Michael McManus Award

Transcriptomic analysis implicates ABA signaling and carbon supply in the differential outgrowth of petunia axillary buds

Zhiwei Luo^{1,2}, Dan Jones³, Sarah Philp-Wright², Joanna Putterill², Kimberley Snowden¹

¹The New Zealand Institute for Plant and Food Research Limited, Auckland, New Zealand.

²School of Biological Sciences, University of Auckland, Auckland, New Zealand.

³NetValue Limited, Hamilton, New Zealand.

Abstract

Shoot branching of flowering plants is determined by the activity of axillary meristems, which in turn is influenced by endogenous and exogenous cues such as nutrients and light. In many species, not all buds on the main shoot develop into branches despite favorable growing conditions. In *Petunia hybrida*, basal buds 1-3 typically do not grow out to form branches, while buds 6 and 7 are competent to grow.

The genetic regulation of buds was explored using transcriptome analyses of petunia axillary buds at different positions on the main stem. Using RNA-seq, we found many (> 5000) differentially expressed genes between bud 6/7, and bud 2. Interestingly, more genes were differentially expressed when we transferred the plants from low phosphate (P) to high P media, compared to shifting from high P to low P media. Buds 6 and 7 had increased transcript abundance of cytokinin and auxin-related genes, whereas bud 2 and to a lesser extent bud 3 had higher expression of strigolactones, ABA, and dormancy-related genes, suggesting the outgrowth of these basal buds was actively suppressed. Consistent with this, the expression of ABA associated genes decreased significantly in bud 6/7 after stimulating growth by switching the media from low P to high P. Comparisons between our data and transcriptome data from other species suggest that the suppression of outgrowth of bud 2 was correlated with a limited supply of carbon to these axillary buds. Candidates were identified and will be the focus of future work to investigate their ability to alter shoot branching.

NZ Hubs Session 1 - Thursday

NbPTR1 confers resistance against Pseudomonas syringae pv. actinidiae in kiwifruit

Shin-Mei Yeh¹, Minsoo Yoon¹, Sidney Scott¹, Abhishek Chatterjee¹, Lauren M. Hemara^{1,2}, Ronan K.Y. Chen³, Tianchi Wang¹,Kerry Templeton⁴, Erik H.A. Rikkerink¹, Jay Jayaraman¹, Cyril Brendolise¹

¹The New Zealand Institute for Plant & Food Research Limited (PFR), Mt Albert Research Centre, Auckland 1025, New Zealand^{, 2} School of Biological Sciences, University of Auckland, Auckland 1010, New Zealand, ³ The New Zealand Institute for Plant and Food Research Limited (PFR), Palmerston North 4474, New Zealand, ⁴ The New Zealand Institute for Plant and Food Research Limited (PFR), Motueka 7198, New Zealand

* shin-mei.yeh@plantandfood.co.nz

Abstract

Pseudomonas syringae pv. actinidiae biovar 3 (Psa3) causes devastating canker disease in yellow-fleshed kiwifruit (*Actinidia chinensis*). The effector HopZ5 from Psa, the agent responsible for the bacterial canker in kiwifruit, triggers immunity in *Nicotiana benthamiana* but is not recognised in susceptible *A. chinensis* cultivars. In a search for non-host resistance genes against HopZ5, we found that the nucleotide-binding leucinerich repeat (NLR) protein PTR1 from *Nicotiana benthamiana* is involved in the recognition of HopZ5, likely involving targeting of guarded plant RIN4 proteins. RPM1-interacting protein 4 (RIN4) orthologues from *N. benthamiana* and *A. chinensis* formed a complex with NbPTR1 and HopZ5 activity was able to disrupt this interaction. No functional orthologues of Nb*PTR1* were found in *A. chinensis*.

Transformation of the Nb*PTR1* gene into Psa3-susceptible *A. chinensis* var. *chinensis* 'Hort16A' plants introduced HopZ5-specific resistance against Psa3. This study suggests that expressing NbPTR1 in Psa3-susceptible kiwifruit is a viable approach to acquiring resistance to Psa3 and provides valuable information for boosting resistance of susceptible kiwifruit against canker disease.

Analysis of role of *MtFULa* and *MtFULb* in Medicago growth, flowering and seed barrel yield

Cristian Lee-Hsiao¹, Lulu Zhang¹, Joanna Putterill¹

¹ School of Biological Sciences, University of Auckland/Auckland 1142, New Zealand

* clee820@aucklanduni.ac.nz

Abstract

The increasing demand for food is creating a need to improve crop production. From promoting soil health to producing nutritious seeds, legumes are a desirable crop target for improvement, as their poor yield constrains them. Flowering time is a crucial trait affecting crop plants' yield and productivity. This trait has been well studied in other plant models, such as Arabidopsis thaliana (Arabidopsis), but less is understood in legumes. One of the genes related to flowering time and seed yield in Arabidopsis is the FRUITFULL (FUL) gene. Medicago truncatula (Medicago), a model legume plant, was found to have two similar FUL genes, MtFULa and MtFULb, however, their role in Medicago is still unknown. Mtful Medicago mutants with genes edited using CRISPR-Cas9, singly or in combination, were analyzed via genotyping (PCR and sequencing analysis) and phenotyping. *Mtfula/b* double mutants did not display a significant difference in their flowering time compared to wild type, however, they kept flowering for a longer time. Moreover, the double mutants were found to exhibit decreased yield. Compared to wild type, all mutant plants produced a higher number of seed barrels, but the barrels where significantly smaller in size, resulting in a ~40% lower yield (measured by weight). This study suggests that the MtFULa and MtFULb genes in Medicago plays a role in the plant's fruit development, and disruption of these genes affects this trait negatively. On the other hand, loss of MtFULa/b function leads to delayed proliferative arrest. Results of RNA-seq will be presented and similarities and differences to FUL function in garden pea will be discussed.

Gillenia trifoliata provides a unique perspective on apple and Rosaceae

<u>Hilary Ireland</u>¹, Ruiling Wang¹, Cecilia H. Deng^{1,2}, Chen Wu^{1,2}, Elena Hilario^{1,2}, Amandine Cornille³, William Marande⁴, Elena López-Girona⁵, Toshi Foster⁶, Ria Rebstock¹, Paul Sutherland¹, Peter McAtee¹, Cyril Hamiaux¹, Ting-Hsuan Chen¹, Ali Saei^{2,5}, Slyvia Erasmuson⁷, Wendy Hall¹, Ross Crowhurst^{1,2}, Jia-Long Yao¹, David Chagné^{2,5}, Karine M. David⁸, Robert J. Schaffer^{6,8}

¹ The New Zealand Institute for Plant and Food Research Ltd, Private Bag 92169 Auckland 1142, New Zealand; ² Genomics Aotearoa, % Department of Biochemistry, University of Otago, PO Box 56, Dunedin 9054, New Zealand; ³ Université Paris Saclay, INRAE, CNRS, AgroParisTech, GQE-LeMoulon, 91190 Gif-sur-Yvette, France; ⁴ INRAE, CNRGV, 31326 Castanet-Tolosan, France, ⁵ The New Zealand Institute for Plant and Food Research Ltd, Batchelar Road, Fitzherbert, Palmerston North 4474, New Zealand; ⁶ The New Zealand Institute for Plant and Food Research Ltd, 55 Old Mill Lane, Motueka, New Zealand; ⁷ The New Zealand Institute for Plant and Food Research Ltd, Private Bag 4704, Christchurch Mail Centre, Christchurch 8140, New Zealand; ⁸ School of Biological Sciences, The University of Auckland, Private Bag 91629, Auckland 1142. New Zealand

* hilary.ireland@plantandfood.co.nz

Abstract

Rosaceae exhibits a diverse array of commonly consumed fruits including berryfruit, summerfruit and pipfruit, and yet also exhibits high degrees of genome synteny. Gillenia trifoliata, one of two species in the *Gillenia* genus, is sister taxon to the apple (Maleae) tribe bearing an unduplicated genome, has an ancestral chromosome complement (x=9), and putatively ancestral characteristics (e.g., sub-shrub habit, annual bearing, dryfruit type), making it useful for comparative genomics. We are using Gillenia to understand different aspects of Rosaceae biology, including self-incompatibility, genome dynamics related to growth, and fleshy fruit development. Rosaceae bears two different mechanisms of self-incompatibility (SI) – a genetic strategy employed by many plants to promote outcrossing. Using comparative genomics between *Gillenia*, *Prunus* and Maleae, we have found that the Maleae ancestor lost the self-recognition SI mechanism it shared with Prunus, then regained a nonself-recognition SI using a rudimentary structure found in the ancestor of Gillenia and Maleae, which demonstrates the labile nature of evolution of nonself-recognition SI mechanisms that control reproductive outcomes in many angiosperms. In exploring genome dynamics related to growth we identified clade contractions/expansions in MADS-box and NAC transcription factors correlated with growth habit and reproductive phenotypes. A fleshy-fruit development study of apple, and using Gillenia as a negative control, suggests the regulation for flesh development is controlled by AE-type MADS-box genes which promote spatiotemporal cell division, cell expansion, and cell wall modification, while also suppressing processes common to dry fruit types. These examples demonstrate the opportunities of using Gillenia to better understand Rosaceae biology.

Genome assembly and annotation of host-specific necrotrophic fungus *Ciborinia camelliae* ICMP19812

<u>Deny Kumar Shrestha</u>¹, Christopher Brown², Tina Sehrish¹, Carl Mesarich¹, Ron van Toor^{3,4*}, Paul Dijkwel¹

¹ Massey University/Palmerston North, ² University of Otago/Dunedin, ^{3,4*} StatsWork 2022 Ltd/Lincoln, ⁴ The New Zealand Institute for Plant and Food Research Limited (PFR), Lincoln, NZ, ^{*} Formerly at PFR.

Email: denykumarshrestha@gmail.com

Abstract

Ciborinia camelliae, a host-specific necrotrophic pathogen from the Sclerotiniaceae family, causes camellia flower blight. This fungus is under-explored despite it being a major problem for ornamental camellia plants. Using PacBio and Illumina technology, we have sequenced the genome of C. camelliae isolate ICMP19812, which was collected from the Palmerston North area of New Zealand. The PacBio reads were assembled using Flye and polished with PacBio and Illumina reads using Medaka and Pilon, respectively. The assembled genome is 44.98 Mb in size across 37 contigs. BUSCO analysis indicated the assembly had 98.7% completeness out of 758 searched groups ensuring the quality of the assembly. The genome was annotated using Funannotate, which predicted 11,691 gene models, of which 11,484 were predicted to encode proteins. Of these, 986 were predicted by SignalP6 to be secreted, from which 308 were also predicted by EffectorP3 to be effector proteins. Alphafold2 has been used to predict the 3D structures of these predicted effector proteins. These will now be clustered according to structural similarity to help identify effectors with similar functionals in promoting host colonization. This study contributes as a reference to other isolates or related species and also for the study of fungal evolution of the Sclerotiniaceae.

Flavones in *Marchantia polymorpha*: Discoveries that tell a story of evolution

<u>Rebecca Yorker</u>¹, John van Klink², Tony McGhie¹, Sarah Cordiner¹, Samarth Kulshrestha¹, Jennifer Tate³, Kevin Davies¹, Nick Albert¹

¹ The New Zealand Institute for Plant and Food Research Limited, Private Bag 11600, Palmerston North 4442, New Zealand, ² Department of Chemistry, Otago University, The New Zealand Institute for Plant and Food Research Limited, Dunedin 9054, New Zealand, ³ School of Agriculture and Environment, Massey University, Palmerston North 4442, New Zealand

* Rebecca.yorker@plantandfood.co.nz

Abstract

Flavones are a major class of plant secondary metabolites that evolved during land plant colonisation to provide UV-B light tolerance. They can undergo various modifications due to different hydroxylation and glycosylation capabilities of plants. Flavones have evolved several times throughout plant evolution, leading to the acquisition of several biosynthetic enzymes; angiosperms use either FLAVONE SYNTHASE I (FNSI) or II (FNSII), whereas in vitro assays suggest that bryophytes contain an FNSI enzyme structurally different from those in angiosperms. An FNSI candidate has been identified in Marchantia polymorpha (hereafter, Marchantia), a model species for the bryophyte lineage, and CRISPR/Cas9 mutagenesis revealed that it is solely required for flavone biosynthesis in Marchantia. This suggests that the use of an FNS enzyme for flavone biosynthesis may be a conserved characteristic among land plants. Chemical analysis of Marchantia sporangia allowed for the identification of a unique yellow flavone called isoscutellarein, discovered to also be synthesised by FNSI. This compound is absent in the thallus, which raises the question of whether it functions in UV-B tolerance or if it was acquired for a different purpose. Another unique characteristic of Marchantia is the presence of a 3' GLYCOSYL TRANSFERASE (3'GT) enzyme that glycosylates the flavone luteolin. While GTs are common in plants, no 3'-specific GT has been characterised in planta until now. These findings have significantly improved our understanding of the evolution of flavone production and biosynthesis among land plants and highlights that flavone production has remained important throughout land plant evolution for UV-B protection.

The *Medicago truncatula*- rhizobium symbiosis confers drought tolerance to the host

<u>Ramadoss Dhanuskodi^{1,2,3}</u>, Cory Matthew², Tsanko S. Gechev³, Michael T. McManus², Paul P. Dijkwel²

1 AgResearch, Grasslands Research Centre, Palmerston North, New Zealand, 2School of Agriculture and Environment, Massey University, Palmerston North, New Zealand, 3 Department of Molecular Stress Physiology, Centre for Plant Systems Biology and Biotechnology, Plovdiv, Bulgaria. * rama.dhanuskodi@agresearch.co.nz

Abstract

Legumes can fix nitrogen (N) through symbiotic N-fixation (SNF) through association with rhizobia soil bacteria. However, this incurs a high energy cost to the plant due to the energy requirements of the fixation reaction. N uptake from soil requires comparatively less energy, and we therefore hypothesised that under drought stress, plants obtaining N through SNF would be more affected by stress than N-fertilised plants.

To test this, *Medicago truncatula* seedlings were either inoculated with a rhizobium strain or fertilised with N. Drought stress was applied 20 days post inoculation by withholding water for 12 days. We measured water status, symbiosis, and plant growth to assess the influence of drought on growth and development. Additionally, we performed RNA sequence analyses on leaves before and after drought to investigate stress-induced transcriptomic responses.

We found that drought affected the leaf water status in both rhizobium-inoculated and N-fertilised plants similarly. However, rhizobium-inoculated plants maintained better plant growth, as shown by more vigorous growth and reduced leaf death. Transcriptomic analysis revealed that the *rhizobium*-inoculated plants preactivated stress-response and metabolite pathways such as jasmonic acid and trehalose biosynthesis at the onset of drought and further upregulated stress tolerance hormones and proline synthesis as drought continued. By contrast, the N-fertilised plants upregulated ethylene biosynthesis and senescence processes.

Contrary to our hypothesis, these results suggest that rhizobium inoculation induced a priming-like response leading to drought tolerance. By contrast, drought treatment of N-fertilised plants resulted in growth cessation and leaf death; a survival mechanism that reduces plant water requirement.

ASPS/NZSPB Joint Session: NZ talks

Roger Slack Award

Genomics for restoring a critically threatened tree species in the rohe of Rangitāne o Manawatū

Colan Balkwill1, Keith Funnell2, Emily Koot2, Julie Deslippe1, Alana Nuku3, Paul Horton3, Wayne Blissett3, David Chagné2,4

1 Victoria University of Wellington, Wellington

2 Plant & Food Research, Papaioea

3 Rangitāne o Manawatu, Papaioea

4 Genomics Aotearoa

Swamp maire (Syzygium maire; maire tawake) is an endemic tree species of Aotearoa's swamp forests that is currently listed as nationally critical due to habitat loss and, most recently, infection by myrtle rust. With fewer than twenty mature trees of swamp maire remaining within the Rangitāne o Manawatū rohe, including a remnant population under threat from the construction of Te Ahu a Turanga Manawatū Tararua, a Mana Whenua-led project was set up for conserving the species in the rohe, in accordance with Rangitānenuiarawa (Rangitāne o Manawatū tikanga). Genome sequencing of naturally occurring trees and seedlings from within the rohe was performed to generate knowledge of genetic diversity. A high-quality reference genome was assembled for the species, becoming the first genome sequence to be named by an indigenous group (Ngā Hua o te la Whenua). This genomics-based mahi focused on understanding the past and current population structure, how much inbreeding has occurred and how related trees are to each other and to other populations in Aotearoa. This research contributed to developing a restoration plan integrating Mātauranga Māori, genetic diversity and habitat suitability for replanting.

Fast flowering as a tool for gene discovery in woody perennials

<u>Allan, A.C.1,2</u>

1Plant & Food Research, Mt Albert, Auckland, New Zealand 2School of Biological Sciences, University of Auckland, Auckland, New Zealand

Plants should be considered as a third of the solution to the climate crisis, as they fix CO2 and make all our food (directly or indirectly). Moving to a more plant-based economy requires both new crops and enhanced climate-resistance of existing crops.

New Zealand's horticultural sector is based on temperate perennials. Breeding woody perennials requires a very long-term program. However, can genetic gain be quick enough in crops which have long generation cycles (seed-plant-seed)? New Breeding Technologies (NBTs) use molecular methods that quickly provide step changes in traits. We are using NBTs to make novel crosses with plants that are more floral.

The question remains of how NZ will respond to such plants, which have no additional DNA and harbour only new variants of genes which are identical to "natural" variants already in the environment. In most countries (but not NZ) these resulting plants are not regulated. NZ must quickly decide if NBTs will play a part in our response to a changing climate.

FRSNZ presentation

The Evolution Of Flavonoid Biosynthesis

<u>Kevin M. Davies¹</u>, Nick W. Albert¹, Yanfei Zhou¹, Samarth Kulshrestha¹, Rubina Jibran², John W. van Klink³, David Chagné¹, Marco Landi⁴, Peter Schafran⁵, Fay-Wei Li⁵, Stefan J. Hill⁶, John L. Bowman⁶

¹ Plant and Food Research, Private Bag 11600, Palmerston North 4442, NZ; ² Plant and Food Research, Private Bag 92169, Auckland Mail Centre, Auckland 1142, NZ; ³ Plant and Food Research, Department of Chemistry, Otago University, Dunedin 9054, NZ; ⁴ Department of Agriculture, Food and Environment, University of Pisa, Italy; ⁵ Boyce Thompson Institute, Ithaca, NY 14853, USA; ⁶ Scion, Private Bag 3020, Rotorua 3046, NZ; ⁷ School of Biological Sciences, Monash University, Melbourne, VIC 3800, Australia * kevin.davies@plantandfood.co.nz

Abstract

The flavonoid pathway is characteristic of land plants and a central biosynthetic component enabling life in a terrestrial environment. It is one of the most studied plant characters, and the subject of >15,000 journal articles each year. However, it is only with the recent advent of model systems for non-seed plants, that we have started to understand how and why the pathway may have evolved. The phylogenetic and functional data on non-seed plants challenge the idea of a canonical flavonoid pathway inherited from the Last Common Ancestor (LCA) of all land plants. Rather, it suggests extensive gene losses and gains within each lineage.

Some flavonoid pathway branches are well conserved and may have been present in the LCA. In particular, the UVR8/HY5-mediated induction of colourless flavonoids for tolerance of UVB-light is strongly conserved between Arabidopsis and the liverwort *Marchantia polymorpha*. In contrast, the stress-related red pigments have striking biosynthetic and functional diversity. Notably, the red pigments of liverworts are a previously unreported flavonoid type 'auronidins' that are cell-wall located polymers that provide protection against abiotic and biotic stresses. One lineage, the hornworts, has lost flavonoid biosynthesis entirely. Yet genome sequencing for eight hornwort genera found a single 'canonical' flavonoid biosynthetic gene in the phylogenetic outlier hornwort species. Thus, the hornwort ancestor may have inherited the flavonoid pathway but the biosynthetic and regulatory genes were lost during lineage-specific evolution. The results illustrate the importance of extending studies out from the usual suspects of plant models and across the embryophyte diversity.

NZ Hubs Session 2 - Friday

Movement of in vitro kiwifruit plants: Strategic measures to overcome Psa-3 Challenges

<u>Azadeh Esfandiari¹, Vishant Tomar¹</u>, Jayanthi Nadarajan¹, Ed Morgan¹, Claudia Wiedow¹, Philippa Barrell², Falk Kalamorz ², Alison Duffy³, Phil Martin⁴

¹ The New Zealand Institute for Plant and Food Research Limited, Palmerston North 4410, New Zealand

² The New Zealand Institute for Plant and Food Research Limited, Lincoln 7608, New Zealand

³ The Kiwifruit Breeding Centre Limited (KBC), Mt Albert 1025, New Zealand

⁴ The Kiwifruit Breeding Centre Limited (KBC), Te Puke 3182, New Zealand

* Email address :Azadeh.esfandiari@plantandfood.co.nz

Abstract

The arrival of Pseudomonas syringae pv. actinidiae (biovar 3; Psa-3) in New Zealand in 2010, and its limited distribution, has provided opportunity for the kiwifruit industry to put in place protocols to prevent further spread of this pathogen to unaffected growing areas. This has required the imposition of regulations to govern plant movement within New Zealand, enforced by Kiwifruit Vine Health (KVH) and the Ministry for Primary Industries (MPI). These measures restrict the movement of plants from areas where Psa-3-infected plants are found ("recovery regions") to Psa-3-free regions ("exclusion regions"). To transfer plants from recovery regions (mainly in the North Island) to exclusion regions (such as the South Island), rigorous testing of tissue-cultured plants and protocols for managing plants are required both to confirm and to maintain Psa-3-free status. This includes PCR testing for Psa-3, and repeated screening of in vitro plants, as well as testing and monitoring of plants in greenhouse and outdoor growing conditions. Since 2019, 26 kiwifruit genotypes have been successfully sent to the South Island for both the Kiwifruit Breeding Centre and Zespri. This has included Actinidia chinensis var. chinensis 'Zes008' (marketed as Zespri RubyRed[™] Kiwifruit), previously not been available to South Island growers. This presentation will cover the protocols, laboratory procedures, and challenges in producing, maintaining and moving Psa-free healthy plants to support the New Zealand kiwifruit industry.

Using common plant secondary metabolites to disrupt the infection process of Sclerotiniaceae fungi *Botrytis cinerea* and *Ciborinia camelliae*

Gabriela Ricardo¹, Nikolai Kondratev¹, Svetla Sofkova-Bobcheva¹, Paul Dijkwel¹

¹ School of Agriculture and Environment, Massey University, Palmerston North, New Zealand

* gricardo@massey.ac.nz

Abstract

Phytopathogenic fungi have major impacts in plant development including post-harvest. These fungi have optimized the acquisition of nutrients from their hosts resulting in a huge economical loss. The Sclerotiniaceae fungal family includes a large number of these pathogens, including the generalist *Botrytis cinerea* – the causal agent of grey mould, which affects many crops worldwide with high financial impact – as well as Ciborinia camelliae, a specialist that causes Camellia Petal Blight uniquely on Camellia blooms. From our previous studies it is known that *Camellia lutchuensis* is inherently resistant to C. camelliae and this is associated with the early activation of the phenylpropanoid pathway upon infection. We tested if these secondary metabolites could inhibit the infection of susceptible Camellia hybrid 'Nicky Crisp' with C. camelliae, and additionally in Arabidopsis thaliana with B. cinerea. We applied nine phenylpropanoids by means of droplet incubation, whole petal/leaf sprays, and by feeding through the vascular system. Five phenylpropanoids effectively inhibited mycelial growth and lesion progression. Spores of both fungi were unable to germinate, and mycelium development and the capability to penetrate the upper epidermis also decreased. Furthermore, we have shown that these metabolites reduce sporemembrane attachment, suggesting that spore cell wall integrity is compromised by their application.

Intergeneric hybrids between apple and pear

<u>Claudia Wiedow¹</u>*, Giulia Pasqualetto², Vincent G.M. Bus³, Ross G. Atkinson⁴, Edwige J.F. Souleyre⁴, David Chagné¹, Susan E. Gardiner¹, Satish Kumar³

¹ The New Zealand Institute for Plant and Food Research Limited (Plant & Food Research), Palmerston North 4410, New Zealand

² Meristema srl Società Agricola, Loc. Riaccio snc, 56032 Cascine di Buti (PI), Italy

³ Plant & Food Research, Havelock North 4157, New Zealand

⁴ Plant & Food Research, Auckland 1142, New Zealand

* Email address: Claudia.Wiedow@plantandfood.co.nz

Abstract

Intergeneric hybrids between Malus and Pyrus may provide a unique germplasm resource for cultivar development. Both genera are closely related, with highly co-linear genomes; however, they are characterised by many phenotypic differences, including distinct patterns of secondary metabolites, disease resistances, fruit texture, flavour and shape, and tree architecture.

Following analysis using flow cytometry and single sequence repeat (SSR) marker alleles 27 hybrids were identified in 2014. Four of these hybrids showed the Dwarfing 1 (Dw1) allele, the principal dwarfing locus in 'Malling 9' apple rootstock. Metabolomics analysis showed the presence in one 'M27' x 'Comice' hybrid of metabolites typical of both pear (arbutin) and apple (phloridzin). Analysis of a further 51 putative hybrid plants using High Resolution Melting analysis, SSR, single nucleotide polymorphism markers, and metabolomics, confirmed hybridity of a further 41 'Cox's Orange Pippin' x 'Old Home', five P265R232T018 x apple seedlings and five 'Fuji' x P125R095T02 progeny. The findings of this work will enhance and accelerate the breeding of novel tree fruit crops that benefit producers and consumers, by introgression of desired traits between pear and apple.

Climatic influences on genetic differentiation in bilberry (*Vaccinium myrtillus*) populations from Europe

<u>Astra Heywood</u>¹, Emily Koot², Laura Jaakola³, Lara Giongo⁴, Richard Espley⁵, David Chagné²

¹ The New Zealand Institute for Plant and Food Research Limited, Lincoln, New Zealand

² The New Zealand Institute for Plant and Food Research Limited, Palmerston North, New Zealand

³ The Arctic University of Norway, Tromso, Norway

⁴ Fondazione Edund Mach, Trento, Italy

⁵ The New Zealand Institute for Plant and Food Research Limited, Mt Albert, New Zealand

* astra.heywood@plantandfood.co.nz

Abstract

Bilberry (*Vaccinium myrtillus*) belongs to the same genus as North American blueberry. While blueberries have undergone domestication and are in commercial cultivation, bilberries are primarily wild foraged from woodlands and heathlands across Europe. Bilberries are sensitive to environmental changes, and their populations can serve as indicators of ecosystem health. Despite their cultural significance and ecological value, the genetic diversity of bilberries is not yet well understood. This study collected samples of 328 plants from various locations across Europe to better understand the genetic, phenotypic, and environmental relationships among bilberry populations.

Whole-genome sequencing of the collected plants identified 1,037,260 high-confidence variants across the genome. Population structure analysis indicated there are likely three ancestral populations across bilberry's natural range. Accessions from Scandinavia and Western Spain clustered genetically apart from the other European accessions. The other European accessions form a dense cluster spanning a wide geographical range from the UK to Bulgaria. Hybridisation of sympatric populations for each cluster with the other European accessions were observed for plants of the Scandinavian and Western Spanish clusters in Scotland and the French Pyrenees, respectively.

To explore the relationship between climate and genetic variation among populations, climatic variables such as temperature and precipitation were analysed using genetic variants as features in a series of Random Forest models. This analysis identified the importance of cold for six genetic variants in genes involved in flower development, chloroplast function, and cell growth. Here, we present our methodologies and findings regarding the climatic associations of genetic differentiation in bilberry. This knowledge is useful for bilberry conservation in the face of climate change, and for informing hybridization with blueberry.

Trait response in diverse red clover germplasm populations under water stress and three-year field trials.

Angus D. Heslop^{1,2}, Rainer W. Hofmann¹, Zulfi Jahufer³

¹ Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, New Zealand. ² AgResearch Limited, Lincoln Research Centre, Christchurch, New Zealand. ³ School of Agriculture and Food Sustainability, The University of Queensland, St Lucia, Brisbane Qld 4072, Australia. * angus.heslop@agresearch.co.nz

Abstract

Red clover (Trifolium pratense L.) is known for its large taproot and ability to produce quality forage. Being an outcrossing species, red clover populations contain a high level of genetic diversity. If evaluated and utilized correctly, this can be used to develop productive, adaptive cultivars that can strengthen temperate pastures. In this study, using glasshouse physiological experiments, multi-location, multi-year field trials, we were able to assess both above and below ground trait response to water stress, evaluate plant yield, plant persistence and root structure of diverse red clover germplasm populations. Under water deficit, the levels of all aboveground morphological traits decreased. This resulted in an increase in root to shoot ratio, which indicated a shift to prioritizing root maintenance by sacrificing shoot growth, a trait attributed to plant water deficit tolerance. Under waterlogging, the importance of root morphology for waterlogging was highlighted with low performance of red clover. After three years growth in field conditions the biomass production of most of the germplasm populations compared to the cultivar controls was low. However, we did observe key relationships between root structure and plant persistence and plant production. With plants seeming to have either an expansive, compact or a mixture of both root systems. Our study identifies the importance of evaluating diverse germplasm for addition into breeding programs and highlights the adaptability needed by traits to ensure peak performance and survival.

NZ Hubs Session 3 – Friday

Phytohormone metabolism modulates the pre-climacteric phase of 'Hass' avocado (*Persea americana* Mill.) fruit after harvest

<u>C.S. Günther¹</u>, D.J. Jensen¹, D. Billing², T.M. Trower¹, Lucia Ying¹, J.M. Cooney¹ and J. Burdon²

The New Zealand Institute for Plant and Food Research Limited.

¹ Ruakura Research Centre, Bisley Road, Hamilton 3214, New Zealand

² Mt Albert Research Centre, Auckland 1025, New Zealand

* Email address: Catrin.Guenther@plantandfood.co.nz

Abstract

The 'Hass' avocado fruit (*Persea americana* Mill.) is strongly climacteric, with clear preclimacteric and climacteric phases during ripening. In contrast to most fruits, avocados do not ripen on the tree, possibly due to an inhibitor transmitted from the tree to the fruit. The pre-climacteric phase (PCP) tends to decrease with the fruits' increasing physiological age, and thus may differ substantially among fruit from a single harvest. Better knowledge of phytohormones coordinating the PCP of avocados may provide increased opportunities to homogenise the onset of ripening.

In this study, seven phytohormone classes were monitored in individual fruit using biopsy samples taken at harvest, the following three days thereafter, when fruit develop ethylene sensitivity, and when fruit started producing ethylene marking the end of the PCP. Through this approach, changes in the metabolism of abscisic acid (ABA), auxin, gibberellins (GA), salicylates, jasmonates, cytokinins and, for the first time in avocado, brassinosteroids, were revealed. Findings support that ABA may expedite ripening of avocado in concert with ethylene, while phytohormones associated with plant growth and defence likely extend the PCP duration. Brassinosteroid metabolism was negatively associated with the climacteric phase, although the accumulation of 6-deoxocastasterone, a key intermediate of castasterone biosynthesis, was strongly positively correlated with ABA content. Variation in PCP-duration among the individual fruit allowed for the segregation of fruit phytohormone profiles. Avocados with long PCP showed higher contents of bioactive GAs at harvest than fruits with short PCPs, indicating that GA metabolism may coordinate ethylene sensitivity early in the PCP.

Characterisation of sugar transporters in kiwiberry

<u>M. Jaudal1^{1*}</u>, H. Boldingh², T. Pereira³, J. Cho¹, S.M. Bulley³, T. Wang¹, A.C. Richardson⁴, S. Nardozza¹

The New Zealand Institute for Plant and Food Research Limited, ¹Mt Albert Research Centre, Auckland New Zealand, ²Ruakura Research Centre, Ruakura New Zealand, ³ Te Puke Research Centre, Te Puke New Zealand, ⁴ Kerikeri Research Centre, Kerikeri New Zealand

* Mauren.Jaudal@plantandfood.co.nz

Abstract

Fruit sugar content contributes to sweetness and flavour, which is an important consumer quality trait. In the history of kiwifruit breeding, sweetness has been a major driving force in domestication. In kiwiberry (Actinidia arguta), adequate storage of soluble sugars such as starch or dry matter (DM) reserve is important to produce fruit with acceptable flavour. However, the molecular mechanisms of sugar transport and accumulation in kiwifruit remain obscure. Sugar transporters have important roles in driving the transport of sugars from photosynthetic source leaves to sink organs and are mainly classified into three main groups: the monosaccharide transporter-like (MST) gene family with members like Sugar Transport Proteins (STPs); the sucrose transporters (SUCs); and the Sugars Will Eventually be Exported Transporter (SWEET). In this study, we showed that AaSTP3, AaSTP14 and AaSWEET1 are expressed in fruit. Kiwiberry has two copies of AaSWEET1 and disruption of these sugar transporter genes using RNAi lines contributes to changes in sugar unloading and other aspects of flower and fruit growth, development, and physiology. These findings may provide opportunities for use of markers or strategies in breeding programmes that aim to enhance selection or development of varieties with improved fruit sweetness and flavour.

Strigolactone precursor biosynthesis genes regulate asexual reproductive growth in *Marchantia polymorpha*

<u>Rubina Jibran</u>¹, Jibran Tahir¹, Christelle M Andre¹, Bart J. Janssen¹, Revel S.M. Drummond¹, Nick W. Albert², Yanfei Zhou², Kevin M. Davies², Kimberley C. Snowden¹

¹The New Zealand Institute for Plant and Food Research Limited, Auckland, New Zealand, ²The New Zealand Institute for Plant and Food Research Limited, Palmerston North, New Zealand * rubina.jibran@plantandfood.co.nz

Abstract

Strigolactones (SLs) play crucial roles controlling shoot and root architecture in flowering plants, regulating underground communication with symbiotic arbuscular mycorrhizal fungi. While the functions of core SL genes have been characterized in many plants, their roles in non-tracheophyte plants like liverworts require further investigation. We employed the model liverwort species Marchantia polymorpha, which lacks detectable SL production and orthologs of key SL biosynthetic genes but does retain some SL components, including DWARF27 (D27) and CAROTENOID CLEAVAGE pathway DIOXYGENASE 7 (CCD7). To help elucidate the function of these remaining components, knockout mutants were generated for MpD27–1, MpD27–2 and MpCCD7. Phenotypic comparisons of these mutants with the wild-type control revealed a novel role for these genes in regulating the release of gemmae from the gemma cup and the germination and growth of gemmae in the dark. *Mpd27–1*, *Mpd27–2*, and *Mpccd7* mutants showed lower transcript abundance of genes involved in photosynthesis, such as EARLY LIGHT INDUCED, and stress responses such as LATE EMBRYOGENESIS ABUNDANT but exhibited higher transcript levels of ETHYLENE RESPONSE FACTORS and SL- and carotenoid-related genes, such as TERPENE SYNTHASE, CCD7 and LECITHIN-RETINAL ACYL TRANSFERASE. Furthermore, the mutants of M. polymorpha in the SL pathway exhibited increased carotenoid content. This unveils a role for MpD27-1, MpD27-2 and MpCCD7 in controlling release, germination, and growth of gemmae in response to varying light conditions. These discoveries enhance our comprehension of the regulatory functions of SL biosynthesis genes in non-flowering plants.

Increasing *Vitis Vinifera* 'Sauvignon Blanc' Transformation Efficiency of Tissue Culture Through Somatic Embryogenesis Using BabyBoom as a Candidate Gene

Tan, Lun¹, Sugiyama, Suguru², Winefield, Christopher³

^{1, 2, 3} Lincoln University* Lun.Tan@lincolnuni.ac.nz (presenting author)

Abstract

Vitis Vinifera is highly recalcitrant to somatic embryogenesis (SE) transformation. The traditional hormone base tissue culture recovery is inefficient for most variety within the *Vitis Vinifera* species. High success rate recovery of certain grape varietals is condition specific, as most transformation requires reproductive base cells such as anther filaments or ovaries of the grapevine plant. This means that it's time specific, requiring once a year for an opportunity to gather new material for tissue culture base cloning. While low success rates see's less than a 1.49% in recovery from the total culture created. An example being of 1,000 anther filament culture less than 15 is expected to be transformed. Sauvignon Blanc is one of the varietals that is within the mentioned 1.49% low recovery group.

However, many species have been successful in improving the rate of transformation utilizing transcription factor base approaches. Crops such as *Gossypium herbaceum* (cotton), *Theobroma cacao* (cocoa), and Oryza sativa (rice) have seen such improvements through the overexpression of BabyBoom, one of many gene that is known to improve SE transformation efficiency and overcome its recalcitrant transformation nature.

Our results have yet to confirm such success within grapevine, as it is still undergoing research. However, understanding the interactions between genes that respond to, stimulates by or induce biosynthesis for auxin may be the key to the success in overcoming SE transformation in recalcitrant transformation nature of *Vitis vinifera*. Additionally, this research hopes to resource of plant organs that is abundant, which are leaves and/ or petioles. To our current hypothesis SE transformation seems to require a multigene expression approach. As other genes within the AP2/ERF gene superfamily and WUSCHEL-related homeobox gene family; are of importance to the maintenance of auxin before the induction of SE. The levels of expression of certain SE inducing genes are different for different species.

The presentation will cover aspects of SE transformation requirement. It will provide a framework for the expectation every plant must undergo before SE transformation within leaf expat(s). The functions, molecular interactions, cascade and pathway of which SE inducing genes undergoes, and the interest in BBM within the transcription factor for SE based transformation research.

Activation and translocation of plant membrane-associated NAC transcription factors

Mercer, C.¹, Curtis, T.¹, DeClercq, I.⁴, Meisrimler, C.N.^{1,2,3}

¹Department of Biology, University of Canterbury, Christchurch, NZ, ²Biomolecular Interaction Centre, University of Canterbury, Christchurch, NZ, ³Bioprotection, Lincoln University, Christchurch, NZ. ,⁴VIB Department of Plant Systems Biology, Ghent University, Belgium. * claudia.meisrimler@canterbury.ac.nz

Abstract

Membrane-associated transcription factors are pivotal for eukaryotic cell adaptation and stress response signalling in plant cells. Land plants encode over 300 variants of these transcription factors, highlighting their significance in environmental sensing. Tethered to cellular membranes, these transcription factors need to undergo activation and cleavage as initial functional steps. This is often induced by environmental cues, involving post-translational modifications and protein interactions. The subsequent step is the translocation of the cleaved transcription factor domain to the nucleus, where they regulate gene expression.

Here we investigate activation and translocation mechanisms of membrane-associated NACs (NAM, ATAF1/2, and CUC2). We focus on the Arabidopsis ANAC013 and ANAC017 cluster within the membrane-associated NACs, which are key regulators of cellular reactive oxygen species levels and organelle signalling. The inactive forms of these proteins are tethered to the endoplasmic reticulum via a C-terminal transmembrane domain. Proteolytic cleavage, mediated by rhomboid proteases, releases the transcription factor domain. However, the activation processes leading to cleavage and translocation are not well understood. Our data suggest that the translocation of these NACs, induced by certain pathogen associated molecular patterns and drought stress, depends on phosphorylation at a conserved site and interaction with 14-3-3 proteins. We hypothesize that this phosphorylation is essential for rhomboid protease cleavage and subsequent nuclear translocation. We will further discuss our results within the context of plant immunity and signalling crosstalk.

Flash talks

Revel Drummond:

Can transient leaf assays be used to quantitatively assess Cas enzyme activity?

Sarah Philip-Wright

ChatGPT, make my plants better! Testing RFdiffusion-designed binding proteins to alter plant hormone signalling and shape

Nishadi Liyanage

Decoding the Kiwifruit Flowering Fate

Ingrid Lindeman

Dawn Light Composition Impacts Perennial Crop Growth in Controlled Environment Agriculture

Erikan Baluku

Beyond Dormancy: How Resurrection Plants Rewire Their Organelles to Stay Alive

Allan Wu

Tales from the TEM: building a 3D model of chloroplast biogenesis one stack at a time

Greg Dawson

Legume nitrogen fixation: exploring the genetic basis of inhibition by nitrate fertilisers

Ayodele Fakoya

Progress towards the development of a non-heading perennial ryegrass (Lolium perenne L.) cultivar

Madison Hall

Need for speed: Investigating the role of CO variants in ryegrass flowering time variation

Baeli Spedding-Devereux

Assessing CRISPR/Cas13 as a tool for plant biology

Jihwi Jang

Determination of nitrate (NO3-) toxicity against Pinus radiata: a pre-requisite to determine NO3- phytoremediation potential of P. radiata in NO3-contaminated soils

Storm Voyce-McCulloch

It's all about self-love: understanding self-incompatibility in Clover

Bea Fulton

Using Confocal Microscopy to Study Ovule Development in Mutant Apomicts

Lei Wang

Enhancing Development of Sauvignon Blanc Embryos

Nicole Samuel

Investigations into pollen protein interactions

Michelle Thompson

A potential role for ellagitannins found in grapevine rootstocks as a deterrent against feeding by Citrophilus mealybugs (Pseudococcus calceolariae)