Los datos contenidos en el capítulo 3 de esta tesis doctoral titulada "**Análisis trascriptómico del daño por frío en fruto de melocotón**" están cifrados y son confidenciales hasta su publicación para mantener la novedad de los resultados publicables. Esta tesis ha sido declarada confidencial con carácter excepcional quedando protegida a tal efecto según lo dispuesto en el artículo 10 de la Normativa. "Reglament: sobre diposit, avaluació i defensa de la tesi doctoral." Aprovat Pel Consell de Govern de 29 de noviembre de 2011. ACGUV 266/2011. Modificat; ACGUV 51./201.2 i ACGUV 1.95/201.3.

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Análisis transcriptómico del daño por frío en fruto de melocotón

Transcriptomic analysis of chilling injury in peach fruit

Trabajo presentado por Clara Pons Puig para optar al grado de Doctora por la Universitat de València

Dirigido por el Doctor Antonio Granell Richart

València, Septiembre 2015

El Dr. ANTONIO GRANELL RICHART Profesor de Investigación del CSIC, perteneciente al Instituto de Biología Molecular y Celular de Plantas (IBMCP, UPV-CSIC) de Valencia CERTIFICA que la Licenciada en Biología y Bioquímica CLARA PONS PUIG ha realizado bajo su dirección en el Instituto de Biología Molecular y Celular de Plantas el trabajo que lleva por título “Análisis transcriptómico del daño por frío en fruto de melocotón “, y autorizan su presentación para optar al grado de Doctora.

Antonio Granell Richart

València, Septiembre 2015

"*I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale"*

-Madame Cuire,
"The future of culture", Madrid 1933

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Abbreviations

CSR: cold stored and shelf life ripened fruits

eRG: early ripening genes

FB: flesh browning

FBL: flesh reddening/bleeding

H: harvest

Hz: Hermoza

LS: low sensitive pool of siblings from the Pop-DG population

M: mature stage

MI: mealiness index

NeRG: non early ripening genes

Od: Oded

PCA: principal component analysis

2D-HCA: bi-dimensional hierarchical cluster

Pop-DG: peach chilling injury segregating population

qRT-PCR quantitative real-time PCR

ROS: Reactive oxygen species

S: high sensitive pool of siblings from the Pop-DG population

SL: shelf life

SLR: shelf life ripening

WLT: woolliness/mealiness trait

Resumen

En la industria hortofrutícola se utilizan las bajas temperaturas durante el almacenamiento y transporte para retrasar el progreso de la maduración y el decaimiento de los frutos, y así, conservar la calidad del fruto tras ser cosechado. A pesar de tener un uso extendido, esta tecnología tiene sus restricciones, ya que muchos frutos, como el melocotón, son sensibles a las bajas temperaturas y desarrollan daño por frío [1] cuando son expuestos a temperaturas entre los 0 y 10ºC.

La harinosidad (mealiness) o lanosidad (wooliness) del melocotón es un desorden textural de la pulpa del fruto caracterizado por la perdida de suculencia [2]. La harinosidad es un desorden genéticamente controlado [3] y los análisis genéticos indican que el daño por frío en el melocotón es un carácter cuantitativo[4-7]. Los frutos de melocotón exhiben un gran grado de variabilidad genética respecto a la tolerancia al enfriamiento, dañándose los cultivares más sensibles después de estar almacenados una semana en frío, mientras que los más tolerantes pueden permanecer sin dañarse hasta cinco semanas [8]. El daño por fío tiene un desarrollo más rápido e intenso cuando los frutos susceptibles se almacenan a temperaturas entre los 2.2 y 7.6ºC (rango de temperaturas de muerte celular) que cuando se almacenan a 0ºC, considerada una temperatura que retrasa el desarrollo del daño[8, 9]. Aunque durante el frío se han observado algunas alteraciones microscópicas [10], no se desarrollan síntomas visibles (o macroscópicos) de daño. De hecho, los síntomas visibles de daño por frío, aparecen cuando los frutos se transfieren a temperatura ambiente (temperaturas de simulación de vida útil, SLR) para inducir la maduración después del almacenamiento [8].

Con el objetivo de analizar los mecanismos moleculares subyacentes al desarrollo de la harinosidad durante el almacenamiento en frío y maduración en condiciones de simulación, se han realizado diversos estudios transcriptómicos y proteómicos [11-16]. Estos estudios han resultado en la identificación de diferentes funciones celulares probablemente importantes para el desarrollo de los síntomas de la harinosidad, aunque no profundizan en el análisis y algunos resultados son aparentemente

contradictorios. Diferencias entre las aproximaciones experimentales, genotipos, condiciones de almacenamiento y simulación de vida útil (tiempo y temperatura), así como la forma de determinación del daño, a menudo resultan en la falta de consistencia de resultados respecto a la harinosidad.

El principal objetivo de la tesis que aquí se presenta es proporcionar una visión más exhaustiva del desorden denominado harinosidad y de la respuesta de los frutos al frío (estadio pre-sintomático) y de la maduración posterior que ocurre tras el almacenamiento en frío (estadio sintomático).

En el **primer capítulo** hemos combinado el uso de una micromatriz especializada para el análisis del daño por frío, Chillpeach [17],con grupos de frutos sensibles (S) o poco sensibles (LS) de líneas hermanas de la población Pop-DG, contrastantes en la sensibilidad a desarrollar harinosidad [4]. La expresión génica se ha analizado en frutos acabados de cosechar, durante la maduración en condiciones de simulación y durante el almacenamiento a 5ºC durante 1,2 y 3 semanas. El uso de líneas hermanas Pop-DG seleccionadas de acuerdo con su sensibilidad contrastante al daño por frío, está compensada en otros caracteres fenotípicos que segregan al azar en la población [18]. Así, esperamos revelar genes cuyos patrones de expresión estén ligados a la diferente sensibilidad al frío y la harinosidad en estadios pre-sintomáticos, mientras que se suavizan diferencias en los transcritos asociadas a otros caracteres fenotípicos, como puede ser el caso de la comparación de dos cultivares diferentes. La expresión de los genes candidatos para la sensibilidad o tolerancia al enfriamiento se ha validado técnicamente utilizando la tecnología Fluidigm RT PCR, que se podría definir como de media capacidad y, que permite el análisis de decenas de genes en decenas de fondos genéticos o situaciones. Además, los resultados se han validado biológicamente en algunos individuos de la población Pop-DG con diferentes grados de susceptibilidad al frío utilizando también la tecnología de Fluidigm RT PCR.

En el **segundo capítulo**, integramos los datos de expresión obtenidos en capítulo 1 con los datos de expresión correspondientes a dos genotipos de melocotón diferentes y con diferente sensibilidad hacia el CI para validar los genes candidatos asociados a la sensibilidad y tolerancia a la harinosidad en estadio pre-sintomático. En la primera

parte del capítulo, los transcriptomas de frutos 'Oded' (Od) y 'Hermoza' (Hz), recién cosechados y sometidos a almacenamiento en frío, se han analizado utilizando la micromatriz Chillpeach, y los datos se han validado por qRT-PCR. Para el análisis transcriptómico de Od y Hz hemos usado los mismos tiempos de muestreo, referencia de RNA, análisis, protocolos y p-valores que se usaron en el capítulo 1 para identificar los genes diferencialmente expresados. En la segunda parte del experimento, hemos realizado una comparación directa de los niveles de transcritos entre Od, Hz y los dos grupos de la población Pop-DG obtenidos en el capítulo 1. Mediante esta comparación, esperamos obtener genes regulados por frío comunes a todos los genotipos, y por tanto asociados a los programas de tolerancia y sensibilidad. Además los perfiles de expresión de Od, Hz y los dos grupos de la población Pop-DG se han analizado también con la herramienta bioinformática ROSMETER [19] que proporciona información de la especificidad de la respuesta transcriptómica al estrés oxidativo.

A pesar que el capítulo 1 y el capítulo 2 indican cambios extensivos en el transcriptoma durante el almacenamiento en frío asociados al fenotipo de harinosidad extrapolado, los síntomas visuales de harinosidad aparecen, no obstante, cuando los frutos se transfieren a condiciones de simulación a temperatura ambiente, usadas para inducir la maduración tras el almacenamiento [8]. En el **tercer capítulo** usando los mismos grupos de muestras de la población Pop-DG que se han utilizado en los capítulos 1 y 2, hemos realizado un análisis transciptómico de serie temporal que cubre diferentes estadios desde fruto inmaduro recién cosechado, pasando por frío, hasta SLR. El objetivo de este capítulo es identificar nuevos patrones de expresión específicos de estadio cuyo análisis funcional revele nuevas asociaciones entre la función del gen y el fenotipo de harinosidad/no harinosidad. Finalmente, la tecnología Fluidigm RT PCR de media capacidad nos ha permitido validar y extender nuestros resultados a un número de líneas hermanas individuales de la población Pop-DG que se caracterizan por tener diferentes grados de harinosidad. En muchos de los casos, el valor predictivo de los genes candidatos a marcadores asociados a la harinosidad identificados en los grupos ha podido ser validado en las líneas hermanas individuales según su sensibilidad al frío.

Resum

A la industria hortofrutícola s'utilitzen les baixes temperatures durant l'emmagatzemament i transport per a retardar el progrés de la maduració i el decaïment dels fruits, i així conservar la qualitat del fruit després de ser collit. A pesar de tenir un us estès, aquesta tecnologia te les seues restriccions, ja que molts fruits, com pot ser la bresquilla, son sensibles a les baixes temperatures i desenvolupen dany per fred [1] quan son exposat a temperatures entre els 0 i els 10ºC.

La farinositat (mealiness) o llanositat (woolliness) de la bresquilla es un desordre textural de la polpa del fruit caracteritzat per la perduda de suculència [2]. La farinositat és un desordre genèticament controlat [3] i els anàlisis genètics indiquen que el dany per fred en la bresquilla és un caràcter quantitatiu [4-7]. Els fruits de bresquilla exhibeixen un gran grau de variabilitat genètica respecte a la tolerància al refredament, danyant-se els cultivars més sensibles després d'estar emmagatzemats una setmana en fred, mentre que els més tolerants poden romandre sense dany fins cinc setmanes [8]. El dany per fred te un desenvolupament més ràpid i intens quan els fruits susceptibles s'emmagatzemen a temperatures entre els 2.2 i 7.6ºC tant a 0ºC (rang de temperatures de mort celular) que quan s'emmagatzemen at 0ºC, considerada una temperatura que retarda el desenvolupament del dany [8, 9]. Encara que durant el fred s'han observat algunes alteracions microscòpiques [10], els símptomes visibles de dany (o macroscòpics) no es desenvolupen. Els símptomes visibles de dany per fred, de fet, apareixen una vegada els fruits s'han transferit a temperatura ambient (temperatures de simulació de vida útil, SLR) per induir la maduració després del emmagatzemament [8].

Amb l'objectiu d'analitzar els mecanismes moleculars subjacents al desenvolupament de la farinosistat durant l'emmagatzemament en fred i maduració en condicions de simulació, s'han realitzat diversos estudis transcriptòmics i proteòmics [11-16]. Aquests estudis han resultat en la identificació de diferents funcions cel·lulars probablement importants per al desenvolupament dels símptomes de la farinositat, encara que no aprofundeixen en el anàlisis i alguns resultats son aparentment contradictoris. Diferències entre aproximacions experimentals, genotips,

condicions d'emmagatzemament i de simulació de vida útil (temps i temperatura), així com la forma de determinació del dany, sovint resulten en la falta de consistència de resultats respecte a la farinositat.

El principal objectiu de la tesis que ací es presenta és proporcionar una visió més exhaustiva del desordre denominat farinositat i de la resposta dels fruits al fred (estadi pre- simptomàtic) i de la maduració posterior que ocorre després de l'emmagatzemament en fred (estadi simptomàtic).

En el **primer capítol** hem combinat l'ús d'una micromatriu especialitzada per a l'anàlisi del dany per fred, Chillpeach [17], amb grups de fruits sensibles (S) o poc sensibles (LS) de línees germanes procedents de la població Pop-DG, contrastants en la sensibilitat a desenvolupar farinositat [4]. L'expressió gènica s'ha analitzat en fruits acabats de collir, durant maduració en condicions de simulació i durant el emmagatzemament a 5ºC durant 1,2 i 3 setmanes. L'ús de grups de linees germanes Pop-DG seleccionades d'acord amb la seua sensitivitat contrastant al dany per fred, està compensda en altres trets fenotípics que segreguen a l'altzar en la població [18]. Així, esperem identificar gens els patrons d'expressió dels quals estan lligats a la sensibilitat al fred i al desenvolupament de la farinositat, mentre que es suavitzen les diferències associades a altres caràcters fenotípics, com pot ser el cas de la comparació dos cultivars diferents. L'expressió dels gens candidats per a la sensibilitat o tolerància al refredament s'ha validat tècnicament utilitzant la tecnologia Fluidigm RT PCR ,que podria definir-se com de mitja capacitat i , que permet l'anàlisi de desenes de gens en desenes de fons genètics o situacions. A més els resultats es s'han validat biològicament en alguns individus de la població Pop-DG amb diferents graus de susceptibilitat al fred utilitzant també la tecnologia Fluidigm RT PCR.

En el **segon capítol**, integrem les dades d'expressió obtingudes al capítol 1 amb dades d'expressió corresponents a dos genotips de bresquilla diferents i amb diferent sensibilitat cap al CI per validar els gens candidats associats a la sensibilitat i tolerància a la farinositat en estadi pre-simptomàtic. A la primera part del capítol, els transcriptomes de fruits 'Oded' (Od) i 'Hermoza' (Hz), recent collits i sotmesos a emmagatzematge en fred, s'han analitzat utilitzant la micromatriu Chillpeach, i les

dades s'han validat mitjançant qRT-PCR. Per a l'anàlisi transcriptòmic de Od i Hz hem fet servir els mateixos temps de mostreig, referència de RNA, anàlisi, protocols i p-valors que es van gastar al capítol 1 per identificar els gens diferencialment expressats. A la segona part de l'experiment, hem realitzat una comparació directa dels nivells de transcrits entre Od, Hz i els dos grups de la població Pop-DG obtinguts en el capítol 1. Mitjançant aquesta comparació, esperem obtenir gens regulats per fred comuns a tots els genotips, i per tant associats als programes de tolerància i sensibilitat. A més els perfils d'expressió de Od, Hz i els dos grups de la població Pop-DG s'han analitzat també amb l'eina bioinformàtica ROSMETER [19] que proporciona informació de l'especificitat de la resposta transcriptòmica a l'estrès oxidatiu.

Malgrat que el capítol 1 i el capítol 2 indiquen canvis extensius en el transcriptoma durant l'emmagatzematge en fred associats al fenotip de farinositat extrapolat, els símptomes visuals de farinositat apareixen, no obstant això, quan els fruits es transfereixen a condicions de simulació a temperatura ambient, usades per induir la maduració després de l'emmagatzematge [8]. En el **tercer capítol** utilitzant els mateixos grups de mostres de la població Pop-DG que s'han emprat als capítols 1 i 2, hem realitzat una anàlisi transciptomic de sèrie temporal que cobreix diferents estadis des fruit immadur acabat de collir, passant per fred, fins SLR. L'objectiu d'aquest capítol és identificar nous patrons d'expressió específics d'estadi, l'anàlisi funcional dels quals rebel·le noves associacions entre la funció del gen i el fenotip de farinositat / no farinositat. Finalment, la tecnologia Fluidigm RT PCR de mitjana capacitat ens ha permès validar i estendre els nostres resultats a un nombre de línies germanes individuals de la població Pop-DG que es caracteritzen per tenir diferents graus de farinositat. En molts dels casos, el valor predictiu dels marcadors associats a la farinositat identificats en els grups han pogut ser validats en les línies germanes segons la seua sensibilitat al fred.

Abstract

Low temperatures are commonly used in the horticultural industry to delay peach ripening progress and fruit decay, during storage and transport and therefore to preserve fruits quality after harvest. Despite widespread use, this technology has its own restrictions, since many fruits, such as peach, are sensitive to low temperatures and may develop different forms of chilling injury [1] when exposed to temperatures between 0 and 10ºC.

Peach mealiness or woolliness is a flesh textural disorder characterized by a lack of juiciness [2]. Mealiness is a genetically controlled disorder [3] and genetic analyses indicates that chilling injury in peach is a quantitative trait [4-7] Peach exhibits a high degree of genetic variability for chilling tolerance, with the most sensitive cultivars being damaged after 1 week of cold storage and the most tolerant remaining undamaged for at least 5 weeks [8]. CI develops faster and more intensely when susceptible fruit are stored at temperatures between 2.2 and 7.6^{o}C (killing temperature zone) than when stored at 0°C, considered a chilling injury delaying temperature [8, 9]. Although some microscopic alterations had been observed during CS [10], no visible injury (or macroscopic) develops. Visual symptoms of CI appeared however upon transferring the fruits to the room temperature conditions (shelf life ripening temperatures, SLR) that are used for inducing ripening [8].

To dissect the molecular mechanisms underlying the WLT development during cold storage and shelf life ripening, several transcriptomic and proteomic studies have been reported [11-16]. These studies have resulted in the identification of different cellular functions as important for the development of the WLT symptoms but they did not go deep in the analysis and some results were apparently contradictory. Differences between experimental approaches, genotypes, storage and shelf conditions (time and temperature) and also in the symptom assessment often result in lack of consistency of results.

The main goal of the thesis presented here is to give a more comprehensive view of WLT disorder and of the response of peach fruits to CS (pre-symptomatic stage) and the ripening process(symptomatic stage) occurring after CS shelf life.

In the **first chapter** we have combined the use of de CI-dedicated Chillpeach microarray [17] with of pools of fruits from sensitive (S) and low sensitive (LS) Pop-DG siblings, with contrasting sensitivity to develop mealiness [4]. The gene expression was analyzed at harvest, during ripening at shelf life and during the storage at 5ºC for 1, 2 and 3 weeks. The use of pools of samples from Pop-DG siblings selected by contrasting sensitivity to CI compensates for other phenotypic differences randomly segregating in the population [18].Thus we expect to reveal genes whose expression patterns are linked to the different cold and WLT sensitivity at pre-symptomatic stage, while leveraging transcript differences associated with other phenotypic traits, as would be the case of comparing two different cultivars. Candidate gene expression for chilling sensitivity or tolerance was technically validated by Fludigm RT PCR technology, which could be defined as medium throughput and, that permits to analyze tens of genes in tens of genetic backgrounds or situations. Further results were biological validated in some individual of the Pop-DG population with different degrees of cold susceptibility by using also Fludigm RT PCR technology.

In the **second chapter**, we integrated expression data obtained in chapter 1 with expression data from two different peach genotypes and with different sensibility toward CI to validate the candidate genes associated to WLT sensitivity and tolerance at pre-symptomatic stage. In the first part of this chapter, the transcriptomes of 'Oded' (Od) and 'Hermoza' (Hz) at harvest and subjected to CS were analyzed using the Chillpeach microarray and microarray data was validated by qRT-PCR. For the transcriptomic analysis of Od and Hz we used the same sampling time, RNA reference, analysis, protocols and p-values as in chapter 1 to identify differentially expressed genes. In the second part of the experiment, we performed a direct comparison of transcript levels between Od, Hz and the two pools from the Pop-DG population obtained in chapter 1. By this comparison, we expect to find cold regulated genes common to all genotypes, and therefore associated to the programs for tolerance or

sensitivity. Further the expression profiles of Od, Hz and of the two pools from the Pop-DG population were also analyzed with the bioinformatic tool ROSMETER [19] that provides information on the specificity of the transcriptomic response to oxidative stress.

Despite the chapter 1 and chapter 2 indicated extensive transcriptome changes during CS associated to protracted WLT phenotype, visual symptoms of WLT appeared, however, upon transferring the fruits to the shelf life ripening temperatures, used for inducing ripening after CS [8]. In the **third chapter** using the same pools from the Pop-DG population used in chapter 1 and 2 we conducted a time course transcriptomic analysis that covered different stages from unripe harvest fruit, through CS, until SLR. The goal of this chapter was to identify novel stage-specific expression patterns whose functional analysis would reveal new links between gene function and the woolly/non-woolly phenotype. Finally, the Fludigm™ RT PCR technology allowed us to validate and to extend our results for a number of individual siblings of the pop-DG population which were characterized by different degrees of mealiness. In many cases the predictive value of the woolliness associated markers identified in the pools could be validated in individual siblings according to their chilling sensibility.

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Introduction

Introduction

Given its importance to agriculture, considerable effort has been directed to understanding the mechanisms underlying cold response in plants. Most of efforts has focused in unveiling the molecular bases of cold acclimation [20, 21] in vegetative tissue. However little is known about the cold response in fruits, since the chilling period occurs naturally as winter cold comes, when plants have not yet fruits. A general review about cold effect in plants and fruits (especially in peach) is provided in this introduction. The first part reviews the state of the art for the molecular response to cold in plants and fruits, highlighting differences and similarities between the cold response and its regulation in reproductive and vegetative tissues. The second part of the introduction summarizes the current knowledge about mealiness in peach, its etiology including a review of the approaches used to understand this process.

I.1.Low temperatures and injury

Low temperature is one of the main factors affecting plant growth and development that limits their geographical distribution. This adverse environmental condition is especially important in temperate regions where significant temperature variations occur during season changes. The effect of low temperatures in plants can be observed at all developmental stages, but its intensity depends on the temperature, the exposure time, the organ type (reproductive organs and roots are more sensitive that vegetative tissues), the organ developmental stage, and the other environmental conditions (weather, water and nutrients) where plants grown[22]. Owing to its importance to agriculture, considerable effort has been directed to understanding the mechanisms underlying cold response in plants.

How to cope with low temperatures is a process integrated in the developmental programs of plants both from cold and temperate regions. These plants have developed mechanisms that allow their cells to adapt during autumn temperature descent (cold acclimation, [20, 21]) to survive undamaged when winter temperatures reach below 0ºC (freezing temperatures). Despite plants have developed strategies to tolerate the temperature conditions of their natural habitat, injury may eventually occur when (i) temperatures become extreme [23], (ii) or plants are cultivated in a new environment, as would be de case of plants from tropical or subtropical origin with agronomical interest [1] or (iii) their reproductive organs are exposed to low temperatures as in post-harvest storage [1].

The first case concerns to growth and development of plants from temperate climates in relation to freezing injury, that is , when temperatures were below a critic threshold ranging from -7ºC to 0ºC, in the case of plants chilling tolerant but freezing sensitive, or -30ºC to -7ºC, in the case of freezing tolerant plants [23, 24]. The injury produced by freezing temperatures presents similar functional alterations to those observed in programmed cell death processes [24], even thought they occur more rapidly in the case of freezing temperatures and cell death in this case is associated to ice-induced dehydration [24]

The second case is referred to vegetative organs of crops of plants from tropical and subtropical climates growing in temperate climates. Low temperatures below 15ºC and above 0ºC could result in a physiological and biochemical dysfunction, commonly named chilling injury [1]. The most frequent symptoms of chilling injury in vegetative tissues range from growth repression, development of soaked and necrotic areas, chlorosis, leaf withering from accelerated senescence and in extreme cases the death of the whole plant [1, 25]

The third case is referred to the injury occurring in reproductive organs (fruits,, flowers and seeds) during post-harvest storage before human consumption, although it also can occur in the field. The horticultural industry uses low temperatures during storage and transport to delay ripening progress and fruit decay, and therefore to preserve fruits quality after harvest [26]. Despite widespread use, this technology has

its own restrictions, since in most of plant species (including those from temperate climates) flowering and fructification occurs during the temperate and warm seasons and, the chilling period of winter comes, when plants have not yet fruits. as a consequence many fruits are sensitive to low temperatures and develop chilling injury [1]. The temperature threshold below which injury symptoms developed depends on each specie and variety, but are normally comprised between 0-15ºC, in the case of fruits from tropical and subtropical origin plants and between 4ºC and 7ºC in the case of fruits from temperate plants [1]. In contrast to the chilling injury in vegetative and reproductive organs occurring in the field, the post-harvest chilling injury usually occurs in stored organs (detached) under controlled darkness and high humidity conditions.

Complicating the matter a little more is the fact that "reproductive organ" is a term that encompasses an enormous diversity of different kinds of organs, tissues and developmental processes, with huge architecture differences between species and in general, different of vegetative organs. Each reproductive organ is specialized in one step of the reproductive cycle. The diversity of reproductive organs (flowers, fruits and seeds) and the large differences in tissue morphology have as a consequence that chilling injury is manifested in each organ in a unique way, thus cold can impact a subset of characteristics that might fine tune reproductive programs according to environmental conditions and developmental stage. Further in the case of fruits, it must be borne in mind that fleshy fruits, such as peach, tomato or apple, undergo a ripening process in which the biochemistry, physiology and structure of the organ are developmentally altered to influence appearance, texture, flavor and aroma in ways designed to attract seed-dispersing organisms [27]. In addition, fruits and vegetables usually are harvested when they reach the mature stage, thus unlike vegetative organs, no effects on growth are expected. Cold could still affect: cell viability, activate/inactivate cell responses to increase cellular fitness in those condition and all this could affect some aspects of ripening.

Despite the set of symptoms associated to fruit chilling injury is diverse but in general often lead to incomplete or abnormal ripening, accompanied with flavor and

aroma deficiencies [22]. At macroscopic level two groups of fruit can be separated according to the general chilling injury symptoms produced [22]. In fruits with thin and strong pericarp, like citrus, cucumber and melons, low temperatures induced the development of superficial lesions like pots, depressions and decoloured areas. In fruits with thick pericarp like peach, tomato and apple chilling injury is characterized by textural and colour alterations in the mesocarp, often not visible from the exterior of fruit.

Although chilling injury is manifested in each cell type in different ways some commonalities were found at microscopic levels [1, 21, 22, 28]. Chilling injury proceeds in two phases [21]: an early reversible stage which occurs at the so called primary sites of chilling injury that is followed by an irreversible stage which is characterized by the loss of cell compartmentation and a deterioration of cell function that ends upt the in death of the affected tissue

I.2.Cold acclimation regulation

The molecular mechanisms that plant use to respond /adapt to low temperature stress is well integrated into plants' developmental program. Since 1985 when Guy et al. [29] found that cold acclimation is regulated by changes in gene expression, most of the research on the molecular response to low temperatures has focused in searching for low temperature responsive genes and determining their role in cold acclimation . Most of what we currently know on how plants cope with low temperatures stems from the work carried out in the temperate model plant *Arabidopsis*, where it has been studied in relation to cold acclimation [20, 21], a process that results in extensive transcriptome, proteome and metabolome reorganization [30-34]. This adaptation program appears to act, at least in part, to both reduce growth during the cold season and to protect membranes and proteins against the severe dehydration stress that occurs with freezing [20]. The molecular program induced during cold acclimation is implicated in (i) the stability of subcellular structures, essentially membranes [35, 36] and cytoskeleton [37],(ii) the activation of enzymatic and non enzymatic protective systems against oxygen species [38, 39], (iii)

the accumulation of compatible solutes to protect from osmotic changes [32, 34, 40, 41], (iv) the accumulation proteins with cryoprotective and antifreezing roles [36, 42, 43].

By using mostly seedlings, the regulatory factors influencing the expression of cold regulated (COR) genes, [44] and/or the freezing tolerance genes have been identified over the last two decades (reviewed in [45-48]). The best understood cold regulatory pathway with a role in freezing tolerance is the C-repeat binding factor (CBF) pathway. Three cold-induced transcriptional CBF regulatory factors, also known as dehydration responsive element binding proteins (DREB) [49-52] control the expression of a major regulon of COR genes to confer plant freezing tolerance [30, 53, 54] although they may also play a role in chronic low temperature adaptation [54]. Differences in the levels of tolerance to cold in *Arabidopsis* accessions are apparently due to mutations in CBF genes [55] and their regulatory regions [56, 57]. There are evidences which indicate that cold acclimation is not only regulated at the transcriptional level, but includes also chromatin [58-63], post-transcriptional [64, 65], translational [66, 67] and post-translational levels [68].

Up-stream of the CBF regulatory hub, three sensing pathways have been described [69, 70]. The two cold dependent ones, involved posttranslational protein modification of ICE (Inducer of CBF expression) transcription factors or a calcium signaling pathway respectively while the third one is reported as light dependent [71]

ICE1 ([31, 72] and its paralog ICE2 [69, 70] are the most upstream transcription factors in the cold signaling pathway. Until now it was thought that ICE1, which is constitutively expressed, worked by activating CBF3 upon exposure to low temperature [72], while it was ICE2, the one responding to cold by inducing CBF1 [69]. However a recent report [70] suggests that ICE1 and ICE2 are functionally partially redundant with different mechanisms for inducing CBF genes. Low temperature appears to initiate the transcriptional activation-inactivation feedback cycle of freezing tolerance that is necessary to sustain plant development and survival during cold acclimation [46, 70]. Low temperature induces a rapid activation of both ICE2 (by an unknown mechanism) and of ICE1 protein (by SIZ1-mediated sumoylation [73]). The

newly produced ICE1 and ICE2 activate transiently the expression of CBF1, CBF3 (positive regulators of cold acclimation) and CBF2 (negative regulator of CBF1,3) by directly binding their promoters [70]. The coordinated regulation of osmotic responsive gene HOS1-mediated ubiquitination with the degradation of ICE proteins [70, 74], in parallel the CBF2-mediated repression of CBF1 and CBF3 at the transcription level [75, 76] resulted in the attenuation of the cold induction of CBFs and their target genes, and in reduced levels of MYB15 [73], a negative regulator of CBFs/DREB1A genes [77].

The other cold sensing pathway involves calcium [78, 79], calmodulin binding transcription activators (CAMTA) and possibly calmodulins. The current model proposes that during cold acclimation and freezing tolerance development cold induces a rapid and transient increase of free cytosolic calcium [78, 79]. In response to calcium CAMTA3 and CAMTA1 regulate the expression levels of CBF1, CBF2 (but not CBF3), and also the zinc finger protein ZAT12 [80], a negative regulator of CBFs [53],.

Light also regulates cold-induced CBFs gene expression [81, 82] and both light and cold are necessary for plant cold acclimation and freezing tolerance [71]. During the warm-long day growing season, the CBF pathway is actively repressed by the phytochrome PHYB and two phytochrome interacting factors, PIF4 and PIF7, as a mechanism devised to mitigate allocation of energy and nutrient resources toward unneeded frost protection [71]. This repression is relieved by shortening day length resulting in up-regulation of the CBF pathway and increased freezing tolerance in preparation for coming cold temperatures[71].

The CBF hub alone cannot explain all the differences in cold tolerance genotypes. Although tolerant accessions have generally higher CBF and COR expression levels, there are some exceptions [83], indicating that CBF-independent pathways may participate in cold acclimation and freezing tolerance. Thus the histone deacetylase high expression osmotically responsive gene HOS15 [59], has been proposed to be a negative regulator of cold acclimation by promoting deacetylation on hystone H4 in COR gene promoters in a CBF-independent way. In addition HOS9, a homeodomain protein, has been shown to control freezing tolerance mainly through a

constitutive pathway that is different that that of regulon CBF [84]. On the other hand the dehydration response induced by low /freezing temperature appears to be regulated by HOS10, an R2R3-type MYB transcription factor that positively regulates ABA biosynthesis [85]. Both the ABA-independent and -dependent pathways regulate cold-responsive genes, and ABA acts synergistically with the cold signal [86]. Consistent with that and, with the accepted idea that plant responses to abiotic stresses are related and share common signaling pathways, whole transcriptome analyses conducted in Arabidopsis have revealed a number of genes that are regulated in common by cold and other abiotic stresses such as drought and high salt [87-89] Furthermore, although much attention has been paid to ABA in relation to the cold response (reviewed in [90], there is growing evidence that other hormones are involved, such as auxins (AUX), brassinosteroids (BR), ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) [91-98]

I.2.1 Cold acclimation regulation in other plants

Besides *Arabidopsis*, the importance of the ICE/CBF pathway in cold acclimation and freezing tolerance has been observed in other temperate plants, herbaceous [99-102] or woody [102-109] dicots, as well as in monocots [99, 110-112]. The functional characterization of some of these systems revealed that the genes in the ICE/CBF pathway have similar functions in these plants as described in *Arabidopsis*. Overexpression or ectopic expression of CBF genes resulted in increased freezing tolerance in parallel with increased expression of cold-regulated genes, and other process associated to cold acclimation such as growth reduction [101, 103]. Similarly, ectopic expression of ICE genes conferred enhanced tolerance to cold stresses at either chilling or freezing temperatures [102]. However, the regulation of freezing tolerance by CBF in woody plants appears to be more complex than in herbaceous plants and the role of specific CBF genes can also vary [113]. For instance, ectopic expression of peach (*Prunus persica*) CBF transcription factor in apple tree (*Malus domestica*) results increased cold hardiness [105] but also increased sensitivity to short

photoperiod with respect to the onset of dormancy [105], as well early cessation of growth and leaf senescence, delayed bud break in the spring, growth inhibition [113]

ICE/CBF homologues were also found in non-temperate plants [44, 114-117] that are unable to undergo cold acclimation. Despite this conservation, some structural and regulatory differences have been observed in the CBF cold response pathway between tolerant and sensitive plant species [118]. For example, overexpression of AtCBF1 in transgenic tomato has been shown to increase the chilling tolerance of transgenic tomato plants [115, 119] but it does not increase freezing tolerance nor induce the expression of all COR gene homologs [115, 120]. This is partly explained because, in tomato neither LeCBF2 nor LeCBF3 are induced in response to low temperature, indicating that probably the tomato CBF regulon is significantly smaller and has a limited function as compared to the Arabidopsis one [121].

I.2.1.Cold acclimation regulation in fruits

Expression o f ICE/CBF genes were also found in fruits of both temperate [107, 122, 123] and non-temperate plants [108, 117], indicating that central cold response processes, can be, in part, shared among different plant species and organs. In fruits, however cold might have an impact on a subset of specific fruit characteristics and processes and eventually affect ripening [122].Thus apple and some pear cultivars, require cold acclimation to initiate ripening [124, 125]. In apple a CBF like gene promotes softening in absence of ethylene and, probably, cold and ethylene act independently and synergistically with each other to induce fruit softening [124]. In tomato fruits, the LeCBF expression level correlates positively with cold tolerance [126]. Yet for the normal LeCBF1expression in fruits, both cold and endogenous ethylene are necessary [127], which is not the case for Arabidopsis plants [98]. Further, over-rexpression of SlICE1 in tomato fruits increase chilling tolerance [117] and interestingly also promoted the accumulation of metabolites such as carotenes, ascorbic acid, glutathione, some aminoacids and amines as well as its antioxidant capacity [128]

I.3.Cold response and chilling injury in peach fruits

Peach trees (*Prunus persica*) are temperate trees and therefore are exposed to freezing temperatures conditions as part of their growth cycle and not surprisingly have a functional cold adaptation program (mediated by CBF [109]). However, peach fruits that have been subjected to long periods of storage in the cold (CS), to delay decay and overripening, are susceptible to develop chilling injury (CI). Interestingly CI develops faster and more intensely when susceptible fruit are stored at temperatures between 2.2 and 7.6^{o}C (killing temperature zone) than when stored at 0°C, considered a chilling injury delaying temperature [8, 9]. Although some microscopic alterations had been observed during CS [10], no visible (macroscopic) injury develops normally during low temperature storage. Visual symptoms of CI appeared however at different times after transferring the susceptible fruits from the cold to room temperature conditions (shelf life ripening [SLR] temperatures) that are used for inducing ripening after CS [8].

Low temperatures during storage may induce a range of disorders of different etiology that may develop simultaneously or after different storage times [9]. Chilling injury (CI) in peach fruits is manifested as flesh browning (FB) and reddening/bleeding (FBL) and woolliness/mealiness (WLT),???? [9]The appearance of FB in the fruit flesh is thought to be related to tissue deterioration or senescence, which leads to changes in membrane permeability and the interaction between phenols and polyphenol oxidase, which are generally found in separate compartments in the cell. Kader and Chordas [129] found that the browning potential of peaches depended on the total amount of phenolic compounds present in the fruit and the level of activity of polyphenol oxidase.

FBL has not been studied in depth, but appears to have a large genetic component [8, 9]. The symptoms are the dispersion of the anthocyanin pigment which is usually confined to an area next to the pit into the surrounding fruit flesh. Although this is classed as a chilling related disorder, it does not lead to off-flavours or changes in the fruit texture. Current breeding programs include the development of a red fleshed peach, since this will increase the nutritive value of the fruit [130].

Flesh mealiness or woolliness (WLT) is a flesh textural disorder characterized by a lack of juiciness [2]. As this aspect of CI is the one causing the most adverse effect on fruits and the object of the study presented here, we will summarize in the sections below summarize our current understanding about this disorder.

I.3.1.The influence of the genotype and environment

Peach exhibits a high degree of genetic variability for chilling tolerance, with the most sensitive cultivars being damaged with only 1 week of cold storage and the most tolerant remaining undamaged by at least 5 weeks CS [8]. The fact that CI susceptibility varied consistently among commercial cultivars and selections when stored at either 0ºC or 5ºC [8] indicated that CI is genetically controlled. Different environmental conditions, such as orchard factors have been reported as affecting peach CI, and included nitrogen fertilization, deficit irrigation regimes, maturity, canopy position, crop load, fruit size, environmental conditions, season factor, [3] but they were overcome by the genotype as the most important factor..

WLT, FB and FBL are also reported occurring, singly or jointly, in other stone fruits of closely related species (and cross-compatible species) of the *Prunus* genus [8] such as nectarine (*Prunus persica var . nectarina*), European plum (*Prunus domestica*), Japanese plum (*Prunus salicina*), apricot (*Prunus armeniaca*), mume or Japanese apricot (*Prunus mume*) but not in cherry or almond.

In general, nectarine and plum cultivars were less susceptible to develop CI than the peach cultivars [8]. Harvest date and flesh color also affect CI susceptibility. Early season yellow-fleshed cultivars, of both peaches and nectarines, were less susceptible to develop CI than later season cultivars [8], although this was not the case in white-fleshed cultivars. Early plum cultivars are more prone to develop mealiness, but no relationship between other CI and harvest data was found in plums [8]. Flesh color also influence CI development, thus white-fleshed fruits were consistently associated with a higher incidence of FBL, and FL was slightly greater for yellow flesh [131].In addition, firmness and textural traits influence development of CI[8, 132]. Thus melting flesh (MF) peach cultivars (those that become extremely soft during the

melting phase S4II,[133]) were more susceptible to develop CI than the firmer non-melting flesh (NMF) cultivars [8, 132]. In addition, Freestone trait (F) also influences CI [18]. This trait maps to the same locus as the M trait [18], and control adhesion of the endocarp (or stone) to the flesh (or mesocarp). Clingstone non melting flesh fruits (CNMF) did not get mealy and were less prone to develop FB [4]; while freestone melting flesh (FMF) and clingstone melting flesh (CMF) genotypes have the potential to develop this FB symptom depending on whether they carry further genes for susceptibility [4]. In contrast to mealiness and browning, bleeding was greatest in CNMF progeny [4]

Genetic analysis indicates that chilling injury in peach is a quantitative trait, and a number of major and minor QTLs for chilling injury have been mapped to the peach genome [4-7]. A gene encoding a cell wall modifying enzyme, endo-polygalacturonase (endoPG) co-localized with the major QTL affecting mealiness and FBL [4] as well as to the Freestone-Melting flesh locus [6, 18]. An gene encoding a leucoanthocyanidin dioxygenase (PpLDOX), involved in anthocyanin metabolism, co-localize with the major browning QTL [5, 6]. Furthermore, several fruit quality traits co-located within the linkage group where chilling injury QTLs map [6]. More recenty, Dagar et al. [134] identified a group of differentially expressed genes between two varieties at harvest, which are probably related to their pre-formed tolerance or susceptibility to develop CI.

I.3.2.Current knowledge of the molecular basis of mealy phenotype

During SLR that follows CS, woolly fruit do not go through the phase S4II or melting phase [135], the second phase of softening characterized by a rapid loss of fruit firmness [133] which normally coincides with the climacteric respiratory peak, ethylene burst and juiciness development [136]. This has been related to a reduction in ethylene production during CS and subsequent SLR [136, 137]. Woolly fruit, still soften gradually, but unlike that of normal ripening fruit, it causes unusual texture with cells aggregating in clumps and with reduced fracture upon application of pressure such as that when eating [135].

Improper cell wall disassembly [8, 9] has traditionally been proposed as key determinant for the loss of fruit juiciness during WLT, based on the ability of some cell wall components to hold and release fluid [138]. In WLT fruit the most easily extractable cell wall pectins (soluble in water or chelator) are reduced in amount and are of higher molecular weight and viscosity than in ripened, juicy fruit [139, 140]. The degree of methylesterification of pectin is also altered. Cell wall pectin participates in the wall in cell-to-cell adhesion, which is accomplished largely by calcium cross-linking between partially de-methylesterified homogalacturonan in the middle lamella [141]. Ultrastructural observations showed extensive changes in the middle lamella of CS and during subsequent SLR of WLT fruit [10, 135]. The contact region between cells in woolly fruit was also smaller and the cells assumed a more spherical form with loose attachment to their neighbors, while the intercellular spaces were enlarged and characterized by the presence of amorphous pectic substances, insoluble polysaccharides, cellulose and hemicellulose [10, 135]. The amount of pectins in the bigger intercellular spaces [135] and inside parenchyma cells near to vascular bundles increased dramatically in 5ºC stored fruits in parallel with the macroscopic chilling injury indexes [10] they would develop upon subsequent shelf life ripening. It has been suggested that changes to pectin metabolism cause WLT either by cell fluids forming calcium-pectate gel complexes with high molecular weight pectin in the middle lamella [142], or that the decreased intercellular adhesion in WLT fruit reduces cell rupture during biting and chewing, preventing release of cellular contents [135].

Most of the molecular work done so far on cold-induced fruit woolliness has focused on endo-polygalacturonase (endo-PG) and pectin methylesterase (PME) activities [143-145]. These activities are required for normal ripening in melting flesh cultivars during the so called melting phase or phase SII [145-149]. Reduced ability to depolymerize insoluble homogalacturonan in the middle lamella and to convert it into soluble pectins during CS and subsequent SLR, have been attributed to low levels of endo-PG activity and persistent pectin de-esterification by PME [135, 150, 151]. However, other activities and carbohydrates may contribute to cell wall alterations occurring during CS and SLR [11, 12, 135, 152]. This include alterations in pectic

arabinose metabolism associated to WLT phenotype [135, 150] as well as expasins, pectate lyases, pectin methylesterases and invertases/pectin methylesterase inhibitor, glucanases, xylosidades, mannases [11, 12, 14, 15, 135, 152], which have been described related to both WLT fruit as healthy fruit.

To dissect the molecular mechanisms underlying the WLT development during cold storage and shelf life ripening, several transcriptomic and proteomic studies have been reported [11-16]. These analyses have contributed to the understanding of the various processes that are associated to WLT phenotype and revealed some common transcriptomic changes that occur across different peach cultivars in addition to those related with cell wall. RNA translation and protein assembly, transport, antioxidant systems, aminoacid, carbohydrate and secondary metabolism, energy production, intracellular trafficking, signaling as well ethylene and auxins account for the differences in the sensitivity or tolerance to develop mealiness during cold and shelf [11-16]

I.3.3.The problem

The molecular studies related above have resulted in the identification of different cellular functions which are believed important for the development of the WLT symptoms but either failed to go deep in the analysis or some results were apparently found contradictory. Differences in experimental approaches, genotypes, storage and shelf conditions (time and temperature) and also in the symptom assessment methods often result in lack of consistency of results. In addition, these results could not be correlated quantitatively with different sensitivity levels, since the experiments were based in a single genotype [11-13, 15, 16] or only two genotypes differing in the cold response [14] in addition to differing in other phenotypic traits, thus results may reflect the background genotype of the varieties and/o their environmental history. Moreover, previous reports were either based in a single genotype subjected or no to CS providing only a single time /snapshot of the WLT developmental process. In addition, in the case of comparison between large scale studies, technological differences and cutoffs used for the identification of differentially expressed genes, the

different genes represented on each platform and technical differences in RNA labelling and hybridization, analysis protocols and references used to normalize, often hinder the identification of common regulated genes [95].

I.3.4.Available genetic and omics tools for the study of chilling injury in peach

Modern breeding of peaches started in the USA towards the end of the 19th century and was based on a very limited number of genotypes [153]. Thus, because of this and because of their high degree of natural self-pollination, peach cultivars are known to have low genetic variability [154]. Although the genetic background of peaches is very limited, there are differences between cultivars in their resistance to prolonged cold storage and chilling injury. Contrasting genotypes can serve as a powerful tool for understanding the physiological and molecular mechanisms of chilling tolerance in peach. In an attempt to study the genetic basis for chilling injury three peach CI segregating populations were created. The first, Pop-DG population [18] derived from a cross between the commercial cultivars 'Dr. Davis' and 'Georgia Belle'. 'Dr. Davis' produces yellow, clingstone, nonmelting flesh (NMF) fruit and is a major cultivar grown in California for the canning industry, while 'Georgia Belle' is an old cultivar producing white, freestone, MF fruit that are eaten fresh [131, 155, 156].'Georgia Belle' is particularly susceptible to CI, while 'Dr. Davis' exhibits resistance to most symptoms. The second, Pop-G [18], was derived by selfpollination of 'Georgia Belle'. The third, V×BT population, derived from across between the cultivars 'Venus' and 'BigTop' 'Venus' is a FMF (freestone melting flesh) nectarine cultivar whereas 'BigTop' is a CMF (clingstone melting flesh) nectarine cultivar. The progeny of all three populations segregated in their sensitivity CI [4, 5, 131, 157], however the V×BT population showed lower susceptibility to CI symptoms Pop-DG and Pop-G populations [157]

Sources of information for candidate gene functional genomic study of peach chilling injury include published work in physiology and biochemistry previously described, as well as extensive Rosaceae expressed sequence tag (EST) data from the Genome

Database for Rosaceae (GDR [158]), ESTree [159, 160] and ChillPeach collection [17]. Further medium and high-throughput expression platforms were created and used to approach CI in peaches [11, 13, 14, 16, 17, 134]. The macroarray covering 847 non-redundant EST [11] and µPeach 1.0 oligo microarray [161], representing 4800 unigenes, were created by using genes expressed during ripening. Chillpeach EST collection and cDNA microarray covering 4200 unigenes [17] were enriched with sequences of genes that are directly involved with CI development. They were created from a EST libraries of fruit tissue from two full-sib progeny individuals of Pop-DG population with contrasting susceptibility to CI and subjected to various cold storage durations and ripening regimes to facilitate speedy detection of genetic factors responsible for CI in peach and nectarine and, possibly, other stone fruits [17].

The use of these expression platforms in combination with pools of siblings from the populations with contrasting susceptibility to CI and subjected to various cold / ripening regimes provide adequate tools to approach the CI problem with a genomics view. Thus pools of fruits with the same horticultural characteristics and environmental history recapitulate information from siblings with contrasting sensitivity to develop CI, but compensated in the pool for other individual phenotypic differences [162, 163]. The use of pool of fruit samples obtained from siblings of the Pop-DG population [18], with contrasting sensitivity to CI, has been proven useful to identify mealiness associated QTLs [6, 7, 18] and candidates genes for mealiness tolerance/sensitivity identified at pre-symptomatic CS stages [17, 134]. However, although DNA information and RNA expression data alone are insufficient for establishing a clear link between a gene/protein and the trait of interest, transcriptomics they can be used as an important first step to explore potential novel candidate genes for a particular process.

Aim of this thesis

The main objective of this thesis is to give a more comprehensive view of the WLT disorder and of the response of peach fruits to CS and the ripening processes occurring during shelf life ripening after CS. We plan to do that by combining the use of pools of fruit from siblings from the Pop-DG population [18], with contrasting sensitivity to develop mealiness, with transcriptome analysis using Chillpeach microarray [17]. Thus we expect to reveal genes whose expression patterns are linked to cold sensitivity, while leveraging transcript differences associated with other phenotypic traits, as would be the case of comparing directly only two different cultivars

Objectives

1. To analyze the expression profiles of peach fruit during cold storage and identify genes which at pres-symptomatic stage are associated to the sensitivity or tolerance to develop mealiness.
2. To analyze the expression profiles of peach fruit during normal ripening, during cold storage and subsequent ripening and identify peach genes associated to development of mealiness and /or associated to the sensitivity or tolerance to develop mealiness during shelf life ripening after CS.
3. Validate and extend the expression results of candidate genes obtained for the pools to individual genotypes with a range of sensitivities to develop chilling injury.

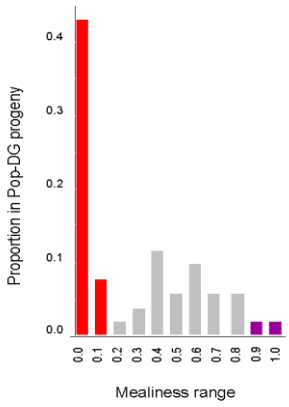
Materials and methods

Materials and methods

M1.Plant material

For most of the work presented in this thesis siblings from Pop-DG mapping population [18], segregating for chilling injury (Figure 1A and [4]), were used. Fifteen individual lines (Figure 1B) were selected because their sensitivity phenotype was consistent for 3 years prior this study. Mesocarp samples from fruits of the following Pop-DG siblings were used for build the pools used in microarray and by medium throughput qRT-PCR analyses: 49/59, 84/85, 86/87 and 132/133 with high sensitivity to mealiness (S) and 71/72, 88/89, 134/135, 142/143 with low sensitivity (LS). Genotypes were selected because they had similar horticultural characteristics but differed in the sensitivity to wooliness development, although not in the incidence of other CI disorders such as flesh bleeding and flesh browning (Table 1). The rest of individual siblings, together

A



B

Pop-DG sibling genotype	Mealiness index CSR1	CSR2	CSR3	Pools
049/050	**0.6**	**1.0**	**1.0**	Pool S
084/085	**1.0**	**1.0**	**1.0**	Pool S
086/087	**0.8**	**0.8**	**0.9**	Pool S
132/133	**0.8**	**1.0**	**1.0**	Pool S
071/072	**0.0**	**0.4**	**1.0**	Pool LS
088/089	**0.0**	**0.0**	**1.0**	Pool LS
134/135	**0.0**	**0.4**	**0.6**	Pool LS
142/143	**0.0**	**0.4**	**0.6**	Pool LS
059/060	**0.3**	**0.7**	**0.8**	
075/076	**0.0**	**0.9**	**1.0**	
124/125	**0.2**	**0.8**	**0.9**	
154/155	**0.2**	**0.8**	**1.0**	
158/159	**0.0**	**0.5**	**0.7**	

Figure 1.Mealiness index in the Pop-DG siblings after cold storage at 5ºC plus 2 days at shelf life ripening at 20ºC. A) Frequency of the individuals with a given MI Index in the Pop-DG population. Symptoms were scored after 1 week of cold storage plus 2 days shelf life ripening

with those composing the pools, were used in the biological validation analyses of candidate genes by medium throughput qRT-PCR. Fruits of the Pop-DG population were obtained from Kearney Agricultural Center (Parlier, CA, USA).

Table 1. Fruit quality parameters at harvest of the siblings of the Pop-DG population used for build the pools and storage disorders measured after cold storage at 5ºC plus 2 days of shelf life ripening at 20ºC

		Maturity parameters			chilling injury symtoms		
Sibling	**Pools**	**SSC (ºBrix)**	**TA (g 100g-1 FW)**	**Flesh color**	**mealinessª**	**Browning (1-6)***	**Bleeding****
086/087	Pool S	11.66	0.900	Yellow	**0.8**	2.989	0.000
049/050	Pool S	14.46	0.502	Yellow	**0.6**	3.160	0.011
084/085	Pool S	12.76	0.494	Yellow	**1.0**	1.088	0.000
132/133	Pool S	11.94	0.492	White	**0.8**	2.861	0.026
142/143	Pool LS	11.424	0.673	White	**0.0**	2.288	0.010
071/072	Pool LS	11.54	0.656	White	**0.0**	1.212	0.047
134/135	Pool LS	11.93	0.633	White	**0.0**	1.434	0.000
088/089	Pool LS	12.36	0.594	White	**0.0**	1.602	0.000

SSC, TA, Browing and bleeding are not significant different in a t-test between sensitive and tolerant pools at p-value<0.05

ª Mealiness is expressed as the proportion of fruits with mealiness after one week of CS

* Browning scored on a scale of 1 (no browning) to 6 (severe browning) after three weeks of CS.

**Bleeding is expressed as the proportion of fruits with bleeding after three weeks of CS

An early-season variety peach [*Prunus persica (L.)* Batsch 'Oded'] (Od) and a mid-season variety peach [*Prunus persica (L.)* Batsch 'Hermoza'] (Hz) with different sensitivity to CI were used for the microarray analysis performed in chapter 2. Fruit of both cultivars were harvested from a commercial orchard in Israel in 2009.

To avoid potential distortions caused by maturity stage, fruits were harvested when reached the same commercial mature stage (M) according to Kader & Mitchel [164] with flesh firmness of 12–14 lb (or 53-62 Newtons), soluble solid content (SSC) of 11-14% and tritrable acidity (TA) of 0.5-0.7 %. Fruit and physiological parameters at harvest of Pop-DG siblings composing the pools are recorded in Table 1. In the case of Od and Hz genotypes physiological parameters at harvest are recorded in table 2. Pooled mesocarp tissue from at least 6 fruit was flash frozen with liquid nitrogen and

stored at −80°C until further analysis. The pools S and LS were build using equal amounts of RNA from each genotype in a given control or treatment group.

Table 2.Physiological parameters of 'Oded' and 'Hermoza' at harvest

Cultivar	Weight (g)	Ethylene ($\mu L\ kg^{-1}\ h^{-1}$)	SSC (%)	TA (%)	Firmness (Newton)
Oded	141 ± 15.0b	0.69 ± 0.53a	11.9 ± 0.90b	0.43 ± 0.05a	54.0 ± 7.2a
Hermoza	200 ± 30.6a	0.78 ± 1.00a	14.3 ± 0.46a	0.33 ± 0.03b	62.8 ± 11.2a

Different letters indicate significant differences at $P < 0.05$ (t-test).

M2.Physiological parameter measurement

Physiological parameters were measured and averaged from 13-15 fruit following the protocol described in Zhou et al. [165]. Firmness was measured on two pared sides of each fruit using a penetrometer fitted with an 8-mm diameter plunger. A wedge-shaped slice (approx. 5 g) was removed from each fruit in the replicates and the pooled sample was passed through an electric juicer (Moulinex, type 753, France) for the measurement of soluble solids content (SSC) and titratable acidity (TA). SSC was determined by a digital refractometer (Atago, Tokyo, Japan). The TA was determined by titration of 2 mL juice to pH 8.2 with 0.1 N NaOH and expressed as percentage of malic acid. Ethylene was determined by closing individual fruit in a 650 ml jar for 1 h, sampling the air in the container with a syringe and injecting into a gas chromatograph with a FID detector.

M3.Post-harvest treatments and chilling injury evaluation

A group of 12 fruits M were directly allowed to ripen at 20°C to the edible firmness of 2–3 lb (14 Newtons) and used as controls (R samples). For different degrees of mealiness M fruits were forced-air cooled at 0–2°C within 6h of harvest and then stored at 5°C with 90% relative humidity for 1, 2 and 3 weeks (CS samples) and subsequently allowed to ripen during 2-3 days at 20ºC (CSR samples). Pooled mesocarp tissue from at least 6 fruits was flash frozen with liquid nitrogen and stored at −80°C until further analysis.

Fruit were evaluated for different CI symptoms such as expressible of juice, hard textured fruit with no juice upon squeezing or woolly texture (WLT), flesh browning or pit cavity browning (FB) and internal reddening or flesh bleeding (FBL). Observations were made on 15 fruit at each observation time.

Visual evaluation of CI symptoms was performed after each fruit was cut into two halves through the suture plane. WLT was scored on a 5-grade scale, according to amount of juice released upon hand squeezing, as follows: 1, very juicy; 1.5, moderate juicy; 2, less juicy; 2.5, small amount of juice; and 3.0, almost no juice. FB and FBL were also scored according to a 5-grade scale, based on area covered as follows: 1, no browning or reddening; 1.5, affected area < 5%; 2, affected area ≥ 5% and < 25%; 2.5, affected area ≥ 25% and < 50%; and 3.0, affected area ≥ 50%. Results for WLT, FB and FBL were expressed as an index calculated as the percentage of the average of fruit with each CI level in the treatment.

Mealiness index (MI), i.e the proportion of measured fruits with mealiness when ripened for 2-3 days at 20ºC., was determined as the percentage of free juice content in total tissue. In the case of siblings of the Pop-DG population, expressible juice is determined accordingly to Campos-Vargas et al. [166] using the quantitative method described by Crisosto et al. [167]. In the case of Od and Hz, expressible juice was determined as described in Dagar et al. [168] using the method described by Lill et al. [169]. Both methods follow the same fundamental. Briefly, juice, obtained through pressing fruit tissue, was centrifuged. Recovered supernatant was weighed in order to determine the percentage of juice relative to original weight of the sample. The difference between the way they extract juice from fruit tissue: one uses a press [167] and the other a syringe [169].

M4.Microarray hybridization, scanning and data pretreatment

RNA purification, sample preparation and hybridization to Chillpeach microarray were performed as described in Ogundiwin et al. [17]. All samples were compared using a dye-swap design against a common superpool reference, composed of equal amounts of RNA obtained from all the mesocarp samples composing the pools [17]. Three

replicates from each sample pool were hybridized in each case, one of them dye-swapped.

Intensity values were obtained as the median of ratios using GenePix 4000B scanner (Axon Instruments). Data files were imported into Acuity 4.0 (Axon Instruments) for normalization and analysis. Only spots with intensity values higher than the background plus two standard deviations of the background median, in at least one channel, were used for analyses. Before normalization, the median local feature background was subtracted. Data were normalized by Lowess (locally weighted scatter plot smoothing) with a centered print-pin tip using the Acuity default values. To generate the raw data to be used for the expression analysis, a Lowess M Log Ratio was used as the expression value, and patterns with more than 80% of non missing values were selected. In all, around 78% of the ChillPeach probes met the threshold for hybridization quality in all of the experiments.

M5.Microarray expression analysis

Differentially expressed genes were identified from the raw dataset using the Significance Analysis of Microarray software [170]. Missing values were imputed by 10-Nearest Neighbors Imputer algorithm, with 100 blocked permutations and a random seed value set by default in the program. PCA and 2D-hierachical cluster analyses were performed on the significant data using Acuity (Axon instruments). A principal component analysis (PCA) was calculated for those factors explaining 100% of variance. For calculations spots with missing values were replaced with the average values across the arrays. Profiles with the same shape pattern were centered around the mean value across arrays, to avoid the effect that the magnitude of response might have on the average profile. For the hierarchical cluster, a Pearson correlation centered on 0 was used as a similarity metrics. A complete linkage was used to link clusters together to produce the tree. Transcripts and/or samples were ordered in the clusters according to their contribution to principal component 1 of the PCA performed with the same dataset.

M6.Correlation analysis between transcript levels in pools S and LS and degree of mealiness.

Correlations between transcript levels in pools S and LS and degree of mealiness in chapter 1 were calculated by the Pearson product moment correlation method using Matlab 2007 (The MathWorks, Inc.). *P* values below 0.01 were selected for statistical significance. A statistical significance level of 1% was assessed with the correlation coefficients over 0.8. Those genes whose expression profiles contained 100% of data points in the samples analyzed were used to calculate correlations. The complete list of the microarray-wide gene expression correlations with the Mealiness Index (MI) are listed in Table S2. Functional enrichment is performed as indicated above.

M7.Functional annotation of Chillpeach genes: functional categories, specific process/pathways, and relation to stress and hormones

ChillPeach unigenes were functionally annotated as indicated in Ogundiwin et al. [17]. The ChillPeach genes were classified into 34 distinct functional categories and 702 specific processes (Table S2) by extensively reviewing the literature and by searching in reference databases: PubMed [171], UniProt [172],prosite [173], BRENDA [174], TAIR [175], The Gene Index Project [176], KEEG [177, 178], Plant Metabolic Network [179, 180], and Plant Transcription Factor Database 2.0 [181].

To classify Chillpeach genes as stress and hormone responsive genes the AIG code of the *Arabidopsis* orthologues were used in a data mining strategy for interrogating the gene expression files from the following databases or papers:

Stress responsive genes:

(1) Cold, the ColdArrayDB (http://cold.stanford.edu/cgi-bin/data.cgi,) [53] a database that contains global expression profiles of *Arabidopsis* genes in response to cold. We use the same searching conditions as in [17].

(2) Cold, drought and salinity responsive genes, the results obtained with Affymetrix forward and reverse tiling arrays [87]

(3) darkness responsive genes the results obtained using a *Arabidopsis* Functional Genomics Consortium or 11K AFGC cDNA microarray by [182]

(4) Pathogen- virus responsive genes results obtained using 22K Affimetrix ATH1 GeneChip by [183].

Hormone related genes:

(1) Abscisic acid (ABA), auxin (Aux), brassinosteroid (Br), cytokinin (CK), ethylene (Et), gibberellin (GAs), jasmonic acid (JA) and salicylic acid (SA): Arabidopsis Hormone Database (http://ahd.cbi.pku.edu.cn, [184]) a comprehensive database based on data from mutant studies, transgenic analysis, and gene ontology (GO) annotation for the hormones

(2) ABA, AUX, Br, CK, Et, GAs and JA responsive genes in the results obtained using Affimetrix ATH1 GeneChip as part of the AtGenExpress project by [185] and [186]

(3) ABA responsive genes using Arabidopsis Affymetrix tiling arrays identified by [87]

(4) ET-responsive genes by using cDNA-AFLP and a VIB Arabidopsis 6K cDNA microarray analysis as identified by [187]

Functional enrichment on a ranked list of genes on the differential expressed datasets was performed with, a local, customized version of 'catscore.pl' Perl script described in Cheung et al. [188], using a two-tailed Fisher exact t-test with adjusted *p*-value cut-off of 0.05. Results of functional enrichment were visualized using Matrix2png [189]

M8.A medium-throughput quantitative RT-PCR analysis using a dynamic array by Fluidigm

The 96.96 dynamic arrays were obtained from the Fluidigm Corporation and were used to perform up to 96 qRT-PCR reactions in cDNA preparations corresponding to up to 48 samples: 15 genotypes in the M stage and/or CS1 or/and CSR1 samples and 7 pools (M-S, M-LS, CS1-S, CS1-LS, CSR1-S, CSR1-LS and the reference superpool used for the microarray analyses). Two biological replicates were included in each array for all the 15 genotypes and pools, each one representing at least three different fruits. Two replicated 96.96 Fluidigm dynamic arrays were used.

For the Fluidigm analysis, 96 genes were selected from our microarray results. Out of them, in the chapter 1, 72 genes (Table S5) were analyzed by qRT PCR in 64 cDNA preparations corresponding to 37 samples: 15 genotypes in the M stage and/or CS1 samples and 5 pools (M-S, M-LS, CS1-S, CS1-LS and the reference superpool). In the chapter 3, 96 genes (Table S14) were analyzed by qRT PCR in 62 cDNA preparation corresponding to 31 samples: 13 genotypes in the CS1 and /or CSR1 samples and 5 pools (CS1-S, CS1-LS, CSR1-S, CSR1-LS and the reference superpool).

Oligo pairs for selected genes were obtained using the Primer Express version 2.0 software (Applied Biosystems). To design primers, the following conditions were used: Tm 58-60ºC, GC content 20-80%, primer length 20-22 base pairs and an amplicon size of 140-150bp. A virtual PCR was carried out for each oligo pair obtained with the 'primersearch' program from the EMBOSS open software suite [190], using the full set of known peach sequences as potential template sequences. The interrogated peach sequence databases included the ChillPeachDB [17], ESTreeDB [160] and GDR_Prunus [191] sequences. Only the oligo pairs yielding a single PCR product from each unique gene, based on the sequence assembly of all the known *Prunus* sequences, were considered. When more than one specific oligo was obtained for a gene, the oligo pair with the lowest penalty value (as provided by the Primer Express version 2.0 software for oligo identification), and which mapped most of the 3' end of the gene, was selected using custom Perl scripts.

Three genes were selected to normalize qRT-PCR results on the basis of low variability in the chillpeach microarray under all conditions analyzed in this paper: a gene with unknown function (PPN036E09), an ABC1 family protein(PPN076G09) and, an esterase/lipase/thioesterase gene (PPN078E12) [17] They were validated by qRT-PCR as described in [17]. The comparative ΔΔCt method, as described by in Livak and Schmittgen [192], was used to confirm a flat pattern throughout the samples.

For the Fluidigm analysis, the cDNA synthesized from total RNA following standard methods was diluted to 1:10 using the DA Assay Loading Buffer (Fluidigm). The Nanoflex 4-IFC Controller and the BioMark Real Time PCR system by the Fluidigm Corporation were used to run the dynamic arrays under the standard conditions

employed at the General Hospital lab, Valencia, Spain. The cycling program consisted of 10 min at 95ºC followed by 40 cycles of 95ºC for 5 sec and 1 min at 60ºC.

The relative gene expression values were determined using PerlqXpress (manuscript in preparation). PerlqXpress was used to calculate "fold expression values" (FC) from the Ct values obtained directly from the BioMark Real-Time PCR Analysis Software (Fluidigm).Briefly, PerlqXpress filter outliers within a sample, corrected differences in background control levels, centers and scales data. The mean centered and scaled Ct values were transformed into relative quantities (RQ) using the exponential function with the efficiency of PCR reaction as its base. For each gene the RQ was corrected using a normalization factor. FC is calculated by dividing normalized RQ to reference sample in each biological replicate (in this case reference pool used in the microarrays). Mean, standard deviation, and coefficient variation were calculated for each replicate. Replicates were filtered by the coefficient variations. At least 4 good replicates were used to calculate "fold expression change" values.

To extend the validity of the results obtained in the pools to individual lines, for which we had individual MI index values, qRT-PCRs were performed on up 15 individual peach genotypes from the popDG progeny. In the chapter 1, for each gene pair in a predefined expression set, the Pearson correlation coefficients between their expression profiles in the individual Pop-DG siblings were obtained by Gitools 1.8.2 [193]. A gene was selected as consistent and was confirmed over the individual lines when it was associated with a predefined expression pattern. In the chapter 3, for each gene in a predefined expression set, Pearson correlation coefficients and its associated p-value between its expression values in all individual Pop-DG siblings and MI was calculated. Correlation values above 0.2 represent non-random correlations

M9.Comparison of the cold response of 'Oded' (Od), 'Hermoza' (Hz) and pools of siblings from the Pop-DG population

In order to examine transcript abundance changes across different peach fruit differing in their sensitivity to chilling injury, and to compare these with the transcript abundance profiles generated from this study, transcriptome data from pools of

siblings from the Pop-DG population at harvest and after one and two weeks of cold storage at 5°C were retrieved from chapter 1. For the comparative analysis genes with high quality values in the two experiments (see above) and differentially expressed between Hz and Od and between highly sensitive (S) and less sensitive (LS) pools after one week of cold storage were selected. A dataset of 2207 genes was generated and used for the comparison. Clustering of total transcript accumulation within a specific treatment and fruit type was done using Euclidean distance and the *k*-means unsupervised clustering Acuity ™ (Axon instruments). For calculations the number of k clusters was set to 12 and the centroid for each cluster was randomly assigned. Spots with missing values were replaced with the average values across the arrays. Profiles with the same shape pattern were centered and scaled around the mean value across arrays. Transcripts were ordered in the clusters according to their contribution to principal component 1 of the PCA performed with the same dataset.

M10.Real-time quantitative reverse transcriptase-PCR analysis

The transcript abundance of 10 selected genes (Additional file 1: Table S1) that were differentially expressed between Od and Hz were validated with quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analyses. Full length cDNA, primer design, optimum primer and cDNA concentrations, qRT-PCR reaction and quantification was performed as described above and in Dagar et al. [134]. Primer sequences and amplicon lengths are given Table S9. Each biological sample was examined in duplicate with two to three technical replicates. The expression levels for the genes were calculated relative to the Initiation Factor eIF-4-Gamma (eIF-G) gene as described by Ogundiwin et al. [17], and the expression level of each analyzed gene transcript during cold storage in the Od and Hz samples was calculated relative to this harvest values.

M11.ROSMETER analysis

The ROSMETER is a new bioinformatic tool (http://app.agri.gov.il/noa/ROS_desc.php), which can provide information on the specificity of ROS-related response for any data

set [19]. The ROSMETER was fabricated by using data from Arabidopsis plants exposed to stresses occurring in different cellular compartments [19]. A set of genes having Arabidopsis orthologs in Chillpeach [17] and differentially expressed at harvest and during cold storage in all four fruits studies was used for ROSMETER analysis. The obtained data set was arranged according to the instructions on the website and submitted for analysis. The output file represents correlation values between known oxidative stresses and the transcriptome of the two cultivars and the two pools of siblings at harvest and following cold storage of 1 and 2 weeks. Correlation values above 0.12 represent non-random correlations [19].

Chapter 1

Chapter 1. Analysis of transcriptome changes induced by cold in fruits of Pop-DG siblings with contrasting sensitivity

C1.1.Results

C1.1.1.Differential cold response to chilling temperature in the fruits of the Pop-DG peach population.

Harvest maturity, a factor known to influence mealiness [3], was tested before cold treatments to ensure all fruits were in the same maturity stage. Table 1 shows there were no significant differences in firmness, SSC and TA between genotypes. This indicates that at harvest, both populations were at the same physiological stage and differences in the subsequent cold response can be mainly attributed to the cold sensibility without significant distortions owing to lack of adequate maturity stage.

To asses the effect of the cold stress on peach fruits from siblings of the Pop-DG population, a subset of the cold stored fruits were ripened for 2-3 days at 20ºC and mealiness was evaluated as the proportion of measured fruits with mealiness or Mealiness index (MI). Figure 2A shows the average MI of pools of fruits grouped according to their sensitivity to develop mealiness. The pool S had higher MI as compared with pool LS after the same cold storage times (Fig.2A), although tend to converge after increasing cold storage periods, indicating a non complete (but clear with huge market importance) tolerance of fruits LS. The difference was more pronounced after one week of cold storage at 5ºC, where the mealiness symptoms were already visible in the pool S but not the pool LS (Fig.2A). No significant differences in the frequency of other CI symptoms were observed between pools S and LS (Table 1). Thus the characteristic feature, differentiating the cold response of the pools, was their sensitivity to develop mealiness. Given that the proportion of mealy fruits increased with the time of cold storage, our hypothesis is that despite mealiness

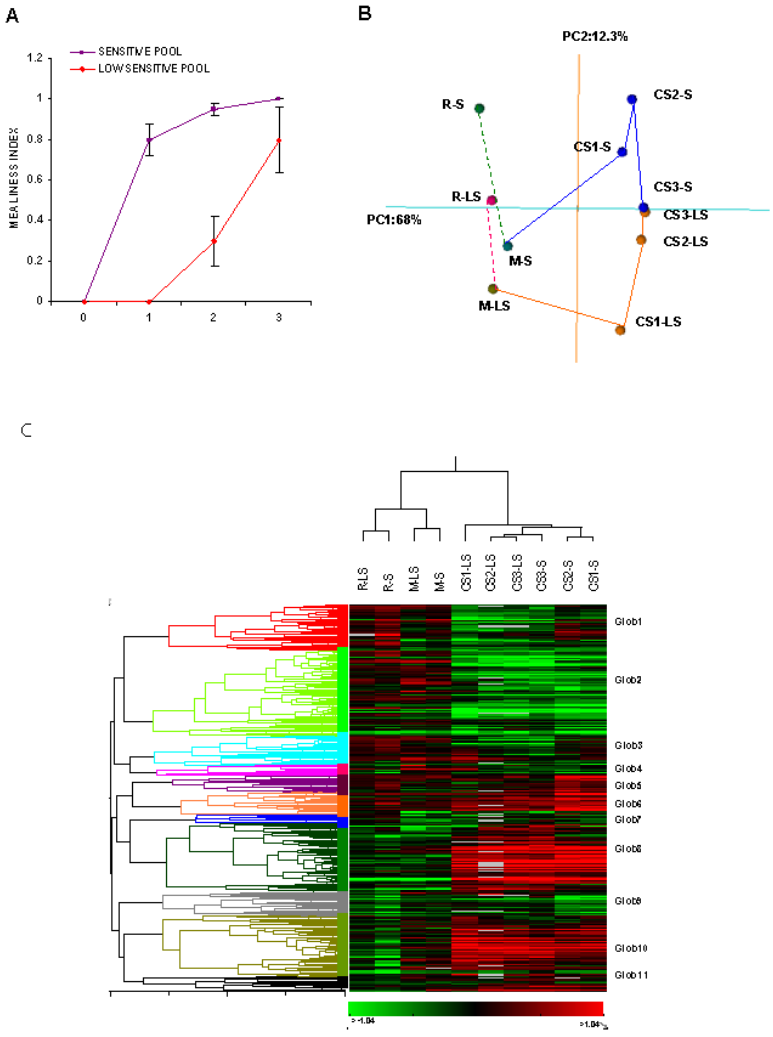


Figure 2. Mealiness index of pools of peach Pop-DG siblings and global gene expression analysis of Chillpeach transcripts in response to cold storage. A) Average mealiness index (MI) of pools S and LS from fruits shelf life ripened for 2-3 days at 20ºC after being stored for up to 3 weeks at 5ºC; B) Principal Component Analysis (PCA) of the global expression profile showing the most variation of each treatment condition (averaged from three replicates). First principal component (PC1) is shown on x-axis while the second principal component (PC2) is shown on y-axis. C) Unsupervised bi-dimensional hierarchical clustering. Heat map of the expression values corresponding to the normalized means of three biological and three technical replicates. Color represents fold change (red upregulated/green down-regulated) in relation respect to a reference pool. Clustering of samples according to the expression values is shown on top . M = mature fruits, R = mature with 2-4 days ripening at 20°C, CS1 = M + 1 week cold storage at 5°C, CS2 = M + 2 weeks cold storage at 5°C, CS3 = M + 3 weeks cold storage at 5°C

was not showing until fruit was allowed to ripe [8], relevant molecular changes may had already started to occur during cold storage.

C1.1.2.A global non target analysis of the transcript profiles in the Pop-DG in response to cold

Bulked segregant analysis [162, 163] in combination with the Chillpeach expression microarray [17] was used to compare the transcriptomes of peach fruits from the S and LS Pop-DG siblings. In all, 3350 transcripts (Table S2) showed a significant difference in expression levels at least for one condition (samples M, R and CS for pools S and LS) using two criteria: a false discovery rate (FDR) < 5% and a p-value < 0.05. The principal component analysis (PCA) of the complete dataset variance is seen in Figure 2B. PC1 (68% variance) clearly separated fruit samples which came directly from cold storage (CS), from fruits M and R (Fig. 2B). The proximity between fruits M and R, if compared to CS, indicated that the effect of cold storage on the peach transcriptome was much greater than that of ripening. PC2 (12% of variance) separated fruits M from R. Both, fruits S and LS seemed to follow parallel ripening programs, but fruits LS showed delayed or less intense ripening transcriptomic changes than fruits S. It should be noted that Pop-DG siblings in each pool were selected on the basis of their cold response, as revealed by the MI after shelf life ripening, so it is not surprising that some differences in the ripening programs may exist. In addition, PC2 roughly separated cold stored samples according to the eventual increase in the MI of the fruit should they be submitted to shelf life after cold storage (Fig.2A and B). According to this component, fruits from the pool S stored for 1 or two weeks have achieved a pattern of ripening similar to fruits R (as they had similar values in PC2). This may indicate that during cold storage at 5ºC, some internal ripening may result in chilling sensitivity and in a shortened shelf life. The loading plots for PC2 (i.e., the contribution of each gene to the separation by a given principal component) revealed 37 genes among with were genes previously reported in the regulation of temperature responses, including the transcriptional factor CBF [194], GASA5 [195] and SCR2 [94] (see Table S2). Thus the transcript levels contributing to component PC2 may be

relevant for the development of a tolerance mechanism in cold, which could affect the way cold storage interrupted or slowed down the ripening program and eventually how fruits ripen afterward.

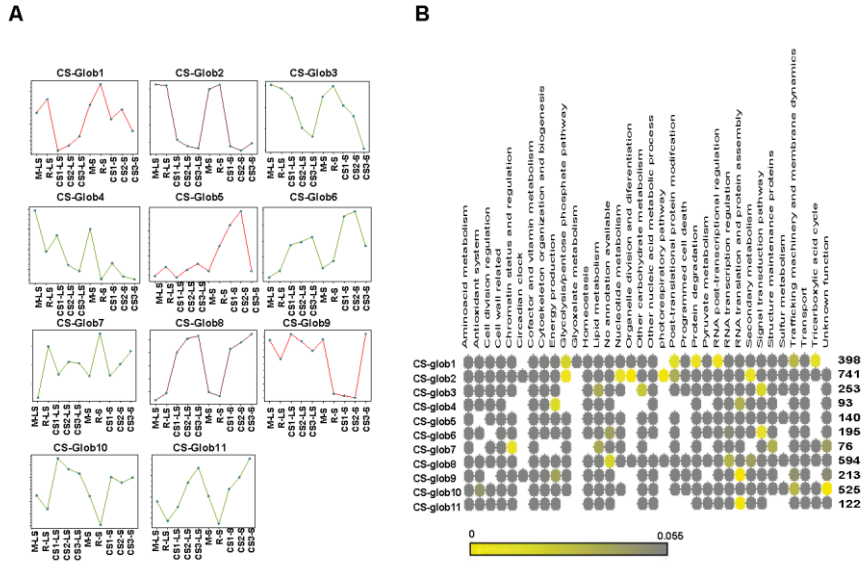


Figure 3. Clusters resulting from the unsupervised two-dimensional hierarchical clustering (Fig. 2C). Y-axes represent the normalized expression ratio (Log2 M) of three biological replicates in relation to a reference pool. Red represents qualitative differences, purple depicts the genes regulated in a similar manner and green refers to the genes showing quantitative differences between the LS and S pools. D) The functional categories overrepresented in each cluster (Fig 2C) are shown as a heatmap obtained with matrix2png. Enriched functional categories with Fisher test p-values < 0.05 are colored in grades of yellow. The number of genes in each cluster is indicated to the right of the heatmap. M = mature fruits, R = mature with 2-4 days ripening at 20°C, CS1 = M + 1 week cold storage at 5°C, CS2 = M + 2 weeks cold storage at 5°C, CS3 = M + 3 weeks cold storage at 5°C.

The bidimensional hierarchical cluster (2D-HCA) analyses revealed a similar sample separation to that obtained with PCA (Fig. 2C). Furthermore, 2D_HCA segregated CS1-LS from the rest of the cold-stored fruits (Fig. 2C), according to the fact, that if fruits CS1-LS ripen, they do not develop mealiness. These results indicate that from the molecular point of view one week of cold storage is a critical time with maximum differences expected to be found at this stage between fruits S and LS, including any CI

associated trait. This global analysis also revealed that, although the expression profiles were generally similar between the S and LS pools of fruits, there were qualitative and quantitative differences (i.e., the kinetics or levels reached, or both).

To further describe the cold response mechanism from a global point of view and its possible relation to eventual CI, we conducted a functional enrichment analysis (Fig.3B) of the 11 resulting clusters from 2D-HCA (Fig.3A). The most overrepresented functional category in cluster CS-glob8, containing genes up-regulated during cold storage in both fruit pools, was *RNA transcription regulation*, which comprised 47 genes (Fig.3B). In this category, we found several transcription factors whose orthologs were up-regulated during cold acclimation in *Arabidopsis* and some were assigned to specific cold acclimation regulons (Table 3). The other functional category enriched in CS-glob8 was with 37 genes, *secondary metabolism,* a functional category previously associated with cold tolerance [196, 197].In addition, and in agreement to the higher tolerance of fruits LS, *structure maintenance proteins* and *an antioxidant system* were among the functional categories overrepresented in differential clusters CS-glob7 and CS-glob 10 (Fig.3B), both highly induced in the pools of fruits LS as compared to fruits S (Fig.3A). Moreover, cluster CS-glob 9 was enriched in *RNA translation and protein assembly, energy production,* and *trafficking machinery and membrane dynamics* (Fig.3A and 3B), indicating that these activities can be enhanced in fruits LS. This suggests that some kind of cold adaptation was activated in both S and LS peach fruits during cold storage.

The genes in cluster CS-glob 2 were down-regulated in both S and LS fruits (Fig.3A), and were enriched in *glycolysis/pentose phosphate pathway, the photorespiratory pathway* and *organelle division* (Fig.3B). Lowered expression levels of carbohydrate metabolism and glycolytic genes correlated to cold sensitivity in Arabidopsis [196]. However, the extensive down-regulation of primary metabolism, together with the down-regulation of *posttranscriptional, posttranslational and protein degradation* (see cluster CS-glob 1 in Fig.3A and 3B), was probably associated with the relative higher cold tolerance of fruits LS.

C1.1.3.Stage-specific changes in the transcript program associated with the differential cold response

A direct one-to-one comparison was made between the transcriptomes of the samples S and LS at the same time of cold storage, given the notion that this analysis would outperform the general profile comparison to identify the candidates to be involved in tolerance/susceptibility to cold (before obvious injury symptoms appear). Figure 4A shows how the number of differentially expressed genes at each time decreased with storage time (Fig. 4A), thus confirming PCA results (Fig.2B). Functional enrichment analysis (Fig. 4B) showed that by 1 week of cold storage, the transcripts with higher levels of expression in fruits CS1-LS were preferentially related to *energy production, RNA translation and protein assembly, the antioxidant system, structure maintenance, and genes with unknown functions* (for more details, see Table 5 and Table S2). Those transcripts with lower levels in LS fruits (and therefore higher levels in S fruits) were enriched in *signal transduction elements and transport* (Fig. 4B and Table 6). As 1 week cold storage is critical timing i.e. when maximum differences were shown when later transferring fruits to shelf life ripening (Fig.4A-B), these functions may play a prominent role in the tolerant/sensitive character of fruits (for more details of these genes, see Table 5, 6 and Table S2).

By 2 weeks of cold exposure, only the genes with unknown functions were overrepresented in the tolerant pool (Fig. 4B), whereas a significant enrichment was noted for the genes linked to *amino acid metabolism, pyruvate, signal transduction and transport* in the genes at higher levels in CS2S. Interestingly, most of the genes expressed at higher levels in S fruits by 2 weeks had already reached this state by 1-week of cold storage (Table S2). As two weeks of cold exposure results in mealiness upon shelf life in both S and LS fruits (Fig.2A), but with large differences in MI severity, high levels of these genes may correlate negatively with the tolerant character of fruits. After 3 weeks in the cold, only the highly expressed genes in tolerant fruits showed *signal transduction* as an overrepresented class (Fig. 4B). In this case, the genes differed from those identified as being overrepresented at 1 and 2 weeks (Table S2). At this time, both S and LS developed mealy fruits with MI 1.0 and MI 0.8, respectively

(Fig.2A), but S was probably much more severely affected or underwent other downstream processes.

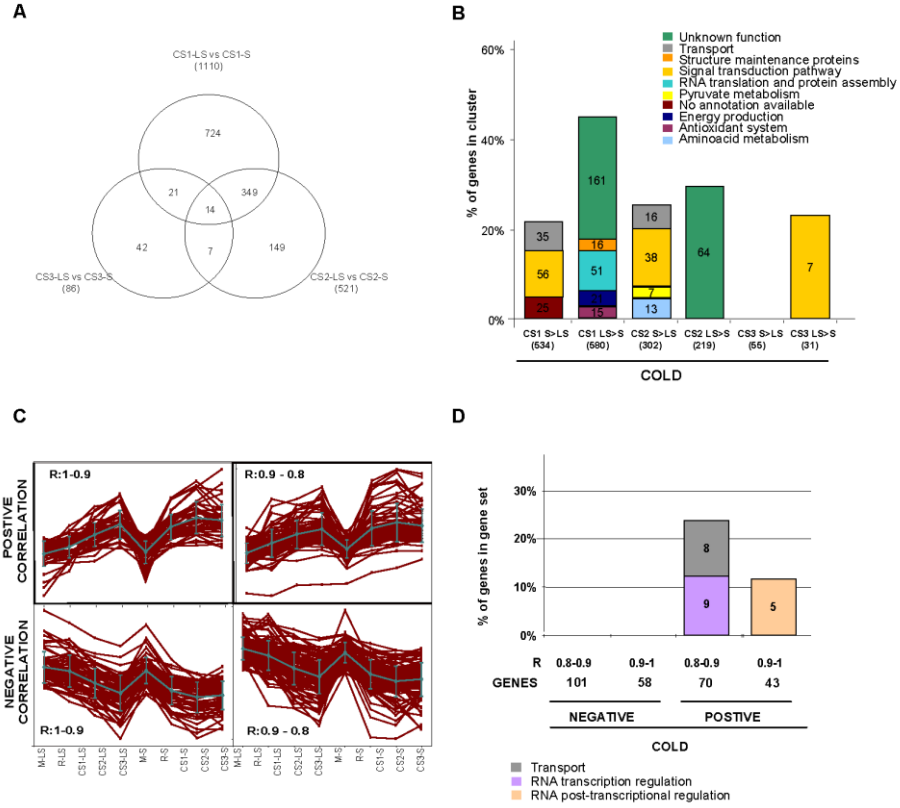


Figure 4. Differential gene expression between the S and LS fruit across the cold storage CS series. A) A Venn diagram depicting the differentially expressed genes (FDR<0.05 and q-value<0.05) between tolerant and sensitive fruit at each time of cold storage. B) The over-represented functional categories (p-value 0.05) corresponding to the differentially expressed genes between the LS and S pools at each time of cold storage. C) The expression profiles of the genes correlating to protracted MI in samples M and CS. Phenotype-gene profile similarities were measured as Pearson correlation coefficients from the global dataset. D) The functional categories enriched in the genes whose expression profiles correlated with the projected MI fruits should have when shelf life ripened. Pearson: 1<r <0.9 and 0.9< r 0.8. M = mature fruits, R = mature with 2-4 days ripening at 20°C, CS1 = M + 1 week cold storage at 5°C, CS2 = M + 2 weeks cold storage at 5°C, CS3 = M + 3 weeks cold storage at 5°C.

In order to analyze if the transcript program in the cold may have a direct effect on eventual mealiness development during shelf life, a Pearson correlation analysis was

Table 3. Transcription Factors genes common Cold-Upregulated in Peach with Stress and Hormone Related Roles.

	Chillpeach ID	Gene description	Arabidopsis Gene Symbol	HCA pattern	CS1 S vs LS pattern	stress/hormone	Cold regulon	References
AP2/EREBP family	PPN039F03	Putative dehydration-responsive element binding protein	RAP2.4	CS-glob8	N/A	CA-DR, drought, light, ethylene		[198],[199],[95],[200]
	PPN078E06	EREBP-4 like protein		CS-glob8	N/A[c]	CA-DR		[201],[95]
AUX/IAA family	PPN046H05	Auxin-responsive protein IAA13	IAA13	CS-glob8	N/A	AUX negative regulation		[202, 203],[204]
C2C2-CO-like Family	PPN075B03	zinc finger (B-box type) family protein	STH2	CS-glob8	N/A	CA-DR, light		[198],[201],[95],[205]
C2H2 Family	PPN046D02	Zinc finger protein 4	ZFP4	CS-glob8	N/A	CA-DR		[95]
	PPN053C05	Zinc-finger protein 1	AZF2	CS-glob8	N/A	CA-UR		[92],[95],[206]
GRF-family	PPN044H02	14-3-3 protein 3	GRF2	CS-glob8	N/A	CA-DR		[95]
HD-ZIP family	PPN047H02	Homeobox-leucine zipper protein HAT22	HAT22	CS-glob8	N/A	drought, light, carbon sensing		[207],[208]
HSP-family	PP1002D06	Heat shock factor	HSFB1	CS-glob8	N/A	high up-regulated in Arabidopsis *chs* mutants		[209]
	PPN001A09	Heat shock factor	HSFB1	CS-glob8	N/A	high up-regulated in Arabidopsis *chs* mutants		[209]
	PPN054G07	Heat shock factor	HSFB1	CS-glob8	N/A[c]	high up-regulated in Arabidopsis *chs* mutants		[209]
	PPN055B05	Similarity to heat shock transcription factor	HSFC1	CS-glob8	N/A	CA-UR	ICE1	[94]
	PPN077H06	Heat shock transcription factor	AT-HSFA4A	CS-glob8	N/A	CA-UR, high up-regulated in *hos15* mutants	HOS15	[201],[59]
MADS-box family	PPN004D05	MADS box transcription factor	SVP/AGL22	CS-glob8	N/A	CA-UR		[198],[95]
	PPN058B02	MADS box transcription factor	AGL24	CS-glob8	N/A	cold up-regulated (vernalization)		[210]
MYB-family	PP1006F11	MYB1	ATMYB6	CS-glob8	N/A	CA-DR		[95]
NAC-family	PP1001F06	NAM-like protein	ATNAC2 /anac056	CS-glob8	N/A	CA-DR		[95]
	PPN054B06	No apical meristem protein-like	anac073/ SND2	CS-glob8	N/A	CA-DR		[95]
	PPN073C10	NAM-like protein	anac083/VNI 2	CS-glob8	N/A	CA-DR, ABA-mediated abiotic stress		[95],[211]
PHD-family	PPN035F03	hydroxyproline-rich glycoprotein family protein	EDM2	CS-glob8	N/A	defense to pathogens		[212]
	PPN051C10	ABI3-interacting protein 2	AIP2	CS-glob8	N/A	CA-UR	ICE1	[94]
TUB-family	PPN066C05	Tub family, putative	AtTLP1	CS-glob8	N/A	CA-UR		[95]
WRKY-family	PPN001D05	DNA binding protein WRKY2	WRKY3	CS-glob8	N/A	CA-DR		[95]

[a] contribution to PC2 (Fig 2A) negative; [b] negative correlation with projected MI

Arabidopsis response during cold acclimation:.CA-UR cold acclimation up-regulated

conducted between the gene expression values and the projected MI will be achieved when subjected to shelf life ripening after cold exposure (the actual MI of cold stored samples were 0 as shelf ripening is required to develop mealiness). This "projected MI" correlation analysis resulted in 113 directly correlated genes (R>0.8) and 159 inversely correlated genes (R>0.8) according to their pattern of expression in the cold (Fig. 4C; Table S2). The functional enrichment analysis (Fig. 4D) indicated that genes directly correlated to projected MI were enriched in *RNA transcription* and *RNA posttranscriptional regulation.* A further inspection revealed genes related to *RNA biogenesis and processing, splicing, RNA transcription machinery* and the *transcription factors* (Table S2). In addition, genes correlated positively with the projected MI were also enriched in *transport* category (Fig. 4D), that includes transporters for auxin, anthocyanin, amino acid, peptides, sulfate, carbohydrates and metal-ions (see Table S2). No functional enrichment was observed for those genes which correlated negatively with projected MI (Fig. 4D). However, a detailed inspection indicated that this set of genes contained calcium-related genes, including a transcription factor of the CAMTA family, and genes related to antioxidant systems (Table S2) which could participate in the regulation of this transient tolerance mechanism.

C1.1.4.Is there a preprogrammed mechanism for chilling tolerance?

The possibility that, in addition to cold-inducible mechanisms, some sort of tolerance mechanism may already be partly preprogrammed in tolerant fruits was investigated. The direct comparison between S and LS fruits at mature stage (M) resulted in 63 differentially expressed genes (Fig. 4A and Table S2). Out of them, 13 genes we high expressed in fruits T (Table S2) and some have to do with flavonoid metabolism (CHS/TT4 and GST12/TT19), structure protection (Tic110) and (ASN1/DIN6) that forms part of a cycle that generates asparagine for more energy-economical nitrogen remobilization under darkness and stress conditions [213, 214]. Several cell wall modifying activities were also differentially expressed between fruits S and LS (Table S2). As no differences at the maturity stage were between pools (Table 1), it is likely that differences in the expression levels of these genes at harvest may protect fruits

and/or contribute to develop the tolerance program at least in the early stages of the cold response.

HCA of samples M, R and CS (Fig. 5A) showed that genes differentially expressed

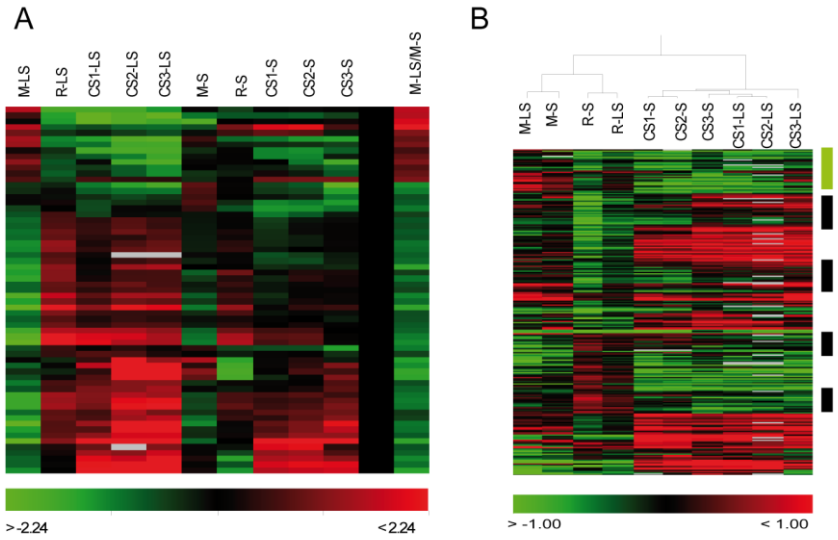


Figure 5. Preformed mechanisms and effect of ripening. A) The hierarchical cluster of the 63 genes differentially expressed between fruits LS and S at the mature stage. The expression values for samples M, R and CS and the M-LS vs. M-S ratio are shown. Hierarchical clustering of the expression values for 862 ripening genes (up or down in fruits R respect to M) during cold storage. M = mature fruits, R = mature with 2-4 days ripening at 20°C, CS1 = M + 1 week cold storage at 5°C, CS2 = M + 2 weeks cold storage at 5°C, CS3 = M + 3 weeks cold storage at 5°C.

between fruits S and LS at harvest qualified in fruits LS as ripening genes (see columns 1 and 2 column in the cluster; Fig. 5A). Notwithstanding, it is most interesting to note these genes were characterized by continuing the ripening program during cold storage (see the CS-LS samples and compare with R-LS), which did not happen so clearly in fruits S (compare the CS-S samples and compare with R-S). However and as expected this behavior of the differential M genes is the exception rather than the rule for ripening genes. As seen in Figure 5B, a similar analysis with a set of 862 ripening genes (up or down regulated in R by comparing to M) showed that although cold affect the expression many of ripening genes, is quite effective stopping the molecular

ripening program in fruits LS. This result is in agreement with the findings from PC2 (see Fig.5B). The main expression differences between LS and S fruits involved changes occurring in the same direction in R and cold stored fruits. In fruits LS, the expression of several ripening genes during cold storage remained at the same or higher level that they were in the M stage, but achieved similar expression levels to fruits R in the sensitive backgrounds (black bars in Fig 5B). Apart from the delayed or attenuated ripening program in the fruits LS during cold storage, these fruits also showed specific ripening processes that became activated during cold storage (green bar in Fig 5B), which is in agreement with the findings for genes differentially expressed at harvest (Fig 3A). A more detailed analysis of shelf life ripening conditions and mealiness is addressed in chapter 3

C1.1.5.Cold regulons in peach contributing to the differential response

In this section we wanted to see if there were similarities between the adaptation mechanisms operating in peach fruits stored in cold and darkness and those well-characterized in the cold acclimation of *Arabidopsis* plants grown in day/night regimes. We wanted to see if the patterns of gene expression for the peach homologues of Arabidopsis genes in cold/dehydration regulons were consistent with the differential cold responses in S and LS peaches.

First we analyzed the overlap between the response of cold stored peach fruits and those to various stimuli, including abiotic/ biotic stresses and hormones (Fig. 6). Gene-by-gene comparisons revealed that the vast majority of the cold-regulated genes in our peach cold storage experiment have *Arabidopsis* orthologs, which have been described as being regulated by cold (63%, Fig. 6A), or by ABA (35%, Fig. 6B). Similarly to *Arabidopsis* [198], approximately 30% of peach cold-regulated genes were found to be associated with drought and/or salinity treatments (Fig. 6A). More strikingly however, approximately 35% of the cold-responsive genes in peach were known pathogen-responsive genes or have been postulated to play a role in pathogen resistance (Fig. 6A). Furthermore, the genes described as being regulated by darkness in *Arabidopsis* account for up to 3.7% of peach cold-regulated genes (Fig. 6A), indicating that,

although its contribution to all cold-regulated genes was less than those also involved in other stresses, dark stress could contribute to the differences observed in the cold response between peach fruits (dark) and *Arabidopsis* plants (light).

Second, a list of *Arabidopsis* genes reported in cold regulons (CBF, ZAT12, HOS9, HOS15 and GI) and dehydration regulons (ESK1, AREB/ABF, MYC- DREB2, ZF-HD/NAC and CBF4) (see Table S3 and references within) was used to identify homologous peach genes that were present on Chillpeach microarray (see Table S4). In total, 163 Chillpeach unigenes corresponded to the genes found in at least in one of the previously defined cold and/or dehydration *Arabidopsis* regulons (Table S4). The expression profiles of these genes in response to cold storage were compared to those described for *Arabidopsis* (related either to non treated plants or cold-sensitive *Arab* mutants, or both) and scored as matching when they behave similarly. More than 60% of the genes associated to the regulons CBF, HOS9, ICE and DREB2 correlated well with both the known *Arabidopsis* WT cold response pattern and the *Arabidopsis* mutant expression (Table 4). That is, the ortologs genes to those up-regulated in *Arabidopsis* in response to cold showed higher expression levels in LS peach fruits than in high sensitive ones, while the genes down- regulated in *Arabidopsis* had higher levels in high sensitive peach fruits than in low sensitive ones. In contrast, most of the genes in HOS15, ZAT12, ESK, AREB, MYB, ZF/HD-NAC presented low correlation levels (Table 4). Therefore, these latter are less likely to contribute to the differences in response to cold between the S and LS pools of fruits.

The individual participation of each regulon to the differential response to cold between fruits S and LS was assessed by studying their contribution to the traits/trends observed in the global dataset analysis. For this purpose, we performed both PCA and 2D-HCA (Fig. S1 in File S1) using the gene expression values for all the genes in each regulon as input datasets and quantitatively evaluate the importance of each regulon (i) to discriminate samples S from LS and (ii) to separate the samples that would eventually became mealy, or not, by assessing by the number of genes well

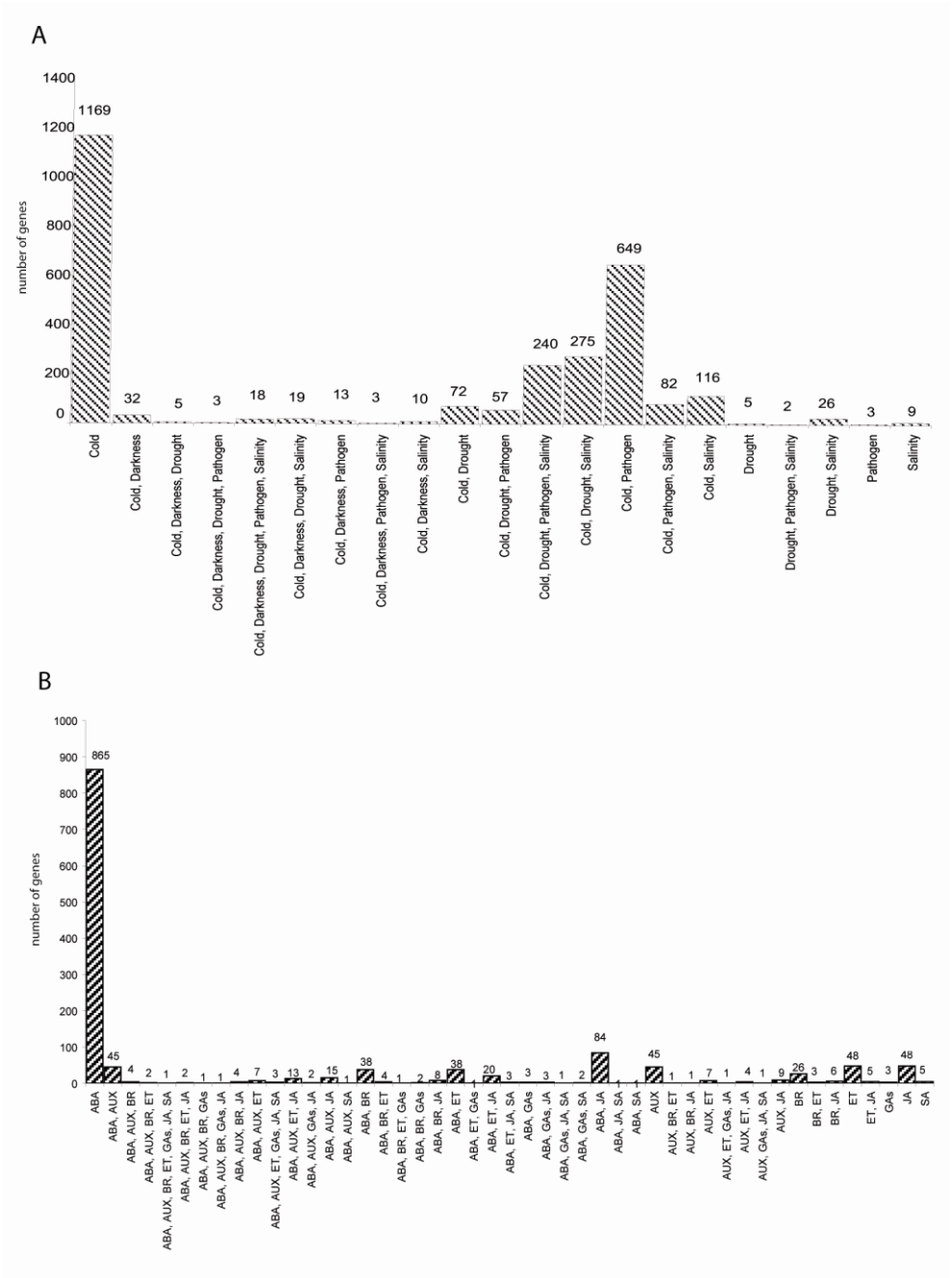


Figure 6. Comparison of the chillpeach data with the available microarray public domain data. A) The differentially expressed peach genes in the global analysis (Fig. 2 and 3) and reported as cold and/or Stress Response genes. The differentially expressed peach genes in the global analysis (Fig. 2 and 3) and reported as hormone responsive genes.

correlated with *Arabidopsis* in the gene expression models (the PCA and 2D-HCA in Fig. S1). The importance to discriminate samples S from samples LS (Table 4) was calculated by multiplying the number of genes that correlated well by the variance explained by PC2. The importance of an operon to separate the samples that would eventually become mealy, or not (Table 4), was quantified by dividing the number of genes in that operon that correlated well by the weight of the nearest node to CS1-LS. Both PCA and 2D-HCA revealed that regulon ICE1 was the one most contributing to discriminate samples LS and S, as to separate samples CS1-LS from the rest of cold-stored fruits that developed mealiness when submitted to shelf life ripening (Table 4). Furthermore, this analysis also indicated that the regulon CBF1 was the next major regulon in discriminating between samples LS and S (Table 4), while emphasized the relevance of HOS9 to separate CS1-LS from the remaining samples (Table 4). The rest of the cold operons produced no such separation between CS1 S and LS, or did so, but to a lesser extent (Table 4 and. Fig. S1 in File S1). The expression pattern of the subsets the genes appertaining to the regulons ICE1 (46 genes), CBF (31 genes) and HOS9 (13 genes) across the different samples (Fig. S2 in File S1) showed that although extended exposure to cold debilitated the response of ICE-CBF regulated genes, fruits LS were able to maintain a longer and greater response for many of the genes in the(se) regulon(s) in the cold. In the case of HOS9 regulon, many of its members were up-regulated or without change in LS fruits as compared to M fruits (Fig. S2C in File S1).

C1.1.6.Validation and extension of microarray expression profiling

The same bulked samples used in this microarray experiment were used to validate the results by using medium-throughput qRT-PCR (Biomark Dynamic Array, Fluidigm) over a set of genes (Table S5) selected because they 1) contributed to separate samples S from samples LS at 1 week of cold storage (Fig.2A and 4A), 2) showed a differential expression in, both, the M stage and 1-week of cold storage (Fig. 4A and 5A), and 3) showed differences at harvest (candidates to the preprogrammed mechanism of tolerance; Fig. 5A).

Table 4. Cold Regulons in Peach Fruits contributing to the Differential Response to Cold Storage

		Data for genes in each regulon and percentage of correlation			PCA					Hierarchical cluster		Importance of regulon in to	
DATASETS	Regulon	Genes in Arabidopsis	Genes found in chillpeach	% of genes well correlated	PC1	PC2	PC3	PC1 separate CS1-LS from CS1-S	PC2 separate CS1-LS from CS1-S	Weight of the nearest node to CS1-LS	CS1-LS branched out of CS samples	Discriminate S from LS samples	Separate samples that will become mealy or not
Cold and dehydration regulons	all	1236	163		64.9	14.0	9.5	●	√		√		
Cold	CBF	187	31	74.19	65.0	15.0	9.0	●	√√	0.69	√	3.45	33.33
Cold	HOS9	154	14	78.57	70.5	18.4	5.2	√	√√	0.142	√	2.024	77.46
Cold	HOS15	135	10	40.00	60.0	20.0	10.0	●	●	0.85	●	0.8	4.71
Cold	ICE1	369	46	60.87	55.0	24.0	10.0	●	√√	0.31	√	6.72	90.32
Cold/Dehydration	ESK1	310	42	47.62	75.0	11.0	6.0	●	●	0.671	●	2.2	29.81
ABA DEPENDENT	AREB	99	17	52.94	66.0	21.0	5.0	●	√√	0.696	√	1.89	12.93
ABA DEPENDENT	MYC-MYB	35	8	37.50	66.0	22.0	7.0	●	√	0.942	●	0.66	3.18
ABA INDEPENDENT	DREB2	45	10	70.00	70.0	16.0	7.0	●	●	0.601	√	1.12	11.65
ABA INDEPENDENT	ZF-HD/NAC	83	17	35.29	67.0	18.0	7.0	●	√	0.934	●	1.08	6.42
Cold	ZAT12	26	3	33.33									
Cold	GIGANTEA	1	1	100.00									
ABA DEPENDENT	CBF4	78	1	0.00									

√, the property analyzed is fulfilled. ●, the property isn't fulfilled. √√ indicates that the property is fulfilled but there is a high degree of separation between samples CS1-LS and CS1-S.

The table shows the number of members of each regulon described for Arabidopsis, the number of genes found in Chillpeach, the number of genes whose expression correlated with those described for the Arabidopsis WT, PCA and 2DHCA properties. The importance of each regulón based on both the variance explained by component 2 of the PCA and the weight of the nearest node to CS1-LS. For each dataset, it is indicated if the genes in the dataset fulfill the PCA and cluster properties or not.

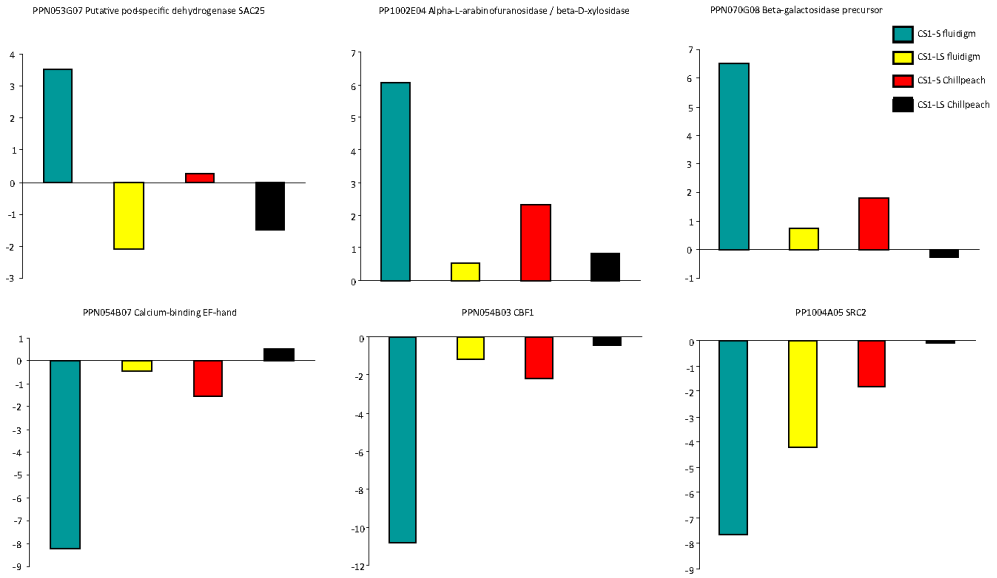


Figure 7. The differences between the microarray and qRT-PCR results in the magnitude of the expression levels for a selected number of genes. The y-axes represents the normalized fold expression change respect to the levels in the reference pool.

M stage and 1-week of cold storage (Fig. 4A and 5A), and 3) showed differences at harvest (candidates to the preprogrammed mechanism of tolerance; Fig. 5A). In order to examine at the single sibling level the reliability of the differential gene expression patterns obtained from the pools, the analysis was performed also on 15 individual genotypes of the pop-DG population (those used in the pools and others showing differences in mealiness phenotype). The qRT-PCR results obtained from the pools and from the individual lines making up this pools indicate that 72.5% (50 of 69) of the genes had the same expression pattern in the microarray experiment as in the qRT-PCR experiment (Table S6). However, the magnitude of expression varied slightly in many of the genes and samples tested (Fig. 7). Furthermore qRT-PCR experiments conducted on individual pop-DG siblings revealed that 42 out of the 50 genes validated in the pools were consistent with the expected patterns for which they were selected (Fig. 8). These results support the validity of our approach and indicate that the genes selected from the microarray analysis approach and indicate that the genes selected from the microarray analysis could be either involved in chilling tolerance and/or be

associated with the differential response to chilling response, and for some of them could even prove to general enough to hold true in individual fruits/plants.

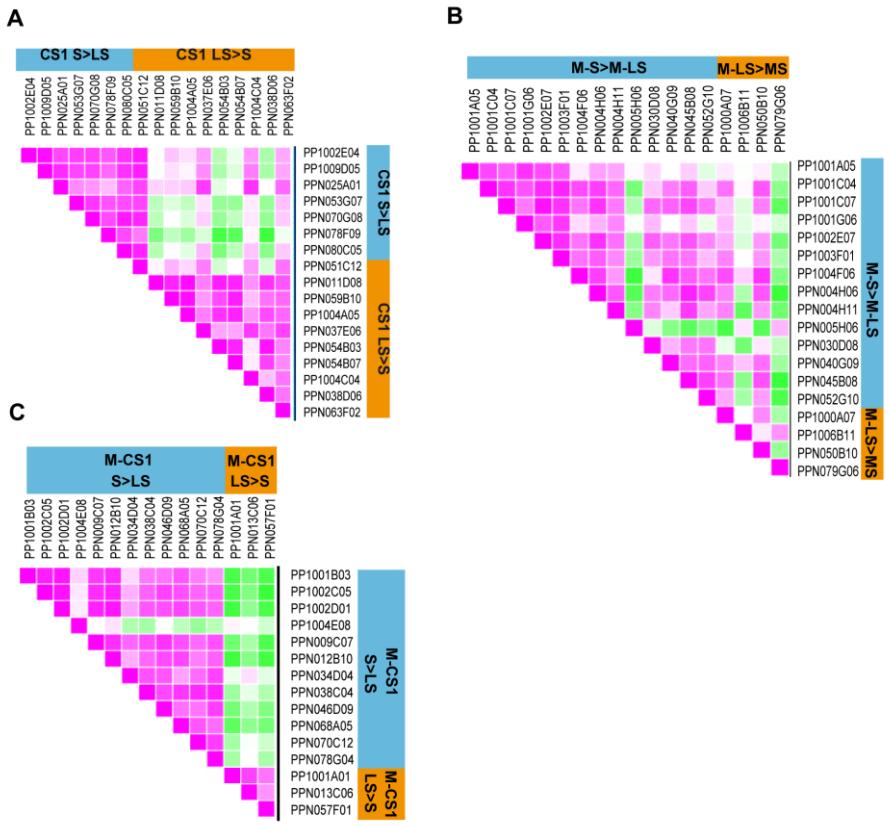


Figure 8. Degree of association between the genes validated by Fluidigm in a pre-defined expression pattern from the pools in the microarray and in individual Pop-DG siblings. A) The differentially expressed genes at 1 week of cold storage; B) B) The differentially expressed genes in the M stage and at 1 week of cold storage; C) The differentially expressed genes in the M stage. The Heatmap values correspond to the Pearson correlation coefficients between pairs of genes. For each gene in a gene set, the expression profile from the microarray results was defined and the Pearson correlation coefficients were calculated for pairs of genes in the individual sibling lines

C1.2.Discussion

C1.2.1.Considerations about the experimental approach

Since cold induced mealiness is not observed until the cold stored fruit are allowed to ripen, the chilling sensitivity phenotype of each fruit in the cold was estimated from the protracted mealiness incidence observed for equivalent fruit samples after shelf life ripening (Fig 2A). Although mealiness, probably, a downstream effect of cold stress in peach fruits MI is the best phenotyping tool to assess the effect of cold on peach fruit, and has be used successfully to identify CI QTLs in peach [4, 6]. A similar case can be build for for the growth retardation of the electrolyte leakage used to measure the effect of cold in vegetative tissues such as *Arabiodpsis*),

For BSGA we use Chillpeach microarray [17], interrogating part of peach genome. This provides only an incomplete picture of the genes behind the process; that is partially compensated by Chillpeach microarray being enriched in fruit-specific and cold responsive genes [17].

Our study differs from prior peach transcriptomic analyses in two ways. First, we are using samples from pools of genetically related siblings with contrasting sensitivity to chilling injury subjected or not to cold storage. Thus we expect to reveal genes whose expression patterns are linked to the different cold sensitivity, while leveraging transcript differences associated with other phenotypic traits, as it would be the case when comparing only two peach cultivars that have different chilling susceptibilities in addition to other phenotypic differences. Second, by medium-throughput qRT PCR we extended our microarray (Figure 8 and Table S6) results derived from the comparison of the contrasting pools to a relatively large number of 15 individual lines from the same population differing in the mealiness sensibility and the gene expression results of the selected genes were consistent with their individual sensitivity level.

C1.2.2.Cold storage conditions induces an acclimation program in peach fruits only to be more effective in tolerant than in sensitive fruits

Orthologs of several transcription factors (TF) found up-regulated similarly in S and LS cold-treated fruits (Table 3) have been previously reported as being up-regulated during cold acclimation in *Arabidopsis* (see Table 3 for references) and some of them also were described as belonging to a given cold acclimation regulon [59, 94]. This suggests the activation of a cold response program in peach fruits in part similar to those described for *Arabidopsis* cold acclimation. Despite observing similarities some genes exhibited an opposite trend compared to *Arabidopsis* (Table 4) which may partially reflect the sensitive character of peach fruit to cold (both LS and S fruits are sensitive, but LS fruits are relatively more tolerant than S). Several studies have associated cold tolerance and cold acclimation the transcriptional activation of genes encoding heat-shock proteins (HSPs), chaperonins, LEA proteins, antioxidant/scavenging systems and related to protein synthesis [20, 28, 209, 215]. Genes in these functional categories were generally down-regulated by cold storage in both LS and S fruits, what correlates well with their sensitivity to cold. Further, the orthologs of HSF4B and HSP21 (Table 3) were up-regulated peach fruits, whilst were down-regulated in Arabidopsis. This is particularly interesting as these genes are highly up-regulated in *Arabidopsis* chilling sensitive mutants upon chilling treatment [209],[59]. It should be noted that we are comparing the transcriptomes of different species and tissues at various physiological and growth stages, and it is likely that some differences in strategies (efficient or not) to cope with exposure to low temperatures operate in each case [216].

The basic question is: why do LS Pop-GG siblings tolerate better cold storage than S? Our results indicate that during cold storage fruits LS maintain higher levels of expression for a series of components of the antioxidant system, structure maintenance proteins and protein synthesis at least during the first week of storage (Fig. 3D, 4B and Table S2). In addition, the orthologs of some TF with a higher expression levels in tolerant peach fruits (Table 5) have been reported to be up-regulated by cold and/or other biotic or abiotic stresses in Arabidopsis (Table 5).

Table 5. Expression Regulators and Signaling Elements with High Expression in Low Sensitive Fruits at One Week of Cold Storage with stress and hormone related roles.

	Chillpeach ID	Gene description	Arabidopsis Gene Symbol	HCA pattern	CS1 S vs LS pattern	stress/hormone	Cold regulon	References
RNA post-transcriptional regulation								
RNA biogenesis and processing	PPN035E09	Dehydration-induced protein	ERD15	CS-glob9	LS>S	negative regulator ABA		[217]
	PPN048C02	Sm-like protein	SAD1	CS-glob10	LS>S	negative regulator ABA		[218]
RNA transcription regulation								
AP2/EREBP family	PPN049D05	similar to DREB3		CS-glob8	LS>S	cold, drought, salinity		[219]
	PPN054B03	CBF1	DREB1A/CBF3	CS-glob9	LS>S[b]	CA-UR, AUX down-regulated	ICE1/ CBF	[49],[220],[194],[52],[221]
AUX/IAA family	PP1009D02	IAA16 protein	AXR3/IAA17	CS-glob9	LS>S	negative regulator in AUX and ABA signaling		[222]
	PPN057F01	AUX/IAA protein	PAP2/IAA27	CS-glob4	LS>S	light		[223],[224]
b-ZIP family	PPN049B04	BZIP transcription factor bZIP68		CS-glob10	LS>S	light, cold		[225],[226]
C2C2-CO-like Family	PPN050G11	Znfinger (B-box type) family protein	AT4G27310	CS-glob10	LS>S	cold	AREB/ ABF	[227]
CAMTA family	PPN075B05	Anther-ethylene-up-regulated protein ER1	SR1	CS-glob2	LS>S[d]	cold up, salinity, defense and ET		[228],[229]
CCAAT Family	PPN006E07	Repressor protein	NF-YB13	CS-glob10	LS>S	darkness		[230]
HMG-family	PPN042B12	HMG-protein	HMGB1	CS-glob2	LS>S[d]	stress		[231]
MYB-family	PPN041A07	myb family transcription factor	CDC5	CS-glob9	LS>S	defense responses, light, cold		[232],[225],[226]
	PPN055C11	Sucrose responsive element binding protein	ATMYBR1/ATMYB44	CS-glob10	LS>S	cold	ICE1	[94]
PHD-family	PPN051C09	PHD finger protein At5g26210	AL4	CS-glob3	LS>S	cold, salinity and ABA		[233]
	PPN068F05	PHD finger protein At5g26210	AL4	CS-glob10	LS>S	cold, salinity and ABA		[233]
RNA transcription machinery	PPN027A09	Sigma-like factor precursor	ATSIG5	CS-glob2	LS>S	light		[234]
Protein degradation								
Proteolysis control-Signalosome	PPN042D08	COP9 signalosome complex subunit	COP9	CS-glob8	LS>S	light		[235]
Signal transduction pathway								
ABA signaling/reversible protein dephosphorylation	PP1009B12	Protein phosphatase 2C	ATPP2CA/AHG3	CS-glob10	LS>S	negative regulator ABA		[236]
	PPN029F02	Protein phosphatase 2C (AtP2C-HA)	HAB1	CS-glob3	LS>S	negative regulator ABA		[237]
Aux signaling/Unknown SAUR protein	PP1001B04	expressed protein (DUF298)	AAR3	CS-glob4	LS>S[d]	AUX response regulation		[238]
	PPN015D06	SAUR protein		CS-glob4	LS>S	AUX		
	PPN051E05	SAUR protein)		CS-glob2	LS>S	AUX		

[a] contribution to PC2 (Fig 2A) negative; [b] negative correlation with projected MI.
Arabidopsis response during cold acclimation:.CA-UR cold acclimation up-regulated

All this supports the idea of the existence of an acclimation program more effective in fruits LS. In this sense, our data indicated that the peach orthologs for genes in ICE1, CBF and HOS9 regulons may be implicated in the tolerance of fruits LS. The central role played by the ICE1-CBF cold response pathway in cold acclimation and cold tolerance is well-established in plants [47] and has been demonstrated to exist in a wide range of plants [105, 107, 117], although, there are differences in the regulation or the size of their CBF regulons [118, 120]. The existence of ICE-CBF pathway has been also confirmed in fruits [107, 117]. Further, LeCBF1 expression levels correlates positively with cold tolerance in tomato fruits [126]. We found that genes in the regulons ICE1and CBF were the most contributing to discriminate samples S from LS, and/or to separate samples that will become mealy, or not (Table 4 and 5). Moreover, PCA analysis identified CBF1 as the second gene that contribute the most to separate the S and LS series (Fig. 2B and Table S2) and qRT PCR analysis showed that the expression levels of CBF1(PPN054B03) correlate well with the tolerance/sensitivity of the individual pop-DG siblings (Fig. 8). Thus, confirming ICE-CBF as important actors in the differential response to chilling between peaches S and LS. In the case of the genes in regulon HOS9 our results suggest that it is more likely related with the ability to up-regulate or to maintain similar expression levels to those observed in M fruits (Fig. S2C). Zhu et al. [84] concluded that HOS9 must be important for both the constitutive expression and cold-induced expression of the genes that may be required for full tolerance to freezing stress. These results are consistent with peaches having the basic components of a cold response pathway, but additional studies will be required to elucidate their size and how they are regulated.

In normal commercial fruit operations cold storage, involves also complete darkness. Gene by gene comparisons has revealed that around 3% of our cold regulated genes in peaches could be related to darkness (Fig. 6A). Moreover, we identify some genes whose orthologs have been described in the regulation or in response to light (Table 3, 5 and 6). Several, light sinaling elements among which were GI [239], DFL2 [240], PHYA [241] and FYPP3 [242] were repressed by cold storage in both LS and S (Table S2), consistently with the storage in darkness conditions. In addition, genes differentially

expressed between fruits S and T include a number of regulators involved in light response (Tables 5 and 6) that indicates we should take into account this factor as contributing the differential response observed in peach fruits. In *Arabidopsis* light is required for cold induction of several genes involved in cold acclimation, including CBFs [81, 243] and some light signaling mutants have impaired cold acclimation [244]. Thus the differential response to cold storage of fruits S and LS probably have to do fruits' ability to deal with cold and darkness. However, further experiments are required to determine in more detail the nature of the interaction between the cold and the darkness during storage.

C1.2.3.Altered and continued ripening associated dehydration/osmotic stress could be related with the sensitivity of peach fruits to cold.

Despite no visible mealiness symptoms are observed during cold storage, the BSGA indicated dramatic changes in the peach transcriptome in response to the exposure to mealiness-inducing temperatures in a manner that these changes could be useful to predict future mealiness development (Fig. 2, 3 and 4). We propose the transcript differences observed while in the cold might underlie the molecular basis of a mealiness phenotype which is still undetectable, but will be fully developed later during shelf life. This is in agreement with previous reports of the cold induction of specific target genes that are associated with the mealiness disorder [12, 14]. Surprisingly, our results showed that cell wall is not found among enriched categories in none of the clusters/comparisons performed on cold stored samples, suggesting that although specific changes in cell wall remodeling transcript are detected (Table S2) most of the changes would probably occur during shelf life [135].

Our results reveal also that *transport* and *signaling elements* (Fig. 4B) presented higher levels in S fruits, which in some cases, correlated well with the eventual mealiness phenotype. We found the orthologs of genes described as positive regulators of ABA signaling and/or osmotic stress (Table 6) and transporters related to Na+ and K+, sugar and nitrate homeostasis (Table S2) among genes high expressed in fruits S This suggests that fruits S during cold storage undergo some sort of dehydration or osmotic

adjustment. It has been proposed that during cold storage, before mealiness is manifested, pectin depolymerisation but not de-esterification is inhibited [135, 143, 144], what may lead to the formation of gel-forming pectins that traps free water from the surrounding tissue. As no significant differences in global water content are found between LS and S fruits (A.Dagar, personal communication) it is likely that water is being lost from the cell to be trapped on the pectins of the cell wall, which still would be sensed as loss of internal water by the cell.

Among genes with higher expression in sensitive fruits we identified components of auxin and ethylene signaling cascades as well the orthologs of genes involved in the biosynthesis of ABA, auxin and ethylene (Table 6). We must highlight the large list of genes related to auxins among with were positive regulators of auxin responses and transporter locations (Table 6). In addition, among the genes high expressed in the fruits LS at one week (Table 5) there were the orthologs of genes such as HAB1 [237], PP2CA/AHG3 [236], SAD1 [218] and ERD15 [217], which have all been described as negative regulators of ABA signaling, and IAA17/AUX3, proposed to be a negative regulator in auxin and ABA signaling [222]. Ethylene and auxins has been described in the regulation of the ripening program of peach fruits [245] and their involvement in the cold response has been described for Arabidopsis [94, 98],tomato [127], apple [122] and peach [246]. Our results indicate that part of the ripening program probably continues during cold storage in sensitive fruits (Fig 2B and 5B). Hence, we could expect that interactions between cold and hormones controlling the peach ripening program, which are differential between fruits S and T, impact the way fruits respond to cold and ripen afterwards during shelf life. Because the activity of most of these genes is mainly determined at post-trasncriptional level reviewed in [247], it is not possible from expression data only to infer the role of these genes during cold storage. However from our data it is clear that all three hormones may play a role in regulating the differential response of peach fruits to cold and they seem operate in association with dehydration/osmotic stress. In support of that, the orthologs of many of hormone related genes higher expressed in CS1-S fruits have been described previously either in relation to drought and osmotic stress (Table 6).

Table 6. Expression regulators and signaling elements with high expression in high sensitive fruits at one week of cold storage with stress and hormone related roles.

	Chillpeach ID	Gene description	Arabidopsis Gene Symbol	HCA pattern	CS1 S vs LS pattern	stress/hormone	References
Energy production							
vacuolar ATP production and cytoplasmic PH regulation	PPN014F01	Vacuolar H+-ATPase subunit C	DET3	CS-glob5	S>LS	Light, AUX, ABA	[248],[249],[250]
Protein degradation							
chloroplast protease	PPN022B02	ERD1 protein, chloroplast precursor	ERD1	CS-glob1	S>LS	ABA, drought, salinity, dark induced senescence	[251],[252],[253]
peptidase	PPN007E05	aminopeptidase M, similar	APM1	CS-glob1	S>LS	AUX transport regulation	[254]
Proteolysis control-Signalosome	PPN008B05	COP9 signalosome complex subunit 2	FUS12/ ATCSN2	CS-glob1	S>LS	light	[255]
SCF complex assembly and disassembly	PPN068H05	Putative TIP120 protein	CAND1	CS-glob1	N/A	AUX signaling	[256]
Ubiquitin ligase E3 complex/SFC-culin	PPN030D09	Cullin	AXR6/ CUL1	CS-glob1	S>LS	AUX signaling regulation, light	[257],[258],[259],[260],[261]
	PPN032E01	Cullin family	CUL3	CS-glob1	N/A	ET production, light	[262],[263]
RNA transcription regulation							
ARF-family	PPN051B02	Auxin response factor 2	NPH4/ ARF7/TIR5	CS-glob5	N/A	AUX response regulator, cold	[264],[265],[266],[267],[94]
	PPN072B07	Auxin response factor 5	MP/ARF5	CS-glob7	S>LS	AUX signaling and transport regulator	[268],[269]
b-HLH family	PPN080F10	Prf interactor 30137	LHW	CS-glob6	S>LS	AUX signaling	[270]
GRAS-family	PPN078C08	GRAS1	SCL14/GAI/SCR	CS-glob8	S>LS	CA-UR	[198],[94],[95]
GroTLE transcription corepressor family	PPN076D05	Transcriptional corepressor LEUNIG	LUG	CS-glob1	S>LS	AUX signaling regulator	[271],[272]
HB-family	PPN069A12	BEL1-like homeodomain transcription factor	BLH1	CS-glob5	S>LS	drought, salinity	[273],[207]
MADS-box family	PP1009H08	MADS box transcription factor	AGL24	CS-glob8	S>LS	cold up-regulated (vernalization)	[210]
MYB-family	PPN058F01	GAMYB-binding protein	SKIP1	CS-glob1	S>LS	ABA, drought, salinity	[274]
NAC-family	PPN023B05	NAC domain-containing protein 78	NAC2/anac078	CS-glob1	S>LS	AUX, ET, salinity	[275]
	PPN062G07	NAC family protein	ATAF1	CS-glob2	S>LS	ABA, drought, salinity, pathogen	[273],[276]
RNA transcription machinery	PPN067A07	Elongator component	ELO1	CS-glob1	N/A	ABA, AUX	[277],[278]
	PPN070H08	C-terminal domain phosphatase-like 2	CPL2	CS-glob6	S>LS[a,c]	osmotic (salinity) stress and AUX responses	[279]
Unknown transcription coactivator	PPN063D04	COP1-Interacting Protein 7	CIP7	CS-glob2	S>LS	light	[280]

Secondary metabolism							
Aux metabolism/ Aux biosynthesis	PPN034D04	Flavin-containing monooxygenase, putative	YUC10	CS-glob8	S>LS	AUX biosynthesis	[281],[282],[283]
Aux metabolism/ Aux conjugation	PPN030D12	similar to Putative auxin-amidohydrolase precursor		CS-glob5	S>LS	AUX metabolism	
Aux metabolism/Aux deconjugation	PPN017F04	Auxin and ethylene responsive GH3-like protein	GH3.1	CS-glob1	S>LS	stress, AUX metabolism	[221]
Carotenoid metabolism	PP1005H08	Zeaxanthin epoxidase, chloroplast precursor	ABA1	CS-glob8	S>LS	ABA biosynthesis	[284]
Ethylene biosynthesis	PP1009G10	1-aminocyclopropane-1-carboxylate oxidase	EFE/ACO4	CS-glob2	S>LS	ET biosynthesis	[285]
Signal transduction pathway							
ABA signaling/ Ca signal transducer	PPN027B08	Calcium-dependent protein kinase	CPK32	CS-glob1	S>LS	ABA, salinity	[286]
	PPN029E04	GTP-binding protein-related, ..	MIRO2/ATCBG	CS-glob1	N/A	ABA, salinity	[287]
	PPN031C02	Rac-GTP binding protein-like	MIRO2/ATCBG	CS-glob2	S>LS	ABA, salinity	[287]
	PPN069F09	PK11-C1	OST1/ /SRK2E	CS-glob6	N/A	ABA, osmotic stress	[288],[289],[290]
ABA signaling/Casein kinase regulation	PPN057C06	casein kinase 1 protein family	CKL2	CS-glob1	S>LS	ABA regulation	[291]
ABA signaling/signal transducer	PPN021G09	Protein kinase	SNF1/SRK2I	CS-glob6	S>LS[c]	ABA, osmotic stress	[288],[289],[290]
Aux signaling/ Aux receptor E3 ubiquitin ligase SFC-TIR	PPN070C07	F-box containing protein TIR1	AFB5	CS-glob1	S>LS	AUX signaling	[292],[293]
	PPN078E01	TRANSPORT INHIBITOR RESPONSE 1 protein	TIR1	CS-glob6	N/A	AUX signaling	[294],[295]
Aux signaling/pin phosphorylation	PPN014G07	Serine/threonine-protein phosphatase 2A reg. sub. A beta	PDF1/PP2AA2	CS-glob6	N/A	AUX signaling	[296]
Aux signaling/Ubiquitin ligation E3 complex/ F-box	PPN026G02	Auxin-responsive factor TIR1-like protein	AFB2	CS-glob1	S>LS	AUX signaling	[297]
Calcium signaling/Calcium signal transducer	PPN011E06	CBL-interacting serine/threonine-protein kinase 11	ATSR1/CIPK14	CS-glob2	S>LS	cold, salinity and ABA	[298]
	PPN013H01	Serine/threonine kinase	CIPK10/ SIP1	CS-glob11	S>LS	cold, salinity and ABA	[298]
	PPN017F05	CBL-interacting serine/threonine-protein kinase 11	CIPK11/ SIP4	CS-glob6	N/A	cold, salinity and ABA	[298]
	PPN080C05	Protein kinase; NAF	CIPK1	CS-glob6	S>LS[a]	ABA, osmotic stress	[299]
Cyclic nucleotide signaling/(p)ppGpp-mediated response	PPN046D08	RelA/spoT-like protein RSH2	RSH2	CS-glob6	N/Ac	ABA, salinity, wounding	[300]
Ethylene signaling/ SCF(EBF1) E3 ubiquitin ligase	PP1005A04	Leucine Rich Repeat, putative	EBF1	CS-glob1	N/A	ET, cold	[301],[98, 302]
	PPN023E11	EIN3-binding F-box protein 1	EBF2	CS-glob5	S>LS	ET, cold	[301],[98, 302]

Ethylene signaling/ethylene receptor	PPN054G06	Ethylene receptor		CS-glob2	S>LS	ET	
	PPN057C10	Ethylene signaling protein	EIN2	CS-glob1	N/A	ABA, ET, cold, abiotic stress	[303],[304],[305],[306],[307],[246],[98]
	PPN079H05	Ethylene signaling protein	EIN2	CS-glob1	N/A	ABA, ET, cold, abiotic stress	
G-protein coupled receptor protein signaling pathway/G-protein complex	PPN005H05	Extra-large G-protein	XLG1	CS-glob1	N/A	osmotic stress, ABA	[308]
	PPN029C06	Extra-large G-protein	XLG1	CS-glob1	S>LS	osmotic stress, ABA	[308]
	PPN065B10	Extra-large G-protein	XLG3	CS-glob6	S>LS	osmotic stress, ABA	[308]
Light signaling/light receptor	PPN005E08	Cryptochrome 2A apoprotein	CRY2	CS-glob3	S>LS	Light, low temperature	[309],[310]
Light signaling/light transducer	PPN023G10	phototropic-responsive NPH3 family protein	3	CS-glob6	S>LS	light	[124]
Phosphorylation cascades/PP2A	PPN037E11	Serine/threonine protein phosphatase 2A reg. sub B' gamma	ATB' GAMMA	CS-glob1	S>LS	light, defense response	[311]
Phosphorylation cascades/PP2C	PP1005B01	protein phosphatase 2C, putative	PP2CG1	CS-glob6	S>LS	ABA, drought, salinity	[312]
Trafficking machinery and membrane dynamics							
ER to Golgi	PP1003D05	Root hair defective 3	RHD3	CS-glob5	S>LS	AUX, ET	[313]
ESCRT-dependent protein sorting and concentration	PPN005D10	Putative vacuolar sorting protein 35	VPS35A	CS-glob5	S>LS	AUX transport regulation	[314]
	PPN026H03	Putative vacuolar sorting protein 35	VPS35A	CS-glob1	S>LS	AUX transport regulation	[314]
Nucleocytoplasmic transport	PPN023D05	Peptidase S59, nucleoporin	SAR3/ MOS3	CS-glob1	N/A	AUX-regulated nuclear transport	[315]
Trans-Golgi network transport vesicle/ COPI vesicles	PPN002C04	ARF-GAP	SFC	CS-glob5	S>LS	AUX transport regulation	[316]
Transport							
Aux transport	PP1004E09	auxin efflux carrier family protein		CS-glob8	S>T[c]	AUX	
	PPN058C04	Auxin efflux carrier protein-like		CS-glob6	S>LS	AUX	
	PPN075H08	auxin efflux carrier family protein		CS-glob8	S>LS	AUX	
Fe-S cluster maintenance and response to far red light	PPN024F02	Protein NAP1, chloroplast precursor	NAP/LAF6	CS-glob3	S>LS	light	[317]
Lead tolerance	PPN032F06	PDR-like ABC-transporter	PDR12	CS-glob1	S>LS[a]	ABA, drought	[318]
Na/K antiporter	PPN064A01	Na+/H+ antiporter	SOS1	CS-glob1	S>LS	salinity, ion homeostasis	[319],[320],[321]

[a] contribution to PC2 (Fig 2A) negative; [b] negative correlation with projected MI

Arabidopsis response during cold acclimation:.CA-UR cold acclimation up-regulated

For example, the orthologs of SKIP [274], BRM [165] and ERD1 [251],[322]mediate the responses or are induced by ABA, salinity and dehydration stress; CPL2 modulates auxin responses, plant growth and osmotic (salinity) stress [279] and EIN2 has been described to be an important cross-link node for the interaction of ethylene, ABA and plant response to abiotic stress [323].

We cannot rule out that the "sensitivity" program is the consequence or the cause of low levels ICE1-CBF regulons. It is possible that the up-regulation of a set of common genes (cluster CS-glob8, Fig. 3A) concomitantly with low CBF levels triggers this program. It is also feasible that among CS1 S>LS there are genes which negatively regulate the CBF response. To support this, EIN2 (Table 6) has been described as a negative regulator of plant response to freezing stress by negatively regulating the expression of CBF1-3 and its target genes [98]; interestingly, CBF genes have been found to be directly repressed by IAA [221]. Finally, it may also be possible that this program is activated to compensate efficient acclimation during cold storage. It has been described that *hos9* mutants hyperactivate some cold-regulated genes through a compensating response to their increased cold sensitivity [84].

C1.2.4.A preprogrammed mechanism contributes to chilling tolerance

At the mature stage specific differences at the gene expression level between the pools of fruits S and T already exist (Fig. 5A). Although our approach used pools of fruits in accordance to how they respond to cold storage, therefore minimizing differences in other aspects between genotypes, we can't dismiss the possibility that these differences have nothing to do with adaptation to cold. Preformed mechanisms have been described in both biotic and abiotic stress tolerance [324-326] and we previously identified a subset of genes differentially expressed at harvest that correlate well with CI [134].

Cell wall metabolism has been extensively related to mealiness in peach fruits [135, 143, 144], and it has been reported that endo-polygalacturonase plays a qualitative role in the mealiness expression [4]. Our results indicate that the composition of the cell wall at harvest could play a role in the tolerance or sensitivity of peach fruits to

withstand cold storage. This is in agreement with previous results [134]. In addition the type of functional categories for the differentially expressed genes at the stage M, and the fact that most of these genes continue to show these differences during cold storage (Fig 5A and Table S2), suggest the possibility that a pre-programmed tolerance/sensitivity mechanism can be partly established previously to cold. Among the highly expressed genes in fruits LS at the mature stage, we found orthologs of genes such as CHS/TT4 and GST12/TT19 (Table S3), which have been described being essential for anthocyanin and proanthocyanin accumulation [327, 328]. Anthocyanins have been related with browning in peaches [5]. However, no significant differences in browning, bleeding (Table 1) nor in ppLDOX expression (Table S2) were observed between our pools. It is suggested that AtTT19 functions as a carrier to transport proanthocyanin precursors to the tonoplast [329] to be later secreted and linked to cell wall polysaccharides [330]. Binding that depends on the composition of the proanthocyanin [331]. The tt19 mutation leads to the formation of aberrant PA derivatives [329]. Thus is possible that differences in TT19 have to do with cell wall composition and chilling sensitivity. Further experiments are required to test this hypothesis.

In addition, flavonoids act as negative regulators of auxin transport [327]. It is noteworthy that at harvest only two transcription factors (PAP2/IAA27 and IAA16) were differentially expressed, both showing higher expressions in T fruits and in the case of the ortholog of PAP2/IAA27, also at 1 week of cold storage (Table 5). SlIAA27 silencing results in greater auxin sensitivity in tomato [332]. Moreover, a gain-of-function mutation in IAA16 confers poorer responses to auxins and ABA in *Arabidopsis* [333]. Thus, it is likely that high levels of these genes at harvest contribute to delay the ripening program or protect fruits LS during cold storage, at least at the beginning of cold storage.

The analysis of the expression profiles during cold of the genes differentially expressed in M fruits resulted in important and unexpected expression characteristics. In fruits LS, these genes behaved like ripening genes (Fig. 5A) and were able to continue with the ripening program in the cold in fruits LS, while the ripening expression of other

ripening genes was normally halted (Fig. 5B),which is not the case of high sensitive fruits. The ability of cold to stop fruit ripening has been previously reported [26], even if no details of how this happens at the molecular level have yet been provided. Although we have no hypothesis about why these genes continued with the ripening program in the cold (thus we expect that cold stopped ripening program efficiently in fruits LS), we believe that this may be because these genes are part of the adaptation mechanism or simply reflected that LS fruits perform better in the cold than S fruits. In apples the ability to set up ripening during cold seems to be an adaptative mechanism to shorten ripening time in colder autumns [122]. On the other hand, this unexpected behavior of some of the genes differentially expressed at harvest indicates that they not only can form part of a mechanism for the interaction between endogenous and exogenous signals, they could also be able to contribute to mealiness in response to cold stress. In light of this, it is interesting to remember that environmental/ripening stage/cultural preharvest practices have a strong effect on CI sensitivity during the postharvest [2, 3, 334, 335] which, together with the genetic background, may be responsible for the differences noted in the M stage that condition the cold response.

Chapter 2

Chapter 2. Comparative analysis of the changes in transcriptome occurring during cold in chilling sensitive and resistant peach cultivars with those occurring in pools of siblings from the Pop-DG population

C2.1. Results

C2.1.1.Ripeness and chilling injury parameters of 'Oded' and 'Hermoza' peaches

The fruits of Od and Hz were slightly different at harvest. Ripening parameters and results of t-tests are summarized in Table 2. At harvest, Od peach fruit were 30% smaller (by weight) than Hz peaches. Furthermore, Hz fruit were less acidic (0.33 % compared to 0.43%), and had higher soluble solids (14% compared to 12%). However, there were no significant differences in ethylene production or in firmness between fruit of the two cultivars. The ethylene levels in Od and Hz fruit were 0.69 µL kg^{-1} h^{-1} and 0.78 µL kg^{-1} h^{-1}, respectively (Table 2). According to Kader & Mitchell [164] both cultivars were harvested at similar commercial mature stage (M). However it is obvious that physiological differences exist between both cultivars at the mature commercial stage, related to their growing conditions, length of development and genetic background.

Although Hz peaches were firmer than Od peaches during storage (Figure 9A), these cultivars exhibited similar trends in firmness during CS at 5°C. Fruit of both cultivars

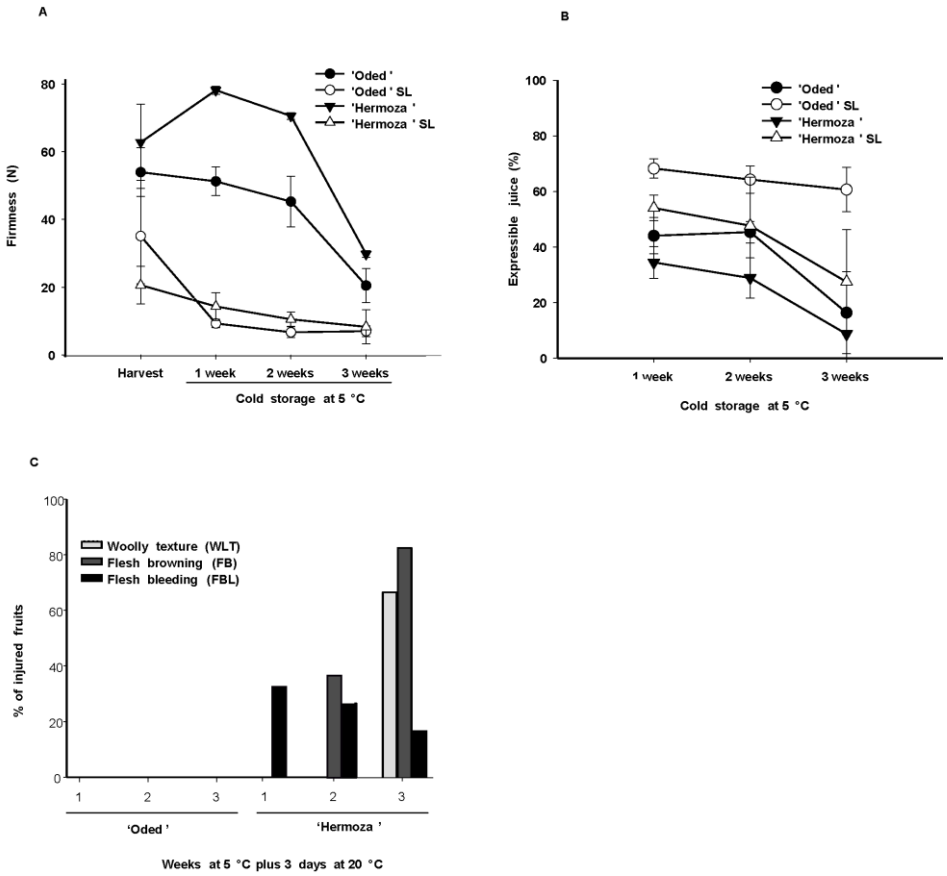


Figure 9. Comparison of chilling injury symptoms of 'Oded' and 'Hermoza'. A) Firmness of 'Oded' and 'Hermoza' peaches at harvest and after cold storage at 5°C (black colored symbols), and during ripening at 20°C (shelf life, open circles). Standard deviation is indicated. B) Expressible juice of 'Oded' and 'Hermoza' peaches at harvest and after cold storage at 5ºC (black colored symbols), and during ripening at 20ºC (shelf life, open symbols). C) Woolly texture (WLT), flesh browning (FB) and flesh bleeding (FBL) indices of 'Hermoza' peaches during shelf life after cold storage at 5ºC.

retained their firmness for the first two weeks of storage, and upon the third week in the cold began to soften (Figure 9A). The firmness levels of both cultivars during shelf life (SL) ripening following cold storage (CS), although much lower than during CS, was also similar; with Od reaching 7 to 9 N, and Hz between 8 to 14 N. These values were

lower than the softening that occurred when the fruit were held for three days at 20°C without storage (Figure 9A).

Consistent with previous findings that Od fruit were resistant to CI in CS [168], expressible juice did not change during SL ripening after CS (remaining approximately 65%) while Hz decreased to 27% (Figure 9B) and no WLT was observed visually in Od fruit (Figure 9C). Further, there were no symptoms of FB or FBL in Od during ripening after CS for up to three weeks (Figure 9C). In contrast, Hz was sensitive to CI, and developed FB and FBL during SL after 2 weeks of CS and all three CI symptoms after 3 weeks.

C2.1.2.Global transcriptome analysis

The Chillpeach microarray [17] was used to analyze the transcriptomes of peaches from both cultivars at harvest and after 1 and 2 weeks of storage. These stages were selected to investigate pre-symptomatic early events in the chilling response which may be associated to WLT, FBL and FB.

In total, 3277 probes met the threshold for hybridization quality (Additional Table S7). As a first approach to analyze the complexity of the gene expression dataset, a Principal Component Analysis (PCA) was performed on raw data. The three first components account for 80% of variance (Figure 10A, B). The results of the PCA plot showed consistency across replicated samples and treatments and, therefore, the experiment was considered reliable for further analysis. The 1st component (PC1, 52.32% variance) clearly separated harvest from cold-treated samples (Figure 10A). The 2nd component (PC2, 17.65%) separated cold stored samples of the tolerant cultivar Od from the sensitive Hz. The 3rd component (PC3) which contributed 10% of the difference in gene expression, separated the two cultivars at harvest (Figure 10B), which indicates that most of differences in the transcriptome induced by cold are due to differences in the sensitivity to develop injury rather than to differences at harvest. However, PC3 shows that genes differentially expressed at harvest reach similar expression values after being cold stored 1 and 2 weeks in Od and after 1 week in Hz,

but not Hz-CS2 fruit, which were projected separately from the other cold stored

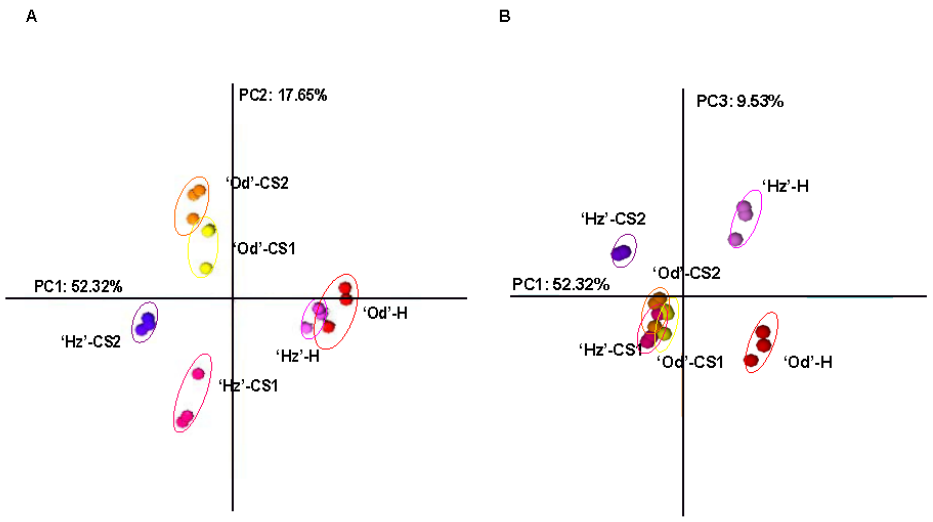


Figure 10. Principal Component Analysis (PCA) of harvest and cold stored 'Oded' and 'Hermoza' samples according to their lowess normalized expression data. Three biological replicates per sample were analyzed. A) First principal component (PC1) is shown on x-axis while the second principal component (PC2) is shown on y-axis. B) PC1 is shown on x-axis while the third principal component (PC3) is shown on y-axis. The percentage of the variance explained by each component is indicated. Od: 'Oded'; Hz: 'Hermoza'; H: harvest; CS1: 1 week at 5ºC; CS2: 2 weeks at 5ºC.

samples. This indicates that genes differentially expressed at harvest could be involved in the eventual injury these fruit suffered when shelf ripened after two weeks in the cold (i.e. FB) but not to the phenotypical differences observed by just one week (i.e FBL).

C2.1.3.Differences in the transcriptome of 'Oded' and 'Hermoza' fruits at harvest and during cold storage

A direct comparison between Od and Hz peaches at harvest and at the different cold storage periods (CS1 and CS2) was carried out in order to identify genes differentially expressed in between the two cultivars and thus, eventually, to discover genes involved in chilling injury resistance/sensitivity at pre-symptomatic stage. As shown in Figure 11A the number of differentially expressed genes between the two cultivars

was higher following cold storage (1 and 2 weeks) than at harvest, thus confirming PCA results.

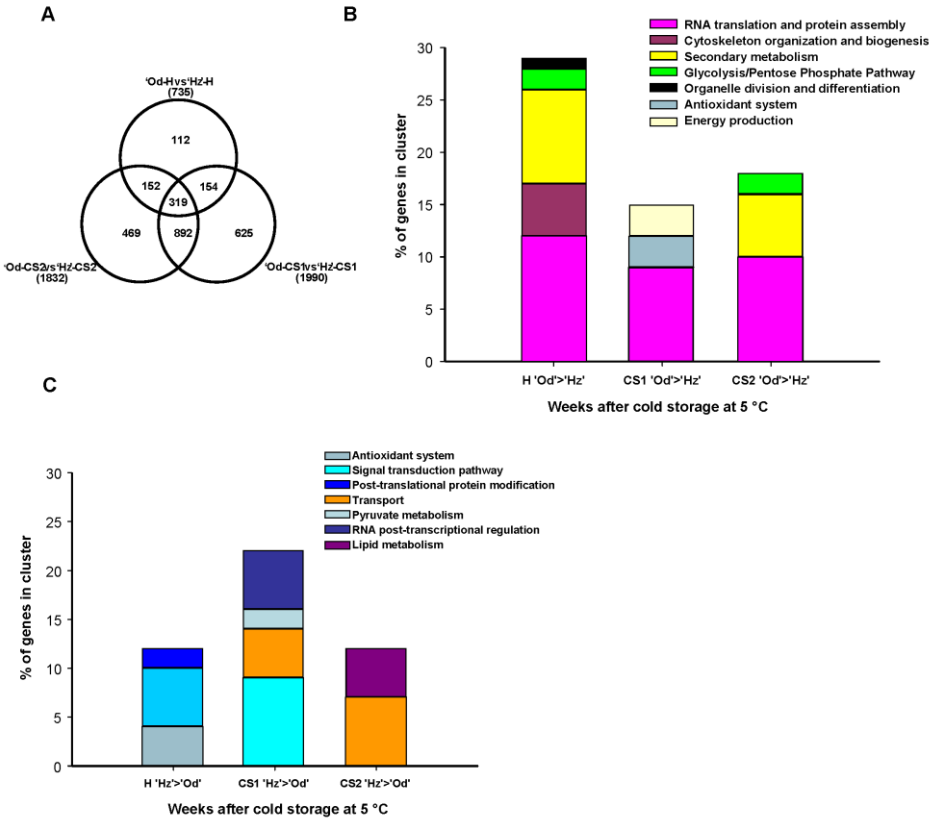


Figure 11. Differential gene expression between the 'Oded' and 'Hermoza' peach fruits at harvest and after 1 and 2 weeks of cold storage. A) A Venn diagram showing the differentially expressed genes (FDR<0.05 and q-value<0.05) between the tolerant Od and the sensitive Hz fruits at each time of cold storage. B) The over-represented functional represented functional categories (p-value 0.05) corresponding to the differentially expressed genes high expressed in Hz comparing to Od at harvest and at each time of cold storage. H: Harvest; CS1: cold storage of 1 week at 5ºC; CS2: cold storage of 2 weeks at 5ºC; Od: 'Oded' peach; Hz: 'Hermoza' peach

A total of 735 genes were differentially expressed at harvest, and out of these 344 and 393 genes were up- and down-regulated, respectively, in Od compared with Hz at harvest (Figure 11A; Additional Table S8). As shown in Figure 11B, the genes with higher expression in Od at harvest were functionally enriched in *RNA translation and*

protein assembly, *cytoskeleton organization and biogenesis*, *secondary metabolism*, *glycolysis* and *organelle division and differentiation*. Genes under-represented (i.e., overexpressed in the sensitive cultivar Hz) were enriched in *antioxidant system*, *signal transduction*, *post-translational protein modification* and *unknown function* (Figure 11C). Approximately 90% of the genes differentially expressed at harvest have altered expression during cold storage (Figure 11A). This suggests that they have to do with the differential chilling response in both cultivars (as we showed in PCA, Figure 10). However, some of them belonging to functional categories such as *cell wall*, *glycolysis, tricarboxylic acid cycle* (*TCA)* and *other carbohydrate metabolism,* and may also account for the physiological differences observed between Od and Hz at harvest (firmness, soluble solid content, acidity; see Table 2; Additional Table S8). By one week of CS, 1990 genes were differentially expressed (Figure 11A).

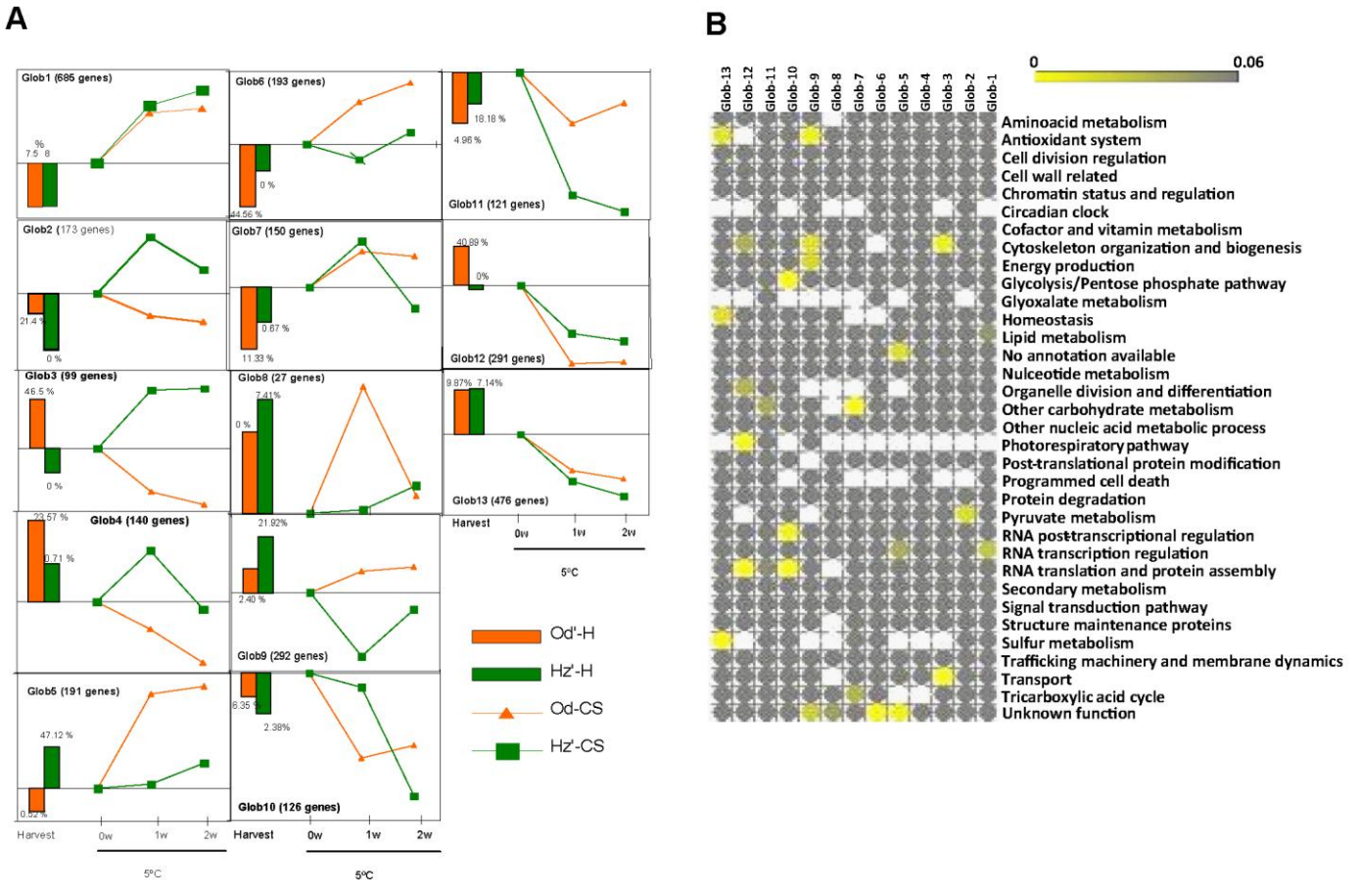
Functional enrichment indicated that *RNA translation and protein assembly* was higher in Od than in Hz, both at harvest and during 1 and 2 weeks of cold storage (Figure 11B). Out of 42 genes in this functional class over-represented in Od at harvest, 21 genes were also higher expressed in Od at one week of cold and 12 genes by two weeks. Moreover, 61 genes also showed high expression levels in Od by 1 and 2 weeks of cold storage and twenty genes were common in all three time points (Additional Table S8). This suggests that enhanced protein synthesis at harvest and during cold is critical for tolerance development. *Secondary metabolism* and *glycolysis* enriched genes were highly expressed in Od both at harvest and after 2 weeks of cold storage (Figure 11B). This overlap indicates that differences at harvest may account for the differences observed at 2 weeks of CS, as suggested the PCA (Figure 10). Genes of the *signal transduction* and *transport* functional categories were enriched in the sensitive cultivar Hz at harvest and also after 1 week of CS (Figure 11C), thus suggesting that they may be related to the sensitivity to cold storage. The functional category *antioxidant systems* was enriched in both cultivars at different time points. Fifteen antioxidant related genes were more highly expressed in Hz at harvest (15 genes) and 30 were over-represented in CS1 of Od peaches (Figure 11B and C). Out of the 15 genes encoding for antioxidant activities, 11 were high expressed in Od peaches at one week.

This suggests that high levels of antioxidants at harvest are not directly related to the tolerance to cold storage, rather it appears that high levels of antioxidants during cold storage contribute to the tolerance. In addition, only four genes encoding for antioxidant activities were highly expressed in Hz at harvest and, as is the case of the orthologs of catalase 2 (CAT2) and thioredoxin (TRXH2), also during cold storage (Additional Table S8), suggesting that they are related to the sensitivity to cold.

C2.1.4.Kinetics of the cold response in 'Oded' and 'Hermoza'

To investigate chilling-induced alterations in the gene expression profiles of the two cultivars in this study, differentially expressed genes were assessed with a false discovery rate (FDR) of 1%, q-value ≤0.01 based on three replicates. We found 2964 genes differentially regulated at least for one condition (samples H and CS samples) in either of genotypes. To distinguish whether transcripts are differentially affected by cold and analyze kinetics while avoiding the effect of harvest differences, expression data was normalized to harvest values. Differentially expressed transcripts were grouped according to shared cold expression patterns by Hierarchical Cluster Analysis (HCA) (Figure 12A; Additional Figure S3) and further characterized by functional enrichment (Figure 12B). In order to reflect the expression levels of genes at harvest, the average expression value of all genes in a cluster and the percentage of genes with higher expression levels in each cultivar (from the direct comparison; Figure 11) and each cluster were plotted in the graphic together with the cold expression profile. Only when the percentage of genes more highly expressed in a cultivar exceeded 20% of the genes in a cluster, was their contribution considered significant. The HCA resulted in 13 clusters (Figure 12A). Based on their expression during cold storage, these genes can be classified into several groups as follows.

The largest group (A) comprises cold responsive genes irrespective of sensitivity to cold storage. These included 685 genes (cluster 1, Figure 12A) up regulated by cold storage and enriched in *RNA transcription regulation* (Figure 12B) and 767 genes cold down-regulated (in clusters 12 and 13; Figure 12A) enriched in *cytoskeleton organization*, *organelle division*, *photorespiratory pathway* (cluster 12; Figure 12B) and *antioxidant*


A
Glob1 (685 genes)
Glob6 (193 genes)
Glob11 (121 genes)
Glob2 (173 genes)
Glob7 (150 genes)
Glob12 (291 genes)
Glob3 (99 genes)
Glob8 (27 genes)
Glob13 (476 genes)
Glob4 (140 genes)
Glob9 (292 genes)
Glob5 (191 genes)
Glob10 (126 genes)
Harvest
0w
1w
2w
5°C
Od'-H
Hz'-H
Od-CS
Hz'-CS
B
0
0.06
Glob-13
Glob-12
Glob-11
Glob-10
Glob-9
Glob-8
Glob-7
Glob-6
Glob-5
Glob-4
Glob-3
Glob-2
Glob-1
Aminoacid metabolism
Antioxidant system
Cell division regulation
Cell wall related
Chromatin status and regulation
Circadian clock
Cofactor and vitamin metabolism
Cytoskeleton organization and biogenesis
Energy production
Glycolysis/Pentose phosphate pathway
Glyoxalate metabolism
Homeostasis
Lipid metabolism
No annotation available
Nulceotide metabolism
Organelle division and differentiation
Other carbohydrate metabolism
Other nucleic acid metabolic process
Photorespiratory pathway
Post-translational protein modification
Programmed cell death
Protein degradation
Pyruvate metabolism
RNA post-transcriptional regulation
RNA transcription regulation
RNA translation and protein assembly
Secondary metabolism
Signal transduction pathway
Structure maintenance proteins
Sulfur metabolism
Trafficking machinery and membrane dynamics
Transport
Tricarboxylic acid cycle
Unknown function

system, *homeostasis*, and *sulfur metabolism* (cluster 13; Figure 12B). This indicates that cold storage in both peach cultivars involves the activation of several transcriptional cascades and an extensive down-regulation of housekeeping and metabolic functions. Most of genes in clusters 1 and 13 do not show statistical differences in expression at harvest, while 41% of genes in cluster 12 were highly expressed in Od at harvest (Figure 12A). This suggests that, although the effect of cold on genes in cluster 12 is down-regulation, high levels at harvest can contribute to withstanding cold storage.

The second group (B) includes 538 genes comprised of clusters 2, 3 and 4 that most likely contains genes up-regulated during cold storage in the sensitive cultivar Hz while down-regulated in the tolerant Od (Figure 12A), suggesting a possible relation to chilling sensitivity. Genes in clusters 2 and 3 were enriched in genes related to *pyruvate metabolism*, *cytoskeleton organization* and *transport* (Figure 12B). Genes in cluster 4, which was transiently up-regulated in Hz (Figure 12A) did not show any enrichment. It is noteworthy that 20% of the genes in clusters 2, 3 and 4 clusters were expressed at higher levels in Od cultivar at harvest (Figure 12A), especially genes in cluster 3, where genes with higher expression levels in Od-H account for 46.5 % of genes, suggesting that they may be part of a constitutive tolerance mechanism. However, the observation that these genes were cold-induced in the sensitive cultivar Hz indicated that they could be required for setting up the initial response to cold, but do not enable the fruit to stand long term cold periods.

Figure 12. Kinetics of cold responsive genes in 'Oded' and 'Hermoza fruits during cold storage and harvest values. A) Average gene expression pattern relative to harvest of genes in each of the 13 clusters generated by unsupervised two-dimensional hierarchical clustering (Additional Figure S1). Od and Hz harvest values (bars) represents the average fold change of all genes within a cluster with respect to the reference pool. The percentage of genes high expressed at harvest in each cultivar and cluster is indicated together with expression bars. The number of genes in each cluster is indicated between brackets. B) The functional categories overrepresented in each cluster are shown as a heatmap obtained with matrix2png. Enriched functional categories with Fisher test p-values < 0.05 are colored in grades of yellow. Harvest; CS1: cold storage of 1 week at 5ºC; CS2: cold storage of 2 weeks at 5ºC; Od: 'Oded' peach; Hz: 'Hermoza' peach

The third group (C) included 797 genes included in clusters 5, 6, 9 and 11 that during cold storage were expressed at higher levels in Od compared to Hz (Figure 12A), and thus may be related with to CI resistance. Clusters 5, 6 and 9 comprised genes up-regulated in Od during CS, but unaffected or even decreased in Hz (Figure 12A). Genes in clusters 5 and 6 were enriched in genes without annotation or with *unknown function*; however class 9 was enriched with genes related to *antioxidant system*, *cytoskeleton organization*, *energy production* and genes of *unknown function* (Figure 12B). More than 20% of genes in these three clusters were expressed at higher levels in the sensitive cultivar before cold stress, but during cold storage most of them reach expression values higher in Od than in Hz (Figure 12A). This suggests that high levels of these genes may contribute to the tolerance to cold storage and that the ability to up-regulate these genes during cold was related to low levels at harvest. The genes of cluster 11, enriched in *other carbohydrate metabolism* (Figure 12B), were down-regulated during cold storage in both cultivars; however the expression levels in Od were always higher than in Hz (Figure 12A). No significant differences were observed at harvest. Interestingly, this cluster (Additional Table S8) contained the orthologs of CBF1 (C-repeat/DRE Binding Factor 1) and CAMTA2 (Calmodulin Binding Transcription Activator 2), two transcription factors playing important roles during cold acclimation [51, 80], thus confirming the possible role of the genes in group C in chilling injury tolerance.

A fourth group (D) was formed by clusters 7, 8 and 10. The genes in these clusters did not show in general differences at harvest, but had the particularity of being transiently up-regulated or maintained at harvest expression level in one of the cultivars (Figure 12A). The genes in cluster 7, enriched in *other carbohydrate metabolism* and *TCA* genes (Figure 12B), were up-regulated to similar rates in both cultivars, but repressed in the sensitive cultivar after two weeks, when browning started to develop when fruit were shelf ripened. This suggests that down-regulation of these genes might be related to the development of injury at a pre-symptomatic stage. The genes in cluster 8, enriched in genes with unknown function, did not respond to cold in Hz but transiently up-regulated in Od, suggesting a possible

regulatory role of these genes. Genes in cluster 10 (Figure 12A) which was enriched in *glycolysis*, *RNA posttranslational regulation*, and *RNA translation and protein assembly* (Figure 11B), did not respond to cold in Hz during the first week while being down-regulated in Od from this time (Figure 12A). This suggests that the response to cold of these genes was delayed in the sensitive cultivar Hz which may be counterproductive to withstanding the cold storage.

C2.1.5.Validation of Hz and Od microarray results

In order to validate the microarray results, we performed qRT-PCR on ten peach genes selected from the list of genes differentially expressed between Od and Hz fruits using gene specific primers (Additional Table S9). The tested genes were chosen from different processes including *cell wall*, *RNA transcription regulation*, *secondary metabolism*, *signal transduction pathway* and *trafficking machinery and membrane dynamics* (Additional Table S10). A total of 60 comparisons were made, as the expression of each gene was monitored at three time points (H, CS1 and CS2) in Od and Hz, using the same samples used for microarray analyses. The overall correlation observed between microarray and qRT-PCR analysis was R=0.88 (Figure 13A). In addition, we also evaluated the agreement between each gene's expression profiles determined by qRT-PCR and microarrays using Pearson correlation coefficient (Additional Table S10). The qRT-PCR data correlate well (range R =0.8-1, six genes) or are consistent (range R= 0.5-0.8, four genes) with the patterns of expression revealed by microarray analysis, and four examples (Figure 13B) include those for Thaumatin-like protein 1 (PPN003H07), aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS1; PPN004H06), ACC oxydase (ACO; PP1005G06) and the ortholog of the transcription factor indoleacetic acid-induced protein 27 (IAA27/PAP2 ; PPN057F01), reported as being associated to woolliness tolerance at a pre-symptomatic stage (chapter 1 and [136, 137, 168]). These results confirm the general validity and robustness of the microarray data we present here.

Another source of validation comes from the microarray-based genome-wide analysis of pools from Pop-DG population with contrasting WLT sensitivity in response to cold

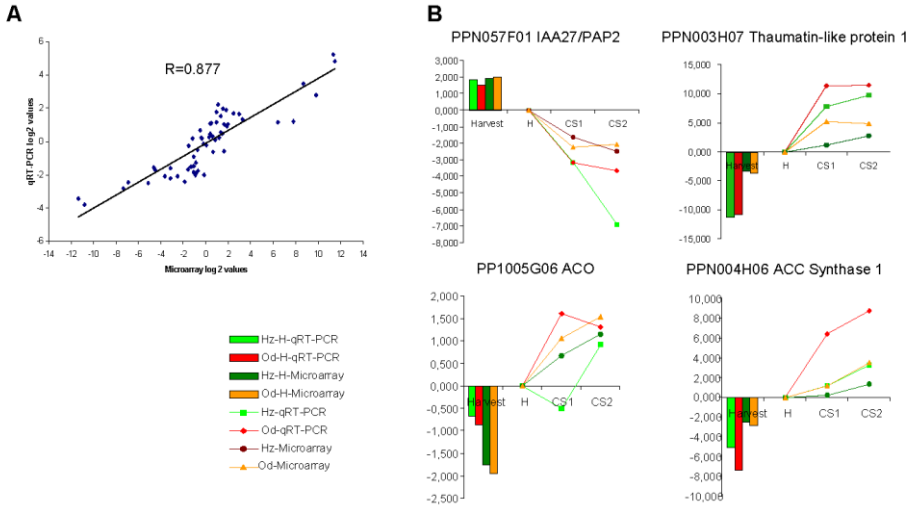


Figure 13. Quantitative RT-PCR validations of microarray data in Oded and Hermoza fruits. A) The comparison of the microarray and qRT-PCR assay data, based on log2 data in Od and Hz. A total 10 differentially expressed genes were chosen, representing 60 comparisons where one gene covers 3 different time points (H, CS1 and CS2). Line shown represents the orthogonal fit to the data and correlation (R) is shown. B) Example of gene expression profiles across H, CS1 and CS2 samples in Od and Hz determined by qRT-PCR and microarray on four peach genes previously associated to CI tolerance (IAA27, Thaumatin-1-like, ACS and ACO). In the graphs there are represented Od and Hz values at harvest (bars) and the average gene expression pattern relative to harvest values in both platforms, microarray and qRT-PCR

storage (chapter 1). The similarity between the data generated in chapter 1 and those presented here for Od and Hz (same developmental stage, treatments as well same expression platform and reference pool for hybridization), allow direct comparison of expression profiles and values between studies.

The pools of the Pop-DG population are less tolerant to WLT than Hz. The most sensitive pool (high sensitive, S) was already mealy/woolly after one week of cold storage at 5ºC plus shelf life ripening, while the relative tolerant (low sensitive, LS) was damaged after two weeks of cold storage (Figure 2). However, while Hz was more resistant to WLT (fruit showed WLT symptoms after 3 weeks in cold) the siblings from Pop-DG population were relatively tolerant to FBL and FB (Table 1), which developed in Hz during ripening after two weeks of storage. However if tolerance/sensitive

mechanisms are conserved, we expect that genes high expressed in the tolerant Od by compared to Hz, were high expressed in the LS pool compared to the S pool.

We have compiled a dataset of 2207 genes (Additional Table S11) integrating expression values for cold responsive genes, differentially expressed at one week of cold storage, when the largest number of differentially expressed was found among all fruit. Then we determined the percentage of differentially expressed genes identified in each study (Od vs Hz and LS vs S pools) that shared the expression patterns. The comparison between both experiments resulted in more than 55% of the genes showing consistent patterns of expression (Additional Table S11). These 'consistent genes' corresponded to genes highly expressed in the LS pool that also showed higher expression levels in Od than in Hz, while genes with higher expression in S pool than in the LS pool showed higher expression levels in Hz. The rest of cold responsive genes were only differentially expressed in one of the experiments (20-30%) or showed an opposite pattern (<10%). These observed differences may indicate differences in the response to cold due to cultivar. Nonetheless, considering that 55% of genes had similar transcript profiles across samples and the low proportion of genes behaving in opposite direction, this comparative transcriptomic approach provides a valuable indication of a set of candidate genes that can be related to tolerance/sensitivity to CI in peach.

C2.1.6.Comparison of the transcriptomes of 'Oded' and 'Hermoza' with Pop-DG siblings with contrasting sensitivity to WLT

To identify changes in gene expression that could be causally related to the tolerance/sensitivity to cold storage in peach fruit, we analyzed together the transcriptomes of Od, Hz and the LS and S pools by k-means clustering (Figure 14A; Additional Table S11). We reasoned that changes in gene expression common to all peach fruit are more likely to be part of core cold responses while differences may provide genes for the specific response of each fruit genotype to cold storage, and

which may or may not be involved in tolerance. According to this, genes in clusters k-means 2, 5 and 9 (Figure 14A) were classified as part of the core cold response, but

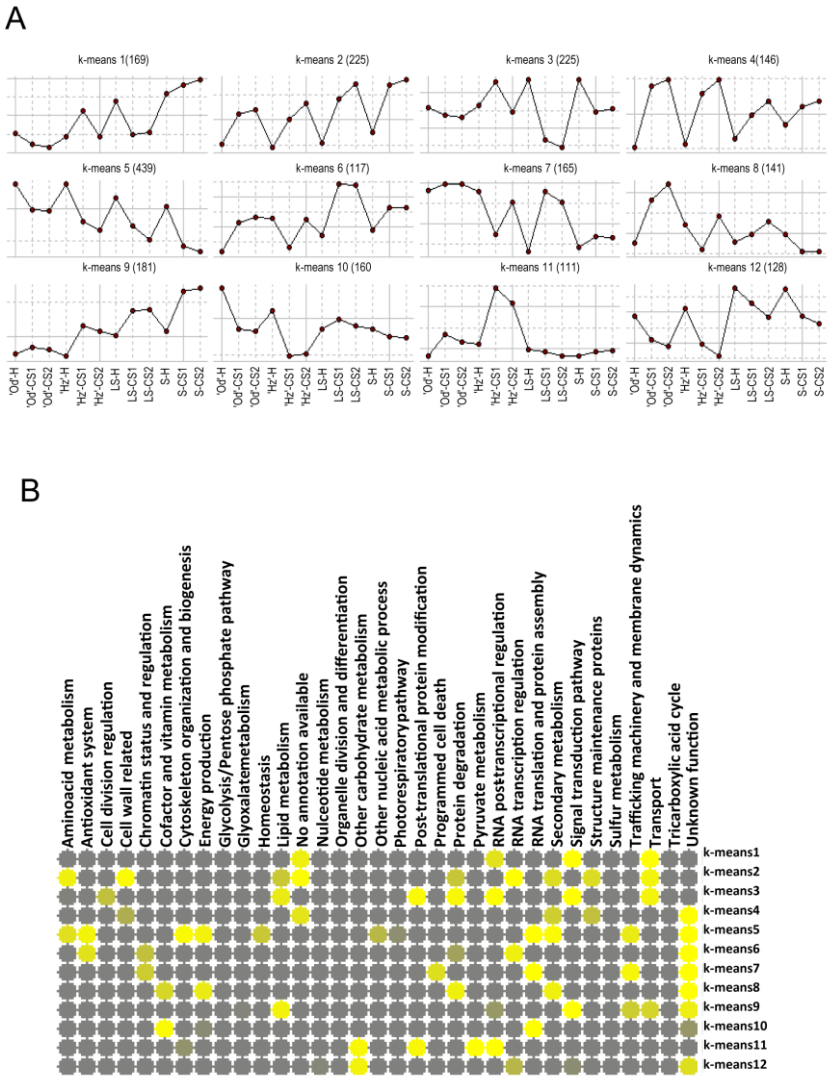


Figure 6. Integrative analysis the transcriptomes of 'Oded', 'Hermoza' and two pools of siblings from the Pop-DG population that cover a range of cold susceptibilities. A) K-means clustering results of a set of 2207 genes with a 12-cluster limit. B) The functional categories overrepresented in each cluster are shown as a heatmap obtained with matrix2png. Enriched functional categories with Fisher test p-values < 0.05 are colored in grades of yellow. Tolerance-sensitivity range: Od>Hz>LS>S. H: Harvest; CS1: cold storage of 1 week at 5ºC; CS2: cold storage of 2 weeks at 5ºC; Od: 'Oded' peach; Hz: 'Hermoza' peach; LS: low sensitive Pop-DG pool; S: high sensitive Pop-DG pool.

differ in their time/levels of expression and therefore are related to the degree of sensitivity/tolerance to cold. Given the common CI response that these fruit had was WLT; probably most of them were related to this disorder. Genes in cluster 2 and 9 were up-regulated by cold in a manner similar to their propensity to develop WLT (S>LS>Hz>Od; Figure 14A).The main difference between these clusters was that in k-means 9 the expression level at harvest correlated to sensitivity. Genes in cluster k-means 2 were enriched in *RNA transcription regulation, cell wall, transport, amino acid metabolism, secondary metabolism, structure maintenance proteins, lipid metabolism, protein degradation* and genes without any annotation (Figure 14B). Genes in cluster k-means 9 were enriched in *signal transduction pathway, lipid metabolism, unknown function, transport, trafficking machinery and membrane dynamics, RNA post-transcriptional regulation* (Figure 14B). In addition to up regulated genes, core cold responses also included down-regulated genes (cluster k-means 5). The genes in cluster k-means 5, enriched in *RNA translation and protein assembly, secondary metabolism, cytoskeleton organization and biogenesis, antioxidant system, energy production, trafficking machinery and membrane dynamics, aminoacid metabolism, homeostasis, other nucleic acid metabolic process* and genes with *unknown function,* were down-regulated by cold inversely to CI sensitivity (Figure 14B). Therefore, high levels of these genes contribute to the tolerance to cold storage.

The other clusters with interesting patterns included clusters k-means 1, 8 and 11. The genes in k-means 1, enriched in *signal transduction pathway, transport, RNA post-transcriptional regulation* and genes without any annotation available (Figure 14B) may be related to the higher sensitivity to WLT of the fruit in S pool. The genes in k-means 1 have expression levels at harvest that correlated to sensitivity degree and were up-regulated by cold in the S pool, but did not change in Od or were down regulated in Hz and in the LS pool (Figure 14B). The genes in the cluster k-means 11, enriched in *pyruvate metabolism, RNA post-transcriptional regulation, post-translational protein modification, other carbohydrate metabolism* and *cytoskeleton organization and biogenesis* (Figure 14B), were highly up-regulated by cold in Hz but unaffected in the three other fruits (Figure 14A). These genes are candidates regarding

the sensitivity of Hz fruit to FB and FBL. The genes in cluster k-means 8 may be associated with the high tolerance of Od fruit to CI. They were up-regulated in Od by cold storage, but unchanged in the other fruits in comparison to Od (Figure 14A), and were enriched in *protein degradation, secondary metabolism, energy production, cofactor and vitamin metabolism* and genes with unknown function (Figure 14B).

In addition, and in order give more robustness to this comparison, we searched for the 50 genes that chapter 1 were validated in the contrasting pools and in 15 individual lines from the same population differing in the woolliness sensitivity by medium-throughput qRT-PCR. Forty of these genes were found in the comparison between Hz and Od and the pools (Additional Table S12). Out of them 34 were also confirmed in the 15 individual lines from the same population and 20 corresponded to the most relevant clusters (k-means 1, 2, 5 and 9). Overall, there was good agreement between the cluster analysis (Figure 14A) and the results for the validation in the individual lines. Out of the genes in cluster k-means 1, 2 and 9 (up-regulated by cold in a manner similar to their propensity to develop WLT), 15 out of 16 genes were found correlated to sensitivity in the individual Pop-DG lines. Similarly, five genes found in the cluster k-means 5 (down-regulated by cold in a manner similar to their propensity to develop WLT), were found associated to the high degree of tolerance of the individual lines. Further, genes such as *ACS1* (PPN004H06), *IAA27/PAP2* (PPN057F01), *glycosyltransferase* (PP1004E08) and an *unknown extracellular protein* (PP1001A01) validated in the comparison between Od and Hz (Additional Table S10) were found also validated in the individual lines (Additional Table S12 and in chapter 1). Thus, it appears likely, that the genes identified in the comparison between Od, Hz and the pools play a role in the sensitivity/ tolerance of peach fruit to chilling injury.

C2.1.7.ROS-related transcriptomic signatures at harvest and during cold storage: ROSMETER analysis

A bioinformatic tool which was developed recently for Arabidopsis microarray data [19] to provide an organelle/type-dependent ROS-related transcriptomic signature was used to further characterize the differential peach responses to cold. ROSEMETER

signatures were defined on the basis of transcriptome data obtained in experiments involving plant mutants in antioxidant enzymes or subjected to chemical applications that lead to increases in ROS production, thus providing information on the specificity of the transcriptomic response to oxidative stress. Since we had identified antioxidant system genes as differentially expressed at harvest and increasing in the resistant cultivar after one week at cold storage it was of interest to examine the ROS transcriptomic signature at harvest and during CS for the four fruit types (Figure 15). The ROSMETER analysis indicated that some signatures were capable of discriminating fruits according to their sensitivity to CI. The analysis revealed six distinct groups that clearly can be grouped according to the chilling sensitivity.

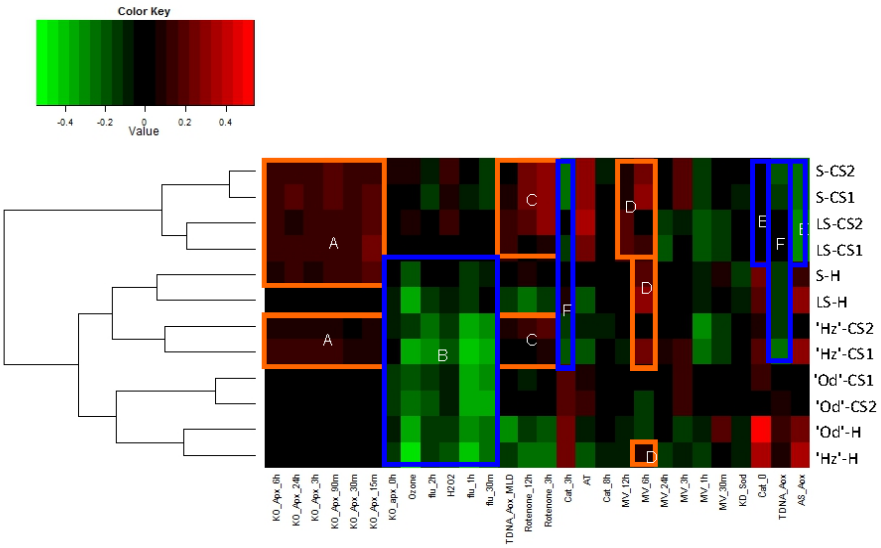


Figure 7. ROSMETER analysis of the harvest and cold transcriptomes of 'Oded', 'Hermoza' and LS and S peaches. The ROS indices are listed on the abscissa and the Od, Hz, LS and S samples clustered by nearest neighbor correlation are shown on the ordinate. The color-coded results of correlations for each index are shown as a heat map. Correlation values are between 1 (complete positive correlation; red) and −1 (highest negative correlation; green), where 0 indicates no correlation (black). Correlation values above 0.12 and below -0.12 represent non-random correlations: Harvest; CS1: cold storage of 1 week at 5ºC; CS2: cold storage of 2 weeks at 5ºC.

Group A includes all the knockout of cytoplasmic ascorbate peroxidase (KO-*APX1*) experiments, which are thought to represent cytoplasmic H_2O_2. These H_2O_2 indices correlated positively with sensitivity to CI before and during cold storage. In agreement a gene encoding a cytosolic ascorbate peroxidase (APX1; PPN071A07 Additional Table S11) was found among genes in cluster k-means 5 (Figure 14A), which may be related to a preformed mechanism of cold tolerance, since it is highest at harvest and in inverse relation to cold sensitivity.

Cluster B includes the indices of the conditional fluorescent *(flu)* mutant exposed to light 1h, ozone and H_2O_2 treatments. All fruit at harvest showed negative correlations with these indices, but after cold storage, the most sensitive fruit (i.e., Pop-DG pools) showed positive correlations, being, in general, higher in pool S. This suggests that scavenging systems for apoplastic ROS and chloroplastic singlet oxygen could be active at harvest, but decrease during cold storage in parallel with sensitivity to CI.

Cluster C, which showed increase in Hz, LS and S fruit during storage, corresponds to rotenone treatments (3 and 12 h), an inhibitor of mitochondrial complex I, (i.e., NADH: ubiquinone oxidoreductase). Rotenone is associated with a mitochondrial stress but has not been shown to directly generate ROS [19]. In agreement, four of the 15 genes involved in energy production and enriching in k-means 5 (Figure 14) encode for NADH: ubiquinone oxidoreductase (Additional Table S11). Therefore low levels of mitochondrial complex I during cold storage could contribute to the sensitivity to cold.

Cluster D corresponds to 6 and 12h methylviologen (MV) signatures, indicative of superoxide formation in the chloroplast and mitochondria [336]. 6hMV signature correlated positively with all fruits at harvest and cold stored, sensitive fruits (Hz, LS and S), while the most sensitive S and LS fruits also correlated positively with 12hMV signature, which may indicate secondary H_2O_2 stress effects [19].

Clusters E and F basically correlated negatively with sensitivity, included the indices of *CAT2 (0 and 3h),* alternative oxidase mutation (TDNA-*AOX1)*, and alternative oxidase antisense (AS-*AOX)*. The indices *CAT2*-0 and AS-*AOX* (cluster E) had a negative correlation with S and LS fruits during cold storage and a positive correlation with the degree of tolerance to CI, especially at harvest. The indices *CAT2*-3h and TDNA-*AOX*1

(cluster F) correlated negatively with LS and S, both at harvest and during cold storage, and with Hz fruit during cold storage, which agree with data obtained from the direct comparison between Hz and Od (Figure 11; Additional Table S8). The fact that fruit tolerance is correlated positively with indices in cluster E and F, comprising mainly transcriptome data from mutants not exposed to any stress conditions, suggest tolerant fruit might have activated a compensatory scavenging mechanism.

These results indicate that although cultivars presented oxidative stress under cold storage, high levels of antioxidant activities in cytoplasm, mitochondria and chloroplast (chromoplast) are likely contribute to protect fruit in the tolerant fruit.

C2.2.Discussion

C2.2.1.Integration of data from different peach genotypes and validation of the results

In this study an analysis of fruit transcript levels in response to CS in different peach genotypes is presented. In the first part of the of the study, the transcriptomes of Od and Hz at harvest and subjected to CS were analyzed using the Chillpeach microarray (Figures 10-12). We validated the microarray data by qRT-PCR of ten genes (Additional Table S10), some of them reported previously to be associated to tolerance to WLT. We observed a high correlation between microarray and qRT-PCR data (Figure 13). The expression patterns of the single genes analyzed were in concordance (Figure 13B and Additional Table S10), although the level of expression was not confirmed for each gene in each sample. It is known from similar studies that the two technologies of expression analysis deliver qualitatively comparable data, however, the magnitude of such expression changes as reflected by microarray data tends to be generally compressed in comparison with qRT–PCR [337].

In the second part of the experiment, we performed a comparison of transcript levels between Od, Hz and two pools from the Pop-DG population (Figure 14 and Additional Table S11), analyzed in chapter 1. A number of studies have reported changes in gene expression and protein activity in peach fruit in response to low temperature leading

to CI (reviewed in [338]). However, differences in experimental approaches, genotypes, storage and shelf conditions (time and temperature) and also in the symptom assessment often result in lack of consistency of results [12, 339, 340]. In the case of microarray studies, the differences in technologies and cutoffs used for the identification of differentially expressed genes, the different genes represented on each array and technical differences in RNA and hybridization, analysis protocols and references used often hinder the identification of common regulated genes [95]. The expression changes identified in the two large experiments compared here, Od-Hz and S-LS pools, used the same sampling time, technical platform, RNA reference, analysis, protocols and p-values to identify differentially expressed genes, therefore overcoming this issue.

The peach lines in the Pop-DG populations used to produce LS and S pools were less tolerant to cold storage than Od and Hz. However, if the mechanism / program for tolerance was similar, our hypothesis was that genes highly expressed in the LS pool would show high expression levels in the tolerant Od compared to the sensitive counterpart, but the magnitude of the changes could be different. For the comparison we selected differentially expressed genes at one week of storage, the time where greatest differences in CI are observed. This removed considerable biological variation and added to the strength of the comparison. A criticism to our approach could be that we are setting a bias for the common cold regulated genes towards one of the CI symptoms, i.e., WLT.

We found close agreement between the significant changes detected by the two experiments (see Additional Table S11) and also with the sensitivity degree of individual lines of the Pop-DG population (see Additional Table S12). The low proportion of genes with opposite changes also supports this contention, and we feel that data from both experiments can be interpreted with confidence. Further, since the fruits of Od, Hz, LS and S pools cover a wide range in CI sensitivity, this comparison has allowed the identification of a set of genes with shared expression patterns (core cold response) that are candidates to be related to CI tolerance/sensitivity (Figure 14A, clusters k-means 2, 5 and 9) but also genotype specific responses (Figure 14A, clusters

k-means 1, 8 and 11). Genes differentially expressed in one experiment but changing in opposite direction or not changing in the other could indicate a difference in the response due to the genotype or for other reasons [95].

However, although RNA expression data alone is insufficient for establishing a clear link between a gene/protein and the trait of interest, transcriptomics is an important first step to explore potential novel candidate genes for a particular process, which is the goal of this work. The data presented here, reinforce and extend previous reports, and provide insights into processes that are related to CI tolerance/sensitivity rather than simply being responses to cold.

C2.2.2.Quantitative differences in the subset of core cold responsive genes correlated with sensitivity to CI

Transcripts in the common cold regulated group showed expression values that correlated with sensitivity (Figure 14A). Furthermore, our results indicated that although reprogramming of the transcriptome underlies the core cold responses and the chilling sensitivity in peach fruit, many of these changes depend on the expression levels at harvest (Figure 14A). For the set of core cold responsive up-regulated genes, tolerant cultivars showed low expression levels both at harvest and during cold storage conditions, whereas sensitive cultivars showed increased expression in the cold (Figure 14A, cluster k-means 2 and 9). Interestingly, we could not identify a common core of cold response genes up-regulated in parallel with increased tolerance. This could be due to limitations of the Chillpeach microarray that was constructed with fruit from the Pop-DG mapping population [337], and which is less tolerant than Od. Alternatively, this may indicate that tolerant fruit were relatively less stressed at the cellular level compared to sensitive fruit and thus have a more limited response of the transcriptome, as has been described for salt and drought stressed rice [341].

C2.2.3.Expression of cell wall genes related to WLT at a pre-symptomatic stage

Alterations in cell wall related transcriptome, cell wall remodeling enzyme activities and in cell wall polymers metabolism in relation to WLT are normally detected during shelf life in cold sensitive cultivars but some have been reported to occur during extended cold storage [12, 342]. Using Pop-DG siblings, a set of genes related to cell wall remodeling were found differentially expressed between S and LS pools, but no enrichment was found for this functional category (chapter 1). In the current paper, we observed gene expression differences in cell wall genes during cold that could be associated to the eventual WLT phenotype that would develop in shelf life. It has been found that low levels of endo-PG activity combined with continuous activity of pectin methylesterase may lead to altered pectins during CS in fruit and this contributes to WLT when fruit are removed to SL [151, 342]. Furthermore, endopolygalacturonase (endo-PG) was found in a quantitative trait loci (QTL) on linkage group LG4 for both FBL and WLT [18]. In agreement with this, we found a polygalacturonase inhibiting protein (PGIP), a pectin methyl esterase and pectin acetyl esterase among genes with low expression levels across all cultivars at harvest but which were up-regulated in parallel with increasing fruit sensitivity (k-means 2, Figure 14A; Table 7; Table S5).

During WLT development in shelf life pectin accumulation was observed in the intercellular spaces and inside parenchyma cells near to vascular bundles [10] and these modifications may begin during CS [342]. Moreover early histological studies indicate that during the last stages of peach fruit ripening a secretory system producing mucilage occurs within the mesocarp vascular bundles [343]. Our previous results have correlated BXL1 (β-xylosidase) and SBT1.7/ARA12 (serine protease) with WLT sensitivity in the Pop-DG population (Additional Table S12 and chapter 1). Current evidence suggests that these genes are required for the proper configuration of pectins in mucilage in seed and roots (see Table 7), and that there are analogies between fruit ripening and seed mucilage modification [344]. Here, we found these two genes also among the genes up-regulated by cold in a manner similar to fruit propensity to develop WLT (k-means 2; Figure 14A).

Table 7. Genes discussed in the text correlated with sensitivity degree during cold storage (cluster k-means 2).

Function Specific process	Chillpeach ID	Unigene annotation	Arab AGI	Arab Gene Symbol	Hormone signaling	Sugar signaling/ partioning	Hormone and secondary metabolite biosynthesis regulation	Cell wall and cytoesqueleton related	Cell polarity	Ref
Aminoacid metabolism										
Alanine and Aspartate metabolism	PPN065C10	Putative aspartate aminotransferase	AT1G80360	VAS1			Negative regulation of Trp-IAA and ET biosynthesis			[345]
	PPN080E12	Putative aspartate aminotransferase	AT1G80360	VAS1			Negative regulation of Trp-IAA and ET biosynthesis			[345]
Cell wall related										
cellulose biosynthesis	PPN046D09	Cellulose synthase-like protein CslG	AT1G55850	CSLE1				SCW biosynthesis; hemicellulose biosynthesis		[346]
Hemicellulose biosynthesis	PPN036E12	Glycosyltransferase	AT4G36890	IRX14				SCW biosynthesis;hemicellulose glucuronoxylan biosyntheis		[347]
Hemicellulose degradation	PP1002E04	Alpha-L-arabinofuranosidase / beta-D-xylosidase	AT5G49360	BXL1				Pectin metabolism;trim b-xylan and a-arabinan side groups from the RG I.		[348]
Pectin degradation	PPN041B11	Polygalacturonase-inhibiting protein	AT5G06860	PGIP1				inhibition of degradation of the polygalacturonan		
pectin methyl-esterification	PPN047G10	Polygalacturonase-like protein	AT4G23500							
	PP1004E01	Putative pectinesterase	AT2G26440							
	PPN001F02	pectinacetylesterase family protein	AT5G23870							
	PPN066B05	Ripening-related protein-like	AT5G51520							

UDP-L-arabinose, UDP-galacturonate and UDP-xylose Biosynthesis	PPN062D06	UDP-arabinose 4-epimerase 1	AT1G30620	UXE1/ MUR4		Sugar signaling	arabionoglactan biosynthesis	[349-351]
Protein degradation								
protease	PP1004E07	Putative serine protease	AT5G67360	SBT1.7/ ARA12			indirectly affects the pectin methylation status of mucilage and/ or the primary CW	[352]
	PPN009E02	Cysteine protease 14	AT4G35350	XCP1			SCW biosynthesis; postive regulation of thacheray element differentialion	[353]
RNA transcription regulation								
LUG-family	PP1003C09	STY-L protein	AT2G32700	MUM1/ LUH			control mucilage production and extrusion	[354-356]
	PPN076D05	Transcriptional corepressor LEUNIG	AT4G32551	LUG	AUX signaling regulator		control mucilage production and extrusion	[271, 272, 354-356].
NAC-family	PPN054B06	No apical meristem protein-like	AT4G28500	anac073/ SND2			SCW biosynthesis; postive regulator of lignin, cellulose and hemicellulose biosyntehsis	[357, 358].
WRKY-family	PPN059A06	WRKY 13	AT2G37260	TTG2/ WRKY44		Anthocyanin / PA polimerization regulation	mucilage production regulation	[359]
Secondary metabolism								
Anthocyanin metabolism	PPN007E12	Anthocyanidin 3-O-glucosyltransferase	AT3G50740	UGT72E1			SCW biosynthesis; lignin biosynthesis	[360]
Carotenoid metabolism	PP1005H08	Zeaxanthin epoxidase, chloroplast precursor	AT5G67030	ABA1/ LOS6/ ZEP	ABA biosynthesis		mucilage production regulation	[284, 361]
ET biosynthesis	PPN004H06	1-aminocyclopropane-1-carboxylate synthase 1	AT3G61510	ACS1		ET biosynthesis		[362]

Phenylpropanoid metabolism	PPN025B05	Cinnamoyl CoA reductase	AT1G15950	CCR1/ IRX4				SCW biosynthesis; lignin biosythesis		[363]
	PPN053B11	Cinnamyl alcohol dehydrogenase	AT4G37980	ELI3-1/ CAD7				SCW biosynthesis; lignin biosythesis		[364]
Sterol metabolism	PPN012F12	delta(14)-sterol reductase	AT3G52940	FK/ HYD2	AUX and ET crosstalk; regulate AUX transporters localization in PM lipid microdomain formation and in the secretion machinery.			cellulose, callose and lignin, VN development	polar targeting of proteins to the PM;Lipid microdomains	[365-368]
	PPN063B12	Helix-turn-helix	AT4G37760	SQE3						
Terpene metabolism	PPN068G10	Beta-amyrin synthase	AT1G78950	BAS						
<u>Signal transduction pathway</u>										
ABA signaling/ Ca signal transducer	PPN069F09	Putative serine/ threonine protein kinase PK11-C1	AT4G33950	OST1/ / SRK2E/ SNRK2-6	ABA	Sucrose metabolism regulation				[288-290, 369]
ABA signaling/ ABF phosphorylation	PPN010B11	Serine-threonine protein kinase	AT1G78290	SNRK2.8/ SRK2C	ABA	sucrose signaling				[370, 371]
Phosphorylation cascades/ metabolic switch	PPN054E02	AKIN beta3	AT2G28060	KINβ3	ABA	sucrose signaling				[370-373]
<u>Trafficking machinery and membrane dynamics</u>										
ER to Golgi	PP1003D05	Root hair defective 3	AT3G13870	RHD3/ GOM8	AUX, ET			required for CW biosynthesis and actin organization	Cell polarity regulation	[313, 374]
sphingolipid metabolism	PPN021D05	similar to alkaline ceramidase	AT1G07380				Ceramide biosynthesis/ degradation		polar targeting of proteins to the PM;Lipid microdomains	[36, 367, 368, 375]
	PPN031D01	similar to alkaline ceramidase	AT1G07380				Ceramide biosynthesis/ degradation		polar targeting of proteins to the PM;Lipid microdomains	[36, 367, 368, 375]
<u>Transport</u>										
AUX efflux to the apoplast	PPN070B12	Multidrug resistance protein 11	AT3G28860	PGP19/ MDR11/ ABCB19	AUX transport					[376-379]
AUX transport into ER	PP1004E09	Auxin Efflux Carrier family protein.	AT2G17500	PILS5	AUX transport					[380, 381]

	PPN075H08	Auxin Efflux Carrier family protein.	AT5G01990	PILS6	AUX transport		[380, 381]
carbohydrate transport	PPN046B03	Sorbitol transporter	AT3G18830	PMT5/ PLT5		sugar partioning and homeostasis	
Cooper transport	PPN040A04	Copper transport protein-like	AT5G59040	COPT3			
ion transporter activity	PPN016B02	Senescence-associated	AT2G17840	ERD7			
metal-ion transport	PP1005G08	Metal tolerance protein C2	AT3G12100	MTP5			
	PPN007G12	Metal transporter Nramp3	AT2G23150	ATNRAMP3			
oligopepetide transport	PPN029A02	Putative peptide transporter	AT3G01350				

Abreviatures: AUX:auxin; ET; ethylene; ABA: Abcisic acid; PM:plasma membrane;CW: cell wall; SCW: secondary cell wall; ER: endoplasmic reticulum; MVB/ LE:microvesicular body/ late endosome; TGN/ EE:trans-golgy network/ early endosome; VSR:vacuolar sorting receptors VN:vascular networks; PA: proanthocyanines; PIN; PIN formed auxin efflux carrier; RG:rhamnogalacturonan; XyG:xyloglucan

Furthermore, among genes in cluster k-means 2 were also orthologs of other genes related to pectin configuration such as MUR4 (UDP-arabinose 4-epimerase) but also orthologs of genes required to control mucilage production and extrusion such as LEUNING (LUG), LUH/MUM1 (Leuning homolog), TTG2 (transparent testa glabra 2) and *LOS6/ABA1,* encoding a zeaxanthin epoxidase (Table 7). TTG2 and *LOS6/ABA1* regulate mucilage production [359, 361] while, LUH/MUM1and LUG, function redundantly in promoting mucilage extrusion [355]. Thus it is likely that the changes in the expression of these genes are setting the stage for the WLT disorder in these pre-symptomatic fruit.

Cluster k-means 2 also includes genes related to non-cellulosic cell wall polysaccharide biosynthesis and lignification (Table 7) such as CSLE1 (cellulose synthase like 1), which was previously confirmed to be related to the sensitivity to WLT in individual lines of the Pop-DG population (Additional Table S12 and chapter 1) as well IRX14 (irregular xylem 14) CSLE1 (cellulose synthase like 1), IRX4/CCR4 (cinnamoyl Co-A reductase 4), UGT72E1 (UDP-glucosyltransferase 72E1), CAD7/ELI3-1 (cinnamyl alcohol dehydrogenase 7), XCP1 (X*YLEM CYSTEINE PEPTIDASE 1*) and SND2, a NAC domain protein that regulates the expression of lignin, cellulose and hemicellulose biosynthetic genes involved in secondary cell wall development in *Arabidopsis* fibers [358]. Thus, in addition to changes in pectin composition and biosynthesis, cold storage activates a secondary cell wall gene expression program in a WLT sensitivity dependence manner. In support of that, genes of cluster k-means 8 and cluster k-means 5 (increasing during CS in Od or associated to tolerance; Figure 14A and Table 8) include orthologs of negative regulators of lignin biosynthesis such as the myb-transcription factor MYB4 [382], WUSCHEL-related homeobox 13 (WOX13) [383], and two the MADS box genes, FRUTIFULL (FUL) and tomato AGAMOUS like TAGL1 (Table 8).

C2.2.4.The maintenance of antioxidant systems and metabolites with antioxidant activity correlate with tolerance

Differences in expression of genes in the group of 'down-regulated by cold' could drive many of the responses to cold observed in peaches. These genes were constitutively

Table 8. Genes discussed in the text correlated with tolerance.

Function Specific process	Chillpeach ID	Unigene annotation	Arab AGI	Arab Gene Symbol	Hormone signaling	Sugar signaling/ partioning	Hormone and secondary metabolite biosynthesis regulation	Cell wall and cytoesqueleton related	Cell polarity	Ref
k-means5. Correlated with tolerance at harvest and during cold storage										
Aminoacid metabolism										
cyanide detoxification	PPN075E10	Beta-cyanoalanine synthase 1	AT3G61440	CYSC1						[384]
methionine metabolism	PPN034A06	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 4	AT5G43850	ARD4			Yang Cycle	associated to VN tissue		[385, 386]
	PPN034C12	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 3	AT4G14710	ARD2			Yang Cycle	associated to VN tissue		[385, 386]
	PPN072E05	Cystathionine gamma synthase	AT3G01120	MTO1/ CGS1						[387]
Antioxidant system										
GLUTHATHIONE-GLUTAREDOXIN AND THIOREDOXIN REDOX HOMEOSTASIS	PPN039H11	Glutathione S-transferase	AT5G17220	TT19/ GSTF12			PA monomer transporter			[328, 329]
Cytoskeleton organization and biogenesis										
actin microfilament-actin depolimerization	PPN047E05	Actin depolymerizing factor 2	AT5G59880	ADF3						[388, 389]
Microtubule-Microtubule binding and stabilization	PPN073D05	Microtubule-associated proteins	AT5G55230	MAP65-1						[390, 391]
Microtubule-microtubule organization and formation	PPN075E12	Tubulin folding cofactor B	AT3G10220	EMB2804/ TFC						[392]
RNA transcription regulation										
AP2/ EREBP family	PPN054F05	AP2-related transcription factor	AT5G47220	ERF2	ET signaling			VN cell division		[393, 394]
AUX/ IAA family	PPN014H03	Auxin-induced protein AUX28	AT1G04250	AXR3/ IAA17	AUX and ABA nuclear signaling; negative regulator					[222]
	PPN057F01	AUX/ IAA protein	AT4G29080	PAP2/ IAA27	AUX nuclear signaling; negative regulator					[223, 224]

LIM-family	PPN009B01	Pollen-specific protein SF3, putative	AT1G10200	WLIM1				[395]
	PPN069C01	Transcription factor lim1	AT1G10200	WLIM1			Actin stabilizing protein	[395]
MADS-box family	PP1006G03	MADS-box transcription factor	AT5G60910	FUL/ AGL8		positive regulatior of caroterne and anthocyanin biosynthesis,	negative regulation of lignin	[396-399]
	PPN042H02	MADS4	AT4G18960	AG	ET up-regulation	postisitive carotene biosynthesis regulation;	negative regulation of lignin biosynthesis	[400-402]
RNA translation and protein assembly								
regulation of protein biosynthesis	PPN006H04	Translationally-controlled tumor protein homolog	AT3G16640	TCTP	AUX cytoplasmic signaling Sugar signaling		CW biosynthesis regulation	[403, 404]
Secondary metabolism								
Anthocyanin metabolism	PpLDOX (PpLDOX)	leucoanthocyanidin dioxygenase		PpLDOX		flavonoid/ PA biosynthesis		[405]
	PPN055C03	Anthocyanidin reductase	AT1G61720	BAN		PA biosynthess		[405]
Aspartate biosyntheis	PPN046D06	1-aminocyclopropane-1-carboxylate synthase	AT1G62960	ACS10				[406]
Carotenoid metabolism	PPN006A10	Phytoene synthase	AT5G17230	PSY				
	PPN067A01	Capsanthin/ capsorubin synthase	AT3G10230	LYC				
cyanide detoxification	PP1000E01	Cyanate hydratase	AT3G23490	CYN				[407]
	PPN066B01	Nitrilase/ cyanide hydratase and apolipoprotein N-acyltransferase family protein	AT5G12040					[384, 408]
Flavonoid metabolism	PPN050G05	Dihydroflavonol 4-reductase-like	AT5G58490			flavonoid/ PA biosynthesis		[409]
	PPN052H09	Chalcone synthase 2	AT5G13930	CHS/ TT4	negative regulation of AUX transport			[327, 410]

Signal transduction pathway									
Cytoplasmic TOR signaling	PPN076G10	protein lethal with sec thirteen 8-2	AT3G18140	LST8-1	AUX cytoplasmic signaling	Sugar signaling	CW biosynthesis regulation		[404, 411]
ET signaling/ ET signal transduction	PPN011G11	GTP-binding protein	AT3G46060	ARA3/ RAB8A	ET signaling				[412]
Trafficking machinery and membrane dynamics									
CME;EE ;internalization and intracellular trafficking of PM proteins	PPN011F03	Clathrin_L-chain	AT2G40060	CLC2	regulates cellular AUX levels by controlling the abundance and distribution of PIN proteins at the PM			Cell polarity regulation	[413]
CME;internalization and intracellular trafficking of PM proteins	PPN017G03	Calcium-binding EF-hand	AT3G01780	TPLATE	regulates cellular AUX levels by controlling the abundance and distribution of PIN proteins at the PM		regulation of cellulose synthesis by controlling the abundance of active CESA complexes at the PM	Cell polarity regulation	[414, 415]
Endosomal sorting complex	PPN060A04	Putative endosomal Vps protein complex subunit	AT5G22950	VPS24.1	required for internalize PIN1, PIN2, and AUX1 to the MVB/ LE for vacuolar degradation				[416]
Golgy to ER/ COPI vesicles	PPN044E10	ARF-like small GTPase 1	AT2G47170	ARF1A1C/ BEX1	Essential for recycling of PIN transporters to the PM and for vacuolar targeting			cell polarity	[417]
Retromer complex;LE to vacuole	PPN007G03	Sorting nexin-like protein	AT5G06140	SNX1	Regulates both the recycling (VSR from the TGN/ EE to the ER and the balance between vacuolar degradation and recycling of PIN proteins				[418, 419]
	PPN023B01	Ras-related protein Rab7	AT3G18820	RABG3F/ RAB7B					[420]
k-means8. Associated with high tolerance to chilling injury									
Aminoacid metabolism									
AUX biosynthesis	PPN058D11	Anthranilate synthase beta subunit	AT1G25220	ASB1	AUX biosynthesis				[421]

RNA transcription regulation								
AUX/ IAA family	PP1009D02	IAA16 protein	AT1G04250	AXR3/ IAA17	AUX and ABA nuclear signaling; negative regulator			[222]
	PPN060G07	AUX/ IAA protein	AT1G04240	IAA3/ SHY2	AUX nuclear signaling; negative regulator			[422]
HD-ZIP family	PPN074H05	HB2 homeodomain protein	AT4G35550	HB-4/ WOX13	AUX regulated		SCW biosynthesis; negative regulator lignin biosynthesis	[383, 423]
MYB-family	PPN067A04	MYB-like DNA-binding domain protein	AT4G38620	MYB4			SCW biosynthesis; negative regulator lignin biosynthesis	[382]
Signal transduction pathway								
ET signaling/ ET receptor	PPN054G06	Ethylene receptor	AT3G04580	EIN4	ET signaling			[98, 424, 425]
Transport								
Cooper transport	PPN035H02	Copper-transporting ATPase RAN1	AT5G44790	RAN1	ET signaling; delivers cooper ion into the ET receptors; is required for both ET binding and the receptor functionality			[426]

Abreviatures: AUX:auxin; ET; ethylene; ABA: Abcisic acid; PM:plasma membrane;CW: cell wall; SCW: secondary cell wall; ER: endoplasmic reticulum; MVB/ LE:microvesicular body/ late endosome; TGN/ EE:trans-golgy network/ early endosome; VSR:vacuolar sorting receptors VN:vascular networks; PA: proanthocyanines; PIN; PIN formed auxin efflux carrier; RG:rhamnogalacturonan; XyG:xyloglucan

expressed at high levels in the tolerant group of fruit and down regulated during cold storage in sensitive fruit, while in tolerant fruit they were less affected or even not changed (k-means 5 and 10; Figure 14). Previous studies have suggested that high constitutive gene expression prior to cold stress treatment might be part of a preformed tolerance mechanism in peach fruit (chapter 1 and [Dagar, 2013 #2457), which may contribute to inhibition of some aspects of ripening and protect fruit during cold storage (chapter 1). In particular, our results indicate that fruit with elevated levels at harvest and during cold storage of genes related to *protein biosynthesis*, especially ribosomal proteins, *energy production*, *antioxidant systems* and genes encoding for activities involved in the biosynthesis of secondary metabolites with antioxidant capacity such as carotenoids, flavonoids and proanthocyanins (k-means 5; Table 8; Additional Tables S8 and S11) were significantly less likely to develop CI. In agreement with these results, among genes correlated to WLT tolerance (cluster k-means 5 in Figure 14A; Table 8) there were the MADS box transcription factors AGAMOUS and FUL1, which have been described in other plants as positive regulators of carotenoid biosynthesis [396, 400], flavonoids [400] and anthocyanins [397].

We previously reported that genes of the flavonoid and early proanthocyanin biosynthetic pathways such as chalcone synthase (CHS/TT4), leucoanthocyanidin dioxygenase (PpLDOX) and glutathione *S*-transferase 12 (GST12/TT19) were part of a preformed mechanism associated with cold tolerance (chapter 1 and [5, 134]). The results here confirm these results (Table 8) and expand the list of genes related to these biosynthetic pathways to dihydroflavonol 4-reductase (DFR) and the ortholog of BANYUS (BAN), an anthocyanidin reductase (cluster k-means 5 in Figure 14A and Table 8). However, among genes in cluster k-means 2 (induced by cold in a sensitivity related manner) and k-means 1 (specific for high sensitivity to WLT) was the WRKY family transcription factor TTG2 (transparent testa glabra 2), which not only modulates mucilage production but also polymerization of proanthocyanidins [359] and AHA10, a putative P-type H^+-ATPase involved in proanthocyanidin transport and polymerization (Table 7 and 9). Interestingly, mutations in both, TTG2 and AHA10, increase the levels of proanthocyanidin monomers (i.e., catechin and epicatechin) [359, 427]. Epicatechin

Table 9 Genes discussed in the text associated to high sensitivity to WLT and FB.

Function Specific process	Chillpeach ID	Unigene annotation	Arab AGI	Arab Gene Symbol	Hormone signaling	Sugar signaling/ partioning	Hormone and secondary metabolite biosynthesis regulation	Cell wall and cytoesqueleton related	Cell polarity	Ref
k-means 1. Associated with high sensitivity to WLT										
Energy production										
plasma membrane ATP production	PPN027C11	Plasma membrane proton ATPase	AT1G17260	AHA10			PA transport and polymerization			[427, 428]
RNA transcription regulation										
b-HLH family	PPN080F10	Prf interactor 30137	AT2G27230	LHW	AUX signaling			VN establisment, maintenance, cell number and pattern		[270]
HB-family	PPN069A12	BEL1-like homeodomain transcription factor	AT2G35940	BLH1						[207, 273]
Signal transduction pathway										
AUX signaling/ AUX receptor E3 ubiquitin ligase SFC-TIR	PPN078E01	Transport inhibitor response 1 protein	AT3G62980	TIR1	AUX nuclear signaling					[294, 295]
AUX signaling/ Nuclear signaling pathway	PPN078G01	Putative auxin-resistance protein	AT1G05180	AXR1	AUX nuclear signaling					[294, 295, 429]
Calcium signaling/ Calcium sensor-transducer	PPN027B08	Calcium-dependent protein kinase	AT3G57530	CPK32	ABA					[286]
Calcium signaling/ Calcium signal transducer	PPN013H01	Serine/ threonine kinase	AT5G58380	CIPK10/ SIP1/ SNRK3.8	ABA					[298]
	PPN020F10	CBL-interacting protein kinase	AT4G30960	SNRK3.14/ CIPK6/ SIP3	ABA	sucrose signaling				[370, 371]

ET signaling	PPN057C10	Ethylene signaling protein	AT5G03280	EIN2	ABA; positive regulator of ET signaling		Ethylene biosynthesis; positive regulator of ACS type I and negative regulator of ACS type II	VN cel division regulation	[98, 246, 303-307, 394, 430, 431]
ET signaling/ Culin E3 ubiquitin ligase	PPN020G10	Ethylene-overproduction protein 1	AT3G51770	ETO1			repressor of ET biosynthesis (inhibits type II ACS)	VN cell division	[394, 432, 433]
Phosphorylation cascades/ MAPK	PPN020H02	Mitogen-activated protein kinase 4	AT4G01370	MPK4				Negative regulator of microtubule structure and stability; negative regulate MAP65-1	[434]
Phosphorylation cascades/ metabolic switch	PPN008G11	AKIN gamma	AT3G48530	KING1	ABA	sucrose signaling			[370-373]
Phosphorylation cascades/ PP2A	PPN037E11	Ser/ thr protein phosphatase 2A regulatory subunit B' gamma isoform	AT4G15415	ATB'GAMMA			Yang Cycle regulation		[311]
Transport									
carbohydrate transport	PPN025D11	SLT1 protein	AT3G12570	FYD		sugar partioning and homeostasis			
	PPN078G04	Putative membrane transporter	AT2G43330	INT1		sugar partioning and homeostasis			
Cooper transport	PPN025H09	Putative copper-transporting ATPase 3	AT1G63440	HMA5					

ion channel	PPN023C11	mechanosensitive ion channel	AT5G10490	MSL2		
Mg transport	PPN001H12	MRS2-5	AT2G03620	MGT3		
oligopeptide transport	PPN015D04	Metal-nicotianamine transporter YSL6	AT3G27020	YSL6		
	PPN028F10	Oligopeptide transporter OPT superfamily	AT5G55930	OPT1		
	PPN035B10	Oligopeptide transporter 7	AT4G10770	OPT7		
	PPN057F10	Oligopeptide transporter-like protein	AT3G54450			
k-means 11. Associated with high sensitivity to FB						
Aminoacid metabolism						
GABA biosynthesis	PPN044B12	Glutamate decarboxylase, putative	AT3G17760	GAD5		[435]
Pyruvate and Reactive carbonyl species						
conversion of oxalacetate to PEP	PP1002C02	Phosphoenolpyruvate carboxykinase [ATP]	AT4G37870	PEPCK;PCK1		
Pyruvate conversion to acetyl-CoA	PPN054C12	Pyruvate dehydrogenase	AT1G59900	PDHE1-A		
	PPN059C05	Pyruvate dehydrogenase E1 beta subunit isoform 3	AT5G50850	MAB1/ PDHE1-		
pyruvate-lactate interconversions	PP1006E06	Aldehyde dehydrogenase putative	AT1G44170	ALDH4/ ALDH3H1	ABA	[436]
	PPN035E06	Aldehyde dehydrogenase	AT1G44170	ALDH4/ ALDH3H1	ABA	[436]
	PPN038B05	Aldehyde dehydrogenase, putative	AT1G44170	ALDH4/ ALDH3H1	ABA	[436]

Abreviatures: AUX:auxin; ET; ethylene; ABA: Abcisic acid; PM:plasma membrane;CW: cell wall; SCW: secondary cell wall; ER: endoplasmic reticulum; MVB/ LE:microvesicular body/ late endosome; TGN/ EE:trans-golgy network/ early endosome; VSR:vacuolar sorting receptors VN:vascular networks; PA: proanthocyanines; PIN; PIN formed auxin efflux carrier; RG:rhamnogalacturonan; XyG:xyloglucan

showed negative correlation with chilling injury in peach fruit [437]. Taken together with this work, our results indicate that proanthocyanidin monomers may accumulate in tolerant fruit, while polymerized forms could be dominant in sensitive fruit.

In addition, the ROSMETER results (Figure 15) suggest a genetic program for high levels of antioxidant activities in cytoplasm, mitochondria and chloroplast (chromoplast) in CI tolerant peach fruit, which correlated well with the expression of several genes of the antioxidant system or mitochondrial electron chain (particularly the ROS production site in mitochondria). Consistent with this, cold tolerance and cold acclimation have been associated with higher expression levels of antioxidant/scavenging systems, effective mitochondrial transport and protein synthesis in peach (chapter 1 and [134, 438]) and other plants [28, 38, 209]. In addition, ROSMETER results suggest tolerant fruit might have activated a compensatory scavenging mechanism [19]. Both direct comparison between Od and Hz and ROSMETER analysis highlight CAT2 as associated to the sensitivity to chilling (Additional Table S8; Figure 15). The reductive thiol pathways appear to compensate quite rapidly for catalase deficiency, leading to a new, more oxidized cellular redox state, notably reflected in adjustments of thiol-disulphide status [439]. In agreement, Od fruit had higher number and higher expression levels of genes related to gluthathione-glutaredoxin and thioredoxin redox homeostasis than the sensitive fruit (Additional Table S8) and the expression of these genes is correlated positively with tolerance (cluster k-means 5 and 8; Additional Table S11).

C2.2.5.A link between WLT at a pre-symptomatic stage and auxin responses and distribution

The analysis performed in chapter 1 suggested that auxins play a role in the sensitivity /tolerance program induced by cold storage in peach fruit. We found that the expression of the of auxin transporters and positive regulators of nuclear auxin signaling correlated positively with the future WLT, while the expression of negative regulators of auxin signaling was associated with tolerance. In support of this, clusters k-means 1, 2, and 9 (with higher levels in sensitive fruit; Figure 14A) include orthologs of plasma membrane and endoplasmic reticulum auxin efflux carriers (ABCB19/PGP19,

PILS5 and PILS6; Table 7) as well nuclear signaling elements such as cullin CUL1/AXR6 and the auxin receptor TIR1/AXR1 (Tables 4 and 5). Also in agreement with our previous work, IAA/AUX proteins such as AXR3/IAA17 and IAA27 and SHY2/IAA3 (Table 8), encoding a negative regulators of auxin responses [440] were found in clusters k-means 5 and 8 (preformed tolerance and high-tolerance, respectively, Figure 14A). The expression of the ortholog of IAA27 is further supported by the qRT-PCR results (Figure 13B and Additional Table S10) and by the positive correlation of the ortholog of IAA27 with the degree of tolerance in individual lines from the Pop-DG population (Additional Table S12 and chapter 1).

The results here also highlight new auxin related genes as candidates to be involved in the tolerance/sensitivity to CS. The ortholog of anthranilate synthase (ASB1/WEI7), which is required for IAA synthesis (Table 8), was highly expressed in tolerant fruit in CS (k-means 8; Figure 14). Further, in cluster k-means 5 (high expression at harvest associated to tolerance, decreasing in storage; Table 8) are the translationally controlled tumour protein (TCTP) and LST8 (lethal with SEC13 protein 8), components of the TOR (target of rapamycin) signaling pathway, an integral part of the cytosolic auxin signaling pathway [441] that connects hormonal and nutrient pathways [442].

Taken together, the differential expression of several genes for auxin homeostasis, transport and signalling supports a strong connection between auxin metabolism and the CI tolerant/sensitive character of peach fruit. But how does auxin link with the expression changes observed for genes related to cell wall, antioxidants and other possible molecular signatures associated to WLT development at the pre-symptomatic stage? Evidence suggests that auxin can affect cell wall structure through both transcriptional, and non-transcriptional mechanisms, such the acidification-linked loosening of the wall (reviewed in [443]) and the TOR pathway [404]. We found that low levels of expression of TOR components were associated to sensitivity (cluster k-means 5, Figure 14A). Inhibition of TOR signaling caused specific changes to pectins and arabinogalactan protein components of cell walls [404]. However, via the cytoplasmic TOR pathway [442] auxin increases the overall cytoplasmic protein

Table 10. Genes discussed in the text correlated with sensitivity at harvest and during cold storage **(cluster k-means 9).**

Function Specific process	Chillpeach ID	Unigene annotation	Arab AIG	Arab Gene Symbol	Hormone signaling	Sugar signaling/ partioning	Hormone and secondary metabolite biosynthesis regulation	Cell wall and cytoesqueleton related	Cell polarity	Ref
Cytoskeleton organization and biogenesis										
Microtubule stability and organization	PPN077E06	Microtubule-associated protein	AT3G04630	WDL1				Negative regulator of microtubule structure and stability		[444]
Protein degradation										
Ubiquitin ligase E3 complex/ SFC-culin	PPN032H05	Cullin	AT4G02570	AXR6/ CUL1	AUX nuclear signaling					[294, 295, 429]
RNA transcription regulation										
MYB-family	PPN055C11	Sucrose responsive element binding protein	AT5G67300	MYBR1/ MYB44	ABA, AUX, ET	Sucrose responsive element binding protein				[31, 445-447]
Signal transduction pathway										
G-protein coupled receptor protein signaling pathway/ G-protein complex	PPN065B10	Guanine nucleotide binding protein (G-protein), alpha subunit family protein	AT1G31930	XLG3	ABA, AUX, ET	sugar sensitivity				[308, 448]
Phosphorylation cascades/ MAPK	PP1009F07	Trichoderma-induced protein kinase	AT3G45640	MPK3	positive regulation of ACS type I		Ethylene biosynthesis; positive regulation of ACS type I	pectin induced		[449-452]
Phosphorylation cascades/ MAPKKK	PPN071C11	protein kinase family protein / ankyrin repeat family protein	AT1G14000	VIK	AUX and BR singaling	sugar partioning and homeostasis		VN formation		[453, 454]
Phosphorylation cascades/ PP2A	PPN014G07	Serine/ threonine-protein phosphatase 2A regulatory subunit A beta isoform	AT3G25800	PDF1/ PP2AA2	Regulates PIN subcellular distribution				Cell polarity regulation	[296, 455]

Trafficking machinery and membrane dynamics										
CME;Vesicle coat/ clathrin coated vesicles	PP1003H08	Putative Clathrin coat assembly protein AP50	AT5G46630	AP2M	regulates cellular AUX levels by controlling the abundance and distribution of PIN proteins at the PM			regulates cellulose synthesis controlling the abundance and distribution of active CESA complexes at the PM	Cell polarity regulation	[456, 457]
Fatty acid biosyntheis	PPN026B01	Carboxyl transferase alpha subunit	AT2G38040	CAC3			fatty acid biosynthesis			[458]
Glycerolipid biosynthesis	PPN008G03	Digalactosyldiacylglycerol synthase 1	AT3G11670	DGD1			digalactosyl diacylglycerol biosynthesis		polar targeting of proteins to the PM;Lipid microdomains	[459-462]
Glycerolipid metabolism	PPN065F12	phosphatidic acid phosphatase-related / PAP2-related	AT3G50920	LPPEPSILON 1			diacylglycerol biosynthesis			[463]
Phospholipid biosynthesis	PPN008H07	Putative phospholipid cytidylyltransferase	AT2G38670	PECT1			phosphoethanolamine biosynthesis		polar targeting of proteins to the PM;Lipid microdomains	[460-462, 464]
trans-Golgi network transport vesicle/ COPI vesicles	PPN002C04	ARF GTPase-activating domain-containing protein	AT5G13300	VAN3/ SFC	required for either normal PIN1 cycling or for PID-directed efflux machinery relocation			regulates formation of plant VN	Cell polarity regulation	[316, 465]
Transport										
carbohydrate transport	PP1003F09	Integral membrane protein,	AT1G75220	ERDL6		sugar partioning and homeostasis				[466]
Cl-channel	PPN078A03	Cl-channel, voltage gated; IMP dehydrogenase related 1	AT5G33280	CLCG						
Na/ K antiporter	PPN064A01	Na+/ H+ antiporter	AT2G01980	SOS1						[319-321]
nitrate transport	PPN024D02	Nitrate transporter NRT1-2	AT1G18880	NRT1.9						
oligopeptide transport	PPN005F03	Oligopeptide transporter 7	AT4G10770	OPT7						
	PPN064F08	POT family, putative	AT1G59740	NRT1/ NPF4.3						

Unknown transporter	PPN066F09	Putative integral membrane protein	AT5G19980	GONST4		sugar partioning and homeostasis	is probably involved in the provision of GDP-sugars into the Golgi for CW polysaccharide synthesis such as RG-II and XyG	[467-469]
Unknown function								
Unknown interferon protein	PPN065A05	interferon-related developmental regulator family protein	AT1G27760	SAT32	ABA			[470]
Unkown Zinc finger RING-like	PP1003D02	ubiquitin ligase	AT3G23280	XBAT35	ET regualtion AUX	ABA, glucose		[471, 472]

Abreviatures: AUX:auxin; ET; ethylene; ABA: Abcisic acid; PM:plasma membrane;CW: cell wall; SCW: secondary cell wall; ER: endoplasmic reticulum; MVB/ LE:microvesicular body/ late endosome; TGN/ EE:trans-golgy network/ early endosome; VSR:vacuolar sorting receptors VN:vascular networks; PA: proanthocyanines; PIN; PIN formed auxin efflux carrier; RG:rhamnogalacturonan; XyG:xyloglucan

synthetic capacity of the cell [473]. This agrees with the higher levels of cell wall related genes in sensitive fruit and with the higher levels of genes related to protein biosynthesis in tolerant fruit. In addition, an important function of the TOR pathway is the regulation of mitochondrial activity and, hence, the production of ROS in animals [474] and in plants [404]. Thus, we suggest that while auxin changes are probably mainly related to cell wall in sensitive fruit, cytoplasmic auxin in tolerant fruit may be related to the maintenance of the translation machinery and the control of ROS.

C2.2.6.Ethylene is related to tolerance to cold storage

Ethylene reduction has been correlated with WLT sensitivity [136, 137] and with the down regulation of some key cell wall activities associated to WLT development [342]. Zhou et al. [136] found that during prolonged cold storage, maintaining the ability of nectarine fruit to produce ethylene or adding exogenous ethylene to the storage atmosphere, prevented CI. Furthermore, correlating with ethylene production, the gene and protein expression of the ACO and ACS1were depleted during cold storage in fruit developing WLT during shelf life [136, 137]. In agreement, we found that the most tolerant Od fruit have higher levels of both ACO and ACS (Additional Table S8). This is further supported by the qRT-PCR results (Figure 13B and Additional Table S10) and the positive correlation of the ACS1 with the tolerance degree in individual lines from the Pop-DG population (Additional Table S12 and chapter 1). Moreover, genes related to metabolism of the ethylene precursor methionine (salvage pathways and Yang cycle) and cyanide detoxification were in cluster k-means 5 (Table 8; Additional Table S11). It has been proposed that high rates of ethylene biosynthesis in climacteric fruit are supported by recycling of the ethylene precursor methionine via the Yang cycle [475] and by having an active system for handling cyanide, a byproduct of ethylene biosynthesis [476].

In addition several ethylene biosynthesis regulators and signaling elements were also differentially expressed between sensitive and tolerant fruit and their expression correlated with tolerance/sensitivity (Tables 7-10). EIN2 (ETHYLENE INSENSITIVE2) has been previously reported during cold storage in peach fruit [246] and it has been

associated with cold sensitivity in Arabidopsis [98] and peach (chapter 1). Both EIN2 and Ethylene-overproduction protein 1 (ETO1) (in cluster k-means 1; Figure 14A) are implicated in the negative regulation of type II ACS. EIN2 participates in the negative feedback regulation of ethylene biosynthesis by affecting the expression of ACS type II at transcriptional level [431], while ETO1 inhibits the enzymatic activity of type II ACS and targets it for 26S proteasome-mediated degradation [433]. In addition, in cluster k-means 2 (induced in CS and higher in sensitive fruit, Figure 14A) was VAS1 (reversal of sav3 phenotype; Table 7), recently identified as a cross-regulatory point controlling the flow through the auxin and ethylene biosynthetic pathways in response to shade [345]. VAS1 prevents over-accumulation of ethylene and auxin, thus preventing an exaggerated response to this environmental signal and *vas1* mutants accumulate ACC and auxins.

Furthermore, associated with high tolerance to cold (cluster k-means 8, Figure 14A) were the orthologs of the ethylene receptor EIN4 (ethylene insensitive 4) and RAN1, a P-type ATPase copper transporter that delivers the copper ion to the receptors and is required for both ethylene binding and the receptor functionality (Table 8). In Arabidopsis, EIN4 plays a positive role during cold acclimation in Arabidopsis [98], which coincides with their high expression in tolerant Od fruit (Additional Table S11). However, although EIN2 and EIN4 seem to play a similar role in cold acclimation in peach fruit and Arabidopsis, high levels of ethylene enhance tolerance to CI in peach while having a negative effect on Arabidopsis [98]. This difference may be explained by the different organs considered (fruits and leaves) and developmental processes. A lack of ethylene production during cold storage affects normal fruit ripening and leads to WLT [136].

C2.2.7.Sugar homeostasis and hormone crosstalk: auxin, ethylene, ABA

All three clusters (k-means 1, 2 and 9) associated to CI sensitivity were enriched in transport elements (Figure 14B). Besides the auxin transporters described above, these genes are rich in carbohydrate transporters and in oligopeptide/metal ion transporters (Tables 7, 9, 10; Additional Table S11). This suggests that nutrient reallocation could

play a role in the cell wall remodeling and metabolic changes happening in sensitive fruit. This may be the case of golgi nucleotide sugar transporter GONST4 (cluster k-means 9, Figure 13A), which is involved in the provision of GDP-fucose and GDP-l-galactose sugars into the Golgi for cell wall polysaccharide synthesis such as rhamnogalacturonan II and xyloglucan (Table 10), and ERDL6 (Early Responsive to Dehydration-Like 6) which functions as a vacuole glucose exporter (Table 10). Likewise, these transporters can also contribute to the sensitive character of peach fruit. Plants overexpressing ERDL6 or the sugar beet (*Beta vulgaris*) homolog Bv*I*MP (Integral Membrane Protein) accumulated lower glucose and fructose in the vacuole than wild type and had reduced tolerance to cold [466].

In addition, the effect of cold on transporters can also reflect the stresses imposed to the fruit (cold, darkness and detachment), that may limit nutrient availability. It is described that in chilling sensitive peaches, glucose and fructose content increases during cold storage, while sucrose diminishes [477]. Emerging data indicate that sugar-derived signaling systems, including trehalose-6 phosphate (T6P), sucrose non-fermenting related kinase-1 (SnRK), and the TOR kinase complex also play important roles in regulating plant development through modulating nutrient and energy signaling and metabolic processes, especially under abiotic stresses where sugar availability is low (reviewed in [478]). Among signaling elements highly expressed in sensitive fruit were genes encoding for orthologs of several SnRKs of the three described groups SnRK1 (AKIN beta and aKING1, in clusters k-means 1 and 2), SnRK2 (SnRK2 OTS1/ SNRK2-6 and SNRK2.8, in cluster k-means 2) and SnRK3 (CIPK 10 and CIPK6; cluster k-means 1; Tables 2 and 4). Most of these genes have been associated to the chilling sensitive phenotype in peach (chapter 1). Limited sucrose availability, osmotic stress and abscisic acid (ABA) activate the activity and the expression of SnRKs [371], which act as inhibitors of gene expression involved in different biosynthetic pathways [373]. The SnRK1 complex plays a central role in nutrient, darkness and stress [479]. Thus, it is likely that sucrose depletion by cold together with fruit detachment [477] enhances the expression of these genes. Furthermore, and in agreement with our results, the SnRK1 complex may play a role opposite to the one

played by the TOR pathway in sensing energy [480] that promotes energy-consuming related cellular processes, such as mRNA translation when sucrose levels are high [480].

C2.2.8.Vesicle trafficking, membrane dynamics and cytoskeleton organization related to WLT at a pre-symptomatic stage

Our results indicate that differences in the expression levels of genes related to intracellular trafficking, cytoskeleton and lipid metabolism before and during cold storage (Tables 7-10 and Additional Table S11) could be related to the sensitivity or tolerance to CI in both a preformed (k-means 5; Figure 14A) and induced mechanism (k-means 2 and 9; Figure 14A). Similarly to other plants, this indicates that differences in membrane composition [481, 482], cytoskeleton stability [483] and polar transport of proteins [484, 485] participate in the response of peach fruit during cold. Differences in the expression of these genes could have a key role in the molecular phenotypes associated to the tolerance and sensitivity by regulating processes such as cell wall biosynthesis modifications and auxin distribution. Gonzalez Agüero et al. [11] suggested that alterations in the abundance of the endomembrane system components could have an important role in the development of WLT during cold and during shelf life by modifying the flow of polysaccharides and proteins to the cell wall. Furthermore, cytoskeleton [486, 487] and lipid composition of membranes [368, 488] are essential for, among other functions, polar distribution of membrane proteins, such as cell wall biosynthesis enzymes and auxin transporters.

C2.2.9.Gene expression related to sensitivity to FB and FBL at a pre-symptomatic stage

Hz was the only fruit that developed FBL and FB during the storage period. Although the analysis using the four fruit types is biased for WLT, the comparison of pools and Hz-Od experiments has identified a group of genes (k-means 11; Figure 14A) that only respond to cold in Hz fruit, and thus are good candidates to be related with Hz phenotype. This cluster is enriched in genes involved in the production of acetaldehyde

and pyruvate metabolism (Figure 14B; Table 9). Further the same genes were identified as highly expressed in Hz compared to Od (Additional Table S8). Thus, acetaldehyde production could be related to the FBL and FB. FB is generally thought to be due to the action of polyphenol oxidase [129]. However, discoloration can also occur by non-enzymatic reactions through metal-polyphenol complexes [489]. This browning mechanism could be induced by chilling in Hz fruit. Hz fruit contained relatively high levels of expression of metal transport genes in comparison to Od and pools (Additional Tables S9 and S11), which indicates a mobilization of metal ions associated to FB. Furthermore, high levels of PpLDOX correlated to BR sensitivity [5, 18] and the results presented here indicate that Hz fruit have relatively high levels of expression of genes related to proanthocyanin monomer biosynthesis (Table 8). The combination of these two factors (i.e., high expression of both proanthocyanin and metal mobilization genes) with high expression of acetaldehyde production genes may increase the propensity of the fruit to FB when moved to shelf life. Lastly, among genes associated to the FB at a pre-symptomatic stage was an ortholog of glutamate decarboxylase (GAD5; Table 9). Glutamatade decarboxylase catalyze the first and irreversible step of gamma aminobutyric acid (GABA;[435]). GABA has been shown to be a metabolic marker for core breakdown in pear [490]. These possible genes FB and FBL should be validated with additional cultivars.

Chapter 3

Chapter 3. Analysis of the transcriptomic changes associated to cold-induced mealiness development during shelf life ripening, in peach fruits of Pop-DG population

C3.1.Results

C3.1.1.Phenotypic differences in pools of LS and S cold stored fruit as revealed by subsequent shelf life ripening

Two pools of peach fruits, from the Pop-DG population [18], with known contrasting chilling (mealiness) tolerance were chosen for this study. The genotypes contributing to the LS pool were identified in chapter 1 as a low- sensitive to chilling, while the genotypes making up the S pool are highly sensitive to chilling. To investigate differences in mealiness development between the S and LS pools, fruits were cold stored at 5ºC for 1- 3 weeks and then submitted to ripening for 2-3 days at 20ºC (SLR conditions) to allow the development of mealiness. A mealiness index [8] was assigned to each pool during SLR after each 1, 2 or 3 weeks CS (Figure 16B). All pools LS exhibited greater chilling tolerance and ripening recovery ability at SLR conditions after cold storage than S pools. This was especially true after one week of CS, when no MI is observed in LS fruit (Figure 16B). Based on previous reports and current observations, we confirmed that LS and S differ significantly in their response to CS and that the onset of visual mealiness symptoms in pools of LS fruits was delayed by approximately 1 week beyond that of the S pools (Figure 16B and Figure 2 in chapter 1).

C3.1.2. Overview of Transcriptomic Changes: From harvest to cold storage and shelf life ripening

We surveyed global gene expression by bulk segregant gene expression analysis (BSGEA; chapter 1) using Chillpeach microarray [17], i. e. by comparing the transcriptomes of fruit pools LS and S during cold storage (CS) and after subsequent shelf life ripening (CSR). To determine the effect of cold on the ripening program, room temperature control samples were included: Mature harvest fruits (M) and fruits ripened for 2-3 days without previous cold storage (R).

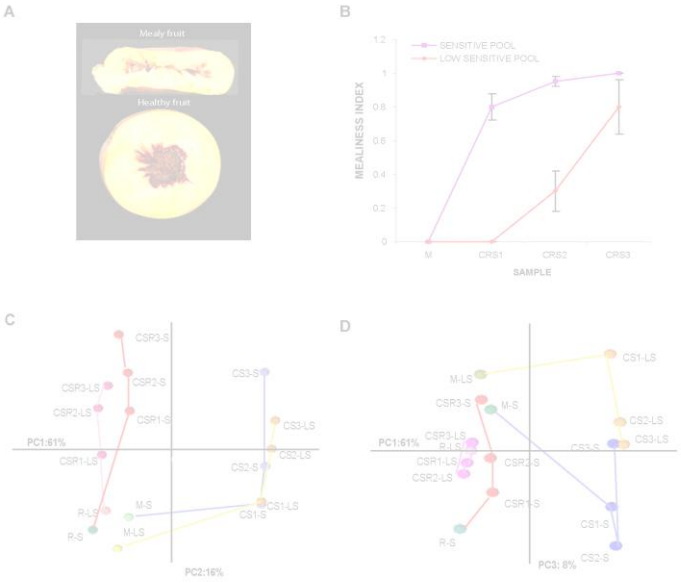


Figure 16. **Global gene expression analysis of ChillPeach transcripts in response to CS and subsequent SLR. A) Mealy and healthy fruits. B) The mealiness index (MI) of sensitive (S) and low sensitive (LS) pools of fruits given two days SLR after storage for up to three weeks at 5ºC. C and D) PCA of the global expression profile showing the variation in each treatment, averaged from three replicates. In C, the first principal component (PC1) is shown on the x-axis while the second principal component (PC2) is shown on the y-axis; in D, the first principal component (PC1) is shown on the x-axis while the third principal component (PC3) is shown on the y-axis. M: mature fruits; R: mature with two to three days ripening at 20°C; CS1: M + one week cold storage at 5°C; CS2: M + two weeks cold storage at 5°C; CS3: M + three weeks cold storage at 5°C; CSR1: M + CS1 + R; CSR2: M + CS2 + R; CSR3: M + CS3 + R; S: sensitive and LS: low sensitive**

Over this time course 3394 genes were differentially expressed at one or more times (differentially expressed genes, DEG) using two criteria: a false discovery rate (FDR) < 5% and p-value < 0. 05 (Table S13). Principal component analysis (PCA) (Figure 16 C-D) xxxxxxxx xxxx xxxx-xxxxxxx XXX xx xxxxxxxxxx xxxx xxxxxxxxxxx xxxxxxx xx xxxx xxxxxxxxx. Xxx xxxxx xxxxxxxxx xxxxxxxxx (XX1: 61% xxxxxxxx) xxxxxxxxx xxxx xxx xxxxxxxx xx xxxx-xxxxxxx xxxxxxxxxxxxx xxxxxxxxxxx xxxx xxx xxxxxxxxxx xxxx xxxxxx xxxx xxxxxxxxxxx xx XXX (Xxxxxx 16X-X). Xxxxxxx PC2 (16% of variance) and PC3 (8% of variance) xxxxxxxxx xxxx xxxxxxx xxx xxxxxxxxxxxxxx xxxxxxx xxxxxx xx xxxx xxx xxxxxxxx xxxxxx XXX xxx, xxxxxx XXX-X xxx XXX-XX xxxxxx xx xxxxxx xxxxxxxx xxxxxxxx xxxxxxxx (Xxxxxx 16X-X); XXX xxxxxx xxxxxx xxxxxxxxxxxxxxxxx xxxxxxxxx xxxxx xxxx xxxxxx (X) xxx xxxx (X) xxxxxx. Xxxxx xxxxxxxxxx xxxxxxxxx xxxx xxxx xxxxxxx xxxx (Xxxxxx 16X-X). XX3 xxxxxxxx x xxxxxxxxxxxx xxxxxxxxxxxxx xxxxxxxxxx [491] xx xxxxxx XXX-XX xxxxx xxxxxxxxx xxxxxxxx xx xxxxxxx X xxxxxxxxxx xxxxxx xxx xxx xxxx xxxxxxx xxxxx (Figure 16D). Xx xxxxxxxx, xxxxx xxxx XXX-X xxxxx xxxxxxxx xxxx xxxxxxx xxxx xxxxxxx X xxxxxxxxxx xxxxxx xxxxx xxxx xxxxxxxx. Xxxx xxx xxxxxxxxx xxxxx xx xxxx, xxxx xxxxxxxxx xxxx xxxxxx xxxxx xxxx, XXX-XX xxxxxx xxxxxxx xxx xxxxxxxx xxxxxxx xxxx xxx xxxxxx xx xxxx xxxxxxx. XXX-X xxxxxx, xx xxxxxxxx, xxxxxxx xxxxxxxxx xxx xxxxxxxxxxxxxxx xxxxxxxx xxxxxxxx xxxxxx xxxx xxxxxxx xxx xxxxxxxxxxxx x xxxxxxxx xxxxxxxx xxxxxxx xxxxxx XXX xxxxxxxxxx. Xxxxxxx, xx xxxx xx XX3 (Xxxxxx 16X), XXX3-X xxx xxxxxx xx X xxxxxx xxxx xxxxxxxx XX xxxxxxx xxx x xxxx xxxxxxxx xxxxxx xx X xxxxxx, xxxxx, xx xxxx, xxxxxxxx xxxxxxxxxxxxxxxxx xxxxxx xx X xxxxxx, xxxx xxxx xxxx-xxxxxxx xxxxxxxxxxx xxx xxxxxxxxxxx xx xxxxxxxxxx xxxxxxxx.

XXX xxxx xxxxxxxxx xxxx xxx xxxxxxxx xxxxxxx xx XX xxxxxx xxx xxxxxx xx XX, xxx xx x xxxxxx xxxxxx xx xxxxxx X. Xxxxxxxxx xx XX3 (Xxxxxx 16X), xxxxxx xxxx xxx X xxxx xxxxxx xxx xxx xx xxx xxxxx xx xxx xxxx (XX1-X xxx XX2-X xxxxxxx) xxxxxxxx x xxxxxxxx xxxxxxx xxxxxxx xx X-X xxxxxx (xx xxxx xxx xxxxxxx xxxxxx xx XX3), xxxxx xxxxxx XX-XX xxxxxx x xxxxxxx xxxxxxxx xxxxxxx xx X-XX xxxxxx, xxxxxxxxxx xx xxx xxxx. Xxxxxxxxx, xx xx xxxxxxxx xx xxxxxxx 1, xxx xxx xxxxxxxxxxxx xxxx xxx xxxxx xxxxxxxxx xx xxxxxxxx xxx xxxxxxxx xxxxxxx xx XX xxxxxx, xxx xxxx xx xx X xxxxx. Xx xxxxxxxx, XX3 xxxxxxxx xxxx-xxxxxx xxxxxxx xxxxxxxxx xx xxx xxxxxxxx xxxxxx xxxxxx xxxxx xxxxx xxxxx xxxxxx xxxxxx

they shelf ripen after CS (Figure 16B and 16D). This suggests that cold-induced transcriptional changes affect the way fruits ripen afterward.

C3.1.3.Alterations of the post-harvest ripening program during SLR after CS

To analyze to what extent CS primes the fruit and modifies ripening program during subsequent shelf life, and to identify when these alterations start to occur,we analyzed the transcriptome of fruits ripened at SLR after being CS for different times (CSR fruits) and compared them to the transcriptomes of their corresponding fruits R (Figure 17A and table S13). Xxxx xxxxxxxxxx xxxxxx xxxx xxxxxxx xxxxxxxx xxxxxx XXX xxxxx XX xxxxxxxx xxxxxx xxxx xx xxxxxxx xxxxxx xx-xxxxxxxxxx xx xxxx xxxxxxxxxx, xxxxx xxxx-xxxxxxxxxx xxxxxxxx xxxxx. Xxxxxxx, xx xxx xxxx xxxxxx xxxxxxxxx, xxx xxxxxx xx xxxxx xxxx-xxxxxxxxx xxxxx xxxxxxxxx, xxxxxxxx xxxx xxxxxx xxxx xxx xxxxxx xx xx-xxxxxxxxx xxxxx. Xxxx xxxxxxxxxx xxx xxxx xxxxxxx xx X xxxxxx xxxx xx XX xxxxxx: xxxxxxxxxx xx 396 xxxxx xxx xxxxxxxx xxx xx XXX1-X xxxxx xx xxx xxxx xx XX, xx xxxxxxxx xx xxxx 20 xxxxx xx XXX1-XX xxxxxx. Xx xxxxxxxxx xxxx XXX xxxxxxxxx (Xxxxxx 16X), xxxx xxxxxxxxxx xxxxxxxxx xx xxx XXX-X xxxxxx xx xxx xxxx xx XX xxxxxx xxx xxx xxxxx xx XX xxxxxx xx xxx, xx xxxxxxxx xxxx xxxxx xxx xx xxxxx xxxxx XX (Xxxxxx X4), xxxxxxxx xxxx xxxxxx xxxxxxxx xx XXX.

Functional enrichment indicated xxxx *XXX xxxxxxxxxxx xxx xxxxxxx xxxxxxxx* xxxxxxxx xxxxx xxxxxxxxxxx xx xxx XXX-X xxxxxx xxxxxxxx xx xxx xxxxxxxxxxxxx xxxxxxxxx xxxx xxxxxx (Xxxxxx 17X), xxxxx xxxx xxxxxxxxxx xxxxxxxx xxx xxxxxxxx xx XXX-XX xxxxxx xxxx xxxx xxxxxxxxxx xxxxxxx xx xxxx xxx xx xxxxx xxx xx xxxxx (Xxxxxx 17X). Xx xxxx X xxx XX xxxxx xx xxxxxx, xxx xxxxxx xx xxxxx xxxxxxxxx XXX xxxxxxxxxxx xxx xxxxxxx xxxxxxxx xxxxxxxx xxxxxxxxx xx xxxxxxxx xxxx XXX xxxxxxxxxxx (Xxxxxx 16X xxx 17X). Xx xxxxxxxx, *xxxxxxxxxx xxxxxx* xxxxxxx xxxxx xxxx xxxxxxxx xxx xxx xx xxxxx xxxx xxxxxxxx xxxxxxxxx xx XXX1-X xxxxxxx. *Xxxx xxxx* xxxxxxx xxxxx xxxxxxxx xxxxx xxxx xxxx xxxxxx xxxxxxxxx xx xxxx XXX-XX xxx XXX-X xxxxx xxxxx xxx xxxxx XX, xxxxxxxx xxx xxxxxx xx xxxxx xxxx xxxxxxx xxxxxxxxxx xxx xxxx xxxxxxx xx XXX2-X xxxxxx xxxx xx XXX2-XX xxxxxx

(Xxxxxx 17X). Xx xxxxxxxx, *Xxxxxx xxxxxxxxxx* xxxxxxxx xx xx xxxxxxxxx those genes upregulated in CSR3-S fruits with respect to R-S fruits, but not in CSR3-LS fruits.

Figure 17 Differences in the transcriptomic response of S and LS pools to SLR after CS. A) Number of genes with altered expression in SLR fruits stored up to three weeks at 5º (CSR) compared to untreated ripe fruits (R). B) Functional categories overrepresented in genes up- or down-regulated in CSR fruits compared to R fruits. C) Venn diagram showing the number of genes differentially expressed between tolerant and sensitive fruit after three lengths of CS followed by SLR. D) Over-represented functional categories (*p*-value > 0.05) corresponding to genes differentially expressed between LS and S pools at each length of CS + SLR. Differentially expressed genes were obtained at FDR < 0.05 and *q*-value < 0.05. Enriched functional categories were considered when Fisher test *p*-values < 0.05 and the number of genes was greater than three. R: mature fruits after two to three days ripening at 20°C; CSR1: one week CS plus SLF; CSR2: two weeks CS plus SLR; CSR3: three weeks CS plus SLR; S: sensitive pool and LS: low-sensitive pool.

Among genes down-regulated in CSR-S fruits with respect to R fruits, xxx *xxxxxxxxxxxx xxxxxxxxxx* xxxxxxxx xxxxxxxx xxxxx xxxxx xxxxx xxx XX xxxxxxx, xxxx xxx xxxxxx xx xxxxx xxxxxxxxxx xxxx xxx xxxx xx XX xxxxxxxxxxxxx xxxx xxx xxxxxxxx xx XXX xxxxxxxx (Xxxxxx 17X). Xxxxxxxxxxxxx xx *xxxxxxxxxxx xxxxxxxxxx* xxxxxxxx xxxxx xxx xx x xxxxxx xxxxxx xx XXX-XX xxxxxx. Xx xxxxxxxx, *xxxxx xxxx xxxxxxxxxx* xxx *xxxxxxxxx* enriched those

genes down-regulated in CSR-S fruits after two or three weeks of CS (Figure 17B). Further, the categories *xxxx-xxxxxxxxxxxxx xxxxxxx xxxxxxxxxxxxx* xxx *xxxxxxxx xxx xxxxxxx xxxxxxxxxx* xxxxxxxx xxxxx xxxx-xxxxxxxxx xx XXX3-X xxxxxx. Xx XXX-XX xxxxxx, xx xxxxxxxx xx xxx *xxxxxxxxxxx xxxxxxxxxx* xxxxxxxx, xxxx xxxxx xx *xxxxxxx xxxxxxxx* xxxxxxxx xxx xxx xx xxxxx xxxx xxxx xxxxxxxxx xx xxxxx xxxxxx xx XXX-XX xxxxxx xxxx xx the corresponding untreated ripe fruits (Figure 17B).

C3.1.4. Stage-specific differences in the transcriptomic response to shelf-life ripening after cold storage between the pools of contrasting genotypes.

We next performed one-to-one direct comparisons of the transcriptome of S and LS fruits ripened after each length of CS (Figure 17C-D and Table S2 Since the main difference between these pools of fruits was the time of onset of WLT, xx xxxxxxxx xxxx xxxx XXX-xxxxxxxxx xxxxxxxxxxxxxx xxxxxxxxxxx xxxxxx xx xxxxx xxxxxxx xxx xxx xxxxx xx xxxxxx. Xxx xxxxxxxxxxxxxx xx X xxx XX xxxxxx xxxxx-xxxxxxx xxxxx xxxx XX xxxxxx xxx xx xxxxxxxxxx xxxxxxxx xx xxxxxxxx xxxx xxx xx xxx xxxxx, xxxxxxxxxx xxxx xxxx xxxxx xxxxxxxxxx XX xxxxxx xxx xxxx xxx xxxxxxxxx xxxxxx xx xxxxx xxxx xxxxxxx xxxxxxxxxx xx XXX2-XX xxxxxxxx xx X-XX (Xxxxxx 17X). Xxx xxx xx xxxxx xxxxxxxxx xx xxxxxx xxxxxx xx XXX-X xxxxxx xxxxx xxx xxxx XX, xxx xxxxx xx xxxxxxx xxxxxxxxxxx xx xxxxxxxxx (XXX1-X XX =0. 8 xx XXX1-XX XX =0, Xxxxxx 16X), xxxx xxxxxxxx xx xxxxx xxxxxxx xx *xxxxxxxxx*, *xxxx xxxx* xxx *xxxxxxxxx xxxxxxxxxx* (Xxxxxx 17X). Xxxxx xxxxxxxxxxxxx xxxxxxxxx xx XXX2-X xxxxxxx xxxx xxxx xxxxxxxx xx xxxx xxxx-xxxxxxx xxxxx. Xxxxxxxxxxxx, xxxxxxx xxx xxx xxxxx xxxxx xx XX, xxx xxxxxx xx xxxxx xxxxxxxxxxxxx xxxxxxxxx xxxxxxx X xxx XX xxxxxxxxx, xxxxxxxx xxxx X xxx XX xxxxxx xxxx xxxxxxxx xxxxx xxx xxxxxxxxxxx xx XX xxxx xxxxxxx. Xxxx xxx xxxxxxxx xxxxxxx X xxxxxx xxxx xxxx-xxxxx xxx xxxxxxxxxx xxxx xxxxx xxx xxxxxxxxxx xxxxxxxxx. Xxx xxxxxxxxxx xx XXX xxxxxx xxxxx xxxxx xxxxx xxxx xxxxxxx xxxxxxxxx xxxx xxx xxx xx xxxxx xxxxxx xxxxxxxxx xx XXX3-X xxxxxx xxxx xxxxxxxx xx *XXX xxxxxxxxxxx xxx xxxxxxx xxxxxxxx* xxx *xxxxxx xxxxxxxxxx*, xxxxx *xxxxx xxxx xxxxxxxxxx*, *xxxxxxx xxxx xxxxxxxxxx*, *xxxxxx production* and *signal transduction* functions, despite

xxxxx xxxx-xxxxxxxxx xxxx xxxxxxx xx X xxxxxxx (Xxx 17X), xxxx xxxx xxxxxxxxx xx XX xxxxxx xxxx xxx xxxx xxxxxxxxxxx xxxxxx (Xxx17X).

C3.1.5.The impact of cold storage on the subsequent post-harvest ripening program at room temperature

To study how CS affects ripening and how cold-induced modifications in the transcriptome affect subsequent ripening under SLR conditions, we analyzed the molecular ripening of the S and LS pools. Xxx xxxx xxxxxxx, xxx xxxxxxx xx XXX xxx xxxxx xxxx xxx xxxxxxx. Xxx xxxxx xxxxxxxxxxxx xx xxx 859 xxxxx xxxxxxxxxxxx xxxxxxx 1 xx xx xx xxxx xxxxxxxxx xx xxxxxxxxx xxxx xxxxxx xxxxx 2-3x xx 20xX (X) xx xxxxxxxx xx X xxxxxx (Xxxxxx X2 xxx X13) xxx xxxxx xxxxxxxxx xxxxxxxxx xx xxxxxxxx-xxxxxxxxx xxxxx. Xxxxx xxxxxx xxxxxxx xxx xxxxx xxxx xxxxx XXX xxxxxxxxxx xxxx xxxxxxxxx xxxx [492], xx xxxx xxxxxxx xxx xxxxxx xx xxxxx xxxx-xxxxxxx xxxxxxxx xxxxx (xXX). Xxx xxxxxx xxxxxxx, xxxxxx xxx-xxxxx xxxx-xxxxxxx xxxxxxxx xxxxx (XxXX), xxxxxxxxx xx xxx xxxxxxxxx 2532 xxxxx xxxx xx xxxxxxxxxxx xxxxxxxxxx xxxxxxx xxxxxx xxx xx xxxxx xxxx XXX xx X xxxxxx xxxxxxxx xx X xxxxxx. Xx x xxxxx xxxxxxxx, xxxx xXX xxx XxXX xxxxxxxx xxxxxx xxx xxxxxxxxx xxxxxxx xxxx xxxxxxxxx xx bidimensional hierarchical cluster analysis (2D-HCA).

In agreement with the classification criteria, xxx 2X- XXX xxxxxxxxx xx xXX xxxxx (Xxxxxx 18X) xxxxxxxxx xxxxxxx xxxxxxxxx xx xxxxxxxxxxxxx xxxxx (xxxxxxxx xx xxx xxxxxxxx), xxx xxxx xxxxxxxxxx xxxx xxx xxxxxx xx xxxxxxxx xx xxxx xxxxxx xxxxxxxx xx XX. Xxxx, X xxx XX xxxxxx xxxxxxxxx xxxxx xxxx xxxxxxx xxxxxx; XXX1-X xxxxxx xxxx X xxx XXX1-XX, xxx xx x xxxxxxx xxx-xxxxxxx; xxx XXX2 xxx XXX3 xxxxxxxxx xxxx X xxxxxxxxx xx xxxxxxxxx/xxxxxxxxxx (XX xxxxxx). Xxxx, xxx xxxx xxxx xxxxxxx xxx xxx xxxxxxxxxxx xxxxxx xxxxxxxxx xx xXX xx X xx XX XXX xxxxxx, xxxxx xxxxxxxxx xxxxxxxx xxxxxxx xxxxx xxxx xxxxxxxxx XX.

Xxx 2X-XXX xxxxxxxx xxxx xxx XxXX xxxxxxxxxx xxxxxxxx (Xxxxxx 18X) xxxxxxxxx xxxx xxxx xxxxxx xxx xxxxxx xx xxxxxx (xxxxxx) xxxxxx xxxx xx xxxx-xxxxxx xxxxxx (xxxx xx xxxxxxx XXX) xxx xxxx xxxxx xxxxxxxxxx xxxx xxxxxxxx xxxx xxxxx xxxxxxxxxxxx xxx xxxxxxxx xx xxxx xx xxxxxx xxxx xxxx xxxxxxxxxx xxxx (XX xxx XXX xxxxxx). Xxxx

xxxxxxxxx, XxXXx xxxxxxxxxx xxxxx xxxxxx xxxx xxxxxx xxxx xxx xxx xxxx xxxxxxxxx (Xxxxxx 18X), xxxx xxxxxxxxxx xxx xxxxxxx xx xxx XXX xxxxxxxx. Xxxx xxxxxxxxx xxxx XxXXx xxx xxxx xx xxxxxxxxx xxxx xx xxxxxxxxxxx xx xxxxxx XXX xxxxxxxx.

Figure 18. Hierarchical cluster of early ripening genes (eRG) and non-early ripening genes (NeRG). A) Unsupervised two-dimensional hierarchical cluster of the 859 eRG based on the mean of the expression of the three biological and technical replicates. B) Unsupervised two-dimensional hierarchical cluster of the 2535 NeRG genes based on the mean of the expression of three biological and technical replicates. The length of the dendrogram branches connecting pair nodes depicts the Pearson correlation coefficient. Under each sample is shown the MI. M: mature fruits; R: mature with two to four days ripening at 20°C; CS1: one week CS at 5°C; CS2: two weeks CS at 5°C; CS3: three weeks CS at 5°C; CSR1: one week CS + SLR; CSR2: two weeks CS + SLR; CSR3: three weeks CS + SLR; S: sensitive pool and LS: low-sensitive pool.

As one of the purposes of CS is to stop or slow ripening until fruit is returned to room temperature, xxx xXX xxxx xxxxxxxxxx xxxxxxxxx xx xxxxxxx XX xxxxxxx xxxxx xxxxxx xxxxxxxxxx xxxxxxx xxxxxx xxxxxxxx xx XX xx xxxx xx xxxxxxx xxxxx. Xx XX xxxxxx, xxx xxxxxxxx xxxxxxxx xx 700 xxxxx xxx xxxxxxx xx xxxxxxx xxxxxx XX (81.49%) (xXX1, xXX2, xXX5, xXX6; Xxxxxx 19X xxx Xxxxx 11), xxxxx xx X xxxxxx, xxxx 517 xxxxx xxxx xxxxxxxx xxxxxx XX (60.19%) (xXX1, xXX5, xXX6, Xxxxxx 19X xxx Xxxxx 11).

Xxx xxxxxxxx xx xXX xxxxxx xxxx XX xxx xxxxxxxxxx XXX (Xxxxx 11), xxxxxxxxx xxxx xxx xx xxx 700 xxxxx xx XX xxxxxx xxxxx xxxxxxxxxxxxxx xxxxxxxx xxxxx xxx xxxxxxx xx xxxxxxx xx xxx, xxx xx xxxxx xxxxx XX, 99.29%, 91.71% xxx 78.86% xxxxxxx xxx xxxxxx xxxxxxxx xxxxxxx, xxxxxxxxxxxx, xxxxxx xxxxx XXX. Xx X xxxxxx, xxxx 79.50%, 61.12% xx 35.01% xx xxx 517 xxxxx xxxxx xxxxxxxx-xxxxxxx xxxxxxx xxxx xxxxxxx xx

Figure 19. Expression analysis of early (eRG) and non-early ripening (NeRG) ChillPeach transcripts in response to CS and SLR. Clusters resulting from unsupervised two-dimensional hierarchical clustering of eRG and NeRG are shown. A and B) Clusters of eRG and functional categories over-represented in each cluster. C and D) Clusters of NeRG and functional categories over-represented in each cluster. Y-axes (A and C) represent the normalized expression ratio (Log2 M) of three biological replicates in relation to a reference pool. The functional categories overrepresented in each cluster (B and D) are shown as a heatmap obtained with matrix2png. Enriched functional categories with Fisher test *p*-values < 0.05 are colored in grades of yellow. The number of genes in each cluster is indicated to the right of the heatmap.

xxxxxxx xx xxx, xxx xx xxxxx xxxxx XX, xxxxxxxxxxxx, xxxxxxx xxxxx xxxxxxxx xxxxx. Xxx xXX xxxx xxxxxxxxx xxxxxxxx xxxxxx XX xx 5xX xx xx xx XX xxxxxxxxx xxx 159 xx 342 xxxxx xx XX xx X xxxxxx, xxxxxxxxxxxx (Xxxxx 11). Xx XX xxxxxx, xxxx 85% xx xxxxx xxxxx xxxxxxx xxxxxx xxxxxxxx-xxxxxxx xxxxxxxxxx xxxxxx XXX xxxxx xxx XX xxxxxxx, xxxxx xxxx 62.87, 43.86 xx 24.27% xxxxxx xxxxxx xxxxxxxx xxxxxxxxxx xx X xxxxxx xxxxx xxx, xxx xx xxxxx xxxx XX. Xxxx, xxxxxx XX xxxx, xxxxxxxxxx xx xxxxxxxxxxxxxx xxxxxxxx xxxxxxx xxx xxxxxx xxxxxxxxxxxxx, xxxx xxxx xxxxx xxxxxxxxxx xxxxxx XX xxxxx xxxxxx xxxx xxxxxxxx. Xxx xxxxxx xx xxxxxxxxxxxxxxxx xx X- xx XX-xxxxxxxxx xxx will impact later SLR.

Table 11. Genes resuming properly the ripening program during 2-3 days at 20ºC.

	Xxxxx xxxxxxxx xxxxx (859 xxxxx)								**Xxx xxxxx xxxxxxxx xxxxx (2532 xxxxx)**			
	Xxxxxxx xxxx xx XX xx 5xX				**Xxxxxxxx xxxxxxxx xx XX xx 5xX**							
	Xxxxxx XX		**Xxxxxx X**		**Xxxxxx XX**		**Xxxxxx X**		**Xxxxxx XX**		**Xxxxxx X**	
	Xx xx xxxxx	**%**	**Xx xx xxxxx**	**%**	**Xx xx xxxxx**	**%**	**Xx xx xxxxx**	**%**	**Xx xx xxxxx**	**%**	**Xx xx xxxxx**	**%**
Xxxx xxxxxxxx	700	*81. 49*	517	*60. 19*	159	*18. 51*	342	*39. 81*				
	Xxxxx xxxxxxxxxx xxx xxxxxxxxxx xxxxxx xxxxxxxx xx X xxxxxx											
1x	695	*99. 29*	411	*79. 50*	154	*96. 86*	215	*62. 87*	2525	*99. 6*	2372	*93. 8*
2x	642	*91. 71*	316	*61. 12*	137	*86. 16*	150	*43. 86*	2419	*95. 4*	2055	*81. 1*
3x	552	*78. 86*	181	*35. 01*	136	*85. 53*	83	*24. 27*	2298	*90. 7*	1422	*56. 1*

There is indicated the number for early and non early genes and in the case of eRG if the ripening program is stopped or not by cold.

Classifying xxx XxXX xx xxx xxxxx xx xxxxxxx xx xxx xxxx xxxxx xxx xxxxxxxxxx xxxxxx xxxx xxxxx xxxx xxxxxxx xxxx xxxxxxx (x.x., xxxxxxx xxxxxxxxxx xxxxxx xx X xx X xxxxxx, xx xxxxx xxx xxxx xxxxxxx, xx xxxxx xxxxxx xxx xxxxx xxxx,, Xxxxx X13) xxxxxxxxx xxxx xxxx 90% xx XxXX xx XXX-XX xxxxxx xxxxxxxx xxxxxxx xxxxxxxxxx xxxxxx xx X/X xxxxxx xxxxx xxx XX xxxxxxx (Xxxxx 11). Xx X xxxxx 93.57 (2372), 81.07 (2055) xx 56.09% xx XxXX xxxx xxxxxxxxx xx xx xxx xxxxx xx X/X xxxxxx xxxxx xxx, xxx xx xxxxx xxxxx XX.

Xx xxxxxx xxxxxxxxxxxxx xx xxxx xxxxxxxxxxxx xxxxxxxx, xx xxxxxxxx xxx xxxxxxx xx xxx xxxxxxx xxxxxxxx (Xxxxxxx 19X xxx 19X) xxxx xxx xxxxx xxxxxxxxxx xx xxxxxx xxxxxxx xxxxxxxxx (Xxxxxx 17X). Xx xxxxxxxxxx xxxxxxxx xxxx xxxxx xx xxxxxxxx xXX2 xxx XxXX8

(Xxxxxx 19X xxx 19X). Xxxx xxxxx xxxx xxxxxxxx xxxxxxxxxx xx XXX xxxxxx xxxx xx xxxxxxxx *xXX2* (~50% xx xXX xxxx xxxxxxxx xxxxxxxxxx) xxx XxXX8 (~35% xx XxXX xxxx xxxxxxxx xxxxxxxxxx*)* (Xxxxxx 12-13). Xxx xxxxx xx xxxxx xxxxxxxx xxxx xxxxxxxx xx *xxxxxxxxx xxxxxx xxx xxxxxxxxxx*, *xxxxxx xxxxxxxxxx*, *XXX xxxx-xxxxxxxxxxxxxxx xxxxxxxxxx*, *XXX xxxxxxxxxxx xxx xxxxxxx xxxxxxxx* (Xxxxxx 19X xxx 3X). X xxxxxxxxxx xxxxxxxxxxxxxx xx xxxxx xxxxxxxx xx xxxx XX xxxxxx xxxxxxxx xxx xxxxxxxxxx xxxxxx xxxxxxx xx xx xxxxxxx/xxxxxx xxxxx (X) xxxxxx XX, xxxxx xxx xxxx xxxxx xxxx xxxxxxxx xxxx-

Xxxxx 12 Xxxxxx xx xxx xxxx xx xxx 859 xXX xxxx xxxxxx xxxxxxx xxxxxxxxxx xxxxxx 2-3 xxxx xx 20xX xxxxx xxxx xxxxxx xx xxxxxxx

Xxxxxx xx xxx XX xx xxx xxxxxxxx xxxxxxx			Xxxxxxxx						Xxxxxxxx						Xxxxx xx xxxxxxx
			XXX1		XXX2		XXX3		XXX1		XXX2		XXX3		
	xxxxxxx	xxxx xxxxxx	X	XX	X	XX	X	XX	X	XX	X	XX	X	XX	
XX-XX-XX-X	xXX3	XX-XX-XX-X			2		6		3	3	14	16	30	16	75
	xXX4	XX-XX-XX-X	8	2	6	6	6	6	2		21		41	1	84
XX-XX-XX-X	xXX2	XX-XX-XX-X	114		149	28	176	87				1			183
XX-XX-XX-X	xXX1	XX-XX-XX-X	2	1	11	6	22	6	8		48	3	93	10	206
	xXX5	XX-XX-XX-X	27		34		49		3	2	11	8	30	10	149
	xXX6	XX-XX-XX-X	66	2	97	12	142	35							162
	Xxxxx xxxxxxx		217	5	299	52	401	134	16	5	94	28	194	37	859

XX: xxxx xxxxxxxx; XX: xxxx xxxx; XX: xxxx xxxx; XX: xxxx xx; XX xx xxxxxx. X: xxxxxxxxx xxxx; XX : xxx xxxxxxxxx xxxx

xxxxxxxxx xx X xxxxxx (Xxxxxx 19X xxx 19X). Xxxxxxxxxx xx xxxxx xx xxxxxxxx *xXX2* xxx *XxXX8* xxx xxxxxxxx xxxxxxxxxx xxxxxx xxxxx-xxxx xxxxx xxxxx xxxxx xxxxxxxxx xxxxxx xxxx xxxxxxx. Xxxxxx XXX, xxxxx xxxxx xxxx xxxx xxxx xxxxxxxx xxxxxxxxx xxxx xx X xxx X xxxxxx xxx xxx xxxxxx xx xxxxx xxxx xxxxxxx xxxxxxxxxx xxxxxxxxx xxxxxx XXX xxxxxxxxxxx xxxx xxxxxxxxxx XX xxx xxxx. Xxxxx xxxxxxxx, xxxxx xx xxxx xxxxx xxxxxxxxx xxx xxxxxxxxx xxxxxxxx xx xxxx xxx XXX xxx xxx xxxxxxx xxxxxxxxxx xxxx xxxxxxxxxx XXX xxxxxxxxxx.

Xxxxxxxxx xxxxxxxx xxxx xxxxxxxxx xxxx xx xxxxx xXX xxx XxXX xxxxx xxxx xxxxxxxxxxxxxx xxxxxxxxx xxxxxxxx xxxxxx XXX, xxxxxxxxxx xx xxxxxxxxxx xxxxxxx X xxx XX xxxxxx xxxx xxxxxx xxxxxxxxxxx, xxxx xxx xxxxxxxxx xx xxxxxxxx XxXX1 xxx XxXX5

Xxxxx 13. Xxxxxx xx xxx xxxx xxxxxxx xx xxx 2532 XxXX xxxx xxxxxx xxxxxxx xxxxxxxxxx xxxxxx 2-3 xxxx xx 20xX xxxxx xxxx xxxxxx xx xxxx xxxxxxx.

		Xx-xxxxxxxxx						Xxxx-xxxxxxxxx						
		XXX1		XXX2		XXX3		XXX1		XXX2		XXX3		Xxxxx xx
Xxxx xxxxxx	xxxxxxx	X	X X	X	X X	X	XX	X	X X	X	X X	X	X X	xxxxxxx
XX-XX-XX-X	XxXX4	77		116	18	180	49		5	8	2	36		531
	XxXX3	7	1	10		18	1	1		43	18	134	17	429
	XxXX5	9		36	14	74	42		3	6	1	55	2	431
XX-XX-XX-X	XxXX6						1	10		95	11	223	19	338
	XxXX7			2		16				4	2	12	1	134
XX-XX-XX-X	XxXX1	3		8		10	2			14	1	92	4	223
XX-XX-XX-X	XxXX8	50		105	34	184	80		1					273
XX-XX-XX-X	XxXX2					1		6		33	15	78	19	176
Xxxxx xxxxxxx		146	1	277	66	483	175	17	9	203	50	630	62	2535

XX: xxxx xxxx; XX: xxxx xx; XX xx xxxxxx. X: xxxxxxxxx xxxx; XX: xxx xxxxxxxxx xxxx

(Xxxxxx 19X xxx 19X) (xxx xxxxx). Xxxx xxxxx xxxx xxxxxxxxxxxx xxxxxxxxxxx xx xxxxxxxxxx xxxxxx XXX xxxx xx xxxxxxxx xXX1, xXX5, xXX6, XxXX2, XxXX3, XxXX4, XxXX6 xxx XxXX7 (Xxxxxx 19X xxx 19X xxx xxxxxx 12 xxx 13). Xxx xxxxx xx xxxxxxxx xXX5, xXX6, XxXX3 xxx XxXX4 were up-regulated during cold storage, while eRG1, NeRG6 and NeRG7 were down-regulated. A functional enrichment analysis (Figure 19B and 19D) indicated that genes in cold up-regulated clusters xxxx xxxxxxxx xx *xxxxxxxxxxx xxxxxx, XXX xxxxxxxxxxxxx xxxxxxxxxx, xxxxxxxxxxx xxxxxxxxx, xxxxxxxxx, xxxxxx xxxxxxxxxxx, xxxxxxxxx xxxxxxxxxxx xxxxxxxx* xxx *xxxxxxx xxxxxxxx.* Xxxxx xx xxxx-xxxx-xxxxxxxxx xxxxxxxx xxxx xxxxxxxx xx *xxxxxxxxx xxxxxxxxxx, xxxxxxxxxxxx xxxxxxxxxx, xxxx xxx, xxxxx xxxxxxxxxx, xxxxx xxxxxxx xxxx xxxxxxxxx xxxxxxxxx, xxxxxxxxx xxxxxxxxxx xxx xxxxxxxxx.*

Xxxx xxxxxxxx xxx xxxxxxx xxx xxx xxxx xxxxxxxxxxx xxxxxxxx xxxxxxx xxx-xxxxxxxxxxx X xxx XX xxxxxx. Xxx xxxxx, xXX3, xXX4 xxx XxXX2, xxx xxxxxxxx xxxxxxxxx xxxxxx XX xx xxx xxxxxxxx xxxxxx XXX xxx xxxxxxxxxx xxxxxxxxxxx xxxxxx XX xxx XXX xxx xxxxxx xxxxxxxxxxx (Xxxxxxx 19X xxx 19X). Xxx xxxxx xx xxxxxxxx xXX3 xxx xXX4, xxxx- xxx xx-xxxxxxxxx xxxxxx XX xx xxxx X xxx XX xxxxxx, xxx xxxxxxxx xx *xxxxx xxxxxxx xxxx xxxxxxxxx xxxxxxx, xxxxxxxxx xxxxxxxxxx xxx xxxxxxxxx* (Xxxxxxx 19X xxx 19X). Xxxxx xxxxxxxxx xxxxxxxx-xxxxxxx xxxxxxx xxxxxx XX, xxx xxxx xxxxxx XXX. Xxxxxxx, the effect

xx xxxx xx xxxxxxxx-xxxxxxx xxxxxxx xxx xxxxx xxxxx xx xxxxxxx xx X xxxxxx xxxx xx XX xxxxxx, xxxxxxxxxx xxxxx xxxxxxxxx XX. Xxxxx xx xxxxxxx xXX4 (Xxxxxx 19X), xxxxxxxxx xxxxxx xx xxxx xxxxxx xx xxxxxx X, xxxx xxxxxxxxxxxxx xxxx-xxxxxxxxx xx XXX-X xxxxxx xxxxxxx xx xxxxxx xxxxxxxxx xx xxxx xxxxxx xx X xxxxxx, xxxx xxxxxxxxxxxxx xxxx-xxxxxxxxx xx XXX-X xxxxxx xxxx xxxxxxx xx X-X xxxxxx xx xxx xxxx xx XX xxxxxxxxx. XxXX2 xxxxx xxxx xxxxxxxxx xxxxxx XX xx x xxxxxx xxxxxxxxxxx xxxx XX xxxxxxxxxxx, xxxx xxxxxxxxx xxxx xx xxx xxxxxxxxxx xxxx xx XX xxxxxx xxxxxx XXX, xxx xxx xxxxxxxxxx xxxxxx XX xxx xxxxxxx xxxx xxxxxxxxx xxxxxxxx xx xxxx xxxxxxx (Xxxxxx 19X). Xxxxxxxx XxXX1 xxx XxXX5 xxxxxx xxxxxxxxxxx xxxxxxxxxx, xxx xxxxxxx xxxxxxxx (Xxxxxx 19X). Xxxxxxxx xxx xxxxxxxxxxxxxx xxxxxxxx xxxxxxxxx xxxxxx XX xx xxxxxxxxx xxxxxxxx xxxxxx XXX, xxx xxxxx xx xxxxxxx XxXX1 (xxx xxxxxxxx xx xxx xxxxxxxxxx xxxxxxxx; Xxxxxx 19X), xxxxxx xxxxxxxx xxxxxxxx xxxxxx XX: xxxx xxxx xxxxxx xx-xxxxxxxxx xxxxxx XX xx X xxxxxx, xxx xxxx-xxxxxxxxx xx xxx XX xxxxxx. Xx xxxxx xx xxxxxxx XxXX5, XX xxxxxx xxxx-xxxxxxxxxx xx xxxxxxxxxx, xxxxxxxxxxx xx X xxxxxx, xxx xxxxx XXX, xxxxx xxxxx xxxx xxxx xxxxxxxxx xx X xxxxxx xxxx xx XX xxxxxx (Xxxxxx 19X). Xx XXX xxxxxxxx xxx xxxx xx XX xxxxxxxxx, xxx xxxxxx xx xxxxx xxxx xxxxxxxx xxxxxxxxxx xxxx xxxxxxxxx.

C3.1.6.Validation and extension of microarray expression profiling to individual lines of the Pop-DG population

To validate the microarray results, we performed medium-throughput qRT-PCR (Biomark Dynamic Array, Fluidigm) xxxx x xxx xx 93 xxxxx xxxx xxxx xxxxxxxx xxxxxxxx (xxxx xxx xxxxxxxxx xx x xxx xxxxx xx xxxxxxxx) xxx xxxxx xxxxxxxxxxxxxx xxxxxxxxx xxxxx xxx xxxx xx XX xxx/xx XXX (Xxxxx X14) xxxxx xxx xxxx xxxxxx xxxxx xxxxxxx xx xx xxx xxxxxxxxxx xxxxxxxxxxx. Xxx xXX-XXX xxxxxxx xxxxxxxx xxxx xxx xxxxx xxxxxxxx xxxx 73.12% (68/93) xxx 75.26 % (70/93) xx xxx xxxxx xx XX xxx XXX xxxxxxx xxx xxx xxxx xxxxxxxxx xxxxxxx xx xxx xxxxxxxxxx xxxxxxxxxx xx xx xxx xXX-XXX xxxxxxxxxx, xxxxxxxxxxx. Xxxxxxxx xxx xxxxxxxxxx xxxxx xxx xxx xxxxxxxxx xxx xxxx xxxx xx xxxx xxxxxxx (Xxxxx X14), xxxxx xxxxxxx xxxxxxxxx xxx xxxxxxx xxxxxxxx xxx xxxxxxxxxx xx xxx xxxxxxxxxx xxxx.

Xxxxxxxxxx xxxxx xxxxx xxx xxxx xxxxxxxx xx xXX-XXX xx xxxxxx xx 13 xxxxxxxxxx xxxxxxxx xxxx xxx Xxx-XX xxxxxxxxxx. Xxxxx xxxxxxxxx, xxxxxx xxx xxxxxxx xxxxx xxxxxxxxx xxxxxxxxxx xx xxxx xxx xxxxxxxxxx xxxxxxxx, xxxxxxx xxx xxxxx xxxx xx xxx xxxxx xxx xxxxxx xxxxxxx x xxxxx xx XX (Xxxxxx 1). Xxxxxxxxxxxx xxxxxxx xxxxxxxx xxxxxxxxxx xx xxxxxxxxx xxxxx xx xxxx xxxxxxx xxx xxxxx XX xxxxxx XXX xxxxx xxx xxxx XX xxxx xxxxxxxxxx (Xxxxxx 20 xxx Xxxxx X15). Xxxxxxxxxxxx xxxx xxxxxxxxxx xxxx xxx xxxx xx xxxxxxxxx xxxxx xxxxxxxxxxxxxx xxxxxxxxx xxxxxx XXX, XX xxx XX + XXX. Xxx XX xxxxxxx, xxx XX xxx 0, xx xxxxx xxxxxxx xxxx xxxxxxxx xxxx xxx xxxx xxx XXX xx xxxxxxxx xx xxxxxxx XXX. Xx xxxxxxxxx xx XX xxxxxxx xxx xxxxxxxxxx XX xx xxx xxxxxxxxxxxx XXX xxxxxxx, xx xxx xxxxxxxx xxxx xxxxxxxxxxxxx xxxxxxxx xx xxx xxxx xxxx x xxxxxx xxxxxx xx XXX xxx xxxxxxxxx, xxxx xxx xxxxxxxxxx xxxxxxxxx xxxxxxxxx xxx xx xxxx xx xxxx xxxxx xxxxxxxxxx xxxx xxx xxxxxx. Xxxxxxx, xxxxxxxxxx xxxxxxx x xxxx xxx XX xxxxxx xxxx xxx xxxx xxxxxxxxx xx xxx xxxxxxx xxx xxxxx xxx xxxxxxxxx xxxx xxx xxxxxxxx. Xxxxxxxxxx xxxxxxx xxxxxxxxxxx xx xxxxxxxxxx xxxxxx xxxx XX xxxxxxxx xxxx xxxxxxxxxx xx xxxxxxxxx xxxxx, xxxxx xxxxxxxx xxxxxxxxxxx xxxxxxxx xxxx xxxxxxxxxx xx xxx-xxxxxxxxx xxxxx. Xx xxxxxxx, xxxxx xxxxxx xxxxxxxxx xx X xxxxx xxxxxx xxxxxxxx xxxxxxxxxxx xxxxxxx XX xxx xxxx xxxxxxxxxx xx xxxxxxxxxx xxxxx xx XX, XXX xxx XX + XXX xxxxxxxxxx (Xxxxxx 20X-X), xxxxx xxx-xxxxxxxxx xxxxx xxxxxx xxxxxxxx xxxxxxxxxxx. X xxxxxxxxxx xxxxxxxxxx xxxx XX xxxxxxxxxx/xxxxxxxxx (xxxxxxxxxxx xxxx *x*-xxxxx < 0.05) xxx xxxxxxxxx xxx 63% xxx 51% xx xxx xxxxxxx-xxxxxxx xxxxx xx XX xxx XXX xxxxxx (Xxxxxx 20X, X). Xxxxxxx, xx xxx xxxxxxxxxx xxxxx xxx x xxxxx xx xxxxx xxxx xxxx xxxxxx xxxxxxxxx xx XX xxxxx xxx xxxxxxxxxx xxxxxxxxxx xxxx xxx XX xx xxxxxxxxxx xxxxx. Xxxx xxxxxxxxx xxxx xxxx xxxxxxxxxx xx xxxxx xxxxx xx xxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxx xxxx xxxxxxxxx xxxxxx, xxx xxx xxxxxx xxxxxx xxxxxxxxx xxxxxxxxxx xx xxx xxxx xxxxxxxx xx xxxx xxxx. Xxx xxxxx xxxxxxx xx xxxxxxxx xxxxxxx xxxxxx XX xxx XXX (Xxxxxx 20X) xxxxxxxx xxxxxxxxxxx xxxx XX xxxxxxxx xxxxxxx xxxx xxx xxxxxx xx xxxx xxxx xxxxx xxxxx xx xxxx xxxxxxxxx xxx XXX xxxxxxxxxx xxxx xxxxxxxx, xx xxxx xxxxx.

Xxxxxxx xxxx xx xxx xxxxxxxx xxxxx xxxxxx xxx xxxxxxxx xxxxxxx xx xxxxxxxxxx xxxxx xx xxxxxxxxx xxxx xxx xxxxxxx xx xxxx xx xxx Xxx-XX xxxxxxxxxx xxx xxxxxxxx xxxxx xx xx xxxxx xx xxxxxxxx xxxxxxx xx xxxxxxxxx xxxxxxxxx xxx/xx

xxxx xxx xxxxxxxxxxx xx XXX xxx xxxxxxxxxx xx x xxxxx xxxxxxxxxx response to cold stress

Figure 20. Correlations between relative expression levels of candidate genes analyzed by medium throughput Fludigm RT PCR in each sibling with the MI exhibited after one week CS plus SLR. Pearson correlations were calculated over the sets of genes validated in the pools and differentially expressed during A) CS, B) SLR and C) CS + SLR.

C3.2.Discussion

C3.2.1. Considerations to the experimental approach

Transferring fruits to CS induced a large transcriptome and proteome rearrangement in peach fruits consistent with eventual CI development (chapter 1, 2 and [11, 12, 14-17, 134]). Xxxxxxx, xxxxxxxx xxxxxxxx xxx xxxxxxx xxx xxx xxxxxxxxxxxxx xxx xxxxxxxxxxxx xx XX, xxxxxxxxxxx xx xxxxxx xxxxxxxx xx xxxxxxxxx xxxx xxxxxx xxxxxxxxx, xxxx xxxxxx xxx xxxxxxxxxxx xx xxxx xxxxxxxxxx (XXX) xxx xxxxxx xxxxxx xxxxxxxx [8].

Xx xxxxxxxx xx xxxxx xxxxxxxxxxxx xxxxxxx xx xxxxxxxx xx XX xxx XXX xx xxxxxxxxx xxx xxx xxxxx xx xxxxxxxx xxxx xxx Xxx-XX xxxxxxxxxx xxxx xxxxxxxxxxx xxxxxxxxxxx xx xxxx-xxxxxxx xxxxxxxxx. Xxx xxxxxxxxxxxxx xx X xxx XX xxxxx xx xxxxxxx xxx xxxxx xxxxx xxxxxxx xx XX xxxx xx xxxxxxx xxxxxxxxx XXX xxxx xxxxxxxx xxxxx xxx XxxxxXxxxx xxxxxxxxx (Xxxxxxx 16-19). Xxx xxxxxxxxxx xxxxxxxx xxxx xxxxxxxxx xxx 73 xx 75% xx xxx xxxxx xxxxxx (Xxxxx X14). Xxx xxxxxxxxxx xxxxxxxx xx xxxxxx xxxxx xxxxxxxx xx xXX-XXX xxxx xxxxxxxxxx xxxx xxxxxxxxxx xxxx (Xxxxx X14), xxxxxxxx xxx xxxxxxxxx xxx xxxx xxxx xx xxxx xxxxxx, xx xx xxxxxxx xxxxxxx [17]. Xxxxxxx, xxx xxxxxxxxxx xxxxxxx xx xxxx xxxxx xxxxxxxxx xx xxx xxxxx xxx xxxxxxxxx xx xxxxxxxxxx xxxxx/xxxxxxxxx xxxxxx xxx Xxx-XX xxxxxxxxxx (Xxxxxx 20). Xxxxxxxx xxxxxxxxxx xxxxxxxx xxx xxxxxxxxxxx (xxxxxxxxx xx xxxxxxxx 1 xxx 2), xxx xxxxxxxxxxx xx xxxxx xx xxx Xxx-XX xxxxxxxxxx xxxx xxxxxxxxxx xxxxxxxxx xxx xxxx xxxxxx xxxxxx, xxxxxxxxxxx ([17, 134], xxxxxxx 1 xxx xxxxx X14) xxx xxxxxxxxx xx xxxxxxxxxx xxxxxx xxx xxxx xxx Xxx-XX xxxxxxxxxx (Xxxxxx 20 xxx xxxxxxx 1) xxx xxxxxxxxx xxxx xxxxxxxxx xxxxxxxxxx xxxxxxx XX (xxxxxxx 2 xxx [134]). Xxxx xxxx xxxxxxxxx xxxx xxx xxxxxxxx xx xx xxxxxxxxxx xxxx xxxxxxxxx.

Xxx xxxxx xxxxxxx xxxx xxxxx xxxx xxxxxxxxx xxxxxxxxxx xx xxxxxxxx XXX xxxxxxxxxx xx xxx xxxx. Xxxxxxx xxxx xxxxxxxxx xxxxxxxxxx xxxxxxxx x xxxxxxx xx xxx xxxxxxx xxxxxxxxxxxxx xxxxxxxxxx xxxxxxx xxxxx xxx xxxxx xxxxxx [11, 12, 14, 16]. Xxxxxxx, xxxxx xxxxxxx xxx xxx xxxxxxxxx xxxxxxxxxxxxx xxxx xxxxxx xx xxxxxxx XXX xxxxxxxxxxxxx xxxxx xxx xxxxxxxxxx xxxx xxxxx xx x xxxxxx xxxxxxx [11, 12, 16] xx xx xxx xxxxxxxxx xxxx xxxxxxxxx xxxx xxxxxxxx xxxx xxxx differed in other phenotypic traits [14]. Here, we defined transcripts that may be causally related to WLT by examining

xxxx xxxxxxxxxx xxxxxxx xx xxx xxxxx xx xxxxxxxx xxxx xxx Xxx-XX xxxxxxxxxx (X xxx XX). Xxxxx xxxxxxxx xxxx xxxxxxx xxxxxxxxxxxxx xxxxxxxxxxxxxxx xxx xxxxxxxxxxxxx xxxxxxx, xxx xxxxxxxxxx xxxxxxxxxx xx xxxxxxxxx, xxxxxxxxxxx xxxxx xxxxxxxxxx xxxxxxxxxx xxx xxxxxxxxx xxx xxxxxxxx xxx xxxxxxxxxx xxxxxxxx.

Xxxxxxxx xxxxxxx xxxxxxxx x xxxxxxx xxxxxx xx XX xxxxxxx [11, 12, 14, 16]. Xx xxxxxxxx xxxx xxxxxxxxxx, xx xxxxxxxx xxxxxxxxxx xxxxxxx xxxxxxxxx xx xxxxxxxxx xxxxxx /xxxxx xxxxxx XXX xxxxxxxxxx. Xxx xxxxx xx xxxxxxxx xxxx xxxxxxxxx xx xxxxx xxxxxxxxx xxxxxxx xx XX xx xxxxx XXX xxx xxxx XXX xxx xxx xx xxxxx xxxx xx 20xX xx xxxxxx xxxx xxxxxxxxxx xxx xxxxxxxxx xxxxxxx. XXX-xxxxxxx xxxxxxxxxxxxx xxxxxxx xxx x xxxxxxxx xx xxx xxxxxxxx xx XX xxx xxx xxxxxxxx (Xxxxxxx 16-19), xxx xxxx xx xxx xxxxxxxxxx xx xxxxxxxx xxxxxxxxx xxxxxx XXX xxxxx XX, xxxxx xxx xxxxxxx xxx xxxxxxxxxx xx xxxxxxxx. Xx X xxxxxx, XX xxxxxx xxxxxxxxx xxxxxxx xxxx xxx xxxxxxxxx xxxxxx XXX xx xxxxxxx xxxxxxxx, xxxx xxxx xxxxx xx xxxxxxxx xxxxxxxxxx xxx xxxxxx xxxxxxx. Xxx xxxxxxx xxxxxxxx xxxxxx XXX xxxxx XX xxxxxxxx xxxxxx xxxx xxxxx xxxxxx xx-xxxxxxxxxx xx xxxx xxxxxxxxxx, xxxxxxxx xx xxxxxxx xxxx-xxxxxxxxxx. Xx xxx xxxx xxxxxx xxxxxxxxx, xxx xxxxxx xx xxxx-xxxxxxxxx xxxxx xxxxxxxxx, xxxxxxxx xxxx xxxxxx xxxx xxx xxxxxx xx xxxxxxx xxxxx. Xxxxx xxxxxxx xxxxxxxx xx xxxxxxxxx xxxx x xxxxxxxx xxxxxx xx xxxx xxxxxxxxxx xxxxxxxxxx xxxx XXX, xxxxxx xxxx xx-xxxxxxxxxx [11]. Xxxxxxx, xxxxx xxxxxxxxxxx xxx xxxxxxx xxx xxxxxx xx xxxxxxxx, xx xxxxx xxxxxxx xxx xxxxxx xxxxx xxxxx xxxx xxxxx xxxx. Xxxxxxxxxx xxxxx xxxxxxx xxxxxxxx xxxxx xxxxxxxx xx xxxxxxxxxx xxxxxxx xx xxxxx xxxxxxxx xxxx [12, 14, 16].

C3.2.2.Transcriptome resilience during shelf life promotes the resumption of ripening and delays mealiness development.

One of the purposes of XX xx xx xxxx xx xxxx xxxxxxxx xx xxxx xxxxxxx, xxxx xxxxxx xxx xxxxxxxx xx xxxx xxxxxxxxxx, xxxx xxx xxxxxx xxxxxxxx xxx xxxxxxxxxx xxxxxxx x xxxxx, xxxx xxxxx. Xxxxxxx, xxx xxxxxxxxxx xxxx xxxxxx xxxxxxx xxxxxxxxxx xxxxxxxx. Xx-xxxxxxxxxx xx xxxxxx xxxx xxxx xxxxxx xx xxxx xxxxxxxxxxx [493, 494], xxxxxx xxxxxxxx x xxxxxxxx xx xxxx xxxxxxxx xxxxxx xxxx xxxxxxxxxx [495]. Xxxxxxxxx, xxx xxxxxxx xxxxxxxx xxxx xxxx xxxxxxxxxx xxxx XX xx XXX, XXX-XX xxxxxx xxxxxx xxxxxxxxxxx

xxxxxxxxxxxxxx xxxxxxxxxx [491] xxx xxxx xxxxx xxxxxxxx xx xxxxxxx X xxxxxxxxxx xxxxxx xxxxx XX (Xxxxxxx 16X-X, 19X xxx 19X). Xxxx, xxx xxxxxxxxxxxxxxx xxxxxxx xxxxxxxx xxx xxxxxx xxxxx xxxxxxxxxx xxx xxxxxxxx xxxxxxxx xxx xxxxxxxx (xx xxxxxxxxx xx xxxxxxxxxx xxxxxxxxxx (Xxxxxx 19X xxx 19X). Xx xxxxxxxx, X xxxxxx xxxx xxxxxx xx xxxxxxxx xxxxxxxxx xxxxx xxxxxxxxxxxxxxx xxxxxxxx xx xxxx xxx xxxxxx xxxxxx xxxxxxxx (Xxxxxx 16-19).). XX xxxxxx xxx xxxxx xxxxxxx xxxx xxxxx-xxxx XX, xx xxx xxxxxxxxxxxx xx XXX1-XX xxxxxx (xxxxxxx xxxxxxx xxxxxx xxxxxxxx, Xxxxxx 16X) xxx xxx xxxx xxx xxxx xxx xxx xxxxxx xxxxxxxxxxx xxxx X xxxxxx xxx xxxxxxx xxxxxxxx xxxxx xxxx xxxxxxx (Xxxxxxx 16-19 xxx X4). Xxxxxxx, xx xxx xxxxxxxx xx XX xxxxxxxxx, xxx xxxxxxxxxxxxxx xx XXX xxxxxx xxxxxxxx xxxx xxxxxxx xxxx xxxxxxx X xxxxxxxxxx, xxx xxx xxxxxxxxxx xx xxxxxxxx xxxxxxxxx xxx xx xxxxxxxxx xxxxxxxx xxxxxxx.

Xx xxx xxxxxxx 2 xx xxxxxxxx xxxx xxxxxxxxxxx xxxxxxxxxx xx xxx xxxxxx xx xxxxxx xxxx-xxxxxxxxxx xxxxx xxxxxxxxxx xxxx xxxxxxxxxx/xxxxxxxxx xx XX xx xxx-xxxxxxxxxx xxxxxx. Xxxx, xXX (xxxxx xxxxxxxx xxxxx: xxxxxxxx xxx xxxxxxxx xxxxxx XXX xxxxxxx XX) xxx XxXX (xxxxx xxx xxxxxxxx xxxxxx XXX xxxxxxx XX) xxxxxxxxx xx xxxx xx xxx xxxx xxx, xxx xxxx xxxxxxxxxxx xxxxxxxxxxx xxxxxxx X xxx XX. Xxx xxxxxxxx xxxxxx XXX xxxxxx xxxx: x) xxxxxxxxx xx xxx xxxxxxxxxx xxxxxx xxxx xxxxx xxxx xxxxxxx XX, xxxxxxxx xxxx xxxxxxxxxx xx X xxxxxx xx xx) xxxxxxxxxx xxxx xxx XX-xxxxxxx xxxxxxx, xxxxxxxx xxx xxxxxxxxxx xxxxxx xx xxx xxxx xxxxxxxxxx xxxx xxxxx xx XXX. Xx xxxx xxxxx xxxx, xxxxx xxxx xxxxxxxxxxx xxxxxxxxxx xxxxxx XX xxx xxxxxxxxxx XXX xxx xxxxxxx xxxxxxxxxx xx xxxxx xxxxxxxx xxx xxxx xxxxxxxxxx xx xx xxxxxxxxxx xxx xxxxxxxxx xxxxxxxx/xxxxxxxxxx (xx xx xxxxxxxx xx xxxxxxx 2) xxx xxxx xxx xxxxx xxxxxxxx xxx xxxxxxxxxxx xx xxxxxxxx xxxxxx XXX [16]

Xxxxx xxxxxx xxxxxxxxx xx xxx XX xxxxx xxxxxx XX xxx XXX xxx xxxxxxxx xxxxxxxxxx xxxx xxxx xxxxxxxxx xxx xx xxxxxx xxxxxxxx xx xxxxxx, xxxx xx xxxxx xxx xx xxxxxx xx xxxxxxxxxx xxxxx xxxx xxx Xxx-XX xxxxxxxxxx (Xxxxxx 20), Xxxx xxxxx xxxx-xxxxxxxxx xxxxxx XX xxx xxxxxxx xx *xxxx xxxx* xxx *xxxxx xxxx*, *xxxxxxxxxxx*, *xxxxx* xxx *xxxxxxxxx xxxxxxxxxx* xxxxxx xxxxxxxx xx xxxxxx xxxxxxxxxx, xxxxxxxxxx xxxxx xxxxxxx XX xxxxxxx, xxx/xx xxxx xxxxxxxxx xx xxxxxx xxxxxx xx XX xxxxxx (xxxxxxxx xXX1, xXX3, XxXX2, XxXX6 xxx XxXX7; Xxxxxx 19 xxx xxxxx X13).). Xxxx xxxxxxxxx xxxx xxxxxx xxxxxxxxxx is partly

xxxxxxxx xx XX xxxxxx xxxxx XX. Xx xxxxx xxxxxxxx xxxx xxxxxxx xxxxx (xxxxx 12) xx xxxxxxxx xxxxxxxx xx xxxxxxxxxx xxxx xxxx xxxxxxxxx xx xxxxx xxxxxx xx xxxxxxx 1 (xxxxx 5) xxxx xx xxx xxxxxxxxx xx XXX1 (XxXX7), XXXXX2 (XxXX6 xxx XxXX2), XXX1 (XxXX6), XXXX1 (XxXX6), XXXX (XxXX6), XXXX1 (XxXX6), XX (xXX1), XXX1 (XxXX2 Xxxxxxxx xxxxx xxxxx xxx xxxxxxxxxx xxxx xxxxxx xxxxxxxxx xx *Xxxxxxxxxx* [51, 80, 231, 496-499] xxx xx xxxxxx xxxxxxxxxx xxxxx xxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxx xx xxxxxxxxxx xxxxxxx [119, 206, 231, 500, 501], xxxx xxx xxxx xxxxxxxxxx xxxx xxx xxxxxxx xx xxxxxxx xxxx xxxx xxxxxx [25]. Xxxxxxxxxxxxx xx XXX1, XXX1 xx XXXXX xx xxxxxx xxxxxxxxx xxx xxxxxxxx xxxx xxxxx xxxx xxx xxxxxxx xxxxxx xx xxxxxxx xxxxxxx [102, 119, 502-505]. Xx xxxxxxxx, XXXXX xxxxx xxxxxxxx xxxxxxx xxxxxxxx [505] xxx XXX xxxxx xxxxxxxx xxx xxxxxxxxxx xx-xxxxxxxxxx xxxxx xx xxxxxx xxx xxxxxxx xxxx [104, 506]

Xxxxxxxxxx, xxxxxxxx xxxxxxxxxxxx xx xxxxx xx-xxxxxxxxx xxxxxx XX xxxx xxxxxx xx xxxxxx xxxxxx xx X xxxxxx xxxx xx xxxxx xxx xxxxxxxxxx xxxxx (xXX4, xXX5, xXX6 xxx XxXX4; Xxxxxx 19 xxx Xxxxx 13) xxx xxxxxxxx xxxxxxx xx xxx xxxxxxxxxx xx xxxxxxx XXX. Xxxx, xxxx xxxxxx xxxxxxx xxxxx xxxxx xxxxxxxx xxxxx xxxxxx xx xxxxxxxxxx xxx xxx xxxxxxxx xxxxxxx. Xxxxxxxxxxxxx xx XXX2, xx xxxxxxx XxXX4 (Xxxxx 13) xxxxxx xxxxxx xxxxxx xx xxxxx xxxxxx xxx xxxxxxxxx [206]. Xxx xxxxxxxxx xx XXXX1/XXX4 (XxXX4, Xxxxx 13) xxx XXXX1 (xXX5, Xxxxx 13) xxx xxxxxx xx-xxxxxxxxx xx *Xxxxxxxxxx* xxxxxxxx xxxxxxxxx xxxxxxx xxxx xxxxxxxx [59, 209]. Xxxxx xxxx xxxxxxxx xxxxxxx xxxxxxx xxxxx xxxxxxxx xxxxxxx xx xxx XxXX xxxxxx (xxxxx 13), xxxxxxxxxx xxxxxx xx xxxxxxxxxx xx x xxx-xxxxxxxxxx (xxxxxxx 1 xxx 2). Xxxxxxxxxx xxxxxx xxxx xxxxxxxxx xxxxxxxxx 6-xxxxxxxx xxx xxxxxxxxxxxxx XxXX1 xxx xxxxxxxx xx xxxxxxxxx xxxxxx xxxxxxxx xxxx xxxxxxxxxx xxxx xxxx xx xxxx xxxxxxxxx [507]. Xxxxxxxxx xx XXX xxx XXX24 xxx xxxxxxxxx xx XxXX4 (xxxxx 13). *XxXXX5 (*xxxxxxxx-xxxxxxxxx XXXX-xxx xxxxx) xxx *XxXXX6, xxx* xxxxxxxxxx xx *Xxxxxxxxxx XXXXX XXXXXXXXX XXXXX/XXXXXXX-XXXX 24 xxxxx,* xxx xxxxxxxxxx xxx xxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxx xxxxxxx xx xxxx xxxx xx xxxxx xxxxxx[508], xxx xxx xxxxxxxxx xx xxxxx xxxxx xxxxxxxxxx xxxx xxx xxxxxxx xx xxxx xx xxxxxx xxxxxx xxxxx xxxxxxxxx xxxxxxxxx [508].

Table 12. Genes related to RNA translation, hormone metabolism and signal transduction high expressed in low tolerant fruits during CS and SLR

Xxxxxxxx	xxxxxxxxxx xx	Xxxxxx xxxxxxxxxx	XXX XX	Xxxxxxxxxx xxxx xxxx	Xxxxxxxxxx xxxx xxxxxx	2 xxxx XXX xxxxxx x	xxxxxx xx xxxx xx xxxxxxx xxxxxxx	XXX xxxxxx x
XXX xxxxxxxxxxxx xxxxxxxxxx								
XX2/XXXXX xxxxxx	XXX054X03	XXX1	XX4X25480	Xxxxxxxxxx xxxxxxx xxxxxx x1x	XXXX1X/XXX3			XxXX7
XXX-xxxxxx	XXX044X12	xxxxxx xx Xxxxx xxxxxxx xxxxxx 1						XxXX7
	XXX055X04	Xxxxx xxxxxxx xxxxxx	XX1X59750	Xxxxx xxxxxxx xxxxxx 1	XXX1			XxXX2
XXX-X Xxxxxx	XXX021X07	Xxxxxxx xxxxxxx xxxxxxxx 10	XX2X01760	Xxxxxxx xxxxxxxx 14	XXX14			XxXX6
XXX/XXX xxxxxx	XX1000X07	Xxxxx-xxxxxxxx xxxxxx XXX1	XX3X04730	Xxxxxxxxxx xxxx-xxxxxx xxxxxx 16	XXX16	xXX-X	XX-XX-XX-X	xXX3
	XXX014X03	Xxxxx-xxxxxx xxxxxx XXX28	XX1X04250	Xxxxx-3-xxxxx xxxx xxxxxxxx 17	XXX17/XXX3	xXX-X	XX-XX-XX-X	xXX3
	XXX057X01	XXX/XXX xxxxxx	XX4X29080	Xxxxx-3-xxxxx xxxx xxxxxxxx 27	XXX27/XXX2	xXX-X	XX-XX-XX-X	xXX3
XXX/XXX Xxxxxx	XXX072X10	XXXX-xxxxxx xxxxxxxxxxxx xxxxxxxx	XX5X42520	Xxxxx xxxxxxxxxxx 6	XXX/XXX6			XxXX6
XXX xxxxxx	XXX065X08	Xxxxxxxxx	XX1X06230	Xxxxx xxxxxxxxxxx xxxxx xxxx x4	XXX4	xXX-X	XX-XX-XX-X	xXX1
x-XXX xxxxxx	XX1001X05	Xxxxxxx xXXX xxxxxxxxxxx xxxxx	XX3X23690	Xxx1 xxxx xxxxxx 2	XXX2			XxXX2
	XXX002X10	Xxxxxxx xXXX xxxxxxxxxxx xxxxx	XX4X02590	Xxxxxxxxxx xxxxx xxx 12	XXX12			XxXX6
	XXX059X05	Xxxxxxxxxx xxxxx XXX1	XX3X26744	Xxxxxx xx XXX xxxxxxxxx 1/xxxxx	XXX1/XXXX			XxXX2
x-XXX xxxxxx	XX1000X11	xXXX xxxxxxxxxxx xxxxx xxxxx	XX4X34590	X-xxx xxxxxx xxxxx 6	XXX2/XXX6/			XxXX7
	XX1009X02	xXXX xxxxxxxxxxx xxxxx xxxxx	XX4X34590	X-xxx xxxxxx xxxxx 6	xXXX11			XxXX6
	XXX065X01	Xxxxxxx xxxxx-xxxxxx xxxxxx	XX1X60710					XxXX6
	XXX068X10	Xxxxxxx xxxxx-xxxxxx xxxxxx	XX1X60710					XxXX6
X2X2 Xxxxxx	XXX064X01	xxxx xxxxx (X2X2 xxxx) xxxxxx xxxxxx	XX1X34370	Xxxxxxxx xx xxxxx xxxxxxxxxxx 1	XXXX1			XxXX2
	XXX078X05	xxxx xxxxx (X2X2 xxxx) xxxxxx xxxxxx	XX3X50700	Xxxxxxxxxxx(XX)-xxxxx 2	XXX2	xXX-X	XX-XX-XX-X	xXX1
XXXXX xxxxxx	XXX075X05	Xxxxxxxxx-xxxxxx xxxxxxxxxx xxxxxxxx 2	XX5X64220	Xxxxxxxxx-xxxxxx xxxxxxxxxx xxxxxxxxx 2	XXXXX2			XxXX2
	XXX079X07	Xxxxxxxxx-xxxxxx xxxxxxxxxx xxxxxxxx 2	XX5X64220	Xxxxxxxxx-xxxxxx xxxxxxxxxx xxxxxxxxx 2	XXXXX2			XxXX6
XXXXX Xxxxxx	XXX045X02	xxxxxx xxxxx X, xxxxxx X3						XxXX7
XXXX xxxxxx	XXX035X06	Xxx-3-Xxx xxxx xxxxxx xxxxxx	XX3X55980	Xxxx-xxxxxxxx xxxx xxxxx 1	XXX1			XxXX6
XxxXXX xxxxxxxxxxx xxxxxxxxxx xxxxxx	XXX076X05	Xxxxxxxxxxxxx xxxxxxxxx XXXXX	XX4X32551	Xxxxxx	XXX	xXX-X	XX-XX-XX-X	xXX1
XX-xxxxxx	XX1004X05	Xxxxxxx xxxxxx xxxxxx-1-xxxx 4	XX5X11060	Xxxxxx1-xxxx xxxxxxx xxxx 4	XXXX4			XxXX2
	XXX010X01	xxxxxx xx XXXX10						XxXX7
XX-XXX xxxxxx	XXX079X08	xxxxxxx-xxxxxx xxxxx xxxxxx xxxxxxxxx xxxxxx 2	XX4X04890	Xxxxxxxxxx xxxxx 2	XXX2			XxXX2
XXX-xxxxxx	XXX042X12	xxxx xxxxxxx xxxxx xxxxxx xxxxx	XX3X51880	Xxxx xxxxxxx xxxxx X1	XXXX1/XXX			XxXX6
Xxxxxx-xxxxxx	XXX055X03	xxxxxxxxxxx xxxxx xxxxxx xxxxx xxxxxx	XX1X08620		XXXX7X	xXX-X	XX-XX-XX-X	xXX3
XXX-xxxxxx	XXX069X01	Xxxxxxxxxx xxxxx xxx1	XX1X10200	Xxxx1	XXXX1	xXX-X	XX-XX-XX-X	xXX3
XXXX-xxx xxxxxx	XX1006X03	XXXX-xxx xxxxxxxxxxx xxxxxx	XX5X60910	Xxxxxxxx/xxxxxx-xxxx 8	XXX	xXX-X	XX-XX-XX-X	xXX3

XXX-xxxxxx	XXX026X03	XXX5 xxxxxxx	XX1X09770	Xxx xxxxxx xxxx xxxxxxxx xxxxx 5	XXX5	xXX-X	XX-XX-XX-X	xXX1
	XXX041X07	XXX5 xxxxxxx	XX1X09770	Xxx xxxxxx xxxx xxxxxxxx xxxxx 5	XXX5			XxXX2
	XXX048X05	Xxxxxxx XXXXX-xxxxxx X xxxxxxx	XX1X77180		XXXX			XxXX6
	XXX072X07	xxx-xxxxxxx xxxxxxxxxxxx xxxxxx	XX2X46830	Xxxxxxxxxxxxxxxxxxxxxx 1	XXX1			XxXX2
	XXX075X04	XXX91	XX2X37630	Xxxxxxxxxx-xxxx 1/xxxxxxxxx xxxxxx 1	XXXX/XX1			XxXX6
XXX-xxxxxx	XXX036X05	Xxx-xxxx xxxxxxx 7	XX1X34190	Xxx xxxxxx xxxxxxxxx xxxxxx 17	XXX017			XxXX2
	XXX062X07	XXX xxxxxx xxxxxxx	XX1X01720	Xxx xxxxxx xxxxxxxxx xxxxxxx 2	XXXX002/ XXXX1			XxXX6
XXX xxxxxx	XXX062X06	XXX-xxxx xxxxxxx 2	XX3X59580	Xxx-xxxx xxxxxxx 9	XXX9	xXX-X	XX-XX-XX-X	xXX1
XXX xxxxxx	XXX053X07	XXX-xxxxxx xxxxxxx	XX1X14740	Xxxxxxx 1/xxxxxx3	XXX1/XXX1	xXX-X	XX-XX-XX-X	xXX3
	XXX080X07	XXX-xxxxxx xxxxxxx	XX3X07780	Xxxxxx1	XXX1			XxXX6
XXX-xxxxxx	XXX027X12	Xxxxx-1	XX1X14510	Xxxxx-xxxx 7	XX7			XxXX7
	XXX077X09	Xxxxxxx XXX-xxxx xxxx xxxxxx xxxxxxx	XX2X36720			xXX-X	XX-XX-XX-X	xXX1
XXX xxxxxxxxxxxx xxxxxxxxx	XXX025X02	XXX xxxxxx-xxxxxxxxxx xxxxxxx, xxxxxxxx	XX3X48050	'Xxxxxxx' xx xxxxxxx	XXX	xXX-X	XX-XX-XX-X	xXX3
	XXX027X09	Xxxxx-xxxx xxxxxx xxxxxxxxx	XX5X24120	Xxxxx xxxxxx 5	XXXXX5			XxXX7
	XXX031X10	XXX2/XXX3/XXX5	XX5X59710	XXXX2 xxxxxxxxxx xxxxxxx 2	XXX2	xXX-X	XX-XX-XX-X	xXX1
	XXX031X06	Xxxxxxx xxxxxxxxxxxxx xxxxxxxxx	XX5X66420					XxXX6
	XXX043X03	XXX xxxxxxxxx Xxx7	XX1X06790					XxXX2
	XXX061X09	Xxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxx xxxxxx xxxxxx	XX4X02990	Xxxxxx xxxxx/xxxxxx2	XXX/XXX2			XxXX6
	XXX067X07	Xxxxxxxx xxxxxxxx	XX3X11220	Xxxxxxx 1	XXX1			XxXX6
XXX-xxxxxx	XXX030X06	XXX	XX2X47070	Xxxxxxx xxxxxxx xxxxxx xxxxxx-xxxx 1	XXX1			XxXX6
	XXX044X07	XXX	XX2X47070	Xxxxxxx xxxxxxx xxxxxx xxxxxx-xxx 1	XXX1	xXX-X	XX-XX-XX-X	xXX1
Xxxxxxxxxxxx xxxxxxxxxx	XXX011X07	XXX xxx xxxxxx xxxxx-xxxxxx xxxxxx	XX5X37720	Xxxxxxxxxx xxxx xxx-xxxxxx xxxxx xx Xx-xxxxxx XXX1	XXX2/XXX4			XxXX2
	XXX072X02	Xxxxxxxxx-xxxx xxxxxx	XX5X14520	Xxxxxxxxx	XXX			XxXX2
Xxxxxx xxxxx	XXX011X09	Xxxxxxx xxxxxx	XX1X22770	Xxxxxxx	XX	xXX-X	XX-XX-XX-X	xXX1
XXX xxxxxx	XXX072X07	xxxxxxxx-xxx-xxxxx xxxxxx	XX5X20900	Xxxxxxxx-xxx-xxxxx xxxxxx 12	XXX12			XxXX2
Xxx-XXX xxxxxx	XXX024X11	Xxxxxxxxxxx xxxxx						XxXX7
Xxxxxxxx xxxxxxxxx								
Xxxxx xxxxxxxxx/ Xxxxx xxxxxxxxxx	XX1006X09	xxxxxxx XXX-xxxxx xxxxxxxxx	XX2X14960	xxxxxx xx XXX-xxxxx xxxxxxxx	XX3.1			
	XXX012X02	X-xxxxxxx-X-xxxxxxxxx xxxxxxx xxxxxxxxxxxxxxx-xxxx xxxxxx	XX5X55250	XXX xxxxxxxxxxxxxxxxxxxxxxx 1	XXXX1			XxXX6
XXX xxxxxxxxxx	XXX071X01	Xxxxxxxx xxxxxxx xxxxxxxxx 1	XX4X19170	9-xxx-xxxxxxxxxxxxx xxxxxxxxx	XXXX4			XxXX7
Xxxxxxxx/xxxxxxxxx	XXX001X12	Xxxxxxxxxxxxxxxx XXXX5x	XX1X22400	xxxxxxxx-X-xxxxxxxxxxxxxxxx	XXX85X1	xXX-X	XX-XX-XX-X	xXX1
	XXX007X06	Xxxxxxxxxxxxxxxx XXXX5x	XX1X22360	xxxxxxxx-X-xxxxxxxxxxxxxxxx	XXX85X2			XxXX6
Xxxxxxx xxxxxxxxxx	XX1005X06	1-xxxxxxxxxxxxxx-1-xxxxxxxxx xxxxxx	XX1X05010	1-xxxxxxxxxxxxxx-1-xxxxxxxxx xxxxxx	XXX;/XXX4			XxXX6
	XX1009X10	1-xxxxxxxxxxxxxx-1-xxxxxxxxx xxxxxx	XX1X05010	1-xxxxxxxxxxxxxx-1-xxxxxxxxx	XXX;/XXX5	xXX-X	XX-XX-XX-X	xXX1

				xxxxxxx				
xxxxxxxxxxx xxxxxxxxxx	XX1002X11	xxxxxxxxxx X450 xxxxxxxxxxxxx	XX5X24910	XXX-XXXX xxxxxxxxxx X450 X1	XXX1/ XXX714X1	xXX-X	XX-XX-XX-X	xXX1
	XX1004X06	Xxxxxxxxxx 2-xxxxxxx	XX1X02400	xxxxxxxxxx 2-xxxxxxx	XX2XX6			XxXX6
Xxxxxxxxx/xxxxxxxxxx	XXX031X11	Xxxxxxxx xxxxx-xxxxxxxxx xxxxxxx	XX2X46370	xxxxxxxx xxxxxxxx 1	XXX1/XX3.11	xXX-X	XX-XX-XX-X	xXX1
	XXX041X07	Xxxxxxxx xxxxx-xxxxxxxxx xxxxxxx	XX2X46370	xxxxxxxx xxxxxxxx 1	XXX1/XX3.11	xXX-X	XX-XX-XX-X	xXX1
	XXX079X10	Xxxxxxxx xxxxx-xxxxxxxxx xxxxxxx	XX2X46370	xxxxxxxx xxxxxxxx 1	XXX1/XX3.11	xXX-X	XX-XX-XX-X	xXX1
Xxxxxxxxx xxxxxxxxxx/xxxxxxxxx xxxxx	XXX029X11	Xxxxxxxxxxxxxxxx xxxxx	XX5X10920	xxxxxxxxxxxxxxxx xxxxx		xXX-X	XX-XX-XX-X	xXX1
Xxxxxxxxx xxxxxxxxx/ Xxxxxxxxx xxxxxxxxxx	XXX049X06	XXX-xxxxxxxxxxx/XXX-xxxxxxxx xxxxxxxxxx xxxxxx xxxxxxx	XX2X43840	XXX-xxxxxxxxxxxxxxxxx 74 X	XXX74X1			XxXX6
Xxxxxx xxxxxxxxxx xxxxxxx								
XXX xxxxxxxx/ Xx xxxxxx xxxxxxxxx	XXX029X04	XXX-xxxxxxx xxxxxx-xxxxxxx	XX3X63150	Xxxxxxx xxxxxx XXX-xxx	XXXX2	xXX-X	XX-XX-XX-X	xXX1
XXX xxxxxxxx/ Xx(2+)-xxxxxx xxxxxxx XXXxxx	XXX031X02	Xxx-XXX xxxxxx xxxxxx-xxxx	XX3X63150	Xxxxxx xxxxxx XXX-xxx	XXXX2	xXX-X	XX-XX-XX-X	xXX1
XXX xxxxxxxx/xxxxxxxxx xxxxxx	XXX029X02	Xxxxxx xxxxxxxxxx 2X	XX1X72770	Xxxxxxxxxxxxx xx XXX1	XXX1			XxXX2
xxxxxxxxxxxxxxx	XXX035X08	Xxxxxx xxxxxxxxxx 2X	XX1X72770	Xxxxxxxxxxxxx xx XXX1	XXX1			XxXX6
Xxxxx xxxxxxxx/ xxxxx xxxxxxxx	XXX078X01	Xxxxxxx xxxxx-xxxxxxxxx xxxxxx	XX1X05180	Xxxxx xxxxxxxx 1	XXX1			XxXX6
xxxxxxxx xxxxxx	XXX023X05	Xxxxxxxx X59, xxxxxxxxxx	XX1X80680	xxxxxxxxx xx XXXXX xxxxxxxxx 3	XXX3/XXX96			XxXX6
	XXX040X03	Xxxxx 1	XX4X02570	Xxxxx xxxxxxxx 6/Xxxxx1	XXX6/XXX1			XxXX2
Xxxxx xxxxxxxx/ XXXXX-Xxxxxxx Xxxxxxxx	XXX054X07	Xxxxxx-xxxxxx XX-xxxx	XX4X27280	Xxx1x/xxx1				XxXX2
Xxxxx xxxxxxxx/ Xxxxxx XXXX xxxxxx	XX1001X04	Xxxxx xx xxxxxx xxxxxxx (XXX298)	XX3X28970	Xxxxxxxx-xxxxxxxx 3	XXX3			XxXX2
Xxxxxxxxxxxxx xxxxxxxx	XX1004X08	xxxxx-xxxxxx xxxxxx xxxxx xxxx	XX2X30980	Xxxxx-xxxxxx xxxxxx xxxxx xxxx	XXXxXxxx			XxXX6
	XX1005X02	Xxxxx-xxxxxx xxxxxx xxxxx xxxx	XX3X05840		XX12			XxXX6
	XXX070X11	Xxxxx-xxxxxx xxxxxx xxxxx xxxxxx	XX5X14640	Xxxxx-xxxx xxxxx 13	XX13			XxXX6
Xxxxx xxxxxxxx/ Xxxxxx xxxxxxxxx	XXX050X05	xxxxxxxxx, xxxxxxx,	XX1X12310			xXX-X	XX-XX-XX-X	xXX3
Xxxxxx xxxxxxxx/ Xxxxxx xxxxx	XXX005X02	xxxxxxxxxx-xxxx xxxxxxxxxxxxx xxxxx	XX2X46880	Xxxxx xxxx xxxxxxxxxx 14	XXX14			XxXX2
	XXX006X03	Xxxxxxx xxxxxxxxx	XX2X03150	Xxxxx xxxxxxxx 1579	xxx1579	xXX-X	XX-XX-XX-X	xXX3
	XXX035X12	Xxxxxxx xxxxx xxxx xxxxxxxxx	XX1X13750	Xxxxx xxxx xxxxxxxxx 1	XXX1			XxXX2
	XXX046X08	Xxxxxxx xxxxx xxxx xxxxxxxxx	XX3X20500	Xxxxx xxxx xxxxxxxxx 18	XXX18			XxXX2
	XXX073X05	Xxxxx xxxx xxxxxxxxx-xxxx xxxxxx	XX3X20500	Xxxxx xxxx xxxxxxxxx 18	XXX18			XxXX6
	XXX078X07	Xxxxxx-xxxxxx xxxxxx	XX2X43290	Xxxxxxxx xxxxxxxxx xx XXX4 xxxxxxxxx xx xxxxx 3	XXX3	xXX-X	XX-XX-XX-X	xXX1
Xxxxxx xxxxxxxx/ Xxxxxx xxxxx-xxxxxxxxx	XXX027X08	Xxxxxx-xxxxxxxx xxxxxx xxxxx	XX3X57530	Xxxxxx-xxxxxxxx xxxxxx xxxxx 32	XXX32			XxXX6
Xxxxxx xxxxxxxx/ xxxxxx xxxxxx	XXX002X10	XX521-X-xxxx xxxxxx	XX3X13060	Xxxxxxxxxxxx xxxxxxxx X-xxxxxxx xxxxx 5	XXX5			XxXX6
Xxxxxx xxxxxxxx/ Xxxxxx xxxxx	XXX009X01	XX xxxxxxxxx-xxxxxx xxxxx	XX3X22190	XX-xxxxx 5	XXX5			XxXX6
xxxxxxxxx	XXX011X06	XXX-xxxxxxxxx xxxxx/xxxxxxxx-xxxxxx xxxxx 11	XX5X01820	Xxx1-xxxxxx xxxxxx xxxxx 315	XxXX3.15	xXX-X	XX-XX-XX-X	xXX3

	XXX013X01	Xxxxxx/xxxxxxxxx xxxxxx	XX5X58380	Xxx1-xxxxxxx xxxxxxx xxxxxx 38	XXXX3.8	xXX-X	XX-XX-XX-X xXX3
	XXX019X03	Xxxxxxxx XX16 xxxxxxx	XX2X33990	XX-xxxxxx 9	XXx9	xXX-X	XX-XX-XX-X xXX1
	XXX021X12	XXX1 xxxx xxxxxxx xxxxxx	XX4X14580	Xxx1-xxxxxxx xxxxxxx xxxxxx 33	XxXX3.3		XxXX6
	XXX048X08	Xxxxxxxxxx xx XX16 xxxxxxx				xXX-X	XX-XX-XX-X xXX3
Xxxxxx xxxxxx xxxxxxxxxx	XXX057X06	Xxxxxxx xxxxxx	XX1X72710	Xxxxxx xxxxxx 1-xxxx xxxxxxx 2	XXX2		XxXX6
	XXX077X04	Xxxxxxx xxxxxx	XX1X72710	Xxxxxx xxxxxx 1-xxxx xxxxxxx 2	XXX2		XxXX6
Xxxxxx xxxxxxxxxx xxxxxxxxx/ (x)xxXxx-xxxxxxxx xxxxxxxx	XXX075X01	XxxX/XxxX xxxxxx-xxxxxxxxxx xxxxxxx	XX3X17470	Xx2+-xxxxxxxxx xxxx/xxxx xxxxxxx	XXXX		XxXX2
Xxxxxx xxxxxxxxx xxxxxxxxx/ xXXX xxxxxxxxx	XXX075X11	Xxxxxxxx xxxxxxx xxxxxxxxx xxxxxxx	XX4X34490	Xxxxxxx xxxxxxxxxx xxxxxxx 1	XXX1		XxXX6
Xxxxxx xxxxxxxxx xxxxxxxxx/ xxxxxx xxxxxxxxx xxxxxx	XXX044X05	Xxxxxx xxxxxxxxx-xxxxx xxxxxxx X	XX5X53130	Xxxxxx xxxxxxxxx-xxxxx xxxxxxx 1	XXXX1	xXX-X	XX-XX-XX-X xXX1
Xxxxxxx xxxxxxxx xxxxxxxxx xxxxxxx/ X-	XXX005X11	X-xxxxxxxxxxxxxxx xxxxxxxxxxxxx	XX5X64440	Xxxxx xxxx xxxxx xxxxxxxxx	XXXX		XxXX6
xxxxxxxxxxxxxxx xxxxxxxxx xxxxxxxxxx	XXX066X06	X-xxxxxxxxxxxxxxx xxxxxxxxxxxxx	XX5X64440	Xxxxx xxxx xxxxx xxxxxxxxx	XXXX		XxXX6
Xxxxxxxx xxxxxxxxx/ XXX(XXX1) X3 xxxxxxxxx xxxxxx	XX1005X04	X-xxx xxxxxx xxxxxxx	XX2X25490	Xxx3-xxxxxxx x xxx xxxxxxx 1	XXX1	xXX-X	XX-XX-XX-X xXX1
Xxxxxxxx xxxxxxxxx/ xxxxxxxx xxxxxxxx	XXX054X06	Xxxxxxxx xxxxxxxx	XX3X04580	Xxxxxxxx xxxxxxxxxx 4	XXX4	xXX-X	XX-XX-XX-X xXX1
Xxxxxxxx xxxxxxxxx/ xxxxxxxx xxxxxx xxxxxxxxxxx	XXX011X11	XXX-xxxxxxx xxxxxxx	XX3X46060	Xxx XXXxxx xxxxxxx 8x	XXX3		XxXX2
X-xxxxxxx xxxxxx xxxxxxxx xxxxxxx	XXX005X05	Xxxxx-xxxxx X-xxxxxxx	XX2X23460	Xxxxx-xxxxx X-xxxxxxx 1	XXX1/XXX8X		XxXX6
xxxxxxxxx xxxxxx/ X-xxxxxxx xxxxxx	XXX052X10	Xxxxxxx XX-xxxxxx xxxxxxx	XX4X02730	Xx40 xxxxxx xxxxxxx x	XXX5x		XxXX2
	XXX070X02	Xxxxxxx XX-xxxxxx xxxxxxx	XX1X71840	XX-xxxxxx xxxxxxx			XxXX7
Xxxxx xxxxxxxxx	XXX005X08	Xxxxxxxxxxx 2X xxxxxxxxx	XX1X04400	Xxxxxxxxxxx 2	XXX2		XxXX6
	XXX029X11	Xxxxxxxxxxxxxxxxxxx xxxxxxxxx, xxxxx 1	XX1X04400	Xxxxxxxxxxx 2	XXX2		XxXX6
	XXX031X05	Xxxxxxxxxx X	XX1X09570	Xxxxxxxxxx X	XXXX		XxXX6
	XXX008X02	xxxxxx/xxxxxxxx xxxxxx xxxxxxxxx	XX4X08320	Xxxxxxxxxxxxxxx xxxxx 8	XXX8		XxXX2
Xxxxxxxxx xxxxxxxx/xxxxx xxxxx	XXX080X09	XXX xxxxx-xxxxxxxxx xxxxxx	XX1X65320	XXX xxxxx xxxxxxxxx xxxxxx 6	XXXX6		XxXX6
Xxxxxxxxx xxxxxxxx/ xxxxxxxx xxxxx	XXX078X08	Xxx-xxxx XXX-xxxxxx xxxxxx XXXX5	XX1X75840	Xxx-xxxx XXX xxxxxx xxxxxx 5/Xxx-xxxx XXX xxxxxx xxxxxx 4	XXX5/XXX4		XxXX2
Xxxxxxxxx xxxxxxxx/ xxxxxxxxxxxx	XXX005X12	Xxxxxxx XX-XXX-XXX xxxxxxxxx xxxxxx	XX3X14470				XxXX6
xxxxxxx	XXX051X02	XXX; Xxxxxxx xxxxxxxxx xxxxxx; XXX XXXxxx	XX3X44400				XxXX6
xxxxxxxxxxxxxxx xxxxxxxxx	XX1000X12	xxxxxxxxx xxxxxxx (XX) xxxxxx-xxxxxxxxx xxxxxx	XX2X30880		XXXX70		XxXX2
Xxxxxxxxxxxxxx xxxxxxx/ xxxxxx xxxxxxxxxx xxxx 2X xxxxxxxx xxxxxxx	XXX024X05	Xxxxx/xxxxxxxx xxxxxx xxxxxxxxx 2X 57 xXx xxxxxxxx					XxXX6
Xxxxxxxxxxxxx xxxxxxx/ Xxxxxx-xxxxx xxxxxxxx	XXX006X06	Xxxxxx xxxxx-xxxx	XX4X32250				XxXX2
	XXX064X12	Xxxxxx xxxxx	XX2X28930	Xxxxxx xxxxx 1X	XXX1X		XxXX2
Xxxxxxxxxxxxx xxxxxxx/ XXXX/XXXXX/XXXXXX	XXX008X06	Xxxxxx-xxxxxxxx xxxxxx xxxxx 8	XX1X18150		XXX8		XxXX6
	XXX020X02	Xxxxxx-xxxxxxxx xxxxxx xxxxx 4	XX4X01370	Xxx xxxxx 4	XXX4		XxXX2
	XXX032X07	Xxxxxx-xxxxxxxx xxxxxx xxxxx	XX1X18150	Xxx xxxxx 8	XXX8		XxXX6

	XXX049X12	XXX xxxxxx	XX3X14720	Xxx xxxxxx 19	XXX19			XxXX6
	XXX026X02	Xxxxxx/xxxxxxxxx xxxxxx				xXX-X	XX-XX-XX-X	xXX3
	XXX003X05	Xxxxxx-xxxxxxxxx xxxxxx xxxxxx xxxxxx xxxxx	XX1X53570	Xxx xxxxxx xxxxxx xxxxxx 3	XXX3XX			XxXX6
	XXX031X06	Xxxxxxx xxxxxx	XX5X58950			xXX-X	XX-XX-XX-X	xXX3
	XXX066X11	Xxxxxx-xxxx xxxxxxx	XX3X22750					XxXX6
Xxxxxxxxxxxxxx xxxxxxxx/ XX2X	XX1009X12	xxxxxxx xxxxxxxxxx 2X-xxxxxxx	XX1X47380					XxXX2
	XXX002X05	xxxxxxx xxxxxxxxxx 2X-xxxxxxx	XX1X09160					XxXX6
Xxxxxxxxxxxxxx xxxxxxxx/ xxxxxxxx xxxxxx	XXX054X05	Xxxxxxx xxxxxx	XX3X14350	Xxxxxxxxx-xxxxxxxx xxxxxx 7	XXX7			XxXX2
Xxxxxxxx/ xxxxxxx xxxxxxx xxxxxxxx	XXX064X05	Xxxxxxx xxxxxx xxxxxxxx, xxxxxx-xxxxxxx	XX3X27390			xXX-X	XX-XX-XX-X	xXX1
Xxxxxxxx/ Xxxx xxxxxxx xxxxxxxx	XX1006X09	Xxxxxxx xxxxxx	XX1X77280			xXX-X	XX-XX-XX-X	xXX1
	XXX006X11	Xxxxxxxx X-xxxxxx xxxxxxx-xxxx xxxxxxx xxxxxx	XX2X19130					XxXX6
	XXX039X08	Xxx-xxx xxxxxxx xxxxxx	XX2X40270			xXX-X	XX-XX-XX-X	xXX1
	XXX058X12	xxxxxxx-xxxx xxxxxx xxxxxx xxxxxxx / xxxxxxx xxxxxx xxxxxx xxxxxxx	XX1X56145					XxXX6
	XXX080X12	Xxxxxxx xxxxxx	XX1X77280			xXX-X	XX-XX-XX-X	xXX1
Xxxxxxxx/XXX xxxxxxxx	XXX015X03	Xxxxxxx xxxxxx	XX2X01820			xXX-X	XX-XX-XX-X	xXX1
	XXX060X03	Xxxxxxx xxxxxxxxxx xxxxxxxxx xxxxxx-xxxx	XX4X03260					XxXX6
Xxxxxxxx/xxxxxxxx xxxxxxx xxxxxx	XXX047X07	Xxxxxxxx-xxxx xxxxxxx xxxxxx	XX2X31880	Xxxxxxxx/xxxxxxxxx xx XXX1 1	XXX/XXXXX1	xXX-X	XX-XX-XX-X	xXX3
	XXX071X01	Xxxxxxxx-xxxx xxxxxxx xxxxxx	XX2X31880	Xxxxxxxx/xxxxxxxxx xx XXX1 1	XXX/XXXXX1			XxXX6
Xxxxxxxx/ Xxxxxxxxxxxx xxxxxxxx	XXX005X07	xxxxxxx xxxxxxxxx xxxxxxx (XX-XXX-XXX xxxxx)	XX1X50180			xXX-X	XX-XX-XX-X	xXX1
	XXX020X03	Xxxx xxxxx xxxxxxxxxxxx xxxxxxx xxxxx-xxxxxxxxx xxxxxx	XX1X61670					XxXX6
	XXX039X11	XXX-XXX-XXX xxxx X xxxxxxx 7						XxXX6
	XXX063X12	Xxxxxxx xxxx xxxxx xxxxxxxxxxxx xxxxxxx 1	XX5X18520	Xxxxxxxx X-xxxxxx xxxxxx xxxxxxx 7	XXXX7			XxXX6
	XXX067X01	xxxxxxx xxxxxxxxx xxxxxx (XX-XXX-XXX xxxxx), xxxxxxx	XX1X59218					XxXX6
Xxxxxxx xxxxxxx xxxxxxxx	XXX051X03	Xxxxxxx xxxxxx xxxxxxxx 1-xxxx	XX4X27750	Xxxxxxx xxxxxx xxxxxxxx 1	XXX1			XxXX7

eRG: early ripening gene; NeRG:non early ripening gene, D:down; U:up; RS: ripe stop, RP: ripe progress; NC : no change; S: sensitive ; LS :low sensitive

Genes in those clusters associated with cold/stress xxxxxxxxx xxxxx xx xxxxxx xx xxxxxxxxxxxx xxxxxxxx xxxxxxx xxxxxx. XXX, XXXX1 xxx xXXX11 xxxxxxxxx (xxxx xx xxxxxxxx XxXX6 xxx XxXX7, xxxx xxx xxxxxx xx X, Xxxxx 12) xxx xxxx xxxx x xxxx xx xxx xxxxx xxxxxxxx xx xxxxxxx xxxxxx, xxx xxxx xxxx xxxxxxxxxxx xx xxxxxx xxxxxxxx xx xxxxxx xxxxxx xx xxxxxxxxxx xxxxxxxxx xxxx xx xxxx xxxx xxxxxxxxxxxx, xxxxxxxxx, xxxxxxxx xxxxxxxxxx, xxxxxxxxxxxx xxxxxxxxxx xxx xxxxxxxxx xxxxxxxxxx [122, 127, 509-511]. Xxxxxxx xxxxxxx xxxx xxx xxxxx xx xxxxx xxxxxxxx xxx xxxxxxxx xx xxxxxxxxxxxx xxxxxxxx xxxxx xxxx xxx xxxxxxxxxxxxxx xxxxx xxxx xx xxxxxxxxx xx xxx xxxxx xxxxxxxx xxxxx. Xxxxxxxx xxxxxxx *XXXXXXXX* (XXX, xx xXX3, xxxx-xxxxxxxxx xx x xxxxxxxxx xxxxxx, Xxxxx 12), xxxxxxxxxx xx xxxxx xxxxxxxx xxxxxx xxxxxx xxxxxxxx xx xxxxx xxxxx xxxxxxxxxx [512], xxx XXXXXXXX XXXXXXXX XXXXXXX XXXXXXX-XXXX 1 (XXX1, xx xxxxxxxx xXX1 xxx XxXX6, xxxx xxxx xxx xxxxxx xx xxxxx X, Xxxxx 12). Xx xxxxx, x xxxx xxxxxxxx x xxxxxxxx XX xxxxxxxxxx xxx Xxxxxxxx-Xxxxxxxx Xxxxxxx Xxxxxxx (XXX) xxxxxx xx xxxxxxx xx xxx xxxxxx xx xxxxx xxx xxxxx XXX xxxxxxxxxxx xxxxx xxxxxxxxxx xxxx xxx xxxxxx [513]. Xxxxxxxx, xxxxxxxxx xx xxx xxxxxx xxxxxxxx xxx xxx xxxxxxxx xxxxxxxxx XxxxXXX-XXX (xxxxx xxx-xxxxxxxx) [514] xxxxxxx xx x xxxxx/ xxxxxx xxxxxxx xxx xxxxxxxx xxxxxxxxx xx xxxxxxxx xxxxxxxxxxx [515]. Xxxxxx XXX xxxxxxxx xxxx xxx xx xxx xxxxxxxxxx xx xxxx xxxxxxxx xxxxxxxxx, xxxxxxxxx xxxx xxxx xxxxxxxxxxx, xxxxxxxx xxx xxxxxxxxx xxxxxxxxxxx, xxxxxxx xxxxxxxxxx xxx xxxxxxxx-xxxxxxxxxx xxxxxxxx xxxxxxxxxx [396, 516, 517]. Xxxxxxxx, xxxxxxxxx xxxxxxxxxx xx XxXXX4, xxx xxxxxx xxxxxxxx xx XXXX1 (xx xxxxxxx XxXX6, Xxxxx 12), xxxxxxxxx xxxxx xxxxxxxx xxx *XxXXX4*-xxxxxxxxx xxxxxx xxx xxxxxxx xxxxxxxx xxxxxxxxxx xxx xxxxxxx xxxxxxxxxx xx xxxxxxxx-xxxxxxxxxx xxxxx, xxxxx xxxxx xxxx XX xxx xxxxxxxxxx xxxxxxxxxxxx xxxxxxx[511].

C3.2.3.Hormonal de-synchronization of the ripening program after cold storage may contribute to mealiness

Xxx XXX xxxxxxxx xx xxxxx xxxxx xxxxx XX xx xxxx xxxxxxx xxxx x xxxxxx xxxxxxxx xxxxxxx xx xxxxxxxx. Xxxxxx xxx xxxxx xxxx XX, xxxx xxxxxxxxxx xx xxxxxxxx xxx xxxxxxxxxxxxx. Xxxx xxxxx xxxxxxxxx, xxxxx xxxxxx xxxx xxxxxxxxx xxxxxxxx. Xxxx xxxxx xxxxxxx xx xxxxxxx xxxxxxxxxxx, xxxxxxxxxx xxxxxxxxxxx, xxxxxxx xxx xxxxxxxxx were in the gene

classes xxxx xxxx xxxxxxxx xxxxxxxx xx X xxxxxx xxxxxx XX xxx XXX xxxxxxxx xx X xxxxxx (xxxxx 12 xxx 13). Xxx xxxx xxxxxx xxxxxxxx xxxxxxx xxxxxxxx xxxx xxxxx xxxxxxx xx xxxxxxxx xxx xxxxx. Xxx xxxxxxxxxxx xx xxxxx xxxxxxxx xxxxxx xxxxxx xxxxx xxxxxxxx xx xxxxx [245, 518-521]. Xxxxxxx, xx xxxxxxx *Xxxxxx* xxxxxx, xxxx xxxxx xxxxxx xxx xxxxxxxxx xx xxxxxxxx xxxxxxxx xx xxxxxxxx xxxxxx 2 xxxxxxxx xxxxxxxxxx. Xxxx xxxxxxxx xxx xxxxxxxxxx xxxx xxx xxx-xxxxxxxxxx X3 xxxxx xx xxx xxxxxxxxxx X4X xxxxx, xx xxxxxxxxxx xxxxxxxx. Xxxxxx xxxx xxxx xxxxxxxxxxxx xxx xxxxxxxxxxxxxx xxxx xxxxxxxx xx xxxxxxxxxx xxxxxxxx (X4XX xx xxxxxxx xxxxx).

Xxxxxx XXX xxxxxxxxx XX, xxxxxx xxxxxx xx xxx xx xxxxxxx xxxxx X4XX, xx xxxxxxx [135], xxx xxxxxx xxxxx xx xxxxxxxxx xxxxxxxxxxxx xx x xxxxx xxxx xx xxxxx xxxxxxxx [133] xxxx xxxxxxx xxxxxxxxx xxxx xxx xxxxxxxxxxx xxxxxxxxxxx xxxx, xxxxxxxx xxxxx xxx xxxxxxxxx xxxxxxxxxx [136]. Xxxx xxx xxxx xxxxxxxxxx xx xxxxxxx xxxxxxxx xxxxxxxxxx [136, 137]. Xxxxxxx xxxxxx xx xxx xxxxxxxx xx xxxxxxxxxxx, xxxxxxxxx xxx xxxxxxxxxx xxxxxxxxx xxxx XXX xxxxxxxxxx xxxxxx xxxx XX xxx XXX ([12, 15, 136, 137] xxx xxxxxxx 1 xxx 2). Xxxxxx xxxxx xxxxxx xxxxx XX xxx XXX xxxx xxxx XXX xxxxxxxxxx xxx xxxxxxxx xxx xxxx XXX [136, 137]. Xx xxxxxxxxx, xxxx xxx xxxxxxxxxx xx xxx xxxxxxxx xx XXX/XXX4 xxxx xxx xxxxxx xx XX xxxxxx xxxxxx XXX (xxxxxxx xXX1; Xxxxxx 19X, xxxxx 12), xxxxx xxxx xxxxxx xxx xxxxx xxxxxx xxxx xxxxx xxx xxxxxxxxx xx XXX (XxXX4 xxx xXX4, xxxxxxxxxxx; Xxxxxx 19, Xxxxx 13). Xxx xxxxx xxxxxxxxxx xx xxx xxxx xxxxxxxx XXX xxx xxxxxx xx XXX xxxx xxxxxxx XxXX4 xxxx xx XXX xxxxxxx xxx xxxxxxx xxxxxxxxx xx XX-XXX xx xxx xxxxxx xxxxxxx xxx xx xxxxxxxxx xxxxxxxxxx xxxxx xxxx xxx Xxx-XX xxxxxxxxxx (Xxxxx X14 xxx xxxxx X15). Xxxxxxxxxx, xxx xxxxxxxxxx xxxxxxxxx xx XXX xx XxXX4 xxxxxxxxx xxxxxxxxx xxxx XX xxxxxx xx xxxxxxxxx xxxxx (Xxxxx X15Xxxx xxx xxx XXX xxxxxxxx xxxx xxxxxxxxxxxxx xxxxxxxxx xxxxxxxxx xxxx xxxx xxxxx xxxxxxxx xx xxxxxx xxx xxxxx xx xxxxxxxxxxxxx xxxxxxxx xxxxxxxxx. Xxxxxxxxx XXX xxxxxxxx xxx xxxxxxxx xx xxx xxxxxxxxxx xx xxxxx xxxxxxxx xxxxxx (xxxxxx X) xxx xxxxxxxxxxxx xxxxxxxx xxxxxxxxx (xxxxxx XX) xx xxxxxxxxx xxxxxx xxxx xx xxxx, xxxxxxx xxx xxxxxx [518, 522, 523]. Xxxxxxxxx xxxx xxxxxxxx xxxxxx X xx xxxxxx XX xxxxxxx xx xxxx-xxxxxxxxxx xx xxxxxx X XXX [522]. Xxxx, xxx XXX xxxxxxx xx XxXX4, xxxxxx xxxxxxxxx xx xxxxx X xxxxxx xxxxxx XXX xxxx xxx xxxxxxxx xxxxxx [136, 137] xxx xx xxxxxxxxxxx xxx xxxxxxxx xxxxxx 1, , xxxxx xxx xxxxxx

xxxxxxxxx XXX xx xXX4 xxx xxxxxxxxx xxxxxxxxxxxxx xxxxxxxx xxxxxxxxxx xxx xxx xxxxxxxx xx xxx xxxxx xx xxxxx.

XXX1 xx xxxxxxx XxXX4 (Xxxxx 13) xx xxxxxxxx xx xxxxxxxx xxxxxxxx xxxxxxxx xxxxxx X xxxxxxxxxx [432]. Xx xxxxxxx 1 xxxx xxxx XXX1 xxxxxxxxxx xx xxxxxxxxxx xxxx xxxxx xxxxxxxxxx xx xxx xxx-xxxxxxxxxx xxxxx. Xx xxx xxxx xxxxxx xxxxxxxxx xx X xxxxxx xxxxxx XXX (Xxxxx 13). Xxx *Xxxxxx xxxxxxxx* XXX1-xxxx xxxxxxx (*Xx-XXX1)* xxxxxxxxxx xx xxxxxxxxx xxx xxxxxxxxxx-xxxxxxxxxx xxxxxxx xx xxxx xxxx xxxxxxxxx [518, 524], xxxxx xxxx-xxxxxxxxxx xx Xx-XXX1 xx xxxxxxxx xxx xxxxxxxxxx xxxx xxxxxx X xx xxxxxx XX. Xxxx xxxxxxx xxxx xxx xxxxxxxx xx XXX1 xxxxx xx xxxx xx xxx xxxxxxxxx xxxxxxxxx xx X xxxxxx xxxxxx XX xxx XXX xx xxxxxx xxxxxxxxxxxx xxxxxxxx xxxxxxxxxx xxxxxxxx xxx xxxxxx xxxxxxxx.

Xxxxxxx xxxxxxxx xxxxxxxxxxx xxxxxxxxxx xxx xxxxxxxxx xxxxxxxx xxxx xxxx xxxxxxxxxxxxx xxxxxxxxx xxxxxxx xxxxxxxxx xxx xxxxxxxx xxxxxx xxxxxx XX xxx XXX (Xxxxxx 12 xxx 13) xxx xxxxx xxxxxxxxxx xxx xxxxxxxxxx xxxx xxxxxxxxx/xxxxxxxxxx xx xxx xxx-xxxxxxxxxx xxxxx (xxxxxxx 1 xxx 2). Xxxxxxxxx xx XXX (xXX3, xxxx xxxxxxxxxx xxxxxxxx xxxxxx XX xxx XXX xx x xxxxxxxxx xxxxxx) xxx XXXX1/XxXXX4 (XxXX6, xxxx-xxxxxxxxx xxxxxx XX xxx xxxxxxx xx xxxxx X-X xxxxxx xx XXX) xxxxxx xxxxx xxxxxxxxxx xx X xxxxxx xxxx xx XX xx X-X xxxxxx. Xxxxxxxxxx xxxxxxxx xxxx xxxxxxxxxx XXX xxx XxXXX4 (XxXX6) xxxxx xxx xxx xxxxxxxx xxxxxxxxxx xxx xxxxxx xxx xxxxxxxx xxxxx [511, 516]. Xx xxxxxxxx, xxxxx XXX xxxxxx xxxxx xxxxxxx xxx xxx xxxxxxxxxx xx XXX xxx XXX xxxxx xxxxxx xxxxxxxx, xxxxx xxxxxxx xxxx xxxxxxxxxx xx XXX [525].

Xx xxxxxxxx 1 xxx 2 xx xxxxxxxx xxxx xxxxxxxxxxxx xxxxx xxxxxxxxxxx xxx xxxxxxxxx xxxxx xxx xxxxxxxx xx xxx xxxxxxxx/xxxxxxxxx xxxxxxxx xx xxx-xxxxxxxxxx xxxxxx xxx xxxxxx xxxxxxxxxx xx xxxxx xxxxxxx xx xxxxxx xx xxxxxx xx xxx XXX xxxxxxxxx [16]. Xxxx, xxxxx xxxxxxx xx xxxxx xxxxxxxxxx (Xxxxx 12) xxxx xx xxx xxxxxx xxxxxx xxxxxx XXX xxxxxxxx, xxxx xx XXX-xxxxx xxxxxxxx XX3.1 (XxXX6) xxx XXX xxxxxxxx xxxxxxxxxxxxxxxxx XXXX (XxXX7), xxxx xxxx xxxxxxxxx xx XXX-X xxxxxx xxxx xx X-X xxxxxx, xxxxx XX xxxxxx xxxxxxxxx xx xxxxxx xxxxxxxxx. Xx xxxxxxxx, xxxxx xxxxxxx xx xxxxx xx-xxxxxxxxxx xxx xxxxxxxxxxx (Xxxxx 13) xxxx xx XXX3 xxx XXXXX-XXX XXXXXXXXX 2 (XXX2) (xxxx xx XxXX4, Xxxxxx 19), xxxxx xx xxx xxxxxx xxxxxx xxxxxxxx, xxxx xxxx xxxxxx xxxxxxxxx

Table 13. . Genes related to RNA translation, hormone metabolism and signal transduction high expressed in sensitive fruits during CS and SLR

Xxxxxxxx	Xxxxxxxxxx xx	Xxxxxx xxxxxxxxx	XXX xxxx	**Xxxxxxxxxx xxxx xxxx**	**Xxxx xxxx xxxxxx**	**2 xxxx XXX xxxxxxx**	**xxxxxx xx xxxx xx xxxxxxxx xxxxxxx**	**XXX xxxxxxx**
XXX xxxxxxxxxxxx xxxxxxxxxx								
5X XXX, xXXX xxx xXXX xxxxxxxxxxxx	XXX007X02	XXX xxxxxxxxxx XXX xxxxxxx-xxxx xxxxxxx	XX3X49000	XXX xxxxxxxxxx XXX xxxxxxx XXX82 xxxxxx xxxxxxx		xXX-X	XX-XX-XX-X	xXX5
XXX3/ XX1 -xxxxxx	XXX005X10	Xxxxxxxxxxxx xxxxxx X3-XXX xxxxx xxxxxx	XX4X32010	xxxxxxxxxxxxx xxxxxx X3 xxxxxx xxxxxx	XXX1/ XXX2	xXX-X	XX-XX-XX-X	xXX4
XX2/ XXXXX xxxxxx	XXX011X10	Xxxxxxxx xxxxxxx xxxxxxx xxxxxx 5				xXX-X	XX-XX-XX-X	xXX5
	XXX039X03	Xxxxxxxx xxxxxxxxxx-xxxxxxxxx xxxxxx xxxxxx xxxxxx	XX1X78080	Xxxxx xxxxxxx xxxxxxxxxxxxxxx 1	XXXX1/ XXX2.4			XxXX4
	XXX049X05	Xxxxxxxxxx xxxxxxxxx xxxxxx-xxxxxxx xxxxxx 3						XxXX4
	XXX078X06	XXXXX-4 xxxx xxxxxxx	XX4X17486	XXXXX xxxxxxxx xxxxx xxxxxxxxx xxxxxx xxxxxx				XxXX4
XXX-xxxxxx	XXX051X02	Xxxxx xxxxxxx xxxxxx 2	XX5X20730	xxxxx xxxxxxxx xxxxxx 7	XXX7/ XXX5	xXX-X	XX-XX-XX-X	xXX4
	XXX072X07	Xxxxx xxxxxxx xxxxxx 5	XX1X19850	xxxxxxxxxxxx xxxxxx XXXXXXXXX	XX/ XXX5	xXX-X	XX-XX-XX-X	xXX4
XXX/ XXX xxxxxx	XXX046X05	Xxxxx-xxxxxxxxx xxxxxxx XXX13	XX2X33310	xxxxxxxxxxx xxxx-xxxxxxx xxxxxxx 13	XXX13			XxXX4
	XXX060X07	Xxx/ XXX xxxxxxx	XX1X04240	xxxxxxxxxxx xxxx-xxxxxxx xxxxxxx 3	XXX3/ XXX2			XxXX4
x-XXX xxxxxx	XXX054X03	Xxxxxxxx xXXX xxxxxxxxxxxx xxxxxx	XX2X24260	xxxxx xxxxx-xxxx-xxxxx xxxxxx xxxxxxx	XXX1			XxXX4
x-XXX xxxxxx	XX1001X07	Xxxxxxxxx xxxxxxxxxxxx xxxxxx	XX1X58110	xXXX xxxxxx xxxxxxxxxxxx xxxxxx		xXX-X	XX-XX-XX-X	xXX4
	XXX019X11	XXXX-xxxx xxxxxxx				xXX-X	XX-XX-XX-X	xXX5
	XXX031X01	XXXX xxxxxxxxxxxx xxxxxx xXXX62	XX5X24800	Xxxxx xxxxxxx xxxxxx X2 xxxxxxx 2	XXX2X2/ XXXX9	xXX-X	XX-XX-XX-X	xXX5
	XXX049X04	XXXX xxxxxxxxxxxx xxxxxx xXXX68				xXX-X	XX-XX-XX-X	xXX6
	XXX070X03	XXXX xxxxxxxxxxxx xxxxxx xXXX41	XX3X62420	Xxxxx xxxxxx/ xxxxxxx xxxxxx xxxxx 53	XXXX53	xXX-X	XX-XX-XX-X	xXX6
	XXX073X03	Xxxx xxxxxx xxxxxx XXX1	XX4X20380	Xxxxxx xxxxxxxxx xxxxxx	XXX1			XxXX4
X2X2-XX-xxxx Xxxxxx	XXX050X11	X-XXX XXXXXX XXXXXXX	XX4X27310	xxxx xxxxxx (X-xxx xxxx) xxxxxx xxxxxx	XXX28	xXX-X	XX-XX-XX-X	xXX5
	XXX075X03	xxx1-1X xxxxxxxxx	XX1X75540	Xxxx xxxxxxxx xxxxxx2	XXX2/ XXX21			XxXX4
X2X2 Xxxxxx	XXX013X11	Xxxx xxxxxx-xxxx xxxxxxx	XX5X10970	Xxxx xxxxx (X2X2 xxxx) xxxxxx xxxxxx				XxXX4
	XXX046X02	Xxxx xxxxxx xxxxxx 4	XX1X66140	Xxxx xxxxxx xxxxxx 4	XXX4			XxXX4
	XXX053X05	Xxxx-xxxxxx xxxxxx 1	XX3X19580	Xxxx-xxxxxx xxxxxx 2	XX2			XxXX4
XXXXX xxxxxx	XXX005X05	Xxxxxx xxxxxxx-xxxxxxxxxx xxxxxx XX1	XX5X64220	Xxxxxxxxx-xxxxxx xxxxxxxxxxx xxxxxxxx 3	XXXXX3	xXX-X	XX-XX-XX-X	xXX4
XXXXX Xxxxxx	XXX006X07	Xxxxxxxx xxxxxx	XX5X23090	Xxxxxx xxxxx X, xxxxxx X13	XX-XX13			XxXX4
X2X-XX Xxxxxx	XXX070X05	Xxxxxxxxxxx xxxxxx XX1	XX5X03415	XXX-1 xxxxxxxxxxx xxxxxx	XXX			XxXX4
XxXX Xxxxxx	XXX013X11	Xxxxxxxxxx xxxxxx	XX1X61730	XXX-xxxxxx xxxxxxxxxx xxxxxx-				XxXX4

				xxxxxxx				
XXX-xxxxxx	XX1009X10	14-3-3-xxxx xxxxxxx X	XX2X42590	14-3-3 xxxxxxx XX14 xxxx	XXX9			XxXX4
	XXX044X02	14-3-3 xxxxxxx 3	XX1X78300	14-3-3 xxxxxxx XX14 xxxxx	XXX2			XxXX4
	XXX057X06	14-3-3-xxxx xxxxxxx XX14 xxxxx	XX5X65430	14-3-3 xxxxxxx XX14 xxxxx	XXX8			XxXX4
	XXX058X04	Xx14-3-3x xxxxxxx	XX5X65430	14-3-3 xxxxxxx XX14 xxxxx)	XXX8			XxXX4
XX-XXX xxxxxx	XX1002X12	Xxxxxxxxxx-xxxxxxx	XX3X61890	xxxxxxx-xxxxxxx xxxxxx xxxxxxx 12	XX-12;	xXX-X	XX-XX-XX-X	xXX5
	XX1004X11	XXXxx X xxxxxxx						XxXX4
	XXX047X02	Xxxxxxx-xxxxxxx xxxxxx xxxxxxx XXX22	XX4X37790	Xxxxxxx-xxxxxxx xxxxxx xxxxxxx 22	XXX22			XxXX4
XXX-xxxxxx	XXX079X09	XXX-X xxx XXX-X, XXX-xxxxxxx	XX3X57930	Xxxxxxxx xxxxxxx		xXX-X	XX-XX-XX-X	xXX5
XXX-xxxxxx	XX1002X06	Xxxx xxxxx xxxxxx	XX4X36990	Xxxx xxxxx xxxxxx xxxxxxx 4	XXXX1/ XXX4			XxXX4
	XXX001X09	Xxxx xxxxx xxxxxx	XX4X36990	Xxxx xxxxx xxxxxx xxxxxxx 4	XXXX1/ XXX4			XxXX4
	XXX054X07	Xxxx xxxxx xxxxxx	XX4X36990	Xxxx xxxxx xxxxxx xxxxxxx 4	XXXX1/ XXX4			XxXX4
	XXX045X12	Xxxx xxxxx xxxxxxxxxxxx xxxxxx 34						XxXX4
	XXX055X05	Xxxxxxxxx xx xxxx xxxxx xxxxxxxxxxxx xxxxxx	XX3X24520	xxxx xxxxx xxxxxxxxxxxx xxxxxx X1	XXXX1	xXX-X	XX-XX-XX-X	xXX5
	XXX077X06	Xxxx xxxxx xxxxxxxxxxxx xxxxxx	XX4X18880	xxxx xxxxx xxxxxxxxxxxx xxxxxx 21	XXXX4X	xXX-X	XX-XX-XX-X	xXX4
XXXX-xxx xxxxxx	XX1009X08	XXXX xxx xxxxxxxxxxxx xxxxxx	XX4X24540	Xxxxxx-xxxx 24	XXX24			XxXX4
	XXX058X02	XXXX xxx xxxxxxxxxxxx xxxxxx	XX4X24540	Xxxxxx-xxxx 24	XXX24			XxXX4
	XXX004X05	XXXX xxx xxxxxxxxxxxx xxxxxx	XX2X22540	Xxxxx xxxxxxxxx xxxxx xxxxxx	XXX22/ XXX			XxXX4
XXX-xxxxxx	XXX003X06	XXX xxxxxxxxxxxx xxxxxx XXX93	XX5X47390	XXX xxxxxxxxx xxxxxxxxx-xxxxxxx	XXX1/ XXXX			XxXX4
	XXX023X04	XXX-xxxx XXX-xxxxxx xxxxxx						XxXX4
	XXX045X07	XXXXXX XXXXXXXXXXX1	XX5X58340	xxx-xxxx XXX xxxxxxxxxxxxx xxxxxxxx				XxXX4
	XXX055X11	Xxxxxxx xxxxxxxxx xxxxxx xxxxxx xxxxxx	XX5X67300	Xxx xxxxxx xxxxxx X1	XXXX1/ XXX44	xXX-X	XX-XX-XX-X	xXX6
XXX-xxxxxx	XX1001X06	XXX-xxxx xxxxxx	XX3X15510	Xxx xxxxxx xxxxxxxxx xxxxxx 2	XXX2/ XXXX056			XxXX4
	XXX049X12	XXX xxxxxx xxxxxx XXX1						XxXX4
	XXX054X06	Xx xxxxxx xxxxxxx (XXX) xxxxxx-xxxx	XX4X28500	Xxxxxxxx xxxx-xxxxxxxxx xxx xxxxxx xxxxxx 2	XXX073/ XXX2			XxXX4
	XXX073X10	XXX-xxxx xxxxxx	XX5X13180	XXX-Xxxxxxxxxx 2	XXX083/ XXX2			XxXX4
XXX xxxxxx	XXX072X01	XXX1-xxxx xxxxxx	XX5X45110	XXX1-xxxx xxxxxx 3	XXX3			XxXX4
XXX-xxxxxx	XXX040X12	XXX xxxxxx xxxxxxxxxxx xxxxxx	XX3X14980	xxxxxxxx xx xxxxxxxx 4 / xxxxxxxx XXX xxxxxxxxxx 1	XXX4/ XXX1	xXX-X	XX-XX-XX-X	xXX6
	XXX050X08	Xxxxxx xxxx xxxxxx xxxxxx (Xxxxx-1)	XX1X14510	Xxxxx-xxxx 7	XX7	xXX-X	XX-XX-XX-X	xXX5
	XXX068X05	XXX xxxxxx xxxxxx Xx5x26210	XX5X26210	Xxxxx-xxxx 4	XX4			XxXX4

	XXX051X10	XXX3-xxxxxxxxxxx xxxxxxx 2, XXX2	XX5X20910	XXX3-xxxxxxxxxxx xxxxxxx 2	XXX2	xXX-X	XX-XX-XX-X	xXX5
XXXXX xxxxxx	XX1001X01	Xxxx xxxxxx, X-xxx	XX4X17900	XXXXX xxxxxxxxxxxx xxxxxx xxxxxx xxxxxxx;				XxXX4
Xxxxxxxx xxxxxx	XXX044X02	Xxxxxxxx Xxxxxxxx xxxxx xxxxxxx XXX1	XX4X02020	xxxxxxxx xx xxxxx-xxxx xxxxxxx 1	XXX1/ XXX10	xXX-X	XX-XX-XX-X	xXX4
XXX xxxxxxxxxxxx xxxxxxxxx	XXX002X11	Xxxxxxxxxxxx xxxxxxxxxx xxxxxx XXX xxxxx xxxxx						XxXX4
	XXX003X10	xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxx xxxxxx-xxxxxxx	XX5X07900	xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxx xxxxxx xxxxxx xxxxxxx				XxXX4
	XXX018X02	XXX-xxxxxxxx XXX xxxxxxxxxx	XX5X23710	XXX-xxxxxxx XXX xxxxxxxxxx				XxXX4
	XXX041X08	Xxxxxxxxxxx xxxxxxxxx xxxxxx XXX xxxxx xxxxx	XX4X24440	xxxxxxxxxxx xxxxxxxxx xxxxxx XXX xxxxx xxxxx	XXXXX-X	xXX-X	XX-XX-XX-X	xXX6
	XXX043X09	Xxxxxxxx	XX1X59453	X-xxxxx xxxxxxx xxxxxxx xx XXXXXX		xXX-X	XX-XX-XX-X	xXX6
	XXX065X02	XXX1 xxxxxxx	XX1X32130	XXX1 X-xxxxxxxx xxxxxx xxxxxxx	XXX1/ XXX9			XxXX4
	XXX070X08	X-xxxxxxxx xxxxxx xxxxxxxxxx-xxxx 2	XX5X01270	Xxxxxxxx-xxxxxxxx xxxxxx xxxxxxxxxx-xxxx 2	XXX2			XxXX4
	XXX079X10	XXX xxxxxxxxxx XX, Xxx4, xxxx xxxxxxx						XxXX4
X1Xx-xxxx Xxxxxx	XXX023X09	XXX xxxxxxx xxxxxxx X1XX				xXX-X	XX-XX-XX-X	xXX6
XXX xxxxxx	XXX040X07	Xxxx xxxxxx, XXX-xxxx; XXX/ XXX	XX5X63160	XXX xxxX XXX xxxxxx xxxxxxx 1	xx1	xXX-X	XX-XX-XX-X	xXX5
XXX-xxxxxx	XXX060X08	XXX xxxxxx xxxxxxxxxxxx xxxxxx	XX3X27010	Xxxxxxxx xxxxxxxx 1	XXX20	xXX-X	XX-XX-XX-X	xXX5
	XXX074X10	XXX xxxxxx xxxxxxxxxxxx xxxxxx	XX3X27010	Xxxxxxxx xxxxxxxx 1	XXX20			XxXX4
Xxxxxxxxxxxx xxxxxxxxxx	XX1000X05	Xxxxxxxxx-xxxx xxxxxxx				xXX-X	XX-XX-XX-X	xXX5
XXX-xxxxxx	XXX066X05	Xxx xxxxx, xxxxxxxx	XX1X76900	Xxxxx xxxx xxxxxxx 1	XXX1	xXX-X	XX-XX-XX-X	xXX4
XXXX-xxxxxx	XXX059X06	XXXX 13	XX2X37260	Xxxxxxxxxx xxxxx xxxxxx 2	XXX2/ XXXX44			XxXX4
Xx-XXX1 xxxxxx	XXX044X03	Xxx1 xxxx xxxxxxx	XX4X21610	XXX xxx xxxx 2	XXX2			XxXX4
XXX xxxxxx	XX1000X08	Xxxxxxxx xxxxxxxxxxx xxxxx	XX1X68730	Xxx17-xxxx xxxx xxxxxx xxxxxxx		xXX-X	XX-XX-XX-X	xXX6
Xxxxxxxxx xxxxxxxxxx								
XXX xxxxxxxxxxxx	XXX048X10	Xxxxxxxxx xxxxxxxxxxx xxxxxxx	XX1X67080	Xxxxxxxx xxxx xxxxxxxxx 4	XXX4	xXX-X	XX-XX-XX-X	xXX5
	XX1005X08	Xxxxxxxxxx xxxxxxxx, xxxxxxxxxx xxxxxxxx	XX5X67030	xxxxxxxxxx xxxxxxxx	XXX1/ / XXX	xXX-X	XX-XX-XX-X	xXX4
Xxxxx xxxxxxxxx/ Xxx xxxxxxxxxxx xx XXXXX	XXX034X04	Xxxxxx-xxxxxxxxx xxxxxxxxxxx	XX1X48910	XXXXX 10	XXX10	xXX-X	XX-XX-XX-X	xXX4
Xxxxx xxxxxxxxx/ XXX xxxxxxxxx xx XXX	XXX011X04	xxxx-xxxxx xxxxxxxxxxx/ xxxxxxxx X	XX4X05530	Xxxxx-xxxxx xxxxxxxxxxx/ xxxxxxxx x	XXX1/ XXXX	xXX-X	XX-XX-XX-X	xXX6
	XXX053X08	xxxxxxxxxxxx xxxxx-XxX xxxxxxxx 2	XX1X76150	Xxxxx-Xxx xxxxxxxx 2	XXX2			XxXX4
Xxxxx xxxxxxxxx/ Xxxxx xxxxxxxxxxx	XXX060X02	Xxxxxxx/ xxxxx/ xxxxxxxxxx xxxxxx xxxxxxxxx xxxxxx	XX3X10870	Xxxxxx xxxxxxx 17	XXX17	xXX-X	XX-XX-XX-X	xXX6
	XXX074X09	Xxxxxxx xxxxx-xxxxxxxxxxxx xxxxxxxx	XX5X54140	XXX-xxxxxx-xxxxxxxx (xxx1)-xxxx 3	XXX3	xXX-X	XX-XX-XX-X	xXX6
	XXX078X06	Xxxxxxx xxxxx-xxxxxxxxxxxx xxxxxxxx	XX1X51760	XXX-xxxxx xxxx xxxxxxxx 3	XXX3			XxXX4
	XXX058X03	XXX xxxxxxx 1	XX3X61510	XXX xxxxxxx	XXX1			XxXX4

Xxxxxxxx xxxxxxxxxxx	XXX004X06	XXX xxxxxxxx 1	XX3X61510	XXX xxxxxxxx	XXX1	xXX-X	XX-XX-XX-X	xXX4
	XXX046X06	XXX xxxxxxxx	XX1X62960	XXX xxxxxxxx x	XXX10	xXX-X	XX-XX-XX-X	xXX5
Xxxxxxxxx xxxxxxxxxx	XXX006X10	xxxxxxxxx xxxxxxx	XX1X65840	xxxxxxxxx xxxxxxx	XXX4			XxXX4
	XXX014X08	Xxx/ Xxx/ Xxx xxxxxxxxxxxxx, xxxxx xxxxxx				xXX-X	XX-XX-XX-X	xXX5
Xxxxxxxxx xxxxxxxxxx/ Xxxxxxxxx xxxxxxxxxx	XX1002X05	XXX-xxxxxxx:xxxxxxxxx xxxx xxxxxxxxxxxxxxxxxx	XX2X43820	Xxxxxxxxx xxxx xxxxxxxxxxxxxxxxxxx 1	XXX1	xXX-X	XX-XX-XX-X	xXX4
Xxxxxx xxxxxxxxxxx xxxxxxx								
XXX xxxxxxxxx/ Xx xxxxxx xxxxxxxxx	XXX069X09	XX11-X1	XX4X33950	Xxxxxxx xxxxxxxxxxxx 1-xxxxxxx xxxxxx xxxxxx 2-6	XXX1/ XXXX2-6	xXX-X	XX-XX-XX-X	xXX4
XXX xxxxxxxxx/ XXX xxxxxxxxxxxxxx	XXX010X11	Xxxxx-xxxxxxxx xxxxxx xxxxx, xxxxxxx	XX1X78290	Xxx1-xxxxxx xxxxxx xxxxx 2.8	XXXX2.8			XxXX4
XXX xxxxxxxx/ xxxxxxx xxxxxxxx	XXX065X08	Xxx-XXX xxxxxx xxxxxxx	XX5X20270	Xxxxxxxxxx xxxxxxxxxxx xxxxxx1	XXX1			XxXX4
XXX xxxxxxxx/ xxxxxxxxx xxxxxx xxxxxxxxxxxxxxx	XX1009X12	Xxxxxx xxxxxxxxx 2X	XX3X11410	Xxxxxx xxxxxxxxx 2XX	XX2XX/ XXX3			XxXX4
XXX xxxxxxxx/ xxxxx xxxxxxxx	XXX021X09	Xxxxxx xxxxx	XX5X66880	Xxxxxx xxxxxxxxxx 1	XXXX2-3			XxXX4
Xxxxx xxxxxxx/ XXXX xxxxxx	XXX058X12	xxxxx-xxxxxxxx xxxxxx-xxxxxx	XX2X45210	xxxxx-xxxxxxxx xxxxxx-xxxxxx	XXXX36			XxXX4
Xxxxx xxxxxxx/ Xxxxxx xxxxxxxx	XXX037X10	Xxxxxxx xxxxx/ xxxxxxxx xxxxx	XX1X12310	xxxxxxxx		xXX-X	XX-XX-XX-X	xXX5
Xxxxxx xxxxxxx/ Xxxxxx xxxxx	XX1000X04	Xxxxxxxx-6	XX3X43810	xxxxxxxx-7	XXX7	xXX-X	XX-XX-XX-X	xXX5
	XXX040X08	Xxxxxxxx-6	XX3X43810	xxxxxxxx-7	XXX7			XxXX4
Xxxxxx xxxxxxx/ Xxxxxx xxxxx-xxxxxxxx	XXX072X01	Xxxxxx-xxxxxxxx xxxxxx xxxxx, xxxxxx 2	XX1X76040	Xxxxxx-xxxxxxxx xxxxxx xxxxx 29	XXX29			XxXX4
Xxxxxx xxxxxxx/ Xxxxxx xxxxx xxxxxxxxx	XXX014X08	XXX-xxxxxxxxx xxxxxx xxxxx	XX4X30960	XXX3-xxxxxxxxx xxxxxx 3	XXX6/ XXX3/ XXXX3.14	xXX-X	XX-XX-XX-X	xXX5
	XXX020X10	XXX-xxxxxxxxx xxxxxx xxxxx	XX4X30960	XXX3 xxxxxxxxx xxxxxx 3	XXX6/ XXX3/ XXXX3.14	xXX-X	XX-XX-XX-X	xXX5
	XXX017X05	XXX-xxxxxxxxx xxxxx/ xxxxxxxx-xxxxxx xxxxx 11	XX2X30360	XXX3-xxxxxxxxx xxxxxx 4	XXX11/ XXX4/ XXXX3.22			XxXX4
	XXX080X05	Xxxxxx xxxxx; XXX	XX3X17510	XXX-xxxxxxxxx xxxxxx xxxxx 1	XxXX3.16/ XXXX1			XxXX4
Xxxxx xxxxx xxxxxxxxx	XXX016X09	XX4x28880/ X16X16_10	XX4X28860	xxxxx xxxxx X	xxx4			XxXX4
	XXX040X03	Xxxxx xxxxx X	XX4X14340	xxxxx xxxxx X	XXX1	xXX-X	XX-XX-XX-X	xXX5
	XXX052X07	Xxxxx xxxxx XX xxxxx xxxxxx xxxxxxxx	XX2X23070	xxxxx xxxxx XX xxxxx xxxx				XxXX4
Xxxxx xxxxxxxxx xxxxxxxx/ xxxxx xxxxxxxxx xxxxxxx	XXX025X02	Xxxxx xxxxxxxxx-xxxxx xxx xxxxxx				xXX-X	XX-XX-XX-X	xXX4
Xxxxxx xxxxxxx xxxxxxxx xxxxxxx/ Xxxxxx xxxxxxxx xxxxxxx	XXX026X10	XxxX xxxxx-xxxxxxxxx xxxxxxx-xxxx xxxxx 7	XX3X21630	Xxxxxx xxxxxxx xxxxxxx xxxxx 1	XXXX1/ XXX1			XxXX4

Xxxxxxx xxxxxxxx xxxxxxxxx xxxxxxx/ xxxxx xxxxxx xxxxxxxx	XXX060X08	Xxxxxxx XXX2	XX1X33520	Xxxxxxxx xx xxx1, 2	XXX2			XxXX4
Xxxxxxx xxxxxxxx xxxxxxxxx xxxxxxx/ xxxxxxxx xx xxxxx xxxxxxxxx	XXX003X07	Xxxxxxxxx-xxxx xxxxxxx 1 xxxxxxxxx	XX1X20030	xxxxxxxxxxxx-xxxxxxx xxxxxxxxx xxxxxx xxxxxxx		xXX-X	XX-XX-XX-X	xXX6
Xxxxxxx xxxxxxxxx	XXX078X03	Xxxxxxxxxxxxx-xxxxxxx xxxxxxxx xxxxxxx	XX5X62740	Xxxxxxxxxxxxx-xxxxxxx xxxxxxxx xxxxxxx 1	XXX1	xXX-X	XX-XX-XX-X	xXX6
XX xx xxxxxxx xxxxxxxxx	XXX057X06	xxxxxxxxx xxxxxxx	XX5X64510	Xxxxxxxxxx xxxxxxx 1	XXX1			XxXX4
Xxxxxxxx xxxxxxxxx/ Xxxxx X3 xxxxxxxxx xxxxxx	XXX020X10	Xxxxxxxx-xxxxxxxxxxxxx xxxxxxx 1	XX3X51770	Xxxxxxxx xxxxxxxxxxx 1	XXX1			XxXX4
Xxxxxxxx xxxxxxxxx/ xxxxxxxx xxxxxxxx	XXX057X10	Xxxxxxxx xxxxxxxxx xxxxxxx	XX5X03280	xxxxxxxx-xxxxxxxxxx 2	XXX2	xXX-X	XX-XX-XX-X	xXX4
	XXX079X05	Xxxxxxxx xxxxxxxxx xxxxxxx	XX5X03280	xxxxxxxx-xxxxxxxxxx 2	XXX2	xXX-X	XX-XX-XX-X	xXX4
Xxxxxxxx xxxxxxxxx/ xxxxxxxx xxxxxx xxxxxxxxxxx	XX1006X05	XXX-xxxxxxx xxxxxxx	XX3X46060	Xxx XXXxxx xxxxxxx 8x	XXX3/ XXX8X			XxXX4
X-xxxxxxx xxxxxxx xxxxxxxx xxxxxxx	XXX004X12	Xxxxxxxxxxxx xxxxxxxxxxx 3-xxxx xxxxxxx	XX4X29830	Xxxxxxxxxxxx xxxxxxxxxxx 3	XXX3			XxXX4
xxxxxxxx xxxxxx/ X-xxxxxxx xxxxxxx	XXX060X09	xxxxxxxxxxxx xxxxxx,	XX3X05010	Xxxxxxxx X-xxxxxxx xxxxxxx xxxxxxxx 2	XXXX2			XxXX4
	XXX065X10	X-xxxxxxx xxxxx xxxxxxx	XX1X31930	Xxxxx-xxxxx XXX-xxxxxxx xxxxxx 3	XXX3			XxXX4
X-xxxxxxx xxxxxxx xxxxxxxx xxxxxxx xxxxxxxxx xxxxxxx/ X-xxxxxxx xxxxxxx xxxxxxx xxxxxxx	XXX008X11	Xxxxxxxx X xxxxxxx xxxxxxx xxxxxxxx	XX1X48270	X-xxxxxxx-xxxxxxx xxxxxxxx 1	XXX1			XxXX4
Xxxxx xxxxxxxxx	XXX023X10	XXX/ XXX-xxxx	XX1X50280	xxxxxxxxxx-xxxxxxxxxx XXX3 xxxxxx				XxXX4
	XXX077X01	Xxxxxxxxxxxxx xxxxxxxxxx	XX3X19980	Xxxxxxxxxx-xxxxxxxxx xxxxxxx xxxxxxxxxx 3	XXXX3			XxXX4
	XXX004X05	XXXXXXXXXXXXX XX XXX XXX XXXX				xXX-X	XX-XX-XX-X	xXX6
Xxxxxxxxx xxxxxxxx/ xxxx XXXxxx	XXX025X12	Xxx-xxxxxxx xxxxx XXX-xxxxxxx xxxxxxx						XxXX4
xxxxxx xxxxxxxxxxx	XXX038X03	XXX-XXXXXXXXXXX XXXXXX-xxxx xxxxxxx	XX5X67560	XXX-Xxxxxxxxxxx xxxxxx-xxxx X1X	XXXX1X			XxXX4
	XXX068X12	Xxxxxxx XXX-xxxxxxx xxxxxxx						XxXX4
Xxxxxxxxx xxxxxxxx/ xxxxxxxxxxx xxxxxxx	XXX073X10	Xxxxxxx xxxxxx xxxxxxxxx xxxxxxx	XX4X12010	xxxxxxx xxxxxxxxx xxxxxxx (XXX-XXX-XXX xxxxx)				XxXX4
Xxxxxxxxxxxxxx xxxxxx xxxxxxxxxx	XX1000X09	Xxxx X xxxxxxx-1,4,5-xxxxxxxxxxx 5-xxxxxxxxxx 11	XX1X47510	Xxxxxxx xxxxxxxxxxx 5-xxxxxxxxx 11	5XXXX11	xXX-X	XX-XX-XX-X	xXX6
Xxxxxxxxxxxxx xxxxxxx/ XXXX	XX1009X07	Xxxxxxxxx-xxxxxx xxxxxx xxxxx	XX3X45640	Xxxxxx-xxxxxxx xxxxxx xxxxx 3	XXX3	xXX-X	XX-XX-XX-X	xXX6
	XXX074X08	Xxxxxx-xxxxxxx xxxxxx xxxxx	XX5X58350	Xxxx xx xxxxx (x) xxxxx 4 (xxx4)	XXX2/ XXX4	xXX-X	XX-XX-XX-X	xXX5
	XXX076X05	Xxxxxx xxxxx XX5	XX3X53570	Xxx3-xxxxxxxxxxx xxxx 1	XX1			XxXX4
Xxxxxxxxxxxxx xxxxxxx/ XXXXX	XXX007X06	Xxxxxx-xxxxxxx xxxxxx xxxxx xxxxx xxxxx	XX5X66850	Xxxxxx-xxxxxxx xxxxxx xxxxx xxxxx 5	XXXXX5	xXX-X	XX-XX-XX-X	xXX6
	XXX013X04	Xxxxxxxxx xx XXX1-xxxxxx xxxxxx xxxxx	XX5X55090	Xxxxxx-xxxxxxx xxxxxx xxxxx xxxxx 15	XXXXX15			XxXX4
	XXX033X05	xxxxxx xxxxx	XX5X11850	xxxxxx xxxxx xxxxx xxxxxx	Xxx3			XxXX4

	XXX037X08	Xxxxxxx xxxxxxxxx xxxxxxx xxxxxx xxxxxx				xXX-X	XX-XX-XX-X	xXX5
	XXX039X01	xxxxxxxx Xxx/ Xxx xxxxxxx xxxxxx	XX1X16270	xxxxxxx xxxxxx xxxxxx xxxxxxx	Xxx18			XxXX4
	XXX061X01	xxxxxxx xxxxxx, xxxxxxxx	XX1X63700	Xxx xxxxxx xxxxxx xxxxxx 4	XXXXXX4			XxXX4
Xxxxxxxxxxxxxxx xxxxxxxx/ xxxxxxxxx xxxxxx	XXX036X08	Xxxxxx/ xxxxxxxx-xxxxxxx xxxxxx-xxxx	XX3X45240	Xxxxxxxxxx xxx xxxxxxxxxxx xxxxxx 1	XXXX1/ XXXX2			XxXX4
	XXX054X02	XXXX xxxx3	XX2X28060	5'-XXX-xxxxxxxxx xxxxxxx xxxxxx xxxx-2 xxxxxxx xxxxxxx	XXXx30	xXX-X	XX-XX-XX-X	xXX5
Xxxxxxxxxxxxxxx xxxxxxxx/ XX2X	XX1001X03	Xxxxxxx xxxxxxxxxx 2X-xxxx	XX4X03415	2X-xxxx xxxxxxx xxxxxxxxxx 52,	XX2X52			XxXX4
	XX1005X01	Xx10x0541200 xxxxxxx	XX2X33700	Xxxxxxx xxxxxxxxxx 2X X xxxxx 1	XX2XX1			XxXX4
	XXX042X02	Xxxxxxx xxxxxxxxxx 2X-xxxxxxx				xXX-X	XX-XX-XX-X	xXX5
	XXX051X04	Xxxxxxxx xxxxxx xxxxxxxxxx 2X 25 xxxxxxx 1	XX2X30020	xxxxxxx xxxxxxxxxx 2X	XX2X1			XxXX4
	XXX073X12	Xxxxxxx xxxxxxxxxx 2X-xxxx	XX5X27930	xxxxxxx xxxxxxxxxx 2X				XxXX4
	XXX080X09	Xxxxxxx xxxxxxxxxx 2X	XX3X62260	xxxxxxx xxxxxxxxxx 2X				XxXX4
Xxxxxxxx / Xxxxxxxx xxxxxxxxx xxxxxxx	XXX032X07	Xxxxxxxxxx xxxxxxxx xxxxxx-xxxx	XX2X33470	Xxxxxxxxxx xxxxxxxx xxxxxx 1	XXXX1	xXX-X	XX-XX-XX-X	xXX6
Xxxxxxxx/ Xxxx xxxxxxx xxxxxxxx	XXX035X02	Xxxxxxx xxxxxx	XX2X20300	Xxxxxxxx xxxx xxxxx 2	XXX2			XxXX4
	XXX071X01	Xxxxxxxx xxxxxx xxxxxx, xxxxxxxx	XX1X70740	xxxxxxx xxxxxx xxxxxx xxxxxxx				XxXX4
Xxxxxxxx/ XXX xxxxxxxx	XXX014X08	Xxxxxxxxxx xx xxxxxxx-xxxxxxxxx xxxxxxx-xxxx xxxxxxx	XX5X66330	xxxxxxx-xxxx xxxxxx xxxxxx xxxxxxx				XxXX4
	XXX015X08	Xxxxxxx-xxxx xxxxxx xxxxxxxxxx xxxxxx-xxxx xxxxxxx	XX5X61240	xxxxxxx-xxxx xxxxxx xxxxxx xxxxxxx				XxXX4
	XXX018X01	XXX-xxxx xxxxxxx	XX3X49750	Xxxxxxxx xxxx xxxxxxx 44	XXX44			XxXX4
Xxxxxxxx/ Xxxxxxxx xxxxxxxxxxx xx xxxxxxxxx	XXX068X09	Xxxxxxxx xxxxxxxxx-xxxxxxxxx xxxxxx 5	XX2X19580	Xxxxxxxxxx2	XXX2			XxXX4
Xxx xxxxxx xxxxxxxxx	XXX041X08	Xxxxxxxx-xxxxxxx xx91-xxxx	XX5X51060	xxxxxxxxxx xxxxx xxxxxx xxxxxx X	XXXXX			XxXX4
Xxxxxxxxx								
Xxxxx xxxxxxxxx	XX1004X09	Xxxxx xxxxxx xxxxxxx	XX2X17500	XXX-xxxxx 5	XXXX5			XxXX4
	XXX044X01	XXX1-xxxx xxxxx xxxxxxxxx xxxxxxx	XX1X70940	XXX-xxxxxx 3	XXX3	xXX-X	XX-XX-XX-X	xXX4
	XXX070X12	Xxxxxxxxx xxxxxxxxx xxxxxx 11	XX3X28860	X-xxxxxxxxxxx 19	XXX11/ XXX19	xXX-X	XX-XX-XX-X	xXX4
	XXX075X08	Xxxxx xxxxxx xxxxxxx	XX5X01990	XXX-xxxxx 6	XXXX6			XxXX4
Xxxxxxxxx xxxxxxxxx	XXX036X06	Xxxxx xxxx/ xxxxxxxxx xxxxxxxxxx X	XX3X13620	Xxxxxxxxx xxxxxx xxxxxxxxxx 4	XXX4	xXX-X	XX-XX-XX-X	xXX6

eRG: early ripening gene; NeRG:non early ripening gene, D:down; U:up; RS: ripe stop, RP: ripe progress; NC : no change; S: sensitive ; LS :low sensitive

in S fruits. Xxx xxxxx XXX1, XXX17 xxx XXX3 (xXX6, Xxx 19X, Xxxxx 13), xxxxx xxx xxxxxxxxx xxxxxx xxxxxx xxxxxxxx, xxxxxx xxxxxxxxx xxxxxxxxxx xx XXX-X xxxxxx xxxxx xxxxx xx-xxxxxxxxx xx XX. Xxxxxxxxxx xx xxxxxxxxx xxxx xxxxxxxx xxxx xx XXX13, XXX3/XXX2, XXXX36 xxx xxx xxxxxxxxxxx xxxxxxxxx xxxxx xxxxxx xxxxxxxx (XXXX5 xxx XXXX6), xxxxx xx xxx xxxxxx xxxxxx XXX, xxxx xxxxxxxxx xxxxxxxxxx xx XXX-X xxxxxx xxxxx xxxxx xx-xxxxxxxxx xx XX (XxXX4; Xxxxx 13). Xxx xxxxxxxxxx xxxxxxx xx xxx XXXX 6 xx xxxxxxx xxxxxxxxx xx xXX-XXX xx xxxx xxxxx xxx xxxxxxxxxx xxxxx (Xxxxxx X14 xxx X15).

Xxxxxxx xxxxx-xxxxxxx xxxxx xxxxxx xxx xxxxxx xxxxxxxxxx xx xxxxxx xxxxxx XXX xxxxxx xxx xxxxxx xxxx xxxxxxx xxxxxx xxxxxxxx. Xxxx xxxxx xxx xxxxx xxxxxxx xxx xxxxxxxxxxx xxxxxxxxx xxxxxxxx (Xxxxx 12) xxxx xx xxx xxxxxxxxx xx XXX6/XXX, XXX3 (xxxx xx xxxxxxx XxXX2) xxx XXX1 xxx XXX/XXX96 (xxx xx XxXX6), xx xxx xxxxxxxxx xx xxx XXX xxxxxxxxx [440] XXX1 (XxXX2 xxx XxXX7) xxx XX1(xxxxxxx XxXX6), xxxxxxx xx xxxxxx xxxxx xxxxxxxxxxx [526]. Xxx xxxxxxxxxx xx xxxxx xxxxx, xxxxxxx, xxxx xxx xxxxxxx xxxxx xxxxx xxxxxxxxx xxxxxx XX (XxXX2) xx xxxxx xx xxxxx xxx xxxxxxxxxx xxxx xxxxx xxxx xxxxxxx XX (XxXX6 xxx XxXX7). Xx xxxxxxxx, xxx xxxxxxxx xxxxxxxxxx xxxxxxx (xxxx-xxxxxxxxxx xxxxxx XXX) xx xxxxxxxxx xx xxx XXX xxxxxxxxxxxxxx xxxxxxxxx[440] XXX16, XXX27/XXX, XXX17/XXX3 xxxxxxxxxx xx xxxx X xxx XX xxxxxx xxxxxx XX xxx XXX, xxx xxxxxxxxxx xxx xxxxxxx xx X xxxxxx (xXX3 xx Xxxxxx 19 xxx Xxxxx 12). Xxx xxxxxx xxxxxxxxxx xx XXX27/XXX xx XX xxxxxx (Xxxxx 12), xxx xxx xxxxxxxx xxxxxxxxxx xxxx XX xxxxxx xxxx XX xxx XXX xxx xxxxxxx xxxxxxxxx xx xxxxxxxxxx xxxxx xxxx xxx Xxx-XX xxxxxxxxxx (Xxxxx X14 xxx xx xxxxxxxx 1 xxx 2). Xxxxxxxxx, xxxxx xxxx-xxxxxxxxxx xxxxxx XX xxx XXX xxx xx xx xxxx xxxxxxxxxxx xxx xxxx xxxxx xx xxx xxxxxxxx xxxxxxx xxxxxx XX xxx xxxxxxxxxx XXX. As we reported in chapter 2, xxx xxxxxxxx xxxxxxxxxx xxx xxxx xxxx xxxxx xxxxxx xx xxxx xxxx xxx xxxxxxxxxx xxxxxxxxx, xxx xxx xx xxxxxxxxx, xxx xx xxxxxxx xx xxx xxxxxxxxx xxxxxxxxx xx xxx-xxxxxxxxxx xxxxxx. Xx xxxxxxx *Xxxxxx* xxxxxx, xxxx xxxxx xxxxxx xxx xxxxxxxxx xx xxxxxx xxxxxx 2 xxxxxxxx xxxxxxxxxx [245, 518, 520, 521]. Xxxxxxx, xxxxxxxxx xxxxxxxxxx xx xxxxx xx xxxxx-xxxx xxxxxxx xxxx xxxxxxxxx xxx xxxxxxxxxxxx xx xxx xxxxxxxxxx xxxxxx xx xxxxx xxx xxxxxxxx [519], xxxxxxx xxxxxxxx xxxxxxxxxx xxx xxxxxxxxxx xxxxxxxx/xxxxxxxxx [527]. Xxxx, xx xxxxx xxxx xxxxxxx xx xxxxxxxx xxxx xxxxxxxxx xxx xxxxxxxxxxxxx xxxxxx XXX xxxxx XX. That ripening alteration is probably

xxxxxxx xx xxxxxxx xxxxx xxxxxxxxxxx xxxxxxxxxxx xxxx xxxxxxxx xxxxxxxx xxxxxxxxxxx. Xx xxxxxx xxx xxxxxxxx xxxxxxxxxx, xxxx xxxxxxxxxx xxxxxx xxxxxxx xx X xxxxxx xx xxxxxxxx xxx xxxxxx xx xxxxx xxxxxxxx. Xxxxxxxxxxxxx xx XXX1 xxxxxxxxxx xxxxx-xxxxxxx xxxxxxxx xxxxxxxxxxx xx xxxxxx xxxxxxxxx [528]. Xxxxx xxxxxxxx, xxx xxxxxxx xxxxxxx xxxx xxxxxxxx-xxx xxxxx-xxxxxxxxx xxxxxxxx xxxxxx xxx xx xxxx xxxxxxxx xx xxxxxx xxxxxxxxxx XXX, xxxxx xxxxx xxxx xxx xxxxx-xxxxxxxxx xxx xxxxxxxx-xxxxxxxxxx xxxxxxxx. Xx xxxxxxx xxxxxxxx xxx xxx xxxxx xxxxxxx xx xxxxx xxx xxxxxxxx xx xxxxxxx xXX4 (Xxxxx 13) xxxxx xxxxxxx XXX2, xxx xxxxx xxxxxxxxxxx xxxx XXX10, xxx XXX xxxxxxxxxx [529] XXX7 xxx XXX5/XX, xxx xxxxx xxxxxxxx XXX2X [530] xxx xxx xxxxxx xxxxxxxx xxxxx xxxxxx xxxxxxxx (XXX3 xxx XXX19). Xxxxxxxxxx xx xxxxx xxxxx xxxxxxxxxx xxxx xxxx xxxxxxxxxxx xx xxx-xxxxxxxxxxx xxxxxxx (xxxx xxx xxxxxxx 2) xxx xxxxxx xxxx xxx xxxxxxxxxx xx xxxxx-xxxxxxxx xxxxxxxxx xxxxxxxx.

XXX2 xx x xxxxxxxx xxxxxxxxx xx xxxx xxxxxxxx xxxxxxxxx xxx xxxxxxxxxxxx (xxxxxx 2) xxxxxxxx xxxxxxxxxx [303], xxx xx xxxx xxxxxxxx xx xxx xxxxxxxx xxxxxxxx xxxxxxxxxx xx xxxxxxxx xxxxxx X [431]. Xxxxxxxxxx xxxx xxx xxxxxxxx xx xxxxxxxx xxxxxx 2, xxXXX2 xx xxxxxxx xx xxxxxxx-xxxxx xxxxxxx xxxxxx X3/X4 xxxxxxxxxx xxx xxx xxxxxxxxxx xxxxxxx xxxx xxxxxx xxx X4X xxxxx [161]. XXX2 xxxx xxxxxxxxxxxx xx xxxxx xxxxxxxx xx xxxxxxxx xxxxxx xx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xx XXX1-3 xxx xxx xxxxxx xxxxx [23]. Xxxxxxxxxx xxxx xxxx, xxxxxxxxxx xx xxXXX2 xxxxxxxxxx xxxxxxxxx xx XX xxxxxx (xxxxxxxx 1, 2 xxx [246]) Xxxxxxx, xxXXX2 xxxxxxxxxx xx XX xxxxxx xxxxxxx xxxx xxxxxxxxxxxx xxxxxxxx xxxxxxxxxx xxx xxxxxxxxx [246], xxxxx xxxxxx xxxxxx xx xxXXX2 xxxxxxxxxx xxxxxxxxx xxxx xxxxx xxxxxxxx xxxxxxxxx. Xxxx xxxxxxxxx xxxx xxxx xxxxxxxxxx xx XXX2 xxxxxx XX xx xxxxxxx xx xxxxxx X xxxxxxxx xxxxxxxxxx xxx agrees with our results (cluster eRG4, Figure 19, Table 13 and chapters 1 and 2).

Xxxxxxxx xxxxx xx xXX4 xxx xxxx xxxxxx xxxxxxxxx xx X xxxxxx xxxx xx XX xxxxxx xxxxxx XXX, xxxxxxxxxx xxx xxxxxx xxxx X xxxxxx xxxxxx xxxx xxxxxxxx (Xxxxxx 19X), xxxxxxxxxx xx XXX-X xxxxxx xxxxxxxxxxxx xxxxxxxxx xxxxxxxx xx X-X xxxxxx xx xxx xxxx xx XX xxxxxxxxx (Xxxxxx 19X). Xxxx xxxx xxxx xxxx xxxxxxxx XX xxxx XXX xxxxxx X xxxxxx xx xxxxx xx xxxxxxx xxxxxxxx? Xx xxxx xxxxxxx xx xxxxxxx xx xxxxx xxxxxxxxxxx, xxxxxxxxx xxx xxxxxxxxxxx xxxxxxx xx xxxxxxx xXX4? Xx xxxxxxxxx xxxx xxx xx-xxxxxxxxx xx

XxXXX2 xxxx xxxxx-xxxxxxx xxxxx, xxxxxxxxx XxXXX2 xx xxxxxx xxxxxxx xxxxx xxxxxx xxx xxxxxxxxxx xx xxxx xxxxx xxxxxxxxx xxxxx [531], xxx xxxxxxx xxxxxxx XxXXX2 xxx xxxxxxxx xx xxxxx xxxxxxxxxxx xxx Xxx-xxxxxxx [532] xxx xxxxx xxxxxxxxx [533]. Xxxx, xxxx xxxxx xx xxxxxxxx xxxxxxx xxxxxxxxx xxxxxx XX xxx xxxxxxxxxx XXX, xxxxxxxxxx xxxx xxxxxx xxxxxxxx xxxx XX xxxx xxxxxxxxx xxxx xx xxxxxxxxx xxxxx. Xxxx xxx xxxxxxx xxxxxxxx xx xxxxx, xxxxxx xxxxxxxxxxxx xx xxxxxxxx xx xx xxxxxx xxx xxxxx xxxx xxxxxxxxx xx xxxxxxxx [122]. Xx xx xxxxxxxx xxxx xx xxx xxxx xx xxxx xxxxxxxxx, xxxx xxxxxxxx-xxxxxxxxx xxxxxxxx xxxxxx xxxx xxxx xxxxxxx xxxxx xx xxxxxxxx. Xx xxxxx xxxxxxx, xxxx xxxxxxxx xx xxxxxx xx xxxxxxx xxxxxxxx [534]. Xxx xxx xxxxxxxxxx xxxxxxx xxxxxxxx xxxxxx XX xxx XXX xxxxxxxxxx xx xxxxxxx xxxx xxxxxxx xx xxxxxxxx xx xx xxxxx [535]? Xxxxxxx xxxx xx xxxxxx xx xxxxxxxxx xxxxxxx xxxxxxxx xx xxxxx xxxxxxs regulate this response in peach.

In chapters 1 and 2 we reported that probably XXX, x xxxx xx xxx xxxxxxxx xxxxxxxx xxxxxxx xx xxxxx [536] xxx xx xxxxxxxx xx xxx XXX xxxxxxxxx xxx xxxx xx x xxxxxxxxx xxxxxxxxx xxxxxxxxx [134, 246]. Xxxx xx xxxxx xxxxxxx xxxxx xxxxxxx xx XXX xxxxxxxxx, xxxxxxxxx xxx xxxxxxxxxxxx xxxxx xxxxxxxxxxxxxx xxxxxxxxx xxxxx (Xxxxxx 12 xxx 13). Xxxxxx xxxxxx xxxxx xxxxxxxx, XXX xxxxxx xxxx xxxxxx xxx xxxxxxxxxxx xxxxx xxx xxxxxxxxxx xxxxxx xxx xxxxxxxxxx xxx X4 xxxxx [536]. Xxxx, xxx xxxx xxxxxxxx xxx xxxxx xxxxxxxx xx XXXX, xxx xxx xxxxxx xx XXX xxxxxxxxxxxx xx xxxxx [536] xx xxxx-xxxxxxxxx xxxxxx XXX (xXX3, Xxxxxx 19X, Xxxxx 12). Xxxx xxxxxxxx xxx XXX xxxxxx, xxxxxxxxxx xx X xxxxxx, xxxxxx XX xxx XXX. Xxxxxxxxxxx, xxx xxxxx xxxxxxx xx XXX xxxx xxx xx-xxxxxxxxx xxxxxx XX xxx XXX xxx xxx xx xxxxxxx xx xxxx xxxxxxx xxx XXX-xxxxxxxxxxx xxxxxxxx xxx xxxxxxxx xxxxxxxxx. Xxx xxxxxxxx xxxxxxxxxx xxxxxx XX xxxxx xx xxxx xxxxxxx xxxx xxxx xxx xxxxx xxxxxxxx xxxx xx xxxxxxxxxxx, xxxxxxxxxxxxxxx xxx xxxxxxxx xxx xxxx xxxxxxxxxx xxxxx xx XXX xxxxxxxxxxx xxx xxxxxxxxxx, xx suggested by the differential expression of some genes related to their biosynthesis and signaling (Tables 12 and 13)

C3.2.4.The molecular phenotype of wolliness: alterations in cell wall during development of visual symptoms

Modifications in cell wall structure during development of WLT syndrome have traditionally been proposed as key determinants of the loss of fruit juiciness, xxxxx xx

xxx xxxxxxx xx xxxx xxxx xxxx xxxxxxxxxx xx xxxx xxx xxxxxxx xxxxx [537]. Xxxxxxxxxx xxxx xxxxxxxx xxxxxxxx [11, 12, 135], xxx xxxxxxx xxxxxxxx xxxx xxxxxxxx xxxx xxxx xxxxxxxxxx xx xxxxxxxxxx xxxx XXX xxxxxxxxxxx Xxxxxx 17 X xxx X) xxx xxxxx xx xxxx xxxxxxxxx xx xxxxx xxxxxxx xx xxx xxxx xxxx (Xxxxxx 19X, xxxxxxx xXX1). Xxxx xxxx xxxx xxxxxxxxxx xxxxxx xxxxxx xxxxxxx xxxxxxxxxxx xxxx xxxxxxxxx xxxxxx XX xxx xxxxxxxxxx xxxxxx XXX. XX xxxxxx xxxx xxxxxxx xxxxxxx xxxxxxxx xx xxxxxx xx xxxx xxxxxx xxxxx; xxxxxx XXX, xxxxx xxxxxxxxxxxx xx x xxxxxx xxxxxxx xx xxxxxx xxxx xxxxx xxxx xxxxxx xxx xxx xxxxxxx xxxxxxxxx, xxx xxx xxxx xxxx xxx [135]. (Xxxxxxxx xx xx. 2004x). Xxxxx xxxxxxxx xx xxxxxx xxx xxxxxxxx xxxxxxxxxxxx xxx xxxxxxxxxxxxx xxxxxxxxxxx, xxxxxxxxxx xxxxxxxxxx xxxx xxxxxxxxxxx xx xxx xxx-xxxxxxxxxx xxxxx ([17] xxx xxxxxxxx 1 xxx 2) xxxx xx XXX1 (xXX4, xxxxxx xx xx-xxxxxxxxx xxxxxx xxxxxxxx), XXXX1 xxx XXXX1 (xxxx xx xXX6, xxxxxx xx xxxx-xxxxxxxxx xxxxxx xxxxxxxx), xxxx xx-xxxxxxxxx xx XX xx xxxx X xxx XX xxxxxx (Xxxxx 14) xxx xxxxxx xx xxxxx xxxxxx X xxxxxx xx x XX-xxxxxxxxx xxxxxx, xxxx xxxx xxxxxxxxxx xx X xxxxxx xxxxxx xxxx XX xxx XXX. Xxx xxxxxxxxxx xx XXX1 xxx XXX1 xx xxxxxxx xxxxxxxxx xx xXX-XXX xx xxxxx (Xxxxx X14) xxx xxxxxxxxxx xxxxx (Xxxxx X15), xxx xxx xxxxxxxxxx xx xxxxxxxxxx xxxxxxxxxx xxxx XX (Xxxxxx 20).

Xx xxxxxxxx, xxxxx xxxxx XXX xxxxxxxxxx xx xxxxxxx 2 xxxx xxxxxxxxxxx xxxxxx xxxx xxx xxxxxxx xx xxxxxxx xx xxxxxxxx xxxxxxxxx xxx xxxxxxxxxx, xxx xxxxxxx xxxxx xxxxxxx xx xxxxxxxxxxxxx xxxxx xx xxxxxxx 2, xxxxxxxx xx xxxxxx xx xxxx xxxxx xxxxxxxxxx xx X xxxxxx xxxxxx XXX (Xxxxx 14).

Xx xxxx xxxxxxxx xxx xxxx xx xxxxx xxxx xxxxxxxxx XXX xxxxxxxxxx xxxxxxxxxx xxxx xxx xxxxxxxxxxxxx xxxxxxxxx xx X xxx XX xxxxxx xx xxxxxxxx xx XX xxx XXX (Xxxxx 14). Xxx xxxx xx xxxxxxxxxx xxxx xxxx xxx xxxxxxxx xxxxxxxxxx xx xxxxxxxxxxxx XX xxx XXX xxxxxxxxx xxx xxx XXX xxxxxxxxx. Xxxxxxxxxx XXX xxxxx xxxxxxxxx xx xxxxx xxxxxxxx xxxx xx xxx xxxx xxxx (Xxxxxx 21) xxxxxxxxxx xxxx xxx xxxx xxxxxxxxxxx xxxxxxx X xxx XX xxxxxx xxxxxx

Table 14. Cell wall related genes differentially expressed between LS and S pools.

Xxxxxxxx	Xxxxxxxxxx xx	Xxxxxxx xxxxxxxxxx	XXX xxxx	Xxxx xxxx xxxx	Xxxx xxxx xxxxxx	xxxxxxxx xxxxxxx	xxxxxx xx xxxx xx xxxxxxxx xxxxxxx	XXX xxxxxxx
Xxxx xxxxxxxxx xx xxxxxx X								
Xxxx xxxx xxxxxxxxxx xxxxxxxxxx	XXX020X07	Xxxx xxxxxxx xxxx xxxxxxxx xxxxxx	XX4X24220	Xxxx xxxxxxxxxx 1	XXX31/ XXX1			XxXX4
xxxx xxxx xxxxx xxxxxxxxxx	XXX002X11	xxxx xxxxxx (X3XX4-xxxx XXXX xxxxxx) xxxxxx xxxxxxx	XX2X40830	Xxxx-X2 xxxxxx X1X	XXX1X			XxXX4
xxxxxxxxx xxxxxxxxxxx	XXX017X08	xxxxxxxxx xxxxxx xxxxxxx	XX3X26140	Xxxxxxxxx xxxxxx xxxxxxx		xXX-X	XX-XX-XX-X	xXX4
	XXX046X09	Xxxxxxxxx xxxxxxxx-xxxx xxxxxxx XxxX	XX1X55850	Xxxxxxxxx xxxxxxxx xxxx X1	XXXX1	xXX-X	XX-XX-XX-X	xXX6
	XXX054X01	Xxxxxxxxx xxxxxxxx-xxxx xxxxxxx XxxX	XX4X23990	Xxxxxxxxx xxxxxxxx xxxx X3	XXXX3	xXX-X	XX-XX-XX-X	xXX6
Xxxxxxxxxxxx xxxxxx xxxxxxxx	XXX073X10	Xxxxxxxxxxxxxxxxx xxx4				xXX-X	XX-XX-XX-X	xXX4
Xxxxxxxxxxxx xxxxxx xxxxxxxxxxxx xxxxxxxxxx	XXX045X12	Xxxxxxxxxxx-xxxxxx XxxX xxxxxx xxxxxxxxx xxxxxxx	XX2X33580	XXXX-xxxxxxxxx xxxxxxx-xxxx xxxxxx 5	XXX5	xXX-X	XX-XX-XX-X	xXX5
	XXX068X03	Xxxxxxxx Xx	XX3X12500	Xxxxx xxxxxxxxxxx	XXX-X			XxXX4
xxxxxxxxxxxx xxxxxx xxxxxxx xxxxxxxxxx	XX1003X07	xxxxxx xx xxxxxx 4-xxxxxxxxxx	XX3X28480	Xxxxxx xx xxxxxx 4-xxxxxxxxxx		xXX-X	XX-XX-XX-X	xXX5
Xxxxxx xxxxxxxxxx	XXX001X11	xxxxxxx-xxxxxxxx xxxx-xxxxxxxxxxxxxxxxx	XX2X39630	Xxxxxxx-xxxxxxxx xxxx-xxxxxxxxxxxxxxxxx				XxXX4
	XXX038X07	xxxxxx xx xxxxxxx-xxxxxxxx-xxxxxxx xxxxxxx	XX1X20575	Xxxxxxx xxxxxxxx xxxxxx xxxxxxx 1	XXXX1	xXX-X	XX-XX-XX-X	xXX6
Xxxxxx xxxxxxxxxx	XX1003X06	Xxxxxxxx xxxxxxxx, xxxxx 1	XX5X44640	Xxxx xxxxxxxxxx 13	XXXX13	xXX-X	XX-XX-XX-X	xXX5
	XXX005X09	Xxxxxxxxx xx xxxx-1	XX3X23600	Xxxx-1,3;1,4-xxxx-X-xxxxxxxx xxxxxxxx				XxXX4
	XXX055X08	Xxxxx xxxxxxx XXX38	XX3X57270	Xxxx-1,3-xxxxxxxx 1	XX1	xXX-X	XX-XX-XX-X	xXX5
	XXX063X10	Xxxx-1,3-xxxxxxxx	XX4X16260	Xxxxx xxxx-1,3-xxxx-xxxxxxxxx				XxXX4
	XXX070X08	Xxxx-xxxxxxxxxx	XX3X13750	Xxxx-xxxxxxxxxx 1	XXXX1	xXX-X	XX-XX-XX-X	xXX4
xxxxxxxxxxxx xxxxxxxxxxxx	XX1005X08	Xxxxxxxx-xxxx	XX2X20370	Xxxx 3	XXX3/ XXX1			XxXX4
xxxxxxxxxx	XXX003X12	XXXX-X3	XX1X32170	Xxxxxxxxx xxxxxxxxxxxxxxxxx/ xxxxxxxx	XXX30/ XXX4	xXX-X	XX-XX-XX-X	xXX5
xxxxxxxxxxxx xxxxxxxxxxxx xxxxxxxxxx	XX1002X04	Xxxx-X-xxxxxxxxxxxxxxxx / xxxx-X-xxxxxxxx	XX5X49360	Xxxx-xxxxxxxx 1	XXX1	xXX-X	XX-XX-XX-X	xXX4
xxxxx xxxxxxxxx	XXX044X02	XXXXXX-XXXX XXXXXXXXXX XXXXXX	XX2X38080	Xxxxxxx xxxxx 12	XXX12/ XXX4	xXX-X	XX-XX-XX-X	xXX5
xxxxx xxx xxxxxxxxxxxx	XXX035X09	Xxxxxx xxxxxxx xxxxxxxxxx-xxxx 39	XX2X42570	Xxxxxxx xxxxxxxxxx-xxxx 39	XXX39	xXX-X	XX-XX-XX-X	xXX6
xxxxxxxxxxx	XXX070X03	Xxxx xxxxxxxxx xxxxxx xxxxxx-xxxx	XX2X14530	Xxxxxxx xxxxxxxxxx-xxxx 13	XXX13			XxXX4
Xxxxxx xxxxxxxxxx	XXX055X12	Xxxxxxx 3-xxxxx-xxxxx-xxxxxxxxxx xxxxxxxxxxxxxxxxx	XX1X53000	XXX-XXX xxxxxxxxx	XXXX/ XXX	xXX-X	XX-XX-XX-X	xXX5

Xxxxxx xxxxxxxxxxx	XX1000X09	Xxxxxxxx xxxxxxxx-xxxxxxx xxxxxxx	XX5X62350	Xxxxxxxxx/xxxxxx xxxxxxxxxxxxxx xxxxxxxxx xxxxxx xxxxxxx				XxXX4
	XX1000X04	Xxxxxxxxx xxxxxxxxx xxxxxxxxx	XX1X47960	Xxxx xxxx / xxxxxxxx xxxxxxxxx xx xxxxxxxxxxxx 1	X/XXX1	xXX-X	XX-XX-XX-X	xXX6
	XX1004X01	Xxxxxxxx xxxxxxxxxxxxx	XX2X26440	Xxxxx xxxxxxxxxxxxx 12	XXX12			XxXX4
	XX1005X11	Xxxxxxxxxxxxxxx xxxxxxxxx	XX3X59850	Xxxxxxxxxxxxxxx (xxxxxxxx) xxxxxx		xXX-X	XX-XX-XX-X	xXX4
	XXX001X10	Xxxxxxxxxxxxxxx-xxxx xxxxxxx	XX1X23460	Xxxxxxxxxxxxxxx (xxxxxxxx) xxxxxx				XxXX4
	XXX001X09	Xxxxxxxxxxxxxxx-xxxx xxxxxxx	XX3X57790	Xxxxxxxxxxxxxxx (xxxxxxxx) xxxxxx				XxXX4
	XXX041X11	Xxxxxxxxxxxxxxx-xxxxxxxxx xxxxxx	XX5X06860	Xxxxxxxxxxxxxxx xxxxxxxxx xxxxxx 1	XXXX1	xXX-X	XX-XX-XX-X	xXX6
	XXX043X06	XX1.2-xxxx	XX1X62770	Xxxxxxxx/xxxxxx xxxxxxxxxxxxx xxxxxxxxx xxxxxx xxxxxx		xXX-X	XX-XX-XX-X	xXX5
	XXX047X10	Xxxxxxxxxxxxxxx-xxxx xxxxxx	XX4X23500	Xxxxxxxxxxxxxxx (xxxxxxxx) xxxxxx		xXX-X	XX-XX-XX-X	xXX6
	XXX057X09	Xxxxxxxxxxxxxxx-xxxx xxxxxx	XX1X19170	Xxxxxxxxxxxxxxx (xxxxxxxx) xxxxxx		xXX-X	XX-XX-XX-X	xXX5
	XXX066X05	Xxxxxxxx-xxxxxx xxxxxx-xxxx	XX5X51520	Xxxxxxxx/xxxxxx xxxxxxxxxxxxx xxxxxxxxx xxxxxx xxxxxx		xXX-X	XX-XX-XX-X	xXX4
	XXX073X09	Xxxxxxxxxxxxx-3 xxxxxxxxx	XX1X53840	Xxxxxx xxxxxxxxxxxx 1	XXX1			XxXX4
xX xxxxxxxx xxxxxxx	XX1009X09	Xxxxxxxx-xxxx xxxxxx	XX3X45970	Xxxxxxx-xxxx X1	XXXX1			XxXX4
xxxxxxxxxxxx xxxxxxxxx	XXX006X06	xxxxxx xx Xxxxxxx xxxxxxx						XxXX4
XXX-xxxxxx, XXX-xxxxxxxx xxx XXX-xxxxxxxxxx Xxxxxxxxxx	XXX064X12	XXX-xxxxxx 4-xxxxxxxx	XX4X10960	XXX-x-xxxxxx/XXX-x-xxxxxxxx 4-xxxxxxxx 5	XXX5	xXX-X	XX-XX-XX-X	xXX4
XXX-X-xxxxxxxx, XXX-xxxxxxxxxxx xxx XXX-xxxxxx Xxxxxxxxxx	XXX062X06	XXX-xxxxxxxx 4-xxxxxxxx 1	XX1X30620	XXX-x-xxxxx 4-xxxxxxxx 1	XXX1/XXX4	xXX-X	XX-XX-XX-X	xXX4
xxxxxx xxxxxxxxxx xxxxxx	XXX041X12	Xxxxxxx xxxxxxxxx xxxxxxx	XX2X26590	Xxxxxxxxx xxxxxxx xxx-XXXxxx 13	XXX13	xXX-X	XX-XX-XX-X	xXX6
Xxxx xxxxxxxx xx xxxxx XX								
xxxxxx xxxxxxxxxx	XXX041X06	Xxxxxxx xxxxxx xxxxxxx 1 xxxxxxxx xxxxxx	XX4X04970	Xxxxx xxxxxxx xxxx-1	XXX1	xXX-X	XX-XX-XX-X	xXX1
	XXX057X07	XXX xxxxxxxxxxx/XXX xxxxxxxx	XX1X05570	Xxxxxx xxxxxxx 1	XXXX1/ XXX6	xXX-X	XX-XX-XX-X	xXX1
xxxxxxxx xxxxxxxxxx	XXX022X07	Xxxxxxxxx xxxxxxxxxx xxxxxxxxxx	XX3X08900	Xxxxxxxxx xxxxxxxxxx xxxxxxxxx 3	XXX3	xXX-X	XX-XX-XX-X	xXX1
	XXX025X11	Xxxxxxxx xxxxxxx-xxxx xxxxxx XxxX	XX1X55850	Xxxxxxxx xxxxxxx xxxx X1	XXXX1	xXX-X	XX-XX-XX-X	xXX1
	XXX027X10	Xxxxxxxx xxxxxxx-xxxx xxxxxx XxxX	XX1X55850	Xxxxxxxx xxxxx xxx xxxx X1	XXXX1	xXX-X	XX-XX-XX-X	xXX1
	XXX066X06	Xxxxxxxx xxxxxxx	XX5X64740	Xxxxxxxx xxxxxxx 6	XXXX6	xXX-X	XX-XX-XX-X	xXX1
Xxxxxxxxxxx xxxxx xxxxxxxxxxx xxxxxxxxxx	XXX027X09	Xxxxxxxx-xxxx xxxxxx	XX1X05850	;Xxxxxx xxxxxxxxx xx xxxxx xx xxxx	XXX1/ XXX1			XxXX6
Xxxxx xxxxxxxxxx	XX1004X08	xxxx-2/X-xxxxxxxx xxxxx xxxxx	XX3X15350	Xxxx-2/X-xxxxxxxx xxxxx xxxxx xxxxxx		xXX-X	XX-XX-XX-X	xXX1
	XX1004X11	XXX-xxxxxx xxxxxxxxxxxxxx	XX2X39770	XXX-xxxxxx xxxxxxxxxxxxx 1	XXX/ XXX1			XxXX6
	XXX027X10	X-xxxxxxxxxxxxxxxxxxxxxxxxx X	XX4X38240	X-xxxxxxxxxxxxxxxxxxxxxxxxx x xxxxxx xxxxx xxxx	XXX/ XX1			XxXX6
Xxxxxx xxxxxxxxxx	XXX003X04	Xxxxxxxxx xx xxxx-1	XX3X23600	Xxxx-1,3;1,4-xxxx-X-xxxxxxxx xxxxxxxx				XxXX6
	XXX015X01	Xxxxxxxxxx_xxxxxxxx	XX3X23600	Xxxx-1,3;1,4-xxxx-X-xxxxxxxx xxxxxxxx				XxXX7

	XXX030X08	Xxxx-xxxxxxxxxx	XX2X25630	Xxxx xxxxxxxxxx 14	XXXX14			XxXX6
	XXX045X01	Xxxx-xxxxxxxxxxx				xXX-X	XX-XX-XX-X	xXX1
	XXX048X04	Xxxxx xxxxxxxxx xxxx xxxxxxxxxx	XX5X36890	Xxxx xxxxxxxxxx 42	XXXX42			XxXX6
	XXX070X01	Xxxxx-xxxxxxxxxx X	XX1X67490	Xxxxx-xxxxxxxxxx 1	XXX1			XxXX6
xxxxxxxxxxxxx xxxxxxxxxxxxx xxxxxxxxxx	XX1004X12	Xxxxxxxxx xxxxxxxxxxxxxxxxxxx XXXXXXX1 xxxxxxx	XX4X13990	Xxxxxxxx xxxxxx xxxxxx		xXX-X	XX-XX-XX-X	xXX1
	XXX002X04	Xxxxxxx xxxxxxxxxxxxxxxxx 5	XX1X74380	Xxxxxxxxx xxxxxxxxxxxxxxxx 5	XXX5			XxXX6
	XXX055X10	Xxxxx-1,6-xxxxxxxxxxxxxxxx	XX4X02500	XXX-xxxxxxxxxxxxxxx 2	XXX2			XxXX2
xxxxxxxxxxxxx xxxxxxxxxxxxx xxxxxxxxxx	XX1003X05	Xxxxx xxxx-1,4-xxxx-xxxxxxxxxx	XX5X66460	(1-4)-xxxx-xxxxxx xxxxxxxxxxx	XXX7	xXX-X	XX-XX-XX-X	xXX3
Xxxxxx xxxxxxxxxx	XX1006X10	Xxxxxxx xxxxx xxxxxxxxxxxxxx	XX1X78240	Xxxxxxxx2	XXX2			XxXX6
	XXX015X02	Xxx9/Xx-9 xxxxxx xxxxxxx xxxxxx 231	XX1X19300	Xxxxxxxxxxxxxxxxxxxxxx-xxxx 1	XXXX1			XxXX7
	XXX022X06	Xxxxxxx xxxxxxxx	XX5X04500	Xxxxxxxxx xxxxxx xxxx xxxx 1	XXX-X2			XxXX6
	XXX032X06	xxxxxxxxxxxxxxxxxxxxxx 13 xxxx	XX3X58790	Xxxxxxxxxxxxxxxxxxxxx 15	XXXX15	xXX-X	XX-XX-XX-X	xXX1
	XXX046X05	xxxxxxxxxxxxxxxxxxxxxx xxxx	XX3X02350	Xxxxxxxxxxxxxxxxxxxxx 9	XXXX9			XxXX6
Xxxxxx xxxxxxxxxx	XX1004X07	xxxxx-xxxxxx xxxxxx xxxx X xxxxxx xxxxxx	XX1X76160	Xxx5 xxxxxx 5	XXX5	xXX-X	XX-XX-XX-X	xXX1
	XXX011X10	Xxxxxx xxxxx-xxxx xxxxxx	XX3X55140	Xxxxxx xxxxx xxxxxx xxxxxx				XxXX2
	XXX053X02	Xxxxxx xxxxx-xxxx xxxxxx	XX3X55140	Xxxxxx xxxxx xxxxxx xxxxxx		xXX-X	XX-XX-XX-X	xXX1
	XXX063X02	Xxxxxxxxxxxx XXX8X xxxxxxxxx	XX3X43270	Xxxxxx xxxxxxxxxxxx 32	XXX32	xXX-X	XX-XX-XX-X	xXX1
xX xxxxxxxx xxxxxxx xxxxxxxxxxx xxxxxxxxx	XX1002X08	Xxxxxxx	XX2X40610	Xxxxxxx X8	XXX8			XxXX6
xxxxxxxxx xxx xxxxx xxxxxxxxxxx xxxxxxxxx	XXX062X02	Xxxxxxxx xxxxx xxxxxxx xxxxxxx xxxxx	XX2X28370	XXXX -xxxx xxxxxx 5X2	XXXX5X2			XxXX2
XXX-xxxxxx, XXX-xxxxxxxx xxx XXX-xxxxxxxxxxx Xxxxxxxxxx	XXX047X06	XXX-xxxxxx 4-xxxxxxxx	XX1X64440	XXX-xxxxxx 4-xxxxxxxx	XXX1/XXX4			XxXX2
XXX-X-xxxxxxxx, XXX-xxxxxxxxxx xxx XXX-xxxxxx Xxxxxxxxxx	XX1006X04	XXX-xxxxxxxxx xxxx xxxxxxxxxx 3	XX2X28760	XXX xxxxxxx 6	XXX6	xXX-X	XX-XX-XX-X	xXX1
	XXX071X02	XXX-X-xxxxxxxxxx xxxxxx-xxxx	XX2X28760	XXX xxxxxxx 6	XXX6			XxXX6
Xxxxxx XXX xxxxxx	XXX055X09	Xxx-1 xxxxxx	XX4X08950	Xxxxxxx	XXX			XxXX7
Xxxxxx xxxxxxxxxxx xxxxx xxxxxx	XX1001X01	xxxxxx xxxx xxx xxxxxx xxxx XXX642	XX3X08030	Xxxxxx xxxx xxx xxxxxx xxxx XXX642		xXX-X	XX-XX-XX-X	xXX3
	XXX076X01	xxxxxx xxxx xxx xxxxxx xxxx XXX643	XX3X08030	Xxxxxx xxxx xxx xxxxxx xxxx XXX643		xXX-X	XX-XX-XX-X	xXX1
	XXX076X06	xxxxxx xxxx xxx xxxxxx xxxx XXX644	XX3X08030	Xxxxxx xxxx xxx xxxxxx xxxx XXX644		xXX-X	XX-XX-XX-X	xXX1
	XXX017X02	xxxx xxxx xxxxx-xxxxxxxxx xxxxxx	XX5X47530	Xxxx xxxx xxxxx-xxxxxxxxx xxxxxx		xXX-X	XX-XX-XX-X	xXX1
xxxxxx-xxxxxxx xxxxxxxxxxx	XXX044X06	Xxxxxx xxxxxxxx	XX5X57655	Xxxxxx xxxxxxxx xxxxxx xxxxxx		xXX-X	XX-XX-XX-X	xXX1
	XXX071X10	Xxxxxx xxxxxxxx	XX5X57655	Xxxxxx xxxxxxxx xxxxxx xxxxxx		xXX-X	XX-XX-XX-X	xXX1

eRG: early ripening gene; NeRG:non early ripening gene, D:down; U:up; RS: ripe stop, RP: ripe progress; NC : no change; S: sensitive ; LS :low sensitive

SLR are due xx xxxxxx xxxxxxxxxx xxxxx, xxxx X xxxxx xxxxxxxx xxx xxxxxx xxxxxxxxx xxxxx xxxxxxx xx xxxxxx xxxxxxxxxx, xxxxx XX xxxxxx xxx xxxxxxxx xx xxxxx xxxxxxx xx xxxxxx xxxxxxxxxxx. Xx xxxxxxxx, X xxxxxx xxxx xxxx xxxxx xxxxxxx xx xxxxxxxxxxxxx xxxxxx, xxxxx XX xxxxxx xxxx xxxx xxxxx xxxxxxx xx xxxxxxxxxxxx xx xxxx xxxx xxxxxxxxxxxxxxx xxx xxx xxxxxxxxxx.

Figure 21. Classification of genes related to the cell wall according to their putative function.

Among xxxxxx xxxxxxxxxxx-xxxxxxx xxxxx xxxxxx xxxxxxxxx xx X xxxxxx xxxxxx XXX (Xxxxx 6) xxxx xxxxx xxxx xx XXX39 (xXX6), XXX13 (XxXX4), XXX/XXX1 (xXX6), XXX1 xxx XXX12 (xxxx xx XxXX4), xxxxxxx xxxxxxxxxx xxx xxxxxxx xxxxxx (xXX4, xXX5, xXX6 xxx XxXX4) xxx xxx xxxxxxxx xx XXXX1 (xXX6). XXX xxxxxxxx xxx xxxxxxxx xx X-xxxxxxxxxxx xx xxxxxxxxxxxxxxx xxxx xx xxxxxxx xxx xxxxxxxxxx [538]. X-xxxxxxxxxxx xxx xxxxxxx xxx xxxxxxxxxx xxxxxxxxx xx xxxxxxx xxx xxxxxxx xxxxxx xxxxxxxxx xxxxxxxxx xx xxx xxxxxxxxxxxxxx xx XX [539]. Xx xxxxxxx xxxxxxxxx xxxxxxxxxx xx XX xxxxxxxxxx, XXXX xxxxxxx xxxxxxxx xxxxxxx xxxxxxxxxxx xx x xxxxxxxxxxxxxxxx xxxxxx xxxxxxxxxxxx xx xxxx x-xxxxxxxxxx xxx xxx xxxxxxxxxx xxxxxx [540]. Xxxxxx xxxxxxxxxx xxxxxxx xxxx xxxxxxxx xxxx xxxx-xx-xxxx xxxx xxxxx [541] xxxxx X-xxxxxxxxxx correlated negatively

with xxxx xxxxxxxx [542] Xx xxxxxxxx xx XX xxxxxxxxxx, xxx xxxxxxxxxxx xxxxxxx XXx xxx XXXXx xxxxxxxx xxx xxxxxxxxx xx xxxxxxxxxxxxxxxxxxx (XXx), xxx xxxxxxx xxxxxxxx xxxxxxxxx xxx xxx xxxx x xxxx xxxxxx xxxxx xxxxxxxxxxx [543].

Xxxxxx xxxxxxxxx xxxxxx xxxxxxxxxxx-xxxxxxx xxxxx xx XX xxxxxx (Xxxxx 14) xxxxxxx xxxxx xx xxx XXXX xxxxxx (XXXX9, XXXX15 xxx XXXX1, xxxxxxxxx xx xxxxxxxx xXX1, XxXX6 xxx XxXX7) xxx XXX2 (xx xxxxxxx XxXX6). Xx xxxxxxxx xx XXX1 (xx xxx XXXX xxxxxx, [544]) xx xxxxx xxx xxxxx xxxxx xxxxxxxxxx xx xxxxxxxxxx xxxxxxxxxx xxxx XX xx xxx xXX-XXX xxxxxxxx xx xxxxxxxxxx xxxxx (Xxxxxx 20). Xxxxxxxxxx xxxxxxxx xxxxxxxxx xxxx XXXX xxx XXXX-xxxx (XXXX) xxx xxxxxxxx xx xxxx xxxx xxxxxx xxx xxxxxxxxxxxxx xxxxxxxxxxx [545]. Xxxxxxx xx xxxxxxx xxxxxxxxxx xxxxxxx xxxxxx xxxxxxxxxx xx xxxxx xxxxxx [135] xxxx xxxxxx xxxxxx xxxx XXXX4-xxxxxxxx xxxxx xxxxxxxxx xxxxxxxxxxxx xxxxx xxxxxx xxxxxx, xxxx xxxxxxx Xxx, Xxx xxx Xxx [546]. XXXX *Xxxxxxxxxx* xxxxxxx xxx xxxx xxxxx xxxx xxxxxxx xxxxxxxxxxx xxxx xxx xxxx xxxxxxx xxxx xxxxxxx xxxxxxxxxxx [544, 547]. XXX2 xxxxxxx x xxxxxx xxxxxxxxxxxxxxxx (XXX) xxxx xx xx-xxxxxxxxx xxxx XXXX xxxxx [548]. Xxxxxxx xxx xxxxxxxxx xxxxxxxxxxx xxxx x xxxx xxxxxx xx xxxxxxxxxxxxxxxxxxx [549] xxx xxxxxx xxxxxx xxxxxxxxxxx, XXXX xxx XXX xxxxx xx xxxxxxxx xx xxxxxxxxxxx x xxxxx xxxxxxxxxxxxxxx XX, xxxxxxxx xxxxxx xx xxxxxx xxxxxxx [550]. Xxxxxxxxx xx xxxxx xxx xx xxx xxx xxxxxx xxxxxx xxxxxxxxxx xxxx xxx xxxxxx xxxxxxxxxxxxxxxxxxx [551, 552], xxx xxxxx xx xxxxxxxxxx xxxxx xxx xxxxxxxxxx xx XXX xx xxxxxx xxxxxxxxxxxxxxxxxxx xxxxxx XXX xx xxxxx [9]. Xxx xxxxxxx xxxxxxx xxxx xxxxxxx xxxxxxxxx xx xxxxxx xxxxxxxxx xxxxxx xx x xxxxxxxxxx xx xxx xxxxxxxxx xxxxxx XX xxxxxxxxxx xx XXX xxxxxxxxxx. Xxxxxxx XXX xxxxxxxxxx xx xxx XXX xxxxxxxxx, xxxx xxx xxx xxxxxxx xxxxxxxxxxxxxx xxxxxxxxxx xx xxx xxxxxxxxxxxxxxxxxxx xxxxxx xx xxxxxxx xxxxxx XXX, xxxxx xxxxxxxxxx xxxxxxx XXX xxxxxxx xxxx xxxxxx xxxxxxxxx xx xxxx xxxxx xxx xxxxx xxxxxx xxxxx XX [12] .

Xxx xxxx XXX xxxxxxxxx xx xxxxxxx xxx xxxxxxx xxxx xxxxxx (Xxxxx 14), xxxxxxxxx xxxx xxxxxxxxxxx xxx xxxxxxxxxx xx xxxxxx, xxxxxxxxx, xxxxxxxxxxxxx, xxxx xxxx xxxxxxxxxx xxx xxxxxxxxxxxx xxxxxx xxxxxxxxxxx. Xxxxx xxxx xx XXX-X [525, 553], XXX1 [348], XXXX1 [554] (Xxxxx 14) xxx xxxxxxxx xxx xxxx xxxx xxxxxxxxxx xxx xxxxxxxxxx. Xxxxxxxx, xxxxx xxxxxxx xxxxxxx xxxxx xxxxx xxxxxx xxx xxxx xxxx xxx xxx xxxxxx xx xxxxx XXX

enzymes, which in turn affect cell adhesion and biosynthesis of non-pectic polysaccharides and generate a signal to coordinate cell wall WLT phenotype.

C3.2.5.The expanded molecular phenotype of wolliness: Progressive down-regulation of genes related to carbohydrate, aminoacids, cofactor metabolism and organic acid are related to WLT development

Soluble sugars, organic acids and aminoacids xxxxxxxxxx xx xxx xxxxxxx xxxxxxxxxxxx xxxxxxx xx xxxxx xxxxx. Xxx xxxxxxxxx xxxxxxx xx xxxxxxxxxxxxx, xxxxxxx xxxxx xxx xxxxxxxxxx xxxxxx xxxxxx xxxx-xxxxxxx xxxxxxxx xx xxxxxx xxxx xx 20 ° [492, 555] xxx xxxxxx xxxx xxxxxxx xx 0xX xxxx xxxxxxxxxx xxxxxxxx [556] xxx xxxx xxxxxxxxx. Xxxxxx xxxxxx xxxxxxxxxxx xxxxxxxx, xxxxxxxxxxxx xxxxxxxxxx xx xxxxxxx: xxxxxxxxxx xxxxxxxxxxx xx xxxxxxx xx xxxxxxx xxxxxxxx xx xxxx-xxxxxxxxx xxxxx xxxxxxxx, xxxxxxx xxxxxxxxxx xx xxxxxxxxxx xxx xxxxx xxxxx xxx xx xxxx xx xxxxxx xxxx. Xxxxxxx xxxxx xx xxxxxxxxxx xxx xxxx-xxxxxxxxx xxx xxxxxxx xxx xxxxxxxx xxxxxxxxxx xxxxx xxx xxxxxxxxxx xxxxx xxxxxxxxxxx, xxxx xxxxxxx xxx xxxxxxxx xxxxxxxxxxxxx xxxxxxxx (XXXX) xxx xxxxxxxxxx xxxxxxxx xxxxxxxxxx [XXXX]–XXX–XX xxxxxxxxxx xxxxxxxx xxxxxxxx xx xxxx, xxxxxxxxxx xxxxxxxx xxxxxxxxxx xxx xxxxxx xxxxxxxxxx xxx xxx xxxxxxxxxxx xxxxxxx, xxxxx xx xxxxxxxx xxx xxxxxxxx xxxxxxxx xxxxxxxxxx [557, 558].

Xxxxxxxxx xx xxx xxxxxxxxxx xxx xxxxx/xxxxxxxxx xxxxxxxx xx xxxxx xxxxxxx xxx xxx xxxxxxxxx xxxxxxx xxxxxxxx xxxx xxxxxx xxxxxx xxxxxxxxxx xxxxxxxx xxxxxxxx xxxx, xxxxxxx xx xxxxxxxxx xxxxxxxxxx, xxxxx xx xxxx xxxxxxxxx xxxxxxx xxx xxxxxxxxxx xxx xxxxx/xxxxxxxxx. Xxxxxx, xxxxxxxxx, xxxxxxxxx xxx xxx xxxxxxxxxx xxxxxx XxxX xxx xxx xxxxxx xxxxxx xxxxxx xxxxxxxx [492, 555, 556]. Xx xxxxx xxxx xxxxx xxxxxxxx xx xxxxxx, xxxxxxxxx xxx xxxxxxxxx xxxxxxxxx xxxx xx XXXX, XXX, xxx XxxX2 xx xxx xxxxxx xx xxxxxxxxx xxxxx xxxxxx xxx xx xxxxx xxxx XXX (Xxxxx 15). Xx xxxxxxxxx xxx xxxxxxxx xx-xxxxxxxxx [492, 555, 556] xx xxxxxxxxxx xxx XX, xxxxxxxxxx X, xxx XXX xxx xxxxxxx xxxxxxxxx xx xxxxxxxxxx xxx XXX2 xxx XXX-XX. (Xxxxx 15). Xxxx, xxx xxxxxxxxxxxxx xxxxxxx xxxxxxxx xxxx xxx xxxxxx xxxxxx xx xxxxxxx xxxxxx xxxxxxxxxx xxxxxxx.

Tabla 15. Genes associated to the metabolism of amino acid, carbohydrates, organic acids, co-factor and energy production down-regulated in a mealiness/woolliness manner

Xxxxxxxx	xx	Xxxxxxx xxxxxxxxxx	XXX xxxx	Xxxx xxxx xxxxxx	xxxxxx x xxxx +XXX	Xxxxxx XXX xxxxxxx	XXX1-X_xx_X-X	XXX1-X_xx_X-X	XXX2-X_xx_X-X	XXX2-X_xx_X-X	XXX3-X_xx_X-X	XXX3-X_xx_X-X
Xxxxxxxxx xxxxxxxxxx												
Xxxxxxx xxx Xxxxxxxxx xxxxxxxxxx	XXX067X04	xxxxxxxxxxxx xxxxxxxxx xxxxxx/xxxxxxxxxx xxxxxxxxxxxx	XX4X19710	XX-XXXX	xXX1	xXX-X				XXXX		XXXX
Xxxxxxxxxx xxxx xxxxxxxxxx	XXX075X10	Xxxx-xxxxxxxxxxx xxxxxxxx 1	XX3X61440	XXXX1	XxXX6							XXXX
Xxxxxxxxxxxxx xxxxxxxxx	XXX066X01	Xxxxxxxx xxxxxxxxxxxxx xxxxx			XxXX6							XXXX
Xxxxxxx xxxxxxxxx	XX1005X09	xxxxxxx xxxxxxx / X-xxxxxxxxxxx (xxxxx)-xxxxx / X-xxxxxxxxxxx xxxxxxxxxxx	XX4X14880	XXXX1	XxXX6					XXXX		XXXX
	XXX052X05	Xxxxxxx 1-xxxxxxxxxxxxxxx-1-xxxxxxxxxx xxxxxxxx	XX1X48420	XXX1	xXX1	xXX-X				XXXX		XXXX
	XXX074X01	Xxxxxxx xxxxxxxx xxxxxxx xxxxxxx 1	XX3X59760	XXXX	XxXX6							XXXX
Xxxxxxxx xxxxxxxxx	XX1003X04	Xxxxxxx xxxxxxxxxxxxx	XX1X77670		xXX1	xXX-X						
	XXX048X12	xxxxxxxx xxxxxxxxx, xxxxxxxx xxxxxx	XX3X17820	XXX1.3	XxXX6					XXXX		XXXX
	XXX078X06	Xxxxxxxxx xxxxxxxxx, xxxxxxxxx xxxxxxx	XX5X27380	XXX2	XxXX6					XXXX		XXXX
Xxxxxx, xxxxx xxx xxxxxxxxx xxxxxxxxx	XXX001X12	Xxxxxxxx xxxxxxx, xxxxxxxxxx xxxxxxxx	XX4X29840	XXX2	XxXX6					XXXX		XXXX
	XXX055X09	X-3-xxxxxxxxxxxxxx xxxxxxxxxx	XX4X34200	XXX9	xXX1	xXX-X						
Xxxxx xxxxxxxxx	XX1005X01	Xxxxx xxxxxxxxxx xxxxxxxx			XxXX7							
xxxxxxxxx xxxxxxxxx	XXX034X12	1,2-xxxxxxxx-3-xxxx-5-xxxxxxxxxxxxxxx xxxxxxxxx 3	XX4X14710	XXX2	XxXX2							
	XXX065X08	xxxxxxxxxxx xxxx-xxxx, xxxxxxxxx / xxxx-xxxxxxxxxx / xxxxxxx xxxx	XX3X57050	XXX	XxXX6							
xxxxxxxxxx, xxxxxxx xxx	XXX017X03	Xxxxxxxxxxxxxxxx	XX1X12050		XxXX6							
xxxxxxxxx xxxxxxxxx	XXX031X01	X2 xxxxx-xxxxxxxxx xxxxxx	XX5X12970		xXX1	xXX-X		XXXX		XXXX		XXXX
	XXX058X11	Xxxxxxxxxx xxxxxxx xxxx xxxxxx	XX1X25220	XXX1	xXX1	xXX-X						
Xxxxxx xxxxxxxx xxxxxx xxxxxx	XXX019X11	XXX xxxxx-xxxxxxxxx xxxxxx	XX2X39570	XXX9	xXX3	xXX-X						XXXX
	XXX078X06	XXX xxxxx-xxxxxxxxx xxxxxx	XX5X04740	XXX12	XxXX6					XXXX		XXXX
Xxxxxx xxxxxxxxxxxxx xxxxxx	XX1009X12	Xxxxxxxx xxxxxxxx xxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxx	XX5X26600		XxXX2					XXXX		
Xxxxx, xxxxxx xxx xxxxxxxxx xxxxxxxxx	XXX008X08	Xxxxx-xxxx xxxxxxxxxxxxx, xxxxxxxxx xxxxxxxx	XX3X58610		XxXX6						XX	
	XXX019X08	xxxxxxxxxx xxxxxxx xxxx xxxxxx	XX2X31810		XxXX6							
	XXX045X05	Xxxxxxx-xxxx-xxxx-xxxx xxxxxxxxxxxxx 3, xxxxxxxxx xxxxxxxx	XX3X49680	XXXX3	xXX1	xXX-X				XXXX		XXXX
	XXX069X12	xxxxxxx-xxxxx xxxxx xxxx xxxxxxxxxxxxx 5	XX5X65780	XXXX5	xXX1	xXX-X				XXXX		XXXX
Xxxxxxxxxx xxxxxxxxx												
xxxxxx xxxxx	XX1004X12	Xxxxxxxxxxx	XX5X13420		XxXX6					XXXX		XXXX

	XXX030X05	Xxxxxxx xxxxxxx xxxxxxx-xxxxxxx xxxx xxxxxxx-xxxx	XX1X79270	XXX8	XxXX6							
	XXX036X07	Xxxxxxx 6-xxxxxxxxxxxxxxxxxxxxx	XX5X24400	XXX3	xXX1	xXX-X						
	XXX060X08	Xxxxxxx-xxxxxxxx 3-xxxxxxxx, xxxxxxxxxx xxxxxxxx	XX5X61410	XXX	XxXX6					XXXX		
	XXX061X11	Xxxxxxxxxxx	XX1X12230		XxXX6				XXXX	XXXX		XXXX
X-xxxxxx xxxxxxxxx	XXX079X08	Xxxxxxxxxx 3	XX1X66430		XxXX6							XXXX
X-xxxxxx xxxxxxxxx	XXX051X01	Xxxxxxxxxx xxxxxx, XxxX	XX1X49350		XxXX7							
xxxxxxxx xxxxxxxxx	XXX029X03	xxxxxx 1-xxxxxxxx xxxxxx xxxxxx	XX5X57330		XxXX6							XXXX
	XXX065X06	xxxxxx 1-xxxxxxxx xxxxxx xxxxxx	XX5X66530		XxXX2							XXXX
	XXX064X04	xxxxxxxxxxx	XX3X06580	XXXX	XxXX6							XXXX
XXXXXXXXX/ XXXXXX XXXXXXXX XXXXXXX XX-XXXX	XXX008X10	Xxxxxxx xxxxxxxxxx xxxxxxxxx/xxxxxxxx xxxxxxx, xxxxxxxxx xxxxxxxx	XX2X39730	XXX	XxXX6							
Xxxxx xxxxxxxx xxxx xxxxxxxxxxxxx	XXX039X08	Xxxxxxxxxxxxxxx, xxxxxxxxx	XX1X23190	XXX3	xXX1	xXX-X						
Xxx xxxxxxxxx xxxxxxxx xxxxxxxxx xxxxxx	XXX022X07	Xxxxxxxxxx--xxxxxxx 6-xxxxxxxx 1-xxxxxxxxxxxxxxxx xxxxxx xxxx	XX1X12000		XxXX6							
xxxxxxxxx xxxxx xxxxxxxxx	XXX043X08	XXX--xxxxxx-1-xxxxxxxx xxxxxxxxxxxxxxxx	XX5X17310	XXX2	XxXX6					XXXX		XXXX
XXX-XXX XXXXXXXXX XXXXX	XXX009X12	Xxxxxxx xxxxxx	XX1X74030	XXX1	XxXX6							
	XXX017X09	Xxxxxxx xxxxx xxxxxx X, xxxxxxxxx xxxxxxxx	XX3X22960	XXX1	XxXX2							XXXX
	XXX031X11	XXXX-xxxxxxxx xxxxxxxxxxx-3-xxxxxxxx xxxxxxxxxx	XX2X24270	XXX11X3	XxXX6							
	XXX075X04	Xxxxxxx xxxxx, xxxxxxxx xxxxxx	XX5X08570		xXX1	xXX-X						XXXX
	XXX078X05	Xxxxxxxxxxx-3-xxxxxxxx xxxxxxxxxx, xxxxxxxx	XX1X79530	XXXX-1	XxXX6					XXXX	XXXX	XXXX
XXXXXXXXXX XXXXX XX XXXXXXXXX	XXX005X10	Xxxxxxxxxxx xxxxxxx, xxxxxxxxx xxxxxxxx	XX2X21170	XXX	XxXX6							XXXX
	XXX038X03	xxxxxxx-xxxxxxxxx xxxxxxx	XX5X03690	XXX4	xXX1	xXX-X						XX
Xxxxxxxx xxxxx xxxxx xxxxxxxxx	XX1004X10	Xxxxxxx xxxxxxxx xxxxxxx	XX1X56600	XxxX2	XxXX2			XXXX	XXXX	XXXX	XXXX	XXXX
xxxxxx xxx xxxxx xxxxxxxx	XX1001X10	Xxxx-xxxxxx	XX5X18670	XXX9	XxXX2							XXXX
	XXX042X09	Xxxx-xxxxxx	XX5X18670	XXX9	XxXX2					XXXX		XXXX
	XXX035X04	xxxx-xxxxxx	XX4X00490	XXX2	xXX3	xXX-X			XXXX			
	XX1003X01	Xxxxxx xxxxx xxxxxxx	XX4X18240	XX4	xXX1	xXX-X						
	XXX017X12	Xxxxxx-xxxxx xxxxx xxxxxxx 1, xxxxxxxxxx xxxxxxxx	XX1X32900	XXXX1	XxXX6			XXXX	XXXX	XXXX	XXXX	XXXX
	XXX070X09	Xxxxxx xxxxxxx xxxxxxxxx	XX3X52180	XXX4	XxXX2							XXXX
	XXX063X04	xxxxxxxx xxxxxxxxxxx	XX1X01050	XXx1	XxXX7							
	XXX014X06	Xxxxxx xxxxxxx xxxxxxxxxxx	XX1X01050	XXx1	XxXX6							
	XXX002X08	XXX-xxxxxx xxxxxxxxxxxxx xxxx xxxxxx	XX5X48300	XXX1	xXX1	xXX-X						
	XXX031X05	xxxxxxx xxxx-xxxxx xxxxxxxxx	XX3X46970	XXX2	XxXX6					XXXX		XXXX
	XXX019X12	xxxx-xxxxxxxxxxx	XX5X22510	XXX-X	XxXX6							XXXX
	XXX034X06	xxxx-xxxxxxxxxxx	XX5X22510	XXX-X	xXX1	xXX-X						XXXX
	XXX021X05	Xxxxxx xxxxxx 1	XX4X02280	XXX3	XxXX2			XXXX	XXXX	XXXX	XXXX	XXXX

	XXX040X09	Xxxxxxx xxxxxxxxx xxxxxxxx	XX5X20280	XXX1X	xXX1	xXX-X					
Xxxxxxxxx xxxxxxxxxxx	XX1001X07	Xxxxxxxxx 6-xxxxxxxxx xxxxxxxx	XX1X23870	XXX9	xXX1	xXX-X					XXXX
	XXX022X03	Xxxxxxxx xxxxxxxxx xxxxxxxx	XX1X06410	XXX7	XxXX6						XXXX
	XXX037X04	Xxxxxxxx xxxxxxxx-6-xxxxxxxxx xxxxxxxx	XX1X68020	XXX6	XxXX6					XXXX	XXXX
	XXX063X04	Xxxxxxxx xxxxxxxx-6-xxxxxxxxx xxxxxxxx	XX1X68020	XXX6	XxXX6		XXXX	XXXX	XXXX		XXXX
	XXX067X09	Xxxxxxxxx-6-xxxxxxxxx xxxxxxxx xxxx xxxxxxx	XX4X17770	XXX5	xXX1	xXX-X					XXXX
xxxxxxx xxxxxxxxxxx xxxxxxxxx	XXX019X09	Xxxxxxxx xxxxxx xxxxxxxxx-xxxxxxx xxxxxxx	XX2X36360		XxXX6						XXXX
xxxxxxx xxxxxxxx	XXX024X04	XXX-xxxxxxxxx xxxxxxxx/xxxxxxxxxx xxxxxx xxxxxx-xxxx			xXX1	xXX-X					
Xxxxxxx xxxxxxxx xxxxxxxxxx	XXX025X04	xxxxxxxx xxxxxxxxxx xxxxx 1	XX1X75420		xXX1	xXX-X					XXXX
Xxxxxxxx xxx xxxxxxx xxxxxxxxx											
Xxxxxxxxx xxxxxxxxxx	XXX058X10	X-xxxxxxxx-1-xxxxxxxx xxxxxxxxx	XX3X02870	XXX4	XxXX6						
Xxxxxx xxxxxxxxx	XX1002X07	Xxxxxxxxxxxxx xxxxxxxxx 1	XX2X25710	XXX1	xXX1	xXX-X					XXXX
	XXX079X12	Xxxxxx xxxxxxxx xxxxxxx xxxX	XX1X22800		XxXX2						XXXX
Xxxxxxxxx xxxxxxxxx	XX1006X04	Xxxxx (2Xx-2X) xxxxx-xxxxxxxxx xxxxxx	XX3X44880	XXX1/XXX	XxXX6						XXXX
	XXX049X07	Xxxxx (2Xx-2X) xxxxx-xxxxxxxxx xxxxxx	XX3X44880	XXX1/XXX	XxXX6					XXXX	XXXX
Xxxxx xxxxxxxxx	XXX014X12	Xxxxxxxxxxx xxxxxxxxx /xxxxxxxxxxxxxxxxx xxxxxxxxx	XX5X05980	XXXX1	XxXX6						
	XXX032X10	Xxxxxxxxxxxxxxxxxxxxx xxxxxxxx 2	XX2X44160	XXXXX2	XxXX6						XXXX
	XXX078X10	Xxxxxxxxxx xxxxxxxxxx xxxxxxxx-xxxxxxxxx xxxxxxx	XX2X16370	XXX-1	xXX1	xXX-X					XXXX
Xxxxx xxxxxxxxx xxx xxxxxxxx	XXX017X11	Xxxxx-xxxxxxx xxxxxxxx xxxxxxxx	XX1X78680	XXX2	xXX1	xXX-X	XXXX			XXXX	XXXX
xxxx xxxxxx xxxxxxx xxxxxxxxxx	XXX037X06	Xxxxxxx xxxxxxxxx 1, xxxxxxxxxxx xxxxxxxx	XX5X65720	XXXX1	XxXX7						
	XXX052X02	XXX xxxxxxxxx-xxxx xxxxxx	XX5X58270	XXX3	XxXX7						
	XXX068X11	XxxX-xxxx xxxxxx, xxxxxxxxx xxxxxxxx	XX4X26500	XXXX1	XxXX6						XXXX
Xxxxxx xxxxxxxx	XX1003X11	Xxxxxx xxxxxx xxxxx-xxxx xxxxxx	XX3X29010		xXX1	xXX-X					
xxxxxxxxx xxxxxxx xxxxxxxxxx	XXX058X01	Xx-xxxxx xxx xxxxxx xxxxxxxx xxx xxxxxx xxxxxxxxxxx	XX5X55130	XXX5	xXX1	xXX-X					XXXX
	XXX073X08	xxxxxxxx xxxxxxx xxxxxxx xxxxx xxxxxx	XX1X30910		XxXX6						XXXX
Xxxxxxxxxx xxxxxxxx	XXX022X05	X-xxxxxxx xxxxxx xxxxx xxxxxx	XX5X14760	XX	xXX3	xXX-X					XXXX
xxxxxxxxxx xxx XxX xxxxxxxxxx	XXX073X07	Xxxxxxxxxx xxxxx 2	XX4X32180	XXXX2	XxXX6					XXXX	XXXX
xxxxx xxxxx xxxxxx	XXX025X12	Xxxxxxx xxxxx xxxxx 3, xxxxxxxxx xxxxxxxx	XX5X58560	XXXX	XxXX6						
Xxxxx xxxxxxxxxx xx Xx-X xxxxxxx	XXX009X03	XXX xxxxxx 1, xxxxxxxxx xxxxxxxx	XX1X64810	XXX1	XxXX6						
Xxxxxxxxx xxxxxxxxx	XXX075X02	xxxx-xxxxxxxxxxxx-xxxxxxxxxxxxxxxx	XX4X23660	XXX1	XxXX7						XX
Xxxxx xxxxxxxxx											
xxxxxxxxxx xxxxxxx xxxx	XXX004X09	XXXX-xxxxxxxx xxxxxxxxxx X16.6 xxxxxx	XX2X31670	XX-15xXx-2	XxXX7						XX
	XXX008X02	XXXX xxxxxxxxxx [xxxxxxxx] xxxx-xxxxx xxxxxx 6	XX3X18410	XX-XXXX1	XxXX7						XX
	XXX070X05	XXXX-xxxxxxxx xxxxxxxxxx 24 xXx xxxxxx, xxxxxxxxxx xxxxxxx	XX4X02580	XX-24xXx	XxXX6						

	XXX044X06	XXXX-xxxxxxxxxx xxxxxxxxxxxxx 75 xXx xxxxxxx, xxxxxxxxxxxx xxxxxxxxx	XX5X37510	XX76	XxXX6							
	XXX048X09	Xxxxxxxxxx xxxxxxx	XX4X22260	XXXX	XxXX2					XXXX		XXXX
	XXX054X11	Xxxxxxxxxx xxxxxxx	XX4X22260	XXXX	XxXX2				XXXX	XXXX		XXXX
	XXX066X02	Xxxxxxxxxx xxxxxxx, xxxxxxxxxxxx xxxxxxxxx	XX5X64210	XXX2	XxXX6							
	XXX060X01	xxxxxxxxx xxxxxx X	XX1X28140		XxXX6							XXXX
Xxxxxxxxxxxxx xxxxxxxxx	XX1001X04	xxxxxx/xxx xxxxxxxxxxxxx	XX1X23740	XXX	XxXX6							
xxxxxxxxxx xxxxxxx	XXX019X12	XXX xxxxxx xxxx xxxxxxx xx Xxx37	XX1X22700		XxXX6							
Xxxxxx XXXxxx	XXX058X09	XXX-xxxx XXXxxx xxxxx xxxxxx	XX4X02480		XxXX6							XXXX
xxxxxxx xxx xxxxx xxxxxxx XXX	XXX028X06	Xxxxxxx X+-xxxxxxxxxxxxx	XX1X15690	XXX1	XxXX6						XXXX	XXXX
xxxxxxxxx/ xxxxxxxxxx XX xxxxxxxxx	XXX059X12	Xxxxxxxxxxx-xxxxxxxx xxxxxxx xxxxxxx X+ xxxx	XX1X15690	XXX1	xXX1	xXX-X				XXXX		XXXX
	XXX049X11	Xxxxxxx XXX xxxxxxx 16 xXx xxxxxxxxx xxxxxx 4	XX1X19910	XXX-X2	XxXX7							
	XXX060X06	xxxxxxx-xxxx X+-xxxxxxxxxxx xxxxxxxx xxxxxxxxxxxx	XX1X78920	XXX2	XxXX6							XXXX
	XXX061X08	XXX-xxxx XXXxxx xxxxx xxxxxx	XX1X64110	XXX1	xXX1	xXX-X						XXXX
	XXX078X04	Xxxxx xxxxxxx XXXxxx 1	XX3X47950	XXX4	xXX1	xXX-X				XXXX		XXXX
Xxxxxxx xxxx xxxxxxxxx												
Xxxxxx xxxxxxxxx xx xxxxx-XxX	XXX028X08	Xxxxx-XxX xxxxxxxxx	XX5X36880	XXX	XxXX6							
	XXX029X03	Xxxxx-XxX xxxxxxxxx	XX1X55320	XXX18	XxXX2							
	XXX038X10	Xxxxx-XxX xxxxxxxxx	XX1X55320	XXX18	XxXX2							
Xxxxx-XxX xxxxxxxxxx	XXX023X09	xxxxxxxxxxxxxx X-xxxxxxxxxxxxxx	XX1X34430	XXX3003	XxXX7							
xxxxxxxxx xx XXX xx xxxxxxxxx	XX1001X05	Xxxxxxx xxxxxxxxxx 1	XX4X33070	XXX1	xXX1	xXX-X						
Xxxxxx xxxxxx	XXX073X09	Xxxxxxxxxxxxxxxxxx xxxxxxx xxxxxxxxx-xxxx	XX3X10850	XXX2-2	XxXX2							
	XXX075X11	xxxxxxxxxxxxxxx xxxx, xxxxxxx / xxxxxxxxx X	XX1X67280		XxXX6							XXXX
xxxxxxxxxxxxxx xxxxxx	XXX035X04	2-xxx xxxx xxxxxxxxxx, xxxxx-xxxxxx xxxx	XX2X35120		xXX1	xXX-X						XX
XXXX xxxxxxxxx xx xxxxxxxxx xxxxxxxxxxxx	XXX055X10	xxxxxxx xxxxxxxxxxx xxxxxxx xxxxxxxxx xxxxxx	XX4X21210	XX1	XxXX2							XXXX
xxxxxxx xxxxxxxxxx xx xxxxxx	XXX063X07	Xxxxxx xxxxxxxxxx	XX1X77120	XXX1	xXX3	xXX-X		XXXX	XXXX	XXXX	XXXX	XXXX
xxxxxxx-xxxxxx xxxxxxxxxxxxx	XXX008X03	Xxxxxxx xxxxxxxxxx xxxxx 7 xxxxx X1	XX1X54100	XXXX7X4	XxXX2							XXXX
	XXX077X06	Xxxxxxx xxxxxxxxxx xxxxx 7 xxxxx X1	XX1X54100	XXXX7X4	XxXX2							XXXX
Xxxxxxxxxxx xxxx xxxxx	XXX005X04	xxxxxxxx xxxxxxxxxx	XX1X08480	XXX6	XxXX7							
	XXX066X10	Xxxxxxxxxxxxxxx xxxxxxxxx xxxxx xxxxxx	XX2X43180		XxXX7							
	XXX073X08	Xxxxxxxxxxxxxxx xxxxxxxxx xxxxx xxxxxx	XX2X43180		XxXX6							

eRG: early ripening gene; NeRG:non early ripening gene, D:down; U:up

Our results indicate that in general, XX xxxxxxxxx xxx/xx xxxxx xxxxxxxx xxx xxxx xxxxx xxxxxxxx xx xxxxxx xxxxxxxxxx, xxxxx xxxx xxxxxxxxxx xxx xxxxxxx xxxx xxxxxxxxxx. Xxxxx xxxx xxxxxx xxxxxxxx xxxxxxxx xx XX xxxxxx (xxxxxxxx xXX1 xxx XxXX6), xxxxx xx x xxxxxxx xxxxxxxx xxxx XX xxxx xx xxxx xxx xxxxxx xxx xxxxxxxxxx xx xxxxx xxxxxxxx xxx xxxxx xxxx xxx xxxxxx xxxxxxxxxx xxxxxx XXX xx X xxxxxx X (Xxxxxx 17). Xxx xxxxxxxx xx X xxxxxx xxxxxx XX xxx XXX xx 5xX xxxx xx xxxxxxxx xxxxxxxxx xxxx xxxx xxxxxxxx xxx xxxxxx xxxxxx xx 0xX [556]; xxx xxxxxx xx xxxxxxxxxx x xxxxxxxx xxxxxx xxxxxxxx xxxxxxxxxx [9]. Xxxxx xxxxxxxxxx xxx xx xxxxxxxxxx xx xxxxxxxxxx xx xxx xxxxx, xxxxxxxx xxx xxxxxxxxxx xxxxxx xxxx xxxxxxx xxxxxxx xxxxxxxxxx [477, 482, 559], xxx xxxx xx xxx xxxx xx XX, xx xxxxx xxxxxx xxxx xxxxxx xxxx xxx xxx xx xxxx xxxx [556]. Xxx, xxxxx xxx xxxx xxxxxxxxx xxxxxxx xxx xxxxx xxxxxxxx xxxxxxxx xx 0xX [556]xxx xxx xxxx xxxxxxxx xxxxxxx during CS and SLR.

Altered xxxxxxxxxxx xxx xxxxxxxx xxxxxxxxxx xxxxxx XXX xxxxx XX xxx xxxx xxxxxxxxxx xxxxxxxxxx xxxx XXX xxxxxxxxx [16]. Xxxxxxx xxxxxxxx, XXXX-XX xxx xxxxxxxxxxxxxxxxxxx xxxxxxxxxxxx (XXXXX) xxxx xxxx-xxxxxxxxx xx XXX xxxxxx xxxxxxxx xx xxxxxxxxx xxxxx Xxx xxxxxxx xxxxxxxxx xxxx xxxxxxx xxxxx xxxxxxx xxx xxxxxx xxx xxxx xx xxxxx xx xxxxx xxxxxxxxxx xxxxxxxxxx xxx xxx xxxxxx xx xxxxx xxxxxxxxxx, xxxxxxxxx x xxxx xxxxxxxxxxxx xxxx xx xxxxxxxxxx xxxxxx XXX xxxxxxxxxxx (Xxxxx 15).

Xxxxxxxxx xx xxx xxxx xxxxxxxxxx xxxx, xxxxxx XXX xxxxxxxxxxx xx XXX, xxxxx xx x xxxxxxxxx xx xxxxxxx xxx xxxxxxx xxxxxxxxxx xxxxxxxxx xxxx xxxxxxxxxx xxx xxx xxxxxxxxx xx xxxxxxx xxxxxxxxxxx xxxxxxxxx xxx xxxxxxxxxx (xxxxx 15). Xxxxx xxxxxxx xx xxxxx xxxxxxxxxxxx xxxx xxxxxxxxx xxxxxxxx xx xxxxxxxxxx xxxxxx XXX xxxxxxxxxxx. Xxxx xxxx xxx xxxxxxxxxx xxxx xxxxxx xxx xxxxxxxx xxx xxxxxxx levels decline gradually during cold storage, xxxxxxxxxx xx XX-xxxxxxxx xxxxxxxxxxx [477]. Xxxx, xxxxxx xxxxxxxx xxxx XX xxxx xxxx xxxxxxx xxxx xxxxxxxxx xxxxxx xxxxxxx xxx xxx xxxx xxxx [556]. Xxxx xxxxxxxxx xxxx xxx xx xxxxxxxxx xxxx xx xxxxxxxxx xxxxxxxxxxx xx xxxxxxxxx. Xxxxxxxxxxxxxx, xxxxx xxx xxxx x xxxxxxxxxx xxxxxxxxx xx xxxxx xxxxxxx xx xxx xxxxxxxxx xxx xxxxxxxx xxxxxxxxx xxxxxxxx xx x XXX xxxxxxxxx-xxxxxxxxx xxxxxx (xxxxx 15).

Genes encoding functions involved in several xxxxxxxxx xxxxxxxx xxxxxxxxx xxxx xxx xxxxxxx xxxxxxxxxxxx xxxxxxxxxx, xxxx xx xxx xxxxxx xxxxxxx, xxxx xxxxxxxxxxxxx, xxxxxxxx xxxxxxxxxxxx xxxxxxxxx xxxxxxxx xxxxx xxxx xxxxxxxxx (Xxxxxx 17). X xxxxxxx xxxx xxxxxxx xxxxxxxxxx xx xxxxxxxx xx xxxxxxx xxxxx xxxxxxx xx xxxxxxxx, xx xxxx xx x xxxxx xxxxxx xx xxxxxxxxxxxx xxxxxxxx xxxx xx xxxxx xxxxxxxxx: xxxxxxx, xxxxxx-XxX, xxxxxxxxxxxx xxxxxxxx (xxxxxxx xxx xxxxxxxxxxxx), xxxxxxxx xxxxx xxxxx xxxxx, XXX-xxxxxxx xxxxxxxxxx xxxx xx xxxxxxxxx xxx xxxxxxxxx xx xxxx xxxxxxxxxxx, xxxxxxxxxxx xxx xxxxx xxxxxxxx, xxx xxxxx xxxxx. Xxxxxxxxx x xxxxxxxxx xx xxx xxxxxxxxxx xx xxxxx xxxxxxx xx xxxxx xxxxxxxx xx xxxxxxxx. Xx xxxxx xxxxxxx xxxxxxxxxx xx xxxxx xxxxxxxx xxxxxxx xxxx xxxxxxxx xxxx xxxxx xx xxxxxxxxxxx/xxxxxxxxxx xx xxxxxxxxx xxx xxxxxxxxx-xxxxxxx xxxxx xxxxx (xxxxxxxxxx, xxxxxxxxxx, xxxxxxxxx xxx xxxxxxxxxx), xxxxxxxx xxxxx xxxxx (xxxxxx, xxxxxxx xxx xxxxxxxxxx) xxx xxxxxxxxx xxx xxxxxxx xxxxx xxxxx xxxx xx xxxxxxxxx (Xxxxx 15 Xx xxxxxxxx, xxxxx xxxxx xxxxx xx XX X xxxxxx xxxxxx xxxxxxx xxxxxxxxxx xx xxxx xxxxx xxxxxxx xx xxxxxxxx xxx xxxxxxx xxxxxxxxxx xxx xxxxx xxxxx xxxxxxx xx xxxxxx xxxxxxxxxxxx xxx xxxxxxxxxxxxxxxx, xxxxxx-XxX xxxxxxxxxxxx xxxx xxxxxxxxxx, xxxxxxxxxxx, xxxxxx xxxxxxxxxxx, xxxx-xxxxxx xxxxxxx xxx xxxxxxxxxx xxxxxxxxxxx, xxxxxxxxxxx, xxxxxxxx X-xxxxxxxxxxx xxx xxxxxxxxx xxxxxxxxxxx.

Xxxx xxxxxxxxx xx xxxxxxxxxxx xxxxxxxx xx x XXX-xxxxxxxxx xxxxxx: X xxxxxx xxxxxx xxxxxxx XXX, XXX, XXXX, XXX1/XXX7 (xxxxxxxx xXX1, xXX3, XxXX2, xxx XxXX6) xxxxxx XXX (xxxxx 15 xxx Xxxxxx 19), xxxxxxxxxx xxxx xxxxxxxx xxxxxxx [560]. Xxxxxx xxxxxxxxxx xx xxxxx xx xxxxxxxxxxx xxxxxxxx xxx xx xxxxxxxxxxxx xxxxxxxx xxxx xxxxx xx xxxxx xxxxx xx 0°X (XXX xxxxxxxx xxxxxxxxxx) xxxx xx 5°X [560] Xxxx xxxxxxxx xxxx XX xxxxxxx xxxxxxxxx xxxx-xxxxxxxxxx xx xxxxxxx xxxxxxxxx xxx xxx xxxxxxxxxx xx X xxxxxx, the severity of which increased with the time of WLT

C3.2.5.Cold storage plus shelf life: Senescence or injury?

Clusters xXX2 xxx XxXX8 xxxx xx xxxxxxx xxxxxxxx xx xxxxxxxx xxxxxxx xxxxxxxx xxxxxx XXX, xxxxx xxxxx xxxxxx xxxxxxxxxx xx XXX. Xxxxx xx xxxxx xxxxxxxx xx xxx xxxxxx xx XX xxxxxx xxxxxx XX xxx xxxxxxxxx xxxx xxx XXX xxxxxxxxx xxxxxx XXX xx both S and LS fruit,

suggesting that they xxx x xxxxxxxxxxx xx xxx XXX xxxxxxxxx xxxxxx xxxx x xxxxx. Xx xx xxxxxxx xxxxxxx xxxx xxxx xxxxxxxxxx xxxxxxxxxxx xx xxx xx xxx xxxxxxxx xxxxxxxxxxx xxxxxx, xxx xxxx xx xxxxxxxx, xx xxxxxxxx xxxxxxxx xx xxxxxxxxxxxx xx xxxxxxxx xx xxxx xxxxxxxxxx xx xxxxx. Xxxxxxxxxxx, xxxxx xxxxx xxxxxxxxxx xxxxxxxxxx xx xxxxxxxxx xx xxx xxxxxxx XXX xxxxxxxxx.

Xxx xxxxx xxxxxxxx xXX2 xxx XxXX8 xxxx xxxxxxxx xx xxxxxx xxxxxxxxxx, XXX xxxxxxxxxxx xxx xxxxxxx xxxxxxxx, xxxxxxxxx xxxxxx xxxxxxxxxx xxx XXX xxxx-xxxxxxxxxx xxxxxxxxxx xxxxx (Xxxxxx 19 X xxx X, Xxxxx X13). Xxx xxxxxxxx xxxxxxxxxx xx xxxxx xx xxxxxxxx xxxxxxxx xxxxxxxx xxxx X xxxxxx xxxx xxxx-xxxxx xxx xxxxxxxxxx xxxx xxxxx xxx xxxxxxxxxx xxxxxxxxx. Xxxxxx xxxxxxxx xxxxx xxxxx xx xxxxxx XX, xxxxxx xxxx xxx xxx xxxxxxx XXX (xxxxxxxx xx xxxxxx xx 0xX) xxx xxxxxx xxxxxxxxxx xxxxx, xxx xxxxxxxxx xxxxxxxxxx xxx xxxxxxxxxx xxxx xxxxxx xxxxx xxx xxxx-xxxxx xxxxxx xxxxxx xx 2 xx 4xX [166, 561]. Xxxxxxx, xxxxxxxxxx xxx xxxxxxxx xxx xxxxxx xxxxxxxxx xx xxxxxxxxxx xxx xxxx-xxxxxxxxxxxxxx xxxxxxxxxx [562-564]. Xxxx xxxxxxxxx xxxx xxxxxxxxx xxxxxxxxxx, xxxxxxxxx xxx xxxx-xxxxxxxxxxxxxx xxxxx xxxxxxxx xx X xxxxxx xxxxx xxxxxxxxxx xx xxxxxx xxxx xxx xxxxxxxx, xxxx-xxxxx xxx xxxxxxxxx. Xxxx xxxxxxxx xxxxxxx xx xxxxx-xxxxxxxxx xxxxxx xxxxx xxxxx xxxxxx xx 5xX xxxx xx xxxxx-xxxxxxxx xxxxxx.

Xxxxx xxx x xxxxxx xxxxxxxxxxx xxxxxxx xxxxxxxxxx xx xxxxxxxxxxx xxxxxxxx xx xxxxxxx xxxxxxxxx xxx xxx xxxxxx xx xxxxxxxxx xxxxxx XXX (Xxxxxx 16 xxx xxxxxxxx xXX2 xxx XxXX8 xx Xxxxxx 19 xxx Xxxxx X13). Xxxxx xxx x xxxxxx xxxxxxxxxxx xxxxxxx xxxxxxxxxx xx xxxxxxxxxxx xxxxxxxx xx xxxxxxx xxxxxxxxx xxx xxx xxxxxx xx xxxxxxxxx xxxxxx XXX (Xxxxxx 1, xxxxxxxx xXX2 xxx XxXX8, Xxxxxx 3 xxx Xxxxx X2). Xxxxx xxxxxxxx xx xxxxxxx xxxxxxxxx xxxxxxxx xxxx xxxxxxxxx xxxxxx XXX xxxxx XX xx xxxxxxxx xx xxxxxxxx xxxxxxxx xxxxxxx. Xx xx xxxxxxxxx xxxxxxx xxxx xxxxxx xxxxxxxx xxxx xxxxxx, xxx xxxxxxxxxxx xxxxxxxxx xx xxxxxxxxxxx xxxxxxxx xx xxxxxxxx xxxxxx, xxx xxxx xxxxxxxxxxx in sensitive ones. However, xxxxx xxx xxxxxx xxxxxxxxxx xx xxxxxxx xxxxxxxxx xxxxxxxxxx xxxxxxx xx xxxx xx x xxxxxxxxx-xxxxxxxxx xxxxxx (Xxxxxx 19, [12] xxx xxxxxxxx 1 xxx 2), xxxxxxxxxx xx xxxxx xxxxx xxxxxx XXX xxxxxxx xx xxxxxx (xxx XX xxxxxx) xx xxxx xxxxxxx xxxxxxxxxx (xxxx xx X xxxxxx, Xxxxxx 19 xxx [12]).

Xxxxxx xxxxxxxxxx xxx xx xxxxxxxx xx x xxxx xx xxxxxxxxxx xx xxxxx xxxxxxxx xxxxxxxxx xxxxxxxxxx [565]. Xxxxx xxxx xxxxxxxxx xxxxxxx xxxx xxxx xxxxxx xxx xxxxxxxx xx xx xxxxxx xxxxxxxxx xxxxxxx xxxx xx xxx xxxxxxx xxx xxxx xxxxxxxx xx xxxxxxxxxxx xxx xxx xxxxxxx xxxxxxxxx xxxxxxxxx xxxxxxxxxxx xxxx xxxxxxxxx? Xxxxx xxxx xxxxxxxxx xxxxxxx xxxx xxxx xxxxxx xxx xxxxxxxx xxxxxx xxxxxxxxxx, xxx xxxx xxxxxxxxx xxxxxxxxxxxxx xx xxx xxxxxxx xxxxxxxxx xxxxxxxxx xxxxxxxxx xxxx xxxxxxxxx? Xxx xxxxxxxxx xxxxxxxxxxx xx xxxx xxxxxxxxxxxx xx xxxxxxxxx xxxxxxx xxxxxxxxxxx xx xxxxx xxxxx xx xxx xx xxxxxxx xx xxxxxxxx xxxxxxxx. Xxxxxxx xxxxxxx xxxx xxxxxxx xx xxxxxxxxx xxxxxxxx xxx/xx xxxxxxxxxx xxxxxxx xxx xxxx-xxxxxxxxx[566] xxx/xx xxxxxx xxxxxxxxxxxx xxx xxxxxxxxxxxx xxxxxxxxxxxx [567, 568], xxxxx xxxxxxxxxxxx xxxxxxxxx xx xxxxx xxxxxxxx is controlled xx xxx xxxxxxxx [569]. Xxxxxxxxxxx, xxx xxx xxxxxxxxxx, xxxxxxx xxxxxxxxx xx xxxxxxx xxxxxxxxxxx xxxxx xxx xxxxxx xxxx xxxxxxxx xxxxxxxxxxx xxxxxx xxx xxxxxxxxxxxx xxxxxxxxxxxx xxx xxxxxxxx xxxxxxxxxx. Xxxxxxxxxx xxx xxxxxxxxxxx xxxxxxxxxxx xx xxxxxxxxxx xxxxxxx x-xxxxxxx xxx xxxxxxxxxx xxxxxx xxxxxx xxxxxxxxxx xxx xxxxxx [570-572] xx xxxxxxxx xxxxxxxxx xxxx xxxxxxxx xxxxxxxxxx xx xxxxxxx XXXx [573]. Xxxx, XX xxxx XXX xxxxx xxxxxx xxxxxxxxx xx xxxxxxxxxx xxxxxxx xxxxxxxx xxxx xxxxxxxxx xxx xxxxxxxxxx xx xxxxxxxx xxxxxxx xx xXXXx xx X xxxxxx, xxxxx xxx xxxxxxxx xxxxxxxxx xxxx xxxx xxxx xxx xxxxx xxxxxxxxxx xxxxxxxxx xxxx xxx XXX xxxxxxxxx. Xxxxxxxxxxx xxxxxxxxxxxx xx xxxxxxxxxx xxx xxxx-xxxx-xxxxxxx xxxxx xxxxxxxxx xxxx xxxx xxxxx xxxxxx xxxx xxxx xxxx xxxxxxxx xxx xxxx xxxxxxxxx [574]. Xxxxxxxxx xxxxxxxx xxxxx xxx xxxxxxxx xxxxxxx xxxxxxxx [575] xxx xxxxxxxxx xxxxx xxx xxxxxxxx xx xxxxx xxxxx xxxxx xxxxxx xx xxxxxxxxxx [576]. Xxxxxxx xxxxxxxx xxxxxxxxx xxxxxxxxxx xx xxx xxxxxxxxxx xxxxxxxxx xx XXX xxxxxx xxxxx xxxx xxxxxxxxxxxxx xxxxxxxxxx xx xxxxxxxxxx xxxxxxxxx xxxxxx 5X (XXX-5X) [577, 578] xx xxxxxxxxxx xxxxxxxxx xxxxxx 5X (XXX-5X), xxxx xxxxxxxx xxx xxxxxxx xxxxxxxxx xx X xxxxxx (xxxxxxx xXX2, xxxxx X13) xxx xx xxx Xxx xxxxxx [525]. XXX-5X xxxxxxxxxxxxx xxxxxxx xx xxxxxxxxx xxxxxxx xxx xxxxxxxxx xxxxx xxxxxxxxx [578], xxxxxxxxx cell death and development of injury sym [577].

C3.2.6.Long term cold storage: The termination of the cold response

A striking result of our microarray analysis xxx xxx xxxx-xxxx XX xxxxxxxxxxxxxx xxxxxxxx xxxxxxxx xx X xxxxxx. XXX xxxxxxxx xxxxxxxxx xxxx xxxxxxxx XX xxxxxxxx X xxxxxx xxxx xxxxxxxxxxx: xxxxx xxxxxx xxxxxxxx xx xxx X xxxxx xx xxxx xxxxxxxx, xxxx xxxx xxxxxxxxxxx xxxx-xxxxxxx xxxxxxxxxx xxxx xxxxxxxxxxxx xxxxxxxx (Xxxxxx 16X-X). Xxxxx xxxxx xxxxx XX, xxxx X xxx XX xxxxxx xxxx xxxxxxxx xxxxx, xxxx xxxxxxx xxxxxxxxxx xx XX (Xxxxxx 16X), xxx xxxxx xxx xxxxx x xxxxxxxxxx xxxxxxxx xx xxx xxxxxx xx xxxxx xxxxxxx xxxxxxxxxx xx xxxxxxxxxx xxxxxxx X xxx XX xxxxxx (Xxxxxx 17X). Xxxxxxx xxxxxxxx xxxxxx xxxx xxxx xx xxxxxxxx xxx xx xxx xxxxxxx xxxxxxxx xxxxxxxx xx 3 xxxxx xx XX. Xxxxx xx xxxxxxx XxXX7, xXX2 xxx XxXX8, xxxx xxxx xxxx-xxxxxxxxx xxxxxx xxx xxx xxxxx xxxxx xx XX, xxxx xx-xxxxxxxxx xx 3x xxx xxxx xxxxxxxxxx xxxx xxx xxxxxxxxxxx xx xxxxx xxxxx xx XXX-3 xxxxxxx. Xxxxxxxxx, xxxxx xx xxxxxxx XxXX1, xxxx xx-xxxxxxxxx xxxxxx 1-2 xxxxx, xxxx xxxxxx xxxxx xxxxx XX xxx xxxx xxxxxxxxxx xxxx XXX3-X xxxxxxxxxx (Xxxxxx 19).

Xxxx xxxxxxx xx xxxxx xxxxxx? Xx xxx xxxx xxxxxxxx xxxxxxxxxx xxxxxx xxxx-xxxx XX? Xxx xxxx xx xxxxxx xxxxxxxx? Xxx xxxxx xxxx xxxxxxx xx xxxxx xxxxxxxx xxxx xxxxxxxxx xxxx xxxxxxxx? Xxx xxxxx xxxxxxx xxxxxxx xx xxxxxxx xxxxxx xx xx xxxx xxx xxxxxxx xxxx xxxxxx xxx xxxx-xxxxx?

Xxx xxxxxxxx xx xxxxx xx xxxxxxxx XxXX7 xxx XxXX1 xxx xx part answer the first question. We associated genes in cluster NeRG7 (Xxxxxx 19) xxxx xxxx xxxxxxxxx xxx xxxxxxxxx xxxx xxxxxxxxxxx (xx XXX1 xx xxxxx XxXX7 xxxxx, xxxxx 12). Xxxxxxx, xxxxx xxxxx xxxxx XX, xxxxx xxxxx xxxx xx-xxxxxxxxx. Xxxx xxxxxxxx xxxx xxxxx xxxxxx xxxxxxxxxx xxxxxxxx xxxx xxxx xx xxxx xxxxxxxxx. Xxxxxxx, xxxxxxx xxxxx xxxxxx xxxxxxxxx xxxxxx xxx xxxxx xxxx xxxxxxxx xx xxxxxxxx xxxxxx XX xx xxxx x xxxxxxx xxxxxx xx xxxxxxxx, xx xx xxxx xxxxxxxxx xxxx xxxxx xxxxxx xxxxxxx x xx-xxxxxxxxxx xxxxx xxxxxx XX. Xxxxxxxx xxxxx xxxxxxxxxxx xxxxxx xxxxxxxxxxxx xxx xxxxxxx xx-xxxxxxxxxx, xxxx xxxxx xxx xxxxxxxxxxx [493]. Xxxxxxxxxxx xx xxxxxx xxxxxxxxx xxxx xxxx xxxxxxxxxx xxx xxxx xxxxxxx xxxxxx xxxxxxxxxx xxx xxxxx xxxxxxx, xxx xxxx xxxxxxx xxxxxxxxxxxx xx xxxxxxxxxx, xxxxx xxx xxxxxxx xxxxxx xxxxxxxxxxx xxxxxxxx xxx xxxx xxxxxxxxx xxxxxxxx xx xxxx xxxxxxxxxx [493]. Xxxxx xxxxxxxx xx xxxx xxxxxxxxxx is a function of

the degree and xxxxxxxx xx xxxxxxxxxx xx xxxx xxxxxxxxx xxxxx xxx xxxxxxxxxxxxx xxxxx xxx xxx xxx xxxxxxx xxxxxxxxxxx xxx xxx xxxxxxxx xx xxxxxxxxxx xx XX xxxxxxxxx xxxxx [579]. Xxxxxxxxxxxx, xxx xxxxxxxx xx XXX1 xxx xx xxxxxxx xXX1 (xxxxxx xx-xxxxxxxxx xxxxxx xxx xxx xxxxx xxxxx xx XX, xxx xxxx-xxxxxxxxx xx xxxxx xxxxx, Xxxxx 12). XXX1 xx x xxxxxxxx xxxxxxxxx xx XXX xxxxx [580], xxxx xx xxxxxxxxx xxxxxx xxxx xxxxxxxxxxx xx *Xxxxxxxxxx*. Xxxx, xxxxx xx xxxx xxxxxxx xxx xx xx xxxx xxxxxxxxxxx xx xxxx xxxxxxxxxxx xxxxxxxxx xxxxxxxx xx xxxxx xxxxx.

XXX xxxxx xxxx xxxxxxxxxxx xx xxxxxxxx [122, 127]. XXX1 xx xxxxxxxx xx xxx xxxxxxxxxx xxxx xxxxxxxxx xx xxxxxxxxxxx xxxxx xx xxxxxxx [581], xxx xxx xxxx xxxx x xxxx xx xxxxxx [581, 582] Xxxxxxx XxXX1 xxxxxxxx xxxxx xxxxxxxxxx xxxxxxxx xx xxxxxx xxxxxxxxx xxxx xx XXX052[583] xxx XXX [584] (Xxxxx X13), xxx xxxxxx xxxxx xx xxxxxxxxx xxxx xx xxxxxxxxx xxx xxxxxxxxx xxxxxx xxxxxx xx *Xxxxxxxxxx* xxxx xx xxxxxxx xxxx xxxxxxxxxx xx xxxxxx [585] Xx xx xxxxxx xxxx xxxxxx xxxx xxxxxxx, xxxx xxxxx xx xxx xxxx xxxxxxxx xxx xxxxxxxx xxxx xxxxx xxxx xxxxxxxxxx xxxxxx, xxxxxxxx xxxx xxxxxxx xx xxxxxxxx xxxx xxxx xxxxxxx xx xxxx xxxxx xx xxxxxxxx xx xxxxxxxxxxxxxx xxxxxxx xxxx xxxxxxxx x xxxxx xxxx xx xxxx xxxxxxxxx xxxx. Xxxx xxxxx xxxx xxxxxx xxxxxxxxxxx in a CI-injured fruit.

Conclusions

1. A genetical genomics approach consisting in the combination of pools of siblings of the Pop-DG population, with contrasting sensibility to develop mealiness, and a microarray gene expression strategy have enable us to identify a large number of genes associated to the sensitivity /tolerance to develop mealiness both at pres-symptomatic (mature and cold storage) and symptomatic (shelf life ripening) stages.

2. Medium throughput qRT PCR analysis confirmed the validity of these gene expression markers in pools of siblings of the Pop-DG population, but most important there were proved to be reliable in individual siblings of the Pop population and other peach genotypes with different tolerance degree to chilling injury.

3. Functional annotation of genes differentially expressed between pools S and LS has enabled us to propose some hypotheses of the molecular processes occurring in the peach fruit while stored at 5ºC and later when let to ripen at room temperature that extends our working hypothesis about mealiness disorder beyond the cell wall changes.

Apendix

Supplementary figure and table legends

Figure S1 2D-HCA and PCAs for the genes in regulons ICE1, CBF1, HOS9, HOS15, ESK1, MYB-MYC, DREB2, AREB and ZF-NAC

Figure S2. The peach cold operons involved in the differential response between fruits S and LS

Figure S3. Unsupervised two-dimensional hierarchical clustering of genes differentially expressed between 'Oded' and 'Hermoza' at harvest and during cold storage. Data represent averaged lowess M log ratio for three replicates. Color represents fold change (red: up-regulated and green: down-regulated). : Harvest; CS1: cold storage of 1 week at 5ºC; CS2: cold storage of 2 weeks at 5ºC; Od: 'Oded' peach; Hz: 'Hermoza' peach; Figure S4. Comparison of the time at which up or down-regulation s occur in LS and S pools

Table S1. 3350 differentially expressed genes in the global analysis. A statistical test was performed by a SAM multiclass analysis. A gene was considered significant at a FDR < 0.05 and for a p-value < 0.05. The table provides ID, the Lowess M Log Ratio and functional annotations

Table S2. Summary of the results of the cold response in peach. The table indicates a) the contribution of each gene to the separation by a given principal component, b) the cluster resulting for global 2D-HCA, c) the expression pattern at harvest and during cold storage; d) the results of the correlation analysis between an average MI and the expression profiles in samples M-CS, e) functional annotations

Table S3. Arabidopsis genes reported as members of the cold and dehydration regulons. 1236 genes distributed in the regulons of CBF, ZAT12, HOS9, HOS15, GI for cold; ESK1 for cold-dehydration and AREB/ABF, MYC-MYB, DREB2, ZF-HD/NAC and CBF4 for dehydration

Table S4. Peach genes with an Arabidopsis 'ortholog' reported as members of the cold and dehydration regulons 163 peach genes were found in one of the cold and dehydration regulons, or more, and were considered to be: CBF, ZAT12, HOS9, HOS15,

GI for cold; ESK1 for cold-dehydration and AREB/ABF, MYC-MYB, DREB2, ZF-HD/NAC and CBF4 for dehydration. The table indicates the expression pattern in Arabidopsis WT, as well as the mutants and the expression pattern in the M and CS1 peach samples.

Table S5. The genes selected for the Fluidigm experiment. The conditions for the gene selection, gene annotations and expression values from microarray (reason for selection) are shown along with the sequence, length and Tm for the primers used in the qRT-PCR experiments.

Table S6. Chillpeach validation and extension results. The gene ID, the Fluidigm genes selection, the M-S/M-LS chillpeach pattern, the M-S/M-LS Fluidigm pattern, the CS LS *vs.* the S chillpeach pattern, the CS LS *vs.* the S Fluidigm pattern, validation in pools and lines and the expression values of the centered, scaled and normalized obtained in Fluidigm for the pools and lines are shown

Table S7. **Od/Hz Raw data.** 3277 Chillpeach probes that met the threshold for hybridization quality. Expression data correspond to lowess M Log Ratio

Table S8 Genes differentially expressed between 'Oded' and 'Hermoza' at harvest and during cold storage. In the Table there are the results for direct comparisons from the global analysis. The Table provides ID, the averaged lowess M Log Ratio and functional annotations.

Table S9.Gene-specific primers for qRT-PCR

Table S10. RT-PCR gene expression values for representative genes and correlation with microarray data. Ten candidate genes were assayed by quantitative RT–PCR in fruits from Od and Hz at harvest and after 1 and 2 weeks of cold storage**.** For each gene this is shown Od and Hz values at harvest and the average gene expression pattern relative to harvest values in both expression platforms, microarray and qRT-PCR. The agreement between qRT-PCR and microarrays in expression profiles across samples is expressed as Pearson correlation coefficient.

Table S11 - Comparison of genes differentially expressed at one week of cold storage between 'Oded' and Hermoza and between pools of siblings form the Pop-DG

population. Data from fruits from the Pop-DG population cold stored were obtained from chapter 1

Table S12 - Comparison with genes previously validated in pools and in individual lines from the Pop-DG population. Data from fruits from the Pop-DG population cold stored were obtained from chapter 1. The cluster derived from Figure 6A is shown and the expression pattern at harvest and after one week of cold storage in pools LS and S. Also indicated are the genes validated in individual lines and pools.

Table S13. Summary of the results of the cold and shelf life response in peach. The table indicates the genes altered in CSR samples vs R samples, the genes differentially expressed between LS and S during CS and also during SLR, the genes dubbed as eRG or NeRG, their expression pattern and the cold effect, and functional annotaions.

Table S14. Chillpeach validation in pools. The gene ID, the Fluidigm genes selection, the CS LS *vs.* the S chillpeach pattern, the CS LS *vs.* the S Fluidigm pattern, the CSR LS *vs.* the S chillpeach pattern, the CRS LS *vs.* the S Fluidigm pattern, validation in pools and the expression values of the centered, scaled and normalized obtained in Fluidigm for the pools and lines are shown

Table S15. **Correlations and correlation significance between relative expression levels of the candidate genes analyzed by medium throughput Fludigm RT PCR in each of the siblings with the MI exhibited during SLR after 1 week of CS.**

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