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BARD Project Number: Research Grant Award No. US-4027-07

Date of Submission of the report: January 28, 2012

Project Title: A biological systems approach to developing mealiness-free peach and nectarine fruit

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Total Requested Budget (in US \$) \$394,000.00

Investigator Details:

Position in proposal	Name	affiliated institution
1) Principal Investigator (PI)	Carlos Crisosto	Univ. of California, Davis
2) co-PI	Susan Lurie	ARO, The Volcani Center
3) Collaborating	Haya Friedman	ARO, The Volcani Center
4) Collaborating	Ebenezer Ogundiwin	Univ. of California, Davis
5) Collaborating	Cameron Peace	University of California, Davis
6) Collaborating	George Manganaris	Univ. of California, Davis

Budget: IS: \$173,000

US: \$221,000

Total: \$ 394,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Appendix G6a

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Abstract Peach and nectarine production worldwide is increasing; however consumption is flat or declining because of the inconsistent eating quality experienced by consumers. The main factor for this inconsistent quality is mealiness or woolliness, a form of chilling injury that develops following shipping periods in the global fruit market today. Our research groups have devised various postharvest methods to prolong storage life, including controlled atmosphere and delayed storage; however, these treatments only delay mealiness. Mealiness texture results from disruption of the normal ripening process involving disassembly of cell wall material, and creates a soft fruit texture that is dry and grainy instead of juicy and smooth. Solving this problem is a prerequisite for increasing the demand for fresh peach and nectarine.

Two approaches were used to reveal genes and their associated biochemical processes that can confer resistance to mealiness or woolliness. At the Volcani Center, Israel, a nectarine cultivar and the peach cultivar (isogenetic materials) from which the nectarine cultivar spontaneously arose, and at the Kearney Agricultural Center of UC Davis, USA, a peach population that segregates for quantitative resistance to mealiness was used for dissecting the genetic components of mealiness development. During our project we have conducted research integrating the information from phenotypic, biochemical and gene expression studies, proposed possible candidate genes and SNPs-QTLs mapping that are involved in reducing peach mealiness susceptibility.

Numerous genes related to ethylene biosynthesis and its signal transduction, cell wall structure and metabolism, stress response, different transcription factor families were detected as being differentially accumulated in the cold-treated samples of these sensitive and less sensitive genotypes. The ability to produce ethylene and keep active genes involved in ethylene signaling, GTP-binding protein, EIN-3 binding protein and an ethylene receptor and activation of ethylene-responsive fruit ripening genes during cold storage provided greater resistance to CI. Interestingly, in the functional category of genes differentially expressed at harvest, less chilling sensitive cultivar had more genes in categories related to antioxidant and heat shock proteins/chaperones that may help fruit to adapt to low temperature stress.

The specific objectives of the proposed research were to: characterize the phenotypes and cell wall components of the two resistant systems in response to mealiness- inducing conditions; identify commonalities and specific differences in cell wall proteins and the transcriptome that are associated with low mealiness incidence; integrate the information from phenotypic, biochemical, and gene expression studies to identify candidate genes that are involved in reducing mealiness susceptibility; locate these genes in the *Prunus* genome; and associate the genes with genomic regions conferring quantitative genetic variation for mealiness resistance. By doing this we will locate genetic markers for mealiness development, essential tools for selection of mealiness resistant peach lines with improved fruit storability and quality. In our research, QTLs have been located in our peach SNPs map, and proposed candidate genes obtained from the integrated result of phenotypic, biochemical and gene expression analysis are being identified in our QTLs as an approach searching for consistent assistant markers for peach breeding programs.

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– List the original objectives, as defined in the approved proposal and any revisions made at the beginning or during the course of project.

Main Goal was to identify controlling genetic factors and their associated biochemical processes that determine resistance/susceptibility of peach to mealiness/wooliness.

System approaches were used to identify genetic factors and their associated biochemical processes to chilling injury. In Israel, at the Volcani Center, we used three different systems: gibberellin treatment of the susceptible peach ('Oded') compared to non-treatment; 'Oded' peach and its nectarine mutant 'Yuval' which is more resistant to mealiness; 'Oded' peach compared to a very susceptible cultivar 'Hermoza'. In USA, at Kearney Agricultural Center of UC Davis, a peach population with 3 year phenotyping data on quantitative resistance to mealiness was genotyped and available for dissecting the genetic factors and biochemical processes that control peach mealiness development.

The specific objectives of our proposed research were to:

- Characterize the phenotypes and cell wall components of three systems examined in Israel with regard to chilling injury-inducing conditions. It was found that gibberellin treatment of peach helped to prevent flesh browning and reddening, but did not have a significant effect on mealiness (Dagar, submitted). 'Yuval' nectarine and the peach progenitor 'Oded' were compared over two seasons, and the nectarine was found to be more chilling resistant than the peach (Dagar et al., 2010). 'Hermoza' and 'Oded' peaches were compared and their differential sensitivity to chilling injury used in objective C (see below).
- Identify specific cell wall proteins and cell wall components that are associated with low mealiness incidence. Identify specific cell wall proteins and cell wall components that are associated with low mealiness incidence. We developed a protocol to isolate cell walls that minimized contamination by cellular proteins (a major problem). Although there were a number of proteins that were differentially expressed during storage, a major protein that appeared to be correlated with chilling resistance was thaumatin-like protein (Dagar et al., 2009). This was also found independently in the ChillPeach database of expressed genes during mealiness (Ogundiwin et al., 2008).
- Identify transcriptome changes in that are associated with low mealiness incidence using a ChillPeach microarray platform. We have used the ChillPeach microarray to compare 'Oded' peach to the nectarine mutant 'Yuval', and also to compare 'Oded' to 'Hermoza'. We found that all these cultivars were more resistant to chilling than the resistant progeny that were used to create the microarray. However, when comparing the transcriptome changes of 'Oded' and 'Yuval' together with the changes found in the fruit used to make the microarray, we found 41 genes that can explain 67% of the variance among the different fruit (Dagar et al., submitted). These are being checked further in other fruit and examined

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concerning the QTLs developed in the project.

- Integrate the information from phenotypic, biochemical, and gene expression studies to propose possible candidate genes that are involved in reducing mealiness susceptibility. As mentioned in C, we have found a number of genes that appear to be markers for differences in chilling susceptibility. These are being investigated further.
- Locate and identify the genes for reducing mealiness susceptibility on the QTL linkage map of *Prunus* (This investigation is on progress).

Background to the topic.

Chilling Injury Symptoms: Temperature management during postharvest handling of fruits and vegetables is critical to maintain quality and reduce losses. Temperature management before, during, and after fruit ripening determines the final quality of the product. Initial produce quality is a critical part of establishing a reliable and successful fruit delivery program. Many tropical or subtropical commodities are sensitive to chilling injury, which occurs when commodities such as poinsettia, bananas, and tomatoes are held at temperatures above their freezing points. Some important temperate-zone crops or cultivars are also susceptible to chilling injury. For example, chilling injury or internal breakdown of some peach, plum, and nectarine cultivars occurs below 50°F and is greatest at about 41°F. Unfortunately, the effect of chilling is cumulative in some commodities. Low temperatures in transit or in the field shortly before harvest add to the total chilling that occurs in cold storage. Some chilled fruits will appear sound while stored at low temperatures but chilling injury symptoms develop upon transfer to warmer temperatures or during/after ripening. Thus, failure of mature fruits to ripen properly, lack of uniform ripening, and/or visual problems can be related to chilling injury during postharvest handling prior to ripening at the production or distribution points. No ice forms in the cells during chilling injury development, but changes in the tissue's chemical composition, especially in the membrane phospholipids, lead to chilling damage. Many physiological and biochemical changes occur before chilling injury symptoms are apparent such as cessation of cytoplasmic streaming, changes in membrane permeability, disorganization of cytoplasm, loss of mitochondrial activity, and alteration of respiratory metabolism ending in cellular dysfunction. At chilling temperatures, tissues are weakened by the inability to maintain normal metabolism. When chilling stress is prolonged, these cell alterations lead to development of chilling injury symptoms. Failure of mature fruits to ripen, surface pitting, surface lesions, external and/or internal discoloration, appearance of water-soaked areas, development of necrotic areas, increased susceptibility to decay, shorter storage life, cessation of growth, loss of sprouting ability in propagules, and loss of characteristic flavor and aroma are associated with chilling injury. Maturity at harvest and degree of ripeness can affect chilling sensitivity in some fruits like avocados, honeydew melons, and tomatoes. Riper fruit are less susceptible to chilling injury. Injury will develop in these commodities after a period of exposure to chilling temperatures below 50 to 59°F, but well above freezing.

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Expression of chilling injury symptoms such as failure to ripen properly is a function of temperature and time. A fruit may be able to withstand temperatures just below the chilling threshold temperature. However, if produce is exposed to temperatures even further below its chilling threshold, the development of chilling injury will be more severe and can affect ripening. A longer exposure results in irreversible damage that affects the ripening and final consumer quality. Thus, it is critical to store fruit at its optimum storage temperature and with proper handling practices throughout postharvest life. The time of exposure at a given temperature that triggers particular chilling injury symptoms is specific to cultivar, maturity, production area, and other conditions. For example, skin pitting, scalding, and blackening are the main symptoms on mature-green avocados kept at 32-35°F for more than seven days before transfer to warm temperatures. Avocados exposed to 37-41°F for more than two weeks may exhibit internal flesh browning, failure to ripen, and increased susceptibility to pathogen attack. Failure to ripen, surface discoloration, and dark brown sub-epidermal streaks result from exposing mature-green bananas for one hour to 50°F, five hours to 53°F, 24 hours to 54°F, or 72 hours to 55°F. Exposure of mature-green mangos to temperatures below 55°F and exposure of ripe mangos to temperatures below 50°F causes uneven ripening. In peach cultivars highly susceptible to internal breakdown, a three-day exposure to the “killing” temperature range (36 to 46°F) can induce failure to ripen, off flavors, flesh browning, and mealiness. Failure to ripen, sunken surface areas, and pitting develop several days after storage at 35.5°F in cantaloupe and after storage at 45°F for honeydew melon and watermelons. Failure to ripen, lack of flavor, premature softening, and increased decay occur when tomatoes have been stored below 50°F for longer than two weeks or at 41°F for longer than six to eight days.

Several treatments, including partial ripening (sometimes with ethylene) after harvest but before cold storage, exposure to high or low but non-chilling temperatures prior to cold storage, cold storage under controlled or modified atmospheres, or treatments with abscisic acid, methyl jasmonate, gibberellic acid, or other natural compounds can reduce chilling injury.

Peach Internal breakdown or Chilling Injury: Internal breakdown and chilling injury (IB and CI) in peach are genetically controlled and triggered by storage temperature. Symptoms include dry, mealy, woolly, or hard-textured fruit (not juicy), flesh or pit cavity browning, and flesh translucency usually radiating through the flesh from the pit. Development of an intense red color in the flesh (“bleeding”), usually radiating from the pit, may be associated with this problem in some peach cultivars. Recently released cultivars rich in skin red pigment showed flesh bleeding that does not affect fruit taste. The development of this symptom is associated with fruit maturity rather than storage temperature. In susceptible cultivars, flavor is lost before visual CI symptoms are evident (Crisosto and Labavitch, 2002). There is large variability in IB susceptibility among peach cultivars (Crisosto et al., 1999c; Mitchell and Kader, 1989a). In general, mid-season and late-season peach cultivars are more susceptible to CI than early-season cultivars (Mitchell and Kader, 1989a), although as new cultivars are released from a new genetic pool, susceptibility to CI is becoming more random among new cultivars (Crisosto, 2002; Crisosto et al., 1999c). Expression of chilling injury symptoms develops faster and more intensely when susceptible fruit are stored at temperatures between 2.2° and 7.6°C (killing temperature zone) than when stored at 0°C or below but above their freezing point (Harding and Haller, 1934; Mitchell and Kader, 1989a). Therefore, market life is dramatically reduced when

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fruit are exposed to the killing temperature zone (Crisosto et al., 1999c). In addition, the severity of CI depends on the ripening stage at harvest: higher incidence was reported for 'Maycrest' peaches picked at more advanced ripening stages (Valero et al., 1997), although the opposite was found in other cultivars (Von Mollendorff et al., 1992).

Several treatments to delay and limit development of this disorder have been tested: controlled atmosphere (CA) environment, calcium applications, warming cold storage interruptions (Anderson, 1979; Garner et al., 2001; Nanos and Mitchell, 1991), plant growth regulators, and controlled delayed cooling. The major benefits of CA during storage/shipment are retention of fruit firmness and ground color. Conditions of 6% O₂ + 17% CO₂ at 0°C promote a modest reduction of internal breakdown during shipment of yellow-fleshed (Crisosto et al., 1999a) and white-fleshed cultivars (Garner et al., 2001). The CA efficacy depends on cultivar (Mitchell and Kader, 1989a), preharvest factors (Crisosto et al., 1997), temperature, fruit size (Crisosto et al., 1999a), marketing period, and shipping time (Crisosto et al., 1999c). Another tool that reduced CI in peach is modified atmosphere packaging (MAP). Thus, 'Paraguay', a flat type cultivar, had reduced CI severity when packed in polypropylene standard film with steady state atmosphere of 12% CO₂ and 4% O₂, or in oriented polypropylene with 23% CO₂ and 2% O₂ (Fernández-Trujillo et al., 1998). A preconditioning treatment (Crisosto et al., 2004) prior to storage/shipment delayed IB symptoms and is successfully being used commercially on Californian and Chilean fruit shipped to U.S. and English markets (Crisosto et al., 2004). The 'Paraguay' cultivar subjected to intermittent warming cycles of one day at 20°C for every six days of storage at 2°C also showed fewer CI symptoms, although scald and translucency occurred (Fernández-Trujillo and Artés, 1998).

Biological basis of chilling injury: Numerous studies on the biochemical basis for mealiness have identified factors which may be important in the development of the symptoms, although considerable discrepancy exists among studies. Mealiness has been associated with pectin methylesterase (PME) activity that was reduced (Buescher and Furmanski, 1978), increased (Ben-Arie and Sonogo, 1980), or unchanged (Obenland and Carroll, 2000; Zhou et al., 2000a). Similarly, mealy fruit had reduced exo-PG activity in some studies (Zhou et al., 2000a), but not in others (Artes et al., 1996). Reduced endo-PG activity during cold storage is commonly observed (Artes et al., 1996), although mealiness develops not in cold storage but during subsequent ripening at warm temperatures (Buescher and Furmanski, 1978). If, on the other hand, the cold period exceeds a certain critical length, or the ripening period is short, no increase in endo-PG activity occurs during ripening and mealiness results (Ben-Arie and Sonogo, 1980). During ripening, fruit may develop mealiness with low extractable juice, but upon extended ripening become juicy (Von Mollendorff and de Villiers, 1988; Von Mollendorff et al., 1989, 1993). However, this apparent restoration of free juice may be due to tissue breakdown and senescence. In mealy fruit, the most easily extractable cell wall pectins (those soluble in water or chelator) are less abundant and are of higher molecular weight and viscosity than in ripened, juicy fruit (Dawson et al., 1992; Lurie et al., 1994; Von Mollendorff and de Villiers, 1988). The degree of methylesterification of pectin may also be altered (Ben-Arie and Lavee, 1971; Lurie et al., 2003). Cell wall pectin participates in cell-to-cell adhesion through calcium cross-linking between partially de-methylesterified homogalacturonan in the middle lamella (Jarvis et al., 2003). Changes to pectin metabolism could cause mealiness either by cell fluids forming

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calcium-pectate gel complexes with high molecular weight pectin in the middle lamella (Ben-Arie and Lavee, 1971; Zhou et al., 2000b), or through decreased intercellular adhesion in mealy fruit which reduces cell rupture during biting and chewing, preventing release of cellular contents (Brummell et al., 2004). Other enzymes and cell wall proteins are being examined for links to the development of mealiness in peaches. Endo-1,4-glucanase activity and mRNA increases after chilling storage, unlike fruit from treatments which delayed the appearance of mealiness (Zhou et al., 2000c). Expansin protein and mRNA decreased as peaches became mealy (Obenland et al., 2003). Other enzymes, including endo-1,4- β -mannase, β -galactosidase, and α -arabinosidase, were less active in mealy fruit than in ripe, juicy fruit (Brummell et al., 2004).

The other CI symptom that gives fruit a dry texture and uneven ripening is leatheriness. Some fruits remain firm and others become soft after a few days at 20°C following cold storage. In general, soft fruit with dry texture are mealy (woolly), while firm fruit with dry texture are leathery (Luza et al., 1992). Under the electron microscope, leathery fruit had consistently thicker cell walls than mealy or juicy fruit (Luza et al., 1992). A study of three 'Huangjin' peach harvests, early, commercial, and late, found that early harvested fruit were most prone to leatheriness (Ju et al., 2000). These peaches did not soften as much as juicy or mealy fruit. The activities of ethylene synthesis pathway enzymes (ACC synthase and ACC oxidase) were lower in these fruit, as were the cell wall modifying enzymes PG and β -galactosidase, while insoluble pectin concentrations were higher than in juicy or mealy fruit. A more recent study examined cell wall polymer depolymerization, cell wall polysaccharide sugar composition, enzyme activity, and tissue physical characteristics during the development of leatheriness (Brummell et al., 2004). Increasing time of cold storage was used to induce increasingly severe CI symptoms and examine which changes in cell wall polymers or enzyme activities precede or accompany symptom developments. After three or four weeks in cold storage, some fruit developed a leathery texture. This was similar to mealiness, but with less free juice and more flesh browning, and the texture of the flesh was firm rather than grainy (Brummell et al., 2004). Fruit developing leatheriness had reduced chelator-soluble pectin concentrations and more tightly bound matrix PG, similar to mealy fruit. Leathery fruit showed a greater arrest of depolymerization than in mealy fruit, as indicated by the molecular weight distribution of chelator-soluble pectin. Leathery fruit possessed lower activities of exo-PG, endo-PG, endo-1,4- β -mannase, β -galactosidase, and α -arabinosidase than similar stored mealy fruit. The activities of PME and endo-1,4- β -glucanase were similar in fruits with both disorders (Brummell et al., 2004).

The appearance of brown discoloration in the fruit flesh, or flesh browning, occurs sooner at temperatures of 2°C to 5°C than at the optimal storage temperature of 0°C. In fact, less flesh browning developed at -0.5°C than at +0.5°C (Ceretta et al., 2000). The disorder may be related to tissue deterioration or senescence, which alter membrane permeability, and to the interaction between phenols and polyphenol oxidase, which are generally found in separate compartments in the cell. The browning potential of peaches depended on the total concentration of phenolic compounds and on the polyphenol oxidase activity (Kader and Chordas, 1984). The concentration of polyphenols differed among white-fleshed peach cultivars (Manabe et al., 1979). Controlled atmosphere (CA) storage alleviated or prevented the development of this disorder in some cultivars (Ceretta et al., 2000; Crisosto et al., 1995). During normal air storage,

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a lack of juiciness indicative of either mealiness or leatheriness is observed before flesh browning develops to any degree (Crisosto et al., 1999b).

Many peach and nectarine cultivars have cells near the stone that contain anthocyanin. When fruit is halved, these appear as red rays extending out from the center which contains the pit (flesh bleeding). Following extended storage, these sharply delineated rays disappear and the whole area around the pit has a reddish appearance. This red color can extend throughout the flesh when the disorder is severe. There has been almost no research conducted on the causes of this disorder. It may be a consequence of tissue senescence, since it is inversely correlated with decreased organic acids in the tissue (Lurie, unpublished) and CA storage prevented its development (Lurie et al., 1992). Alternatively, it may be a consequence of the arrest of normal ripening, since the ethylene action inhibitor, 1-methylcyclopropene (1-MCP), greatly enhanced the development of this disorder (Dong et al., 2001). However, application of exogenous ethylene during 0°C storage for 30 days did not affect the development of internal reddening or flesh bleeding (Crisosto et al., 2001).

Genotype influence on chilling injury: Peach is a self-pollinated diploid species ($2n=16$) with a small genome of approximately 5.9×10^8 base pairs/diploid nucleus, about twice the size of *Arabidopsis* (Arumuganathan et al., 1991). In spite of the small genome, progress in developing molecular markers has been very modest. The linkage maps in the diploid species peach and nectarine (*P. persica*, $2n=2x=16$), almond (*P. dulcis*, $2n=2x=16$), and sweet cherry (*P. avium*, $2n=2x=16$) are the most extensive (Arus et al., 1994; Chaparro et al., 1994; Dirlewanger and Bodo, 1994; Foolad et al., 1995; Rajapakse et al., 1995; Stockinger et al., 1996; and Viruel et al., 1995). There is a large variation among peach and nectarine selections and/or cultivars in susceptibility to CI when stored at either 0°C or 5°C (Mitchell and Kader, 1989). This variation may allow geneticists and breeders to understand the genetic inheritance of CI, isolate the genes responsible, and develop stone fruit cultivars free of CI. In a large-scale study in California, 25 nectarine and 32 peach cultivars were evaluated for storage potential. Early season yellow-fleshed cultivars were less susceptible to CI than later-season cultivars (Crisosto et al., 1999a; Lurie, unpublished), but white-fleshed cultivars were not. Among newly released cultivars developed from a new genetic pool, susceptibility to CI is random (Crisosto, 1999a; 2003; Crisosto, 2002). In general, nectarine cultivars were less susceptible to CI than peach cultivars. Also, melting flesh peach cultivars were more susceptible than those with firmer, non-melting flesh (Brovelli et al., 1998; Crisosto et al., 1999a). Non-melting flesh cultivars have reduced endo-PG activity (Lester et al., 1996). The genetic locus for freestone appears to contain a cluster of endo-PG genes (Callahan et al., 2004). Cold storage is a postharvest practice which is necessary to extend fruit shelf life but which can damage fruit firmness, texture, and color (Crisosto et al., 1999, 2006). Mealiness, browning, and bleeding are major symptoms of chilling injury, a physiological disorder occurring during cold storage or after subsequent ripening. Genetic inheritance of these three symptoms is quantitative and several related quantitative trait loci (QTLs) have been localized (Ogundiwin et al., 2007; Peace et al., 2006). A major QTL controlling both mealiness and bleeding co-located with the endoPG gene (Ogundiwin et al., 2007; Peace et al., 2005, 2006). The progeny population (Pop-DG) used to localize this QTL had only FMF and CNMF individuals. EndoPG co-segregated with FMF/CNMF in this population. Mealiness was observed exclusively in FMF progeny, while cold-induced bleeding was observed

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almost exclusively in CNMF progeny. This segregation pattern showed co-localization of both the mealiness and bleeding major QTL with endoPG. A new peach genetic linkage map (Pop-DG; Figure 2) that is co-linear with the *Prunus* reference 'Texas' × 'Earlygold' (T×E) map (Ogundiwin et al., 2007; Peace et al., 2005) has been developed. The genomic locations of over 50 fruit softening candidate genes have been determined either by fine mapping onto Pop-DG or bin mapping onto the T×E reference map (Peace et al., 2006). An extensive database of expressed genes during mealiness development known as "ChillPeach" has been developed (Ogundiwin et al., 2008b). This database contains 8,144 enriched, full-length expressed sequence tags (ESTs), out of which ~2,000 are new to *Prunus*. All sequences have been deposited to GenBank and GDR. Seventy ChillPeach SSRs novel to *Prunus* were mapped directly to the expanded Pop-DG map or bin-mapped to the T×E reference map. A ChillPeach microarray containing around 4,200 unigenes has been printed. This microarray has been used to identify genes differentially expressed between cold treated and normal peach mesocarp tissues (Ogundiwin et al., 2008b). Over 80 similarly differentially regulated ChillPeach unigenes were similarly mapped to the *Prunus* genome using Pop-DG and T×E maps (unpublished data). The positions of these mapped genes may coincide with known QTLs.

Major conclusions, solutions, achievements.

- Genetic basis for chilling injury, inheritance of the major CI symptoms mealiness, flesh browning, and flesh bleeding were examined over three years in two related peach progenie.
- A peach SNP map consists of 738 SNPs markers, distributed among 11 linkage groups. The linkage groups were covered with at least 15 markers (linkage group 8). This map covers >70% of the peach genome (estimated at 519 cM; Aranzana et al., 2003). The markers were well-dispersed throughout the genome of peach, and the average distance between markers was 1.02 cM.
- Numerous genes related to ethylene biosynthesis and its signal transduction, cell wall structure and metabolism, stress response, different transcription factor families were detected as being differentially accumulated in the cold-treated samples of the two cultivars.
- The ability to produce ethylene and keep active genes involved in ethylene signaling, GTP-binding protein, EIN-3 binding protein and an ethylene receptor and activation of ethylene-responsive fruit ripening genes during cold storage provided greater resistance to CI.
- The higher expression of the genes related to cell wall structure and its catabolism in the might enhanced prolonged low temperature storage. There were 22 genes involved in cell wall disassembly or structure and all of them were more up regulated in the less chilling susceptible. Three genes of pathogen inhibition involved in chitin degradation. Other

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genes included those involved in cell wall disassembly; five in pectin degradation, in hemicellulose degradation and three in glycan degradation, as well as cell wall synthesizing enzymes; xylosyltransferase, glucosyl transferase, and cellulose synthase.

- A recurring hypothesis to explain woolliness in peach supports that it results from disruption of the normal ripening process involving disassembly of cell wall material. This disruption results either from a defect in the program of cell wall degradation that occurs at low temperatures or from a difference in a basic structure of a cell wall.
- 20 genes related to *stress and cellular homeostasis* involved in antioxidant activity were differentially transcribed in the cold-treated and be higher in less chilling sensitive. Among these, 11 genes were related to glutathione-glutaredoxin and thioredoxin redox homeostasis (thioredoxin, glutathione S-transferase, glutathione peroxidase), 1 Cu/Zn superoxide dismutase and 1 Mn superoxide dismutase involved in dismutation of superoxide, 1 metallothionein-like protein involved in regulation of Zn levels and ROS scavenger, and 1 ascorbate peroxidase involved in peroxide detoxification. It appears that the higher expression levels of the antioxidant genes during cold storage might allow coping better with the low temperature storage and thus resulted in enhanced resistance to CI.
- 34 Transcription factors (TFs) genes were differentially expressed during cold-treated samples were higher in less chilling susceptible. These covered members of different transcription factor families and encompassed: 2 genes from MADS-box family, 3 genes from AP2/EREBP family, 1 gene from AUX/IAA family, 2 genes from bHLH family, 1 gene from LIM family, 1 gene from HB family, 2 genes from NAC family, 1 gene from MYB family and 1 gene from HD-ZIP family.
- Functional category of genes differentially expressed at harvest, less chilling sensitive cultivar had more genes in categories related to antioxidant and heat shock proteins/chaperones that may help fruit to adapt to low temperature stress.
- Interval mapping (IM) and multiple QTL model (MQM) mapping were utilized to detect any significance association between chilling injury symptoms and marker loci in the data sets. Two highly-significant QTL (qML1 and qML4) were detected for mealiness on LG1 and LG4, respectively. The qML1 was detected on the same region of the marker UCD_SNP_1032. The qML1 explained 75.2 of the observed phenotypic variance. The qML4 was detected on the region near marker UCD_SNP_1084 and explained 58.8% of the observed phenotypic variance for mealiness.
- QTLs have been identified in our peach SNPs mapping and proposed candidate genes obtained from the integrated result of phenotypic, biochemical and gene expression analysis are being located in our QTLs as an approach searching for consistent assistant markers for peach breeding programs.

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Implications, both scientific and agricultural.

Marker-Assisted Selection: Some important agronomic characters in fruit trees behave as major genes, including chilling injury. Their simple inheritance makes them obvious targets in the search for tightly linked markers for early selection, particularly for characters that require complex analysis that cannot be evaluated until the plant matures.

Molecular Biology Information: Finally, the results found in the present study provide a possible explanation to chilling resistance and suggest that resistance to chilling injury in peaches. Moreover, the ChillPeach microarray has established to be an important means for elucidating postharvest storage potential of peaches.

Mapping QTLs: Quantitatively inherited characters constitute most of the variability selected during the breeding process in fruit trees, as in most cultivated species. Characters related to plant growth and architecture, yield, blooming and harvesting times, and fruit quality are usually quantitative traits, and they have been analyzed with the help of molecular markers in several fruit species. An international consortium led by the Clemson University (Clemson, SC) has developed tools to characterize the *Prunus* genome. Using RFLPs on the TxE map and a BAC library of peach cv. Nemared, a physical map has been partially assembled.

Genomic Databases: Genomic databases are a collection of data arranged in a systematic way to be publicly searchable. Genomic data include nucleic acid and protein sequence variants (including neutral polymorphisms, susceptibility alleles to various phenotypes, and pathogenic mutations) and polymorphic haplotypes. The information comes from genomics, proteomics, metabolomics, microarray gene expression, and phylogenetics. It includes gene function, structure, localization (both cellular and chromosomal), clinical effects of mutations, similarities of biological sequences and structures, gene sequences, textual descriptions, attributes and ontology classifications, citations, and tabular data. These are often described as semi-structured data, and can be represented as tables, key delimited records, and XML structures. Cross-references among databases using accession numbers are common. The Genome Database for Rosaceae (GDR) is a curated and integrated web-based relational database supported by the NSF Plant Genome Program and by the USDA Specialty Crop Research Initiative. It contains comprehensive data on the genetically anchored peach physical map, an annotated peach EST database, Rosaceae maps and markers, and all publicly available Rosaceae sequences. It contains annotations of ESTs include contig assembly, putative function, simple sequence repeats, and anchored position to the peach physical map where applicable. An integrated map viewer allows graphical interface to the genetic, transcriptome, and physical mapping information. ESTs, BACs, and markers can be queried by category and the search result sites are linked to the integrated map viewer. Users can browse and query the database, then compare their sequences with annotated GDR sequences via a dedicated sequence similarity server running either the BLAST or FASTA algorithm. The goals of the GDR were (1) to develop an organized and integrated web resource for peach genomics data to facilitate gene discovery in other *Prunus* species through comparative mapping, (2) to collect and integrate all Rosaceae genomics data, and (3) to develop online tools and resources for the Rosaceae community. GDR will be

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expanded to include whole genome sequences and annotations for apple, peach, and strawberry, transcript data, metabolic pathways, large scale phenotype and genotype data, breeding data, controlled vocabularies, and new analysis tools, all implemented within the open source infrastructure.

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	2	4	1	7
Submitted, in review, in preparation	1		2	3
Invited review papers				
Book chapters		1		1
Books				
Master theses				
Ph.D. theses	1			1
Abstracts				
Not refereed (proceedings, reports, etc.)			1	1

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	Carlos Crisosto Eben Oguindiwin	Anu Dagar Susan Lurie		
Longer Visits (Sabbaticals)				

Description Cooperation:

The cooperation consisted of frequent consultation via e-mail and phone. During these teleconferences, issues about the conduct of the research and preliminary results were discussed. The teams used e-mail messaging as the most frequent means of communication. An exchange of protocols between the two teams for handling individual experiments continue – tissue sampling for cell wall proteome analysis, mapping, etc. Plant samples were sent from Israel to the US for genetic marker analysis. In addition, the Israeli's visited the US lab twice (each time different scientists) and the US scientists visited Israel once. The Israeli group had access to the microarray developed by the US group together with collaborators in Spain which they used for the

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data which are in the two papers being prepared. Recently, upon the completion of our SNPs-QTL mapping (chilling injury), paper in preparation, the Israeli group received access to this novel peach SNPs QTL mapping.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Publications supported by BARD

Dagar, A., Friedman, H. and Lurie, S. 2009. Thaumatin-like proteins accumulate at high levels in cold storage of peach and nectarines. *Postharvest Biology and Technology*, 57, 77-85.

Ogundiwin, E.A., C.P. Peace, T.M. Gradziel, D.E. Parfitt, F.A. Bliss, and C.H. Crisosto. 2009. A fruit quality gene map of *Prunus*. *BMC Genomics*.

Dagar, A., A. Weksler, H. Friedman, E.A. Ogundiwin, C.H. Crisosto, R. Ahmad and S. Lurie. 2011. Comparing ripening and storage characteristics of 'Oded' peach and its nectarine mutant 'Yuval'. *Postharvest Biology and Technology* 60:1-6.
Doi:10.1016/j.postharvbio.2010.11.002.

A. Dagar, A., Friedman, H., Lurie, S. and Crisosto, C.H. 2011. Utilization of ChillPeach microarray platform for comparing chilling injury susceptible 'Heremoza' and chilling injury resistant 'Oded' peaches. *Acta Hort.*

Martinez-Garcia, P.J., C.P. Peace, D.E. Parfitt, E.A. Ogundiwin, J. Fresnedo-Ramirez, A.M. Dandekar, T.M. Gradziel, C.H. Crisosto. 2011. Influence of year and genetic factors on chilling injury susceptibility in peach (*Prunus persica* (L.) Batsch). *Euphytica*. DOI 10.1007/s10681-011-0572-1.

Martinelli, Federico, C.H. Crisosto, and A. Dandekar. 2011. Genomics of Fruit Quality and Disorders. Chapter 8. *Omics Technologies: Tools for Food Science*. K11677_C008.indd.

Ahmad R, Parfitt D, Fass J, Ogundiwin E, Dhingra A, Gradziel T, Lin D, Joshi N, Martínez-García PJ, Crisosto C (2011) Whole genome sequencing of peach (*Prunus persica* L.) for SNP identification and selection. *BMC Genomics* 2011:12-569.

Pedro J. Martínez-García, Dan E. Parfitt, Ebenezer A. Ogundiwin, Joseph Fass, Helen M. Chan, Riaz Ahmad, Thomas M. Gradziel, and Carlos H. Crisosto. 2012. High Density SNP Mapping of Two Peach (*Prunus persica* L.) Populations and Evaluation of SNP Effects. *Tree Genetics and Genomes* (in press).

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- Dagar, A., Puig, C.P., Ibanez, C.M., Granell, A., Ziliotto, F., Bonghi, C., Crisosto, C.H., Friedman, H. and Lurie, S. 2012. Comparative transcript profiling of a peach and its nectarine mutant at harvest; differences in gene expression related to storability, (submitted).
- Anurag Dagar, A., Weksler, A., Friedman, H. and Lurie, S. 2012. Gibberellic acid (GA₃) application at the end of pit ripening: effect on ripening and storage of two harvests of 'September Snow' peach, (submitted).
- Pedro J. Martínez-García¹, D.E. Parfitt, E.A. Ogundiwin, A.M. Dandekar, T.M. Gradziel, Lurie, S., C.H. Crisosto. 2012. Mapping of QTLs associated with fruit quality characteristics using a high-density SNP map develop from a segregating population of peach (*Prunus persica* L.), (in preparation).