Chapter 1

Fruits Forever
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1.1 Introduction

Since Adam’s first visit to the fruiteress in the Garden of Eden, humankind has been totally dependent upon the angiosperms flower and the fruit it bears. Fruits are not only an enjoyable component of a healthy diet but also a valuable source of vitamins, minerals and fiber. Much of food and clothing is derived from flowers and their fruits. One of the vital aspects of fruit production is ripening which is characterized by a series of coordinated changes in the biochemistry and physiology of the tissues involved (Giovannoni, 2004) and also a protracted form of senescence. When fruits attain physiological maturity, the growth ceases and post harvest ripening starts, to attain organoleptic characteristics to be consumed. Among these, texture is the principal quality attribute for consumer acceptability. After palatability other important aspects which depend on texture are shelf life, transport capability and disease resistance (Manrique and Lajolo, 2004). The main factor in determining the post-harvest deterioration in fruit crops is the rate of softening of fruits that accompanies ripening of commercially important fruits, exacerbates damage incurred during handling which influences shelf life and increases pathogen susceptibility. Thus post harvest biologists have studied fruit softening to identify ways to manage ripening and optimize fruit quality. One of the important and foremost characteristics of ripening is progressive modification of cell wall architecture and the polymers of which it is composed. Therefore for plants, the cell wall is an important structure that determines cell shape, glues cells together, and provides essential mechanical strength, rigidity, and acts as a critical barrier against pathogens (Cosgrove, 2000). Moreover it is an important component of the fruit contributing to the texture and shelf life. This dynamic organization consists mainly of carbohydrates and proteins encoded by multigene families. The carbohydrate component of the wall accounts for about 90% of the dry weight and constitutes a framework of cellulose microfibrils embedded in a matrix of hemicelluloses and pectin (Cosgrove, 1997). The softening that occurs during the ripening of many fruits is presumably the result of enzymatic modifications of cell wall polysaccharides. The polysaccharide network is undoubtedly an important component of the cell wall which contains bonds that are susceptible to a variety of hydrolytic enzymes. During ripening of many fruits, wall polysaccharides are hydrolyzed by a suite of digestive enzymes whose genes are specifically expressed during the stages of fruit development (Rose and Bennett, 1999).

Reduction in fruit firmness is accompanied by the increased expression of numerous cell wall degrading enzymes, like polysaccharide hydrolases/glycoside hydrolase, transglycosylases, lyases and expansins (Brummell, 2006). The structural and functional diversity of especially
glycosides is mirrored by a vast array of enzymes involved in their synthesis (glycosyltransferases), modification (carbohydrate esterases) and breakdown (glycoside hydrolases and polysaccharide lyases). All these classes of enzymes are grouped under CAZymes (Carbohydrate Active Enzymes) (Henrissat et al., 2001).

Many examples are evident to summarize the importance of CAZymes among the plant species and within the genome. Recently about 1,600 genes encoding carbohydrate-active enzymes (CAZymes) were identified based on sequence homology, annotated, and grouped into families of glycosyltransferases, glycoside hydrolases, carbohydrate esterases, polysaccharide lyases, and expansins in *Populus trichocarpa* (Torr. & Gray) genome (Geisler-Lee, 2006). Carbohydrate active enzymes are also known to be involved in the secondary cell wall biogenesis in hybrid aspen (Aspeborg, 2005). Additionally, *Arabidopsis* genome contains more than 730 genes encoding putative glycosyl transferases and hydrolases (Henrissat et al., 2001) implicated in wall biosynthesis or function. Moreover proteomic analysis of *Arabidopsis* cell wall further revealed that large portion of the proteins are carbohydrate active enzymes (Bayer et al., 2006) which are essential constituents of plant cell wall, and are known to be involved in modification of cell wall components, wall structure signaling and interacting with the plasma membrane proteins.

Among the Carbohydrate active enzymes, Glycosyl hydrolase constitute a major chunk. These are known to perform vast array of functions but are mainly examined for their roles in fruit softening, seed germination, tissue development (Ross et al., 1994; Smith and Gross, 2000; Martinez et al., 2004; Minic et al., 2004; Esteban et al., 2005) and freezing tolerance (Thorlby et al., 2004). However recent reports reveal that glycosyl hydrolases are involved in degradation of cell wall during sugar starvation (Lee E-J, 2007). In *Arabidopsis* out of the total 29% of proteins acting on cell wall polysaccharides, 21% belong to glycoside hydrolases (Jamet et al., 2006) revealing their importance in the plant genome. Although their general catalytic activity can be inferred from sequence, the precise enzymatic function and biological role of most of these proteins are still unknown (Fry, 2004).

Therefore the 'experimental landscape' must be broadened, taking advantage of insights that can be provided by molecular and physiological analysis and the conventional wisdom supporting the idea that ripening associated softening of fleshy fruits is a direct consequence of enzyme-mediated cell wall degradation. The linkage between cell wall metabolism and fruit softening needs to be revised and the new connections must be explored so as to optimize ripening/softening that does not lead to decrease in other aspects of fruit quality.
1.2 Review of literature

1.2.1 Fruits: A Developmental perspective

In their constant effort to yield subsequent generations of viable and competitive progeny, plant species have evolved numerous mechanisms for seed dispersal. Fruits are an integral part of this endeavor and can be narrowly defined as mature carpels (Giovannoni, 2001). Embryonic development in many angiosperms occurs concomitantly with the development of the ovary into a specialized organ, the fruit which provides a suitable environment for seed maturation and often a mechanism for the dispersal of mature seeds (Gillaspy et al., 1993). Most fruits develop from a gynoecium that contains one or more carpels. In most plants, early fruit development can be divided into three phases. The earliest phase involves the development of the ovary and the decision to abort or to proceed with further cell division and fruit development, which is generally referred to as fruit set. The second phase involves cell division, seed formation, and early embryo development. The third phase begins when cell division ceases. During this phase, fruit growth continues, mostly by cell expansion, until the fruit reaches its final size. All the above mentioned stages during tomato fruit development and their associated hormonal changes are depicted in Fig 1.1. Recently these phases have been further divided into five stages, including organogenesis,
Figure 1.1 Tomato Fruit development and hormonal changes during fruit development.

(1) Longitudinal section through the ovary within the flower at anthesis. Arrow indicates the pericarp.
(2) Cross-section of a fruit 0.3 cm in diameter. Arrow points to vascular tissue within the placenta.
(3) Cross-section of a fruit 0.5 cm in diameter. Arrow indicates the presence of locular tissue, which has differentiated from the placenta.
(4) Part of a cross-section through a fruit 1.2 cm in diameter. Arrow points to the gradient zone of differentiation between placenta and locular tissue.
(5) Cross-section through a developing seed from a fruit 1.2 cm in diameter. Arrow points to the developing embryo within the seed.
(6) Changes in hormone levels throughout tomato fruit development are indicated by white diamonds.
expansion, maturation, ripening and senescence (Alba et al., 2005). The major developmental changes that occur during tomato ripening are depicted in the figure 1.2 (Giovannoni, 2004).

1.2.2 Ripening of fruits: Practical and scientific importance

Ripening is a vital aspect of fruit production (Causier et al., 2002). Fruit development and ripening are process unique to plant species, development and maturation of fruit tissues represent a final phase of floral development typically proceeding and signaled by successful pollination. Fleshy fruits undergo a natural stage of development known as ripening. From the stand point of agriculture, ripening confers both positive and negative attributes to the resulting commodity. Although ripening imparts desirable flavor, color and texture, considerable expense and crop loss result from negative ripening characteristics like, ripening related increase in fruit pathogen susceptibility which is a major contributor to fruit loss both before and after harvest. The ripe phenotype is the summation of biochemical and physiological changes that occur at the terminal stage of fruit development and render the organ palatable and desirable to seed dispersal by animals (Giovannoni, 2001) and typifies non-dehiscent fruits. Ripening also imparts values to fruit as agricultural commodities. These changes, although variable among species, generally include modification of cell wall ultra structure and texture, conversion of starch to sugars, increased susceptibility to post-harvest pathogens, alterations in pigment biosynthesis and accumulation and heightened levels of flavor and aromatic volatiles. Ripening also imparts various critical aspects to mature fruit, including fiber content and composition, lipid metabolism, and the levels of vitamins and various antioxidants. (Ronen et al., 1999).

Several ripening attributes translate to decreased shelf-life and high input harvest, shipping and storage practices, particularly as a result of changes in firmness and the overall decrease in resistance to microbial infection of ripe fruit (Giovannoni, 2001). The ability to understand key control points in global ripening regulation or within specific ripening processes will allow for manipulation of nutrition and quality characteristics associated with ripening. The most convincing argument for the promotion of safe plant-genetic engineering will be the development of products with direct consumer impact and appeal, such as quality, shelf life and nutritionally enhanced fruits. But scientists still argue that the genetically regulated change in fruit physiology will increase the use of pesticides, post harvest fumigants, and controlled atmosphere storage and shipping mechanism in attempts to minimize loss. In addition to being wasteful of energy and potentially harmful to the environment, such practices represent major expenses in fruit production.

There are two characteristic types of fruit ripening that show different patterns of respiration:
Figure 1.2 Major developmental changes during tomato fruit development and ripening.
• **Non-climacteric** fruit ripening-refers to those fruits which ripen only while still attached to the parent plant. Their eating quality suffers if they are harvested before they are fully ripe because their sugar and acid content does not increase further. Respiration rate slows gradually during growth and after harvest. Ethylene production is only of basal level and maturation and ripening are a gradual process. Examples are: cherry, cucumber, grape, lemon, pineapple, Capsicum.

• **Climacteric fruit** ripening-refers to fruits that can be harvested when mature but before ripening has begun. These fruits may be ripened naturally or artificially. Here ethylene is necessary for the coordination and completion of ripening in climacteric fruit which was found out via analysis of inhibitors of ethylene biosynthesis and through examination of the *Never ripe* (*Nr*) ethylene receptor mutant of tomato. The start of ripening is accompanied by a rapid rise in respiration rate, called the respiratory climacteric. After the climacteric, the respiration slows down as the fruit ripens and develops good eating quality. Examples are: apple, banana, melon, papaya, tomato.

A clarification of the common genetic regulatory elements that are shared among climacteric and non-climacteric species is central to a full understanding of fruit ripening. (Giovannoni, 2001)

1.2.3 Importance of shelf life

Estimates of the post-harvest losses of food grains in the developing world from mishandling, spoilage and pest infestation are put at 25 %; this means that one-quarter of what is produced never reaches the consumer for whom it was grown, and the effort and money required to produce it are lost-forever. Further, fruit, vegetables and root crops are much less hardy and are mostly quickly perishable than the food grains, and if care is not taken in their harvesting, handling and transport, they will soon decay and become unfit for human consumption. Estimates of production losses in developing countries are hard to judge, but some authorities put losses of sweet potatoes, plantain, tomatoes, bananas and citrus fruit sometimes as high as 50% which includes losses due to ripening which farmers can not control (FAO, 1989). Reduction in this wastage, particularly if it can economically be avoided, would be of great significance to growers and consumers alike.

1.2.3.1 What are the principal causes of losses?

All fruits, vegetables and root crops are living plant parts containing 65 to 95 percent water, and they continue their living processes after harvest. Their post-harvest life depends on the rate at which they use up their stored food reserves and their rate of water loss. When food and water reserves are exhausted, the produce dies and decays. Anything that increases the rate of this
process may make the produce inedible before it can be used. The principal causes of loss are discussed below, but in the marketing of fresh produce they all interact, and the effects of all are influenced by external conditions such as temperature and relative humidity.

a) Physiological deterioration

An increase in the rate of loss because of normal physiological changes is caused by conditions that increase the rate of natural deterioration, such as high temperature, low atmospheric humidity and physical injury. Abnormal physiological deterioration occurs when fresh produce is subjected to extremes of temperature, of atmospheric modification or of contamination. This may cause unpalatable flavors, failure to ripen or other changes in the living processes of the produce, making it unfit for use.

b) Mechanical damage (physical injury)

Careless handling of fresh produce causes internal bruising, which results in abnormal physiological damage or splitting and skin breaks, thus rapidly increasing water loss and the rate of normal physiological breakdown. Skin breaks also provide sites for infection by disease organisms causing decay.

Injuries caused can take many forms:

- splitting of fruits or roots and tubers from the impact when they are dropped;
- internal bruising, not visible externally, caused by impact;
- superficial grazing or scratches affecting the skins and outer layer of cells;

c) Diseases and pests

All living material is subject to attack by parasites. Fresh produce can become infected before or after harvest by diseases widespread in the air, soil and water. Some diseases are able to penetrate the unbroken skin of produce; others require an injury in order to cause infection. Damage so produced is probably the major cause of loss of fresh produce. The influences of all three causes are strongly affected by the various stages of post-harvest operations.

d) Respiration

Respiration is the process by which plants take in oxygen and give out carbon dioxide. Oxygen from the air breaks down carbohydrates in the plant into carbon dioxide and water. This reaction produces energy in the form of heat. Respiration is a basic reaction of all plant material,
both in the field and after harvest. It is a continuing process in the growing plant as long as the leaves continue to make carbohydrates, and cannot be stopped without damage to the growing plant or harvested produce (FAO, 1989). After harvest, fruits start ripening at faster rate leading to softening and further damage caused due to pathogens, which reduce the price of the crop.

1.2.4 Fruit ripening and ethylene

In 1858, Fahnestock attributed the deterioration of a collection of plants cultivated in a Philadelphia greenhouse and showing signs of senescence and leaf abscission to the presence of illumination gas, but although he detected the presence of hydrocarbons he was unable to identify the component responsible for such effects (Chaves and de Mello-Farias, 2006). Some years later, in 1864, Girardin verified that trees growing close to places where illumination gas was leaking showed the same symptoms of senescence, and also demonstrated that ethylene was present in this gas. In fact, illumination gas contained 5% ethylene and its physiological effects on plants had been observed for many years without being formally attributed to illumination gas (Zegzouti, 1997). In 1886 Neljubov discovered that ethylene was the biologically active component of illumination gas when he noticed that illumination gas was responsible for the horizontal growth of etiolated pea seedlings which he had been cultivating. In 1930s most of the physiological effects of ethylene on plants had already been described (Pech et al., 1992). Further it was demonstrated that a strong increase in ethylene production was associated with peak in respiration during ripening (Zegzouti, 1997). All these reports on biological activity of ethylene led scientist to consider this endogenous growth regulator as a plant hormone (Abeles et al., 1992). The ethylene biosynthetic pathway has now been completely elucidated as depicted in the Figure 1.3. Several theories on ethylene signal perception and transduction have been proposed to explain the mechanisms by which ethylene receptors could promote signal transduction through a cascade involving several components (Bleecker and Kende, 2000; Kieber, 1997) (Fig. 1.4).

Ethylene plays a major role in regulating ripening and softening of climacteric fruits (Hadfield et al., 2000). Two approaches have been used to determine the precise role of ethylene in fruit ripening and softening: transgenic suppression of ethylene production and the use of volatile inhibitors of ethylene action. Genetically engineered tomatoes and melons with suppressed ethylene biosynthesis exhibited delayed and reduced fruit softening (Guis et al., 1997 and Flores et al., 2001) establishing the importance of ethylene. Further the expression of some of the ripening-related and cell wall-associated genes like Polygalacturonase (Hiwasa et al., 2003a), expansins (Rose et al., 1997) and EGases (Lashbrook et al., 1994) were regulated by ethylene. Recently it has been proved that fruit softening and cell wall disassembly in charentais melon is ethylene dependent (Nishiyama et al., 2007; Inaba, 2007). Transcriptome profiling by cDNA microarray analysis identified 869 genes that are differentially expressed in developing tomato of
Figure 1.3. Ethylene biosynthesis pathway
Figure 1.4 Genetic interaction and biochemical identities of the ethylene signal transduction pathway components.
which 37% of the gene are altered in Nr mutant (Alba et al., 2005). Further they reported that transcript accumulation in tomato fruit was observed to be extensively coordinated and often completely dependent on ethylene, unlike Never-ripe (Nr) which reduces ethylene sensitivity and inhibits ripening. These results demonstrate multiple points of ethylene regulatory control during tomato fruit development and provide new insights into the molecular basis of ethylene-mediated ripening (Fig.1.5). Considering all the mutants available in ripening, Giovannoni (2004) further explained the model summarizing the developmental, hormonal, and environmental regulation of ripening control in tomato fig. 1.6.

In regard to the variety of physiological responses of plant to ethylene, the number of genes reported to be induced are very less. For this reason, novel early ethylene-regulated (ER) genes from late immature green tomato fruit have been isolated using the differential display technique, in order to obtain a broader understanding of the molecular basis by which ethylene coordinates the ripening process (Zegzouti et al., 1999). A large set of ER gene have been isolated which show homology to genes involved in transcriptional and post-transcriptional regulation, signal transduction components, stress-related proteins and primary metabolism. The latest data have indicated that ER50 is a CTR-like clone, potentially involved in the ethylene transduction pathway (Adams-Phillips et al., 2004) and ER24 is homologous to a multi-bridging factor involved in transcriptional activation (Tournier et al., 2003) while ER49 is a putative mitochondrial translation elongation factor that could be involved in the stimulation of mitochondrial activity by ethylene during the climacteric increase in respiration (Benichou et al., 2003).

1.2.5 Tomato as Model Plant

Tomato has emerged as the primary model for climacteric fruit development and ripening for combination of scientific and agricultural reasons. The importance of tomato as agricultural commodity resulted in public and private breeding efforts that have yielded numerous spontaneous and induced mutations that affect fruit development and ripening. Simple diploid, small genome (0.9 pg per haploid genome (Arumuganathan and Earle, 1991), short generation time, easy system for transgenic analysis, significance as food source and diverse germplasm (Vrebalov, 2002) ease of transformation, commercial importance and the availability of basic genetic, genomic and biochemical information (Brummell and Harpster, 2001). Availability of mapping population, mapped DNA markers (Tanksley et al., 1992), extensive EST collections, publicly available microarray, and a developing physical map render among the most effective model crop systems. Moreover availability of single gene mutants that regulate fruit size, shape, development and ripening combined with dramatic and readily quantifiable ripening phenotypes (ethylene colour index, carotenoids and softening) have enhanced its use as the model system. Moreover, most of the transgenic plants that have been characterized for altered fruit softening are
Figure 1.5 Model for C$_2$H$_4$ regulatory control in tomato fruit. Time-series transcriptome analysis, morphometric analysis, and selective metabolite profiling reveal novel regulatory points in pathways associated with ripening and identified numerous candidate regulatory loci.
Ripening:
Softening
Flavor/Aroma
Nutrition
Chlorophyll Loss
Carotenoid Accumulation
Pathogen Susceptibility

Figure 1.6 Model for the molecular regulation of tomato fruit ripening. Fruit harboring homozygous mutations for the indicated genes or loci are shown. The nor, rin, Nr, Cnr, hp1, and hp2 mutants are all nearly isogenic with cv Ailsa Craig. The r and B mutants are from reported introgression lines (Eshed and Zamir, 1994) nearly isogenic with cv M82 (WT). A triple phytochrome (PHY) mutant deficient in PHYA (fri), PHYB1 (tri), and PHYB2 (B72) with associated modification of carotenoid accumulation is also shown.
from tomato, and several single gene ripening-impaired mutants of tomato are available to understand many aspects of development, ripening/softening and pigmentation which have been well characterized (Table 1.1).

1.2.6 Cell walls: Structure and signals

The plant cell wall in general is strong fibrillar network that gives each cell its stable shape. To enlarge, cells selectively loosen this network, enabling it to yield to the expansive forces generated by cell turgor pressure (Deniel and Cosgrove, 2001). There are many cell wall models proposed by different scientists. Keegstra et al., 1973 proposed that matrix polymer, consisting of xylogulcan, pectic polysaccharides, and structural proteins, were covalently linked to form a giant macromolecular network where the matrix is bounded via H-bonding. The most popular one is “tethered network” model (Carpita and Gibeaut, 1993) (Fig. 1.7) where cellulose microfibrils may be tethered together directly via long xyloglucan chains. But there is no definitive evidence at present favoring the tethered network model over the others. Out of the many differences, the models share a common concept that cellulose microfibrils are coated with xyloglucan. McCann et al., (1990) and Daniel et al., (1999) suggested that Xyloglucan coats and cross-linking cellulose microfibrils and disruption of the cellulose/xyloglucan matrix may be a key element in regulation of wall integrity. Additional hemicellulosic polymers, including xylans, arabinoxylans, mannans, and galactoglucomannans, have been detected in different fruit species.

A representative structure for an Arabidopsis leaf primary cell wall that is consistent with more specialized models (Vincken et al., 2003) as viewed from electron microscope is depicted in (Fujino et al., 2000; McCann et al., 1990) (Fig. 1.8). The complexity of the image underscores the challenge associated with understanding the structure, function, and synthesis of plant cell walls. According to this model Arabidopsis cell wall has three major classes of polysaccharides: cellulose, hemicellulose and pectins. Cellulose is long unbranched fibrils composed of approximately 30 to 36 hydrogen bonded chains of beta-1-4-glucose. Hemicelluloses are branched polysaccharides with neutral sugar backbones forming hydrogen bonds with cellulose fibrils. Pectins are made of uronic acids. The simplest of these is homogalacturonan (HG), an unbranched polymer of (1-4) α-D-galacturonic acid. Rhamnogalacturonan I (RGI) has a backbone composed of alternating (1-2) α-L-rhamnose and (1-4) α -D-galacturonic acid decorated primarily with arabinan and galactan side chains. It has recently been suggested that RGI functions as a scaffold to which other pectins, such as rhamnogalacturonan II (RGII) and HG, are covalently attached as side chains (Vincken et al., 2003).
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Activity</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rin, ripening-inhibitor</td>
<td>Transcription factor</td>
<td>Comprehensive ripening</td>
<td>Tigchelaar et al., 1978.</td>
</tr>
<tr>
<td>Nor, non-ripening</td>
<td>Transcription factor</td>
<td>Comprehensive ripening</td>
<td>Tigchelaar et al., 1978.</td>
</tr>
<tr>
<td>Nr, Never-ripe</td>
<td>C_{2}H_{4} receptor</td>
<td>Ethylene signaling</td>
<td>Wilkinson et al., 1995.</td>
</tr>
<tr>
<td>Hp-2, high pigment-2</td>
<td>DET1 homolog</td>
<td>Light signaling</td>
<td>Mustilli et al., 1999.</td>
</tr>
<tr>
<td>cr, crimson</td>
<td>Lycopene cyclase</td>
<td>Carotenoid metabolism</td>
<td>Ronen et al., 1999.</td>
</tr>
<tr>
<td>B, Beta</td>
<td>Lycopene cyclase</td>
<td>Carotenoid metabolism</td>
<td>Ronen et al., 1999.</td>
</tr>
<tr>
<td>hp-1, high pigment-1</td>
<td>NA</td>
<td>Light signaling</td>
<td>Wann et al., 1985.</td>
</tr>
<tr>
<td>alc, alcobaca</td>
<td>NA</td>
<td>Comprehensive ripening</td>
<td>Kopeliovitch et al., 1981.</td>
</tr>
<tr>
<td>Gr, Green ripe</td>
<td>NA</td>
<td>Comprehensive ripening</td>
<td>Kerr, 1981.</td>
</tr>
<tr>
<td>Cnr, Clear non ripe</td>
<td>NA</td>
<td>Comprehensive ripening</td>
<td>Thompson et al., 1999.</td>
</tr>
<tr>
<td>Gf</td>
<td>NA</td>
<td>Comprehensive ripening</td>
<td>Akhtar et al., 1999.</td>
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<tr>
<td>t, tangerine</td>
<td>NA</td>
<td>Carotenoid metabolism</td>
<td>Rick and Butler, 1956.</td>
</tr>
<tr>
<td>at, apricot</td>
<td>NA</td>
<td>Carotenoid metabolism</td>
<td>Jenkins and Mackinney, 1955.</td>
</tr>
<tr>
<td>ACO</td>
<td>ACC oxidase</td>
<td>C2H2 Biosynthesis</td>
<td>Picton et al., 1993.</td>
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<td>LeETR4</td>
<td>Ethylene receptor</td>
<td>Ethylene signaling</td>
<td>Tieman et al., 2000.</td>
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<tr>
<td>TCTR1</td>
<td>Putative MAPKKK</td>
<td>Ethylene signaling</td>
<td>**</td>
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** Adams, Kannan, Barry, and Giovannoni, unpublished.

Table 1.1 Tomato germplasm altered in ripening.
Figure 1.7 Two models of the plant cell wall
Figure 1.8 (A) General model of plant cell wall. (B) Scale model of the polysaccharides in an Arabidopsis leaf cell wall. The amount of the various polymers is shown based approximately on their ratio to the amount of cellulose. The amount of cellulose shown was reduced, relative to a living cell for clarity. The figure is an elaboration of a model originally presented by McCann et al., 1990.
Ripening in many fruits is associated with textural changes that are believed to result from disassembly of the primary cell wall. This includes modification of the structure and composition of the constituent polysaccharides that have been correlated with the expression of a range of hydrolases and transglycosylases (Lashbrook et al., 1997).

1.2.7 Cell wall metabolism and softening

Plant cell growth is constrained by the cell wall, whose rigid cellulose microfibrils are held together by three structurally independent but interacting matrices (Carpita and Gibeaut, 1993). The cell wall is the target for many hydrolytic enzymes which modify the polysaccharide content resulting in disassembly or softening. Fruit softening is a complex process that could involve three sequential steps: loosening of cell wall mediated by expansins, depolymerisation of hemicellulose, and finally polyuronide depolymerisation by polygalacturonase or other hydrolytic enzymes (Brummell et al., 1999a). During fruit softening, pectins (Lashbrook et al., 1997) and hemicelluloses typically undergo solubilisation and depolymerisation that are thought to contribute to wall loosening and disintegration, although the relative extent and timing vary between species.

Since the isolation of the tomato fruit POLYGALACTURONASE (PG) gene, ripening research has focused on ripening-related cell wall metabolism and associated textural changes. Antisense repression in unripe tomato indicated that PG alone is not sufficient for softening. Nevertheless, a reduction in ripe fruit susceptibility to postharvest pathogenesis in antisense PG fruit led to the commercialization of PG antisense tomatoes. After the collapse of the hypothesis that PG represented the primary determinant of tomato fruit softening caused attention to turn to the isolation and functional analysis of alternative cell wall-associated and/or metabolizing proteins (Brummell and Harpster, 2001). Antisense repression of pectin methyl esterase (PME) increased juice viscosity attributable to the retention of preripening pectin chain length, and did not have any impact on softening (Tieman et al., 1992). Further CEL1 and CEL2 altered pedicel and fruit abscission but did not influence fruit softening (Lashbrook et al., 1998). But β-galactosidase gene did have a modest impact on fruit softening when repressed via antisense (Smith et al., 2002). Many more efforts to reveal the molecular basis of changes in firmness, which are a major contributing factor to fruit quality, have focused on cell wall-associated enzymes, which are believed to mediate and/or contribute to cell wall breakdown, therefore, understanding about the enzymes involved in fruit cell wall metabolism and softening is important for genetic engineering for shelf life improvement. Although considerable progress has been made in determining the biochemical contribution of specific cell wall proteins during fruit
ripening, the molecular basis of fruit softening is still poorly understood and remains an active area of investigation.

The wall contains many enzymes able to modify matrix polysaccharides. These include various endoglycanase that may cleave the backbone of matrix polysaccharides; glycosidases that may remove side chains, thus allowing greater interactions between polysaccharide backbones; transglycosylases that may cut polysaccharides and ligate them together; esterases that can remove methyl groups from pectins and cleave ester linkages between polysaccharide chains; and peroxidases that may form or break phenolic linkages in the wall (Deniel and Cosgrove, 2001). These enzymes offer many possibilities for altering wall structure, and thereby modulating wall expansion. Table 2 lists all the genes targeted and the technology used to investigate their role in ripening and or softening. Here we run into a subtlety of what constitutes wall loosening. Wall hydrolytic enzymes may physically weaken the wall, yet not induce wall extension (Cosgrove, 1999).

1.2.8 Enzymes involved in cell wall disassembly

Many different cell wall modifying enzymes are present in fruit. Some activities are present throughout development and increase or decrease during ripening, whereas others are ripening specific and appear only during ripening. Fruit ripening, or senescence, is a complex process involving ultra-structural modifications of the cell wall arising through the combined activities of cell wall-associated enzymes. Ripening leads to softening which is accompanied by alterations both in the architecture and physicochemical properties of the cell wall, and the polymers.

1.2.8.1 Expansins

Expansins were first isolated in 1992 as the mediators of "acid growth" which refers to the increase in growth rate when placed in acidic solution. Such stimulation comes about because the cell wall becomes more extensible at acidic pH. Expansins are known to disrupt hydrogen bonding between cellulose fibers (McQueen-Mason and Cosgrove, 1994). Expansin activity is also stimulated and inhibited by the same chemical agents that affect acid growth of walls (Reinhardt et al., 1998). The detailed work on expansins mediate acid-induced extension was done in cucumber hypocotyl walls (Cosgrove, 1999). Expansins characteristically induce extension and stress relaxation of isolated cell walls, but they do not hydrolyse the major polysaccharides of the wall matrix (McQueen-Mason et al., 1992). The evidence for expansins as significant wall loosening enzymes (Cosgrove, 1999) came from the fact that they can induce extension of walls in-vitro; their applications to living cells induces faster growth; their genes are expressed at the right time and in the right place to function in growth control. Further the
reduction of expansin gene expression by antisense methods resulted in growth inhibition. Rose and Bennett, (1999) found high expression of an expansin gene during the last stages of fruit ripening. The time course for expansin action was studied which revealed that walls began extending within seconds of protein addition, without the lag expected of a hydrolytic enzyme that acts by changing matrix viscosity. Expansin appears to act catalytically, rather than stoichiometrically (Cosgrove, 1999).

Most of the expansins are known only from sequence, not from their biological activities. It remains to be seen whether the functional definition of expansin and the sequence definition are fully congruent with each other. Till date two families of expansins are recognized which are called α- and β-expansins (Cosgrove, 1998 and Cosgrove, 1997). The knowledge of how expansins make the wall more extensible is still limited, but most evidence points to a subtle mechanism, such as destabilization of glycan-glycan interactions, rather than hydrolysis of matrix polymers. Studies indicate that α-expansins lacks significant hydrolytic activity against the major polysaccharides of the wall (McQueen –Mason and Cosgrove, 1995). Confirmatory of this conclusion, α-expansins does not lead to a progressive, time-dependent weakening of the wall, as would be expected of a hydrolytic enzyme. Further analysis of expansin genes suggests that they encode three functional domains, separated by introns. The N terminus of the primary transcript has a signal peptide of ~22-25 amino acids. The C terminus has a series of conserved tryptophans with spacing similar to that of some bacterial CBDs (carbohydrate binding domains) (Din et al., 1994). Several references have accumulated relating to the activity and expression of expansins. Among them four α-expansins isolated from rice internodes demonstrated to have classical expansin activity, i.e. they induced wall extension at acidic pH (Cho and Kende 1998). α-expansin genes were highly expressed during the period of maximal growth of the fiber in cotton (Shimizu et al., 1997). Cosgrove, 1999 reported nine different α-expansin genes in tomato, six in tobacco and 22 in Arabidopsis. An in-situ hybridization study of the tomato shoot apical meristem indicated that an expansin gene is locally expressed in the cells underlying the site of future outgrowth of leaf primordia and is turned on in sub epidermal cells prior to primordial emergence; this appears to be earliest markers of leaf initiation (Reinhardt et al., 1998).

The second class, β- Expansins are more divergent in sequence and perhaps in function, they share only ~25% amino acid identity with α-expansins, but they appear to be homologous to α-expansins in structure (Cosgrove, 1999). Only one member of this family of expansins has been analyzed for its action on the cell wall, and the properties of this protein, Zea m 1, may not be typical of the whole family. In pear fruit multiple expansin genes showed differential expression and hormonal regulation during fruit development and among them at least six expansins had overlapping expression during ripening (Hiwasa et al., 2003b). Some of these expansins may be preferentially related to elongation and widening of leaves after integrating several spatial, environmental, genetic, and developmental cues in maize (Muller et al., 2007). Recent report
revealed the involvement of expansins in strawberry fruit softening (FaEXP1, FaEXP2, FaEXP4, FaEXP5 and FaEXP6) in three cultivars which differed in fruit firmness and also found to have a correlation between mRNA expression levels and fruit firmness (Dotto et al., 2006).

### 1.2.8.2 Endoglucanases (EGase)

These enzymes are often referred to as cellulases, but in most of the higher plants the cellulose binding domains is absent unlike the microbial cellulases. Of the numerous hydrolytic activities in the wall, most growth-related studies have focused on enzymes capable of breaking down matrix glucans, specifically xyloglucan and the link 1,3: 1,4-β-glucan, which are abundant in the growing cell walls of dicots and grass seedlings, respectively. Endoglucanases (EC 3.2.1.4) can hydrolyse glucosidic bonds at a site in the middle of the glucan. No structure has yet been determined for plant-derived glucanase that degrade β-glucans with a pure 1, 4-linked backbone. Numerous studies led circumstantial support to the idea that endoglucanases may be involved in cell expansion (Cosgrove, 1999) and wall loosening, however the pattern of its activity raises some doubts on this point. For example, endoglucanase activity reached maximum levels after coleoptile growth ceased, rather than at the time of maximal extension rate. Perhaps the strongest argument in favor of wall-loosening activity of glucanase has been from experiments in which specific antibodies against these proteins were found in auxin induced growth (Hoson et al., 1992).

Multiple isoforms of EGase have been detected in ripening fruit (Kanellis and Kalaitzis, 1992) with varied expression among the stages. In tomato, EGase activity is present throughout fruit development, with highest levels in young expanding green fruit and during ripening (Hall, 1964) but its role in cell wall disassembly is still unclear. In one of the study, ripening-related pepper endo-1, 4-beta-D-glucanase (EGase) CaCell was over-expressed in tomato plants under the control of the constitutive 35S promoter to investigate the effects on plant growth, fruit softening and cell wall- degrading activity. Transgenic fruits appeared the same as the control or slightly firmer at both green and red developmental stages and no differences in plant vegetative growth was observed (Harpster et al., 2002). Some reports even support the idea that the tomato Cell EGase responds to pathogen infection and presume to have a relationship between EGases, plant defense responses and fruit ripening (Real, 2004).

### 1.2.8.3 Xyloglucan Endotransglycosylase (XET)

This enzyme, which has been called endoxyloglucan transferse or XEGT (Nishitani, 1997), catalyses a kind of molecular grafting reaction in which the backbone of a xyloglucan is cleaved and one of the resulting half-chains is added to the non-reducing end of second xyloglucan chain. XET was proposed to have wall loosening activity (Fry et al., 1992), but direct tests of its ability to catalyse wall extension in-vitro have not supported this hypothesis. Several
other possible functions for this enzyme, including incorporation of newly secreted xyloglucan into the wall, as well as rearrangement of the xyloglucan-cellulose network during wall assembly and growth, have been suggested by Nishitani (1997). XET may be responsible for the shifts in xyloglucan size recorded in several studies (Nishitani and Masuda, 1982; Talbott and Pickard, 1994). Plant XET proteins are moderate sized (~33kD) and, at least in some cases are N-glycosylated. Glycosylation seems to be important for enzyme activity. Recent studies have identified XETs as a subset of a larger family of related proteins called XRPs, for xyloglucan-related proteins (Nishitani, 1997). In arabidopsis more than 16XRPs, which may have distinct pattern of expression in the plant have been reported.

Among the Xyloglucan endotransglucosylase/hydrolases (XTHs) (Rose et al., 2002), one of the cell wall-modifying enzyme from tomato (SIXTH5) was of particular interest as it was expressed abundantly during ripening in tomato (Saladié et al., 2006). A very recent report on comprehensive bioinformatics analyses of XTH gene products, together with detailed kinetic data, strongly suggest that xyloglucanase activity has evolved as a gain of function in an ancestral GH16 XET to meet specific biological requirements during seed germination, fruit ripening, and rapid wall expansion (Baumann, 2007).

1.2.8.4 Polygalacturonase (PG)

The tomato fruit ripening is accompanied by an increase in polygalacturonase (EC 3.2.1.15) activity, which was the first cell wall hydrolase to be examined using transgenic methods, which depolymerizes the pectin of the middle lamellae between the cells of the pericarp (Hobson, 1964). Much was not known about the regulation and expression of this enzyme (Fisher and Bennett, 1991 and Hobson and Grierson, 1993) as it was thought that it may not play a major role in the softening of fruit in-situ (Brummell and Labavitch, 1997). Chromatographic separation of the extract of ripe fruits revealed two isoenzymes, PG1 and PG2, which act randomly as endoenzymes, prefer lower-esterified pectins for higher activity, and have pH optima of 3.8-4.5, depending on substrate size and ionic strength of the medium (Pressey and Avants, 1973). PG1 and PG2 show molecular weight of 100 kD and 42-46 kD respectively (Tucker et al., 1980). At the start of ripening PG1 was found exclusively, followed by a decrease or further increase (Knegt et al., 1988). In green fruits a presumably heat-stable factor is found, which can convert PG2 in-vitro into a form that resembles PG1 in several respects (Tucker et al., 1981). Ali and Brady, (1982) reported three isozymes:PG1, PG2A and PG2B, but PG2A and PG2B appear to function as monomeric catalytic subunits of 45 kD and 46 kD, respectively, with the size difference arising from a difference in extent of glycosylation (DellaPenna and Bennett, 1988). Most work on polygalacturonase has focused on characterizing the PG2A and PG2B catalytic subunits, which function both as monomers and as subunits of PG1 (Katherine et al., 1990). The biosynthesis and maturation of these monomers is themselves complex. Both PG2A and PG2B appear to be the
products of a single gene and are synthesized as a larger molecular weight precursor that undergoes a number of processing events during conversion to the mature protein. DellaPenna and Bennett (1988) suggested that the differences in glycosylation between PG2A and PG2B result from differences in co-translational core glycosylation rather than from differential processing of the glycan side chains. PG1 as an artifact of extraction, PG2A and PG2B being the only isozymes present in-vivo was reported by Pressey (1988), whereas Knegt et al (1988) presented data contrary to this view and proposed that PG1 is the physiologically functional isozyme in-vivo. Although a correlation between polygalacturonase accumulation and tomato fruit softening has been documented (Huber, 1983), later it was indicated that, by itself, degradation of polyuronides by polygalacturonase is insufficient to cause tomato fruit softening (Giovannoni et al, 1989). Although early models implied that PG-catalysed pectin degradation represent the fundamental process underlying fruit softening (Crookes and Grierson, 1983), however, molecular genetic approaches subsequently revealed that PG-dependent pectin degradation is not essential for fruit softening (Giovannoni et al., 1989), but may play a role in other aspects of fruit quality. This suggests that other wall polymers contribute significantly to fruit firmness.

Daniel et al., (1999) reported that polygalctouronase (β-subunit) is expressed in tomato leaves in response to wounding. Further, polygalacturonase mediated pectin disassembly was reported by Hadfield et al. (2000) in ripe melon fruit, contradicting the earlier report (Hobson; 1962) of lack of PG activity in melon fruit. The activity of polygalacturonase in banana during ripening been positively correlated with softening of the fruit tissue and differential expression of this gene is suspected to be regulated by the plant hormone ethylene (Asif and Nath, 2005). Recent report revealed that non-specific lipid transfer proteins (nsLTPs) is capable of converting the gene product PG2 into a more active and heat-stable form, which increases PG-mediated pectin degradation in-vitro and stimulates PG-mediated tissue breakdown in planta. This finding suggests a new, not previously identified, function for nsLTPs in the modification of hydrolytic enzyme activity and modulating PG activity during tomato fruit softening (Tomassen et al., 2007).

1.2.8.5 Endo-β-mannanase

Mannose-containing polysaccharides are widely distributed in cell walls of higher plants. During endosperm mobilization in germinated tomato seeds (1-->4)-beta-mannan endohydrolases (EC 3.2.1.78) participate in the enzymic depolymerization of these cell wall polysaccharides. Overall, the enzyme exhibits only 28-30% sequence identity with fungal (1-->4)-beta-mannanases, but more highly conserved regions, which may represent catalytic and substrate-binding domains. (Bewley et al., 1997). The tomato fruit cell wall is composed of protein and three major polysaccharide components, pectin, hemicelluloses and cellulose (Gross, 1984). The hemicellulose polymers include xyloglucans, glucomannans and glucactomannans which are covalently linked to pectin and hydrogen bonded to cellulose (Fisher and Bennett, 1991). Since
mannans are a component of tomato fruit cell walls the activity of endo-β-mannanase (EC 3.2.1.78), a high salt-soluble enzyme which hydrolyses these polymers, has been studied and it has been suggested that it plays a role in fruit ripening. There are reports on the presence of this enzyme in ripening tomato fruit (Sozzi et al., 1996). Endo-β-mannanase is very active in tomato seeds during and following germination and it is highly soluble occurring in many isoforms (Viogt and Bewley, 1996). Bewley et al (2000) showed that the outer pericarp loses its integrity and becomes very soft during the late stages of ripening. The enzyme activity increased during the breaker/turning stage, and activity was associated with the skin and the region immediately beneath it throughout fruit ripening. The molecular weight was about 40 kD as determined by SDS-PAGE.

1.2.8.6 Pectate Lyase

Rapid softening during ripening is one of the main causes of the short postharvest shelf life of climacteric fruits therefore; any improvement of softening behavior could have a significant commercial importance. Histological analysis of ripe fruit showed a cell wall thinner than unripe fruit and with little contact and separated by considerable intercellular space (Redgwell et al., 1997). The largest changes in the plant cell wall during ripening occur in the pectin component. The percentage of water-soluble pectins increases during ripening but total quantity of polyuronide residues (Redgwell et al., 1997) and polyuronide length are slightly modified. Alternative to the role of pectin modification in softening, several authors have focused on the degradation of the cellulose matrix as the primary cause of the loss of fruit firmness. Along this line, Knee et al. (1977) observed that the cell wall became swollen during fruit development and this higher hydration was attributed to the change in the neutral sugars of the cell wall fractions resulting in the degradation of the hemicellulose and cellulose.

Pectate lyase gene which was isolated from ripe strawberry has been proposed as a new candidate for pectin degradation, contributing to the loss of fruit firmness (Medina-Escobar et al., 1997) Pectate lyases have been extensively studied in pathogenic bacteria, which secreted this enzyme causing depolymerization of pectins in middle lamella and primary cell wall of higher plants, and consequently the maceration of plant tissues (Henrissat et al., 1995). The degradation of pectins by pectate lyase occurs by a β-elimination reaction in contrast to the hydrolytic mechanism of polygalacturonase. Contrary to the abundant literature about the role of polygalacturonase in fruit ripening, no data on pectate lyase activity in fruit have been reported so far (Jimenez-Bermudez et al., 2002). Thus, the role of these genes in fruit softening remains uncertain. In other plant species, pectate lyase-like genes have been isolated, of which most of them are related to pollination because they are highly expressed in mature pollen grains and pollen tubes (Dricks et al., 1996). In case of strawberry, the reduction of the steady-state levels of pectate lyase mRNA resulted in increase in firmness of full ripe fruit and reduced the post harvest
softening, without affecting other fruit characteristics such as weight, colour, or soluble solids. Thus, this gene is an excellent candidate for biotechnological improvement of strawberry fruit softening.

1.2.8.7 Pectin Methylesterase (PME)

Pectin methylesterase (EC 3.1.1.11) is a cell-wall metabolizing enzyme responsible for the demethylation (de-esterification) of galacturonic acid residues in high molecular weight pectin. It is characterized by the existence of multiple isoforms, and up to eight different isoenzymes have been detected in tomato fruit, depending up on the variety, and method of isolation (Pressey and Avants, 1972; Hall et al., 1994). In tomato PME is present throughout fruit development, with activity increasing two to three fold during ripening (Hobson, 1963). Ray et al. (1988) have isolated two major isozymes (PME1 and PME2) and the ratio of these two enzymes changes during ripening, with PE2 becoming more dominant as ripening progresses.

PME is an enzyme found in all plants and in many plant pathogenic bacteria and fungi (Huber, 1983), it demethoxylates pectin to form a carboxylated pectin while releasing methanol and a proton. It catalyzes the de-esterification of galactosyluronate methyl esters of pectin to their free carboxyl groups. PME activity has been detected in most plant tissues; however, the enzyme is particularly associated with ripening fruit, abscission zones, and maturing cell walls (Northcote, 1986). It has been suggested that the carboxyl groups of pectins are highly methylesterified when they are synthesized, but the esters are later cleaved by PME (Roberts, 1990). The role of PME in plant cell growth and development is still not yet clear. It has been suggested that demethoxylation is necessary for the formation of Ca\(^{2+}\) across bridges in pectins, leading to stabilization of the middle lamella (Northcote, 1986).

Although several cell wall hydrolase have been implicated in fruit softening, special attention has been paid to the degradation of polyuronides (Fisher and Bennett, 1991). PME may play a role in fruit softening by increasing in vivo susceptibility of pectins to polygalacturonase during ripening (Koch and Nevins, 1989). Increase in PME activity has been reported during ripening of several fruits, including tomato (Harriman et al., 1991). Unlike polygalacturonase, which is produced during ripening, PME is produced during development of tomato fruit (Harriman et al., 1991). When the antisense and sense chimaric PME gene was introduced in to tomato, the transgenic plants expressing high levels of antisense PME RNA showed <10% of wild-type PME enzyme activity and undetectable levels of PME protein and mRNA. Lower PME activity in fruits from transgenic plants was associated with an increased molecular weight and methylesterification of pectins and decreased levels of total and chelator soluble polyuronides in cell walls. The fruits of transgenic plants with antisense construct also contained higher levels of soluble solids than wild-type fruits (Denise et al., 1992).

In grapes the expression of PME mRNAs started accumulating from one week before the onset of ripening until complete maturity, indicating that this transcript represents an early marker
of veraison and could be involved in berry softening (Barnavon et al., 2001). Pectinmethylesterase and polygalacturonase are known to operate in tandem to degrade methylesterified polyuronides in avocado and tomato during fruit ripening (Wakabayashi et al., 2003).

1.2.8.8 β-Galactosidase

Among the other known pectin modification that occur during fruit development, one of the best characterized is the significant net loss of galactosyl residues that occurs in the cell walls of many ripening fruit (Seymour and Gross, 1996). Although some loss of galactosyl residues could result from the action of PG, β-galactosidase (EC 3.2.1.23) is the other only enzyme identified in higher plants capable of directly cleaving β (1-4) galactan bonds, and probably plays a role in galactan side chain loss (Carrington and Pressey, 1996). The view that β-galactosidase is active in releasing galctosyl redsidues form the cell wall during ripening is supported by the dramatic increase in free Gal, a product of β-galactosidase II activity in toamtoes during ripening. β-galactosidase has pl values of 5.0, 5.1 and 5.2, and molecular weight of 41, 49 and 54 kDa, respectively (Ian De Veau et al., 1992). This enzyme can be separated into three forms, termed β-galactosidase I, II and III (Pressey, 1983). In tomato, β-galactosidase are encoded by a gene family of at least seven members (Smith and Gross, 2000) and the genes have been termed TBG1-TBG7. Beta-galactosidase is thought to be involved in fruit softening through cleaving beta (1->4) galactan bonds in cell wall hemicellulose. Recently in banana the northern analysis showed that the MA-Gal transcripts in pulp were at low level during pre-climacteric stage, but an increasing progression was observed during fruit ripening, and the highest transcript was found at the post-climacteric stage. These results suggest that MA-Gal may play a role during banana fruit ripening and softening (Zhuang et al., 2006). During papaya ripening, alpha-galactosidase activity increased concomitantly with firmness loss (Lazan et al., 2004, Soh et al., 2006) again indicating its role in fruit ripening and/or softening.

1.2.8.9 α-D-Mannosidase

α-Mannosidases are widely distributed in nature. Purified from various vegetable and animal species, the enzymes can serve as a structural reagent for those glycoproteins having D-mannose as a common constituent (Curdel and Petek, 1980). Interest in these enzymes was increased by the discovery that mannosidase deficiency in mammals leads to a hereditary disease that can be related to other lysosomal storage diseases such Hurler’s, Tay-Sachs and Sandhoff’s diseases.

α-D-Mannosidase (EC 3.2.1.24), a glycosidase shows consistent increase in activity with the process of softening in Capsicum annum (Priya seth and Prabha, 1997) and tomato (Suvarnalatha and Prabha, 1999). Interestingly it also showed an activity peak during ripening/softening of mango, banana, papaya, bell pepper etc. This enzymes cleaves the short chain oligo-mannose residues (8~10) present in oligosaccharides and glycoproteins. The Physiological role of most of the glycosidase is not known. Further, no correlation between α-D-
Mannosidase activity and physiological function has been reported and there is no known natural substrate identified for this enzyme \textit{in vivo}. In Capsicum only one isoform is reported but tomato is known to have two isoforms of the enzyme. Till date no experimental data is available to prove their involvement in fruit ripening.

1.2.9 Fruit texture analysis

Texture can be regarded as a manifestation of the rheological properties of a food (Pomeranz and Meloan, 1994). It is an important attribute which affects processing and handling (Charm, 1962), influences food habits, and affects shelf-life and consumer acceptance of foods (Matz, 1962). Characterization of food texture commonly falls into two main groups, based on sensory and instrumental methods of analysis. Sensory analysis includes use of the senses of smell, taste, sound and touch. Evaluation of food texture by touch includes the use of the fingers, as well as the lips, tongue, palate and teeth in the mouth. As would be expected, sensory methods of analysis are subject to wide variability, though this variability can be reduced by using trained assessors. It is sometimes preferable to use instrumental methods of assessing food texture rather than sensory analysis because they can be carried out under more strictly defined and controlled conditions. Furthermore, problems of experimental variability are more likely to be caused by sample heterogeneity than by instrumental imprecision. Another reason for instrumental analysis may be that often changes in ingredient levels cause several simultaneous changes in product characteristics. Some of these changes are difficult to mask and thus tend to make sensory analysis difficult, e.g. variation in cake firmness due to sugar content. Therefore a main goal of many texture studies is to devise one or more mechanical tests with the capacity to replace human sensory evaluation as a tool to evaluate food texture (Peleg, 1983).

Instrumental techniques of studying the rheological behaviour of foodstuffs can be classified into three groups, Fundamental tests, Empirical tests and Imitative tests, according to the type of information obtained by the test (Voisey, 1976). Fundamental tests determine one or more physical constants to describe exactly the properties of the food in terms of well defined rheological parameters. Empirical tests usually measure parameters which are poorly defined in rheological terms but which, from practical experience, have been found to relate closely to the property of interest. The third group, imitative tests, aims to reproduce the mechanical operations applied in human evaluation and which have been shown to correlate closely with sensory assessment. The numerous probes and fixtures, for use on the TA.XT Plus texture analyser, which are based on empirical principles, may be classified according to the type of action involved. Like Compression, Puncture & Penetration Cutting & Shearing Extrusion Tension, Fracture & Bending/Snapping and adhesion.
1.2.10 Plant volatiles: the invisible bouquet
Of the thousands of different metabolites that plants can produce, may form a cloud around the plant. These volatile compounds reflect the metabolic complexity of plants and also serve a diversity of functions. Many volatiles are produced in plant tissues at specific developmental stages— for example, during flowering, ripening, or maturation (Goff and Klee, 2006). Tomato being the model for fruit development, more is known about the chemicals contributing to tomato flavors. These volatiles or their precursors have antimicrobial or other health-promoting activities. Thus, flavor volatiles can be perceived as positive nutritional signals. Many volatile organic compounds are also produced in the grape which determines the wine quality. The major chemical determinants of flavor and wine quality in grape berries are predominantly localized to mesocarp (flesh) or exocarp (skin). These include terpenes, geraniol, linalool, terpineol, nerolidol and many more (Lund and Bohlmann, 2006). Many of the volatiles are known to be stored as amino acid conjugates, which are needed to be broken for volatilization of the compounds for perception. This is done by the glycosidases and peptidases, which cleave the conjugates that play a vital role in the timing and production of natural fruit flavors. These volatiles play a major role in signal transduction within a plant or between a plant in response to a particular condition or a process. The modulators of volatile organic compounds include pathogen/virus, herbivores, pollinators, temperature, humidity and radiations (Baldwin et al., 2006).

1.3 Strategies to improve shelf life

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1.3.1 RNA silencing
RNA silencing is a novel gene regulatory mechanism that limits the transcript level by either suppressing transcription (transcriptional gene silencing [TGS]) or by activating a sequence-specific RNA degradation process (posttranscriptional gene silencing [PTGS]/RNA interference [RNAi]) (Agrawal et al., 2003). The phenomenon of RNAi first came into the limelight following the discovery by Fire et al., 1998, who unequivocally demonstrated the biochemical nature of inducers in gene silencing by introducing purified dsRNA directly into the body of Caenorhabditis elegans. Although there is a mechanistic connection between TGS and PTGS, TGS is an emerging field while PTGS is undergoing an explosion in its information content. As PTGS is very relevant to our work, we discuss it in detail.
1.3.2 Posttranscriptional gene silencing (PTGS)/RNAi

RNA interference (RNAi) is a well-recognized, potent and highly specific gene-silencing phenomenon or pathway initiated or triggered by double-stranded (ds) RNA and involved in cellular defense against viral invasion, transposon expansion, and post-transcriptional regulation. This evolutionarily conserved phenomenon is observed in nearly every eukaryote studied thus far, and represents a unique form of post-transcriptional gene silencing (PTGS) (Rana, 2007). The discovery of dsRNA as potent trigger of gene silencing in the nematode Caenorhabditis elegans (Fire, 1998) was an important achievement. Since these early observations, several groups have contributed to elucidating the post-transcriptional gene silencing mechanisms (Fig. 1.9) (Kurreck, 2003) in eukaryotes and RNAi pathway, diagrammed in Fig. 1.10A. Posttranscriptional gene silencing occurs in plants and fungi transformed with foreign or endogenous DNA and results in the reduced accumulation of RNA molecules with sequence similarity to the introduced nucleic acid (Vaucheret et al., 1998; Cogoni and Macino, 1997). During the process of RNAi/PTGS, double-stranded RNA (dsRNA) molecules, which cleave the inducer molecules into smaller pieces first (Bender, 2001) and eventually destroy the cellular or viral cognate mRNA molecules (called the target) (Bernstein et al., 2001) act as inducers or activators of this process. As a result, the target mRNAs cannot accumulate in the cytosol, although they remain detectable by nuclear run-on assays (Fagard and Vaucheret, 2000). PTGS can be suppressed by several virus-encoded proteins (Pruss et al., 1997; Anandalakshmi et al., 1998; Kasschau and Carrington 1998; Brigneti et al., 1998; Beclin et al., 1998) and is closely related to RNA-mediated virus resistance and cross-protection in plants (Lindbo et al., 1993; Ratcliff et al., 1997; Plasterk, 2002). Therefore, PTGS may represent a natural antiviral defense mechanism and transgenes might be targeted because they, or their RNA, are perceived as viruses (Covey et al., 1997). PTGS could also represent a defense system against transposable elements and may function in plant development (Flavell 1994, Jorgensen et al., 1998 and Voinnet et al., 1998). A preferred method for delivering dsRNA to plants for inducing RNA interference (RNAi) is to introduce transgenes engineered to express self-complementary transcripts that can ‘fold back’ to form dsRNA molecules (Waterhouse and Helliwell 2003). The principal advantage of integrated transgenes over transiently introduced dsRNA is that the silencing phenotype is sexually transmissible, and so permanent collections of stable RNAi lines can be produced and archived in public repositories.

RNA silencing in plants has emerged as a topic of general interest in recently years. But the first report on RNA silencing was published as long as 1928 by Wingard (Wingard, 1928). In his paper he showed upper leaves had somehow become immune to the virus and consequently were asymptomatic and resistant to secondary infection. Further the RNA silencing story unfolded serendipitously during a search for transgenic petunia flowers that were expected to be purpler. In 1990, R. Jorgensen’s laboratory wanted to up regulate the activity of a gene for
Figure 1.9 Five mechanisms of post-transcriptional gene silencing in eukaryotes. (A) An antisense oligonucleotide, either single-stranded DNA or RNA, may bind complementary target mRNA and thereby block translation. (B) Protein activity may be silenced by an inhibitor that binds the protein directly. (C) A catalytically active oligonucleotide may promote degradation of target mRNA molecules. (D) Short double-stranded RNA molecules, known as small interfering RNA or siRNA, may incorporate into the RNA Induced Silencing Complex (RISC) and catalyze cleavage of complementary mRNAs. (E) Endogenous microRNAs (miRNA) may incorporate into the RISC to repress mRNA translation—a mechanism that is not yet fully understood.
chalcone synthase (chsA), an enzyme involved in the production of anthocyanin pigments. Surprisingly, some of the transgenic petunia plants harboring the chsA coding region under the control of a 35S promoter lost both endogene and transgene chalcone synthase activity, and thus many of the flowers were variegated or developed white sectors (Napoli et al., 1990) this was attributed to silencing. Three natural pathways of RNA silencing in plants are described by genetic and molecular investigation (Baulcombe, 2004). All these pathways involve the cleavage of a double stranded RNA (dsRNA) into short 21-26 nucleotide RNAs by an enzyme Dicer that has RNAase III domains. The first pathway describes about the cytoplasmic siRNA silencing (Hamilton and Boulcombe, 1999). This pathway is important in virus-infected plant cells where the dsRNA could be a replication intermediate or a secondary-structure feature of single-stranded viral RNA. The second pathway is the silencing of endogenous messenger RNAs by miRNA. These miRNA negatively regulate gene expression by base pairing to specific mRNAs, resulting in either RNA cleavage or arrest of protein translation. The third pathway of RNA silencing in plants is associated with DNA methylation and suppression of transcription. Recent findings indicate that siRNA-directed DNA methylation in plants is linked to histone modification (Zilberman et al., 2003).

Although RNA silencing is relatively recent topic of research which has completely changed our view of RNA as a regulatory molecule in eukaryotic cells and further it is likely that this view will continue to evolve as discoveries emerge about the diversity of silencing mechanisms.

1.3.2.1 siRNA: Synthesis, mechanism and gene knockout
RNA silencing refers to small regulatory RNA-mediated processes that repress gene expression. A variety of pathways are implicated in RNA silencing, but they all share certain core biochemical features. RNA silencing starts with the processing of double-stranded RNAs (dsRNAs) or imperfectly self-folded hairpin precursors into small interfering RNA (siRNA) or miRNA duplexes, by RNase III-type Dicer enzymes. The small RNA duplexes are then incorporated into a ribonucleoprotein complex called RNA-induced silencing complex (RISC) that degrades any RNA complementary to small RNAs (Bartel, 2004; Tomari and Zamore 2005). The core component of the RISC complex is an Argonaute (AGO) protein, which has a PAZ domain that provides a 2-3 nucleotide overhang recognition pocket and a PIWI domain that confers endonucleolytic activity (Hall, 2005; Song and Joshua-Tor, 2006). The key steps in siRNA processing are depicted in figure 1.10B

1.3.2.2 RNA silencing: tool to study fruit ripening
RNAi system was developed in which gene specific sense and antisense sequences with a spacer sequence in between could induce post transcriptional gene silencing (PTGS) efficiently and it
Figure 1.10 The RNA interference pathway. (A) The pathway is triggered by double-stranded RNA molecules that share sequence specific homology to the target mRNA. The mediators of RNAi are 21-25 nucleotide small interfering RNA duplexes (siRNAs) derived from the digestion of the ds RNA triggered by a RNase III like enzyme known as Dicer. The siRNAs are incorporated into RISC. RISC is then activated by ATP and probes the target mRNA for complementary sequences. (B) Key steps in siRNA processing.
worked for both viral and endogenous genes. To investigate any gene function, introduction of long dsRNA, which is a triggering molecule of PTGS, is a method of choice. Virus induced gene silencing (VIGS) offers an attractive technology to down regulate specific gene expression in plants (Dineshkumar et al., 2003). One of the viral vectors developed to study gene function which overcame the limitations of host range and meristem exclusion was vector based on tobacco rattle virus (TRV) (Liu et al., 2002, Ratcliff et al., 2001). Using this vector several reporter genes related to ethylene responses and fruit ripening, including LeCTR1 and LeEILs genes, were successfully silenced during fruit development. Further silencing of the LeEIN2 gene resulted in the suppression of tomato fruit ripening (Fu et al., 2005). Recently, tobacco rattle virus (TRV) based VIGS vector was used to silence LeACS2 gene which delayed post harvest ripening and senescence of tomato (Xie et al., 2006). In another report where, LeEIN2-silenced tomato fruits were developed using a virus-induced gene silencing fruit system to study the role of LeEIN2 in tomato fruit ripening (Zhu et al., 2006). Silenced fruits were delayed in fruit development and ripening, due to greatly reduced expression of ethylene-related and ripening-related genes in comparison with those of control fruits. These results together indicate that VIGS techniques can be successfully applied in tomato and is a valuable tool for studying functions of the relevant genes during fruit developing.

1.3.3 Antisense technology

Messenger RNA (mRNA) is a single stranded molecule that is used as the template for protein translation. It is possible for RNA to form duplexes, similar to DNA, with a second sequence of RNA complementary to the first strand. This second sequence is called antisense RNA. The formation of double stranded RNA can inhibit gene expression in many different organisms including plants, flies, worms and fungi. The suppression of protein synthesis by introducing antisense RNA into a cell is very useful. A gene encoding the antisense RNA can be introduced fairly easily into organisms by using a plasmid vector or using a gene gun that shoots microscopic tungsten pellets coated with the gene into the plant cells. Once the antisense RNA is introduced, it will specifically inhibit the synthesis of the target protein by binding to mRNA. This is a quick way to create a knockout organism to study gene function. Using antisense RNA as a tool in this way is an exciting prospect for many molecular biologists.

Antisense RNA methods have also been used for commercial food production. The best example is Flavr Savr tomato. This tomato was developed by Calgene Inc. of Davis, California in 1991 and was approved by the U.S. FDA in 1994. Since then many attempts were made to use this technology to study fruit ripening. The list of all the genes employed and their effects on the plant have been tabulated in the Table 1.2.
<table>
<thead>
<tr>
<th>Crop</th>
<th>Gene targeted</th>
<th>Technology used</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>Polygalacturonase (PG)</td>
<td>Antisense</td>
<td>Suppression of PG mRNA accumulation was ca.99% in homozygous progeny.</td>
<td>Smith et al., 1988; Sheehy et al., 1988</td>
</tr>
<tr>
<td>Tomato</td>
<td>Ethylene (pTOM13)</td>
<td>Antisense</td>
<td>Ethylene synthesis reduced in a gene dosage-dependent manner.</td>
<td>Hamilton et al., 1990</td>
</tr>
<tr>
<td>Tomato</td>
<td>Pectin Methylesterase (PME)</td>
<td>Antisense</td>
<td>PME expression in pericarp is highly regulated during fruit development and delayed protein synthesis in mutants(Nr, rin, nor).</td>
<td>Harriman et al., 1991</td>
</tr>
<tr>
<td>Tomato</td>
<td>Polygalacturonase</td>
<td>Antisense</td>
<td>Transgenic fruit had just 0.5-1% of PG activity</td>
<td>Krammer et al., 1992</td>
</tr>
<tr>
<td>Tomato</td>
<td>PME(PME2 and PEC2)</td>
<td>Antisense</td>
<td>PME2 activity was undetectable. PME activity was reduced by 10% of the wild. And the degree of pectin methylesterification in transgenic Antisense PME fruit was higher than controls by 15-40% throughout ripening.</td>
<td>Tieman et al., 1992</td>
</tr>
<tr>
<td>Tomato</td>
<td>Polygalacturonase (PG)</td>
<td>Antisense</td>
<td>Transgenic plants with the antisense did not exhibit autolysis and solubalization of pectins.</td>
<td>Carrington et al., 1993</td>
</tr>
<tr>
<td>Tomato</td>
<td>Pectin Methylesterase (PE)</td>
<td>Antisense</td>
<td>PE enzyme activity was reduced up to 93%, as a result there was inhibition of PE mRNA accumulation from endogenous gene/genes. No major difference in fruit development and ripening were apparent.</td>
<td>Hall et al., 1993</td>
</tr>
<tr>
<td></td>
<td>Plant</td>
<td>Description</td>
<td>Antisense Effect</td>
<td>Reference</td>
</tr>
<tr>
<td>---</td>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>8</td>
<td>Tomato</td>
<td>Polygalacturonase (PG)</td>
<td>Fruit suppressed in PG were slightly firmer than non-transgenic controls at all ripening stages.</td>
<td>Langley et al., 1994</td>
</tr>
<tr>
<td>9</td>
<td>Tomato</td>
<td>β-subunit of PG</td>
<td>β-subunit plays a significant role in regulating pectin metabolism by limiting the extent of pectin solubilisation and depolymerisation.</td>
<td>Watson et al., 1994</td>
</tr>
<tr>
<td>10</td>
<td>Tomato</td>
<td>Polygalacturonase (PG)</td>
<td>Plant with excision allele showed reduction in polygalacturonase activity.</td>
<td>Cooley and Yoder., 1998</td>
</tr>
<tr>
<td>11</td>
<td>Tomato</td>
<td>β-galactosidase (TBG3)</td>
<td>Antisense suppression resulted in a reduction in extractable exo-galactanase activity up to 75%.</td>
<td>De Silva and Verhoeven, 1998</td>
</tr>
<tr>
<td>12</td>
<td>Tomato</td>
<td>1-aminocyclopropane-1-carboxylic acid (ACC)</td>
<td>Transgenic fruit when treated with ethylene(0.1-1μL/L) induced PG mRNA accumulation, suggesting that PG mRNA is ethylene regulated and low concentration of ethylene is sufficient for the induction.</td>
<td>Sitrit and Bennet, 1998</td>
</tr>
<tr>
<td>13</td>
<td>Tomato</td>
<td>Expansin (Exp1)</td>
<td>Expansin suppression up to 3%, lead to firmer fruits compared to wild type</td>
<td>Brummell et al., 1999a</td>
</tr>
<tr>
<td>14</td>
<td>Tomato</td>
<td>Endogulcanases (EGases)</td>
<td>mRNA accumulation of the EGases was reduced.</td>
<td>Brummell et al., 1999b</td>
</tr>
<tr>
<td>15</td>
<td>Tomato</td>
<td>LaRab11a (GTPase) which is induced by ethylene</td>
<td>Antisense fruit changed color but failed to soften normally, accompanied by reduced levels of PE and PG</td>
<td>Chungui Lu et al., 2001</td>
</tr>
<tr>
<td>No.</td>
<td>Fruit</td>
<td>Gene</td>
<td>Technology</td>
<td>Result</td>
</tr>
<tr>
<td>-----</td>
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</tr>
<tr>
<td>16</td>
<td>Strawberry</td>
<td>Pectate Lyase</td>
<td>Antisense</td>
<td>An analysis of firmness at three different stages of fruit development (green, Breaker, and red) showed that the highest reduction of softening in fruit occurred during the transition from the white to the red stage. At the stage of full ripen, no differences in color, size, shape, and weight were observed between transgenic and control fruit. However, in most of the transgenic lines, ripened fruits were significantly firmer than controls.</td>
</tr>
<tr>
<td>17</td>
<td>Tomato</td>
<td>LePG or LeExp1</td>
<td>Suppression by antisense</td>
<td>Fruits significantly firmer throughout ripening and were less susceptible to deterioration during long-term storage. Juice prepared from the transgenic tomato fruit with reduced LePG and LeExp1 expression was more viscous than juice prepared from control fruit.</td>
</tr>
<tr>
<td>18</td>
<td>Tomato</td>
<td>ACC synthase and ACC oxidase</td>
<td>Double antisense</td>
<td>The transgenic plants showed the characteristics of prolonged shelf life over 50 d. The amount of ethylene released from the fruits was reduced significantly to about 9.5% of that released by non-transformed controls.</td>
</tr>
<tr>
<td>19</td>
<td>Apple</td>
<td>ACC synthase and ACC oxidase</td>
<td>Gene silencing by RNAi</td>
<td>Fruits were significantly firmer than controls and displayed an increased shelf-life. No significant difference was observed in sugar or acid accumulation suggesting that sugar and acid composition and accumulation is not directly under ethylene control.</td>
</tr>
<tr>
<td>20</td>
<td>Tomato</td>
<td>ACC oxidase</td>
<td>RNAi</td>
<td>Fruit shelf life increased more than 120 days</td>
</tr>
</tbody>
</table>

*Table 1.2* List of genes targeted, technology used and the result obtained due its manipulation in plants to elucidate its role in ripening/softening.
1.4 Carbohydrate-active enzymes (CAZymes)

Plants contain far more carbohydrate-active enzymes than any organism sequenced to date. But why so many carbohydrate-active enzymes are present in plants? This can be explained by the complex structure of the plant cell wall and moreover the carbohydrates in the form of glycosides are central to many biological pathways, from cell wall structure to energy, signaling and defense (Coutinha et al., 2003).

Glycosides are made from activated sugars by glycosyltransferases and are degraded by glycosyl hydrolases (glycosidases). Genes encoding glycosidases and glycosyltransferases in all organisms (all data available form the Carbohydrate-Active enZymes server at http://afmb.cnrs-mrs.fr/CAZY/) are currently analyzed and listed based on the classification of glycosidases (Henrissat and Davis., 1997) and glycosyltransferases (Coutinho et al., 2003b) in sequence based families. At present 110 families of Glycosidases are reported. Figure 1.11 A showes a global correlation between the number of glycosidase and glycosyltransferases-related genes and the total number of genes in the organism. Arabidopsis is a clear outliner with almost 800 glycosidase and glycosyltransferases-related genes comprising > 3.3% of its genes compared to human genome with only 350 glycosidases and glycosyltransferases-related genes (Figure 1.11 A). Another reason of so many CAZymes in plants can be due to genome duplication that was recently established in Arabidopsis (Bowers et al., 2003). Among the CAZymes we restrict our discussion to Glycoside hydrolases (GHs) as they were our main focus. Glycoside hydrolases (EC 3.2.1.-) are a widespread group of enzymes which hydrolyse the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety. The IUB-MB Enzyme nomenclature of glycoside hydrolases is based on their substrate specificity and occasionally on their molecular mechanism. But such a classification does not reflect the structural features of these enzymes. A classification of glycoside hydrolases in families based on amino acid sequence similarities has been proposed a few years ago. Because there is a direct relationship between sequence and folding similarities, such a classification:

(i) Reflects the structural features of these enzymes better than their sole substrate specificity,
(ii) Helps to reveal the evolutionary relationships between these enzymes, and
(iii) Provides a convenient tool to derive mechanistic information. (Henrissat, 1991; Henrissat and Bairoch, 1993; Henrissat and Bairoch, 1996)

The Catalytic Mechanism in most cases, the hydrolysis of the glycosidic bond is performed by two catalytic residues of the enzyme: a general acid (proton donor) and a nucleophile/base (Davies and Henrissat 1995). Depending on the spatial position of these catalytic residues, hydrolysis occurs via overall retention or overall inversion of the anomeric configuration (Fig. 1.11 B-C). For each family listed in the CAZy database, the stereochemical outcome of the reaction catalyzed as well as the type of amino-acid residues acting as a nucleophile/base and as a proton donor is given. In some cases, the catalytic nucleophile is not borne by the enzyme, and is
Figure 1.11 (A) Correlation between the number of glycosidase –(GH) and glycosyltransferase –(GT) related genes and the total number of genes in 86 bacterial and nine eukaryotic genomes. Bacteria are indicated by open diamonds and eukaryotes are indicated by red diamonds. Owing to massive gene loss, parasites tend to appear below the average, for instance, Plasmodium falciparum (a), whereas 'excess' is found in Arabidopsis (b) and in Bacteroides thetaiotamicron (c). (B-C) Depending on the spatial position of these catalytic residues, hydrolysis occurs via overall retention (B) or overall inversion (C) of the anomeric configuration in glycosyl hydrolase.
replaced by the acetamido group at C-2 of the substrate (Terwisscha van scheltinga et al., 1995). A completely unrelated mechanism has been demonstrated recently for a family of glycosidases utilizing NAD+ as a cofactor (Rajan et al., 2004).

Retaining glycoside hydrolases operate via a double displacement mechanism that leads to the retention of the configuration at the anomeric carbon of the sugar ring undergoing catalysis. These enzymes often display transglycosylating abilities. The catalytic machinery of these enzymes involves two catalytic carboxylates located on opposite sides of the sugar plane and that perform two separate chemical steps. In the first step (glycosylation), a carboxylic group provides general acid-catalysed leaving group departure simultaneously with a nucleophilic attack by the second carboxylate to form a glycosyl-enzyme intermediate. In the second step (deglycosylation), the first residue functions as a general base to activate the incoming nucleophile (a water molecule in the case of hydrolysis, and an alcohol in the case of transglycosylation) which hydrolyzes the glycosyl-enzyme. The distance between the two carboxylates is approximately 5.5 Å. Many retaining glycoside hydrolases hydrolysing beta-N-acetyl glucosaminic bonds utilise a double-displacement mechanism in which the nucleophile is donated not by the enzyme, but by the C-2 N-acetamido group of the substrate itself (Terwisscha van scheltinga et al., 1995).

Inverting glycoside hydrolases lead to an inversion of the anomeric configuration via a single nucleophilic displacement. Hydrolysis of a beta-glycosidic bond thus creates a product with the alpha-configuration or vice-versa. The catalytic machinery of these enzymes involves two catalytic carboxylates in order to provide (i) general acid-catalysed leaving group departure and (ii) general base-assistance to nucleophilic attack by a water molecule from the opposite side of the sugar ring. The distance between the two carboxylates is less constrained as for the retaining enzymes and is in the range 6.5-9.5 Å. Figure represents pictorially the above discussed catalytic mechanisms.

Glycosidases are believed to play important roles in many diverse processes including chemical defense herbivory, lignification, hydrolysis of cell wall-derived oligosaccharides during germination, and control of active phytohormone levels. Glycosyl hydrolase and glycosyltransferases are also responsible for processing N linked oligosaccharides in the secretary pathway (Dupas et al., 2006).

1.5 New Players in the field
The recent isolation of the transcription factors NOR and LeMADS-RIN, which participate in ethylene-independent signaling in tomato, and the discovery that a homologue of the RIN gene is expressed in non-climactric fruit has suggested that common regulatory cascades may operate in all the fruits (Vrebalov et al., 2002). The elucidation of the molecular basis of such early and common events represents an active frontier in fruit ripening research. MADS box genes are known to be involved in numerous developmental processes which are depicted in the fig.1.12.
Figure 1.12 A MAD pathway from root to fruit. (Left) MADS-box transcription factors are involved in numerous steps in plant's development. (Right) The LeMADS-RIN transcription factor directs ripening of the tomato fruit. LeMADS-RIN is depicted to be acting together with other unknown MADS-box factors. Members of the MADS-box family bind to DNA as dimers, heterodimers, or heteromultimers to regulate the expression of target genes.
This knowledge could enable generic strategies to manipulate the ripening of any fruit. Such work has been complemented by transcriptional profiling during the development and ripening of both climacteric and non-climacteric fruit (Aharoni and O'Connell, 2002; Moore et al., 2002; Seymour et al., 2002) which may disclose more common regulatory elements. In addition, genomics tools have also been immensely useful in identifying and confirming the genes involved in fruit quality, and in defining the biochemical and molecular bases of texture flavour, colour, and aroma.

1.6 Aim and Scope

Major transitions in fruit development and metabolism accompany the initialization of fruit ripening. In addition to alterations in pigment biosynthesis and production of volatile compounds, tomato fruit undergoes significant changes in texture during ripening. To understand the ripening phenomenon, many glycosyl hydrolases like Polygalacturonase, Pectinmethylesterase, cellulase, β-galactosidase, β-mannanase have been targeted to understand their role in fruit ripening/softening. Although there was little success in reducing the ripening phenomenon but a considerable success was gained in understanding the cell wall disassembly.

Alpha-mannosidase is one among the glycosidases whose function has not yet been explored. This enzyme belongs to the glycosyl hydrolase family 38, which is known to break the glycoside bond between the carbohydrates or between carbohydrate and non-carbohydrate. Although reports of isolating several glycosidases like alpha-galactosidase, alpha-mannosidase from cultured sycamore had thrown light on the function of these enzymes as early as 1970 (Keegstra and Albersheim, 1970) but it remained unexploited. Since then numerous evidences have accumulated wherein α-mannosidase was associated with cell wall (Greve and Ordin, 1977; Wilden and Chrispeels, 1983 Ahmed and labavitch, 1980; Yashoda et al., 2007) and also known to perform varied functions like digestion of high mannose glutelin in rice (Kishimoto T, 2001). This enzyme shows high activity in many fruits including tomato and capsicum but till date its purification, characterization and its physiological role in ripening has not been elucidated.

Capsicum and tomato, apart from being commercially important vegetable fruits, behave differently during ripening. For these crops the texture, in particular crispness is an important quality attribute to consumers (Priya sethu and Prabha, 1997). Moreover the major post-harvest problem with these crops is excessive softening that may cause shrinkage, drying and pathological disorders which severely reduce the quality and acceptability of the product. Considering all these points we framed some objectives which targeted alpha-mannosidase from capsicum and tomato.

The present study has the following objectives:

1) Purification and characterization of α-D-Mannosidase protein from capsicum and tomato and raising antibodies against it.

2) Molecular cloning of α-D-mannosidase from capsicum and tomato.
(3) Developing transgenic tomato by antisense and RNAi technology to elucidate the role of α-D-Mannosidase gene in ripening.

(4) Promoter cloning and in-silico analysis.

This is an exciting time to study fruit ripening/softening and to address the association of hydrolases with cell wall disassembly. A number of dogmatic views in the cell wall/fruit softening literature have been overturned and laid to rest, and many questions are being asked that are bringing researchers from diverse fields that have not previously had a connection with cell wall biology. With the little research done to understand the nature and cell wall disassembly in vivo, has dramatically expanded the experimental toolbox to further explore the nature with the help of novel techniques and resources. With this information and novel techniques we need to hunt for more genes and test them individually or in combination to decipher their role in fruit ripening and/or softening. As the understanding of the biology of fruit ripening has improved, so has the ability to manipulate fruit for improved nutritional and organoleptic quality. Long may this vital trend continue.