



## MAICh Conference Center

### Chania, Crete, Greece

The second Annual International Conference of the COST ACTION FA1106 QualityFruit has been organized by a Committee headed by Prof. Panagiotis Kalaitzis, the Chair, Mondher Bouzayen and the Co-chair of the Action, Mario Pezzotti and the Grant Holder Jean-Marc Routaboul in close collaboration with the scientific committee.

The main part of this meeting has been dedicated to scientific and networking activities. With 80 participants, 31 speakers (9 were Early Stage researcher) from 15 different countries (5 Keynote speakers from non COST countries were also represented: South-Africa, Argentina, New-Zealand, Australia and United-State see below) the science oriented QualityFruit 2013 meeting reached its objectives giving the largest information about “Fleshy fruit development”, the major theme of our Action.

The scientific program included three sessions: Fruit development and ripening, Fleshy fruit metabolism and Molecular breeding for fruit quality traits. The program covered different aspects of the molecular and genetic regulatory mechanisms underlying the buildup of fruit quality traits. The meeting focused on the two model fruit species, tomato and grape, but few communications were dedicated to other fleshy fruit species such as apple.



Don Grierson

## Thoughts on Control of Fruit Development & Ripening

University of Nottingham, UK

## James Giovannoni

*Boyce Thompson Institute for Plant Research; Department of Plant Biology, Cornell University. Ithaca, NY 14853 USA, jjg33@cornell.edu*

Tomato is a primary model for fruit development and shelf-life in addition to a vegetable crop of increasing production and nutritional importance the world over. Annual per capita tomato consumption in the US alone is near 70 pounds making tomato primary sources of vitamins A and C in US diets. Pioneering work in tomato biology has elucidated mechanisms of pathogen response, ethylene hormone synthesis and perception, carotenoid metabolism and transcriptional control of fruit ripening. Translational biology of tomato discoveries has, for example, demonstrated conservation of nutrient metabolism and ripening mechanisms in a range of species and suggests that additional discoveries will have wide ranging effects on food security and nutrient content. Novel and well characterized germplasm resources, efficient transformation and a high quality genome sequence have accelerated the pace of tomato biology facilitating genome, expression and metabolite/trait analyses and the ability to exploit systems approaches toward biological discovery with practical implications to crop improvement. In highlighting these resources and their applications, the roles of multiple transcriptional regulators of ripening and nutrient content will be described along with

preliminary data suggesting a role for epigenome modification in control and manifestation of the ripening process.

**Chris Davies**

*CSIRO Plant Industry, Adelaide, Australia*

There are obvious differences in the morphology and metabolism of grapes (non-climacteric) and tomatoes (climacteric) and how they develop. Similarly, there are numerous examples of differences in the roles of various hormones during fruit development and in particular ripening. Close examination however, reveals some common elements underlying the way various hormones participate in the ripening of these two important fruit. Interactions between the biosynthesis and perception pathways of the different hormones are important in both fruit and the role of different hormones is strongly dependent upon developmental stage. In grapes, levels of abscisic acid (ABA) and the brassinosteroid castasterone increase rapidly at veraison. ABA and castasterone application before veraison can advance ripening and an inhibitor of brassinosteroid biosynthesis delayed ripening. ABA may also act as a promoter of tomato ripening and appears to be involved in cell wall changes. As in grapes, brassinosteroids can advance tomato disc ripening. The role of ethylene in grape berry ripening is less clear than in tomatoes. An increase in ethylene evolution at veraison is so small as to be difficult to measure. However, much of the 'machinery' of synthesis and perception are similar in both fruit species. Increasing evidence demonstrates that ethylene can play a role in grape berry development albeit a less dominant role than that played in climacteric fruit. Grapes appear to lack autocatalytic ethylene production and exhibit a biphasic response to ethylene during the first half of development. Auxins delay ripening in tomato and other climacteric fruit if applied early enough in development to avoid the induction of a climacteric response. In all cases the timing of hormone application is crucial to effect. Grape ripening is also delayed by auxin application pre-veraison. The pattern of auxin accumulation is similar in both being higher in young fruit decreasing to low levels prior to the onset of ripening. The family of indole-3-acetic acid (IAA)-amido synthetases (GH3 enzymes) important for auxin homeostasis appears to be a critical factor in the control of grape berry ripening and there is an accumulation of the IAA-Asp conjugate after veraison. A similar situation occurs in tomatoes and two GH3 genes are upregulated at the onset of tomato ripening. We have recently elucidated the 3-D structure for one of the grape IAA-amido synthetases that provides insights at the molecular level into an important mechanism involved in auxin homeostasis.

Department of Plant Sciences, University of California, Davis, CA, USA

Developmental processes contribute to the outcomes of interactions between plants and pathogens. Regulated in part by transcription factors, early development of fruit in tomato (*Solanum lycopersicum*), establishes structural and metabolic environments within the fruit flesh that are reconfigured later as other transcription factors and hormones activate and regulate maturation and ripening programs. Ripening leads to significant losses of mature and harvested products from the maceration or rotting of flesh of the fruit caused by microbial infections and growth. As much as 80% of the ripened fruit from over 200 species are destroyed due to their susceptibility, in particular to necrotrophic fungal pathogens, such as the filamentous ascomycete *Botrytis cinerea*. No robust genetic source of resistance to this pathogen has been identified. The limited growth of *B. cinerea* on unripe fruit is altered by developmental and ripening processes in the fruit and by the differences in the infection strategies deployed by the pathogen on ripe and unripe fruit.. Although unripe tomato fruit are typically resistant to infections by *B. cinerea*, the pathogen expresses virulence functions that eventually enable aggressive hyphal growth and the precocious activation of selected aspects of fruit ripening. As tomato fruit ripen around mature seeds, the changing physiology and cell wall polysaccharide architecture of the epidermis and pericarp flesh contribute to the increasing susceptibility of fruit. The susceptibility of ripe fruit could simply be a default outcome of the entire syndrome of ripening. However, tomato lines with mutations that affect fruit development and ripening provide evidence of the key roles of selected aspects of early development and subsequent ripening in the acquisition of susceptibility in ripened fruit. Mutations in ripening regulators reveal that specific ripening processes and changes in the polysaccharides within the cell walls of the softening flesh are required to render ripe fruit supportive of active *B. cinerea* growth. Some non-ripening mutants are susceptible to *B. cinerea*, leading to the conclusion that not all ripening processes are required for fruit to become susceptible to infections. By identifying the regulators of fruit ripening and fungal virulence processes which are crucial for the susceptibility of ripened fruit, acceptably mature fruit with reduced rotting may eventually be developed and yield improved fresh and stored products.

*Plant and Food Research, 120 Mount Albert Road Auckland New Zealand*

In fleshy fruit species that have a strong requirement for ethylene to ripen, ethylene is synthesised auto-catalytically, producing increasing concentrations as the fruit ripen.

Suppression of the ethylene biosynthesis gene, ACC OXIDASE 1 (*ACO1*) in apples, stops autocatalytic ethylene production at fruit maturation. Using these apples, applied with different concentrations of ethylene, we have shown ripening traits are controlled in an ethylene sensitivity-dependency manner. Some early traits such as starch breakdown have a high sensitivity to ethylene, but low dependency, while others such as flesh softening have a lower

sensitivity, yet high dependency on ethylene. Further investigation of this model suggests that apple fruit responds to ethylene in a dose-by-time mechanism, with sustained lower ethylene concentrations giving the same response as shorter periods of high ethylene. Focussing on fruit softening, at the molecular level, high levels of ethylene ( $>100 \mu\text{L.L}^{-1}$ ) causes multiple waves of cell wall gene expression. This contrasts to expression patterns observed in receptor genes, which are rapidly and consistently up regulated by these concentrations. At lower ethylene concentrations ( $>1 \mu\text{L.L}^{-1}$ ) the expression patterns become more variable, especially with early response waves. This data suggests multiple levels of transcriptional control during fruit ripening that are carefully orchestrated to a set program of ripening steps.

M.A. Vivier

*Institute for Wine Biotechnology, Stellenbosch University, Stellenbosch, South Africa*

Terpenes are a large group of organic compounds that are involved in diverse functions in plants that range from defense responses to phytohormone production. Carotenoids are  $\text{C}_{40}$ -tetraterpenes and their metabolism in plants and fruits has received much attention. From a human perspective carotenoids are an important source of antioxidants and serve as vitamin A (retinol) precursors in our diet. From a plant perspective, they fulfill crucial roles in photosynthesis (i.e. light harvesting and photo-protection). Similarly, carotenoid catabolism (via carotenoid cleavage) has received much attention due to the production of the phytohormones ABA and strigolactone; as well as the formation of norisoprenoids, impact flavour and aroma compounds in a number of commercially important fruits and flowers. The carotenoid metabolic pathway is well characterised in plants and the orthologues of the pathway members have been identified in the grapevine genome and selected enzymes characterised<sup>1</sup>.

The availability of the grapevine genome and associated tools such as transcriptional and metabolite profiling, facilitated a carotenoid pathway analysis, using berries from a model (highly characterised) Sauvignon blanc vineyard. Methods have been optimised for profiling the carotenoids (carotenes and xanthophylls) and chlorophylls<sup>2</sup>; as well as the flavor and aroma related volatile apocarotenoids<sup>3</sup>. During berry development in grapevine, the major carotenoids (i.e.  $\beta$ -carotene and lutein) decrease in a developmental pattern that closely mirrors chlorophyll decline. Specific carotenoids (especially the xanthophylls) were shown to increase during development depending on the environmental conditions. Carotenoid degradation via enzymatic cleavage is catalysed by the carotenoid cleavage dioxygenases (CCDs). Grapevine possesses orthologues for VvCCD1 and VvCCD4 (involved in norisoprenoid production), VvCCD7 and VvCCD8 (involved in strigolactone formation) and NCEDs (involved in ABA production). A transcriptomic analysis of Sauvignon blanc berries harvested in three stages of development showed that VvNCED3 expression corresponded with the sudden increase in ABA levels in ripening berries<sup>1</sup>. The expression profile, functional analysis and substrate specificity of VvCCD1, VvCCD4a and b are providing important clues to their potential *in planta* roles in berries. The transcriptomic profiling, as well as the pigment and apocarotenoid volatile profiling from the same samples during berry development and ripening allow the possibility to evaluate carotenoid metabolism in a holistic manner. The results obtained for grapevine fruits will be discussed with reference to what is known in tomato and other fruits.



*Organization Committee:*

**Panagiotis Kalaitzis**

**Mondher Bouzayen**

*Scientific Committee:*

**Asaph Aharoni**

**Mondher Bouzayen**

**Mario Pezzotti**

**Jean-Marc Routaboul**

**Arnaud Bovy**

**Serge Delrot**

**Allisdair Fernie**

**Giovanni Giuliano**

**Hernani Geros**

**Toni Granell**

**Panagiotis Kalaitzis**

**Angelos Kanellis**

**Cathie Martin**

**Mario Pezzotti**

**Graham Seymour**

**Jose Martinez-Zapater**