ASPS21 ACT

Genome-wide association study uncovers novel alleles of two rice blast resistance genes

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Genome-wide association studies (GWAS) are frequently used to determine the association between traits and genomic loci in diverse genetic populations. However, GWAS are rarely applied to crop plants and very few studies confirm the underlying genetic determinant of a particular trait. A major limitation to the efficacy of GWAS in the context of gene discovery and subsequent plant breeding is the limited availability of high quality and high density molecular markers across a diverse population. Of the major grass crop species which provide a large proportion of the calories consumed by humans, rice has the most abundant genetic resources. The 3000 rice genomes project has provided researchers with millions of polymorphic nucleotides across the rice genome that can be leveraged for GWAS. By screening a subset of the 3000 rice genomes for resistance or susceptibility to multiple strains of Magnaporthe oryzae, the causal agent of rice blast, we uncovered several candidate regions which are associated with rice blast resistance. We then performed genomic sequencing of a small subset of accessions which carry the resistance associations, allowing for the extraction of genotype specific candidate gene sequence. The resistance functions of novel resistance alleles of Ptr, an atypical membrane bound Arm-repeat protein that behaves as a race specific resistance gene, and Pia, a resistance locus underpinned by a pair of typical nucleotide-binding site leucine-rich repeat (NLR) resistance genes were then validated using transgenic rice plants and transgenic strains of Magnaporthe Oryza respectively. By matching the alleles of the resistance genes we have discovered to corresponding polymorphisms in the 3000 rice genomes data, genotypes which are likely to contain these, and previously described resistance alleles have been highlighted to inform breeding programs. With the reduced cost of genome sequencing, the diversity in other more genetically complex crops will be able to be leveraged in the same way as is currently possible for rice.

ASPS21 ACT

An Atlas of Plasmodesmata Density Between Photosynthetic Cells Throughout Early Leaf Development of C_3 and C_4 Species

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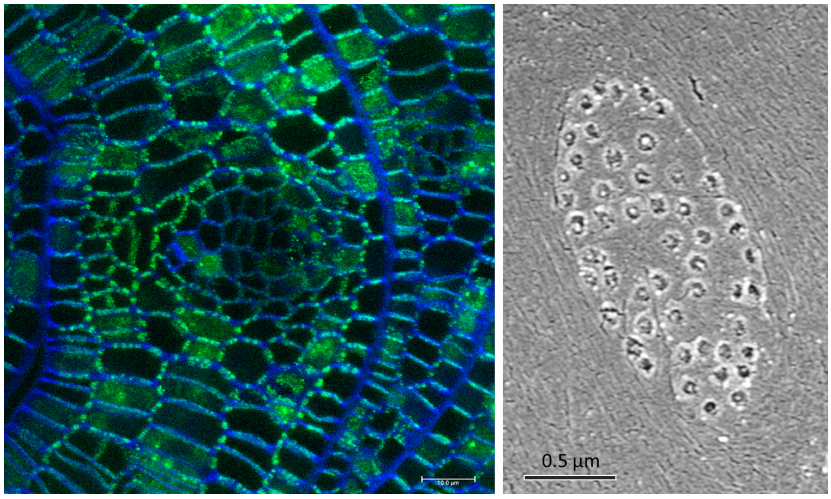
Plasmodesmata are tiny highways between plant cells essential for transport. More photosynthetic efficient C_4 leaves have up to 10x more plasmodesmata between photosynthetic cells compared to C_3 leaves. To investigate what are the genes involved in the formation and regulation of plasmodesmata between photosynthetic types, transgenic lines of rice (C_3) and setaria (C_4) with fluorescent protein-tagged plasmodesmata were generated. Plasmodesmata form at very early stages of cell development. Therefore, these lines were used to build an atlas of plasmodesmata density between photosynthetic cells throughout early leaf developmental stages using confocal microscopy to identify the developmental stage/s where plasmodesmata density differentiation occurs in C_3 and C_4 leaves. There are five early developmental stages of interest identified and RNA samples were collected from these developmental stages for sequencing and analysis to obtain a candidate plasmodesmata-related gene list.



ASPS21 ACT

Disrupted photoperiodic flowering in Revenue wheat

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Wheat (*Triticum aestivum* L.) is generally classified as a vernalisation-sensitive, long-day plant, with reproductive development occurring after exposure to winter cold followed by lengthening days of spring. Adaptation to a range of climates is driven by diversity in phenology, that is, the seasonal timing of the plant life cycle. In wheat, phenology is controlled by major genes including *VERNALISATION-1 (VRN1*) and *PHOTOPERIOD-1 (PPD1*) which underlie responses to temperature and daylength. Genetic variation at these loci has been well-described, though minor effect genes for flowering are still being identified. Australian cultivar Revenue is a late-flowering wheat with unexplained phenology; it flowers later than expected given its alleles of *VRN1* and *PPD1* identified by current molecular markers. This research project was established to determine the genetic basis for Revenue's flowering behaviour and provide information for future cultivar development. Quantitative trait loci (QTL) mapping in a recombinant inbred population in controlled environments and field conditions revealed vernalisation requirement of Revenue was associated with *VRN1*, while an additional gene on chromosome 5A, *HEADING DATE 6 (Hd6)* was associated with photoperiodic flowering response. Cytogenetic analysis revealed chromosome-scale variation was also associated with flowering behaviour. Additional genetic loci including candidate genes from the photoperiodic flowering pathway in rice were also identified, suggesting conservation of an ancestral short-day flowering response contributes to phenology in wheat cultivar Revenue (a long-day plant).

ASPS21 ACT

Parastagonospora nodorum effector ToxA interacts with wheat NHL proteins to induce *Tsn1*-mediated cell death

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The wheat fungal pathogen, *Parastagonospora nodorum*, secretes effector proteins to manipulate host immunity and promote successful infection. One of the major determinants of the disease is ToxA effector protein, which causes cell death in the presence of wheat susceptibility gene *Tsn1*. However, the molecular mechanisms underpinning this interaction and resulting cell death remain unclear. To elucidate the molecular mechanisms of the ToxA effector, we used independent and complementary approaches to identify wheat proteins that interact with ToxA effector. We found that ToxA targets an integral membrane protein of wheat (TaNHL10) and showed that this interaction occurs in the extracellular space of the plant. We further showed that the interaction between ToxA and TaNHL10 is required for *Tsn1*-mediated cell death via mutagenesis studies of the effector. This indicates that TaNHL10 is a linking protein between ToxA and Tsn1 indirect interaction and is involved in *Tsn1*-mediated necrosis. This research also advances our understanding on how ToxA induces cell death during infection and further highlights the importance of host cell surface interactions in necrotrophic pathosystems.

A Selectivity Switch in the Arabidopsis Aquaporin AtPIP2;1: Ion and Water Permeability Differentially Regulated by Kinases

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Phosphorylation is a key post-translational modification that influences plant aquaporin function and has recently been demonstrated to be an important regulator of the Arabidopsis PIP2;1 (AtPIP2;1) water and ion channel function. The phosphorylation status of two serine residues located on the cytoplasmic facing loops B and D of AtPIP2;1 can also influence its water and ion transport. AtPIP2;1 phospho-mimic and phospho-deficient versions of these two residues were expressed in *Xenopus laevis* oocytes and their ionic conductance and water permeability tested. Although phosphorylation is emerging as a strong regulator of ion channel aquaporin function, the specific kinases and the associated signalling pathways controlling ion channel aquaporins are mostly unknown. Members from two plant kinase families involved in signal transduction in plant osmotic stress responses, SnRK2 and Calcium-Dependent Protein Kinases (CDPKs), have been found previously to phosphorylate PIP subfamily aquaporins. Therefore, candidate upstream kinases OST1 (Snrk2.6), and the CDPKs CPK3 and CPK21 were investigated for their capacity to influence AtPIP2;1-associated water and ion transport by their co-expression in *X. laevis* oocytes. The co-expression of candidate plant kinases with AtPIP2;1 were found to have profound effects on its water and ion channel function; co-expression with OST1 and CPK3 reduced both the water and ion transport of AtPIP2;1, whereas CPK21 co-expression only reduced AtPIP2;1 water channel activity. The influence of these kinases on the combination of aquaporin facilitated water and ion conductance may have important implications for osmotic and ionic regulation *in planta*.

ASPS21 ACT

Photosynthetic responses of a model legume under elevated CO_2 concentrations

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Legumes are generally considered to be more responsive to elevated CO2 (eCO_2) conditions due to the benefits provided by symbiotic nitrogen fixation. The responsiveness of nodulation to eCO_2 suggests that photosynthesis and nodulation are closely regulated. In response to high carbohydrate demand from nodules, legumes display autoregulation of nodulation (AON) to restrict nodules to the minimum number necessary to sustain nitrogen supply under current photosynthetic levels. AON mutants supernodulate and typically grow smaller than wild-type plants under ambient CO_2. This study examined photosynthetic and nodulation traits across super-nodulating (*rdn1-1* and *sunn4*) and non-nodulating (*nfp1*) *Medicago truncatula* mutants, compared to the wild-type under elevated CO_2 (eCO_2) conditions. We hypothesised that AON mutants are carbon-limited and can perform better at eCO_2 through improved photosynthesis. Plants were grown in controlled growths room either at ambient (400 ppm) or eCO_2 (700 ppm) for seven weeks under low (1 mM) nitrate with ^{15}N supplementation to measure nitrogen fixation. CO_2 assimilation rate and chlorophyll fluorescence were measured to derive estimates of maximum Rubisco activity, V_{cmax}, and rate of electron transport, J. Under eCO_2 conditions, the improved nitrogen fixation of super-nodulating mutants allowed for greater investment in photosynthetic proteins compared to the wild-type. Super-nodulating mutants also exhibited higher V_{cmax} and J than wild-type and non-nodulating counterparts. Nodulating legumes, especially those with higher nitrogen fixation capability, are likely to out-perform non-nodulating plants under future CO_2 conditions, though long term studies will be necessary in determining whether this effect is vulnerable to acclimation.

ASPS21 ACT

New methods for confocal imaging of infection threads in crop and model legumes

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The formation of infection threads in the symbiotic infection of rhizobia in legumes is a unique, fascinating, and poorly understood process. Infection threads are tubes of cell wall material that transport rhizobia from root hair cells to developing nodules. They form in a type of reverse tip-growth from an inversion of the root hair cell wall, but the mechanism driving this growth is unknown. High resolution, 3-dimensional imaging of infection threads, and cell wall component specific labelling, would greatly aid in our understanding of the nature and development of these structures. To date, such imaging has not been fully utilised, with infection threads typically imaged by bright field, by GFP-tagged rhizobia, and histochemically in thin sections. We have developed new methods of imaging infection threads using novel and traditional cell wall fluorescent labels, and laser confocal scanning microscopy. We have applied the use of a new highly intense and stable periodic Schiff rhodamine-123 label, which has allowed for 3D imaging of infection threads in high detail. Through novel combination of the above method and calcofluor-white staining, we have also succeeded in differentially labelling infection threads, and have visualized infection thread walls and matrices separately. This differential labelling of infection threads and imaging of whole samples by confocal microscopy has made possible a more detailed and accurate quantification of thread phenotypes and numbers than had until now been practicable. Our methods have made the imaging and study of infection threads more efficient and informative, and present exciting new opportunities for future research in the area.

ASPS21 ACT

The identification of flavonoids which can modulate polar auxin transport during nodule development in *Medicago truncatula*

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The ability of plants to undergo a symbiotic relationship with nitrogen-fixing rhizobia is one of the only two possible pathways for plants to gain access to nitrogen through the conversion of atmospheric nitrogen into ammonia by rhizobia. However, only the legume family can undergo this symbiosis, which is important for plants growing in nitrogen-poor soil. The aim of my project is to understand how rhizobia regulate the process of nodule development, in particular, how rhizobial signals control the initiation of cell division in the root. We previously found that rhizobia redirect the polar transport of auxin in the root to localize auxin in dividing cells. Auxin transport control requires the action of flavonoids, as flavonoid-deficient legumes cannot inhibit auxin transport and do not develop nodules. However, the structure of the active flavonoid is unknown. Using Medicago truncatula, a model legume, several flavonoids were tested as potential auxin transport regulators based on; 1) whether they can inhibit auxin transport in the roots, 2) Whether they are upregulated in by nitrogen-fixing bacteria in roots and 3) whether they can rescue nodulation in roots that are flavonoid-deficient. Using a combination of metabolite profiling, direct compound tracing and bioassays, several flavonoid candidates were identified to match these criteria. Future experiments will be directed at finding out how conserved the role of flavonoids as auxin transport regulators during legume nodulation is. This could contribute to answering the overarching question of why some plants can nodulate, but others cannot.

A recurrent selection for increased shoot vigour in wheat modified root hair length and epidermal cell size in the roots and leaves.

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Wheat competitiveness with weeds has been reduced through a century of selection for reduced plant height and increased harvest index. Re-introducing early shoot vigour has been suggested to improve competitiveness without deteriorating the harvest index. A recurrent selection for early shoot vigour in wheat resulted in significant leaf width and shoot biomass increases. However, little is known on the impact of this above-ground selection on below-ground traits. The effect of early shoot vigour on root biomass, rhizosheath size, root hair length, and cell size in the roots and leaves was examined across different selection cycles. It was confirmed that leaf width increased for the first three leaves at every cycle of the recurrent selection. Increased shoot vigour was also associated with greater root biomass, larger rhizosheath size and longer root hairs. Our findings demonstrate that rhizosheath size was a reliable surrogate for root hair length in this germplasm. Examination of the root epidermis revealed that the “cell body” of the trichoblasts (hair-forming cells) and the atrichoblasts (non-hair forming cells) decreased in size as shoot vigour increased. Therefore, in higher vigour germplasm, longer root hairs emerged from smaller trichoblasts so that total trichoblast volume (root hair plus cell body) was generally similar regardless of shoot vigour. Similarly, the sizes of four main cell types on the leaf epidermis became progressively smaller as shoot vigour increased, increasing stomatal density. The relationship between shoot vigour and root traits is considered, and the potential contribution of below-ground root traits to performance and competitiveness of high vigour germplasm is discussed.

Deciphering aquaporin functional roles in salt-secreting mangrove *Avicennia marina*.

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Aquaporins (AQP) are transmembrane proteins with key functional roles in all kingdoms of life. In plants, the complement of AQP roles is fast expanding, encompassing roles in plant growth and resilience to environmental stresses such a salinity. Mangroves are particularly adapted to grow in highly-saline environments, with salt-secreting mangroves such as Avicennia marina having evolved specialised cellular structures in leaves (salt glands) that enable the secretion of salt to the leaf surface. Aquaporins are emerging as candidate mechanisms implicated in mangrove salinity tolerance. We aim to explore the functional roles of aquaporins in A. marina leaves as osmotic regulators, through their dual transport capability of water and/or sodium across cell membranes. We identified 40 full length AQP-encoding genes in the A. marina genome. Several isoforms from the two major AQP sub-families, Plasma membrane Intrinsic Proteins (PIP) and Tonoplast Intrinsic proteins (TIPs), were functionally tested for water and sodium permeabilities using novel high-throughput yeast-based screens. We identified candidate AQPs permeable to water and sodium, implicating them as potential novel players in mangrove salt-secreting mechanisms. These newly-identified sodium-transporting candidates could also pose as genetic-engineering targets to increase salt tolerance of other plant species.

Expression of the CO_2-permeable aquaporin SiPIP2;7 enhances CO_2 diffusion in leaves of C_4 *Setaria viridis*

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A major limitation for assimilating carbon from the biosphere is the diffusion of CO_2 to the primary carboxylases of photosynthesis. Modelling of C_4 photosynthesis have shown that increasing mesophyll conductance (the conductance to CO_2 diffusion from leaf intercellular air space to the mesophyll cytosol) could stimulate CO_2 assimilation rate at low CO_2. The significant contribution of aquaporins, the pore-forming integral membrane proteins that regulate the movement of water and solutes, to increased CO_2 diffusion has been demonstrated in C_3 plants. The role of CO_2 permeable aquaporins in C_4 photosynthesis has not been investigated.

We explored the aquaporin gene family within the C_4 grass *Setaria italica* and screened them for CO_2 permeability utilising a yeast expression system. We identified SiPIP2;7, predominately expressed in roots, as a CO_2 permeable aquaporin. We repurposed its function by expressing SiPIP2;7 in *S. viridis* mesophyll cells and demonstrated its correct insertion into the plasma membrane. We discovered that mesophyll conductance in C_4 plants can be enhanced by expressing CO_2 permeable aquaporins in the mesophyll plasma membrane. Moreover, we demonstrated that greater mesophyll conductance improved CO_2 assimilation at low concentrations of intercellular CO_2, in line with C_4 photosynthesis model predictions.

ASPS21 ACT

A new structural family of effectors from *Fusarium oxysporum* adopt a dual-domain fold

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Plant pathogens secrete proteins, known as effectors, that function in the apoplast and inside plant cells to promote virulence. Despite their importance, our general understanding of fungal effector function and detection by immunity receptors remains poor. One complication often associated with effectors is their high sequence diversity and lack of identifiable sequence motifs enabling prediction of structure or function. As a result, cross-disciplinary approaches involving structural biology and protein biochemistry are often required to decipher and better characterise effector function. Using protein x-ray crystallography aided by a co-expression system within the heterologous host *E. coli* for the high yield production of disulfide-rich effectors, we identify a new structural class of effectors hidden within the secreted in xylem (SIX) effectors from *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). The effectors Avr1 (SIX4), Avr3 (SIX1) and SIX6 represent the founding members of the Fol dual-domain (FOLD) effector class consisting of two distinct domains: A disulfide-rich N-domain and a C-domain consisting of a β-sandwich fold. Using a protein-mediated phenotyping approach, we identify the N-domains of Avr1 and Avr3 are sufficient for recognition by the resistance proteins, I and I-3, respectively. Collectively, these data will aid future studies to understand the molecular basis of *F. oxysporum* effector function and recognition, and by extension, the design and engineering of immunity receptors with novel recognition specificities to help protect plants against *Fusarium* wilt disease.

Enhancing Rubisco catalysis improves plant growth

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The rate of photosynthesis and plant growth is often rate limited by the activity of the CO_2-fixing enzyme Ribulose bisphosphate carboxylase oxygenase (Rubisco). Over half a century there has been significant effort to understand the catalytic chemistry and biogenesis properties of this enzyme towards identifying ways to improve its carboxylation properties.

Kinetic diversity screens have identified natural variants of Rubisco with properties beneficial to photosynthesis in C3-plants like rice, wheat and canola, however there are impediments to introducing these changes into crops. In many cases the levels of catalytic improvement are also insufficient to translate to improvements in growth. In this talk I will discuss new synthetic biology and directed evolution methods that have proved a more feasible pathway towards generating the step change in Rubisco performance needed to visibly improve plant productivity.