UNIVERSIDADE FEDERAL DE PELOTAS

Programa de Pós-Graduação em Ciência e Tecnologia Agroindustrial

Dissertação

LANOSIDADE EM PÊSSEGOS: ASPECTOS FÍSICO-QUÍMICOS E MOLECULARES

Camila Pegoraro

Pelotas, fevereiro de 2010

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CAMILA PEGORARO

Engenheira Agrônoma

LANOSIDADE EM PÊSSEGOS: ASPECTOS FÍSICO-QUÍMICOS E MOLECULARES

Dissertação apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia Agroindustrial da Universidade Federal de Pelotas como requisito parcial para obtenção do título de Mestre em Ciências (área do conhecimento: Ciência e Tecnologia Agroindustrial).

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Resumo

PEGORARO, CAMILA. **LANOSIDADE EM PÊSSEGOS: ASPECTOS FÍSICO-QUÍMICOS E MOLECULARES, 2010. 100f. Dissertação (Mestrado) –** Programa de Pós-Graduação em Ciência e Tecnologia Agroindustrial. Universidade Federal de Pelotas.

Estudou-se a expressão transcricional e traducional de um grupo de genes associados ao metabolismo de parede celular (*Cob, GLS, GalT, CCR, PL, PG, PME, β-Gal, β-Man, α-Ara, Exp1, Exp2, Exp3* e *Exp4*), transporte endomembranas (*SFT2, Vap27-2, ADL1A, ROC7, Rab5, Vamp, Kin, Cla, Syn, ERD2* e *Rab11*), expressão de HSPs (*HSP40-1er, HSP70er, HSP40-2er, HSPCNX1, HSPCRT2, HSP70ch, HSP17.8ch, HSP60mi, HSP26.5mi* e *HSP60-3Bmi*) e síntese de etileno (*ACCO*) em pêssegos tratados e não tratados com ácido giberélico (AG_3), monitorando-se a evolução da maturação e as variações ocorridas após o armazenamento em ar refrigerado (AR) e atmosfera controlada (AC). Como variáveis de caracterização do estádio de maturação dos frutos avaliou-se a produção de etileno, firmeza de polpa, acidez total (AT), sólidos solúveis (SS), relação SS/AT e coloração de fundo da epiderme. Inicialmente avaliaram-se alterações moleculares existentes em frutos em diferentes estádios de maturação. Posteriormente, estudou-se o efeito de diferentes formas de armazenamento sobre o desenvolvimento da lanosidade e, por fim, foi avaliado o efeito da aplicação de AG_3 na pré-colheita sobre o retardamento da maturação e na prevenção da lanosidade. Verificou-se que o amadurecimento de pêssegos é dependente da transcrição de genes codificadores para proteínas envolvidas na síntese e na degradação da parede celular e para proteínas envolvidas no transporte endomembranário. Ao analisarem-se diferentes formas de armazenamento observou-se que a utilização de atmosfera controlada reduziu a incidência de lanosidade, e ao contrário do esperado, verificou-se que a lanosidade não é ocasionada pela redução da expressão de genes relacionados com o transporte via endomembranas. A aplicação de AG_3 antes do endurecimento do caroço não retarda o amadurecimento, mas contribui para aumentar o tamanho e a massa dos frutos, e diminui a incidência de lanosidade. O aumento do tamanho pode ser explicado pela indução de genes codificadores de expansinas e a prevenção da lanosidade pode ser atribuída à indução de genes de proteção a estresses abiótiocos, com destaque para as HSPs.

Palavras-chave: amadurecimento, atmosfera controlada (AC), atmosfera refrigerada (AR), ácido giberélico (AG_3).

Abstract

PEGORARO, CAMILA. **WOOLLINESS IN PEACH: PHYSICAL-CHEMICAL AND MOLECULAR ASPECTS, 2010. 100f. Dissertation (Master Degree in Agroindustrial Science and Technology)**. Universidade Federal de Pelotas.

We studied the transcriptional and translational expression of a group of genes associated with cell wall metabolism (*Cob, GLS, GalT, CCR, PL, PG, PME, β-Gal, β-Man, α-Ara, Exp1, Exp2, Exp3* and *Exp4*), endomembrane transport (*SFT2, Vap27-2, ADL1A, ROC7, Rab5, Vamp, Kin, Cla, Syn, ERD2* and *Rab11*), expression of HSPs (*HSP40-1er, HSP70er, HSP40-2er, HSPCNX1, HSPCRT2 , HSP70ch, HSP17.8ch, HSP60mi, HSP26.5mi* and *HSP60-3Bmi*) and ethylene synthesis (*ACCO*) in peaches treated and not treated with gibberellic acid (GA_3), monitoring the evolution of ripening and the changes occurred after cold storage (CS) and controlled atmosphere (CA). As variables for characterization of ripening fruit evaluated the production of ethylene, firmness, total acidity (TA), soluble solids (SS), SS / TA ratio and background color of the skin. Initially molecular changes were evaluated in fruit at different maturation stages. Later, the effect of different storage conditions on woolliness development was studied, and finally, the effect of GA_3 in delaying maturation and preventing woolliness was evaluated. It was verified that peach ripening is dependent on genes encoding proteins involved in synthesis and degradation of cell wall and proteins involved in the endomembrane transport. Analyzing different forms of storage was observed that the use of controlled atmosphere conditions reduced woolliness, against all expectations, it was found that woolliness is not caused by the reduction in the expression of genes associated with the endomembrane transport. No delay ripening was observed when evaluating the effect of GA_3 application prior to pit hardening but help to increase the size and weight of fruit, and decreases the incidence of woolliness in peaches. Increase in fruit size could be explained by the induction of genes encoding expansins and woolliness prevention could be attributed to the induction of genes encoding proteins associated to stress abiotic response, especially HSPs.

Key-words: Ripening, controlled atmosphere (CA), cold storage (CS), gibberellic acid (GA_3).

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1. Introdução Geral

A produção mundial de pêssegos e nectarinas aumentou 4,4% ao ano na última década, atingindo 17,4 milhões de toneladas em 1,5 milhões de hectares, cultivados no ano de 2008. Em termos de produção mundial, o Brasil ocupa a décima segunda posição, produzindo 238,5 mil toneladas em uma área de 24,2 mil hectares (FAO, 2009). O Rio Grande do Sul é o principal produtor de pêssegos, com 50,5% da produção nacional, ocupando uma área de 14,9 mil hectares, seguido por São Paulo (20,7%), Minas Gerais (14,2%), Paraná (9,5%) e Santa Catarina (4,8%) (IBRAF, 2009). Dentre as cultivares mais plantadas no Rio Grande do Sul estão a Chiripá, a Chimarrita e a Granada (MEDEIROS; RASEIRA, 1998).

Pêssegos da cultivar Chiripá se caracterizam pelo formato redondo ovalado, sutura desenvolvida e ponta pequena, apresentando tamanho médio a grande, polpa branca, caroço aderente, epiderme com coloração de superfície avermelhada, cor de fundo esverdeada e alto potencial de acúmulo de açúcares, em média 15ºBrix (MEDEIROS; RASEIRA, 1998).

A cultivar Chimarrita é bastante difundida no Sul do Brasil devido à alta produtividade e a baixa necessidade de frio. É uma cultivar de polpa branca, fundente, firme e semi-aderente, para consumo *in natura*. O fruto é redondo, sem ponta, com sutura levemente desenvolvida. A colheita é feita entre o final de novembro e início de dezembro, sendo que os frutos apresentam, normalmente, massa superior a 100g e sólidos solúveis totais variando entre 12 e 15ºBrix (MEDEIROS; RASEIRA, 1998).

Pêssegos ‘Granada’ são frutos de dupla finalidade (*in natura* e indústria) em virtude da aparência, época de colheita e aceitação no mercado *in natura*. Esses frutos caracterizam-se pela forma redonda, sutura levemente desenvolvida e peso médio superior a 120g. Destacam-se pela firmeza, pelo tamanho e pela aparência

em relação às outras cultivares de mesma época de maturação. A epiderme é amarela com até 40% de vermelho. A polpa é firme, amarela, aderente ao caroço e de sabor levemente doce-ácido, com sólidos solúveis variando de 8º a 11ºBrix (MEDEIROS; RASEIRA, 1998).

Pêssegos são frutos climatéricos, que apresentam elevada velocidade metabólica, deteriorando-se rapidamente em temperatura ambiente (20-23ºC). Em função disso, o armazenamento refrigerado tem sido utilizado para reduzir o metabolismo (LURIE; CRISOSTO, 2005). Todavia, a temperatura e o período de armazenamento de pêssegos são limitados pela ocorrência de distúrbios fisiológicos como a lanosidade e o escurecimento interno (LURIE; CRISOSTO, 2005).

Os principais estudos de fisiologia de maturação realizados com pêssegos estão concentrados na busca do estabelecimento de condições de armazenagem, de conservação e de resolução de problemas relacionados a distúrbios fisiológicos, como a perda de suculência, a retenção de firmeza, o escurecimento interno e ocorrência de podridões durante o armazenamento refrigerado - principais causas de perda de qualidade sensorial em pêssegos (ARTES et al., 1996; CRISOSTO et al., 1999; Ju et al., 2000; ROMBALDI et al.; 2001, 2002, ZHOU et al., 2001; NAVA; BRACKMANN, 2002; BRACKMANN et al., 2003). Vários autores têm buscado explicar a ocorrência desses distúrbios e encontrar a solução para a problemática, mas, muitos resultados acabam entrando em contradição.

A lanosidade é um distúrbio fisiológico que afeta frutos de caroço que passaram por períodos de armazenamento a frio. Os sintomas são desenvolvidos durante o amadurecimento e se manifestam quando os frutos são retirados da câmara fria, geralmente no momento do consumo (INFANTE et al., 2009). Frutos lanosos caracterizam-se pela redução na suculência da polpa, redução não

decorrente da alteração no conteúdo de água (BEN-ARIE; LAVEE, 1971; ZHOU et al., 2000), mas do seu aprisionamento à fração péctica das paredes celulares (BEN-ARIE; SONEGO, 1980; OBENLAND et al., 2003; BRUMMELL et al., 2004). A incidência de lanosidade (*mealiness* ou *woolliness*) tem causado efeitos negativos na ampliação da área de produção e na infra estrutura de armazenamento de pêssegos de polpa branca, como o 'Chiripá' e o 'Chimarrita', ambos suscetíveis ao distúrbio fisiológico (ROMBALDI et al., 2002; GIRARDI et al., 2005). A prevenção da ocorrência do problema é pré-requisito para a manutenção dessas cultivares no mercado. Entretanto, nas cultivares de polpa amarela, a maioria é pouco suscetível à lanosidade, durante e após o armazenamento refrigerado (BRACKMANN et al., 2007).

Uma das formas de evitar o aparecimento da lanosidade é a diminuição do período de armazenamento, para isso, podem-se utilizar técnicas que visem o atraso da colheita através do retarmento do amadurecimento a campo. O ácido giberélico (AG_3), por exemplo, é um fitohormônio utilizado para atrasar o amadurecimento de pêssegos (JU et al., 1999; MARTINEZ-ROMERO et al., 2000; AMARANTE et al., 2005), nectarinas (ZILKAH et al., 1997), caqui (FERRI et al., 2004), tangerina (MARUR et al., 1999) e morango (MARTÍNEZ et al., 1994).

Em pós-colheita, tecnologias como o uso de atmosfera controlada, aquecimento intermitente, aplicação de etileno durante o armazenamento refrigerado (GIRARDI et al., 2005), condicionamento em baixas temperaturas combinado com a aplicação de metil jasmonato antes do armazenamento (JIN et al., 2009a), pré-condicionamento combinando ar quente com a aplicação de metil jasmonato (JIN et al., 2009b) e aplicação de ácido salicílico após a colheita (WANG et al., 2006) vêm sendo estudadas para a prevenção da lanosidade. Entretanto,

como esse distúrbio é dependente do genótipo (CRISOSTO et al., 1999), do manejo da cultura, da posição das plantas no pomar, do estádio de maturação na colheita, do tamanho do fruto (LURIE; CRISOSTO, 2005) e das variações sazonais (CAMPOS-VARGA et al., 2006), a resposta dos frutos submetidos a essas tecnologias é bastante diferenciada.

Estudos anteriores demonstravam que a lanosidade era resultante do amadurecimento anormal dos frutos devido a alterações na atividade de proteínas de degradação da parede celular, como expansinas, poligalacturonases, pectina metilesterases, pectato liases, beta-galactosidase, beta-manosidades e alfa-arabinofuranosidase (BRUMMELL et al., 2004; LURIE; CRISOSTO, 2005; CAMPOS-VARGAS et al., 2006). Recentemente, Gonzalez-Agüero et al. (2008) ao compararem transcriptomas de pêssegos com e sem lanosidade observaram que além dos genes envolvidos no metabolismo de parede celular, genes associados com o tráfego celular por vesículas também apresentavam expressão reduzida em frutos lanosos. Complementarmente Wang et al., (2006) e Sun et al., (2010) observaram que em frutos lanosos havia menor expressão de *heat shock proteins* (HSPs). Dessa forma, acredita-se que além dos genes associados com o metabolismo de parede celular, genes de transporte endomembrana e genes de HSPs também estão envolvidos com o desenvolvimento da lanosidade (Figura 1).

Em células eucarióticas a transcrição do RNA ocorre no núcleo e a síntese de proteínas acontece no retículo endoplasmático. As proteínas recém sintetizadas devem ser encaminhadas ao seu local apropriado de funcionamento dentro da célula. Quando as proteínas possuem peptídeo sinal de trânsito, como é o caso das proteínas que atuam na degradação da parede celular, o transporte é feito através de vesículas. As vesículas são formadas por brotamento, num processo chamado

de fissão. Existem dois tipos de revestimento, coatômero (COP I e COP II) e clatrina (Cla). Vesículas revestidas por clatrina mediam o transporte seletivo, transportando enzimas hidrolíticas do sítio *trans*-Golgi para o sistema vacuolar e outros compartimentos celulares. O revestimento se dissocia tão logo se forme a vesícula, deixando-a pronta para se fundir com a membrana-alvo. No transporte mediado por clatrinas há a participação de uma GTPase chamada dynamim like protein (ADL1A), que facilita a formação das vesículas e auxilia no transporte vesicular. ADL1A além da importância no tráfego vesicular, tem função regulatória (SEVER et al., 1999), recrutando ligações do maquinário endocítico, lipídeos, moléculas de sinalização e proteínas do citoesqueleto (MC NIVEN, et al.,2000).

As proteínas SNAREs situadas em vesículas (v-SNARE: Vap27-2 e Vamp 722) e membranas-alvo (t-SNARE: Syn) interagem para formar um complexo estável que mantém a vesícula perto da membrana alvo (PRATELLI et al., 2004). As proteínas Rabs são GTPases que regulam o transporte de forma específica, controlando a fusão da vesícula com a membrana alvo correta (ZAINAL et al., 1996). Em alguns momentos, essa fusão é mediada por outra GTPase (SFT2), porém essa proteína não é essencial para a fusão das vesículas (CONCHON, et al., 1999). Quando as proteínas são residentes no retículo endoplasmático, além do peptídeo sinal de trânsito presente na região N-terminal, essas proteínas possuem o motivo KDEL na região C-terminal, o qual é reconhecido por proteínas específicas (ERD2) (LEWIS et al, 1990). As kinesins são proteínas motoras envolvidas no transporte de organelas e vesículas (HIROKAWA, et al., 1998). Algumas kinesins se ligam á Rab6, e complexadas atuam no tráfego de membranas no complexo do Golgi para o retículo endoplasmático, promovendo a mobilidade das vesículas (ECHARD et al., 1998). Cyclophilins são proteínas ubíquas presentes em todos os compartimentos

subcelulares, envolvidas em muitos processos, incluindo o tráfego e maturação de proteínas (FERREIRA, et al., 1996), estabilização do complexo receptor (LEVERSON; NESS, 1998).

Proteínas de choque térmico ou chaperonas moleculares (*Heat Shock Proteins*-HSPs) são proteínas conservadas, induzidas por estresses de frio e/ou calor (LINDQUIST, 1986). HSPs possuem papel fundamental na manutenção da homeostase celular, uma vez que atuam na conformação de proteínas e direcionam proteínas mal formadas para a degradação (BOSTON et al., 1996).

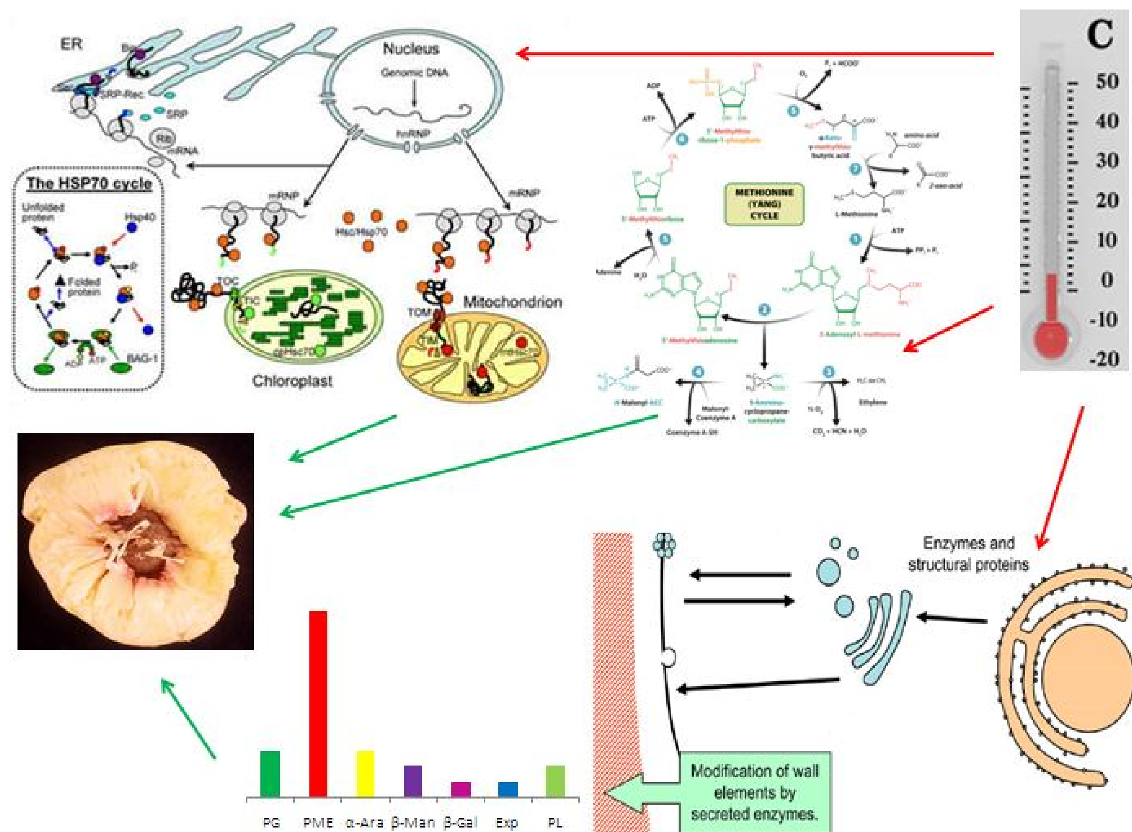

Figura 1: Esquema demonstrando a ação do frio sobre a síntese de etileno, transporte celular por vesículas, metabolismo de parede celular e HSPs. Essas alterações no metabolismo normal de amadurecimento são responsáveis pelo desenvolvimento de lanosidade.

Baseando-se na problemática descrita e nos dados disponíveis na literatura, acreditava-se que o melhor entendimento do processo de amadurecimento pudesse ajudar a compreender o mecanismo responsável pelo desenvolvimento de lanosidade. Além disso, partiu-se do pressuposto que esse distúrbio era ocasionado pela redução na expressão de genes codificadores de proteínas envolvidas no sistema endomembranas, comprometendo a secreção de componentes da parede celular, ocasionando alterações no metabolismo normal de maturação. Ao mesmo tempo, esperava-se que a aplicação de AG_3 na pré-colheita retardasse o processo de amadurecimento e atuasse na prevenção de lanosidade.

Neste contexto este trabalho foi estruturado em três etapas, que resultaram em observações publicadas nos seguintes artigos:

Artigo 1: “Differential expression of genes associated with endomembrane transport and cell wall metabolism during ripening of ‘Chimarrita’ and ‘Granada’ peach”

Artigo 2: “Transcript accumulation of cell wall metabolism and endomembrane transport genes and woolliness in peach”

Artigo 3: “Physiological and molecular changes associated with prevention of woolliness in peach following pre-harvest application of gibberellic acid”

ARTIGO 1

“Differential expression of genes associated with endomembrane transport and cell wall metabolism during ripening of ‘Chimarrita’ and ‘Granada’ peach”

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Title

Differential expression of genes associated with endomembrane transport and cell wall metabolism during ripening of 'Chimarrita' and 'Granada' peach

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Differential Expression of Genes Associated with Endomembrane Transport and Cell Wall Metabolism During Ripening of ‘Chimarrita’ and ‘Granada’ Peach

Abstract: Flesh firmness reduction, caused by enzymes acting on cell wall disassembly, is a typical indicator of fruit ripening in peach [*Prunus persica* (L.) Batsch]. Endomembrane transport through the endoplasmic reticulum, Golgi complex (GC) and vesicles may be important for fruit softening to occur since all genes putatively involved in dissolving cell wall polysaccharides have a transit peptide and several of these proteins are glycosylated in the GC. Differential expression of genes involved in endomembrane transport and cell wall metabolism, particularly in synthesis and degradation of cell wall macromolecules were studied during ripening of two peach cultivars, a white melting flesh cultivar, Chimarrita and a yellow non-melting flesh cultivar, Granada. In both, most genes were up regulated as ripening evolved. In ‘Chimarrita’, overall higher transcript accumulation occurred during the S3II stage, while in ‘Granada’ higher relative transcription was detected during S4I. Although transcript accumulation was correlated with fruit softening, the process does not seem to be totally dependent on the set of studied genes since the peak of RNA abundance occurred when flesh firmness had already decreased.

Keywords: Prunus persica, fruit softening, flesh firmness, transcript accumulation

Introduction

Peach [*Prunus persica* (L.) Batsch] is a climacteric fruit with a high metabolic rate and a short postharvest life. Chimarrita, a white melting flesh, and the yellow non-melting flesh Granada, are among the most widely cultivated and consumed peach cultivars in Southern Brazil. Although possessing different morphological and physiological characteristics, such as different ripening rates, both cultivars undergo fruit softening (Brackmann et al., 2003 and 2007).

Fruit softening or reduction in flesh firmness is one of the most easily perceived alterations during peach ripening in addition to chlorophyll degradation and increased ratio between total soluble solids and total acidity (Trainotti et al., 2003 and 2006, Crisosto et al., 2006, Di Santo et al., 2009). From a sensory perspective, flesh softening can be seen as positive since fruit become juicier and more palatable (Crisosto et al., 2006). However, it bring some limitation because they also become more susceptible to mechanical damage and decay (Heyes et al., 1996, Castillejo et al., 2004, Di Santo et al., 2009). Hence, breeding programs seek cultivars that can combine both sensory attributes and conservation potential after harvest.

Biochemical mechanisms controlling changes in flesh firmness involve the dissolution of cell wall polysaccharides by enzymatic and non-enzymatic proteins (Rose et al., 1998) such as expansins (Hayama et al., 2003), polygalacturonases (PG), pectin methylesterases (PME) (Di Santo et al., 2009), pectate lyases (PL) (Hayama et al., 2006) , β-galactosidases (Lee et al., 2003), α-L-arabinofuranosidases (Brummell, 2006), α-mannosidases (Brummell et al., 2004), and β-D-xylosidases (Di Santo et al., 2009). Various studies have shown ethylene induced transcription of *PL* (Sañudo-Barajas et al., 2009), *PG* (Nishiyama et al., 2007, Cara and Giovannoni, 2008) and *PME* (Cara and Giovannoni, 2008, Bennett and Labavitch, 2008). In peach (Trainotti et al., 2003), melon (*Cucumis melo*) (Nishiyama et al., 2007), tomato

(*Lycopersicon esculentum*) (Cara and Giovannoni, 2008), and strawberry (*Fragaria ananassa*) (Castillejo et al., 2004), flesh firmness reduction is coordinated by ethylene-dependent and ethylene-independent genes; although ethylene may accelerate the process, it is not essential for it to occur (Pech et al., 2008). Trainotti et al. 2003 and 2006 have shown evidence for differential gene transcription during peach ripening and association between changes in transcript levels with physiological responses (ethylene production and flesh firmness), suggesting a complex metabolism during ripening, with ethylene responsive genes being up and down-regulated and both cell wall catabolism and biosynthesis occurring. In addition, González-Agüero et al. 2008 showed a requirement for activation of genes involved in endomembrane transport, such as ER lumen protein retaining receptor 2, ROC7 cyclophilin, clathrin, and associated proteins (such as dynamin-like protein 1A, and proteins associated directly with vesicles such as SNARE-like protein, vesicle-associated membrane protein 722, Rab GTP-binding protein, and Golgi transport protein SFT2-like), in order to obtain adequate synthesis and transport of cell wall acting enzymes with resulting increase in peach juiciness and softening development. Since all genes putatively encoding proteins acting on cell wall polysaccharides have a signal peptide directing to the cell wall (Bayer et al., 2006, Zhu et al., 2006), and several of these proteins are glycosylated in the Golgi complex (GC) (Johnson et al., 2003), it is possible that endomembrane transport involving ER, GC, and vesicles is fundamental for softening to occur (Lycett, 2008). It is proposed that expression of genes in the transport pathway is essential to the adequate development of softening, which is a consequence of the action of proteins and enzymes such as PG, PME and PL involved in cell wall dissolution.

Materials and Methods

Plant Material

The two peach [*Prunus persica* (L.) Batsch] cultivars, Chimarrita and Granada, used in this study were six-year-old plants grafted on ‘Capdebosq’, arranged with 1.5m between plants, with a canopy trained to a Y trellis, located at Centro Agropecuário da Palma of UFPel, Capão do Leão, Rio Grande do Sul. Fruit were harvested at maturation stages S3I (85 days after anthesis), S3II (95 days after anthesis), and S4I (115 days after anthesis) as previously described by Zanchin et al. 1994.

Physicochemical analysis

Flesh firmness was determined in ten fruit using a hand-held penetrometer, with an 8 mm diameter probe. Each fruit was measured twice on opposite sides along the equatorial region after epidermal removal. An average was obtained for each fruit and results were expressed in Newtons (N).

Skin colour was determined using a colorimeter (Minolta 300, D65 with an 8 mm opening, CIE-Lab). Ten fruit were measured twice on opposite sides along the equatorial region. Hue angle (hº), was expressed as degrees using the formula $h^{o} = \tan^{-1} b^*/a^*$.

Soluble solids (SS) were measured using a digital refractometer and results were expressed in ºBrix.

Titratable acidity (TA) was measured diluting ten-fold 10 mL of juice of five fruit in distilled water and titrating with a 0.1 N NaOH solution until pH 8.1. Results were expressed in *meq* of acid 100 mL^{-1}.

SS/TA ratio was obtained from the quotient between the above measured variables.

Physicochemical data was analyzed by ANOVA in SAS (SAS Institute Inc, Cary, NC, USA).

Primer design

Oligonucleotides used here (Table 1) included relevant genes identified in the studies of González-Agüero et al. 2008 as well as other genes (pectin methylesterase, kinesin and syntaxin) based on selected sequences deposited in the National Center for Biotechnology Information, using *Vector*™ (Invitrogen, Carlsbad, CA, USA), according to an expected potential role in fruit ripening. Selection criteria were as follows: size between 20 and 26 bp; melting temperature between 60-65ºC; percent CG bases between 40 and 60%; average size of amplified fragments between 50 and 150 bp; and no more than two C or G bases among the last five nucleotides of the 3' end.

qPCR

Transcriptional expression was studied by quantitative PCR (qPCR). Total RNA extraction was performed using *Concert Plant Reagent* ™ (Invitrogen), followed by treatment with *DNAse I*™ (Invitrogen). cDNAs were obtained using *SuperScript III RT* ™ (Invitrogen). qPCR amplification with *Power kit SYBR Green Master Mix*™ (Applied Biosystems, Foster City, CA, USA), utilized 12.5 µL of *SYBR Green PCR Master Mix*, 2 µM of the oligonucleotide, 1 µL of cDNA (five-fold dilution) and the volume was brought to 25 µL with water. Samples were deposited in 96 well plates covered with optic adhesive, and then placed in a 7500 Real Time PCR System (Applied Biosystems). Reaction conditions were as follows: 50ºC for two minutes, 95ºC for ten minutes, 40 cycles of three stages: 95ºC for 30 seconds, 57ºC for one minutes and 72ºC for one minute, and final extension of 72ºC for five minutes, followed by a standard dissociation curve.

Data analysis

Optic data were analyzed using 7500 *System Software* (Applied Biosystems). Threshold cycle (CT) was obtained during the reaction cycles, and ΔΔCT was calculated based on the exponential PCR reaction using $REL=2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001). REL represents the level of relative expression, threshold cycle (CT) represents the amplification cycle when the sample has an exponential amplification, ΔCT is the difference between the sample CT for the target gene and the CT for the amplified sample of the constitutive gene *18S* (five-fold dilution), while ΔΔCT represents the difference between the sample ΔCT and the ΔCT of the reference sample (S3I). Gene expression data were analyzed using *Multi Experiment Viewer* (MeV), *EASE Expression Analysis Systematic Explorer* version 4.6 according to Saeed et al. 2003 and presented in a colour diagram using the S3I stage as baseline.

Results and Discussion

Fruit ripening stage is determined by attributes such as skin colour, flesh firmness, soluble solids (SS), total acidity (TA) and the ratio between SS/TA. Ripe fruits are usually less green, softer, have higher SS, lower TA and higher SS/TA. In this study, flesh firmness was higher in ‘Chimarrita’ indicating that ‘Granada’ ripened faster, but SS/TA ratio was also higher in ‘Chimarrita’ suggesting the opposite. The lower SS/TA ratio found in ‘Granada’ is a result of the normally high acidity found in this cultivar. Because each cultivar has a distinct skin colour, comparing the ripening level between cultivars based on this attribute is also complicated (Table 3). Therefore all the parameters discussed above serve as indicators of ripening only within a cultivar.

Although the measured variables did not allow comparisons, the molecular events leading to these physicochemical characteristics were similar. Expression of two groups of functional genes was evaluated during ripening of peach cultivars Chimarrita and Granada.

The first group consisted of genes involved in endomembrane transport and the second of genes involved in cell wall metabolism, particularly in synthesis and degradation of cell wall macromolecules. In ‘Chimarrita’ as well as in ‘Granada’ peach most of the studied genes had higher relative transcript levels as ripening developed (Figure 1). Such behavior was expected, since chosen genes had been previously shown to participate in endomembrane transport of proteins associated with cell wall disassembly (Trainotti et al., 2003 and 2006, González-Agüero et al. 2008).

In the case of ‘Chimarrita’ peach (Figure 1), the peak of transcript accumulation for most genes occurred in S3II and coincided with significant reduction in flesh firmness in the transition between S3II (76.98 N) and S4I (26.25 N) (Table 2). However, no up-regulation of *PG* and *PME* was observed with the advance of ripening, which contradicts most reports involving these genes and enzymes (Brummell et al., 2004, Trainotti et al., 2006). On the other hand, *PL*, another cell wall disassembly gene, was up-regulated in S3II suggesting participation in the softening process. *CCR, Cob* and *GLS*, encoding proteins acting on the synthesis of cell wall macromolecules (Ridley et al., 2001, Kawasaki et al., 2005, Brady et al., 2007, Töler et al., 2008), were up-regulated at S3II and S4I, while for *GalT* transcript accumulation increased at S3II but had a slight drop during S4I. Up-regulation of genes encoding structural proteins in the post-climacteric stage, when fruit have reduced flesh firmness, could be explained by the neutralizing action of these proteins during excessive cell wall degradation, preventing a possible cell wall break (Trainotti et al., 2003). *Roc7, Vamp, Rab5, Kin, SFT2, ERD2, ADL* and *Cla*, protein transport associated genes, exhibited an increase in transcript accumulation from stage S3I to S3II, while only *Roc7*, *Rab5*, *SFT2* maintained the levels through S4I. Although the transcript abundance of *Vamp*, *Kin*, *ERD2, ADL* and *Cla* during S4I had a slight drop when compared to S3II, their levels at S4I were higher than in S3I. Meanwhile, *Vap 27-2, Syn* and *Rab11* were up-regulated during S3II and

down-regulated in S4I. These results suggest an association between softening and the transport of proteins whose function is linked to cell wall disassembly.

Although a decline in flesh firmness occurred in 'Granada' peach (Figure 1) during the transition from S3I to S3II, the peak of transcript abundance occurred later during S4I. The highest transcript accumulation for endomembrane transport genes *Roc7*, *Vap 27-2*, *SFT-2 Vamp*, *Rab5, ERD2*, *ADL1A* and *Cla*, occurred at the S4I stage. During S3II, *Roc7*, *Vap 27-2*, *SFT-2, ERD2* and *Cla* had a relative transcript accumulation of up to 20-fold increase when compared to S3I, while *Vamp*, *Rab5*, *Rab11* and *ADL1A* showed an up-regulation of more than 20-fold. *Syn* and *Kyn* maintained the same transcript abundance during S3I and S3II, and during S4I only *Syn* showed increased transcript accumulation. Cell wall associated genes *Cob*, *CCR* and *GalT* exhibited up-regulation of about 20-fold from S3I to S3II, and expression levels in S4I went up approximately 500-fold. *GLS* was up-regulated (nearly 500-fold) in S4I only. *PG* and *PME* were up-regulated in S4I, when flesh firmness was already significantly reduced (Table 2). Among the cell wall disassembly genes only *PL* was up-regulated in S3II, reinforcing *PL* participation in the loss of flesh firmness during ripening. The lower relative transcript abundance for the other cell wall disassembly genes suggest either a secondary role for these genes during ripening or that expression levels seen in S3I for such genes are sufficient to induce softening.

In summary, evidence found here reinforces that development of fruit softening is a complex process yet to be completely understood. Better understanding of the molecular mechanisms associated with the ripening process will provide strategies to increase peach shelf-life. Cell wall disassembly during fruit softening is an event that involves genes associated with the vesicle transport system and cell wall synthesis and degradation, supporting the concept that genes associated with the endomembrane transport participate in the early stages of cell wall catabolism modulating flesh firmness. Although transcript

accumulation was correlated with fruit softening, the process does not seem to be totally dependent on the set of studied genes since the peak of RNA abundance occurred when flesh firmness had already decreased. In addition, the timing and level of transcript accumulation of genes involved in endomembrane transport and cell wall metabolism lead to differential reduction in flesh firmness for the studied cultivars.

Acknowledgements

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Literature Cited

Bayer, E.M., A.R. Bottrill, J. Walshaw, M. Vigouroux, M.J. Naldrett, C.L. Thomas and A.J. Maule. 2006. Arabidopsis cell wall proteome defined using multidimensional protein identification technology. Proteomics 6:301-311.

Bennett, A.B. and J.M. Labavitch. 2008. Ethylene and ripening-regulated expression and function of fruit cell wall modifying proteins. Plant Sci. 175:130-136.

Brackmann, A., C.A. Steffens and R.F.H. Giehl. 2003. Storage of 'Chimarrita'. peach in controlled atmosphere and under ethylene absorption. Ciência Rural 33:431-435.

Brackmann, A., B.C.S. Bordignon, R.F.H. Giehl, I. Sestari and A.C. Eisermann. 2007. Storage of cv. 'Granada' peach fruit in controlled atmosphere aiming the transport for long distances. Ciência Rural 37:676-681.

Brady, S.M., S. Song, K.S. Dhugga, J.A. Rafalski and P.N. Benfey. 2007. Combining expression and comparative evolutionary analysis, The *COBRA* gene family. Plant Physiol. 143:172-187.

Brummell, D.A. 2006. Cell wall disassembly in ripening fruit. Funct. Plant Biol. 33:103-119.

Brummell, D.A., V. Dal Cin, S. Lurie, C.H. Crisosto and J.M. Labavitch. 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. J. Exp. Bot. 55:2041-2052.

Cara, B. and J.J. Giovannoni. 2008. Molecular biology of ethylene during tomato fruit development and maturation. Plant Sci. 175:106-113.

Castillejo, C., J.I. De La Fuente, P. Iannetta, M.A. Botella and V. Valpuesta. 2004. Pectin esterase gene family in strawberry fruit: study of FaPE1, a ripening-specific isoform. J. Exp. Bot. 55:909-918.

Crisosto, C.H., G.M. Crisosto, G. Echeverria and J. Puy. 2006. Segregation of peach and nectarine (*Prunus persica* (L.) Batsch) cultivars according to their organoleptic characteristics. Postharvest Biol. Technol. 39:10-18.

Di Santo, M.C., E.A. Pagano and G.O. Sozzi. 2009. Differential expression of α-L-arabinofuranosidase and α-L-arabinofuranosidase/β-D-xylosidase genes during peach growth and ripening. Plant Physiol. Biochem. 47:562-569.

González-Agüero, M., L. Pavez, F. Ibánez, I. Pacheco, R. Campos-Vargas, L.A. Meisel, A. Orellana, J. Retamales, H. Silva, M. González and V. Cambiazo. 2008. Identification of woolliness response genes in peach fruit after post-harvest treatments. J. Exp. Bot. 59:1973-1986.

Hayama, H., A. Ito, T. Moriguchi and Y. Kashimura. 2003. Identification of a new expansin gene closely associated with peach fruit softening. Postharvest Biol. Technol. 29:1-10.

Hayama, H., T. Shimada, H. Fujii, A. Ito and Y. Kashimura. 2006. Ethylene-regulation of fruit softening and softening-related genes in peach. J. Exp. Bot. 57:4071-4077.

Heyes, J.A. and D.F. Sealey. 1996. Textural changes during nectarine (*Prunus persica)* development and ripening. Sci. Hortic. 65:49-58.

Johnson, K.L., B.J. Jones, C.J. Schultz and A. Bacic. 2003. Non-enzymic cell wall (glyco) proteins, p. 111-154. In: J.K.C. Rose (ed.). Annual plant reviews. Blackwell Publishing Ltd, Oxford, UK.

Kawasaki, T., H. Koita, T. Nakatsubo, K. Hasegawa, K. Wakabayashi, H. Takahashi, K. Umemura, T. Umezawa and K. Shimamoto. 2005. Cinnamoyl-CoA reductase, a key enzyme in lignin biosynthesis, is an effector of small GTPase Rac in defense signaling in rice. Proc. Natl. Acad. Sci. USA 103:230-235.

Lycett, G. 2008. The role of Rab GTPases in cell wall metabolism. J. Exp. Bot. 59:4061-4074.

Lee, D.H., S. Kang, S. Suh and K.B. Jae. 2003. Purification and characterization of a β-galactosidase from peach (*Prunus persica*). Mol. Cells 15:68-74.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25:402-408.

Nishiyama, K., M. Guis, J.K.C. Rose, Y. Kubo, A.K. Bennett, L. Wangjin, K. Kato, K. Ushijima, R. Nakano, A. Inaba, M. Bouzayen, A. Latche, J.C. Pech and A.B. Bennett. 2007. Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. J. Exp. Bot. 58:1281-1290.

Pech, J.C., M. Bouzayen and A. Latché. 2008. Climacteric fruit ripening: Ethylene-dependent and independent regulation of ripening pathways in melon fruit. Plant Sci. 175:114-120.

Ridley, B.L., M.A. O'neill and D. Mohnen. 2001. Pectins, structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry 57:929-967.

Rose, J.K.C., K.A. Hadfield, J.M. Labavitch and A.B. Bennett. 1998. Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. Plant Physiol. 117:345-361.

Saeed, A.I., V. Sharov, J. White, J. Li, W. Liang, N. Bhagabati, J. Braisted, M. Klapa, T. Currier, M. Thiagarajan, A. Sturn, M. Snuffin, A. Rezantsev, D. Popov, A. Ryltsov, E. Kostukovich, I. Borisovsky, Z. Liu, A. Vinsavich, V. Trush and J. Quackenbush. 2003. TM4: a free, open-source system for microarray data management and analysis. Biotechniques 34:374-378.

Sañudo-Barajas, J.A., J.M. Labavitch, C. Greve, T. Osuna-Enciso, D. Muy-Rangel and J. Siller-Cepeda. 2009. Cell wall disassembly during papaya softening: role of ethylene in changes in composition, pectin-derived oligomers (PDOs) production and wall hydrolases. Postharvest Biol. Technol. 51:158-167.

Töler, A., L. Browfield, C. Neu, D. Twell and P. Schulze-Lefert. 2008. Dual function of Arabidopsis glucan synthase-like genes GSL8 and GSL10 in male gametophyte development and plant growth. Plant J. 54:911-923.

Trainotti, L., C. Bonghi, F. Ziliotto, D. Zanin, A. Rasori, G. Casadoro, A. Ramina and P. Tonutti. 2006. The use of microarray μPEACH1.0 to investigate transcriptome changes during transition from pre climacteric to climacteric phase in peach fruit. Plant Sci. 170:606-613.

Trainotti, L., L. Zanin and G. Casadoro. 2003. A cell wall-oriented genomic approach reveals a new and unexpected complexity of the softening in peaches. J. Exp. Bot. 54:1821-1832.

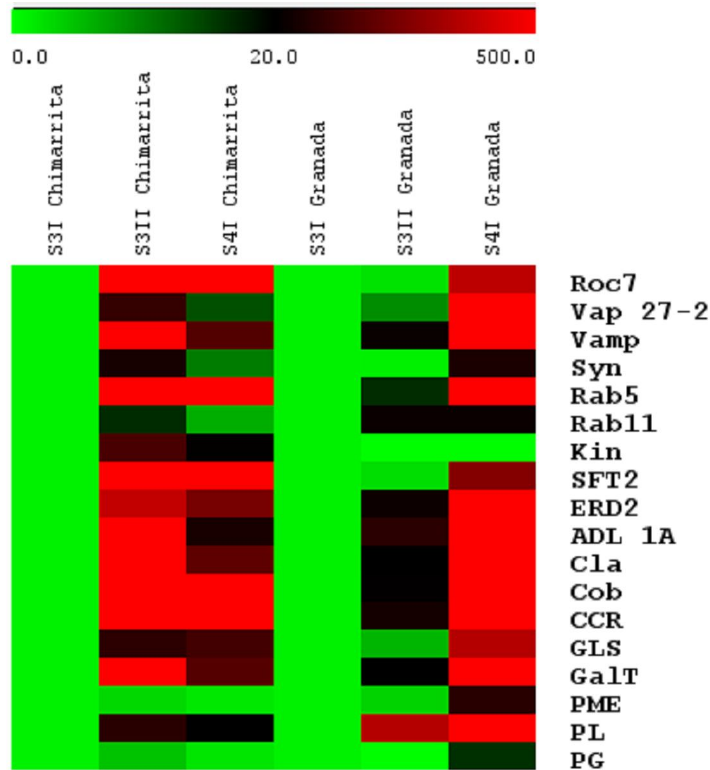
Zanchin, A., C. Bonghi, G. Casadoro, A. Ramina and N. Rascio. 1994. Cell enlargement and cell separation during peach fruit development. Int. J. Plant Sci. 155:49-56.

Zhu, H., B. Zhu, Y. Shao, X. Wang, X. Lin, Y. Xie, Y. Li, H. Gao and Y. Luo. 2006. Tomato fruit development and ripening are altered by the silencing of LeEIN2 gene. J. Integr. Plant Biol. 48:1478-1485.

Table 1. Specific primers used for quantitative PCR analysis of target genes.

Genes	GI	Forward	Reverse
Cell wall Metabolism			
Cobra(*Cob*)	22478530	ACTCATCCAGGAAGCTGTGTAG	ATGGCTGTATCATTTATTGTCGCA
Glucan Synthase (*GLS*)	22478796	TGGGAAACATGGTGGTATGAGGA	CGAGACATTTGAAGTGAGTGAAC
Galactosyl transferase (*GalT*)	22480478	ATGTGAAAAGTGGATGCGGAATG	TTGGATGAGAAGCGGGAAGAGA
Cinnamoyl-CoA reductase (*CCR*)	22481300	ATCAAGTCCAAGACCCCGAGAA	CGCCCAACACGGTGCCAGGA
Pectate lyase (*PL*)	22483439	GCCTTGCCGTACGCTCATGTC	CTTCAGCCTCAACCCCTTCCCT
Endopolygalacturonase (*PG*)	110293962	AAAGGGTGCCCTGGTCAGGTAAGATA	GCTCTTCTAGGTGGAAGCCCAAGAAA
Pectin methylesterase (*PME*)	1213628	AGGTGGCCTCCATTCTCTCTCAGTT	GGGAAGCAGAGAGAGACCAGTTCAA
Endomembrane Transport			
Golgi transport protein SFT2-like (*SFT2*)	22481199	CACTCA AAGGCCCGAAGAATCA	CAACGTCACCTCCCAAAACATC
SNARE-like protein (*Vap27-2*)	22481415	CCAAAGAAATACTGCGTGCGGC	GGAAGAGGGTGGGCTGATGAG
Dynamin-like protein1A (*ADL1A*)	22481576	GTGAACAAAATCCAAAGAGCTTG	GCCAGTTTCTCGATCTGTCTC
Roc7 cyclophilin (*Roc7*)	22481624	CCAGGCAAA GAAGTCAAAGGAG	TCACCTCCCTGAATCATGAAACT
Rab GTP-binding protein (*Rab5*)	22482805	CCATAGGTGCTGCCTTCTTCTC	CCATGTTTGGATTGCCTTGTGATT
Vesicle-associated membrane protein 722 (*Vamp*)	22483019	GCAAAGCAGGTGGTCTCAGG	TTAAGGCTATTGGCAGGGGCT
Kinesin (*Kin*)	51560894	ATCAAACGACCAAAGCGGGCTTA	AATTTCGCCACGAGAACCCACAT
Clathrin-binding protein b-adaptin (*Cla*)	22483877	CTTGGTGATCTGATTGGCATGG	ACTTGTGGAACCTGAAGGGGTC
Syntaxin (*Syn*)	56162812	CTGTGCAAGCTACCTCCCACCTT	GATCACTGCTCAAGCCACCACAA
ER lumen protein retaining receptor 2 (*ERD2*)	22477673	GCCAGTATTTTGGTCCTCCTTC	TCTTGAAATGTGAATTCCTCGTG
RabGTP-binding protein (*Rab11*)	22484722	ATGTTTGTAGGTTATTAGTCGCTTA	CGCTCTTGACCAGTTGTATCCCA
Endogenous Control			
18S	66627320	AAAACGACTCTCGGCAACGGATA	ATGGTTCACGGGATTCTGCAATT

Figure 1. Relative transcription level of genes putatively encoding proteins involved in endomembrane transport and cell wall metabolism in peach cultivars Chimarrita and Granada at different ripening stages (S3I - 85 days after anthesis, S3II - 95 days after anthesis and S4I - 115 days after anthesis).



mRNA abundance is represented, on a scale of 0 to 500, using the Multi Experiment Viewer (TIGR MeV) software. The lower end of the scale (light green colour) indicates the lowest level; black colour in the middle of the scale represents accumulation 20 times higher than the light green end; and red colour in the upper end of the scale represents the highest mRNA abundance, 500 times higher than the lowest. mRNA abundance of each gene S3I stage served as the baseline for determining relative RNA levels.

Table 2 – Total acidity (TA), soluble solids (SS), ratio SS/TA and flesh firmness in peach cultivars Chimarrita and Granada during the ripening stages S3I (85 days after anthesis), S3II (95 days after anthesis) e S4I (115 days after anthesis).

Cultivar	**Ripening Stage**	**TA (*meq* 100mL^{-1})**	**SS (ºBrix)**	**SS/TA**	**Firmness (N)**
Chimarrita	S3I	8.5a*	10.60a	19.48c	94.56a
	S3II	4.8b	10.60a	34.15b	76.98b
	S4I	4.2c	10.23a	38.10a	26.25c
Granada	S3I	16.6a	11.46a	11.34b	63.63a
	S3II	10.9b	12.06a	17.24a	28.92b
	S4I	9.6c	12.06a	18.53a	17.80c

* Means of treatment in the same column, followed by different letters, are statistically different according to Tukey's test ($p \leq 0.05$).

Table 3 – Peach skin colour (L*, a*, b*, h°) of cultivars Chimarrita and Granada during the ripening stages S3I (85 days after anthesis), S3II (95 days after anthesis) e S4I (115 days after anthesis).

Cultivar	**Ripening Stage**	**L***[1/]	**a***	**b***	**°h**
Chimarrita	S3I	65.94b[2/]	-12.36b	37.93a	107.24a
	S3II	71.80a	-9.24ab	36.15ab	104.24ab
	S4I	70.43a	-6.02a	35.20b	99.62b
Granada	S3I	66.55a	-7.00c	50.70c	97.99a
	S3II	65.38a	5.61b	55.71b	84.28b
	S4I	65.82a	11.89a	61.26a	79.02c

[1/]L* (0 = black, 100 = white); a* (+a = red, - a = green); b* (+b = yellow, - b = blue); hue angle hº (0º = red, 90º = yellow, 180º = green, 360º = blue).
[2/]Means of treatment in the same column, followed by different letters, are statistically different according to Tukey's test ($p \leq 0.05$).

ARTIGO 2

"*Transcript accumulation of cell wall metabolism and endomembrane transport genes and woolliness in peach*"

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Key Words	cold storage, chilling injury, woolliness, transcript abundance

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Title

Transcript accumulation of cell wall metabolism and endomembrane transport genes and woolliness in peach

Running Title

Cell wall metabolism and endomembrane transport in woolliness in peach

Authors

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Transcript accumulation of cell wall metabolism and endomembrane transport genes and woolliness in peach

Abstract

Quality of cold stored peach [*Prunus persica* (L.) Batsch] is negatively affected by woolliness, consequence of incomplete cell wall solubilization. Low transcript abundance of genes coding for proteins involved in the endomembrane transport in woolly fruit suggest involvement in woolliness development. In order to test this hypothesis, differential expression of genes involved in transport and cell wall metabolism, particularly in synthesis and hydrolysis of cell wall macromolecules were studied during ripening of cold stored peach cultivars Chimarrita (susceptible to wooliness) and Granada (not susceptible to wooliness). Contrary to expected, endomembrane transport and cell wall metabolism gene transcripts had high relative transcript accumulation in woolly 'Chimarrita' fruit. Although 'Granada' peach developed similar up-regulation, its transcript abundance was lower, suggesting that woolliness prevention is not dependent on the up-regulation of this group of genes.

Keywords: transcript abundance, chilling injury, woolliness, cold storage.

Introduction

Cold storage is one of the most employed methods for extending shelf-life of peach [*Prunus persica* (L.) Batsch]. However, prolonged storage can negatively affect fruit quality causing chilling injury (Lurie and Crisosto, 2005). Its occurrence is genotype specific (Crisosto et al., 1999), moreover it depends on management practices, orchard location, maturation stage, fruit size (Lurie and Crisosto, 2005) as well as on seasonal variations (Campos-Vargas et al., 2006). If harvested at an early maturation stage or stored for extended periods the problem may affect 100% of the fruit (Girardi et al., 2005). In melting flesh peach the most common symptom of chilling injury is woolliness, causing a reduction in the commercial value of the fruit (Rombaldi et al., 2002).

Several authors have sought to explain the causes of this physiological disorder (Brummell et al., 2004, Lurie and Crisosto, 2005, González-Agüero et al., 2008, Ogundiwin et al,. 2008, and Vizoso et al., 2009) and proposed solutions (Girardi et al., 2005, Wang et al., 2006, Jin et al., 2009a, Jin et al., 2009b), however the molecular mechanism leading to woolliness has not been completely understood. Characteristic physiological alterations are lack of juiciness, dry texture, and poor flavor (Arana et al., 2005).

Wooliness has been associated to incomplete solubilization of cell wall macromolecules, as a consequence of low levels of expansins and/or an imbalance in the expression and enzymatic activity of pectin methylesterases (PMEs), polygalacturonases (PGs) and pectate lyases (PLs) (Ben-Arie and Sonego, 1980, Zhou et al., 2000, Lurie et al., 2003, Obenland et al., 2003, Brummell et al., 2004). In addition, studies using differential transcriptomics indicated participation of other mechanisms in the development of this disturbance (Trainotti et al., 2003, 2006, González-Agüero et al., 2008, Ogundiwin et al., 2008 and Vizoso et al., 2009). Trainotti et al. (2003, 2006) studying normal peach ripening

observed an increase in the transcript accumulation of genes coding for proteins associated with the endomembrane transport. González-Agüero et al. (2008), found woolly fruit with low transcript accumulation of genes involved in vesicle transport and cell wall metabolism. Jin et al. (2009a and 2009b) showed the importance of antioxidant enzymes in woolliness prevention. Vizoso et al. (2009) observed alteration in the metabolism of carbohydrates, nucleic acids, aminoacids, lipids, secondary metabolites, in protein translation, cellular transport and signal transduction, and differential expression of genes coding for mitochondrial and plastidial proteins between woolly and juicy fruit. Wang et al. (2006), Ogundiwin et al. (2008) and Pegoraro et al. (unpublished results) related woolliness development to the low expression of genes coding for heat shock proteins (HSPs), suggesting involvement of these proteins in woolliness prevention.

Controlled atmosphere, intermittent warming, and ethylene application during cold storage (Girardi et al., 2005), pre-storage treatment with methyl jasmonate followed by cold storage (Jin et al., 2009a), pre-conditioning with methyl jasmonate and hot air (Jin et al., 2009b) and postharvest salicylic acid application (Wang et al., 2006) are all methods used to prevent woolliness.

Here we focused on quantifying the level of relative mRNA accumulation of candidate genes related to the endomembrane transport and cell wall metabolism in 'Chimarrita' (susceptible to woolliness) and 'Granada' (not susceptible to woolliness) peach stored under cold and controlled atmosphere conditions followed by ripening at room temperature. The hypothesis is that low temperatures down-regulate genes coding for proteins involved in the endomembrane transport leading to low transcript accumulation, affecting transport of proteins and cell wall components, preventing an adequate metabolism of the cell wall, and developing woolliness; while storage at room temperature or storage under controlled

atmosphere combined with cold storage induce up-regulation of the same genes preventing woolliness.

Materials and Methods

Six-year-old plants of two peach [*Prunus persica* (L.) Batsch] cultivars, Chimarrita and Granada, grafted on 'Capdebosq', arranged with 1.5m between plants, with a canopy trained to an Y trellis, located at Centro Agropecuário da Palma of UFPel, Capão do Leão, Rio Grande do Sul, Brasil, were used in this study. 'Chimarrita' peach was harvested with a flesh firmness of 83.61 N, soluble solids content of 11.27 ºBrix, 8.7 *meq* 100 mL^{-1} of organic acids and background color of 107.03ºh, while 'Granada' was harvested with a flesh firmness of 64.08N, soluble solids content of 11.50 ºBrix, 21.5 *meq* 100mL^{-1} of organic acids and background color of 102.96 ºh.

The experimental design was completely randomized, consisting of three treatments with two replicates: (1) control, storage at room temperature (23 ± 3Cº and 75% RH) during five days, (2) cold storage (CS) (4ºC ± 1 and 90% ± 5% RH) during 30 days, followed by five days at room temperature, (3) storage under controlled atmosphere combined with cold storage (CA/CS) (2% of O_2 and 8% of CO_2, 4ºC ± 1, 90% ± 5% RH,) during 30 days, followed by five days at room temperature; sample collections occurred at every 24 hours during storage at room temperature.

A set of 18 genes was selected based on their putative role in cell wall synthesis, recycling and solubilization, and vesicular transport (González-Agüero et al., 2008; Loraine et al., 1996; Trainotti et al., 2003 and 2006; Obenland et al., 2003; Park et al., 2006). Oligonucleotides used here (Table 1) were constructed based on selected sequences deposited on the *National Center for Biotechnology Information*, using *Vector*™ (Invitrogen, Carlsbad, CA, USA). Selection criteria were as follows: size between 20 and 26 bp; melting temperature

between 60-65ºC; percent CG bases between 40 and 60%; average size of amplified fragments between 50 and 150 bp; and no more than two CG bases among the last five nucleotides of the 3' end.

Relative mRNA level was studied by quantitative PCR (qPCR). Total RNA extraction was performed using *Concert* ™ *Plant Reagent* (Invitrogen), followed by treatment with DNAse I™ (Invitrogen). RNA purity was confirmed by PCR using oligonucleotides corresponding to *18S*. cDNAs were obtained using *SuperScript*™ III RT (Invitrogen). qPCR amplification, with Power kit SYBR Green Master Mix™ (Applied Biosystems), utilized 12.5 µL of SYBR Green PCR Master Mix, 2 µM of the oligonucleotide, 1 µL of cDNA and the volume was completed to 25 µL with water. Samples were deposited in 96 well plates (Applied Biosystems) covered with optic adhesive (Applied Biosystems), and then placed in a 7500 Real Time PCR System (Applied Biosystems). Reaction conditions were as follows: 50ºC for two minutes, 95ºC for ten minutes, 40 cycles of three stages (95ºC for 30 seconds, 57ºC for one minute and 72ºC for one minute), and final extension of 72ºC for five minutes, following a standard dissociation curve.

Optic data was analyzed using 7500 *System Software* (Applied Biosystems). Threshold cycle (CT) was obtained during the reaction cycles, and ΔΔCT was calculated based on the exponential PCR reaction using $REL=2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001). REL represents the level of relative expression, threshold cycle (CT) represents the amplification cycle when the sample has an exponential amplification, ΔCT is the difference between the sample CT for the target gene and the CT for the amplified sample of the constitutive gene *18S* (five-fold dilution), while ΔΔCT represents the difference between the sample ΔCT and the ΔCT of the reference sample (harvest = Control + 0d). Gene expression data was analyzed using *Multi Experiment Viewer* (MeV), *EASE Expression Analysis Systematic Explorer* version 4.6 according to Saeed et al. (2003) and presented in a color diagram using the harvest stage as

baseline. Woolliness was determined as percentage according to the method described by Girardi et al. (2005).

Results and Discussion

This study focused on cell wall metabolism and endomembrane transport gene transcript accumulation in peach cultivars Chimarrita and Granada stored under controlled atmosphere and cold temperature followed by ripening at room temperature.

As expected, cold stored woolliness susceptible ‘Chimarrita’ peach, showed high incidence (90%) of woolliness two days after being removed from the cold (Table 2). This disturbance was significantly prevented when ‘Chimarrita’ peach was stored under CA/CS, confirming the efficiency of this conservation method in woolliness prevention (Girardi et al., 2005, Brackmann et al., 2003). Control ‘Chimarrita’ and Control, CS and CA/CS ‘Granada’ peach ripened at RT, without previous cold storage, developed a normal ripening process and did not present woolliness, confirming well documented behavior for these cultivars (Crisosto et al., 1999, Lurie and Crisosto, 2005, Brackmann et al., 2007).

The consensus on woolliness development is that the problem is a consequence of incomplete solubilization of cell wall polysaccharides (Brummell et al., 2004; Lurie and Crisosto, 2005), derived from an imbalance in the activity and gene expression of PGs, PMEs, PLs, expansins, β-galactosidases, α-arabinosidase, β-mannosidase, and other enzymes (Ben-Arie and Sonego, 1980; Zhou et al., 2000; Obenland et al., 2003; Brummell et al., 2004). Here we verified an overall higher gene transcript accumulation for ‘Chimarrita’ (up to 500 fold increase) and for ‘Granada’ (up to 5 fold increase). The higher transcriptional activity in ‘Chimarrita’ peach, whether it had been stored under CS, CA/CS or RT, is in agreement with a higher respiration rate and ethylene production of ‘Chimarrita’ (Brackmann et al., 2003) when compared to ‘Granada’ peach (Brackmann et al., 2007).

Recent publications (González-Agüero et al., 2008, Ogundiwin et al., 2008 and Vizoso et al., 2009) , utilizing ESTs/microarrays identified groups of genes differentially expressed in woolly and juicy. Holistic studies by Trainotti et al. (2003, 2006) and González-Agüero et al. (2008) showed lower mRNA accumulation in CS fruit. González-Agüero et al. (2008) also down-regulation of genes associated with cell wall metabolism and vesicular transport in woolly fruit. In contrast, in this study woolly 'Chimarrita' fruit had high transcript accumulation of genes coding for vesicle transport and cell wall disassembly proteins.

Most of the candidate genes in CS 'Chimarrita' peach had a peak in transcript accumulation starting two days into ripening at room temperature (Figure 1) coinciding with woolliness development (Table 2). Meanwhile, for CA/CS and Control Chimarrita' peach, the peak in transcript accumulation occurred five days into ripening, when peach were starting to senesce. The post-storage up-regulation of these set of genes manifest molecular changes occurring during cold storage that lead to woolliness or woolliness prevention when under CA. A very similar trend was observed for 'Granada' fruit. An early peak in mRNA accumulation was observed in CS fruit while in Control and CA/CS the highest transcript accumulation was observed four days into ripening. Although 'Granada' did not develop woolliness it showed other symptoms of chilling injury such as internal browning.

Normally for most fruit, PG and PME activity increase during ripening, along with increase in fruit softening. Melting flesh peach stored under cold for extended periods has an imbalance in PG and PME activities resulting in woolliness. According to Ben-Arie and Sonego (1980), Zhou et al. (2000), Lurie and Crisosto (2005), Girardi et al. (2005) woolliness is characterized by high PME activity and low PG activity after storage. In our study *PG* transcript accumulation was low while *PME* showed high accumulation in Control, CS and CA/CS 'Granada'. The opposite occurred for 'Chimarrita'. CS, Control and CA/CS 'Chimarrita' peach presented high transcript accumulation of *PG* and low transcript

accumulation of *PME*, suggesting that PG and PME proteins are not the essential for woolliness development. These results are in agreement with Buescher and Furmanski (1978) who verified low PME activity in woolly fruit and Von Mollendorff and de Villiers (1988) who reported low PG activity during storage but increased activity during ripening of fruit that developed woolly characteristics. In addition, Artés et al. (1996) and Manganaris et al. (2008) did not find correlation between PG activity and woolliness development, or differences in PG and PME activity between juicy and woolly fruit.

Pectate lyase (PL) also participates in the cell wall dissolution process during fruit softening, having a fundamental role during peach softening (Trainotti et al., 2003), while the reduction of this enzyme activity can contribute to the development of woolliness in peach (González-Agüero et al., 2008). However, in the current work, *PL* showed high transcript accumulation in woolly fruit. This increased transcript accumulation of *PL* may be related to lower firmness observed in woolly fruit (Table 3), since transcript accumulation of *PG* and *PME* did not differ between fruit juicy and woolly.

Genes coding for Rab GTPases, proteins involved in the vesicle transport, are ethylene dependent and up-regulated during fruit ripening (Park et al., 2006, Abbal et al., 2008). According to Loraine et al. (1996) and Zainal et al. (1996) Rab GTPases regulate transport of enzymes that act on cell wall dissolution during softening. Participation of SNARE proteins is necessary in order to have correct vesicle fusion in the target membrane (Pratelli et al., 2004). González-Agüero et al. (2008) verified that woolly fruit presented a reduction in the transcript level of genes coding for Rabs and SNAREs. However, in the current work, genes coding for Vap 27-2, Vamp (v-SANREs), Syn (t-SNARE), Rab 5 and Rab 11 (GTPases) presented high transcript abundance in woolly fruit.

Non-protein molecules passing the Golgi complex are transported to other cell compartments through vesicles covered with clatrina, which require GTPase ADL1A action,

for its formation (Sever et al., 1999). In this work, however, *Cla* had high relative mRNA accumulation, while *ADL1A* had low transcript accumulation in woolly fruit, which indicates some alteration of normal transport.

Transport *ERD2*, *Roc 7* and *SFT2* and cell wall *Cob*, *CCR*, *GLS* and *GalT* had early high transcript accumulation in CS and late accumulation in CA/CS and Control for both cultivars. Meanwhile *Kin*, coding for the motor protein kinesin, had differential expression between cultivars. In 'Chimarrita' *Kin* had low transcript abundance in all treatments and in 'Granada' it was high in Control and CS.

In summary, results found here suggest that the studied set of genes encoding proteins involved in endomembrane transport and cell wall synthesis and solubilization are not essential to woolliness development in 'Chimarrita' peach. Moreover, 'Chimarrita' had 100 times higher transcript accumulation than 'Granada' in agreement with its higher metabolic rate. Finally, the early increase in transcript accumulation manifested during ripening after cold storage in both cultivars is indicative of molecular changes occurring during storage that lead to chilling injury.

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Literature Cited

Abbal, P., M. Pradal, L. Muniz, F. X. Sauvage, P. Chatelet, T. Ueda, and C. Tesniere. 2008. Molecular characterization and expression analysis of the Rab GTPase family in *Vitis vinifera* reveal the specific expression of a VvRabA protein. J. Exp. Bot. 59:2403-2416.

Artés, F., A. Cano, and J.P. Fernández-Trujjilo. 1996. Pectolytic enzyme activity during intermittent warming storage of peaches. J. Food Sci. 61:311-314.

Arana, I., C. Jarén, and S. Arazuri. 2005. Nectarine woolliness detection by non-destructive mechanical impact. Biosystems Engineering 90:37-45.

Ben-Arie, R. and L. Sonego. 1980. Pectolytic enzyme activity involved in woolly breakdown of stored peaches. Phytochemistry 19:2553-2555.

Brackmann, A., B.C.S. Bordignon, R.F.H. Giehl, I. Sestari, and A.C. Eisermann. 2007. Storage of cv. “Granada” peach fruit in controlled atmosphere aiming the transport for long distances. Ciência Rural 37:676-681.

Brackmann, A., C.A. Steffens, and R.F.H. Giehl. 2003. Storage of ‘Chimarrita’ peach in controlled atmosphere and under ethylene absorption. Ciência Rural 33:431-435.

Brummell, D.A., V. Dal Cin, S. Lurie, C.H. Crisosto, and J.M. Labavitch. 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. J. Exp. Bot. 55:2041-2052.

Buescher, R.W. and R.J. Furmanski. 1978. Role of pectinesterase and polygalacturonase in the formation of woolliness in peaches. J. Food Sci. 43:264-266.

Campos-Vargas, R., O. Becerra, R. Baeza-Yates, V. Cambiazo, M. González, L. Meisel, A. Orellana, J. Retamales, H. Silva, and B.G. Defilippi. 2006. Seasonal variation in the development of chilling injury in ‘O’Henry’ peaches. Sci Hortic 110:79-83.

Crisosto, C.H., F.G. Mitchell, and Z. Ju. 1999. Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. HortScience 34:1116-1118.

Girardi, C.L., A.R. Corrent, L. Lucchetta, M.R. Zanuzo, T.S.da Costa, A. Brackmann, R.M. Twyman, F.R. Nora, L. Nora, J.A. Silva, and C.V. Rombaldi. 2005. Effect of ethylene, intermittent warming and controlled atmosphere on postharvest quality and the occurrence of woolliness in peach (*Prunus persica* cv. Chiripá) during cold storage. Postharvest Biol. Technol. 38:25-33.

González-Agüero, M., L. Pavez, F. Ibáñez, I. Pacheco, R. Campos-Vargas, L.A. Meisel, A. Orellana, J. Retamales, H. Silva, M. González, and V. Cambiazo. 2008. Identification of woolliness response genes in peach fruit after post-harvest treatments. J. Exp. Bot. 59: 1973-1986.

Jin, P., K. Wang, H. Shang, J. Tong, and Y. Zheng. 2009a. Low-temperature conditioning combined with methyl jasmonate treatment reduces chilling injury of peach fruit. J. Sci. Food Agric. 89:1690-1696.

Jin, P., Y. Zheng, S. Tang, H. Rui, and C.Y. Wang. 2009b. A combination of hot air and methyl jasmonate vapor treatment alleviates chilling injury of peach fruit. Postharvest Biol. Technol. 52:24-29.

Livak, K.J., and T.D. Schittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402-408.

Loraine, A.E., S. Yalovsky, S. Fabry, and W. Gruissem. 1996. Tomato Rab1A homologs as molecular tools for studying Rab geranylgeranyl transferase in plant cells. Plant Physiol. 110:1337-1347.

Lurie, S., and C.H. Crisosto. 2005. Chilling injury in peach and nectarine. Postharvest Biol. Technol. 37:195-208.

Lurie, S., H.W. Zhou, A. Lers, L. Sonego, S. Alexandrov, and I. Shomer. 2003. Study of pectin esterase and changes in pectin methylation during normal and abnormal peach ripening. Plant Physiol. 119:287-294.

Manganaris, G.A., A.R. Vicente, C.H. Crisosto, and J.M. Labavitch. 2008. Cell wall modifications in chilling-injured plum fruit (*Prunus salicina*). Postharvest Biol.Technol. 48:77-83.

Obenland, D.M., C.H. Crisosto, and J.K.C. Rose. 2003. Expansin protein levels decline with the development of mealiness in peaches. Postharvest Biol Technol. 29:11-18.

Ogundiwin, E.A., C. Marti, J. Forment, C. Pons, A. Granell, T.M. Gradziel, C.P. Peace, and C.H. Crisosto. 2008. Development of ChillPeach genomic tools and identification of cold-responsive genes in peach fruit. Plant Mol.Biol. 68:379-397.

Park, S., N. Sugimoto, M.D. Larson, R. Beudry, and S. Nocker. 2006. Identification of genes with potential roles in apple fruit development and biochemistry through large-scale statistical analysis of expressed sequence tags. Plant Physiol. 141:811-824.

Pratelli, R., J.U. Sutter, and M.R. Blatt. 2004. A new catch in the SNARE. Plant Sci. 4:187-195.

Rombaldi, C.V., J.A. Silva, A. Parussolo, L. Lucchetta, M.R. Zanuzo, C.L. Girardi, R.F.F. Cantillano. 2002. Armazenamento de pêssegos (*Prunus persica* L.), cultivar Chiripá, em atmosfera controlada. Cienc. Rural. 31, 43-47.

Sever, S., A.B. Muhlberg, and S.L. Schmid. 1999. Impairment of dynamin's GAP domain stimulates receptor-mediated endocytosis. Nature 398:481-486.

Saeed A.I., V. Sharov, J. White, J. Li, W. Liang, N. Bhagabati, J. Brainsted, M. Klapa, T. Currier, M. Thiagarajan, A. Sturn, M. Snuffin, A. Rezantsev, D. Popov, A. Ryltsov, E. Kostukovich, I. Borisovski, Z. Liu, A. Vinsavichi, V. Trush, and J. Quackenbusch. 2003.

TM4: a free, open-source system for microarray data management and analysis. Biotechniques 34:374-378.

Trainotti, L., D. Zanin, and G. Casadoro. 2003. A cell wall-oriented genomic approach reveals a new and unexpected complexity of the softening in peaches. J. Exp. Bot. 54:1821-1832.

Trainotti, L., C. Bonghi, F. Ziliotto, D. Zanin, A. Rasori, G. Casadoro, A. Ramina, and P. Tonutti. 2006. The use of microarray μPEACH1.0 to investigate transcriptome changes during transition from pre-climacteric to climacteric phase in peach fruit. Plant Sci. 170:606-613.

Vizoso, P., L.A. Meisel, A. Tittarelli, M. Latorre, J. Saba, R. Caroca, J. Maldonado, V. Cambiazo, R. Campos-Vargas, M. Gonzalez, A. Orellana, and H. Silva. 2009. Comparative EST transcript profiling of peach fruits under different post-harvest conditions reveals candidate genes associated with peach fruit quality. BMC Genomics 10:410-423.

Von Mollendorf, L.J. and O.T. De Villiers. 1988. Role of pectolytic enzymes in the development of woolliness in peaches. J. Hortic. Sci. Biotechnol. 63:53-58.

Wang, L., S. Chen, W. Kong, S. Li, and D.D. Archbold. 2006. Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. Postharvest Biol. Technol. 41:244-251.

Zainal, Z. bin, G.A. Tucker, and G.W. Lycett. 1996. *A rab11*-like gene is developmentally regulated in ripening mango (*Mangifera indica* L.) fruit. Biochim Biophys. Acta. 1314:187-190.

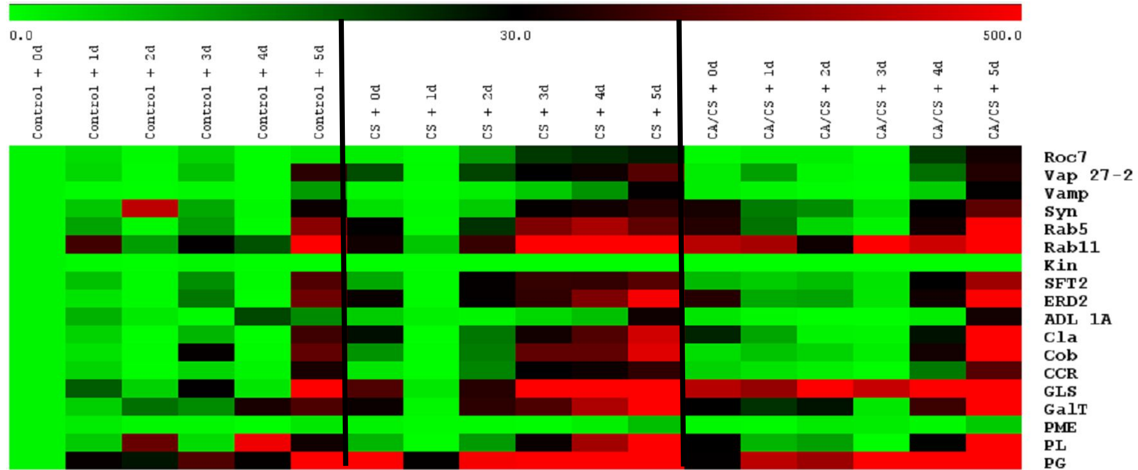
Zhou, H.W., S. Lurie, A. Lers, A. Khatchitski, L. Sonego, and R. Ben Arie. 2000. Delayed storage and controlled atmosphere storage of nectarines: two strategies to prevent woolliness. Postharvest Biol. Technol. 18:133-141.

Table 1. Specific primers used for quantitative PCR analysis of target genes

Genes	GI	Forward	Reverse
Endomembrane Transport			
Roc7 cyclophilin (*Roc7*)	22481624	CCAGGCAAA GAAGTCAAAGGAG	TCACCTCCCTGAATCATGAAACT
SNARE-like protein (*Vap27-2*)	22481415	CCAAAGAAATACTGCGTGCGGC	GGAAGAGGGTGGGCTGATGAG
Vesicle-associated membrane protein 722 (*Vamp*)	22483019	GCAAAGCAGGTGGTCTCAGG	TTAAGGCTATTGGCAGGGGCT
Syntaxin (*Syn*)	56162812	CTGTGCAAGCTACCTCCCACCTT	GATCACTGCTCAAGCCACCACAA
Rab GTP-binding protein (*Rab5*)	22482805	CCATAGGTGCTGCCTTCTTCTC	CCATGTTTGGATTGCCTTGTGATT
RabGTP-binding protein (*Rab11*)	22484722	ATGTTTGTAGGTTATTAGTCGCTTA	CGCTCTTGACCAGTTGTATCCCA
Kinesin (*Kin*)	51560894	ATCAAACGACCAAAGCGGGCTTA	AATTTCGCCACGAGAACCCACAT
Golgi transport protein SFT2-like (*SFT2*)	22481199	CACTCA AAGGCCCGAAGAATCA	CAACGTCACCTCCCAAAACATC
ER lumen protein retaining receptor 2 (*ERD2*)	22477673	GCCAGTATTTTGGTCCTCCTTC	TCTTGAAATGTGAATTCCTCGTG
Dynamin-like protein1A (*ADL1A*)	22481576	GTGAACAAAATCCAAAGAGCTTG	GCCAGTTTCTCGATCTGTCTC
Clathrin-binding protein b-adaptin (*Cla*)	22483877	CTTGGTGATCTGATTGGCATGG	ACTTGTGGAACCTGAAGGGGTC
Cell wall Metabolism			
Cobra (*Cob*)	22478530	ACTCATCCAGGAAGCTGTGTAG	ATGGCTGTATCATTTATTGTCGCA
Cinnamoyl-CoA reductase (*CCR*)	22481300	ATCAAGTCCAAGACCCCGAGAA	CGCCCA ACA CGGTGCCAGGA
Glucan Synthase (*GLS*)	22478796	TGGGAAACATGGTGGTATGAGGA	CGA GACATTTGAAGTGAGTGAAC
Galactosyl transferase (*GalT*)	22480478	ATGTGAAAAGTGGATGCGGAA TG	TTGGATGAGAAGCGGGAAGAGA
Pectin methylesterase (*PME*)	1213628	AGGTGGCCTCCATTCTCTCTCAGTT	GGGAAGCAGAGAGAGACCAGTTCAA
Pectate lyase (*PL*)	22483439	GCCTTGCCGTACGCTCATGTC	CTTCAGCCTCAACCCCTTCCCT
Endopolygalacturonase (*PG*)	110293962	AAAGGGTGCCCTGGTCAGGTAAGATA	GCTCTTCTAGGTGGAAGCCCAAGAAA
Endogenous Control			
18S	66627320	AAAACGACTCTCGGCAACGGATA	ATGGTTCACGGGATTCTGCAATT

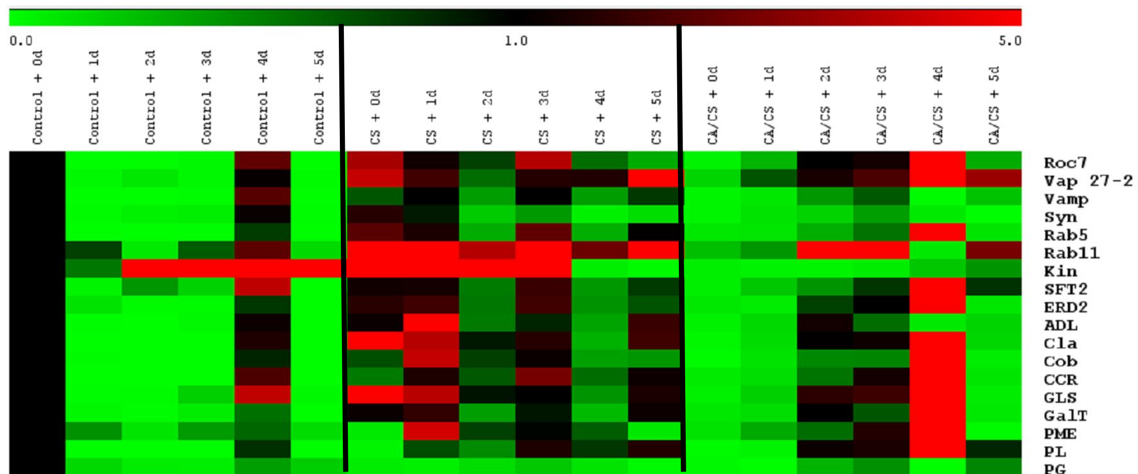
Figure 1. Relative accumlation of gene transcripts putatively coding for proteins involved in endomembrane transport and cell wall metabolism in peach cultivars Chimarrita (**a**) and Granada (**b**) stored under: room temperature (Control), cold storage (CS) and controlled atmosphere/cold storage (CA/CS) followed by ripening at room temperature for five days (0d, 1d, 2d, 3d, 5d)

a. cv. Chimarrita.



Transcript levels are shown on a 0 to 500 scale, in which green color indicates lowest expression level (0), black represents expression 30 times and red 500 times higher than the reference (Control + 0d).

b. cv. Granada



Transcript levels are shown on a 0 to 5 scale, in which green color indicates lowest expression level (0), black represents expression 1 time and red 5 times higher than the reference (Control + 0d).

Table 2. Woolliness incidence (%) in peach cultivars Chimarrita and Granada during five days of ripening at 23ºC after being cold storage with (CA/CS) and without controlled atmosphere (CS)

Cultivars	Storage	Woolliness (%)					
		Days stored at 23ºC after CS or CA/CS					
		0	1	2	3	4	5
Chimarrita	CS	0	20	90	90	80	20
	CA/CS	0	0	10	20	20	0
Granada	CS	0	0	0	0	0	0
	CA/CS	0	0	0	0	0	0

Table 3 – Flesh firmness (Newtons) of peach cultivars Chimarrita, and Granada ripened at room temperature during five days without prior storage (Control), with prior cold storage (CS) at 4ºC, 90% RH, during 30 days, and with prior controlled atmosphere combined with cold storage (CA/CS) 2% O_2 and 8% CO_2, 4ºC, 90% RH, during 30 days at. FAEM/UFPel, Capão do Leão, RS, 2008/09.

		Ripening days					
Cultivar	**Storage**	**0**	**1**	**2**	**3**	**4**	**5**
Chimarrita	Control	84.5 A a*	86.4 A a	75.1 A a	38.2 B a	13.5 C a	0.0 D a
	CS	66.1 A b	38.2 B b	0.0 C c	0.0 C b	0.0 C b	0.0 C a
	CA/CS	90.9 A a	78.3 A a	28.8 B b	6.7 C b	18.0 C ab	9.0 C a
Granada	Control	64.8 A a	47.7 B a	50.4 B a	41.8 B a	46.8 B a	26.1 C b
	CS	40.5 A a	28.3 B b	33.7 AB b	30.6 AB b	31.9 AB b	32.8 AB ab
	CA/CS	64.8 A a	53.1 B a	54.0 AB a	43.6 BC a	27.9 D b	40.0 C a

*Means of treatment within a cultivar in the same column, followed by the same lower case letter, and in the row by the same upper case letter, are not statistically different according to Tukey's test ($p \leq 0.05$).

ARTIGO 3

"*Physiological and molecular changes associated with prevention of woolliness in peach following pre-harvest application of gibberellic acid*"

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Physiological and molecular changes associated with prevention of woolliness in peach following pre-harvest application of gibberellic acid

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Physiological and molecular changes associated with prevention of woolliness in peach following pre-harvest application of gibberellic acid

Abstract

Peach [*Prunus persica* (L.) Batsch, cv. Chiripá] harvested from plants sprayed with gibberellic acid (GA_3), at the beginning (T1) and end of pit hardening (T2), kept under cold storage (CS) and controlled atmosphere (CA/CS), and from plants not sprayed with GA_3 (Control), kept under CS, were evaluated in terms of fruit size, ripening, occurrence of woolliness, and expression of supposedly related genes and proteins. Peach not sprayed with GA_3 and submitted to CS presented high incidence of woolliness, up-regulation of vesicle transport genes, down-regulation of genes associated with cell wall loosening, ethylene biosynthesis and heat shock protein (*HSPs*). Early GA_3 spraying did not delay ripening but induced increase in fruit size. In addition, it also induced a climacteric rise and prevented the occurrence of woolliness after CS. Meanwhile, woolliness prevention induced by either GA_3 or CA/CS treatment resulted in up-regulation of genes associated with cell wall metabolism, mitochondrial *HSPs*, and 1-aminocyclopropane-1-carboxylic acid oxidase (*ACCO*). A unique GA_3 response consisted of up-regulation of genes and/or proteins such as *HSP40-1er*, *HSP40-2er*, *HSPCTR2*, β-mannosidase (*β-Man*), α-arabinosidase (*α-Ara*) and *ADL1A*.

Keywords: GA_3; *Prunus persica*; ripening; cold storage; controlled atmosphere; ethylene; chilling injury.

Introduction

Chiripá peach [*Prunus persica* (L.) Batsch], a late maturing cultivar grown in Southern Brazil yields medium size white melting flesh fruit highly appreciated by consumers. The fruit is harvested in the summer, from mid December through mid January, and requires cold storage (CS) to achieve extended shelf-life. However, the occurrence of woolliness due to a prolonged period of CS is an important constraint for Chiripá peach growers (Rombaldi et al., 2002; Girardi et al., 2005). Woolliness occurs in almost all melting flesh cultivars of peach [*Prunus persica* (L.) Batsch] and nectarine [*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C. K. Schneid], and the prevention of this disorder is essential in order to maintain their marketability (Lurie and Crisosto, 2005; González-Agüero et al., 2008). This chilling injury, characterized by loss of juiciness, has been associated with abnormal cell wall disassembling during ripening (Zhou et al., 2000; Brummell et al., 2004; Girardi et al., 2005; Lurie and Crisosto, 2005; González-Agüero et al., 2008). Normal peach ripening involves a series of cell wall modifications and increased transcription of genes encoding proteins and enzymes and associated with the functionality of the endomembrane system (Trainotti et al., 2003; González-Agüero et al., 2008). Recently, González-Agüero et al. (2008) identified a set of genes differentially expressed in juicy and woolly peaches, including genes putatively involved in intracellular trafficking and cell wall metabolism that were repressed in woolly fruit. In addition, Vizoso et al. (2009) found differential expression of genes associated with plastids, mitochondria, endoplasmic membrane and ribosomes when comparing woolly and juicy fruit. Ogundiwin et al. (2008) also found genes controlling chilling injury differentially expressed between juicy and woolly fruit. In woolly fruit, commonly stress-induced genes, ripening related genes and genes involved in amino acid transport were up-regulated, while *HSPs* genes were down-regulated.

The use of controlled atmosphere (CA/CS), intermittent warming, or ethylene supply during CS are effective measures in preventing woolliness in Chiripá peach (Girardi et al., 2005). However, in contrast to CA/CS, the use of intermittent warming or ethylene supply during storage results in a high incidence of fruit decay (50 % and 25 % respectively) (Girardi et al., 2005). One strategy for extending the period of fruit availability and shelf-life is to delay the ripening process. For example, GA_3 has been shown to delay ripening in peach [*Prunus persica* (L.) Batsch] (Ju et al., 1999; Martinez-Romero et al., 2000; Amarante et al., 2005), nectarine [*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C.K. Schneid] (Zilkah et al., 1997), persimmon (*Diospyros kaki* Thunb.) (Ben-Arie et al., 1996; Ferri et al., 2004), tangerine (*Citrus reticulata* Blanco) (Marur et al., 1999) and strawberry (*Fragaria annanassa* Duch.) (Martínez et al., 1994). Responses to GA_3 action seem to vary according to the species and cultivar involved. GA_3 can induce hydrolytic cell wall enzymes, enhancing polysaccharide solubilization and favouring cell expansion (Thomas et al., 2005). GA_3 is also involved in the protection of the endomembrane system, regulating genes involved in its own biosynthetic pathway, or acting in cell wall disassembly (Hu et al., 2008). In other cases, GA_3 inhibits chlorophyll breakdown (Rosenvasser et al., 2006) and delays the onset of climacteric respiration (Ben-Arie et al., 1996) and the ripening process (Ferri et al., 2004). In addition, previous studies have indicated the stage of pit development as being pivotal in achieving physiological responses with the application of plant growth regulators such as GA_3 (Zilkah et al., 1997; Ju et al., 1999; Amarante et al., 2005).

The current study tested whether GA_3 application at a definite stage of pit development is effective in increasing fruit size and weight, delaying the ripening process, and preventing woolliness in Chiripá peach. Moreover, in order to gain further understanding of the molecular mechanisms associated with these physiological changes, the relative mRNA abundance of genes putatively associated with cell wall metabolism, intracellular trafficking,

heat shock proteins, and ethylene synthesis, as well as the expression of a set of proteins involved in cell wall metabolism and ethylene synthesis were investigated.

Materials and methods

Plant material and experimental design

A preliminary study (2006) was performed on a 6-year-old commercial peach [*Prunus persica* (L.) Batsch cv. Chiripá] orchard, planted in Farroupilha, Southern Brazil. In order to evaluate the effects of gibberellic acid (GA_3) on fruit growth and ripening, three replicates of twenty trees, selected based on size uniformity, were treated as follows: Control, without GA_3; T1 – spraying 400 L ha^{-1} of a GA_3 solution [50 mg L^{-1} of GA_3 (Proggib®) and 0.05 % (v/v) of surfactant (Silwet®), pH 4.5] at the beginning of the pit hardening stage (45 days after anthesis, DAA); T2 – spraying 400 L ha^{-1} of the same GA_3 solution at the end of the pit hardening stage (75 DAA). A similar stage characterization has been applied in previous studies (Zilkah et al., 1997; Ju et al., 1999; Amarante et al., 2005). For each treatment, 84 kg of peach (12 boxes with 7 kg of fruit) were harvested when fruit colour was light green, corresponding to the pre-climacteric stage, between S3 I and S3 II, described by Trainotti et al. (2003). Fruit were evaluated in terms of size (equatorial circumference), mass, skin colour, flesh firmness, soluble solids content (SSC), and ethylene production, and then cold stored at 1.0 °C (± 1.0 °C), 92.0 % (± 5.0 %) relative humidity (RH) for 30 days. Finally, the fruit were exposed to 23.0 °C (± 3.0 °C) and 75.0 % (± 5.0 %) RH for two days then evaluated for woolliness occurrence. Were considered woolly, those fruit that upon hand squeeze did not release any juice. For a detailed description of each analysis refer to Girardi et al. (2005).

A second experiment was carried out in the following year (2007), in which Control, (without GA_3) and T1 (GA_3 sprayed at the beginning of pit hardening) treatments were applied following the same experimental design on the same set of plants. At harvest, 168 kg

of peach (24 boxes with 7 kg of fruit) from Control and 84 kg of peach (12 boxes with 7 kg of fruit) from T1 were collected. Half of the Control fruit were kept under CS (Control CS) and the other half under controlled atmosphere (Control CA/CS) at 2 kPa of O_2, 5 kPa of CO_2, 1.0 °C (± 1.0 °C) and 92.0 % (± 5.0 %) RH (same temperature and humidity used for CS) while T1 fruit were stored under CS only (T1 CS). After 30 days of storage under CS or CA/CS conditions, fruit were exposed to 23.0 °C (± 3.0 °C) and 75 % (± 5 %) RH, for 6 h, 2, 4 and 6 days and then evaluated for mRNA and protein accumulation, ethylene production and occurrence of woolliness.

The experiments were performed in a completely randomized design. Percentage data were normalized according to the equation $f(x) = \text{arcsine } \sqrt{X}$. ANOVA was performed using the F test at the 5 % significance level. Means of treatments were compared using Duncan's test at the 5 % significance level. SANEST (Zonta and Machado, 1991) was used to perform statistical analysis.

Quantitative Real-Time PCR (q-PCR)

RNA was extracted from flesh of peaches following the protocol described for *PureLinK*™ reagent (*Plant RNA Reagent – Invitrogen*™). Total RNA was treated with DNAse I – *Invitrogen*™ and each sample was reverse transcribed into cDNAs using the commercial kit *SuperScript First-Strand System for RT-PCR* (*Invitrogen*™). The quantity and quality of the RNA and cDNA was assessed spectroscopically and by electrophoresis in agarose gel.

Genes from peach, tomato (*Lycopersicon esculentum* Mill.) and arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] putatively encoding proteins involved in cell wall metabolism, intracellular trafficking, heat shock proteins, and ethylene synthesis were selected based on previous work that showed an association between these metabolic

functions and woolliness (Pratt and Toft, 2003; Trainotti et al., 2003; Trainotti et al., 2006; González-Agüero et al., 2008; Iwata et al., 2008; Mueller et al., 2008; Su and Li, 2008; Deacon, 2009). Gene-specific primers were designed from sequences deposited in the GeneBank (Benson et al., 2005) using Vector NTI Advance™ 10 (Invitrogen, 2005). The criteria used for primer selection consisted of: amplicon size between 100 and 150 bp, CG content between 40 and 60 %, 3' ends with less than two C and G bases in the last five nucleotides, and melting temperature ranging from 60 to 65 °C according to Applied Biosystems® recommendations. The sizes of amplification products and their specificity were tested in agarose gels (2 %, w/v) prior to q-PCR. Melting curves were evaluated and only primers giving single peaks were used. Primers used in the current work are listed in Table 1.

q-PCR was performed with a 7500 Real-Time PCR System (Applied Biosystems®) using SYBR® Green. The amplification reaction was carried out in a total volume of 25 µL, containing 2 µM of each primer, 12.5 µL of PCR Master Mix SYBR® Green, 1 µL of cDNA (diluted 5-fold) and water to make up the final volume. Samples were loaded in 96 well optic plates (Applied Biosystems®) and covered with optic adhesives (Applied Biosystems®). Thermal cycle conditions were as follows: denaturing at 50 °C for 2 min and 95 °C for 10 min, followed by 40 three-step cycles (95 °C for 30 s, 57 °C for 1 min and 72 °C for 1 min) and final extension at 72 °C for 5 min. Relative quantification of each single gene expression was performed using the comparative threshold cycle method, as described by Livak and Schmittgen (2001). For each cDNA, *18S* was used as a reference gene to quantify cDNA abundance (at the same dilution as mentioned above). Threshold cycle (CT) was calculated based on the PCR exponential reaction obtained from the relative expression level (REL) formula, $REL = 2^{-\Delta\Delta CT}$. Results were expressed as mRNA abundance in a colour diagram using the *Multi Experiment Viewer* (TIGR MeV) software (Saeed et al., 2003).

mRNA abundance of each gene from Control CS fruit at 6 h served as the baseline for determining relative RNA levels.

Protein immunodetection

Immunodetection of proteins was performed by Western blotting using mouse polyclonal antibodies produced against the recombinant proteins endopolygalacturonase (anti-PG, gi110293962), pectin methylesterase (anti-PME, gi1213628), β-galactosidase (anti-β-Gal, gi157313305), pectate lyase (anti-PL, gi22483439), endo-1,4-β-mannase (anti-β-Man, gi157313309) and α-arabinofuranosidase (anti-α-Ara, gi145338352) expressed by vector pAE (Ramos et al., 2004) and ACC oxidase (anti-ACCO, gi33329719) as previously described by Rombaldi et al. (1994). Frozen peach pulp (3.0 g) was ground into powder and extracted with a solution containing 1.5 mL of 280 mM Tris-HCl (pH 8.3), 0.5 M DTT, glycerol (20 % v/v) and SDS (4 % w/v). β-mercaptoethanol (10 % v/v) was added just before heating the samples at 80 °C for 10 min. Samples were then cooled at 4 °C and centrifuged at 14 000 x *g* for 30 min. Protein quantification was performed according to Bradford (1976) method following precipitation with trichloroacetic acid and solubilization with 0.1 M sodium hydroxide. Equal amounts of total protein (30 μg) were loaded per gel slot onto a denaturing, 1 mm thick, polyacrylamide gel (12 % w/v), according to Sambrook et al. (1989), and run for 30 min at 90 V followed by one hour at 150 V. Proteins were transferred from the polyacrylamide gel to a nitrocellulose membrane (Hybond ECL, GE Healthcare®) using a gel blotter (Bio-Rad), at 4 °C ± 2 °C. The efficacy of the transfer was monitored by colour using Ponceau’s reagent. The membrane was washed with a Tris-buffered saline Tween solution [TBS-T, 20 mM Tris, 137 mM NaCl and Tween-20 (0.1 % v/v), pH 7.6] for one hour at room temperature (RT) with agitation and blocked with ECL Advance Blocking® agent (2 % w/v) in TBS-T. Primary antibodies were diluted in TBS-T at 1:2,000 (anti-β-Man and anti-α-Ara), 1:5,000 (anti-PL,

anti β-Gal and anti-ACCO) and 1:10,000 (anti-PG and anti-PME) and incubated for one hour at RT. Membranes were washed three times for 10 min with TBS-T and incubated for one hour at RT with a 1:50 000 dilution of the peroxidase-labelled rabbit anti-mouse antibody (GE Healthcare®). Membranes were then washed with water and developed using the GE Healthcare® Kit (ECL Advance Western® blotting detection reagents), with an exposure time of about 10 seconds.

Preparation of antibodies

Total RNA was extracted from 50 mg of flesh from Chiripá peach at S1, S2, S3 and S4 ripening stages using Concert Plant RNA Reagent (Invitrogen, USA, catalogue # 12323-012) to produce polyclonal antibodies. The RNAs were mixed and 5 µg was reverse-transcribed using SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen, USA, catalogue # 18080-051). cDNA sequencing was performed using the Applied Biosystems Automated 3730 DNA Analyzer.

The *Bam*HI restriction site was added at the sense end and the *Kpn*I restriction site and stop codon TGA at the antisense end, and additionally the stop codon TCA. The PCR conditions were: denaturing at 95 °C for 3 min, followed by 30 cycles at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 90 s, followed by one cycle at 72 °C for 10 min. The fragments amplified by PCR were purified using the GFX™ PCR DNA system and the Gel Band Purification system (Amersham Bioscience®) and analyzed by electrophoreses in agarose gel (0.8 % w/w). The purified product was sequenced, cloned using the vector pCR2.1-TOPO-TA (Invitrogen®) and transferred to the expression vector pAE (Ramos et al., 2004). Each cloning was carried in duplicate and resequenced prior to *Escherichia coli* BL21 pLyss transformation. Recombinant proteins were affinity-purified on a HisTrap column ÄKTA prime (Amersham Biosciences®) then inoculated into mouse to

obtain the polyclonal antibodies. A solution containing 100 μg of each purified protein in 0.05 mL of sterile 200 mM phosphate buffer (pH 8.0), containing NaCl 0.5 M and imidazole 0.005 M, was mixed with aluminium hydroxide (15 % v/w) prior to intramuscular injection. The second dose was given 21 days later. Serum was collected 63 days after the second injection and immunopurified against the respective recombinant proteins. The specificity and sensitivity of the antibodies was tested against recombinant proteins and total peach protein extracts from the S1, S2, S3 and S4 ripening stages. The antibodies recognized their recombinant protein and reacted specifically with one or few background bands in 30 μg of total peach proteins. Antibody recognition occurred at the same conditions of Western blot, 0.005 pg of recombinant PME, PG, β-Gal, PL, ACCO and 0.025 pg of β-Man and α-Ara protein.

Results

In order to extend the ripening process and increase fruit size, Chiripá peach was treated with gibberellic acid (GA_3) at the beginning and end of the pit hardening stage. Pre-harvest GA_3 spray did not delay ripening of Chiripá peach (Table 2). Fruit from all treatments reached the same ripening stage at the same time without showing any differences in colour (hue angle from 98.12 to 102.01), flesh firmness (ranging from 48.14 to 52.25 N), soluble solids content (ranging from 11.82 to 12.23 %) or ethylene production. At harvest, fruit showed a pre-climacteric ripening equivalent to the S3 I or S3 II stage (Trainotti et al., 2003), with low ethylene production ranging from 0.86 to 1.26 nL g^{-1} h^{-1}. Pre-harvest spraying of GA_3 at the beginning of pit hardening (T1) resulted in a significant increase in fruit size and weight compared to untreated fruit (Control); leading to a 47 % gain in productivity per plant. However, postponing GA_3 application to the end of pit hardening (T2) did not contribute to an increase in fruit size or weight.

Larger fruit, from early GA_3 treatment (T1), showed lower incidence of woolliness when compared to untreated fruit (Control) or late GA_3 treatment (T2) (Table 2). Incidence of woolliness in peach from control treatment (without GA_3) was high after 30 days of cold storage (CS), reaching 85 % of the fruit after 2 days of ripening at room temperature. In contrast, fruit that received GA_3 treatment at the beginning of pit hardening (T1) and followed 30 days of CS presented lower levels of woolliness (12.3 %). However, when treated with GA_3 at the end of pit hardening (T2) woolliness incidence did not differ statistically from the untreated control.

Storage under controlled atmosphere (Control CA/CS), as expected, prevented woolliness (Table 3). Woolliness occurrence in GA_3-treated peach stored under cold (T1 CS) was as low as 29 % and did not differ statistically from woolliness occurrence in untreated peach stored under CA/CS. The highest percentage of woolly fruit (94.2 %) was observed in untreated peach stored under cold during the second and fourth days of ripening. Upon extension of the ripening period, it was observed that woolly fruit became juicy again.

Fruit from treatments with lower occurrence of woolliness (Control CA/CS and T1 CS) developed a classical climacteric behaviour during ripening, while fruit with high occurrence of woolliness (Control CS) did not develop climacteric production of ethylene (Table 4).
In order to assess the effects of pre-harvest GA_3 treatment and storage under CA/CS on woolliness prevention, transcript abundance of a set of 36 genes associated with cell wall structure and disassembly (14), intracellular transport (11), chaperones (heat shock proteins) (10) and ethylene biosynthesis (1) were studied (Fig. 1).

T1 CS and Control CA/CS showed higher relative expression of expansins (*Exp1, Exp2 and Exp3*), *PME*, *PG*, *PL*, *β-Gal*, *β-Man*, and heat shock proteins (*HSP40-1er*, *HSP40-2er*, *HSP70er, HSPCNX1, HSP17.8ch*, *HSP60mi*, *HSP60-3Bmi* and *HSP26.5mi*).

mRNA accumulation of *HSP40-1er*, *HSP40-2er*, *HSPCRT2*, *HSP17.8ch*, *Exp4, β-Man, α-Ara*, *GalT* and *ADL1A*, positively related to woolliness prevention, had different transcript profiles between Control CA/CS and T1 CS. While *HSPs*, *ADL1A*, and *α-Ara* and *β-Man* showed higher relative expression in GA_3-treated peach, *Exp4* and *GalT* expression was high in Control CA/CS peach.

mRNA accumulation of transport associated genes *Roc7*, *ERD2*, *Cla*, *SFT2*, *Kin,* of *HSP70ch* and of cell wall structure associated *Cob*, *GLS*, and *CCR*, were generally unaffected by GA_3 treatment or by storage condition during ripening but *Vap27-2*, *Vamp, Syn*, *Rab5* and *Rab11*, vesicle transport associated genes, showed higher relative expression of transcripts in woolly fruit (Control CS).

In woolly fruit (Control CS), *ACCO* transcript accumulation was lower than in untreated CA/CS and T1 CS fruit (Fig. 1) and protein was not detected (Fig. 2). In contrast, high relative accumulation of *ACCO* transcripts and protein were detected and classic climacteric behaviour was observed in Control CA/CS and T1 CS peach.

In woolly fruit all measured proteins were detected in lower amounts (Fig. 2). PME, PG, β-Gal and PL proteins (Fig. 2) were detected during ripening at room temperature in treatments preventing woolliness (Control CA/CS and T1 CS) before the onset of the climacteric stage (Table 4). The differences between protein accumulation between Control CA/CS and T1 CS rely on PL which started to accumulate later in Control CA/CS when compared to T1 CS and PME which was absent during day 4th in T1 CS and not in Control CA/CS. Moreover β-Man and α-Ara proteins were only detected during days 4 and 6 during ripening on gibberellic acid treated fruit (Fig. 2).

Discussion

Pre-harvest application of GA_3 to peach (Zilkah et al., 1997; Ju et al., 1999; Martinez-Romero et al., 2000; Amarante et al., 2005) and other fruit, such as persimmon (*Diospyros kaki* L.) (Ben-Arie et al., 1996; Ferri et al., 2004), tangerine (*Citrus reticulata* L.) (Marur et al., 1999) and cherry (*Prunus avium* L.) (Kappel and MacDonald, 2002; Usenik et al., 2005) can extend ripening process and as consequence in some cases increase fruit size. In this study, pre-harvest spray with GA_3 did not extend ripening process of Chiripá peach but induced increase in fruit size. Fruit from all treatments reached the same ripening stage at the same time without showing any differences in physicochemical characteristics (Table 2). At harvest, fruit showed a pre-climacteric ripening with an ethylene production ten times less than that found for Chiripá peach at the climacteric stage (Girardi et al., 2005).

However, an important technological improvement was obtained here, with the increase in fruit size and weight upon GA_3 application, leading to a 47 % gain in productivity per plant (51 to 75 kg per plant) equivalent to increasing productivity from 25,500 to 37,740 kg ha^{-1}. Interestingly, the increase in fruit size did not negatively affect peach preservation, with low levels of woolliness after CS. It has been shown that agronomic practices that contribute to an increase in fruit size in Chiripá peaches (i.e. thinning), results in higher susceptibility to woolliness (Rombaldi et al., 2002).

Gibberellic acid has been associated with the prevention of many physiological postharvest disorders (Zilkah et al., 1997; Ju et al., 1999; Martinez-Romero et al., 2000; Kappel and MacDonald, 2002; Amarante et al., 2005; Usenik et al., 2005; Deacon, 2009). According to Yamaguchi and Kamiya (2000) responses derived from exogenous GA supply are associated with synthesis deficiency and/or higher sensitivity to signal reception and transduction. Physiological responses observed in Chiripá peach upon GA_3 treatment indicate a possible deficit of this hormone. In addition, Chiripá peach was only responsive to GA_3,

leading to size increase and preventing woolliness upon cold storage, when sprayed prior to pit hardening.

Woolliness occurrence in GA_3-treated peach (T1 CS) was as low as in untreated control peach stored under CA/CS (Table 3). Upon extension of the ripening period, it was observed that woolly fruit (Control CS) became juicy again (Table 3). According to Lurie and Crisosto (2005), this apparent restoration of free juice is due to tissue breakdown and senescence. Therefore, decrease in occurrence of woolliness seen in late ripening does not represent an actual reduction of the problem but a further decay of the fruit. In addition, signs of internal browning observed six days after CS confirmed the senescence of the fruit at that point.

In order to understand the molecular mechanisms associated with the physiological responses to GA_3 application and storage conditions, relative mRNA accumulation of a set of genes putatively involved in endomembrane transport via endoplasmic reticulum (ER), Golgi complex (GC) and vesicles, cell wall structure and disassembly, stress response (heat shock proteins) and ethylene synthesis were investigated.

Fruit from treatments with lower occurrence of woolliness (Control CA/CS and T1 CS) developed a classical climacteric behaviour during ripening, while fruit with high occurrence of woolliness (Control CS) did not develop climacteric production of ethylene (Table 4). This response could be a consequence of the lower *ACCO* transcript accumulation (Fig. 1) and absence of ACCO protein in woolly fruit (Fig. 2). In contrast, high relative accumulation of *ACCO* transcripts and protein were detected and classic climacteric behaviour was observed in Control CA/CS and T1 CS peach, suggesting ethylene involvement in woolliness prevention. These findings agree with Giehl et al. (2008) who demonstrated that juiciness in peach is an ethylene-dependent process, with CA/CS storage allowing normal ethylene production and reduced woolliness. Moreover, Girardi et al. (2005) reported that supplying ethylene during CS prevents woolliness, while inhibiting ethylene with 1-MCP increases this

chilling injury. In this study, the transcription and translation of genes encoding cell wall proteins were not completely ethylene-dependent. *PME*, *PG*, *β-Gal* and *PL* transcripts (Fig. 1) and proteins (Fig. 2) were detected before the onset of the climacteric stage (Table 4), corroborating transcriptomic findings of Trainotti et al. (2003). In addition, Nishiyama et al. (2007) found that two out of three *PGs* are ethylene-dependent in climacteric melon.

All genes studied here, putatively coding for cell wall associated proteins, have a signal peptide, suggesting that transport to the apoplast occurs via ER and GC, as predicted using iPSORT (Bannai et al., 2002), TargetP (Emanuelsson et al., 2000), and SignalP (Nielsen et al., 1997) software. Therefore, it is expected that protection of the ER, GC and vesicular endomembrane system is essential for the appropriate transport and folding of proteins to occur, in agreement with recent results by Vizoso et al. (2009) and Ogundiwin et al. (2008). The higher relative expression profile of ER genes (*HSP40-1er*, *HSP40-2er*, *HSP70er, HSPCNX1*) in peach from Control CA/CS and T1 CS agrees with this hypothesis. Moreover, recently Sun et al. 2010, found three cytosolic small heat shock proteins with an important role in chilling tolerance in stone fruit. The potential role of these genes in regulating cell homeostasis under abiotic stresses has been previously observed for other species (Pratt and Toft, 2003; Iwata et al., 2008; Mueller et al., 2008; Su and Li, 2008). GA_3 treatment also induced high relative expression of expansins (*Exp1, Exp2 and Exp3*), *PME*, *PG*, *PL*, *β-Gal*, *β-Man* and *α-Ara* genes (Fig. 1) coding for cell wall targeted proteins transported via ER and GC (Nakashima et al., 2004; Lycett, 2008). Moreover, pre-harvest GA_3 treatment contributed to a higher relative expression of chaperone (*HSPs*) transcripts associated with the defense of chloroplasts (*HSP17.8ch*) (Millar et al., 2006; Jarvis, 2008; Kahlau and Bock, 2008) and mitochondria (*HSP60mi*, *HSP60-3Bmi* and *HSP26.5mi*) (Lister et al., 2004; Radhamony and Theg, 2006; Deacon, 2009), indicative of better homeostasis of the metabolism of these organelles in peaches with adequate ripening evolution. According to González-Agüero et al.

(2008), peach with normal ripening generally show up-regulation of genes encoding proteins associated with vesicle transport. In contrast, in Chiripá peach, vesicle transport associated genes *Vap27-2*, *Vamp, Syn*, *Rab5* and *Rab11* showed higher relative expression of transcripts in woolly fruit (Control CS), (Fig. 1). *Rab11* involvement in PME and PG transport and fruit softening has been previously demonstrated in tomato (Lu et al., 2001). Similarly, Lycett (2008) cited *Rab5* participation in the formation of endosomes, with increased transcription during fruit ripening. Additionally, Appezzato-da-Glória et al. (2004) in cytological studies of woolly fruit, observed a macroendocytosis process with endosome individualization containing cell wall materials, probably pectins, suggesting an endocytic transport associated with woolliness.

Although an inverse relationship between the expression of some vesicular genes and the prevention of woolliness was observed, a direct relationship between the mRNA abundance of genes associated with ethylene synthesis, ER, mitochondria and chloroplast protection, and of cell wall metabolism occurred (Fig 1). The lower relative accumulation of proteins involved in cell wall metabolism observed in Control CS (Fig. 2) is in agreement with the down-regulation of relative mRNA levels of these genes (Fig. 1), indicating a coordination between transcription and translation of these genes.

mRNA accumulation of *Cob*, *GLS*, *CCR*, *Roc7*, *ERD2*, *Cla*, *SFT2*, *Kin,* and *HSP70ch* were not affected by GA_3 treatment or by storage condition during ripening (Fig. 1). mRNA accumulation of *HSP40-1er*, *HSP40-2er*, *HSPCRT2*, *HSP17.8ch*, *Exp4*, *β-Man*, *α-Ara*, *GalT* and *ADL1A*, positively related to woolliness prevention, had different transcript profiles between Control CA/CS and T1 CS. While *HSPs*, *ADL1A* and *α-Ara* and *β-Man* showed higher relative expression in GA_3-treated peach, *Exp4* and *GalT* expression was high in Control CA/CS peach. Although both treatments prevented woolliness, differential gene

transcript and protein accumulation suggest differences in the molecular and biochemical mechanisms of woolliness prevention induced by GA_3 and CA/CS.

In summary, GA_3 treatment followed by cold storage (T1 CS) proved as efficient as storage of peach under controlled atmosphere conditions (Control CA/CS) in providing normal ripening and preventing woolliness in Chiripá peach. GA_3 treatment, despite not delaying ripening, induced increase in fruit size and weight when applied at the beginning of pit hardening. The molecular responses associated with woolliness prevention in common between GA_3 and CA/CS treatments, involved high relative expression of genes associated with cell wall metabolism, ER, chloroplast and mitochondria *HSPs*, and *ACCO* and lower relative transcript accumulation of vesicle transport related genes (*Vap27-2*, *Vamp, Syn*, *Rab5* and *Rab11*) when compared to woolly fruit. Although both treatments prevented woolliness, differential accumulation of gene transcript (*HSP40-1er*, *HSP40-2er*, *HSPCTR2, HSP17.8ch, Exp4, β-Man, α-Ara*, *GalT* and *ADL1A*) and protein (β-Man and α-Ara) suggest different molecular and biochemical mechanisms induced by GA_3 and CA.

Studies should follow to further characterize the protective mechanisms induced by GA_3 treatment and controlled atmosphere storage of peach. Currently, a pre-harvest evaluation of gene expression in peach fruit, treated and untreated with GA_3, is underway to characterize early changes leading to woolliness or woolliness prevention.

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References

Amarante, C.V.T. do, Drehmer, A.M.F., Souza, F. de, Francescatto, P., 2005. A pulverização pré-colheita com ácido giberélico (GA_3) e aminoetoxivinilglicina (AVG) retarda a maturação e reduz as perdas de frutos na cultura do pessegueiro. Rev. Bras. Frutic. 27, 1-5.

Appezzato-da-Glória, B., Bron, I.U., Machado, S.R., 2004. Lanosidade em cultivares de pêssego (*Prunus persica* (L.) Batsch): estudos anatômicos e ultra-estruturais. Revista Brasileira Botânica 27, 55-61.

Bannai, H., Tamada, Y., Maruyama, O., Nakai, K., Miyano, S., 2002. Extensive feature detection of N-terminal protein sorting signals. Bioinformatics 18, 298-305.

Ben-Arie, R., Saks, Y., Sonego, L., Frank, A., 1996. Cell wall metabolism in gibberellin-treated persimmon fruits. Plant Growth Regul. 19, 25-33.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Rapp, B.A., Wheeler, D.L., 2005. GenBank. Nucl. Acids Res. 33, D34-D38.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Brummell, D.A., Dal Cin, V., Lurie, S., Crisosto, C.H., Labavitch, J.M., 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. J. Exp. Bot. 55, 2041-2052.

Deacon, J., 2009. Yeast and yeast like-fungi: Saccharomyces, Cryptococcus and Candida albicans. Vol, 2002. The University of Edinburgh.

Emanuelsson, O., Nielsen, H., Brunak, S., von Heijne, G., 2000. Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. J. Mol. Biol. 300, 1005-1016.

Ferri, V.C., Rinaldi, M.M., Silva, J.A., Luchetta, L., Marini, L., Rombaldi, C.V., 2004. Ácido giberélico no retardamento da maturação de caquis (*Diospyrus kaki*, L.), cultivar Fuyu. Ciênc. Tecnol. Aliment. 24, 1-5.

Giehl, R.F.H., Brackmann, A., Eisermann, A.C., Weber, A., Neuwald , D.A., 2008. CA affects juiciness in peaches due to an earlier post-storage ethylene production. In: International Conference on Ripening Regulation and Postharvest Fruit Quality, ISHS Acta Horticulturae 796, 185-189.

Girardi, C.L., Corrent, A.R., Lucchetta, L., Zanuzo, M.R., Costa, T.S. da, Brackmann, A., Twyman, R.M., Nora, F.R., Nora, L., Silva, J.A., Rombaldi, C.V., 2005. Effect of ethylene, intermittent warming and controlled atmosphere on postharvest quality and the occurrence of woolliness in peach (*Prunus persica* cv. Chiripá) during cold storage. Postharvest Biol. Technol. 38, 25-33.

González-Agüero, M., Pavez, L., Ibáñez, F., Pacheco, I., Campos-Vargas, R., Meisel, L.A., Orellana, A., Retamales, J., Silva, H., Gonzalez, M., Cambiazo, V., 2008. Identification of woolliness response genes in peach fruit after post-harvest treatments. J. Exp. Bot. 59, 1973-1986.

Hu, J., Mitchum, M.G., Barnaby, N., Ayele, B.T., Ogawa, M., Nam, E., Lai, W.C., Hanada, A., Alonso, J.M., Ecker, J.R., Swain, S.M., Yamaguchi, S., Kamiya, Y., Sun, T.P., 2008. Potential sites of bioactive gibberellin production during reproductive growth in *Arabidopsis*. Plant Cell. 20, 320-336.

Invitrogen, 2005. Vector NTI Advance™ 10 Quick Start Guide. Invitrogen Corporation, Carlsbad.

Iwata, Y., Fedoroff, N.V., Koizumi, N., 2008. *Arabidopsis* bZIP60 is a proteolysis-activated transcription factor involved in the endoplasmic reticulum stress response. Plant Cell. 20, 3107-3121.

Jarvis, P., 2008. Targeting of nucleus-encoded proteins to chloroplasts in plants. New Phytol. 179, 257-285.

Ju, Z.G., Duan, Y.S., Ju, Z.Q., 1999. Combinations of GA_3 and AVG delay fruit maturation, increase fruit size and improve storage life of 'Feicheng' peaches. J. Hortic. Sci. Biotechnol. 74, 579-583.

Kahlau, S., Bock, R., 2008. Plastid transcriptomics and translatomics of tomato fruit development and chloroplast-to-chromoplast differentiation: Chromoplast gene expression largely serves the production of a single protein. Plant Cell 20, 856-874.

Kappel, F., MacDonald, R.A., 2002. Gibberellic acid increases fruit firmness, fruit size, and delays maturity of 'Sweetheart' sweet cherry. J. Am. Pomol. Soc. 56, 219-222.

Lister, R., Chew, O., Lee, M.N., Heazlewood, J.L., Clifton, R., Parker, K.L., Millar, A.H., Whelan, J., 2004. A transcriptomic and proteomic characterization of the Arabidopsis mitochondrial protein import apparatus and its response to mitochondrial dysfunction. Plant Physiol. 134, 777-789.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C(T)) Method. Methods 25, 402-408.

Lu, C., Zainal, Z., Tucker, G.A., Lycett, G.W., 2001. Developmental abnormalities and reduced fruit softening in tomato plants expressing an antisense Rab11 GTPase gene. Plant Cell. 13, 1819-1833.

Lurie, S., Crisosto, C.H., 2005. Chilling injury in peach and nectarine. Postharvest Biol. Technol. 37, 195-208.

Lycett, G., 2008. The role of Rab GTPases in cell wall metabolism. J. Exp. Bot. 59, 4061-4074.

Martinez-Romero, D., Valero, D., Serrano, M., Burlo, F., Carbonell, A., Burgos, L., Riquelme, F., 2000. Exogenous polyamines and gibberellic acid effects on peach (*Prunus persica* L.) storability improvement. J. Food Sci. 65, 288-294.

Martínez, G.A., Chaves, A.R., Añón, M.C., 1994. Effect of gibberellic acid on ripening of strawberry fruits (*Fragaria annanassa* Duch.). J. Plant Growth Regul. 13, 87-91.

Marur, C.J., Stenzel, N.M.C., Rampazzo, E.F., Scholz, M.B.S., 1999. Ácido Giberélico (GA_3) e maturação de frutos das tangerinas 'Mexerica Montenegrina'e 'Poncã'. Scientia Agricola 56, 517-521.

Millar, A.H., Whelan, J., Small, I., 2006. Recent surprises in protein targeting to mitochondria and plastids. Curr. Opin. Plant Biol. 9, 610-615.

Mueller, S., Hilbert, B., Dueckershoff, K., Roitsch, T., Krischke, M., Mueller, M.J., Berger, S., 2008. General detoxification and stress responses are mediated by oxidized lipids through TGA transcription factors in *Arabidopsis*. Plant Cell 20, 768-785.

Nakashima, J., Endo, S., Fukuda, H., 2004. Immunocytochemical localization of polygalacturonase during tracheary element differentiation in *Zinnia elegans*. Planta 218, 729-739.

Nielsen, H., Engelbrecht, J., Brunak, S., vonHeijne, G., 1997. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng. 10, 1-6.

Nishiyama, K., Guis, M., Rose, J.K.C., Kubo, Y., Bennett, K.A., Wangjin.L., Kato, K., Ushijima, K., Nakano, R., Inaba, A., Bouzayen, M., Latche, A., Pech, J.C., Bennett, A.B., 2007. Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. J. Exp. Bot. 58, 1281-1290.

Ogundiwin, E.A., Marti, C., Forment, J., Pons, C., Granell, A., Gradziel, T.M., Peace, C.P., Crisosto, C.H., 2008. Development of ChillPeach genomic tools and identification of cold-responsive genes in peach fruit. Plant Mol.Biol. 68, 379-397.

Pratt, W.B., Toft, D.O., 2003. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp. Biol. Med. 228, 111-133.

Radhamony, R.N., Theg, S.M., 2006. Evidence for an ER to Golgi to chloroplast protein transport pathway. Trends Cell Biol. 16, 385-387.

Ramos, C.R.R., Abreu, P.A.E., Nascimento, A.L.T.O., Ho, P.L., 2004. A high-copy T7 Escherichia coli expression vector for the production of recombinant proteins with a minimal N-terminal His-tagged fusion peptide. Braz. J. Med. Biol. Res. 37, 1103-1109.

Rombaldi, C., Lelievre, J.M., Latche, A., Petitprez, M., Bouzayen, M., Pech, J.C., 1994. Immunocytolocalization of 1-aminocyclopropane-1-carboxylic acid oxidase in tomato and apple fruit. Planta 192, 453-460.

Rombaldi, C.V., Silva, J.A., Parussolo, A., Lucchetta, L., Zanuzo, M.R., Girardi, C.L., Cantillano, R.F.F., 2002. Armazenamento de pêssegos (*Prunus persica* L.), cultivar Chiripá, em atmosfera controlada. Cienc. Rural 31, 43-47.

Rosenvasser, S., Mayak, S., Friedman, H., 2006. Increase in reactive oxygen species (ROS) and in senescence-associated gene transcript (SAG) levels during dark-induced senescence of Pelargonium cuttings, and the effect of gibberellic acid. Plant Sci. 170, 873-879.

Saeed, A.I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M., Currier, T., Thiagarajan, M., Sturn, A., Snuffin, M., Rezantsev, A., Popov, D., Ryltsov, A., Kostukovich, E., Borisovsky, I., Liu, Z., Vinsavich, A., Trush, V., Quackenbush, J., 2003. TM4: A free, open-source system for microarray data management and analysis. Biotechniques 34, 374-378.

Sambrook, J., Fritsch, E.J., Maniatis, T. 2nd, 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY.

Su, P.H., Li, H.M., 2008. Arabidopsis stromal 70-kD heat shock proteins are essential for plant development and important for thermotolerance of germinating seeds. Plant Physiol. 146, 1231-1241.

Sun, J.-h., Chen, J.-y., Kuang, J.-f., Chen, W.-x., Lu, W.-j., 2010. Expression of *sHSP* genes as affected by heat shock and cold acclimation in relation to chilling tolerance in plum fruit. Postharvest Biol. Technol. 55, 91-96.

Thomas, S.G., Rieu, I., Steber, C.M., 2005. Gibberellin metabolism and signaling. In: Litwack, G. (Ed.), Plant Hormones. Vol. 72. Elsevier Academic Press Inc, Toluca Lake, California, pp. 289-338.

Trainotti, L., Zanin, D., Casadoro, G., 2003. A cell wall-oriented genomic approach reveals a new and unexpected complexity of the softening in peaches. J. Exp. Bot. 54, 1821-1832.

Trainotti, L., Bonghi, C., Ziliotto, F., Zanin, D., Rasori, A., Casadoro, G., Ramina, A., Tonutti, P., 2006. The use of microarray µPEACH1.0 to investigate transcriptome changes during transition from pre-climacteric to climacteric phase in peach fruit. Plant Sci. 170, 606-613.

Usenik, V., Kastelec, D., Stampar, F., 2005. Physicochemical changes of sweet cherry fruits related to application of gibberellic acid. Food Chem. 90, 663-671.

Vizoso, P., Meisel, L.A., Tittarelli, A., Latorre, M., Saba, J., Caroca, R., Maldonado, J., Cambiazo, V., Campos-Vargas, R., Gonzalez, M., Orellana, A., Silva, H., 2009. Comparative EST transcript profiling of peach fruits under different post-harvest conditions reveals candidate genes associated with peach fruit quality. BMC Genomics 10, 423-440.

Yamaguchi, S., Kamiya, Y., 2000. Gibberellin biosynthesis: Its regulation by endogenous and environmental signals. Plant and Cell Physiol. 41, 251-257.

Zhou, H.W., Lurie, S., Lers, A., Khatchitski, A., Sonego, L., Ben Arie, R., 2000. Delayed storage and controlled atmosphere storage of nectarines: two strategies to prevent woolliness. Postharvest Biol. Technol. 18, 133-141.

Zilkah, S., Lurie, S., Lapsker, Z., Zuthi, Y., David, I., Yesselson, Y., Antman, S., Ben Arie, R., 1997. The ripening and storage quality of nectarine fruits in response to preharvest application of gibberellic acid. J. Hort. Sci. 72, 355-362.

Zonta, E.P., Machado, A.A., 1991. Manual do SANEST: Sistema de análise estatística para microcomputadores. DMEC/IFM/UFPEL, Pelotas, RS, Brasil.

Table 1. Specific primers used for quantitative PCR analysis of target genes.

Genes	GI	Forward	Reverse
Ethylene Synthesis			
1aminocyclopropane-1-carboxylate oxidase (*ACCO*)	33329719	GGATTGGGAGCTTGCTTGCAA	TTGCAAGCCCGTGAGATGAT
Cell wall Metabolism			
Cobra(*Cob*)	22478530	ACTCATCCAGGAAGCTGTGTAG	ATGGCTGTATCATTTATTGTCGCA
Glucan Synthase (*GLS*)	22478796	TGGGAAACATGGTGGTATGAGGA	CGA GACATTTGAAGTGAGTGAAC
Galactosyl transferase (*GalT*)	22480478	ATGTGAAAAGTGGATGCGGAA TG	TTGGATGAGAAGCGGGAAGAGA
Cinnamoyl-CoA reductase (*CCR*)	22481300	ATCAAGTCCAAGACCCCGAGAA	CGCCCA ACA CGGTGCCAGGA
Pectate lyase (*PL*)	22483439	GCCTTGCCGTACGCTCATGTC	CTTCAGCCTCAACCCCTTCCCT
Endopolygalacturonase (*PG*)	110293962	AAAGGGTGCCCTGGTCAGGTAAGATA	GCTCTTCTAGGTGGAAGCCCAAGAAA
Pectin methylesterase (*PME*)	1213628	AGGTGGCCTCCATTCTCTCTCAGTT	GGGAAGCAGAGAGAGACCAGTTCAA
Beta-galactosidase (*β-Gal*)	157313305	CGTGTATGGGGCATTGGAGAA	CTCCCGCATTCCATGTCTCAA
Endo-1,4-beta-mannosidase (*β-Man*)	157313309	ACAAGGTTTTCCCATCCATGTTGAA	ATGGGTCAGAAGCCACATACATCAA
Alpha -L- arabinofuranosidase (*α-Ara*)	145338352	ACCTCCACTCCTCCTCTGTCTGATT	TTCTGAGCAACTTCCAAGACTCCAT
Expansin 1(*Exp1*)	16305104	AAACGTTGGTGGTGCCGGTGAT	TTGCTTGCCAACCAGTCCTGGA
Exp2	29466640	TCCAGGACTGGTTGGCAAGCAA	TAGGACACCACTGTGCGGCCAT
Exp3	29466642	GGGTGCATGGGAAGCAGCTCAT	CCATGGTGCCAGAGGCATCAGA
Exp4	21901947	TGAGCTGTGGGGCATGCTATGA	AGGATCACACCAGCCACCTGGT
Endomembrane Transport			
Golgi transport protein SFT2-like (*SFT2*)	22481199	CACTCA AAGGCCCGAAGAATCA	CAACGTCACCTCCCAAAACATC
SNARE-like protein (*Vap27-2*)	22481415	CCAAAGAAATACTGCGTGCGGC	GGAAGAGGGTGGGCTGATGAG
Dynamin-like protein1A (*ADL1A*)	22481576	GTGAACAAAATCCAAAGAGCTTG	GCCAGTTTCTCGATCTGTCTC
ROC7 cyclophilin (*ROC7*)	22481624	CCAGGCAAA GAAGTCAAAGGAG	TCACCTCCCTGAATCATGAAACT
Rab GTP-binding protein (*Rab5*)	22482805	CCATAGGTGCTGCCTTCTTCTC	CCATGTTTGGATTGCCTTGTGATT
Vesicle-associated membrane protein 722 (*Vamp*)	22483019	GCAAAGCAGGTGGTCTCAGG	TTAAGGCTATTGGCAGGGGCT
Kinesin (*Kin*)	51560894	ATCAAACGACCAAAGCGGGCTTA	AATTTCGCCACGAGAACCCACAT
Clathrin-binding protein b-adaptin (*Cla*)	22483877	CTTGGTGATCTGATTGGCATGG	ACTTGTGGAACCTGAAGGGGTC
Syntasin (*Syn*)	56162812	CTGTGCAAGCTACCTCCCACCTT	GATCACTGCTCAAGCCACCACAA
ER lumen protein retaining receptor 2 (*ERD2*)	22477673	GCCAGTATTTTGGTCCTCCTTC	TCTTGAAATGTGAATTCCTCGTG
RabGTP-binding protein (*Rab11*)	22484722	ATGTTTGTAGGTTATTAGTCGCTTA	CGCTCTTGACCAGTTGTATCCCA
Heat Shock Proteins (HSP)			
HSP40-1 er resident (*HSP40-1er*)	186512089	TGCACCTCGCCTGCTTGAAGAT	TTGCGGGGTATAACGGCCATCT
HSP70er	240254046	CCGCGGTCCAAGGTGGAGTATT	TCAAAGGCGCAACATCAAGCAG
HSP40-2er	79318135	CGCCAGCAGCTTCTGCACACAA	TTCTCCGTCGAGCCACGCAAAT
HSP calnexin 1 (*HSPCNX1*)	145359541	TGTCCTCGTCGCCATTGTGGTT	CTTCTTTTCCACAGGTGCCGCC
HSP calreticulin 2 (*HSPCRT2*)	145335312	TCTCCTCGCGGTATATATAAGC	AGACGAGGCTAGGAATCATTTT
HSP70 chloroplast resident (*HSP70ch*)	145359060	CCAACCTCCGCCTTCCTTCGTA	ACCAACGGTGTATCGGGAAGCG
HSP17.8ch	30680121	CCGAGTCACCAGCTTTGCTCAGAA	TGTTGTTGCCGAAGAAGCTTGGAA
HSP60 mitochondria resident (*HSP60mi*)	20466255	ACCTCGCCTCCAAGGCAAGGAT	TTCTGCTGGAAACCTGGCGAGC
HSP26.5mi	186490445	CAATGGCTCTAGCTCGTCTGGCTTT	TTCACCAGCCGAAGTAGCCATGAAT
HSP60-3Bmi	145338881	CCTCGCCTCCAAGGCAAGGATT	TTCTGCTGGAAACCTGGCGAGC
Endogenous Control			
18S	66627320	AAAACGACTCTCGGCAACGGATA	ATGGTTCACGGGATTCTGCAATT

Table 2. Characteristics of peach fruit [*Prunus persica* (L.) Batsch, cv. Chiripá], immediately after harvest from trees sprayed with GA_3, at the beginning (T1) and end (T2) of pit hardening, and from untreated trees (Control)[1, 2].

treatment	fruit equatorial circumference (cm)	fruit mass (g)	fruit colour (hue angle)	flesh firmness (N)	soluble solids content (%)	ethylene production (nL g^{-1} h^{-1})	woolly fruit (%)
Control	19.14 b[3]	102.12 a	98.12 a	50.25 a	12.01 a	1.26 a	85.0 a
T1	26.80 a	150.14 b	102.01 a	48.14 a	11.82 a	1.09 a	12.3 b
T2	18.15 b	100.04 a	100.07 a	52.25 a	12.23 a	0.86 a	90.3 a

[1] The peaches were harvested when the fruit colour was light green, corresponding to the pre-climacteric stage, between S3 I and S3 II, described by Trainotti et al. (2003).

[2] Three replicates of ten trees, selected based on size uniformity, were treated as follows: Control, without GA_3; T1 – spraying 400 L ha^{-1} of a GA_3 solution [50 mg L^{-1} of GA_3 (Proggib®) and 0.05 % (v/v) of surfactant (Silwet®), pH 4.5] at the beginning of the pit hardening stage; T2 – spraying 400 L ha^{-1} of the same GA_3 solution at the end of the pit hardening stage.

[3] Means of treatment followed by different letters are statistically different according to Duncan's test ($p \leq 0.05$). For the variables fruit equatorial circumference, fruit mass, fruit colour, flesh firmness, soluble solids content and woolly fruit, n = 120, and for the variable ethylene production, n = 3.

Table 3. Woolliness incidence in peach fruit [*Prunus persica* (L.) Batsch, cv. Chiripá], harvested from untreated trees (Control) and from trees sprayed with GA_3 at the beginning of pit hardening (T1), stored for 30 days, under cold storage (CS) or controlled atmosphere (CA/CS), and finally exposed to 23.0 °C (± 3.0 °C) and 75.0 % (± 5.0 %) RH for 6 h, 2, 4 and 6 days. [1]

Treatment		woolly fruit (%)			
		period at 23.0 °C (± 3.0 °C) and 75.0 % (± 5.0 %) RH			
GA_3	storage	6 h	2 days	4 days	6 days
Control	CS[2]	24.5 a[4]	94.2 a	94.2 a	48.5 a
Control	CA/CS[3]	0.0 b	23.5 b	23.2 b	0.0 c
T1	CS	0.0 b	28.6 b	9.5 b	19.8 b

[1] The peaches were harvested when the fruit colour was light green, corresponding to the pre-climacteric stage, between S3 I and S3 II, as described by Trainotti et al. (2003).

[2] Cold storage (CS): 1.0 °C (± 1.0 °C), 92.0 % (± 5.0 %) relative humidity (RH).

[3] Controlled atmosphere (CA/CS): 2 kPa of O_2, and 5 kPa of CO_2, 1.0 °C (± 1.0 °C), 92.0 % (± 5.0 %) RH.

[4] Means of treatment (n = 120) in the same column, followed by different letters, are statistically different according to Duncan's test ($p \leq 0.05$).

Table 4. Ethylene production of peach fruit [*Prunus persica* (L.) Batsch, cv. Chiripá], harvested from untreated trees (Control) and from trees sprayed with GA_3 at the beginning of pit hardening (T1), stored for 30 days, under cold storage (CS) or controlled atmosphere (CA/CS), and finally removed from storage and exposed to 23.0 °C (± 3.0 °C) and 75.0 % (± 5.0 %) RH, for 6 h, 2, 4 and 6 days. [1]

Treatment		ethylene production (nL g^{-1} h^{-1}) period at 23.0 °C (± 3.0 °C) and 75.0 % (± 5.0 %) RH			
GA_3	storage	6 h	2 days	4 days	6 days
Control	CS[2]	0.93 a[4]	1.23 c	1.24 c	0.59 b
Control	CA/CS[3]	0.87 a	5.52 b	15.21 b	3.36 a
T1	CS	1.06 a	11.65 a	24.53 a	4.80 a

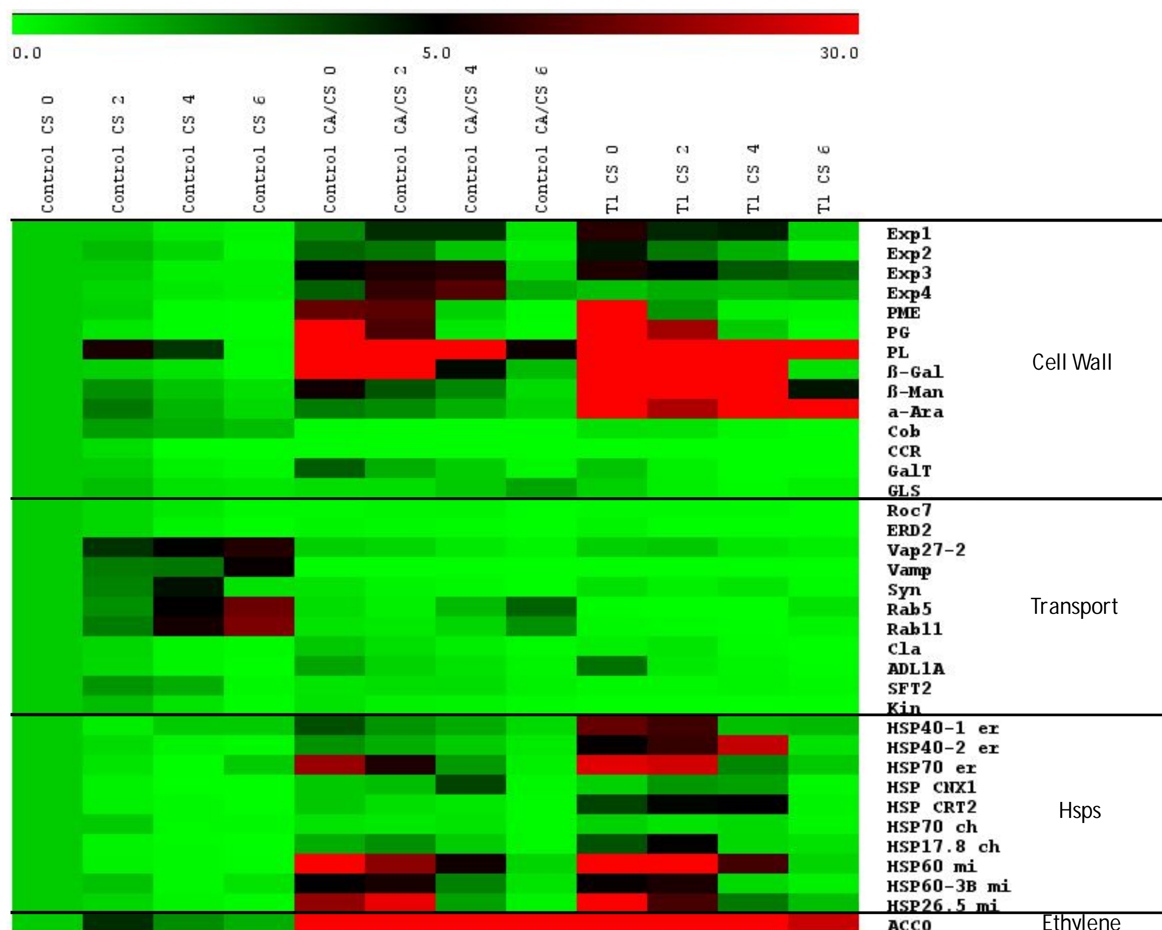
[1] The peaches were harvested when the fruit colour was light green, corresponding to the pre-climacteric stage, between S3 I and S3 II, as described by Trainotti et al. (2003).

[2] Cold storage (CS): 1.0 °C (± 1.0 °C), 92.0 % (± 5.0 %) relative humidity (RH).

[3] Controlled atmosphere (CA/CS): 2 kPa of O_2, and 5 kPa of CO_2, 1.0 °C (± 1.0 °C), 92.0 % (± 5.0 %) RH.

[4] Means of treatment (n = 3) in the same column, followed by different letters, are statistically different according to Duncan's test ($p \leq 0.05$).

Figure 1. Relative mRNA accumulation in peach fruit [*Prunus persica* (L.) Batsch, cv. Chiripá], harvested from untreated trees (Control) and from trees sprayed with GA_3 at the beginning of pit hardening (T1), stored for 30 days, under cold storage (CS) or controlled atmosphere/cold storage (CA/CS), and finally removed from storage and exposed to 23.0 °C (± 3.0 °C) and 75.0 % (± 5.0 %) RH, for 6 h (0), 2, 4 and 6 days.[1, 2, 3]



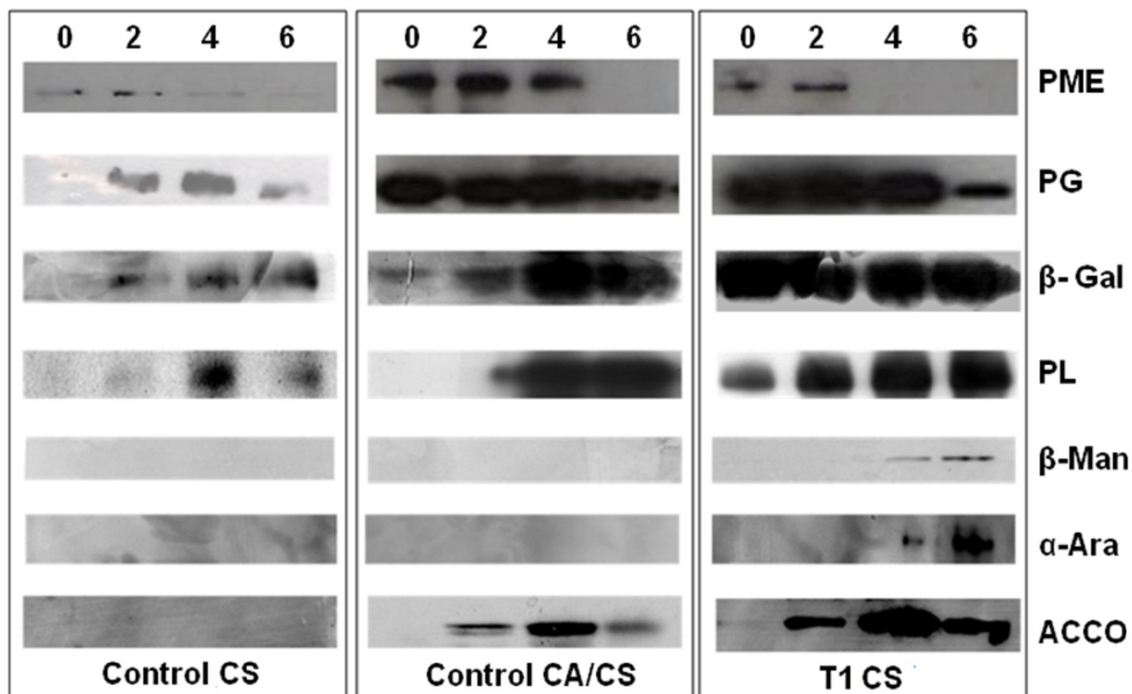
[1] mRNA abundance is represented, on a scale of 0 to 30, using the Multi Experiment Viewer (TIGR MeV) software (Saeed et al., 2003). The lower end of the scale (light green colour) indicates the lowest level; black colour in the middle of the scale represents accumulation 5 times higher than the light green end; and red colour in the upper end of the scale represents the highest mRNA abundance, 30 times higher than the lowest. mRNA

abundance of each gene from Control CS fruit at 6 h served as the baseline for determining relative RNA levels.

[2] Cold storage (CS): 1.0 °C (± 1.0 °C), 92.0 % (± 5.0 %) relative humidity (RH).

[3] Controlled atmosphere (CA/CS): 2 kPa of O_2, and 5 kPa of CO_2, 1.0 °C (± 1.0 °C), 92.0 % (± 5.0 %) RH.

Figure 2. Immunodetection of pectin methyl esterase (PME), endopolygalacturonase (PG), β-galactosidase (β-Gal), pectate lyase (PL), endo-1,4-β-mannase (β-Man), α-arabinofuranosidase (α-Ara) and ACC oxidase (ACCO) in total protein extracts of peach fruit [*Prunus persica* (L.) Batsch, cv. Chiripá], harvested from untreated trees stored for 30 days under cold (Control CS) or controlled atmosphere/cold storage (Control CA/CS), and from trees sprayed with GA_3 at the beginning of pit hardening followed by 30 days under cold storage (T1 CS), and finally removed from storage and exposed to 23.0 °C (± 3.0 °C) and 75.0 % (± 5.0 %) RH, for 6 h (0), 2, 4 and 6 days.



2. Considerações Finais

De maneira geral, pode-se considerar que o desenvolvimento de lanosidade é ocasionado por diferentes mecanismos moleculares. Neste trabalho verificou-se que o aparecimento desse distúrbio está associado pela anormalidade da síntese de etileno, deficiência de enzimas de degradação da parede celular, assim como redução na expressão de genes codificadores de proteínas relacionadas com respostas a estresses (HSPs). Além disso, observou-se que a lanosidade não é ocasionada pelo baixo acúmulo de transcritos de genes codificadores de proteínas associadas ao transporte endomembranas.

No artigo 1 evidenciou-se que durante o amadurecimento normal de pêssegos há aumento no acúmulo de mRNAs para genes codificadores de proteínas envolvidas no transporte endomembranas e no metabolismo de síntese e degradação de parede celular. Entretanto, o processo de amadurecimento não é totalmente dependente dos genes estudados, uma vez que o maior acúmulo de transcritos acontece depois da redução da firmeza de polpa.

No artigo 2 verificaram-se que os genes codificadores de proteínas envolvidas no transporte endomembranas não estão envolvidos com o desenvolvimento de lanosidade em pêssegos ‘Chimarrita’.

No artigo 3 a aplicação de AG_3 antes do endurecimento do caroço não atrasou o processo de amadurecimento, mas proporcionou aumento de tamanho dos frutos e preveniu o desenvolvimento de lanosidade. Esse aumento de tamanho proporcionado pelo AG_3 corresponde a um incremento na produtividade de 47%, ou seja, de 25.500 para 37.500 Kg há$^{-1}$. O maior tamanho dos frutos tratados com AG_3 não afetou negativamente a qualidade após armazenamento em atmosfera refrigerada (AR). A prevenção da lanosidade após AR está associada com o alto

acúmulo de mRNAs de genes codificadores de proteínas de respostas a estresses abióticos – HSPs, proteínas associadas com o metabolismo de parede celular e proteína chave na biossíntese de etileno.

Diante desses resultados, acredita-se que os dados aqui apresentados possam contribuir cientificamente com o assunto abordado, além de levantar novos questionamentos relacionados aos mecanismos associados com o desenvolvimento de lanosidade.

3. Referências Bibliográficas

AMARANTE, C.V.T.D., DREHMER, A.M.F., SOUZA, F.D., FRANCESCATTO, P. A pulverização pré-colheita com ácido giberélico (GA_3) e aminoetoxivinilglicina (AVG) retarda a maturação e reduz as perdas de frutos na cultura do pessegueiro. **Revista Brasileira de Fruticultura**. v. 27, p. 1-5, 2005.

ARTES, F., CANO, A., FERNADEZ-TRUJILO, J. P. Pectolytic enzyme activity during intermittent warming storage of peaches. **Journal of Food Science**. v. 61, p. 311-313, 1996.

BEN-ARIE, R., LAVEE, S. Pectic changes occurring in ‘Elberta’ peaches suffering from woolly breakdown. **Phytochemistry**. v. 10, p. 531-538, 1971.

BEN-ARIE, R., SONEGO, L. Pectolytic enzyme activity involved in woolly breakdown of stored peaches. Phytochemistry. v. 19, p. 2553-2555, 1980.

Boston, R.S., Viitanen, P.V., Vierling, E. Molecular chaperones and protein folding in plants. **Plant Molecular Biology**, v. 32, p. 191-222, 1996.

Brackmann, A., Steffens, C. A., Giehl, R. F. H. Armazenamento de pêssego “Chimarrita” em atmosfera controlada e sob absorção de etileno. **Ciência Rural**. v. 33, p. 431-435, 2003.

BRACKMANN, A., BORDIGNON, B.C.S., GIEHL, R.F.H., SESTARI, I., EISERMANN, A.C. Storage of cv. “Granada” peach fruit in controlled atmosphere aiming the transport for long distances. **Ciência Rural**. v. 37, p. 676-681, 2007.

BRUMMELL, D. A., CIN, V. D., LURIE, S., CRISOSTO, C. H., LABAVITCH, J. M. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. **Journal of Experimental Botany**. v. 55, p. 2041-2052, 2004.

CAMPOS-VARGAS, R., BECERRA, O., BAEZA-YATES, R., CAMBIAZO, V., GONZÁLEZ, M., MEISEL, L., ORELLANA, A., RETAMALES, J., SILVA, H., DEFILIPPI, B. G. Seasonal variation in the development of chilling injury in 'O'Henry' peaches. **Scientia Horticulturae**. v. 110, p. 79-83, 2006.

CONCHON, S., CAO, X., BARLOWE, C., PELHAM, H.R. Got1p and Sft2p: membrane proteins involved in traffic to the Golgi complex. **Embo Journal,** v. 18, p. 3934-3946, 1999.

CRISOSTO, C.H., MITCHELL, F. G., JU, Z. Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. **HortScience**. v. 34, p. 1116-1118, 1999.

ECHARD, A., JOLLIVET, F., MARTINEZ, O., LACAPERE, J.J., ROUSSELET, A., JANOUIEX-LEROSEY, I., GOULD, B. Interaction of a Golgi associated kinesin-like protein whit Rab 6. **Scince**, v. 279, p. 580-585, 1998.

FAO – Food and Agriculture Organization of the United Nations. Base de dados estatísticos – Faostat Agriculture. Disponível em: <http://www.fao.org.br>. Acesso em: 04 maio 2009.

FERREIRA, P.A., NAKAYAMA, T.A., PAK, W.L., TRAVIS, G.H. Cyclophilin-related protein RanBP2 acts as chaperone forred / green opsin. **Nature**, v. 383, p. 637-640. 1996.

FERRI, V.C., RINALDI, M.M., SILVA, J.A., LUCHETTA, L., MARINI, L., ROMBALDI, C.V. Ácido giberélico no retardamento da maturação de caquis (*Diospyrus kaki*, L.), cultivar Fuyu. **Ciência e Tecnologia de Alimentos**. v. 24, p. 1-5, 2004.

GIRARDI, C. L., CORRENT, A. R., LUCCHETTA, L., ZANUZO, M. R., COSTA, T. S. D., BRACKMANN, A. TWYMAN, R. M., NORA, F. R., NORA, L., SILVA, J. A., ROMBALDI, C. V. Effect of ethylene, intermittent warming and controlled atmosphere

on postharvest quality and the occurrence of woolliness in peach (Prunus persica cv. Chiripá) during cold storage. **Postharvest Biology and Technology**. v. 38, p. 25-33, 2005.

GONZÁLEZ-AGÜERO, M., PAVEZ, L., IBÁNEZ, F., PACHECO, I., CAMPOS-VARGAS, R., MEISEL, L. A., ORELLANA, A., RETAMALES, J., SILVA, H., GONZÁLEZ, M., CAMBIAZO, V. Identication of woolliness response genes in peach fruit after post-harvest treatments. **Journal of Experimental Botany**. v. 59, p. 1973-1986, 2008.

HIROKAWA, N., NODA, Y., OKADA, Y. Kinesin and dynein superfamily proteins in organelles transport and cell division. **Cell Biology International**. v. 10, p. 60-63, 1998.

IBRAF - Instituto Brasileiro de Frutas. In: Estatísticas. Disponível em http://www.ibraf.org.br. Acesso: 12 maio 2009.

INFANTE, R., MENESES, C., RUBIO, P., SEIBERT, E. Quantitative determination of flesh mealiness in peach [Prunus persica L.(Batch.)] through paper absorption of free juice. **Postharvest Biology and Technology**. v. 5, p. 118-121, 2009.

JIN, P., WANG, K., SHANG, H., TONG, J., ZHENG, Y. Low-temperature conditioning combined with methyl jasmonate treatment reduces chilling injury of peach fruit. **Journal of the Science of Food and Agriculture**. v. 89, p. 1690-1696, 2009a.

JIN, P., ZHENG, Y., TANG, S., RUI, H., WANG, C. Y. A combination of hot air and methyl jasmonate vapor treatment alleviates chilling injury of peach fruit. **Postharvest Biology and Tecnology**. v. 52, p. 24-29, 2009b.

JU, Z.G., DUAN, Y.S., JU, Z.Q. Combinations of GA_3 and AVG delay fruit maturation, increase fruit size and improve storage life of 'Feicheng' peaches. **Journal of Horticultural Science & Biotechnology**. v. 74, 579-583, 1999.

JU, Z., DUAN, Y., JU, Z. Leatheriness and mealiness of peaches in relation to fruit maturity and storage temperature. **Journal of Horticultural Science & Biotechnology**. v. 75, p. 86-91, 2000.

LEVERSON, J.D., NESS, S.A. Point mutations in v-Myb disrupt a cyclophilin-catalysed negative regulatory mechanism. **Molecular Cell**. v. 1, p. 203-211, 1998.

LEWIS, M.J., SWEET, D.J., PELHAM, H.R. The ERD2 gene determines the specificity of the luminal ER protein retention system. **Cell.** v. 61, p. 1359-1363, 1990.

LINDQUIST, S. The heat-shock response. **Annual Review of Biochemistry**. v. 55, p. 1151-1191, 1986.

LURIE, S., CRISOSTO, C. H. Chilling injury in peach and nectarine. **Postharvest Biology and Technology**. v. 37, p. 195-208, 2005.

MARTINEZ-ROMERO, D., VALERO, D., SERRANO, M., BURLO, F., CARBONELL, A., BURGOS, L., RIQUELME, F. Exogenous polyamines and gibberellic acid effects on peach (*Prunus persica* L.) storability improvement. **Journal of Food Science**. v. 65, p. 288-294, 2000.

MARTÍNEZ, G.A., CHAVES, A.R., AÑÓN, M.C. Effect of gibberellic acid on ripening of strawberry fruits (*Fragaria annanassa* Duch.). **Journal of Plant Growth Regulation**. v. 13, p. 87-91, 1994.

MARUR, C.J., STENZEL, N.M.C., RAMPAZZO, E.F., SCHOLZ, M.B.S. Ácido Giberélico (GA_3) e maturação de frutos das tangerinas 'Mexerica Montenegrina'e 'Poncã'. **Scientia Agricola**. v. 56, p. 517-521, 1999.

MC NIVEN, M.A., CAO, H., PITTS, K.R., YOON, Y. The dynamin family of mechanoenzymes: pinching in new places. **Trends in Biochemical Sciences.** v. 25, p. 115-120, 2000.

MEDEIROS, C. A. B., RASEIRA, M. C. A cultura do pessegueiro. Brasília: Embrapa-SPI; Pelotas: **Embrapa-CPACT**, 251p. 1998.

NAVA, A. G., BRACKMANN, A. Armazenamento de pêssegos (*Prunus persica* (L.) Batsch), cv. Chiripá, em atmosfera controlada. **Revista Brasileira de Fruticultura**. v. 24, p. 328-332, 2002.

OBENLAND, D. M., CRISOSTO, C. H., ROSE, J. K. C. Expansin protein levels decline with the development of mealiness in peaches. **Postharvest Biology and Technology**. v. 29, p. 11-18, 2003.

PRATELLI, R., SUTTER, J., BLATT, M. R. A new catch in the SNARE. **TRENDS in Plant Science**. v. 9, p. 187-195, 2004.

ROMBALDI, C. V., SILVA, J. A., MACHADO, L. B., PARUSSOLO, A., KASTER, L. C., GIRARDI, C. L., DANIELI, R. Ponto de colheita e período de armazenamento refrigerado na qualidade de pêssegos (*Prunus persica*, L.) de mesa, cv. Chiripá. **Ciência Rural**. v. 31, p. 19-25, 2001.

ROMBALDI, C. V., PARUSSOLO, A., LUCCHETTA, L., ZANUZO, M. R., GIRARDI, C. L., CANTILANO, R. F. F. Armazenamento de pêssegos (Prunus persica L.), cultivar Chiripá, em atmosfera controlada. **Ciência Rural**. v. 32, p. 43-47, 2002.

SEVER, S., MUHLBERG, A.B., SCHMID, S.L. Impairment of dynamin‘s GAP domain stimulates receptor-mediated endocytosis. **Nature**. v. 398, p. 481-486, 1999.

TRAINOTTI, L., ZANIN, D., CASADORO, G. A cell wall-oriented genomic approach reveals a new and unexpected complexity of the softening in peaches. **Journal of Experimental Botany**. v. 54, p. 1821-1832, 2003.

TRAINOTTI, L., BONGHI, C., ZILIOTTO, F., ZANIN, D., RASORI, A., CASADORO, G., RAMIRA, A., TONOTTI, P. The use of microarray μPEACH1.0 to investigate

transcriptome changes during transition from pre-climateric to climacteric phase in peachfruit. **Plant Science**. v. 170, p. 606-613, 2006.

WANG, L., CHENA, S., KONG, W., LI, S., ARCHBOLD, D. D. Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. **Postharvest Biology and Technology**. v. 41, p. 244-251, 2006.

ZAINAL, Z. BIN, G.A. TUCKER, AND G.W. LYCETT. *A rab11*-like gene is developmentally regulated in ripening mango (*Mangifera indica* L.) fruit. **Biochimica et Biophysica Acta**. v. 1314, p. 187-190, 1996.

ZILKAH, S., LURIE, S., LAPSKER, Z., ZUTHI, Y., DAVID, I., YESSELSON, Y., ANTMAN, S., BEN ARIE, R. The ripening and storage quality of nectarine fruits in response to preharvest application of gibberellic acid. **Journal of Horticultural Science & Biotechnology**. v. 72, p. 355-362, 1997.

ZHOU, H. W., LURIE, S., LERS, A., KHATCHITSKI, A., SONEGO, L., BEN-ARIE, R. Delayed storage and controlled atmosphere storage of nectarines: two strategies to prevent woolliness. **Postharvest Biology and Technology**. v. 18, p. 133-141, 2000.

ZHOU, H. W., LURIE, S., BEN-ARIE, R., DONG, L., BURD, S., WEKSLER, A., LERS, A. Intermittent warming of peaches reduces chilling injury by enhancing ethylene production and enzymes mediated by ethylene. **Journal of Horticultural Science & Biotechnology**. v. 76, p. 620-628, 2001.

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