EFFECTS OF CHILLING ON TOMATO FRUIT RIPENING

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The alteration of fruit ripening is a common chilling injury (CI) symptom in tomato. To evaluate whether tomato can be used as a model study for an altered fruit ripening associated with CI, the effect of chilling on fruit ripening have been investigated in tomato fruit cv. Trust (*Solanum lycopersicum* L.cv Trust) and tomato introgression line 11-2 (IL 11-2). Tomato fruit were harvested at breaker stage of maturity and ripened at 20 ^{o}C for up to 14 d, or stored at 3 ^{o}C for up to 4 weeks, and then ripened at 20 °C.

In Trust tomato, the effects of chilling on fruit ripening were small, and the mealiness disorder was not detected. Chilling had a marked effect on gene expression, total activity, and protein accumulation of PG. However, pectin solubilization and depolymerization did not seem to be affected much by chilling. The expression of *LeEXP1* was reduced by chilling, but LeEXP1 protein accumulation level was not affected. Post-transcriptional regulation of PG and LeEXP1 affected by chilling was observed. In IL 11-2 tomato, the effects of chilling on fruit ripening and the expression of ripening-related genes were investigated. Genes involved in color development: *PSY1, CRTISO, GGPPS2,* and *DXS*; cell-wall modification: *PG, PE1, TBG4, LeEXP1,* and XTH5; and volatile biosynthesis: *TomloxC, ADH2,* and *ATT*, were down-regulated by chilling. The alteration of ethylene production correlated with the altered *ACS2, ACS4,* and *ACO1* expression. The expression of genes involved in ethylene signal transduction pathway such as *LeETR1, NR, LeETR4,*

LeCTR1, LeEIL3, LeEIL4, and *LeERF3* was altered by chilling. The gene expression of *LeMADS-RIN*, a ripening-regulated transcription factor, was down-regulated by chilling. The microarray analysis suggested other transcription factors may be involved in altered fruit ripening associated with CI. In conclusion, IL 11-2 tomato had the potential of being used as a model to study of the effects of chilling in fruit ripening. How chilling affects fruit ripening at the transcriptional and post-transcriptional levels should be studied in this tomato.

BIOGRAPHICAL SKETCH

Adirek Rugkong was born and grew up in Nakhon Pathom, a small province in central Thailand. He is the youngest of seven children. He received his B.Sc. in Horticulture from the King Mongkut Institute of Technology Ladkrabang (KMITL) and his M.Sc. in Horticulture from Kasetsart University. After finishing his Masters degree, he moved to the south of Thailand to work as a lecturer at the Department of Plant Science of the Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla. After he had worked at PSU for five years, he applied to and was accepted at Cornell University in 2002 to pursue his Ph.D. degree in Pomology under the supervision of Prof. Dr. Chris Watkins.

To my family, friends, and tomatoes

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INTRODUCTION

1.1 Fruit ripening

Ripening is a complex process involving major transitions in fruit development and metabolism leading to changes in the color, softness, aroma and flavor in fleshy fruits (Fischer and Bennett, 1991; Howell, 1998). Fruits can be divided into two groups: climacteric and non-climacteric. Climacteric fruits, such as tomatoes, bananas, apples, pear, and melons, have an increased respiration rate and increased ethylene synthesis at the onset of ripening. In contrast, non-climacteric fruits, such as strawberries, grapes, pineapples, oranges and cherries, do not show significant increases in respiration rate and ethylene production during ripening (Fischer and Bennett, 1991; Brownleader et al., 1999). The other difference between climacteric fruit and non-climacteric fruit is the response of respiration and/or ethylene production to exogenous ethylene or its analogs, such as propylene. In climacteric fruit, exogenous ethylene advances the timing of the respiratory climacteric, autocatalytic ethylene production will continue after removal of ethylene, and the magnitude of respiratory rise does not depend on the concentration of applied ethylene. In non-climacteric fruit, ethylene can increase respiration rate but this increase will not continue after removal of ethylene, and the magnitude of respiration rate depends on the concentration of applied ethylene.

In climacteric fruit, ethylene is a key regulator of ripening. Ethylene action inhibitors can delay the onset or progression of the ripening process. Ethylene is responsible for the occurrence of respiratory climacteric and stimulates other ripening

processes such as color change, softening of fruit flesh, altered sugar metabolism, and the synthesis of aroma volatiles (Grierson, 1986; Barry and Giovannoni, 2007)

1.1.1 Fruit softening

Fruit flesh is composed mainly of parenchyma cells containing primary cell walls (Redgwell and Fischer, 2002), and adjacent cells adhere to each other by the so-called 'glue', a pectin-rich middle lamella (Brownleader et al., 1999). According to the characteristic of fruit softening during ripening, fruit can be divided into two groups. The first group is fruit that softens to melting texture, such as tomato, kiwifruit, plum, avocado, blackberry, persimmon, and strawberry. In this group, the forces holding cells together is weaker than the cell wall, the cell is separated with minimal cell breakage when pressure is applied (as in chewing), giving the melting texture. The second group is fruit that stays crisp during ripening, such as apple, nashi pear, and watermelon. In this group, the forces holding cell together are stronger than the cell wall, and the cell is broken when pressure is applied, giving the feeling of crispiness (Brownleader et al., 1999; Redgwell and Fischer, 2002).

Fruit softening is thought to be the consequence of a decrease in cell turgor pressure (Shackel et al., 1991; Saladie et al., 2007), cell wall disassembly, and reduced cell adhesion during ripening (Brummell, 2006). Cell wall disassembly has been extensively studies in tomato. It involves the solubilization and depolymerization of xyloglucan polysaccharides and pectic polysaccharides (Brummell, 2006). Therefore knowing about the components and structure of cell wall, the changes of cell wall components, and cell wall-modifying enzymes/proteins will be helpful for a better understanding about fruit softening.

1.1.1.1 Cell wall components and structure

The primary cell walls in fruit flesh are composed of numerous polymers which vary among species; however, the following components are usually present (Brummell and Harpster, 2001).

1.1.1.1.1 Cellulose, which is composed of (1→4) β-D-glucan chains assembled together by hydrogen bonding into very long crystalline microfibrils, each ca. 36 glucan chains in cross section but with many thousands of chains in total.

1.1.1.1.2 Hemicelluloses or cross-linking glycans, a class of polysaccharide that can hydrogen-bond to cellulose microfibril. They may coat microfibrils but also are long enough to span the distance between microfibrils and link them together to form a network (Carpita and McCann, 2000). Xyloglucan possesses a (1→4) β-D-glucan backbone like cellulose, but is substituted with α-D-xylose in a regular fashion on three consecutive glucose residues out of four, xylose occasionally being extended with β-galactosyl-α-L-fucose (or arabinose in some species). (Galacto)glucomannan, which has a backbone composed of regions of (1→4) β-D-glucan and (1→4) β-D-mannan in approximately equal amount, with occasional side chain of single units of terminal α-D-galactose. Glucuronoarabinoxylan, which has a backbone of (1→4) β-D-xylan with side chains of single units of reducing terninal α-L-arabinose and D-glucuronic acid.

1.1.1.1.3. Pectins, a mixture of heterogeneous, branched, and highly hydrated polysaccharides rich in D-galacturonic acid (Carpita and McCann, 2000). Homogalacturonan (HGA) is composed of long chains of (1→4) β-D-galacturonic acid, and is initially highly methyl-esterified. Rhamnogalacturonan I (RG I) is made of alternating α-D-rhamnose and α-D-galacturonic acid residues, with long side-chain attached to the rhamnose residues of either unbranched (1→4) β-D-galactan or branched α-L-arabinans or type I arabinogalactans. Rhamnogalacturonan II (RG II) is

made of a backbone of (1→4) β-D-galacturonic acid like homogalacturonan, but with complex side chains of several type of neutral sugar.

1.1.1.4. Structural proteins found in cell wall include hydroxyproline-rich glycoproteins (extensin), proline-rich glycoproteins, glycine rich proteins, and arabinogalactan proteins.

The primary cell wall is made up of three structurally independent but interacting networks (Carpita and McCann, 2000). The fundamental framework of cellulose and cross-linking glycan (hemicellulose) lies embedded in a second network of matrix pectic polysaccharides. The third independent network consists of structural proteins or a phenylpropanoid network. There are two cell wall types, Type I and Type II, which are different in chemical composition and are associated with distinct plant taxa. Type I, the walls of most dicots and the non-commelinoid monocots, contain about equal amounts of xyloglucans and cellulose. Type II walls of commelinoid monocots, including bromeliads, palms, gingers, cypresses, and grasses, contain cellulose microfibrils similar to those of the Type I walls. However, major cross-linking glycans found in Type II wall is glucuronoarabinoxylan instead of xyloglucan

In type I walls, the cellulose microfibrils are interlocked by xyloglucan to form cellulose-xyloglucan network embedded in a matrix of pectic polysaccharides, HGA and RG I. Pectic polysaccharide networks are formed in several ways. The chains of HGAs can condense by cross-linking with Ca^{2+} to form 'junction zone', thereby linking two anti-parallel chains. The junction zone can only be formed at the unmethylated region in HGA. Since HGA is secreted to cell wall as a highly methyl-esterified polymer, it must be cleaved some methyl groups by pectin methylesterase (PME). Some HGAs and RGs are cross-linked by ester linkage to other polymers. RG II monomers can dimerize as boron di-esters of the apiose residues (Carpita and McCann, 2000).

1.1.1.2 Cell wall disassembly in ripening fruit

During ripening, pectic polysaccharides undergo solubilization and depolymerization. Pectin depolymerization is the lowering of the molecular mass of pectin polymers, resulting from the cleavage of either the rhamnogalacturonan backbone and/or the neutral galactose-arabinose side-chains, and the de-aggregation of pectic polysaccharide complexes held together by non-covalent bonds. The loss of galactose from cell wall polymers, as part of pectin depolymerization, often occurs during fruit ripening, but it may not be involved in pectin solubilization and changes in fruit texture. In addition to pectin depolymerization, xyloglucan is also depolymerized in many fruits. However, the side-chains of xyloglucan are not as susceptible to degradation as they are in pectic polysaccharides. Xyloglucan depolymerization occurs early in fruit softening and could have a role in cell wall loosening. In theory, pectic polysaccharides can cross-link with adjacent polymers by forming covalent bonds, and some from those, could be broken during ripening, leading to a destabilization of the matrix (Redgwell and Fischer, 2002).

A general model for ripening-associated cell wall disassembly has been proposed by Bennett (2002). In this model, cell wall disassembly can be divided into at least two distinct and sequential stages. The first stage occurs in early fruit ripening and is associated with disassembly of xyloglucan polymers, particularly of that fraction of xyloglucan that is bound to the cellulose surface. The second stage of softening occurs in the overripe stage and is associated with disassembly of the pectin network.

1.1.1.3 Cell wall-modifying enzymes/proteins

The following enzymes/proteins are thought to be involved in the disassembly of pectic and hemicellulosic polysaccharides during fruit ripening.

1.1.1.3.1 Polygalacturonase (PG) is an enzyme that catalyzes the hydrolysis of the linear α-1,4,D-galacturonan backbone of pectic polysaccharides. There are two types of PG: exo- and endo-PG. Exo-PG (EC 3.2.1.67) removes single galacturonic acid units from the non-reducing end of polygalacturonic acid, whereas the endo-PG (EC 3.2.1.15) cleavages polymers at random. The substrate for PG is mainly demethylated homogalacturonans. Because homogalacturonans are secreted to the cell wall in a highly methyl-esterified form, they must be de-esterified before becoming available as a substrate for PG (Brummell and Harpster, 2001, Redgwell and Fischer, 2002).

1.1.1.3.2 Pectin methylesterase (PME, EC 3.1.1.11) is an enzyme that catalyzes demethylation of the C6 carboxyl group of galacturonosyl residues. Demethylation of pectin to their free carboxyl groups changes the pH and charge in the cell wall, allows the aggregation of polyuronides into a calcium-linked gel structure, and makes the polyuronides susceptible to degradation by PG (Brummell and Harpster, 2001; Redgwell and Fischer, 2002).

1.1.1.3.3 Pectate lyases (PL, EC 4.2.2.2) are enzymes catalyze the cleavage of unesterified α-1→4-galacturonosyl linkages by β-elimination mechanism, rather than by hydrolysis (Rose et al., 2003; Bennett and Labavitch, 2008). Cleavage by PL requires the presence of calcium ions and generates oligosaccharides with unsaturated galacturonosyl residues at their non-reducing ends (Marin-Rodriguez et al., 2002).

1.1.1.3.4 β-Galactosidase (β-gal, EC 3.2.1.23) is an enzyme that hydrolyzes terminal non reducing β-D-galactosyl residues from β-D-galactosides which are the side chains in RG I, therefore β-Gal is regarded as a pectin degrading enzyme (Brummell and Harpster, 2001; Redgwell and Fischer, 2002).

1.1.1.3.5 Endo 1,4-β-D-glucanases (EGase, EC 3.2.1.4) are enzymes that degrade the soluble substrate carboxymethyl cellulose (Cx-cellulose) (Redgwell and Fischer, 2002). EGases hydrolyze internal linkages of (1→4)β-D-linked glucan chains adjacent

to unsubstituted residues (Hatfield and Nevins, 1986). In the plant cell wall, their substrates probably include xyloglucan, integral and peripheral regions of non-crystalline cellulose (particularly the outer layers of cellulose microfibrils where glucan are interwoven with xyloglucan chains), and possibly glucomannan where sufficient consecutive (1→4)β-*D*-linked glucan residues occur for substrate binding (Brummell and Harpster, 2001).

1.1.1.3.6 Xyloglucan endotransglucosylase-hydrolases (XTH, EC 2.4.1.207) cleave internal linkages of the (1→4)β-D-glucan backbones of xyloglucan and transfers the newly formed potentially reducing end to the C-4 position of the glucose unit at the non-reducing end of another xyloglucan polymer or oligosaccharide (Smith and Fry, 1991) or act as hydrolases (Saladie et al., 2006)

1.1.1.3.7 Expansins are cell wall localized proteins that probably act by causing a reversible distruption of hydrogen bonding between cellulose microfibrils and matrix polysaccharides, particularly xyloglucan, resulting in a loosening of the cell wall (McQueen-Mason et al., 1992; Rose et al., 1997).

1.1.1.4. Cell wall disassembly in tomato fruit ripening

The tomato primary cell wall is considered a Type I cell wall that contains xyloglucan as a principal hemicellulose. Xyloglucan in solanaceous species is lower in substitution with xylose and possesses side chains with terminal galactose or arabinose than galactosyl-fucose (Carpita and McCann, 2000). During ripening, tomato fruit undergoes cell wall polysaccharides degradation, especially of the pectic and hemicellulosic polysaccharides (Huber, 1983; Carrington et al., 1993; Maclachlan and Brady, 1994).

PG is one of the cell wall modifying proteins responsible for polyuronide degradation in tomato fruit during ripening. Study of transgenic tomato repressed in

PG activity found that PG is responsible for pectin depolymerization (Smith et al., 1990; Brummell and Labavitch, 1997) but not responsible for pectin solubilization (Brummell and Labavitch, 1997). However, the repression of PG activity has a little effect on fruit softening (Smith et al., 1988; Brummell and Labavitch, 1997). Furthermore, a mutant complementation study in transgenic *rin* tomato showed the expression of PG gene and the accumulation of active PG enzyme found that PG did not affect fruit softening, even though the degradation of cell wall polyuronide occurred in this fruit (Giovannoni et al., 1989). Collectively results indicate that PG activity alone is not sufficient for fruit softening in tomato. Two PG isozymes can be extracted from ripe fruit: PG2 which is a single catalytic PG polypeptide and PG1 which is composed of PG2 tightly associated with the non catalytic β-subunit. PG2 is responsible for pectin depolymerization in vivo, whereas β-subunit protein plays a significant role in regulating pectin metabolism by limiting the extent of pectin solubilization and depolymerization that can occur during ripening (Watson et al., 1994). Transgenic tomato fruit suppressed in β-subunit protein accumulation showed increased fruit softening (Chun and Huber, 2000).

PME also plays a role in pectin solubilization and depolymerization. In antisense PME tomato, which showed <10% of wild type PME activity and undetectable levels of PME protein and mRNA, lower PME activity in transgenic fruit was associated with an increased molecular weight and methylation of pectins and decreased levels of total and chelator soluble polyuronides in cell walls (Tieman et al., 1992). However, reduced PME activity had a little effect on fruit firmness during ripening (Tieman and Handa, 1994).

β-galactosidase II is an enzyme responsible for the loss of galactosyl residues from the cell wall of ripening tomato (Carrington and Pressey, 1996; Smith and Gross, 2000). This loss begins early and increases with ripening (Gross and Wallner, 1979).

β-gal is encoded by gene *TBG4* (Smith and Gross, 2000). Suppression of *TBG4* expression in antisense fruit was correlated with a reduction in extractable exo-galactanase activity, and the antisense fruit were firmer than the control fruit (Smith et al., 2002).

EGase that is encoded by gene *Cel2* may be involved in ripening-asscociated cell wall changes in ripening tomato (Gonzalez Bosch et al., 1996). However, its role seems to contributes to cell wall disassembly occurring in cell separation during fruit abscission rather than in fruit softening or textural changes (Brummell et al., 1999a).

XTH, which catalyze the cleavage and concomitant transfer of one xyloglucan molecule to another, is thought to be an important component of cell wall metabolism in expanding tissue and ripening fruit (Arrowsmith and De Silva, 1995). Xyloglucan endotransglucolysase (XET) activity increases during ripening (Maclachlan and Brady, 1994), however suppression of *LeXETB1* in transgenic tomato fruit showed no correlation between *LeXETB1* mRNA abundance and fruit softening (de Silva et al., 1994; Brummell and Harpster, 2001). *LeXTH5* encodes xyloglucan endohydrolase5 (XTH5) was reported to be a XTH with endohydroxylase activity that may contribute to xyloglucan breakdown and fruit softening (Saladie et al., 2006).

Expansin proteins are thought to play an indirect role in pectin polymerization, but the accumulation of this protein is correlated with fruit softening (Brummell and Harpster, 2001). The most abundant expansin mRNA in ripening tomato is *LeEXP1* (Rose et al., 1997; Brummell et al., 1999a). Fruit in which EXP1 protein accumulation was suppressed to 3% that of wild type levels were firmer than those of controls throughout ripening. Suppression of EXP1 protein also inhibited polyuronide depolymerization late in ripening fruit but did not prevent the breakdown of structurally important hemicelluloses. In contrast, fruit that over-express high levels of recombinant EXP1 protein were much softer than control fruit, even in mature green

fruit before ripening commenced. This softening was correlated with the precocious and extensive depolymerization of structural hemicelluloses, whereas polyuronide polymerization was not altered (Brummell et al., 1999b). LeEXP protein accumulation was ethylene-regulated and matched the expression of mRNA, suggesting that expression is not regulated at the level of translation (Rose et al., 2000). Fruit with suppressed expression of *LeEXP1* has improve shelf life and process properties (Brummell et al., 2002).

1.1.2 Color changes

Ripening is frequently signaled by a change in color. Color changes may involve the destruction of chlorophyll and a synthesis of carotenoids in plastids, or the production of anthocyanins that accumulate in vacuoles. Sometimes color changes are distributed throughout the flesh, as in tomato, but in other fruits, such as apple and banana, are confined to the surface layers (Grierson, 1986).

In tomato fruit, the total carotenoids, consisting mainly of lycopene and β-carotene, increase with the concomitant decrease in chlorophyll during ripening (Fraser et al., 1994). Carotenoid biosynthesis takes place within the plastid (Cunningham and Gantt, 1998) and follows the scheme in Figure 1.1 (Ronen et al., 1999)

The accumulation of lycopene is regulated by the differential expression of carotenoid biosynthesis genes; (Giuliano et al., 1993; Ronen et al., 1999). Accumulation of lycopene begins at the breaker stage of fruit ripening. At this ripening stage, mRNA levels of phytoene synthase (*PSY*) and phytoene desaturase (*PDS*) genes also increase (Giuliano et al., 1993; Fraser et al., 1994; Ronen et al., 1999). In contrast, mRNA levels of Crt-b and Crt-e , which encodes lycopene β-

cyclase and lycopene ε-cyclase, respectively, decrease at this ripening stage (Ronen et al., 1999). Psy and Pds are the lycopene-producing enzymes, while lycopene β-cyclase and lycopene ε-cyclase are enzymes which convert lycopene to either β-carotene or δ-carotene (Figure 1.1). Therefore, differential gene expression play a major role in accumulation of lycopene in tomato fruit by elevating the concentration of its biosynthetic enzyme and blocking the synthesis of enzymes that convert it to cyclic carotenoids (Ronen et al., 1999).

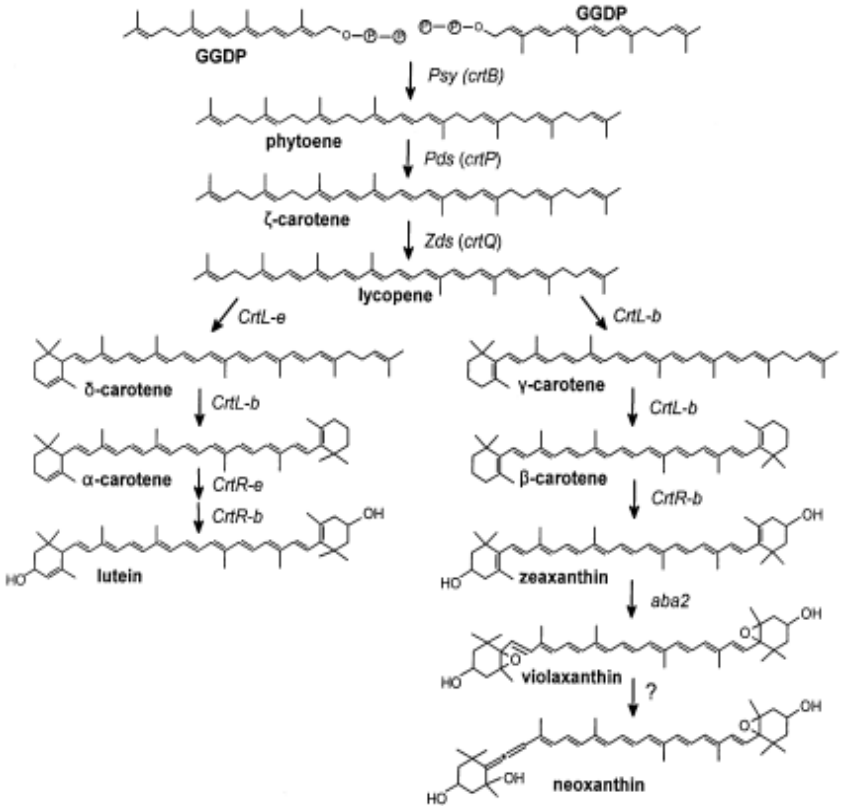

Figure 1.1 Pathway of carotenoid biosynthesis in plants and algae (Ronen et al., 1999). Enzymes are indicated by their gene assignment symbols: aba2, zeaxanthin epoxidase:CrtL-b, lycopene β-cyclase; CrtL-e, Lycopene ε-cyclase; CrtR-b, β-ring hydroxylase; CrtR-e, ε-ring hydroxylase: Pds, phytoene desaturase; Psy, phytoene synthase; Zds, ζ-carotene desaturase. GGDP, geranylgeranyl diphosphate.

2. Chilling injury

Chilling injury is a physiological damage that occurs when plants or plant parts are exposed to low, but non-freezing, temperatures (Lyons, 1973; Raison and Lyons, 1986). Symptoms of chilling injury generally develop after removal from the chilling temperature to non-chilling temperatures, and they vary with the plant tissue and the severity of injury (Lyons, 1973). The commonly occurring symptoms include cellular change, altered metabolism, reduced plant growth and death, loss of vigor, and especially in the case of harvested products, surface lesions, water-soaking of tissue, internal discoloration, accelerated senescence, increased susceptibility to decay, and failure to ripen normally (Saltveit and Morris, 1990).

The generally accepted model of development of chilling injury (Figure 1.2) is based on primary and secondary events (Raison and Lyons, 1986; Raison and Orr, 1990). The primary event might be a change in membrane lipid structure, a conformational change in some regulatory enzyme or structural protein or an alteration in the cytoskeletal structure of the cell. The primary event occurs when the tissue is exposed to temperature below the critical or threshold temperatures which correlates with the onset of chilling injury. The secondary events, which follow the primary event, can include the metabolic and ionic imbalances, the loss of cellular integrity, and similar events that lead to visible symptoms of injury. Secondary events are both time and temperature dependent. In the short term, they are reversible if chilling stress is removed. However, if the stress is maintained, the imbalances and/or loss of cellular integrity become excessive and the process becomes irreversible. After irreversible stage, warming to non-chilling temperatures exacerbates the symptoms of injury. The rate of development and magnitude of the visible symptoms of injury depend on the metabolic status of the tissue at the time the chilling stress is imposed.

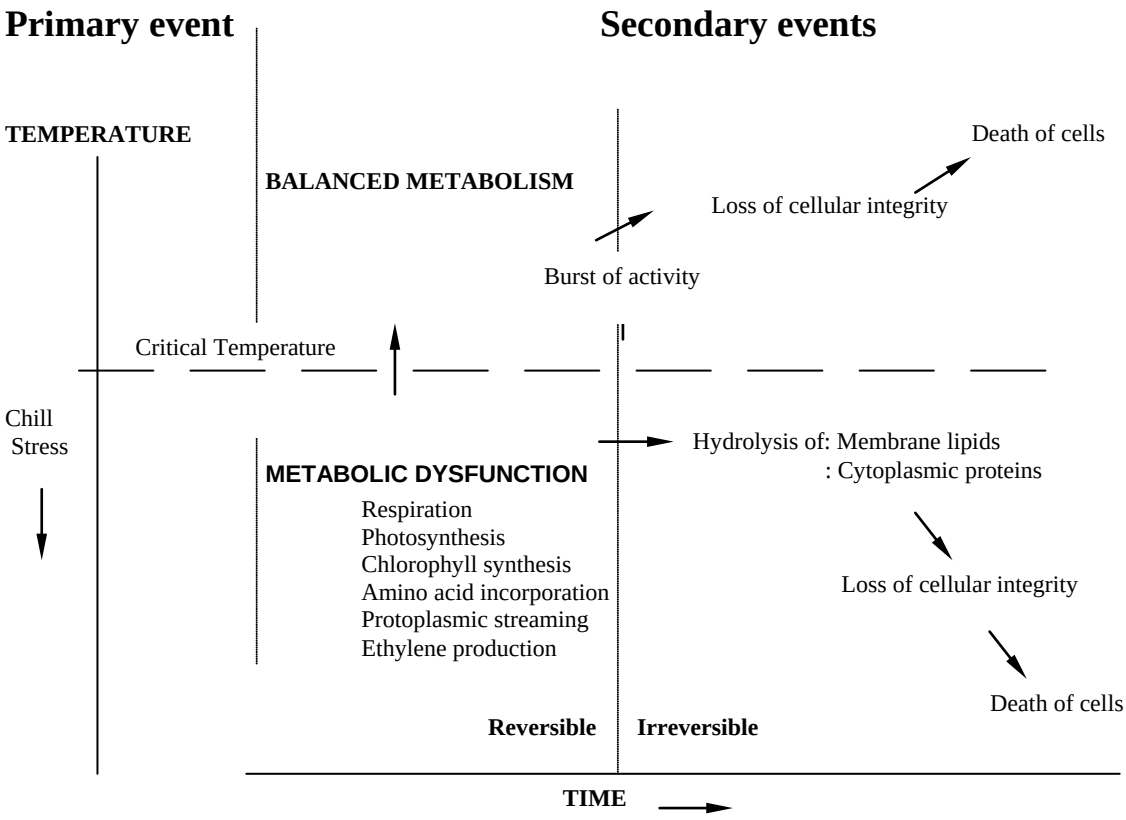

Figure 1.2 A schematic representation of the relationship between the primary and secondary event of chilling injury (Raison and Orr, 1990).

2.1 Mealiness in peaches and nectarines

Chilling injury associated with altered cell wall metabolism has been studied most extensively in peaches and nectarines. Mealiness or woolliness, characterized by lack of juiciness, dry and mealy texture, is a well-known chilling injury symptom in peaches and nectarines (Dawson et al., 1992; Luza et al., 1992). It has been suggested that mealiness is caused either by prevention of the normal cell breakage and the release of juice or cellular contents during biting and mastication (King et al., 1989;

Brummell et al., 2004b), or by the presence of calcium-pectate gels containing high molecular weight pectins that can absorb water or cell contents released from ruptured cells (Zhou et al., 2000a; Lurie et al., 2003).

Microscopic observations show that juice collected from crushed mealy fruit contains more intact cells than that collected from crushed juicy fruit, and tissue fragments into clumps across enlarge intercellular spaces in mealy fruit (Brummell et al., 2004b). Mesocarp cells, in mealy fruit appear to be separated throughout the area of the cell wall (Luza et al., 1992; Lurie et al., 2003; Brummell et al., 2004b) and have larger intercellular spaces than juicy fruit (Brovelli et al., 1998; Lurie et al., 2003; Brummell et al., 2004b), and accumulation of pectin substances is found in intercellular spaces (Luza et al., 1992). Cell wall of fruit that become mealy during ripening after cold storage show extensive breakdown in the middle lamella area, but less degradation of outer cell walls, whereas in the cultivar that does not develop mealiness, cell wall degradation is extensive throughout the entire wall (King et al., 1989). The cellulosic component of cell wall does not change much in mealy fruit (Luza et al., 1992).

Cell wall analysis data shows that solubilization and rapidly depolymerization of pectin polymer and loss of arabinan and galactan side chains found during ripening in juicy fruit does not occur in mealy fruit (Dawson et al., 1992; Dawson et al., 1995; Brummell et al., 2004a; Brummell et al., 2004b). Chelator soluble-polyuronides are partially depolymerized during cold storage, and are not depolymerized further during the ripening period, resulting in accumulation of high molecular weight (M_r) pectic polymers. Depolymerization of the matrix glycans shows only minor differences between juicy and mealy fruit (Brummell et al., 2004b). During cold storage, the degree of esterification of cell wall decrease in fruit become mealy during subsequent ripening (Lurie et al., 1994; Lurie et al., 2003). The accumulation of high molecular

weight (M_r) and de-esterified pectic polymer may form calcium-pectate gel complexed in cell wall and cause mealiness. Calcium and magnesium contents in cell wall material are also higher in mealy fruit than in non-mealy fruit (Manganaris et al., 2005).

During normal ripening in peaches, activities of exo-and endo-PG, PME, EGase, endo-1,4-β-mannanase, α-arabinosidase, and β-galactosidase increase during ripening, but the timing and extent of the increases differed between enzymes (Brummell et al., 2004a). Compared with juicy fruit, mealiness has been found associated with a reduction in PME activity (Buescher and Furmanski, 1978; Manganaris et al., 2005), or with an increase (Ben Arie and Sonego, 1980; Zhou et al., 2000a) or with unchanged levels of PME activity (Obenland and Carroll, 2000; Zhou et al., 2000a). PME activity is lower in slightly mealy and higher in very mealy fruit (Brummell et al., 2004b). PME activity is not inhibited during cold storage (Zhou et al., 2000a; Zhou et al., 2000c; Brummell et al., 2004b). In normally ripened fruit, transcript abundant of PME mRNA is abundant at harvest and decreases during ripening, while in mealy fruit PME mRNA increases during ripening after storage (Zhou et al., 2000c). Exo-PG activity is lower in mealy fruit during ripening (Zhou et al., 1999; Zhou et al., 2000c; Manganaris et al., 2005). However, lack of correlation of exo-PG activity and mealiness has been reported (Artes et al., 1996; Brummell et al., 2004b). A reduction in endo-PG activity has commonly been observed during subsequent ripening in mealy fruit (Buescher and Furmanski, 1978; Ben Arie and Sonego, 1980; Von Mollendorff and De Villiers, 1988; Zhou et al., 1999; Zhou et al., 2000a; Brummell et al., 2004b; Manganaris et al., 2005). However, PG mRNA level increases during ripening of both normally ripened fruit and mealy fruit. The observation that PG mRNA transcript levels do not correspond with PG activity suggests that regulation of cell wall degradation in mealy fruit does not appear to be by enzyme synthesis (Zhou et al.,

2000c; Zhou et al., 2001b). EGase acitivity and the abundance mRNA are higher in mealy fruit than in juicy fruit (Zhou et al., 2000c; Zhou et al., 2001b). However, reduced EGase activity is also found in mealy fruit (Brummell et al., 2004b). Other enzymes including endo-1-4-β-mannase, β-galactosidase and α-arabinosidase have lower activities in mealy fruit than in ripe, juicy fruit (Brummell et al., 2004b). Expansin protein and mRNA abundance are lower in mealy tissue (Obenland et al., 2003).

Methods used to alleviate mealiness of stonefruit include intermittent warming, delayed cold storage, and controlled atmosphere (CA) storage. Intermittent warming alleviates mealiness by promoting pectic polymerization (Dawson et al., 1995). PG activity increases (Artes et al., 1996; Zhou et al., 2001b) and PME activity decreases (Artes et al., 1996) in fruit treated with intermittent warming. In delayed storage, fruit continue cell wall degradation during cold storage, which allows normal ripening to proceed after removal from storage (Zhou et al., 2000c). PG activity is higher (Von Mollendorff and De Villiers, 1988; Zhou et al., 2000b) while that of PME is lower (Zhou et al., 2000b) in treated fruit than in control fruit that developed mealiness at removal from storage. PG activity increases further during ripening, as fruit normally soften (Zhou et al., 2000b). CA storage appears to alleviate mealiness via a different mechanism than delayed storage. PG activity is repressed during CA storage, but recovers during subsequent ripening (Zhou et al., 2000b).

Development of mealiness is associated with reduced ethylene production by the fruit (Dong et al., 2001; Zhou et al., 2001a) as a result of the decreased 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) activity (Zhou et al., 2001b). Furthermore, adding ethylene in storage atmosphere can reduce the development of mealiness (Dong et al., 2001; Zhou et al., 2001a), and fruit treated before storage with 1-methylcyclopropene (1-MCP), an ethylene action inhibitor, develop more severe

mealiness than untreated fruit (Dong et al., 2001). It is suggested that ethylene is essential for promoting the proper sequence of cell wall hydrolysis necessary for normal fruit softening (Dong et al., 2001; Zhou et al., 2001a).

2.2 Chilling injury in tomato fruit

Symptoms of chilling injury in tomato fruit can be expressed differently depending on the severity of chilling injury (Hobson, 1987). During storage at chilling temperatures, membrane permeability, as indicated by greater electrolyte leakage, increases with storage time (Autio and Bramlage, 1986; Bergevin et al., 1993; Saltveit, 2002). Increased membrane permeability is interpreted as due to either low temperature adaptation or membrane damage (Bergevin et al., 1993). In contrast, after removal to non-chilling temperatures, electrolyte leakage decreases (Bergevin et al., 1993). This decrease in electrolyte leakage may be due to the increase in demethylated pectin binding calcium ions, resulting to the loss of cations from the solution and the decrease in electrolyte leakage.

Chilling temperatures can affect ultrastructural changes of tomato fruit. In chilled fruit, the conversion of chloroplasts to chromoplasts is interfered and plastids and mitochondria are swollen and degenerated (Moline, 1976).

Chilling temperatures can result in increased ethylene production (Lurie and Klein, 1991), decreased ethylene production (Watkins et al., 1990; Lurie et al., 1996), or unchanged level of ethylene production during subsequent ripening at non-chilling temperatures. These differences in ethylene production response may be due to the difference of the exposure times and temperatures (Cheng and Shewfelt, 1988). Accumulation of ACC oxidase mRNA (pTOM 13) was increased during storage at chilling temperature and declined rapidly after transfer to ripening temperature (Watkins et al., 1990). Post-chilling respiration, measured as carbon dioxide

production, of chilled fruit immediately increases after removal to non-chilling temperatures (Cheng and Shewfelt, 1988; Lurie and Klein, 1991). Alterations of respiration processes occur after the alteration of ethylene production (Cheng and Shewfelt, 1988).

Ripe tomato fruit stored at chilling temperatures lose color during storage as a result of loss of total carotenoids (Hall 1961). Chilling temperatures inhibit color development in fruit after transfer to non-chilling temperatures (Cheng and Shewfelt, 1988; Efiuvwevwere and Thorne, 1988; Lurie et al., 1996; Chomchalow et al., 2002). Lycopene synthesis is inhibited during storage at chilling temperature, and its accumulation is reduced in chilled fruit (Watkins et al., 1990). The mRNA level of phytoene synthase, an enzyme in carotenoid biosynthesis, is lower in chilled fruit than non-chilled fruit after subsequent ripening (Lurie et al., 1996). Cell wall metabolism is also altered by chilling temperatures, leading to excessive softening (Marangoni et al., 1995a) and development of mealiness (Jackman et al., 1992)

Titratable acidity increases with storage time at chilling temperatures, but decreases at non-chilling temperature (Thorne and Efiuvwevwere, 1988). After subsequent ripening, titratable acidity in chilled fruit is higher than in non-chilled fruit (Hall, 1968). Citric acid concentration in chilled fruit is also higher in chilled fruit (Buescher, 1975; Thorne and Efiuvwevwere, 1988). In contrast, glucose and fructose levels in chilled fruit are lower than in non-chilled fruit after subsequent ripening (Buescher, 1975)

3. Using tomato as a model system

Tomato has been long used as a primary model for study in ripening of climacteric fruit because it has a short generation period and can be grown all year round in

greenhouses (Giovannoni, 2001). Genetic information associated with fruit ripening is available, as in an expressed sequence tag (EST) database (http://ted.bti.cornell.edu). Such tools may contribute to the success of using gene expression analysis approaches to investigate the effects of chilling on fruit ripening.

Alterations of cell walls in peaches and nectarines have been studied at the level of gene expression to some proteins involved in cell wall metabolism. However, these studies are limited because of the lack in genetic information for these fruits. An extensive knowledge in cell wall modification during ripening in tomato will be an effective tool for study of alteration of cell wall metabolism during ripening. Cell wall metabolism is also affected by chilling in tomato fruit. Enhanced softening and development of mealiness of tomatoes have been reported (Jackman et al., 1992; Marangoni et al., 1995b). Therefore, it would be worthwhile to study in alteration of cell wall metabolism caused by chilling temperatures in tomato and to use it as a model system.

3. Hypothesis

Tomato can be used as a model system to study the effects of chilling on fruit ripening.

4. Objectives

The objectives of the research reported in this thesis are to investigate the effects of chilling on fruit ripening using tomato as a model system, with focus on changes in cell wall metabolism associated with chilling in tomato fruit, and the expression of ripening-related genes affected by chilling injury.

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CHAPTER 2

CHANGES IN CELL WALL METABOLISM ASSOCIATED WITH CHILLING IN TOMATO FRUIT

Abstract

The effects of chilling on cell wall metabolism of tomato fruit (*Solanum lycopersicum* L. cv. Trust) have been investigated. Fruit were harvested at the breaker stage of maturity and ripened at 20 ^{o}C for 0 to 12 d, or stored at 3 ^{o}C for 0 to 3 weeks, and then ripened at 20 ^{o}C. The effects of cold storage on fruit ripening were small. Pericarp tissues from fruit stored for 2 weeks had only slightly reduced pectin solubilization and depolymerization but polygalacturonase (PG) mRNA levels, PG protein accumulation and PG activity in the tissues were reduced by chilling. A reduction of PG protein abundance and PG activity occurred to a greater extent than that of PG mRNA levels, suggesting that chilling affected post-transcriptional regulation. Expression of expansin1 (*LeEXP1*) gene was also reduced by chilling, but LeExp1 protein accumulation levels were not affected by chilling. β-galactosidase activity was highest in chilled fruit during cold storage and during early ripening, but expression of a galactosidase gene (*TBG4*) was unaffected. While chilling had no effect on pectin methylesterase (PME) activity, expression of *PME1* in tissues from cold stored fruit was lower. Endo-β-1,4-glucanase (EGase) activity and the expression of endo-β-1,4-glucanase *Cel1* were not affected by chilling. The predominant effect of chilling on the activity, protein accumulation, and gene expression of *PG* did not correlate with pectin solubilization and depolymerization.

Keywords: Chilling injury; Tomato; Cell wall metabolism; *Solanum lycopersicum* L.

2.1 Introduction

Chilling injury (CI) is a physiological disorder that limits the storage of susceptible fruit and vegetables at low, but non-freezing, temperatures. Because susceptible produce must be stored above temperatures that result in injury, metabolic activity cannot be reduced as much as for chilling-insensitive fruit and thus storage periods can be restricted. CI symptoms include skin pitting, flesh browning and blackening, water-soaking, failure to ripen normally, increased susceptibility to decay, and accelerated senescence (Saltveit and Morris, 1990).

Deleterious effects on texture are also a symptom of CI and can affect fruit such as peaches and nectarines. Prolonged exposure of fruit to low storage temperatures can result in alteration of cell wall metabolism, which is expressed as mealiness or wooliness, characterized by lack of juiciness, dry, and mealy texture when fruit ripen. Mealy fruit contain a higher proportion of insoluble pectic material with a higher molecular weight and a lower degree of esterification than juicy fruit (Ben-Arie and Lavee, 1971; Dawson et al., 1992; Brummell et al., 2004). Most research on mealiness has focused on the activity of the cell wall enzymes polygalacturonase (PG) and pectin methylesterase (PME), although the possible roles of other enzymes and cell wall proteins are beginning to be examined. Increased endo-β-1,4-glucanase activity and mRNA are observed in mealy fruit compared with fruit from treatments which delay the appearance of mealiness (Zhou et al., 2000). Decreased expansin protein and mRNA are associated with mealiness in peaches (Obenland et al., 2003). Also, activity of enzymes including endo-β-1,4-mannase, β-galactosidase and α-arabinosidase are lower in mealy fruit than in ripe, juicy fruit (Brummell et al., 2004).

Cell wall metabolism is also affected in other chilling susceptible fruit and vegetables, including tomato (Hobson, 1987). Effects of chilling on firmness of tomato varies according to the length of the storage period with greater softening

(Hall, 1961), inhibited softening (Efiuvwevwere and Thorne, 1988; Watkins et al., 1990), or both excessive softening and persistent firmness (Hobson, 1987), being reported. However, relatively little is known about the effects of chilling temperatures on cell wall metabolism in this fruit. Enhanced softening and development of mealiness of tomatoes was associated with higher PME activity for the first 2 d after removal of tomatoes from storage, but cell wall changes were not related to PG activity (Jackman et al., 1992; Marangoni et al., 1995). Expression of a gene encoding PG remained low during cold storage and transcript accumulation was inhibited after prolonged storage (Watkins et al., 1990). However, pectin dissolution in chilling injured fruit appears to be independent of PG action and trends in polymeric neutral sugars suggest that differences in pectin disassembly exist (Almeida and Huber, 2008).

The objective of the current study was to investigate the effect of chilling temperature on tomato fruit ripening with focus on cell wall metabolism. Tomato has been long used as a model system for fruit ripening in climacteric fruit, and cell wall metabolism of tomato during ripening has been extensively studied (Giovannoni, 2001; Brummell, 2006). Our initial question was to ask if tomato fruit could be used to investigate cell wall metabolism that would increase understanding of mealiness development in fruit such as peaches and nectarines or show differences from previous studies of stone fruits.

2.2 Materials and Methods

2.2.1 Plant materials

Tomato fruit (*Solanum lycopersicum* L. cv. Trust), grown in a commercial greenhouse at Danby, New York, USA, were harvested at the breaker stage of maturity. Fruit were transported to Ithaca within 1 h of harvest where they were divided randomly into 28 groups of five fruit. One group of five fruit was used for

evaluation on the day of harvest. The remaining fruit were either kept at 20 °C for 1, 3, 5, 7, 9 and 12 d, or placed in storage at 3 °C for 1, 2 and 3 weeks plus 1, 3, 5, 7, 9 and 12 d at 20 °C. At each storage time, and shelf life periods, fruit were used for measurements of ethylene, color and firmness, and then the pericarp tissues used for measurement of extractable juice or frozen for later extraction of enzymes, RNA and cell wall materials.

2.2.2 Ethylene production, color, firmness and extractable juice

To measure ethylene production, fruit were sealed individually in 1L containers for 1 h at 20 °C or 3 h at 3 °C. Headspace gas samples were analyzed in duplicate using a Hewlett Packard 5890 series II gas chromatograph (Hewlett Packard Co., Wilmington, Delaware, USA), equipped with a stainless steel column packed with 60/80 mesh alumina F-1 (internal diameter: 2m × 4mm) and a flame ionization detector. Operating conditions were as follows: oven temperature 180 °C, injector temperature 230 °C, and detector temperature 250 °C. Flow rates for nitrogen, hydrogen and air were 45, 30, and 200 mL min^{-1}, respectively. The ethylene production rate was expressed as mL kg^{-1} h^{-1}.

The color of each fruit was measured three times around the equator, using a Minolta Chroma Meter, Model CR-300 (Minolta, Mahwah, New Jersey, USA) and expressed as hue angle, where, 0^{o} = red-purple color, 90^{o} = yellow, 180^{o} = bluish-green, and 270^{o} = blue (McGuire, 1992).

Whole fruit firmness was measured with a Precision Penetrometer (GCA/Precision Scientific, Chicago, Illinois, USA) using 0.1 mm divisions. Fruit were held in a V-shape hollow base, and fruit deformation was measured after a 0.5 kg force was applied at the fruit equator for 5 s (Manzano-Mendez et al., 1984). Firmer fruit have lower deformation values than softer fruit.

Extractable juice was assessed using a modification of the method developed for stone fruit (Lill and Vandermespel, 1988). Fruit pericarp tissues from each fruit were ground into powder in liquid nitrogen. About 1.2 g of powder was added into a pre-weighed microcentrifuge tube, the tube with powder reweighed, and then centrifuged at 13,000 × g for 10 min. The supernatant was removed and the tube weighed again. The percent extractable juice was calculated.

2.2.3 Cell wall extraction

Alcohol-insoluble solids (AIS) were prepared using the Tris-buffered phenol method as described in Huber and O' Donoghue (1993). Briefly, fruit pericarp tissue was ground into powder in liquid nitrogen, homogenized in ice-cold 95 % ethanol using Polytron homogenizer, and then filtered through Miracloth (CalBiochem, San Diego, CA, USA). The residues were washed with ice-cold 80% ethanol, stirred in Tris-buffered phenol, pH 7.0, at room temperature for 30 min, adjusted to 80% of ethanol, reprecipitated at -20 ^{o}C for 1 h, and then filtered through Whatman Grade GF/C Glass Microfibers filter paper. The residues were washed with 95% ethanol, stirred in chroroform:methanol (1:1, v/v) at room temperature for 30 min, and then filtered through the GF/C filter paper. The residues were washed with acetone, dried in the oven at 34 ^{o}C for 12 h and then stored in a desiccator at room temperature.

The water-soluble and water-insoluble pectin fractions were sequentially extracted from AIS as described by Manganaris et al. (2006). Five mg of AIS was extracted twice in 5 mL of distilled water for 30 min and centrifuged at 17,000 × g for 30 min. The supernatants were collected, combined, and designated as the water-soluble pectin fraction. The pellet was dissolved in 2 mL of concentrated H_2SO_4, stirred for 5 min, and the volume was adjusted to 10 mL with distilled water, and designated as the water-insoluble fraction. Aliquots of these fractions were used for uronic acid

determination by the *m*-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973), using galacturonic acid as standard.

For size exclusion chromatography, cell wall pectic fractions, including water-soluble, CDTA-soluble, and Na_2CO_3-soluble fraction, were sequentially extracted from 300 mg of AIS as described in Rose et al. (1998). The AIS residue was homogenized and extracted in 0.02% NaN_3 at room temperature for 4 h, centrifuged at 6,000 × g for 20 min, and re-extracted in the same solution for 20 min. The supernatants were combined, lyophilized, resuspended, and stirred in dimethyl sulfoxide (DMSO):water (9:1, v/v) for 24 h, filtered through Miracloth, oven-dried at 30 ^{o}C, stored in a desiccator as the water-soluble fraction. The water-insoluble pellets were homogenized and extracted twice in 50 mM CDTA and 50 mM $NaC_2H_3O_2$, pH 6.5 for 12 h each. After centrifugation, the supernatant were combined and exhaustively dialyzed against distilled water for 2 d at 4 ^{o}C, lyophilized, stored in a desiccator, and designated as the CDTA-soluble fraction. The CDTA-insoluble pellets were washed once with CDTA buffer solution, twice with 80 % ethanol, twice with acetone, extracted twice with Na_2CO_3 and 20 mM $NaBH_4$ at 4 ^{o}C as for the CDTA extraction, and designated as the Na_2CO_3-soluble faction.

Aliquots (approximately 4 mg) of each fraction were dissolved in 0.5 mM ammonium formate, pH 5.0 and separated on a Superdex-75 HR 10/30 (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA), eluted at 0.6 mL min^{-1} with 0.5 mM ammonium formate, pH 5.0 using Dionex BioLC HPLC system (Dionex Corp., Sunnyvale, CA, USA) . The elutant was monitored with a Knauer differential refractometer (Knauer, Berlin, Germany).

2.2.4 Cell wall protein extraction

Frozen pericarp tissue was ground into powder in liquid nitrogen. Then, 5 g of the powder was extracted with 15 mL of extraction buffer (0.05 M HEPES pH 7.0, 5 mM DTT, 3 mM sodium metabisulfite, 2 mM EDTA, 2 % Polyvinylpyrrolidone (PVPP) and 1.5 M sodium chloride). The suspension was centrifuged at 20,000 x g for 20 min and proteins in the supernatant were precipitated at 80% ammonium sulfate saturation. After centrifugation at 30,000 × g for 10 min, the pellet was dissolved in 10 mL of 50 mM HEPES pH 7.0 and dialyzed overnight in dialysis buffer (50 mM HEPES pH 7.0 and 50 mM NaCl). After dialysis, the enzyme extract was divided to several tubes and then stored at -80 ^{o}C.

Protein concentration was measured using the method of Bradford (1976). The reaction mixture consisting of 0.8 mL of sample solution and 0.2 mL of Bio-Rad Protein Assay Dye Reagent Concentrate were mixed by vortexing, and incubated at room temperature for 5-60 min. The absorbance at 595 nm was then determined. A standard curve of protein concentration was obtained using 0 to 10 μg of bovine serum albumin (BSA).

2.2.5 Measurement of cell wall enzyme activity

2.2.5.1 Polygalacturonase (PG)

PG activity was measured using the method of Gross (1982). The reaction mixture consisted of 200 μL of the reaction solution containing 0.2 % (w/v) of polygalacturonic acid dissolved in 37.5 mM sodium acetate pH 4.4, and 50 μL of the enzyme extract. After 30 min of incubation at 30 ^{o}C, the reaction was stopped by adding 1.0 mL of cold 0.1 M borate buffer (pH 9.0), then, 0.2 mL of 1 % (w/v) 2-cyanoacetamide was added and sample was immersed in boiling water for 10 min. The absorbance at 276 nm was then determined against a blank consisting of 50 mL of

50 mM HEPES pH 7.0 in the reaction mixture instead of the enzyme extract. A standard curve of 0 to 200 nmole of galacturonic acid was obtained. One unit of PG activity was defined as the amount of enzyme required to release 1 nmole of reducing group per min from polygalacturonic acid.

2.2.5.2 Pectinmethylesterase (PME)

PME activity was measured by the method of Hagerman and Austin (1986). The reaction mixture consisted of 2.0 mL of pectin pH 7.5, 0.8 mL of distilled water pH 7.5, 150 μL of bromothymol blue pH 7.5, and 50 mL of enzyme extract. For the blank, 50 μL of 50mM HEPES pH 7.0 was used instead of the enzyme extract. A change in absorbance at 620 nm from 10 to 60 s was measured after the reaction was started. A standard curve was obtained from using 0 to 250 nmole of galacturonic acid dissolved in 50 mM HEPES (pH 7.0). One unit of PME activity was defined as the amount of enzyme required to produce 1 μmole of acid per min.

2.2.5.3 β-galactosidase (β-gal)

β-gal activity was measured by the method of Sozzi et al. (1998). The reaction mixture consisted of 0.5 mL of 0.1 M citrate buffer pH 4.0, 0.4 mL of 0.1 % BSA, 0.1 mL of enzyme extract and 0.4 mL of 13 mM of ρ-nitrophenol-β-D-galactopyranoside. After 15 min at 37 ^{o}C, the reaction was stopped by addition of 2 mL of 0.2 M sodium carbonate. The release of ρ-nitophenol was measured by the absorbance at 400 nm. A standard curve was obtained by using 0 to 300 nmole of ρ-nitrophenol standard solution. One unit of β-gal activity was defined as the amount of enzyme necessary to release 1 nmole of ρ-nitophenol per min.

2.2.5.4 Endo-β-1,4-glucanase (EGase)

EGase activity was measured by using disodium 2, 2′-bicinchoninate (BCA) method (Gracia et al., 1993). The reaction mixture consisted of 0.5 mL of 0.05% of carboxymethylcellulose (CMC), 0.475 mL of 0.05 M sodium acetate buffer pH 5.0 and 25 μL of the enzyme extract. After 10 min of incubation at 40 ^{o}C, the reaction was stopped by adding 0.25 mL of the alkaline solution. Then, 0.2 mL of BCA reagent was added. The samples were heated at 80 ^{o}C for 30 min. After the samples were cooled, the absorbance at 560 nm was read against the blank consisting of 25 μL of 0.1 M HEPES, pH 7.0, in the reaction mixture instead of the enzyme extract. A standard curve of 0 to 50 μM glucose was obtained. One unit of enzyme activity is defined as the amount of enzyme required to hydrolyzes 1 nmole of β-1,4 glycosidic bonds per min.

2.2.6 Northern blot analysis

Total RNA was isolated from 7.5 g tomato pericarp tissue according to Chang et al. (1993). Aliquots of total RNA (10 μg per lane) were separated by electrophoresis on a 1% agarose-formaldehyde gel, visualized with ethidium bromide to confirm equal loading, and transferred onto Hybond N nylon membrane (Amersham Biosciences, Piscastaway, NJ, U.S.A.). cDNA specific probes for PG, PME1.9, LeExp1, TBG4, and Cel1 were labeled with [α-^{32}P]-dCTP using the Ready-To-Go DNA Labeling Kit (Amersham Biosciences, Piscataway, NJ, USA) and purified with ProbeQuant G-50 Micro Columns (Amersham Biosciences). The membranes were hybridized at 42 ^{o}C in 50% (w/v) formamide, 6X SSPE, 0.5% (w/v) SDS, 5X Denhardt's solution and 100 mg mL^{-1} sheared salmon sperm DNA with the radiolabeled probes and washed twice in 2× SCC/0.1% SDS at 65 ^{o}C and twice in 0.1× SCC/0.1% SDS at 65 ^{o}C for 15 min (Lee et al., 2006). Full length cDNA probes for *PG* (SGN-U312703) , *PME1.9* (SGN-

U312953), and *LeExp1* (SGN-312702) were obtained from a tomato cDNA library, where as probes for *TBG4* and *Cel1* were created by using RT-PCR-amplification; nucleotides 2220 to 2499 for *TBG4*, and nucleotides 1329 to 1615 for Cel1.

2.2.7 Western Bot Analysis

Protein extracts (20 μg per sample) were precipitated in 20 % trichloroacetic acid (TCA) for 30 min on ice, and then centrifuged at 15,000 × g for 5 min. The supernatants were decanted and the pellets were washed twice with 200 μL cold acetone, dried and resuspended in 20 μL of 2X SDS loading buffer. The samples were separated by SDS-PAGE on 4% to 20% polyacrylamide gels, and transferred to nitrocellulose membrane (Sigma-Aldrich, St. Louis, MO, USA). Membranes were blocked in 5% of non-fat dried milk in PBS-Tween and sequentially incubated with either PG or LeExp1 antiserum (diluted 1:1,500 in PBS-Tween), followed by a 1:10,000 dilution of the horseradish peroxidase-linked whole antibody, and chemiluminescent reagents, using the ECL western blotting detection reagents and analysis system (Amersham Biosciences), before exposure to Hyperfilm ECL (Amersham Biosciences)

2.2.8 Statistical analysis

Analysis of variance (ANOVA) was calculated in each time point using Minitab Statistical Software (Minitab Inc, State College, PA, USA). The means were compared by the Fisher's LSD test at a significance level of 0.05.

2.3 Results

2.3.1 Visible defects

Surface pitting was observed at the bottom of fruit on the stem end side within 1 d in fruit stored for 3 weeks at 3 °C after transfer to 20 °C. Development of sunken water soaking tissue with skin breakage and fungal infection was observed at the same area within 3 d (Figure 2.1).

Figure 2.1 Visible chilling injury symptoms of tomato fruit stored for 3 weeks at 3 °C, then ripened at 20 °C for 3 d.

2.3.2 Ethylene production, color, firmness and extractable juice

Ethylene production was similar in fruit without chilling and those stored at 3 ^{o}C for 1 and 2 weeks (Figure 2.2A). However, ethylene production of fruit stored for 3 weeks was markedly higher for the first 3 d before decreasing over time. During cold storage at 3 ^{o}C, the hue angle decreased slightly over time as fruit changed from green to yellow (Figure 2.2B). During ripening at 20 ^{o}C after cold storage, fruit from all treatments reddened. The effects of storage temperature on hue angle were small, but the hue angle was higher in fruit stored for 2 and 3 weeks compared with fruit that

were not chilled or those stored for 1 week after ripening for 7 and 9 d, and fruit needed 12 d to reach full red color compared with 7 d in non-chilled fruit.

Firmness of fruit decreased during storage at 3 ^{o}C, being softer after 2 and 3 weeks than after 0 and 1 week (Figure 2.2C). Thereafter, chilled fruit tended to be softer than non-chilled fruit for the first day of ripening at 20 ^{o}C, but not consistently so. The percent extractable juice of the pericarp was higher in non-chilled fruit and fruit stored for 3 weeks than in fruit stored for 1 and 2 weeks but only at 1 d at 20 ^{o}C (Figure 2.2D).

2.3.3 Water-soluble pectin and water-insoluble pectin contents, and size exclusion chromatography

To investigate the effects of chilling on pectin solubilization and polymerization, the water-soluble pectin contents and the size distribution of pectic fractions extracted from cell wall materials of fruit at harvest, after ripening at 20 ^{o}C for 7 d, after cold storage at 3 ^{o}C for 2 weeks, and after 2 weeks of cold storage and ripening at 20 ^{o}C for 7 d, were compared. Two weeks of cold storage was chosen because CI was induced without pathogen infection (Figure 2.2B).

The water-soluble pectin content in the fruit stored at 3 ^{o}C for 2 weeks was similar to that of the fruit at harvest (Table 2.1). After ripening at 20 ^{o}C for 7 d, both the non-chilled fruit and the fruit stored at 3 ^{o}C for 2 weeks showed an increase in water-soluble pectin contents. However, the water-soluble pectin content in chilled fruit was lower than that in those without chilling when they ripened for 7 d. The water-insoluble pectin content decreased after 2 weeks of cold storage and further decreased after ripening at 20 ^{o}C for 7 d, the water-insoluble pectin contents of the non-chilled and the chilled fruit after ripening were similar.

Table 2.1 Uronic acid content of the water-soluble and water-insoluble pectin fractions extracted from the alcohol insoluble (AIS) fraction of cell wall of tomato fruit at harvest, after cold storage at 3 ^{o}C for 2 weeks, and after ripening at 20 ^{o}C for 7 d.

Treatments	Uronic acid content (μmole/g AIS)	
	Water-soluble	Water-insoluble
At harvest	0.28 c	2.76 a
20 ^{o}C 7 d	0.78 a	1.96 bc
3 ^{o}C 2 weeks	0.28 c	2.34 b
3 ^{o}C 2 weeks + 20^{o}C 7 d	0.62 b	1.63 c

Mean values within a column followed by the same letters are not significantly different at the 0.05 level by using Fisher's LSD.

The size distribution profiles of the water-soluble pectin fractions (Figure 2.3A) showed that these fractions consisted mostly of high molecular weight pectins with size close to rhamnogalacturonan I (RG I) in endopolygalacturonase solubilized (EPG-sol) fraction from Arabidopsis leaf cell wall (Figure 2.3D). The fruit that stored for 2 weeks at 3 ^{o}C showed a similar size distribution profile as fruit at harvest. After ripening at 20 ^{o}C for 7 d, the size of water-soluble pectins in the non-chilled fruit was smaller than that in the chilled fruit.

Unlike the pectins in water-soluble fractions, the pectins in CDTA- and Na_2CO_3-soluble fractions consisted of a very high amount of oligogalacturonic acid, and a small amount of pectins with larger size (Figure 2.3B and 2.3C). Since there were the differences in the amount of the larger size pectins, it was difficult to tell if the difference in size exclusion profiles of the larger pectins in CDTA- and Na_2CO_3-soluble fractions was really due to the size difference of pectins.

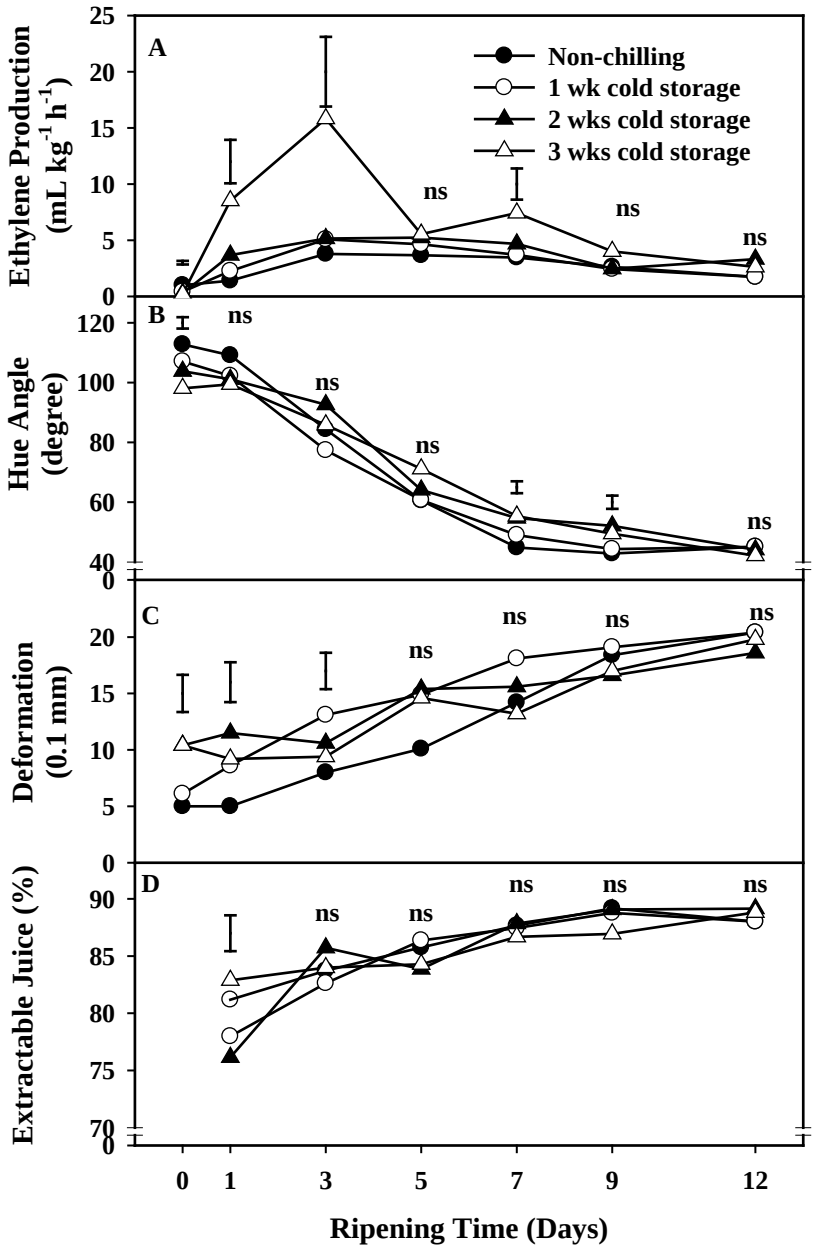

Figure 2.2 Ethylene production (A), color (B), firmness (C), and extractable juice (D) of tomato fruit without chilling or after storage at 3 ^{o}C for 1, 2, and 3 weeks, then ripened at 20 ^{o}C for 12 d. Vertical bars at each time point represent the LSD at the 0.05 level of significance, and ns = no significant difference.

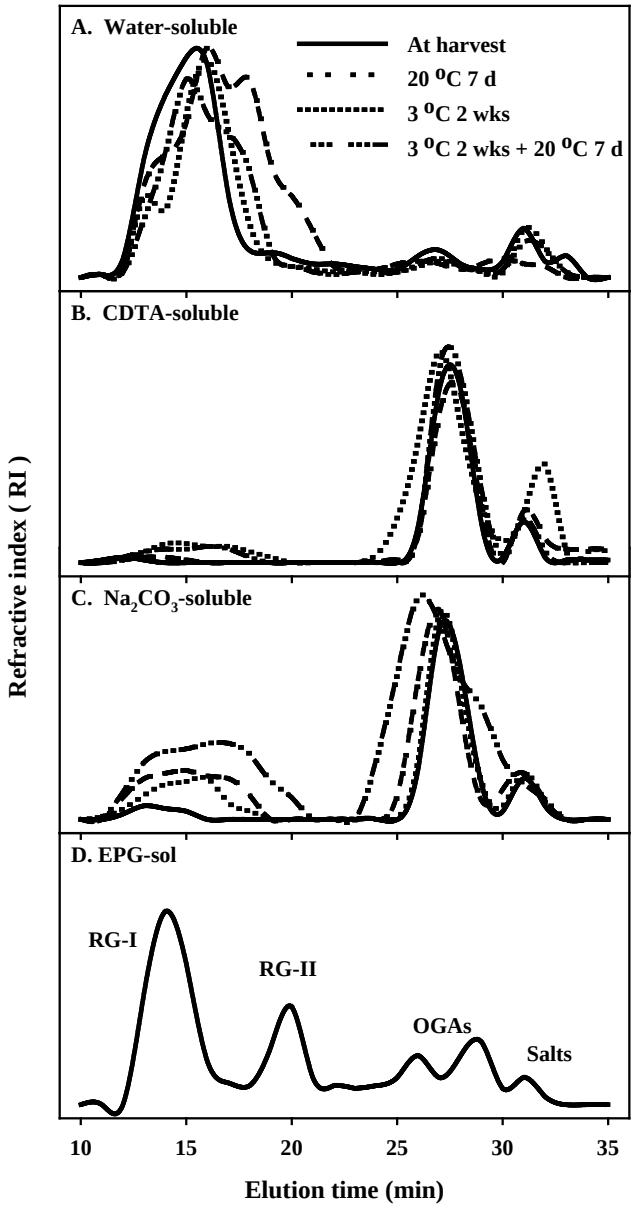

Figure 2.3 Size distribution profiles of water-soluble (A), CDTA-soluble (B), Na_2CO_3-soluble (C) pectins from tomato fruit at harvest, after ripening at 20 ^{o}C for 7 d, after 2 weeks of cold storage at 3 ^{o}C, with and ripening at 20 ^{o}C for 7 d. EPG-sol : an endopolygalacturonase solubilized fraction from Arabidopsis leaf cell wall; RG I : rhamnogalacturonan I; RG II: rhamnogalacturonan II; OGAs: oligogalacturonic acid.

2.3.5 Enzyme activity

PG activity of the non-chilled fruit increased after 3 d of ripening at 20 ^{o}C (Figure 2.4A). PG activity was reduced by about 50% after 1 week of cold storage, and 75% after 2 and 3 weeks of cold storage. PME activity was not affected by cold storage (Figure 2.4B). β-gal activity was similar between fruit at harvest and those stored at 3 ^{o}C for 1 week, but the activity was higher in fruit stored for 2 and 3 week at 3 ^{o}C (Figure 2.4C). During ripening at 20 ^{o}C, β-gal activity was similar in all treatments during the first 3 d of ripening, but then β-gal activity of the non-chilled fruit was lower than that in chilled fruit. EGase activity was not affected by treatments, although there was a trend of lower activity in fruit stored for 3 weeks (Figure 2.4D).

2.3.6 Northern blot analysis

During storage at 3 ^{o}C, *PG* mRNA level in tomato fruit increased slightly after 1 week of cold storage, and then decreased after 2 weeks and 3 weeks of cold storage (Figure 2.5). During ripening at 20 ^{o}C, *PG* mRNA level of non-chilled fruit increased within 1 d, and reached a maximum level after 5 d of ripening. Compared with *PG* mRNA level of fruit without chilling, fruit stored for 1 week at 3 ^{o}C showed slightly decreased of *PG* mRNA level at 5 d after ripening. However, *PG* mRNA level of fruit that stored at 3 ^{o}C for 2 and 3 weeks decreased markedly after 1 and 5 d of ripening. *PME* mRNA level was high in fruit at harvest and gradually decreased during ripening at 20 ^{o}C until 5 d of ripening, then decreased after 12 d of ripening.

The *PME* mRNA of fruit decreased after 1 week of cold storage and decreased markedly after 2 and 3 weeks. However, the level of *PME* mRNA in fruit stored at 3 ^{o}C for 1 week increased to similar levels as fruit without chilling during ripening at 20 ^{o}C. The level of *PME* mRNA in fruit stored at 3 ^{o}C for 2 and 3 weeks was lower

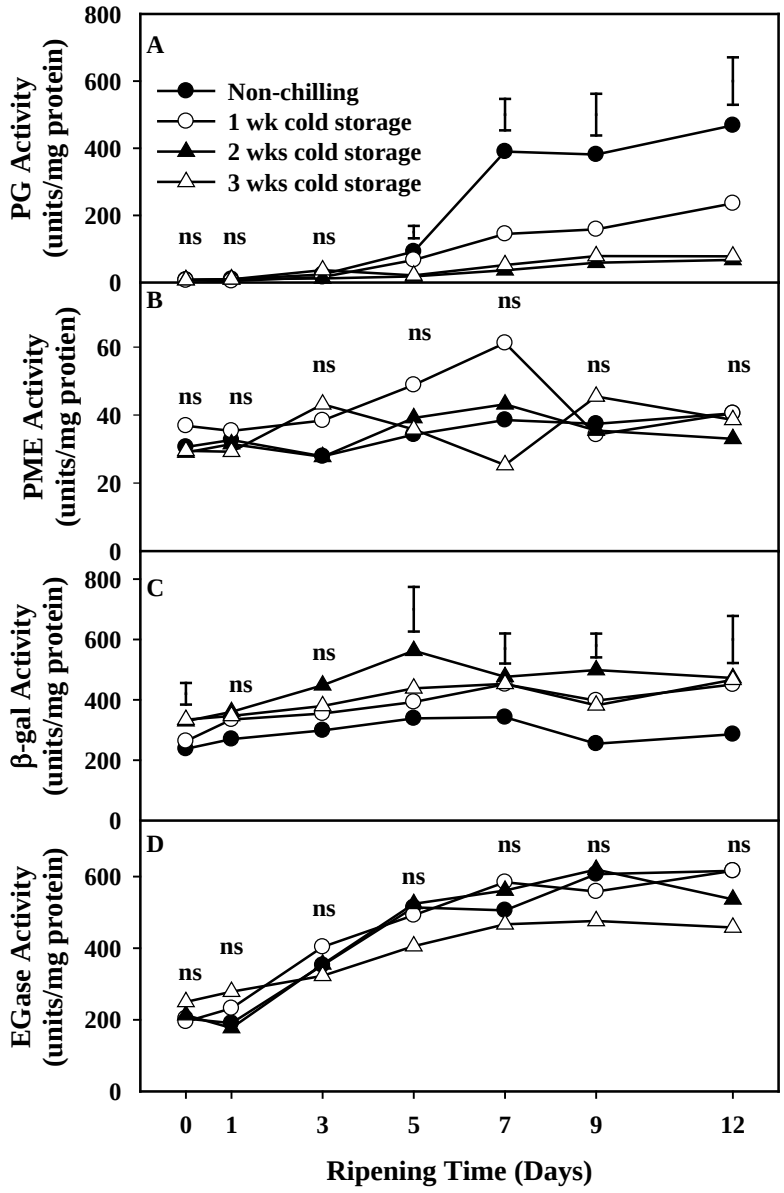

Figure 2.4 Activities of polygalacturonase (PG) (A), pectin methylesterase (PME) (B), β-galactosidase (β-gal) (C), and endo-β-1,4-glucanase (EGase) (D) in tomato fruit without chilling or after storage at 3 ^{o}C for 1, 2, and 3 weeks, then ripened at 20 ^{o}C for 12 d. Vertical bars at each time point represent the LSD at the 0.05 level of significance. ns; no significant difference.

compared with the fruit stored at 3 ^{o}C for 1 week after 1 d of ripening, and slightly lower after 5 d of ripening.

In fruit without chilling, *LeEXP1* mRNA increased to highest level at 5 d of ripening at 20 ^{o}C, then slightly decreased at 12 d after ripening. During cold storage at 3 ^{o}C, expression of *LeEXP1* decreased to undetectable level after 1 week of cold storage and remained undetectable through 3 weeks of cold storage. Expression of *LeEXP1* in fruit stored for 1 week increased to similar levels of non-chilled fruit during ripening. However, fruit stored at 3 ^{o}C for 2 and 3 weeks showed lower levels of *LeEXP1* mRNA compared with fruit without chilling during ripening at 20 ^{o}C.

During ripening at 20 ^{o}C, the mRNA of *Cel1* in the non-chilled fruit was high at 5 d of ripening and was barely detectable at 12 d of ripening. Fruit stored for 1 and 2 weeks showed slightly higher level of *Cel1* mRNA than those without chilling at 12 d. The mRNA level of *Cel1* in fruit stored for 3 weeks at 3 ^{o}C was lower at 5 d of ripening and slightly higher at 12 d of ripening compared with that in fruit without chilling. The *TBG4* mRNA levels in the non-chilled and chilled fruit were similar both during cold storage and ripening at 20 ^{o}C.

2.3.7 Western blot analysis

Expansin protein abundance was similar in non-chilled and chilled fruit during cold storage at 3 ^{o}C and ripening at 20 ^{o}C. PG protein was detected at 12 d of ripening in fruit stored for 1 week and fruit without chilling with the similar levels of PG protein accumulation. In contrast, PG protein was absent in fruit stored at 3 ^{o}C for 2 and 3 weeks before and after ripening (Figure 2.6).

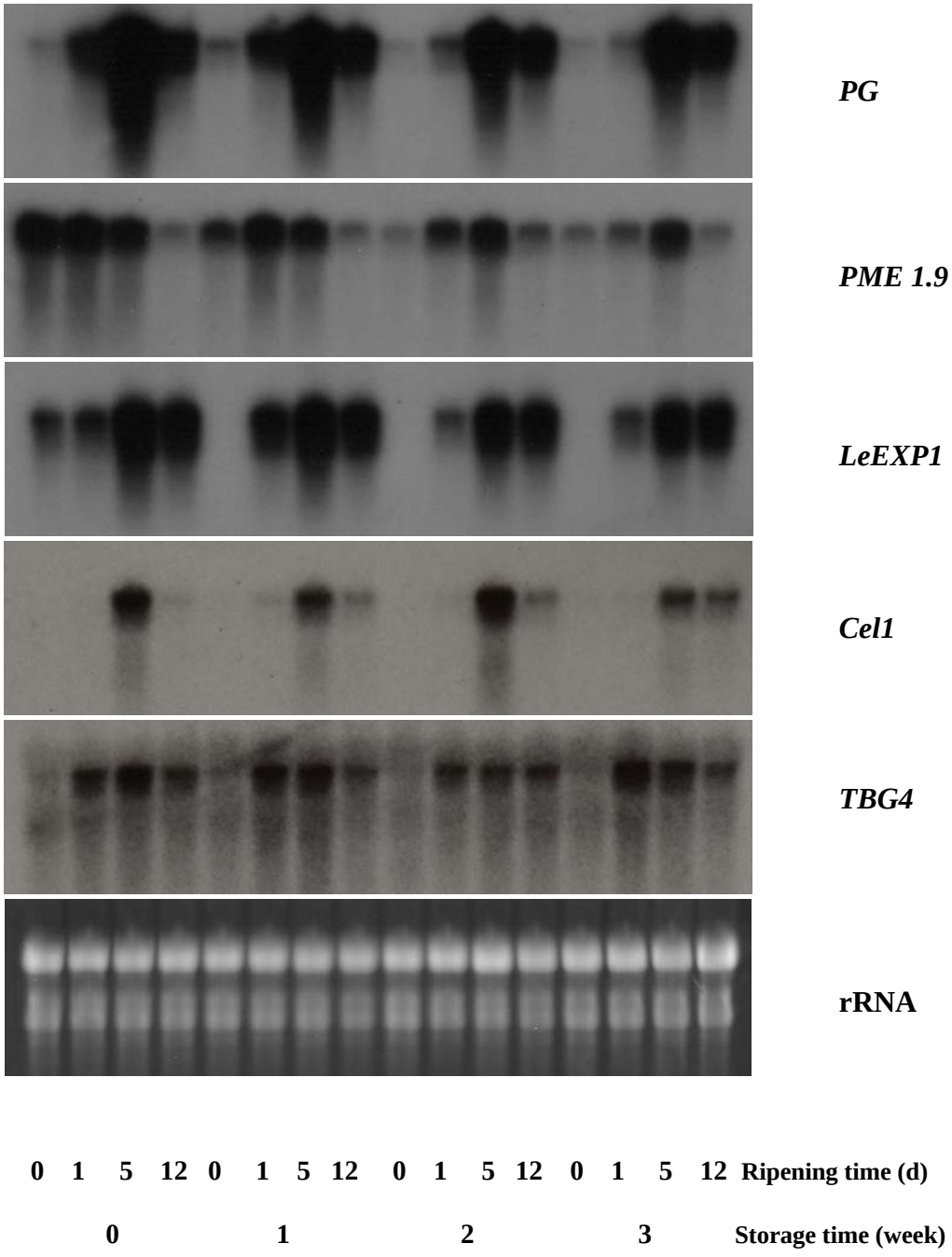

Figure 2.5 Northern blot analysis of polygalacturonase (*PG*), pectin methylesterase (*PME1.9*), expansin (*LeEXP1*), EGase (*Cel1*), and β-galactosidase *(TBG4)* genes of tomato fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for up to12 d.

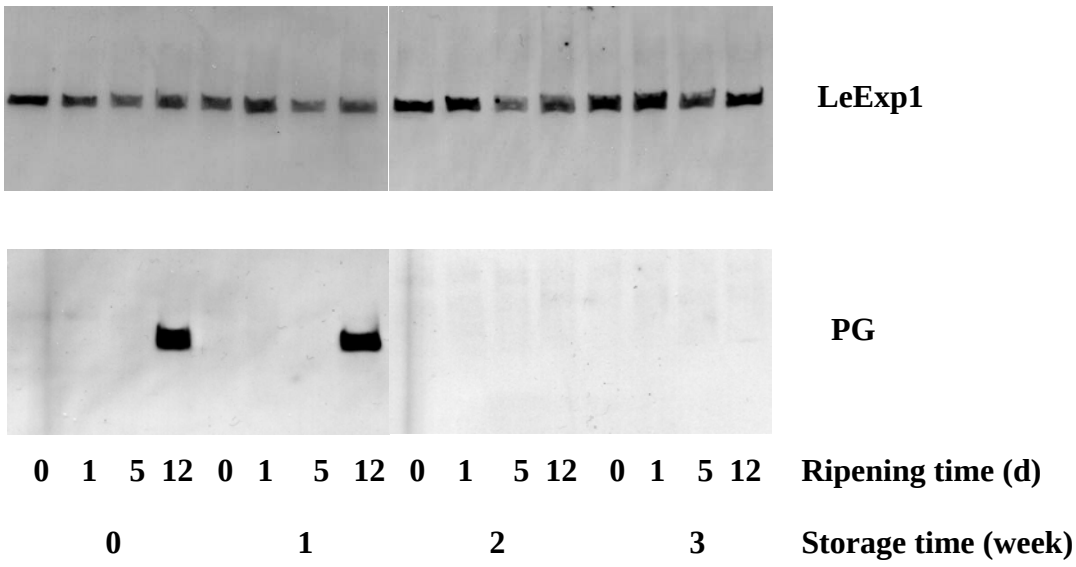

Figure 2.6 Western blot analysis of expansin (LeExp1) and polygalacturonase (PG) proteins of tomato fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for up to 12 d.

2.4 Discussion

The original concept behind this study was that cell wall metabolism in chilled tomato could provide a model system to investigate mealiness development in fruit. However, while mealiness has been reported as a CI symptom in tomato (Jackman et al., 1992), and has been described as a dry, grainy, coarse look at the cut surface (Ahrens and Huber, 1988), the disorder was not detected either by measurements of extractable juice or by visual observation. Nevertheless, the current study indicates that cell wall metabolism of tomato fruit is affected by exposure to CI-inducing temperatures. These effects are comparable to changes associated with mealiness development in stone fruit, but not consistently so.

To investigate the effects of chilling on cell wall metabolism, pectin solubilization and depolymerization of isolated cell walls were examined. Chilled fruit showed no increase in pectin solubilization during storage at 3 °C for 2 weeks as indicated by little change in the water-soluble pectin fraction (Table 1). The pectin solubilization of chilled fruit, however, increased after transfer to 20 °C with only slightly reduced pectin solubilization compared with that of non-chilled fruit. In contrast to pectin solubilization, pectin depolymerization was observed during cold storage as indicated by size exclusion profiles of water soluble pectin (Figure 2.3A). Pectin depolymerization continued after removal of fruit to 20 °C, but to a lesser extent in chilled fruit than in non-chilled fruit. Reduced pectin solubilization and polymerization in chilled fruit has been observed in other fruit such as peaches (Brummell et al., 2004), nectarines (Dawson et al., 1995), and plum (Manganaris et al., 2008), although unchanged solubilization was reported in mealy nectarines compared with juicy fruit (Dawson et al., 1992). In tomato, Almeida and Huber (2008) found greater reduction of pectin solubilization and polymerization than in my study. However, I used fruit stored for 2 weeks that on the basis of color development

showed only slight CI (Hobson, 1987), in contrast to the study of Almeida and Huber (2008) who used fruit with obvious ripening impairment and yellowing. This suggests that differences in the extent of CI contribute to the difference in degree of reduction in pectin solubilization and depolymerization. The extent of pectin depolymerization in non-chilled fruit during ripening in current study was similar to that in another study (Saladie et al., 2007) that used a similar method of size exclusion chromatography.

Increasing exposure to chilling temperatures reduced gene expression (Figure 2.5), total activity (Figure 2.4A), and protein accumulation (Figure 2.6) of PG compared with non-chilled tomato fruit. PG activity was affected by chilling after 1 week of cold storage, while PG protein accumulation was affected by chilling after 2 weeks of cold storage. After ripening at 20 °C for 12 d, fruit stored at 3 °C for 2 and 3 weeks showed the lower levels of PG protein than non-chilled fruit and fruit stored for 1 week at 3 °C although the *PG* mRNA level of these non-chilled and chilled fruit were similar, suggesting that chilling had an effect on post-transcriptional regulation of *PG* gene expression as chilling may decrease translation process of PG protein and/or increase protein turnover of PG. Therefore, *PG* mRNA levels may not be a good predictor of PG protein in chilled fruit.

Decreased expression of PG (Watkins et al., 1990) and reduced PG activity (Marangoni et al., 1995) has been reported in chilled tomato fruit, and other fruit, such as the mango (Ketsa et al., 1999), when exposed to chilling temperatures. The development of mealiness in stone fruit has long been associated with lower PG activity (Buescher and Furmanski, 1978; Ben Arie and Sonego, 1980; Artes et al., 1996; Zhou et al., 1999) and an increase of PME activity (Ben Arie and Sonego, 1980; Lurie et al., 2003; Brummell et al., 2004). Reduced total PG activity in mealy peaches and nectarines formed the basis of the hypothesis that imbalances between PG and

PME activities resulted in mealiness development (Ben Arie and Sonego, 1980). Most often, reduced activity of endo-PG has been observed (Buescher and Furmanski, 1978; Artes et al., 1996; Zhou et al., 1999; Brummell et al., 2004), although reduced (Zhou et al., 1999; Zhou et al., 2001), or unchanged exo-PG activity (Artes et al., 1996; Brummell et al., 2004) have also been reported. However, *PG* mRNA abundance was not affected by chilling in nectarines (Zhou et al., 2000), and even enhanced *PG* gene expression was found in mealy peaches (Zhou et al., 2001).

In tomato, the role of PG in cell wall solubilization has been controversial. While increasing PG activity in transgenic *rin* fruit caused increased pectin solubilization (Giovannoni et al., 1989), and transgenic tomato suppressed in PG activity showed reduced water-soluble pectin (Carrington et al., 1993), other experiments in PG-antisense fruit have shown no correlation between pectin solubilization and PG (Smith et al., 1990; Brummell and Labavitch, 1997; Almeida and Huber, 2008). In contrast, it is thought that PG is responsible for pectin depolymerization (Brummell and Labavitch, 1997; Almeida and Huber, 2008). Therefore, the relatively small effects of chilling on pectin polymerization provide an interesting contrast with the large effects of chilling on PG activity and protein accumulation in current study. However, this contrast seems to be reasonable because PG-antisense fruit in which activity had been suppressed to 1% showed relatively small effects on pectin polymerization (Brummell and Labavitch, 1997).

PME activity was not affected by cold storage (Figure 2.4B), in contrast with increased PME activity reported for tomato by (Marangoni et al., 1995). Reduced PME activity (Buescher and Furmanski, 1978), and increased PME activity (Ben Arie and Sonego, 1980; Lurie et al., 2003; Brummell et al., 2004) also have been reported for chilled stonefruit, while higher PME activity was found in chilled mango fruit (Ketsa et al., 1999). Unlike PME activity, *PME* gene expression was reduced in

chilled fruit during cold storage and during subsequent ripening (Lurie et al., 2003) although greater PME gene expression in mealy peaches and nectarines was reported by Zhou et al. (2000).

In addition, PG is not the only enzyme causing pectin polymerization. PME plays the role in demethylation of pectins and makes them susceptible to PG, whereas, β-galactosidase and α-arabinosidase are thought to cause the role in the loss of galactan and arabinan side chains in pectic polymers, respectively. Furthermore, expansin may also play a role in pectin polymerization by providing the access of pectic degrading enzymes to their substrates (Brummell et al., 1999). Increased β-gal activity (Figure 2.4C), together with, unchanged PME activity (Figure 2.4B) and expansin protein accumulation (Figure 2. 6) in chilled tomato fruit compared with non-chilled fruit may be associated with the modest changes of pectin polymerization of chilled tomato in the current study.

A β-galactosidase activity increased in chilled tomato fruit, although transcript accumulation of TBG4, a gene that encodes β-galactosidase II protein, was not affected by cold storage (Figure 2.5). In contrast, a reduction of β-gal activity was observed in mealy peaches (Brummell et al., 2004) and chilled mangoes (Ketsa et al., 1999) during ripening. Neither EGase activity nor *Cel1*gene expression was affected by chilling (Figure 2.4D and 2.5). Decreased EGase activity has been reported in mealy peaches (Brummell et al., 2004), although increased EGase activity and its gene expression has been reported for mealy peaches (Zhou et al., 2000). In this study, transcripts of *Cel5* and *Cel8* mRNA, with homology to EGase gene in peaches, were undetectable (data not shown). *LeEXP1* gene expression in chilled fruit was inhibited during cold storage and reduced during subsequent ripening. However, LeExp1 protein accumulation was not substantially affected by chilling. Since the amount of LeExp1 protein was unchanged during cold storage while the levels of *LeEXP1*

mRNA were not detectable after 1 week of cold storage, it is suggesting that LeExp1 protein may have a long half-life during cold storage. In contrast, expansin gene expression and protein accumulation was reduced in mealy tissue in peaches (Obenland et al., 2003). Unchanged EGase activity and LeExp1 protein accumulation may contribute to fruit softening of chilled fruit during cold storage at 3 ^{o}C and during early stage of ripening after transfer of fruit to 20 ^{o}C (Figure 2.1C).

In conclusion, chilling had a marked effect on gene expression, total activity, and protein accumulation of PG. However, pectin solubilization and depolymerization did not seem to be affected much by chilling compared with the reduced PG activity or protein accumulation. Chilling had an effect on *PG* gene expression at both the transcriptional and post-transcriptional level. The differences in response of the other cell wall modification proteins to chilling among fruit species could contribute to differences in how fruit exhibit the CI symptom associated with the chilling-altered cell wall metabolism, such as mealiness, excessive softening, and failure to soften.

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CHAPTER 3

THE EXPRESSION OF RIPENING-RELATED GENES AFFECTED BY CHILLING IN TOMATO FRUIT

Abstract

The effects of chilling on fruit ripening and the expression of ripening-related genes have been investigated in the tomato introgression line 11-2 (*Solanum lycopersicum* × *S. pennelli*) (IL 11-2). Fruit were harvested at breaker stage, stored at 3 °C for 0, 1, 2 and 4 weeks, and then transferred to ripen at 20 °C for 0 to14 d. Fruit ripened normally, as assessed by red color development and softening at 20 °C, if stored for 1 week, but ripening was delayed or inhibited in fruit stored for 2 and 4 weeks. The extent of ripening inhibition was more severe in fruit stored for 4 weeks than fruit stored for 2 weeks. Climacteric ethylene production was greatest in fruit stored for 1 week, but reduced in fruit stored for 4 weeks. The expression of ripening-related genes affected by chilling was assessed using reverse transcription polymerase chain reaction (RT-PCR). Genes involved in color development, phytoene synthase1 (*PSY1*), carotenoid isomerase (*CRTISO*), geranyl-geranyl diphosphate synthase 2 (*GGPPS2*), and 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*) were reduced by chilling, as were polygalacturonase (PG), pectin esterase1 (PE1), β-galacturonase *TBG4*, expansin1 (*LeExp1*), and xyloglucan endotransglucosylase/hydrolase 5 (*XTH5*), associated with cell wall-modification. Alcohol dehydrogenase 2 (*ADH2*) and alcohol acyltransferase (*AAT*) gene expression was also reduced by chilling. Fruit stored for 1 week showed an increased or unchanged gene expression of *ACS2*, *ACS4*, whereas reduced *ACS2* and *ACS4* expression was observed in fruit stored for 4 weeks. *ACO1* expression increased in chilled fruit during cold storage, but decreased after

removal to 20 °C. Fruit stored for 1 and 2 weeks showed a transient increase in *NR* expression, while the reduced *NR* expression was observed in fruit stored for 4 weeks through ripening. In contrast, *LeETR1* was induced by chilling during cold storage, and then decreased after removal to 20 °C. Reduced *LeETR4* expression was observed in fruit stored for 4 weeks, while fruit stored for 1 and 2 weeks showed slightly reduced *LeETR4* during early state of ripening. The expression of *LeCTR1*, encoding a LeCTR1 protein was induced during cold storage, but the expression levels decreased after removal to 20 °C. The expression of genes involved with positive control of the ethylene transduction pathway, such as *LeEIL3*, *LeEIL4*, and *LeERF3* was slightly reduced by chilling during ripening at 20 °C. The expression of *LeMADS-RIN*, encoding a ripening-specific transcription factor Le-MADS-RIN, was increased after 1 week of cold storage but was reduced after 4 weeks. Fruit stored for 4 weeks showed reduced *LeMADS-RIN* expression during ripening, while fruit stored for 1 week showed reduced *LeMADS-RIN* after 3 d of ripening. Microarray analysis showed that the gene expression of transcriptional repressors such as C2H2-type zinc finger proteins was induced by chilling. The effect of chilling on ethylene biosynthesis, ethylene perception, the expression of ripening control transcription factors, and transcriptional repressors may contribute to the alteration of fruit ripening in tomato.

Keywords: Chilling injury; Tomato; Gene expression; Fruit ripening, Ethylene receptor, Le-MADS-RIN.

3.1 Introduction

Horticultural crops of tropical and subtropical origin are usually susceptible to chilling injury (CI), after exposure to low, but higher than freezing point,

temperatures. Susceptibility of fruits and vegetables to CI is an important postharvest problem because the extent to which low temperature storage can be used control ripening and deterioration for storage and transport is limited; 13 -15 °C is usually an optimum storage temperature for CI-sensitive crops (Couey, 1982; Raison and Lyons, 1986). Tomato fruit are susceptible to CI if the fruit are exposed to temperatures below 12.5 °C, commonly visible CI symptoms being an alteration of ripening process as indicated by delayed or even total failure of fruit color development and softening (Hobson, 1987; Cheng and Shewfelt, 1988; Efiuvwevwere and Thorne, 1988; Lurie and Klein, 1991)

Fruit ripening is a developmental process in which fruit undergo physiological and metabolic changes, such as changes in color, texture, flavor, aroma, nutrition, that render fruit to be attractive to potential consumers in order to promote seed dispersal (Giovannoni, 2007). Tomato fruit ripening is accompanied by changing of color from green to red, softening, and increasing of tomato flavor and aroma. The change from green to red color during ripening is due to chlorophyll degradation and accumulation of lycopene, a major carotenoid in tomato that provides red color (Ronen et al., 1999). Tomato fruit softening is thought to be the consequence of a decrease in cell turgor pressure (Shackel et al., 1991; Saladie et al., 2006), cell wall disassembly, and reduced cell adhesion during ripening (Brummell, 2006). Cell wall disassembly has been extensively studies in tomato. It involves the solubilization and depolymerization of xyloglucan polysaccharides and pectic polysaccharides (Brummell, 2006). Cell wall-modifying enzymes/proteins thought to be involved in this process include polygalacturonase (PG), pectin methylesterase (PME), β-galactosidase (β-gal), endo-1,4- β-glucanase (EGase), xyloglucan endotransglucosylase/hydrolase (XTH), and expansin (Rose and Bennett, 1999; Bennett and Labavitch, 2008)

Fruit ripening is regulated by ethylene and developmental factors (Barry and Giovannoni, 2007; Giovannoni, 2007). Ethylene is a key regulator hormone for fruit ripening. Tomato is considered as a climacteric fruit in which an increased ethylene production and respiration rate occurs during ripening (Barry and Giovannoni, 2007). Control of endogenous ethylene synthesis or ethylene perception can affect fruit ripening. Transgenic tomato with suppression of ACC synthase, a rate-limiting step enzyme in ethylene biosynthesis, fails to ripen in the absence of exogenous ethylene (Hamilton et al., 1990). Also, applying 1-MCP, an inhibitor of ethylene action, delays tomato fruit ripening (Hoeberichts et al., 2002; Tassoni et al., 2006).

Study of ripening control in tomato mutants, such as *Ripening–inhibitor* (*rin*) and *Colorless non-ripening* (*Cnr*) has revealed that the underlying mutations are associated with ripening control transcription factors that act upstream of ethylene and have non-ethylene mediated activities, since the mutants fail to produce climacteric ethylene, and fail to ripen with the present of ethylene although the mutants show the response to ethylene as indicated by induction of ethylene-inducible genes (Giovannoni, 2007). In the *rin* mutant, the failure to ripen phenotype is due to a mutation in the *Le-MASD-RIN* gene, which encodes a MADS-box transcription factor necessary for fruit ripening (Vrebalov et al., 2002).

While the effect of chilling on fruit ripening has been reported in many studies, few have examined the effects of chilling on the expression of ripening-related genes. Watkins et al. (1990) and Lurie et al. (1996) found that the expression of some ripening-related genes, including those that encode PG, ACC oxidase and phytoene synthase, were altered by chilling in tomato. The expression of genes encoding cell wall-modifying enzymes/proteins that may involve in developing of mealiness in peaches and nectarines has been examined (Dong et al., 2001; Lurie et al., 2003), and increased expression of cell wall modifying genes by cold storage has been reported

(Fonseca et al., 2005). In avocado, the effect of chilling on gene expression during cold storage has been studied (Dopico et al., 1993). Reduced expression of genes encoding expansins was observed in cold-stored bananas (Yong et al., 2006)

The objective of the current study was to examine the effects of chilling on a wider range of metabolic processes than those involved in cell wall disassembly. To meet this objective we first carried out an initial microarray analysis of chilled and non-chilled fruit using the long oligo-based TOM2 cDNA array that allows the detection of differential expression for about 12,000 independent tomato genes at the same time. On the basis of these comparisons, we chose genes involved in various metabolic pathways including ethylene biosynthesis and perception, and cell wall changes, and aroma.

3.2. Materials and methods

3.2.1 Plant material

Fruit were harvested at the breaker stage of maturity from a tomato introgression line 11-2 (IL 11-2), derived from a cross between *Solanum lycopersicum* and *S. pennellii*, grown in a greenhouse at the Guterman Bioclimatic Laboratory, Ithaca, New York, and divided randomly into 20 groups of 6 fruit. One group was used for evaluation on the day of harvest. The remaining fruit were either kept at 20 ^{o}C for 1, 3, 7 and 14 d, or placed in storage at 3 ^{o}C for 1, 2 and 4 weeks. After each storage period, 4 groups of fruit were transferred to 20 ^{o}C and evaluated at 1, 3, 7, and 14 d. At each storage time, and shelf life period, fruit were used for measurements of ethylene production, color and firmness, and then the pericarp tissues were frozen and stored at -80 ^{o}C for later extraction of RNA.

3.2.2. Ethylene production, color, whole fruit firmness, and pericarp firmness

To measure ethylene production, fruit were sealed individually in 470 mL containers for 1 h at 20 ^{o}C or 4 h at 3 ^{o}C. Headspace gas samples were analyzed in duplicate using a Hewlett Packard 5890 series II gas chromatograph (Hewlett Packard Co., Wilmington, Delaware, USA), equipped with a stainless steel column packed with 60/80 mesh alumina F-1 (2mX4mm, i.d.) and a flame ionization detector. Operating conditions were as follows: oven temperature 180 °C, injector temperature 230 °C, and detector temperature 250 °C. Flow rates for nitrogen, hydrogen and air were 45, 30, and 200 ml min $^{-1}$, respectively. The ethylene production rate was expressed as µL kg $^{-1}$ h $^{-1}$.

The color of each fruit was measured three times around the equator, using a Minolta Chroma Meter, Model CR-300 (Minolta, Mahwah, New Jersey, USA) and expressed as hue angle, where 0 ^{o} = red-purple color, 90 ^{o} = yellow, 180 ^{o} = bluish-green, and 270 ^{o} = blue (McGuire, 1992)

Whole fruit firmness was measured by a compression test using a Precision Penetrometer (GCA/Precision Scientific, Chicago, Illinois, USA) using 0.1 mm divisions. Fruit were held in a V-shape hollow base, and fruit deformation was measured after a 0.5 kg force was applied at the fruit equator for 5 s (Manzano-Mendez et al., 1984). Firmer fruit have lower deformation values than softer fruit.

Pericarp firmness was measured by a puncture test on opposite pared sides around the equator of each tomato using a Force Five pressure tester (Model FDV-30, Wagner Instruments, Greenwich, CT, USA.) fitted with a 7.9 mm diam. flathead probe. The skin (epicarp) of each tomato was removed before measuring pericarp firmness.

3.2.3 RNA isolation

Total RNA was isolated from 7.5 g of frozen pericarp tissue as described by Chang et al. (1993). Briefly, pericarp tissue was ground in liquid nitrogen, then homogenized in 15 mL prewarmed (65 ^{o}C) CTAB extraction buffer using a Ultra-Turrax T25 Homoginizer (IKA Works, Inc, Willington, NC, USA) for 1 min, then,15 mL of chloroform :isoamylalcohol (24:1, v/v) was added. The mixture was mixed by vortexing for 2 min, and then centrifuged at 8000 × g for 10 min at room temperature. The aqueous phase (the top phase) was transferred and filtered through Miracloth (CalBiochem, San Diego, CA, USA), and extracted again with chloroform:isoamylalcohol (24:1, v/v) as described above. The aqueous phase was filtered, a 0.25 volume of 10 M LiCl was added, and the total RNA was precipitated overnight at 4 ^{o}C. After centrifugation at 8000 × g for 20 min, the RNA was dissolved in 500 μL of prewarmed (65 ^{o}C) SSTE buffer, and extracted once with 500 μL of chloroform:isoamylalcohol (24:1, v/v). The aqueous phase was transferred to new tubes and 0.5 volume of ice-cold absolute ethanol was added. RNA was precipitate at -80 ^{o}C for 30 min, and after centrifugation at 10,000 × g for 20 min at 4 ^{o}C the pellet was dissolved in DEPC water. Aliquots of RNA samples were treated with DNase I to remove DNA I using the TURBO DNA-free kit (Ambion, Austin, TX, USA).

3.2.4 Microarray analysis

The SuperScript Indirect cDNA Labeling Kit (Invitrogen Corp., Carlsbad, CA, USA) was used for first-strand cDNA synthesis from total RNA, extracted from tomato pericarp, and cDNA labeling with fluorescent dye (Cy3- and Cy5-dye). Then, Cy-labled cDNA wash hybridized to the TOM2 oligo-array, obtained from The Center of Gene Expression Profiling at Boyce Thompson Institute for Plant Research (http://ted.bti.cornell.edu/). See Appendix A for details of the protocols used for first-

strand cDNA synthesis, cDNA labeling, pre-hybridization, hybridization, washes, and subsequent scanning of TOM2 microarray.

Two microarray comparisons were carried out: (1) fruit stored at 3 °C for 4 weeks and non-chilled fruit; and (2) before and after ripening at 20 ^{o}C for 7 d to examine the effects of chilling on gene expression during cold storage and subsequent ripening, respectively. Each comparison was performed in triplicate. The microarray data were log transformed (log_2) and presented as the expression ratio.

3.2.5 Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR)

A two-step RT-PCR was performed using the RETROscript kit (Ambion, Austin, TX, USA). First strand cDNAs were synthesized from 1.5 μg of total RNA with random hexamers as primers in RT reactions. One μL of RT reaction was used for PCR amplification. cDNAs were denatured at 94 ^{o}C for 5 min. PCR cycling conditions were 95 ^{o}C for 30 sec, 60 ^{o}C for 30 sec, and 72 ^{o}C for 30 sec, followed by 7 min of extension at 72 ^{o}C. QuantumRNA Universal 18S Internal Standard (Ambion, Austin, TX, USA) was used as internal standard, and the ratio of 18S primers: competimers used in PCR reactions. Gene specific primers, number of PCR cycles, and the 18S primer: competimer ratio used for PCR amplification of each gene is shown in Table 1. Ten μL of PCR product was fractionated in 1.5 agarose gel in 1× TBE buffer, stained with ethidium bromide (0.5 μg/mL), and digitally photographed using a UVIpro gel documentation system (UVItec, Inc, Cambridge, UK). The band intensity was quantified using a Bio-Rad Quantity One software program (Bio-Rad, Hercules, CA, USA). Signal intensity was normalized with the signal intensity of 18s internal standard from the same reaction to obtain relative signal intensity. The gene expression was reported as the relative gene expression as the realative signal was normalized to the relative signal from fruit at harvest.

Table 3.1 Primer sequences, number of PCR cycle (cycle #), and 18S primers : competimers ratio (18s primer ratio).

Gene	Accession	Primer sequences (5′ – 3′)		Cycle #	18S primer ratio
		Forward	Reverse		
PSY1	M84744	ATGGTGCTTTGTCCGATACA	ACCAAAGATGCCCATACAGG	25	1:5
CRTISO	AF416727	AGACATTTTGGC GGAATCAACTA	GAGAGTCCCTCCCAATCTTCAAT	33	1:8
DXS	AF143812	TTCTGATGAAGCGGAGCTATTTC	GCTGCAATGTGAGATGGTGTTAG	36	1:10
GGPPS2	DQ297903	AAGCCGACGAATCATAAAGTGTA	CAAATTCCTTAGCCTTTTCCAAT	36	1:11
PG	X04583	ACCTTTTCAGGTCCATGCAGAT	CCATGACCTGGACCACAAGTAA	25	1:5
PE1	X74638	GCAGTCGGCCAAGGATTTATAC	GCACCAGGTCCATTGTTCATAA	27	1:8
TBG4	AF020390	AGGTGGCTATGCAAGGATTTGT	TAGTTGCAATGAAAAGCCCTGA	30	1:7
LeExp1	AF548376	TCCTGGAAACCCTTCCATTTTA	ATTGCCAATGAGAAGGAACCAT	27	1:8
XTH5	AY497475	AGTACTCAATGGATGTGCAATGG	TCATTTTTCACCCTTCACTCTGA	36	1:11
TomLoxC	U37839	CACATTGGAGATAAATGCCTTAGC	CAGTTGTTGGCCTATTTGGAAAG	30	1:7
ADH2	M86724	AGGAGTGTGGAATGTACTGGTCA	AAGAGATATGGCAAAGCACAACA	30	1:7
AAT	AY534531	GCGTGGGAATCTATGGAAGATAA	GCCTTTAAGATCCCTCCAAAAAT	33	1:8
ACS2	AY326958	GGTTAGGTAAAAGGCACAAACAT	GAATAGGTGACGAAAGTGGTGAC	27	1:8
ACS4	M63490	GATTTGCGGTCATTGTTGAAAG	GCCTGGGCGAATCTAGTTTATTT	27	1:8
ACO1	AF532976	TGGAAAAGCTCAATGGAGGAGATGA	GGGCTTAGGACATGGTGGATAG	25	1:5
ACO6	EF501822	GACAAAGTCAGTGGTCTCCAACT	AGTTGCACAAAGCAAGATAAAGC	36	1:11
LeETR1	AF043084	TGTTGGCAATGCTGTAAAGTTCT	TAACACCATTCTCATCCATCACC	35	1:9
NR	U38666	TTGTCATCCTCCTGAAATGTTCC	TTTGTCACACCAATGTCCTTTTG	27	1:8
LeETR4	AF118843	GAATCTTCTGATCATTCGCATCC	CACTCCTATAAGGCACCGTCAAC	27	1:8
LeCTR1	AY079048	CTGGTGCCAGCAGTAGTTGAATAA	ACAATGGTTCAAAGAAATGGA	33	1:8
LeEIL1	AF328784	AAAGGCAAAATAGGGGGAACATT	ATTGGCAAGTATTTGAGGGGTGT	35	1:9
LeEIL2	AF328785	CCAACATTTCTCACAACTCCA	AATATCTCCGAAACCGATTGA	33	1:8
LeEIL3	AF328786	ATGAGCACAACACAGTTTTTCCTC	ATATCCTGCTTTGTCAGCTGTTCC	33	1:8
LeEIL4	AB108840	CTTCAGTTGCTCTGCCTGTTAAT	GCTATATCTATGGAAGCCCCGACT	30	1:7
LeERF2	AY192368	CTAAGATGTGTGGTGGTGCAAT	AACAGGTTCAACGTTCTCGATT	30	1:7
LeERF3	AY192369	TTGATTCATCATCGCCGTTA	GCGATGGCTCTTCAGTTTTC	35	1:9
MADS	DQ157796	TGCAGCAATTCAAGTATGTCCAA	ATGTGTTGATGGTGCTGCATTT	27	1:8

3.2.6 Statistical analysis

Analysis of variance (ANOVA) was calculated for storage and shelf life period with Minitab Statistical Software (Minitab Inc, State College, PA, USA). The means were compared by the Fisher's LSD test at a significance level of 0.05.

3.3 Results

To select tomato variety used in this study, thirty-three tomato introgression lines (ILs), cultivated tomato *Solanum lycopersicon* containing a single homozygous restriction fragment length polymorphism (RFLP)-defined chromosome segment introduced from the green-fruited species *Solanum pennellii* (Eshed and Zamir, 1994), were screened for the alteration of ripening process as a symptom of CI. Tomato line IL 11-2 was chosen in this study because: (1) non-chilled fruit of this line ripened normally within 7 d, as indicated by red color development and fruit softening, (2) its ripening process was obviously altered by chilling, and (3) there was no incidence of pathogen infection in chilled fruit after ripening (Appendix Table B1).

To investigate the effects of chilling on tomato fruit ripening, fruit color, fruit firmness, and ethylene production were measured. Also, the expression of genes associated with fruit ripening was analyzed using RT-PCR. The candidate genes were chosen from two sources: (1) ripening-associated genes obtained from the previous published work and (2) genes affected by chilling using microarray analysis.

The list of genes differentially expressed more than two fold in fruit stored for 4 weeks at 3 °C before and after ripening at 20 °C for 7 d were presented in Appendix Table C1 and C2, respectively. At the end of cold storage at 3 °C for 4 weeks, 352 genes were up-regulated by chilling, whereas 321 genes were down-regulated. After the chilled fruit were ripened at 20 °C for 7 d, there were 180 and 126 genes up-

regulated and down-regulated by chilling, respectively. Table 3.2 shows the number of gene affected by chilling and categorized by gene function.

Table 3.2 Number of genes up-regulated (Up) and down- regulated (Down) by chilling in fruit stored for 4 weeks at 3 °C before and after ripening at 20 °C for 7 d, and obtained from microarray analysis.

Gene function category	Number of genes			
	3 °C, 4 weeks		20 °C, 7 d	
	Up	Down	Up	Down
1. Transcriptional regulation	47	25	10	12
2. RNA processing	8	1	3	1
3. Protein synthesis	34	2	61	2
4. Protein modification	8	13	2	7
5 Protein degradation	25	13	11	5
6. Nucleotide metabolism	3	-	1	-
7. Amino acid metabolism	8	11	-	3
8. Carbohydrate metabolism	11	16	5	8
9. Lipid metabolism	3	20	2	7
10. Transporters	11	11	7	4
11. Secondary metabolism	9	22	5	13
12. Cell wall	3	11	-	7
13. Signal transduction	10	4	8	-
14. Pathogenesis related	9	4	8	-
15. Stress	12	4	10	0
16. Ethylene	-	7	0	5
17. Others	115	133	40	40
18. Unknown	24	17	13	5
19. No hits found	12	9	2	4
Total	**352**	**321**	**180**	**126**

3.3.1 Effects of chilling on color development

Fruit color changed from green to yellow during storage at 3 ^{o}C for 2 and 4 weeks. Red color development, as indicated by decreasing Hue angles, was similar in fruit kept at 20 ^{o}C at harvest and after a week of cold storage, but was inhibited in fruit stored for 2 and 4 weeks (Fig 3.1).

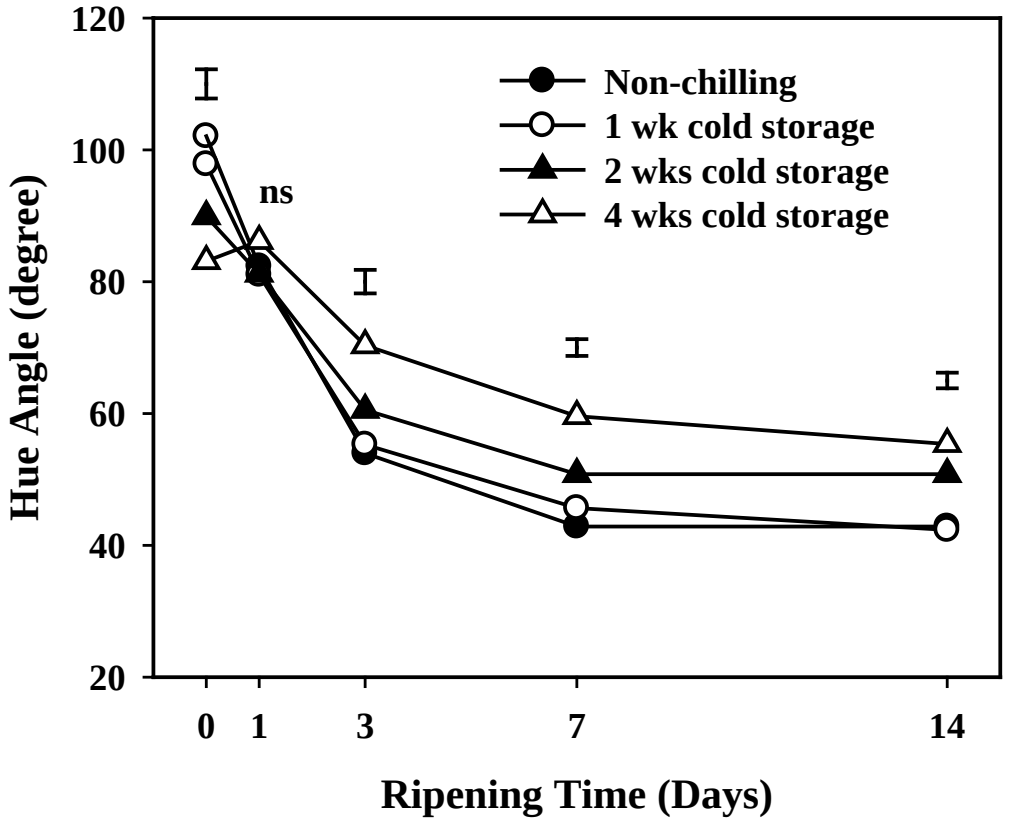

Figure 3.1 Color (hue angle) of tomato fruit stored at 3 ^{o}C for 0, 1, 2, and 4 weeks and then ripened at 20 ^{o}C for 14 d. At each time point, vertical bars represent the LSD at the 0.05 level of significance; ns = not-significant.

The expression of PSY1, encoding phytoene synthase 1, and CRTISO, encoding carotenoid isomerase, in non-chilled fruit was up-regulated during ripening at 20 ^{o}C without cold storage (Figure 3.2A and 3.2B). In general, the expression patterns of these two genes were similar in response to chilling, with progressively reduced expression during cold storage at 3 ^{o}C compared with fruit at harvest.

Expression of *GGPPS2* , which encodes geranylgeranyl pyrophosphate synthase, was similar in fruit stored for 1 week and in non-chilled fruit during ripening at 20 ^{o}C (Figure 3.2C). Fruit stored for 2 weeks showed an increase in *GGPPS2* expression, but then a decline after 3 d of ripening after storage. In contrast, the expression in fruit stored for 4 weeks remained low throughout ripening at 20 ^{o}C. The effects of chilling on expression of *DXS*, which encodes 1-deoxy-D-xylulose-5-phosphate synthase, were similar with those found for GGPPS2, except that chilling had less effect on DXS during storage (Fig 3.2D).

3.3.2 Effects of chilling on fruit softening

To investigate the effects of chilling on fruit softening, fruit firmness was measured using two different methods: compression test to measure whole fruit firmness, and the puncture test to measure fruit pericarp firmness.

According to the compression test, fruit stored for 1 week showed an increase in fruit softening at the early stage of ripening at 20 ^{o}C compared with non-chilled fruit, whereas, fruit stored for 2 and 4weeks failed to soften to the same levels as non-chilled fruit during ripening (Figure 3.3A). Unlike whole fruit firmness, the pericarp firmness in fruit stored for 1 week was similar to that in non-chilled fruit during ripening at 20 ^{o}C. Pericarp firmness in fruit stored for 2 and 4 weeks were higher than non-chilled fruit during ripening at 20 ^{o}C, and fruit stored for 4 weeks were firmer than fruit stored for 2 weeks during ripening after storage.

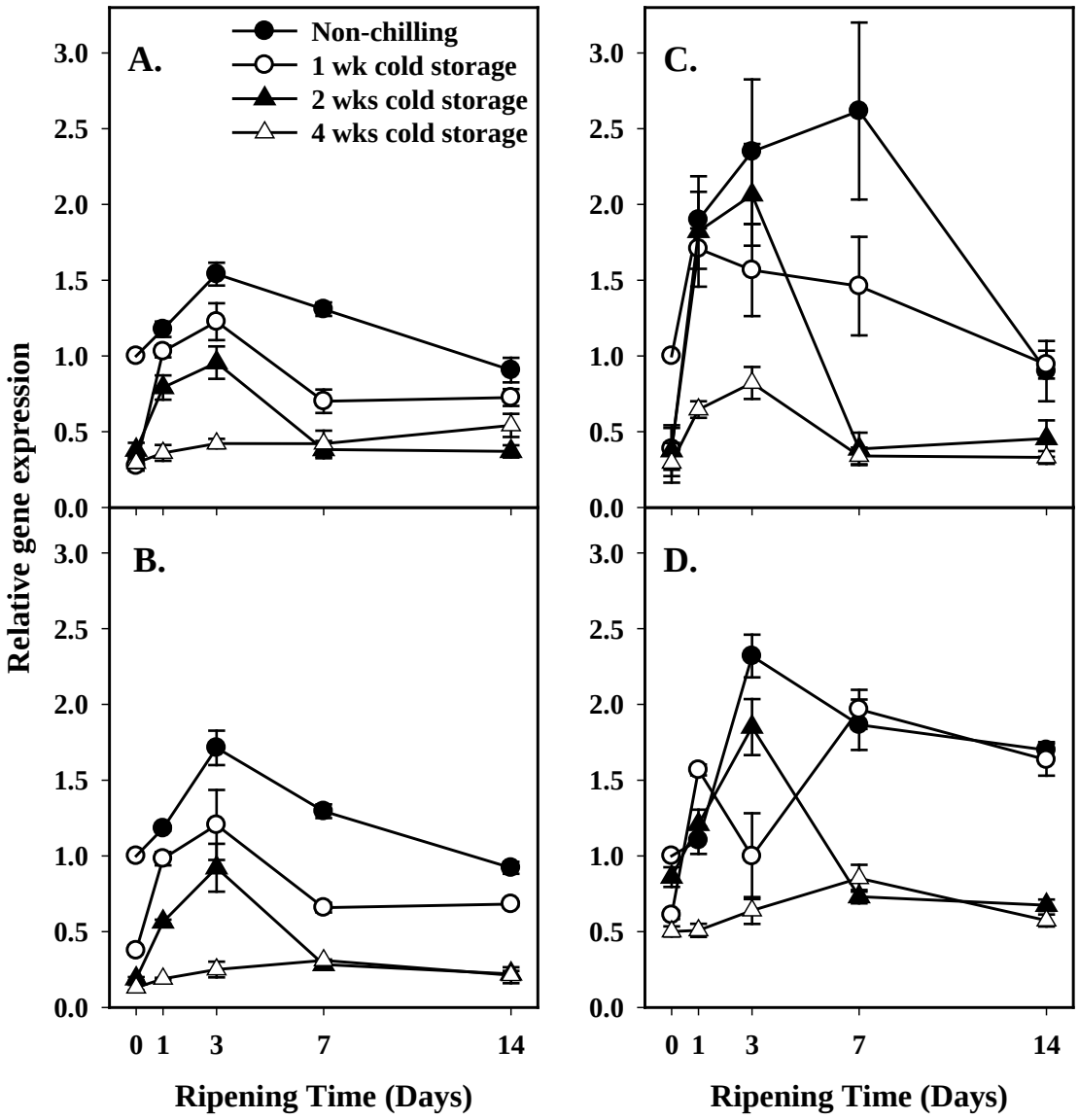

Figure 3.2 RT-PCR analysis of (A) phytoene synthase 1 (*PSY1*), (B) carotenoid isomerase (CRTISO), (C) geranylgeranyl pyrophosphate synthase 2 (GGPPS2), and (D) 1-deoxy-D-xylulose 5-phosphate synthase (DXS) expression levels in fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars represent SE of three independent PCR amplification experiments.

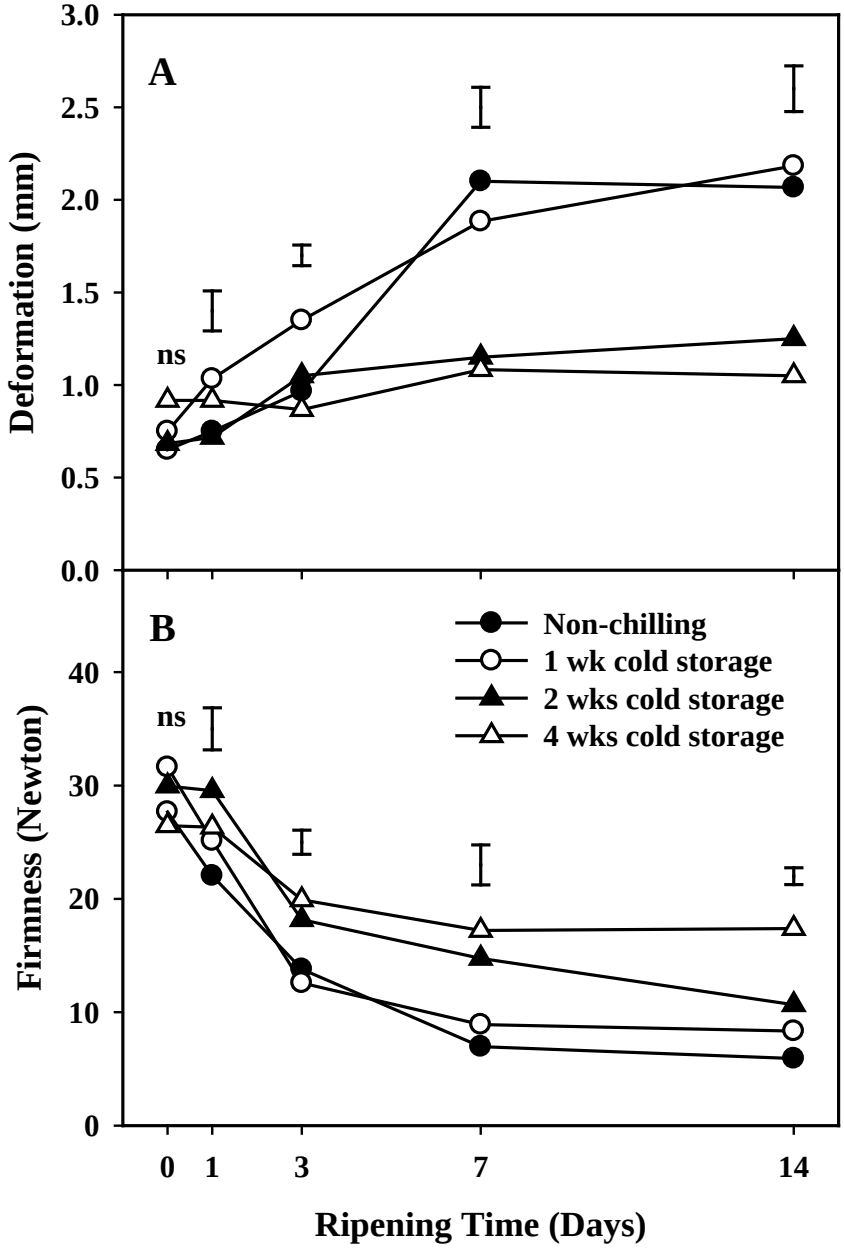

Figure 3.3 Whole fruit firmness (A) and pericarp firmness (B) of fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars at each time point represent the LSD at the 0.05 level of significance. “ns” means no statistical difference between treatments at each time point at the 0.05 level.

The expression of *PG, PE1, TBG4, EXP1* and *XTH5*, encoding polygalacturonase, pectin esterase, β-galactosidase II, expansin, and xyloglucan endotransglucosylase/ hydrolase, respectively, were used as candidate genes to study the effects of chilling on cell wall metabolism.

The expression of *PG* was not affected during cold storage, but the effects of chilling on PG were shown after transfer of fruit to 20 ^{o}C (Fig 3.4A). Fruit stored for 1 and 2 weeks showed similar patterns of *PG* expression to that shown in non-chilled fruit but with lower gene expression levels. Little increase was observed in fruit stored for 4 weeks during ripening at 20 ^{o}C. In non-chilled fruit, the expression *PE1* increased and then declined after 1 day of ripening at 20 ^{o}C (Figure 3.4B). *PE1* expression was reduced during storage. Expression increased after transfer of fruit to 20 ^{o}C, but was still lower than that of non-chilled fruit. The expression of *TBG4* was reduced during storage, but it at 20 ^{o}C (Figure 3.4C). However, the expression levels in chilled fruit were still lower than those of non-chilled fruit.

The expression of *EXP1* was reduced during storage, the extent of reduction being *greater increasing time (Figure 3.5A). EXP1 expression in chilled fruit increased* slightly after transfer of fruit to 20 ^{o}C.. The expression of *XTH5* in non-chilled fruit was up-regulated during ripening at 20 ^{o}C (Figure 3.5B). Unlike the other cell wall-associated genes, the *XTH5* expression was induced in chilled fruit during cold storage. After removal to 20 ^{o}C, the expression of XTH5 declined to similar levels of non-chilled fruit at the early stage of ripening. While the expression of XTH5 in fruit stored for 1 week still remained similar to non-chilled fruit, the expression in fruit stored for 2 and 4 week were lower at later stages of ripening.

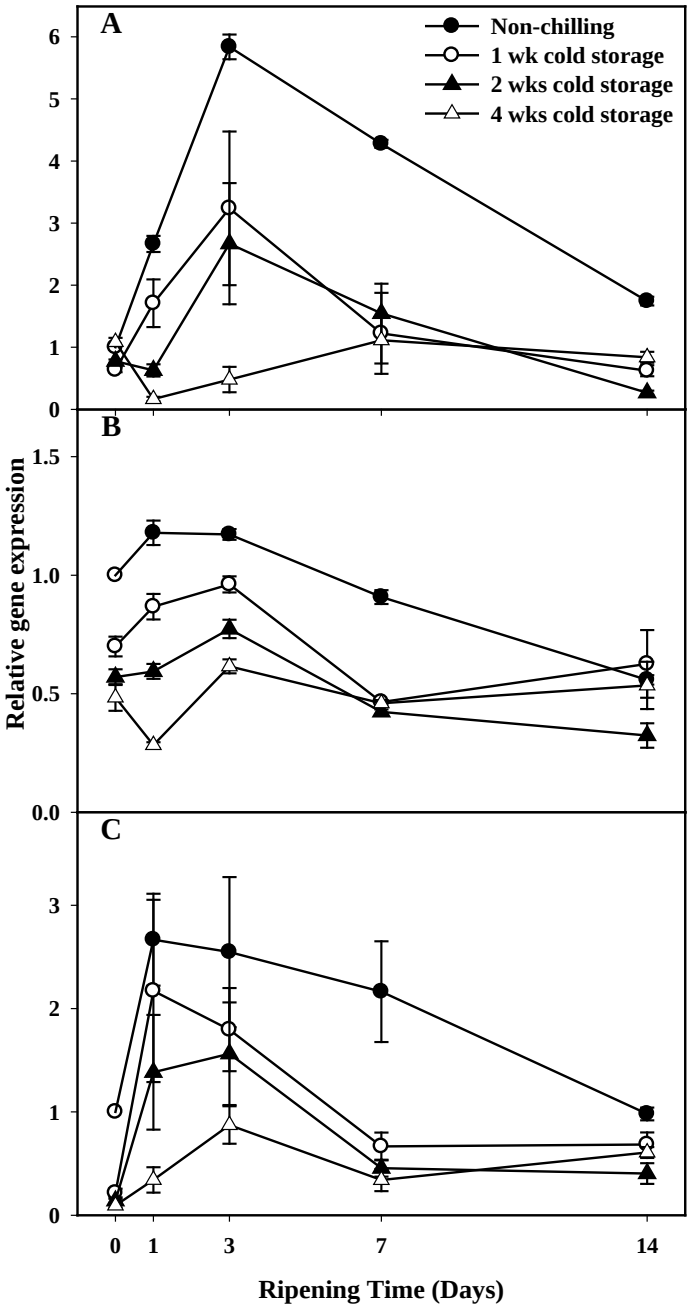

Figure 3.4 RT-PCR analysis of (A) polygalacturonase (*PG*), (B) pectin methylesterase 1(*PE1*), and (C) β–galactosidase 4(*TBG4*) expression levels in fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars represent SE of three independent PCR amplification experiments.

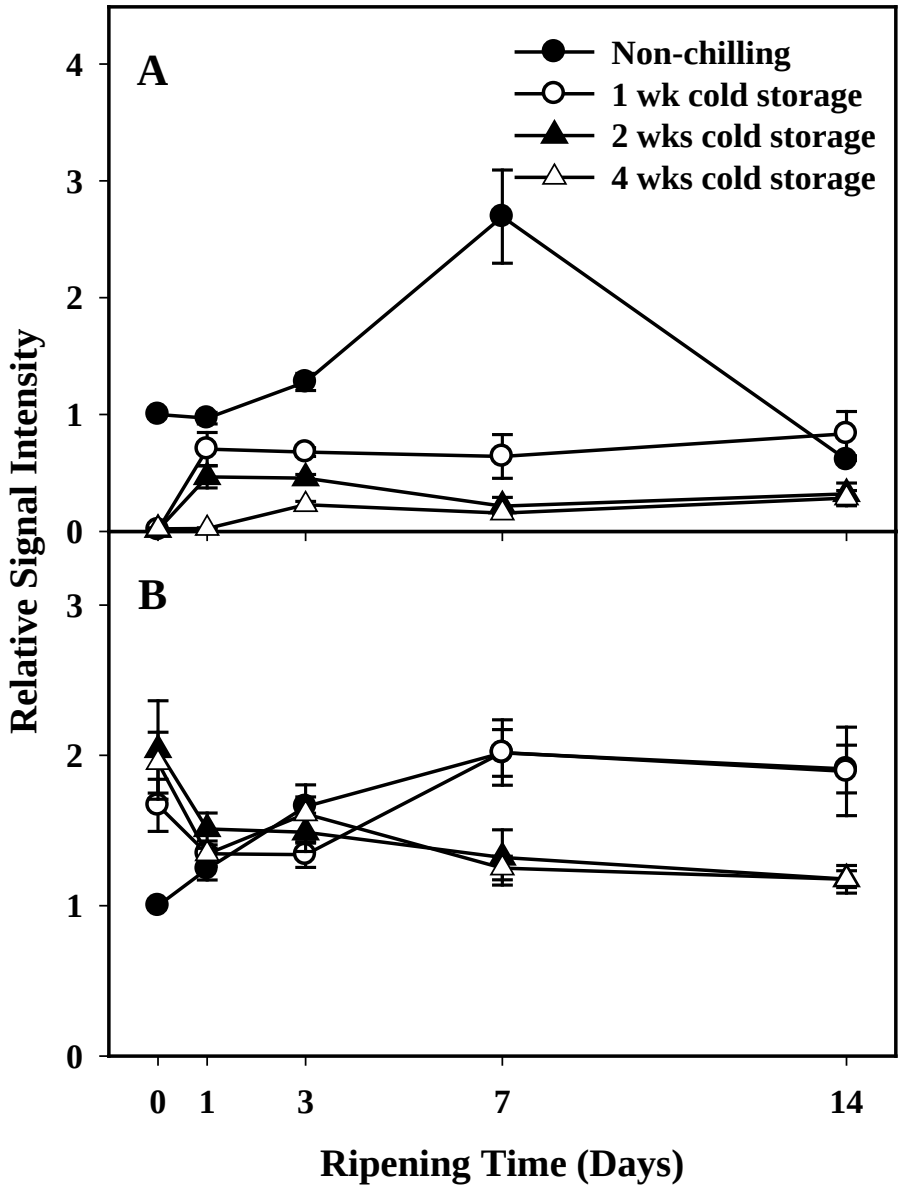

Figure 3.5. RT-PCR analysis of (A) expansin1(*LeExp1*) and (B) xyloglucan endotransglucosylase/hydrolase 5 (*XTH5*) expression levels in fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars represent SE of three independent PCR amplification experiments.

3.3 Effects of chilling on the expression of genes in volatile metabolism

The expression of *TomloxC* was reduced during storage at 3 °C (Figure 3.6A). After removal to 20 °C the expression in fruit stored for 4 weeks remained low, while the expression in fruit stored for 1 and 2 weeks increased to the similar level in non-chilled fruit. However, *TomloxC* gene expression decreased after 3 d of ripening in fruit stored for 2 weeks. In general, the expression patterns of *ADH2* and *AAT* in response to chilling were similar. The expression of *ADH2* and *AAT* was reduced during storage (Figure 3.6B and 3.6C). After removal to 20 ^{o}C, the expression of these genes in fruit stored for 1 and 2 weeks increased during the early stage of ripening, then declined and remained lower than the expression of these genes in non-chilled fruit at the later stage of ripening. The expression of these genes in fruit stored for 4 weeks slightly increased during ripening after cold storage, but expression levels were much lower than in non-chilled fruit.

3.4 Effects of chilling on ethylene biosynthesis metabolism

Ethylene production of tomato fruit responded differently to chilling depending on the length of chilling exposure time. While the ethylene production in fruit stored for 1 week markedly increased after transferring to 20 ^{o}C, the ethylene production in fruit stored for 2 weeks was similar to that of non-chilled fruit at the early state of ripening (Figure 3.7). Compared with non-chilled fruit, the ethylene production in fruit stored for 4 weeks was lower during the early state of ripening.

ACS2 expression increased after 1 week of cold storage, declined to similar levels as the fruit at harvest after 2 week, and then its expression was reduced after 4 weeks of storage (Fig 3.8A). After transfer of fruit to 20 ^{o}C, fruit showed a transient increase of *ACS2* expression. The *ACS2* expression levels in fruit stored for 1 week was still higher than those in non-chilled fruit after 1 d, but then declined to lower

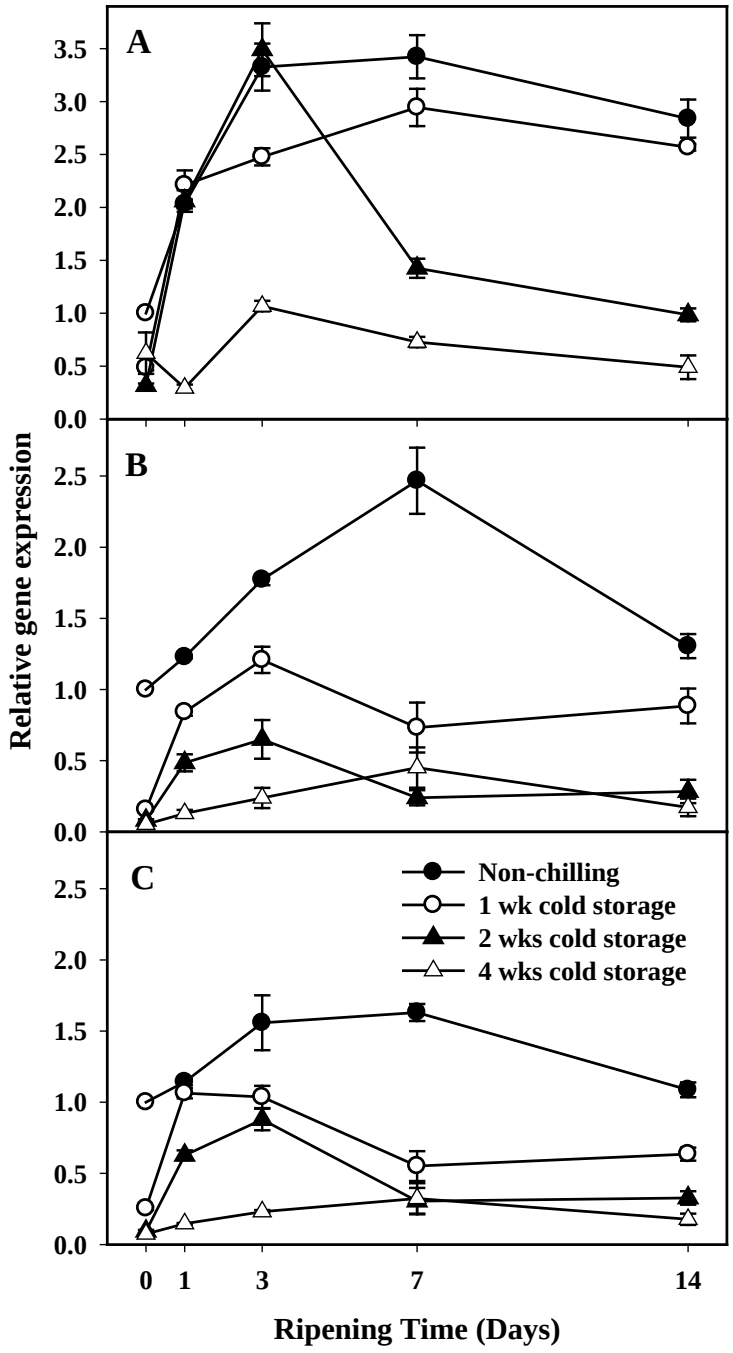

Figure 3.6 RT-PCR analysis of (A) lipoxygenase C (*TomloxC*), (B) alcohol dehydrogenase (*ADH2*) and (C) alcohol acyl transferase (*ATT*) expression levels in fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars represent SE of three independent PCR amplification experiments.

levels after 3 d. The gene expression of fruit stored for 2 weeks was still similar to that in non-chilled fruit at 1 d of ripening, but the levels of gene expression were lower after that. Markedly reduced *ACS2* expression was observed in fruit stored for 4 weeks relative to that of non-chilled fruit.

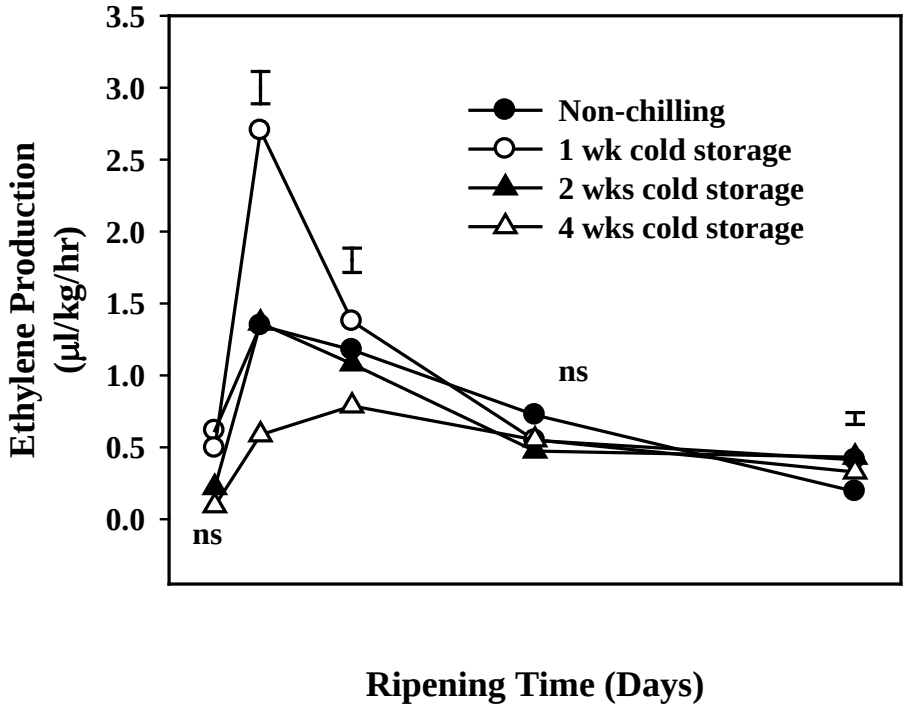

Figure 3.7 Ethylene production of tomato fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars at each time point represent the LSD at the 0.05 level of significance. "ns" means no statistical difference between treatments at each time point at the 0.05 level.

Chilling had no effect on *ACS4* expression at 1 week of cold storage, but the expression was down-regulated at 2 and 4 weeks of cold storage (Figure 3.8B). After removal to 20 ^{o}C, fruit stored for 1 week showed the similar expression levels to those in non-chilled fruit in the first 3 d. Expression levels in fruit stored for 2 and 4 weeks were lower than those in non-chilled fruit, with the greatest reduction in fruit stored for 4 weeks.

The expression of *ACO1* increased slightly during storage, but declined after transfer of fruit to 20 ^{o}C (Figure 3.8C). Chilled fruit had reduced *ACO1* expression compared with non-chilled fruit during ripening at 20 ^{o}C, and the fruit stored for 4 weeks showed the highest reduction in the gene expression.

The expression of *ACO6* in non-chilled fruit was high at harvest and down-regulated during ripening (Figure 3.8D). ACO6 expression was markedly reduced by 4 weeks of cold storage. Expression levels were low during ripening at 20 ^{o}C in both non-chilled and chilled fruit.

3.5. Effects of chilling on the expression of genes in the ethylene signal pathway

LeETR1, *NR* and *LeETR4* expression showed different responses to chilling. While gene expression of ETR1 was induced during storage (Figure 3.9A), *NR* gene expression was reduced (Figure 3.9B). After transfer of fruit to 20 ^{o}C, fruit stored 4 weeks showed the reduction in both of *LeETR1* and *LeETR3* gene expression, while fruit stored for 1 and 2 week at 3 ^{o}C showed increased gene expression to similar levels of non-chilled fruit at some points during ripening.

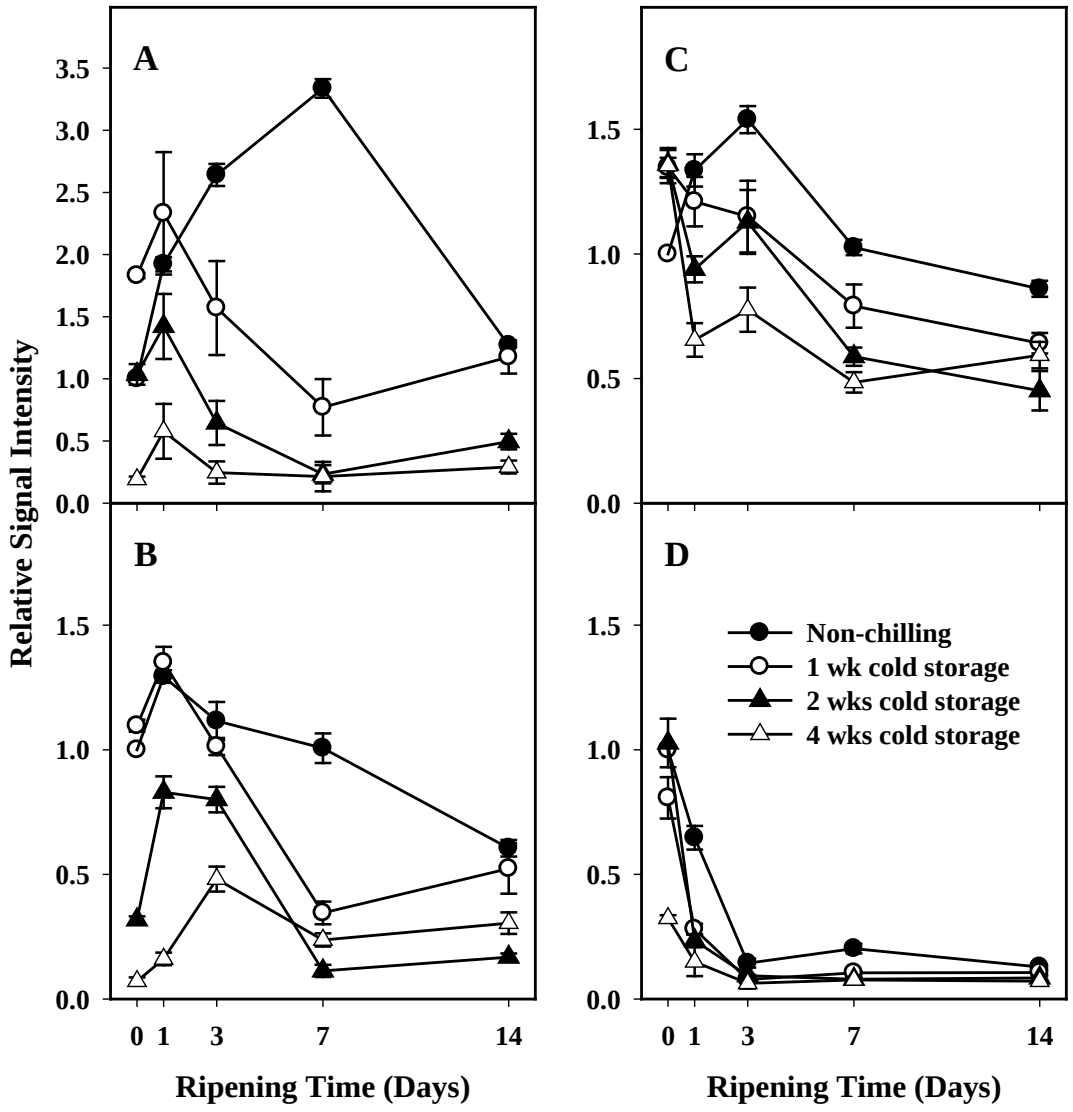

Figure 3.8 RT-PCR analysis of (A) 1-aminocyclopropane 1-carboxylate (ACC) synthase 2 (*ACS2*), (B) ACC synthase 4 (*ACS4*), (C) ACC oxidase 1 (*ACO1*), and (D) ACC oxidase 6 (*ACO6*) expression levels in fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars represent SE of three independent PCR amplification experiments.

The expression of *LeETR4* gene decreased after 1 week of storage, but its expression was restored after 2 weeks, and reduced again after 4 weeks at cold storage (Figure 3.9C). The expression of *LeETR4* in fruit stored for 4 weeks remained low during ripening at 20 ^{o}C. Expression in fruit stored for 1 and 2 weeks increased to similar levels as those of non-chilled fruit for the first few days after removal to 20 ^{o}C, and then declined to lower level compared with non-chilled fruit.

Chilling also increased gene expression of *LeCTR1* during storage (Figure 3.9D). However, the level of gene expression declined after transfer of fruit to 20 ^{o}C and became lower than in non-chillled fruit within a few days.

The expression of *LeEIL1* was induced after 2 and 4 weeks during cold storage, and the expression level still higher than that of non-chilled fruit during the first 3 d of ripening after storage (Fig 3.10A). Chilling had no effect on *LeEIL1* expression in fruit stored for 1 week or after subsequent ripening. *LeEIL2* expression increased slightly after 1 week of cold storage, and its expression declined after removal to 20 ^{o}C (Figure 3.10B). The expression levels of *LeEIL2* in fruit stored for 4 weeks were higher than those in non-chilled fruit during the first 3 d of ripening. *LeEIL3* expression was also up-regulated during cold storage, but the expression declined to the lower levels than non-chilled fruit during ripening after storage (Figure 3.10C). *LeEIL4* gene expression was slightly affected by chilling after 2 weeks of cold storage (Fig. 3.10D). However, after transfer of fruit to 20 ^{o}C, *LeEIL4* gene expression in all chilled fruit remained lower than that of non-chilled fruit.

Fruit stored for 1 and 2 weeks showed an increased *LeERF2* gene expression compared with fruit at harvest (Fig 3.11A), while the expression in fruit stored for 4 weeks slightly increased at the end of cold storage. Increased *LeERF2* gene expression occurred in non-chilled fruit was not shown in fruit stored at 3 ^{o}C for 4 weeks. Chilling had no effects on gene expression of *LeERF3* (Figure 3.11B).

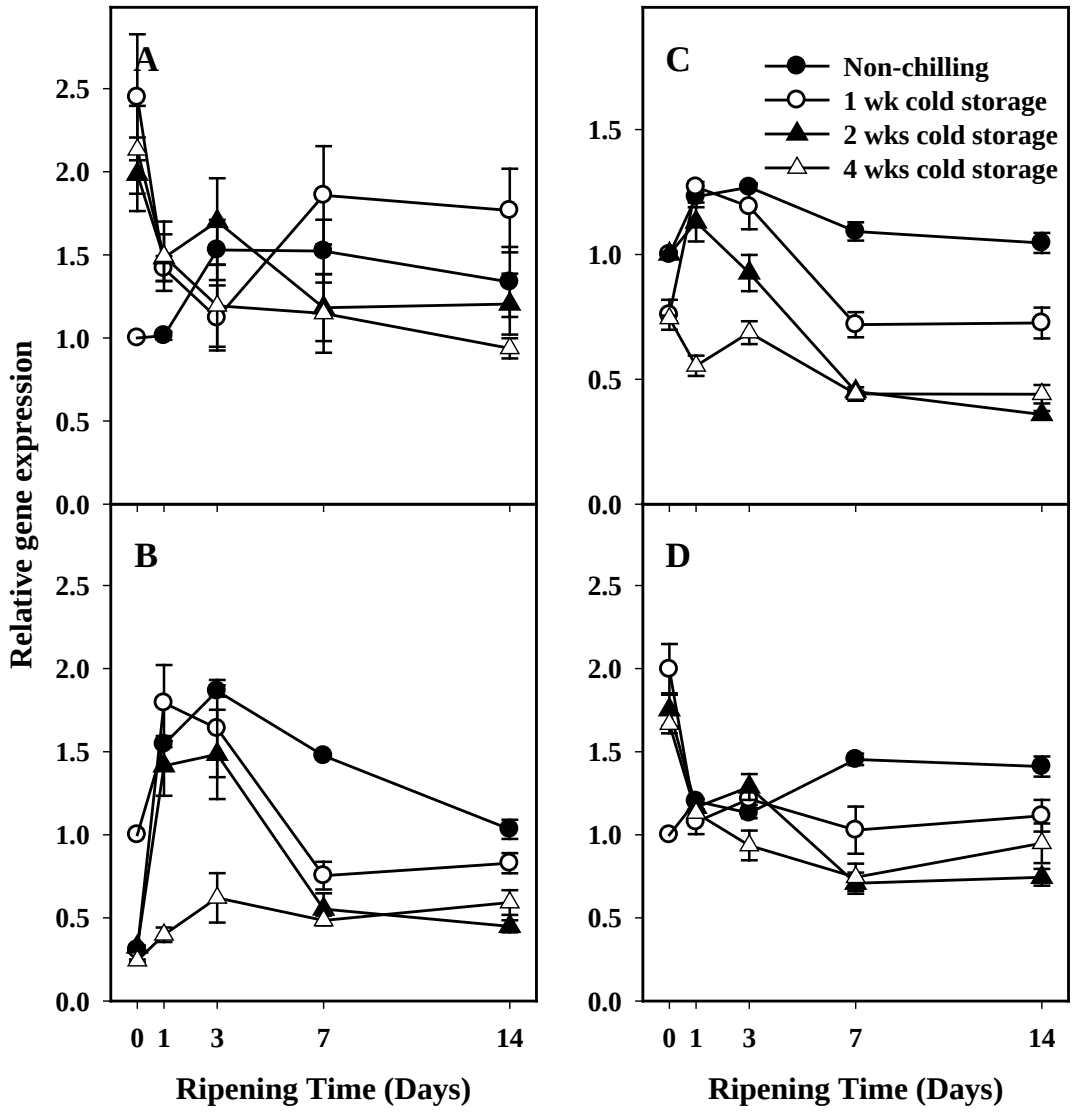

Figure 3.9 RT-PCR analysis of ethylene receptors: (A) *LeETR1*, (B) *NR*(*LeETR3*), and (C) *LeETR4*, and (D) *LeCTR1* expression levels in fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars represent SE of three independent PCR amplification experiments.

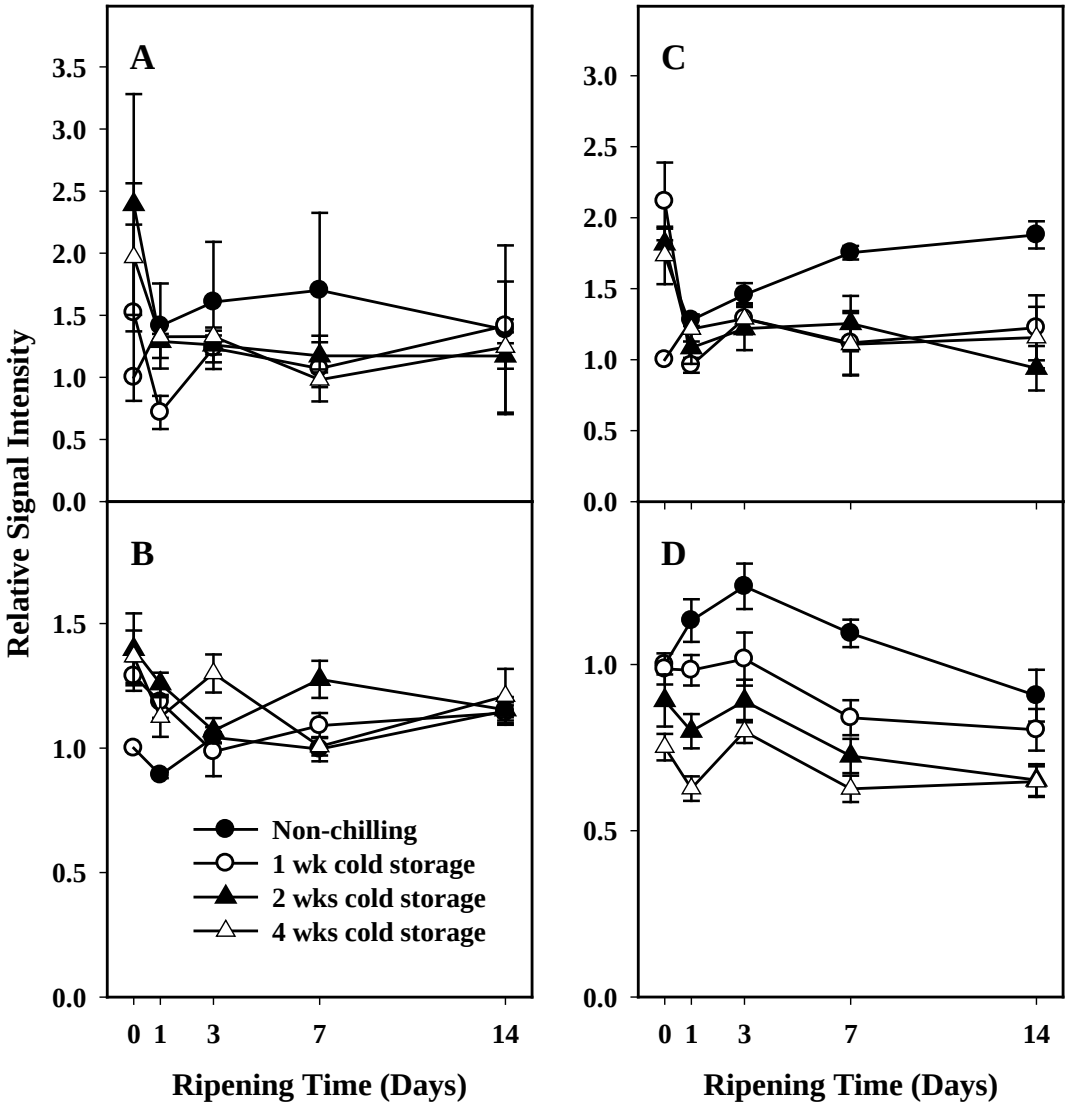

Figure 3.10 RT-PCR analysis of tomato ethylene-insensitive3 (EIN3)-like: (A) *LeEIL1*, (B) *LeEIL2*, and (C) *LeEIL3*, and (D) *LeEIL4*, expression levels in fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars represent SE of three independent PCR amplification experiments.

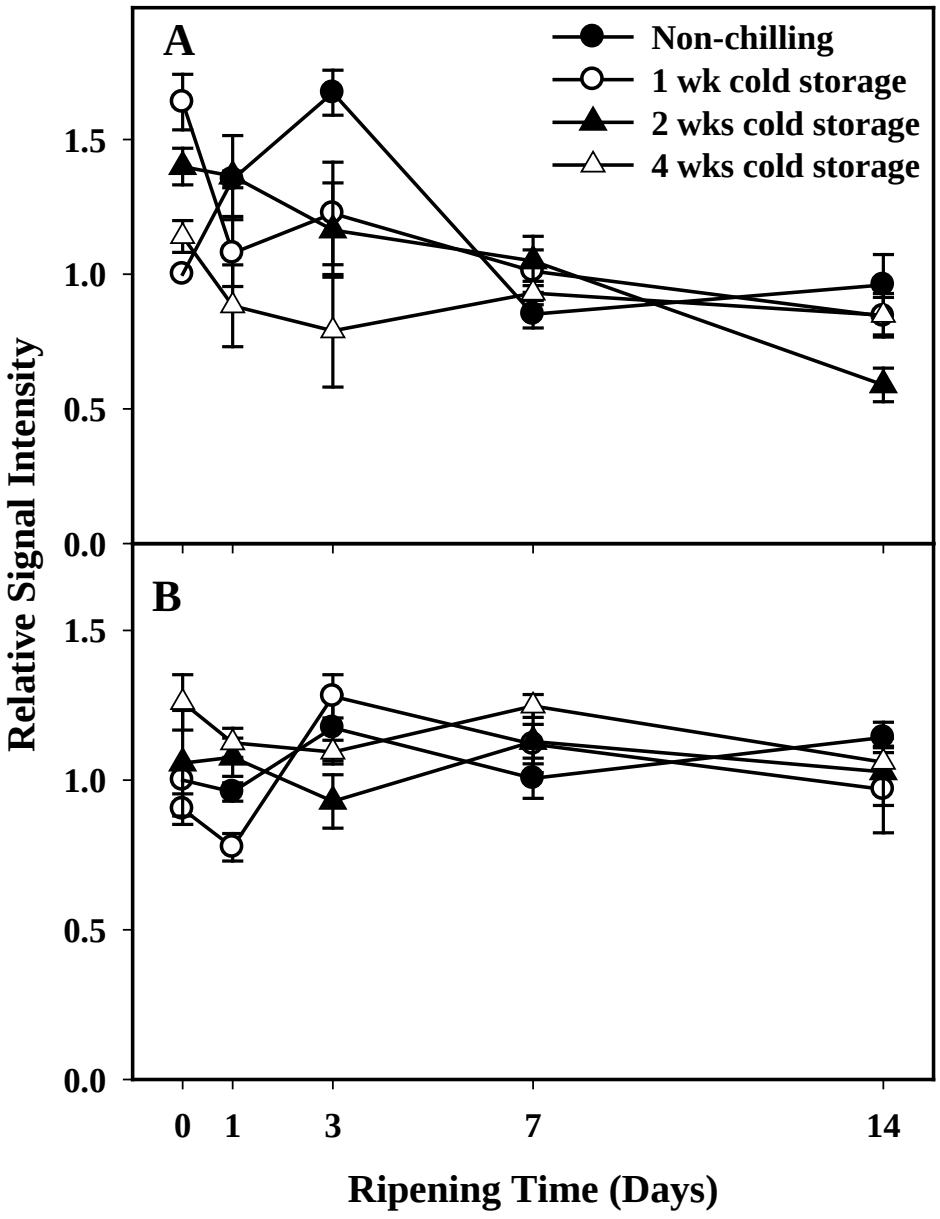

Figure 3.11 RT-PCR analysis of (A) ethylene response factor 2 (*LeERF2*) and (B) ethylene-responsive factor 3 (*LeERF3*) expression levels in fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars represent SE of three independent PCR amplification experiments.

3.6. Effects of chilling on the expression of LeMADS-RIN

The expression of *LeMADS-RIN* was down-regulated in fruit stored for 4 weeks, and the expression levels were lower than those in non-chilled fruit after transfer of fruit to 20 ^{o}C (Fig. 3.12). Fruit stored for 1 and 2 weeks at 3 ^{o}C responded slightly differently to chilling as the gene expression was higher than fruit at harvest at the end of cold storage, and a reduction of *LeMADS-RIN* gene expression was observed after 3 d after transferring to 20 ^{o}C.

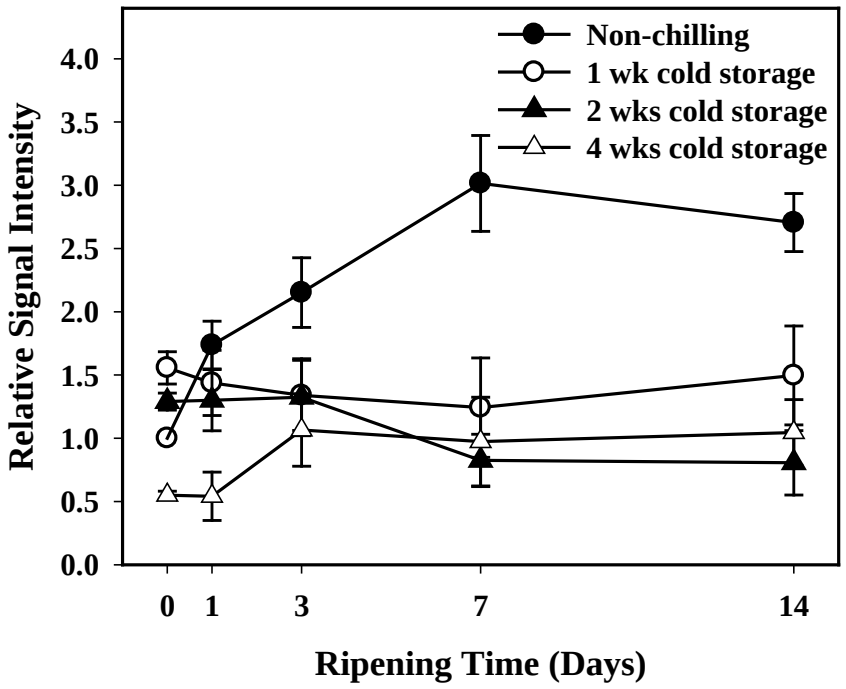

Figure 3.12 RT-PCR analysis of *LeMADS-RIN* expression levels in fruit stored at 3 ^{o}C for 0, 1, 2, and 3 weeks then ripened at 20 ^{o}C for 14 d. Vertical bars represent SE of three independent PCR amplification experiments.

3.4 Discussion

Exposure to temperatures below the critical chilling temperatures causes metabolic imbalances in plant cell that lead to the development of visible CI symptoms (Raison and Lyons, 1986). Metabolic dysfunction occurs in a temperature- and time-dependent manner, and it can be reversible if the chilling stress is removed before the process becomes irreversible. In this study, fruit stored at 3 °C for 1 week ripened normally at 20 °C compared with fruit without chilling, but fruit stored for 2 and 4 weeks showed inhibition of red color development (Figure 3.1) and delayed fruit softening (Figure 3.3A and 3.3B), suggesting that the imbalances of metabolic processes caused by chilling could not be restored after 2 weeks of storage at 3 °C. Failure to ripen normally is a common symptom of CI in tomato fruit (Hobson, 1987). In addition, the degree of CI in fruit stored for 4 weeks was more severe than that in fruit stored for 2 weeks, as the fruit were firmer than fruit stored for 2 weeks, exhibited uneven pigmentation, and had pitting on the surface (Figure 3.13).

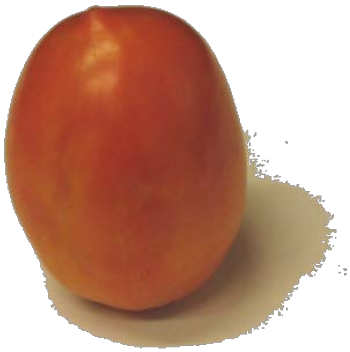

Figure 3.13 Visible chilling injury symptoms (uneven pigmentation and pitting) of tomato fruit stored for 4 weeks at 3 °C, then ripened at 20 °C for 14 d.

During ripening, tomato fruit color changes from green to red as the result of lycopene accumulation and chlorophyll degradation (Fraser et al., 1994). Loss of chlorophyll in the fruit during cold storage was indicated by yellowing of fruit stored for more than 2 weeks (Figure 3.1). Delayed color development as an effect of chilling has been reported by others (Cheng and Shewfelt, 1988; Efiuvwevwere and Thorne, 1988; Sharom et al., 1994; Lurie et al., 1996).

Inhibition of red color development in chilled fruit was the result of reduced lycopene accumulation (Watkins et al., 1990). In my study, at least four carotenoid biosysthesis-associated genes were affected by chilling: *PSY1* and *CRTISO*, from the carotenoid biosynthesis pathway, and, *GGPPS2* and *DXS* from pathways located upstream of carotenoid biosynthesis. *PSY1* encodes fruit-specific phytoene synthase, an enzyme that catalyzes the first step of carotenoid biosynthesis (Fraser et al., 2000) in which phytoene was produced from the condensation of two molecules of geranylgeranyl diphosphate (GGPP). CRTISO encodes carotenoid isomerase, an enzyme that isomerizes poly-cis-lycopene to all-trans lycopene (Isaacson et al., 2004) and thereby provides red color in tomato. *GGPPS2* is involved in carotenoid biosynthesis as it encodes an enzyme, GGPP synthase, producing GGPP, a precursor of phytoene. *DXS* encodes 1-deoxy-D-xylulose 5-phosphate synthase, the first enzyme in methylerythritol-4-phosphate (MEP) pathway involved in synthesis of isopentenyl pyrophosphate, a precursor of GGPP. Reduced expression of these genes in response to chilling would contribute to the reduced lycopene accumulation resulting in inhibition of red color development in chilled fruit.

Fruit stored for 2 and 4 weeks were firmer than non-chilled fruit during ripening at 20 °C as according to whole fruit compression and fruit pericarp firmness (Fig 3.3A and 3.3B). Interestingly, fruit stored for 1 week exhibited chilling-associated softening at the early stage of ripening based on whole fruit firmness (Figure 3.3A),

but not in the fruit pericarp firmness measurements (Figure 3.3B) indicating that the fruit softened to a similar degree as non-chilled fruit. The strength of fruit skin may contribute to this difference in fruit firmness between two methods because fruit skin (epicarp) was removed before measuring the pericarp firmness. Reduced skin firmness has been observed in cold stored fruit (Jackman and Stanley, 1994). In contrast, chilling-associated softening assessed by puncture method with skin intact has been reported in tomato (Marangoni et al., 1995).

Even though, fruit firmness is influenced by cell turgor pressure (Shackel et al., 1991; Saladie et al., 2007) and cell wall integrity (Brummell, 2006) only the expression of genes involved in cell wall disassembly was examined in current study using RT-PCR. Since the total RNA used for RT-PCR analysis was extracted from pericarp tissue, the fruit pericarp firmness was used to investigate the correlation between fruit softening and the gene expression patterns. The delay in fruit softening in chilled fruit was correlated with the reduced expression of *PG*, *PE1*, *TBG4*, and *LeExp1*. These genes encode a group of proteins involved with cell wall disassembly, and it is suggesting that these enzymes/proteins work in the cooperative manner to disassemble cell wall. PG cleaves the homogalacturonan backbone, and PME play the role in pectin demethylation that make the pectin susceptible to PG. β-gal II, encoded by TBG4, play a role in degradation of galactan side chains of pectin polysaccharides, and expansin protein play a role in cell wall loosening and provides the assess for other cell wall enzymes to their substrates. XTH5 was another enzyme affected by chilling as the expression was induced during cold storage. XTH5 has xyloglucan endohydrolase activity that can cut matrix glycan resulting in matrixglycan depolymerization (Saladie et al., 2006). However, the increase in *XTH5* expression may not affect fruit softening because the expression of other cell wall-modifying genes were reduced. In tomato, reduced *PG* expression by chilling has been observed

(Watkins et al., 1990; Lurie et al., 1996). Also, PG expression was reduced in avocado during cold storage (Dopico et al., 1993).

It has been reported that red-ripe tomato fruit stored at the temperatures 12.5 °C or lower have reduced ripe aroma and tomato-like flavor (Maul et al., 2000). Levels of five flavor volatiles were lower in chilled than non-chilled fruit (McDonald et al., 1999), and two of them, hexanal and geranylacetone, were related to ripe aroma and tomato-like flavor (Baldwin et al., 1998; Maul et al., 2000). The expression of *TomloxC*, *ADH2*, and *ATT*, involved in volatile biosynthesis, was reduced by chilling (Figure 3.6). *TomloxC* is a fruit-specific gene encodes lipoxygenase (LOX) in tomatoes (Chen et al., 2004), and LOX catalyzes the conversion of linoleic (18:2) and linolenic (18:3) acids to hexanal and cis-3-hexanol, respectively. Transgenic tomato fruit with suppressed expression of *TomloxC* had lower levels of hexanal, cis-3 hexenal, and hexenol. Transgenic tomato fruit in which *AHD2* over-expressed showed increased hexanal and Z-3-hexenol levels with a more intense "ripe fruit" flavor (Speirs et al., 1998). ATT is capable of combining various alcohols and acyl CoAs, resulting in the synthesis of a wide range of esters. Aliphatic esters contribute to aroma of nearly all fruits and are emitted by vegetative tissues (Schwab et al., 2008)

Because the ripening of climacteric fruits is controlled by ethylene biosynthesis, the effect of chilling on ethylene production and the expression of genes in ethylene biosynthesis was examined. Fruit stored for 1 week showed an increase in ethylene production compared with non-chilled fruit at the early stage of ripening (Figure 3.7). Increased ethylene production in fruit stored for 1 week appears to be the result of either increased or unchanged *ACS2*, *ACS4* and *ACO1* expression levels during cold storage and 1 d after ripening compared with non-chilled fruit. Fruit stored for 4 weeks had lower rated of ethylene production, which correlated with the reduction of *ACS2*, *ACS4* and *ACO1* expression. Even though chilling had an effect on *ACO6*, this

gene was expressed as very low levels, as indicated by the high cycle number used for PCR, and its expression decreased during fruit ripening. In fruit stored for 1 week exhibiting normal ripening, the increased ethylene may play role to restore the normal ripening process. Increased ethylene production in response to chilling has been reported (Autio and Bramlage, 1986; Cheng and Shewfelt, 1988). Also, an increase in *ACO1* gene expression during cold storage has been reported in tomato (Watkins et al., 1990; Lurie et al., 1996) and avocado (Dopico et al., 1993). However, in the case of fruit stored for 2 weeks in which ethylene production is in similar levels as those in non-chilled fruit, but showed delayed ripening, ripening regulatory pathways than the level of endogenous ethylene may have been affected by chilling. The ethylene sensitivity of fruit might be altered by chilling since a higher ethylene concentration was required to restore the fruit ripening. To play the role in plant growth and developmental processes, ethylene perception in which ethylene binds to its receptors is required, and then the signal transduction occurs through the subsequent signal transduction pathway (Hall et al., 2007).

The primary mechanism of chilling is thought to involve effects of temperature on cell membranes (Lyons, 1973). Since ethylene receptors predominantly localized to the endoplasmic reticulum (ER) membrane (Chen et al., 2002; Ma et al., 2006; Zhong et al., 2008), changes in cell membrane physical properties could affect the ethylene perception. Results for the expression of genes involved in ethylene transduction signal show that *LeETR1* and *NR* receptor gene were markedly altered by chilling during cold storage, while the expression of *LeETR4* receptor gene was slightly affected (Figure 9A, B, and C). According to a recent model for *Arabidopsis*, the receptor interacts with CTR1, a raf-like kinase protein that actively represses ethylene responses (Hall et al., 2007). In the absence of ethylene, the receptor-CTR1 complex acts as a negative control of ethylene responses, however, binding of ethylene by the

receptor induces a conformation change in CTR1 that relieves the repression of ethylene responsive pathway. Subfamily-1 receptors have a greater role in signaling than subfamily-2 receptors relating to their ability to activate CTR1, and the interaction between CTR1 and the receptors is required for the signal output (Hall et al., 2007). In tomato, the receptor LeETR1, LeETR2, and NR belong to subfamily-1 receptors, while the receptor LeETR4, LeETR5, and ETR6 are members of subfamily-2 receptors (Barry and Giovannoni, 2007). A recent study of the interaction between LeCTRs and tomato ethylene receptors shows that only subfamily-1, not subfamily-2 receptors, are able to bind to LeCTR1, LeCRT3, and LeCTR4 (Zhong et al., 2008), suggesting that subfamily-1 receptors in tomato may contribute to ethylene signaling output. Since, *NR LeETR4*, and *LeETR6* are abundantly expressed during fruit ripening (Kevany et al., 2007), NR may have a more important role in ethylene signaling during fruit ripening.

It has been reported that ethylene perception is required to continue the ripening process when is been initiated at breaker stage. Tomato fruit harvested at either breaker or orange stage and then treated with 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, exhibited delayed color development, softening, and ethylene production (Hoeberichts et al., 2002; Tassoni et al., 2006). Of the receptors that are abundantly expressed in tomato during ripening, NR is the most likely to play the role in ethylene perception during fruit ripening because: (1) only tomato subfamily-1 receptors are able to bind to LeCTRs (1, 3 and 4) (Zhong et al., 2008); (2) although NR protein decreases at the onset of ripening, NR protein levels increased as ripening progresses; and (3) transgenic fruit with suppression of NR show delayed color development (Tieman et al., 2000). Therefore a reduction in NR expression in chilled fruit (Figure 3.9B) may be an important cause of delayed ripening.

Unlike *NR*, *LeETR1* expression was induced during cold storage and the expression levels were higher than those in non-chilled for the first few days after removal to ripen at 20 °C (Figure 3.9A). As a member of ethylene receptors subfamily-1, LeETR1 also binds to LeCTRs. If LeETR1 play the same role as NR does, the increased *LeETR1* expression may not compensate the NR action because *LeETR1* is expressed at much lower levels than *NR*, as indicated by the higher number of PCR cycles required for *LeETR1*. Repression of *LeETR1* expression in transgenic tomato resulted in reduction in ethylene response, but had no effect on fruit ripening (Whitelaw et al., 2002). In contrast, Bao et al., (2007) found increased ethylene responses including accelerated fruit ripening in response to the suppression of *LeETR1*. Suppression of *LeETR4* in transgenic tomato resulted in early-ripening tomato fruit without the effect on color development after the onset of ripening (Kevany et al., 2008). Therefore, reduced *LeETR4* expression may not increase sensitivity in chilled fruit because breaker fruit were used in this experiment. However, it has been reported that receptor *NR*, *ETR4* and *ETR6* proteins were rapidly degraded in the presence of ethylene, while the gene expression was induced by ethylene. Care must be taken in interpreting the effects of receptor function on the gene expression level. The expression of *LeCTR1* (Figure 3.9D) was induced during cold storage, this could contribute to the decreased in ethylene sensitivity. The reduction of some positive transcription factors expression, such as *LeEIL3* (Figure 3.10C), *LeEIL4* (Figure 3.10D), and *Le ERF2* (Figure 3.11A) was shown suggesting the reduction of ethylene transduction signal.

The expression patterns of some genes that are directly regulated by ethylene, such as *PG* (Sitrit and Bennett, 1998) and *DXS* (Alba et al., 2005) did not correlate with the levels of ethylene production during ripening, suggesting that other ripening regulated factors were also altered by chilling. The microarray results showed that *LeMADS*-

RIN expression was affected by chilling. Since *LeMADS-RIN* encodes a ripening control transcription factor that is necessary for fruit ripening (Vrebalov et al., 2002), fruit ripening was expected to be affected by CI. Indeed, fruit stored for 4 weeks with the highest reduction of *LeMADS-RIN* expression showed the highest degree of fruit ripening inhibition. The expression *ACS4*, a ripening associated gene regulated by *Le-MADS-RIN* and ethylene independent (Barry et al., 2000), was affected by chilling, indicating that the reduction in *Le-MADS-RIN* expression by chilling is possibly involved with the delayed ripening. While *Le-MADS-RIN* expression was reduced at 4 weeks during cold storage, the gene expression was induced at 1 week of cold storage. Since Le-MADS-RIN control climacteric ethylene production, this increased *Le-MADS-RIN* expression during short-term storage may result in the increased ethylene production during early stages of ripening in fruit stored for 1 week, and restoration of normal ripening in the fruit. However, other ripening- regulated transcription factors may involved with chilling-associated delayed ripening, for example, the microarray results showed the differential expression of other MADS-box genes that homolog to MADS-box gene in *Capsicum annuum*, and TDR4 transcription factor, a MADS-box gene that is down-regulated in Colorless non-ripening (*Cnr*) mutant. Comparison of the expression patterns of these genes between fruit stored for 1 week and 4 weeks may provide more information about how chilling affects fruit ripening.

As mentioned above, most of ripening-related genes were down-regulated by chilling. It is possible that transcriptional repressors induced by chilling may play the role in the transcriptional repression of these genes. It has been studied that C2H2-type zinc-finger proteins could function as a key transcription repressors involved in the defense and acclimation response of plants to different environmental stress conditions (Ciftci-Yilmaz and Mittler, 2008). The microarray results showed that four genes up-regulated by chilling after 4 weeks of cold storage homolog to genes that

encode C2H2-type zinc-finger proteins, including SGN-U213848, SGN-U233285, SGN-U224735 and SGN-U225727 (Appendix Table C1). These genes may repress the expression of ripening-related genes including ripening-regulated transcription factor genes. In *Arabidopsis*, C2H2-type zinc-finger protein ZAT 12 and STZ/ZAT10 function as repressor and the expression of these genes were induced by cold stress (Sakamoto et al., 2004; Vogel et al., 2005; Mittler et al., 2006). Among four transcription factors, homologous to C2H2-type zinc-finger proteins, the gene SGN-U233285, homologous to a histone deacetylase 2a (HD2a) of *Solanum chacoense,* was induced by chilling both after 4 weeks of cold storage and after subsequent ripening at 20 °C (Apendix Table C2). The AtHD2A protein could function as a transcriptional repressor in *Arabidopsis* (Wu et al., 2003). Therefore, the role of these C2H2-type zinc-finger proteins on tomato fruit ripening should be investigated.

In conclusion, fruit ripening in tomato was altered by chilling after two weeks of cold storage, as indicated by an inhibition of red color development and delayed fruit softening. The expression of genes involved in color development and cell wall disassembly was reduced by chilling. Ethylene production was differently affected by chilling depending on the length of chilling-exposure time as indicated by an increased climacteric ethylene production in fruit stored for 1 week, and reduced ethylene production in fruit stored for 4 weeks. The alteration of ethylene production correlated with the altered *ACS2*, *ACS4*, and *ACO1* expression. The expression of genes involved in ethylene signal transduction pathway such as *LeETR1*, *NR, LeETR4, LeCTR1, LeEIL3, LeEIL4,* and *LeERF3* was altered by chilling, suggesting that ethylene perception and sensitivity was affected by chilling. Chilling had an effect on the gene expression of a ripening-regulated transcription factor, *LeMADS-RIN*. The reduction of LeMADS-RIN could contribute to the delayed ripening caused by chilling. However, other ripening-regulated transcription factors affected by chilling

may involve this phenotype as well. The microarray results showed that some transcriptional repressors were induced by chilling, suggesting that these transcription factors may be involved in the alteration of fruit ripening.

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CHAPTER 4

CONCLUSIONS AND FUTURE DIRECTIONS

The alteration of ripening caused by storage at cold storage was investigated using tomato as a model system, with focus on changes in cell wall metabolism and the expression of ripening-related genes affected by chilling.

In the CHAPTER 2, investigations on changes in cell wall metabolism in tomato cv. Trust to evaluate the possibility whether this tomato can be used as a model study of mealiness development associated with chilling are described. The results showed that chilling had a small effect on fruit ripening, and mealiness was not detected in this study either by measurements of extractable juice or visual observation. Nevertheless, cell wall metabolism in this tomato was affected by chilling, as chilling had a marked effect on gene expression, total activity, and protein accumulation of PG. However, pectin solubilization and depolymerization did not seem to be affected much by chilling compared with the reduced PG activity or protein accumulation. The expression of PG and PG protein accumulation was not correlated, suggesting post-transcriptional regulation by chilling. The expression of *LeEXP1* was reduced by chilling, but LeExp1 protein accumulation levels were not affected.

To investigate the effects of chilling on tomato fruit ripening, tomato introgression lines (ILs) have been screened for the lines that exhibited obviously altered ripening processes by chilling. Tomato IL 11-2 was the only line that its ripening was greatly affected by chilling without showing any visual sign of pathogen infection. Therefore, as described in the CHAPTER 3, IL 11-2 tomato fruit were used to investigate the effects of chilling on fruit ripening. The results showed that fruit ripening in tomato IL 11-2 was altered by chilling after two weeks of cold storage, as indicated by an inhibition of red color development and delayed fruit softening. Ethylene production

was differently affected by chilling depending on the length of chilling-exposure time as indicated by an increased climacteric ethylene production in fruit stored for 1 week, and reduced ethylene production in fruit stored for 4 weeks.

The expression of ripening-related genes affected by chilling was assessed using semi-quantitative RT-PCR. Genes involved in color development: *PSY1, CRTISO, GGPPS2*, and *DXS*; cell-wall modification: *PG, PE1, TBG4, LeEXP1*, and XTH5; and volatile biosynthesis: *TomloxC, ADH2*, and *ATT*, were down-regulated by chilling. The alteration of ethylene production correlated with the altered *ACS2*, *ACS4*, and *ACO1* expression. The expression of genes involved in ethylene signal transduction pathway such as *LeETR1, NR, LeETR4, LeCTR1, LeEIL3, LeEIL4,* and *LeERF3* was altered by chilling, suggesting that ethylene perception and sensitivity was affected. The expression of *LeMADS-RIN,* a ripening-regulated transcription factor, was down-regulated by chilling. The reduction of LeMADS-RIN could contribute to the delayed ripening caused by chilling. The microarray analysis suggested that other ripening-regulated transcription factors, such as TDR4 and other MADS-box proteins affected by chilling may involve this phenotype as well. Also, some transcriptional repressors, such as C2H2-type zinc-finger proteins, induced by chilling, may be involved in the reduction of ripening-regulated gene expression.

In conclusion, the tomato cv. Trust may be not suitable as a model study for mealiness development or altered fruit ripening associated with chilling injury, whereas the IL 11-2 tomato had the potential of being used as a model study for the altered fruit ripening. Based on the results in this thesis, the follow-up studies may be done in IL 11-2 tomato to provide more information about the effects of chilling on fruit ripening. Besides fruit color development, fruit softening, and ethylene biosynthesis, the RT-PCR analysis suggested that volatile biosynthesis and ethylene perception may be also affected by chilling. However, more research to support these

effects of chilling should be examined, for example the measurements of volatile profiles. The expression pattern of other ripening-regulated transcription factors should be examined using RT-PCR to confirm the microarray analysis. The results in the CHAPTER 2 suggested that chilling affected post-translational regulation. Therefore, if possible, the abundance of protein encoded by genes of interest should be measured to confirm the effects of chilling at the protein expression level. Also, how chilling affects post-transcriptional regulation should be also studied. The microarray analysis revealed that some transcription repressor genes were up-regulated by chilling. As transcriptional repressors, they might suppress the expression of ripening-regulated genes directly or act upstream of ripening-regulated transcription factors, such as LeMADS-RIN, so the role of these transcriptional repressors associated with fruit ripening should be investigated.

APPENDIX

Appendix A: Protocols for microarray analysis

The following protocols are developed by Rob Alba and Ryan McQuinn at Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY, USA.

A1. SuperScript Indirect cDNA Labeling from total RNA

1. First-Strand cDNA Synthesis using Invitrogen's SuperScript Indirect cDNA Labeling Kit (Catalog numbers L1014-01 and L1014-02).

The following protocol is used for cDNA synthesis from total RNA and indirect cDNA labeling prior to microarray hybridizations. Although I have made a few non-trivial modifications have been made, the protocol below is similar to that in the instruction manual provided with this kit.

1.1 First-Strand cDNA Synthesis Rxn.

1. Mix and briefly spin each kit component before use.
2. Prepare rxns as follows:

   X µl DEPC-H_2O

   X µl total RNA (15 to 20 µg/rxn; typically I use 15 µg/rxn)

   2 µl Anchored Oligo(dT)20 Primer (2.5µg/ µl)

   Total Volume = 18 µl
3. Incubate tubes at 70^{o}C for 10 min, and then place on ice for 5 min.
4. add the following to each rxn tube on ice (add in order):

   6 µl 5X First-Strand buffer

   1.5 µl 0.1 M DTT

   1.5 µl 10-mM dNTP mix

1 µl RNaseOUT (40 µl)

2 µl SuperScript III RT (400 µl)

Total Volume = 30 µl

5. Mix gently and spin briefly. Incubate tube at 46^{o}C overnight (~16hrs).
6. Add 15 µl of 1 N NaOH to each rxn tube and mix thoroughly.
7. Incubate tube at 70^{o}C for 10 min.
8. Add 15 µl of 1N HCl; mix gently.
9. Add 20 µl 3M sodium acetate (pH 5.2); mix gently.

1.2 Purifying First-Strand cDNA.

1. Add 500 µl of Loading Buffer to the cDNA (from Step 9) and mix well.
2. Place a SNAP Column on a collection tube and load your cDNA on the column.
3. Spin at 14,000g at room temp for 1 min; discard the flow-through.
4. Place the SNAP Column onto the same collection tube and add 500 µl of Wash Buffer.
5. Spin at 14,000g at room temp for 1 min; discard the flow-through.
6. Repeat Steps 4 and 5 twice more, for a total of three 500 µl washes.
7. pin one more time at 14,000g at room temp for 1 min sec; discard the flow-through.
8. Place the SNAP Column onto a new 1.5-ml tube.
9. Add 50 µl of DEPC-treated water to the SNAP Column and incubate at room temp for 1 min. Elute the cDNA via spin at 14,000g at room temp for 1 min.
10. Repeat Step 9, using the same 1.5-ml tube.
11. Add 10 µl of 3 M sodium acetate (pH 5.2) to the eluent from steps 9 and 10.
12. Add 2 µl of glycogen (20mg/ml) to the tube and mix.

13. Add 300 µl of ice-cold 100% EtOH, and incubate the tube for 45 min to 1hr at -80^{o}C or overnight at -20^{o}C.

14. Spin the tube at 14,000 g at 4^{o}C for 20 min. Carefully remove the supernatant.

15. Add 500 µl of ice-cold 70% EtOH and spin the tube at 14,000g at 4^{o}C for 5 min. Carefully remove the supernatant.

16. Spin down briefly and pipette off excess supernatant and then allow to air dry the for ~5 min; ensure that all EtOH is removed.

17. Warm the 2X Coupling Buffer at 37^{o}C for 5 min and re-suspend the cDNA sample in 5 µl of warm 2X Coupling Buffer. Heat the cDNA/Coupling buffer at 50^{o}C for 10 min and vortex well. Ensure that your cDNA pellet is fully re-suspended in the 2X Coupling Buffer.

2. Labeling with Fluorescent Dye.

When preparing the rxn, be careful to minimize exposure of the dye solution to light. Also, DMSO is hygroscopic and will absorb moisture from the air, which will react with the NHS ester of the dye and significantly reduce the coupling rxn efficiency. Keep the DMSO supplied in the kit in an amber screw-capped vial at -20^{o}C, and let the vial warm to room temperature before opening to prevent condensation. Use only the DMSO provided with this kit.

1. Open one packet of Cy3- or Cy5- dye and add 40 µl of DMSO directly to the dye vial.
2. Add 5 µl of the DMSO/dye solution to the tube from Step 17.
3. Mix well and incubate the tube at room temp in the dark for 2 hr.
4. Add 20 µl of 3 M Sodium Acetate (pH 5.2) to the dye-coupled cDNA solution.
5. Add 500 µl of Loading Buffer to the cDNA solution. Mix well by vortexing.

6. Place a SNAP Column onto a clear collection tube and load the cDNA/buffer solution.

7. Spin at 14,000g at room temp for 1 min; discard the flow-through.

8. Place the SNAP Column on the same collection tube; add 500 µl of Wash Buffer to column.

9. Spin at 14,000g at room temp for 1 min; discard the flow-through.

10. Repeat Steps 8-9 three times, for a total of four 500 µl washes.

11. Spin one more time at 14,000g at room temp for 1 min; discard the flow-through.

12. Place the SNAP Column onto a new amber collection tube.

13. Add 63 µl of DEPC-water to the SNAP Column and incubate at room temp for 1 min. Spin at 14,000g at room temp for 1 min and collect the flow-through. The flow-through should contain 60 µl of your purified dye-coupled cDNA.

3. Assessing the Labeling Procedure.

Conduct a spec assay to assess each Cy-cDNA labeling reaction using the procedure described in TIGR's Standard Operating Proceedure #M004 (http://pga.tigr.org/sop/M004_1a.pdf). This technique is also described briefly in the Appendix of the Instruction Manual for the Invitrogen cDNA labeling kit. Calculate the total pmol of synthesized cDNA, the total pmol of incorporated Cy dye for each labeling reaction, and the nucleotide/dye ratio for each reaction. Optimal labeling reactions generate >6000 pmols of cDNA (per 60 µl), >200 pmol of Cy dye (per 60 µl), and a nucleotide/dye ratio that is <30.

A2. Hybridizing Cy-labeled cDNA Targets to the TOM2 Oligo-array

The following protocol is used for pre-hybridization, hybridization, washes, and subsequent scanning of our TOM2 microarrays. The pre-hybridization and wash protocols derive from the lab of John Quackenbush at TIGR (http://atarrays.tigr.org/PDF/Probehyb.pdf), with a few modifications. Hybridization buffer from NC state.

*1. Reagents/Materials Required-(*made fresh each time)*

A.*Pre-Hyb Soln, (5X SSC, 0.1% SDS, 1% BSA)

B.*Wash Soln #1 (1X SSC, 0.2% SDS; pre-heat to 43 ^{o}C)

1. Filter wash solution using a 0.2μm SFCA filter unit

C. *Wash Soln #2 (0.1X SSC, 0.2% SDS; room temperature)

1. Filter wash solution using a 0.2μm SFCA filter unit

D.*Wash Soln #3 (0.1X SSC; room temperature)

1. Filter wash solution using a 0.2μm SFCA filter unit

E.*Microarray Hyb Soln (calculated per rxn)

1. NCSU Hyb Soln : per rxn-formamide-13.5 μl, 20x SSC 11.25 μl, 50x denhardt's 4.5 μl, PolyA (10ug/μl) 0.45 μl, water 14.85 μl, 10% SDS 0.45 μl)

F. Isopropyl alcohol

G. 0.1% SDS

H. Milli-Q® H_2o

I. Coplin staining jars

J. LifterSlips^{TM} (Erie Scientific Co.; catalog #22X50I-2-4711)

K. 50mL Falcon^{TM} tubes (place the cap from a 14mL Falcon^{TM} culture tube at the bottom of each 50mL Falcon^{TM} tube to elevate the arrays during centrifugation)

L. Corning Hybridization Chambers (catalog #2551)

M. 0.1% SDS

N. 100% Ethanol

2. Treatment of slides prior to prehybridization (immediately prior to prehybridization):

1. Rehydrate slide (barcode side up) over a 65 ^{o}C waterbath

2. UV treat slides at 65 mJ

3. Move on immediately to **Pre-hybridization** step

3. Pre-hybridization/Blocking the Arrays

1. Pre-heat ~60mL of the filtered Pre-Hyb Soln to 43 ^{o}C.

2. Incubate arrays in warm Pre-Hyb Soln for 45min.

3. Rinse the "blocked" arrays via 10 dips in Milli-Q® H_2o, followed by 10 dips in fresh Milli-Q® H_2o, followed by 10 dips in isopropyl alcohol.

4. Quickly dry the rinsed arrays by centrifugation (30 sec; 1500 x rpm) in a 50mL Falcon^{TM} tube.

a. Do not let arrays dry out prior to centrifugation.

b. Excessive centrifugal force will crack array slides.

c. Use arrays immediately after pre-hybridization.

5. During the pre-hyb step, wash and rinse a sufficient number of LifterSlips^{TM}.

a. 5-10 dips in Milli-Q® H_2o.

b. 5-10 dips in EtOH.

c. Dry the clean LifterSlips^{TM} on a lint-free Kimwipe®

4. Preparation of Cy-Labeled cDNA Targets

1. Conduct a spec assay to asses each Cy-cDNA labeling reaction using the procedure described in TIGR's SOP #M004 (http://pga.tigr.org/sop/M004_1a.pdf). Calculate the total pmol of synthesized cDNA, the total pmol of incorporated Cy dye for each labeling reaction, and the nucleotide/dye ratio for each reaction. Optimal labeling reactions generate >6000 pmols of cDNA (per 60ul), >200 pmol of Cy dye (per 60 ul), and a nucleotide/dye ratio that is <30.

2. For each Cy-labeled cDNA, calculate the volume of Cy5-cDNA and Cy3-cDNA equivalent to 200 pmol of incorporated Cy5 or 200 pmol of incorporated Cy3.

3. Combine the volumes calculated in step 2 in a microfuge tube and minimize the volume using a roto-evaporator (45 ^{o}C).

a. Avoid drying the Cy-cDNA samples completely.

4. Re-suspend the combined cDNA targets in 65µl of Hyb Soln.

5. Incubate the re-suspended cDNA targets at 95 ^{o}C for 4 min

6. Centrifuge at max speed for 1 minute (room temperature).

7. Place "blocked" array in a Corning Hybridization Chamber; fill humidity wells as per the manufacturer's instructions.

8. Carefully pipette all of re-suspended cDNA targets onto array surface; ensure that no bubbles are present.

9. Carefully cover the "loaded" array with a clean dry LifterSlips^{TM}.

10. Seal the array chamber (without moving the LifterSlips^{TM}) as per the manufacture's instructions.

11. Incubate the sealed chamber containing array at 42 ^{o}C for @16 hours.

a. Conduct the hybridization in the dark.

5. Washing Arrays after Hybridization (better if more wash buffer per array-use medium glass chambers for up to 4 slides)

1. Fill a glass washing chamber with pre-heated Wash Soln #1.

2. Remove the LifterSlips™ from the array surface by dipping arrays in the Plastic Coplin staining jar containing Wash Soln #1; the LifterSlips™ should slide off the array easily.

3. **(twice)** Place the slides in the glass chamber with pre-heated Wash Soln#1 (~43^{o}C)

4. Incubate arrays in Wash Soln #1 for 3 minutes at RT ^{o}C on a rocker mixer

5. Transfer arrays using the glass slide holder to a new glass chamber containing Wash soln #2.

6. Incubate arrays in Wash Soln #2 for 3 minutes at room temperature

    a. Rock arrays gently during wash step again on the rocker.

7. Dip slides individually in Wash Soln #3 in a plastic Coplin jar. Then transfer arrays to a new glass chamber containing Wash Soln #3 using the glass slide holder.

8. Incubate arrays in Wash Soln #3 for 3 minutes at room temperature

    a. Rock arrays gently during wash step.

9. Repeat step 7 twice in new wash #3 each time (for a total of 3 times).

10. Dip in Wash Soln #3. Then immediately dry the washed arrays via gentle centrifugation in a 50mL Falcon™ tube.

    a. Do not let the arrays dry out prior to the centrifugation step.

    b. 2 min at 1500 x rpm

11. Place arrays in foil-covered slide tray until scanning.

6. Scanning Arrays

We scan our arrays immediately after they are washed/dried using a two-channel confocal microarray scanner (ScanArray5000; GSI Lumonics, MA) and the associated ScanArray software (v3.1 Packard BioChip Technologies, MA). After laser focusing and balancing of the two channels, scans are conducted at a resolution of 10 μm with the laser power set at 90% of maximum and the photomultiplier tube typically set at 60-85% of maximum.

Excitation/emission settings are 543 ηm/570 ηm and 633 ηm/670 ηm for the Cy3 and Cy5 fluors, respectively. Raw fluorescence image data is saved as .tif files, which are converted to numerical signal data (.txt files) using ImaGene software (v4.2, BioDiscovery Inc., CA).

Appendix B: Chilling injury in tomato introgression lines

To screen for chilling injury in tomato introgression lines, fruit of tomato introgression lines were harvested at breaker-stage of maturity, stored at 3 °C for 3 weeks, and then transferred to ripen at 20 °C. After ripening at 20 °C for 7 d, fruit ripening was assessed by measuring fruit color (hue angle) and pericarp firmness compared with fruit ripened without chilling. Also, chilling injury symptoms as pitting and susceptibility to decay were visually assessed after 1 d and 7 d, respectively.

Chilling injury index was evaluated as severity of surface pitting as following five-stage scale: 0 = no pitting; 1 = scattered pitting; 2 = pitting covering < 5% of the fruit surface; 3 = pitting covering 5-25% of the fruit surface; 4 = pitting coring > 25% of the fruit surface). Severity of decay also was measure as decay index (0 = no decay; 1 = slightly decay; 2 = moderately decay; 4 = severely decay.

Appendix Table B1. Fruit color (hue angle) and fruit pericarp firmness of tomato introgression lines ripened at 20 °C for 7 days after harvest (nonchilled) or ripened after storage at 3 °C for 3 weeks.

Lines	Hue Angle		F-test	Pericarp firmness (N)		F-test
	Nonchilled	Chilled		Nonchilled	Chilled	
IL 1-1	48.57	83.07	**	9.96	17.67	*
IL 1-4	56.72	75.24	*	16.38	19.35	ns
IL 1-4-18	54.78	81.96	**	10.44	13.95	ns
IL 2-1	50.70	74.87	**	5.08	7.92	ns
IL 2-1-1	47.35	67.00	**	10.54	17.31	*
IL 2-4	62.42	58.70	ns	9.92	14.21	ns
IL 2-6	47.64	60.38	**	7.20	11.04	**
IL 2-6-5	51.63	67.38	**	8.58	14.71	**
IL 3-2	91.23	90.14	ns	8.54	14.65	*
IL 3-3	48.87	62.52	**	10.33	18.79	*
IL 4-1	50.63	73.55	**	8.88	16.71	**
IL 4-1-1	50.30	71.73	**	10.21	15.04	ns
IL 4-2	59.40	76.43	**	7.20	11.13	ns
IL 5-5	50.77	68.72	**	11.29	17.54	ns
IL 6-1	48.78	72.48	**	7.63	14.88	**
IL 6-2	63.08	61.20	ns	5.65	7.65	ns
IL 7-1	48.22	68.98	**	8.50	13.06	*
IL 7-4	75.98	73.80	ns	15.54	16.19	ns
IL 7-5	52.97	67.63	*	8.25	12.38	*
IL 7-5-5	52.52	62.57	ns	11.13	16.17	*

Appendix Table B1. (Continued)

IL 8-1-2	52.10	57.75	ns	10.21	12.42	ns
IL 8-2	59.93	78.30	*	10.08	13.56	ns
IL 8-3	56.27	74.05	*	8.29	12.19	ns
IL 8-3-1	51.86	82.08	**	11.40	22.25	*
IL 9-1-2	62.80	73.88	ns	14.79	20.75	ns
9-2-5	48.43	64.02	**	7.83	13.20	*
IL 10-1	53.83	81.00	**	5.21	14.08	**
IL 10-1-1	53.67	79.35	**	7.67	14.29	ns
IL 11-2	45.93	62.25	**	7.04	20.92	**
IL 11-3	44.45	54.12	**	6.96	9.83	*
IL 12-1	44.62	64.93	**	11.04	15.75	*
IL 12-1-1	56.87	70.04	ns	14.63	19.75	ns
IL 12-2	68.42	78.48	**	8.00	14.45	**

ns : not significant

* : significantly different at P< 0.05

** = significantly different at P< 0.01

Appendix Table B2. Chilling injury index and decay index of tomato introgression lines stored at 3 °C for 3 weeks, then ripen at 20 °C.

Lines	Chilling injury index		Decay index	
IL 1-1	1.17	cdef	1.50	abcdefgh
IL 1-1-2	1.00	def	1.17	bcdefgh
IL 1-4	1.17	cdef	1.33	abcdefgh
IL 1-4-18	0.83	def	1.17	bcdefgh
IL 2-1	1.33	cdef	1.40	abcdefgh
IL 2-1-1	0.33	def	1.00	cdefgh
IL 2-2	1.33	cdef	0.50	efgh
IL 2-3	0.83	def	1.00	cdefgh
IL 2-4	0.00	f	0.00	h
IL 2-6	0.00	f	0.33	fgh
IL 2-6-5	0.50	def	0.33	ghf
IL 3-1	0.20	ef	1.00	cdefgh
IL 3-2	1.50	bcdef	0.67	defgh
IL 3-3	0.67	def	0.00	h
IL 3-4	3.33	a	2.17	abcd
IL 3-5	1.00	def	1.50	abcdefgh
IL 4-1	0.33	def	0.50	efgh
IL 4-1-1	1.50	bcdef	1.00	cdefgh
IL 4-2	1.33	cdef	2.00	abcde
IL 4-3	1.80	bcd	2.33	abc
IL 4-3-2	0.50	def	0.83	cdefgh
IL 5-1	1.33	cdef	1.17	bcdefgh

Appendix Table B2. (Continued)

IL 5-2	0.83	def	1.00	cdefgh
IL 5-3	1.33	cdef	1.00	abcde
IL 5-4	0.83	def	0.00	h
IL 5-5	0.67	def	0.83	cdefgh
IL 6-1	0.17	ef	0.50	efgh
IL 6-2	0.33	def	0.50	efgh
IL 6-3	0.83	def	0.50	efgh
IL 6-4	0.83	def	0.83	cdefgh
IL 7-1	0.83	def	1.50	abcdefgh
IL 7-2	1.17	cdef	1.33	abcdefgh
IL 7-3	0.00	f	0.17	gh
IL 7-4	1.67	bcde	1.50	abcdefgh
IL 7-4-1	2.83	ab	2.80	a
IL 7-5	1.33	cdef	1.50	abcdefgh
IL 7-5-5	1.67	bcde	1.50	abcdefgh
IL 8-1	1.00	def	2.67	ab
IL 8-1-1	0.50	def	1.83	abcdef
IL 8-1-2	0.00	f	0.67	defgh
IL 8-2	1.00	def	1.60	abcdefgh
IL 8-2-1	2.50	b	0.50	abcde
IL 8-3	0.67	def	1.50	abcdefgh
IL 8-3-1	1.17	cdef	1.17	bcdefgh
IL 9-1	0.50	def	0.50	efgh
IL 9-1-2	0.50	def	1.50	abcdefgh
IL 9-1-3	0.83	def	0.50	defgh

Appendix Table B2. (Continued)

IL 9-2-5	0.67	def	1.33	abcdefgh
IL 9-2-6	1.20	cdef	0.83	cdefgh
IL 9-3	0.20	ef	1.80	abcdef
IL 9-3-2	1.40	cdef	1.67	abcdefg
IL 10-1	0.33	def	1.67	abcdefg
IL 10-1-1	0.33	def	1.00	cdefgh
IL 10-2	1.20	cdef	1.00	cdefgh
IL 10-2-2	1.17	cdef	1.00	cdefgh
IL 10-3	0.67	def	0.17	gh
IL 11-1	0.33	def	0.17	gh
IL 11-2	0.00	f	0.50	abcdef
IL 11-3	0.67	def	0.67	defgh
IL 11-4	1.17	cdef	1.83	abcdef
IL 11-4-1	1.83	bcd	0.67	defgh
IL 12-1	1.00	def	1.00	cdefgh
IL 12-1-1	0.83	def	0.33	fgh
IL 12-2	1.00	def	1.00	cdefgh
IL 12-3	1.33	cdef	1.80	abcdef
IL 12-3-1	0.50	def	0.83	cdefgh
IL 12-4	1.60	bcde	1.80	abcdef
IL 12-4-1	1.33	cdef	0.67	defgh

Mean values within a column followed by the same letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Appendix Table C1. List of genes differentially expressed (more than 2 folds) in tomato fruit stored at 3 °C for 4 weeks compared with fruit at harvest obtained from microarray analysis and categorized by putative gene function.

Unigene	Genebank best hit	Expression ratio (Log2)
	>2 fold up-regulated	
	1. Transcription Regulation	
SGN-U213216	AAW28573 putative NAC domain protein NAC2 [Solanum demissum]	5.45433
SGN-U227453	ABE94188 Helix-loop-helix DNA-binding [Medicago truncatula]	4.82991
SGN-U213219	AAW28573 putative NAC domain protein NAC2 [Solanum demissum]	4.4031
SGN-U215384	NP_195392 DNA binding / transcription factor [Arabidopsis thaliana]	4.40285
SGN-U215724	AAB32591 MybSt1 [Solanum tuberosum]	3.59007
SGN-U215004	Q40168 Floral homeotic protein AGAMOUS (TAG1)	3.58525
SGN-U240829	NP_567811 ANAC074; transcription factor [Arabidopsis thaliana]	3.47946
SGN-U223608	AAO43165 DRE binding protein 1 [Gossypium hirsutum]	3.46347
SGN-U219590	AAM34767 nam-like protein 4 [Petunia x hybrida]	3.42322
SGN-U213848	AAU12056 zinc-finger protein [Solanum tuberosum]	3.32717
SGN-U217359	AAD46402 ethylene-responsive transcriptional coactivator [Lycopersicon esculentum]	3.15825
SGN-U225100	NP_189074 MYB305; DNA binding / transcription factor [Arabidopsis thaliana]	3.09398
SGN-U220052	NP_195487 DNA binding / transcription factor [Arabidopsis thaliana]	3.06485
SGN-U213502	P41379 Eukaryotic initiation factor 4A-2 (ATP-dependent RNA helicase	3.03806

Appendix Table C1. (Continued)

SGN-U219331	AAF04915 jasmonic acid 2 [Lycopersicon esculentum]	2.92838
SGN-U219819	AAR88435 NAC domain protein [Lycopersicon esculentum]	2.85694
SGN-U240026	ABF69979 transcriptional factor B3 family protein [Musa acuminata]	2.82197
SGN-U219020	NP_201280 DNA binding / transcription factor [Arabidopsis thaliana]	2.79319
SGN-U230490	AAM98190 putative Ap2 domain protein [Arabidopsis thaliana]	2.75177
SGN-U216769	NP_189461 PMZ [Arabidopsis thaliana]	2.71638
SGN-U233379	AAB58314 Cpm7 [Craterostigma plantagineum]	2.61346
AF011555	AAF04915 jasmonic acid 2 [Lycopersicon esculentum]	2.6133
SGN-U233285	Q6V9I6 Histone deacetylase 2a (HD2a) (ScHD2a)	2.59268
SGN-U226453	NP_001046176 Os02g0194200 [Oryza sativa (japonica cultivar-group)]	2.59045
SGN-U219135	AAK95688 ethylene-responsive factor 1 [Lycopersicon esculentum]	2.56823
SGN-U224735	BAA05079 zinc-finger protein [Petunia x hybrida]	2.55986
SGN-U213908	AAX14501 putative zinc finger protein [Gossypium hirsutum]	2.46803
SGN-U221734	P27484 Glycine-rich protein 2	2.41315
SGN-U225727	BAA21923 ZPT2-14 [Petunia x hybrida]	2.38243
SGN-U221748	AAM34771 nam-like protein 8 [Petunia x hybrida]	2.37756
SGN-U235872	AAF17682 F28K19.13 [Arabidopsis thaliana]	2.34734
SGN-U217757	NP_563839 transcription factor [Arabidopsis thaliana]	2.33985
SGN-U217388	AAC49740 Pti5 [Lycopersicon esculentum]	2.22752
SGN-U228269	ABL63125 MYB transcription factor [Catharanthus roseus]	2.22191
SGN-U218511	CAA67368 TFIIA [Arabidopsis thaliana]	2.19422
SGN-U215297	NP_193524 binding [Arabidopsis thaliana]	2.18888
SGN-U221925	NP_920425 putative hydroxyproline-rich glycoprotein [Oryza sativa (japonica cultivar-group)]	2.15207

Appendix Table C1. (Continued)

SGN-U219687	NP_189170 ribonuclease/ transcriptional repressor [Arabidopsis thaliana]	2.15174
SGN-U234231	BAC23031 WRKY-type DNA binding protein [Solanum tuberosum]	2.1264
SGN-U218023	AAD39439 PHAP2A protein [Petunia x hybrida]	2.11406
SGN-U230369	AAC04719 MYB-like DNA-binding domain protein [Gossypium hirsutum]	2.10577
SGN-U215579	AAQ24532 histone deacetylase [Solanum chacoense]	2.08625
SGN-U213429	BAE46417 double WRKY type transfactor [Solanum tuberosum]	2.06655
SGN-U218504	NP_174279 WRKY71; transcription factor [Arabidopsis thaliana]	2.01448
SGN-U214235	NP_172363 DNA binding [Arabidopsis thaliana]	2.01385
SGN-U221029	XP_477619 putative RNA helicase [Oryza sativa (japonica cultivar-group)]	2.0084
SGN-U216022	AAC50047 Pti4 [Lycopersicon esculentum]	2.00439
	2. RNA processing	
SGN-U233205	ABC01901 3'-5'-exoribonuclease/RNA binding-like protein [Solanum tuberosum]	4.24388
SGN-U220979	AAN72051 cell cycle control crn (crooked neck) protein-like [Arabidopsis	3.18796
SGN-U213732	NP_566199 DCL2; ATP binding / ATP-dependent helicase/ RNA binding / double-stranded RNA binding / helicase/ nucleic acid binding / ribonuclease III [Arabidopsis thaliana]	2.92465
SGN-U217217	NP_176007 NOP56 [Arabidopsis thaliana]	2.74979
SGN-U214618	ABA99370 DEAD/DEAH box helicase, putative [Oryza sativa (japonica cultivar-group)]	2.23502
SGN-U217498	ABE81433 RNA-binding region RNP-1 (RNA recognition motif); Zinc finger,	2.18545
SGN-U219086	BAD27986 putative small nuclear ribonucleoprotein polypeptide F [Oryza sativa (japonica cultivar-group)]	2.13454

Appendix Table C1. (Continued)

SGN-U217201	NP_567724 FIB2 (FIBRILLARIN 2) [Arabidopsis thaliana]	2.12216
	3. Protein synthesis	
SGN-U212847	BAA09709 elongation factor-1 alpha [Nicotiana tabacum]	23.27547
SGN-U213207	ABB29933 P0 ribosomal protein-like [Solanum tuberosum]	3.58704
SGN-U216442	AAM64816 Ribosomal protein L7Ae-like [Arabidopsis thaliana]	3.54749
SGN-U213040	AAR17783 ribosomal protein L3 [Lycopersicon esculentum]	3.17876
SGN-U212838	CAA37212 elongation factor 1-alpha [Lycopersicon esculentum]	3.11296
SGN-U214078	AAD13388 ribosomal protein L27a [Petunia x hybrida]	3.10828
SGN-U217325	XP_215404 PREDICTED: similar to Brix domain containing 1 [Rattus norvegicus]	3.07572
SGN-U213998	CAA49175 ribosomal protein YL16 [Mesembryanthemum crystallinum]	2.72614
SGN-U212918	CAC27136 40S ribosomal protein S2 [Picea abies]	2.71216
SGN-U213175	AAK25759 ribosomal protein L18a [Castanea sativa]	2.56823
SGN-U216648	ABE87988 ribosomal protein S3 [Medicago truncatula]	2.41913
SGN-U213730	ABB55361 40S ribosomal protein S10-like [Solanum tuberosum]	2.39414
SGN-U212719	CAA54095 ribosomal protein S4 [Solanum tuberosum]	2.39254
SGN-U212651	P93847 60S ribosomal protein L10 (EQM)	2.38641
SGN-U213195	AAR89617 40S ribosomal protein S5 [Capsicum annuum]	2.37539
SGN-U213093	NP_849818 LOS1; GTP binding / translation elongation factor/ translation factor, nucleic acid binding [Arabidopsis thaliana]	2.33626
SGN-U214018	AAL09401 ribosomal protein [Petunia x hybrida]	2.29067
SGN-U214558	AAD32206 60S ribosomal protein L1 [Prunus armeniaca]	2.28009
SGN-U214017	AAL09401 ribosomal protein [Petunia x hybrida]	2.27538

Appendix Table C1. (Continued)

SGN-U213562	NP_850665 structural constituent of ribosome [Arabidopsis thaliana]	2.13592
SGN-U217711	ABB86256 eukaryotic translation initiation factor 2 beta subunit-like	2.12268
SGN-U213047	ABB86257 40S ribosomal protein S15-like [Solanum tuberosum]	2.11329
SGN-U213169	AAP80667 ribosomal Pr 117 [Triticum aestivum]	2.10897
SGN-U213045	ABB86269 40S ribosomal protein S15-like [Solanum tuberosum]	2.10687
SGN-U213293	AAQ96375 60S ribosomal protein L13 [Solanum brevidens]	2.09344
SGN-U214582	ABB02632 ribosomal protein L38-like [Solanum tuberosum]	2.07088
SGN-U213298	ABA46790 60S ribosomal protein L13a-like protein [Solanum tuberosum]	2.06597
SGN-U215950	NP_198129 structural constituent of ribosome [Arabidopsis thaliana]	2.04266
SGN-U215149	BAD95794 similar to 60S ribosomal protein L35 [Lycopersicon esculentum]	2.00642
SGN-U217855	AAU06579 eukaryotic initiation factor iso4E [Nicotiana tabacum]	2.20525
SGN-U225523	ABB17004 ribosomal protein S7-like protein [Solanum tuberosum]	2.20514
SGN-U219782	AAP80667 ribosomal Pr 117 [Triticum aestivum]	2.19285
SGN-U212967	BAD28853 putative ribosomal protein L10a [Oryza sativa (japonica cultivar-group)]	2.18942
SGN-U213468	NP_564417 structural constituent of ribosome [Arabidopsis thaliana]	2.06498
	4. Protein modification	
SGN-U212665	AAC14412 calcium dependent protein kinase [Arabidopsis thaliana]	9.8049

Appendix Table C1. (Continued)

SGN-U223335	NP_200709 ATP binding / protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase [Arabidopsis thaliana]	2.96394
SGN-U218775	BAD46607 peptidylprolyl isomerase [Oryza sativa (japonica cultivar-group)]	2.65303
SGN-U235458	CAA58594 Petunia Shaggy kinase 4 [Petunia x hybrida]	2.48497
SGN-U231291	AAG52362 putative receptor protein kinase; 10992-14231 [Arabidopsis thaliana]	2.41356
SGN-U213278	ABJ91212 CBL-interacting protein kinase 4 [Populus trichocarpa]	2.2377
SGN-U230452	NP_191271 ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase [Arabidopsis thaliana]	2.10615
SGN-U215175	AAK83088 Pin1-type peptidyl-prolyl cis/trans isomerase [Malus x domestica]	2.09034
	5. Protein degradation	
SGN-U213642	AAF23126 cystatin [Lycopersicon esculentum]	4.68978
SGN-U214620	AAZ94198 Kunitz-type protease inhibitor precursor [Solanum tuberosum]	4.26474
SGN-U214987	NP_188902 PBD1 (PROTEASOME SUBUNIT PRGB); peptidase/ threonine endopeptidase [Arabidopsis thaliana]	3.50742
SGN-U215388	XP_470419 putative proteasome regulatory non-ATPase subunit [Oryza sativa (japonica cultivar-group)]	3.24548
SGN-U215765	NP_176633 peptidase [Arabidopsis thaliana]	3.07734
SGN-U214642	ABA40428 26S proteasome AAA-ATPase subunit RPT4a-like protein [Solanum tuberosum]	3.04301
SGN-U214535	BAD33488 putative ubiquitin conjugating enzyme 7 interacting protein 4 [Oryza sativa (japonica cultivar-group)]	3.01951
SGN-U216307	NP_566818 PBB1; endopeptidase/ peptidase/ threonine endopeptidase [Arabidopsis thaliana]	2.98702
SGN-U215184	CAA57510 cyprosin [Cynara cardunculus]	2.89472

Appendix Table C1. (Continued)

SGN-U218610	NP_915446 putative AAA-metalloprotease [Oryza sativa (japonica cultivar-group)]	2.88067
SGN-U214388	ABB16990 proteasome-like protein alpha subunit [Solanum tuberosum]	2.70649
SGN-U213693	NP_190937 ubiquitin-protein ligase/ zinc ion binding [Arabidopsis thaliana]	2.6706
SGN-U218743	NP_910636 proteasome inhibitor-like protein [Oryza sativa (japonica cultivar-group)]	2.65982
SGN-U215903	NP_564149 PBC1; peptidase/ threonine endopeptidase [Arabidopsis thaliana]	2.61919
SGN-U214901	NP_918654 putative 26S proteasome subunit [Oryza sativa (japonica cultivar-group)]	2.51962
SGN-U219136	AAM28274 PFE18 protein [Ananas comosus]	2.40034
SGN-U232043	ABA46785 ubiquitin extension protein-like protein [Solanum tuberosum]	2.37197
SGN-U214928	O24361 Proteasome subunit beta type 5 precursor (20S proteasome subunit E) (Proteasome epsilon chain)	2.3519
SGN-U213081	CAA78403 pre-pro-cysteine proteinase [Lycopersicon esculentum]	2.27859
SGN-U216637	NP_568750 ERD1 (EARLY RESPONSIVE TO DEHYDRATION 1); ATP binding / ATPase/ nucleoside-triphosphatase/ nucleotide binding / protein binding [Arabidopsis thaliana]	2.26208
SGN-U214367	NP_564011 ubiquitin conjugating enzyme/ ubiquitin-like activating enzyme [Arabidopsis thaliana]	2.10033
SGN-U214847	NP_174210 ATS9 (19S PROTEOSOME SUBUNIT 9) [Arabidopsis thaliana]	2.08678
SGN-U216576	AAC19402 26S proteasome regulatory subunit S5A [Mesembryanthemum crystallinum]	2.06672
SGN-U213988	NP_568389 ATSUG1; ATPase [Arabidopsis thaliana]	2.04678
SGN-U212672	AAM98141 polyubiquitin UBQ10 [Arabidopsis thaliana]	2.02974
	6. Nucleotide metabolism	

Appendix Table C1. (Continued)

SGN-U218467	AAR06289 5-phosphoribosyl-1-pyrophosphate amidotransferase [Nicotiana tabacum]	2.29218
SGN-U228906	XP_481632 putative GMP synthetase [Oryza sativa (japonica cultivar-group)]	2.22242
SGN-U213330	AAZ85394 cytosolic nucleoside diphosphate kinase [Solanum chacoense]	2.14694
	7. Amino acid metabolism	
SGN-U218881	P17570 Nitrate reductase [NADH] (NR)	5.55087
SGN-U219935	NP_180763 N-acetyltransferase [Arabidopsis thaliana]	5.49589
X14060	P17570 Nitrate reductase [NADH] (NR)	5.11148
SGN-U225781	P17570 Nitrate reductase [NADH] (NR)	5.00745
SGN-U225530	AAL36888 NADH-glutamate dehydrogenase [Lycopersicon esculentum]	2.94439
SGN-U225652	CAA05895 dihydrofolate reductase-thymidylate synthetase precursor [Daucus	2.76436
AF403178	AAL36888 NADH-glutamate dehydrogenase [Solanum lycopersicum]	2.38378
SGN-U221509	NP_914412 putative aspartate aminotransferase [Oryza sativa (japonica cultivar-group)]	2.14609
	8. Carbohydrate metabolism	
SGN-U221349	BAD93688 glucosyltransferase NTGT4 [Nicotiana tabacum]	17.85441
SGN-U232321	CAA09593 sucrose synthase [Solanum lycopersicum]	13.37126
SGN-U214429	CAA59450 twi1 [Lycopersicon esculentum]	12.82747
SGN-U213118	AAO34668 sucrose synthase 2 [Solanum tuberosum]	9.28727
SGN-U215276	NP_001065749 Os11g0148500 [Oryza sativa (japonica cultivar-group)]	4.21255
SGN-U238930	CAA73334 invertase inhibitor homologue [Nicotiana tabacum]	3.97809
SGN-U221214	AAK84008 beta-amylase PCT-BMYI [Solanum tuberosum]	3.77987

Appendix Table C1. (Continued)

SGN-U218015	NP_181910 UDP-glycosyltransferase/ transferase, transferring glycosyl groups / transferase, transferring hexosyl groups [Arabidopsis thaliana]	3.55905
SGN-U212954	AAM22410 cell-wall invertase [Lycopersicon esculentum]	3.0277
SGN-U236488	CAB39974 glyceraldehyde-3-phosphate dehydrogenase [Nicotiana tabacum]	2.28928
SGN-U218153	CAC80375 glyceraldehyde-3-phosphate dehydrogenase [Capsicum annuum]	2.15052
	9. Lipid metabolism	
SGN-U215025	AAL54884 cytochrome P450-dependent fatty acid hydroxylase [Nicotiana tabacum]	12.39058
SGN-U214192	AAT72296 microsomal omega-6-desaturase [Nicotiana tabacum]	2.09047
SGN-U218568	AAQ13595 putative squalene epoxidase [Lycopersicon esculentum]	2.00003
	10. Transporter	
SGN-U214604	AAM47580 putative ABC-transporter-like protein [Sorghum bicolor]	11.35936
SGN-U214603	NP_001051311 Os03g0755100 [Oryza sativa (japonica cultivar-group)]	7.30117
SGN-U219674	NP_201341 antiporter/ drug transporter/ transporter [Arabidopsis thaliana]	5.91924
SGN-U220732	AAL07089 putative nonsense-mediated mRNA decay protein [Arabidopsis thaliana]	3.50772
SGN-U216406	NP_566854 amino acid permease [Arabidopsis thaliana]	3.1297
SGN-U212911	ABB29945 ADP/ATP translocator-like [Solanum tuberosum]	3.09762
SGN-U221177	NP_001031018 transporter [Arabidopsis thaliana]	2.47161
SGN-U212977	P38547 GTP-binding nuclear protein Ran2	2.17646
SGN-U219833	AAC49791 MRP-like ABC transporter [Arabidopsis thaliana]	2.12854

Appendix Table C1. (Continued)

SGN-U216748	AAX36074 non-intrinsic ABC protein [Nicotiana benthamiana]	2.03728
SGN-U218067	CAA63223 TOM20 [Solanum tuberosum]	2.2205
	11. Secondary metabolism	
SGN-U232334	AAZ85221 arginine decarboxylase [Lycopersicon pimpinellifolium]	6.39137
SGN-U213123	CAI39242 arginine decarboxylase [Lycopersicon esculentum]	6.03809
SGN-U221412	BAD77944 UDP-glucuronic acid:anthocyanin glucuronosyltransferase [Bellis perennis]	5.96391
SGN-U214399	CAA91228 caffeoyl-CoA O-methyltransferase [Nicotiana tabacum]	3.61306
SGN-U213084	Q96471 S-adenosylmethionine decarboxylase proenzyme (AdoMetDC) (SamDC) [Contains: S-adenosylmethionine decarboxylase alpha chain; S-adenosylmethionine decarboxylase beta chain]	3.55374
SGN-U221620	CAA11226 chalcone reductase [Sesbania rostrata]	2.90137
L16582	CAB64599 arginine decarboxylase 1 [Datura stramonium]	2.77023
SGN-U214591	AAX15955 cinnamyl alcohol dehydrogenase 1 [Nicotiana tabacum]	2.73749
Z83835	P93236 Zeaxanthin epoxidase, chloroplast precursor	2.55379
	12. Cell wall	
SGN-U212549	CAA54561 cell wall protein [Lycopersicon esculentum]	2.83735
SGN-U213444	AAS46240 xyloglucan endotransglucosylase-hydrolase XTH5 [Lycopersicon esculentum]	2.45724
SGN-U214418	AAA66362 arabinogalactan-protein precursor	2.23507
	13. Signal transduction	
SGN-U216160	AAR87711 salicylic acid-binding protein 2 [Nicotiana tabacum]	29.72096
SGN-U216162	AAR87711 salicylic acid-binding protein 2 [Nicotiana tabacum]	16.20563
SGN-U214230	AAT40481 putative protein kinase [Solanum demissum]	3.28726

Appendix Table C1. (Continued)

SGN-U216642	CAI53895 putative receptor associated protein [Capsicum chinense]	3.19557
SGN-U215155	AAL28022 small GTPase Rab2 [Nicotiana tabacum]	2.28639
SGN-U225549	NP_912583 Putative leucine-rich repeat transmembrane protein kinase [Oryza sativa (japonica cultivar-group)]	2.12473
SGN-U220268	ABE91085 Protein kinase [Medicago truncatula]	2.12176
SGN-U219586	Q40521 Ras-related protein Rab11B	2.09878
SGN-U217677	NP_195311 ATGB2 (GTP-BINDING 2); GTP binding [Arabidopsis thaliana]	2.07658
SGN-U224209	CAD42725 putative rac protein [Nicotiana tabacum]	2.06896
	14. Pathogenesis-related	
SGN-U213790	CAA78846 chitinase [Lycopersicon esculentum]	9.72908
SGN-U213791	Q05540 Acidic 27 kDa endochitinase precursor	9.19763
SGN-U212883	CAA78845 chitinase [Lycopersicon esculentum]	9.08246
SGN-U212884	AAF25602 class I chitinase [Solanum tuberosum]	7.61553
SGN-U214259	CAA50596 PR-1a1 [Lycopersicon esculentum]	7.48444
SGN-U213451	AAC06244 basic PR-1 protein precursor [Capsicum annuum]	4.49173
SGN-U213160	AAN52931 hin1-like protein [Solanum tuberosum]	3.72645
SGN-U213935	AAP43673 PR5-like protein [Lycopersicon esculentum]	2.63908
SGN-U213868	AAN87262 xyloglucan-specific fungal endoglucanase inhibitor protein precursor [Lycopersicon esculentum]	2.36441
	15. Stress	
SGN-U214049	AAD51854 stress related protein [Vitis riparia]	5.94451
SGN-U212639	AAR12195 molecular chaperone Hsp90-1 [Lycopersicon esculentum]	4.73226
SGN-U228034	CAA37971 heat shock protein cognate 70 [Lycopersicon esculentum]	4.53666
AJ225049	O82013 17.3 kDa class II heat shock protein (Hsp17.3) (Hsp20.2)	4.41471
SGN-U223323	NP_568276 heat shock protein binding / unfolded protein binding [Arabidopsis thaliana]	2.95718

Appendix Table C1. (Continued)

SGN-U213773	AAX13276 USP family protein [Triticum aestivum]	2.85761
SGN-U214952	CAA50218 chaperonin 60 [Cucurbita cv. Kurokawa Amakuri]	2.62858
SGN-U212696	BAA32547 mitochondrial small heat shock protein [Solanum lycopersicum]	2.51018
X54029	P24629 Heat shock cognate 70 kDa protein 1	2.39724
SGN-U212643	AAR12195 molecular chaperone Hsp90-1 [Lycopersicon esculentum]	2.18002
SGN-U224254	AAG43551 Avr9/Cf-9 rapidly elicited protein 146 [Nicotiana tabacum]	2.00605
SGN-U215229	NP_187434 ATP binding / unfolded protein binding [Arabidopsis thaliana]	2.1768
	16. Ethylene	
	-	
	17. Others	
SGN-U217204	AAG43551 Avr9/Cf-9 rapidly elicited protein 146 [Nicotiana tabacum]	15.31806
SGN-U220325	AAC78508 putative phloem-specific lectin [Arabidopsis thaliana]	14.91427
SGN-U212850	NP_181250 peroxidase [Arabidopsis thaliana]	13.86794
SGN-U213431	AAA33106 cytochrome P-450 protein [Catharanthus roseus]	12.30305
SGN-U215855	AAX84672 aldo/keto reductase AKR [Manihot esculenta]	9.41643
SGN-U217960	BAD46173 putative calcium binding protein [Oryza sativa (japonica cultivar-group)]	8.22206
SGN-U214504	DAA00284 TPA: putative phytosulfokine peptide precursor [Lycopersicon esculentum]	7.70533
SGN-U215827	ABB02383 temperature-induced lipocalin' [Lycopersicon esculentum]	7.56645
SGN-U213881	ABC69414 CYP72A57 [Nicotiana tabacum]	7.27132
SGN-U214481	CAA45741 C-7 [Nicotiana tabacum]	6.57359
SGN-U216310	NP_565876 binding [Arabidopsis thaliana]	6.49416

Appendix Table C1. (Continued)

SGN-U232919	tpg|DAA00284.1| TPA: TPA_exp: putative phytosulfokine peptide precursor	5.52215
SGN-U219896	AAA33106 cytochrome P-450 protein [Catharanthus roseus]	5.23966
SGN-U214691	AAK58482 alternative oxidase 1a [Lycopersicon esculentum]	5.08933
SGN-U216028	CAB61840 putative glycine and proline-rich protein [Sporobolus stapfianus]	4.98528
SGN-U218950	Q8SAG3 Actin-depolymerizing factor (ADF)	4.98205
SGN-U226077	NP_001065462 Os10g0572300 [Oryza sativa (japonica cultivar-group)]	4.82185
SGN-U227544	NP_200485 histone acetyltransferase [Arabidopsis thaliana]	4.75545
SGN-U233072	ABF59516 putative spindle disassembly related protein CDC48 [Nicotiana	4.71346
SGN-U212756	AAG16757 putative glutathione S-transferase T2 [Lycopersicon esculentum]	4.68079
SGN-U213948	AAS98165 hypersensitive-induced reaction protein [Capsicum annuum]	4.64298
SGN-U219666	NP_201058 SRO5 [Arabidopsis thaliana]	4.52497
SGN-U217364	NP_173769 SRO2 [Arabidopsis thaliana]	4.43239
SGN-U215033	BAD33248 mitochondrial glycoprotein-like [Oryza sativa (japonica cultivar-group)]	4.20929
SGN-U221629	NP_568580 catalytic [Arabidopsis thaliana]	4.13659
SGN-U231632	AAB80714 phosphoenolpyruvate carboxylase 1 [Gossypium hirsutum]	4.12828
SGN-U213747	P37122 Cytochrome P450 76A2 (CYPLXXVIA2) (P-450EG7)	4.09407
SGN-U213406	CAA05894 CYP1 [Lycopersicon esculentum]	3.92805
SGN-U214040	NP_200265 unknown protein [Arabidopsis thaliana]	3.71703
SGN-U219566	XP_450774 putative Avr9/Cf-9 rapidly elicited protein 276 [Oryza sativa (japonica cultivar-group)]	3.69073

Appendix Table C1. (Continued)

SGN-U216385	AAB80714 phosphoenolpyruvate carboxylase 1 [Gossypium hirsutum]	3.68827
SGN-U222648	NP_180763 N-acetyltransferase [Arabidopsis thaliana]	3.67577
SGN-U215032	BAD33248 mitochondrial glycoprotein-like [Oryza sativa (japonica cultivar-group)]	3.63494
SGN-U212940	Q40460 Ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplast precursor (RuBisCO activase 1) (RA 1)	3.57214
SGN-U223576	CAA85426 catalase [Nicotiana plumbaginifolia]	3.54628
SGN-U217225	CAD56690 proliferating cell nuclear antigen [Solanum lycopersicum]	3.48819
SGN-U214507	BAD82322 copine III-like [Oryza sativa (japonica cultivar-group)]	3.39682
SGN-U221418	AAK19054 UVI1 [Pisum sativum]	3.25663
SGN-U213072	CAB85628 putative ripening-related protein [Vitis vinifera]	3.22578
SGN-U238097	CAA09852 cyclin D2.1 protein [Nicotiana tabacum]	3.2099
SGN-U213506	BAA76895 LeArcA1 protein [Lycopersicon esculentum]	3.11778
SGN-U215254	CAA42660 luminal binding protein (BiP) [Nicotiana tabacum]	3.04788
SGN-U219978	AAH84467 Igf2r-prov protein [Xenopus tropicalis]	3.01155
SGN-U239936	AAC78592 Hcr2-0A [Lycopersicon esculentum]	2.94668
AY547273	AAS92268 early light inducible protein [Lycopersicon esculentum]	2.91716
SGN-U223312	NP_176732 calcium ion binding [Arabidopsis thaliana]	2.90296
SGN-U213956	BAC02896 tobacco nucleolin [Nicotiana tabacum]	2.90281
SGN-U213728	NP_195526 HD1; histone deacetylase [Arabidopsis thaliana]	2.89075
SGN-U217037	CAB39974 glyceraldehyde-3-phosphate dehydrogenase [Nicotiana tabacum]	2.87429
SGN-U215038	CAC39620 ferredoxin-thioredoxin-reductase catalytic subunit B [Solanum tuberosum]	2.81329

Appendix Table C1. (Continued)

SGN-U215571	NP_200118 nucleic acid binding [Arabidopsis thaliana]	2.72557
SGN-U215620	NP_568846 NMT1 (N-MYRISTOYLTRANSFERASE 1) [Arabidopsis thaliana]	2.70166
SGN-U232827	NP_001049049 Os03g0162200 [Oryza sativa (japonica cultivar-group)]	2.66173
SGN-U213566	NP_181673 rhodopsin-like receptor [Arabidopsis thaliana]	2.63067
SGN-U219910	NP_193880 NADH dehydrogenase [Arabidopsis thaliana]	2.62417
SGN-U233939	NP_568253 catalytic [Arabidopsis thaliana]	2.59494
SGN-U224758	NP_563703 nucleotide binding [Arabidopsis thaliana]	2.58127
SGN-U215374	CAA56599 34 kDA porin [Solanum tuberosum]	2.55988
SGN-U213913	ABA40457 TSJT1-like protein [Solanum tuberosum]	2.55211
SGN-U225469	AAK43897 putative helicase [Arabidopsis thaliana]	2.53874
SGN-U218985	XP_465274 nuclear protein-like [Oryza sativa (japonica cultivar-group)]	2.53296
SGN-U216099	NP_193524 binding [Arabidopsis thaliana]	2.51948
SGN-U216139	BAD93797 integral membrane protein -like [Arabidopsis thaliana]	2.51417
SGN-U219133	XP_480007 putative RNA recognition motif (RRM)-containing protein [Oryza sativa (japonica cultivar-group)]	2.50416
SGN-U215677	NP_910332 putative plastid protein [Oryza sativa (japonica cultivar-group)]	2.44004
SGN-U219663	AAP32203 latency associated nuclear antigen [Saimiriine herpesvirus 2]	2.42696
SGN-U215029	BAC81649 glutathione S-transferase [Pisum sativum]	2.39874
SGN-U214601	AAK94781 gamma hydroxybutyrate dehydrogenase [Arabidopsis thaliana]	2.37141
SGN-U215928	NP_917385 putative nifU-like protein [Oryza sativa (japonica cultivar-group)]	2.36594
SGN-U218943	XP_550386 putative BRI1-KD interacting protein 128 [Oryza sativa (japonica cultivar-group)]	2.35335
SGN-U227536	NP_190662 ATP binding / ATPase/ nucleoside-triphosphatase/ nucleotide binding [Arabidopsis thaliana]	2.34934

Appendix Table C1. (Continued)

SGN-U213836	AAL54858 tetratricoredoxin [Nicotiana tabacum]	2.34339
SGN-U213010	ABE77454 Phenazine biosynthesis PhzC/PhzF protein [Medicago truncatula]	2.34121
SGN-U213802	NP_194480 PPI1 (PROTON PUMP INTERACTOR 1) [Arabidopsis thaliana]	2.34074
SGN-U221805	ABA99397 SRP40, C-terminal domain, putative [Oryza sativa (japonica cultivar-group)]	2.33261
SGN-U235619	NP_188791 nucleotide binding [Arabidopsis thaliana]	2.31024
SGN-U217967	NP_176647 catalytic [Arabidopsis thaliana]	2.30585
SGN-U227474	BAA06012 RNA binding protein, RZ-1 [Nicotiana sylvestris]	2.29895
SGN-U218727	NP_001055174 Os05g0316200 [Oryza sativa (japonica cultivar-group)]	2.29836
SGN-U217550	NP_917471 cytosolic aldehyde dehydrogenase [Oryza sativa (japonica cultivar-group)]	2.28414
SGN-U222935	ABE77687 RNA-binding region RNP-1 (RNA recognition motif) [Medicago	2.2696
SGN-U231432	NP_190695 ATP binding / protein kinase [Arabidopsis thaliana]	2.26607
SGN-U225054	ABB47985 protein kinase, putative [Oryza sativa (japonica cultivar-group)]	2.26267
SGN-U216992	NP_179460 ATTPS11; transferase, transferring glycosyl groups [Arabidopsis thaliana]	2.26061
SGN-U231000	NP_182175 importin-alpha export receptor/ protein transporter [Arabidopsis	2.23546
SGN-U220355	NP_001046260 Os02g0208600 [Oryza sativa (japonica cultivar-group)]	2.23525
SGN-U229834	XP_493889 putative protein kinase [Oryza sativa]	2.22243
SGN-U215219	ABC69418 CYP92B3 [Nicotiana tabacum]	2.18718
SGN-U216798	NP_568630 metal ion binding [Arabidopsis thaliana]	2.17405
SGN-U227514	BAA83713 RNA binding protein [Homo sapiens]	2.17344

Appendix Table C1. (Continued)

SGN-U215469	CAI62049 UDP-xylose phenolic glycosyltransferase [Lycopersicon esculentum]	2.17008
SGN-U214066	NP_849770 ALDH3H1; aldehyde dehydrogenase [Arabidopsis thaliana]	2.16898
SGN-U230686	NP_192182 nucleotide binding [Arabidopsis thaliana]	2.16368
SGN-U231227	NP_174182 carboxylic ester hydrolase/ hydrolase, acting on ester bonds [Arabidopsis thaliana]	2.14998
SGN-U235481	NP_680393 ATP binding / DNA binding / DNA-dependent ATPase [Arabidopsis thaliana]	2.14416
SGN-U216067	AAM63205 stomatin-like protein [Arabidopsis thaliana]	2.12071
SGN-U213822	AAL92873 glutathione S-transferase-like protein [Lycopersicon esculentum]	2.10098
SGN-U214358	AAQ56349 putative CBS domain containing protein [Oryza sativa (japonica cultivar-group)]	2.0964
SGN-U213877	BAA76896 LeArcA2 protein [Lycopersicon esculentum]	2.0929
SGN-U216091	NP_060791 BRIX [Homo sapiens]	2.08949
SGN-U220345	BAD53258 breast carcinoma amplified sequence 3-like protein [Oryza sativa (japonica cultivar-group)]	2.07871
SGN-U239712	AAV98199 phenylalanine ammonialyase 1 [Petunia x hybrida]	2.06813
SGN-U218756	AAG16759 putative glutathione S-transferase T4 [Lycopersicon esculentum]	2.06035
SGN-U217018	AAO42464 phosphorybosyl anthranilate transferase 1 [Arabidopsis thaliana]	2.05377
SGN-U212830	XP_469739 putative RNA-binding protein [Oryza sativa]	2.04642
SGN-U225406	XP_856861 PREDICTED: similar to cell division cycle 2-like 1 (PITSLRE proteins) isoform 2 isoform 3 [Canis familiaris]	2.04457
SGN-U219849	ABE78291 Helicase, C-terminal [Medicago truncatula]	2.04146
SGN-U218762	NP_568123 nucleobase:cation symporter [Arabidopsis thaliana]	2.03155

Appendix Table C1. (Continued)

SGN-U223182	ABG90382 lateral organ boundaries domain protein [Caragana korshinskii]	2.01936
SGN-U219732	AAX96259 PB1 domain, putative [Oryza sativa (japonica cultivar-group)]	2.28299
SGN-U212908	ABA81857 ripening regulated protein-like [Solanum tuberosum]	2.20537
SGN-U215770	XP_696293 PREDICTED: similar to Tetratricopeptide repeat protein 11 (TPR repeat protein 11) (Fis1 homolog) [Danio rerio]	2.19082
SGN-U221322	AAO41900 putative VAMP protein SEC22 [Arabidopsis thaliana]	2.18074
SGN-U213660	CAI44933 N-rich protein [Glycine max]	2.13878
SGN-U222659	CAA55812 Sn-1 [Capsicum annuum]	2.08176
	18. Unknown	
SGN-U239113	NP_174417 unknown protein [Arabidopsis thaliana]	10.35004
SGN-U224395	ABA94064 expressed protein [Oryza sativa (japonica cultivar-group)]	6.54581
SGN-U213689	XP_470153 unknown protein [Oryza sativa]	4.72791
SGN-U217907	NP_198681 unknown protein [Arabidopsis thaliana]	4.72258
SGN-U219293	AAM65077 unknown [Arabidopsis thaliana]	4.57663
SGN-U214998	XP_476340 unknown protein [Oryza sativa (japonica cultivar-group)]	4.32527
SGN-U221858	NP_683304 unknown protein [Arabidopsis thaliana]	4.16532
SGN-U216552	AAL92309 similar to Dictyostelium discoideum (Slime mold). Phosphatidylinositol 3-kinase 3 (EC 2.7.1.137) (PI3-kinase) (PtdIns-3-kinase) (PI3K) (Fragment)	4.15548
SGN-U228776	NP_568275 unknown protein [Arabidopsis thaliana]	3.77841
SGN-U220091	NP_973399 unknown protein [Arabidopsis thaliana]	3.37819
SGN-U221155	NP_201232 unknown protein [Arabidopsis thaliana]	3.15158
SGN-U215417	NP_850751 unknown protein [Arabidopsis thaliana]	3.14479
SGN-U217062	NP_566314 unknown protein [Arabidopsis thaliana]	2.98418
SGN-U231790	AAM13859 unknown protein [Arabidopsis thaliana]	2.77728

Appendix Table C1. (Continued)

SGN-U229052	AAQ62582 unknown [Glycine max]	2.68253
SGN-U223909	NP_563993 unknown protein [Arabidopsis thaliana]	2.58463
SGN-U213387	ABB29921 unknown [Solanum tuberosum]	2.55535
SGN-U215418	NP_850751 unknown protein [Arabidopsis thaliana]	2.45317
SGN-U218348	NP_973861 unknown protein [Arabidopsis thaliana]	2.34559
SGN-U217919	NP_565383 unknown protein [Arabidopsis thaliana]	2.30829
SGN-U225064	NP_190603 unknown protein [Arabidopsis thaliana]	2.17472
SGN-U216917	ABA81880 unknown [Solanum tuberosum]	2.10596
SGN-U215303	NP_565562 unknown protein [Arabidopsis thaliana]	2.04197
SGN-U213989	ABA81865 unknown [Solanum tuberosum]	2.18022
	19. No hits found	
SGN-U214357	No hits found	4.97396
SGN-U224525	No hits found	3.67778
SGN-U226787	No hits found	3.45458
SGN-U214360	No hits found	2.92582
SGN-U225268	No hits found	2.7226
SGN-U222282	No hits found	2.493
SGN-U226053	No hits found	2.4269
SGN-U234169	No hits found	2.4209
SGN-U232786	No hits found	2.39647
SGN-U222017	No hits found	2.36261
SGN-U214356	No hits found	2.35144
SGN-U218327	No hits found	2.32693
	>2 fold down-regulated	
	1. Transcriptional regulation	
SGN-U227525	AAM34774 nam-like protein 11 [Petunia x hybrida]	0.11418
SGN-U216213	AAU43923 NAC domain protein [Lycopersicon esculentum]	0.30122
SGN-U213417	CAA64417 homeobox [Lycopersicon esculentum]	0.32428
SGN-U215261	AAT76423 putative HSF-type DNA-binding protein [Oryza sativa (japonica cultivar-group)]	0.35142

Appendix Table C1. (Continued)

SGN-U229870	AAW67002 WRKY transcription factor-c [Capsicum annuum]	0.35973
SGN-U223525	AAX85983 NAC6 protein [Glycine max]	0.36122
SGN-U232941	AAO12211 MADS11 [Nicotiana tabacum]	0.36209
SGN-U212614	AAZ83588 MADS-box transcription factor [Lycopersicon esculentum]	0.36472
SGN-U218652	NP_177686 transcription factor/ zinc ion binding [Arabidopsis thaliana]	0.37066
SGN-U232570	AAK95687 transcription factor JERF1 [Lycopersicon esculentum]	0.37114
SGN-U214549	AAF22139 MADS box protein [Capsicum annuum]	0.38454
SGN-U213383	AAD39439 PHAP2A protein [Petunia x hybrida]	0.38724
SGN-U217708	NP_567861 WRKY32; structural constituent of ribosome [Arabidopsis thaliana]	0.41101
SGN-U232621	CAB67118 homeodomain protein [Solanum lycopersicum]	0.41727
SGN-U220658	NP_176620 DNA binding / transcription factor [Arabidopsis thaliana]	0.42569
SGN-U212613	AAF77579 pepper MADS-box protein [Capsicum annuum]	0.43971
SGN-U213637	AAF61864 DNA-binding protein 4 [Nicotiana tabacum]	0.45839
SGN-U219120	AAS72389 ethylene response factor 5 [Lycopersicon esculentum]	0.46482
SGN-U219797	NP_174087 transcription factor [Arabidopsis thaliana]	0.4694
SGN-U217437	BAA88221 myb-related transcription factor LBM1 [Nicotiana tabacum]	0.47274
SGN-U213659	AAM33098 TDR4 transcription factor [Lycopersicon esculentum]	0.47308
SGN-U214883	AAQ91334 JERF3 [Lycopersicon esculentum]	0.47576
SGN-U220789	BAB61098 phi-2 [Nicotiana tabacum]	0.49066
SGN-U227950	NP_190996 ATP binding / ATP-dependent helicase/ DNA binding / helicase/ nucleic	0.49603

Appendix Table C1. (Continued)

SGN-U213578	AAN03622 BEL1-related homeotic protein 11 [Solanum tuberosum]	0.49709
	2. RNA processing	
SGN-U216725	NP_001030817 3'-5'-exoribonuclease/ RNA binding [Arabidopsis thaliana]	0.30803
	3. Protein synthesis	
SGN-U213801	ABB29934 acidic ribosomal protein P1a-like [Solanum tuberosum]	0.31618
SGN-U222310	NP_191121 EMB1624; translation initiation factor [Arabidopsis thaliana]	0.49265
	4. Protein modification	
SGN-U218864	BAF44192 SNF1-related kinase [Solanum lycopersicum]	0.19352
SGN-U216434	AAO32318 phosphoenolpyruvate carboxylase kinase 2 [Lycopersicon esculentum]	0.29125
SGN-U232483	AAF86506 CBL-interacting protein kinase 2 [Arabidopsis thaliana]	0.31317
SGN-U241632	CAE55203 protein kinase 1 [Nicotiana tabacum]	0.31809
SGN-U216432	AAO32318 phosphoenolpyruvate carboxylase kinase 2 [Lycopersicon esculentum]	0.31849
SGN-U218475	AAF91323 receptor-like protein kinase 2 [Glycine max]	0.33926
SGN-U230817	CAA57872 pyruvate,orthophosphate dikinase [Mesembryanthemum crystallinum]	0.34387
SGN-U228333	ABE88713 Protein kinase [Medicago truncatula]	0.36251
SGN-U215938	CAA57872 pyruvate,orthophosphate dikinase [Mesembryanthemum crystallinum]	0.39754
SGN-U230827	NP_187120 ATP binding / kinase/ protein kinase/ protein serine/threonine	0.48642
SGN-U216366	NP_177231 ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase [Arabidopsis thaliana]	0.49336
	5. Protein degradation	
SGN-U213770	AAP41846 cysteine protease [Anthurium andraeanum]	0.2869

Appendix Table C1. (Continued)

SGN-U223542	AAP85546 putative RING-H2 zinc finger protein [Oryza sativa (japonica cultivar-group)]	0.3115
SGN-U213363	AAC37397 auxin-induced proteinase inhibitor	0.33986
SGN-U217032	AAO45753 RING/C3HC4/PHD zinc finger-like protein [Cucumis melo]	0.34899
SGN-U222040	AAP32310 putative FtsH protease [Lycopersicon esculentum]	0.35497
SGN-U233700	AAL05932 SKIP5-like protein [Lycopersicon esculentum]	0.36837
SGN-U215158	BAC54828 vacuolar processing enzyme-1b [Nicotiana tabacum]	0.37909
SGN-U223216	XP_468249 putative hematopoietic-specific IL-2 deubiquitinating enzyme [Oryza sativa (japonica cultivar-group)]	0.40501
SGN-U219218	AAO18731 cysteine protease [Gossypium hirsutum]	0.41345
SGN-U216580	Q40143 Cysteine proteinase 3 precursor	0.48443
SGN-U220591	AAM51209 polyubiquitin [Cercomonas edax]	0.45555
SGN-U225323	AAK25839 putative subtilisin serine protease [Arabidopsis thaliana]	0.46423
SGN-U213409	ABB86270 ubiquitin-conjugating protein 13-like [Solanum tuberosum]	0.47336
	6. Nucleotide metabolism	
	-	
	7. Amino acid metabolism	
SGN-U212592	CAA50719 histidine decarboxylase [Lycopersicon esculentum]	0.06552
SGN-U212594	P54772 Histidine decarboxylase (HDC) (TOM92)	0.09807
SGN-U212595	CAA50719 histidine decarboxylase [Lycopersicon esculentum]	0.12047
SGN-U212615	CAA50719 histidine decarboxylase [Lycopersicon esculentum]	0.13379

Appendix Table C1. (Continued)

SGN-U213373	AAN14410 bifunctional lysine-ketoglutarate reductase/saccharopine dehydrogenase [Gossypium hirsutum]	0.33596
SGN-U232452	AAN14410 bifunctional lysine-ketoglutarate reductase/saccharopine	0.37226
SGN-U213860	P54767 Glutamate decarboxylase (GAD) (ERT D1)	0.38298
SGN-U222566	NP_181121 SCPL26; serine carboxypeptidase [Arabidopsis thaliana]	0.39611
SGN-U212562	AAC39483 glutamate decarboxylase isozyme 2 [Nicotiana tabacum]	0.44341
SGN-U215965	BAD54126 aspartate transaminase precursor, mitochondrial [Oryza sativa (japonica cultivar-group)]	0.48029
SGN-U233373	P13443 Glycerate dehydrogenase (NADH-dependent hydroxypyruvate reductase)	0.48869
	8. Carbohydrate metabolism	
SGN-U212901	CAA78063 beta-fructofuranosidase; vaculolar invertase [Lycopersicon pimpinellifolium]	0.0635
SGN-U212902	CAA78063 beta-fructofuranosidase; vaculolar invertase [Lycopersicon pimpinellifolium]	0.08244
SGN-U214669	S39507 glucuronosyl transferase homolog, ripening-related - tomato (fragment)	0.15827
SGN-U214904	AAY85180 pectate lyase [Gossypium hirsutum]	0.27707
SGN-U221226	NP_849285 transferase, transferring glycosyl groups / transferase, transferring hexosyl groups [Arabidopsis thaliana]	0.29446
SGN-U217223	AAX33231 plastid alpha-amylase [Malus x domestica]	0.32558
SGN-U213712	CAH60892 1,4-alpha-glucan-maltohydrolase [Lycopersicon esculentum]	0.3437
SGN-U230638	AAX33233 plastid alpha-amylase [Actinidia chinensis]	0.35825
SGN-U216742	AAZ05069 pyruvate decarboxylase [Citrus sinensis]	0.3646
SGN-U212899	P29000 Acid beta-fructofuranosidase precursor (Acid sucrose hydrolase) (Acid	0.39418

Appendix Table C1. (Continued)

SGN-U213844	CAH60890 carbonic anhydrase [Solanum lycopersicum]	0.40193
SGN-U214522	P21343 Pyrophosphate--fructose 6-phosphate 1-phosphotransferase beta subunit (PFP) (6-phosphofructokinase, pyrophosphate dependent) (Pyrophosphate-dependent 6-phosphofructose-1-kinase) (PPi-PFK)	0.40443
SGN-U224541	AAU89740 At1g69830/T17F3_14-like [Solanum tuberosum]	0.44006
SGN-U214700	P21342 Pyrophosphate--fructose 6-phosphate 1-phosphotransferase alpha subunit (PFP) (6-phosphofructokinase, pyrophosphate-dependent) (Pyrophosphate-dependent 6-phosphofructose-1-kinase) (PPi-PFK)	0.48024
SGN-U237471	NP_190284 hydrolase, hydrolyzing O-glycosyl compounds [Arabidopsis thaliana]	0.48794
SGN-U216715	CAA09420 tomato invertase inhibitor [Lycopersicon esculentum]	0.49483
	9. Lipid metabolism	
SGN-U215592	AAL54886 cytochrome P450-dependent fatty acid hydroxylase [Nicotiana tabacum]	0.09001
SGN-U216841	XP_465295 C2 domain-containing protein-like [Oryza sativa (japonica cultivar-group)]	0.19483
SGN-U216734	ABA93963 GDSL-like Lipase/Acylhydrolase [Oryza sativa (japonica cultivar-group)]	0.21276
SGN-U214849	AAB65766 lipoxygenase	0.23521
SGN-U214561	AAR83862 elicitor-inducible protein EIG-J7 [Capsicum annuum]	0.25976
SGN-U224441	AAP83137 lipoxygenase [Nicotiana attenuata]	0.27515
SGN-U216420	AAM82606 putative non-specific lipid transfer protein StnsLTP [Solanum tuberosum]	0.28826
SGN-U234711	CAA65268 13-lipoxygenase [Solanum tuberosum]	0.30551

Appendix Table C1. (Continued)

SGN-U215645	P46253 Acyl-[acyl-carrier-protein] desaturase, chloroplast precursor (Stearoyl-ACP desaturase)	0.35659
SGN-U218305	NP_195581 phospholipase C [Arabidopsis thaliana]	0.37115
SGN-U222218	P93224 Nonspecific lipid-transfer protein 2 precursor (LTP 2)	0.37167
SGN-U232114	AAA74393 lipoxygenase	0.37899
SGN-U214750	BAB40450 long-chain acyl-CoA synthetase [Arabidopsis thaliana]	0.38732
SGN-U213037	AAS80139 arachidonic acid-induced DEA1 [Lycopersicon esculentum]	0.39154
X56040	P27056 Nonspecific lipid-transfer protein 1 precursor (LTP 1)	0.39155
SGN-U224013	NP_180255 hydrolase, acting on ester bonds [Arabidopsis thaliana]	0.40076
U37839	AAB65766 lipoxygenase	0.45765
SGN-U212783	AAG21691 lipoxygenase [Lycopersicon esculentum]	0.4604
SGN-U236212	NP_193091 triacylglycerol lipase [Arabidopsis thaliana]	0.46626
SGN-U227728	NP_564662 esterase/lipase/thioesterase family protein [Arabidopsis thaliana]	0.49609
	10.Transporters	
SGN-U213547	NP_193555 ATPUP10; purine transporter [Arabidopsis thaliana]	0.3029
SGN-U215368	ABB02390 temperature-induced lipocalin [Lycopersicon esculentum]	0.33977
SGN-U217780	CAD59598 MRP-like ABC transporter [Oryza sativa (japonica cultivar-group)]	0.34257
SGN-U224652	NP_199567 ATSDAT/ATTDT; malate transporter/ sodium:dicarboxylate symporter [Arabidopsis thaliana]	0.34603
SGN-U213111	ABB29939 major intrinsic protein 2-like [Solanum tuberosum]	0.39804
SGN-U217251	NP_179836 binding / transporter [Arabidopsis thaliana]	0.42533

Appendix Table C1. (Continued)

SGN-U219704	NP_922837 putative carnitine/acylcarnitine translocase [Oryza sativa (japonica cultivar-group)]	0.44132
SGN-U232089	P27056 Nonspecific lipid-transfer protein 1 precursor (LTP 1)	0.45163
SGN-U232997	CAA54046 H()-transporting ATPase [Solanum tuberosum]	0.4565
SGN-U214683	AAF98344 plasma membrane H+ATPase [Lycopersicon esculentum]	0.46645
SGN-U220938	ABB90028 ATP synthase CF1 alpha chain [Solanum tuberosum]	0.49849
	11. Secondary metabolism	
SGN-U213739	NP_171723 CER1 (ECERIFERUM 1) [Arabidopsis thaliana]	0.16783
SGN-U212843	P08196 Phytoene synthase 1, chloroplast precursor (Fruit ripening-specific protein pTOM5)	0.17895
SGN-U217447	AAO18664 wax synthase isoform 1 [Vitis vinifera]	0.19888
M84744	P08196 Phytoene synthase 1, chloroplast precursor (Fruit ripening-specific protein pTOM5)	0.2296
SGN-U212842	CAA47625 mutant phytoene synthase [Lycopersicon esculentum]	0.28184
SGN-U213861	XP_482840 glutamate decarboxylase [Oryza sativa (japonica cultivar-group)]	0.31341
SGN-U213371	XP_482984 putative flavonol synthase [Oryza sativa (japonica cultivar-group)]	0.33256
SGN-U213594	AAT90376 DWARF1/DIMINUTO [Lycopersicon esculentum]	0.35658
SGN-U222998	AAD38941 1-D-deoxyxylulose 5-phosphate synthase [Lycopersicon esculentum]	0.36895
SGN-U215117	CAA63056 NAD(P)H oxidoreductase, isoflavone reductase homologue [Solanum tuberosum]	0.39046
SGN-U215115	P52578 Isoflavone reductase homolog (CP100)	0.41804

Appendix Table C1. (Continued)

SGN-U232158	P08196 Phytoene synthase 1, chloroplast precursor (Fruit ripening-specific protein pTOM5)	0.42005
SGN-U214174	P31684 4-coumarate--CoA ligase 1 (4CL 1) (4-coumaroyl-CoA synthase 1)	0.4204
SGN-U214110	AAL16927 3-hydroxy-3-methylglutaryl CoA reductase [Lycopersicon esculentum]	0.42681
AF143812	AAD38941 1-D-deoxyxylulose 5-phosphate synthase [Lycopersicon esculentum]	0.43294
SGN-U218721	AAD38941 1-D-deoxyxylulose 5-phosphate synthase [Lycopersicon esculentum]	0.4493
SGN-U223568	CAA56554 geranylgeranyl diphosphate synthase [Capsicum annuum]	0.45554
SGN-U233769	ABB29850 carotene isomerase [Nicotiana langsdorffii x Nicotiana sanderae]	0.47013
SGN-U232476	ABA55732 ABA 8'-hydroxylase CYP707A1 [Solanum tuberosum]	0.47445
AF416727	Q8S4R4 Carotenoid isomerase, chloroplast precursor (CrtISO) (Protein tangerine)	0.47864
SGN-U242720	ABB82555 geranylgeranyl pyrophosphate synthase 2 [Lycopersicon esculentum]	0.48079
SGN-U218068	AAY41879 cinnamoyl-CoA reductase [Lycopersicon esculentum]	0.49887
	12. Cell wall	
SGN-U212774	BAE48664 Pectate lyase [Prunus mume]	0.0752
SGN-U212775	AAY85180 pectate lyase [Gossypium hirsutum]	0.08038
SGN-U213213	CAA32235 polygalacturonase [Lycopersicon esculentum]	0.18677
SGN-U215711	AAQ12264 expansin 1 protein; LeExp1 [Lycopersicon esculentum]	0.24103
SGN-U213186	CAA52703 pectin esterase [Lycopersicon esculentum]	0.31611
SGN-U213702	AAK97760 endo-beta-mannanase [Lycopersicon esculentum]	0.31635
SGN-U214121	CAA10175 ss-galactosidase [Lycopersicon esculentum]	0.32954

Appendix Table C1. (Continued)

SGN-U214120	AAC25984 beta-galactosidase [Lycopersicon esculentum]	0.36455
SGN-U214119	AAC25984 beta-galactosidase [Lycopersicon esculentum]	0.42666
SGN-U216984	AAB35284 arabinogalactan-protein; AGP [Nicotiana alata]	0.45511
SGN-U215153	AAG00902 xyloglucan endotransglycosylase LeXET2 [Lycopersicon esculentum]	0.49429
	13. Signal transduction	
SGN-U227339	NP_197640 protein binding / zinc ion binding [Arabidopsis thaliana]	0.21975
SGN-U221524	XP_480822 putative S-receptor kinase (EC 2.7.1.-) homolog 2 precursor [Oryza sativa (japonica cultivar-group)]	0.40762
SGN-U214100	AAQ72787 putative GTP-binding protein [Cucumis sativus]	0.44782
SGN-U232939	CAA49849 phosphoprotein phosphatase type 2A [Medicago sativa]	0.47852
	14. Pathogenesis--related	
SGN-U212990	BAD95797 similar to pathogenesis-related protein STH-2 [Lycopersicon esculentum]	0.24092
SGN-U224571	ABE85366 pathogenesis-related protein-like protein [Medicago truncatula]	0.25097
SGN-U224778	Q9S8M0 Chitin-binding lectin 1 precursor (PL-I)	0.283
SGN-U214985	CAA41439 pathogenesis-related protein P2 [Lycopersicon esculentum]	0.37555
	15. Stress	
SGN-U212929	AAA34193 ORF	0.4817
SGN-U214795	AAT01418 putative stress-responsive protein [Tamarix androssowii]	0.48682
SGN-U212930	AAA86424 heat shock protein	0.49796
SGN-U220751	AAV92895 Avr9/Cf-9 rapidly elicited protein 76 [Nicotiana tabacum]	0.49822
	16. Ethylene	

Appendix Table C1. (Continued)

SGN-U222201	AAC02213 ethylene receptor homolog [Lycopersicon esculentum]	0.29489
SGN-U216896	CAA41855 1-aminocyclopropane 1-carboxylate synthase [Lycopersicon esculentum]	0.31896
SGN-U220379	P29535 1-aminocyclopropane-1-carboxylate synthase 4 (ACC synthase 4) (S-adenosyl-L-methionine methylthioadenosine-lyase 4) (ACS-4) (Le-ACS4)	0.32735
SGN-U214919	AAK68076 1-aminocyclopropane-1-carboxylate oxidase [Solanum tuberosum]	0.35972
SGN-U232734	CAA90808 ethylene receptor [Solanum lycopersicum]	0.3673
SGN-U222410	AAR21569 putative ethylene receptor [Solanum tuberosum]	0.44717
SGN-U214004	AAU34075 ethylene receptor neverripe [Lycopersicon esculentum]	0.47103
	17. Others	
SGN-U240060	NP_199780 transferase, transferring glycosyl groups [Arabidopsis thaliana]	0.05705
SGN-U214670	S39507 glucuronosyl transferase homolog, ripening-related - tomato	0.0751
SGN-U213509	AAK29646 putative short-chain type alcohol dehydrogenase [Solanum tuberosum]	0.0755
SGN-U212764	CAA37333 alcohol dehydrogenase [Solanum tuberosum]	0.08049
SGN-U212765	CAA54450 alcohol dehydrogenase [Lycopersicon esculentum]	0.12195
SGN-U214258	NP_196094 amino acid binding [Arabidopsis thaliana]	0.12581
SGN-U213696	O04397 Ferredoxin--NADP reductase, root-type isozyme, chloroplast precursor (FNR)	0.12755
SGN-U218249	NP_566150 unknown protein [Arabidopsis thaliana]	0.15803
SGN-U212798	AAB71139 E8 protein homolog [Lycopersicon esculentum]	0.17089
SGN-U214037	AAG49032 ripening regulated protein DDTFR18 [Lycopersicon esculentum]	0.17776

Appendix Table C1. (Continued)

SGN-U214037	AAG49032 ripening regulated protein DDTFR18 [Lycopersicon esculentum]	0.17776
SGN-U214091	NP_197954 catalytic [Arabidopsis thaliana]	0.17901
SGN-U213423	ABA55732 ABA 8'-hydroxylase CYP707A1 [Solanum tuberosum]	0.17978
SGN-U212578	AAS48091 alcohol acyl transferase [Lycopersicon esculentum]	0.18704
SGN-U214044	BAC23045 monooxygenase [Solanum tuberosum]	0.1906
SGN-U214036	AAG49032 ripening regulated protein DDTFR18 [Lycopersicon esculentum]	0.19399
SGN-U214038	AAG49032 ripening regulated protein DDTFR18 [Lycopersicon esculentum]	0.20028
SGN-U215006	AAP37967 seed specific protein Bn15D1B [Brassica napus]	0.20183
SGN-U213424	ABA55732 ABA 8'-hydroxylase CYP707A1 [Solanum tuberosum]	0.20674
SGN-U222911	NP_922606 putative kinesin-related protein [Oryza sativa (japonica cultivar-group)]	0.20734
SGN-U213339	BAD33601 fibroin heavy chain precursor (Fib-H) (H-fibroin)-like protein [Oryza sativa (japonica cultivar-group)]	0.21065
SGN-U217797	CAA55813 Sn-2 [Capsicum annuum]	0.21438
SGN-U219932	NP_001050156 Os03g0360700 [Oryza sativa (japonica cultivar-group)]	0.21978
SGN-U213064	P68172 Adenosylhomocysteinase (S-adenosyl-L-homocysteine hydrolase) (AdoHcyase) (Cytokinin binding protein CBP57)	0.21979
SGN-U212885	AAP03875 putative chloroplast thiazole biosynthetic protein [Nicotiana tabacum]	0.23171
SGN-U214292	BAD17856 gibberellin 2-oxidase 2 [Nicotiana tabacum]	0.24186
SGN-U227535	AAL32440 SH3 domain-containing protein 3 [Arabidopsis thaliana]	0.24415

Appendix Table C1. (Continued)

SGN-U235284	YP_257531 thiamine biosynthesis protein ThiI [Pseudomonas fluorescens Pf-5]	0.2448
SGN-U234083	AAA34148 chlorophyll a/b-binding protein Cab-3C	0.24824
SGN-U234086	AAA34148 chlorophyll a/b-binding protein Cab-3C	0.25072
SGN-U212898	CAA50312 P450 hydroxylase [Solanum melongena]	0.25212
SGN-U232745	AAG49032 ripening regulated protein DDTFR18 [Lycopersicon esculentum]	0.2526
SGN-U220183	NP_195201 calmodulin binding [Arabidopsis thaliana]	0.25351
SGN-U214289	BAD17856 gibberellin 2-oxidase 2 [Nicotiana tabacum]	0.2565
SGN-U216838	AAM63491 putative kinesin light chain [Arabidopsis thaliana]	0.25748
SGN-U215211	BAB33412 putative senescence-associated protein [Pisum sativum]	0.26489
SGN-U215178	P12372 Photosystem I reaction center subunit II, chloroplast precursor	0.26541
SGN-U224073	NP_181190 ROS1 [Arabidopsis thaliana]	0.2708
SGN-U213282	AAO85557 photosystem I subunit XI [Nicotiana attenuata]	0.279
SGN-U236860	BAD14933 monodehydroascorbate reductase [Brassica oleracea]	0.28622
SGN-U212915	CAA44736 photosystem II 23 kDa protein [Lycopersicon esculentum]	0.2928
SGN-U225681	ABA54896 NBS1 [Arabidopsis thaliana]	0.29861
SGN-U219302	JQ0398 wun1 protein - potato	0.30869
SGN-U212863	P14278 Chlorophyll a-b binding protein 4, chloroplast precursor (LHCII type I CAB-4) (LHCP)	0.30952
SGN-U213372	BAE54520 anthocyanidin synthase [Spinacia oleracea]	0.31367
SGN-U218904	P07370 Chlorophyll a-b binding protein 1B, chloroplast precursor (LHCII type I CAB-1B) (LHCP)	0.31764
SGN-U221916	Q8LK56 Transcriptional activator DEMETER (DNA glycosylase-related protein	0.31919
SGN-U241613	AAX11684 perakine reductase [Rauvolfia serpentina]	0.31922

Appendix Table C1. (Continued)

SGN-U217123	NP_179899 CYP96A1; heme binding / iron ion binding / monooxygenase/ oxygen binding [Arabidopsis thaliana]	0.32156
SGN-U212753	P32111 Probable glutathione S-transferase (Pathogenesis-related protein 1)	0.33061
SGN-U213381	AAP03873 photosystem I reaction center subunit X psaK [Nicotiana tabacum]	0.3311
SGN-U214148	P37218 Histone H1	0.33525
SGN-U214689	AAM66054 ethylene-responsive protein, putative [Arabidopsis thaliana]	0.33557
SGN-U214576	No hits found	0.33575
SGN-U231815	NP_182185 ATP binding / ATPase/ nucleoside-triphosphatase/ nucleotide binding	0.33612
SGN-U226145	AAF79656 F5O11.10 [Arabidopsis thaliana]	0.33723
SGN-U214024	AAF75749 dehydration-induced protein ERD15 [Lycopersicon esculentum]	0.33865
M14444	AAA34148 chlorophyll a/b-binding protein Cab-3C	0.34122
SGN-U218844	BAD29526 dehydration-responsive family protein-like [Oryza sativa (japonica cultivar-group)]	0.34211
SGN-U215966	NP_180337 CYP94C1; heme binding / iron ion binding / monooxygenase/ oxygen binding [Arabidopsis thaliana]	0.34347
SGN-U222502	BAB85644 dynamin like protein 2a [Arabidopsis thaliana]	0.34456
BT012820	AAK62346 elicitor-inducible cytochrome P450 [Nicotiana tabacum]	0.34689
SGN-U214992	Q43512 Metallothionein-like protein type 2	0.34979
SGN-U213568	XP_464429 putative thioredoxin peroxidase [Oryza sativa (japonica cultivar-group)]	0.35649
SGN-U212897	CAA50312 P450 hydroxylase [Solanum melongena]	0.35976
SGN-U215479	CAA76346 putative arginine/serine-rich splicing factor [Medicago sativa subsp. x varia]	0.35977
SGN-U218911	P07369 Chlorophyll a-b binding protein 3C, chloroplast precursor (LHCII	0.36112

Appendix Table C1. (Continued)

SGN-U213374	AAN14410 bifunctional lysine-ketoglutarate reductase/saccharopine dehydrogenase [Gossypium hirsutum]	0.36444
SGN-U221489	AAK69274 unknown [Glycine max]	0.36583
SGN-U218912	P07369 Chlorophyll a-b binding protein 3C, chloroplast precursor (LHCII type I CAB-3C) (LHCP)	0.36995
SGN-U215124	AAS58469 ultraviolet-B-repressible protein [Gossypium hirsutum]	0.37054
SGN-U212938	CAA42818 LHCII type III [Lycopersicon esculentum]	0.37134
SGN-U212805	AAP03872 putative photosystem I subunit III precursor [Nicotiana tabacum]	0.37391
SGN-U213223	CAA32197 chlorophyll a/b-binding protein [Lycopersicon esculentum]	0.37676
SGN-U216140	NP_565524 SEP2 (STRESS ENHANCED PROTEIN 2) [Arabidopsis thaliana]	0.37921
SGN-U212985	AAT39459 protein disulfide isomerase [Ipomoea batatas]	0.3846
SGN-U219607	AAX96261 PB1 domain, putative [Oryza sativa (japonica cultivar-group)]	0.38573
SGN-U232086	P27524 Chlorophyll a-b binding protein CP24 10A, chloroplast precursor	0.38733
SGN-U213440	NP_179568 protein binding [Arabidopsis thaliana]	0.39428
SGN-U214730	NP_567276 ATALN; allantoinase/ hydrolase [Arabidopsis thaliana]	0.39473
SGN-U214274	BAD46080 molybdenum cofactor sulfurase protein -like [Oryza sativa (japonica cultivar-group)]	0.40107
SGN-U225708	NP_565167 unknown protein [Arabidopsis thaliana]	0.40186
SGN-U234591	AAL13436 anaphase promoting complex subunit 11 [Arabidopsis thaliana]	0.40291
SGN-U218195	CAK22419 B12D-like protein [Beta vulgaris]	0.40672
SGN-U236449	ABE91595 Arf GTPase activating protein [Medicago truncatula]	0.40765

Appendix Table C1. (Continued)

SGN-U228384	AAK52092 WD-40 repeat protein [Lycopersicon esculentum]	0.41025
SGN-U218110	NP_194650 gamma-glutamyltransferase [Arabidopsis thaliana]	0.41056
SGN-U215282	ABE93077 2OG-Fe(II) oxygenase [Medicago truncatula]	0.41204
SGN-U214232	AAP37970 seed specific protein Bn15D17A [Brassica napus]	0.41378
SGN-U242862	YP_874735 photosystem I P700 apoprotein A2 [Agrostis stolonifera]	0.41857
SGN-U213688	CAC14888 putative extensin [Nicotiana sylvestris]	0.41916
SGN-U232863	AAB97152 Mg protoporphyrin IX chelatase [Nicotiana tabacum]	0.42294
SGN-U218320	BAD15365 nitrite reductase [Nicotiana tabacum]	0.42411
SGN-U213892	AAW02789 aluminum-induced protein [Codonopsis lanceolata]	0.42487
SGN-U213262	AAU03361 photosystem II oxygen-evolving complex protein 3 [Lycopersicon esculentum]	0.42529
SGN-U217229	CAC87643 alcohol oxidase [Arabidopsis thaliana]	0.42628
SGN-U218102	NP_974452 catalytic [Arabidopsis thaliana]	0.42679
SGN-U214550	NP_199516 ATP binding / shikimate kinase [Arabidopsis thaliana]	0.43393
SGN-U216932	NP_190882 O-methyltransferase [Arabidopsis thaliana]	0.4363
SGN-U213411	AAR92031 cystathionine gamma synthase [Lycopersicon esculentum]	0.43845
SGN-U216849	AAP06759 auxin response factor-like protein [Mangifera indica]	0.43859
SGN-U213395	CAA59409 protein of photosystem II [Spinacia oleracea]	0.43868
SGN-U222937	ABC69711 auxin response factor 2 [Lycopersicon esculentum]	0.43903
SGN-U212547	CAA74359 ferredoxin--NADP() reductase [Nicotiana tabacum]	0.44067
SGN-U238084	P06290 ATP synthase B chain (ATPase subunit I)	0.44398

Appendix Table C1. (Continued)

SGN-U222296	NP_187168 catalytic [Arabidopsis thaliana]	0.44821
SGN-U214329	AAB97152 Mg protoporphyrin IX chelatase [Nicotiana tabacum]	0.44916
SGN-U221852	BAD15364 nitrite reductase [Nicotiana tabacum]	0.45273
SGN-U212812	BAD42919 similar to senescence-associated protein [Arabidopsis thaliana]	0.45426
SGN-U214185	AAM64572 gda-1, putative [Arabidopsis thaliana]	0.45433
SGN-U213598	CAA75558 chloroplast drought-induced stress protein, 34 kD) [Solanum tuberosum]	0.45453
SGN-U220629	AAR14273 predicted protein [Populus alba x Populus tremula]	0.45459
SGN-U219802	NP_001048806 Os03g0123600 [Oryza sativa (japonica cultivar-group)]	0.45767
SGN-U212700	BAA77604 plastidic aldolase NPALDP1 [Nicotiana paniculata]	0.45851
SGN-U231837	CAA55143 pyruvate,orthophosphate dikinase [Mesembryanthemum crystallinum]	0.46049
AF126021	NP_009204 prohibitin 2 [Homo sapiens]	0.46161
SGN-U217836	NP_916297 P0665A11.10 [Oryza sativa (japonica cultivar-group)]	0.46343
SGN-U212937	CAA42818 LHCII type III [Lycopersicon esculentum]	0.47367
SGN-U213292	NP_001066887 Os12g0515600 [Oryza sativa (japonica cultivar-group)]	0.47525
SGN-U215881	BAB44155 hydroxypyruvate reductase [Bruguiera gymnorrhiza]	0.47694
SGN-U224187	BAD54110 auxin-induced protein-like [Oryza sativa (japonica cultivar-group)]	0.47768
SGN-U224830	AAL87345 putative chloroplast nucleoid DNA-binding protein [Arabidopsis thaliana]	0.47799
SGN-U217525	NP_564022 catalytic/ hydrolase [Arabidopsis thaliana]	0.47803
SGN-U233653	NP_190882 O-methyltransferase [Arabidopsis thaliana]	0.4796

Appendix Table C1. (Continued)

SGN-U212733	P27524 Chlorophyll a-b binding protein CP24 10A, chloroplast precursor	0.47965
SGN-U215810	XP_449995 cyclase-like protein [Oryza sativa (japonica cultivar-group)]	0.48042
SGN-U223227	XP_470337 putative cytochrome P450 [Oryza sativa (japonica cultivar-group)]	0.48042
SGN-U215144	AAB65163 glutathione S-transferase, class-phi [Solanum commersonii]	0.48727
SGN-U225740	AAS02074 auxin and ethylene responsive GH3-like protein [Capsicum chinense]	0.48745
SGN-U215654	NP_181672 calcium ion binding [Arabidopsis thaliana]	0.48778
SGN-U213768	1407140B acetolactate synthase SuRB	0.4904
SGN-U225200	BAD87450 integral membrane protein-like [Oryza sativa (japonica cultivar-group)]	0.49385
X57709	CAD21956 neomycin phosphotransferase [Hepatitis GB virus B]	0.4939
SGN-U214278	YP_635638 photosystem I P700 apoprotein A2 [Solanum tuberosum]	0.49443
SGN-U212951	AAB93772 SRE1b [Solanum tuberosum]	0.49794
	18. Unknown	
SGN-U214282	AAT76982 protein of unknown function [Oryza sativa (japonica cultivar-group)]	0.11375
SGN-U214690	NP_850563 unknown protein [Arabidopsis thaliana]	0.28699
SGN-U218406	NP_195300 unknown protein [Arabidopsis thaliana]	0.31351
SGN-U223249	NP_192765 unknown protein [Arabidopsis thaliana]	0.33494
SGN-U224635	NP_190080 unknown protein [Arabidopsis thaliana]	0.36077
SGN-U222728	ABE87128 Protein of unknown function DUF581 [Medicago truncatula]	0.36878
SGN-U215475	NP_198805 unknown protein [Arabidopsis thaliana]	0.39449
SGN-U218003	CAB78866 putative protein (fragment) [Arabidopsis thaliana]	0.4019
SGN-U215002	NP_565167 unknown protein [Arabidopsis thaliana]	0.42158

Appendix Table C1. (Continued)

SGN-U225777	NP_200205 unknown protein [Arabidopsis thaliana]	0.42596
SGN-U220997	NP_194558 unknown protein [Arabidopsis thaliana]	0.42702
SGN-U238513	NP_181371 unknown protein [Arabidopsis thaliana]	0.43524
SGN-U216324	NP_198805 unknown protein [Arabidopsis thaliana]	0.46562
SGN-U214076	ABE87525 Protein of unknown function XH; Protein of unknown function XS;	0.47018
SGN-U218848	NP_568828 unknown protein [Arabidopsis thaliana]	0.47401
SGN-U216535	BAB03164 unnamed protein product [Arabidopsis thaliana]	0.49248
SGN-U215736	NP_001060517 Os07g0658100 [Oryza sativa (japonica cultivar-group)]	0.49257
	19. No hits	
SGN-U240396	No hits found	0.18928
SGN-U225964	No hits found	0.31391
SGN-U221141	No hits found	0.3434
SGN-U230465	No hits found	0.35273
SGN-U222458	No hits found	0.39706
SGN-U224913	No hits found	0.46007
SGN-U241529	No hits found	0.46589
SGN-U217116	No hits found	0.46882

Appendix Table C2. List of genes differentially expressed (more than 2 folds) in tomato fruit stored at 3 °C for 4 weeks, then ripened at 20 °C for 7 d compared with fruit ripened at 20 °C for 7 d without chilling obtained from microarray analysis and categorized by putative gene function.

Unigene	Genebank best hit	Expression ratio (Log2)
	>2 fold up-regulated	
	1. Transcriptional regulation	
SGN-U235872	AAF17682 F28K19.13 [Arabidopsis thaliana]	2.85109
SGN-U217359	AAD46402 ethylene-responsive transcriptional coactivator [Lycopersicon esculentum]	2.81029
SGN-U219135	AAK95688 ethylene-responsive factor 1 [Lycopersicon esculentum]	2.61658
SGN-U240026	ABF69979 transcriptional factor B3 family protein [Musa acuminata]	2.61601
SGN-U217210	NP_567875 transcription factor [Arabidopsis thaliana]	2.3813
SGN-U218511	CAA67368 TFIIA [Arabidopsis thaliana]	2.26733
SGN-U221734	P27484 Glycine-rich protein 2	2.152
SGN-U213502	P41379 Eukaryotic initiation factor 4A-2 (ATP-dependent RNA helicase	2.10588
SGN-U216769	NP_189461 PMZ [Arabidopsis thaliana]	2.03733
SGN-U233285	Q6V9I6 Histone deacetylase 2a (HD2a) (ScHD2a)	2.03011
	2. RNA processing	
SGN-U216265	BAB41076 MAR-binding protein [Nicotiana tabacum]	2.89779
SGN-U217217	NP_176007 NOP56 [Arabidopsis thaliana]	2.44733
SGN-U213732	NP_566199 DCL2; ATP binding / ATP-dependent helicase/ RNA binding / double-stranded RNA binding / helicase/ nucleic acid binding / ribonuclease III [Arabidopsis thaliana]	2.25592

Appendix Table C2. (Continued)

	3. Protein synthesis	
SGN-U212847	BAA09709 elongation factor-1 alpha [Nicotiana tabacum]	19.16812
SGN-U213207	ABB29933 P0 ribosomal protein-like [Solanum tuberosum]	3.64235
SGN-U215714	AAT38711 Ribosomal protein L34e [Solanum demissum]	3.47577
SGN-U214078	AAD13388 ribosomal protein L27a [Petunia x hybrida]	3.39717
SGN-U214286	NP_850328 structural constituent of ribosome [Arabidopsis thaliana]	3.13975
SGN-U225523	ABB17004 ribosomal protein S7-like protein [Solanum tuberosum]	3.1027
SGN-U215061	NP_194847 ATP binding / aspartate-tRNA ligase/ nucleic acid binding / tRNA ligase [Arabidopsis thaliana]	2.99043
SGN-U213040	AAR17783 ribosomal protein L3 [Lycopersicon esculentum]	2.8923
SGN-U213556	1909359A ribosomal protein S19	2.87553
SGN-U214092	P46301 40S ribosomal protein S25	2.79729
SGN-U232889	CAA05365 high mobility group protein [Solanum tuberosum]	2.74775
SGN-U212918	CAC27136 40S ribosomal protein S2 [Picea abies]	2.7402
SGN-U214558	AAD32206 60S ribosomal protein L1 [Prunus armeniaca]	2.70932
SGN-U213998	CAA49175 ribosomal protein YL16 [Mesembryanthemum crystallinum]	2.64894
SGN-U212838	CAA37212 elongation factor 1-alpha [Lycopersicon esculentum]	2.64713
SGN-U225530	AAL36888 NADH-glutamate dehydrogenase [Lycopersicon esculentum]	2.62132
SGN-U213093	NP_849818 LOS1; GTP binding / translation elongation factor/ translation factor, nucleic acid binding [Arabidopsis thaliana]	2.53507
SGN-U213175	AAK25759 ribosomal protein L18a [Castanea sativa]	2.51285
SGN-U213045	ABB86269 40S ribosomal protein S15-like [Solanum tuberosum]	2.48439
SGN-U213260	AAK25760 ribosomal protein L33 [Castanea sativa]	2.46476

Appendix Table C2. (Continued)

SGN-U213258	AAT40505 Elongation factor 1-beta' , putative [Solanum demissum]	2.44423
SGN-U213468	NP_564417 structural constituent of ribosome [Arabidopsis thaliana]	2.41255
SGN-U216442	AAM64816 Ribosomal protein L7Ae-like [Arabidopsis thaliana]	2.3979
SGN-U217764	ABB29934 acidic ribosomal protein P1a-like [Solanum tuberosum]	2.37706
SGN-U212719	CAA54095 ribosomal protein S4 [Solanum tuberosum]	2.36102
SGN-U213094	CAC12818 elongation factor 2 [Nicotiana tabacum]	2.35962
SGN-U215361	CAA72721 PRT1 protein [Nicotiana tabacum]	2.35037
SGN-U213502	P41379 Eukaryotic initiation factor 4A-2 (ATP-dependent RNA helicase	2.3484
SGN-U220355	NP_001046260 Os02g0208600 [Oryza sativa (japonica cultivar-group)]	2.34564
SGN-U213502	P41379 Eukaryotic initiation factor 4A-2 (ATP-dependent RNA helicase	2.33947
SGN-U212837	AAR83865 elongation factor 1-alpha [Capsicum annuum]	2.3299
SGN-U212651	P93847 60S ribosomal protein L10 (EQM)	2.32531
SGN-U219782	AAP80667 ribosomal Pr 117 [Triticum aestivum]	2.31411
SGN-U212996	ABB87108 putative elongation factor 1-gamma-like [Solanum tuberosum]	2.29463
SGN-U213265	ABA40469 60S ribosomal protein L21-like protein [Solanum tuberosum]	2.27425
SGN-U213294	AAQ96375 60S ribosomal protein L13 [Solanum brevidens]	2.26842
SGN-U213379	AAD47346 ribosomal protein S26 [Pisum sativum]	2.24688
SGN-U213097	ABB72816 ribosomal protein L24-like protein [Solanum tuberosum]	2.24306
SGN-U214017	AAL09401 ribosomal protein [Petunia x hybrida]	2.22259
SGN-U213309	CAI48073 60S ribosomal protein L37a [Capsicum chinense]	2.20103

Appendix Table C2. (Continued)

SGN-U216648	ABE87988 ribosomal protein S3 [Medicago truncatula]	2.1805
SGN-U213067	ABB29931 P40-like protein [Solanum tuberosum]	2.1778
SGN-U218050	AAK49947 TGF-beta receptor-interacting protein 1 [Phaseolus vulgaris]	2.16315
SGN-U213293	AAQ96375 60S ribosomal protein L13 [Solanum brevidens]	2.14125
SGN-U213562	NP_850665 structural constituent of ribosome [Arabidopsis thaliana]	2.13209
SGN-U213730	ABB55361 40S ribosomal protein S10-like [Solanum tuberosum]	2.12101
SGN-U214018	AAL09401 ribosomal protein [Petunia x hybrida]	2.12014
SGN-U213298	ABA46790 60S ribosomal protein L13a-like protein [Solanum tuberosum]	2.11382
SGN-U214373	AAG53649 eukaryotic translation initiation factor 5A-3 [Lycopersicon esculentum]	2.10942
SGN-U213827	ABA40434 60s acidic ribosomal protein-like protein [Solanum tuberosum]	2.10532
SGN-U214582	ABB02632 ribosomal protein L38-like [Solanum tuberosum]	2.10237
SGN-U214063	BAA96367 ribosomal protein L27 [Panax ginseng]	2.0834
SGN-U215780	BAD95794 similar to 60S ribosomal protein L35 [Lycopersicon esculentum]	2.0697
SGN-U214203	ABB29934 acidic ribosomal protein P1a-like [Solanum tuberosum]	2.05541
SGN-U213174	AAK25759 ribosomal protein L18a [Castanea sativa]	2.04328
SGN-U213169	AAP80667 ribosomal Pr 117 [Triticum aestivum]	2.03479
SGN-U212967	BAD28853 putative ribosomal protein L10a [Oryza sativa (japonica cultivar-group)]	2.0312
SGN-U212722	CAA54095 ribosomal protein S4 [Solanum tuberosum]	2.02855
SGN-U213152	AAU93594 putative ribosomal protein [Solanum demissum]	2.02848

Appendix Table C2. (Continued)

SGN-U214093	NP_179752 structural constituent of ribosome [Arabidopsis thaliana]	2.02678
SGN-U214742	ABB72809 60S ribosomal protein L7A-like protein [Solanum tuberosum]	2.00385
	5. Protein modification	
SGN-U215175	AAK83088 Pin1-type peptidyl-prolyl cis/trans isomerase [Malus x domestica]	2.092
SGN-U218135	NP_564750 CW14 [Arabidopsis thaliana]	2.02344
	2.4 Protein degradation	
SGN-U214535	BAD33488 putative ubiquitin conjugating enzyme 7 interacting protein 4 [Oryza sativa (japonica cultivar-group)]	3.32415
SGN-U216576	AAC19402 26S proteasome regulatory subunit S5A [Mesembryanthemum crystallinum]	2.99885
SGN-U212678	CAA77735 ubiquitin monomer/ribosomal protein [Solanum tuberosum]	2.80396
SGN-U214642	ABA40428 26S proteasome AAA-ATPase subunit RPT4a-like protein [Solanum tuberosum]	2.56525
SGN-U214791	AAZ20285 ubiquitin fusion protein [Arachis hypogaea]	2.56042
SGN-U232043	ABA46785 ubiquitin extension protein-like protein [Solanum tuberosum]	2.54384
SGN-U213406	CAA05894 CYP1 [Lycopersicon esculentum]	2.47177
SGN-U215388	XP_470419 putative proteasome regulatory non-ATPase subunit [Oryza sativa (japonica cultivar-group)]	2.29217
SGN-U215184	CAA57510 cyprosin [Cynara cardunculus]	2.28975
SGN-U215765	NP_176633 peptidase [Arabidopsis thaliana]	2.08365
SGN-U229808	BAC43404 putative aspartyl aminopeptidase [Arabidopsis thaliana]	2.04256
	6. Nucleotide metabolism	
SGN-U213330	AAZ85394 cytosolic nucleoside diphosphate kinase [Solanum chacoense]	3.48658
	7. Amino acid metabolism	

Appendix Table C2. (Continued)

	-	
	8. Carbohydrate metabolism	
SGN-U213118	AAO34668 sucrose synthase 2 [Solanum tuberosum]	5.55049
SGN-U221214	AAK84008 beta-amylase PCT-BMYI [Solanum tuberosum]	2.60041
SGN-U217037	CAB39974 glyceraldehyde-3-phosphate dehydrogenase [Nicotiana tabacum]	2.37846
SGN-U212825	CAA41115 enolase [Lycopersicon esculentum]	2.2409
SGN-U231265	NP_178071 NAD binding / glyceraldehyde-3-phosphate dehydrogenase	2.05992
	9. Lipid metabolism	
SGN-U215025	AAL54884 cytochrome P450-dependent fatty acid hydroxylase [Nicotiana tabacum]	7.33638
SGN-U214192	AAT72296 microsomal omega-6-desaturase [Nicotiana tabacum]	2.87813
	10. Transporters	
SGN-U214604	AAM47580 putative ABC-transporter-like protein [Sorghum bicolor]	6.49124
SGN-U214603	NP_001051311 Os03g0755100 [Oryza sativa (japonica cultivar-group)]	2.93874
SGN-U212911	ABB29945 ADP/ATP translocator-like [Solanum tuberosum]	2.58784
SGN-U218028	NP_195030 electron transporter/ thiol-disulfide exchange intermediate [Arabidopsis thaliana]	2.40408
SGN-U215374	CAA56599 34 kDA porin [Solanum tuberosum]	2.25985
SGN-U212977	P38547 GTP-binding nuclear protein Ran2	2.22398
SGN-U231000	NP_182175 importin-alpha export receptor/ protein transporter [Arabidopsis	2.10565
	11. Secondary metabolism	
SGN-U221412	BAD77944 UDP-glucuronic acid:anthocyanin glucuronosyltransferase [Bellis perennis]	6.86325

Appendix Table C2. (Continued)

SGN-U213123	CAI39242 arginine decarboxylase [Lycopersicon esculentum]	4.32959
SGN-U232334	AAZ85221 arginine decarboxylase [Lycopersicon pimpinellifolium]	3.61401
L16582	CAB64599 arginine decarboxylase 1 [Datura stramonium]	2.57623
SGN-U213084	Q96471 S-adenosylmethionine decarboxylase proenzyme (AdoMetDC) (SamDC) [Contains: S-adenosylmethionine decarboxylase alpha chain; S-adenosylmethionine decarboxylase beta chain]	2.55003
	12. Cell wall	
	-	
	13. Signal transduction	
	-	
	14. Pathogenesis-related	
SGN-U214259	CAA50596 PR-1a1 [Lycopersicon esculentum]	14.43462
SGN-U213451	AAC06244 basic PR-1 protein precursor [Capsicum annuum]	11.18276
SGN-U212884	AAF25602 class I chitinase [Solanum tuberosum]	7.51013
SGN-U217204	AAG43551 Avr9/Cf-9 rapidly elicited protein 146 [Nicotiana tabacum]	6.80935
SGN-U213791	Q05540 Acidic 27 kDa endochitinase precursor	4.98906
SGN-U213935	AAP43673 PR5-like protein [Lycopersicon esculentum]	2.99863
SGN-U212881	Q05538 Basic 30 kDa endochitinase precursor	2.6875
SGN-U219566	XP_450774 putative Avr9/Cf-9 rapidly elicited protein 276 [Oryza sativa (japonica cultivar-group)]	2.05124
	15. Stress	
SGN-U215254	CAA42660 luminal binding protein (BiP) [Nicotiana tabacum]	2.84385
SGN-U216469	AAC14577 class II small heat shock protein Le-HSP17.6 [Lycopersicon	4.33238
AJ225049	O82013 17.3 kDa class II heat shock protein (Hsp17.3) (Hsp20.2)	3.76146

Appendix Table C2. (Continued)

SGN-U228034	CAA37971 heat shock protein cognate 70 [Lycopersicon esculentum]	3.57787
SGN-U212639	AAR12195 molecular chaperone Hsp90-1 [Lycopersicon esculentum]	2.60574
SGN-U213773	AAX13276 USP family protein [Triticum aestivum]	2.43256
SGN-U213202	AAS57912 70 kDa heat shock cognate protein 1 [Vigna radiata]	2.38285
SGN-U212696	BAA32547 mitochondrial small heat shock protein [Solanum lycopersicum]	2.37048
SGN-U212643	AAR12195 molecular chaperone Hsp90-1 [Lycopersicon esculentum]	2.32226
X54029	P24629 Heat shock cognate 70 kDa protein 1	2.138
	16. Ethylene	
	-	
	17. Others	
SGN-U212665	AAC14412 calcium dependent protein kinase [Arabidopsis thaliana]	7.86074
SGN-U213431	AAA33106 cytochrome P-450 protein [Catharanthus roseus]	7.16342
SGN-U220325	AAC78508 putative phloem-specific lectin [Arabidopsis thaliana]	7.07268
SGN-U215855	AAX84672 aldo/keto reductase AKR [Manihot esculenta]	6.22005
SGN-U215827	ABB02383 temperature-induced lipocalin' [Lycopersicon esculentum]	5.42295
SGN-U216139	BAD93797 integral membrane protein -like [Arabidopsis thaliana]	4.46152
SGN-U216028	CAB61840 putative glycine and proline-rich protein [Sporobolus stapfianus]	4.15581
SGN-U219935	NP_180763 N-acetyltransferase [Arabidopsis thaliana]	3.74581
SGN-U213010	ABE77454 Phenazine biosynthesis PhzC/PhzF protein [Medicago truncatula]	3.38736
SGN-U213011	NP_192195 catalytic [Arabidopsis thaliana]	3.3718

Appendix Table C2. (Continued)

SGN-U217225	CAD56690 proliferating cell nuclear antigen [Solanum lycopersicum]	3.22681
SGN-U212756	AAG16757 putative glutathione S-transferase T2 [Lycopersicon esculentum]	3.17651
SGN-U227544	NP_200485 histone acetyltransferase [Arabidopsis thaliana]	3.16814
SGN-U218950	Q8SAG3 Actin-depolymerizing factor (ADF)	3.11984
SGN-U213948	AAS98165 hypersensitive-induced reaction protein [Capsicum annuum]	3.11909
SGN-U233072	ABF59516 putative spindle disassembly related protein CDC48 [Nicotiana	3.04071
SGN-U213934	AAU95238 osmotin-like protein [Solanum phureja]	3.00076
SGN-U226077	NP_001065462 Os10g0572300 [Oryza sativa (japonica cultivar-group)]	2.99569
SGN-U227453	ABE94188 Helix-loop-helix DNA-binding [Medicago truncatula]	2.98003
SGN-U221629	NP_568580 catalytic [Arabidopsis thaliana]	2.8686
SGN-U218015	NP_181910 UDP-glycosyltransferase/ transferase, transferring glycosyl groups / transferase, transferring hexosyl groups [Arabidopsis thaliana]	2.66194
SGN-U212687	P30264 Catalase isozyme 1	2.64065
SGN-U223576	CAA85426 catalase [Nicotiana plumbaginifolia]	2.61232
SGN-U213877	BAA76896 LeArcA2 protein [Lycopersicon esculentum]	2.58512
SGN-U213506	BAA76895 LeArcA1 protein [Lycopersicon esculentum]	2.56017
SGN-U219896	AAA33106 cytochrome P-450 protein [Catharanthus roseus]	2.46997
SGN-U213881	ABC69414 CYP72A57 [Nicotiana tabacum]	2.45975
SGN-U216642	CAI53895 putative receptor associated protein [Capsicum chinense]	2.45012
SGN-U223260	NP_001050723 Os03g0636700 [Oryza sativa (japonica cultivar-group)]	2.43754
SGN-U217723	NP_195566 monooxygenase [Arabidopsis thaliana]	2.42368

Appendix Table C2. (Continued)

SGN-U213843	ABB29936 deacetylase-like protein [Solanum tuberosum]	2.42281
SGN-U214235	NP_172363 DNA binding [Arabidopsis thaliana]	2.27556
SGN-U222659	CAA55812 Sn-1 [Capsicum annuum]	2.20912
SGN-U213828	CAD58675 putative peroxisomal membrane protein PEX11-1 [Arabidopsis thaliana]	2.15742
SGN-U215032	BAD33248 mitochondrial glycoprotein-like [Oryza sativa (japonica cultivar-group)]	2.14718
SGN-U213072	CAB85628 putative ripening-related protein [Vitis vinifera]	2.12676
SGN-U213660	CAI44933 N-rich protein [Glycine max]	2.12344
SGN-U213743	XP_465491 endosperm specific protein-like [Oryza sativa (japonica cultivar-group)]	2.06342
SGN-U221531	AAC18566 2,4-D inducible glutathione S-transferase [Glycine max]	2.06159
SGN-U223136	P30173 Actin-101	2.0556
	18. Unknown	
SGN-U213689	XP_470153 unknown protein [Oryza sativa]	4.5991
SGN-U229052	AAQ62582 unknown [Glycine max]	4.32678
SGN-U214998	XP_476340 unknown protein [Oryza sativa (japonica cultivar-group)]	3.57246
SGN-U217907	NP_198681 unknown protein [Arabidopsis thaliana]	3.48512
SGN-U242761	AAX27911 unknown [Schistosoma japonicum]	2.96331
SGN-U219293	AAM65077 unknown [Arabidopsis thaliana]	2.92409
SGN-U213387	ABB29921 unknown [Solanum tuberosum]	2.469
SGN-U220091	NP_973399 unknown protein [Arabidopsis thaliana]	2.23352
SGN-U221418	AAK19054 UVI1 [Pisum sativum]	2.20122
SGN-U213989	ABA81865 unknown [Solanum tuberosum]	2.19956
SGN-U215417	NP_850751 unknown protein [Arabidopsis thaliana]	2.07411
SGN-U231790	AAM13859 unknown protein [Arabidopsis thaliana]	2.07084
SGN-U218943	XP_550386 putative BRI1-KD interacting protein 128 [Oryza sativa (japonica cultivar-group)]	2.00162
	19. No hits found	
SGN-U212829	No hits found	9.25524

Appendix Table C2. (Continued)

SGN-U214357	No hits found	4.01964
	>2 fold down-regulated	
	1. Transcriptional regulation	
SGN-U227525	AAM34774 nam-like protein 11 [Petunia x hybrida]	0.18243
SGN-U213417	CAA64417 homeobox [Lycopersicon esculentum]	0.2825
SGN-U216213	AAU43923 NAC domain protein [Lycopersicon esculentum]	0.32154
SGN-U227950	NP_190996 ATP binding / ATP-dependent helicase/ DNA binding / helicase/ nucleic	0.33261
SGN-U231815	NP_182185 ATP binding / ATPase/ nucleoside-triphosphatase/ nucleotide binding	0.33497
SGN-U238731	NP_176107 transcription factor [Arabidopsis thaliana]	0.35263
SGN-U212614	AAZ83588 MADS-box transcription factor [Lycopersicon esculentum]	0.39889
SGN-U215261	AAT76423 putative HSF-type DNA-binding protein [Oryza sativa (japonica cultivar-group)]	0.42082
SGN-U213659	AAM33098 TDR4 transcription factor [Lycopersicon esculentum]	0.44822
SGN-U232621	CAB67118 homeodomain protein [Solanum lycopersicum]	0.46217
SGN-U214549	AAF22139 MADS box protein [Capsicum annuum]	0.48791
SGN-U213644	AAK95687 transcription factor JERF1 [Lycopersicon esculentum]	0.49599
	2. RNA processing	
SGN-U215479	CAA76346 putative arginine/serine-rich splicing factor [Medicago sativa subsp. x varia]	0.38594
	3. Protein synthesis	
SGN-U235037	Q42948 Dihydrodipicolinate synthase, chloroplast precursor (DHDPS)	0.35388
SGN-U213374	AAN14410 bifunctional lysine-ketoglutarate reductase/saccharopine dehydrogenase [Gossypium hirsutum]	0.43531

Appendix Table C2. (Continued)

	4. Protein modification	
SGN-U232483	AAF86506 CBL-interacting protein kinase 2 [Arabidopsis thaliana]	0.40302
SGN-U215938	CAA57872 pyruvate,orthophosphate dikinase [Mesembryanthemum crystallinum]	0.43833
SGN-U218526	XP_463364 protein phosphatase 2C-like protein [Oryza sativa (japonica cultivar-group)]	0.46913
SGN-U216432	AAO32318 phosphoenolpyruvate carboxylase kinase 2 [Lycopersicon esculentum]	0.47901
SGN-U218864	BAF44192 SNF1-related kinase [Solanum lycopersicum]	0.49265
SGN-U214714	NP_566580 CIPK1 (CBL-INTERACTING PROTEIN KINASE 1); ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase [Arabidopsis thaliana]	0.49605
SGN-U241632	CAE55203 protein kinase 1 [Nicotiana tabacum]	0.49848
	5. Protein degradation	
SGN-U223542	AAP85546 putative RING-H2 zinc finger protein [Oryza sativa (japonica cultivar-group)]	0.30087
SGN-U233700	AAL05932 SKIP5-like protein [Lycopersicon esculentum]	0.30484
SGN-U222566	NP_181121 SCPL26; serine carboxypeptidase [Arabidopsis thaliana]	0.40521
SGN-U215505	BAD81083 putative COP9 signalosome complex subunit 2 [Oryza sativa (japonica cultivar-group)]	0.49158
SGN-U217032	AAO45753 RING/C3HC4/PHD zinc finger-like protein [Cucumis melo]	0.49897
	6. Nucleotide metabolism	
	-	
	7. Amino acid metabolism	
SGN-U212594	P54772 Histidine decarboxylase (HDC) (TOM92)	0.20249
SGN-U212615	CAA50719 histidine decarboxylase [Lycopersicon esculentum]	0.25527

Appendix Table C2. (Continued)

SGN-U213373	AAN14410 bifunctional lysine-ketoglutarate reductase/saccharopine dehydrogenase [Gossypium hirsutum]	0.44344
	8. Carbohydrate metabolism	
SGN-U214669	S39507 glucuronosyl transferase homolog, ripening-related - tomato (fragment)	0.18789
SGN-U212902	CAA78063 beta-fructofuranosidase; vaculolar invertase [Lycopersicon pimpinellifolium]	0.2181
SGN-U214670	S39507 glucuronosyl transferase homolog, ripening-related - tomato	0.25051
SGN-U212903	AAL75450 minor allergen beta-fructofuranosidase precursor [Lycopersicon	0.2681
SGN-U212901	CAA78063 beta-fructofuranosidase; vaculolar invertase [Lycopersicon pimpinellifolium]	0.30737
SGN-U212899	P29000 Acid beta-fructofuranosidase precursor (Acid sucrose hydrolase) (Acid	0.33535
SGN-U221226	NP_849285 transferase, transferring glycosyl groups / transferase, transferring hexosyl groups [Arabidopsis thaliana]	0.41101
SGN-U230638	AAX33233 plastid alpha-amylase [Actinidia chinensis]	0.43546
	9. Lipid metabolism	
SGN-U216734	ABA93963 GDSL-like Lipase/Acylhydrolase [Oryza sativa (japonica cultivar-group)]	0.23985
SGN-U215592	AAL54886 cytochrome P450-dependent fatty acid hydroxylase [Nicotiana tabacum]	0.24917
SGN-U214849	AAB65766 lipoxygenase	0.29596
SGN-U234711	CAA65268 13-lipoxygenase [Solanum tuberosum]	0.35688
U37839	AAB65766 lipoxygenase	0.40897
SGN-U224441	AAP83137 lipoxygenase [Nicotiana attenuata]	0.42648
SGN-U217502	NP_182144 oxidoreductase [Arabidopsis thaliana]	0.44231
	10. Transporters	

Appendix Table C2. (Continued)

SGN-U213547	NP_193555 ATPUP10; purine transporter [Arabidopsis thaliana]	0.2892
SGN-U217780	CAD59598 MRP-like ABC transporter [Oryza sativa (japonica cultivar-group)]	0.38032
SGN-U215692	AAK70407 pol polyprotein [Citrus x paradisi]	0.38057
SGN-U219704	NP_922837 putative carnitine/acylcarnitine translocase [Oryza sativa (japonica cultivar-group)]	0.43184
	11. Secondary metabolism	
SGN-U212595	CAA50719 histidine decarboxylase [Lycopersicon esculentum]	0.11095
SGN-U212592	CAA50719 histidine decarboxylase [Lycopersicon esculentum]	0.14065
SGN-U212843	P08196 Phytoene synthase 1, chloroplast precursor (Fruit ripening-specific protein pTOM5)	0.22241
SGN-U213739	NP_171723 CER1 (ECERIFERUM 1) [Arabidopsis thaliana]	0.23129
M84744	P08196 Phytoene synthase 1, chloroplast precursor (Fruit ripening-specific protein pTOM5)	0.26078
SGN-U212842	CAA47625 mutant phytoene synthase [Lycopersicon esculentum]	0.28287
SGN-U223568	CAA56554 geranylgeranyl diphosphate synthase [Capsicum annuum]	0.37827
SGN-U222998	AAD38941 1-D-deoxyxylulose 5-phosphate synthase [Lycopersicon esculentum]	0.42855
SGN-U242720	ABB82555 geranylgeranyl pyrophosphate synthase 2 [Lycopersicon esculentum]	0.42981
SGN-U218721	AAD38941 1-D-deoxyxylulose 5-phosphate synthase [Lycopersicon esculentum]	0.44054
SGN-U217447	AAO18664 wax synthase isoform 1 [Vitis vinifera]	0.44973
AF143812	AAD38941 1-D-deoxyxylulose 5-phosphate synthase [Lycopersicon esculentum]	0.45148

Appendix Table C2. (Continued)

SGN-U215117	CAA63056 NAD(P)H oxidoreductase, isoflavone reductase homologue [Solanum tuberosum]	0.49413
	12. Cell wall	
SGN-U215711	AAQ12264 expansin 1 protein; LeExp1 [Lycopersicon esculentum]	0.15888
SGN-U212775	AAY85180 pectate lyase [Gossypium hirsutum]	0.18673
SGN-U212774	BAE48664 Pectate lyase [Prunus mume]	0.19396
SGN-U213213	CAA32235 polygalacturonase [Lycopersicon esculentum]	0.38679
SGN-U214121	CAA10175 ss-galactosidase [Lycopersicon esculentum]	0.39984
SGN-U214904	AAY85180 pectate lyase [Gossypium hirsutum]	0.4192
SGN-U214119	AAC25984 beta-galactosidase [Lycopersicon esculentum]	0.49441
	13. Signal transduction	
SGN-U227339	NP_197640 protein binding / zinc ion binding [Arabidopsis thaliana]	0.26743
SGN-U220183	NP_195201 calmodulin binding [Arabidopsis thaliana]	0.33115
SGN-U213440	NP_179568 protein binding [Arabidopsis thaliana]	0.4855
	14. Pathogenesis-related	
	-	
	15. Stress	
	-	
	16. Ethylene	
SGN-U216896	CAA41855 1-aminocyclopropane 1-carboxylate synthase [Lycopersicon esculentum]	0.25072
SGN-U220379	P29535 1-aminocyclopropane-1-carboxylate synthase 4 (ACC synthase 4) (S-adenosyl-L-methionine methylthioadenosine-lyase 4) (ACS-4) (Le-ACS4)	0.46323
SGN-U212800	P10967 1-aminocyclopropane-1-carboxylate oxidase homolog (Protein E8)	0.48417
SGN-U222201	AAC02213 ethylene receptor homolog [Lycopersicon esculentum]	0.44662
SGN-U232734	CAA90808 ethylene receptor [Solanum lycopersicum]	0.45261
	17. Others	

Appendix Table C2. (Continued)

SGN-U213509	AAK29646 putative short-chain type alcohol dehydrogenase [Solanum tuberosum]	0.10548
SGN-U213696	O04397 Ferredoxin--NADP reductase, root-type isozyme, chloroplast precursor (FNR)	0.17997
SGN-U212765	CAA54450 alcohol dehydrogenase [Lycopersicon esculentum]	0.21482
SGN-U214044	BAC23045 monooxygenase [Solanum tuberosum]	0.27033
SGN-U227535	AAL32440 SH3 domain-containing protein 3 [Arabidopsis thaliana]	0.2806
SGN-U212764	CAA37333 alcohol dehydrogenase [Solanum tuberosum]	0.28135
SGN-U220155	AAK14060 major latex-like protein [Prunus persica]	0.28737
SGN-U219932	NP_001050156 Os03g0360700 [Oryza sativa (japonica cultivar-group)]	0.3005
SGN-U212798	AAB71139 E8 protein homolog [Lycopersicon esculentum]	0.30414
SGN-U215966	NP_180337 CYP94C1; heme binding / iron ion binding / monooxygenase/ oxygen binding [Arabidopsis thaliana]	0.30803
SGN-U215368	ABB02390 temperature-induced lipocalin [Lycopersicon esculentum]	0.31276
SGN-U222502	BAB85644 dynamin like protein 2a [Arabidopsis thaliana]	0.31347
SGN-U214258	NP_196094 amino acid binding [Arabidopsis thaliana]	0.3158
SGN-U213381	AAP03873 photosystem I reaction center subunit X psaK [Nicotiana tabacum]	0.31836
SGN-U217797	CAA55813 Sn-2 [Capsicum annuum]	0.36466
SGN-U228384	AAK52092 WD-40 repeat protein [Lycopersicon esculentum]	0.37794
SGN-U216932	NP_190882 O-methyltransferase [Arabidopsis thaliana]	0.38613
SGN-U216434	AAO32318 phosphoenolpyruvate carboxylase kinase 2 [Lycopersicon esculentum]	0.3896
SGN-U212898	CAA50312 P450 hydroxylase [Solanum melongena]	0.3944
SGN-U212578	AAS48091 alcohol acyl transferase [Lycopersicon esculentum]	0.39973

Appendix Table C2. (Continued)

SGN-U230817	CAA57872 pyruvate,orthophosphate dikinase [Mesembryanthemum crystallinum]	0.39988
SGN-U214091	NP_197954 catalytic [Arabidopsis thaliana]	0.40159
SGN-U213844	CAH60890 carbonic anhydrase [Solanum lycopersicum]	0.40371
SGN-U214464	NP_192249 DFL2 (DWARF IN LIGHT 2) [Arabidopsis thaliana]	0.40648
SGN-U217836	NP_916297 P0665A11.10 [Oryza sativa (japonica cultivar-group)]	0.40822
SGN-U212915	CAA44736 photosystem II 23 kDa protein [Lycopersicon esculentum]	0.41297
SGN-U213064	P68172 Adenosylhomocysteinase (S-adenosyl-L-homocysteine hydrolase) (AdoHcyase) (Cytokinin binding protein CBP57)	0.41326
SGN-U212897	CAA50312 P450 hydroxylase [Solanum melongena]	0.4184
SGN-U214689	AAM66054 ethylene-responsive protein, putative [Arabidopsis thaliana]	0.41842
SGN-U232745	AAG49032 ripening regulated protein DDTFR18 [Lycopersicon esculentum]	0.42093
SGN-U233653	NP_190882 O-methyltransferase [Arabidopsis thaliana]	0.42191
SGN-U216838	AAM63491 putative kinesin light chain [Arabidopsis thaliana]	0.43355
SGN-U217229	CAC87643 alcohol oxidase [Arabidopsis thaliana]	0.44855
SGN-U214038	AAG49032 ripening regulated protein DDTFR18 [Lycopersicon esculentum]	0.44921
SGN-U220673	AAO86692 small blue copper protein Bcp1 [Boea crassifolia]	0.46069
SGN-U213993	AAB23481 fruit-ripening gene [Lycopersicon esculentum]	0.47425
SGN-U212832	AAQ90151 putative Rieske Fe-S protein precursor [Solanum tuberosum]	0.47475
SGN-U212803	CAA31789 E8 protein [Lycopersicon esculentum]	0.47955

Appendix Table C2. (Continued)

SGN-U215783	AAZ23261 senescence-associated protein [Nicotiana tabacum]	0.48508
SGN-U212812	BAD42919 similar to senescence-associated protein [Arabidopsis thaliana]	0.49935
	18. Unknown	
SGN-U218249	NP_566150 unknown protein [Arabidopsis thaliana]	0.31647
SGN-U225777	NP_200205 unknown protein [Arabidopsis thaliana]	0.34735
SGN-U214690	NP_850563 unknown protein [Arabidopsis thaliana]	0.35087
SGN-U220997	NP_194558 unknown protein [Arabidopsis thaliana]	0.35177
SGN-U215475	NP_198805 unknown protein [Arabidopsis thaliana]	0.37836
	19. No hits found	
SGN-U225964	No hits found	0.30241
SGN-U230465	No hits found	0.38121
SGN-U233539	No hits found	0.40367
SGN-U217116	No hits found	0.42731