QMB Abstracts Plant Biology

P1: Evolution of land plant body plans

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The origin of land plants (embryophytes) was one of the most important evolutionary events in the earth's history. The spread and diversification of a land flora changed the biosphere and made possible the subsequent colonization of land by metazoans, allowing the origin of complex terrestrial ecosystems. Molecular phylogenetic and ultrastructural data indicate that land plants are most closely-related to charophycean alga; land were thus likely derived from freshwater, aquatic ancestors from which they inherited numerous developmental, biochemical, and cell biological features. However, the origin and diversification of embryophytes involved dramatic evolutionary changes in life history, physiology, and body plan that allowed for more complex forms adapted to life on land. Some of the key features associated with land plant evolution were the origin of a multicellular diploid sporophyte from a retained zygote, a gametophytic apical meristem with an apical cell that divides in multiple planes producing 3-dimensional tissues, a sporophytic shoot apical meristem (SAM) with a capacity for branching, and the origin of roots and mechanisms to regulate gas exchange and water loss. We have been developing *Marchantia polymorpha*, a liverwort, as a model system to investigate the evolution of key features of shoot development associated with the colonization of land.

P2: Goods and evils of plant genomic instability

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Genome integrity is dependent on controlling and balancing DNA replication, recombination, cell division, mating, mutation, and transposition. The orchestration of these processes can fail during stress resulting in genome instability, an outcome whose nature and consequences are not clearly understood. Significantly, plants are likely to tolerate instability better than animals, both because of flexible development, lack of predetermined germline and frequent polyploidy. As a consequence, plants may be more likely to retain karyotypic novelty. We are studying genome instability resulting from stresses. One is haploid induction, a sexual process resulting in uniparental progeny with a haploid genome complement, which we are studying in Arabidopsis and potato. Haploid induction is often associated with genome elimination, such as when modification of centromeric histone 3 (CENH3), the major epigenetic determinant of centromere function, results in postzygotic malfunction leading to chromosome missegregation and elimination. Another stress results when protoplasts are regenerated into plants. The resulting “somaclonal variation” has uncertain causes. Last, we are studying the outcome of induced dsDNA breaks. By characterizing the structural outcome of instability, our results identify potential similarities between these processes and provide a framework for understanding and engineering of plant genomes.

P3: SHOOT BRANCHING: Role of strigolactones and interactions with other signals

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Shoot branching occurs due to the regulation of the outgrowth of axillary buds which are embryonic shoots in the axil of leaves. Long-distance signaling is central to this regulation and mainly involves strigolactones, cytokinins, auxin and sugars. The sugar role may be at least partly due to sugar signalling and to involve trehalose 6-phosphate. It also appears that the growth of axillary buds from a state of very slow growth or dormancy, to sustained growth involves a number of stages during which the emerging shoots show differential sensitivity to growth stimulus and inhibition. For example, there are substantial differences in responses to different hormones at different periods after shoot tip removal. This could be due to differences in hormone signaling and downstream responses as well as due to changes in the vasculature of the growing buds. We will present our latest unpublished findings on the interaction of signals during bud outgrowth. In addition to providing a new mechanism for how plants respond to shoot tip removal, this work is provides a better understanding of how plants achieve diverse architecture in response to the environment.

P4: Investigating the formation and function of boundary-localized organizers in plant development.

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A fundamental process in plant development is the patterning of cell polarity and morphogenesis. Recently we found that in the shoot, Auxin Response Factor 5 (ARF5) or MONOPTEROS (MP), acts as a cell polarity cue and promoter of organ formation through its role in mediating auxin transcriptional response (Bhatia et al., 2016). However, the response to auxin doesn't just depend on MP. It also depends on transcription factors involved in leaf dorsoventrality, including the dorsally expressed Class III HD-ZIP and ventrally expressed KANADI transcription factors. These transcription factors act negatively to restrict auxin response to narrow boundary regions in between their expression domains where their expression is absent (Caggiano et al., 2017). According to established dogma however, direct juxtaposition of dorsal and ventral cell types should provoke organogenesis, without necessarily requiring a region or “middle domain” separating the two cell types (Waites and Hudson, 2005). To investigate this conundrum we used live imaging to observe the consequences of direct juxtaposition of HD-ZIPIII and KAN expression in the ventral and dorsal cells of leaf primordia respectively. While our findings further support a cell autonomous role for HD-ZIPIII and KAN expression in repressing organogenesis, they also reveal that HD-ZIPIII activity induces long-range signaling that promotes growth and auxin-related gene expression. Thus our results help clarify our understanding of dorsoventral boundaries by revealing how opposing activities coordinate to regulate morphogenesis.

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P5: Cell Wall sensors and their roles in plant growth and development

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In addition to chemical signals such as hormones and peptides the role of physical signals, also known as mechanics, to control plant growth is being increasingly recognised. Despite extensive knowledge of the mechanical stress sensors and signaling pathways in the animal extra-cellular matrix and yeast cell wall, relatively few components of the signalling pathways have been identified in plants. Plant cell wall sensors are proposed to regulate responses to mechanical stress by remodeling their cell walls to maintain integrity during growth. Our work focuses on two classes of putative cell wall sensors, the arabinogalactan protein (AGP) family of extracellular glycoproteins and DEFECTIVE KERNEL1 (DEK1), a plant-specific CALPAIN protease. DEK1 has been shown to associate with a mechanically activated calcium channel *in planta*, suggesting it is involved in perception of mechanical stress[1]. We have shown that altered levels of *DEK1* results in changes in cell wall structure and composition leading to growth defects (Amanda et al., 2016; 2017). Phenotypic analysis of the shoot apical meristem of a *dek1-4* mutant and lines with inducible overexpression of the CALPAIN domain show these lines have defects in floral primordia initiation and sepal development. These data suggest DEK1-regulation of the cell wall impacts shoot apical meristem development and further investigation of DEK1 will reveal its important roles during plant growth.

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P6: Male germ-line specification in flowering plants

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The pollen grain is responsible for the production and delivery of the sperm cells to the female gametes in flowering plants. The male germline that gives rise to these sperm cells is specified early in pollen development. This specification depends on an asymmetric division of the microspore, suggesting that there is unequal segregation of a germline-fate determinant. It is hypothesized that this determinant leads to the expression of the transcription factor DUO1 specifically in the germ cell. DUO1 then controls the differentiation of the germline through activating a suite of genes required for cell cycle progression and sperm cell function. This early germ-cell expression of DUO1 is a key step in the specification of the male germ-line.

We are now investigating the mechanisms that control DUO1 expression. Using a combination of phylogenetic foot-printing, deletion analysis and promoter reporter fusions we have identified *Regulatory Region of DUO1* (*ROD1*), a novel *cis*-regulatory module that is both necessary and sufficient for expression of DUO1. *ROD1* has a distinct architecture with several repeated *cis*-regulatory elements which play distinct roles in regulating transcription. Further, *ROD1* displays a remarkable level of sequence similarity for a regulatory region, and is functionally conserved in the eudicots. This conservation highlights the importance of *ROD1* in ensuring DUO1 is expressed early in male germline development and functional sperm cells are specified within each pollen grain.

P7: A *SOC1-like* gene *MtSOC1a* promotes flowering and primary stem elongation in the model temperate legume Medicago

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Medicago truncatula (Medicago) flowering, like winter annual Arabidopsis, is promoted by vernalisation and long days, but alternative mechanisms are predicted because Medicago lacks the key regulators CO and FLC. The *SOC1* gene is an important integrator of different flowering time pathways in Arabidopsis. *SOC1-like* genes regulate flowering in some plants while others have different functions. Three Medicago *SOC1-like* genes, including *MtSOC1a,* were previously implicated in flowering control, but no legume *soc1* mutants with altered flowering were reported yet[1]. This study focuses on the characterization of the *MtSOC1a* gene in Medicago[2]. We showed that the timing and magnitude of *MtSOC1a* expression was regulated by long days and the flowering promoter *FTa1*, while *in situ* hybridisation indicated that *MtSOC1a* expression increased in the shoot apical meristem during the floral transition. By reverse genetics, we found a *Mtsoc1a* mutant line that showed delayed flowering and short primary stems. Overexpression of *MtSOC1a* partially rescued the delayed flowering of *Mtsoc1a,* but caused a dramatic increase in primary stem height, well before the transition to flowering. *MtSOC1a* promotes internode cell elongation in the primary stem. However, while the hormone gibberellin (GA) classically stimulates stem elongation in dwarf peas, both wild type and *Mtsoc1a* elongated in response to GA3 sprays, indicating the mutant was not defective in GA responsiveness. These results indicate that *MtSOC1a* may function as a floral integrator gene and promotes primary stem elongation. Overall, this study suggests, that apart from some conservation with the Arabidopsis flowering network, *MtSOC1a* has a novel role in regulating aspects of shoot architecture.

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P8: The implications of gene editing technology for New Zealand

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The revolution in gene editing technologies is making it easier to make targeted changes in the genomes of animals, plants and microorganisms. The development of these new technologies has huge potential benefits in many sectors including healthcare, agriculture and conservation. However, the technology to carry out gene editing and the ideas about how it might be applied are, in many cases, moving well ahead of public understanding and consideration of the proposed changes, and any consensus on how this technology might be used.

To explore the implications of gene editing technology for New Zealand, the Royal Society Te Apārangi has convened a multidisciplinary panel of experts, supported by a Māori reference group, to consider the social, cultural, legal and economic implications of gene-editing technologies for New Zealand.

The terms of reference for the panel are to:

- Raise awareness of the current gene editing technologies, their recent development and what they are being used for
- Outline the technologies' opportunities and risks, including current global practice
- Provide insight and advice for public, business and government audiences on the future implications of these new technologies for New Zealand

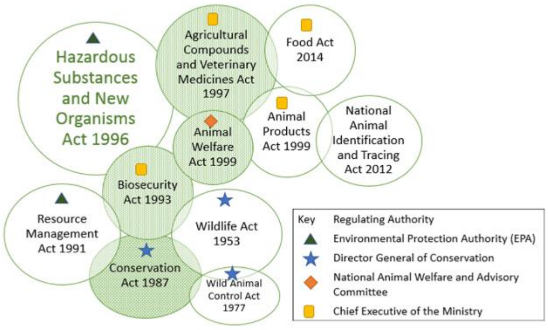
The approach taken by the panel has been to generate a series of discussion papers containing various gene-editing scenarios to initiate a conversation with the NZ public. To date, papers on gene editing in healthcare and pest control have been released and a series of meetings held to canvas views of policy makers, industry, scientific and community organisations, and high school students. Two additional pieces of work are in progress on *The use of gene editing in the primary industries* and the *Implications for the New Zealand regulatory framework*.

This talk will provide an overview of the activities of the panel, progress to date, major issues encountered and future challenges that lie ahead.

Reference
https://royalsociety.org.nz/major-issues-and-projects/gene-editing-in-aotearoa/

P9: Gene editing in Aotearoa – legal considerations

Julie Everett-Hincks and Mark Henaghan. Law Faculty, University of Otago, Dunedin, New Zealand.



Gene edited crops and animals pose significant new challenges for regulation. Under current NZ legislation (Hazardous Substances and New Organisms Act, 1996) and a judicial ruling on interpretation of the legislation and regulations, the status of gene-edited crops and animals in New Zealand are considered genetically modified. A precautionary approach is employed for regulating these new organisms. The implications from the effects of differing legislation and regulatory authorities has been investigated, identifying legal and policy issues requiring consideration for gene editing use in our primary industries.

Professor Mark Henaghan and Dr Julie Everett-Hincks have been working with the Gene Editing Panel for Royal Society Te Apārangi, providing legal advice as to the potential use of gene editing in the areas of human healthcare, pest control and primary industries. Royal Society Te Apārangi is encouraging New Zealanders to consider and share their views on some potential uses of gene editing in New Zealand. To assist public discussion, two papers have been produced outlining scenarios for the use of gene editing for both pest control and healthcare. A further paper with scenarios for the use of gene editing in primary industries will be published soon, along with a paper examining current legislation and regulation. The papers have been produced by a multidisciplinary expert panel convened by the Society and co-chaired by Professor Barry Scott, who is also Vice President of the Society and a Professor of Molecular Genetics at Massey University.

For further information please contact Dr Julie Everett-Hincks jeh@otago.ac.nz
More information can be found by visiting the Royal Society Te Apārangi website: https://royalsociety.org.nz/major-issues-and-projects/gene-editing-in-aotearoa/

P10: Social License or Cultural License: Is there a difference?

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Gaining a social license from the public for the adoption of new biotechnologies has become the focus of increasing attention. The level of trust the public has in the scientific community to make responsible choices in the public interest has seemingly declined over a number of years. Gene editing is the most recent addition to the genetic engineering toolbox to challenge public perceptions and ethical sensitivities. Understanding and mitigating their concerns is an important part of social license but how are cultural rights and interests reflected in this dialogue? This presentation will reflect some of the emerging Māori perspectives on gene editing and discuss what would be required to gain a cultural license.

P11: Social license in the marine environment: Dissecting the discourse

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The term “social license to operate”, or SLO, has increasingly featured in public discussion about commercial operations in the marine environment. Choice of wording and sentence structure can affect power relations between people and groups, so how the term is used matters. We analysed grey literature documents such as company reports, government policy documents and press releases to examine the implications of how SLO is defined and deployed with respect to NZ’s marine industries. We show that this discourse has been dominated by industry and central government voices, who frequently vest agency over SLO with industry and then state or imply that industry already has SLO and just needs to maintain or improve it. Whether inadvertent or intentional, this choice of language empowers industry at the expense of communities and iwi. Industry and government could change their wording to send a different, more empowering message to iwi and community groups about seeking their acceptance and trust. This would help achieve the vision of a blue economy, increasing benefits from the ocean environment while sustaining communities and marine ecosystems.

P12: Conflicts between agricultural and tourism sectors: evidence for solutions

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Synthesising our research findings on international importers' trust in NZ agricultural exports[1, 2], crises recovery in food products[3], international market acceptance of food production technologies[4, 5] and country food production technologies on inbound tourism markets[6] with pervasive current affairs discourses surrounding NZ tourism sector and proposed agricultural production strategies[7] we noted evidence for solutions that might met all sectors' needs. We found no evidence that the choice of either agricultural, or any other sector amongst production technologies supressed inbound tourist demand[6].

Evidence for consumer or inbound tourist resistance to some agricultural production technologies offered by actors outside of the agricultural sector based on stated preferences we found to be seriously flawed[8]. This has long distracting discourse from more substantial issues such as damage to recreation land and water ecologies by pests and other agricultural management strategies which are far riskier to inbound tourist demand and highly incongruous with the positioning of New Zealand to international tourists. We find that, paradoxically, opportunities to efficiently deal with those real problems with biotechnology have been needlessly overlooked.

Furthermore, we find evidence that the profitability of both sectors can be substantially enhanced and protected with the judicious application of biotechnology to agricultural production, land and water ecology management.

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P13: The regulation of GMOs in New Zealand

Strabala, T. J.

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The concept of a ‘new organism’ in the New Zealand context came into being with the Hazardous Substances and New Organisms Act, 1996 (The HSNO Act), and is defined as any organism not present in New Zealand on or immediately before 29 July 1998, as well as any genetically modified organism. The Environmental Protection Authority is responsible for administering the HSNO Act, and as such regulates all New Organisms, including GMOs.

A GMO in New Zealand has a broad definition, but refers to Regulations specifying those organisms that meet the definition, but are considered exempt. These regulations were reviewed and redrafted in 2016 in response to a High Court challenge to the result of another of EPA’s functions, the statutory determination of whether or not any organism is a New Organism under the HSNO Act.

The EPA has the power to grant various types of approvals for GMOs, which mostly involve the importation into containment, or the development of GMOs in containment. However, the EPA also has given approvals for the field trials and release of various GMOs since the inception of the HSNO Act. I will discuss the aspects of the law that EPA must consider in coming to a decision for any approval for the release of a GMO, or a determination as to whether or not an organism is or is not a GMO for the purpose of the HSNO Act, using recent EPA decisions as examples.

P14: Gene editing technologies: how they fit in New Zealand’s policy framework

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Withdrawn

P15: Drought-induced senescence of *Medicago truncatula* nodules involves serpin and ferritin to control proteolytic activity and iron levels*

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Drought is a major constraint for legume growth and yield. Senescence of nitrogen-fixing nodules is one of the early drought responses and may cause nutrient stress in addition to water stress in legumes. For nodule senescence to function as part of a drought-survival strategy, we propose that the intrinsically destructive senescence process must be tightly regulated.

Medicago truncatula protease inhibitor and iron scavenger-encoding genes, possibly involved in controlling nodule senescence, were identified. RNA interference (RNAi) lines were constructed in which expression of a *serpin* or *ferritins* was knocked down. Both wild-type and RNAi lines were subjected to drought stress and nodule activity and plant physiological responses were measured.

Drought caused *M. truncatula* to initiate nodule senescence before plant growth was affected and before an increase in papain-like proteolytic activity and free iron levels was apparent. Knock-down expression of *serpin6* and *ferritins* caused increased protease activity, free iron levels, early nodule senescence and reduced plant growth.

The results suggest that *M. truncatula* nodule-expressed *serpin6* and *ferritins* mediate ordered drought-induced senescence by regulating papain-like cysteine protease activity and free iron levels. This strategy may allow the drought-stressed plants to benefit maximally from residual nitrogen fixation and nutrient recovery resulting from break down of macromolecules.

*Dhanushkodi, R. , Matthew, C. , McManus, M. T. and Dijkwel, P. P. (2018). *Drought-induced senescence of Medicago truncatula nodules involves serpin and ferritin to control proteolytic activity and iron levels.* New Phytologist.doi:10.1111/nph.15298.

P16: Transcriptomic response to a short day to long day shift in the reference legume *Medicago truncatula*

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Flowering marks a shift between the vegetative and reproductive growth phases of the plant. The precision with which flowering is induced aligns the life-cycle of the plant with seasonal environmental variation facilitating successful pollination and the maximizing of crop productivity and yield. *Medicago truncatula* is a long-day photoperiod (LD) and vernalisation responsive reference legume for which understanding of the molecular regulation of flowering is incomplete. Temperate legumes like *M. truncatula* possess multiple homologs of *FLOWERING LOCUS T* (*FT*) with one, *FTa1,* having been demonstrated to be a key component of the transition to flowering[1]. However the role of other *FT-like* loci present in *M. truncatula* remains unclear. Furthermore orthologs of core *FT* regulators in *Arabidopsis thaliana* are absent *s*uggesting significant differences exist between the two species. Shifting young *M. trunatula* plants grown in short-day photoperiod conditions to LD has previously been shown to induce flowering and induce *FT*-like genes *FTa1*, *FTb1* and *FTb2*[1]*.* Here two RNA-Seq experiments generated from plants subjected to such shifts were combined to analyse the transcriptomic response to a change in photoperiods in the first part of the day which coincides with the expression of *FTa1* and *FTb* genes. Modelling and clustering were employed to stratify genes by the manner in which their expression changed resulting in the identification of potential candidate genes for future interrogation. The results complement parallel reverse genetics approaches to understand flowering time in *M. truncatula* targeting putative flowering time genes.

[1]Laurie, R. E., Diwadkar, P., Jaudal, M., Zhang, L., Hecht, V., Wen, J., Tadege, M., Mysore, K.S., Putterill, J., Weller, J. L., Macknight, R. C. (2011). *The Medicago FLOWERING LOCUS T homolog, MtFTa1, is a key regulator of flowering time*. Plant Physiology, 156(4), 2207–2224. DOI: 10.1104/pp.111.180182

P17: Identification of active transposable elements *in Pinot noir* embryogenic callus

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Transposable elements (TEs) contribute to considerable proportions of eukaryotic genomes. Although TEs are predominantly suppressed by epigenetic silencing systems, TE transposition can lead to an increase of genetic variation within and among populations, or give rise to a new variety of crops by affecting the expression of nearby genes[1,2]. However, genome-wide surveys of TE activity are rarely reported and the interaction between TEs and co-localized genes remains unclear. Besides, the repetitive characteristic of TE sequences has added difficulties in distinguishing transcriptionally active TEs from non-active ones of the same TE family.

By applying our new pipeline we're able to collect expressed candidates of TEs in *Pinot noir* embryogenic callus. Among the 223411 annotated TEs comprising about 40% of grapevine genome, only 1.7% of them (3845 TEs) were potentially expressed in the cell culture, of which >90% were fragmented. Over three-quarters of the 338 full-length expressed candidates were retrotransposons. By looking into the sequence features of each full-length expressed candidate, 7 LTR-retrotransposons were found to be potentially expressed autonomous TEs. Furthermore, the observation of transcription of putative unannotated TE insertions suggesting a substantial divergence between *P. noir* and the PN40024 *Vitis vinifera* reference genome.

Following treatment with two different biotic stimuli, live yeast and *Botrytis* extract, the embryogenic calli responded with different sets of transcriptionally active TE candidates and showed different preference of expression pattern over treated time period. Biotic stress is known to induce TE transposition[1,3], and ten and four autonomous TE candidates were specifically responsive to live yeast and *Botrytis* extract, respectively. The majority of expressed candidates were found co-localizing with expressed genes.

Our findings support that TE activation can be stress-specific and that given the high prevalence of TE sequences in the genome, few are capable of transposition. Also, TEs are more likely to be expressed when located within expressed genes.

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P18: Molecular control of skin formation in kiwifruit

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The surface of most fruits are typically protected by a waxy cuticle. There are a few exceptions where the fruit surface are composed of cells and tissue layers that are heavily suberized or lignified. The berry fruit of *Actinidia species* (kiwifruit) is a suitable model for the study of fruit skin (exocarp) formation due to the variation of exocarp morphology within the germplasm. There are two distinct exocarp phenotypes found within *Actinidia* those that have epidermal exocarp 'live skin' and those that have a peridermal exocarp 'dead skin'. The morphological development of the two exocarp types has been previously reported[1], however little is known about the molecular factors involved in the control of exocarp development. In dead skinned fruit the periderm layers formed during development are produced by secondary meristematic tissue known as cork meristem. In this study, the development of this secondary meristem and its subsequent programmed cell death and suberisation was analysed by extensive microscopy paired with mRNA sequencing methods. A comparison of gene expression in live skin fruit compared to dead skin fruit have highlighted potential regulators of development, and key genes and enzymes involved in the biosynthesis of either periderm or cuticle. Secondly, a segregating population of live skinned and dead skinned fruit was used to identify the genomic regions controlling skin type. A genotyping by sequencing (GBS) study was conducted to map the genomic regions related to skin formation, with several significant QTL's being identified. The Molecular factors that lie within these genomic regions and show clear differential expression in my gene expression study are being selected for testing as candidates for the control of skin formation in fruit.

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P19: Large scale structure variation (SV) profiling reveals genetic diversity in peach

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Genomic structure variation (SVs) including large insertions (INSs), deletions (DELs), duplications (DUPs), inversions (INVs) and translocations may be associated with disease resistance, affect agronomic traits, and is a major source of genetic diversity in plants. In this study, we sequenced the genome of 336 peach accessions ranging from wild, landrace, to elite cultivars collected from around the world. Based on these datasets, genome-wide SVs were detected using multiple software packages such as LUMPY, DELLY, Manta, and GRIDSS. A core collection of peach SVs was developed with each identified by at least two tools. The database consists of 127,821 SVs which are larger than 50bp, with DELs and DUPs further filtered with a maximum length of 500 KB. Fifty randomly chosen SVs verified through PacBio long read sequencing achieved 82% validation rate. The results of a genome-wide association study (GWAS) utilizing SVs and phenotypic data for 14 agronomic traits in peach are being analysed. An inversion controlling flat vs round peach fruit shape was found through GWAS with SVs. This pool of high quality SVs effectively represents genetic diversity in peach and provide an importance resource for future research.

P20: Molecular markers for resistance to myrtle rust in Australian Myrtaceae

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Austropuccinia psidii, causal agent of myrtle rust, was discovered in Australia in 2010 and has since become established on a wide range of species within the Myrtaceae. There are concerns for myrtle rust impacts on commercial industries; such as forestry, cut-flower and native foods, as well as natural ecosystems dominated by species in the Myrtaceae. Resistance, in the form of localised necrosis, has been identified in some individuals indicating early pathogen recognition. Gene expression profiles from inoculated *Syzygium luehmannii,* a commercial species endemic to the east coast of Australia, were examined and found to be significantly different in resistant versus susceptible phenotypes. Resistant plants significantly regulated 439 transcripts (185 up-regulated) 48 hours after pathogen exposure, compared to three in susceptible plants. Most significantly up-regulated in resistant plants were gene homologues coding for transcription factors, receptor-like kinases and enzymes in the secondary metabolite pathway and defence. Interestingly, putative *G-type lectin receptor-like kinase* transcripts were found exclusively in resistant plants prior to inoculation. Significant differential expression of two transcripts incorporating Toll/interleukin-1, nucleotide binding site and leucine-rich repeat domains were identified in resistant plants at both 24 and 48 hours post inoculation. These putative receptor genes have been computationally re-assembled with higher stringency, using expression data from each plant, and primers developed to screen plants based on resistant phenotypes. The development of a molecular markers will provide a useful tool for plant breeders and land managers.

P21: Epigenetics and environmental perception: WLC is required for the memory of winter

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Like many plant species, overwintering Arabidopsis requires a prolonged period of winter cold (vernalization) before achieving competence to flower. Perception of winter in Arabidopsis has recently been shown to comprise both low temperatures, but also, surprisingly, the absence of warmth. Vernalization is registered by epigenetic regulation of *FLC*, a master repressor of flowering that prevents *FT* induction by activating cues. *FLC* expression prevents flowering during vegetative development, but its transcription is gradually silenced during winter cold. Quantitative silencing at the whole-plant level reflects a cell-autonomous, bi-stable switch between active and silenced chromatin states at *FLC*, allowing the repressed state to be maintained in the warmth of spring. The low-probability, stable nature of this epigenetic switch provides both a mechanism for determining the duration of winter experienced, and also effectively buffers against short-term temperature fluctuations.

To date, mutants defective in their ability to “remember” winter cold have been characterized as components of the Polycomb Repressive Complex 2 (PRC2) or accessory proteins. We have recently identified *wlc1* as a novel flowering time mutant, reflecting mis-regulation of both *FLC* and *FT* in addition to other PRC2 targets. WLC interacts with the PRC2 in IP-MS experiments, and the *wlc1* mutant displays vernalization phenotypes characteristic of PRC2 mutants along with global changes in histone modifications. Unusually, *wlc1* also has altered patterns of DNA methylation. We show that WLC is key to the memory of winter, facilitating active chromatin mark removal at *FLC* alongside PRC2-driven silencing.

P22: Regulation of the flavonoid pathway in the liverwort *Marchantia polymorpha* for abiotic stress tolerance

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The flavonoid pathway is ubiquitous in extant land plants. It is hypothesized to have evolved during land colonization for protection against abiotic stress, particularly UVB-light. In angiosperms, flavonoid production can be induced by excessive UVB- or white-light, nutrient deprivation, cold, and senescence. R2R3MYB transcription factors are key for regulating these responses in angiosperms, but it is not known whether the functions of flavonoids and the pathway regulation mechanisms are conserved across land plants. To address these questions we are characterising the flavonoid pathway of the liverwort *Marchantia polymorpha*. Liverworts are one of the basal land plant groups and can provide information on characters that may have an ancient evolutionary origin.

We identified marchantia flavonoid biosynthetic and regulatory gene candidates then analysed their importance to abiotic stress tolerance using transgenic over-expression and CRISPR/Cas9 mutagenesis, in combination with physiological, metabolic and transcriptomic analyses. Mp*MYB14* was identified as essential for production of the red flavonoid pigment riccionidin A in response to nutrient deprivation or high-light stress. Although Mp*MYB14* also activated biosynthesis of flavones, it was not required for their production in response to UVB. For UVB, induction of flavones occurred via the UVR8-photoreceptor/HY5 pathway, and their production was essential for UVB tolerance. Mutant lines with reduced flavone production or interruptions in the UVR8/HY5 pathway had increased sensitivity to UVB, while plants with enhanced flavone production were more tolerant. Our results suggest that stress-induced flavonoid production is a character conserved across land plants and may have been an early adaptation to life on land.

P23: Interactions between the parasitic plant Cuscuta and its tomato host

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Parasitic angiosperms directly attach to host plants using specialized organs known as haustoria, which function as physiological bridges to extract nutrients and water from their hosts. *Cuscuta* species (dodders) are common and agriculturally destructive flowering stem parasitic plants. Many *Cuscuta* species are listed in the Federal or State Noxious Weed lists, including *Cuscuta pentagona* (*C. pentagona*). Reports have shown a 50–72% reduction in tomato yield due to *Cuscuta*. Because of the intimate physiological connection between host plants and parasites, most traditional herbicides and control methods have not been effective or are too costly. We used transcriptomics to identify genes upregulated in *Cuscuta* upon attachment to host. Expression of key upregulated genes was reduced using host-induced-gene-silencing and haustorium formation monitored. Reduction in expression of some of the identified genes attenuated parasitism. While most tomato cultivars can be parasitized by *C. pentagona*, we obtained some Heinz hybrid cultivars, which exhibited resistance to dodders. Local lignification in the stem cortex upon dodder attachment led to resistance to haustorium penetration in the resistant cultivars. Key resistance genes included an *AP2*-like transcription factor, a *MYB* transcription factor and an *NBS-LRR* (a gene encoding a nucleotide-binding site leucine-rich repeat protein). The function of these genes was deciphered using virus based gene expression. The results of this study may help develop a parasite-resistant system in crops to reduce economic losses in agriculture.

P24: Conserved metabolic pathways and associated regulatory networks for the synthesis and secretion of nectar

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Plants attract mutualistic animals by offering a reward of nectar. Specifically, floral nectar (FN) is produced to attract pollinators, whereas extrafloral nectar (EFN) mediates indirect defenses through the attraction of mutualist predatory insects to limit herbivory. Nearly 90% of all plant species, including 75% of domesticated crops, benefit from animal-mediated pollination, which is largely facilitated by FN. Moreover, EFN represents one of the few defense mechanisms for which stable effects on plant health and fitness have been demonstrated in multiple systems, and thus plays a crucial role in the resistance phenotype of plants producing it. In spite of its central role in plant-animal interactions, the molecular events involved in the development of both floral and extrafloral nectaries (the glands that produce nectar), as well as the synthesis and secretion of the nectar itself, have been poorly understood until recently. To date, a holistic and coordinated characterization of nectar secretion from a comparative genomic and molecular perspective has been lacking. Toward this end, we have evaluated the transcriptomes and proteomes of floral and extrafloral nectaries throughout development across twelve dicotyledonous species and identified core sets of genes and modules involved in the synthesis and secretion of nectar across species, as well as its regulation. Key conserved modules include hormonal biosynthesis and response pathways, as well as genes involved in carbohydrate metabolism and transport. Similarly, metabolite profiling coupled with transcriptomic and reverse genetics approaches identified specific loci responsible for nectar characteristics that influence mutualist visitation. For example, genes and pathways required for the synthesis of sucrose-rich and proline-rich nectars across species were identified.

P25: Inhibition of strigolactone receptors by *N*-phenylanthranilic acid derivatives

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The strigolactone (SL) family of plant hormones regulates a broad range of physiological processes affecting plant growth and development and also plays essential roles in controlling interactions with parasitic weeds and symbiotic fungi. Recent progress elucidating details of SL biosynthesis, signalling, and transport offer many opportunities for discovering new plant growth regulators via chemical interference. Here, using high throughput screening and downstream biochemical assays, we identified *N*-phenylanthranilic acid derivatives as potent inhibitors of the SL receptors from petunia (DAD2), rice (OsD14) and *Arabidopsis* (AtD14). Crystal structures of DAD2 and OsD14 in complex with inhibitors further provided detailed insights into the inhibition mechanism, and *in silico* modeling of 19 other plant strigolactone receptors suggested that these compounds are active across a large range of plant species. Altogether, these results provide chemical tools for investigating SL signaling and further define a framework for structure-based approaches to design and validate optimized inhibitors of SL receptors for specific plant targets.

P26: Epigenetically activated small RNAs are modified in pollen and mediate chromosome segregation and dosage.

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Chromosome dosage plays a significant role in reproductive isolation and speciation in both plants and animals, but underlying mechanisms are largely obscure. We have found that a highly conserved microRNA in plants, miR845, targets the tRNA^{Met} primer-binding site (PBS) of LTR-retrotransposons in *Arabidopsis* pollen, and triggers the accumulation of 21 to 22-nucleotide small RNA in a dose dependent fashion via RNA polymerase IV. We show that these epigenetically activated small-interfering RNAs (easiRNAs) mediate hybridization barriers between diploid seed parents and tetraploid pollen parents ("the triploid block"), and that natural variation for miR845 may account for "endosperm balance" allowing formation of triploid seeds. Targeting the PBS with small RNA is a common mechanism for transposon control in mammals and plants, and provides a uniquely sensitive means to monitor chromosome dosage and imprinting in the developing seed. Loss of easiRNAs in DNA methylation mutants results in severe mitotic chromosome mis-segregation, strongly reminiscent of RNAi mutants in *S.pombe,* which lacks DNA methylation. We have found that easiRNA are specifically modified in pollen, along with certain miRNA. We are determining the biogenesis and function of these modifications.

P27: Gender in kiwifruit

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Hermaphroditism has been a key reproductive trait for the domestication of crops. *Actinidia chinensis* (Kiwifruit) is a dioecious plant. For 30 years, kiwifruit breeders have tried to generate hermaphrodites with flowers containing functional male and female organs. However, there are still no commercial hermaphrodites, largely due to poor genetic understanding of dioecy and extensive inbreeding in any hermaphrodites produced.

There are two genes in kiwifruit that determine the gender of male and female flowers. One of these genes suppresses the formation of the ovary and the other controls pollen fertility. Both of these genes are located in a non-recombining region known as the sex determining region (SDR). The gene that suppresses ovary formation (SuF) has recently been identified [1]. SuF is dominant and present only in males. However, in the PFR research orchard there are some fruiting males, which have reduced suppression of ovary development, suggesting that this dominance has been compromised, and the structure of the kiwifruit sex locus and the mechanism leading to the evolution of recombination suppression in this region remain unknown.

Now, using our kiwifruit germplasm resource of rare kiwifruit genders such as fruiting males and hermaphrodites, combined with improved genetic techniques, and advances in kiwifruit genomic sequencing and annotation [2] at a range of ploidies, we have had the opportunity to further resolve the kiwifruit SDR and to investigate the recombination rates around the locus.

1. Akagi, T., et al., *A Y-encoded suppressor of feminization arose via lineage-specific duplication of a cytokinin response regulator in kiwifruit.* The Plant Cell, 2018.
2. Pilkington, S.M., et al., *A manually annotated Actinidia chinensis var. chinensis (kiwifruit) genome highlights the challenges associated with draft genomes and gene prediction in plants.* BMC Genomics, 2018. **19**(1): p. 257.

P28: A single complex locus is responsible for the production of multiple flavour-related terpenes in ripe kiwifruit

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Terpene volatiles are important flavour and aroma compounds in many fruit including citrus, grape and strawberry. In some kiwifruit varieties, the monoterpene 1,8-cineole has been suggested to be a key flavour and aroma compound[1]. Using flavour recombinates presented to sensory panels we have shown that 1,8-cineole contributes a characteristic mint-like note to the aroma of ripe gold-fleshed kiwifruit (*Actinidia chinensis* var. *chinensis*). To understand the genetics of 1,8-cineole production, a segregating population was screened by Fast-GC. Two QTL for 1,8-cineole production were identified in the 'Red5' kiwifruit genome, one of which co-located with a complex cluster of putative terpene synthase (TPS) genes. Transient expression in tobacco and analysis of recombinant proteins expressed in *E. coli* showed that four genes in the cluster (*AcTPS1a-d*) encoded functional TPS enzymes producing sabinene, 1,8-cineole, nerol and an unknown sesquiterpene respectively. The expression of *AcTPS1a* and *AcTPS1b* correlated with accumulation of sabinene and 1,8-cineole during fruit development and ripening. Transient over-expression of *AcTPS1b* in *A. eriantha* fruit confirmed this gene produced 1,8-cineole in *Actinidia*. Structural analysis showed that *AcTPS1a* and *AcTPS1b* are natural variants at key TPS catalytic site residues previously shown to change enzyme specificity in vitro[2]. Our results demonstrate how variation at a single locus can produce a wide diversity of terpene products in planta and provides a route for applying marker aided selection for targeted flavour improvement in kiwifruit.

[1] Friel, E.N., Wang, M., Taylor, A.J., and MacRae, E.A. (2007). *In vitro and in vivo release of aroma compounds from yellow-fleshed kiwifruit*. J Agric Food Chem 55, 6664-6673.

[2] Kampranis, S.C., Ioannidis, D., Purvis, A., Mahrez, W., Ninga, E., Katerelos, N.A., Anssour, S., Dunwell, J.M., Degenhardt, J., Makris, A.M., et al. (2007). *Rational conversion of substrate and product specificity in a Salvia monoterpene synthase: structural insights into the evolution of terpene synthase function*. Plant Cell 19, 1994-2005.

P29: Developing a new generation of grapevine cultivars: markers for consumer traits.

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Most of the modern-day winegrape cultivars were being used by the late 1700’s and there is evidence that Pinot noir existed as far back as the late 1300’s. So it is clear that there has been only limited success in the improvement of grape germplasm in the last 200 years. Significant barriers to the development of new grape cultivars include: the long life cycle of conventional grapevines which pushes generation times out to 2-3 years; the space required to grow a population; and the difficulty in obtaining flowers and fruit in growth-rooms and glasshouses. However, the discovery of a rapid-cycling grapevine mutant (microvine), which has a limited response to gibberellins, has enabled more targeted breeding strategies to be developed. The microvine is also useful for marker discovery and the elucidation of the genetics behind important consumer traits. The ultimate aim is to bring together traits important for more efficient production systems with desirable consumer characteristics to kick-start the development of a new generation of winegrape cultivars.

P30: Role of the 5'-3' mRNA decay pathway in fine-tuning of the circadian clock in the model plant *Arabidopsis thaliana*.

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Organisms as diverse as humans, plants and microorganisms have an internal time-keeping mechanism called the circadian clock, which generates daily biological rhythms. The clock allows organisms to anticipate changes in the environment, and adjust biological processes to occur at an appropriate time of day or season. These biological rhythms are controlled by a complex gene network —called the central oscillator—, in which genes interact mainly by transcriptional feedback loops, to maintain an approximate 24h rhythm. In plants, the clock controls many important physiological processes such as growth, flowering, metabolic regulation and stress responses.

There is increasing evidence supporting the importance, not only of transcriptional but post-transcriptional regulation as a key step for the correct oscillation of the clock. We are particularly interested in the post-transcriptional regulation through 5’-3’ messenger RNA (mRNA) decay. This regulatory pathway includes genes encoding proteins involved in translation arrest, mRNA degradation, poly (A) tail shortening and decapping.

We characterised different outputs of the clock in mutants for genes involved in this pathway. We observed longer circadian period of leaf movement and bioluminescent reporters, and a late flowering time phenotype compared to wild type plants. When analysing gene expression patterns in the mutants, de-regulation of mRNA levels of some core clock genes and changes in their half-lives were detected.

These results confirm the importance of this regulatory layer for the correct oscillation of clock genes. Nevertheless, further transcriptomic analysis will be performed in order to deeper understand the role of mRNA decay in fine-tuning of circadian rhythms.

P31: Dissecting GxE Effects on Wheat Development

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The availability of genome-wide typing methods for wheat raises the possibility of predicting adaptive fitness of candidates for accelerating and redesigning breeding strategies under a changing climate. In order to develop methods for linking genotype and environment with developmental phenotypes we designed an efficient phenotyping strategy using controlled environments based on an integrative model we previously proposed[1]. Six wheat genotypes with diverse developmental phenotypes were grown under sort day (SD) or long day (LD) treatments with or without prior vernalisation. Final leaf number (FLN) was used to quantify four developmental phenology parameters: minimum leaf number, photoperiod response, SD vernalisation sensitivity, and cold vernalisation sensitivity. Foliar expression of the key regulators *Vrn-1, Vrn-2 and Vrn-3* was measured by qPCR across the duration of the trial. Development phenology parameters correlated well with allelic composition of the cultivars at key adaptive loci and with temporal patterns of *Vrn* gene expression. This research suggests scope for improvement of existing models and also for application of these phenotyping methods for dissection of wheat adaptive physiology.

1. Brown, Hamish E., Peter D. Jamieson, Ian R. Brooking, Derrick J. Moot, and Neil I. Huth. "Integration of molecular and physiological models to explain time of anthesis in wheat." Annals of botany 112, no. 9 (2013): 1683-1703.

P32: New breeding technologies for fruit trees

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Many studies have focused on manipulating levels of secondary metabolites in fruit through both conventional breeding and GM approaches. However, New Breeding Technologies (NBTs) offer ways of providing large changes consumer traits (in addition to grower traits) if the public will accept the resulting fruit.

Annualization of woody perennials has the potential sped the breeding and production of fruit crops and rapidly improve horticultural species. Kiwifruit (*Actinidia chinensis*) is a recently domesticated fruit crop with a short history of breeding and tremendous potential for improvement. We used CRISPR/Cas9- mediated manipulation to target mutation of CENTRORADIALIS (CEN)-like genes. Targeting these genes transformed a climbing woody perennial, which develops axillary inflorescences after many years of juvenility, into a compact plant with rapid terminal flower and fruit development. These changes have made kiwifruit amenable for accelerated breeding, indoor farming and cultivation as an annual crop. Using these plants, crossing has begun targeting higher levels of anthocyanins and carotenoids for future cultivars.

P33: Biotechnology and Forest Trees in New Zealand

Charleson Poovaiah, Sanjeev Raikar, Lorelle Phillips, Steffi Fritsche, Catherine Reeves and Glenn Thorlby.

Scion, Rotorua, NZ,

The large stature of forest trees, long periods of juvenile (pre-reproductive) growth, and delayed expression of traits, such as wood quality result in breeding programmes being long and expensive. Biotechnology provides solutions to mitigate these breeding challenges, particularly through the use of new breeding technologies such as gene editing which allow rapid and precise trait modifications. We will illustrate the potential benefits of integrating gene editing into forest tree breeding programmes using engineered sterility as an example trait. Wildings, non-native invasive conifer tree species that have spread from planted forests, are a major problem in New Zealand. Their control costs in excess of $15M per year and they have been described by the government as “the most significant weed problem New Zealand faces”. The production of trees that are not able to generate wildings would provide a tool to mitigate the environmental and socio-economic damage they cause.

Uncertainly over the global regulatory status of gene editing remains a barrier to its integration into breeding programmes. New Zealand is one of the few countries where the regulatory status of gene editing has been clarified. In 2014 the NZ Environmental Protection Authority ruled that plants produced via gene editing, where no transgene was used in the editing process, would not be regulated as GMOs. However, following a challenge in the High Court, this decision was overturned such that NZ currently regulates the products of gene editing as GMOs. The regulatory process which led to gene editing’s current GMO classification in NZ will be discussed.

P34: Developing Gene editing technology in conifers

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The CRISPR/Cas9 nuclease system is a powerful and flexible tool for genome editing. CRISPR/Cas9 has been demonstrated to edit genomes in various plant species including woody species but has not yet been developed in conifers. Scion is developing gene editing technology in conifers, as we believe it will be an invaluable tool in accelerating breeding in these relatively undomesticated species. For a commercial release, it is likely that it will be beneficial to produces edited trees that do not contain a transgene. The long breeding cycles of conifers would make it challenging to uses crossing to remove a transgene. We are, therefore, developing protocols to directly edit protoplasts using a non-transgenic procedure and to regenerate trees from these edited protoplasts. We have consistently isolated protoplasts and have carried out successfully transformation. We will test the efficiency of various RNA polymerase III promoters for use in gene editing. Preliminary results show protoplasts can be used for gene targeting in conifers and will provide mutations for tree improvement and functional studies.

P35: CRISPR Fruit: three bites at the problem

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Actinidia is a long lived woody perennial with excessive vegetative vigour that can contribute to an unacceptably low yield in an otherwise promising cultivar. Creating a dwarfed cultivar via GA insensitivity is a potential route to improving yield in such a plant. We are using three CRISPR-Cas9 based strategies that will produce respectively; a small deletion event, a gene replacement event and a base editing event, that lead to the removal or inactivation of the DELLA domain from an otherwise functional RGL gene. In this plant the T-DNA locus is likely to be unlinked to the RGL gene allowing its segregation away from the edited allele during subsequent breeding. The regulatory constraints on the use of these plants will vary between both the methods used and the jurisdictions where the plants are grown despite similarly precise and limited changes to the plant's genome.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institution
P36	Low-input transcriptomics of developing pollen isolated from a single anther	Chakkatu S.P	University of Otago
P37	The molecular control of fruit size in kiwifruit (*Actinidia chinensis*)	Read, N.C	University of Auckland
P38	Understanding pollen abortion in female kiwifruit	Le Lievre L.G	University of Otago
P39	Transcriptomic profiling of mast flowering Celmisia lyallii plant	Samarth Kulshrestha	University of Canterbury
P40	Identification and characterization of MYBA from blueberry (Vaccinium spp.)	Blue Plunkett	The New Zealand Institute for Plant and Food Research Limited
P41	Variation at S- and Z-genes in Ryegrass	Herridge, R	University of Otago
P42	Genetic mapping and characterisation of the barley leaf rust resistance gene Rph26 introgressed from *Hordeum bulbosum*	Paul A. Johnston	The New Zealand Institute for Plant and Food Research Limited
P43	High-throughput transposon genotyping in a large mutant population	Lizamore, D.	Lincoln University
P44	Manually annotating the *Actinidia chinensis* (kiwifruit) genome	Schaffer, R.J.	The New Zealand Institute for Plant and Food Research Limited
P45	De novo transcriptome assembly for estimating fruit crop genetic diversity and improving genome annotations	Rodrigues, J.A.	The New Zealand Institute for Plant and Food Research Limited
P46	Characterisation of CAROTENOID CLEAVAGE DIOXYGENASE 4 alleles in yellow and white flowered kale and radish.	Braun, R.H.	The New Zealand Institute for Plant and Food Research Limited

P36: Low-input transcriptomics of developing pollen isolated from a single anther

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Pollen are the male gametophytes of plants that deliver the sperm cells to the female gametes. Development of pollen from a unicellular microspore to a mature triploid pollen grain is a highly complex process involving precise and co-ordinated regulation of cell cycle, specification and differentiation of two different cell types. A key challenge in plant reproductive biology is to understand the genes that participate in this complex regulation. However, transcriptomic analysis of developing pollen has been a challenge due to their relative inaccessibility within an anther and also because of a tough outer pollen wall. Due to these challenges, previous studies of pollen transcriptomes have used either whole anthers (comprising parental tissue) or pollen from multiple plants encompassing a broad developmental window.

To enable transcriptomic analysis on pollen at a particular developmental stage and from a small number of plants, we have developed a protocol to analyze pollen from a single anther in Arabidopsis thaliana. This protocol involves removal of an anther from a bud. The anther is ruptured and the released pollen is washed before being frozen. The remaining anther material with pollen is fixed and later stained to determine its developmental stage. The frozen pollen is chemically lysed and used in direct cDNA synthesis. We will report on our development and testing of the method for RT-qPCR, digital droplet PCR and RNA sequencing and its use to study gene expression in pollen.

P37: The molecular control of fruit size in kiwifruit (*Actinidia chinensis*)

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Kiwifruit are an increasingly important crop for the New Zealand (NZ) and world economy, with NZ aiming to reach a return of 4.5B NZD by 2025. An important factor in determining market value is fruit size, with small fruit being rejected from the supply chain. Additionally, when breeding new varieties, a minimum fruit size is needed for the new cultivar to be considered. At present little is known about the molecular regulation of fruit size in kiwifruit. In model fruit species such as tomato it has been shown that final cell number within the mature ovary (pre-fertilisation) and young fruit (post-fertilisation), are key determinants of fruit size. This suggests that the rapid cell division phase during early fruit development is crucial for fruit growth. Plant hormones regulate these phases in an as yet, unknown mechanism. Using a multipronged approach, we aim to better understand the regulation of fruit size in kiwifruit. Firstly, mapping populations that segregate for fruit size will be used to identify genetic regions controlling fruit size. Secondly there are a growing number of genes that have been implicated in controlling organ size in other plant species. Candidate genes based on homology were identified in the newly re-annotated *Actinidia chinensis* (genotype Red5) genome[1]. Combining genetic control locations with candidate genes will identify potential regulators of fruit size. Finally, the effect of exogenous hormones on fruit size will be determined using physiological and cellular based analyses. Using this hormone-based approach, candidate genes will be tested for differential expression during early fruit development. Understanding the mechanisms controlling fruit size in kiwifruit can help develop new cultivars and molecular tools to increase breeding efficiency, and give tools for growers to ensure that a larger proportion of the crop can be marketed.

1. Pilkington SM, Crowhurst R, Hilario E, et al., (2018). *A manually annotated Actinidia chinensis var. chinensis (kiwifruit) genome highlights the challenges associated with draft genomes and gene prediction in plants*. BMC Genomics. 1-19.

P38: Understanding pollen abortion in female kiwifruit

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Kiwifruit (genus *Actinidia*) is dioecious and so exhibits separate male and female plants. Dioecy incurs unique problems in breeding and production of kiwifruit as growers must dedicate approximately 10% of orchards to non-fruiting pollen-donor male vines.

Kiwifruit plants produce flowers with either fertile stamens and degenerate gynoecia (male-fertile), or fertile gynoecia and developed stamens that produce non-viable pollen (male-sterile). Studies comparing morphology of male and female anthers throughout early development suggest pollen abortion in female flowers is due to a disruption of the tapetal cells that support developing pollen[1].

This project will compare gene expression and cytological changes in anthers from male and female kiwifruit flowers across pollen development. We will induce flowering in dormant budwood, and excise anthers of males and females throughout key stages of development from individual flowers. Morphology of the anthers harvested will be analysed by confocal microscopy and sequencing will be done using low-input RNA methods to compare gene expression at precise developmental stages in both whole anthers and isolated pollen.

We will then identify the genes and pathways first altered in female kiwifruit, enabling us to determine the cause of pollen abortion in female kiwifruit flowers. Results from this study will lead to identification of genes important for pollen fertility and may inform strategies to induce pollen fertility in females.

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P39: Transcriptomic profiling of mast flowering *Celmisia lyallii* plant

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Mast flowering is synchronised highly variable flowering by populations of perennial plants, such as *Celmisia lyallii* (Asteraceae), over a wide geographical area. The ΔT model hypothesises that the size of the temperature difference between successive summers determines each year's flowering intensity. Currently, in model plant species we know that the flowering process is regulated by various transcription factors and the process is responsive to temperature. Researchers have also established microRNAs 156 and 172 as key regulators of flowering time. The current study deals with the identification of essential flowering pathway genes that might have a role in the masting syndrome of *Celmisia* plants. To initiate flowering, *Celmisia* plants were transplanted from their original base at Mt Hutt to the University of Canterbury. This resulted in perception of higher summer temperatures by the plants which activated the flowering process. Samples were collected over time and retrospectively sorted into those leaves supporting flowers and those leaves in which plants remained vegetative. Gene expression analysis of *CONSTANS, GIGANTEA, APETELA2, SHORT VEGETATIVE PHASE,* and *TRIOSE PHOSPHATE SYNTHASE* 1 showed conservation of the model flowering pathway in *Celmisia.* The data suggests that the master regulator of the masting syndrome could be upstream to the molecular flowering pathway. To investigate further, RNA-seq was employed to sequence the transcriptome of leaves from flowering and non-flowering *Celmisia* samples. The RNA-seq data will be assembled and analysed using Trinity and various R packages to identify key differentially expressed gene(s). Expression of selected genes obtained from RNA-seq data analysis will be then validated using RT-qPCR.

P40: Identification and characterization of *MYBA* from blueberry (*Vaccinium* spp.)

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The *Vaccinium* genus consists of around 450 species and includes blueberry, bilberry, cranberry, huckleberry, and lingonberry. Commercially, the most important are the blueberries, such as *V. angustifolium* (lowbush blueberry), *V. ashei* (‘rabbiteye’ blueberry) and *V. corymbosum* (northern highbush blueberry). There is an increasing body of evidence for human health benefits of a diet containing blueberries. This is due, at least in part, to anthocyanins, the pigmented compounds found in blueberries. This has elevated blueberries to ‘superfood’ status. The genetic pathway which produces anthocyanins is transcriptionally regulated by the MBW complex; consisting of an R2R3MYB, a bHLH transcription factor, and a WD-repeat protein. In blueberries, anthocyanin is restricted to the skin and the flesh is white/colourless. However, some *Vaccinium* species, such as bilberry, develop colour in the flesh. Understanding this difference in spatial accumulation of anthocyanin will be useful in breeding commercial blueberry varieties with anthocyanin in the flesh. The genes comprising the MBW complex have been identified in many plant species, but not to date in blueberry. To investigate this we performed RNA-seq from blueberry skin and flesh and using this data isolated a MYB from blueberry (*V. corymbosum*), *VcMYBA*, which is homologous to known anthocyanin promoting R2R3MYBs from other species. Using biolistics we show that MYBA was able to compliment an anthocyanin mutant in *Antirrhinum*. Further, MYBA could induce anthocyanin production in tobacco leaves when transiently expressed with a bHLH co-factor. Using a reporter assay we also show that MYBA was capable of activating the promoter of the anthocyanin biosynthesis gene, *DFR*. We tested expression of MYBA in a range of blueberry tissues and confirmed that it was most highly expressed in anthocyanic tissue. Taken together, these results suggest that MYBA is a key regulator of anthocyanin biosynthesis in blueberry.

P41: Variation at S- and Z-genes in Ryegrass

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Ryegrass is an obligate outcrosser – self pollen aborts during germination through an interaction with the stigma. This self-incompatibility is dependent on two genomic loci, S and Z, which have components expressed in the stigma and the pollen. Pollen abortion occurs when the pollen S- and Z-alleles both match to S- and Z-alleles of the stigma. Recently the genes underlying this effect in the pollen have been identified (1, 2). Each locus contains a gene encoding a DUF247 protein which displays high levels of sequence polymorphisms between individuals in a population. The function of these proteins and the effect of sequence polymorphism in self-incompatibility is unknown. We examined sequences of these genes from *Lollium perenne* and *Lollium multiflorum* in published datasets and New Zealand varieties to uncover variability in nucleotide and protein sequence.

S- and Z-DUF247 protein sequences from two available reference genomes were compared to each other. Certain regions of the protein were conserved, particularly the C-terminal trans-membrane domain, while others tended to diverge heavily, suggesting they may be involved in differentiating self pollen. Transcriptome sequences from pollen and stigma were also available for the S-gene, revealing alterations at the trans-membrane domain in the pollen sample. Partial S-DUF247 sequences from several ryegrass varieties revealed regions that were conserved between alleles, and several new alleles were discovered. The S- and Z-DUF247 homologs from *Brachypodium distachyon* (which is self-fertile) were examined, uncovering a premature stop codon in the Z-DUF247 homolog likely causing a loss of the transmembrane domain. The high level of variability, and mutation in self-compatible species, agrees with previous results suggesting these proteins are responsible for mediating self-incompatibility. This knowledge will inform future work involving functional studies of the proteins.

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P42: Genetic mapping and characterisation of the barley leaf rust resistance gene *Rph26* introgressed from *Hordeum bulbosum*

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Genetic resistance is considered one of the best methods of reducing the effects of crop pathogens and limiting the reliance of farmers on chemical control measures. Wild species have often been used as sources of genetic resistance for cultivated crops.

Unfortunately, there are often limited marker and sequence resources available for these secondary species to assist in the genetic mapping and transfer of disease resistance into more adapted genetic backgrounds. The advent of genotyping-by-sequencing (GBS) (Elshire et al. 2011) has made it much easier to generate large amounts of single nucleotide polymorphism (SNP) data from a greater number of species and genotypes. Using this technique, a set of 145 introgression lines between cultivated barley and the wild species *H. bulbosum* were used to develop a marker resource for barley breeding (Wendler et al. 2015).

One of these introgression lines, coded 200A12, featuring an introgression on the distal end of barley chromosome 1HL, was identified in field experiments as conferring quantitative, ‘slow rusting’, resistance to barley leaf rust (*Puccinia hordei*). A set of 47 markers was developed within this introgression using the SNPs generated by GBS. Screening 1368 plants from a backcross F_2 population, using two flanking markers, identified 19 interspecific recombinant lines. Phenotyping and genotyping was completed using homozygous F_3 lines derived from each of these recombinants, resulting in the resistance gene *Rph26* being mapped to the proximal region of the original introgression. The effect of *Rph26* was to reduce the infection frequency (IF) and prolong the latent period (LP) of *P. hordei* in glasshouse trials. *Rph26* resistance increased over the course of plant development, resulting in very low severity of infection in adult plants. This type of resistance gene action is considered more durable than hypersensitive type resistance, especially when multiple genes are pyramided in the same line.

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2. Wendler N, Mascher M, Himmelbach A, Johnston P, Pickering R, Stein N (2015) *Bulbosum to go: a toolbox to utilize Hordeum vulgare/bulbosum introgressions for breeding and beyond*. Molecular Plant 8:1507-1519

P43: High-throughput transposon genotyping in a large mutant population

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Though typically silenced by host epigenetic systems, the mutagenic activity of transposable elements (TEs) can be highly elevated in plant cells through exposure to environmental stresses. Having identified biotic stressors that can be used to stimulate transposition in grapevine somatic embryo cultures, we are producing a population of several thousand vines harbouring novel transposition events. This endogenous mutagenic potential, along with a small, well-assembled reference genome, makes grapevine a useful model for functional genomics studies.

To provide utility to the growing population of vines, we have developed a semi-automated strategy build a searchable database of TE loci for each plant. Our protocol involves pooling genomic DNA in a two-dimensional array prior to short-read library synthesis. The libraries are then enriched for fragments spanning TE end-sequences with a bespoke set of 400 probes and sequenced by paired-end sequencing. Using a newly developed software tool (TEFingerprint), we map reads spanning TE flanks to non-repetitive regions of the reference genome. The read clusters produced are used to define bins representing TE loci across the genome, which can be compared among libraries.

The strategy we have established allows genotyping of approximately 60,000 TE sites per genome for around $70 per plant, with unique transposition events trackable back to individual plants. These data will be cross-referenced with seasonal phenotypic data in a searchable database to allow for both forward and reverse gene function studies.

P44: Manually annotating the *Actinidia chinensis* (kiwifruit) genome

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Most published genome sequences are drafts, and most are dominated by computational gene prediction. Draft genomes typically incorporate considerable sequence data that are not assigned to chromosomes, and predicted genes have no quality confidence measures. The original *Actinidia chinensis* (kiwifruit) 'Hongyang' draft genome[1] had 164 Mb of sequence unassigned to pseudo-chromosomes, and omissions were identified in the gene models. A new genome of an *A. chinensis* (genotype Red5) was fully sequenced. This new sequence and combined genetic mapping data, resulted in a 554.0 Mb assembly with all but 6 Mb assigned to pseudo-chromosomes. RNA sequencing data from 12 tissues and ab initio analysis was used to identify genomic regions that contained possible genes. Using the WebApollo tool[2], an international consortium of 99 researchers undertook a year-long manual annotation process, checking, and modifying where necessary, each gene model. These new models were then checked a second time with a small group of annotators for quality and consistency. Each gene model was scored for quality allowing future researchers to gauge how confident the annotator was for the completeness of each gene model. In total, 33,257 gene loci represented by 33,492 isoforms were identified, named and tagged for quality[3]. Approximately 93% of the manually edited gene models differed from those in presented in the 'Hongyang' assembly[1]. The quality of the manually annotated gene models was tested by fully sequencing 550 cloned cDNAs confirming an increased quality using a manual annotation strategy. Our study highlighted the need to take a cautious approach to draft genomes and computationally predicted genes. The improvement demonstrated that a WebApollo-like manual checking step of gene models is necessary to improve computer predicted gene models, especially for certain types of gene families. This high quality gene set is now being used to supply the kiwifruit research community with a new tool for genomics and other comparative analysis.

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3. Pilkington S, Crowhurst R, Hilario E, *et al. An improved, manually edited Actinidia chinensis var. chinensis (kiwifruit) genome highlights the challenges associated with draft genomes and gene prediction in plants.* BMC Genomics 2018,

P45: *De novo* transcriptome assembly for estimating fruit crop genetic diversity and improving genome annotations

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As next generation sequencing advances, genomic resources once limited to model organisms are increasingly available for crop species. However, unlike model organisms, crops are often highly heterozygous and vary in ploidy. Here we analyse strand-specific RNA-sequencing data from several crops with published genomes, including apple, blueberry, grape, and kiwifruit, comparing results from genome-guided analysis using the STAR aligner[1] to those from *de novo* assemblies generated by Trinity[2], Oases[3], SOAP[4] and rnaSPAdes[5] and refined by either Mikado[6], PASA[7] or EvidentialGene[8] pipelines. We conclude that *de novo* assembly enhances detection of non-coding RNA sequences, as genome mapping excludes some divergent untranslated regions and regulatory non-coding transcripts. To a lesser extent, *de novo* assembly highlighted sequencing errors or divergence of coding regions, where interrupted open reading frames are missed in genome annotations. However, *de novo* assembly is prone to errors and limited by low coverage, with necessary refinement adding to computational costs. The utility of *de novo* assembly in crop RNA-sequencing analysis should be evaluated on a case-by-case basis, depending on experimental goals and the divergence between target cultivars and published genomes.

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P46: Characterisation of *CAROTENOID CLEAVAGE DIOXYGENASE 4* alleles in yellow and white flowered kale and radish.

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Flower colour is one of the major visual signals used by plants to attract insect pollinators, and the yellow colour in flowers of Brassicas such as canola (*B.napus*) and kale (*B.oleracea* var *acephala*) has been mainly attributed to carotenoids. In ancestral Brassica lineages a *CAROTENOID CLEAVAGE DIOXYGENASE 4* (*CCD4*) gene catalysed the breakdown of carotenoids leading to a white (wild type) flower phenotype. The disruption of this gene by transposons or indels has recently been shown to restore the yellow flowered phenotype in many of modern lineages seen today. Radish (*Raphanus sativus* L.) is a closely related member of the Brassica family and is normally white flowered, and along with forage kale has become increasingly important in New Zealand's supplementary feed sectors. In this work we were interested to determine which alleles of *CCD4* were present in NZ adapted kale and radish cultivars and to which of the published *CDD4* lineages they belong.