Transcriptome Analyses and Postharvest Physiology of Peaches and Nectarines

C. Bonghi, M. Begheldo, F. Ziliotto and A. Rasori
Department of Environmental Agronomy and
Crop Science, University of Padova
Viale dell' Università 16
35025 Legnaro (Padova)
Italy

L. Trainotti and A. Tadiello
Department of Biology
University of Padova
Viale G. Colombo 3
35121 Padova
Italy

R. Tosetti and P. Tonutti
Scuola Superiore Sant'Anna Piazza Martiri
della Libertà 33
56127 Pisa
Italy

Keywords: auxin, ethylene, fruit, gene expression, genomics, 1-MCP, microarray, *Prunus persica*, ripening, storage, woolliness

Abstract

Genomics approaches and transcriptome analyses are increasingly being used to elucidate mechanisms that regulate complex developmental processes such as fruit ripening and the evolution of quality-related biochemical changes during the postharvest phase. In peach (*Prunus persica* L.), projects aimed at isolating ESTs corresponding to genes expressed in different fruit tissues and the construction and use of tools such as microarrays has resulted in identification of basic information concerning hormonal regulation, in particular on the role of ethylene and auxin and their interplay and cross-talk during ripening. Functional genomics approaches have been undertaken for studies specifically targeted to identify and characterize genes involved in the peculiar response of peaches and nectarines to applications of 1-MCP and in the development of storage disorders.

INTRODUCTION

Climacteric fruit exhibit an increase of ethylene biosynthesis during ripening and the hormone regulates many of the changes in the transcriptional profile of genes during this period (Giovannoni, 2004; Cara and Giovannoni, 2008). Such coordinated and programmed modulation of gene expression leads to changes in fruit texture, colour, taste, and aroma, the most important quality parameters considered by consumers together with the increasing interest in nutritive value and safety for human health. These quality attributes are the result of many chemical and structural modifications that are genetically programmed during ripening, when fruit become edible and attractive for consumption, as well as during the postharvest phase. Specific aspects of fruit biochemistry have received great attention due to their importance in fruit quality: elucidation of biosynthetic pathways of pigments, cell wall architecture and composition, sugar and organic acid metabolism. The application of genome-scale gene expression profiling tools allows better elucidation of the basic mechanisms and interactions occurring during ripening and the postharvest phase in different fruit species including peach (Bonghi and Trainotti, 2006; Tonutti and Bonghi, 2009).

TRANSCRIPTOME CHANGES DURING THE TRANSITION FROM PRE-CLIMACTERIC TO CLIMACTERIC STAGE IN PEACH FRUIT

The Italian Consortium for Genomics studies in *Rosaceae* species (ESTree Consortium) has developed the first microarray (named µPEACH1.0) containing about 4,800 oligonucleotide (70mer) probes corresponding to genes expressed in fruit throughout development (ESTree Consortium, 2005). Microarray hybridizations indicate

that among the genes present in the microarray slide, 267 and 109 genes are up- and down-regulated, respectively during the transition from pre-climacteric to climacteric stage of nectarine fruit ('Fantasia', Trainotti et al., 2006). Among the up-regulated genes, the marked increase of genes encoding protein with enzymatic activity clearly demonstrates that changes in metabolic pathways represent an important feature of the ripening syndrome also in peach fruit. Microarray hybridization data analyses established that a member of the peach ethylene receptor complex (named *Pp-ETR2*) showing high homology to tomato *Le-ETR4* seems deeply involved, together with other receptors already described by Rasori et al. (2002), in the transition from pre-climacteric to climacteric stage. Developmental stage transition is paralleled by changes in expression of 19 genes encoding transcription factors (TFs) belonging to several families including MADS-box, AUX/IAA, bZIP, bHLH, HD and Myb. Six AUX/IAA proteins were up-regulated during the pre-climacteric/climacteric transition.

The μPEACH1.0 has been used to specifically investigate the role played by auxin in the regulation of the climacteric fruit ripening (Trainotti et al., 2007). Interestingly, several Auxin Response Factors (ARF) and at least nine different Aux/IAA genes (transcriptional modulators of the hormone response) have been found to have increased expression at the onset of ripening (i.e., S3II-S4I transition), and could therefore may be involved in this process. Two Aux/IAA genes appeared to be up-regulated by exogenous 1-naphthalene acetic acid (NAA) application, whereas four of them resulted induced by both exogenous ethylene and auxin treatments. Other IAA-related genes have been shown to be up-regulated at the transition: in particular two putative TIR1 (encoding for auxin receptors) and one PIN 1 (encoding a putative auxin efflux facilitator protein) that is induced at the onset of ripening. PIN1 expression is strongly induced by exogenous ethylene demonstrating that also in peaches an active cross-talk between auxin and ethylene is important for the regulation of ripening. This is confirmed by analyzing the expression pattern of the two ethylene biosynthesis key genes (ACS1 and ACO1) following treatments with ethylene and auxin. While the expression of ACO1 appears to be up-regulated mostly by ethylene, in the case of the ACS1 gene the highest up-regulating effect is caused by auxin (Trainotti et al., 2007). These findings highlight a novel aspect of the regulatory networks that operate during the ripening of climacteric peach fruit, thereby demonstrating the existence of an important cross-talk between auxin and ethylene, with genes of the auxin domain regulated by ethylene and genes in the ethylene domain regulated by auxin.

Another relationship studied using the peach microarray is that between ethylene and the jasmonates (JAs), ubiquitous signalling molecules that mediate plant responses to environmental stress (Wasternack, 2007) and play a role during fruit development (Pena-Cortés et al., 2005). Transcriptome profiling of ripening peaches treated with methyl jasmonate (MJA) in planta showed that the delayed development of the ripening syndrome and the reduced ethylene production are associated to a down-regulation of several genes that are strongly induced during ripening and involved in ethylene biosynthesis (ACO1), transcriptional regulation (IAA7) and cell wall metabolism/fruit softening (PG) (Ziosi et al., 2008). An opposite effect (transcription up-regulation) was observed for specific stress/defence-related genes. Taken together these genomics data support the notion that MJA counteracts ripening in peach fruit by altering the expression of specific ripening- and ethylene-related genes.

PEACH POSTHARVEST PHYSIOLOGY AND TRANSCRIPT PROFILING

Peach is up to now one of the few fruit species where transcriptomic approaches have been used to elucidate specific aspects and processes characterizing the postharvest phase. It is well known that peaches and nectarines ripen and deteriorate quickly and even at refrigerated temperature. Their storage life, differently from other climacteric fruit (e.g., apples), rarely exceeds 4-5 weeks making these fruit types interesting for physiological comparative studies. Extended storage of peaches and nectarines induces a decrease of quality parameters (taste, texture) and the appearance of chilling injury (CI)

and physiological disorders, as woolliness (Lill et al., 1989; Lurie and Crisosto, 2005). Therefore, the distance the fruit can be shipped to markets and/or stored before marketing is limited (Campos-Vargas et al., 2006). A macroarray containing 847 non-redundant ESTs from a ripe peach fruit cDNA library has been used to better elucidate the molecular basis of woolliness (Gonzales-Aguero et al., 2008). A total of 106 genes appeared differentially expressed in juicy and woolly fruit. Molecular changes occurring in fruit affected by the physiological disorder involve genes associated with cell wall metabolism and endomembrane trafficking. A database (CHILLPEACH) that includes a collection of about 8,000 full-length cDNAs from sensitive and tolerant peach selections has been developed and a CHILLPEACH microarray has been printed (Granell et al., 2007) and used to identify cold-responsive genes in peach fruit (Ogundiwin et al., 2008). Stress-induced genes such as dehydrin, chitinase, RING zinc finger, ankyrin protein, ABA-inducible protein, BZIP TF, Chalcone synthase, protein kinase resulted up-regulated in cold-stored fruit, whereas genes encoding for heat shock proteins (HSP) were down-regulated. This set of molecular information is important to expedite CI-resistance gene discovery and improve the precision of candidate gene mapping.

Another feature of peaches and nectarines is the limited effects of 1-methylcyclopropene (1-MCP), an antagonist of ethylene action (Blankenship et al., 2003), in slowing ripening and prolonging the postharvest storage life (Dal Cin et al., 2006). In contrast to apples, the effect of 1-MCP on peaches is limited to a few hours after the end of the incubation period and the difference might be due to differences existing between these two fruit species in terms of ratio, expression pattern and/or turn-over of the ethylene receptors affecting the sensitivity to ethylene and its inhibitors (Dal Cin et al., 2006). To better elucidate the relationships existing between peach ripening/postharvest physiology and ethylene, the μPEACH1.0 has been used to analyze the effect of exogenous ethylene and propylene (analogous to ethylene) treatments (24h) in pre-climacteric peach fruit ('Redhaven'). A total of 196genes appeared up-regulated by the treatment whereas the transcription of 169 was repressed: considering that, as previously reported, 219 were the genes induced and 188 those down-regulated in the transition pre-climacteric/climacteric stage (Trainotti et al., 2007). Taken together these data confirm that both ethylene-dependent and ethylene-independent gene regulation mechanisms are present also in ripening peaches. Among ethylene/propylene-stimulated targets, microarray analysis reveals that a number of genes encoding transcription factors (e.g., bZIP. AUX/IAA, AP2, MADS-box) are present, indicating a marked modification of peach fruit physiology induced by the treatment.

In addition to peaches treated with ethylene/propylene, large-scale transcriptome analyses have been performed on peach mesocarp sampled at the end of the 24h incubation period with 1-MCP and in air (control) (Ziliotto et al., 2008). A total of 53 and 50 genes resulted up- and down-regulated by the chemical, respectively, indicating that the presence of 1-MCP is perceived by peaches and leads to changes in the expression pattern of specific genes. A comparison with genes differentially expressed (43 up- and 47 down- regulated) after the same period (24h from harvest) in air shows that 17 genes induced by 1-MCP are also induced by ripening, whereas 30 genes down-regulated by ripening are also induced by the ethylene antagonist. The former set of shared genes resulted stimulated by propylene, whereas genes belonging to the second group appeared repressed by the ethylene analogue.

Considering specific genes involved in quality-related processes, a polygalacturonase, an expansin, and putative pectin methylesterase and pectin acetylesterase genes showed reduced expression at the end of the 24h incubation period with 1-MCP. Some genes involved in ethylene biosynthesis/perception/signal transduction appeared negatively affected by the ethylene antagonist. Considering these and other results, it might be hypothesized that the limited effect of 1-MCP on peaches is not due to a lack of binding activity to (all) ethylene receptors but other factors are probably responsible for the quick recovery of ripening parameters following 1-MCP treatment. Improvement in gene annotation and growth of sequence databases together

with functional analysis approaches will shed light on this and other mechanisms controlling the fruit ripening syndrome and modulation of the postharvest evolution of quality parameters of peaches and nectarines.

FINAL CONSIDERATIONS AND FUTURE PERSPECTIVES

The expansion of genomics resources and the rational organization of databases will facilitate a systems approach and a wider use of bioinformatics platforms for studying ripening and other postharvest processes in several fruit species including peach. For this purpose, integration of genomics datasets resulting from the application of transcript and protein abundance, metabolite accumulation and metabolic flux analysis, will be crucial to unravel the mechanisms that link genotypes to phenotypes and are responsible for quality traits. In this context, the ongoing *Prunus persica* genome sequencing project, together with the development of deep-sequencing technologies (e.g., RNA-Seq), will represent formidable advancements and powerful tools to identify and characterize peach genes and track gene expression changes during the last phase of development and in relation to different postharvest conditions.

Literature Cited

Blankenship, S.M. and Dole, J.M. 2003. 1-Methylcyclopropene: a review. Postharvest Biol. Technol. 28:1-25.

Bonghi, C. and Trainotti, L. 2006. Genomics tools for a better understanding of the fruit ripening process. Stewart Postharvest Review [online], April 2006, vol2, no.3. Available from: http://www.stewartpostharvest.com/April_2006/Bonghi.pdf.

Campos-Vargas, R., Becerra, O., Baeza-Yates, R., Cambiazo, V., González, M., Meisel, L., Orellana, A., Retamales, J., Silva, H. and Defilippi, B.G. 2006. Seasonal effects on the development of chilling injury development in peach (*Prunus persica* [L.] *Batsch*) cv. O'Henry. Scientia Hort. 110:79-83.

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

Dal Cin, V., Rizzini, F.M., Botton, A. and Tonutti, P. 2006. The ethylene biosynthetic and signal transduction pathways are differentially affected by 1-MCP in apple and peach fruit. Postharvest Biol. Technol. 42:125-133.

ESTree Consortium. 2005. Development of an oligo-based microarray (μPEACH 1.0) for genomics studies in peach fruit. Acta Hort. 682:263-268.

Giovannoni, J.J. 2004. Genetic regulation of fruit development and ripening. Plant Cell 16:170-180.

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

Granell, A., Pons, C., Marti, C., Forment, J., Royo, C., Gradziel, T.M., Peace, C.P., Ogundiwin, E. and Crisosto, C.H. 2007. Genomic approaches - Innovative tools to improve quality of fresh cut produce. Acta Hort. 746:203-211.

Lill, R.E., O'Donoghue, E.M. and King, G.A. 1989. Postharvest physiology of peaches and nectarines. Hort. Rev. 11:413-452.

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

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

Pena-Corés, H., Barrios, P., Dorta, F., Polanco, V., Sanchez, C., Sanchez, E. and Ramirez, I. 2005. Involvement of jasmonic acid and derivatives in plant responses to pathogens and insects and in fruit ripening. J. Plant Growth Regulation 23:246-260.

Rasori, A., Ruperti, B., Bonghi, C., Tonutti, P. and Ramina, A. 2002. Characterization of two putative ethylene receptor genes expressed during peach fruit development and

abscission. J. Exp. Bot. 53:2333-2339.
Tonutti, P. and Bonghi, C. 2009. Advanced technologies and integrated approaches to investigate the molecular basis of fresh produce quality. P561-582. In: W.J. Florkowski (ed.), Postharvest Handling: A Systems Approach (2nd edition), Elsevier Inc., Amsterdam.
Trainotti, L., Bonghi, C., Ziliotto, F., Zanin, D., Rasori, A., Casadoro, G., Ramina, A. and 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.
Trainotti, L., Tadiello, A. and Casadoro, G. 2007. The involvement of auxin in the ripening of climacteric fruits comes to age: the hormone plays a role of its own and has an intense interplay with ethylene in ripening peaches. J. Exp. Bot. 58:3299-3308.
Wasternack, C. 2007. Jasmonates, an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann. Bot. 100:681-697.
Ziliotto, F., Begheldo, M., Rasori, A., Bonghi, C. and Tonutti, P. 2008. Transcriptome profiling of ripening nectarine (*Prunus persica* L. Batsch) fruit treated with 1-MCP. J. Exp. Bot. 59(10):2781-2791.
Ziosi, V., Bonghi, C., Bregoli, A.M., Trainotti, L., Biondi, S., Sutthiwai, S., Kondo, S., Costa, G. and Torrigiani, P. 2008. Jasmonate-induced transcriptional changes suggest a negative interference with the ripening syndrome in peach fruit. J. Exp. Bot. 59:563-573.