The XI International Symposium on the Plant Hormone Ethylene
June 2-6, 2018, C₂H₄ANIA, Crete, GREECE

PROGRAM
and
BOOK OF ABSTRACTS

Conveners of ETHYLENE2018
Angelos K. Kanellis, Mondher Bouzayen, Panagiotis Kalaitzis

Conference Center of the Mediterranean Agronomic Institute of Chania,
Crete, GREECE
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Ethylene in root growth in Arabidopsis

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PROGRAM

June 2, 2018

16:00-18:30 Registration

18:30-18:40: Angelos K. Kanellis, Mondher Bouzayen, Panagiotis Kalaitzis
Introduction to the Symposium

Opening Talk

18:40-19:20 Caren Chang (invited talk)
(University of Maryland, College Park, USA)

Past, Present and Future: New signaling roles for EIN2 and ACC

TOPICS, CHAIRPERSONS AND INVITED SPEAKERS

I. History and important steps in ethylene biology and biotechnology
   (Don Grierson, Mark Tucker)

19:20-19:30 Introduction by Chairs

19:30-19:55 Don Grierson (invited talk)
(Zhejiang University, Hangzhou, PR China
University of Nottingham, Sutton Bonington, UK)

Ethylene: Old & New- What’s left to discover & where should we look?

19:55-20:20 Jean Claude Pech (invited talk)
(University of Toulouse, Toulouse, France)

The pantheon of ethylene biologists

THE WINE OF CRETE

20:20-20:40 Stefanos Koundouras (invited talk)
Aristotle University of Thessaloniki, Thessaloniki, Greece

The vineyards and wines of Crete

20:40-23:00 Wine Tasting and Welcome Reception
June 3, 2018

II. Ethylene Biosynthesis, Signal Transduction and Responses
(Julien Pirrello, Caren Chang)

08:30-08:35 Introduction by Chairs

08:35-09:00 Brad Binder (invited talk)
(University of Tennessee, Knoxville, USA)

Unconventional Ethylene Receptor Signaling

09:00-09:25 Julien Pirrello (invited talk)
(Université de Toulouse, INRA, Castanet-Tolosan, France)

Ethylene Response Factors: key regulators of ethylene-related ripening process

09:25-09:50 Hong Qiao (invited talk)
(University of Texas, Austin, USA)

Transcriptional repression in ethylene response

09:50-10:05 Ranran Zhang (selected talk)
(Chinese Academy of Sciences, Shanghai, China)

A Conserved role of Arabidopsis ECR1 and human tumor suppressor HsECR1 in translational control of ctr1-10

10:05-10:20 Wangshu Mou (selected talk)
(Zhejiang University, University of Maryland, College Park, USA)

The ethylene precursor, ACC, is an extracellular cue in the guidance of pollen tubes toward ovules in Arabidopsis thaliana

10:20-10:50 Coffee Break

II. Ethylene Biosynthesis, Signal Transduction and Responses
(Julien Pirrello, Caren Chang) continued

10:50-11:05 Christian Chervin (selected talk)
(Toulouse INP/ENSAI/INRA, Castanet-Tolosan, France)

Study of the seven ethylene receptor proteins over the tomato fruit ripening by targeted mass spectrometry, in wild type and Never Ripe backgrounds
A systematic approach for dissecting the mechanisms of EIN3-dependent regulation of ethylene response in *Arabidopsis thaliana*
IV. Ethylene in Vegetative Growth-Development
(George Eric Schaller, Jose Alonso)

14:30-14:35 Introduction by Chairs

14:35-15:00 George Eric Schaller (invited talk)
(Dartmouth College, Hanover, USA)

Ethylene signal transduction and the regulation of cell proliferation

15:00-15:25 Jose Alonso (invited talk)
(North Carolina State University, Raleigh, USA)

From ethylene signaling to translation regulation

15:25-15:50 Gloria Muday (invited talk)
(Wake Forest University, Winston Salem, USA)

ETR1-dependent root development and transcriptional responses in light grown Arabidopsis seedlings

15:50-16:05 Carolin Seyfferth (selected talk)
(Umeå Plant Science Centre, Umeå, Sweden)

PttERF85 affects cell division and growth in tree stems potentially through an effect on ribosome biogenesis

16:05-16:20 Shangwei Zhong (selected talk)
(Peking University, China, Beijing, China)

Plant seedling soil emergence: regulation of EIN3 protein stability

16:20-16:35 Andria Harkey (selected talk) NSF Travel Awardee
(Wake Forest University, Winston-Salem, USA)

Computational and genetic approaches to uncover ethylene transcriptional networks that regulate root development

16:35-17:00 Coffee Break

V. Cross-talk between Ethylene and other Hormones
(Hongwei Guo, Dominique Van Der Straeten)

17:00-17:05 Introduction by Chairs

17:05-17:30 Hongwei Guo (invited talk)
(Southern University of Science and Technology, Shenzhen, Guangdong, China)
Coordinated regulation of apical hook formation by ethylene and other signals

17:30-17:55 Dominique Van Der Straeten (invited talk)  
Ghent University, Ghent, Belgium

Ethylene restricts Arabidopsis growth via the epidermis

17:55-18:10 Rongfeng Huang (selected talk)  
Chinese Academy of Agricultural Sciences, Beijing, China

The regulation of ethylene in rice primary root elongation and salt response

18:10-18:25 Yuying Chen (selected talk)  
Chinese Academy of Sciences, Shanghai, China

Overexpression of an Arabidopsis histidine kinase-like protein leads to cytokinin-induced ethylene responses

18:25-18:40 Gyeong Mee Yoon (selected talk) NSF Travel Awardee  
Purdue University, West Lafayette, USA

Maintenance of ethylene homeostasis by brassinosteroid-mediated mutual degradation of E3 ubiquitin ligases in Arabidopsis

18:40-18:55 Livio Trainotti (selected talk)  
University of Padova, Padova, Italy

Peach secreted peptide hormones interact with auxin and ethylene to regulate plant development

18:55-19:10 Thomas Depaepe (selected talk)  
Ghent University, Gent, Belgium

The small molecule ACCERBATIN mimics the triple response phenotype and acts through disruption of auxin and ROS metabolism

19:10-20:10 Poster session

Dinner at your own
June 4, 2018

VI. Ethylene on Cell and Organ Identity Specification
(Teva Vernoux, Keith Lindsey)

08:30-08:35 Introduction by Chairs

08:35-09:00 Teva Vernoux (invited talk)
(INRA, CNRS, ENS, Universite de Lyon, Lyon, France)

Shaping a flower with hormonal signals

09:00-09:25 Keith Lindsey (invited talk)
(University of Durham, Durham, UK)

Ethylene in root growth in Arabidopsis

09:25-09:50 Abdel Bendahmane (invited talk)
(IPS2-INRA, Gif-Sur-Yvette, France)

Ethylene is a master regulator of sex determination in cucurbits

09:50-10:05 Sonia Philosoph-Hadas (selected talk)
(Agricultural Research Organization, Rishon LeZion, Israel)

Ethylene plays opposite or dual roles in various physiological processes
operating in cut flowers

10:05-10:35 Coffee Break

VII. Ethylene in abiotic stresses
(Autar Mattoo, Angelos Kanellis)

10:35-10:40 Introduction by Chairs

10:40-11:05 Autar Mattoo (invited talk)
(USDA-ARS, Beltsville, USA)

Multiple interactors regulate plant response to abiotic stresses - the
story of ethylene and polyamine dynamics in tomato

11:05-11:20 Bram Van de Poel (selected talk)
(KU Leuven, Leuven, Belgium)

Root hypoxia-induced epinasty is ontogenetically regulated by ethylene
in tomato

11:20-11:35 Zeguang Liu (selected talk)
(Utrecht University, Utrecht, Netherlands)
The early flooding signal ethylene prepares Arabidopsis for hypoxia tolerance: mechanism and significance

VIII. Ethylene in Pathogenesis and Disease Resistance
(Dov Prusky, Abdel Bendahmane)

11:35-11:40 Introduction by Chairs

11:40-12:05 Dov Prusky (invited talk)
(Agricultural Research Organization, Rishon LeTzion, Israel)

Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity in phytopathogenic fungi

12:05-12:20 Carlew Scott (selected talk) NSF Travel Awardee
(University of Tennessee, Knoxville, USA)

Elucidating the role of a newly discovered ethylene receptor in the plant growth-promoting rhizobacterium Azospirillum brasilense

12:20-12:35 Thomas Svoboda (selected talk)
(University of Natural Resources and Life Sciences, Tulln, Austria)

*Fusarium graminearum* is able to produce ethylene, and to degrade its precursor ACC

12:35-12:50 Songkui Cui (selected talk)
(Nara Institute of Science and Technology, Ikoma, Japan)

Ethylene is involved in in plant parasitism by modulating development and function of the haustorium, an invasive organ in parasitic plants

12:50-13:05 Peter Marhavy (selected talk)
(University of Lausanne, Lausanne, Switzerland)

Single cell damage elicits local, nematode-restricting ethylene responses in roots

13:05-13:20 Anna Stepanova (selected talk) NSF Travel Awardee
(North Carolina State University, Raleigh, USA)

A single-locus biosensor for simultaneous monitoring of multiple plant hormones

13:20-15:00 Lunch
IX. Ethylene in Senescence and Abscission of Plant Organs
(Panagiotis Kalaitzis, Shimon Meir)

15:00-15:05 Introduction by Chairs

15:05-15:30 Reidunn B. Aalen (invited talk)
(University of Oslo, Oslo, Norway)
Orthologues of Arabidopsis INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) and its receptors promote cell separation in mature abscission zones of leaves, fruits and seeds of diverse species

15:30-15:55 Shimon Meir (invited talk)
(Agricultural Research Organization, Rishon LeZion, Israel)
Ethylene is the initial inducer of organ abscission in plants

15:55-16:15 Panagiotis Kalaitzis (invited talk)
(Mediterranean Agronomic Institute at Chania, Greece)
Suppression and over-expression of a prolyl 4 hydroxylase results in alterations in tomato abscission program

16:15-16:35 Mark Tucker (invited talk)
(USDA-ARS, Beltsville, USA)
A study of the role of IDA-like gene expression in soybean and tomato abscission – IDA is expressed in abscission but may not be essential

16:35-17:05 Coffee break

IX. Ethylene in Senescence and Abscission of Plant Organs
(Panagiotis Kalaitzis, Shimon Meir) continued

17:05-17:20 Amnon Lers (selected talk)
(Agricultural Research Organization, Rishon LeZion, Israel)
T2-type Ribonuclease function in ethylene associated processes

17:20-17:35 Sara Patterson (selected talk) NSF Travel Awardee
(University of Wisconsin, Madison, USA)
Characterization of morphology, biochemistry and ethylene associated gene expression during fruit development in cold hardy grapes

17:35-17:50 Zora Singh (selected talk)
(Curtin University, Perth, Australia)
1-Hexylcyclopropene fumigation inhibits ethylene induced abscission of floral organs in cut waxflower (Chamelaucium spp.)

17:50-18:50 Poster session
Dinner at your own
June 5, 2018

X. Postharvest Physiology and Quality
   (Antonio Granell, Bo Zhang)

08:30-08:35 Introduction by Chairs

08:35-09:00 Antonio Granell (invited talk)
   (CSIC, Universitat Politècnica de València, Valencia, Spain)

   Postharvest fruit ripening and quality within the European Traditional Pool of Tomato Varieties: Effect of temperature and 1-MCP

09:00-09:25 Bo Zhang (invited talk)
   (Zhejiang University, Hangzhou, China)

   Regulation of fruit flavor during postharvest cold storage

09:25-09:40 Fabrizio Costa (selected talk)
   (Fondazione Edmund Mach, San Michele all'Adige, Italy)

   The interference of the ethylene perception system leads to a transcriptional re-programming involved in hormonal cross-talk and protection to superficial scald in apples

09:40-09:55 Me-Hea Park (selected talk)
   (National Institute of Horticultural and Herbal Science, Wanju, Korea)

   Deciphering the role of CO₂ treatment on chilling injury in tomato: Effect on transcriptome profiling

09:55-10:10 Clara Mata (selected talk)
   (KU Leuven, Leuven, Belgium)

   Transcriptomic and targeted MS proteomic quantification of the first ethylene signaling elements: ethylene receptors, CTRs and EIN2 in tomato fruit ripening

10:10-10:40 Coffee break

XI. Ethylene and Storage of Perishable Produce
    (Chris B. Watkins, Jean-Claude Pech)

10:40-10:45 Introduction by Chairs

10:45-11:10 Chris B Watkins (invited talk)
   (Cornell University, Ithaca, USA)

   To infinity (1-methylcyclopropene) and beyond!
11:10-11:35  **Ron Porat** (invited talk)  
(Agricultural Research Organization, Rishon LeTzion, Israel)  
Effects of the ethylene-action inhibitor 1-methylcyclopropene on postharvest quality of non-climacteric fruit

11:35-11:50  **Yasutaka Kubo** (selected talk)  
(Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan)  
Comparative analysis of ethylene–induced and low temperature–modulated ripening in kiwifruit

11:50-12:05  **Xiaoyang Zhu** (selected talk)  
(South China Agricultural University, Guangzhou, China)  
Characterization of genes in ethylene signal transduction pathways in papaya fruit under various experimental conditions

12:05-12:20  **Oscar W. Mitalo** (selected talk)  
(Okayama University, Okayama, Japan)  
Probing the role of ethylene and low temperature in the modulation of flavedo colour change in Satsuma mandarins (*Citrus unshiu* Marc) fruit

12:20-13:20  Poster session

13:20-14:30  Lunch

**EXCURSION in the afternoon**

20:30 Gala dinner at MAICH
June 6, 2018

XII. Ethylene interplay with other hormones in controlling secondary metabolism
(Andrew Allan, Jin-Song Zhang)

08:30-08:35 Introduction by Chairs

08:35-09:00 Andrew C. Allan (invited talk)
(Plant and Food Research, Auckland, New Zealand)

An elevated anthocyanic response in apple upregulates ethylene

09:00-09:25 Avtar Handa (invited talk)
(Purdue University, West Lafayette, USA)

Transcriptional cross talk between ethylene and spermidine during tomato fruit ripening

09:25-09:50 Jin-Song Zhang (invited talk)
(Chinese Academy of Sciences, Beijing, China)

Ethylene signaling in rice - novel insights

09:50-10:15 Tahira Fatima (selected talk) NSF Travel Awardee
(Purdue University, West Lafayette, USA)

Ethylene interaction with higher-polyamines in regard to defense-related secondary metabolites demonstrated in field-grown transgenic tomato genotypes

10:15-10:30 Eleni Tsantili (selected talk)
(Agricultural University of Athens, Athens, Greece)

The ability of ethylene to regulate the concentration of phenolic compounds and textural changes in harvested olives

10:30-11:00 Coffee Break

XII. Ethylene interplay with other hormones in controlling secondary metabolism
(Andrew Allan, Jin-Song Zhang) continued

11:00-11:15 Juan Wang (selected talk)
(Chinese Academy of Agricultural Sciences, Beijing, China)

Ethylene promotes ascorbic acid biosynthesis via the regulation of EIN3 and ABI4 on VTC2 transcription
XIII. Biotechnological Control of Ethylene Action and Biosynthesis
(Hiroshi Ezura, Zhengguo Li)

11:15-11:20 Introduction by Chairs

11:20-11:45 Hiroshi Ezura (invited talk)
(University of Tsukuba, Tsukuba, Japan)

Engineering shelf life of tomato fruits via targeted mutagenesis of ethylene receptor genes by the Target-AID technology

11:45:12:10 Zhengguo Li (invited talk)
(Chongqing University, Chongqing, China)

Overexpression a single SlMYB75 could affect fruit ripening related process and flavor metabolism in transgenic purple tomato

12:10-12:35 Satoko Nonaka (invited talk)
(University of Tsukuba, Tsukuba, Japan)

Control of ethylene biosynthesis via genome editing technology in melon

12:35-12:50 Houben Maarten (selected talk)
(University of Leuven, Heverlee, Belgium)

Screening for novel regulators of ethylene biosynthesis: focusing on ACC-oxidase

12:50-13:05 Michal Karady (selected talk)
(Umeå Plant Science Centre, Umeå, Sweden)

1-aminocyclopropane-1-carboxylic acid (ACC) and other compounds profiling in plant tissues using ultra-performance liquid chromatography – tandem mass spectrometry

13:05-14:45 Lunch

XIV. Chemical Control of Ethylene Action (Bart Nicolai, Athanasios Molassiotis)

14:40-14:45 Introduction by Chairs

14:45-15:10 Bart Nicolai (invited talk)
(KU Leuven, Leuven, Belgium)

Chemical ethylene control during growth and postharvest storage of climacteric fruit: an engineering perspective
15:10-15:35  Athanasios Molasiotis (invited talk)
(Aristotle University of Thessaloniki, Thessaloniki, Greece)
Towards systemic view for superficial scald in apple fruit: an ethylene perspective

15:35-15:50  Namrata Pathak (selected talk)
(Leibniz-Institut für Agrartechnik und Bioökonomie e.V., Potsdam, Germany)
Investigation of ethylene removal in fresh produce storage using advanced oxidation methods

15:50-16:05  Marcelo Menossi (selected talk)
(University of Campinas, Campinas, Campinas State University, Campinas, Brazil)
Transcriptome analysis reveals hormonal regulation of Ethephon-induced ripening in field-grown sugarcane

16:10-16:40  Coffee break

16:40-17:40  GENERAL DISCUSSION AND CONCLUSION
OPENING TALK TO THE SYMPOSIUM

INVITED TALKS

Past, Present and Future: New Roles for EIN2 and ACC

Chang Caren, Michard Erwan, Shemansky Jennifer, Qing Dongjin, Coleman Andrew, Ahtesham Uzair, Simon Alexander, Clay John, Kao Yun-Ting, Feijó José

University of Maryland, College Park, USA

In the past 3 decades, tremendous advances have been made in elucidating the ethylene-signaling pathway. However, several underlying mechanisms of ethylene signal transduction remain unknown. In particular, the molecular functions of ETHYLENE INSENSITIVE2 (EIN2), a central positive regulator in the ethylene-signaling pathway are just beginning to be uncovered. Recent findings in Arabidopsis have revealed that the C-terminal domain of EIN2 is regulated by phosphorylation and cleaved from the N-terminal domain in the presence of ethylene to control downstream signaling. Much less is known about the EIN2 N-terminal domain. The N-terminal domain localizes to the endoplasmic reticulum (ER) membrane and has sequence similarity to Nramp metal ion transporters known to transport Fe$^{2+}$ and Mn$^{2+}$, but whether EIN2 transports a metal has been undetermined. We now have evidence that the N-terminal domain functions as a proton-coupled antiporter capable of transporting Ca$^{2+}$ across the ER membrane, based on expression of the Arabidopsis EIN2 Nramp-like domain in yeast and mammalian cells and the use of specialized reporters, such as yellow cameleon (a FRET-based Ca$^{2+}$ sensor) and pHluorin (a pH-sensitive GFP). EIN2 is the only known Nramp-like protein with the ability to transport Ca$^{2+}$. We found that Ca$^{2+}$ transport is blocked by an ein2 missense allele that confers ethylene insensitivity. We therefore conclude that Ca$^{2+}$ transport by EIN2 is required for ethylene signaling. While ethylene has been known as a plant hormone for over a century, its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), has not been generally considered as a distinct signaling molecule. ACC levels are correlated with ethylene levels, and therefore ACC treatment is frequently used in place of ethylene in studies of ethylene responses. Unexpectedly, we found that ACC and ethylene responses can be uncoupled in Marchantia polymorpha and Arabidopsis thaliana. Moreover, we found that micromolar quantities of ACC can activate Ca$^{2+}$ currents in root protoplasts. The activation of Ca$^{2+}$ currents by ACC is
dependent on glutamate receptor-like (GLR) channels, which are controlled by amino acids. Our findings suggest that ACC may be an evolutionarily conserved plant hormone, distinct from its role in ethylene biosynthesis, and that ACC is perhaps a ligand for GLRs. These findings could require a reevaluation of the ACC-based ethylene literature.

I.

History and important steps in ethylene biology and biotechnology

INVITED TALKS

The pantheon of ethylene biologists

PECH Jean-Claude

Université de Toulouse, INP-ENSA Toulouse, Génomique et Biotechnologie des Fruits, Toulouse, France

The aims of the presentation are to honor the eminent ethylene biologists that have made important breakthroughs in the knowledge ethylene biology. The biological effects of ethylene have been discovered long time ago at the beginning of the 20th century. However, the elucidation of the biosynthetic pathway has been made much later in the years 1960s to 1980s. The use of novel experimental designs, such as reverse biochemistry, has played a crucial role in these discoveries. The concept of ethylene has been formulated quite early and has led to the synthesis of antagonists of ethylene action. One of them, 1MCP, has been released in the market and has revolutionized the handling of a number of agricultural and horticultural commodities. While direct biochemical techniques have failed for the isolation of the receptor, the use in the years 1990s, of Arabidopsis mutants has given the key for elucidating the mechanisms of perception and signaling transduction pathways. Lately the mode of action of ethylene in the regulation of gene expression at the transcriptional level has been described with much detail. This historical review teaches us that the involvement of brilliant scientists that had a good knowledge of the literature, had sometimes a bit of chance and have used well adapted strategies has been crucial for making of ethylene one of the best known, if not the best known, of all plant hormones.
Ethylene Old & New-
What's Left to Discover & Where Should We Look?

Don Grierson

Zhejiang Provincial Key Laboratory of Horticultural Plant Integrative Biology, Zhejiang University, Zijingang Campus, Hangzhou 310058, PR China and Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

110 years after publication of the effect on carnation flowers of ethylene in illuminating gas (1), we have a good general understanding of ethylene synthesis, perception and response systems in plants. Key components have been identified by work on Arabidopsis and crops, particularly fruits and flowers, focusing on processes such as infection, abscission, ripening and senescence.

In this presentation I shall discuss some deficiencies in this general understanding (2-5), identify results of research that have generated new insights (6-8) and suggest areas, such as flowering, ripening, and hormone interactions (9-12), where additional investigation could improve and broaden our understanding of the functioning of ethylene in controlling different aspects of the plant life cycle.

The vineyards and wines of Crete

Koundouras Stefanos

Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment School of Agriculture, Laboratory of Viticulture, Thessaloniki, Greece

The island of Crete is divided into the northern and the southern part by big mountain ranges (elevating up to 2,456 m) that cross the island. The climate of Crete is characterized by intense sunshine, high temperatures and low precipitation during summer and early autumn. However, the biggest part of the vineyards lies in altitude, at the cooler north-facing slopes of the mountains, which also provide a natural barrier from the warm south winds. This mountain influence is combined with that of the Cretan Sea offering beneficial cool breezes during the warm summer months. The winemaking tradition of Crete started almost 4000 years ago during the Minoan Civilization. The unique Cretan ecosystem provides an ideal terroir for the production of the Protected Designation of Origin (PDO) wines of Archanes, Peza, Dafnes and Sitia, in addition to several regional wines (Protected Geographical Indication or PGI). The most popular indigenous grapes are the red Kotsifali, Mandilaria and Liatiko and the white Vilana, Vidiano, Plyto, Dafni and Thrapsathiri as well as Muscat blanc, Chardonnay, Syrah and Cabernet-Sauvignon among several international varieties.

Most of Cretan viticulture and wine production is situated in the geographical area of Heraklion which is dominated by the indigenous red grape varieties 'Kotsifali', a pale color but intensely flavored variety, 'Liatico' which shares similar characteristics with Kotsifali but with stronger spicy and herbal aromas and an oxidative tendency, and 'Mandilaria' which offers deep color but astringent tannins and is used as a blending agent. The PDO zone of Peza is the eastern of the three successive PDO zones of Heraklion and produces a traditional 75% Kotsifali and 25% Mandilaria red wine. The area is also the only one in Heraklion to produce a single-variety white PDO wine from the local grape 'Vilana'. The PDO zone Archanes is located in the north-central part of the Heraklion area, in a historical region, south of the archaeological site of Knossos, and is the middle of three successive PDO zones. The area produces only red wines which, although of the same varietal composition as the neighboring PDO Peza, are more structured due to the increased amount of the Mandilaria variety. The vineyards of the PDO Dafnes extend to the western part of the region of Heraklion, at the eastern foothills of Psiloritis mountain (2456m) and is the largest of the three successive zones,
exclusively cultivating the 'Liatiko' grape. This variety produces light colored wines but, due to its oxidative aroma profile, has a particular use for sweet wines. Finally, in the eastern part of the Crete, in the Lassithi region and mainly on the plateau of Lefki at an average altitude of 620m, lies the PDO zone of Sitia, cultivated with the red Liatiko and Mandilaria and the white Vilana and Thrapsathiri.

II.
Ethylene Biosynthesis, Signal Transduction and Responses

INVITED TALKS

Unconventional Ethylene Receptor Signaling

Binder Brad, Lacey Randy, Allen Cidney, Carlew Scott, Rodriguez Celeste

University of Tennessee Knoxville, Knoxville, USA

A major focus of research in my lab is focused on non-canonical ethylene receptor functions in plants and ethylene receptors in bacteria. In this talk I will focus on ethylene receptors in bacteria. In bacteria, ethylene receptors have diverse domain structures indicating multiple signaling pathways. Our research indicates that in the model cyanobacterium Synechocystis, the ethylene receptors signal as canonical two-component receptors to affect the cell surface. This has diverse physiological outcomes. I will summarize our recent results characterizing ethylene signaling and responses in Synechocystis and discuss putative ethylene receptors in other bacteria where they may function to mediate plant-microbe interactions.

Ethylene Response Factors: key regulators of ethylene-related ripening process

Liu Mingchun¹, Lima Gomes Bruna², Mila Isabelle², Zouine Mohamed², Bouzayen Mondher², Julien Pirrello²

¹Key Laboratory of Bio-Resource and Eco-Environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu, China, ²GBF, Université de Toulouse, INRA, Castanet-Tolosan, France, Auzeville-Tolosane, France,

The plant hormone ethylene plays a key role in climacteric fruit ripening. Studies on components of ethylene signaling have revealed a linear transduction pathway leading
to the activation of ethylene response factors. However, the means by which ethylene selects the ripening-related genes and interacts with other signaling pathways to regulate the ripening process are still to be elucidated. Ethylene Response Factors (ERFs) are downstream components of ethylene signaling, known to regulate the expression of ethylene-responsive genes. Although fruit ripening is an ethylene-regulated process, the role of ERFs remains poorly understood. A large set of RNA sequencing (RNA-Seq) data available for multiple tomato cultivars was mined at the genome-wide scale using the newly developed bioinformatics platform TomExpress (http://gbf.toulouse.inra.fr/tomexpress), indicating that out of the 77 ERFs present in the tomato genome, 27 show enhanced expression at the onset of ripening while 28 display a ripening-associated decrease in expression, suggesting that different ERFs may have contrasting roles in fruit ripening. Using the CRES-T strategy we shed new light on the role of Sl-ERF.B3 in the transcriptional network controlling the ripening process and uncover a means towards uncoupling some of the main ripening-associated processes. In addition, our data support a model in which the ethylene-responsive Sl-ERF.B3 integrates ethylene and auxin signaling via regulation of the expression of the auxin signaling component Sl-IAA27. A comprehensive expression profiling of tomato ERFs in wild-type and tomato ripening-impaired tomato mutants (Never-ripe [Nr], ripening-inhibitor [rin], and non-ripening [nor]), leads to the identification of a small subset of ERF genes, from the subclass E, displaying a consistent ripening-associated expression pattern. Transactivation assay data illustrate the high complexity of the regulatory network connecting RIN and ERF.E. Assigning specific roles to ERF members will open new avenues toward engineering fruit development and ripening via targeted approaches, especially when aiming to enhance some desirable traits and metabolic pathways and to reduce unwanted ones.

**Histone deacetylases and ethylene-induced transcriptional repression**

Qiao Hong Qiao

University of Texas, Austin, USA

Ethylene plays pleotropic roles in plant growth, plant development, and stress responses. Although the effects of ethylene on plants are well documented, little is known about molecular-level events that result in transcriptional repression during the ethylene response. In this study, we found that two histone deacetylases, SRT1 and
SRT2, interact with ENAP1, which associates with EIN2 in the nucleus. Genetic and transcriptome analyses revealed that SRT1 and SRT2 are required for negative regulation of certain ethylene-responsive genes. The acetylation of histone 3 at K9 (H3K9Ac) is specifically regulated by SRT1 and SRT2 in ethylene-repressed genes. In addition, the srt1srt2 double mutation in Arabidopsis suppresses both the ENAP1ox and the EIN3ox constitutive ethylene-response phenotypes, and the ethylene-induced transcriptional repression observed in EIN3ox plants is de-repressed in the EIN3ox/srt1srt2 mutant. SRT2 and ENAP1 both bind to promoter regions of genes negatively regulated by ethylene reducing H3K9Ac levels and resulting in transcriptional repression. This work establishes a novel mechanism by which histone deacetylases SRT1 and SRT2 interact with ENAP1 to mediate transcriptional repression by regulating the levels of histone 3 acetylation in the ethylene response.

**ORALS**

**A Conserved Role of Arabidopsis ECR1 and Human Tumor Suppressor HsECR1 in Translational Control of ctr1-10**

*Zhang Ranran, Wen Chi-Kuang*

National Key laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China

The weak *ctr1-10* mutation, resulting from a T-DNA insertion at the 5'-UTR, confers a relatively weak constitutive ethylene response phenotype. An enhancer screen isolated ENHENCING CTR1-10 ETHYLENE RESPONSE1 (*ECR1*), and ECR1 shares a high degree of sequence identity with the human tumor suppressor *HsECR1*, which has an in vitro hydrolase activity. The exact molecular mechanism of *HsECR1* tumor suppression is to be unveiled. Expression of the transgenes respectively encoding the wild-type and a hydrolase activity-dead HsECR1 complemented *Arabidopsis ecr1*. Sub-cellular localization studies indicated ECR1 and HsECR1 to be associated with the ER. CTR1 levels were reduced in *ctr1-10*, compared with the wild type, and to a greater extent in *ctr1-10 ecr1*. Transgenes with mutations that eliminated the upstream open reading frames (uORFs) of the ctr1-10 transcript greatly rescued the *ctr1-10 ecr1* phenotype, lending support to the argument that the uORFs may alleviate the CTR1 translation in
the absence of ECR1. A yeast two-hybrid screen isolated 34 independent clones that encode ECR1-ASSOCIATED PROTEIN1 (EAP1), which is known for a role in protein translation. Early termination mutations of EAP1, by CRISPR-Cas9, enhanced ctr1-10 mutant phenotype to ctr1-1 levels, and eap1 effects on ethylene response phenotype were not prominent. Various evidence reveals that uORFs have a role in the control of the translation of the main ORF. Our present data suggest a conserved role of ECR1 and HsECR1 tumor suppressor in protein translational control at the 5'-UTR with multiple uORFs, and the control may also involve EAP1. The result is consistent with studies in mammals that translation of tumor-related proteins is alleviated by a longer 5'-UTR.

The ethylene precursor, ACC, is an extracellular cue in the guidance of pollen tubes toward ovules in Arabidopsis thaliana

Mou Wangshu1, Li Dongdong1, Wudick Michael M.2, Ying Tiejin3, Feijó José A.2, Chang Caren2

1College of Biosystems Engineering and Food Science, Zhejiang University; 2Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, USA, 3College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, China

It is well established that ethylene is synthesized by a simple two-step reaction starting from conversion of S-adenosylmethionine (AdoMet) to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS), followed by conversion of ACC to ethylene by ACC oxidase (ACO). Numerous ethylene response studies in flowering plants are based on treatments with ACC (which can be directly added to growth media) in place of using ethylene gas, since exogenous ACC is taken up and readily converted to ethylene gas by ACO. However, our findings here provide an example in which the ACC-ethylene relationship is uncoupled. The Arabidopsis genome contains 12 ACS genes annotated as ACS1-12, and 8 of these genes (ACS2, ACS4–9, and ACS11) are functional. Tsuchisaka et al. (2009) constructed an acs octuple mutant (acs2-1 acs4-1 acs5-2 acs6-1 acs7-1 acs9-1 amiRacs8acs11) with 6 ACS genes knocked out by T-DNA insertions and 2 ACS genes knocked down by a single artificial microRNA. The authors observed that the acs octuple mutants have shorter siliques as well as fewer seeds than wild type (WT), but that ethylene treatment failed to rescue this phenotype. Interestingly, this phenotype is not detected in any ethylene-insensitive mutants. The lack of correlation with ethylene-insensitive phenotypes raised the possibility that ACC itself could be an independent signal in regulating reproduction in
Here, we analyzed the defect more closely, starting with a series of reciprocal crosses that revealed the reproduction defect in the *acs* octuple mutant derives from the female sporophyte and is dominant over WT. Based on hand crosses and aniline blue staining of pollen tubes, we discovered that in *acs* octuple mutant pistils, WT and mutant pollen tubes do not turn toward the ovules as well as they do in WT pistils. In addition, pollen tubes of ethylene-insensitive mutants can turn normally in WT pistils but exhibit less turning in mutant pistils, suggesting that turning of pollen tubes toward the ovules is affected by ACC, not by ethylene. Moreover, a semi-in vivo pollen tube guidance assay with WT/*acs* octuple ovules in competition indicated that the sporophytic tissue of *acs* octuple mutant ovules is responsible for the defect in pollen tube attraction. We found that pre-treating the ovules with ACC could rescue this defect in the semi-in vivo assay. Our findings identify ACC as an extracellular cue for pollen tube guidance from the female sporophyte, indicating a novel role for ACC that is independent of its role as the ethylene precursor in *Arabidopsis*.

Study of the seven ethylene receptor proteins over the tomato fruit ripening by targeted mass spectrometry, in wild type and *Never Ripe* backgrounds.

Chen Yi¹, Gil Julie¹, Nosarzewska Joanna¹, Rofidal Valerie², Hem Sonia², Berger Nathalie², Demolombe Vincent², Bouzayen Mondher¹, Santoni Véronique², Chervin Christian¹

¹Toulouse INP/ENSAT/INRA, Castanet-Tolosan, France, ²INRA Montpellier, Montpellier, France

Ethylene receptors are the critical first step in ethylene perception. In tomato, until now, the proteins have been studied by western blot with a limited number of antibodies (for 3 receptors only), and some results have been contradictory. We have optimized the extraction process and developed a targeted mass spectrometry approach that enable us to quantify the seven ethylene receptor proteins of the tomato. We analysed the proteins and the mRNA, on the same samples, over the ripening period over 4 stages: immature green, mature green, breaker and breaker + 8 days. We also performed a label-free protein analysis on the same samples. The protein variation followed the mRNA profile, confirming the results by Kamiyoshihara et al. (2012), infirming the results by Kevany et al. (2007), who wrote that protein and mRNA profiles were anti-correlated. The ETR3 and ETR4 are the proteins showing the biggest relative increase over the ripening inception, matching the mRNA variations. Considering the good coverage of ETR1 and
ETR4 in the label-free approach, the results suggest that ETR1, ETR3 and ETR4 may be key players of ethylene perception over tomato ripening. ETR7, a novel ethylene receptor discovered since the tomato genome release, was also analysed and showed a slight increase over the ripening period. Further works by Chen et al. (poster in this conference) show it is a functional ethylene receptor. We also compared the seven receptor proteins in wild type and Never Ripe backgrounds. We observed that the limitation of ETR signal, in the NR mutant, leads the plant to produce even more NR (mutated ETR3), thus reinforcing the blockade, as NR is a gain-of-function mutant. Thus, this approach gave us critical insights about ETR regulation at the protein level. More studies should arise from this methodological breakthrough.

A systematic approach for dissecting the mechanisms of EIN3-dependent regulation of ethylene response in Arabidopsis thaliana

Zemlyanskaya Elena, Levitsky Victor

Novosibirsk State University; Institute of Cytology and Genetics SB RAS, Novosibirsk, Russian Federation

The plant hormone ethylene regulates numerous developmental processes and stress responses [1]. Ethylene signaling proceeds via a linear pathway, which activates EIN3 transcription factor [2]. EIN3 influences gene expression upon binding to a specific sequence in gene promoters. However, in many cases EIN3 binding to gene promoter is not enough to trigger transcriptional response [3]. Here we apply a systematic bioinformatics approach to dissect the factors essential for EIN3 functioning. We extracted EIN3 binding regions (EBRs) from publicly available ChIP-seq data on EIN3 binding in Arabidopsis thaliana [3] and used RNA-seq data on ethylene-induced transcriptomes [3] to determine ethylene responsiveness of EIN3 target genes. To characterize EIN3 binding regions with respect to the epigenetic status we used previously published genome-wide map of nine chromatin states in A. thaliana [4]. The subsequent analysis was conducted to investigate the impact of DNA-binding context, its position relative TSS and epigenetic status on the ethylene sensitivity of genes bound by EIN3. The analysis of ChIP-seq data showed bimodality of distribution of EIN3 binding regions in gene promoters. We found that the implicit distal peak was associated with a specific chromatin state (referred to as chromatin state 4 in the primary source), which was just poorly represented in the pronounced proximal peak.

POSTERS

CTR1-independent ETR1 Receptor Signaling May Involve SNS1 and EIN2

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The Raf-like CTR1 protein has a crucial role in mediating ethylene receptor signal output to repress EIN2-mediated ethylene signaling, and loss-of-function mutations of CTR1 lead to strong constitutive ethylene responses. On the other hand, the strong constitutive ethylene response by ctrl mutations can be greatly suppressed by expression of the N-terminus (etr11-349) of the ETR1 receptor, indicative of a regulation of ethylene signaling independent of CTR1. Present studies suggest that EIN2-mediated ethylene signaling is constitutively activated in the absence of CTR1,
and we investigated how the receptor N-terminus could repress ethylene signaling in ctr1 mutants. We artificially mutated residues on the CTR1-interacting ETR1 HK-domain; expression of these mutated, ethylene-insensitive etr1-1 isoforms conferred ethylene insensitivity in etr1 ers1 etr2 ein4 ers2 and ctr1-1. A suppressor screen for etr1-11-349 ctr1-1 isolated loss-of-function mutations of SUPPRESSING N-TERMINAL SIGNALING1 (SNS1), and etr1-11-349 ctr1-1 sns1 resembled ctr1-1. SNS2 is homologous to SNS1, and sns2 effects on etr1-11-349 ctr1-1 were minor. The strong ctr1-3 allele was constitutive ethylene responsive; the ctr1-3 phenotype was greatly reversed by a heterogonous ein2 (i.e., ein2/EIN2), and ctr1-3 ein2/EIN2 was fully responsive to ethylene. Those data indicate: (1) mutations at the HK-domain could convert the receptor to a conformation capable of signal output independent of CTR1, (2) SNS1 plays a major role in the CTR-independent pathway, and (3) EIN2 activation of ctr1-3 ein2/EIN2 in part requires ethylene, in the absence of CTR1-mediated phosphorylation/inactivation. Our results indicate a regulation of ethylene signaling, possibly via the N-terminus, without CTR1 via the integration or coordination of the receptor and EIN2, involving SNS1. We are in the progress of examining possible cooperations of EIN2 with the CTR1-independent ethylene signaling regulation and discuss other possibilities about the current model.

Identification and quantitative measurement of proteins of ethylene biosynthesis using MRM assays by mass spectrometry

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ACC synthase (ACS) is the rate-limiting enzyme in the ethylene biosynthesis pathway. ACS families are many isozymes. These isozymes are classified into 3 types by C-terminal phosphorylated sequence. Type 1 has CDPK and MAPK phosphorylation sites. Type 2 has only CDPK sites, and type 3 has no phosphorylation sites. We previously reported that SIACS2, a wound-inducible ACS in tomato, is phosphorylated at Ser-460 by CDPK and at additional Ser residues by MAPK, that is type 1. On the other hand, there is another isozyme, SIACS4, in ripening tomato fruits. SIACS4 has no phosphorylation sites, that is type3. However, we cannot detect SIACS2 and SIACS4 change using Western blotting by each antibody because each ACS protein content level was very low. Here, we have developed a highly sensitive analysis for protein
identification and quantitative measurement of proteins based on multiple reaction monitoring (MRM) assays by mass spectrometry. Our method, depended on the report by Matsumoto et.al (Nature Methods, 14: 251, 2017), comprised MRM assays of mass tag (mTRAQ)-labeled peptides to measure the abundance of target proteins using LC/MS/MS analysis coupled triple quadrupole mass spectrometer (QTRAP 5500 Scix). We used the target sample tryptic peptides labeled by mTRAQ Δ0 and recombinant ones as the internal standard labeled by mTRAQ Δ4. Theses mTRAQ labeled peptides were combined and then analyzed MRM assay by mass spectrometry. As the result of this method, SIACS proteins were detected and quantitatively measured.

Membrane protein MHZ3 stabilizes OsEIN2 in rice by interacting with its Nramp-like domain

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The ethylene signaling pathway has been extensively investigated in Arabidopsis. Rice is a monocotyledonous model plant that exhibits different features in many aspects compared with the dicotyledonous Arabidopsis. Thus, rice provides an alternative system for identification of novel components of ethylene signaling. Here, we identified a stabilizer of OsEIN2 through analysis of the rice ethylene-response mutant mhz3. Loss-of-function mutations lead to ethylene insensitivity in etiolated rice seedlings. MHZ3 encodes a previously uncharacterized membrane protein localized to the endoplasmic reticulum. Ethylene induces MHZ3 gene and protein expression. Genetically, MHZ3 acts at the OsEIN2 level in the signaling pathway. MHZ3 physically interacts with OsEIN2, and both the N- and C-termini of MHZ3 specifically associate with the OsEIN2 Nramp-like domain. Loss of mhz3 function reduces OsEIN2 abundance and attenuates ethylene-induced OsEIN2 accumulation, whereas MHZ3 overexpression elevates the abundance of both wild-type and mutated OsEIN2 proteins, suggesting that MHZ3 is required for proper accumulation of OsEIN2 protein. The association of MHZ3 with the Nramp-like domain is crucial for OsEIN2 accumulation, demonstrating the significance of the OsEIN2 transmembrane domains in ethylene signaling. Moreover, MHZ3 negatively modulates OsEIN2 ubiquitination, protecting
OsEIN2 from proteasome-mediated degradation. Together, these results suggest that ethylene-induced MHZ3 stabilizes OsEIN2 likely by binding to its Nramp-like domain and impeding protein ubiquitination to facilitate ethylene signal transduction. Our findings provide new insight into the mechanisms of ethylene signaling.

Screening of rice accessions with altered ethylene responses

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Ethylene plays an important role in rice growth and environmental adaptation. Unlike the classical "triple response" of Arabidopsis thaliana, rice has a typical "double response", including ethylene-promoted coleoptile elongation and ethylene-inhibited root growth. However, the mechanism for ethylene promotion of rice coleoptile elongation is largely unknown. Recent research shows that a single genome does not represent the genome of the entire species well, and it does not reflect the variation of the entire species. In particular, some elite traits of crops are attributed to rare mutations. Through analysis of the ethylene response of 1459 rice varieties, we found that two rice varieties, YC45 and SE68, had abnormal coleoptile ethylene response. The coleoptile of YC45 is completely insensitive to exogenous and endogenous ethylene, while the coleoptile of SE68 is only insensitive to exogenous ethylene. Ethylene treatment inhibited root growth in dark-grown YC45 and SE68 rice seedlings, which is similar to that in dark-grown Nipponbare. The ethylene responsiveness of YC45 and SE68 was further confirmed at the molecular level by examining the expression of ethylene-inducible genes. The expression of three genes, receptor-like kinase (SHR5), AP2 domain-containing protein (ERF073) and cupin domain-containing protein (Germin-like) was abolished or hampered in YC45. While in SE68, only the expression of Germin-like was abolished or hampered. Through map-based cloning, the YC45 locus was mapped to the short arm of chromosome 3 between markers Idl3-24.933 and Idl3-24.994. The location of SE68 was mapped to a 34-kb genomic DNA region between markers dCAPS 6-29.158 and dCAPS 6-29.192. Confirmation of the two candidate genes is ongoing. The analysis of the molecular mechanisms underlying the above-mentioned ethylene abnormalities in rice varieties will expand our understanding of rice ethylene signal transduction.
Copper transfer in the ethylene signaling pathway probed by NMR spectroscopy

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The ethylene signaling pathway controls fundamental developmental processes such as fruit ripening or senescence. Ethylene is perceived by membrane receptors located at the surface of plants endoplasmic reticulum (ER). For high affinity binding of the plant hormone a copper (I) cofactor is essential. Integration of the metal cofactor into the receptor is a challenge as copper (I) is hardly soluble in aqueous solutions and may participate in its free form in harmful Haber-Weiss/Fenton reactions leading to the formation of reactive oxygen species (ROS). Therefore, all domains of life have developed tightly controlled chaperone systems for copper (I) storage and transfer throughout the cell. In Arabidopsis thaliana Copper Chaperone (CCH) belonging to the Antioxidant 1 (ATX1)-family is able to bind copper (I) with a femtomolar affinity. Compared to ATX1 from Arabidopsis thaliana and homologs from other species CCH has a C-terminal extension of about 45 residues. In this study we have cloned CCH from Arabidopsis thaliana in E. coli, expressed the chaperone in the bacterial host and purified AtCCH from the bacterial cells to homogeneity. Solution NMR was then used to study copper (I) binding to the purified recombinant chaperone in vitro. In these experiments the copper-bound and the apo form of CCH showed significant differences in chemical shifts for almost all residues except those of the C-terminal extension. Transition of the apo- to the copper-bound form and vice versa was studied using BCA2-Cu(I) or DTT as donor or acceptor for copper (I), respectively. Addition of DTT to copper bound CCH shifted the spectra back to those previously recorded for apo-CCH. Vice versa addition of BCA2-Cu(I) to apo-CCH resulted in spectra similar to those of copper-bound CCH. Moreover, further structural information on the C-terminal extension of CCH was gained in our NMR studies. So far, the C-terminal extension was thought to form an amphipathic helix based on the unusual repetitive sequence of positively and negatively charged residues in this part of the protein. Now our NMR studies reveal that both, the apo- and the copper-bound form, are mainly unstructured, as indicated by a characteristic clustering of signals in the corresponding HSQC spectra. Current NMR studies in our lab aim to resolve copper transfer from CCH to its potential targets in the ethylene signaling pathway.
FRET-based sensors for studying metal binding at the ethylene receptor family

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The plant hormone ethylene is an important regulator in plant growth and development. Signal perception and response to the plant hormone are mediated by a small family of integral membrane receptors localized at the ER and the Golgi network. The biological function of the receptors, which mark the start of the ethylene signaling cascade at the endomembrane network and control signal transfer to further downstream elements, relies on a protein-bound copper cofactor. The determinants for assembly and integration of this copper cofactor in the integral membrane receptors are still largely unknown. To resolve the molecular basis of copper transfer as well as conformational changes related to the binding of the essential cofactor we developed FRET-based sensors of the ethylene receptor family consisting of the receptors transmembrane sensor domain in concert with fluorescent protein monomeric turquoise 2 (mT2). Furthermore, a tetracysteine motif was engineered in the receptors sensor domain to provide a specific binding site for the small biarsenical fluorophore FlAsH - a membrane permeable, fluorescein derivative showing fluorescence only when bound to a tetracysteine motif. The FRET sensor was cloned in E. coli, expressed in the bacterial host and purified form the bacterial cells to homogeneity. FlAsH labeling of the purified ETR-mT2 completed the FRET-sensor of the ethylene receptor sensor domain. Quantitative binding data of the receptor such as stoichiometry, specificity and affinity were obtained from donor-acceptor fluorescence resonance transfer studies upon titration of the purified detergent-solubilized sensor with different transition metal ions (e.g. copper or gold). These data indicate preferred interaction with copper (I) and a protein:metal ratio of 1:1. Time-resolved studies will gain additional information on the dynamics of metal binding in the ethylene receptor family.
Ligand-induced transitions in the phosphorylation status of ripening-related ethylene receptors in tomato fruit

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The plant hormone ethylene is perceived by a membrane-associated receptor family which is similar to the bacterial two-component histidine kinase receptors. Since ethylene receptors negatively regulate the signaling, the suppression is canceled upon ethylene binding, permitting responses including fruit ripening. Although receptors are known to have autophosphorylation activity, the mechanism whereby signal transduction occurs has not been fully elucidated. Here we demonstrate that SlETR4, a crucial receptor for tomato (*Solanum lycopersicum*) fruit ripening, is phosphorylated in vivo and the phosphorylation level is dependent on ripening stage and ethylene action. Although only phosphorylated isotypes were detected in immature and mature green fruits, the non-phosphorylated isotype appeared after ripening started. Furthermore, treatment of preclimacteric fruits with ethylene resulted in accumulation of SlETR4 with reduced phosphorylation while treatments of ripening fruits with ethylene antagonists, 1-methylcyclopropene and 2,5-norbornadiene, induced accumulation of the phosphorylated isotypes. A similar phosphorylation pattern was also found in another ripening-related receptor SlETR3. Interestingly, in *Never ripe* mutant in which SlETR3 is unable to perceive ethylene because of a point mutation, ethylene-induced dephosphorylation of SlETR4 was partially prevented. This result implies that the functional defect in SlETR3 somehow influences the SlETR4 phosphorylation state. Alteration in the phosphorylation state of receptors is likely to be an initial response upon ethylene binding since treatments with ethylene and 1-methylcyclopropene rapidly influenced the phosphorylation state. The SlETR4 phosphorylation state was closely related to ripening progress, suggesting that the phosphorylation state of receptors is implicated in the ethylene signal output in the fruits. This receptor phosphorylation potentially play a role as a regulator for the interaction with downstream components in ethylene signal transduction.
The ethylene precursor, ACC, itself may function as a plant hormone in the liverwort *Marchantia polymorpha*

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The plant hormone ethylene functions in numerous aspects of development and environmental responses. In higher plants, it is well established that ethylene is synthesized from the precursor 1-aminocyclopropane-1-carboxylic acid (ACC) by the activity of ACC oxidase (ACO). Interestingly, ACO homologs capable of efficiently converting ACC to ethylene are found only in higher vascular plants, whereas the synthesis of ACC appears to be well conserved, even in basal land plants, raising the question of what role ACC plays in these plants. We addressed this question using the model system *Marchantia polymorpha* (liverwort), a basal land plant. We discovered that treating *Marchantia* with ACC induces a phenotype that is quite distinct from that of ethylene treatment. In *Marchantia gemmalings*, ethylene treatment increases overall plant size. *Mp-ctr1* knockout mutants created by CRISPR/Cas9 are larger than the wild type (WT), consistent with a constitutive ethylene response, while *Mp-ein3* knockout mutants are smaller than WT, consistent with ethylene insensitivity. In contrast, ACC treatment during the early stages of gemmaling development results in severe inhibition of cell differentiation and growth not seen with ethylene treatment. These ACC effects are non-toxic and reversible. A knockout of one of the two ACC synthase (ACS) gene homologs in *Marchantia, Mp-acs1*, is interestingly larger in size compared to the WT. We have thus unmasked a reversible ACC response that is distinct from ethylene response, leading us to propose that ACC itself serves as plant hormone in *Marchantia*. We speculate that ACC may be an important signaling molecule that evolutionarily predated the ability of higher land plants to efficiently convert ACC to ethylene.
Generation of tomato ethylene receptor loss-of-function mutants and functional analyses

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Ethylene regulates several aspects of plants growth and development, which are mediated by ethylene receptors (ETRs): seed germination, fruit ripening, stress responses and so on. In tomato, lots of work have been done about ethylene receptor function and mechanism. Now we know that there are seven ETRs in tomato, but it is still not clear whether they are functionally redundant or not. For studying each ETR function and their redundancy, we generated ETR loss-of-function mutants in a similar genetic background, Micro-Tom. By CRISPR-Cas9 strategy, we knocked out these ETR genes. At the moment, we have obtained four ETRs KO single mutants (Sletr2, Sletr3, Sletr4, Sletr7) and two ETRs double KO mutant (Sletr 1/ Sletr 2, Sletr 5/ Sletr 7). The target of the study is to get single mutants for each ETR, and a maximum combination of double mutants. We measured different levels of triple responses in various ETR mutants compare to wild type (WT). For example, single mutants of Sletr7 are more sensitive to 1 μM of ACC than Sletr2 and WT. We also showed that ETR7 loss-of-function mutants tend to germinate faster than WT. Finally, preliminary experiments in fruits showed only slight differences between WT and single mutants, however ethylene production around breaker stage, color change and Brix level were affected. These preliminary results show that ETR7, that has never been described, seems to function as an ethylene receptor. Additionally, we believe that this complete series of ETR loss-of-function mutants will be a great tool for studying the ETR subfunctions in tomato.

Pyrazinamide and derivatives block ethylene biosynthesis by inhibiting ACC oxidase

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The gaseous phytohormone ethylene plays important roles in plant growth and development, defense response, as well as adaption to stress. Ethylene also involves in the regulation of fruit ripening and senescence, which has unique value in the management of postharvest fruits, vegetables and flowers. High level of ethylene
bioproduction will reduce their shelf life, leading to huge postharvest losses and problems of food safety. Therefore, specific ethylene biosynthesis inhibitors would help to decrease postharvest loss. Recently, we identify pyrazinamide (PZA), a clinical drug used to treat tuberculosis, as an inhibitor of ethylene biosynthesis in Arabidopsis thaliana, using a chemical genetics approach. PZA is converted to pyrazinecarboxylic acid (POA) in plant cells, suppressing the activity of 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), the enzyme catalysing the final step of ethylene formation. The crystal structures of Arabidopsis ACO2 in complex with POA or 2-Picolinic Acid (2-PA), a POA-related compound, reveal that POA/2-PA bind at the active site of ACO, preventing the enzyme from interacting with its natural substrates. Our work suggests that PZA and its derivatives may be promising regulators of plant metabolism, in particular ethylene biosynthesis. Ethylene is a plant hormone that promotes fruit ripening. Here, we identify pyrazinamide, a drug used to treat tuberculosis, as an inhibitor of ethylene biosynthesis in Arabidopsis thaliana and present the crystal structure of its active form (pyrazinecarboxylic acid) bound to ACC oxidase.

III.

Ethylene in Reproductive Growth-Development and Fruit Ripening

INVITED TALKS

Ethylene and the cascade of ripening control

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Among the important roles of ethylene in plant development and biological response is a central role in ripening of many fleshy fruit species. The cultivated tomato (*Solanum lycopersicum*) is a tractable and efficient model for fruit development, storage quality and nutrient accumulation, in addition to being a crop of established and expanding production, consumption and culinary importance the world over. Ethylene is a critical component of the tomato ripening process. While recent genetic and molecular research
has focused on identification of genetic regulators of ripening such as transcription factors in addition to the intersection of additional hormone pathways, ethylene and its underlying genes were among the first targets of ripening molecular biology. We have explored the function of ripening transcription factors underlying fruit ripening mutations including those altered in the *rin*, *nor*, and *u* mutations defining fruit development roles for the MADS, NAC and GLK transcription factor families, respectively. Mining of these families provided additional genes effecting fruit development and ripening characterized in transgenic tomato plants. Additional regulators have been uncovered via examination of fruit quality QTLs and genes associated with ripening based on expression profiles. Most if not all of these regulators intersect with ethylene synthesis and response. More recently, the ability to examine specific tissues has allowed for examination of these known and potential interactions in the 3-D context of the whole maturing fruit.

**The network of hormone signaling underlying fruit ripening: allies and opponents**


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The plant hormone ethylene is regarded as the major regulator of climacteric fruit ripening but the assumption that fleshy fruit ripening is driven by a complex hormonal balance in combination with the intervention of developmental factors like RIN and NOR has long been formulated, even though the mechanisms underlying this hormonal interplay remained elusive. Our recent study clearly validated the role assigned to Auxin in the ripening of fleshy fruits based on the observed ripening delay induced by exogenous auxin treatment. Furthermore, using tomato lines expressing a DR5 promoter-driven VENUS (VEN) fluorescent protein revealed that auxin levels and signalling undergo a sharp decrease at the onset of fruit ripening in wild type tomato while it remains in active state in *rin* ripening mutants, suggesting that auxin removal is need to allow ripening to proceed. The down-regulation of *Sl-SAUR69*, which participates in several auxin-dependent processes, leads to a delay in the onset of ripening and, conversely, over-expression of this gene induced premature ripening. Interestingly, these phenotypes were associated to abnormal auxin distribution and
altered ethylene sensitivity of the fruit tissue. Further evidence supporting the active role of auxin signalling in fruit ripening came from the down-regulation of *SlARF2*, a member of the *Auxin Response Factor* (*ARF*) gene family in the tomato, which results in several ripening defects. ARF2 emerges as a new component of the regulatory network controlling tomato fruit ripening. While the link between ethylene and RIN/NOR-dependent mechanisms during climacteric ripening is a commonly accepted, so far, the precise role of Ethylene Response Factors (*ERFs*) in mediating ripening-associated processes remains unresolved. We recently showed that only a small subset of *ERF* genes display ripening-associated expression patterns. In particular, sub-class *E ERFs* might represent the missing link between the climacteric rise in respiration and the autocatalytic ethylene production, considering their reported function as oxygen sensor. Overall, the data support a model in which the regulatory network controlling fruit ripening relies on complex interactions between multi-hormonal signaling and developmental factors.

**Lesson from the fruit ENCODE project: is tomato the right model to study ethylene dependent fruit ripening?**

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Fleshy fruit ripening has evolved many times throughout the angiosperm history, and many require the plant hormone ethylene. Much of what we know about this process came form the study of tomato, where ethylene, MADS-box transcription factors and whole-genome demethylation are critically involved. But the precise molecular mechanism and whether it is conserved in other species remain largely unknown. The fruitENCODE project has systematically profiled gene expression, accessible chromatin, histone modifications and DNA methylation changes in 11 fleshy fruit species to investigate the molecular circuits controlling ripening. We found most climacteric fruits utilize a common angiosperm senescence-related NAC transcription factor to create a positive feedback loop to synthesize the autocatalytic ethylene. For plants undergone recent whole-genome duplication like tomato, apple and pear, their loop depends on neofunctionalization of the duplicated MADS-box transcription factors. Banana, a monocot climacteric species that diverged from eudicot over 100 myr and undergone three recent WGD, uses leaf senescence-related NAC transcription
factor to generate a positive feedback loop and an additional loop with three MADS-box transcription factors that makes its ripening ethylene independent once initiated. It turns out that DNA methylation changes associate with ripening genes could be unique for tomato, while other species utilize H3K27me3 to regulate their genes involved in ripening and autocatalytic ethylene production. The fruitENCODE study has raised important questions regarding how we should interpret complex developmental processes while the data is often derived from a single model species like tomato.

**ORALS**

**Dissecting the ethylene ripening response in apples**

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During apple fruit ripening there is a ripening progression of starch degradation, skin colour change, loss of firmness and the production of aroma volatiles. ‘Royal Gala’ (RG) apples suppressed for the ripening associated ACC OXIDASE1 (ACO1as) exhibited no ethylene related ripening, as 1-MCP treated ACO1as apples were identical to those left at room temperature. While some ripening traits (such as starch degradation) progressed in the absence of ethylene, others (such as volatile production) required ethylene to proceed. By treating ACO1as apples with different concentrations of ethylene, we found that each ripening trait showed a different sensitivity and dependency to ethylene. With early ripening events being less dependent and more sensitive to ethylene, and later ripening events being more dependant and less sensitive to ethylene. This was mirrored in gene expression patterns, with some cell wall related genes such as POLYGALACTURONASE1 (PG1) requiring more ethylene to reach a high transcriptional abundance compared to the B-GALACTOSIDASE1 (BGAL1) gene. Moreover this transcriptional activation was achieved in an ethylene-dose by time mechanism with low concentrations of ethylene taking longer to establish a similar transcriptional state than high concentrations of ethylene. F1 crosses to RG ACO1as
line with pollen from an old variety of rapid softening apples (‘Mr Glastone’), created lines with firm apples that produced no detectable ethylene. Ethylene treatment of these apples resulted in very rapid softening compared to the parental RG lines. This suggests that modern cultivars have been selected for reduced sensitivity to ethylene during the breeding selection process.

Transcriptional co-repressor SITPL3 regulates tomato fruit development and ripening

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TOPLESS genes encode a family of transcriptional co-repressor factors functioning in many hormone signaling pathway by interacting with transcription factors. In Arabidopsis, TPL interacts with AUX/IAA or ERF transcription factors to repress gene expression involved in auxin or ethylene response. In tomato, 6 TOPLESS members are identified. To gain insight on the function of TOPLESS in regulating tomato fruit development and ripening, reverse genetics approach is performed. The study shows that SITPL3 is involved in flower and fruit development. Transcript level expression analyses indicate that SITPL3 is mainly expressed in floral organs. Down-regulation of SITPL3 genes results in flower and fruit associated phenotypes. At the early flower bud stages, the silencing of SITPL3 increases tomato flower organs, fruit locules. Noteworthy, the transgenetic plants with higher numbers of flower organs give bigger and heavier tomato fruit. Moreover, down-regulation of SITPL3 inhibits the ripening process in tomato. The lycopene and sugar contents are lower than wild type. Overall, the phenotypes observed from the silencing lines indicate that down-regulation of SITPL3 disturbs reproductive organs differentiation and identity suggesting that this gene plays a critical role in fruit development from initiation through ripening.
Ethylene for CLM (Crop load management) in stone fruit (plum)

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With ca. 10 mil t, plums are one of the major commodities worldwide. Consumers and fruit trade require a large fruit size for plum marketing, both as fresh dessert fruit as well as for baking purposes, where the fruit halves are displayed to the consumer and uniform and large fruit size are sales determinants. Late frost can destroy the crop of pome and stone fruit trees in year 1 and induce alternate bearing in year 2 in many crops including plum leading to excessive flowering with an excess of small fruit and no fruit in year 3. While a large range of chemicals is suitable and approved for thinning of pome fruit trees, ethylene releasing compounds (Ethephon, Flordimex, Ethrel) are often the only chemical approved for such action in IP/IFP integrated stone fruit production. This contribution summarises the results of five year trials with crop load management (CLM) under different weather regimes in European plum (Prunus domestica). Five year-old spindle cv. ‘Ortenauer’ plum trees were used on dwarfing rootstock St. Julien INRA GF 655/2, with a spacing of 4.25 m x 2.80 in a commercial orchard near Bonn (50°N). Untreated and mechanically thinned trees served as control with the following results. 1) Trees thinned with ethylene-promoting compounds produced fruit of the required large size 2) Yields were reasonable in all five years 3) In 2 out of the 5 years, fruit were slightly softer fruit than the mechanically-thinned or un-thinned control, making them more suitable for local markets or baking. 4) Trees treated with ethylene – promoting compounds showed less alternate bearing. The underlying mechanisms of crop load management under different weather and flowering regimes and direct action of ethylene on flower induction and breaking or preventing alternate bearing are discussed.
Banana fruit transcriptional repressors *MaNAC1* and *MaNAC2* antagonize the RING E3 ligase MaXB3 in the regulation of *MaERF11* and stabilization of *MaACS1* and *MaACO1*

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Ethylene plays a key regulatory role in climacteric fruits ripening, a highly coordinated process involving a large number of transcription factors (TFs). Our previous research has shown that two NAC TFs, *MaNAC1* and *MaNAC2* are ethylene-inducible, and associated with banana fruit ripening. However, the regulatory network of *MaNAC1* and *MaNAC2* co-ordinating ethylene response and fruit ripening is largely unknown. Here, we show that MaNAC1 and MaNAC2 are transcriptional repressors. Electrophoretic mobility shift assay (EMSA), chromatin immunoprecipitation (ChIP) and transient expression assays revealed that MaNAC1 and MaNAC2 repressed the expression of MaERF11, a possible negative regulator of ethylene biosynthesis and fruit ripening, by directly binding to its promoter. Yeast two-hybrid, bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation (Co-IP) assays showed that the RING E3 Ligase MaXB3, interacted with MaNAC2, MaACS1 and MaACO1. Further *in vitro* and *in vivo* experiments indicated that MaXB3 mediated the ubiquitinations of MaNAC2, MaACS1 and MaACO1 for proteasome degradation, and therefore suppressed the transcriptional repression ability of MaNAC2, and ethylene biosynthesis. Over-expression of *MaXB3* in tomato also delayed fruit ripening. Intriguingly, MaNAC1 and MaNAC2 antagonized MaXB3 via directly binding to its promoter, and repressing its expression, suggesting a feedback regulatory mechanism of maintaining the stability of MaNAC2, MaACS1 and MaACO1. Together, our findings provide new information on components in the regulatory network of NAC TFs involving in ethylene biosynthesis and signaling, and fruit ripening process.
Ethylene response factors: the missing link between the respiratory crisis and the autocatalytic ethylene production during ripening of climacteric fruits

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Ripening of fleshy fruits corresponds to the massive physiological/biochemical changes underlying the nutritional and sensory traits that make the fruit appealing to the consumers. In climacteric fruits, such as tomato (Solanum lycopersicum), ripening is characterized by a burst of ethylene production and an increase in respiration, although the link between these two processes remains obscure. The plant hormone ethylene is essential for the initiation and coordination of climacteric ripening and its action is known to be mediated by a linear transduction pathway leading to the activation of Ethylene Response Factors (ERF). While it is commonly admitted that the diversity of ethylene responses takes place at least partly at the level of ERFs, the precise mechanism by which these transcription factors targets downstream ripening-associated genes in a specific way remains unsolved. Members of the tomato ERF.E clade, display a remarkable ripening-associated expression pattern, and are characterized by a methionine-cysteine N-terminal domain that targets these proteins for degradation through the N-end rule pathway. Interestingly, it was reported that low levels of O₂ results in the stabilization of these ERFs in Arabidopsis linking these ERFs to plant adaptation to hypoxia. To gain insight on the putative regulation of ERF.E members by oxygen during tomato fruit ripening, we first assessed the evolution of oxygen concentrations in fruit tissues during the transition from unripe to ripe using a non-destructive probe. The data revealed a dramatic depletion of oxygen concentration in the locular gel tissue leading to hypoxia condition (less than 50µM/L) in this compartment. To check whether ERF.E proteins relocated to another subcellular compartment under hypoxic condition like in Arabidopsis, we performed transient expression assays in Nicotiana benthamiana, revealing that cell localization of ERF.E in tomato is also regulated by oxygen level. In order to further address the role of ERF.E members in ripening, over-expressing lines were generated that display delayed ripening, reduced ethylene production and retarded depletion of oxygen, supporting the hypothesis that
this gene encodes a negative regulator of climacteric ripening. Overall, the study brings new insight on the factors regulating the initiation of the ripening process.

A conflict regulation of ethylene biosynthesis between wounding and ripening by NOR

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Ethylene production greatly affects shelf life and is genetically regulated. Tomato ripening is associated with increased ethylene production, and tomato is used as a model for studying fruit ripening, particularly the ethylene biosynthesis and signaling pathways. The key ripening regulator NOR encodes a NAC domain transcription factor, which is part of a large plant-specific gene family. Both CRES-T and RNAi gene suppression transgenic lines revealed delayed ripening signs. Further evidence indicated possible feedback regulation of NOR and cross-regulation of NOR-like genes. A better understanding of NOR-like genes could provide insights into the complex transcriptional regulation of fruit ripening. Screening of the Micro-Tom ethyl methanesulfonate (EMS)-mutagenized population enabled the selection of alleles responsible for phenotype alterations. Analyses of the transcription levels of ethylene biosynthesis genes ACC synthase (ACS) and ACC-oxidase (ACO) revealed that reduced ethylene production was largely due to transcriptional suppression of ACO1 and ACO3. While ethylene function as a negative regulator on Agrobacterium-mediated gene transfer, and efforts on reducing ethylene production have induced transformation efficiency. The nor fruits failed exhibit ripening-ethylene production that in basal amounts during fruit ripening, however, it showed difficulties for callus formation and relatively lower transformation efficiency. Further investigations indicating the nor produce higher level of ethylene evolution when infected by Agrobacterium tumefaciens. We suggest that variable ethylene production rate regarding the roles of NOR, which could reflect multiple regulation mechanisms in ethylene production. In ripening, decreased ACO failed to oxidize the ethylene precursor, thus producing a non-climacteric phenotype in loss-of-function allele nor. The defense phenomenon at the infection site diverged as NOR-mediated systemic activation of ethylene biosynthesis genes in the nor.
Fruit ripening is controlled by the hormone ethylene and transcription factors that cause the development of nutritional and organoleptic properties valued by humans. Tomato RIN, a MADS-box transcription factor, is a hub regulator of the ripening gene expression network, with hundreds of gene targets including ACS2 and ACS4, required for ethylene production, and others that alter color, flavor, texture, and taste. RIN was identified from a classic ripening inhibitor (rin) mutant, in which a genomic DNA deletion resulted in the fusion of two truncated transcription factors, MADS-RIN and MADS-MC [1]. Originally the fusion was believed to be inactive, which caused the rin phenotype, but our research showed that overexpression of RIN-MC in transgenic wild-type cv Ailsa Craig tomato fruits impaired several ripening processes. Furthermore, down-regulating RIN-MC expression in the rin mutant stimulated the normal yellow mutant fruit to produce a weak red color, suggesting a distinct negative role for RIN-MC in tomato fruit ripening. By comparative transcriptome analysis of rin and rin 35S::RIN-MC RNA interference (RNAi) fruits, a total of 1,168 and 1,234 genes were identified as potential targets of RIN-MC activation and inhibition. The RIN-MC fusion gene was shown to be translated into a chimeric protein that was localized to the nucleus and was capable of protein interactions with other MADS-box transcription factors. These results indicated that tomato RIN-MC fusion encodes a chimeric transcription factor that modulates the expression of many ripening genes, thereby contributing to the rin mutant phenotype [2]. Results by Ito’s group are in partial agreement with our findings [3]. [1] Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, SchuchW, Giovannoni J (2002) A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (rin) locus. Science 296: 343–346 [2] Li S, Xu H, Ju Z, Cao D, Zhu H, Fu D, Grierson D, Qin G, Luo Y, Zhu B (2018) The RIN-MC fusion of MADS-box transcription factors has transcriptional activity and modulates expression of many ripening genes. Plant Physiol 176: 891-909 [3] Ito Y, Nishizawa-Yokoi A, Endo M, Mikami M, Shima Y, Nakamura N, Kotake-Nara E,

IV. Ethylene in Vegetative Growth-Development

INVITED TALKS

Ethylene signal transduction and the regulation of cell proliferation

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Ethylene signal transduction involves the sensing of ethylene by receptors, transmission of the signal through downstream pathways, and the regulation of targets that mediate tissue- and cell-specific responses to this phytohormone. To gain insight into the mechanisms by which ethylene controls growth and development, we and colleagues have employed computational approaches to model the ethylene-binding domain of the receptor ETR1. To test predictions of the computational model, we have generated site-directed mutations in ETR1 and expressed the mutant versions in *Arabidopsis*, taking advantage of the triple-response seedling growth assay as a test for functionality. Results from these analyses identify several amino acids that play a critical role in transducing the ethylene signal from the binding site to the output domain. The major signal-transduction pathway for output from the receptors incorporates CONSTITUTIVE TRIPLE RESPONSE1 (CTR1), ETHYLENE INSENSITIVE2 (EIN2), and the EIN3 family of transcription factors implicated in most ethylene responses. However, additional pathways operating downstream of the receptors have also been identified, potentially allowing for fine-tuning of the ethylene response. We find this to be the case when exploring the role that ethylene plays in the regulation of vegetative growth. Our analysis of ethylene-insensitive and constitutive ethylene-response mutants indicate that these affect cell proliferation, in addition to their known effects on cell expansion. Genetic analysis indicates that the inhibition of root cell proliferation by ethylene involves two pathways, the canonical CTR1-dependent pathway and, to a lesser extent, a His-Asp phosphorelay.
From ethylene signaling to translation regulation

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Plants hormones play a central role in the control and coordination of every aspect of plant growth and development, from seed germination to fruit ripening and organ senescence. Genetic and molecular studies, primarily in the reference plant Arabidopsis, have elucidated many of the key aspects of these hormones’ signal transduction pathways. In general, these signaling cascades are initiated by the binding of the hormone to the receptors, followed by a chain of molecular events that results in the transcriptional changes of hundreds of genes. Our studies on the ethylene signaling pathway have unveiled an additional essential layer of regulation in the plant response to this hormone. Specifically, we have found that the signaling molecule EIN2 and the nonsense-mediated decay proteins UPFs play a central role in a previously uncharacterized ethylene-induced translational response. Currently, we are investigating the role of other plant hormones in gene-specific translational regulation. Our studies are revealing new nodes of interaction between hormones, as well as implicating 3’UTRs and 5’uORFs of specific transcripts in the regulation of plant responses to key growth regulators.

ETR1-dependent root development and ethylene transcriptional responses in light grown Arabidopsis seedlings

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When light grown seedlings are treated with ethylene or with the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), primary root elongation and lateral root formation are inhibited, while the initiation and elongation of root hairs are increased. These developmental changes are mediated by interacting responses with auxin, which acts synergistically in modulating root elongation and root hair formation, but antagonistically in lateral root formation. The effects of ACC are lost in the ethylene insensitive mutants etr1-3 and ein2-5, consistent with the developmental effects of ACC in this tissue acting via its conversion to ethylene. We used gain-of-function and loss-
of-function mutants in the five ethylene receptors to identify the receptors that function in modulating root architecture and find that these developmental processes are largely controlled by ETR1, although EIN4 also contributes to root hair developmental changes. To identify the transcriptional responses that may drive ethylene’s effect on root elongation, lateral root formation, and root hair proliferation, we performed a transcriptomic analysis with high temporal resolution focused on roots from light grown seedlings. The kinetics of transcript abundance changes were examined over 8 time points spanning the first 24 hours after treatment of *Arabidopsis* seedlings with ACC), which were overlaid on time matched developmental changes. This data set was filtered to identify transcripts with significant changes relative to an untreated and time-matched controls and to have consistent pattern and time course of responses, yielding 449 reproducibly regulated transcripts across 3 replicates. Functional annotation of clusters of transcripts with similar temporal patterns revealed rapidly-induced clusters with known ethylene function, and more slowly regulated clusters with novel predicted functions linked to root development. In contrast to studies with dark grown seedlings, where the canonical ethylene response transcription factor, EIN3, is central to ethylene responses, only a subset of these clusters of ethylene-responsive transcripts were enriched in targets of EIN3. Additionally, the roots of *ein3* and *eil1* single and double mutants still respond to ACC. These results are consistent with EIN3/EIL1-independent developmental and transcriptional changes in light grown roots and suggests additional transcription factors may drive ethylene responses in these tissues. The changes in transcript abundance were also compared to transcriptomic data sets performed with dark grown seedlings or roots treated with ethylene and substantial differences were revealed, consistent with different transcriptional programs that demonstrate light and tissue dependent responses. Lastly, we overlaid the ACC responsive transcripts with a simultaneously collected auxin transcriptome data set, identifying 139 common transcripts, with the majority have similar response to both hormones. These results provide new insight into the transcriptional and developmental responses of roots to ethylene and auxin. This work was supported by the U.S. National Science Foundation (NSF-MCB #1716279).
PttERF85 affects cell division and growth in tree stems potentially through an effect on ribosome biogenesis

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Wood formation (xylogenesis) is the result of a highly dynamic and coordinated developmental cascade. It starts with cell division in the vascular cambium surrounding the stem and is followed by cell expansion, secondary cell wall formation and finally programmed cell death. Exogenous application of ethylene stimulates cambial cell division and affects secondary cell wall development, making this plant hormone an excellent compound to promote wood production. Ethylene Response Factors (ERFs) are the last element of the ethylene signaling cascade and thus potential candidates to translate the ethylene signal into developmental outcomes impacting xylogenesis. We hypothesize that ERFs contribute to transcriptional reprogramming during wood formation in hybrid aspen (Populus tremula x tremuloides; Ptt). We first utilized a network analysis of a transcriptome data set that covers all developmental steps during xylogenesis in aspen (“AspWood”). This revealed eight potential PttERFs that could function as hubs and shape the transcriptome in accordance to the developmental gradient during xylogenesis. Overexpression of 20 ethylene-inducible PttERFs in hybrid aspen stems revealed 5 other ERFs (beyond the xylem-hub ERFs) that caused altered cell wall biochemistry, stem diameter and height growth. Among these ERFs, PttERF85 is of particular interest since it is specifically and highly expressed during cambial division and xylem cell elongation, suggesting a potential function in xylem cell production. Overexpression of PttERF85 in the stem of hybrid aspen (PttERF85OE) caused reduced wood density and secondary cell wall thickness, but a general enlargement of xylem cells. Xylem transcriptomes from PttERF85OE trees showed upregulation of genes related to ribosome composition, cell growth and cell division. These genes are co-expressed with PttERF85 during xylogenesis, according to the AspWood database. Thus, PttERF85 is a perfect candidate to reveal the yet unknown molecular mechanisms underlying the coordination of cell cycle activity and cell wall integrity during cambial cell division and xylem cell expansion in trees.
A key question in seedling emergence is how plants sense changing soil conditions and adjust their growth and development accordingly. In our studies, we reveal that the soil overlay quantitatively activates seedlings’ ethylene production, and an EIN3 dependent ethylene response cascade is required for seedlings to successfully emerge from the soil. Seedlings transduce the soil conditions via EIN3 to simultaneously regulate apical hook formation (EIN3-HLS1) (Shen et al., 2016), hypocotyl elongation (EIN3-ERF1) (Zhong et al., 2014), and chloroplast development (EIN3-PIF3-LHCs) (Liu et al., 2017) in orchestrating organ-specific soil responses. Upstream of EIN3, we show that COP1 directly targets the F-box proteins EBF1 and EBF2 for ubiquitination and degradation, thus stabilizing EIN3 (Shi et al., 2016a). COP1 channels the light signals while ethylene transduces the information on soil mechanical conditions, which cooperatively control EIN3 protein levels to promote seedling emergence from the soil (Shi et al., 2016a). Upon reaching the surface, light triggers a dramatic developmental transition termed de-etiolation that requires immediate termination of ethylene responses. We find that photoreceptor phyB directly interacts with EIN3 in a light-dependent manner and acts as a light-reversible "molecular glue" that directly manipulates the substrate-E3 ligase interactions to control the stability of EIN3 (Shi et al., 2016b). Our findings illustrate a mechanistic model of how seedlings emerge from soil by integrating the multiple signals of soil conditions.

Computational and genetic approaches to uncover ethylene transcriptional networks that regulate root development

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Ethylene has profound effects on root development in light grown Arabidopsis seedlings, which include inhibition of root elongation, inhibition of lateral root development, and stimulation of root hair development and elongation. We are exploring the transcriptional networks that control these developmental changes. In many tissues of dark grown seedlings, the ETHYLENE INSENSITIVE3 (EIN3) transcription factor (TF) is absolutely required for ethylene transcriptional and developmental effects. However, we have found that ein3, eil1, and ein3eil1 mutants retain ethylene sensitivity in roots of light grown seedlings (Harkey et al. 2018. Plant Phys. 176(3):2095). This suggests that other transcription factors (TFs) are also important for ethylene responses in the roots of light grown seedlings. Our lab has performed a root-specific time course transcriptomic study using the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), and examined transcriptional changes across a span of 24 hours of treatment that coincides with changes in root development. We identified 449 transcripts with reproducible changes after ACC treatment, which cluster into groups with distinct temporal responses. This dataset includes 26 genes predicted to encode transcription factors that may drive these developmental responses. These TFs show peak abundance change at different times in the time course, and can be grouped into early (0.5-2hrs), middle (4-8hrs), and late (12-24hrs) responders. This pattern suggests a cascade of sequentially activated and/or repressed TFs, where early responders control middle responders, who in turn control late responders. We are utilizing Bayesian modeling of the transcriptomic data to predict likely relationships between TFs, which we can test using qRT-PCR on insertion mutants in one TF to look at downstream changes in the transcript abundance of another TF. Additionally, TF target data obtained through DAP-Seq (O’Malley et al. 2016. Cell. 165(5):1280) overlayed on our dataset has enabled us to predict functional targets of these TFs. These
analyses allowed to predict potential hierarchical TF relationships that we are testing by examining root development in T-DNA insertion mutants in these genes, and identified several mutants which have alterations in one or more root developmental responses. This combination of computational modeling and experimental testing of models has suggested several sub-networks of TFs which regulate root developmental changes in response to ethylene. One such network includes TFs which appear to regulate cell wall biosynthesis/degradation, and may therefore affect lateral root emergence from the primary root, which requires remodeling of the rigid cell wall to allow emergences of the new lateral root. This network, and others like it, which we are now testing, show the value of time-course data for discovering novel pathways. By modeling relationships within our dataset, and utilizing data from other Arabidopsis systems biology researchers, we are uncovering new mechanisms by which ethylene mediates development leading to changes in root architecture. This work was supported by the U.S. National Science Foundation (NSF-MCB #1716279).

POSTERS

Ethylene-induced microtubule reorientation is essential for fast inhibition of root elongation in Arabidopsis

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Microtubule reorientation is a long-standing observation that has been implicated in regulating the inhibitory effect of ethylene on axial elongation of plant cells. However, the signaling mechanism underlying ethylene-induced microtubule reorientation is still elusive. Here, we revealed by live confocal imaging and root elongation kinetic assay that the time courses of ethylene-induced microtubule reorientation and root elongation inhibition well resemble each other, and that proper microtubule reorientation is required for full responsiveness of root elongation to ethylene treatment. Our genetic analysis demonstrated that the effect of ethylene on microtubule orientation and root elongation is mainly transduced through the canonical linear ethylene signaling pathway. We found through pharmacological and genetic analysis that the TIR1/AFBs-
Aux/IAAs-ARFs auxin signaling pathway, but not the ABP1-ROP6-RIC1 auxin signaling branch, is essential for ethylene-induced microtubule reorientation and root elongation inhibition. Together, we present a signaling mechanism for ethylene-induced microtubule reorientation and fast root elongation inhibition in Arabidopsis, which involves essential roles of endogenous auxin biosynthesis, transport and the TIR1/AFBs-Aux/IAAs-ARFs auxin signaling pathway.

V.

Cross-talk between Ethylene and other Hormones

INVITED TALKS

Coordinated Regulation of Apical Hook Formation by Ethylene and Other Signals

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Arabidopsis develops a hook-like structure at the apical part of hypocotyl shortly after germination in darkness, which protects the delicate shoot meristem from mechanical damage while the young seedling pushing forward the surface of soil. The developmental processes of this transient structure, from formation, maintenance to opening, are finely tuned by multiple interconnected phytohormonal signals and environmental stimuli. Among these, the gaseous hormone ethylene plays a predominant role, as manifested by the great exaggeration the hook curvature under excessive ethylene conditions. Our previous works had revealed the importance of the two key transcriptional factors of ethylene pathway, EIN3/EIL1, in integrating different hormonal signals such as GA and JA in regulating HLS1 expression and apical hook development. Particularly, we had demonstrated that DELLA repressors of GA pathway physically interact with the DNA-binding domains of EIN3/EIL1 and repress EIN3/EIL1-regulated HLS1 expression, and that MYC2 transcriptional factor of JA pathway represses EIN3 function by both directly inducing EBF1 expression and physically interacting with EIN3 and inhibiting its DNA binding activity. Nevertheless, negative effects of both DELLA and MYC2 are partially EIN3/EIL1-independent,
implicating other factors mediating the effect of GA and JA. Recently, we found through combinatorial approaches of molecular genetics and real time imaging that EIN3/EIL1 and PIFs function as a transcriptional couple to integrate multiple upstream signals including ethylene, GA, JA and light to regulate HLS1 gene expression and the development of apical hook. Our transcriptome profiling also revealed that EIN3/EIL1 and PIFs cooperatively and independently regulate a wide array of biological processes in addition to apical hook development, which indicates that EIN3/EIL1 and PIFs act as a functional couple to fine-tune adaptive growth in response to hormone and light signals.

Ethylene restricts Arabidopsis growth via the epidermis

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The gaseous hormone ethylene plays a key role in plant growth and development, and is a major regulator of stress responses. It inhibits vegetative growth by restricting cell elongation, mainly through crosstalk with auxins. However, it remains unknown whether ethylene controls growth throughout all plant tissues or whether its signaling is confined to specific cell types. We employed a targeted expression approach to map the tissue site(s) of ethylene growth regulation. The SCF-EBF E3 ubiquitin ligases EBF1 and EBF2 target the degradation of EIN3, the master transcription factor in ethylene signaling. We coupled EBF1 and EBF2 to a number of cell type-specific promoters. Using phenotypic assays for ethylene response and mutant complementation, we revealed that the epidermis is the main site of ethylene action controlling plant growth in both roots and shoots. Suppression of ethylene signaling in the epidermis of the constitutive ethylene signaling mutant ctr1-1 was sufficient to rescue the mutant phenotype pointing to the epidermis as a key cell type required for ethylene-mediated growth inhibition.

Ethylene represents a key regulatory signal for root development and salt response. In rice, ethylene has been described to cause double response: promotes coleoptile growth and inhibits root elongation in dark-grown rice seedling. In order to investigate how ethylene regulates root growth and salt response in rice seedlings, we established a system to screen a large population of rice fast neutron bombardment and ethylmethanesulfonate (EMS)-mutagenized lines. Ethylene-response mutants were selected according to the phenotypes of roots and coleoptile of etiolated seedlings. By this approach, at least 30 lines of ethylene responsive mutants were identified, either showing insensitivity or reduced sensitivity to ethylene in root growth but exhibiting differential response in coleoptile growth, or displaying constitutive root retardation or longer primary root. Here we describe two of these mutants that function in ethylene biosynthesis or ethylene signalling. a DOF transcription factor OsDOF15, which is salt suppressible, negatively controls the expression of ethylene genes and ethylene production. This regulation obviously affects salt tolerance of rice seedlings, evidencing that a DOF transcription factor OsDOF15 negatively controls root primary elongation and ethylene biosynthesis, which further improves salt tolerance of rice seedlings. To further unravel how ethylene signalling control root elongation and salt response, we then investigated the function of an auxin biosynthesis gene YUC8/REIN7, which is essential for ethylene-inhibited root elongation and salt response. Genetic and molecular studies reveal that YUC8/REIN7-mediated auxin biosynthesis directly functions downstream of OsEIL1. Thus, our findings reveal the regulation of ethylene in rice seedling primary root elongation and salt response.
Overexpression of an *Arabidopsis* Histidine Kinase-Like Protein Leads to Cytokinin-induced Ethylene Responses

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In a gene expression profiling we identified an elevation of a candidate gene, namely *ARABIDOPSIS HISTIDINE KLINASE-LIKE1* (AKL1), by ethylene treatment in etiolated Arabidopsis seedlings. Overexpression of AKL1 (AKL1ox) led to a typical constitutive ethylene response phenotype, which was reversed by treatment with silver or the ethylene biosynthesis inhibitor AVG. Ethylene evolution was elevated in the AKL1ox lines. The phenotype, however, was only prominent at seedling stage. ACS5 expression plays a critical role in ACC and ethylene production during early seedling stage and can be induced by cytokinin, prompting us to investigate possible connections of AKL1 overexpression with cytokinin signaling or biosynthesis. AKL1ox calli differentiated into shoots in the absence of exogenous cytokinins, and the wild-type calli did not, lending a support to the possibility that AKL1 could be involved in cytokinin functions. Consistently, expression of cytokinin-induced *ARR* genes was elevated in AKL1ox plants. Genetic introgression of each of the T-DNA-inserted *acs5, mkk9, acs2, acs6* and *acs2 acs6* with AKL1 overexpressor prevented the AKL1 overexpression-induced constitutive ethylene response phenotype and ethylene evolution. Unfortunately, we found that the suppression was a consequence of epigenic silencing of the 35S promoter, a common phenomenon that happens when multiple 35S promoters exist in a same genome. We are investigating if AKL1 could activate cytokine signaling or production to determine AKL1 functions.

**Maintenance of ethylene homeostasis by brassinosteroid-mediated mutual degradation of E3 ubiquitin ligases in *Arabidopsis***

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The plant hormone ethylene influences multiple aspects of plant biology via a tight regulation of the homeostasis of its biosynthesis and responsiveness. Several studies have demonstrated that ACC synthase (ACS), the rate-limiting enzymes in ethylene
biosynthesis, regulate ethylene biosynthesis at the post-translational level. Recent studies in our laboratory indicate that the protein stability of ACS is co-regulated by two independent E3 ubiquitin ligases, Ethylene Overproducer 1 (ETO1)-like 2 (EOL2) and Seven in absentia (SINAT). A series of the biochemical and genetic analysis shows that EOL2 and SINAT E3 ligases undergo a mutual degradation to control the protein stability of ACS in a brassinosteroid (BR)-dependent manner. In addition, analysis of the loss of function mutant sinat demonstrates that SINAT E3 ligases play a role in regulating the size of root meristem through BR-mediated ethylene biosynthesis control. Together, we identified a novel regulatory mechanism by which plants utilize to regulate the protein stability of ACS via the crosstalk with BR. The coordinated negative feedback regulation through the mutual control of the E3 ligases provides a new insight into the mechanism underlying hormone homeostasis regulation and controlled turnover of E3 ligases in plants.

Peach secreted peptide hormones interact with auxin and ethylene to regulate plant development

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Secreted peptide hormones are involved in both short and long distance signaling (Tavormina et al., 2015). Their precursors are secreted in the apoplast and proteolytic processing leads to the delivery of the active form that is perceived at the plasma membrane by leucine-rich repeat receptor-like kinase (LRR-RLK) receptors (Shiu and Bleecker, 2003). Transcriptomic data highlighted the relevance of secreted peptide hormones during fruit development and ripening in peach and suggested their possible roles in mediating an auxin-ethylene crosstalk (Tadiello et al., 2016). Expression of genes coding for peptides belonging to the subfamilies CLAVATA-LIKE (CLE) and ROOT GROWTH FACTOR/GOLVEN (RFG/GLV) is tightly regulated and starts to be detectable at the beginning of ripening. Their expression is modulated by auxin and ethylene, ARE and ERE (Auxin/Ethylene Responsive Elements) are present in their promoters and when exogenously applied to Arabidopsis roots they function as modulators of classical hormone actions. The best candidates were a CLE peptide (Prupe.1G311100, or ppa013758), whose expression, albeit being ripening associated, is induced by ethylene and two RGF/GLV peptides (Prupe.7G256100, or ppa012311m –
CTG134- and Prupe.5G072500 or ppa022084m –CTG512-), again positively regulated by ripening but repressed by ethylene and stimulated by auxin. To have a wider picture of peptide hormones’ action, we looked in the peach genome for possible receptors and selected several candidates based on their expression profiles and sequence similarity to Arabidopsis receptors (LRR-RLK belonging to subfamily XI) and co-receptors (LRR-RLK belonging to subfamily II). Further analyses are underway to associate receptors to their peptide ligands. Peach peptide hormone genes were introduced in model species such as Arabidopsis, tobacco and tomato to gain insights on their functions. Phenotypes in Arabidopsis and tobacco overexpressing lines (Busatto et al., 2017) include effects on morphology and development of root, hypocotyl, capsule, silique and ovule. The most severe lines are impaired in growth. Since several developmental processes in which the action of ethylene and auxin (or their interaction) is well documented show abnormalities, there is experimental support for a role of these peptide hormones in mediating the interactions between the two phytohormones. References Busatto N, Salvagnin U, Resentini F, Quaresimin S, Navazio L, Marin O, Pellegrini M, Costa F, Mierke DF, Trainotti L. 2017. The Peach RGF/GLV Signaling Peptide pCTG134 Is Involved in a Regulatory Circuit That Sustains Auxin and Ethylene Actions. Frontiers in Plant Science 8. Shiu S-H, Bleecker AB. 2003. Expansion of the Receptor-Like Kinase/Pelle Gene Family and Receptor-Like Proteins in Arabidopsis. Plant Physiology 132, 530–543. Tadiello A, Ziosi V, Negri AS, Noferini M, Fiori G, Busatto N, Espen L, Costa G, Trainotti L. 2016. On the role of ethylene, auxin and a GOLVEN-like peptide hormone in the regulation of peach ripening. BMC Plant Biology 16, 44. Tavormina P, De Coninck B, Nikonorova N, De Smet I, Cammue BPA. 2015. The Plant Peptidome: An Expanding Repertoire of Structural Features and Biological Functions. The Plant Cell 27, 2095–2118.

The small molecule ACCERBATIN mimics the triple response phenotype and acts through disruption of auxin and ROS metabolism

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Ethylene plays a pivotal role in a wide array of physiological processes in virtually every stage of plant development. During skotomorphogenic growth, ethylene acts in
concert with an array of signals, both internal and external, to ensure proper seedling development. To further understand ethylene biosynthesis/signaling and its crosstalk with other hormones, we screened a 12,000 compound chemical library based on an ethylene-related bioassay on dark-grown *Arabidopsis thaliana* (L.) Heynh. seedlings. We isolated a quinoline carboxamide designated ACCERBATIN (AEX) that exacerbates the 1-aminocyclopropane-1-carboxylic acid-induced triple response, typical for ethylene-treated seedlings in darkness. Phenotypic analyses revealed distinct AEX effects including inhibition of root hair development and shortening of the root meristem. Mutant analysis and reporter studies further suggested that AEX most probably acts in parallel to ethylene signaling. We demonstrated that AEX functions at the intersection of auxin metabolism and reactive oxygen species (ROS) homeostasis. AEX inhibited auxin efflux in BY-2 cells and promoted indole-3-acetic acid (IAA) oxidation in the shoot apical meristem and cotyledons of etiolated seedlings. Gene expression studies and superoxide/hydrogen peroxide staining further revealed that the disrupted auxin homeostasis was accompanied by oxidative stress. Interestingly, in light conditions, AEX exhibited properties reminiscent of the quinoline carboxylate-type auxin-like herbicides. We propose that AEX interferes with auxin transport from its major biosynthesis sites, either as a direct consequence of poor basipetal transport from the shoot meristematic region, or indirectly, through excessive IAA oxidation and ROS accumulation. Further investigation of AEX can provide new insights into the mechanisms driving etiolated seedling growth and further explore the extensive crosstalk network connecting auxin metabolism with ROS homeostasis and ethylene signaling.

POSTERS

**Transcription Factor Constans-like 4 Possibly Play an Interactive Role between Abscisic Acid and Ethylene in The Regulation of Tomato Fruit Ripening**

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Fruit ripening is modulated by an intricate network of different phytohormones. Ethylene is well known to be involved in tomato fruit ripening, while abscisic acid (ABA) is proposed to be an upstream modulator of ethylene pathway. However, the
interaction between ABA and ethylene signal was not fully understood. Our study showed that a transcriptional factor Constans-like 4 expressed in all tissues of tomato plant and the expression level declined at the onset of fruit ripening. Virus induced gene silencing (VIGS) of Constans-like 4 in detached tomato fruit proved that Constans-like 4 was a negative regulator involved in tomato ripening. In addition, the expression of Constans-like 4 in response to ABA treatment declined within 6 hours after treatment. Meanwhile, a number of ethylene signal-related genes were identified downstream of Constans-like 4 by Chromatin Immunoprecipitation-Deep Sequencing (Chip-Seq). Taken together, Transcription factor Constans-like 4 may act as a negative regulator and interact abscisic acid and ethylene signal involved in the regulation of tomato ripening.

Novel insights into cytokinin-mediated regulation of ethylene biosynthesis, its spatiotemporal specificity and developmental consequences in Arabidopsis

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The genetic and the molecular basis of plant hormones action and interaction have been extensively studied in the recent years and the importance of cytokinin-ethylene hormonal crosstalk in plant development and homeostasis was recognized. Cytokinin induces ethylene production in plants via stabilization of ACC SYNTHASE (ACS) proteins. Importantly, all the three remaining enzymes acting in the ethylene biosynthetic pathway were quickly upregulated by cytokinins leading to prompt increase in the levels of 1-aminocyclopropane-1-carboxylic acid (ACC), the rate-limiting precursor of ethylene biosynthesis specifically in the root. Here we show that cytokinins control the transcription of several ACSs in specific cell files of the Arabidopsis root tip. In line with that, we recognized cell-type specific effects of overproduction of IPTs, the cytokinin biosynthetic genes, on the ACC production and RAM shortening. Finally, we demonstrate that mutations in the cytokinin-regulated ACSs are resistant to the cytokinin-induced shortening of the root apical meristem (RAM) size. Altogether, our data point out that both cytokinin-induced ethylene
production as well as the cytokinin/ethylene-mediated RAM shortening is tissue/cell type specific.

**A DOF transcription factor OsDOF15 mediates root growth by coordinating the interlinking between ethylene and gibberellin**

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Emerging researches have shown that hormonal cross-talk has pivotal roles in root growth. Ethylene and gibberellin perform antagonistic functions on root growth. Here, we report that a DOF transcription factor OsDOF15 performs as a suppressor on ethylene biosynthesis and an activator on gibberellin metabolism, which modulates root growth through different transcriptional regulations. The loss-of-function that osdof15 mutant generated by CRISPR/Cas9 displayed shorter root length than that of wild-type plants, while OsDOF15 overexpression plants showed longer root. The gene expression of OsDOF15 was much higher in root than that in other tissues at seedling stage, suggesting the function of OsDOF15 on primary root development. Phytohormone analysis indicated that the contents of ethylene increased in osdof15 mutants, in which the gibberellin levels reduced. ChIP-sequencing of OsDOF15 showed that OsDOF15 could bind to the promoter of genes encoding ethylene synthesis enzyme ACS1 and gibberellin metabolism regulator OsDSK2a. However, OsDOF15 inhibited expression of ACS1 and promoted expression of OsDSK2a, respectively, suggesting dual transcriptional regulations of OsDOF15 on target genes. We found a typical EAR motif in OsDOF15, which contributes to the repress function of OsDOF15. Moreover, genetic studies indicated that ACS2 and OsDSK2a act downstream of OsDOF15 to modulate root growth. Together, our studies reveal a novel dual-function transcription factor involved in cross-talk between ethylene and gibberellin on root growth.
The interaction of ethylene and auxin in the control of primary root elongation in rice early seedlings

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Rice is an important monocotyledonous crop worldwide; it differs from the dicotyledonous plant Arabidopsis in many aspects. A linear ethylene signaling pathway has been established in Arabidopsis. However, the ethylene signaling mechanism in rice is still unclear. Here, we conducted a screen for ethylene-insensitive mutants with altered root elongation in rice. Through screening a large population of rice mutants, at least 30 ethylene–response mutants were selected according to the phenotypes of roots. Here we report that the transcriptional activation of OsEIL1 on the expression of YUC8/REIN7 and indole-3-pyruvic acid (IPA)-dependent auxin biosynthesis is required for ethylene-inhibited root elongation. One of a rice ethylene-insensitive mutant, rein7-1, in which YUC8/REIN7 is truncated at its C-terminus, was further investigated. Mutation in YUC8/REIN7 reduced auxin biosynthesis in rice, while YUC8/REIN7 overexpression enhanced ethylene sensitivity in the roots. Moreover, YUC8/REIN7 catalyzed the conversion of IPA to IAA, while the truncated version at C-terminal end of the YUC8/REIN7 resulted in significant reduction of enzymatic activity, indicating that YUC8/REIN7 is required for IPA-dependent auxin biosynthesis and ethylene-inhibited root elongation in rice early seedlings. Further investigations indicated that ethylene induced YUC8/REIN7 expression and promoted auxin accumulation in roots. Addition of low concentrations of IAA rescued the ethylene response in the rein7-1, strongly demonstrating that ethylene-inhibited root elongation depends on IPA-dependent auxin biosynthesis. Genetic studies revealed that YUC8/REIN7-mediated auxin biosynthesis functioned downstream of OsEIL1. Thus, our findings reveal a model of interaction between ethylene and auxin in rice seedling primary root elongation, enhancing our understanding of ethylene signaling in rice.
Cross-talk between brassinosteroid and ethylene: regulation of sugarcane ACC synthase in response to brassinosteroid

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Sugarcane (Saccharum hybrids) is a very efficient biomass producer and has the capacity to accumulate high concentrations of sucrose in the stem. Ethylene is a phytohormone that plays an important role in the regulation of growth and sucrose accumulation in sugarcane. The rate-limiting step of ethylene biosynthesis is mediated by the enzyme 1-aminocyclopropane-1-carboxylase synthase (ACS). In several hormone biosynthesis and signaling pathways, protein turnover has emerged as a common regulatory element. Brassinosteroids (BR) are a class of steroid hormone that function as a master regulator of plant growth and belong to a group of factors influencing ethylene biosynthesis through the regulation of ACS stability. Here we investigated the effects of brassinosteroid on ethylene biosynthesis in both Arabidopsis, a dicot plant, and in rice as a closely related species to sugarcane. Our results showed that etiolated wild-type seedlings had an increase in ethylene production in response to brassinolide (BL). Rice bri1 and Arabidopsis bak1, bsk1, bes1 and bzr1 mutants showed a decrease in ethylene production in response to BR in the dark. Interestingly, Arabidopsis gain-of-function bzr1-1D mutant enhanced ethylene levels in the presence of 1 μM BL when compared with etiolated wild-type seedlings. In addition, to determine if ethylene production by BR is the result of an increase in ACS stability, we examined the stability of full length and truncated forms of HA-tagged ACS proteins in response to BL in rice protoplasts. The treatment with BL enhanced the half-life of the full-length type-2 HA-tagged ACS protein in a time-dependent manner. The elucidation of the mechanisms underlying the regulation of ACS via brassinosteroid signaling will provide significant insight into sugarcane growth and ripening, and more generally will contribute to understanding the interaction of these hormones in plant physiology.
The RIN-activated SI-SAUR69 is involved in the initiation of the ripening process in tomato

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In climacteric fruits including tomato, while ethylene is the main plant hormone controlling most aspects of ripening, the role of auxin remains rather obscure. In order to gain better insight on the involvement of auxin in fruit ripening we investigated auxin distribution during the initiation of the ripening process by monitoring the fluorescence of VENUS reported gene driven by the DR5 auxin responsive promoter in WT and ripening impaired rin tomato lines. The data show that auxin signalling displays a dramatic decline in WT fruit at the onset of ripening while it remains constantly high in rin backgrounds, suggesting that RIN might be required to negatively regulate auxin signalling. To further explore how RIN regulates auxin during ripening, we addressed the functional significance of Small auxin-up RNA 69 (SI-SAUR69), auxin-induced gene and previously reported to be a direct gene target of RIN. The expression of SI-SAUR69 exhibit dramatic but transient increase at the onset of ripening. Direct evidence for the involvement of SI-SAUR69 in fruit ripening was revealed via reverse genetics approach showing that over-expressing lines initiate premature ripening while down-regulated lines result in delayed ripening. The phenotypes were caused by a decrease of proton pump activity and polar auxin transport in SI-SAUR69 up-regulated lines. Taken together, the study supports an active role for auxin signalling in the transition from unripe to ripe of tomato fruit tissues with the RIN-activated SI-SAUR69 regulating ethylene sensitivity via alteration of polar auxin transport. Key words: auxin, ethylene, onset of ripening, RIN, SI-SAUR69, polar auxin transport
VI. Ethylene on Cell and Organ Identity Specification

INVITED TALKS

Shaping a flower with hormonal signals

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In multicellular organisms, undifferentiated cells have the competence to respond to endogenous signals and switch identity, resulting in cell fate determination and morphological changes. How these two processes are coordinated is a key question in developmental biology. In plants, leaves and flowers are formed in the periphery of Shoot Apical Meristems (SAMs), from cells that have progressively lost their stem cell identity and acquired the competence to respond to the small signaling molecule auxin. We have used a genome-wide gene profiling meta-analysis to explore the gene network triggered by auxin during flower initiation. This approach allowed us to identify a tissue-specific set of genes involved in early flower development and we will show how this dataset inform us on mechanisms coordinating growth and patterning that shapes the young flower. We will notably discuss a novel growth regulation module mediating the early responses to auxin and the interactions with other hormonal signals during early flower development.

Ethylene in root growth in Arabidopsis

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Plant development contrasts with animal development by exhibiting a high degree of flexibility (plasticity), in which final form is unpredictable. This plasticity represents a mechanism for responding to environmental change, such as variations in availability of water, nutrients, light, or attack by herbivores. While animals respond to such environmental challenges through behavioural change, plants use plasticity in development to adapt and survive, and this is mediated to a significant extent through
the activity of meristems and control of cell elongation. In this talk I will present some of our work on the genetic and signalling mechanisms, and in particular the crosstalk between ethylene, auxin and cytokinin, that control meristem activity and cell elongation and regulate growth during root development in Arabidopsis. I will describe a proposed molecular mechanism by which the POLARIS peptide regulates ethylene receptor function in the Arabidopsis root, and the use of mathematical modelling to predict signalling-gene interactions.

**Ethylene is a master regulator of sex determination in cucurbits**

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How the gender of a flower or plant is determined is an important issue in plant developmental biology. Understanding this process also has practical applications in agriculture and plant breeding, as the gender of a flower or plant often limits how it is bred and cultivated. Sex determination is a process that leads to the physical separation of male and female gamete producing structures in separate flowers on the same plant (monoecious species) or on separate individuals (dioecious species). Several species in the Cucurbitaceae, including melon, have bisexual floral primordia, but often have flowers limited to a single sex. Sex determination occurs by the selective arrest of either the male stamen or female carpel during development. In melon, sex determination is governed by three genes andromonoecious (M), androecious gene (A) and gynoecious (G) and the interplay of alleles of these three genes results in a range of sexual types. In addition, cucurbit sex expression patterns can be modified by hormone treatments and ethylene play a central role. To bring new insights into the molecular mechanisms controlling sex determination in cucurbits we cloned and characterized the M, G and A genes and have shown that the gynoecious (G) gene encodes for a zinc finger transcription factor, CmWIP1, the andromonoecious (M) gene encodes for an ethylene biosynthesis enzyme, CmACS-7 and the androecious gene (A) encodes CmACS11. How CmWIP1, CmACS-7 and CmACS11interact to control carpel and stamina primordia development to lead to unisexual flowers will be discussed.
Ethylene plays opposite or dual roles in various physiological processes operating in cut flowers

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Ethylene is well known in its enhancing effects on senescence and abscission of various organs in cut floriculture crops. This presentation will focus on other roles of ethylene in growth, development, anthocyanin biosynthesis and degradation in four cut ornamental systems, and elucidating its modes of action in affecting these processes, which are separated from ethylene effects on senescence. 1) Enhancement of leaf growth in cut Dodonaea 'Dana' branches: A significant ethylene-enhanced increase in both elongation and widening dimensions was observed only in immature leaves (3-5 cm long), but not in mature leaves (7-12 cm long). This ethylene-enhanced increase in leaf growth was completely abolished by the water channel inhibitor, phloretin, suggesting that the ethylene improving effect on young leaf elongation involves phloretin-sensitive aquaporins. 2) Inhibition of flower opening in several cut rose (Rosa hybrida) cultivars: 1-MCP significantly increased both flower diameter and longevity of two cut rose cultivars which do not open, suggesting that ethylene inhibited flower opening and enhanced their senescence. Unlike this, 1-MCP had no effect on flower diameter of other four rose cultivars, which open regularly in the vase, but it extended their flower longevity, suggesting that ethylene only enhanced their senescence. It seems therefore, that depending on the rose cultivar, ethylene can inhibit the growth process operating in flower opening, separately from its enhancing effect on flower senescence. 3) Differential enhancement and inhibition of shoot growth during the gravitropic bending of cut snapdragon (Antirrhinum majus) spikes: The stimulus transduction events occurring during shoot gravitropism are mediated through differential changes in level and action of auxin and ethylene, associated with differential growth leading to shoot bending within 3 h. The differential expression in favor of the lower shoot flank of auxin responsive genes (Am-Aux/IAA3, Am-Aux/IAA1, and Am-SAUR1) in reoriented spikes was detected 1 h after their reorientation, while that of ethylene biosynthesis-related genes, Am-ACS and Am-ACO, was detected 2 and 9 h, respectively, following gravistimulation. Unlike these patterns of changes, the
ethylene receptor gene, Am-ETR1 was differentially overexpressed in favor of the upper shoot flank during 6-24 h of gravistimulation, which might be related to the gravity-induced shrinkage of the upper shoot flank. These suggest that ethylene plays a dual role in modulating the responsiveness of the tissue to auxin in the gravitropic response of snapdragon spikes, by enhancing the growth of the lower shoot flank, and inhibiting the growth of the upper flank. 4) Enhanced biosynthesis or degradation of anthocyanin pigmentation in cut orchid flowers: In cut mini-Cymbidium florets, ethylene induced rapid labellum redness within two days after harvest, before senescence symptoms were observed. In the Vanda ‘Sansai Blue’ cut flowers, ethylene enhanced petal anthocyanin degradation on day 2, which preceded other senescence-related processes occurring on day 8. This ethylene-induced rapid color fading of florets resulted from a significant reduction in the levels of two anthocyanins, cyanidin and delphinidin. Taken together, our results suggest that ethylene can play different roles in various physiological processes in cut ornamentals, besides its traditional enhancing effects on senescence and abscission.

VII. Ethylene in abiotic stresses

INVITED TALKS

Multiple interactors regulate plant response to abiotic stresses - the story of ethylene and polyamine dynamics in tomato

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Recent advances in stress biology have revealed that abiotic stress tolerance in plants is governed by a signal transduction cascade involving multi-hormonal interactions, metabolites, regulatory proteins and transcription factors. Our laboratory is studying interactions between ethylene and biogenic amines (polyamines), viz., putrescine, spermidine and spermine in regulating the growth, development and fruit ripening in tomato. That ethylene features in plant responses to abiotic and biotic stresses is known for a long time and led to the term “stress ethylene”. Generally, stress initiates biphasic
ethylene production in plants, an initial small peak and later a second, more robust peak, causing among other things growth inhibition, premature senescence and yield reduction. Networks of ethylene-dependent stress regulation have also emerged. Independently, polyamines have been shown to be involved in plant tolerance against stress. We surmised that a crosstalk may exist between polyamines and ethylene during abiotic stresses in tomato and, therefore, utilized three transgenic lines (polyamine-enriched line, ethylene-deficient line, and their genetic cross) along with their azygous control line to address this idea. Following exposure of these transgenic lines and their control to independent abiotic stresses, we mapped transcripts of polyamine/ethylene anabolic and catabolic pathway genes. Specific groups of polyamine anabolic genes were up regulated in response to heat, cold, salt, drought and wounding. Interestingly, specific polyamine catabolic genes were also upregulated. These data indicate that ADC/ODC catabolic and anabolic pathways are modulated during abiotic stresses. A long-term drought imposed on polyamine-enriched and ethylene-deficient lines resulted in higher biomass and fruit yield in both the transgenic plants as compared to their azygous plants. The drought-stressed transgenic high polyamine and low ethylene plants were found to have developed a profound root architecture together with high-water use efficiency, the two physiological processes that are key for sustaining plant growth and development during stress. Specific ACC synthase and ACC oxidase genes were found elevated or downregulated in response to drought, cold, salinity and heat, each stress produced a unique expression signature for each member of ACS and ACO genes. Taken together, these studies favor the development of combinational tomato germplasm with specific polyamine and ethylene biosynthesis/catabolic genes and which can withstand long term stress exposure.
Root hypoxia-induced epinasty is ontogenetically regulated by ethylene in tomato

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Root hypoxia-induced epinasty is ontogenetically regulated by ethylene in tomato. Although a lot of important crops such as tomato, potato and tobacco have the tendency to respond to unfavorable conditions such as root hypoxia by bending down their leaves, the regulation of this epinastic response remains elusive. We have discovered an ontogenetic dependency towards epinasty, which means that younger leaves are less prone to stress induced epinasty compared to older leaves, indicative of a developmentally regulatory pathway. This differential response is caused by a shift in the ethylene metabolism between the different leaves. Young leaves convert 1-aminocyclopropene-1-carboxylic acid (ACC), which is the precursor of ethylene and acts as a root-borne signal during hypoxia, to the conjugate MACC, while in older leaves the conversion of ACC to ethylene predominates. Furthermore, the expression pattern of ACO1 is stimulated in older leaves, indicating that ethylene production itself is also enhanced by the elevated ACC transport from the roots. We have also quantified in real-time important physiological changes to study the dynamic epinastic response during hypoxia. Leaf angle and canopy cover rapidly change in response to root hypoxia, reducing foliar transpiration. There is a close relationship between transpiration and the regulation of stomatal conductance through ABA signaling and the regulation of ACC transport from the roots to the shoot. Notabilis, an ABA deficient tomato mutant, showed elevated ACC levels in the roots and in the older leaves after a 24h hypoxia treatment, suggesting that both signaling pathways are closely intertwined. In order to further unravel the molecular regulation of this signaling mechanism and the ontogenetic differences, are currently performing a genome wide association study of 400 sequenced tomato accessions. So far, we have observed a large variations in the timing and the magnitude of the epinastic response as well as the ontogenetic differentiation thereof, for root hypoxia-induced epinasty.
Floods have a severe effect on plant performance and crop productivity. Climate change has increased the severity and frequency of floods. Plant species differ enormously in their tolerance to flooding, whereas the genetic background of these differences is largely unknown. When flooded, plants typically suffer from 104 times lower gas diffusion in the underwater environment compared to air, leading to restricted availability of carbon dioxide (CO₂) and oxygen (O₂) in flooded plants. Furthermore, light intensity could be reduced if flood waters are muddy and turbid. The limited O₂, CO₂ and light reduce photosynthesis and respiration in plant cells leading to carbohydrate starvation and a severe energy crisis. Oxygen availability in submerged plants is variable and highly dependent on flood water conditions such as clarity of the water. Unlike O₂, ethylene levels within submerged plants increase fast due to physical entrapment regardless of water clarity, light availability and plant tissue. Therefore, ethylene is a more reliable and relatively early flooding signal for plants and it mediates adaptive responses to this stress in many plant species. We observed that pre-exposure of Arabidopsis seedlings to high ethylene concentrations improves subsequent hypoxia survival. Hypoxia tolerance was dependent on survival of not just the hypoxia phase itself, but also the period following hypoxia. Seedlings return to normoxic conditions following hypoxia, induces the production of high quantities of reactive oxygen species (ROS) resulting in cell damage. A transcriptome analyses of ethylene and air pre-treated seedlings during hypoxia and reoxygenation provided an overview of molecular processes that mediate survival of subsequent oxygen deficient conditions (hypoxia). The dynamics of global gene expression changes suggested that ethylene improves survival of both the hypoxic and post-hypoxic phase but the molecular regulatory pathways underlying it might be distinct.
Is ethylene involved in oleuropein increase in harvested olives?

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Olives are non-climacteric fruit. In a previous article, oleuropein (OE) increased substantially in fresh green olives exposed at 20°C for 7 d, but the increases were lower in preharvest treated fruit with an ethylene synthesis inhibitor. The present aim was to investigate whether phenolic compounds, including OE, were affected by ethylene treatment in green harvested olives. Postharvest treatments with the ethylene perception inhibitor, 1-methylcyclopropene (1-MCP) at 1.5 μL L⁻¹ for 12 h, and/or ethylene at 1000 μL L⁻¹ at 20°C for up to 10 d were applied to fruits of ‘Konservolia’ cultivar. The results showed that ethylene and/or 1-MCP had similar effects on total phenolics (TP), total antioxidant capacity (TAC) and OE (as confirmed by HPLC-DAD-ESI-MS). Particularly, in all treated fruit, but not in controls, TP and TAC increased soon after harvest and remained at similar levels throughout storage, whereas OE increased in all fruit, both controls and treated, at later stages and independently of degreening. Although 1-MCP is a specific ethylene inhibitor, there is a gap for its role in non–climacteric. Diverse responses to 1-MCP have been observed in non-climacteric fruit, such as inducing ethylene dependent or independent processes or in other plant tissue ethylene perception in the presence of 1-MCP implied that ethylene and its inhibitor may bind to different sites with different affinities. Here, the TP increases only in treated fruit could be possibly attributed to stress responses since the high ethylene treatment and/or 1-MCP comprise an environment where the fruit have not a previous experience and is received as stressor. During maturation and ripening on tree, OE is degraded to products, such as demethyloleuropein, and/or elenolic acid, and OE-aglycon. The involvement of β-glucosidase activity is also crucial for OE modification. A β-glucosidase, an oleuropein-specific enzyme with kinetics similar to olive native enzyme, participates in a dual defense system, resulting in a glutaraldehyde-like component with protein cross-linking properties, but only in disrupted tissue. This case has been observed in fruit infested by the olive fly larvae along with a burst of ethylene. However, here, the concomitant and similar OE levels in all intact fruit, controls,
ethylene and 1-MCP treated, do not seem to be related to the dual defense system. Also, the results do not indicate that ethylene is involved in changes in OE concentration, at least directly. In practice, OE elevation in short-stored olives at ambient temperature might have a positive or negative impact on olive products quality, depending on cultivar and requirements of fruit quality for further use. The work has been published recently in Journal of Plant Physiology, as Short Communication. DOI 10.1016/j.jplph.2018.03.019

VIII. Ethylene in Pathogenesis and Disease Resistance

INVITED TALKS

Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity in phytopathogenic fungi

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Fruit pathogens can contribute to acidification or alkalization of the host environment. This capability has been used to divide fungal pathogens into acidifying and/or alkalizing classes. Here we show that diverse classes of fungal pathogens—*Colletotrichum gloeosporioides, Penicillium expansum, Aspergillus nidulans*, and *Fusarium oxysporum*—secrete small pH-affecting molecules. These molecules modify the environmental pH that dictates acidic or alkaline colonizing strategies and induce the expression of PACC-dependent genes. We show that in many organisms, acidification is induced under carbon excess, i.e. 175mM sucrose (the most abundant sugar in fruits). In contrast, alkalization occurs under conditions of carbon deprivation, i.e., less than 15mM sucrose. The carbon source is metabolized by glucose oxidase (*gox2*) to gluconic acid, contributing to medium acidification, whereas catalyzed deamination of non-preferred carbon sources, such as the amino acid glutamate, by glutamate dehydrogenase 2 (*gdh2*) results in the secretion of ammonia. Sucrose concentration also affected secondary metabolites accumulation suggesting the importance of sugar content in the fruits on fungal metabolism. The interaction between
ethylene and sugar level during colonization was not fully address. The present results rise the questions on the importance of the interaction between ethylene and sucrose during fruit ripening. It indicates that differential pH modulation by fruit fungal pathogens is a host-dependent mechanism, affected by host sugar content, which modulates environmental pH to enhance fruit colonization.

ORALS

Elucidating the role of a newly discovered ethylene receptor in the plant growth-promoting rhizobacterium Azospirillum brasilense

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The plant growth-promoting rhizobacteria Azospirillum brasilense is widely used in biofertilizers as it is a promiscuous colonizer of many major crops and has been shown to increase yield. We show here that it has a putative ethylene receptor (AzoETR1) that was discovered by sequence comparison against ethylene receptors from Arabidopsis thaliana. This putative receptor contains the conserved amino acids in a transmembrane domain that have been shown necessary for ethylene binding. Consistent with this protein being an ethylene receptor, A. brasilense binds ethylene and when AzoETR1 is disrupted, ethylene binding activity is reduced. Additionally, exogenous expression of AzoETR1 results in the formation ethylene binding sites in yeast. We hypothesize that ethylene perception by A. brasilense plays a key role in regulating root surface colonization of a potential plant host. To test this, we are now examining the role that ethylene perception plays in A. brasilense physiology, colonization, and motility.
Fusarium graminearum is able to produce ethylene, and to degrade its precursor ACC

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Fusarium graminearum is a plant pathogenic fungus with the ability to infect economically relevant small grain cereals. Due to the production of mycotoxins like trichothecenes, the fungus is of concern for food and feed safety. It has been reported that F. graminearum can interfere with ethylene signalling to enhance the susceptibility of the host plants. Furthermore, deoxynivalenol accumulation was shown to be strongly reduced in ethylene-insensitive barley (Chen et al., 2009). F. graminearum is able to produce ethylene in high methionine medium. However, up to now, it is not clear whether the fungus uses the plant pathway via 1-aminocyclopropane-1-carboxylic acid (ACC), the bacterial pathway via 2-keto-4-methylthiobutyric acid (KMBA), or the ethylene forming enzyme (EFE). Two ACC-deaminase candidate genes were bioinformatically identified in the genome of F. graminearum. ACDs degrade the immediate precursor of ethylene, ACC, releasing NH₃ and 2-oxobutyrate. We already characterized one of the genes as ACC deaminase (ACD), while the other gene showed D-cysteine desulphhydrase activity. The ACC deaminase showed a Km value of 3.3 mM, which is well in the range of bacterial enzymes reported to decrease “stress ethylene” (Singh et al., 2015). The characterized ACD gene was knocked out in F. graminearum. Infection experiments using wheat (cv. Apogee and Remus) revealed that the gene is dispensable during infection.

Ethylene is involved in plant parasitism by modulating development and function of the haustorium, an invasive organ in parasitic plants

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Parasitic plants in Orobanchaceae family including Striga and Orobanche genera are agricultural pests worldwide. The heterotrophic lifestyle of parasitic plants is attributed to the convergent evolution of haustorium, a multicellular organ that allows movement
of not only water and nutrient but also RNAs and hormones between host and parasitic plants. With the purpose of elucidating the molecular mechanisms underlying haustorium initiation and development, we performed the forward genetic screening using a model parasitic plant *Ptheirospermum japonicum*, a facultative root parasite belonging to Orobanchaceae, and identified two mutants showing abnormal haustorium development and function. Using whole genome sequencing we identified the mutated loci in each mutant, respectively encoding orthologous genes of *Arabidopsis* ETR1 and EIN2, respectively. *P. japonicum* initiate haustorium formation by stimuli of host cell wall derived phenolic compounds such as 2,6-dimethoxy-p-benzoquinone (DMBQ). In the absence of host attachment, haustorium terminates growth within 2 days and exhibits bump-like structure. In contrast, haustorium of Pjetr1 and Pjein2 grows continuously resulting in elongated haustorium. The extended elongation process is likely linked to modulation of haustorium apex cells via crosstalk between auxin and ethylene signaling as the mutants display prolonged accumulation of auxin in haustorium apex cells. Interestingly, elongated haustorium forms xylem strands inside, resulting in its structural resemblance to the lateral root to some extent. Application of ethylene inhibitors in the wild type mimicked the haustorium morphology of mutants. These results indicate the requirement of ethylene signaling in the suppression of extended haustorium growth in the absence of host plants and maintenance of proper haustorium structure. Upon host attachment, haustorial apex cells undergo cell differentiation toward intrusive cells that allow haustorium to invade host tissue and establish vascular connection with host. Pjetr1 and Pjein2 show strong defects in host root invasion and thus failure in infecting host plants, indicating the importance of ethylene signaling in host recognition. Unexpectedly, *Arabidopsis* ethylene mutants were less invaded by the wild type *P. japonicum*. Our studies reveal the unanticipated role of ethylene signaling in haustorium development and plant-plant interaction.

**Single cell damage elicits local, nematode-restricting ethylene responses in roots**

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Plants are exposed to a variety of insults resulting in cell and tissue damage. This ranges from purely mechanical stresses to herbivore feeding or cell death caused by invading
microbes. The primary wound response at the injured site is communicated to neighbouring and distal tissues by mobile, chemical or electrical, signals (Hilleary and Gilroy 20181). In leaves, crushing of large cell populations activates a long-distance signal, causing production of jasmonates in distal organs. This is mediated by a depolarization wave dependent on GLUTAMATE RECEPTOR-LIKE proteins (GLRs) (Mousavi et al., 2013). Electrical signalling induced by wounding or other stresses has moreover been linked to waves of Ca2+ (Choi et al., 2014) and Reactive Oxygen Species (ROS) production (Miller et al., 2009), thought to work in conjunction for long-distance signal transmission. Here, we report that single cell wounding in roots, induced by laser ablation, elicits distinct responses, notably short-distance surface potential changes, involving both calcium influx and ROS production. Yet, these local responses do not induce jasmonate response markers, but instead robustly activate ethylene-production and -response. We find that initial stages of nematode attack elicit very similar responses and that ethylene signaling antagonises nematode feeding, strongly suggesting that these local signalling events are part of a relevant immune response. That wound signaling of roots is distinct from leaves might be a result of their very different structure and function and their contrasting biotic and abiotic environments.

**A single-locus biosensor for simultaneous monitoring of multiple plant hormones**

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Phytohormones are growth regulators that govern plant development, control interactions with the environment, and orchestrate plant adaptation and survival in ever-changing environments. In the past two decades, a handful of plant biosensors have been developed that enable live or ex vivo imaging of hormone levels and distribution. Synthetic transcriptional reporters, such as EBS for ethylene or DR5 for auxin, have been successfully applied to characterize the effects of various genetic or environmental perturbations on the spatiotemporal activity patterns of individual hormones. The utility of these hormone-specific sensors is, however, limited to detecting one growth regulator at a time. To increase the readout capacity of transcriptional reporters, we are multiplexing several synthetic biosensors in a single construct. Using GoldenBraid molecular cloning technology, we have generated a collection of synthetic hormone-responsive promoters, core promoter elements and terminators, as well as multiple
versions of red, green and blue fluorescent proteins and subcellular localization signals. We are in the process of assembling, testing in transient assays and combining various hormone-specific transcriptional units for in planta expression. Our immediate plan is to generate and characterize in Arabidopsis and tomato a single-locus ACE (auxin/ethylene/cytokinin) sensor, with the ultimate goal to multiplex nine transcriptional reporters for nine major growth regulators (ACE plus ABA, gibberellins, brassinosteroids, salicylic acid, jasmonate, and strigolactones), with an individual hormone readout distinguishable by fluorescent protein color and subcellular localization.

IX.

Ethylene in Senescence and Abscission of Plant Organs

INVITED TALKS

Orthologues of Arabidopsis INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) and its receptors promote cell separation in mature abscission zones of leaves, fruits and seeds of diverse species

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The small peptide IDA signals through the leucine-rich repeat receptor-like kinases HAESA (HAE) and HAESA-LIKE2 (HSL2) to control cell separation events in Arabidopsis thaliana. Genes encoding IDA peptides and HSL proteins are found in flowering plant species from all orders of angiosperms, and the 12 amino acids representing the bioactive peptide in A. thaliana have virtually been unchanged throughout the evolution of the angiosperms (Stø et al., Front Plant Science 2+15). Poplar, oil palm and Brassica rapa were chosen to test whether there also is a functional conservation of IDA-HSL signaling in collaboration with Urs Fischer and Carole Dubreuil (SLU, Umeå, Sweden) Tim Tranbarger (IRD) and Fabienne Morcillo (CIRAD, Montpellier, France, and Lars Østergaard (John Innes Centre, UK). The peptide turns out to enhance abscission processes, however dependent on a mature ethylene-responsive abscission zone. Our newest investigations show that the Brassica
**Ethylene is the initial inducer of organ abscission in plants**

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The discovery that flower organ abscission was delayed but not blocked in ethylene-insensitive mutants suggested that developmental pathways independent of ethylene may regulate floral organ abscission in Arabidopsis. The observations that two receptor-like protein kinases, HAESA (HAE) and HAESA-like2 (HSL2), and the INFLORESCENCE DEFICIENT IN ABCISSION (IDA) peptide ligand are required for Arabidopsis floral organ abscission suggested that the IDA-HAE-HSL2 components activate the ethylene-independent pathway of abscission. Nevertheless, it is well established that although the IDA-HAE-HSL2 components are essential for floral organ abscission, they serve as a signal only for the late stage of abscission (separation) and for post-abscission events, contrary to ethylene which acts as the initial inducer of the process. In contrary to these findings, a recent review on organ abscission signaling in plants (Patharkar and Walker, 2018) suggested that both ethylene and IDA-HAE-HSL2 components are equal inducers of organ (flowers and leaves) abscission, by inducing the expression of the cell wall disassembly genes. However, it was previously reported that ethylene is not involved in the abscission of Arabidopsis cauline leaves in response to water stress followed by re-watering, which was mediated only by the IDA-HAE-HSL2 pathway (Patharkar and Walker, 2016). This conclusion contradicts the well-established concept of using Arabidopsis as a model plant system for physiological and molecular
research, since it is well documented that water stress followed by re-watering induced leaf abscission in various plant species, including cotton, bean, citrus, and poplar, in an ethylene-mediated manner. Therefore, we further investigated the role of ethylene in Arabidopsis cauline leaf abscission in response to water stress, and observed that ethylene is indeed involved in this process. Our conclusion is based on the following new evidence: 1) A cycle of water stress of 40% soil water content (SWC) and re-watering increased the ethylene production rates of cauline leaves; 2) Exposure of water-stressed plants at 60% SWC to 1-MCP inhibited the cauline leaf abscission, which occurred after re-watering at 40% SWC; 3) Cauline leaves in the ethylene-insensitive mutant, ein2-5, did not abscise in response to a cycle of water stress (40% SWC) and re-watering; 4) Exposure of water-stressed plants at different SWC (80-40%) to 5 µl L-1 ethylene for 24 h resulted in enhanced cauline leaf abscission following re-watering, which was positively correlated to the degree of water stress. These results clearly demonstrate that ethylene has an important role in the process of cauline leaf abscission in response to water stress and re-watering in the Arabidopsis model plant, similar to what was reported for many other plant species. Taken together, the literature on Arabidopsis floral organ abscission and our results on the involvement of ethylene in the Arabidopsis cauline leaf abscission system, indicate that the role of IDA-HAE-HSL2 pathway as an inducer of organ abscission was overestimated compared to the primary role of ethylene in abscission signaling in Arabidopsis. We suggest that the ethylene-independent pathway operates only in ethylene-insensitive mutants (etr1, ein2) and/or in delayed-abscission mutants (dab1-5).

**Suppression and over-expression of a prolyl 4 hydroxylase results in alterations in tomato abscission program**

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Proline hydroxylation is a major post-translation modification of hydroxyproline-rich glycoproteins (HRGPs) that is catalyzed by prolyl 4-hydroxylases (P4Hs). Their involvement in plant growth and development has been investigated in Arabidopsis.
mainly during root hair growth and in tobacco and carnation while little is known about their role in tomato. The tomato genome comprises 10 putative P4Hs. Preliminary experiments to partially suppress their expression using Virus Induced Gene Silencing resulted in alterations on cell division and expansion of tomato leaves. Therefore, transgenic tomato plants with suppressed expression of P4H3 by an RNAi construct as well as over-expression lines were produced in order to investigate their physiological significance in flower and leaf abscission and in fruit growth and development. A delay was observed in pedicel abscission of over-ripe RNAi fruits which was associated with expression of key abscission progression genes while no changes were observed in the over-expression lines. Ethylene induced fruit abscission was accelerated in the over-expression lines while was delayed in RNAi lines compared to control. No changes were observed in ethylene induced flower and leaf abscission. However, immunolocalization of Arabinogalactan proteins (AGPs) showed lower expression in flower abscission zones in the RNAi lines. Attempts to investigate the basis of these alterations were initiated. Collectively, these results indicate that the target P4H plays a significant role in tomato fruit abscission.

A study of the role of IDA-like gene expression in soybean and tomato abscission – IDA is expressed in abscission but may not be essential

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In 1994, David Thompson and Daphne Osborne (Plant Physiology 105:341-347) published a paper identifying a diffusible signal produced in the vascular bundle (stele) that was required for initiation of cell separation in the cortex of the bean leaf abscission zone (AZ). In 2003, Melinka Butenko, et al., (Plant Cell 15:2296-2307) published their discovery of a small secreted peptide, IDA, that was necessary for floral organ abscission in *Arabidopsis*. They also reported that sequences similar to *AtIDA* were found in the cDNA database for other plant species. We hypothesized that an IDA-like peptide might be important to abscission in soybean and tomato and that an IDA-like peptide might be the diffusible signal that Thompson and Osborne proposed for bean
abscission. We initially identified twelve *IDA-like* sequences in soybean (a tetraploid) and five in tomato (a diploid). In both soybean and tomato only one gene (*GmIDA2a* and *SlIDA1*, respectively) was significantly up-regulated during abscission. In regard to a diffusible abscission signal, we used a *PG::GUS* reporter gene to determine if a diffusible signal from the stele, like that identified in bean, was necessary for cell separation in the leaf AZ cortex of tomato. We concluded that a diffusible signal from the stele is not required for cortex separation during abscission in tomato. Nonetheless, because IDA-like genes were up-regulated in abscission of soybean and tomato, we proceeded to determine a role for these signaling peptides in abscission. We used a virus-induced-gene-silencing (VIGS) expression system in soybean to determine if expression of *GmIDA2a* expression gene was necessary for soybean leaf abscission. VIGS expression of *GmIDA2a* did not delay abscission but there are complications with VIGS in that it induced viral symptoms. We also transformed tomato with an *SlIDA1* RNAi construct to suppressed *SlIDA1* expression. *SlIDA1* expression in the pedicel AZ was suppressed by 90% but this did not delay abscission. RNAseq results of the *SlIDA1* RNAi compared to control AZ at 20 h post ethylene indicated a mostly similar pattern of gene expression but a few significant differences. The importance of these few differences will be discussed. Important to the tomato study was the recent discovery of 3 additional *IDA-like* genes in the tomato genome. Two of these genes are significantly up-regulated in tomato flower abscission with a different pattern of expression than *SlIDA1* but still may be partially redundant to *SlIDA1*.

ORALS

**T2-type Ribonuclease function in ethylene associated processes**

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T2-type Ribonucleases (RNases) are RNA-degrading enzymes, which function in various cellular processes mostly via RNA metabolism. T2-type RNase encoding genes have been identified in various organisms ranging from bacteria to mammals and reach their highest diversity in plants. The existence of T2-type RNase genes in almost every
organism suggests that they may have an important biological function that has been conserved through evolution. In plants, the T2-type RNases were suggested to be involved in phosphate scavenging and recycling and implicated in defense responses. In non-plant organisms, T2-type RNases were demonstrated to be involved in various important processes and consider to have antitumorigenic & antiangiogenic, as well as pro-apoptotic activities, including ROS propagation during oxidative stress-mediated cell death. Previously, we had shown that suppression of the tomato LX T2-type RNase gene resulted with significant retardation of leaf senescence and flower/leaf abscission. In both of these processes ethylene is well known to have a central regulatory role and cell death processes occur. Further analyses of LX-suppressed tomato transgenic lines revealed retardation of additional biological processes associated with ethylene regulation including cell death involved with the responses to phosphate-starvation and challenge with phytotoxins and pathogens. Transcriptomic analysis of tomato leaves before and after ethylene treatment revealed significant and wide effect resulting with differential gene expression patterns which significantly differ between wild type and LX-suppressed lines. Significant differences in patterns of gene expression between wild type and LX-suppressed lines are observed already before the ethylene treatment while following the treatment significant inhibition of ethylene-induced gene expression was revealed in the LX-suppressed lines. Preliminary results support an involvement of LX in the responses to biotic as well as abiotic stresses. The observations regarding the consequences of suppressing LX gene expression, as well as suppression of its Arabidopsis ortholog, support a central regulatory function for these T2-type RNases.

Experiments for investigating LX mode of function, including possible involvement in ethylene response and signal transduction pathways, are in progress.

**Characterization of morphology, biochemistry and ethylene associated gene expression during fruit development in cold hardy grapes**

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The increased cultivation of cold hardy wine and table grapes in northern climates has resulted in a significant need to develop an improved understanding of shattering in these hybrid grapes due to major losses. The Patterson lab has sampled the abscission
zone of four cold hardy grape varieties (*Vitis vinifera* x *V. riparia*) at three stages of development to compare morphological, physiological and molecular changes associated during development. Specifically, these three stages were selected for sampling based on the Eichorn-Lorenz Phenological Stages (EL 31: 7-10mm berries; EL-35: veraison; and EL 38: fully ripe berries). Phenotypically we observed significant changes in color development, volatiles, organic acids, sugar composition and chlorophyll levels in the developing fruit. Thin sections of the fruit abscission zone show differences between cultivars prone to abscission and those that are not subject to shattering. We will present how the morphological and biochemical changes correlate to changes in gene expression of ethylene and abscission associated genes during these three stages of development.

1-Hexylcyclopropene fumigation inhibits ethylene induced abscission of floral organs in cut waxflower (*Chameliaucium* spp.)

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Various methods of blocking ethylene production or inhibition of ethylene action have been reported to prevent the damaging effects of ethylene in the postharvest phase of ornamental waxflowers. The objective of this study was to test the effectiveness of fumigation with 1-Hexylcyclopropene (1-HCP) as an ethylene antagonist in different genotypes of waxflower ‘WX73’, ‘Purple Pride’, ‘WX56’, ‘WX58’ during 2014 and ‘Purple Pride’ and ‘Hybrid1’ during 2015. The effects of 1-HCP fumigation (1 µM) for 18 h followed by exposure to ethylene (10 µL L⁻¹) for 24 h and also, the effect of three concentrations (0.5, 1.0 or 2.0 µM) of 1-HCP followed by exposure to ethylene on flowers/ buds abscission were also evaluated on different cultivars of Geraldton wax. The flower sprigs were fumigated with 1-HCP using 60 L plastic drums for 18 h followed by 24 h exposure to ethylene with untreated flower stems kept as control. The experiments were one or two- factor factorial completely randomised design with three replicates and three stems per replication. Cumulative abscission of flowers/buds was calculated for four consecutive days following 24 h of ethylene exposure. The percentage of flowers/buds abscission in each replicate was calculated daily for four days, while with the different concentrations experiment the data were calculated one
day after the treatment. Fumigation of 1-HCP (1 µM) followed by single exposure to ethylene (10 µL L-1) significantly (P ≤ 0.05) reduced flowers/ buds abscission to 2.7% ‘WX73’, 4.8% ‘Purple Pride’, 14.6% ‘WX58’ and 23.5% ‘WX56’ compared to ethylene treatment alone, where flowers/buds abscission increased significantly by 95.0% ‘WX73’, 77.4% ‘Purple Pride’, 68.8% ‘WX58’ and 85.9% ‘WX56’. 1-HCP (1 µM) was the most effective in reducing flowers/ buds abscission when fumigated for 18 h followed by exposure to ethylene (10 µL L-1) for 24 h. In conclusion, 1-HCP is an effective ethylene antagonist and substantially reduced flowers/ buds abscission in several cut waxflowers genotypes.

X.

Postharvest Physiology and Quality

INVITED TALKS

Postharvest Fruit Ripening and Quality within the European Traditional Pool of Tomato Varieties: Effect of Temperature and 1-MCP

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Fruit ripening is a highly coordinated and regulated process that involves changes in color, texture, metabolic composition etc. In general, traditional varieties show rapid ripening and shorter post-harvest life than modern hybrid varieties carrying the ripening-inhibitor (rin) mutation in heterozygosis. To evaluate postharvest ripening variability present in the traditional European tomato pool, >220 varieties in a Core
Collection representing the genotypic and phenotypic diversity available in over 1700 accessions in the TRADITOM repository collection, were studied. Postharvest responses were also analyzed in a large number of inbreed lines and commercial hybrids, most of them having rin allele in heterozygosis. Large variation in firmness and external fruit color evolution during fruit ripening were found according to the genotypes within and across the tradition to commercial varieties sets. Furthermore, the effect of combination of low temperature storage and 1-MCP treatment was assessed using a “Valenciano” type one characterized by its fastest ripening behavior. The combined treatment uncoupled different aspects of ripening, including texture, color and metabolite composition. To understand the molecular genetic basis, haplotypes associated to different ripening behavior groups were analyzed and the expression levels of master ripening regulators and key genes involved in the different aspects of ripening were analyzed by using a mid-throughput Fluidigm RT-PCR platform in addition to metabolites associated to different postharvest behaviours.

**Regulation of fruit volatiles during postharvest cold storage**

**Bo Zhang**

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Postharvest cold storage is the most effective tool for extending the storage life of horticultural products. However, this handing results in reduced flavor quality for tomato and peach fruit. Volatile, together with sugar and acids, is one of the important traits for fruit flavor quality. Flavor-related volatiles are sensitive to low temperature, and their loss greatly reduced flavor quality and consumer liking. Cold storage suppressed production of volatiles did not fully recover after transferring to shelf-life at room temperature up to three days. Transcripts for some key volatile synthesis enzymes and most important transcription factors are reduced in response to postharvest cold storage. Those reduction of transcripts are accompanied by major changes in the methylation status of promoter regions. Our analysis provides insight into the molecular mechanisms of fruit flavor loss caused by postharvest cold storage.
ORALS

The interference of the ethylene perception system leads to a transcriptional re-programming involved in hormonal cross-talk and protection to superficial scald in apple

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Ethylene is a gaseous plant hormone playing master regulatory roles in triggering and coordinating the ripening syndrome in climacteric fruits. The control of this hormone represents a key point in the modern horticulture and postharvest management, since the reduction of ethylene can extend the postharvest life of fruits limiting quality decay and general fruit loss. One of the most efficient strategies to limit the effect of ethylene during the postharvest ripening of apple is the exogenous application of 1-methylcyclopropene (1-MCP), a molecule competing with ethylene at the receptor-binding site. The transcriptional signature coded by the application of 1-MCP was further investigated with microarray platforms. Together with an expected gene transcriptional repression, an equal dose of genes was also de-repressed or de-novo activated, underlying elements especially involved in regulatory processes and hormonal cross-talk, in particular with auxin. The re-programming of the auxin perception pathway, correlated with the amount of ethylene produced during normal ripening, was validated by the specific expression pattern of genes involved in conjugation/de-conjugation processes. In this physiological scenario the activation of auxin following the interference with ethylene is thought as an alternative mechanism induced by the fruit in the attempt to re-establish a normal physiological progression towards the completion of ripening. Although 1-MCP is usually applied to delay fruit ripening, it turns out to be also an effective strategy to prevent, in specific apple cultivars, the development of superficial scald, one of the most severe postharvest disorders for this fruit species. To elucidate the role of 1-MCP, a comprehensive investigation coupling large scale RNA-seq based transcriptomic and metabolite profiling was carried out. The exogenous application of 1-MCP in fruit of ‘Granny
Smith’ apple cultivar induced an important series of re-programming events towards the triggering of a cold acclimation process. The transcriptome-metabolite correlation network reveals an induced accumulation of very long chain fatty acid and unsaturated type of fatty acids for protecting the stability of internal membrane against chilling injuries. This protecting mechanism enhances the compartimentation of chlorogenic acid and polyphenol oxidase enzyme, preventing, in the end, the browning phenomenon. Within the cluster of genes stimulated by 1-MCP, the most expressed resulted a sorbitol-6-phosphate-dehydrogenase (S6PDH), known as a limiting step in the biosynthesis of sorbitol, a polyalcohol with cryoprotectant role controlling the osmolarity of the cell. The over-expression of this gene in Arabidopsis transgenic lines validated the role of this gene in the protection from freezing temperatures and chilling injuries phenomenon such as superficial scald.

**Deciphering the role of CO\(_2\) treatment on chilling injury in tomato: Effect on transcriptome profiling**

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A pre-treatment with high CO\(_2\) proved effective in maintaining the fruit quality and alleviating chilling injury in tomatoes during storage. CO\(_2\) treatments at 30% concentration successfully reduced chilling injury as fruits stored at 4°C displayed less pits even after transferring to 20°C for 8 days. The CO\(_2\) treated tomato showed the lower lycopene contents, and higher lutein and \(\beta\)-carotene contents than those of non-treated control. Co-ordinately, the skin color development in tomato was blocked by high CO\(_2\) treatments and at low temperature. An antioxidant activity in tomato stored at 20°C showed non-significant effect between CO\(_2\) treated and non-treated control, in contrast, CO\(_2\) treated tomato stored under chilling temperature (4°C) showed significantly high DPPH scavenge activity compared to non-treated control. Interestingly, ethylene production of CO\(_2\) treated fruits stored at 20°C for 0-3 days both at 30 and 60% concentrations was higher than that of the control, whereas those of 60% CO\(_2\) treated fruits showed a significant decline at 11 days storage. Transcription analysis showed the expression of ethylene signaling genes, LeERF3, LeETR1 immediately increased after CO\(_2\) treatment. However, CO\(_2\) treatment
negatively regulated the expression of ethylene synthesis genes LeACS2, LeACS4. To improve our understanding of the molecular mechanisms involved in the beneficial effect of CO₂ on tomato, a comparative transcriptomic analysis between CO₂ treated and non-treated fruit before and after cold storage and/or shelf-life condition was carried out. RNA Seq analysis detected a large number of differently expressed genes (DEGs) that ranged from 183(CO₂+Cold) to 1330(CO₂). Heat map showed different expression pattern between CO₂ treated and non-treated tomato. Global transcription analysis showed CO₂ treatment enhance the cell wall related genes including xyloglucan endotransglucosylase/hydrolase protein, glycine-rich cell wall structural protein-like, and hormone related genes including brassinosteroid-regulated protein BRU1, ethylene responsive gene transcriptional coactivator, abscisic acid receptor PYL4. CO₂ treatment and cold storage enhanced transcription factors including TGA2.3-like, TCP9, Ethylene responsive (ERF) 109-like, dehydration-responsive element binding protein 1F-like genes, jasmonate ZIM domain gene, and protein inhibitor genes. However, CO₂+Cold storage and shelf life condition treatment down-regulated the ethylene response factor genes, ERF107, ERF 104, ERF5, ERF5 like, ERF3-like, ERF4. KEGG enrichment analysis showed CO₂ responsive DEGs were significantly enriched secondary metabolite, protein processing in endoplasmic reticulum, MAPK signaling pathway, while CO₂+Cold responsive DEGs were significantly enriched metabolic pathways, plant hormone signal transduction. And CO₂+Cold and shelf life condition responsive DEGs were significantly enriched plant-pathogen interaction, biosynthesis of secondary metabolite. Our results provide a global insight into mechanism of CO₂ in regulating tomato quality during cold storage and shelf life condition.
Transcriptomic and targeted MS proteomic quantification of the first ethylene signalling elements: ethylene receptors, CTRs and EIN2 in tomato fruit ripening

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Ethylene is perceived by the ethylene receptors which are the first step in the complex pathway of the ethylene signal transduction. Receptors (ETRs), together with the CTR proteins inhibit the action of EIN2, a key regulator of the signalling pathway which activates ethylene-related transcription factors in the nucleus. This work provides the transcriptomic analysis through RT-qPCR of ETRs and CTRs during tomato fruit ripening on-vine as compared to ripening off-vine either or not treated with the ethylene inhibitor 1-MCP. To fully understand the underlying regulation and physiological responses, there was a need to complement these transcriptomic results with the quantification of the proteins, which are the real effectors of the reactions. However, there was not yet a well stabilised way to identify and quantify these proteins, with only some of the receptors being quantified through Western Blots. This work shows the development of a feasible and reproducible LC-MS based technique, to identify and quantify the ETRs, CTRs and EIN2 in tomato pericarp. The approach started as an MS discovery approach in a highly fractionated tomato peptide sample which allowed the identification of 8,588 proteins, one of the largest dataset described for Solanum lycopersicum L. This information was taken as the starting point to create a targeted MS proteomic assay based on the parallel reaction monitoring mode. As a result, all seven ETRs, three out of the four CTRs and EIN2 could be identified and quantified. Finally, their protein and mRNA levels were quantified in four ripening stages of tomato fruit. Their behaviour revealed that some of the receptors and CTRs could initiate ripening, while others could control the progress of ripening. The levels of EIN2, both mRNA and proteins, decreased during ripening, which might be related to its proteolysis and translocation to the nucleus upon ethylene binding to the receptors.
Abscisic acid induces differential expression of genes involved in wound suberization in postharvest tomato fruit

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Fruit wounding occurred at harvest and transportation requires rapid suberization as a major part of the healing process to prevent infection and desiccation. The focus of this work was to explore the mediation of abscisic acid (ABA) on wound suberization and to determine expression profiles of specific genes involved in wound suberization in tomato fruit. Tomato fruit wounded artificially on surface cuticle was treated respectively with distilled water (Control), ABA, and fluridone (FLD, an inhibitor of ABA biosynthesis) through vacuuming penetration, following stored in certain environmental condition to heal for 2, 4, 6 and 8 days. In the next experiments, histological stain, microscopic observation, quantitative real-time polymerase chain reaction (qRT-PCR) and some physiological analysis approach were implemented. The results showed that the measurements of weight loss and fruit firmness suggested wound suberization was likely to start at 2 d after wounding. Decrease in the respiration and ethylene production after wounding was more gently following ABA application. The suberization process with the accumulation of suberin polyphenolics (SPP) and polyaliphatics (SPA) observed through autofluorescence microscopy and Sudan IV staining was accelerated by ABA. Expressions of SlPAL5 encoding phenylalanine ammonialyase and Sl4CL encoding 4-coumarate ligase involved in the synthesis of SPP reached the highest at 4 and 8 d after wounding following ABA application, respectively. The genes regulating SPA synthesis pathway showed different variation patterns. SlLACS1 and SlLACS2 encoding long chain acyl-CoA synthetases showed the most abundant transcript at 8 and 6 d in ABA group, respectively. However, transcript levels including SlKCSs encoding β-ketoacyl-CoA synthase, SlCYP86B1 encoding fatty acyl ω-hydroxylase, SlFAR3 encoding fatty acyl-CoA reductases and SlGPATs encoding glycerol-3-phosphate acyltransferase were significantly up-regulated at 2 d after wounding by ABA, and then slightly declined at 6–8 d when the healing gradually completed. Evaluation about expression profiles of wound-induced genes that mediate suberin formation would help in understanding the wound-healing process in tomato
fruit. In addition, the wound-induced increase in activities of lipoxygenase (LOX) and polyphenol oxidase (PPO) also suggested roles supporting healing and wound suberization of tomato fruit. The results in this research proved that ABA accelerated the progress of wound suberization and increased the transcript levels of relevant genes in postharvest tomato fruit.

**Excessive water loss induced by internal damage of simulated transport vibration in postharvest kiwifruits**

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Water loss is an important physiological process that affects the main quality characteristics of kiwifruits, including appearance, saleable weight and texture. This study was conducted to interpret the excessive water loss caused by simulated transport vibration in kiwifruits. Kiwifruits (*Actinidia deliciosa* cv. Xuxiang) were harvested during commercial maturity in Hangzhou, Zhejiang, China. Fruits were subjected to simulated vibration in the range 2-30 Hz and 0.7 amplitude for 8 h (simulating 40 hours of real road transport) using an electrodynamic shaker, followed by storing at room temperature in dark for 12 days. Weight loss of fruits, water content in the outer pericarp (OP), inner pericarp (IP) and core (C) tissues were determined. Epidermal cell shrivels, water status and movement in OP, IP and C tissues, and cell ultrastructure in OP were observed using fluorescence microscope, magnetic resonance imaging (MRI) and transmission electron microscopy (TEM), respectively. Skin and epidermal cell shrivels appeared after 8 days of storage in both control and vibrated fruits, and vibration aggravated the symptom. Water loss mainly occurred in OP and IP, and the rate in OP was much higher than that in IP within the first 4 days of storage. Vibrated fruits appeared much more water loss in both OP and IP resulting in significant weight loss during the storage. However, vibration had no significant effect on water content in C. MRI showed that vibration accelerated water diffusion, which mainly occurred in OP and IP and initially taken place in OP (in the first 4 days of storage). TEM imaging showed that vibration directly caused plasmolysis, plasmalemma deformation in OP cells, resulting in obvious cell wall and tonoplast degradation at 12 days of storage. Simulated transport vibration induced fruit epidermal cell shrivel and weight loss due to
the water loss through water diffusion and transference in IP and OP. It could be concluded that vibration-accelerated water loss in kiwifruits is closely related with the cell damage in OP tissue.

**Response of strawberry to UV-C radiation supplemented during growth: Linking plant hormones changes with fruit yield and quality parameters**

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Preharvest ultraviolet-C (UV-C) treatment of strawberry is a very new approach, and little information is available on the effect of this treatment the levels of bioactive phytochemicals. In this study, the effect of preharvest UV-C irradiations at three different doses on strawberry yield, fruit quality parameters, bioactive phenolics and endogenous plant hormones was investigated simultaneously. The overall marketable yield of strawberry was not affected although more aborted fruits in treated plants. The fruits of high dose group were firmer and had approximately 20% higher sucrose content and 15% higher ascorbic acid content. Significant accumulation (p < 0.05) of several flavonoids (25% to 75% increase) was found in the fruits of the low and middle doses groups. At the same time, higher abscisic acid (ABA) content and stimulated flavonoid pathway genes express were found in these two groups. The citric acid content decreased only in the low dose group (reduction of 5.8%), with a concomitant 37% reduction in jasmonic acid (JA) content. In terms of aroma, three volatile alcohols differed significantly among the various treatments with obvious activation of alcohol acyltransferase (AAT) activity. Our study shows that hormetric preharvest UV-C treatment may be used to supplement cultural practices to enhance the bioactive compounds of strawberry fruit, with modification on the plant hormones profiles.
Transcriptome analysis provides new insights into the regulation of chilling–
induced ripening in ‘Passe Crassane’ pear

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Fruit ripening in climacteric fruit is primarily driven by ethylene–regulated changes in gene expression. In most European pears (*Pyrus Communis* L.), a chilling treatment for several weeks is required to induce ethylene production and subsequent ripening. Late-maturing pear cultivars such as ‘Passe Crassane’ require long chilling periods (up to 3 months) for them to ripen normally at room temperature. Although the regulation of pear fruit ripening by low temperature (through induction of autocatalytic ethylene production) has been widely reported in previous studies, the mechanisms behind this phenomenon remain elusive. In this study, ‘Passe Crassane’ fruit were either exposed to propylene (an ethylene analogue) at 20 °C immediately after harvest, or stored at different temperatures (0 °C, 5 °C and 20 °C) for 6 weeks prior to ripening at 20 °C.

Propylene exposure for 9–10 d resulted in significant softening, although endogenous ethylene production was not detected. Fruit stored at 0 °C and 5 °C registered subsequent softening accompanied by a climacteric rise in ethylene production, which was significantly suppressed by 1–MCP (1–methylcyclopropene) treatment. Transcriptome analysis by RNA sequencing revealed three distinct groups of differentially expressed genes that were associated with ripening. Group I (GI) comprised genes that were exclusively regulated by low temperature, as their expression was not inhibited by 1–MCP. These included genes encoding transcription factors such as *PcZinc–finger1*, *PcZinc–finger2*, *PcERF2* and *PcERF3*. Group II (GII) consisted of genes that were regulated by ethylene regardless of any chilling requirements such as *PcPL1*, *PcACO1* and *PcGRAS2*. Finally, group III (GIII) included genes that were first induced by low temperature, prior to regulation by ethylene of which *PcACS1* and *PcACO2* were notable members. Based on the above findings, we suggest a possible model for the regulation of ripening in ‘Passe Crassane’ pears. Adequate chilling exposure initially induces the expression of GI genes (encoding mostly transcription factors) which in turn regulate the expression of GIII genes, triggering autocatalytic ethylene production. The dramatic rise in ethylene production upon transfer to room temperature could be due to increased activity of ethylene biosynthetic enzymes caused...
by accumulation of $PcACS1$, $PcACO1$ and $PcACO2$ transcripts during cold storage. The increased ethylene production could also induce the expression of GII genes, which include those associated with softening such as $PcPL1$.

**Reevaluation of effects of postharvest ethanol treatment on ripening and ethylene production of climacteric fruits**

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Postharvest ethanol treatment affects senescence and ripening of horticultural crops. It is generally reported that it delays ripening and ethylene production of various climacteric fruits. Most of their results were achieved with transient ethanol vapor treatment and they are not necessarily efficient, comparing to continuous treatment. Fruit responses to ethanol are dependent on a lot of factors and especially mode of application and duration of exposure are important and subjects to be resolved. Ethanol vapor treatment with ethanol pads is practical and effective technology of ethanol treatment because it is continuous treatment, simple operation, and easy to regulate the ethanol concentration of atmosphere. In this study, we reevaluated effects of postharvest ethanol treatment, using ethanol pads, on ripening and ethylene production of climacteric fruits, including tomato, banana, kiwifruit and avocado. The mature fruits were sealed with and without (control) ethanol pads (Antimold mild®, Freund Corporation) in a perforated polyethylene bag and stored at 20°C in the darkness. Ethanol concentration of the atmosphere was speculated about 100 pmol / ml from our previous paper. Ethylene was measured with gas chromatography. Color score or flesh firmness were measured as ripening indices. In the case of tomato fruit, while the color score of control fruit developed from mature green to turning during the storage, the score of ethanol treated fruit remained mature green. But, unexpectedly, in ethanol treated fruit ethylene production was drastically stimulated. Furthermore, gene expression of ripening related transcription factors and ethylene biosynthetic enzymes was also stimulated. These results suggest that ethanol could have essential roles in fruit ripening because, though ripening itself was delayed in this system, ethylene biosynthesis and ripening process upstream of ethylene were stimulated by ethanol. To confirm that, we investigated in other fruits. In the case of banana fruit, the color score of ethanol treated fruit started to
develop earlier than that of control fruit by 2 days. Initiation and peaks of ethylene production of ethanol treated fruit were also detected earlier than those of control. In the case of kiwifruit, while the rate of ethylene producing fruit remained low in the control during the storage, the rate in ethanol treated fruit increased and it reached 85% on 16 days in storage (DIS). The firmness on 0 DIS was 2.0 N and that of control fruit on 16 DIS did not change. The firmness of ethanol treated fruit decreased to 1.3 N on 16 DIS. In the case of avocado fruit, ethylene production of ethanol treated fruit started to increase on 3 DIS and reached to the peak on 5 DIS, while that to control fruit started to increase on 5 DIS and reached to the peak on 6 DIS. These results suggest that appropriate ethanol treatment could potentially stimulate ripening and ethylene production of climacteric fruit. Molecular analyses are necessary to confirm the possibility and clarify the mechanism.

**FaMADS1a acted as a mediator to participate in ABA-induced ripening of strawberry fruit**

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Despite the key role of ethylene in climacteric fruit ripening, abscisic acid (ABA) played the important role in non-climacteric strawberry fruit. In this study, octoploid strawberry (*Fragaria × ananassa* Duch. cv. Akihime) fruit at De-green stage were injected with 0.1 mL of 0.1 mM ABA through the pedicel using a sterile microsyringe. The results showed that ABA promoted strawberry fruit ripening by modulating fruit firmness and the contents of anthocyanin, soluble solid and total titratable acid. Meanwhile, *FaMADS1a*, a member of the MADS-box family, was significantly down-regulated. The role of *FaMADS1a* was further studied by transiently modifying gene expression through tobacco rattle virus-induced gene silencing technique. The time taken by the down-regulated fruit to attain the full red stage was much shorter compared
to controls (empty vector). Fruit firmness and protopectin content were reduced with the induction of anthocyanin and soluble pectin in FaMADS1a-RNAi fruit. In parallel with the modified ripening progress, several ripening-related genes, including anthocyanin-related genes (FaPAL6, FaC4H, Fa4CL, FaDFR and FaUFGT), softening-related genes (FaPL and FaXTH), and aroma-related genes (FaQR and FaAAT2), also were induced significantly in the transiently modified fruit. Sequence analysis showed that there were ABA-related cis-elements in the promoter of FaMADS1a. Five transcription factors (ABI5-5, TRAB1, ABI5, ABI5-2 and ABI5-3) involved in ABA signal pathway were selected by yeast one-hybrid system. The results indicated that ABA signal activated FaMADS1a transcription level through ABI5-5, TRAB1 and ABI5, and then the target genes were activated. Taken together, the obvious synergy among yeast one-hybrid experiment, ABA application and FaMADS1a-RNAi suggested that the depression of FaMADS1a might be the essential downstream of ABA signal transduction pathway connected to the activation of anthocyanin-related FaPAL6, FaC4H, Fa4CL, FaDFR and FaUFGT, softening-related FaPL and FaXTH, and aroma-related FaQR and FaAAT2.

Genotype-dependent responses in the oil composition of harvested olives treated with exogenous ethylene

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Fruit of two olive cultivars, Leccino (LE) and Moraiolo (MO), were harvested at two different ripening stages according to Jaén index: 4.58 and 4.17 (less advanced) for LE and MO, respectively, and 5.10 and 4.40 (more advanced) for LE and MO, respectively. Fruit were treated with ethylene (1,000 ppm in air) or air (control) for 24 hours, and before and after incubation were analyzed in terms of firmness and total antioxidant capacity (TAC). The extracted oils, in addition to technological parameters (all oils resulted to be extra-virgin), were analyzed in terms of aroma (VOCs) profiling, total polyphenol content (TPC), and subsequently were evaluated by a panel test. Decreased firmness at the end of the treatment was observed only in more advanced LE fruit. Ethylene negatively affected TAC of MO in both harvests. On the contrary, in LE fruit TAC was found to be significantly increased only when more advanced fruit treated
with ethylene. Regarding the oil, the total polyphenol content was significantly higher when less advanced MO fruit were treated with ethylene. The opposite trend was observed for more advanced MO and for both harvests of LE. Regarding the VOCs composition of the oils obtained from the different cultivars and treatments, Partial Least Squares (PLS) analysis indicated that MO treated with ethylene, regardless of the ripening stage, produced oil associated with C6 aldehydes and alcohols (2-hexenal, 2-hexen-1-ol and 1-hexanol), whereas the oils obtained from LE treated with ethylene were more related to hexanal in both ripening stages. Panel tests revealed changes in the organoleptic perception of the different oils, with those derived from ethylene treated more advanced fruit receiving higher scores for “green” and “herb” when compared to air treated. These preliminary data indicate that the effects of exogenous ethylene on metabolic processes in harvested olives (and quality traits of the resulting oil) are strongly genotype-dependent. Further studies will try to elucidate the physiological and metabolic mechanisms underlying this behaviour.

Effects of postharvest treatment with 1-methylcyclopropene (1-MCP) on fig (Ficus carica L.) fruit during storage

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Fig (Ficus carica L.) fruit are highly appreciated by consumers due to their nice taste. Recent researches have also recommended the consumption of fresh figs because of their high nutritional value. At the fresh state, however, figs are highly perishable fruits. They exhibit a rather climacteric pattern of ripening, but in order to develop the optimum flavor they have to be harvested near the fully ripe state when they become soft and susceptible to deterioration. The objective of this work was to investigate the effect of postharvest treatment with the ethylene perception inhibitor 1-methylcyclopropene (1-MCP) on ripening processes of the dark peel ‘Mavra Markopoulou’ figs stored at low temperature. Particularly, fruit were harvested early in phase III during their development and treated with 0, 0.5, 1.5 and 2.5 μL L-1 1-MCP at 1°C for 12 h. Then, fruit were stored at 1°C with 95% RH for 3, 6 and 14 d. Ethylene and CO₂ production rates, concentrations of 1-amino cyclopropane-1-carboxylic acid
(ACC), fruit firmness, peel color parameters a* and b* near the ostiole, and concentrations of cyanidin-3-glucoside, cyanidin-3-rutinoside, and malvidin-3-glucoside in peel were determined at harvest and after storage followed by exposure at 20°C for 12h. The results showed that firmness decreased progressively during storage, but 1-MCP treated fruit were firmer than controls. However, ethylene, CO₂ and ACC levels increased during storage and similarly in controls and treated fruit. Peel color development and increases in anthocyanin concentrations were prevented by 1-MCP concentrations.

Chemical composition and biological activities of some North African local Plants

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Camphor laurel trees (Cinnamomum camphora) are used in traditional medicine in Algeria for their anti-inflammatory and also anti-rheumatism properties. This introduced species in Algeria is grown in the National Park of El-Kala (Northern East Algeria). The essential oil composition of Cinnamomum camphora was carried out by GCMS and revealed high amounts of Longifolene (17.28%), Caryophyllene (15.32%), β-Selinene (11.06%), Spathulenol (8.73%), Germacrene D (7.55%), Camphor (6.69%) and α-Humulene (5.94%). We investigated the in vivo effect of this essential oil on the anti-inflammatory system of Wistar Rats. Adult male Wistar rats were divided into four groups: Group I: negative control; group II: Ovalbumin sensitized/challenged rats (positive control); group III: received CC Intraperitoneal Injection of essential oil (80mg/kg/day) along the experimental protocol; group IV: received CC Intraperitoneal Injection of essential oil (80mg/kg/day) along the experimental protocol and sensitized/challenged with ovalbumin. After 24 days, blood and tissue samples were collected for haematological and histopathological analysis, respectively. Essential oils from CC exhibited an anti-inflammatory potential and enhanced the reaction of immune system.
XI.

Ethylene and Storage of Perishable Produce

INVITED TALKS

To infinity (1-methylcyclopropene) and beyond!

Watkins Chris
Cornell University, Ithaca, USA

It is just over 22 years that 1-methylcyclopropene (1-MCP) was patented, and 16 years since its first registration for use on food crops. As an inhibitor of ethylene perception, 1-MCP has proven not only to be a powerful research tool, but also has had huge impacts of horticultural industries around the world, most notably for apple. Indeed the apple has been the ideal fruit for 1-MCP usage because minimal changes after harvest are desirable. In contrast, modulating 1-MCP action for many fruits that require extensive ripening changes (color development, softening) has been more difficult. A number of other factors such as scale of industry, compatibility with existing management protocols, and cost-benefit ratios have also affected uptake of the technology, although the impact of these factors can change over time. 1-MCP is now off patent, and new application methods for 1-MCP have been developed for large-scale use, in addition to a number of niche opportunities such sachet applications, liquid applications, and incorporation into plastics. Preharvest applications of 1-MCP as Harvista are now widely used, as well as application of 1-MCP to fruit after storage, including interesting combinations with dynamic controlled atmosphere storage. So, perhaps we will continue without limits, limitless, sky's the limit (e.g. limitless possibilities, etc.). Or maybe not!
Effects of the ethylene-action inhibitor 1-methylcyclopropene on postharvest quality of non-climacteric fruit

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1-Methylcyclopropene (1-MCP) (SmartFresh™) is an ethylene antagonist widely used to retain quality and prolong the postharvest storage period of various climacteric fruits in which ethylene plays a major role in regulation of the ripening process. Nonetheless, it has been found that exposure to 1-MCP may affect certain ripening-related processes also in non-climacteric fruits. In this study, we evaluated the effects of 1-MCP on quality and postharvest storage performances of various non-climacteric fruit crops, including grape, cherry, pomegranate, citrus, pitaya, prickly pear, lychee and loquat. It was found that 1-MCP affects the following aspects in non-climacteric fruit: 1) Inhibition of senescence processes, such as rachis browning in grapes and scale senescence in pitaya 2) Prevention of physiological disorders, such as scald in pomegranate and and internal browning in loquat. 3) Retardation of degreening and color changes in various fruit, including pitaya and prickly pear. Beside these major effects, exposure to 1-MCP had divergent effects on fruit respiration and ethylene production rates, and on decay development, with differing and sometimes contrary effects observed in different crops. Finally, exposure to 1-MCP had relatively minor effects on internal fruit-quality parameters, including nutritional quality and flavor. In the future, it may be worth considering the commercial application of 1-MCP as a means of retaining the green color, reducing physiological disorders, and retarding senescence processes in some non-climacteric fruits.
Comparative analysis of ethylene–induced and low temperature–modulated ripening in kiwifruit

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Kiwifruit is generally considered climacteric since exogenous ethylene/propylene induces rapid ripening as well as endogenous ethylene production. However, it is widely known that the majority of ripening–associated changes in kiwifruit on and off the vine occurs in the absence of any detectable ethylene. This peculiar ripening phenomenon is often attributed to ethylene signaling (that is, system 1 ethylene), since kiwifruit is considered extremely sensitive to ethylene. In this study, the aim was to compare kiwifruit ripening characteristics and the expression profile of associated genes in response to propylene (an ethylene analogue), during storage at different temperatures (in the absence of ethylene) and on the vine. Propylene exposure induced ethylene production, softening, titratable acidity (TA) reduction, soluble solids content (SSC) increase and aroma development within <10 d at room temperature. This was accompanied by increased expression of genes such as AcACS1, AcAAT, AcPG, AcEXP1, AcβAMY1, AcERF6 and AcNAC4. During storage, kiwifruit at 5°C and 10°C ripened faster (to eating quality within four weeks), consistent with increased expression of several genes such as AcEXP1, AcPG, AcβAMY1, AcβAMY2, AcNAC4 and AcMADS2. Significant ripening also occurred at 15°C, although fruit at this temperature required longer exposure periods (eight weeks) to attain eating quality, in concurrence with delayed expression of associated genes. By contrast, fruit at 22°C ripened at the slowest rate and did not attain eating quality even after eight weeks, consistent with very minimal expression of ripening–related genes. On–vine ripening proceeded gradually at the early stages when the average field temperature was ~20°C but the rate increased as the temperature dropped to ≤15°C, accompanied by increased expression of ripening–related genes. Unlike ethylene–induced ripening, low temperature–modulated ripening was not suppressed by frequent treatment of the fruit with 1–MCP (a potent ethylene perception inhibitor). Interestingly, neither ethylene production nor aroma volatile synthesis were observed in fruit ripening during low
temperature storage and on the vine. Together, the above findings suggest that kiwifruit ripening can occur through either ethylene–dependent or low temperature–modulated mechanisms. Furthermore, kiwifruit ripening during cold storage and on the vine appears to be modulated by low temperature independent of ethylene. Characterization of genes in ethylene signal transduction pathways in papaya fruit under various experimental conditions.

Characterization of genes in ethylene signal transduction pathways in papaya fruit under various experimental conditions

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Ethylene play important roles in fruit development, ripening, defence responses and stress signaling pathways. After harvest, the climacteric fruit such as papaya are subject to a range of problems associated with postharvest handling and storage treatments. To investigate the transcriptional mechanisms underlying fruit developmental, ripening and stresses, we cloned genes in ethylene signal transduction pathway from papaya, which including CTR1s, ETR1, EIN2, EIN3s/EIL1, EBF1/2, and ERF1-4. Expression patterns of these genes were examined during fruit development, under 1-MCP treatment, ethephon treatment and temperature stresses. All of these genes displayed differential expression patterns and expression levels under different experimental conditions. 1-MCP treatment significantly repressed the expression of CpCTRs and high concentration of 1-MCP treatment had a more significant effect. CpERF2 and CpERF3 showed a close association with fruit ripening and CpERFs had a high expression level in the earlier stages of the fruit development period. The expression of CpERFs strongly associated with stress response. Chilling injury induced the expression of ACS1/2/3, EIN2, EIN3s/EIL1, CTR1/2/3, and ERF1/3/4 but reduced the transcript level of CpETR1, CpCTR4, CpEBF2, and CpERF2. All together, these results indicate that these genes may play important roles in papaya fruit ripening and stress responses.
Probing the role of ethylene and low temperature in the modulation of flavedo colour change in Satsuma mandarins (Citrus unshiu Marc) fruit

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Citrus fruit, such as Satsuma mandarins, produce very small amounts ethylene (system 1 ethylene) during maturation and are therefore considered non–climacteric. However, evidences indicate that ethylene is involved in the control of maturation in citrus fruit; application of the gas is a common postharvest practice to accelerate and attain uniform peel colour changes in lemon. In Satsuma mandarins, natural peel degreening coincides with the onset of winter, and there is a strong correlation between the peel colour changes and the gradual decrease in environmental temperature. The mechanisms that control this phenomenon are yet to be elucidated and until now, it is not clear whether natural peel degreening in Satsuma mandarins is induced by ethylene or low temperature or both. Currently, we are attempting to uncover peel colour change mechanisms through comparisons between ethylene–induced and low temperature–modulated ripening. In this study, fruit exposed to propylene (an ethylene analogue) rapidly induced peel colour changes within 4 d, indicated by an increase in colour index (CI), and a decrease in the contents of chlorophylls a and b. Equally, storage at 10°C and 15°C resulted in colour change from green to yellow, an increase in CI, and a decrease in the contents of chlorophylls a and b within four weeks. However, there were no significant changes in peel colour, CI and chlorophyll content in fruit during storage at 5°C, 20°C and 25°C. Comparative transcriptome analysis of the flavedo revealed 5612 differentially expressed genes (DEGs) that coincided with the colour changes. Out of these, 1444 DEGs were exclusively regulated by propylene with 393 and 1051 being up– and down–regulated, respectively. Interestingly, a considerable number of DEGs showed little response to propylene, while they were significantly up– or down–regulated by 5°C, 10°C and 15°C. By contrast, insignificant changes in gene expression were recorded in the flavedo of fruit stored at 20°C and 25°C. From these findings, the change of flavedo colour in Satsuma mandarins in response to moderate temperature appears to be distinct from ethylene signalling. Further research is required to unravel the role of the genes identified in this study in regulation of colour change–associated events.
XII.

Ethylene interplay with other hormones in controlling secondary metabolism

INVITED TALKS

An elevated anthocyanic response in apple up-regulates ethylene

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Elevated anthocyanin levels in fruit and vegetables is a breeding target for a number of plant species. In some fruit (e.g. tomato) higher levels of anthocyanins enhance storage and shelf life. In contrast, red-fleshed apples (*Malus x domestica*) have a higher incidence of internal browning flesh disorder (IBFD). To study this, we examined Royal Gala apples over-expressing the anthocyanin-related R2R3 MYB10, compared to standard Royal Gala grown under the same conditions. As Royal Gala is a cultivar with no reported incidence of IBFD, it was apparent immediately that in the same genetic background, 35S:MYB10 Royal Gala apples had a high incidence of this disorder. Metabolites of these apples were compared. Anthocyanins, chlorogenic acid, flavon-3-ols and flavonols (quercetin-derivatives) were all higher in R2R3 MYB10. For flavol-3-ols, epicatechin, rather than catechin, was higher in red-fleshed lines compared to control. In addition, total peroxidase activity was elevated in flesh both pre- and post-harvest. Internal ethylene levels were measured and were significantly higher in red-fleshed lines, and at an earlier developmental stage pre-harvest. Expression analysis of key genes for the phenylpropanoid pathway, ethylene biosynthesis, peroxidases and polyphenol oxidases confirmed the elevation of ethylene production capacity and the mechanism for enhanced browning. Analysis of transcriptional activation by MYB10 showed this transcription factor could activate the expression of apple *ACS* and *ACO* genes, perhaps via up-regulation of other transcription factors. Enhancement of maturity, via ethylene induction, by anthocyanin regulated transcription factors, has implications for breeding and storage of more highly pigmented plant products.
Transcriptional cross talk between ethylene and spermidine during tomato fruit ripening

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Ethylene (Ethy), ripening and pro-senescence hormone, and polyamines (PAs), the pro-growth and anabolic molecules are biosynthesized utilizing a common precursor S-adenosylmethionine (SAM). A cross-talk between Ethy and PAs pathways has therefore been envisioned since they can compete for SAM during plant growth and development. Studies in our laboratories on transgenic tomatoes expressing a heterologous SAM decarboxylase (SAMdc), which resulted in the accumulation of higher polyamines, spermidine (SPD) and spermine (SPM), demonstrated significant enhancement also in ethylene production during ripening. These data suggested that SAM may not be limiting for Ethy production. Interestingly, in spite of higher Ethy production, tomato fruit ripening was attenuated raising strong possibilities of a cross talk between these growth regulators. To elucidate the nature of this cross talk, we examined changes in transcriptional regulation in high SPD/SPM transgenic and isogenic parental wild-type fruits. Our results show that increased SPD/SPM in the transgenic fruit causes a massive shift in transcriptomes during fruit ripening. The SAMdc-transgenic fruit had 2-fold higher expression of 1452 (at Br) and 2540 [at Br+8d (B8)] genes and 2-fold lower expression of 993 (at Br) and 822 (B8) genes, respectively, compared to the parental wild type fruits during the same stages of ripening. Majority of the differentially expressed genes exhibited strong correlations with putrescine (PUT), SPD and SPM, but the correlations coefficients for PUT were generally opposite of that for SPD/SPM. Several Ethy biosynthesis genes were significantly up regulated, including ACS2 and ACO1. These findings provide a molecular basis for the previously observed increased Ethy production in high SAMdc transgenic fruits. Several genes in the Ethy signal transduction pathway were down regulated at Br stage but up regulated at B8 stage of ripening, including an ETR, two EBF1 and four Ein3 genes, but transcript levels of one of the EBF1 genes was upregulated in the transgenic fruit. The transcript levels of two CTR1s and two MAPKs did not change in the high SPD/SPM fruit. In regard to PAs biosynthesis, transcript levels of SAM synthase (SAMS), an ADC, one SAMdc and a PAO-like gene were significantly up regulated at both Br and B8 stages of transgenic fruits. However, it was noted that transcript levels of highly expressed SAMS, SAMdc
and SpdSyn were also up regulated at B8 stage of fruit ripening. Notably, the correlation between transcript levels and PUT were generally opposite of that observed between transcript levels and SPD, SPM and Ethy, suggesting differential roles of different polyamines during fruit development. Collectively, our results indicate a significant cross-talk between PAs and Ethy with SPD/SPM acting as a dominant factor up-stream of Ethy to extend fruit ripening process. Implications of these results will be discussed for ethylene-regulated fruit ripening process. Supported by USDA/NIFA Hatch IND011872. Collaborating Authors: Kexin Wang, Jingxin Chen and Autar K. Mattoo

Ethylene signaling in rice - novel insights

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Rice is an important monocotyledonous crop worldwide and lives in water environment in most of its life cycle. Ethylene as a gas phytohormone plays significant roles in the adaptation of rice to the water environment. However, the understanding of ethylene signaling in rice is very limited. Based a distinct phenotype of root inhibition but coleoptile promotion in rice etiolated seedlings upon ethylene treatment, we have isolated a set of rice ethylene-response mutants from T-DNA insertion and EMS-mutagenized populations, and identified the corresponding genes through map-based cloning. Among these, MHZ7/OsEIN2 and MHZ6/OsEIL1 are similar to those in Arabidopsis. MHZ6/OsEIL1 and OsEIL2 are responsible for the ethylene responses in roots and coleoptiles respectively. Mutation of these genes caused salt tolerance, different from the observation in Arabidopsis. We also identified MHZ5/carotenoid isomerase and MHZ4/ABA4, both of which are involved in ABA biosynthesis, supporting that ethylene signaling acts upstream of ABA pathway to regulate root growth in rice. Through characterization of a gaoyao1 (gy1) mutant exhibiting longer mesocotyl and longer coleoptile compared to its original variety, the GY1 gene was identified to encode a PLA1-type phospholipase. The GY1 functions at the initial step of jasmonic acid (JA) biosynthesis. Ethylene inhibits expression of GY1 and other genes in the JA biosynthesis pathway to reduce JA levels and enhance the growth of mesocotyl and coleoptile through promotion of cell elongation. Through analysis of the re-sequencing data from 3000 rice accessions, we identified a single natural variation of
the GY1 gene, GY1376T, which contributes to mesocotyl elongation in rice varieties and is beneficial for emergence of seedlings in dry land after direct sowing. MHZ3 is a novel membrane protein localized on ER. It can stabilize the OsEIN2 through association with the transmembrane domain of OsEIN2, strengthening the importance of the OsEIN2 transmembrane domain during ethylene signaling. Other recent advances will also be discussed. Our study provides novel insights into the understanding of ethylene signaling in rice. Further characterization of rice ethylene-response mutants and their corresponding genes will help to clarify the whole picture of ethylene signaling mechanisms in plants.

ORALS

Ethylene interaction with higher-polyamines in regard to defense-related secondary metabolites demonstrated in field-grown transgenic tomato genotypes

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Secondary metabolites such as phenolics/flavonoids are known for their role(s) in plant defence. Both ethylene and polyamines (spermidine - SPD and spermine - SPM) have been independently demonstrated to play roles in plant response to abiotic and biotic stresses. However, it is not known whether these growth regulating substances interact to regulate secondary metabolism or impact secondary metabolites accumulation. In addition to phenolics/flavonoids, other widely distributed secondary metabolites such as N-acyethanolamines (NAE fatty acids) are now known to be impacted in response to abiotic/biotic stresses. Our research objective is to elucidate the cross talk between SPD/SPM and ethylene in regulating phenolic-flavonoid pathway and that of NAE fatty acids. Toward that end we quantified the levels of phenolics/flavonoids and NAE fatty acids at different stages of fruit ripening utilizing previously developed transgenic lines either impaired in ethylene production or enriched in high SPD/SPM levels, and their hybrids. These three transgenic lines along with their isogenic azygous control were
cultivated at the Beltsville Agricultural Research Center fields under two different agroecosystems created by using polyethylene (BP) and hairy vetch (HV) mulch for two different years. HPLC and GC-MS analyses of different phenolics/flavonoids and NAE fatty acids in fruits at various stages of ripening from these plants were quantified. Also, total phenolics and antioxidant capacity of these fruit were determined using Folin-Ciocalteu (FC) and ferric ion reducing antioxidant power (FRAP) assays, respectively. Additionally, we carried out RNA-seq analysis of fruit from high SPD/SPM lines at different ripening stages to determine the transcriptional regulation of biosynthesis of phenolics, NAE fatty acids and ethylene. Our results show that an interaction between genotypes and the agroecosystem employed modulates the accumulation of phenolics/flavonoids and NAE fatty acids in ripening fruits. High SPD/SPM genotypes and their cross hybrid with ethylene-deficient line grown in HV were found to significantly accumulate high levels of caffeic acid, naringenin chalcone (the precursor for resveratrol), quercetin 3-O-glucoside and chlorogenic acid than the azygous control. The content of NAE fatty acids also changed significantly among different genotypes grown in the two agroecosystems, providing evidence for interactions between each genotype and a specific agroecosystem. RNA-seq analysis of the structural and regulatory genes for the biosynthesis of phenolic acid/flavonoid pathways showed their upregulation in fruit at early ripening stages while those for the NAE fatty acids’ pathway were found upregulated at the late ripening stage in the high SPD/SPM genotypes. Genes involved in flavonoid pathway were found expressed at significantly higher levels than those for isoprenoid pathways. Collectively, these findings provide evidence of a cross-talk between ethylene and polyamines in regulating levels of secondary and defense-related metabolites.

The ability of ethylene to regulate the concentration of phenolic compounds and textural changes in harvested olives

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Olives are non-climacteric fruit. In a previous article, oleuropein (OE) increased substantially in fresh green olives exposed at 20 oC for 7 d, but the increases were lower in preharvest treated fruit with an ethylene synthesis inhibitor. The present aim was to
investigate whether phenolic compounds, including OE, were affected by ethylene treatment in green harvested olives. Postharvest treatments with the ethylene perception inhibitor, 1-methylcyclopropene (1-MCP) at 1.5 μL L⁻¹ for 12 h, and/or ethylene at 1000 μL L⁻¹ at 20 °C for up to 10 d were applied to fruits of ‘Konservolia’ cultivar. The results showed that ethylene and/or 1-MCP had similar effects on total phenolics (TP), total antioxidant capacity (TAC) and OE (as confirmed by HPLC-DAD-ESI-MS). Particularly, in all treated fruit, but not in controls, TP and TAC increased soon after harvest and remained at similar levels throughout storage, whereas OE increased in all fruit, both controls and treated, at later stages and independently of degreening. Although 1-MCP is a specific ethylene inhibitor, there is a gap for its role in non-climacteric. Diverse responses to 1-MCP have been observed in non-climacteric fruit, such as inducing ethylene dependent or independent processes or in other plant tissue ethylene perception in the presence of 1-MCP implied that ethylene and its inhibitor may bind to different sites with different affinities. Here, the TP increases only in treated fruit could be possibly attributed to stress responses since the high ethylene treatment and/or 1-MCP comprise an environment where the fruit have not a previous experience and is received as stressor. During maturation and ripening on tree, OE is degraded to products, such as demethyloleuropein, and/or elenolic acid, and OE-aglycon. The involvement of β-glucosidase activity is also crucial for OE modification. A β-glucosidase, an oleuropein-specific enzyme with kinetics similar to olive native enzyme, participates in a dual defense system, resulting in a glutaraldehyde-like component with protein cross-linking properties, but only in disrupted tissue. This case has been observed in fruit infested by the olive fly larvae along with a burst of ethylene. However, here, the concomitant and similar OE levels in all intact fruit, controls, ethylene and 1-MCP treated, do not seem to be related to the dual defense system. Also, the results do not indicate that ethylene is involved in changes in OE concentration, at least directly. In practice, OE elevation in short-stored olives at ambient temperature might have a positive or negative impact on olive products quality, depending on cultivar and requirements of fruit quality for further use. The work has been published recently in Journal of Plant Physiology, as Short Communication. DOI 10.1016/j.jplph.2018.03.019.
Ethylene promotes ascorbic acid biosynthesis via the regulation of EIN3 and ABI4 on VTC2 transcription

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Both ethylene and ascorbic acid (AsA) play important roles in plant growth and development, as well as stress response, however, the regulatory relation is still unclear. Here, we report a positive role of ethylene in the regulation of AsA biosynthesis. Firstly, we revealed that endogenous and application of ethylene obviously promoted AsA biosynthesis via an ETHYLENE INSENSITIVE 3 (EIN3)-depended manner. Through transcriptome analysis and qPCR confirmations, VITAMIN C DEFECTIVE 2 (VTC2) was identified as a master regulator in this process, unfortunately EIN3 did not directly bind to the promoter of VTC2. Further investigations revealed that an ERF protein abscisic acid (ABA) INSENSITIVE 4 (ABI4) was identified to fill up this gap. Transient transcriptional activity assay, chromatin immunoprecipitation analysis and electrophoretic mobility shift assay demonstrated that EIN3 binds to ABI4 promoter and ABI4 binds to VTC2 promoter to suppress their target genes’ transcription, respectively. Thus, an EIN3-ABI4-VTC2 transcriptional cascade is proposed in ethylene-promoted AsA biosynthesis. This regulatory pattern is further demonstrated by genetic analysis with double mutant ein3 abi4 and abi4 vtc2. In addition, ethylene and ABA antagonistically regulate the expression level and protein accumulation of ABI4. Therefore, our data establish a novel regulatory pathway that ethylene promotes AsA biosynthesis via EIN3-ABI4-VTC2, which is inhibited by ABA in an EIN3-dependent manner, revealing the regulatory mechanism of AsA biosynthesis in Arabidopsis seedling.
XIII.
Biotechnological Control of Ethylene Action
and Biosynthesis

INVITED TALKS

Engineering shelf life of tomato fruits via targeted mutagenesis of ethylene receptor genes by the Target-AID technology

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Shelf life is an important breeding trait in tomato. Ethylene is a major role in shelf life control of fruits. There are many efforts on elucidating molecular mechanism underlying ethylene biosynthesis and its action. Based on those results, a variety of genetic engineering technologies to extend shelf life of fruits are proposed so far. We also have demonstrated that a mutant with a mutation in the second transmembrane domain of ethylene receptor SlETR1 is a useful material for tomato breeding to improve shelf life of fruits with minimal effects on other important breeding traits. Several fruit-ripening mutants have been reported in tomato and causative genes were shown to act as upstream regulators of the ethylene signaling network. Tomato has seven ethylene receptor genes in its genome. SlETR2 amino acid sequence exhibits highly homology with that of SlETR1 and SlETR2 is suggested it act like SlETR1. According to previous study, we tried to isolate the SlETR2 mutants that have 1 amino acid substitution in its transmembrane domain (ethylene binding domain) from Micro-Tom mutant libraries. However, the mutants were not isolated from over 3000 EMS mutant lines by using TILLING technology. In this study, we aimed to generate mutants that have 1 amino acid substitution in the second transmembrane domains of SIETR1 and SIETR2 by Target-AID (target-activation-induced cytidine deaminase) technology. Target-AID is a base-editing technology, and the construct comprising nuclease-deficient Cas9 (dCas9) or nickase CRISPR/Cas9 (nCas9) fused to Petromyzon marinus cytidine deaminase (PmCDA1) and sgRNAs to introduce point mutations into genomes. Several lines of mutants that were substituted 1 or 2 amino acids in expected region in SIETR1/SIETR2
were isolated from the Target-AID mutated lines. In triple response assay that investigating the ethylene response, any mutants did not show clear ethylene insensitivity or reduced sensitivity phenotype. However, both of \textit{SIETR1} and \textit{SIETR2} mutant fruits at the ripening stage displayed weak prolonged shelf life phenotype. And the double mutants showed delayed fruit ripening and strongly extended shelf life of fruit. Although there are variations in the extent of shelf life extension, all lines of fruits from mutant lines showed extended shelf life. This work suggests that Target-AID is a powerful tool to generate substitution mutants in tomato. It also demonstrates that the gene region encoding the amino acid sequence of second transmembrane domain in ethylene receptors SIETR1/SIETR2 is an effective target for improving shelf life of tomato fruits.

\textbf{Overexpression a single \textit{SIMYB75} could affect fruit ripening related process and flavor metabolism in transgenic purple tomato}

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Various strategies have been used to improve fruits quality, among which, the most commonly method is genetic manipulation of genes to upregulate-specific branches of metabolism pathway. However, there still lacking a single gene could effectively improve the metabolites of different branches or pathways at the same time. We showed here overexpressing a single gene \textit{SIMYB75} could effectively upregulate the anthocyanin content to 1.8 mg/g fresh weight at red-ripening stage, which showing a great significance in molecular assisted breeding. In contrast to wild type (WT), the purple tomato displays a series physiological changes, including higher fruit set ratio, longer ripening time and higher ethylene content. In addition to anthocyanin, the total content of phenolic, flavonoids and soluble solids in purple tomato were upregulated to 2.6, 4 and 1.2 times higher than WT fruit, respectively. More importantly, most of the flavour component, aldehyde, phenylpropanoid-derived and terpenes volatiles in purple tomato were significantly increased, especially some of terpenes volatiles were more than 10 times higher than WT. In accordance with this, transcriptome data showed genes involved in ethylene signaling, phenylpropanoid and isoprenoid pathways were greatly affected in purple tomato. Yeast one-hybrid and dual-luciferase assays indicated \textit{SIMYB75} could directly bind to \textit{MYBPLANT} and \textit{MYBPZM} elements, and the
promoters of LOXC, AADC2 and TPS genes could be trans-activated by SlMYB75. Taken together, our data demonstrated SlMYB75 could regulate fruits quality partly through transcriptional regulation of downstream genes, which might shed light on production of high quality fruits.

**Control of ethylene biosynthesis via genome editing technology in melon**

**Nonaka Satoko**

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Melon (*Cucumis melo*) is a well-produced crop after tomato, watermelon and cucumber. The major producing regions are Asia and Mediterranean coast. Melon has a variety of breeding traits including fruit shape, aroma, fruit color, sex determination, vascular fluxes, ripening process and shelf-life. Especially, shelf-life is an important trait from agricultural view point because it effects on fruit supply, fruit nutritional value and consequently, human health. Therefore, many studies on shelf-life have been carried out and shown that ethylene plays a major role in shelf-life for climacteric type of melon. Reduction of ethylene production via suppression and/or defeat of ACC oxidase gene (*CmACO1*) showed improved shelf-life, suggesting that the ACO1 gene is a target gene for improving shelf-life. In Japan, melon is special fruit. Some Japanese melon with high sweetness, great flavor and beautiful net is expensive (around $300) and used as special gift. ‘Harukei No.3’ (subsp. reticulatus) is utilized as a breeding parent for the expensive Japanese melon. Although 'Harukei No.3' is climacteric group and has many excellent traits like above, the shelf-life is not so long. Therefore, improvement of shelf-life is required. In this study, we attempted to control ethylene biosynthesis to improve shelf-life of 'Harukei No.3'. To control ethylene production, *CmACO1* was mutated by a genome editing technique. The Clustered, Regularly Interspaced, Short Palindromic Repeats (CRISPR)/ CRISPR-associated protein9 (Cas9) system is a useful genome editing technology and is a powerful tool applicable for targeted mutagenesis in various organisms. This system enables to induce double-strand breaks (DSBs) at a specific genome region and induce mutations preferentially via error-prone non-homologous end-joining (NHEJ) pathway. Although there is a requirement that a 20 bp-target sequence must be designed at the region followed by the protospacer-adjacent motif (PAM) sequence (5’-NGG for the type II CRISPR/Cas9 system) on the genome, the advantages in ease of construction and higher mutagenesis efficiency have accelerated
the use of the system in various areas of research. Moreover, genome editing technology via CRISPR/Cas9 system is expected as a powerful tool for plant breeding. Especially, under the certainly genomic information, the breeding speed by CRISPR/Cas9 is faster than conventional mutagenesis and then time and labor could be saved. To improve the shelf-life of 'Harukei No.3', we tried to introduce mutation into CmACO1 by CRISPR/Cas9 system. The target site was selected via CRISPR-P site (http://crispr.hzau.edu.cn/CRISPR2/), and CRISPR/Cas9 binary vector was constructed. The binary vector was transformed through the liquid culture system into melon genome. Five transgenic lines were obtained, and in all of T0 generation, the CmACO1 gene included one nucleic acid insertion or deletion. In the T1 generation, the mutation was detected in CmACO1 gene and some lines were without CRISPR/Cas9, that is, null segregation of CRISPR/Cas9. Characterization of the induced mutants is in progress.

ORALS

Screening for novel regulators of ethylene biosynthesis: focusing on ACC-oxidase

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The gaseous plant hormone ethylene plays an important role in many developmental processes and responses towards various stresses. Past research has strongly relied on genetic screens of ethylene-related mutants to unravel the ethylene biosynthesis and signaling pathways. Despite these advancements, little is known about the regulation ACC oxidase, the final and crucial step of ethylene biosynthesis. We have performed a biochemical and phenotypic characterization of the different aco T-DNA lines and have set up two screens to discover novel regulators of ACC-oxidase. We found several dark and light phenotypes of different single T-DNA knockout mutants of the Arabidopsis ACO gene family. These single aco T-DNA lines also show different levels of reduced ethylene production in dark grown seedlings. Higher order aco knock-out lines will be generated using CRISPR/Cas9. Next, we have performed an EMS genetic screen in the ethylene overproducing mutant (eto1eol1eol2) background. We screened for ethylene insensitive mutants using the Arabidopsis triple response phenotype, and several
candidate mutants were retrieved. These new putative mutants are currently being further characterized. Finally, we are also exploiting the availability of the 1135 genomes of natural accessions of *Arabidopsis thaliana* by means of a Genome Wide Association Study (GWAS). A subset of more than 200 *Arabidopsis* accessions collected from different places around the globe, has been screened for ethylene production using 4-day-old dark-grown seedlings. This way we use natural genetic variability to link ethylene production capacity to different Single Nucleotide Polymorphisms (SNPs) in the genome. These SNPs and EMS mutants will allow us to identify putative candidate genes which might play an important role in the regulation of ethylene biosynthesis (or signaling).

1-aminocyclopropane-1-carboxylic acid (ACC) and other compounds profiling in plant tissues using ultra-performance liquid chromatography – tandem mass spectrometry

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ACC (1-aminocyclopropane-1-carboxylic acid) is a simple non-proteinogenic amino acid, having a central function in ethylene biosynthesis as its direct precursor. Ethylene, as one of the main plant hormones, plays role in a wide range of developmental processes, often intertwined with other phytohormones. ACC synthesis has been shown to be dependent on various developmental, environmental and hormonal signals, with a possibility to further modify and metabolize its molecule by conjugation or deamination reactions. Discovering major functions of ACC represents a quickly developing field of interest, with implications of ACC acting as an ethylene-independent signaling molecule in plants. Here we demonstrate a simple and reliable profiling method to determine ACC, glutamine, glutamic acid, tryptophan, tryptamine, salicylic acid and other compounds in Arabidopsis thaliana plants. By employing a simple extraction, derivatization, ultra-performance liquid chromatography with tandem mass spectrometry and isotope dilution analysis for quantification, we exhibit a detection limit for ACC around 1 fmol during a 15-minute long analysis, requiring 10 mg of fresh weight plant material. This method, although being still an ongoing work, provides
substantial improvements in terms of robustness, sensitivity, selectivity, through-put and cost effectiveness over previous methods published.

POSTERS

Research on methods of improving editing efficiency of CRISPR/Cas9 system on tomato genome

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Modification of genomes in a site-directed manner to improve traits of crop is described to be critical for plant breeding and genetic engineering. The genome edit strategies were first proved to be effective in bacteria and human cell lines, but they have been successfully adapted in plant systems. In recent years, CRISPR/Cas9(Clustered regularly interspaced short palindromic repeats/CRISPR-associated endonuclease 9) system has become the most widely adopted gene editing technology. In animals, by introducing sufficient amount of sgRNA and Cas9 RNA through microinjecting, biallelic mutations can be generated in one-cell-stage embryos efficiently. However, Agrobacterium-mediated transformation of calli is a common method for generating transgenic plants. The transformed cells soon divide allowing relatively short time for generating the germline mutation. On the contrary, it takes several weeks even months to regenerate transgenic crop plants from embryogenic cells, and the sgRNA/Cas9 complex is continuously being expressed during this time period. Such long expression stage might result in the high risk of chimeric and heterozygous mutation in the first generation. Due to the posttranscriptional gene silencing (PTGS) mechanism in plants system, Cas9 protein could not be accumulated excessively. Furthermore, non-transgenic plants could be generated by backcross T1 heterozygous mutants to eliminate unwanted parts of T-DNA, but if the efficacy of CRISPR /Cas9 is strong enough, T1 biallelic mutations can be generated effectively. Therefore, it is an urgent issue that how to increase the expression level of Cas9 protein so as to improve the genome editing efficiency. Tomato is one of the most important crops worldwide and is regarded as an important model plant for studying flower and fruit development, because of the
availability of its entire genome sequence and its well-studied genomics. In this study, we use tomato plants for testing the efficiency of P19m assembled sgRNA/Cas9 system. Our aim is to make it available for generating homozygous mutants in short time period and even manipulate the target region precisely in genome editing by HDR. Using the GoldenBraid cloning strategy designed and constructed SIBES7 (Solyc03g005990.2.1) gRNA-Cas9 vectors with P19m and transformed them into Agrobacterium for stable transformation of tomato plants and analyzed the target region of T1 progeny. We obtained 20 SIBES7 T1 mutation plants, among them, 25% were biallelic mutants that was 100% mutation frequency. 3 of the 5 biallelic mutants were homozygous mutants. The rest were 7 heterozygous mutants and 8 chimeric mutants. Through the analysis of the type of mutations, most of the mutation occurred at gRNA2 target region, including 8 mutation types, the majority of the detected mutations were 2-nucleotide deletions, followed by 1-nucleotide insertions. Surprisingly, there were 4 non-transgenic mutants among 20 mutants. Furthermore, we observed that ethylene synthesis genes were significantly up-regulated in SIBES7 T1 mutation plants.

XIV.

Chemical Control of Ethylene Action

INVITED TALKS

Chemical ethylene control during growth and postharvest storage of climacteric fruit: an engineering perspective

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1-MCP and ethephon are the most important chemical compounds that are used to control ripening in climacteric fruit. While 1-MCP retards ripening by irreversibly binding to the ethylene receptors, ethephon enhances ripening after decomposition to ethylene. In this presentation we will discuss both compounds, with a focus on engineering aspects. Ethephon is used in greenhouse tomato production to promote ripening late in the season and thus avoid the need for further heating the greenhouse. Recently there have some concerns by consumers about possible toxicological effects.
As a consequence, some retail chains are requesting ethephon free tomatoes. Unripe fruit can be directly treated postharvest by ethylene, but this has negative effects on the green parts of the harvested tomatoes. Alternatively, farmers started to apply ethylene directly in the greenhouse. We will show how computational fluid dynamics techniques can be used to optimise this process. 1-MCP was first registered for ornamentals in 1999 and later for a wide range of horticultural products with both preharvest and postharvest applications. The patent has been expired since 2014 and other companies are now commercialising 1-MCP or alternative techniques for in situ 1-MCP generation. We will review these products and also discuss practical aspects such as the distribution of 1-MCP in cool rooms for pome fruit and absorption of 1-MCP by wooden or plastic bins.

Towards systemic view for superficial scald in apple: an ethylene perspective

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Superficial scald is a major physiological disorder of apple fruit (Malus domestica Borkh.) characterized by skin browning following cold storage; however, knowledge regarding the downstream processes that modulate scald phenomenon is unclear. To gain insight into the mechanisms underlying scald tolerance, ‘Granny Smith’ apples after harvest were treated with diphenylamine (DPA) or 1-methylcyclopropene (1-MCP), then cold stored (0°C for 3 months) and subsequently were ripened at room temperature (20°C for 8 days). Phenotypic and physiological data indicated that both chemical treatments induced scald tolerance while 1-MCP inhibited the ethylene-dependent ripening. A combination of multi-omic analysis in apple skin tissue enabled characterization of potential genes, proteins and metabolites that were regulated by DPA and 1-MCP at pro-symptomatic and scald-symptomatic period. Specifically, we characterized strata of scald tolerance responses, among which we focus on selected pathways including dehydroabiestic acid biosynthesis and UDP-D-glucose regulation. Through this approach, we revealed scald-associated transcriptional, proteomic and metabolic signatures and identified both ethylene-dependent and independent components modulated by the common or distinct functions of DPA and 1-MCP. Evidence is also presented supporting that cytosine methylation-based epigenetic
regulation is involved in scald tolerance. To further characterize superficial scald outcomes, ‘Granny Smith’ apples were treated with 1-MCP and exposed to ethylene-free cold storage (0°C) in the absence (control) or presence of ozone (O3, 0.3 μL.L-1); their subsequent scald-related physiognomy was then tested. Ozone exposure strongly induced the scald symptoms while 1-MCP treatment alleviated O3-stimulated scald and shifted the levels of scald to those of 1-MCP-alone treated fruits. Comparative microarray gene expression profiling along with metabolomics and proteomics analysis dissects scald responses, unravels common 1-MCP- and O3-driven metabolic pathways and identifies specific genes/proteins associated with these different scald fates. Molecular components differentially expressed following these treatments were identified through bioinformatics approaches. Given that immature apples scald more rapidly than mature apples, we then try to connect the identified scald-responsive pathways with the on-tree apple fruit maturity physiology. Hence, we harvested apples 3 times at 7-day intervals (designed as early harvest, normal harvest, and late harvest). Following cold storage, a crucial set of on-tree maturity and scald development apple stages with diverse scald physiognomy will also used for multi-omics analysis. The development of Genetically Modified Organism (GMO)-free tools based on exogenous RNA application to selectively downregulate (through RNAi) or upregulate (through RNAa) the most prominent candidate genes that will emerge upon the aforementioned -omics analysis is also presented. Finally, the potential of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the CRISPR-associated protein 9 (Cas9) (CRISPR/Cas9) system to generate valuable apple plant breeding material for scald tolerance is highlighted.
Investigation of ethylene removal in fresh produce storage using advanced oxidation methods

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Fresh fruit and vegetables are highly perishable products. A major cause of rapid ripening and senescence in these products is ethylene. Thus, in order to prolong the shelf life of these commodities, ethylene removal becomes important. Two advance oxidation techniques: photocatalytic oxidation (PCO) with titanium dioxide as the photocatalyst and vacuum ultraviolet light (VUV) photolysis methods were investigated for ethylene removal. Both these processes involve irradiation by ultraviolet light, subsequently, generating reactive oxygen species such as hydroxyl radicals (OH•) and superoxide ions (•O\textsubscript{2}−) that eventually oxidize ethylene. The ethylene removal efficiency of both processes was analysed in a closed system, in a continuous flow-through system as well as in a case study for stored ‘Gala’ apples. The rate of ethylene removal was found to be dependent on the initial ethylene concentration. Based on the model and statistical parameters obtained, PCO process followed Langmuir Hinshelwood kinetics, while VUV followed first order kinetics. Oxygen favored both ethylene removal methods. However, humidity impeded PCO efficacy whereas high humidity enhanced VUV photolysis of ethylene. Temperature (within the range 1 to 21 °C) did not show significant effect on PCO process. On the other hand, the VUV photolysis process slowed down at lower temperature. Apart from these storage parameters, process parameters such as flow rate and ultraviolet light intensity also affected the efficiency of both processes. In the case study with storage of apples in chambers connected to the process reactors, the VUV reactor reduced ethylene concentration in the corresponding storage chamber to 2.6 ppm compared to 24 ppm in the storage chamber connected to PCO reactor at the end of 10 day storage at 1 °C. On the other hand, in the control chamber, without any reactor, the ethylene concentration soared up to 70 ppm at the end of storage. For future perspectives improving the efficiency of these two methods at low...
ethylene concentrations and low temperatures needs to be done. Also, further research into the impact of these processes on the quality of fresh commodities is required.

Transcriptome analysis reveals hormonal regulation of Ethephon induce ripening in field -grown sugarcane

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The hormone ethylene is used as a sugarcane ripener, affecting internode growth and sucrose storage. However, the molecular basis of ethylene action under field conditions has never been investigated in sugarcane. We selected two ripener-responsive sugarcane cultivars that contrast for season maturing (early and mid-late) to which ethephon was sprayed at the maturation stage following standard practices of commercial mills. Ethephon was able to boost ethylene emission levels in leaves and culms and to elicit expected phenotypical responses, including the production of shorter internodes at the culm apex, which probably shifts energy allocation to sucrose accumulation with an impact on sucrose yield. Ethephon-treated cultivars had on average 7.6 Kg sugar t¹ cane and 1% Pol increase when compared to mock treatment at harvest (65 days after chemical spraying). We selected younger fully expanded leaves and upper internodes to conduct a comprehensive transcriptome survey in three-time points after chemical application, generating 72 mRNA libraries sequenced by HiSeq 2500 Illumina platform. Among 4,843 differentially expressed transcripts identified in all comparisons, approximately 13.8% was involved in at least one hormone pathway in leaves or internodes, suggesting that the hormonal balance changes upon ethylene stimuli. The majority of hormone-related genes were induced in internodes, especially those in ethylene, abscisic acid (ABA), and brassinosteroid (BR) pathways. These data corroborate the findings from our previous experiment conducted under greenhouse condition. ABA functions as a ripening signal and potentially modulates sucrose response, while BR, auxin, and gibberellin have major roles in internode growth and development. Our results advanced the understanding of sugarcane ripening through a hormonal perspective, including the provision of a valuable set of ethylene responsive...
genes with potential roles in sucrose accumulation, internode growth, and stress tolerance that should be further investigated.

POSTERS

Application of ultraviolet light and titanium dioxide for ethylene removal in postharvest storage of horticultural commodities

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Ethylene removal is one of the important methods for shelf–life prolongation of horticultural commodities. Use of ultraviolet light and titanium dioxide (TiO2) is an emerging, non-conventional method for ethylene removal. TiO2 is a semiconductor that acts as photocatalyst. When ultraviolet radiations (wavelength <380 nm) fall on TiO2 surface, electron-hole pairs are generated on the catalyst surface that in turn react with surface adsorbed oxygen and water to produce reactive oxygen species (ROS). These ROS are highly reactive and oxidize ethylene to carbon dioxide. This process is called photocatalytic oxidation (PCO). Vacuum ultraviolet light (VUV) photolysis with wavelength <200 nm, without using catalyst, are self-sufficient in dissociating oxygen and water molecules to produce ROS that eventually oxidizes ethylene. A study was done to investigate the efficiency of ethylene removal in PCO and VUV processes. Two steel lab scale reactors (diameter 12 cm and height 11 cm) were developed with three ultraviolet lamps (3W each) placed inside. For photocatalytic oxidation, ultraviolet lamps with radiation at 254 nm were used, on the other hand for VUV photolysis; ultraviolet lamps with major radiation at 254 nm and minor radiation at 185 nm were used. In PCO reactor, there was additionally TiO2 coated onto glass surface. VUV reactor showed faster ethylene removal efficiency than PCO. In a flow through system, a continuous supply of 10 ppm ethylene through the reactor at 0.24 L/min flow rate ethylene removal was 80 % in VUV whereas it was only <20 % in PCO. The VUV reactor connected to fruit storage chamber was able to reduce the ethylene accumulated
in the chamber to a higher extent than PCO reactor. Further research into improving the efficiency of both these techniques through catalyst modification, improved reactor design as well as optimizing process along with storage parameters are still needed for an efficient application in storage and transport of horticultural commodities.