic superoxide dismutase responds not directly to light but to the increased superoxide formation arising from the inefficient transfer of electrons through the photosystems owing to inadequate maintenance of the photosynthetic apparatus during the prolonged dark period.

In addition to superoxide and hydrogen peroxide (and therefore the potential to form hydroxyl radicals), illuminated chloroplasts can produce singlet oxygen by a transfer of excitation energy from chlorophyll to oxygen. Carotenoids can ameliorate this problem because they can react with singlet oxygen at diffusion-limited rates and can also quench the excited triplet states of chlorophyll that lead to singlet oxygen formation (Knox and Dodge 1985).

During normal conditions chloroplasts are likely to be well adapted for minimizing the damage that can occur from misuse of photosynthetic energy transfer. Thylakoid membranes are rich in antioxidants such as α- tocopherol and carotenoids, and the presence of superoxide dismutase and ascorbate peroxidase provides an efficient enzymatic means for eliminating potentially harmful superoxide and hydrogen peroxide. In addition, the levels of these antioxidants can increase if light intensity increases slowly (Gillham and Dodge 1987). However, because of the continuous absorption of light energy by the photosynthetic machinery, any perturbation of electron transport can lead to the donation of electrons to the wrong electron acceptor. If this happens to be oxygen, reactive oxygen species can be generated. Such disturbances can be caused by herbicides that interfere with electron transport or CO_2 fixation and also during conditions of photoinhibition, in which the absorbed light energy exceeds the capacity of the photo systems to direct it through photosynthetic electron transport. Such a condition can arise during high light intensities and also when a temperature stress (chilling or heat) accompanies illumination.

Exposure of plants to high light intensities leads to a reduction of photosynthetic capacity owing to the redirection of photon energy into processes that inhibit photosynthetic capacity. Maintained long enough, this condition leads to the destruction of photosynthetic pigments (commonly referred to as photooxidation). While this pigment bleaching is dependent upon oxygen as well as light and appears to be mediated to some extent by reactive oxyradicals, the reduced photosynthesis that precedes it can occur

largely in the absence of oxygen, hence questioning any involvement of active oxygen species (Bowler et al. 1992). Further evidence for oxygen-dependent and independent events have been obtained from experiments with isolated spinach chloroplasts or thylakoids, which showed that the addition of superoxide dismutase or catalase could only provide partial protection against photoinhibitory conditions (Wild et al. 1990). Consistent with the observations that oxygen radicals play some role in the cellular damage, occurring as a result of photoinhibition, some reports have documented changes in superoxide dismutase activity.

CHAPTER III

MATERIALS AND METHODS

3.1 Plant Material

3.1.1 Apples:

Four commercial apple cultivars (Gala, Fuji, Granny Smith and Red delicious) were selected. Mature ripe fruits were obtained from the local market in College Park, MD. All the fruits were obtained and used between January to May 2003.

3.1.2 Leaves:

Mature source leaves of the Gala cultivar were obtained from three years old apple trees grown in the University of Maryland green house nursery, College Park, MD.

3.2 Enzyme extraction

Superoxide dismutase activity:

The method used, with some modifications, for the determination of SOD activity is based on, Gong et al. 2001.

Frozen apple tissue (5 g) was homogenized with 15 ml (0.05 M) phosphate buffer (pH 7.0) containing 10% PVPP and 0.1 M EDTA. The homogenate was centrifuged at 15000 g for 15 min at 4°C. The supernatant was used for SOD analysis. SOD activity was determined by measuring inhibition of the photochemical reduction of NBT using the method described by Beauchamp and Fridovich (1971). The reaction mixture (3 ml) was composed of 13mM methionine, 0.075 mM NBT, 0.1 mM EDTA, 0.002 mM riboflavin, and 0.1 ml of enzyme extract in 50 mM phosphate buffer (pH 7.8). The mixture in tubes

was placed on a holder in a light source for 15 minutes (light intensity was 175 ± 5 µeinstein per m^2 per second). The absorbance was read at 560 nm with a spectrophotometer (Beckman, model DU-640). One unit of SOD activity was defined as the amount of enzyme that inhibits the NBT photoreduction by 50%. The activity of SOD was presented both as unit / g FW and unit / mg protein.

3.3 Ascorbate concentration

The method used, with some modifications, for the determination of ascorbate concentration is based on Baier et al. (2000).

Leaf discs (0.5 g fresh weight) were frozen in liquid N2 and ground with 1 mL of 1.2 M $HClO_4$ and 0.1 g PVPP. The samples were centrifuged for 5 min at 12000 g and the supernatant was neutralized with 5 M KOH/1 M triethanolamine to obtain pH values between 4 and 5. Ascorbate concentration was measured by the change in absorbance at 265 nm following the addition of ascorbate oxidase. Samples (50 µl) were added to a 0.1 M sodium phosphate buffer (pH 5.6) to obtain a final volume of 990 µl. The absorbance at 265 nm was measured in a spectrophotometer (Beckman, DU-640) and 10 µl (4 units) of ascorbate oxidase were added. The ascorbate concentration measured via the absorption decrease was estimated using an extinction coefficient of 0.010 at 265 nm. The ascorbate concentration of the apple samples were expressed as µm / g FW.

3.4 Protein determination

3.4.1 Assay for Protein determination:

Total protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as the standard and expressed as milligram protein per gram fresh weight of the sampled tissue (mg / g FW).

A known weight of peel and fleshy cortex tissue was sampled and frozen in liquid N_2 and then grounded. It was then washed with 90% acetone (cold) and then filtered to remove the pectins. The washed powder and extraction buffer was homogenized by mixing and then it was centrifuged at 15,000 rpm for 20 min.

3.4.2 Bradford method: This method is based on the ability of proteins to bind the dye coomassie Brilliant Blue G-250 (commonly know as Bradford dye). The binding of the dye to proteins cause a shift in the absorption maximum of the dye from 465 to 595 nm. The concentration of protein in the samples was determined by measuring the increase in absorption at 595 nm and expressed as mg protein /g FW of sampled tissue.

3.4.3 Assay reagents used:

The protein reagent, Coomassie Brilliant Blue G-250 was used. 100 mg of the dye was dissolved in 50 ml of 95% ethanol and then 100 ml of 85% phosphoric acid were added to the reaction mixture. The solution was diluted to a final volume of 1 liter with distilled water.

3.4.4 Protein Standard: A solution of 1 mg/ml Bovine Serum albumin was prepared and was used as a standard for all protein concentration determinations.

3.4.5 Assay procedures:

Standard protein curve was prepared. Five 15 ml test tubes were prepared. To the first tube (Tube number 1) 0.1 ml 5mM (Tris-EDTA) TE buffer was added.

The remaining tubes were prepared as following:

Tube number	Protein solution	TE buffer
2	10 µl	90 µl
3	20 µl	80 µl
4	40 µl	60 µl
5	60 µl	40 µl

Five ml of the protein reagent (Coomassie Brilliant Blue G-250) was added to all the tubes. The tubes were shaken and mixed properly. The optical density was read at 595 nm by transferring 3 ml of the tube's contents to a 3 ml cuvette, and standard curve was prepared which was then used to determine protein concentration in all the apple cultivars.

For measurement of protein concentration in apple cultivars, 100 µl of supernatant was used in 5 ml of Coomassie Brilliant Blue G-250 and then the absorption was measured at 595 nm and expressed as mg protein / g FW.

3.5 Statistical Analysis

Data were subjected to multivariate analysis of variance and means were compared for significant difference. The effect of apple cultivar (Gala, Fuji, Granny Smith and Red Delicious) and, the type of skin tissue (fleshy cortex tissue and peel tissue) were determined using The Proc-GLM procedure in SAS 8.2 (SAS Institute Inc. Gary, NC, USA). Difference in apple cultivars in terms of antioxidant activity of both antioxidants

(ascorbate and superoxide dismutase) were also determined using the Proc-GLM procedure in SAS 8.2 (SAS Institute Inc. Gary, NC, USA). Means were evaluated by Tukey's test and differences at $p \leq 0.05$ were considered to be significant.

CHAPTER IV

RESULTS

4.1 Ascorbate Concentration

4.1.1 Ascorbate concentration in apple fruit fleshy cortex tissue

Figure 7 shows, ascorbate concentration of fruit fleshy cortex tissue for all the four apple cultivars used in this study. Gala fruit fleshy cortex tissue had an ascorbate concentration of 0.073 ± 0.003 (µ mol / g FW). Red Delicious fruit fleshy cortex tissue had an ascorbate concentration of 0.019 ± 0.003 (µ mol / g FW). Fuji fruit fleshy cortex tissue had an ascorbate concentration of 0.053 ± 0.006 (µ mol / g FW). Granny Smith fruit fleshy cortex tissue had an ascorbate concentration of 0.054 ± 0.004 (µ mol / g FW). Gala apple fruit fleshy cortex tissue had the maximum ascorbate concentration of 0.073 ± 0.003 (µ mol / g FW) and Red Delicious fruit fleshy cortex tissue had the minimum ascorbate concentration of 0.019 ± 0.003 (µ mol / g FW), out of the four selected apple cultivars. Table 4 summarizes all the results, which include means and standard deviations.

4.1.2 Ascorbate concentration in apple fruit peel tissue and leaves of gala

Figure 8 shows, ascorbate concentration of fruit peel for all the four apple cultivars used in this study and the ascorbate concentration of source leaves from Gala. Red Delicious fruit peel tissue had an ascorbate concentration of 0.90 ± 0.023 (µ mol / g FW). Gala fruit peel tissue had an ascorbate concentration of 0.99 ± 0.022 (µ mol / g FW). Fuji fruit peel tissue had an ascorbate concentration of 1.02 ± 0.019 (µ mol / g FW). Granny Smith fruit

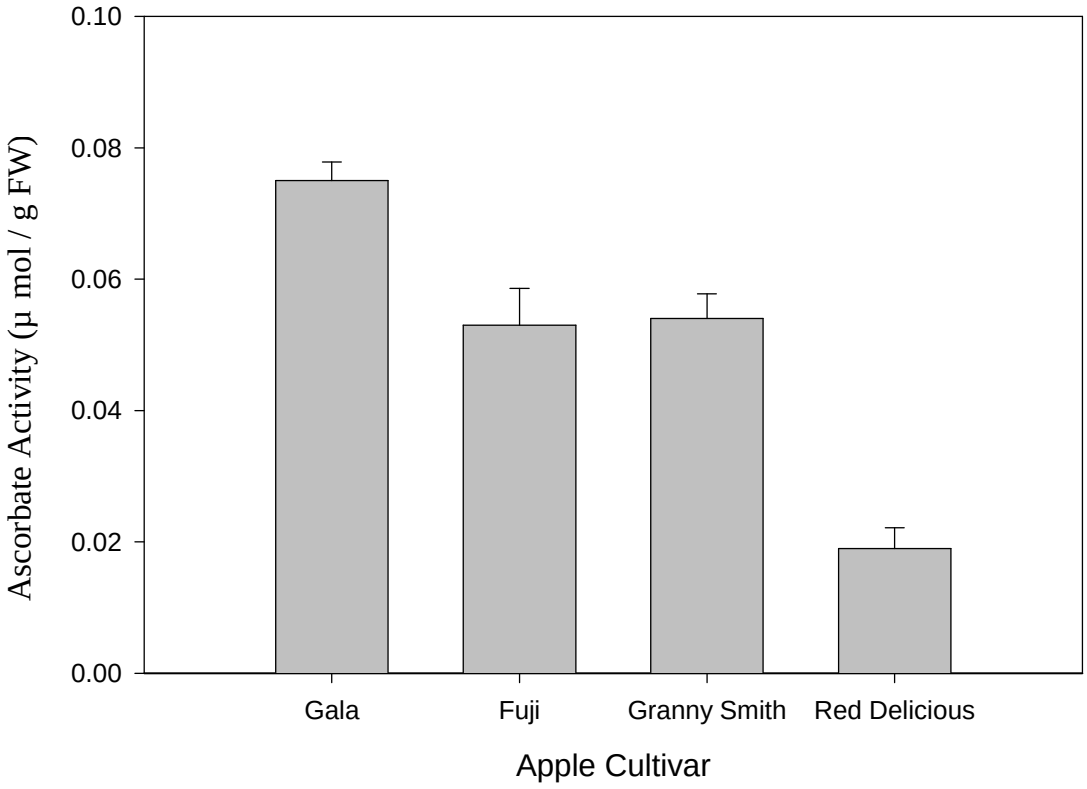

Figure 7: Ascorbate concentration in fruit fleshy cortex tissue of four apple cultivars

Table 4. Ascorbate concentration of the fresh fruit peel and fruit fleshy cortex tissues [a] of four apple cultivars

Cultivar	Skin Tissue	Asc (µmol / g FW)
Red Delicious	Peel Tissue	0.90 ± 0.023
	Fleshy cortex Tissue	0.019 ± 0.003
Gala	Peel Tissue	0.99 ± 0.022
	Fleshy cortex Tissue	0.075 ± 0.003
Fuji	Peel Tissue	1.02 ± 0.019
	Fleshy cortex Tissue	0.053 ± 0.006
Granny Smith	Peel Tissue	1.13 ± 0.023
	Fleshy cortex Tissue	0.054 ± 0.004
Tukey's $_{0.05}$ significance [b]		
Cultivar [C]		**
Skin Tissue [S]		**
[C] × [S] interaction of cultivar and type of skin tissue		**

[a] Data expressed in mean ± SEM (n=15). [b] **, significant at $p \leq 0.05$

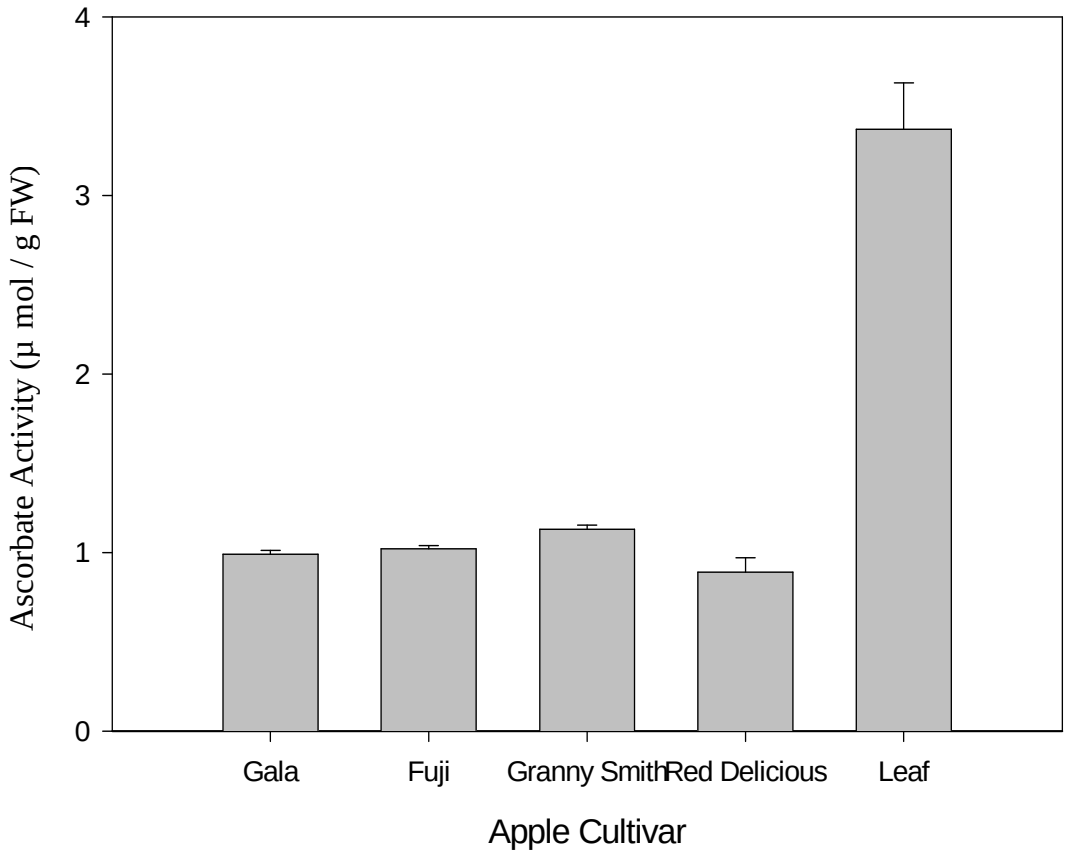

Figure 8: Ascorbate concentration in peel fruit tissue of four apple cultivars compared to source leaves of Gala

peel tissue had an ascorbate concentration of 1.13 ± 0.023 (µ mol / g FW). Granny Smith fruit peel tissue had the maximum ascorbate concentration of 1.13 ± 0.023 (µ mol / g FW), and Red Delicious peel tissue had the minimum ascorbate concentration of 0.90 ± 0.023 (µ mol / g FW) out of four selected apple cultivars. Mature Gala apple tree leaves had more ascorbate concentration than fruit peel tissues of each selected apple cultivar.

4.1.3 Statistical analysis results

Statistical analysis of all the data was done in SAS and results are summarized in Table 4. Tukey's least significant difference (LSD) test ($p \leq 0.05$) showed that ascorbate concentration was significantly different in the different cultivars studied. Fruit peel tissue had more ascorbate concentration than fruit fleshy cortex tissue. Our values ranged from a high of 1.13 ± 0.023 (µ mol / g FW), and a low of 0.90 ± 0.023 (µ mol / g FW). Tukey's LSD test also showed that the interaction was significant of cultivar and type of skin tissue (fleshy cortex and peel tissue). There was also a significant interaction for ascorbate concentration.

4.2 Superoxide Dismutase Activity

4.2.1 SOD activity in apple fruit fleshy cortex tissue

Figure 9 compares SOD activity (in units /g FW) in fruit fleshy cortex tissue of all the four apple cultivars used in this study. Red Delicious fruit fleshy cortex tissue had a SOD activity of 15.8 ± 0.72 (units / g FW). Gala fruit fleshy cortex tissue had a SOD activity of 8.57 ± 0.31 (units / g FW). Fuji fruit fleshy cortex tissue had a SOD activity of 19.47 ±

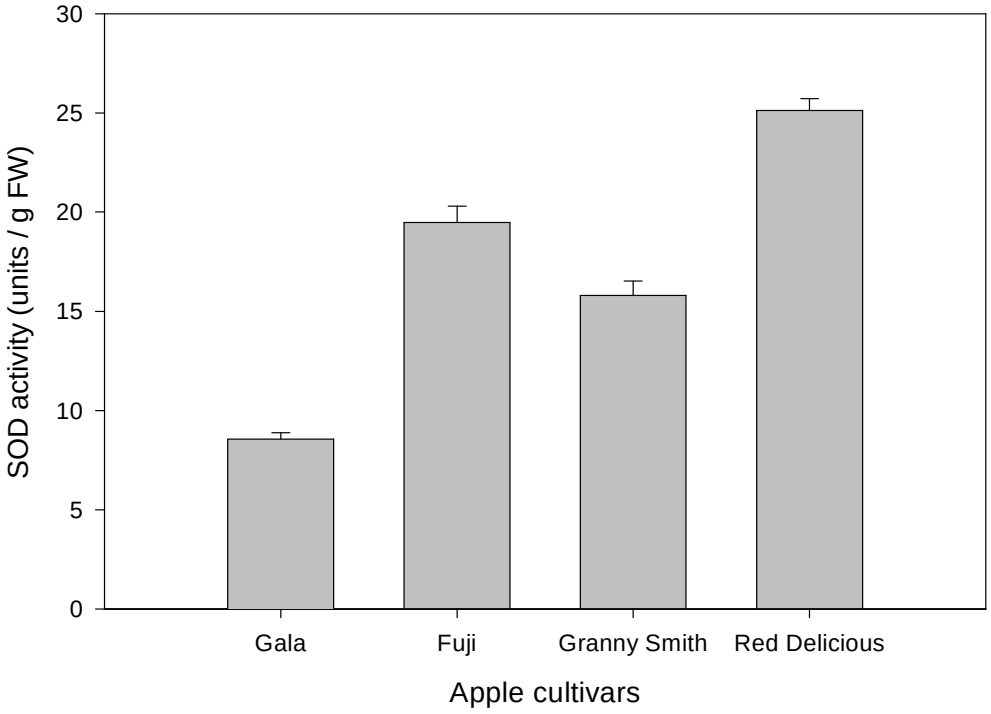

Figure 9: Superoxide dismutase activity in fruit fleshy cortex tissue of four apple cutivars

0.47 (units / g FW). Granny Smith fruit fleshy cortex tissue had a SOD activity of 25.12 ± 0.59 (units / g FW). Red Delicious fruit fleshy cortex tissue has the maximum SOD activity of 15.8 ± 0.72 (units / g FW) and Gala fruit fleshy cortex tissue had a minimum SOD activity of 8.57 ± 0.31 (units / g FW) compared to all the four apple cultivars used in this study. Table 5 presents and summarizes all the results, which include means and standard deviations for SOD activity of all the cultivars.

4.2.2 SOD activity in apple fruit peel tissue and source leaves of Gala.

Figure 10 shows the SOD activity of peel fruit tissues of all the four apple cultivars used in this study and, the SOD activity of source leaves of Gala. Red Delicious fruit peel tissue had a SOD activity of 37.14 ± 1.04 (units / g FW). Gala fruit peel tissue had a SOD activity of 16.46 ± 0.74 (units / g FW). Fuji fruit peel tissue had a SOD activity of 27.5 ± 0.77 (units / g FW). Granny Smith fruit peel tissue had a SOD activity of 38.64 ± 2.15 (units / g FW). Granny smith fruit peel tissue had the maximum SOD of 38.64 ± 2.15 (units / g FW) and Gala fruit peel tissue had the minimum SOD activity of 16.46 ± 0.74 (units / g FW) activity out of four selected apple cultivars in this study. Table 5 gives all the results in units / g FW and units / mg protein. Figure 11 shows a comparison of SOD activity in peel tissue of the four apple cultivars and mature Gala apple fresh leaves. Leaves had higher SOD activity of as compared to fruit peel tissues of each apple cultivar selected in this study.

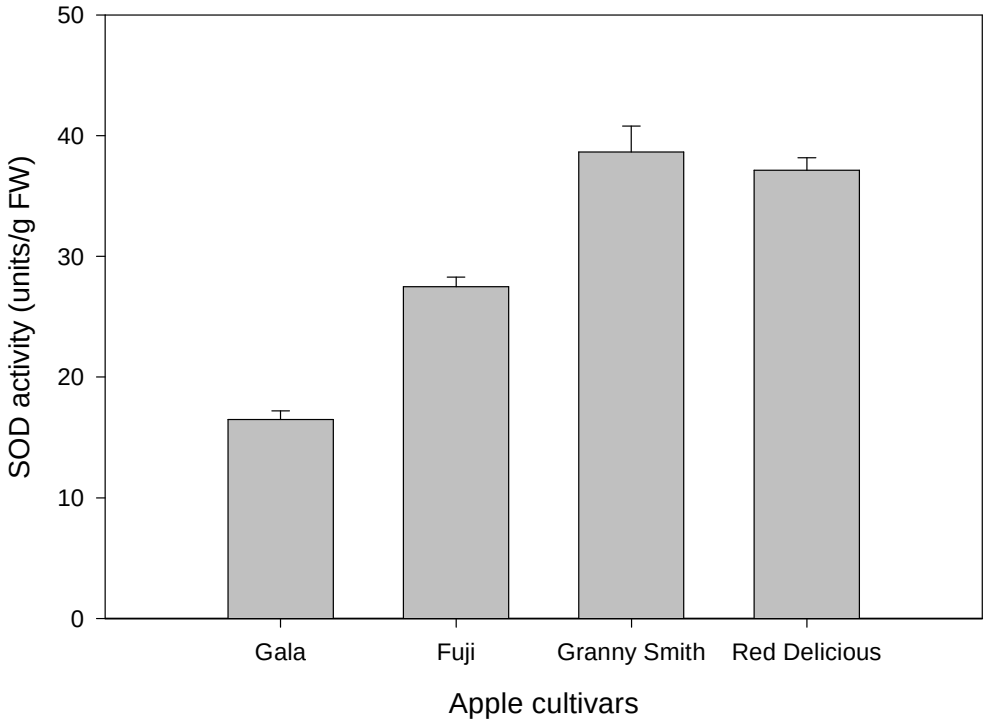

Figure 10: Superoxide dismutase activity in fruit peel tissue of four apple cultivars

Table 5. Superoxide Dismutase activity of the fresh fruit peel and fruit fleshy cortex tissues [a] of four apple cultivars			
Cultivar	Skin Tissue	SOD (units / g FW)	SOD (units / mg proteins) (mean)
Red Delicious	Peel Tissue	37.14 ± 1.04	1125.46
	Fleshy cortex Tissue	15.8 ± 0.72	1322.11
Gala	Peel Tissue	16.46 ± 0.74	49.88
	Fleshy cortex Tissue	8.57 ± 0.31	342.8
Fuji	Peel Tissue	27.5 ± 0.77	105.76
	Fleshy cortex Tissue	19.47 ± 0.47	432.67
Granny Smith	Peel Tissue	38.65 ± 2.15	203.42
	Fleshy cortex Tissue	25.12 ± 0.59	287.27
Tukey's $_{0.05}$ significance [b]			
Cultivar [C]		**	**
Skin Tissue [S]		**	**
[C] × [S] interaction of cultivar and skin tissue		**	**

[a] Data expressed in mean ± SEM (n=15). [b] **, significant at $p \leq 0.05$

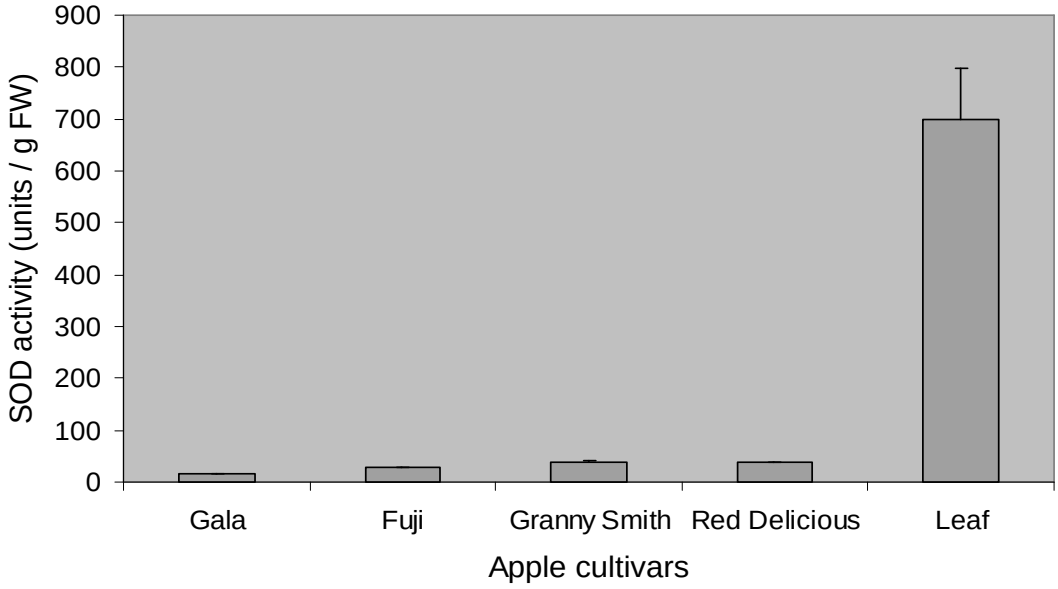

Figure 11: Superoxide dismutase activity in fruit peel tissue of four apple cutivars compared to source leaf tissue of Gala

4.2.3 Statistical analysis results

Statistical analysis of all the data was done in SAS and the results are presented and summarized in Table 5. Tukey's least significant difference (LSD) test ($p \leq 0.05$) showed that SOD activity was significantly different in the different cultivars. Fruit peel tissue had 3 to 4 times more SOD activity than fruit fleshy cortex tissue. Tukey's LSD test also showed that the interaction of cultivar and type of skin tissue (fleshy cortex and peel tissue) was also significant for SOD activity.

4.3 Protein Concentration

Figure 12 shows the protein standard curve which was used to determine protein concentrations in each apple cultivar.

4.3.1 Protein concentration in apple fruit fleshy cortex tissue

Figure 13 shows, protein concentration of fruit fleshy cortex tissue for all the four apple cultivars used in this study. Gala fruit fleshy cortex tissue had a protein concentration of 0.025 ± 0.002 (mg / g FW). Red Delicious fruit fleshy cortex tissue had a protein concentration of 0.019 ± 0.002 (mg / g FW). Fuji fruit fleshy cortex tissue had a protein concentration of 0.045 ± 0.007 (mg / g FW). Granny Smith fruit fleshy cortex tissue had a protein concentration of 0.056 ± 0.005 (mg / g FW). Red Delicious apple fruit fleshy cortex tissue had the minimum protein concentration of 0.019 ± 0.002 (mg / g FW) and Granny Smith fruit fleshy cortex tissue had the maximum protein concentration of 0.056 ± 0.005 (mg / g FW), out of the four selected apple cultivars.

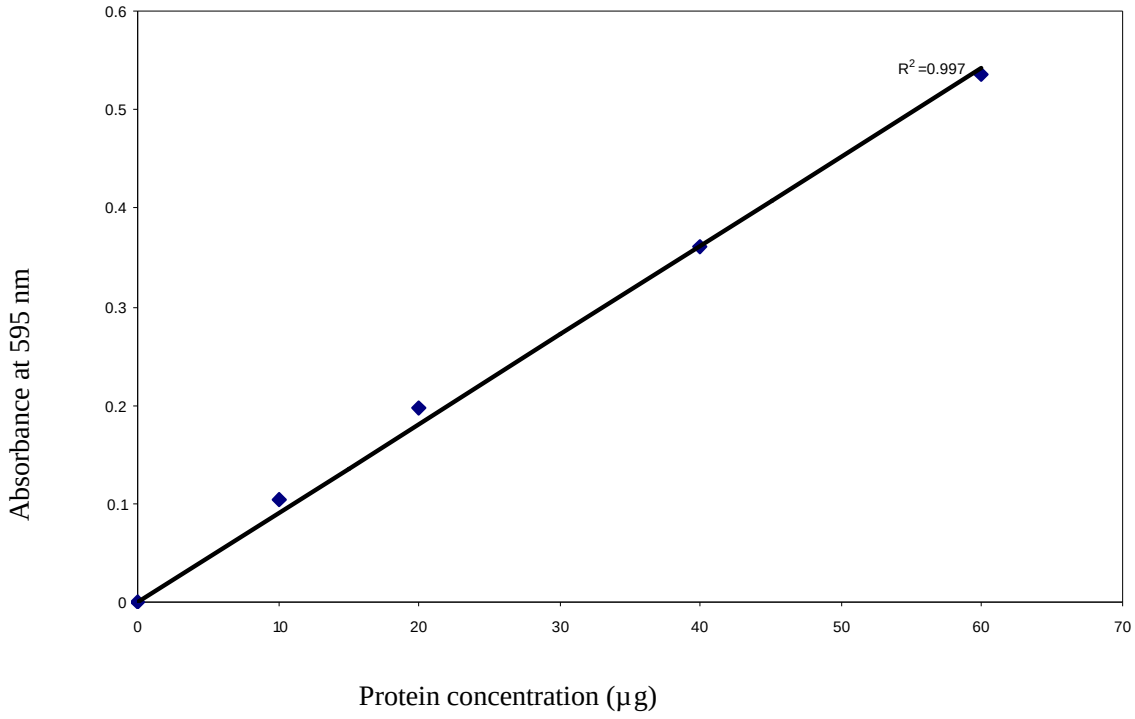

Figure 12: Protein (bovine serum albumin) standard curve

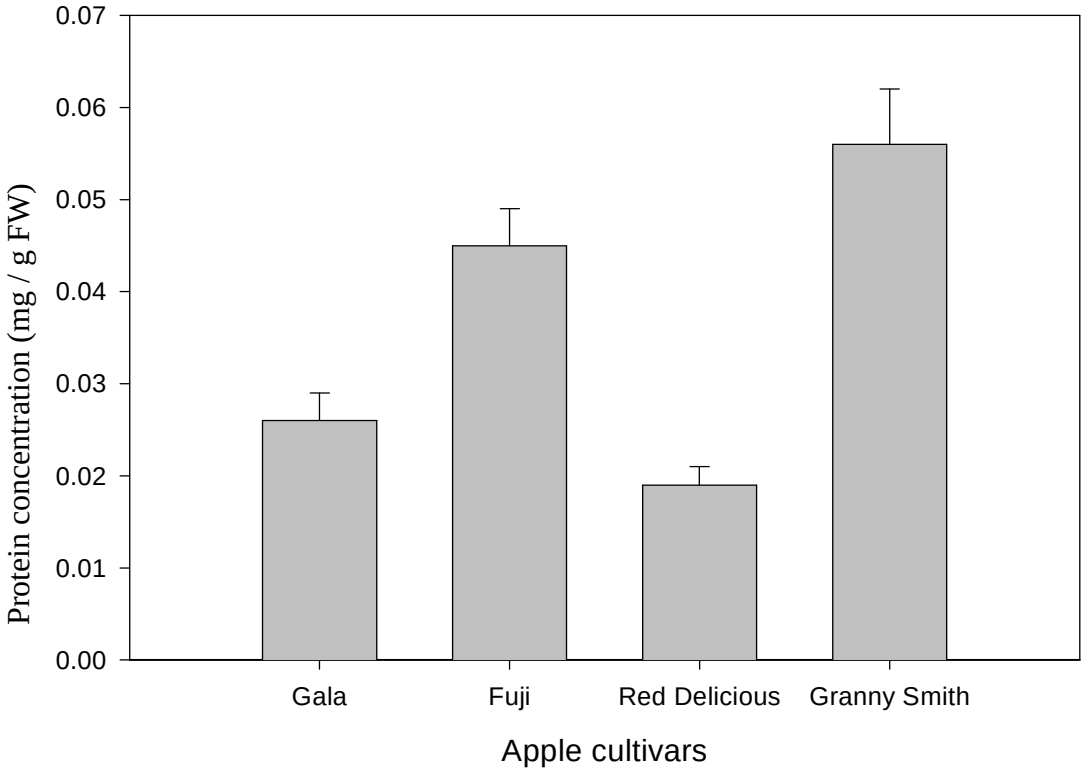

Figure 13: Protein concentration in fruit fleshy cortex tissues of four apple cultivars

4.3.2 Protein concentration in apple fruit peel tissue and leaves of gala

Figure 14 shows, protein concentration of fruit peel for all the four apple cultivars used in this study and Figure 15 shows, protein concentration of fruit peel for all the four apple cultivars used in this study the protein concentration of source leaves from Gala. Red Delicious fruit peel tissue had a protein concentration of 0.033 ± 0.005 (mg / g FW). Gala fruit peel tissue had a protein concentration of 0.33 ± 0.009 (mg / g FW). Fuji fruit peel tissue had a protein concentration of 0.26 ± 0.012 (mg / g FW). Granny Smith fruit peel tissue had a protein concentration of 0.19 ± 0.008 (mg / g FW). Gala fruit peel tissue had the maximum protein concentration of 0.33 ± 0.009 (mg / g FW), and Red Delicious peel tissue had the minimum protein concentration of 0.033 ± 0.005 (mg / g FW) out of four selected apple cultivars. Gala apple tree leaves had more protein concentration than fruit peel tissues of each selected apple cultivar.

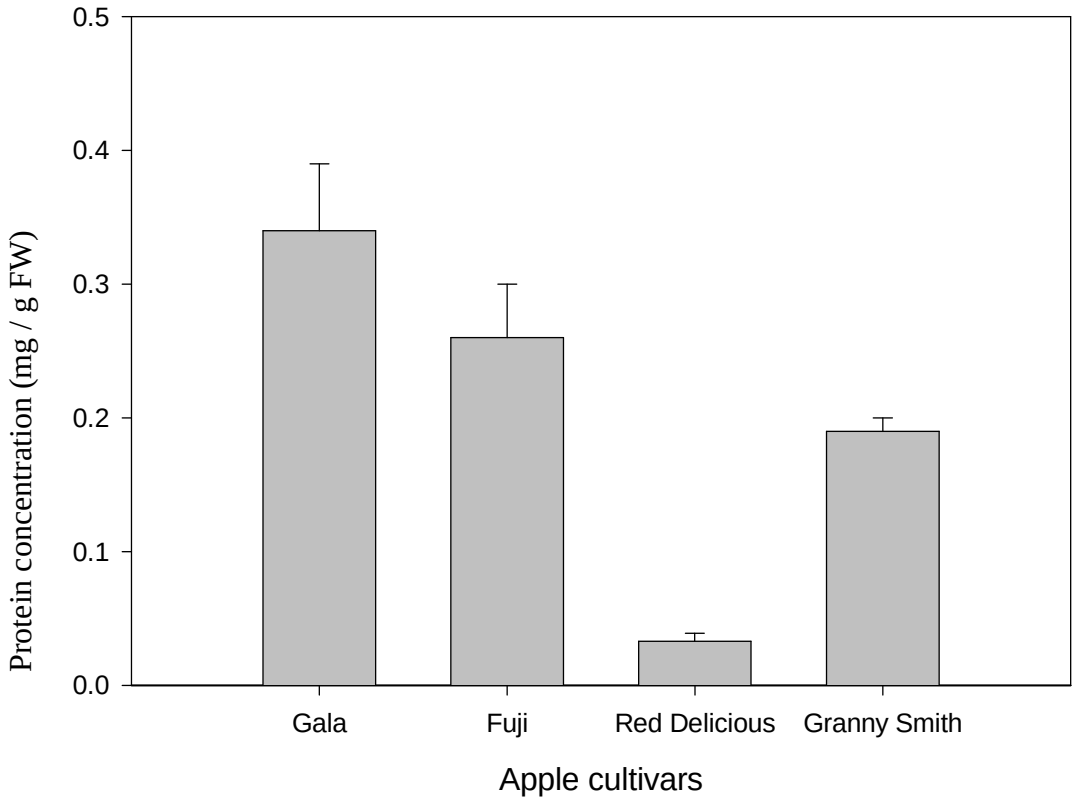

Figure 14: Protein concentration in peel fruit tissues of four apple cultivars

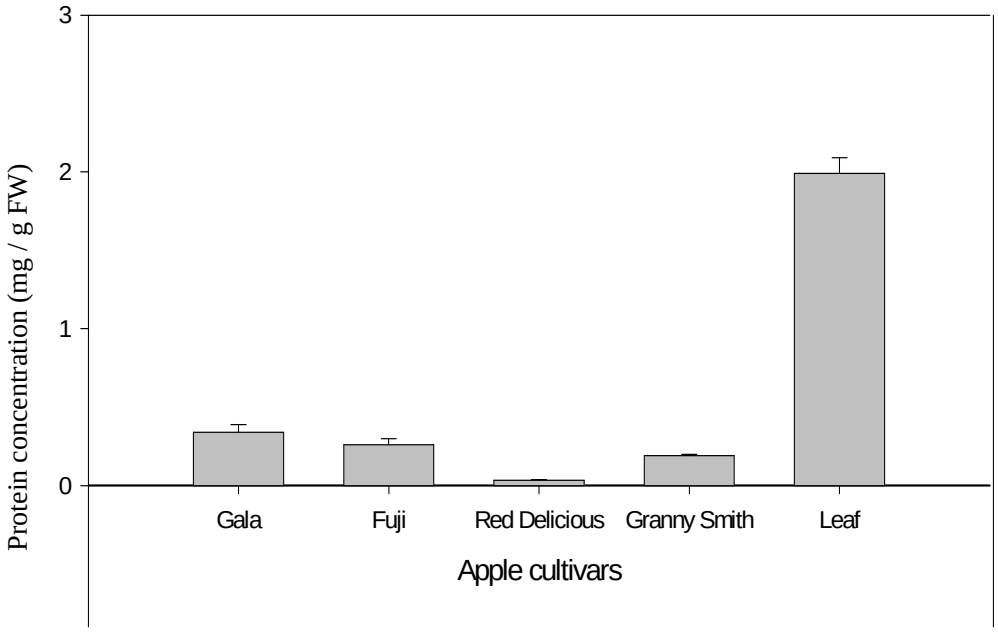

Figure 15: Protein concentration in fleshy cortex tissues of four apple cultivars compared to source leaf tissue of Gala

CHAPTER V

DISCUSSION

While oxygen is fundamental for the survival of all aerobic organisms in plants, it is subjected to in vivo activation into toxic forms. The most damaging forms of active oxygen are free-radicals such as superoxide and hydroxyl (Fridovich 1989) and hydrogen peroxide (Noctor and Foyer, 1998). Superoxide dismutases are considered to be the first line of defense against O_2 reactions. Their reaction products are H_2O_2 and O_2. H_2O_2 is reduced by Ascorbate to water (figure 6).

4.1 Role of ascorbate in fruit ripening

It is clear, that ascorbate is a major primary antioxidant, reacting directly with hydroxyl radicals, superoxide, and singlet oxygen. Ascorbate is also a powerful secondary antioxidant, reducing the oxidized form of α-tocopherol, an important antioxidant in nonaqueous phases (Noctor and Foyer 1998).

Different plant species and tissues have characteristically different ascorbate concentrations, and in leaves there is strong evidence that the ascorbate pool size is light dependent. Leaves normally contain 2-5 μ mol g^{-1} fresh weight although a few species, including some high mountain plants and temperate evergreen species, can contain up to 10 times more. In leaves of a number of species, there is a strong relationship between light and ascorbate concentration. Acclimation to high light increases the ascorbate pool whereas transfer to low light or darkness causes a decrease (Smirnoff et al. 2001).

Current study shows that there is not too much variation in the ascorbate concentration in the fruit peel tissue among the different cultivars studied. Granny Smith has the highest ascorbate concentration of 1.13 ± 0.023 (µ mol / g FW), and Red Delicious peel tissue had the lowest ascorbate concentration of 0.90 ± 0.023 (µ mol / g FW) where as Fuji and Gala fall in the same group according to our Tukey's least significant test. Tukey's test also showed that peel fruit tissue has much higher ascorbate concentration than fleshy cortex tissue for all the cultivars tested in this study. In fruit peel tissue the range of ascorbate concentration was 0.90 to 1.13 (mg / g FW) compared to a range of 0.019 to 0.075 (mg / g FW) in fleshy cortex tissue.

However, there is huge difference in the ascorbate concentration of the leaf tissue as compared to the peel fruit tissues (table 6). A possible reason of this difference could be due to the fact that the leaf tissue is more exposed to oxidative stresses. As reported by Smirnoff et al. 2001, leaves have higher ascorbate concentration then fruits (and their peel) and leaves normally contain 2-5 µ mol g^{-1} fresh weight. Ascorbate is very important in photoprotection and in the regulation of photosynthesis (Noctor and Foyer 1998). Higher photosynthesis is found in leaves and hence it is likely that leaves do have more ascorbate than fruit peel tissue. The photosynthetic role in fruit peel tissues are extremely low or non existent.

Granny Smith is highly susceptible to scald (most susceptible in all the four selected apple cultivars). Hence, more antioxidants are needed to protect by the oxidation of α-farnesene, which causes scald formation. It was reported earlier that ascorbate inhibit oxidation of α-farnesene and thus may block the sequence of events leading to scald formation (Barden and Bramlage 1994).

Table 6. Ratio of ascorbate content in Gala leaf, peel and flesh cortex tissue determined in four apple cultivars		
Cultivar	Leaf tissue / peel fruit tissue	Peel fruit tissue / fleshy cortex tissue
Granny Smith	3.1	20.9
Red Delicious	4.0	46.8
Gala	3.6	13.2
Fuji	3.5	19.3

Other studies have found that Granny Smith produces low ethylene, as compared to the other selected cultivars in this study (Hampson and Kemp 2003). One of the possible reasons of high ascorbate concentration in this cultivar could be due to the fact that ascorbate is utilized as a cofactor of ACC oxidase, the ethylene biosynthesis enzyme, making more ascorbate available.

Other studies have established that titrable acidity in Granny Smith is higher compared to other cultivars (Thomaie et al. 1998; Lavilla et al. 1999), which could be a possible explanation for the high ascorbate concentration in the cultivar Granny Smith.

Du and Brahmlage, 1994 earlier reported that Red Delicious has a relatively high concentration of α-tocopherol, carotenoids and phenolics, and during maturation other antioxidants increase but ascorbate concentration does not increase significantly. A similar trend was also found in our studies. This may explain why red delicious has a low ascorbate concentration.

From our current studies Fuji and Gala were found to have relatively the same level of ascorbate concentration. The possible explanation could be that both cultivars have similar fruit peel color and both of these cultivars are derived from Red Delicious crosses (figure 16).

In all the cultivars studied the fruit peel tissue had higher ascorbate concentration compared to fleshy cortex tissues (table 6). One of the possible explanations could be that,

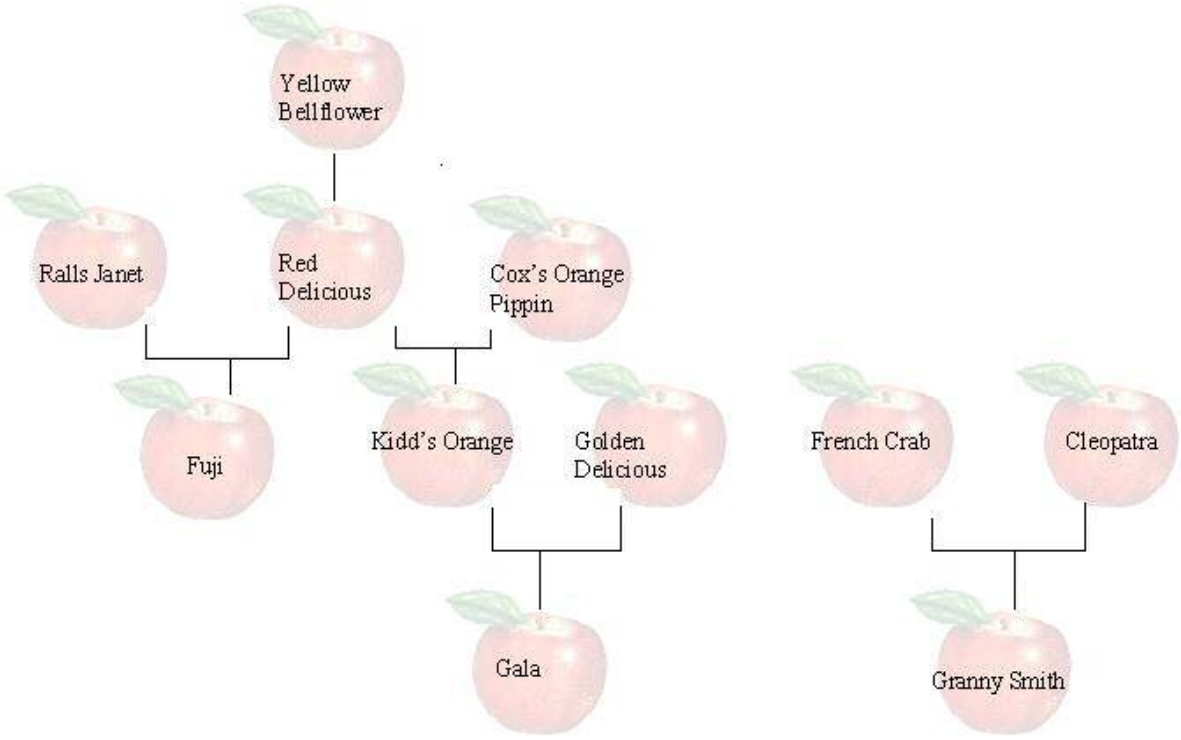

Figure 16: Genetic parentage of the apple cultivars studied.

the fruit peel is an exposed surface and there is direct sunlight on the peel which increases the antioxidant activity (Gong et al. 2001) and also it has more oxidative stress as compared to fleshy cortex tissue. This may be one of the reasons why the fruit peel tissue has more antioxidant concentration than the fleshy cortex tissue. The water concentration in fleshy cortex tissue is higher than peel tissue and has lower concentrations of ascorbate.

Statistical results show that ascorbate concentration in Gala, Fuji and Granny Smith fleshy cortex tissue fall within the same group. Hence, they have relatively the same ascorbate concentration. The ascorbate concentration in red delicious fleshy cortex tissue is low as compared to the other cultivars studied. The overall ascorbate concentration is low in Red Delicious and, the same trend was seen in peel tissue. Red delicious produces maximum internal ethylene C_2H_4. Hence less ascorbate may be available since ascorbate is one of the cofactors for the ethylene biosynthetic enzyme ACC-oxidase.

4.2 Role of superoxide dismutase in plants

Superoxide dismutase is a metalloprotein catalyzing the dismutation of superoxide free radical to molecular oxygen and H_2O_2 (Giannopolitis and Ries 1977). Active oxygen species can lead to tissue damage and losses in many fruits and could be particularly important in fresh products waiting processing. Superoxide dismutase is a ubiquitous defense enzyme against superoxide damage to aerobic organisms (Du and Bramlage 1994).

Rogiers et al. 1998 showed that oxidative stress increased progressively during fruit ripening, probably as a consequence of reduced activities of key enzymes (superoxide dismutase and catalase) responsible for quenching active oxygen species. High temperature and higher light conditions lead to increased superoxide dismutase activity and may protect against sunscald of vegetables and fruits (Rabinowitch and Sklan 1980; Gong et al. 2001).

Leaf tissue has much higher superoxide dismutase activity than fruit peel tissue (table 7). As discussed above leaf tissue is more exposed to light and photoreduction and phosynthesis takes place (as major events) in leaf tissue. According to Gong et al. 2001, with tissue directly exposed to sunlight and with photosynthesis taking place superoxide dismutase activity increases. Also oxidative stress in leaf tissue is higher than fruit peel tissue.

Granny Smith has higher superoxide dismutase activity as compared to all the other selecter cultivars studied. One of the possible reasons could be that Granny Smith is the most susceptible to scald and superoxide dismutase activity protects against scald. Red Delicious has relatively higher susceptibility to scald than Fuji and Gala so it has higher superoxide dismutase activity than Fuji and Gala cultivars. Due to its higher scald susceptibility more antioxidants are needed to prevent scald formation.

According to Tukey's LSD test, Fuji and Gala both fall in the same group for superoxide dismutase activity which could be due to the fact that both the cultivars are derived from Red Delicious crosses (figure 16).

Superoxide dismutase activity has been shown to increase during the fruit ripening process. Overall the activity of superoxide dismutase is higher in the fruit peel tissues as compared to fleshy cortex tissues (table 7). Based on these studies, it can be inferred that the antioxidants are produced more in the fruit peel as compared to fleshy cortex tissue.

The peel is an exposed surface and hence more prone to oxidative stresses. Fleshy cortex tissue, as discussed above, has higher water concentration and which could be one of the reasons why it has lower superoxide dismutase activity.

Table 7. Ratio of superoxide dismutase activity in Gala leaf, peel and fleshy cortex tissue determined in four apple cultivars		
Cultivar	Leaf tissue / peel fruit tissue	Peel fruit tissue / fleshy cortex tissue
Granny Smith	18.1	2.5
Red Delicious	18.8	1.5
Gala	42.4	1.9
Fuji	25.4	1.4

CHAPTER VI

SUMMARY

In the current study, Granny Smith peel tissue had the highest ascorbate concentration of 1.13 ± 0.023 (mg / g FW) and Red Delicious peel tissue had the lowest ascorbate concentration of 0.90 ± 0.023 (mg / g FW). Overall there was not too much variation in the ascorbate concentration in the fruit peel tissues of the four different cultivars used in this study. Fuji and Gala fruit peels were not significantly different in their ascorbate concentration. Leaf tissues of Gala had more ascorbate concentration than fruit peel tissue of each of the four apple cultivars used in our studies.

Fruit peel tissue was found to have higher ascorbate concentration than the fruit fleshy cortex tissue for all the four cultivars tested in this study. Tukey's LSD test showed that Gala, Fuji and Granny Smith did not differ statistically in ascorbate concentration in their respective fruit fleshy cortex tissues. Red Delicious fleshy cortex tissue had the lowest ascorbate concentration of 0.019± 0.003 (mg / g FW.

Leaf tissues of Gala had higher superoxide dismutase activity than fruit peel tissue for all the four cultivars used in this study. Granny Smith fruit peel tissue had the highest superoxide dismutase activity and Red Delicious fruit peel tissue had the second highest superoxide dismutase activity out the four apple cultivars used in this study. Tukey's LSD test showed that Gala and Fuji fruit peel tissue did not differ statistically in their superoxide dismutase activity.

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