EPSO Workshop
Plant Pigments and Human Health

Hotel Parador de Aiguablava, Costa Brava near Gerona, Spain

24-26 May 2011

European Plant Science Organisation
www.epsoweb.org

Abstract Book

Organisers :
Allan White (Plant & Food Research, NZ)
Karin Metzlaff (European Plant Science Organisation, BE)
Cathie Martin (John Innes Centre, UK)
Chiara Tonelli (University Milano, IT)
Manuel Rodriguez-Concepción (CRAG Barcelona, ES)

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The EPSO Workshop Plant Pigments and Human Health is sponsored by:

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PO Box 5762
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New Zealand

Plant & Food Research
Private Bag 1401
Havelock North 4157
New Zealand

Nestlé R&D Centre – Tours
Centre R&D Nestlé
2, rue Ch. Tellier – Beauvais
France

The Scottish Government
Research and Science Division
Saughton House
Broomhouse Drive
Edinburgh EH 11 3XD
United Kingdom

GlaxoSmithKline
Cnr Crosses and St George's Roads
Havelock North 4130
United Kingdom

Workshop programme

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Programme

The workshop will bring together the latest developments in the science of plant pigments and their potential benefits for human health. The primary intention is to identify the gaps between functional plant genomics and human health research and measures required to overcome them. Participants will be from academia as well as from private enterprises. The expected outcome is a white paper describing the current state of the area and suggestions for new directions in research within Europe at European and National level. It is proposed an international network be established amongst attendees to provide a platform for collaboration across the areas of pigment chemistry, functional plant genomics, food science, and health research that will achieve the recommendations in the white paper.

Size of the meeting: maximum 60 attendees
Organisers: Allan White & Roger Hellens (Plant & Food Research, NZ), Karin Metzlaff (EPSO, BE), Cathie Martin (John Innes Centre, UK), Chiara Tonelli (Univ Milano, IT) and Manuel Rodriguez-Concepción, CRAG Barcelona, ES

Programme:

24.5.2011

Registration
1700-1900

Welcome and Introduction – Chairs: Allan White and Karin Metzlaff

1900-1915	Welcome and Introduction from EPSO – **Karin Metzlaff, EPSO**	**01**
1915-1925	Welcome from NZ – **Karla Falloon, Counsellor for Science and Technology, New Zealand Mission to the European Union**	
1925-1940	Plants and Health in FP7-KBBE: a European Perspective – **Chiara Tonelli, EC Food Advisory Group**	
1940-1955	The Joint Programming Initiative on Diet and Health – **Carlos Segovia Perez, Instituto de Salud Carlos III., ES & Spanish delegate to the Management Board JPI Diet and Health**	**02**
2000	Dinner	

25.5.2011
Chairs: Cathie Martin and Manuel Rodriguez-Concepción

Session 1: Plant pigments with Health Benefits: An overview of the properties of pigments, with a focus on anthocyanins and carotenoids that are related to health. Availability in plants and advances in methods of identification of function.

0900-0930	Keynote address - Overview of the properties of pigments, with a focus on anthocyanins and carotenoids that are related to health – **Mary Ann Lila, Plants for Human Health Institute, USA**	**03**
Panel: Screening for bio-active pigments and assessing bioactivity		
0930-0955	Plant pigments and advances in function identification **- Derek Stewart, Scottish Crop Research Institute, UK**	**04**
0955-1020	Other polyphenols - **Roger Corder, Queen Mary University of London, UK**	**05**
1020-1045	Anthocyanins – **Tony McGhie, Institute for Plant and Food Research, NZ**	**06**
1045-1110	Carotenoids: biosynthesis and regulation-**Manuel Rodriguez-Concepción,CRAG Barcelona,ES**	**07**
1110-1130	Break	

Session 2: Anthocyanins and carotenoids, biosynthesis and enhancement of expression

1130-1200 Keynote address - Biosynthesis and role of Anthocyanins in plants, novel methods of increasing expression – **Cathie Martin, John Innes Centre, UK** **08**

Panel: Enhancement of expression of Anthocyanins in plants, transcriptional control/trafficking and sequestration/biochemistry/target crops and transformation

1200-1225	Corn - **Chiara Tonelli, University of Milan, IT**	**09**
1225-1250	Apple - **Roger Hellens, Institute for Plant and Food Research, NZ**	**10**
1250-1315	Berries – **Bruno Mezzetti, Marche Polytechnic University, IT**	**11**
1315-1340	Grapes – **Agnès Ageorges, INRA, FR**	**12**
1340-1405	Basic mechanisms – **Alexander Vainstein, The Hebrew University of Jerusalem, IL**	**13**
1405-1430	Carotenoid Accummulation in plants – **Shizue Matsubara, Research Centre Jülich, DE**	**14**
1430-1530	Lunch	

Session 3: Delivery of pigments to humans: Packaging pigments, pigments in foods, digestion and adsorption and bioavailability

1530-1600	Keynote: Adsorption and bioavailblility - **Paul Kroon, Norwich Institute of Food Research, UK**	**15**

Panel: Delivery to humans, packaging and adsorption

1600-1625	Novel methods of delivery - **Pete Wilde, Norwich Institute of Food Research, UK**	**16**
1625-1650	Adsorption – **David Edward Stevenson, Plant & Food Research Ruakura, NZ**	**17**

1750–1845 General informal discussion

2030 Dinner

26.5.2011

Chairs: Chiara Tonelli and Roger Hellens

Session 4: Human Health Benefit: The role of pigments in diet and nutrition

0900-0930	Keynote address: Pigments in foods – **Lynn Ferguson, University of Auckland, NZ**	**18**

Panel: the role of pigments in nutrition and diet

0930-0955	Obesity, Diabetes, Metabolic Syndrome – **Marco Giorgio, European Institute of Oncology, IT**	**19**
0955-1020	The development of functional foods and their ingredients – **Lesley Stevenson, GlaxoSmithKline Nutritional Healthcare, UK**	**20**
1020-1045	Systems biology approaches to quantify the effect of pigments on human health - **Ben van Ommen, Netherlands Organization for Applied Scientific Research, NL**	**21**

1045-1105 Break

Session 5: Human Health Benefit

1105-1135	Keynote address: The role of pigments in Human Health – **Michel de Lorgeril, University of Grenoble, FR**	**22**

Panel: The role of plant derived pigments in reducing the frequency and ameliorating the effects of human disease.

1335-1200	Plant Pigments and Disease Models – Bridging the Gap – **Jacquie Harper, Malaghan Institute of Medical Research, NZ**	**23**
1200-1225	Inappropriate inflammation – **Roger Hurst, Institute for Plant & Food Research, NZ**	**24**
1225-1250	Anthocyanins and health – **Sonia de Pascual-Teresa, ICTAN, CSIC, ES**	**25**
1250-1315	Neurodegenerative diseases – **Claudia Nunes dos Santos, Instituto de Biologia Experimental e Tecnológica, PT**	**26**

1315-1415 Lunch

1415-1515 Roundtable – Chiara Tonelli and Cathie Martin to integrate the discussions of the five sub-themes with the overall subject of the workshop and the appointment of working group to develop white paper and agreement on setting up of a network of researchers to provide a platform for collaboration on Plant Pigments and Human Health.

1515-1530 Wrap-up and Departure – Karin Metzlaff and Allan White

Speaker abstracts

01 Welcome and Introduction from EPSO

Karin Metzlaff
European Plant Science Organisation, EPSO
Rue de l'Industrie 4
1000 Brussels, Belgium
Tel/Fax: +32-(0)2-21362-60 / 69
Karin.Metzlaff@epsomail.org
www.epsoweb.org

EPSO, the European Plant Science Organisation, is an independent academic organization, who's mission it is to

- Promote plant science and support plant scientists
- Discuss future plant science programmes across Europe
- Provide authoritative source of independent information on plant science
- Promote training of plant scientists to meet the 21st century challenges in breeding, agriculture, horticulture, forestry, plant ecology and sectors related to plant science.

To date EPSO has 223 research institutes, universities and research departments as members. These are from 30 countries in Europe and beyond. In addition, EPSO already has over 2 850 personal members.

The association was founded in 2000 to represent the needs and interests of European plant science. Since then, it discusses with the European Commission, Members of the European Parliament and national politicians recommendations on European science policy. EPSO is a key driver for strategic input into European Research Programmes, in strengthening the ERA-NET on Plant Genomics and the upcoming ERA-CAPS, and one of the three stakeholders of the European Technology Platforms 'Plants for the Future'. EPSO offers input to the emerging Joint Programming Initiatives, such as 'Diet and Health' relevant to todays' workshop.

EPSO has partnerships with industry, NGOs and major European science organisations. To collaborate closer with national learned societies inside Europe, EPSO offers a Memorandum of Understanding to jointly develop with these national societies recommendations on science policy. EPSO is a founding member of the 'Global Plant Council' bringing together plant science societies from all five continents to jointly address the grand challenges our societies are facing.

EPSO organises workshops to foster key / emerging / promising research areas. We bring together plant biologists and other experts across disciplines to discuss the state of the art and develop recommendations on research priorities, needs for education, research infrastructure and policies.
Today's workshop is focused on plant pigments and human health, part of the larger theme plant sciences for healthy nutrition / human health an area that has been identified in the scientific community from academia and industry across sectors as a key element for future progress. You as participants will work towards a white paper articulating the state of art and recommendations for the coming years. In addition you will develop new ideas and collaborations across disciplines and continents.

EPSO holds a biannual conference at which scientists from all parts of the world present and discuss cutting edge science and, together with non-plant scientists, they discuss societal issues and build an interface to new areas. The next conferences will take place in Germany 2012 (jointly with FESPB) and Greece 2013.

EPSO membership is open to universities and research institutions conducting research in the field of plant science (institutional membership) worldwide, as well as to individuals interested in plant science (personal membership). This latest opportunity is open to all nationalities, professions, career stages and age groups worldwide. We are looking forward to welcoming you as a member.

02 The Joint Programming Initiative "A Healthy Diet for a Healthy Life"

Wim Saris
Carlos Segovia

Carlos Segovia Perez
Institute of Health Carlos III
Monforte de Lemos 5
28029 Madrid
Spain
csegovia@isciii.es

One of the challenges of Europe is to build a healthy society. Joint Programming Initiatives are thought to boost European R&D efforts, innovation and competitiveness, as compared to our reference countries, such as the USA, but increasingly also with emerging economic powers like China. European food industry is large and an important economic actor, but R&D investments are insufficient and scattered.

The aim of "A Healthy Diet for a Healthy Life" (HDHL) is to promote research on healthy diet, pooling resources in common activities, filling existing gaps, avoiding unnecessary duplications, and achieving the critical mass we need in Europe to address this research field.

JPI HDHL will take into account the work done in the European Technology Platform on Food for Life. The objective of the JPI HDHL is to change dietary patterns based on developments in food-, nutritional-, social- and health sciences and to develop innovative product formats that will, together with concomitant changes in physical activity, have a major impact on improving public health, increasing the quality of life and prolonging productive life.

JPI HDHL has defined three main areas of research:

- Lifestyle, determinants of food consumption behaviour and physical activity
- Diet and food production
- Diet-related chronic diseases

There are currently 20 countries involved in the JPI HDHL, which is coordinated by The Netherlands. The governing structure is defined including:

- Management Board (with representatives of research funding agencies from participating countries)
- Scientific Advisory Board
- Stakeholder Advisory Board
- Secretariat
- Thematic task forces

JPI HDHL will define the Strategic Research Agenda in 2011.

03 Carotenoids and anthocyanins: Solo and collective impacts on human health maintenance

Mary Ann Lila
600 Laureate Way, Plants for Human Health Institute,
North Carolina State University
Kannapolis, North Carolina 28081
USA
Maryann_lila@ncsu.edu

The natural plant pigments that are responsible for the colourful palette of edible fruits and vegetables are recognized resources for human health maintenance; this evidence is the premise behind the 'color code' for prescriptive, proactive healthful dietary guidelines. These rich pigments protect their plant hosts from biotic and abiotic stressors, and likewise provide metabolic protection to the animals which consume the pigmented food sources, through their antioxidant, anti-inflammatory, and detoxifying mechanisms of action. The carotenoids are best recognized through both epidemiological evidence as well as in vivo bioassays as cancer chemopreventive phytochemicals (notably, consumption as prophylaxis against prostate cancer), but also compelling indications of roles in prevention of macular degeneration and CVD have been documented. Anthocyanins have been cited as key inhibitors of various cancers, CVD, stroke, cataracts, and cognitive impairment, and more recently revealed as effective natural countermeasures against the ravages of metabolic syndrome (type II diabetes and obesity). While these colors serve as effective "beacon lights" heralding the health benefits of certain fruits and vegetables to consumers, few outside of the sciences realize the wealth of potentiating health-protective interactions that occur between key pigments and other phytochemicals, or between the carotenoid and flavonoid classes. Both additive and synergistic interactions have been recently demonstrated. The (apparent) low bioavailability of ingested pigments, often under the limits of detection by current laboratory instruments, begs the question of ***how*** they exert bioactive potential at ostensibly low concentrations. Our collaborating lab teams from North Carolina State University, Rutgers, and Purdue have used two very different yet complementary approaches to assess issues of bioavailability, bioaccessibility, biodistribution, pharmacokinetics, pharmacodynamics, and metabolic tracking for biologically-active health-protective plant pigments. Using the TNO intestinal model (TIM) for the human gastrointestinal tract, we have revealed distinct differences in the bioaccessibility of individual pigment species, which coincided with differences in observed bioactivity in vivo. Radiolabeling of pigments from tomato, grape, and berry germplasm permitted definitive tracking of deposition in organs, transport in serum, and clearance from the body. Finally, early experiments with co-delivery of plant pigments in defined functional food matrices clearly protected the pigments during digestion and enhanced accessibility and potential bioavailability.

04 Plant pigments and advances in functional identification

Derek Stewart
Gordon McDougall

Derek Stewart
Plant Products and Food Quality Programme,
The James Hutton Institute, Dundee, DD2 5DA, Scotland
Derek Stewart@hutton.ac.uk

"*Mere colour, unspoiled by meaning, and unallied with definite form, can speak to the soul in a thousand different ways*." Oscar Wilde; 1854-1900

Our fascination with colour has progressed beyond the unalloyed enjoyment of their existence. Developments in botany, agriculture and food are increasingly aligning with biochemical, biomedical and clinical research to elucidate the beneficial components in our food beyond nutrition. The last 20-30 years has seen an intensification in the research focus on plant pigments, in particular anthocyanins and carotenoids, with the ultimate aim of attributing human health benefits. The targeted benefits have been multiple and largely fall into two classes: enhanced performance such as increased cognitive ability, visual acuity etc and reduction of largely degenerative disease risk (cancer, cardiovascular and neurological diseases etc).

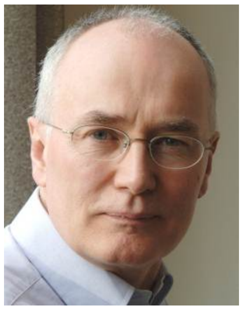
Key to delivery on any research into plant pigment-derived health benefits is the construction and employment of appropriate bioactivity models. Clearly the human is the best model possible! However, if we are looking for longer term disease prevention strategies the effects of pigment consumption may be delicate and the effects lost in the biological noise. Also clinical studies are prohibitively expensive over the long term. However, even short term intervention studies have highlighted benefits with anthocyanins and selected carotenoids.

Ex vivo studies have also proved beneficial but the majority of the effort in establishing pigment bioefficacy has gone into cell based assays and these have increasingly been employed as the 'omic technologies have matured. However, what is often lacking in these approaches is consideration of the gamut of metabolic processes that the pigments are exposed to on entering the body especially those associated with digestion and liver metabolic clearing.

We will consider all of these and look at the way forward for modern plant pigment bioefficacy testing.

05 Screening for bioactive polyphenols using endothelial cell responses to identify bioactivity

Paul W. Caton
Mark R. Pothecary
Noorafza Q. Khan
Roger Corder



Roger Corder
Queen Mary University of London, William Harvey Research Institute,
Barts and the London School of Medicine and Dentistry,
Charterhouse Square, London, EC1M 6BQ,
United Kingdom.
r.corder@qmul.ac.uk

Over the past 20 years there has been increasing awareness of the reduced risk of coronary heart disease in people consuming a diet rich in flavonoids. Based on epidemiological studies and clinical investigations the evidence for this protective effect is strongest for daily consumption of flavanols particularly from cocoa products or red wine [1,2]. This is attributed mainly to improvements in endothelial function, which increase vasodilator responses and can lower blood pressure. Systolic hypertension, risk of myocardial infarction, and mortality from heart failure are all linked to endothelial dysfunction. Hawthorn extract, which is a traditional herbal medicine used to treat heart failure, has a high flavanol content (mainly procyanidins) [3]. Therefore, dietary flavanols are likely to improve the general wellbeing of healthy individuals, and could potentially have a medical benefit, as there are no pharmaceutical products that specifically treat endothelial dysfunction.

Clinical studies of cocoa products have shown dose-dependent improvements in endothelial function [1]. This represents the response to consumption of a mixture of flavanols: monomer (mainly (-)-epicatechin) and procyanidins (dimers to decamer). Although, the effect of cocoa has been attributed to (-)-epicatechin, it was unable to produce the same effect as high flavanol cocoa [4]. This indicates that procyanidins contribute a substantial component of the endothelial response to cocoa flavanols. Studies on isolated blood vessels and cultured endothelial cells have shown consistently the greatest bioactivity is associated with procyanidins in the tetramer to hexamer range, with little or no activity due to monomers [5-9]. However, the lack of data to support bioavailability of procyanidins has led many to doubt the role of procyanidins in mediating the effects on endothelial function. Therefore further research is needed to identify bioavailable procyanidins and their metabolites.

Cultured endothelial cells represent a useful screening tool for identification of bioactive compounds. Assessing changes in endothelin-1 synthesis in response to flavanol extracts has proved a reproducible method to identify bioactivity, which correlates with measurements of endothelial-dependent vasodilatation obtained with isolated blood vessels [5-9]. This led to the hypothesis that a single mechanism mediated via a putative *procyanidin-receptor* underlies these endothelial actions [3,10]. Characterisation of gene expression in endothelial cells treated with native flavanols or metabolites can provide a broader understanding of the biological actions of these compounds [8,11], as well as insights into biomarkers that could be used to assess the response to dietary consumption.

References

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06 Anthocyanin chemistry and health

Tony McGhie
The New Zealand Institute for Plant & Food Research Ltd
Private Bag 11600, Palmerston North 4442,
New Zealand
tony.mcghie@plantandfood.co.nz

Chemistry

Anthocyanins are a group of over 500 compounds that contain a C_6-C_3-C_6 ring structure and are therefore classified as flavonoids. Unlike other flavonoids, anthocyanins contain a 2-phenylbenzopyrylium (flavylium) cation and they are intensely coloured. The colour of anthocyanins can be attributed to the electronic conjugation of the flavylium ion, and colours vary from red-orange to blue depending on the hydroxyl group substitution on the B-ring. Anthocyanins share structural similarities with other flavonoids, such as the flavonols and chemical properties attributed to specific chemical groups e.g. B-ring *o*-dihydroxy groups.

A unique feature of anthocyanins is that their molecular structure is pH dependent. At any given pH, an equilibrium containing at least four different molecular structures occurs. The red-coloured flavylium cation is the dominant form at low pH, whereas other forms such as quinonoids, hemiketals and chalcones dominate at higher pH. The equilibrium present at any given pH is likely to have a major effect on anthocyanin bioactivity. Thus it is often not clear which anthocyanins forms are bioactive or which are subjected to absorption, metabolism and transport *in vivo*.

Diversity

Over 500 anthocyanin compounds have been reported, with variation in the hydroxylation of the B-ring and the type and position of sugar conjugation. Plant foods, both fresh and prepared, contain anthocyanins with a variety of chemical structures. For example, anthocyanins from grape are mainly of the malvidin type, *Rubus* fruits contain cyanidins, whereas blackcurrants contain both cyanidins and delphinidin, and blueberries contain five of the most commonly occurring anthocyanin aglycones. Thus a diet containing plant foods provides a variety of different types of anthocyanins to the human consumer.

Chemistry and Health

The health-related bioactivities of anthocyanins are a result of the unique molecular features of these compounds. A large number of health-promoting properties have been reported for anthocyanins, including anti-inflammatory, anti-carcinogenic, prevention of cardiovascular diseases, vision enhancement, and prevention of obesity. The most studied biochemical activity of anthocyanins is antioxidant capacity, which may be the primary driver for most of bioactivities that have been reported. Anthocyanins have higher antioxidant capacity than other compounds, including vitamin C, when measured by *in vitro* assays. The high antioxidant capacity is directly related to the ability of anthocyanins to donate electrons readily and form a stable compounds with low reactivity.

Anthocyanin biochemistry related to health is complex, as the molecular forms change with pH. The coloured flavylium ion present in fruit and foods is probably not the biologically active molecular form that promotes health. More probably, the hemiketal and chalcone forms are the compounds responsible for bioactivity; however, currently these compounds are not detected by most analytical methods and information on these molecular forms is limited. Therefore, elucidation of the mechanisms of biological activity of anthocyanins and their effects on health still requires further research.

07 Carotenoids: biosynthesis and regulation

Manuel Rodríguez-Concepción
Centre for Research in Agricultural Genomics (CRAG)
Campus UAB Bellaterra, Cerdanyola del Valles
08193 Barcelona
Spain
manuel.rodriguez@cragenomica.es

Carotenoids are one of the most abundant groups of natural pigments found in plants. They provide color to non-photosynthetic roots, flowers and fruits, but also play central roles in photosynthesis and photoprotection. Additionally, their oxidative cleavage generates apocarotenoids such as the hormones abscisic acid (ABA) and strigolactones that regulate plant development and responses to external stimuli. Carotenoids are also important components of the human diet, as precursors of essential retinoids (including vitamin A) and protective antioxidants. But despite their relevance, the molecular mechanisms controlling plant carotenogenesis are not well understood yet.

Plant carotenoids are synthesized in plastids and, like the rest of plastidial isoprenoids, derive from prenyl diphosphate precursors produced mainly by the methylerythritol 4-phosphate (MEP) pathway. The identification and characterization of *Arabidopsis thaliana* mutants resistant to the inhibition of the pathway has provided evidence of several mechanisms controlling the levels and activities of key rate-determining enzymes at the post-transcriptional level. In particular, specific plastidial protease and chaperone systems appear to act together to ensure proper levels of active enzymes of the MEP pathway under normal growth and also in response to environmental challenges. MEP-derived precursors are channelled to the carotenoid pathway by the enzyme phytoene synthase (PSY). Unlike that described for the MEP pathway enzymes, PSY levels are mainly controlled at the gene expression level. Environmental factors such as light and salt stress are important regulators of the only gene encoding PSY in Arabidopsis. We have shown that *PSY* gene expression is repressed in dark-grown seedlings by direct binding of the phytochrome-interacting transcription factor PIF1. Degradation of PIF1 upon interaction with photoactivated phytochromes during deetiolation results in a rapid derepression of *PSY* gene expression and a burst in the production of carotenoids in coordination with chlorophyll biosynthesis and chloroplast development for an optimal transition to photosynthetic metabolism. Our data on the regulation of *PSY* expression by PIFs beyond deetiolation and by other transcripcion factors in response to salt stress will also be presented.

08 Biosynthesis of anthocyanins in plants, novel methods of increasing production

Cathie Martin
Eugenio Butelli
Angelo Santino
Jie Luo
Rakesh Shukla

Cathie Martin
John Innes Centre, Norwich Research Park, Colney,
Norwich NR4 7UH
United Kingdom
cathie.martin@bbsrc.ac.uk

There is increasing evidence that dietary anthocyanins can contribute to health and reduce the risk or impact of chronic diseases including obesity, cardiovascular diseases and cancer. The genes encoding the enzymes of the core pathway for anthocyanin biosynthesis are present in almost all higher plants (Gymnosperms and Angiosperms); the pathway is switched on specifically in different tissues of different plant species to give the huge variety in colours of flowers, leaves and fruits with which we are all familiar. The induction of anthocyanin biosynthesis in different plant cells is under the control of a transcriptional complex (the MBW complex) and increasing evidence suggests that variation in levels and patterns of anthocyanin production is most often (although not exclusively) a function of the activity of the MYB transcription factor in the MBW complex. An independent MYB regulator (AtMYB12) controls the production of flavonols (or related flavonoids) in many species. A good example of natural variation in anthocyanin production in fruit is provided by blood oranges. The activity of different gain-of-function alleles of blood orange and how these alleles impact the ‘anthocyanin trait’ will be described. Because transcription factors naturally determine variation in anthocyanin production, they also provide effective tools to engineer increases in anthocyanin content in foods. This has been demonstrated in tomato where anthocyanin levels high enough to impact health in disease models in preclinical trials have been achieved. High levels of flavonols can be achieved using AtMYB12 in tomato. Combination of the anthocyanin regulators and the flavonol regulator results in tomatoes that produce high levels of both anthocyanins and flavonols. Increased flux of carbon into flavonoid metabolism is achieved through the activity of AtMYB12. The flavonols serve to modify the colour and stabilise the anthocyanins, giving rise to indigo tomatoes through co-pigmentation. The ability of AtMYB12 to switch on primary metabolism to supply the increased requirements of secondary metabolism, means that AtMYB12 can also be used to achieve high level accumulation of other health-promoting polyphenols, including the stilbene, resveratrol. Our objective is to produce a range of tomato varieties enriched in different polyphenols so that their activities in reducing the risk or rate of progression of diseases can be compared in a common food type which provides a matched matrix control.

09 Development of flavonoid-enriched model foods in corn

Chiara Tonelli
Katia Petroni
Roberto Pilu
Elena Cassani
Valentina Calvenzani
Eleonora Cominelli
Marie-Claire Toufektsian
Michel de Lorgeril

Chiara Tonelli
Department of Biomolecular Sciences and Biotechnology
Via Celoria 26, 20133 Milano
Italy
chiara.tonelli@unimi.it

Different epidemiological studies have demonstrated that regular consumption of flavonoid-rich foods and beverages is associated to a reduced risk of chronic diseases, such as cardiovascular diseases, cancer and obesity. Model foods enriched in specific classes of flavonoids can be obtained in corn by breeding strategies exploiting the existing natural biodiversity of ancient corn varieties. Taking advantage of geographic alleles of the *MYB* and *bHLH* regulatory gene families (*C1/Pl1* and *B1/R1*, respectively) controlling activation of the anthocyanin biosynthetic pathway in maize, isogenic lines carrying different levels of anthocyanins in a desired tissue/organ can be obtained. For example, by selecting different *bHLH* genes anthocyanin synthesis can be directed in different tissues of the seed, such as pericarp, aleurone or scutellum (1,2). On the other hand, by using different geographic alleles of the *MYB* regulatory genes, the extent of anthocyanin accumulation can be modulated (3). High levels of other classes of flavonoids can be obtained by selecting branch-specific regulatory genes, such as the *P1 MYB* regulatory gene that activates the synthesis of phlobaphenes (3'-deoxyflavonoids) in maize kernels. Furthermore, combining the anthocyanin regulatory genes (*e.g. R1 C1*) with mutations in some biosynthetic genes, seeds with high levels of specific metabolic intermediates can be obtained. In the frame of EU-funded projects FLORA and ATHENA, anthocyanin-rich and anthocyanin-free isogenic corn lines were generated and used to demonstrate the cardioprotective effect of dietary anthocyanins in rats (4). Evidence was obtained that corn anthocyanins increase the omega-3 levels in blood (5). Novel allelic combinations of regulatory genes are being selected to generate isogenic lines with increasing levels of cyanidin-based anthocyanins in kernels.

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5) Toufektsian et al., *J Nutr* doi:10.3945/jn.110.127225

10 Red-fleshed apples: good enough to eat?

Roger P. Hellens
Richard V. Espley
Cyril Brendolise
Sumathi Kutty-Amma
Kui Lin-Wang
Cara Norling
Jonathan Crawford
Christina Bava
Andy C. Allan

Roger P. Hellens
The New Zealand Institute for Plant & Food Research Limited
Mount Albert Research Centre, Private Bag 92 169
Auckland 1142
New Zealand
roger.hellens@plantandfood.co.nz

Consumers of whole foods such as fruits demand consistent high quality and drive the development of new varieties with enhanced health, convenience, novel taste, and reduced impact on the environment. Conventional breeding of temperate fruit crops such as apples and kiwifruit exploits both existing cultivars and the extensive germplasm collections of related species and novel accessions.

Our genomics research is focused on the key producer and consumer traits. We achieve this by defining the biology of these traits and developing an understanding of the processes in model plants. Our translational genomics research then uses this molecular information in our target crops. To do this, we have utilized an extensive fruit EST sequence database and, through collaboration, the Whole Genome Sequence for these crops.

In our work on fruit colour, we have described both the metabolic and regulatory genes involved in anthocyanin accumulation. In addition, we have analysed novel germplasm of apples with red flesh and shown that this colour is due to the ectopic expression of a MYB regulatory gene. A simple rearrangement in the promoter DNA is sufficient to account for this desirable phenotype. We have genetically modified elite cultivars with this gene, including the alleles from red-fleshed germplasm, and have been able to use these as research tools to learn about the tree growth characteristics and fruit storage of these red-fleshed apples. We have also examined the effect of high anthocyanin apple in the diet of mice, and in human taste trials to assess the influence that high anthocyanin content has on taste and potential consumer perception of such a novel apple.

11 Integrating breeding and Biotech for improving strawberry nutritional quality

Bruno Mezzetti[1]
Jacopo Diamanti[1]
Franco Capocasa[1]
Carlo Rosati[2]
Maurizio Battino[3]

Bruno Mezzetti
[1]SAPROV – Dept. of Environmental and Crop Science, Faculty of Agriculture
Università Politecnica delle Marche,
Ancona
Italy
b.mezzetti@univpm.it

[2] ENEA – C.R. Trisaia – S.S.106, km 419+500
75026 Rotondella (MT)
Italy
carlo.rosati@trisaia.enea.it

[3]Dept. Biochemistry, Medical School, Università Politecnica delle Marche,
Ancona
Italy
m.a.battino@univpm.it

The quality of fruit is considered an extremely complex matter because it is submitted by the subjectivity of the consumers. In the last few years the nutritional value of fruit has been widely studied and requested by the consumers, especially for the general health benefits it can provide. These benefits can be described mainly to the antioxidant capacity of fruit. Both quality and antioxidant attributes are good tools to describe the nutritional quality of fruit.

During our research we have noticed that progenies from strawberry crosses having *F. virginiana glauca* (FVG) as a common parent showed a significant increase in their fruit quality and nutritional features confirming the interest of the wild species to improve cultivated strawberry for several characters. For those reasons we developed a study addressed to compare intra and inter-specific crossing for the selection of new genotypes with improved fruit sensorial, nutritional and nutraceutical quality. With this aim progenies are evaluated by taking in account the following parameters: Firmness, Color, Soluble Solid content, Titratable Acidity, Total Antioxidant Capacity, Anthocyanin Content, Total Phenolic Content and vitamin C. This breeding approach resulted of interest in order to produce new genotypes with high agronomic performance combined with improved fruit sensorial and nutritional and nutraceutical quality.

Flavonoids are widespread plant secondary metabolites involved in many functions such as pigmentation, protection from abiotic and abiotic stress, and plant-environment cross-talks. The anthocyanin subclass comprise the most important plant pigments, responsible for orange, red and blue colours in plants and having antioxidant activity. The last steps of anthocyanidin biosynthesis involve dihydroflavonol 4-reductase (DFR), catalysing the reduction of dihydroflavonols to leucoanthocyanidins, and anthocyanidin synthase (ANS), catalysing the formation of anthocyanidins.

In this study, 35S-driven DFR and ANS constructs were introduced in two strawberry cultivars, ‘Sveva’ (June-bearing) and ‘Calypso’ (everbearing), by *Agrobacterium tumefaciens*-mediated transformation. A gene stacking experiment (DFR+ANS) has also been performed by transforming one ‘Sveva’ DFR line with the ANS gene, under double kanamycin and hygromycin selection. Transgenic lines were obtained using leaf disk regeneration and kanamycin selection, then in vitro proliferated and acclimated. Antibiotic-resistant clones were confirmed as transgenic plants by PCR detection. These new transgenic lines represent unique new material for molecular and biochemical studies to elucidate the regulation of flavonoid pathway and improve the nutritional properties of strawberry.

12 Anthocyanin in grape berries: biosynthesis, decoration, trafficking and storage

Agnès Ageorges
Nancy Terrier
Véronique Cheynier

Agnès Ageorges
INRA, UMR Sciences pour l'Œnologie
2 place Viala
F-34060 Montpellier
France
ageorges@supagro.inra.fr

Anthocyanins are pigments responsible for red to blue colors found in many plant organs and constitute major determinants of the quality of plant-derived foods. They are also widely studied for their known beneficial effects on human health, and are of significant industrial interest as natural colorants. Grapes are an important source of anthocyanins. Red grape (*Vitis vinifera*) anthocyanin composition is mainly 3-*O*-monoglucoside, 3-*O*-acetylglucoside, and 3-*O*-*p*-coumaroylglucoside derivatives of five anthocyanidins: delphinidin, cyanidin, peonidin, petunidin and malvidin. The total amount of anthocyanins and relative abundance of different anthocyanins are extremely variable among red- to blue-skinned cultivars; however, methylated derivatives are usually largely predominant. The anthocyanin composition accumulated in grape berries is therefore critical to determine final color of the red wine.

In grapevine, anthocyanins are synthesized during ripening in the skin of red berries and their chemical stability is dependent on the decoration of the molecules (mainly methylation, glucosylation, acylation). In cells, anthocyanins are synthesized in the cytoplasm and accumulate into the vacuole. In recent years, the anthocyanin biosynthetic pathway in grapes has been thoroughly investigated, and most of the structural enzymes involved in this pathway have been identified. This pathway is controlled by specific Myb-bHLH transcriptional complexes, and in the case of grapevine, the *VvMybA* genes are known to be the key to the control of quantitative anthocyanin accumulation in grape. However, some steps remain to be elucidated such as those involved in the decoration of anthocyanins, their transport and their storage into the vacuole. In order to get insight into the molecular control of these processes, we have recently combined several tools and approaches from cell imaging to quantitative genetics, transcriptomics and plant transformation. In particular, transgenic grapevine plantlets in which ectopic expression of *VvMybA1* was induced, were produced leading to *de novo* production and storage of anthocyanins in all vegetative organs. These tools have led to the identification of new genes involved in anthocyanin biosynthesis. A gene encoding an anthocyanin-O-methyltransferase (AOMT), responsible for methylation of anthocyanins, was characterized by coupling approaches of transcriptomics and functional genomics. Its role was further validated using quantitative and association genetics. This gene is of particular importance in grapes, because the methylation changes the color and increases the stability of anthocyanins, which is fundamental to the quality of red wines. Furthermore, a gene encoding a MATE-type protein, which transports specifically acylated anthocyanins into the vacuole was identified. This gene is located at the tonoplast as well as on small cytoplasmic vesicle-like structures.
The anthocyanin cellular trafficking have been investigated in *MybA1* transformed grapevine hairy roots producing anthocyanins. Anthocyanins accumulate mainly inside the vacuole but also in many small cytoplasmic vesicle-like structures. Grapevine tissues expressing either a MATE-type anthocyanin transporter antisense or Glutathione S-transferase (a molecular actor involved in anthocyanin vacuolar sequestration) antisense display a different intracellular anthocyanin compartmentation, suggesting that different anthocyanin transport mechanisms co-exist in grapevine cells.

What remains to be done is to understand how the genetics and the environment interplay to modulate this pathway in grape berry.

13 Toward rational plant-metabolic engineering

Alexander Vainstein

Institute of Plant Sciences and Genetics in Agriculture
The Robert H. Smith Faculty of Agriculture, Food and Environment,
The Hebrew University of Jerusalem,
P.O. Box 12, Rehovot 76100
Israel
vain@agri.huji.ac.il

Plant secondary metabolites dictate both the health/nutritional and aesthetic value of flowers, fruits, roots, etc. Some of these compounds also play a role in attracting pollinators and seed dispersers, as well as in plant defense. This highly diverse group of metabolites, which includes both pigments and aroma compounds, derives in part from the phenylpropanoid biosynthetic pathway. Despite great progress in detailing this pathway, interactions between its various branches and the machinery regulating the fluxes within it are still far from being understood. For example, cross talk between the pathway's shunts was revealed by enhanced production of volatile benzenoids upon suppression of anthocynin biosynthesis. On the other hand, a number of myb-regulatory factors have been shown to affect production of both anthocyanins and volatile benzenoids. Our efforts to rationally manipulate the pathway toward enhanced production of health-beneficial compounds are hampered not only by a lack of detailed knowledge on the mechanism regulating metabolic flow, but also by limitations in our current ability to modify genome sequences. To this end, I will also present a non-transgenic approach for the delivery of zinc finger nucleases (ZFNs) and the production of mutant plants using a novel Tobacco rattle virus (TRV)-based expression system. Sequence analysis and transmission of the mutations to the next generation confirm the stability of the ZFN-induced genetic changes.

14 Photosynthetic Carotenoids: Serving for Plant Health and Human Health

Shizue Matsubara
IBG-2: Pflanzenwissenschaften, Forschungszentrum Jülich GmbH
D-52425 Jülich
Germany
s.matsubara@fz-juelich.de

Photosynthetic organisms, from bacteria to algae and higher plants, are rich source of natural carotenoids. They synthesise and accumulate carotenoids to capture light energy for driving photosynthesis on one hand, and to protect photosynthetic membranes against light-induced (photo-)oxidative damage on the other hand. Photo-oxidative stress is a serious and inevitable problem that arose when the oxygenic (oxygen-evolving) form of photosynthesis was invented by an ancestor of today's cyanobacteria. Embedding a biological solar powerhouse in a lipid membrane environment, in which light energy is collected by numerous pigment molecules (chlorophylls and carotenoids) in the very vicinity of the protein complex for water-splitting (the so-called oxygen evolving complex), demanded highly efficient defence systems to prevent formation of free radicals and other reactive oxygen species, and when these are formed, to remove them quickly and harmlessly.

Due to their physicochemical properties, carotenoids can rapidly dissipate light energy as heat to prevent generation of free radicals (e.g. $^1O_2^*$). If free radicals are formed in spite of the preventive mechanism, carotenoids can efficiently scavenge these harmful molecules, thereby providing a second line of defence to stop destructive chain reactions of lipid oxidation in membranes. In organisms performing oxygenic photosynthesis, including algae and plants, the importance of carotenoids as photoprotectants and antioxidants outweighs the role of carotenoids for light harvesting. Optimal leaf carotenoid composition can therefore potentially increase stress tolerance of crop plants. Although animals cannot synthesise carotenoids, they can also benefit from similar protective functions of carotenoids by taking up through diet.

Importantly, not all carotenoids naturally occurring in the photosynthetic membranes can fulfil photoprotective and antioxidative functions equally well. For example, cis- and trans-conformation, length of conjugated double bonds and the presence of hydroxyl or epoxy moieties can affect localisation of carotenoid molecules in the lipid membranes and alter the efficiencies of different reactions. In leaves of many tropical plants, β-carotene accumulates strongly at the expense of α-carotene under strong light. Upregulation of xanthophyll biosynthesis, such as zeaxanthin and lutein, is another response typically found in leaves under photo-oxidative stress. Likewise, these carotenoids are concentrated in certain part of human organs, such as in the ovary to confer antioxidative protection (β-carotene) or in the inner surface of eyes to protect this light-sensitive tissue against degeneration (lutein and zeaxanthin).

The apparently small but functionally vital variations in carotenoid molecules are produced by the enzymes in the carotenoid biosynthetic pathway. The regulation of the carotenoid biosynthetic pathway allows selective accumulation of different carotenoid species for protection of the photosynthetic membranes under various environmental conditions. While more research efforts are needed to better understand the regulatory mechanisms of carotenoid biosynthesis and accumulation in photosynthetic cells, enhanced synthesis and accumulation of specific carotenoids can be achieved in leaves by applying well-defined stress stimuli during plant cultivation. Together with genetic manipulations of the carotenoid biosynthetic pathway, such treatments can modify the carotenoid composition in leaves to improve, in the first place, stress tolerance of crop plants, and in addition, their nutritional value.

15 Absorption, metabolism and bioavailability of flavonoid pigments – what is being delivered to human tissues?

Paul A Kroon
Institute of Food Research, Norwich Research Park
NR4 7UA
UK
paul.kroon@bbsrc.ac.uk

Flavonoids are a large and diverse group of ubiquitous plant phenolics that are consumed as part of plant-based diets. Some flavonoids are coloured including flavanols such as quercetin (yellow) and anthocyanins (ACN) that cover a range of colours including red, blue, indigo and violet. Recently published evidence from epidemiological studies supports the notion that increased consumption of ACNis associated with reduced disease risk, particularly for cardiovascular diseases [1,2]. In vitro studies have demonstrated that ACN and anthocyanidins possess a range of activities that are in keeping with cardioprotective effects and other health benefits [3].

The bioavailability of flavonoids varies substantially between classes and compounds, with some compounds such as daidzein (isoflavone), naringenin (flavanone) and (-)-epicatechin (flavan-3-ol) showing quite high bioavailability, while others such as quercetin (flavonol) and (+)-catechin showing poorer bioavailability [4]. However, ACN are the most poorly available flavonoids, and typically less than 1% of the anthocyanin can be recovered in urine as ACN or anthocyanidin metabolites [5,6]. The concentrations of total anthocyanidins observed in peripheral blood after an anthocyanin-rich meal are very low, typically <0.1 μM, and there is little evidence they are accumulated in body tissues except for the gut mucosa. It appears paradoxical that such poorly bioavailable flavonoids as ACN can exert significant health benefits in humans. How can ACN-rich diets be protective against CVD if they are hardly absorbed to the circulation?

It seems likely that the fate of the 'missing' ACN will offer the solution to the 'anthocyanin paradox'. ACN and anthocyanidins are the least stable of the flavonoids, and are especially unstable at near neutral and alkaline pHs. They are prone to breakdown once the plant cells holding them are ruptured, which may occur during commercial or domestic processing (e.g. juicing, blending), storage, chewing or gastrointestinal digestion. Breakdown of ACN during processing does not appear to account for the low levels of ACN in final products [7], and it is likely that losses occurring during storage are important. Recent research has shown that anthocyan(id)ins also breakdown in physiological buffers and in human plasma, and that the products of their breakdown are a phenolic acid formed from the B-ring and an aldehyde formed from the A-ring [8,9]. It was shown that conversion of anthocyanidin to phenolic acid / aldehyde products is quantitative and can reach completion, which makes it likely that the degradation products are likely to commonly be both the major ingested and *in vivo* 'form' of ACN.

Future research should focus on establishing the fate of these ACN breakdown products in the human body, and their biological activities, in order to provide evidence to support a plausible mechanistic link between ACN in plant foods and reduced risk of age-related diseases such as CVD.

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16 Novel methods of delivery

Peter Wilde
Harisun Yaakob
Paul Kroon
Susan Barker
Annalisa Mercuri
Boon Seang Chu

Peter Wilde
Institute of Food Research, Norwich Research Park
Colney Norwich
NR4 7UA
UK
Peter.wilde@bbsrc.ac.uk

The controlled delivery of bioactive compounds is of interest to the food and nutrition field to improve uptake of beneficial dietary compounds with limited bioavailability. Reduced uptake may be due to low aqueous solubility, complexation, chemical modification or poor taste. In addition, the delivery system must also protect the bioactive during processing and storage of the food. As a colloid scientist, I will focus on the use of emulsions as delivery vehicles, but I will also give an overview of the basic principles underlying encapsulation and delivery.

The basic properties of a delivery device is to protect the bioactive during production, processing and storage, and release its payload during digestion at the correct site in the GI tract. There are a range of encapsulation devices which have been used in the pharmaceutical and cosmetic industries. Only some of these are viable for use in foods, due mainly to cost, but also to food regulations. When considering bioavailability, the normal release site would be the duodenum, but some devices are designed for colonic release. The simplest approach is to disperse the bioactive in a glassy, biopolymer matrix such as starch, with defined breakdown and digestion properties. The matrix is designed to resist breakdown in the mouth and stomach, but will breakdown and release the compound in the dueodenum. The trigger could be purely a time dependent dissolution, or it could be in response to specific changes in environmental conditions in the GI tract.

For bioactives with poor aqueous solubility, an effective method is to solubilise the bioactive in the oil phase of an emulsion. This will prevent aggregation/crystallisation or reaction with other water soluble compounds. There is only a limited amount of lipid hydrolysed in the stomach, and most of the dietary lipid is hydrolysed in the duodenum, hence releasing lipophilic nutrients and compounds into the micellar phase ready for uptake. The bioavailability will depend on the partition coefficient of the compound, and the extent of dilution during preparation and consumption.

A novel approach used in the pharmaceutical sector is the use of self-emulsifying drug delivery systems. Here the payload is dispersed in an oil phase, containing a high concentration of emulsifier (typically around 50%w/v), which induces very low interfacial tensions. Hence, when this mixture comes into contact with an aqueous phase, it spontaneously emulsifies, creating a large interfacial area for effective release of the payload into the lumen during digestion. Whilst this is suitable for pharmaceutical applications, the high concentration of emulsifier renders it inappropriate for use in food.

Emulsions can also be used to deliver water soluble compounds, by encapsulating water droplets within conventional lipid emulsion droplets. Water in oil in water (WOW) emulsions can protect water soluble bioactives from reactions with other food components during storage, mask poor flavours, and will release the compounds duodenal lipolysis. However, maintaining the stability of such emulsions during processing and storage can be challenging.

17 Can we improve delivery and do we need to?

David E Stevenson
Janine M Cooney
Arjan Scheepens
Jingli Zhang

David E Stevenson
Plant & Food Research Ruakura
Private Bag 3230, Waikato Mail Centre
Hamilton 3240
New Zealand
David.Stevenson@plantandfood.co.nz

Background

The bioavailability, or efficiency of delivery, of polyphenol pigments (anthocyanins and other flavonoids), i.e., their absorption from the gastro-intestinal (GI) tract and appearance in the bloodstream, as determined from numerous trials, is only a few percent of dietary intake at most. We highlight some of the limiting factors and discuss potential ways to mitigate them, in order to improve pigment delivery from functional foods. Recent reports, however, suggest that polyphenol bioavailability in general and of anthocyanins in particular, may be considerably higher than once thought, so improving delivery may not always be necessary.

Degradative structural modification of pigments

There is little to gain in conducting a human trial on the health benefits of a pigment-rich fruit, if processing radically changes the pigment composition and therefore the potential biological effects. There are many reports of losses of large proportions of anthocyanins during fruit product processing and storage. Understanding pigment loss will be vital to formulation of fruit-based functional foods with good delivery and efficacy.

Food formulation or chemical modification to improve bioavailability

Quercetin is the only readily and inexpensively available pigment in supplement form, but there are serious doubts about the bioavailability of this poorly water-soluble pigment. Recent reports suggest that formulation of quercetin with fat or oil/water emulsions can significantly improve delivery. Another potentially useful approach is suggested by highly methylated flavonoids found in citrus fruit. Their greatly increased hydrophobicity significantly improves intestinal absorption. Chemical methylation of other flavonoids has potential to improve the delivery of many more pigments.

Intelligent combination of pigments

A major limitation to intestinal absorption of pigments is conjugation by Phase II metabolic enzymes and subsequent excretion of the conjugate back into the intestine by Multi-drug resistance pumps. The action of these pumps, however, can be inhibited by a variety of polyphenols, including some pigments and this phenomenon has already been applied to improving the delivery of some pharmaceuticals. These interactions almost certainly occur in many polyphenol-rich foods already and understanding them better could greatly improve bioavailability from a low dose.

We may not be targeting the right compounds for improved delivery technology

The old theory of polyphenols as “antioxidants” is being replaced by a new role as “adaptogens”, which stimulate a reduction in oxidative stress at source. Good *in vitro* antioxidants may not necessarily be good adaptogens.

Is polyphenol bioavailability really as poor as we thought?

Recent reports suggest that ~75% of polyphenols in the blood are bound to cell walls and missed during plasma concentration measurements. There is also evidence that anthocyanins may be absorbed much better than we thought, but degrade quickly and are therefore missed completely by measurements of urinary excretion. Finally, there is evidence that polyphenols may have some biological effects at concentrations well within even current estimates of plasma concentrations.

18 The role of plant pigments in diet and health

Lynnette R. Ferguson,
Discipline of Nutrition, Faculty of Medical & Health Sciences,
The University of Auckland

Classic nutrition textbooks emphasise a dietary role of carotenoids, a common group of yellow, orange, and red pigments synthesized by plants. The most common dietary sources of these are alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, zeaxanthin, and lycopene. These first but not the last three of these can be converted by the body to retinol (vitamin A). With these exceptions, traditional nutrition texts often fail to emphasise a role of plant pigments in diet and health. However, there is increasing evidence that many of these may be important to both. A particular interest in my own laboratory is plant polyphenols, especially anthocyanins, of which a range can be found in New Zealand grown and developed food plants. There has been debate as to whether the biological effects of various plant pigments in humans are related to their antioxidant activity or other non-antioxidant activities. While a number of pigments are free radical scavengers, their ability to upregulate antioxidant enzymes may be more important in protection against damage to DNA and other cellular targets. However, there is large variability in this activity within and between groups of plant pigments, and this property alone appears unlikely to explain effects on human health. Effects on immune response and on genomic instability increasingly seem to be major effects associated with plant pigments, and will be emphasised in this presentation.

19 Dietary mimetics of caloric restriction

Marco Giorgio
Chiara Tonelli
PierGiuseppe Pelicci

Marco Giorgio
European Institute of Oncology
Via Adamello 16
20139 Milan
Italy
marco.giorgio@ifom-ieo-campus.it

Life span and aging is regulated by energetic intake. Indeed, several animal models and human studies established that restriction induces longevity whereas excessive feeding diseases shortening life span. Conserved molecular pathways including insulin signaling, AMPK–TOR-S6K and sirtuins mediate the effect of caloric intake on life span.

The main carbon sources for humans are angiosperms. Fruits, seeds or flowers in the form of sprouts or nectar elaborated by bees constitute the majority of our caloric supply. In addition, together with energetic substrates those plants provide us a variety of compounds that target several processes including the pathways that controls life span.

Angiosperms coevolved with animals to ensure reproduction. These animals (including us) access to important food source and evolved consequently in a strict relation with the plants.
We hypothesize that angiosperms enrich their edible parts with compounds that improve the association with animal. The beneficial content of some food plants evolved under a specific selective pressure. In this view, healthy bioactive compounds are produced on purpose and it is not only by chance that they interfere with our metabolism.

Searching for plant foods active on longevity pathways to identify dietary mimetics of caloric restriction would indicate the best “natural” healthy diet.

20 Considerations in the development of fruit based functional beverages

Dr. Lesley Stevenson
Director, New Product Research
Nutritional Healthcare
GlaxoSmithKline - GSK House
980 Great West Road
Brentford TW8 9GS
United Kingdom
lesley.m.stevenson@gsk.com

The principle of maintaining wellness, and improving the health of the general population rather than treating disease (championed by ageing populations), has fuelled the popularity of functional foods. This provides the opportunity to develop functional foods targeted towards different health areas. The general consensus amongst scientists, consumers and authorities as well as industry is that health claims should be scientifically substantiated. This is in the interests of all stakeholders. However, substantiating a health claim may be difficult and complex depending on the claim and the regulatory authority involved.

Functional foods must generally be made available to consumers in formats/forms that are consumed within the usual daily dietary pattern of the target population. Consumers expect functional foods to have good organoleptic qualities (eg. aroma, taste, texture and visual aspects) and to be of similar qualities to the traditional foods in the market.

The field of functional foods continues to grow in importance to the food industry. Consumers want 'healthier' choices associated with food and functional beverages provide 'healthiness on the go.'

Current issues concerning the development of functional beverages containing fruit polyphenols will be discussed in the context of obtaining the clinical evidence. Mentioning, in particular, those aspects of research and development that need consideration including agronomic impact, prossessing, bioavailability, agronomy and food matrix issues.

21 Systems biology approaches to quantify the effect of pigments on human health

Ben van Ommen
TNO - The Netherlands and the Nutrigenomics Organisation
Utrechtseweg 48
Zeist, The Netherlands
ben.vanommen@tno.nl

The effect of diet and dietary ingredients like pigments on health is hard to quantify. Although the nutrigenomics technology approach provided a wealth of mechanistic insights, more fundamental issues needed to be tackled, and these primarily relate to the function of nutrition: maintaining optimal health by providing the molecular components to our "machinery of physiology" that strives to keep all processes as normal as possible. This provides an interesting paradox: optimal nutrition results in optimal health so no changes in biomarkers will be detected. Thus, a shift from disease- and damage-oriented biomarkers to "biomarkers of health processes" was initiated, together with a renewed focus on homeostasis. The concept of robustness of homeostasis has (re-) emerged, and connected to the overarching processes that are involved in maintaining optimal health: metabolism, oxidation, inflammation, and psychological stress. Many (supposed) anti-inflammatory or anti-oxidant activities of phytochemicals should be (re)viewed in this aspect.
Biomarkers to quantify health optimization are needed since many if not most biomarkers are developed for diseases. Quantifying "normal homeostasis" and developing validated biomarkers are formidable tasks because of the robustness of homeostasis and of inter-individual diversity. The following concepts are central to define the physiology of the healthy individual: (i) responses to a challenge of homeostasis will be more informative than static homeostatic measures; (ii) processes involved in maintaining homeostasis usually are multi-factorial and require quantitative analyses of the many individual components involved; (iii) health includes a large variation in "normality" and the effects of nutritional interventions may remain hidden in this "diversity of robustness," if incompletely analyzed. Specifically, comprehensive multi-parameter ("omics") analysis may lead to a greater understanding of health supporting processes. Perturbation tests that accurately target aspects of the overarching drivers of health (metabolism, oxidation, inflammation, and psychological stress) will be instrumental in creating knowledge for maintaining health and preventing disease through nutrition.

22 The role of pigments in human health - Focus on cardiovascular diseases

Michel de Lorgeril
Patricia Salen
Marie-Claire Toufekstian
François laporte

Michel de Lorgeril
PRETA - TIMC - IMAG
Equipe Coeur & Nutrition
CNRS UMR 5525 - UJF - INP
Faculté de Médecine, Domaine de la Merci, Université de Grenoble
38706 La Tronche
France
michel.delorgeril@ujf-grenoble.fr

The two main plant pigments with potential health effects are anthocyanins and carotenoids. Anthocyanins belong to a group of natural substances with phenolic structures and are found in fruit, vegetables, tea, and wine. These natural products were known for their beneficial effects on health long before anthocyanins were isolated as the effective compounds. Many varieties of anthocyanins have been identified, some of which are responsible for the attractive colors of flowers, fruit, and leaves. Research on anthocyanins received an added impulse with the discovery of the French paradox, the low cardiovascular mortality rate observed in France in association with red wine consumption and a high saturated fat intake. The polyphenols (including anthocyanins) in red wine are responsible, at least in part, for this effect.

Carotenoids are organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants. There are over 600 known carotenoids. In general they absorb blue light. Carotenoids have many physiological functions. Given their structure, they are efficient free-radical scavengers. However, there is at present a lot of confusion regarding the health benefits of dietary antioxidants. The most common carotenoids include lycopene and the vitamin A precursor β-carotene. Whereas epidemiological studies have shown that people with high β-carotene intake and high plasma levels of β-carotene have a significantly reduced risk of certain cancers, studies of supplementation with large doses of β-carotene in smokers have not shown significant health benefits rather an increase in cancer risk. In a randomised, dietary intervention study, healthy non-smoking postmenopausal women consumed lycopene supplements from tomato purée for 1 week. Although lycopene was absorbed and resulted in a significant increase in lycopene plasma levels compared with baseline and the control period (no tomato purée), endothelial-dependent vasodilation was not influenced by this intervention.

Whereas the biological activities of anthocyanins have been characterized in vitro, there were until recently no clear experimental data demonstrating that chronic dietary intake and intestinal absorption of anthocyanins actually protects the heart against ischemia-reperfusion injury. In a recent study, we have tested whether long-term consumption of anthocyanins included in normal food could render the heart of rats more resistant to myocardial infarction. Anthocyanins were significantly absorbed and in Langendorff preparations, the hearts of rats fed the anthocyanins-rich diet were more resistant to regional ischemia and reperfusion insult. Moreover, on an in vivo model of coronary occlusion and reperfusion, infarct size was reduced in rats that ate the anthocyanins-rich diet than in those that consumed the anthocyanins-free diet. Omega-3 (n-3) polyunsaturated fatty acids (PUFA) form fish also have major health benefits and our studies have suggested that wine anthocyanins may interact with the metabolism of n-3 PUFA and increase their blood and cell levels. In a recent study, we have demonstrated, for the first time, that the chronic consumption of anthocyanins increases very long-chain n-3 PUFA levels, an effect we have called “fish-like effect of anthocyanins”.

These findings suggest important potential health benefits of foods rich in anthocyanins and emphasize the need of further studies to develop anthocyanin-rich functional foods with protective activities for promoting human health.

23 Plant Pigments and Disease Models – Bridging the Gap

Jacquie L Harper
Malaghan Institute of Medical Research
PO Box 7060
Wellington 6242
New Zealand
jharper@malaghan.org.nz

Plant pigments (anthocyanins, carotenoids, flavinoids) are an integral component of the makeup of the human diet through the consumption of vegetables, fruits and nuts. These pigments have been shown to exhibit anti-inflammatory, antimicrobial and anticancer activities, predominantly *in vitro*. One of the key steps in determining the downstream benefit of these dietary plant pigments is to investigate the relevance of these effects an in vivo setting. This presentation will introduce examples of how animal models, with a focus on controlling inflammation, may be used to test the health benefits of plant pigments a preclinical context and facilitate the development of whole food or food-based products for improved human health.

24 Plant pigments for modulation of inappropriate inflammation

Hurst, R.D.[1]
Gould, E.[1]
Walton, M.[2]
Mandamika, T.[1]
Harper, J.L.[2]

Roger D. Hurst
[1]Food and Wellness Group
The New Zealand Institute for Plant and Food Research Ltd.
New Zealand.
Roger.Hurst@plantandfood.co.nz

[2]Malaghan Institute of Medical Research
Victoria University
Wellington
New Zealand

Fruit and vegetable consumption is well regarded to be good for human health. Part of this benefit has been attributed to the presence of a variety of natural health promoting pigmented bioactive polyphenolic compounds. Plant pigments have been reported to possess a wide range of biological and pharmacological activities in the prevention of common diseases and illnesses. It is thought that the strong antioxidant properties exhibited by some polyphenolic compounds are responsible for the health benefits of a diet rich in fruits and vegetables. However, large-scale human intervention and meta analysis studies have failed to support this hypothesis and questions the relevance of the antioxidant ability of pigmented polyphenolics for health. More recent studies suggest inflammation-modulatory and immune-modulatory properties of plant polyphenolic compounds may be of greater biological significance to health and associated disease mitigation. In recent years a reduction in antioxidants (particularly polyphenolic compounds) in the diet rather than increasing environmental toxicity and an increased vulnerability of the pulmonary airways to inflammation have been proposed as a hypothesis to explain the rising prevalence of inflammatory lung conditions such as asthma.

A number of observational studies have revealed a positive correlation between fruit nutrient intake and asthma or lung function/disease. Furthermore there is building evidence from cell assays, animal and human intervention trials to indicate benefits to lung health from plant polyphenolics. At the New Zealand Institute for Plant & Food Research Ltd (PFR) we have developed a number of research platforms to evaluate the health benefits of fruit and vegetable polyphenolics. This research investigates the health-promoting properties of fruit and vegetable varieties from breeding programmes with well-defined compositional data utilising a range of cell-based screening and animal/human intervention trials. Our ultimate goal is the provision of appropriate information to plant breeding teams that enables them to select for scientifically proven health-promoting fruits and vegetables as whole fresh functional foods and/or ingredients. At PFR we have a research programme in which we are investigating the effects of polyphenolic compounds on key inflammation responses in lung epithelial cell-based assays and animal models of airway inflammation. We will present here a review of the evidence for modulation of inflammation by plant polyphenolic compounds with a focus upon airway inflammation and review our own research findings.

25 Anthocyanins and Health

Sonia de Pascual-Teresa
María Hidalgo
Maria Garcia-Alonso

Sonia de Pascual-Teresa
Department of Metabolism and Nutrition, ICTAN-CSIC.
C.jose Antonio Novais 10, Ciudad Universitaria
Madrid 28040
Spain.
soniapt@ictan.csic.es

Flavonoids in general and Anthocyanins in particular, as natural pigments present in many plants and derived food products, have gained interest in the research area of Nutrition mainly due to their potential as health protectors which has shown them as potential functional foods or functional ingredients. So far anthocyanins have proved active in different experimental models which include cardiovascular disease, neurological illnesses, cancer and ophthalmic conditions. Some of these potential effects are interrelated and based on their implication on inflammatory processes, redox response, endothelial function or antihypertensive action.

We have conducted several assays to prove that all these mechanisms are somehow modulated by anthocyanins. In assaying anthocyanins bioactivity we have considered not only the parent compounds but also the metabolites formed via intestinal or hepatic metabolism, chemical instability and microbiological metabolism. We have now proved in vitro and in various cellular models the effects of anthocyanins and their metabolites on different parameters involved in atherosclerosis and other conditions, including inflammation, endothelial function, estrogenic/antiestrogenic activity, angiotensin-converting enzyme (ACE) inhibitory activity and matrix metalloproteinases expression and secretion. In vivo, our results suggest potential antioxidative and antiinflammatory properties of anthocyanins at doses which are within the estimated intake in an average human diet.

26 Plant-derived compounds and neurodegenerative diseases

Cláudia Nunes dos Santos,
Lucélia Tavares,
Inês Figueira,
Marta Alves,
Derek Stewart,
Rui Pimpão,
Gary Willianson,
Ricardo Boavida Ferreira

Cláudia Nunes dos Santos
Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa,
Av. da República
2780-157 Oeiras
Portugal
and

Instituto de Biologia Experimental e Tecnológica,
Apartado 12, 2781-901 Oeiras, Portugal.
csantos@itqb.unl.pt

In the developed world the population lifespan is increasing with a concomitant increased of the incidence of many age-related diseases such as cancer, cardiovascular and neurodegeneration. The impact of this at the financial and social level is immense with the health care costs in 2008 for Alzheimer's disease and other forms of dementia recently estimated at €160 billion for the EU27 and €177 billion for the whole of Europe (Wimo et al., Int. J. Geriat. Psychiatry, 2011). Clearly it is paramount that preventative, amelioration and/or, ideally, inhibition strategies are developed to retard or reverse neuronal and behavioral deficits that occur in aging. Indeed, these foci are areas of intense research effort but as yet the delivery of (pharma) products and therapeutic strategies have been limited. Furthermore the mechanisms involved in the behavioral deficits during aging remain to be discerned.

There is intense interest in the studies related to the potential of phytochemical-rich foods to prevent age-related neurodegeneration and cognitive decline. Recent evidence has indicated plant-derived compounds limit the neurodegeneration associated with a variety of neurological disorders and prevent or reverse deteriorations in cognitive performance.

Although the precise mechanisms by which these compounds act within the brain remain unresolved. Flavonoids have potential to protect neurons against neurotoxins, to suppress neuroinflammation and to promote memory, learning and cognitive function. Originally, it was thought that they act by their antioxidant capacity. However, their limited absorption and low bioavailability in the brain suggest that this simplistic view is not reasonable. Recent evidences suggest other molecular mechanisms: interactions with protein kinase and lipid kinase signalling cascades, which regulate transcription factors and gene expression involved in both synaptic plasticity and cerebrovascular blood flow. In conclusion, the elucidation of the molecular mechanism underlying the neuroprotective effects of plant-derived compounds are only now gathering pace.

List of participants

Agnès Ageorges
INRA
UMR 1083 SPO
2, Place Viala
34060 Montpellier cedex, France
ageorges@supagro.inra.fr

Asaph Aharoni
Weizmann Institute of Science
Dept. of Plant Sciences
P.O. Box 26
Rehovot 76100, Israel
Asaph.Aharoni@weizmann.ac.il

Pierre Aymard
Kraft Foods Europe
Avenue de la Vauve, RD 128
91767 Palaiseau Cedex
France
pierre.aymard@kraftfoods.com

Karen Brimmer
Sensient Colors
2515 N Jefferson Ave
St Louis MO 63110
United States
Karen.Brimmer@sensient.com

Roger Corder
Queen Mary University of London
William Harvey Research Institute
Barts & the London School of Medicine & Dentistry
Charterhouse Square London, EC1M 6BQ
United Kingdom
r.corder@qmul.ac.uk

Michel de Lorgeril
University of Grenoble (FR)
Faculty of Medicine
PRETA - TIMC - IMAG
Domaine de la Merci
38706 La Tronche Cedex
France
michel.delorgeril@ujf-grenoble.fr

Sonia de Pascual-Teresa
Institute of Food Science, FoodTechnology and Nutrition ICTAN - CSIC
C. Jose Antonio Novais 10
28040 Madrid
Spain
soniapt@ictan.csic.es

Simon Deroles
The New Zealand Institute for Plant & Food Research Limited
Postal Address: Plant & Food Research Palmerston North
Private Bag 11600, Manawatu Mail Centre, Palmerston North, 4442, New Zealand
Physical Address: Plant & Food Research Palmerston North
Batchelar Road, Palmerston North, 4474, New Zealand
Simon.Deroles@plantandfood.co.nz

Jocelyn Eason
The New Zealand Institute for Plant & Food Research Limited
Private Bag 11600
Palmerston North 4442, New Zealand
jocelyn.eason@plantandfood.co.nz

Karla Falloon
New Zealand Ministry of Research, Science and Technology
7th Floor Avenue des Nerviens 9-31
1040 Brussels, Belgium
karla.falloon@morst.govt.nz

Lynnette Ferguson
The University of Auckland
Private Bag 92019 Auckland
New Zealand
l.ferguson@auckland.ac.nz

José A. Garcia Regueiro
IRTA
Finca Camps i Armet
17121 Monells, Spain
JoseAntonio.Garcia@irta.cat

Marco Giorgio
European Institute of Oncology
Via Adamello 16
Milan, Italy
marco.giorgio@ifom-ieo-campus.it

Mark Goldschmidt
Sensient Colors
2515 N Jefferson Ave
St Louis MO 63110, United States
Mark.Goldschmidt@sensient.com

Jacquie Harper
Malaghan Institute of Medical Research
PO Box 7060
Wellington
New Zealand
jharper@malaghan.org.nz

Roger P. Hellens
The New Zealand Institute for Plant & Food Research Limited
Postal Address: Plant & Food Research Mt Albert
Private Bag 92169, Auckland, 1142, New Zealand
Physical Address: Plant & Food Research Mt Albert
120 Mt Albert Road, Sandringham, Auckland, 1025, New Zealand
roger.hellens@plantandfood.co.nz

Werner Howad
IRTA
Centre de Recerca en Agrigenòmica (CRAG) CSIC-IRTA-UAB
Campus UAB, Edifici CRAG
Bellaterra (Cerdanyola del Vallés)
08193 Barcelona, Spain
werner.howad@cragenomica.es

Roger Hurst
The New Zealand Institute for Plant & Food Research Limited
Food & Wellness Group
Food Innovation Portfolio
Private Bag 11600, Palmerston North 4442, New Zealand
roger.hurst@plantandfood.co.nz

Anne Kathrine Hvoslef-Eide
Norwegian University of Life Sciences
Tårnbygningen, C.M. Falsens vei 16,
1432 Ås, Norway
trine.hvoslef-eide@umb.no

Ingar Janzik
IBG-2: Pflanzenwissenschaften,
Forschungszentrum Jülich GmbH
52425 Jülich, Germany
i.janzik@fz-juelich.de

Cath Kingston
Plant & Food Research
Hawkes Bay Research Centre
Private Bag 1401
Havelock North 4157
New Zealand
Cath.Kingston@plantandfood.co.nz

Paul Kroon
Institute of Food Research
Norwich Research Park, Colney
Norwich, NR4 7UA
United Kingdom
paul.kroon@bbsrc.ac.uk

Benjamin Kuhn
Institute of Plant Biology
University of Zürich
Zollikerstrasse 107
CH-8008 Zürich, Switzerland
bkuhn@access.uzh.ch

Caroline Labrie
Wageningen UR Greenhouse Horticulture
Researcher Quality Greenhouse Vegetables
P.O. Box 20 2665 ZG Bleiswijk
Visitor address : Violierenweg 1, 2665 MV Bleiswijk
caroline.labrie@wur.nl

Maud Lepelley
Nestlé R&D Centre - Tours
101, Avenue Gustave Eiffel - BP 49716
37097 Tours Cedex 2, France
maud.lepelley@rdto.nestle.com

Mary Ann Lila
Plants for Human Health Institute
North Carolina Research Campus
600, Laureate Way
North Carolina 28081
mlila@ncsu.edu

Jessica Lobo
Sensient Colors
2515 N Jefferson Ave
St Louis MO 63110, United States
Jessica.Lobo@sensient.com

Cathie Martin
John Innes Centre
Norwich Research Park
Colney, Norwich, NR4 7UA
United Kingdom
cathie.martin@bbsrc.ac.uk

Shizue Matsubara
IBG-2: Pflanzenwissenschaften,
Forschungszentrum Jülich GmbH
52425 Jülich, Germany
s.matsubara@fz-juelich.de

Tony McGhie
The New Zealand Institute for Plant & Food Research Limited
Private Bag 11600, Palmerston North 4442, New Zealand
tony.mcghie@plantandfood.co.nz

Karin Metzlaff
European Plant Science Organisation
4, rue de l'Industrie
1000 Brussels, Belgium
karin.metzlaff@epsomail.org

Bruno Mezzetti
Marche Polytechnic University
Piazza Roma 22, Ancona,
60121 Marche, Italy
b.mezzetti@univpm.it

Jim Morton
Department of Wine, Food and Molecular Biosciences, Lincoln University
P.O. Box 84
Canterbury, 7647 New Zealand
James.Morton@lincoln.ac.nz

Valentina Naumenko
Institute of Food Biotechnology and Genetics NAS of Ukraine
Institute of Food Biotechnology and Genetics NAS of Ukraine,
2a Osipovsronogo St., Kiev, 04123, Ukraine
Naumenko.Valentina88@gmail.com

Maria Nieves Goicoechea
University of Navarra, School of Sciences
Plant Physiology Dept.
Irunlarrea 1
31008-Pamplona, Spain
niegoi@unav.es

Clàudia Nunes dos Santos
Instituto de Tecnologia Química e Biológica (ITQB)
Av. Da Republica, Qta, do Marquês
2780-157 Oeiras, Portugal
csantos@itqb.unl.pt

Michal Oren-Shamir
Volcani Center
Department of Ornmental Horticulture
P.O. Box, Bet-Dagan
50250 Israel
vhshamir@volcani.agri.gov.il

Francesco Paolocci
IGV-CNR Division of Perugia
National Research Council, Plant Genetics Institute-Perugia, Perugia Division, Via Madonna Alta 130,
06128 Perugia, Italy
francesco.paolocci@igv.cnr.it

Jordi Perez
Centre for Agricultural Genomics (CRAG)
CSIC-IRTA-UAB, Campus UAB Bellaterra
08193 Cerdanyola del Vallès Barcelona
Spain
jpggmp@cid.csic.es

Katia Petroni
University of Milan
Department of Biomolecular Sciences and Biotechnology, Via Celoria 26
20133 Milano
Katia.Petroni@unimi.it

Manuel Rodriguez-Concepción
Centre for Agricultural Genomics (CRAG)
CSIC-IRTA-UAB, Campus UAB Bellaterra
08193 Cerdanyola del Vallès Barcelona
Spain
manuel.rodriguez@cid.csic.es

Carlos Segovia Perez
Instuto de Salud Carlos III.
Ministerio de Ciencia e Innovación / Ministry of Science and Innovation
Monforte de Lemos 5
E-28029 Madrid (Spain)
csegovia@isciii.es

David Edward Stevenson
Plant & Food Research Ruakura
Private Bag 3230, Waikato Mail Centre,
Hamilton 3240, New Zealand
David.Stevenson@plantandfood.co.nz

Lesley Stevenson
GlaxoSmithKline Nutritional Healthcare
980 Great West Road, Brentford,
Middelsex TW8 9G5
United Kingdom
lesley.m.stevenson@gsk.com

Derek Stewart
Scottish Crop Research Institute
Mynfield, Ingergowrie
Dundeee DD2 5DA
Scotland, UK
Derek.Stewart@scri.ac.uk

Emma Timewell
The New Zealand Institute for Plant & Food Research Limited
Postal Address: Plant & Food Research Mt Albert
Private Bag 92169, Auckland, 1142, New Zealand
Physical Address: Plant & Food Research Mt Albert
120 Mt Albert Road, Sandringham, Auckland, 1025, New Zealand
Emma.Timewell@plantandfood.co.nz

Gabriela Toledo
Centre for Agricultural Genomics (CRAG)
CSIC-IRTA-UAB, Campus UAB Bellaterra
08193 Cerdanyola del Vallès Barcelona
Spain
gtogmy@cid.csic.es

Chiara Tonelli
Milan University
Dipartimento di Scienze Biomolecolari e
Biotecnologie
Via Celoria 26
20133 Milano, Italy
chiara.tonelli@unimi.it

Harriet Trewin
Syngenta
Jealott's Hill International Research Centre
Bracknell
Berkshire, RG42 6EY
Harriet.Trewin@SYNGENTA.COM

Alexander Vainstein
Institute of Plant Sciences and Genetics in
Agriculture
The Robert H. Smith Faculty of Agriculture
Food and Environment
The Hebrew University of Jerusalem
P.O. Box 12, Rehovot 76100, Israel
vain@agri.huji.ac.il

Olaf van Kooten
Wageningen University #E1.218
Horticultural Supply Chains group
PO Box 630
NL-6700 AP Wageningen
olaf.vankooten@wur.nl

Ben van Ommen
TNO
Utrechtseweg 48
Zeist, The Netherlands
ben.vanommen@tno.nl

Thomas Vanek
Joint Laboratory of Institute of Experimental Botany
AS CR
v.v.i. and Research Institute of Crop Production,
v.v.i., Rozvojová 263, 165 02 - Prague 6, Czech Republic
vanek@ueb.cas.cz

Robert Veberic
University of Ljubljana
Biotechnical faculty
Jamnikarjeva 101
1000 Ljubljana
robert.veberic@bf.uni-lj.si

Allan White
The New Zealand Institute for Plant & Food
Research Limited
Postal Address: Plant & Food Research Hawkes Bay
Private Bag 1401, Havelock North, 4130, New Zealand
Physical Address: Plant & Food Research Hawkes Bay
Cnr Crosses and St Georges Roads, Havelock North,
4157, New Zealand
Allan.White@plantandfood.co.nz

Peter Wilde
Institute of Food Research, Norwich
Norwich Research Park, Colney
Norwich, NR4 7UA
United Kingdom
peter.wilde@bbsrc.ac.uk

Claudia Zahn
Forschungszentrum Jülich GmbH
52425 Jülich, Germany
c.zahn@fz-juelich.de

European Plant Science Organisation

Rue de l'Industrie 4
B-1000 Brussels, Belgium
Tel/Fax: +32 2 213 62 60/69
Email: epso@epsomail.org
www.epsoweb.org