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Peach and Nectarine

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CONTENTS

List of Abbreviations

CI	Chilling Injury
PME	Pectinmethylesterase
PG	Polygalacturonase
SNP	Nucleotide Polymorphism
QTLs	Quantitative Trait Loci
GA	Gibberellic Acid
ACC	1-amino-cyclopropane-1-carboxylic acid
ACO	1-amino-cyclopropane-1-carboxylic acid oxidase
ABA	Abscisic Acid
IW	Intermittent Warming
HSPs	Heat Shock Proteins
HT	Heat Treatments
SA	Salicylic Acid
MeJA	Methyl Jasmonate
GABA	γ-Aminobutyric Acid
GWAS	Genome-Wide Association Study
GS	Genomic Selection
GEBVs	Genomic Estimated Breeding Values
EBVs	Estimated Breeding Values

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13.1 Introduction

Peaches and nectarines (*Prunus persica* (L.) Batsch) are the most important fleshy stone fruits produced worldwide (Brady, 1993). They have been grown historically in China, the United States, Italy, Spain, and Greece. Fresh-market peaches are produced in the northern hemisphere from April through

September and in the southern hemisphere from November to March. Although the sweet drupes are highly appreciated due to their unique texture and flavor, consumption is low compared with other fresh fruits such as apples and bananas (Crisosto, 2006). Unfortunately, most of the appealing attributes of the fruit are reduced dramatically after long-term storage at low temperatures (Lurie and Crisosto, 2005). Chilling injury (CI) is the main physiological problem limiting the export and consumption of peach and nectarine and a frequent source of complaints by wholesalers and customers. Its symptoms develop during ripening after cold storage and, since externally the fruits appear normal, mealiness is detected during consumption (Crisosto and Labavitch, 2002). This discourages repeated purchasing (Crisosto et al., 1995).

This chapter provides information about the types of CI symptoms in peach and nectarine and their biological basis, main hormone groups associated with cold tolerance and sensitivity, methods for CI assessment, factors affecting CI incidence and severity, and postharvest and genetic strategies to control it.

13.2 Chilling Injury Symptoms and Biological Basis

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CI occurs after extended storage at chilling temperatures and results in fruit lacking in juiciness (Fernandez Trujillo et al., 1998; Zhou et al., 200a,b, Lurie and Crisosto, 2005, Fruk et al., 2014). Symptoms are evident as mealiness (woolliness), internal breakdown (flesh browning), and flesh reddening (Figure 13.1). Sometimes, the symptoms may be evident as hard-textured fruit (leatheriness), while mealiness refers to soft fruit with reduced juiciness (Manganaris et al., 2005a; 2005b).

Mealy (woolly) texture is thought to be caused by altered activity and concentrations of cell wall-degrading proteins (pectinmethylesterase [PME], polygalacturonase [PG], arabinofuranosidase, and expansins) which reduce pectin solubility and increase their *in muro* gelling capacity. Ultrastructural studies showed that mesocarp parenchyma cells collapse in leathery peaches, while in mealiness, increased intercellular spaces are observed (Luza et al., 1992; Brovelli et al., 1998). In spite of their dry sensory perception, mealy peaches and nectarines have similar water content than normal, juicy fruit. The reduced water release in mealy fruit is most likely due to increased water retention (Lurie et al., 1994) caused by gel formation and decreased cell rupture (Brummell et al., 2004).

Early work related mealiness development to abnormal changes in cell wall pectin metabolism, especially to degrading enzymes like PG and PME (Ben Arie and Lavee, 1971). Subsequent studies showed that PG activity was inhibited in cold-stored peaches in contrast to PME, which remained relatively high, yielding pectic polysaccharides with higher molecular weight, lower methyl esterification, and high calcium-binding capacity (Artes et al., 1996). Thus, it was proposed that the lack of juiciness was caused, in part, by pectin gelation (Lurie et al., 1994; Zhou et al., 2000a). Nonetheless, in some stone fruits, mealiness has been shown to occur without alterations in PME and PG activity (Manganaris et al., 2008; Pegoraro et al., 2010a). Several other cell wall-degrading proteins such as endo-1,4-β-glucanase, endo-1,4-β-mannanase, β-galactosidase, α-arabinosidase, and expansin have also been shown to be inhibited in cold-stored peaches (Obenland et al., 2003; Brummell et al., 2006). In 'Fortune' plums, CI correlated with reduced β-1,4-D-galactosidase activity and increased galactose retention in rhamnogalacturonan I polyuronides (Manganaris et al., 2008). Cell wall-associated thaumatin proteins (TLPs), which have been shown to prevent ice crystal formation, growth, and recrystallization (Griffith et al., 2005)

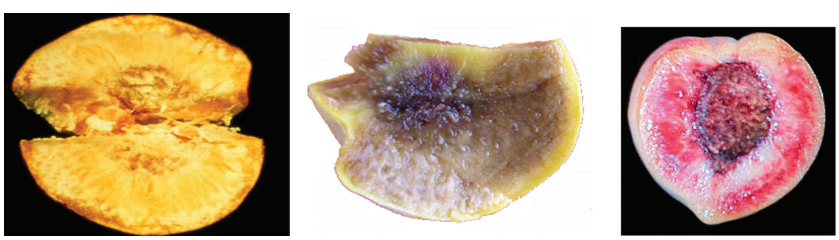

FIGURE 13.1 Main chilling injury symptoms in peach and nectarine fruits, evident as mealiness, internal breakdown, and flesh bleeding.

increased rapidly in CI tolerant cultivars (Dagar et al., 2010). However, whether or not there is a causal relationship or only a temporal association between their accumulation and chilling tolerance remains to be established.

The use of methods to study gene expression, proteins, and metabolites on a global scale has provided a broader view of the changes occurring in fruits in response to low temperatures. The expression of genes associated with vesicle trafficking within the secretory and endocytic pathways was affected during the development of mealiness (Gonzalez-Agüero et al., 2008). Regarding other stresses, the up-regulation of genes involved in the antioxidative response was found at the onset of mealiness development (Pavez et al., 2013). Overall, most recent studies relying on 'omic' approaches concluded that the molecular changes taking place during the development of fruit woolliness include not only changes in the cell wall and membranes, but also alterations in sugars, amino acids, and hormone metabolism (Vizoso et al., 2009; Friedman and Lurie, 2010; Nilo et al., 2010). Altered homeostasis of phenolics (biosynthesis and degradation) has been related to other CI symptoms, such as flesh browning and reddening (bleeding).

As previously mentioned, CI development has been also associated with flesh browning (Kader et al., 1984). Although browning usually develops after mealiness (Lurie and Crisosto, 2005), most cultivars will develop both types of symptoms. Only a few cultivars develop flesh browning without mealiness (Crisosto et al., 1999).

Flesh bleeding, usually radiating from the pit, may be a problem in some peach cultivars (Figure 13.1). This symptom does not affect the taste of fruit but causes rejection in the peach canning industry. There has been almost no research conducted on the causes of fruit bleeding. Some *MYB* transcription factors help to control phenylpropanoid metabolism and specifically activate the transcription of genes encoding enzymes involved in anthocyanin biosynthesis (Ambawat et al., 2013) Transcription factors of *MYB* members can be induced by abiotic stresses, and some are directly involved in chilling tolerance. In *Arabidopsis*, the overexpression of *OsMYB4* significantly enhanced tolerance to chilling (Vannini et al., 2004; Pasquali et al., 2008).

13.3 Main Hormone Groups Associated with Cold Tolerance and Sensitivity

Mealiness was originally associated with decreased ethylene biosynthesis (Zhou et al., 2001b). Ethylene during storage promotes the sequence of cell wall hydrolysis necessary for normal ripening (Dong et al., 2001). However, recent studies underlined that cold-adaptive responses also involve other hormones, such as abscisic acid, gibberellin, and auxin. 1-MCP-treated fruit had more intense browning and failed to soften normally (Dong et al., 2001; Fan et al., 2002). Accumulation of the ethylene biosynthetic enzyme ACC oxidase (ACO) was decreased in mealy peaches (Obenland et al., 2008). Fruit with greater capacity to produce ethylene after cold storage had less severe CI (Gine-Bordonaba et al., 2016). The response of sensitive fruit to cold involved the participation of other hormones such as ABA and auxin (Pons et al., 2014). Gibberellins were also related to cold responses in peaches, since GA_3 application markedly reduced CI (Pegoraro et al., 2010b). Although the mechanism underlying GA-induced protection against CI is unclear, it is suggested that transcriptional changes triggered by GAs during early fruit development may affect subsequent responses to stress after harvest (Pegoraro et al., 2015). Pons Puig et al. (2015) showed that cultivars with improved storage potential (less prone to CI symptoms) had elevated expression of genes related to antioxidant systems already at harvest time.

13.4 Methods for Chilling Injury Assessment

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Mealiness has traditionally been assessed by direct visual inspection and destructive and nondestructive methods (reviewed in Arefi et al., 2015). Centrifugation of homogenized as a way to determine juice extractability was an early empirical assay to quantify mealiness (Lill and Van der Mespel, 1988). Other techniques relied on fruit pressing and centrifugation and related mealiness to the ratio of juice collected relative to the initial sample weight (Crisosto and Labavitch, 2002), while a paper absorption method originally used to evaluate exudates in foods was adapted to estimate mealiness (Infante et al.,

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2009). Reliable strategies for the early detection or prevention of mealiness would be very useful to allow the study of early changes associated with CI. A two-step method combining impact response and near-infrared spectroscopy was developed to identify mealy peaches (Ortiz et al., 2001). Recently, hyperspectral reflectance imaging methods showed a discriminating accuracy of 92–97% (Sun et al., 2017). However, further studies are needed to determine whether these methods can be scaled up and used during line sorting.

13.5 Factors Affecting Chilling Injury

A comprehensive review of the influence of different factors on CI development in peach and nectarine can be found elsewhere (Lurie and Crisosto, 2005). Genotype has a strong influence on tolerance to CI; melting flesh peach cultivars are more susceptible to CI than firmer, non-melting cultivars (Crisosto et al., 1999). As a general rule, only fruit with melting flesh develop mealiness symptoms. Bleeding is more prevalent in white-fleshed cultivars and is not associated with flesh browning (Martínez-García et al., 2012), thus indicative of preprogrammed mechanisms associated with their ability to tolerate postharvest cold-induced stress (Pons et al. 2014, 2016). However, some responses induced by exposure to low temperature that may be also involved in improved cultivar performance upon chilling include plasma membrane enrichment in linolenic acid and N-acylphosphatidylethanolamine, as well as the accumulation of compatible solutes (Zhang et al., 2009, 2010; Bustamante et al., 2014).

Large seasonal variations have been reported in CI (Campos-Vargas et al, 2006). However, the factors causing these variations remain unclear. Low crop loads have been found to increase browning and mealiness, while shaded fruit had lower storage potential and were more prone to postharvest disorders (Lurie and Crisosto, 2005). Early-harvested fruit are more susceptible to CI, especially flesh browning, during storage. For a given genotype and ripening stage, the three main factors determining the incidence and severity of CI symptoms are storage duration, temperature, and atmosphere. As expected, prolonging cold storage increases the incidence and severity of CI symptoms. However, the incidence of bleeding and leatheriness are not markedly affected by storage duration (Cantin et al. 2010). The effect of proper temperature management in CI prevention cannot be overstated. CI develops faster and more intensely in fruit stored at 2.2–7.6°C (peaches) or 2.2–7.8°C (nectarines) than in fruit stored at 0°C or below (Crisosto et al., 1999). Maximum storage life can be achieved at 0°C. Higher concentrations of CO_2 in the atmosphere can protect some peach cultivars from CI (Infante et al., 2009).

13.6 Postharvest and Genetic Strategies to Reduce and Control Chilling Injury

13.6.1 Postharvest Strategies

Contradictory results have been found regarding the efficacy of controlled atmosphere (CA) storage of peaches and nectarines. Elevated CO_2 levels reduced mealiness symptoms during shipping in yellow (Crisosto et al., 1999; Zhou et al., 2000b)- and white-fleshed cultivars (Garner et al., 2001). Fernandez-Trujilio et al. (1998) postulated that storage under 12% CO_2 and 4% O_2 reduced CI and increased peach storability. CA conditions of 6% O_2 and 17% CO_2 are recommended to reduce internal breakdown in peaches. However, the efficacy of this approach is related to cultivar, pre-harvest factors, market life, and shipping duration. Modified atmosphere packaging (MAP) has been tested in several peach cultivars, mostly without success (Zoffoli et al., 2002; Lurie and Crisosto, 2005).

Controlled delayed cooling boosts the flavor and shelf life of peach and nectarine fruit. This strategy has been commercially applied in California by different handlers (Summeripe and others, Figure 13.2). Fruit are harvested at the firm-ripe stage, defined for each cultivar, and subsequently continue ripening at room temperature (20°C) and high relative humidity for 24–48 h. Subsequently, fruits are subjected to forced-air cooling to stop flesh softening, senescence, decay, and weight loss. This treatment increased the minimum market life by up to 2 weeks in the cultivars tested (Figure 13.3, Crisosto et al., 2004). Careful monitoring of weight loss and firmness during delayed cooling and the proper use of fungicides

FIGURE 13.2 Commercial-scale treatment of peach and nectarine fruits after harvest at Summerripe, Fresno, California. Firm–ripe fruit are maintained at 20°C, 90–95% R.H. for 48 h (left), followed by forced-air cooling (right) prior to cold storage.

is highly recommended for this strategy to be successful. This method can also be used to pre-ripen susceptible and non-susceptible peaches to deliver a ready-to-buy product (Crisosto et al., 2004)

Intermittent warming (IW; fruit are subjected to cold storage with interludes at room temperature) was suggested as an alternative to reduce CI in stone fruit. The basis for IW is to remove the fruit from the stress condition before it gets into the phase at which irreversible damage may occur. When 2 days of IW at 20°C was applied every 12 days during 0°C storage, mealiness was reduced (Zhou et al., 2001a). Despite its potential value, this method has practical difficulties for large-scale implementation. Heat treatments (HT) were also evaluated as a strategy to reduce CI (Murray et al., 2007). The improved chilling tolerance imparted by these treatments correlated with the induction of heat shock proteins (HSPs) (Lara et al., 2009). Many metabolites related to protein and membrane protection during heat, drought, oxidative damage, and other types of stress were induced in both cold- and heat-treated peaches, indicating some overlap in the responses to both stresses that could explain the acclimation effect of heat exposure prior to chilling (Lauxmann et al., 2014). Unfortunately, the improvement obtained has not been sufficient to justify the commercial use of HT (Lurie and Crisosto, 2005).

An array of postharvest chemical treatments has been applied to peach to prevent and/or alleviate CI with variable success. These include salicylic acid (SA), methyl jasmonate (MeJA), oxalic acid,

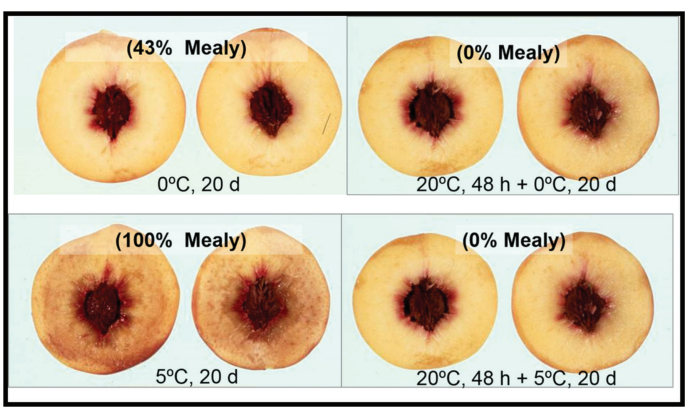

FIGURE 13.3 The effect of conditioning treatments (delayed cooling for 48 h) on postharvest performance of peach fruits. (Source: Crisosto et al., 2004)

γ-aminobutyric acid (GABA), gibberellic acid (GA), and other compounds (Jin et al., 2009, 2014, Yang et al.,ss 2011, 2012, Shan et al., 2016). MeJA enhanced the chilling tolerance of peach fruit by inducing the activity of enzymes related to energy metabolism and maintaining high concentrations of ATP and energy charge (Jin et al., 2013), while the application of SA alleviated CI through the induction of HSPs, dehydrins, and other stress-related proteins (Wang et al., 2006). Such treatments still need to be validated in commercial settings, and are not widely used.

13.6.2 Genetic Strategies

Breeding methods such as self-pollination (or recurrent self-pollination) and intraspecific hybridization are being used to develop new peach cultivars. However, little progress has been made in selecting chilling-resistant genotypes using traditional approaches. During the pre-genome sequencing era, traditional breeding was carried out through phenotype-based selection and the identification of quantitative trait loci (QTLs), using a low number of genetic markers for these traits (Cantín et al., 2010). The markers linked to these traits could be used for marker-assisted selection to identify genotypes not prone to CI at the seedling stage (Lurie and Crisosto, 2005). To improve the precision of candidate gene mapping associated with CI, ChillPeach, a specialized database to target genes expressed during CI development, and a cDNA microarray containing probes for these genes were developed (Ogundiwin et al., 2008a), and the leucoanthocyanidin dioxygenase gene (*PpLDOX*) was proposed as a potential functional marker for cold-storage browning in peach (Ogundiwin et al., 2008b).

With the recent sequencing of the peach genome (Verde et al., 2013), there has been a coordinated effort to develop a high-throughput Illumina Infinium SNP genotyping array (Verde et al., 2012), and other bioinformatics studies to identify additional high-frequency SNPs distributed throughout the peach genome (Ahmad et al., 2011). The large number of SNPs obtained is improving mapping resolution and the systematic identification of genes underlying CI symptoms. A significant QTL associated with mealiness was detected in the bottom of linkage group four (qML4), with two SNP markers significantly associated with qML4 (Martínez-García et al., 2013a). The effects of these SNPs were predicted and a large list of candidates genes associated with mealiness and other CI symptoms was obtained (Martínez-García et al., 2013b). More studies are needed to understand genotype variation in the incidence of CI symptoms. This information will allow geneticists and breeders to develop stone-fruit cultivars free of CI, as well as to understand the genetic inheritance of CI and to isolate the genes responsible for it. A non-significant QTL for flesh browning was identified on linkage group five (spread over a large part of LG5) in peach (qBrL5) and associated with several SNPs markers. The most significant markers explained between 29.2–35.8% of the phenotypic variance for this trait (Martínez-García et al., 2013a). Two non-significant QTLs for flesh bleeding were located on LG1 and LG4. These two QTLs (qBL1 and qBL4) for flesh bleeding explained approximately 30% and 34% of the phenotypic variance observed, respectively; several markers were significantly associated with the QTLs. These loci were also significantly associated with freestone-melting flesh (qF-ML4.4) and mealiness (qML4) on LG4 (Martínez-García et al., 2013a).

Deep sequencing of RNA transcripts (RNA-seq) is emerging as an alternative to microarray studies to quantify gene expression. Several transcriptomic resources have been generated that are enabling scientists to increase the knowledge on the molecular basis of phenotypic variability, identify allelic variants, and find candidate genes associated with reduced susceptibility to CI. The ultimate goal is to incorporate this new knowledge into breeding programs (Carrasco et al., 2013).

Metabolomics has helped to identify potential novel players in CI. Some genes, proteins, and metabolites associated with CI are potential biomarkers for breeding and/or early detection of the disorder. In a recent study of six peach cultivars, the more susceptible genotypes accumulated xylose after chilling, whereas the less susceptible genotypes tended to accumulate raffinose and galactinol (Bustamante et al., 2016). Studies using prolonged cold storage identified this sugar as a potential biomarker for improved tolerance to chilling. Changes in the concentrations of compatible solutes were also speculated as a potential strategy to increase fruit tolerance to postharvest abiotic stresses (Lauxmann et al., 2014).

One approach to improving breeding efficiency is to develop modern breeding programs, realizing the full potential of genomics-assisted breeding through approaches such as a genome-wide association study (GWAS) (Cao et al., 2016) and genomic selection (GS). This may help to clarify the genetic basis

of chilling-related disorders in peach, potentially allowing more efficient breeding to provide resistant cultivars relatively quickly.

GS uses genomic estimated breeding values (GEBVs) as selection parameters, rather than traditional estimated breeding values (EBVs) (de Souza et al., 2000) or breeding values estimated through pedigree-based analysis (Fresnedo-Ramírez et al., 2016). GEBVs are predicted based on dense, genome-wide, single-nucleotide polymorphism (SNP) markers used in a phenotyped training population (van Nocker and Gardiner, 2014). In the second step of GS, individuals from a selection population (validation test) are genotyped (but not phenotyped) and, finally, superior individuals, selected based on GEBVs, are used to advance generations or are evaluated in the field as potential cultivars resistant to CI symptoms.

13.7 Final Remarks

- Chilling injury (CI) is the main physiological disorder limiting the shelf life as well as the short- and long-distance distribution of peaches and nectarines.
- A mealy (wooly) texture is caused by altered expression and activity of several cell wall-degrading proteins.
- Studies in the past decade have indicated that changes accompanying peach chilling go far beyond those occurring on cell wall polyuronides and loosing enzymes and include global alterations in fruit transcriptional and metabolic profiles.

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- Susceptibility to CI is largely dependent on genotype, and is triggered by temperature and time of exposure to chilling temperature.
- Maturity stage at harvest and storage temperature, atmosphere, and duration are the most relevant factors associated with the disorder.
- 'Omic' approaches have identified potential novel players in CI and some genes, proteins, and metabolites associated with the disorder that are potential biomarkers for breeding and/or early detection of CI symptoms.
- The development of tolerant cultivars through breeding is currently more feasible.
- The large number of high-quality SNPs obtained for peach is improving mapping resolution and helping to identify genes underlying CI symptoms.
- Meanwhile, avoiding damaging temperatures, CA, and conditioning are tools available to minimize chilling disorders in peach and nectarine.

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