



ELSEVIER

Abstracts / Comparative Biochemistry and Physiology Part A 132 (2002) S93–S100

www.elsevier.com/locate/cbpa

---

---

**CBP**

---

---

## **P1–FRUIT DEVELOPMENT AND RIPENING**

**Organised by R. Napier, G. Seymour and P. White**

This session is sponsored by The Journal of Experimental Botany

### **P1.1–Analysis of *Arabidopsis* mutants that confer changes in pod senescence**

T. Yang, J.A. Roberts, Biosciences, University of Nottingham; M. Maunder, ATC Cambridge, UK

Senescence is a sequence of biochemical and physiological events that lead to the eventual death of a cell, organ or the whole organism. The time course of the phenomenon is genetically determined, however, in spite of its biological and practical importance, the events that regulate it are unclear.

To increase our understanding of the process we have chosen to focus our research on pod development in *Arabidopsis*. Pod and leaf senescence share a number of features in common, however, the yellowing of the former organ occurs at a much more predictable rate than the latter and as a consequence it may be easier to identify the molecular events that are critical for the process to take place. Initial studies have determined the variation in chlorophyll content during the course of pod development and this is being used as an index of the time course of senescence. Results will be presented of the impact of mutations in the pathway of ethylene action on pod senescence and the characterisation of a number of Recombinant Inbred lines that exhibit attenuations in the process.

### **P1.2–The role of *FRUITFULL*-Like genes in fleshy fruit**

A.H. Popovich, E. Eriksson, G.J. King and G.B. Seymour, Horticulture Research International, Department of Plant Genetics and Biotechnology, Wellesbourne, Warwick, CV35 9EF, UK

In *Arabidopsis*, genes have been identified which are responsible for controlling dehiscence in the siliques [Ferrandiz et al., 2000: Science 289 (2000) 436–438]. The *FRUITFULL* (*FUL*) MADS-box gene, is necessary for fruit valve differentiation, is a negative regulator of other MADS-box genes which promote dehiscence, and constitutive expression of *FUL* is sufficient to prevent the formation of the dehiscence zone. Our hypothesis is that genes involved in regulating ripening in fruits have

been conserved during evolution and that orthologues of *FUL* may have a related function in indehiscent fleshy fruits. We have been investigating the role of likely *FUL* orthologues in tomato and apple fruits. Real-time PCR demonstrated a marked increase in *FUL*-like expression in ripening tomato pericarp, but this increase was absent in the mealy *Cnr* tomato ripening mutant. We have generated transgenic tomato plants to suppress the expression of our *FUL*-like gene in tomato fruit and are in the process of characterising the phenotype of this transgenic material.

### **P1.3–Vascular structure of oilseed rape pods and its importance in valve separation**

J.E. Summers, J. Babij, R.D. Child (IACR-Long Ashton, University of Bristol, UK), D.M. Bruce & J. Farrent, Biomaterials, Silsoe Research Institute, UK

Ripening of oilseed rape (*Brassica napus*) pods is completed by the dehydration of the pod wall and separation of cells in the dehiscence zones (DZ) formed between the lignified margin of the two valves and the replum. Seed release occurs when the two valves separate along these lines of weakness, but only after external forces sever vascular tissue passing through the pod wall and replum. Ripe pods of all commercial cultivars are susceptible to valve loss (shatter) before and during the harvest and this can result in over 20% seed loss. Although some variation in shatter resistance exists in commercial varieties, it is small compared with the increased resistance found in lines resynthesised from the putative parents of *B. napus*. Using scanning electron microscopy and a new microfracture technique, we have exploited the variation in shatter resistance within and between populations of a commercial cultivar and a synthetic line to further the understanding of the mechanisms underpinning resistance. Comparing between lines, differences in the degree of cell separation are of minor importance, although more energy is required to separate synthetic pods that have wider DZs than Apex. In contrast, the synthetic line has more vascular tissue intruding into the DZ at the pedicel end of the pod. This area is more resistant than the main body and here, vascular bundles running the length of the pod merge to form the main vascular bundle. It is the size of this struc-

ture that largely determines the shatter resistance of the entire pod.

#### **P1.4—A physiological role of photoassimilates and ABA in the premature abscission of sweet cherry fruits**

T. Blanus, HRI-East Malling, Lancaster University, UK; M.A. Else and C.J. Atkinson, HRI-East Malling, UK; W.J. Davies, Lancaster University, UK

Premature fruit abscission is a major concern for UK sweet cherry growers, but its causes are not understood. Competition for resources such as photoassimilates and plant hormones could trigger fruit abscission.

Predicting fruit abscission before the visible symptoms develop has, hitherto, not been possible. Sweet cherries develop primarily on short shoots (spurs), simultaneously with their leaves (spur leaves). Spurs generally have similar leaf areas but very different fruit numbers. Patterns of fruit abscission can be modified by spur girdling (removing the phloem connections between the spur and the tree); the disruption of phloem import presumably 'forces' fruit to derive all their assimilates from their spur leaves.

We show here that cherries from girdled spurs with many fruit (eight fruit per spur) abscised within 14 days after girdling (DAG), cherries on spurs with few fruit (two fruit per spur) were retained. Carbon dioxide assimilation of leaves on girdled spurs, with different fruit numbers, was measured over 15 DAG. Fruit were collected for ABA quantifications 3, 5, 7 and 9 DAG. After girdling, net CO<sub>2</sub> assimilation of leaves from spurs with eight fruit was greater than that of leaves from spurs with two fruit. However, spurs with many fruit assimilated 2.5–4-fold less CO<sub>2</sub> per fruit compared to spurs with few fruit. ABA concentration in fruits, from spurs with eight fruit, was greater than in fruits from the spurs with two fruit; differences peaked at four-fold, 5 DAG.

The relationships between leaf-derived photoassimilates, fruit hormonal status and fruit abscission are discussed.

#### **P1.5—A comparison of the optical properties of ripening fruits and senescing leaves**

D.N. Price, S.D. Lane, A. Edmans, K.L. Gazzard, M.E. Donkin, University of Plymouth, UK

Changes in the optical properties of ripening fruit and senescing leaves were followed using a modified Ulbrecht sphere attached to a standard scanning spectrophotometer. In evaluating this potentially non-invasive approach to studying these closely related processes, the percentage transmittance, percentage reflectance and

computed percentage absorbance of appropriate tissues were determined between 350 and 750 nm.

Transmittance proved to be the most effective method for analysing leaf senescence, whilst for most fleshy fruit, percentage reflectance measurement was the only feasible approach. However, an analysis of wavelength sensitivity revealed that 670–680 nm was the most responsive region for both approaches. This clearly relates to the major decline in chlorophyll levels accompanying the two processes; confirmed in a correlation study analogous to that of Carter et al. (2001). The correlation appears to be exponential rather than linear. The method proved to be less sensitive/specific in detecting changes in other fruit/leaf pigments.

This approach to studying ripening and senescence is illustrated using two studies—the effect of temperature on ripening bananas and the wheat leaf senescence bioassay for cytokinins. Its wider exploitation is discussed. Reference: Carter, G.A., Knapp, A.K., 2001. Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. *Am. J. Bot.* 88, 677–684.

#### **P1.6—Molecular and biochemical analysis of broccoli (*Brassica oleracea* var. *botrytis*); a tool towards Quantitative Trait Loci (QTL) for post-harvest senescence**

E. Mathas, V.B.-Wollaston, and D. Pink, Horticulture Research International, Plant Genetics & Biotechnology, Wellesbourne CV35 9EQ, UK; M.J. Kearsey, University of Birmingham, School of Biosciences, Edgbaston B15 2TT, UK

Broccoli (*Brassica Oleracea* var. *Botrytis*) is a very popular and widely consumed vegetable. It has a very high nutritional value and it is currently used in a number of value-added products. However, its commercial value is affected by its relatively short life (5 days). Recent research in our laboratory has shown that there is a genetic determinant that affects shelf life of broccoli. The aim of this project is to identify regions in the genome of broccoli that determine the rate of post-harvest senescence. A number of different double haploid broccoli lines (DH) and F1s were tested in field trials that took place at HRI, Wellesbourne during 2001. The broccoli heads were harvested and stored in the dark at room temperature for 4 days. The phenotypic changes during storage were evaluated daily. Floret material was isolated and used in biochemical and molecular analyses. The expression patterns of different senescence enhanced genes have been compared and biochemical changes have been measured in the good and poor storing broccoli lines. The molecular and biochemical data will serve as a guide/reference for the characterisation of this mapping population.

A number of double haploid lines have been generated through microspore culture from a cross between two broccoli lines showing very different storage characteristics. These will be grown during summer 2002 and their shelf life assessed. This will serve as the mapping population for Quantitative Trait Loci (QTL) analysis.

### **P1.7—The role of calcium signalling in fruit ripening**

K. Harms, D. Grierson, Plant Sciences, University of Nottingham, UK; G.B. Seymour, P.J. White, HRI Wellesbourne, UK

Fruit ripening is initiated by changes in the expression of a highly co-ordinated set of genes leading to distinct biochemical changes in all cell compartments. Expression of many genes increases such as those encoding polygalacturonases (PG), pectinesterases (PE), 1-aminocyclopropane-1-carboxylate oxidase (ACO), while that of others decreases. In climacteric fruit the expression of many ripening-related genes is regulated by the hormone ethylene. There is evidence that  $\text{Ca}^{2+}$  may act as a second messenger in ethylene signal transduction. This project will test whether ripening-specific gene expression is dependent on a change in cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ). The gene encoding the  $\text{Ca}^{2+}$ -reporting protein aequorin has been introduced into wild type Ailsa Craig and the non ripening tomato mutants *ripening-inhibitor* (*rin*) and *never-ripe* (*Nr*) and will also be introduced into *colourless non-ripening* (*cnr*) and *non-ripening* (*nor*) mutants using the cytoplasmic-targeting expression vector pMAQ2. These plants will be analysed for changes in  $[\text{Ca}^{2+}]_{\text{cyt}}$  at different ripening stages. The non-ripening mutants are being used since there is tantalising evidence that these fruit may have altered levels or distribution of  $\text{Ca}^{2+}$  in their cells.

### **P1.8—Characterization of developmental regulatory genes controlling tomato fruit ripening**

J. Giovannoni, P. Payton, S. Moore, and J. Vrebalov, USDA-ARS and Boyce Thompson Institute for Plant Research, Ithaca, NY, USA; Z. Fei, Department of Horticultural sciences, Texas A&M University, College Station, TX, USA

Little is known regarding steps prior to ethylene biosynthesis for the regulation of climacteric fruit ripening. We utilize tomato as a model system for ripening as a number of single-gene mutations in regulatory steps prior to ethylene have been identified. We have focused on isolation of genes at the *ripening-inhibitor* (*rin*) and *non-ripening* (*nor*) loci as corresponding mutations result in fruit, which are capable of synthesizing and responding to ethylene, yet fail to ripen. We interpret this phenotype to be indicative of developmental regulatory steps reg-

ulating ethylene biosynthesis and additional factors required for completion of the fruit maturation process. Positional cloning of the *rin* locus revealed two MADS-box transcription factors that have been functionally characterized in transgenic tomato lines altered in expression of each respective gene. We have isolated homologous genes from additional fruit species (strawberry and banana), and have observed fruit-specific expression for both. We are currently characterizing additional MADS-box family members from tomato for assessment of their potential involvement in fruit development and ripening. Our laboratory is also a participant in the Tomato Genome Project supported by the National Science Foundation (NSF) and current project objectives and publicly available tools will be summarized. We currently are employing tomato microarrays resulting from this project for gene expression profiling of fruit development, and ripening and have additionally tested the utility of said microarrays for gene expression analysis of additional members of the Solanaceae.

### **P1.9—Characterisation of genes regulating ripening in fleshy fruits**

G.B. Seymour and K. Manning, Plant Genetics & Biotechnology Department, Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK

Fleshy fruits are an essential part of the human diet providing vital vitamins, minerals and other health promoting compounds such as lycopene, that have been implicated in reducing the incidence of chronic conditions such as heart disease and certain cancers. The economic value of fruits is estimated at £3.8 billion retail in the UK.

Effective control of the ripening process is very important to deliver to the consumer high quality fresh and processed fruit products and to encourage increased consumption of these products. Currently the industry in the UK and worldwide faces significant problems relating to the control of storage and shelf life and predicting and controlling the quality of the ripe fruit, especially attributes such as texture. A major breakthrough would be identification of generic genes associated with texture and shelf life in fleshy fruits. Our aim is to identify and characterise generic ripening-regulatory genes controlling texture and shelf life in a wide range of fleshy fruits and use this information to develop strategies for improving fruit quality.

In tomato, a small number of single gene mutations exist, such as *rin*, *nor* and *Cnr* which have pleiotropic effects resulting in the reduction or almost complete abolition of ripening. These probably represent lesions in ripening-regulatory genes. For instance *Cnr* (Colourless non-ripening) results in a non-ripening phenotype with two distinct characteristics: (1) firm fruit with reduced cell-to-cell adhesion; and (2) complete abolition of carotenoid biosynthesis in the pericarp [see Thomp-

son et al., *Plant Physiology* 120 (1999) 383–389]. We are using a genetic map-based approach to isolate the *CNR* gene and want to then understand its role in juiciness and colour development.

### **P1.10—An analysis of fruit development in *Arabidopsis thaliana***

C. Ferrandiz, Dept. Biología Aplicada/Genética, Universidad Miguel Hernández de Elche, San Juan, Alicante, Spain; S. Sato, A. Roeder, M. Yanofsky, Department of Biology, University of California at San Diego, La Jolla, CA, USA

The fruit is a highly specialized plant organ that occurs in diverse forms among the angiosperms. Fruits of *Arabidopsis thaliana* develop from a gynoecium that consists of two fused carpels. The mature gynoecium of *Arabidopsis* is composed of an apical stigma, a short style, and a basal ovary that contains the developing ovules. Following fertilization of the ovules, the fruit elongates and differentiates a number of distinct cell types, allowing for the successful maturation and the eventual dispersal of the seeds.

The *FRUITFULL* (*FUL*) MADS-box gene is required for normal fruit development in *Arabidopsis*. All the tissues within the ovary walls are affected by the *ful* mutation: The valves fail to differentiate and expand and frequently break out before maturation of the seeds, the style displays abnormal morphologies, and the replum adopts a zig-zag arrangement (1). To better understand the role of *FUL* in carpel tissue development, we have generated gain-of-function alleles by constitutively expressing *FUL* under the control of the 35S CaMV promoter. 35S::*FUL* lines display a phenotype in which the ovary walls are largely converted into valve tissue. Moreover, the dehiscence of the pod does not occur upon desiccation (2). Dehiscence, or pod shatter, is a carefully orchestrated event that occurs late in fruit development to assist in the dispersal of seeds. This process involves the formation of a dehiscence zone, a region that is only one to three cells wide, and extends along the entire length of the fruit at the valve-replum boundary. The indehiscent phenotype of the 35S::*FUL* fruits resembles the *shatterproof1 shatterproof2* (*shp1 shp2*) double mutants (3). We have investigated the possible interactions between *FUL* and *SHP1/SHP2* at the genetic and molecular level (2). To further explore the function of *FUL*, we have generated transgenic plants that have a constitutively activated version of *FUL* (*FUL:VP16*) under the control of the endogenous *FUL* regulatory sequences. The phenotypic and molecular analysis of *FUL:VP16* gynoecia identify the *SHATTER-PROOF* genes as direct targets of *FUL* repression in fruit valves, whereas other genes regulated by *FUL* seem to be controlled by more indirect ways. The *FUL:VP16*

allele also reveals a role of *FUL* in controlling both inflorescence and floral determinacy.

We have also undertaken a mutagenesis approach to identify additional loci involved in these processes. In an EMS mutagenized *ful* population, a suppressor of *ful* phenotypes in the valves has been identified and cloned. A new gain-of-function mutant obtained in an activation tagging experiment showed phenotypes resembling *ful* effects in fruit development (4). Corresponding loss-of-function alleles have been identified and are currently under functional characterization.

Gu, Q., Ferrandiz, C., Yanofsky, M.F., Martienssen, R., 1998. *Development* 125 1509–1517.

Ferrandiz, C., Liljegren, S., Yanofsky, M., 2000. *Science* 289 436–438.

Liljegren, S., et al., 2000. *Nature* 404 766–769.

Weigel, D.

### **P1.11—Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae**

S. Knapp, Department of Botany, The Natural History Museum, London SW7 5BD, UK

The Solanaceae contains many species of agricultural importance. Several of these are cultivated for their fruits, such as the tomato, the pepper and the aubergine. The family is very diverse in fruit type with capsules, drupes, pyrenes, berries and several sorts of dehiscent non-capsular fruits occurring in the 90+ genera. In this paper I review the recent work on fruit type evolution in angiosperms in relation to dispersal agents and habitat ecology. Defining fruit types in the Solanaceae in a simple five-state system, then mapping them onto a previously published molecular phylogeny based on chloroplast DNA allows discussion of the evolution of these fruit types in a phylogenetic framework. Capsules are plesiomorphic in the family, and although berries are a synapomorphy for a large clade including the genus *Solanum* (tomatoes and aubergines), they have arisen several times in the family as a whole. Problems with homology of drupes and pyrenes are discussed, and areas for future investigation of fruit structure homology identified. The distribution of fruit types in the large and diverse genus *Solanum* is also discussed in the light of monophyletic groups identified using chloroplast gene sequences. This variety is related to recent advances in the understanding of the molecular biology of fruit development. Finally, several key areas of future comparative, phylogenetic investigation into fruit type evolution in the family are highlighted.

### **P1.12—Control of tomato development at the level of ethylene receptors**

H. Klee, University of Florida, Horticultural Sciences Plant Molecular and Cellular Biology Program, P.O. Box 110690, Gainesville, FL 32611, USA

The plant hormone ethylene controls many aspects of development and response to the environment. In tomato, ethylene is an essential component of flower senescence, organ abscission, adventitious root initiation and fruit ripening. Responses to ethylene are also critical for aspects of biotic and abiotic stress responses. Clearly, much of the control of these events occurs at the level of hormone synthesis. However, it is becoming apparent that levels of the ethylene receptors are also highly regulated. The tomato ethylene receptors are encoded by a family of six genes. Levels of expression of these genes are spatially and temporally controlled throughout development. Further, a subset of the receptor genes respond to external stimuli. Genetic and biochemical evidence supports a model in which the ethylene receptors act as negative regulators of downstream responses; in the absence of ethylene, receptors actively suppress expression of ethylene responsive genes. Consistent with this model, reduction in the overall level of receptor increases ethylene responsiveness of a tissue while higher expression of receptor decreases ethylene sensitivity. Evidence to support this model will be presented.

### **P1.13—Ethylene signalling in ripening tomato fruit**

L. Alexander, Z. Lin, G. Chen, S. Kim, R. Hackett, and D. Grierson, Plant Science Division, University of Nottingham, UK; I. Wilson, IACR Long Ashton, UK

Progress in understanding how plants perceive and respond to ethylene has been made by studying *Arabidopsis thaliana* mutants showing disruption of the 'triple response' of dark grown seedlings to ethylene. Cloning of the mutant genes giving rise to these phenotypes has led to identification of five ethylene receptors, which show homology to bacterial two component receptors. Some of these receptors interact with the signalling component CTR1, a protein kinase with similarity to the Raf family of serine/threonine protein kinases. In tomato, a family of six ethylene receptor genes has been identified, *LeETR1*, *LeETR2*, *NR*, *LeETR4*, *LeETR5* and *LeETR6*. However, other putative ethylene signal transduction components from this species have not been described. We recently reported a gene, *TCTR2*, whose encoded protein is 41% identical to CTR1, with the strongest homology over the putative C-terminal kinase domain. Using previously identified components of the ethylene signal transduction pathway as 'baits' in yeast 2-hybrid screen of a cDNA library generated from tomato fruit, we have isolated putative downstream components of the ethylene signal transduction pathway. Specific inter-

actions between the ethylene receptor protein LeETR1 and TCTR2, but not NR, have been characterised, indicating that tomato ethylene receptors may not share a common signal transduction pathway. We have demonstrated that TCTR2 and LeETR1 have protein kinase activity. Seven proteins have been identified that interact with NR, several of which show increased mRNA levels in fruit during ripening. Also, a further four proteins have been shown to interact in vitro with both TCTR2 and LeETR1.

### **P1.14—Ripening-associated transcriptional regulation in the tomato. A case of cross-talk between ethylene and auxin?**

M. Bouzayen, UMR990 INR/INP Toulouse, 'Biologie Moléculaire et Physiologie de la Maturation des Fruits', 31326 Auzeville-Tolosane, France

Fruit development and ripening correspond to a chain of genetically programmed events orchestrated by both endogenous phytohormones and exogenous factors such as light and temperature. Physiological and reverse genetics approaches have clearly demonstrated the role of the plant hormone ethylene in triggering and regulating the ripening of climacteric fruit such as the tomato. However, changes in the levels of other plant hormones strongly suggest their dynamic involvement in this developmental process. In an attempt to identify factors that act in concert with ethylene in regulating tomato fruit development and ripening, we sought to isolate genes encoding transcription factors potentially involved in ethylene and auxin responses. A number of clones encoding putative auxin and ethylene transcription factors differentially regulated either by ethylene or during fruit development, were isolated and subjected to fine characterisation at the molecular and physiological levels. Importantly, several auxin transcription factor homologs (ARF and Aux/IAA) were shown to be rapidly and significantly altered by ethylene suggesting a cross-talk between ethylene and auxin throughout fruit development. Transgenic tomato plants under- and over-expressing these genes were generated in order to explore their potential role in the ripening process. Down-regulation of an Auxin-Response-Factor homolog (DR12) in the tomato resulted in pleiotropic phenotype including dark-green and blotchy ripening fruit, enhanced firmness and increased pigment accumulation at the red-ripe stage. One important clone (ER24) that came out from our initial screening showed significant homology to the MBF1 type of transcriptional co-activators previously shown to contribute, with other proteins, to the formation of the TAF complex (TBP-associated factors). The differential regulation of the ER24 gene during the late stages of fruit development along with the selective interaction of its encoded

protein with some specific transcription factors suggest its participation in the regulation of the ripening-associated gene transcription in the tomato. This study raises the hypothesis that ER24 could be the physical site where the ethylene signalling acts to ultimately control the transcription of a specific set of ethylene-responsive genes.

### **P1.15—Profiling strawberry fruit maturation: from gene expression to metabolic pathways**

A. Aharoni, Business Unit Cell Cybernetics, Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Functional-genomics tools such as Expressed Sequence Tags (ESTs) and whole genome sequencing, gene expression using macro and micro arrays and generation of mutant populations contributed largely to the research on model plants, mainly *Arabidopsis thaliana*. A substantial portion of this 'tool-box' can also be utilised successfully for research on non-model plants, which are more difficult and time consuming to deal with, but allow investigations of unique biological processes such as fruit flavour biogenesis. As a first phase in a strategy to investigate strawberry fruit maturation and to identify key genes associated with fruit quality traits (in particularly aroma and flavour), we generated a collection of more than 1000 ESTs from ripe fruit cDNA library (cv. *Elsanta*). Combining information on the putative identity of the ESTs and gene expression studies was subsequently used to select candidate genes for further investigation. In early studies we used RNA gel-blot to analyse levels of 50 selected genes (selected based on homology). More than 15 transcripts showed ripening-regulated expression pattern and included *FaMYB1*, a member of the R2R3 MYB family of transcription factors. The *FaMYB1* gene was subjected to a more profound investigation, and the results suggested it to function as repressor of late flavonoid biosynthesis genes in the ripe strawberry fruit. To perform a more comprehensive study of gene expression we constructed DNA microarrays representing 1700 strawberry cDNAs and compared gene expression both during fruit development and between receptacle and achene tissues. A major finding in this study was the identification of the *SAAT* gene encoding the ester-forming enzyme from strawberry. Volatile esters are major components of the aroma profiles of most fruit, including strawberry. We also generated a second, dedicated set of arrays, comprising only 384 probes selected on the basis of the first hybridisation results including mainly ripening regulated and receptacle associated cDNAs. This set was used to analyse gene expression in fruit treated with auxin and fruit under oxidative stress conditions. Taken as a whole, microarray experiments have provided us with an exten-

sive and novel insight into the transcriptional programs active in strawberry fruit during maturation. They also led to the identification of several other flavour associated genes which are currently being characterised. As a complementary step for the large-scale analysis of gene expression using microarrays we conducted a set of experiments aimed at identifying key metabolic changes in strawberry fruit during development using a Fourier Transform Ion Cyclotron Mass Spectrometry (FTMS)-based method. The analysis identified changes in the levels of a large range of masses corresponding to known fruit metabolites and revealed novel information on the metabolic transition from immature to ripe fruit. The integration of emerging functional genomic practices will be an invaluable approach both for gene discovery and for understanding the biology of non-model plant species such as strawberry.

### **P1.16—Role of pectate lyase in banana fruit softening**

M.C. Marin-Rodriguez, Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

Perishable horticultural commodities such as banana have a relatively short post-harvest shelf-life during which the fruits undergo changes in texture, colour and flavour. Texture changes are thought to involve significant modifications in cell wall structure, the most apparent of which occur in the pectic fraction with an increase in polyuronide solubilization and depolymerisation. The role of pectate lyase (PEL) in banana fruit softening was studied. The expression of two distinct PEL genes, PEL I and PEL II, was apparent in ripening banana fruit peel and pulp, both genes were ripening-related and their expression was absent in non-fruit tissue. Heterologous expression studies of PEL I in *S. cerevisiae* confirmed that the protein encoded by PEL I had PEL activity. Furthermore, an assay was developed for measuring PEL activity from the pulp of ripening bananas. Transgenic banana plants with a sense fragment of PEL I were analysed. The relationship between PEL expression, PEL activity and firmness will be discussed.

### **P1.17—Development and utilization of tomato microarrays for the solanaceae**

S. Moore, P. Payton, J. Giovannoni

Tomato has long served as the model system for examining climacteric fruit ripening. Years of scientific investigation have resulted in substantial information and genetic resources for studying the biology of this agronomically important plant, including recently developed genomics tools. One interesting aspect of the emergence of such tools is the possibility of their exploitation across species boundaries to yield knowledge to benefit other agriculturally important crops, for which less informa-

tion (and resources) are currently available. We describe here the construction of a cDNA microarray for analysis of tomato fruit ripening and development. A fruit development gene expression profile is currently being created to establish a baseline of gene expression throughout development and especially ripening. Additionally, we have demonstrated the potential use of tomato microarrays for genomics applications in other members of the Solanaceae family. Hybridization of leaf, and in some cases fruit, cDNA probes to tomato microarrays was employed to assess the potential utilization of this tool for gene expression profiling in tobacco, potato, petunia, pepper, and eggplant. Selected gene expression data was confirmed by RNA gel-blot analysis. Global gene expression data, coupled with corresponding proteome, metabolome and physiological variation among developmental stages or phenotypes will eventually provide specific targets for genetic manipulation in tomato, as well as other members of the family Solanaceae, yielding greater understanding of plant development and molecular tools for crop improvement.

#### **P1.18—Increasing antioxidant levels in tomatoes through modification of the flavonoid biosynthetic pathway**

M. Verhoeven, G. Collins, S. Colliver, S. Muir, S. Robinson, Unilever Research Colworth House, Sharnbrook, Bedford MK44 1LQ, UK; A. Bovy, R. deVos, Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Flavonoids are a diverse group of phenolic secondary metabolites that occur naturally in fruits, vegetables, nuts, seeds and flowers and therefore, form an integral part of the human diet. Many of the compounds belonging to this group are potent antioxidants in vitro and epidemiological studies, suggest a direct correlation between high flavonoid intake and decreased risk for cardiovascular disease.

The biochemistry and genetics of the flavonoid pathway has been well studied in plants and many of the key genes have been cloned, thus facilitating modification of the pathway in chosen crops. Novel food plants with elevated levels of antioxidant flavonoids have the potential to be used as raw materials for 'functional foods' which may be beneficial to human health.

Using genetic engineering, we were able to probe the role and importance of a number of key enzymatic steps in the tomato flavonoid pathway. Transformation of tomato with genes encoding key biosynthetic enzymes, either alone or in combinations, gave rise to modified tomato lines displaying a range of novel phenotypes. The most dramatic phenotype was observed when over-expressing the *Petunia chi-a* gene, which encodes chalcone isomerase. Resulting transgenic lines showed up to

a 78-fold increase in fruit flavonols. Several of the other experiments also led to novel tomato varieties with significantly altered flavonoid content and profiles, the detail of which will be presented during the meeting.

#### **P1.19—Genetic analysis of organoleptic quality components in fresh market tomato**

M. Causse, V. Saliba-Colombani, and L. Lecomte, INRA-Centre d'Avignon-Unité de Génétique et d'Amélioration des Fruits et Légumes, Domaine Saint-Maurice, BP 94-84143 Montfavet Cedex, France; M. Buret, INRA, Centre d'Avignon, UMR Sécurité et Qualité des Produits d'Origine Végétale, Domaine Saint-Paul, 84 914 Avignon Cedex 9, France

The organoleptic quality of tomato fruit is a complex characteristics involving a set of components (flavour, aroma, texture), evaluated either by sensory analysis or by instrumental measures. In order to study the genetic control of this characteristic, a recombinant inbred line (RIL) population was developed from an intraspecific cross between a cherry tomato line with a good overall aroma intensity and an inbred line with a common taste but bigger fruits. A total of 38 traits involved in organoleptic quality and fruit composition were evaluated. Physical traits included fruit weight, diameter, colour, firmness and elasticity. Chemical traits were dry matter weight, titratable acidity, pH, and the contents in soluble solids, sugars, lycopene, carotene and 12 aroma volatiles. Trained panels quantified sensory attributes: flavour (sweetness and sourness); aroma (overall aroma intensity, together with candy, lemon, citrus fruit and pharmaceutical aromas); and texture (firmness, meltiness, mealiness, juiciness and embarrassing skin). RILs showed a large range of variation. Molecular markers were used to map a total of 131 quantitative trait loci (QTL) for the 38 traits. They were mainly distributed in a few regions on chromosomes 2, 3, 4, 8, 9, 11 and 12, facilitating marker-assisted selection. Major QTLs ( $R^2 > 30\%$ ) were detected for fruit weight, diameter, colour, and for six aroma volatiles. A few examples are shown to illustrate how the simultaneous analysis of QTL segregation for related traits may help to understand the genetic control of quality traits and pave the way towards QTL characterisation.

#### **P1.20—Regulation of carotenoid formation during tomato fruit development and ripening**

P.M. Bramley, School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, TW20 0EX, UK

Carotenoid formation during tomato fruit development and ripening has been studied extensively. It is a com-

plex process, as it coincides with the differentiation of chloroplasts to chromoplasts and characteristic changes to the organoleptic properties of the fruit. During ripening there is an increase in the concentration of carotenoids of some 10–15 fold, and also the specific accumulation of lycopene in the chromoplasts, giving the characteristic red colour of ripe fruit. Levels of other isoprenoids, such as the gibberellins, plastoquinone and sterols are also changed during tomato fruit ripening.

Although control of gene expression is thought to be the key regulatory mechanism controlling carotenogenesis in fruit ripening, post-transcriptional regulation has been detected, and carotenoids are also sequestered with carotenoproteins in chromoplasts. Feedback inhibition by end products of the pathway has also been reported.

In order to genetically manipulate the levels and types of carotenoids in ripe fruit, it is essential to elucidate the rate limiting steps of the pathway, target transgene expression to the ripening fruit and also avoid deleterious effects on the levels of other isoprenoids. Examples of such genetic manipulations will be given, as well as a discussion of the regulatory mechanisms present in tomato fruit.

#### **P1.21—Influence of harvest date and light integral on the development of strawberry flavour compounds**

T. McBurney, ADAS Rosemaund; R. Watson, C. Wright, A.J. Taylor, and R.S.T. Linforth, Nottingham, UK

The characteristic flavour of fresh strawberry fruit is a complex interaction between a large number of volatile and non-volatile components. The non-volatile compounds, e.g. sugars and acids, are responsible for the sweetness and tartness of the fruit; the volatile compounds, e.g. esters, aldehydes and furanones, are central in producing the distinctive fruity flavour. There is considerable variation in the flavour profile of fruit of the same cultivar harvested at different locations, seasons, and even within a single harvest period of the same crop. This variation results in consumer disappointment with some crops.

Strawberries cv Elsanta were grown in peat bags in a glasshouse and subjected to three shading levels (0, 25

and 47%) for 2 weeks, commencing 1 week prior to first fruit ripening. Fruit was harvested at five intervals and analysed using Atmospheric Pressure Chemical Ionisation (APCI) and LC-MS techniques. Thirteen volatiles implicated in strawberry flavour and three non-volatiles, sucrose, glucose and citric acid, were measured.

Highly significant differences in volatile and non-volatile concentrations existed between harvest dates. Shading had a significant effect on hexanal, hexenal, ethylmethylbutyrate and methylbutyrate concentrations. In general, at each harvest the higher the level of shading the lower the level of the volatile in the fruit. Sucrose concentration showed a steady decrease throughout the harvest period, whereas glucose and citric acid showed less clear trends. Shading had a significant effect on glucose and sucrose concentrations. Sugar concentration was inversely proportional to the amount of shading.

Possible reasons for the variability in strawberry flavour are discussed.

#### **P1.22—Dehiscence zone specific polygalacturonase contributes to pod shatter**

C. Powell, G. Simmons, and W. Paul, Biogemma, Cambridge, UK; K. Elliot, S. Gattolin and J. Roberts, School of Plant Sciences, University of Nottingham, UK

The hydrolytic enzyme polygalacturonase (PG) mediates pectin disassembly, leading to cell wall loosening, followed by a loss of cell cohesion. A distinct *B. napus* clone was previously isolated from a dehiscence zone (DZ) cDNA library. A promoter-GUS fusion showed that expression of this PG is directed to the pod DZ. Northern analysis found that PG expression increased during pod maturing. It is therefore thought that DZPG plays a role in silique dehiscence.

We recently identified a DZPG ‘knock-out’ in an *Ara-bidopsis* t-DNA insertion library. PCR analysis and sequencing confirmed a disruption of the DZPG gene. This mutant line shows a significant reduction in pod shatter, verifying our hypothesis.