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Oral Presentations

Advances in rice biotechnology and genome editing

OP.01

Type: Oral Presentation

Topic: Advances in rice biotechnology and genome editing

High frequency synthetic apomixis in rice

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Transferring apomixis to crops has the potential to revolutionize agriculture, notably for small-holder farmer, by allowing F1 hybrids, with enhanced yield and adaptation potentials, to be clonally propagated by seeds, in an immortalized manner, across generations. Apomixis can be induced by combining i. the conversion of meiosis into mitosis (apomeiosis) producing unreduced, unrecombined male and female gametes, and ii. the parthenogenic development of the diploid egg cell into a clonal embryo. This strategy was successfully applied to engineer synthetic apomixis in rice through CRISPR/Cas9-mediated induction of the triple *pair1/osrec8/ososd1* mutations (that turn meiosis into mitosis: Mieulet et al 2016) in a line (cv.Kitaake) expressing the BABYBOOM1 (BBM1) parthenogenesis inducer driven by an Arabidopsis egg-cell specific promoter (Khanday et al 2019). In that study, the frequency of apomictic plant formation ranged from 10-30% and apomictic trait was stable over 3 generations. We show here, using F1 seeds, that apomixis can be induced in a single step and at a >95% frequency across multiple generations. Clonal apomictic plants faithfully recapitulated the phenotypes of the F1 hybrid, and the stability of their heterozygous genome across generations was directly confirmed. Our results establish that there is no intrinsic barrier preventing very high frequency of apomictic plant formation in rice rendering it compatible with its use in agriculture.

OP.02

Type: Oral Presentation

Topic: Advances in rice biotechnology and genome editing

Neutralization of SARS-CoV-2 by heterologous cyanovirin-N produced in rice endosperm is enhanced by globulins

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel beta coronavirus, the causative agent of coronavirus disease 2019 (COVID-19), that has infected more than 192.6 million people and caused more than 4 million deaths¹. Although currently there are approved vaccines based on mRNA, recombinant viral proteins, and peptides technologies, these can only be used to protect healthy individuals who have not yet been exposed to the virus². For patients in the acute disease phase, who are most at risk of death, drugs that block the viral replication cycle are urgently needed, as well as the means to produce such drugs on a large scale. Lectins are carbohydrate-binding proteins produced by plants, algae and cyanobacteria³. A number of lectins can neutralize viruses displaying surface glycoproteins, offering a promising therapeutic approach for acute infections with virulent β -coronaviruses such as severe SARS-CoV-2^{4,5}. We show that transgenic rice endosperm extracts containing the lectins griffithsin (GRFT) and cyanovirin-N (CV-N) can neutralize SARS-CoV-2 *in vitro*. We also demonstrate that pure CV-N produced in *E. coli* binds more efficiently to the SARS-CoV-2 spike protein (S1) than GRFT and that both lectins compete for the same binding site. CV-N was more potent when was presented in a crude rice seed extract, suggesting an independent neutralizing effect that resolves to the globulin fraction. The rice extract enhanced the effect of CV-N specifically and resulted in synergistic neutralization. Molecular modelling predictions revealed that rice globulins bind simultaneously to S1 and CV-N explaining, at least in part, the synergistic activity of the two proteins against SARS-CoV-2.

OP.03

Type: Oral Presentation

Topic: Advances in rice biotechnology and genome editing

Novel hybrid CRISPR/Cas9 and Cpf1 genome editing yields bacterial leaf blight-resistant rice for Africa

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Bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a destructive rice disease in Asia. In recent years, increasing BLB outbreaks have been reported in Africa, placing rice farmers at risk of losing their yield. More importantly, climate change and the globalization of seed exchange pose a severe BLB threat to Africa.

Xoo strains induce three rice sucrose transporter-encoding genes: *OsSWEET11*, *OsSWEET13* and *OsSWEET14*, using transcription activator-like (TAL) effectors. The transcriptional upregulation of these *OsSWEET* genes likely provides nutrients to the pathogen, allowing it to reproduce and cause the disease. African and Asian *Xoo* strains have distinct sets of TALEs that can upregulate *OsSWEETs*. While Asian strains harbor PthXo1, PthXo2, PthXo3, AvrXa7 to upregulate all three *OsSWEETs*, African *Xoo* strains harbor TalC and TalF specifically target *OsSWEET14*. Editing the TAL effector binding elements (EBEs) on *OsSWEET* promoters prevents TALE-dependent induction of *OsSWEETs*, leading to broad-spectrum resistance against the bacterial pathogen. Hence, editing the TalC and TalF EBEs in *OsSWEET14* would create BLB resistance against Africa *Xoo* strains and editing the EBEs on *OsSWEET11* and *13* would prepare Africa for a putative invasion by Asian *Xoo* strains.

Here, we developed a new hybrid CRISPR/Cas9 and Cpf1 system to simultaneously edit all 6 EBEs on three *OsSWEETs* genes in Komboka, a mega *indica* variety for Africa. The new hybrid CRISPR/Cas9 and Cpf1 offered higher flexibility in designing guide RNA, given the limitation of PAM recognition sites near EBEs. The EBE edited Komboka lines showed broad-spectrum resistance to a set of five representative *Xoo* strains from Asia and Africa harboring all known *OsSWEETs*-inducing TALEs. Our results demonstrate a powerful approach to develop broad-spectrum and durable resistance rice for Africa using genome editing.

OP.04

Type: Oral Presentation

Topic: Advances in rice biotechnology and genome editing

Expression of functional nitrogenase Fe protein (NifH) in rice

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Biological nitrogen fixation (BNF) is the conversion of atmospheric nitrogen to ammonia, carried out by free-living diazotrophs and symbiotic bacteria in the root nodules of legumes and some non-leguminous plants. The integration of nitrogen fixation traits into cereal crops is a long-term goal of agricultural development, but this has been frustrated by the inability to express the nitrogenase protein complex (NifH and NifDK) in an active form, partly due to the extreme oxygen-sensitivity of the Fe protein (dinitrogenase reductase) encoded by *nifH*. We generated rice plants expressing NifH from the extremophile *Hydrogenobacter thermophilus* (Aquificae) targeted to mitochondria together with a putative peptidyl prolyl cis-trans isomerase (NifM). Both Nif proteins accumulated in soluble form and we were able to purify substantial amounts of *H. thermophilus* NifH from rice callus and corresponding regenerated plants. The isolated NifH was capable of electron transfer to NifDK and the *in vitro* activity of NifH confirmed its ability to incorporate endogenous rice mitochondrial Fe-S clusters. The expression and activity of a nitrogenase component in rice represents an important first step toward the expression of a complete functional Nif complex required to achieve BNF *in planta*.

OP.05**Type: Oral Presentation****Topic: Advances in rice biotechnology and genome editing**

Dissecting starch metabolism in rice and generation of specialty rice genotypes through genome editing

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Starch properties can be modified by mutating genes responsible for the synthesis of amylose and amylopectin in the endosperm, in concert with manipulating enzymes that extend the polymer backbone, add new branches, or remove them. The effects of such interventions on wider metabolism are rarely investigated but could help to predict the most optimal metabolic engineering strategies for the creation of specialty starches for industrial or medical applications. We investigated the impact of deactivating individually three genes in the rice endosperm starch biosynthetic pathway on overall starch and broader metabolism, physicochemical properties and phenotype. Inactivation of starch branching enzyme (OsSBEIIb), cytosolic glucose-1-phosphate adenylyl transferase large subunit (OsAPL2) and granule-bound starch synthase 1 (waxy/GBSS1) individually, triggered broad transcriptional reprogramming of starch metabolism, and profound changes in overall metabolism. These responses reflect the adjustment of metabolism to deal with the changes in amylose and amylopectin and resulted in the generation of rice genotypes with nutritional and health-promoting properties.

Future Prospects: Food security and nutrient enhancement

OP.06

Type: Oral Presentation

Topic: Future prospects: Food security and nutrient enhancement

Identification and characterization of *OsTH1*, a gene putatively involved in thiamin biosynthesis in rice

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Food security and nutrition quality are not yet guaranteed to the whole population. Over 2-billion people suffer from hidden hunger and the UN Sustainable Development Goal-2 aims to end all forms of hunger by 2030. Rice is the world's second major staple crop and the primary source of calories in developing countries. However, white rice, the most consumed form of rice, has insufficient concentration of several vitamins and micronutrients. Biofortification via metabolic engineering is thus a powerful strategy to alleviate micronutrient deficiency.

Thiamin (B1) deficiency is one of the most common vitamin deficiencies in developing countries. To guarantee normal metabolic function, humans rely on a sufficient dietary supply of thiamin. Nonetheless, not much is known regarding rice thiamin biosynthesis and, to redesign its pathway, a deep knowledge is required. Aiming to identify putative limiting enzymes of thiamin biosynthesis, we created a kinetic model of B1 pathway. The model revealed *THIC* (HMP-P synthase) as the rate-limiting enzyme of thiamin pathway as well as *THI1* (HET-P synthase) and *TH1* (HMP-P kinase/thiamin monophosphate synthase). *THIC* and *THI1* were previously identified in rice, however, the gene coding for *OsTH1* remains elusive. Here we report the identification and characterization of a putative *OsTH1* gene, involved in thiamin biosynthesis in *Oryza sativa* L.

To identify a putative *OsTH1*, we analyzed the rice genome using blast algorithm, gene expression data (Genevestigator) and phylogenetic analysis. We identified *LOC_Os12g09000* as a putative *OsTH1*. Using yeast knock-out mutants impaired in thiamin production, we could functionally complement the phenotype through heterologous expression of *LOC_Os12g09000/OsTH1*. In conclusion, we (1) identified *THIC*, *THI1* and *TH1* as the limiting enzymes of rice-B1 biosynthetic pathway and (2) identified and characterized the rice *TH1* gene putatively involved in thiamin biosynthesis. Our results provide new insights in B1 biosynthetic pathway, opening doors for its biofortification in rice.

OP.07**Type: Oral Presentation****Topic: Future prospects: Food security and nutrient enhancement**

Promoting food security and human health: making black rice staple food

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Rice grains are rich in diverse nutrients, including protein, unsaturated oil, minerals, vitamins, dietary fibre, flavonoids and polysaccharides, among others. These nutrients are present in the pericarp, seed coat, aleurone, germ and endosperm. Rice grains exist in many different colours. Black rice, rich in anthocyanins in the pericarp and seed coat, is considered in Chinese legend to be a panacea for health and longevity. Indeed, numerous studies have shown that water-soluble extracts of black rice are protective against a wide range of non-communicable diseases including cancers, cardiovascular diseases, diabetes mellitus and metabolic syndrome. Yet, for thousands of years, consumers have preferred polished rice and eat only the endosperm in pursuit of palatability; such habit discards ~80% of the nutrients and beneficial components of the grain as bran. Thus, consumption of whole-grain rice will be necessary to maximize its nutritional values and health benefits. Moreover, eating the whole grain would amount to a >30% increase of the edible portion relative to polished rice, thereby also enormously reducing the pressure on demand thus contributing to food security. However, grains of black rice are in general unpalatable and difficult to cook as whole grains, presenting a major obstacle to their consumption. Recent progress in genomic breeding has produced black rice varieties with good cooking texture and palatability. We propose to change our dietary habit to make black rice staple food in our daily life. Such change will not only make fundamental impacts in our nutrition, health and food security, it will also generate far reaching effects on sustainable agriculture.

Integrative physiology and functional genomics

OP.08

Type: Oral Presentation

Topic: Integrative physiology and functional genomics

NCRx may involve in auxin flow regulation through plasmodesmata in promoting crownroot development in rice

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Rice (*Oryza sativa* L.), an important food crop highly susceptible to environmental stresses, relies on its extensive system of crown and lateral roots for water, nutrient uptake, and for stress response. A Genome-Wide Association Study was conducted on a rice panel including 155 Vietnamese landraces under 5 μ M of exogenous JA-induced stress that pinpoint a QTL associated with the induction of crown root. An expression study of genes located on the interval confidence of this QTL revealed a Germin-Like Protein named NCRx was strongly associated with the increase of crown root number under JA-induced stress. This protein contains a signal peptide that is supposedly targeted to the plasmodesmata, where it assembles into a hexamer anchored to the plasma membrane by the N-terminal transmembrane helix. Transgenic rice carrying the *ProNCRx::GUS* reporter gene shows the consistent pattern in the root system under different hormone-induced stresses and coincided with the auxin distribution pattern. Specifically, the strong expression of *NCRx* in the crown root epidermis, stele and lateral root primordia implied its involvement in all stages of the lateral root development. Moreover, this gene was expressed in the cortex, vasculature, and mostly in the epidermis of crown root primordia that suggests its involvement in crown root development, specifically during primordium maturation and emergence. *Cis*-acting regulatory elements analysis shows that *NCRx* is a hormone-responsive gene that is involved in a variety of stress responses. Taken together, the findings implied that *NCRx* could be involved in regulating plasmodesmata permeability to regulate the auxin flow as a part of different stress responses that results in changes in root development. This study provides further insight information for a better understanding of the regulatory mechanism that controls the monocotyledons root development.

OP.09

Type: Oral Presentation

Topic: Integrative physiology and functional genomics

Cloning and characterization of high temperature tolerance associated microRNA Osa-miR1436 in rice

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In plants, apart from protein-coding genes, non-coding small RNAs are important players in the regulation of stress responses. MicroRNAs (miRNAs) are known key regulators in plant development as well as in biotic and abiotic stresses which act by cleaving target mRNAs or by translational repression. Based on the genome-wide identification of miRNAs in tolerant (Nagina 22) and susceptible (Vandana) rice cultivars (Mangrauthia et al 2017, J Exp Bot) *miR1436* was identified as heat tolerance associated microRNA. In this study, the functional characterization of *miR1436* was carried out by qPCR analysis and genetic transformation into rice. qPCR analysis of stressed RNA sample revealed 9.0 and 1.8 fold up-regulation of *miR1436* in shoot and root of the heat tolerant cultivar N22, while no significant changes were observed in susceptible cultivar Vandana. Also, the expression level of Tyrosine protein kinase domain containing protein, one of the target gene of miRNA1436 was upregulated 7.9 and 1.2 folds in N22 root and shoot respectively while, in case of Vandana 2.9 and 1.2 fold up-regulation was observed in the shoot and root. The gene encoding *miR1436* was amplified from N22 genomic DNA using gene-specific primers and cloned into plant transformation vector pGWB17. The construct pGWB17-miR1436 was genetically transformed into the rice genotype Taipei-309 through *Agrobacterium* mediated transformation. Of the 197 T₀ transgenic plants, 6 plants were confirmed as positive through PCR analysis using *miR1436*, 35S promoter, miR1436+35S promoter and *hpt* gene specific primer combinations. The transgenic plants showed higher chlorophyll a, b and carotenoid content compared to control plants. Notably leaf length and width was also higher in *miRNA1436* transformed plants when compared with control. These *miRNA1436* transformed rice lines will be useful genetic resource for characterization of the function of this miRNA in plant developmental regulation and heat stress response.

OP.10

Type: Oral Presentation

Topic: Integrative physiology and functional genomics

Functional characterization of rice transcription factors regulating C₄ *PEPC* promoters

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Rice is the staple food for over half of the world population and its yield must increase in the next decades. Engineering C₄ photosynthesis into rice (C₃ plant) is thought to help meeting this goal, since C₄ plants outperform C₃ in hot and dry climates. C₄ evolution was preceded by major changes in the regulation of genes encoding key enzymes. A good example is phosphoenolpyruvate carboxylase 1 (*PEPC1*), whose promoter evolved to drive mesophyll cell-specific and high gene expression in C₄ plants. Interestingly, *PEPC1* promoters from different C₄ species can drive mesophyll-specific and diurnal gene expression in rice. It was therefore fundamental to identify/characterize transcription factors (TFs) regulating the activity of C₄ *PEPC1* promoters in rice. Using a Yeast One-hybrid approach, we have identified several rice TFs binding to the C₄ *PEPC1* promoters from maize and *Setaria viridis*. We have identified for instance OsbHLH112, a rice bHLH TF that interacts with the maize *PEPC1* promoter and has two homologues in maize, ZmbHLH80 and ZmbHLH90 ⁽¹⁾. These two TFs act antagonistically and, together with ZmOrphan94 ⁽²⁾, contribute to regulate *PEPC1* cell-specific gene expression in maize. We have also identified OsbHLH60, which binds to the setaria *PEPC1* promoter and seems to act as activator. To test its function in planta, we have produced rice transgenic lines harbouring promSv*PEPC1*::*GUS* and knocked out *OsbHLH60*, using CRISPR-Cas9. These transgenic plants show a clear reduction of *GUS* expression, showing that OsbHLH60 indeed acts as an activator of *PEPC1* expression. We are also investigating the interaction of the rice TFs identified as binding to C₄ *PEPC1* promoters, as well as their homologs in other C₃ and C₄ plants, with different promoters from representative C₃ and C₄ plants. Altogether, our work suggests that a C₃ pre-existing gene regulatory network was co-opted by C₄ plants to regulate *PEPC1* expression.

Molecular breeding under a changing climate

OP.11

Type: Oral Presentation

Topic: Molecular breeding under a changing climate

Marker-assisted introgression of the salinity tolerance locus *SalTol* in temperate japonica rice

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Rice production in worldwide cultivating areas is severely affected by soil salinity. The H2020 project NEURICE “New Commercial EUropean Rice (*Oryza sativa*) harbouring salt tolerance alleles to protect the rice sector against climate change and apple snail invasion” aimed at introducing salt tolerance in susceptible European rice germplasm using the positive effect of the *SalTol* QTL from *indica* rice.

SalTol QTL was introgressed in two high yielding Italian temperate *japonica* rice varieties, Vialone Nano and Onice through Marker Assisted Back-Cross (MABC) Breeding. Three back-crosses (BC₃) followed by selfing allowed the successful *SalTol* QTL introgression. After the cross with the IR64-*SalTol* donor, foreground selection was carried out using SSR markers in the first BC generation and subsequently KASP markers. In addition, 96 genome-wide KASPs polymorphic between donor and recurrent parents were used in background selection. A total of 12 and 14 BC₃F₄ lines were selected from the BCs of Vialone Nano and Onice, respectively. In-depth genotyping by sequencing analysis of the donor, recurrent parents and BC₃F₄ selected lines allowed an estimation of recovery of the recurrent parent genomes ranging from 93.4 to 98.9%.

A selection of 16 BC₃F₄ lines was evaluated in two different non-saline fields in Northern Italy and in two different saline ones in the Po river delta.

The three best field performing lines (O11, VN25 and VN28) were evaluated for salt tolerance (80 mM NaCl) at seedling stage in hydroponic conditions. Compared with sensitive parents, these lines showed lower visual and physiological sufferance symptoms and lower Na⁺/K⁺ ratio, especially in young leaves.

The present study of MABC was done in three years and half (2016-2019) accelerating the development of salt tolerant lines in the genetic background of Vialone Nano and Onice. The selected lines could be used for commercial cultivation in saline affected area.

OP.12**Type: Oral Presentation****Topic: Molecular breeding under a changing climate**

Transposable Element Polymorphisms Improve Prediction of Agronomic Traits in Rice

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Previous work has shown that Transposable Elements Polymorphisms (TIPs) can improve detection of causative loci on agronomic traits in rice. Here we quantify the fraction of variance explained by SNPs vs TIPs and we explore whether TIPs can also improve prediction of phenotypes when compared to using only SNPs. For this purpose, we use eleven traits from five different varieties (Aus, Indica, Aromatic, Japonica and Admixed) that were downloaded from SNP-SEEK database (<https://snp-seek.irri.org>). The final dataset consisted of N = 738 samples, 228871 SNPs, 66367 DNA transposon and 30336 Retrotransposon markers. We assess prediction by cross-validation in two scenarios: a within variety scenario where we predict performance of improved Indica varieties (N = 76), and an across variety scenario where we predicted phenotypes of N=34 admixed aromatic samples. Prediction was measured by computing correlation between predicted and observed phenotypes. In each scenario, we compared Bayes C and a GBLUP like approaches. Bayes C is a variable selection method where markers entering into the model are sampled in each iteration. In GBLUP, a marker relationship matrix is obtained for each marker class separately. Prediction across varieties improved with TIPs in nine out of the eleven traits. In contrast, TIPs improved prediction within Indica only in four phenotypes. Our results confirm that TIP genotyping could improve prediction of complex traits in rice, especially when samples to be predicted are less related to phenotyped samples. We conjecture that this may happen because TIPs are in higher linkage disequilibrium with causative mutations, perhaps because gene regions are enriched in TIPs.

OP.13**Type: Oral Presentation****Topic: Molecular breeding under a changing climate**

Molecular mapping of QTLs governing number of grains and weight of panicles using a high-density linkage map

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The number of grains and the weight of panicles produced by rice plants are important factors determining yield. In an attempt to decipher genetic factors governing yield and to identify molecular markers for breeding, in this study, a genotyping-by-sequencing (GBS) based high-density linkage map of rice was developed using a recombinant inbred line (RIL) mapping population of 192 individuals derived from a cross between Argila and Cops8. These two varieties exhibited different weight and number of grains per panicle. A genetic map of 2455,65 cM was constructed with 7.676 filtered SNPs obtained by GBS. QTL analysis using phenotyping and genotyping information was carried out by two statistic methods, Inclusive Composite Interval Mapping (ICIM) and Multiple QTL Mapping (MQM). The QTL analysis identified a total of 8 QTLs. Of these, three major and one minor QTLs were detected for the number of grains per panicle accounting for 71.2 % of the phenotypic variance and four minor QTLs for the panicle weight explaining 26.3 % of the phenotypic variance. Candidate gene analysis showed that most of them were involved in panicle development and architecture or in spikelet meristem determinacy and floral organ identity. Three QTLs located in chromosomes 11 and 12 were not described previously and constitute novel QTLs governing number of grains in the panicles.

OP.14**Type: Oral Presentation****Topic: Molecular breeding under a changing climate**

Molecular Breeding temperate japonica rice

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Breeding favored the expansion of rice cultivation, modifying varieties to adapt the crop to unfavorable agroclimatic conditions and to show the best performance in new places. During this process, a vast genetic diversity was originated and different genetic groups appeared, defined mostly by the agroclimatic conditions where they were developed. During the intense and long-term breeding process many traits were forsaken due to the lack of interest in a certain moment. Nowadays, it is difficult to reincorporate them because of the genetic distance raised between the different genetic groups. The use of non-adapted varieties in breeding programs is challenging, as the incorporation of a new trait is generally accompanied by undesirable characters that do not meet climate adaptation requirements. Temperate *japonica* rice is adapted to the long days that occur in summer when temperature allows cultivation in temperate regions. Despite the narrow genetic pool due in temperate *japonica*, the region where it is cultivated is wide enough to hold relevant natural diversity uncovering a wide spectrum of morphological and physiological variations. The characterization of genome diversity is crucial for an efficient breeding, since it allows the identification of genetic factors and specific alleles that may introduced improvements by combination while maintaining the favorable components for the local area. Studies of population structure and genetic relationship have evidenced a strong substructure in temperate *japonica* rice, predominantly based on grain type and the origin of cultivars. There is evidence of a relatively high gene flow and elevated rates of admixture between cultivars grown in remote regions, probably favoured by local breeding.

The characterization of this diversity constitutes the basis for genetic association analyses, leads to molecular marker identification, selection appropriate parents for MAGIC populations and offers opportunities to incorporate new traits into local cultivars while conserving those characters responsible for local adaptation.

OP.15

Type: Oral Presentation

Topic: Molecular breeding under a changing climate

Overexpression of Rubisco and Rubisco activase rescues photosynthesis and biomass in rice under heat stress

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Global warming threatens food security by decreasing crop yields through inhibiting the activity of photosynthesis, in which Rubisco activation is extremely sensitive to temperature rise. Overexpressing Rubisco activase, which promotes the catalytic activity of Rubisco, was believed to be able to improve photosynthesis in high temperature. However, the previous studies showed that overexpression of Rubisco activase was always accompanied with decreases in Rubisco content, which resulted in the overall CO₂ assimilation and crop growth. In the present study, we produced rice lines co-overexpressing Rubisco and Rubisco activase to exam whether it is capable of improving photosynthetic and growth performance in high temperatures. Three rice lines were cultured throughout our research—the wild-type (WT), the Rubisco activase–overexpressing line (oxRCA), and the Rubisco- and Rubisco activase–co-overexpressing line (oxRCA-RBCS). Photosynthesis and plant biomass were analyzed at 25 and 40 °C. The content of Rubisco activase was 153% greater in oxRCA and 138% greater in oxRCA-RBCS, and the Rubisco content was 27% lower in oxRCA and similar in oxRCA-RBCS, comparing with WT. The photosynthetic rate of WT was lower at 40 °C than at 25 °C, caused by Rubisco deactivation under heat damage. On the other hand, the photosynthetic rate of oxRCA and oxRCA-RBCS showed no significant reductions at 40 °C, therefore resulting in maintenance of the photosynthetic rate, comparing with WT. Also, oxRCA-RBCS shows 26% higher dry weight compared with WT at 40 °C. Taken together, we concluded that increasing Rubisco activase content while maintaining Rubisco content improves yield and sustainability in rice at high temperature.

Plant-environment interactions: Abiotic stress

OP.16

Type: Oral Presentation

Topic: Plant-environment interactions: Abiotic stress

A Shaker channel improves rice salt tolerance by controlling leaf transpiration and K⁺ secretion to xylem sap

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High soil salinity strongly affects plant production and the magnitude of this constraint is constantly increasing due to salinisation by irrigation and climate change. Considerable efforts have been made over the last two decades to identify salt tolerance genes/alleles and to understand the physiological mechanisms by which they enable plant tolerance. Leaf K⁺/Na⁺ homeostasis is considered a key parameter of plant salt tolerance. A large number of studies have focused on Na⁺ transporters controlling Na⁺ translocation to leaves. Less attention has been paid to the K⁺ transport mechanisms that operate under saline conditions and ensure efficient K⁺ supply to leaves. Here, using a reverse genetics approach, we showed that the rice K⁺ Shaker channel OsK5.2 contributes strongly to the plant's salt tolerance (biomass production, leaf fitness). OsK5.2 is an outwardly-rectifying channel involved both in K⁺ loading of xylem sap for translocation to shoots and in the control of leaf transpiration by mediating K⁺ efflux from guard cells (Nguyen et al. 2017 *Plant Physiology* 174:2409-2418). We show that the contribution of OsK5.2 to plant salt tolerance results from the combined functions of OsK5.2 in roots and shoots. Indeed, OsK5.2 activity in guard cells allows, upon salt treatment, rapid reduction in stomatal opening, decrease in transpiratory water flow and thus reduction in Na⁺ translocation to the shoots. The associated negative effect of OsK5.2 activity on K⁺ translocation to shoots, which results from the reduction in xylem sap flow, is however outweighed by the direct contribution of OsK5.2 to the K⁺ load of xylem sap in roots. Thus, the dual function of OsK5.2 is overall very beneficial to shoot K⁺/Na⁺ homeostasis. In conclusion, our results highlight K⁺ channel-mediated mechanisms of salt tolerance, and provide a possible new target for plant breeders to improve salt tolerance in rice.

OP.17**Type: Oral Presentation****Topic: Plant-environment interactions: Abiotic stress**

Insight into jasmonate-dependent cold signalling of rice

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Despite of global warming low temperature episodes during the vegetation period can cause fatal damage to plants. According to recent studies late-spring frost-risk increased in Europe and Asia in the past 60 years significantly. Therefore, we are interested into the impact of low temperatures on rice plants and focused on jasmonate-dependent responses to cold-stress by studying the difference between wild type and jasmonate(JA)-deficient *allene oxide cyclase (aoc)* mutants. We found that the lack of this plant hormone caused a higher sensitivity to chilling stress suggesting a positive role of jasmonate for cold tolerance, which is reflected by accumulation of less malondialdehyde (MDA), more chlorophyll, a higher dry weight, and was characterized by an overall strong seedling growth under cold stress in the wild type as compared to the mutant. Transcriptional analysis of a cold-responsive gene, JA-responsive genes and hormonal profiling confirmed the positive role of jasmonates in cold tolerance. When comparing the expression profiles in leaves and roots we found organ-specific JA-dependent cold regulation of a cold responsive gene. This difference could be explained only partially by the action of lipid-derived second messengers suggesting the existence of a complex signalling network. Using untargeted metabolite profiling we got deeper inside into the beneficial adaptive responses to cold stress which are regulated by jasmonates.

OP.18**Type: Oral Presentation****Topic: Plant-environment interactions: Abiotic stress**

From the molecular mechanism to natural allele regulating rice grain filling under high temperature

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High-temperature (HT) has been more and more frequent, and became a serious threat to grain setting and filling which determine rice yield and quality. However, the molecular mechanism of grain setting and filling under HT is poorly understood, and the elite natural allele used to breed HT-tolerant rice varieties is few. Here, we used two strategies – pan-genomic and HT-sensitive mutant screening – to study the molecular mechanism regulating grain setting and filling under HT, and mine HT-tolerant natural allele. Here, we show that rice employ a leaf-to-caryopsis ABA transport-based mechanism regulated by defective grain-filling 1 (*DG1*), a multidrug and toxic compound extrusion transporter that effluxes ABA, to ensure normal seed development in response to variable temperatures (Qin et al., 2021b). Specifically, ABA is biosynthesized in both WT and *dg1* leaves, but only WT caryopses accumulate leaf-derived ABA. Our demonstration that leaf-derived ABA activates starch synthesis genes explains the incompletely filled and floury seed phenotypes in *dg1*. Both the *DG1*-mediated long-distance ABA transport efficiency and grain-filling phenotypes are temperature sensitive. Moreover, we identify a natural allele of *DG1* which could increase grain weight and quality under HT. To mine HT-tolerance natural allele more efficiently, we assembled 31 high-quality genomes of genetically diverse rice accessions. Coupling with two existing assemblies, we generate pan-genome-scale genomic resources, including structural variation (SV) and gene copy number variation (gCNV), most of which were not discovered in previous studies based on short-reads sequencing (Qin et al., 2021a). We offer illustrative examples of how these resources can promote to mine HT-tolerance allele, and can deepen our understanding of how SV and gCNV have contributed to environmental adaptation.

OP.19

Type: Oral Presentation

Topic: Plant-environment interactions: Abiotic stress

Genome wide association study for salt tolerance in japonica rice accessions: from germination to flowering

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A panel of 274 *japonica* rice accessions was phenotypically characterized in control or salt conditions by using the salt stress susceptibility (SSI) score of: *i*) four descriptors of germination as the maximum percentage of germination (G_{max}), time to reach 50% of maximum germination ($t_{50\%}$), uniformity of germination (U_{25-75}) and area under the germination curve (AUC); *ii*) four descriptors for visual stress symptoms and the physiological status during vegetative growth as Standard Evaluation Score (SES), plant height (H), flag leaf chlorophyll content (CHL) and flowering time (FL). A marked and continuous variation for all descriptors was observed. A Genome-Wide Association Study (GWAS) were carried out by using the phenotypic results associated with the filtered result of 31,550 SNPs identified by Genotype-by-Sequencing (GBS) analysis. The GWAS analysis uncovered a total of 12 significant Marker Trait Associations (MTAs) in case of germination (8 within, partially overlapping or including QTLs already identified as related to salt-tolerance traits) and a total of 18 significant MTAs in case of vegetative growth (4 within, partially overlapping or including QTLs already identified as related to salt-tolerance traits in rice). The alignment analysis to the *japonica* cv. Nipponbare reference sequence of the genes included in these MTAs highlighted some genes involved in a multiplicity of pivotal cell functions related to the salt stress responses as associated with the different descriptors. Biochemical and physiological analyses supported the involvement in salt tolerance of *OsTPP10*, coding for a trehalose 6-phosphate phosphatase, and *OsOVP6*, coding for the vacuolar H⁺-pyrophosphatase 6. Results provided information useful for the further dissection of molecular and biochemical mechanisms involved in rice tolerance to salt stress and for the development of molecular markers for improving salt tolerance in future breeding programs.

OP.20**Type: Oral Presentation****Topic: Plant-environment interactions: Abiotic stress**

Identification and functional characterization of abiotic stress-responsive RING E3-Ubiquitin ligases in rice

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1) ITQB NOVA

Rice is one of the most important crops worldwide and a traditionally important crop in Portugal. However, its production is highly affected by various abiotic stresses, such as drought and high salinity. Over the last decades, an enormous effort has been made to identify key-players regulating stress responses in rice (mainly transcriptional regulators and functional genes). However, little is known how the proteome pool is affected under stress, especially by the action of Ubiquitin-Proteasome system (UPS). The modulation of key components of the UPS has been established as a potential candidate for crop improvement programs towards abiotic stress tolerance (Melo et al., 2021). Among the different components of the UPS, the E3-ubiquitin ligases are the most abundant group. In rice, still a large number of E3-ubiquitin ligases remain uncharacterized. In our group, we are following a candidate gene approach to identify and functionally characterize E3-ubiquitin ligases involved in abiotic stress response. We selected 16 putative RING E3-ligases showing differential gene expression under stress, and validated their gene expression profile by RT-qPCR using in-house stress assays. Four of these RING E3-ubiquitin ligases were selected for a deepen functional characterization using a transgenic approach either overexpressing or knock-out the gene through CRISPR, and identifying its targets by Yeast-Two hybrid. At the moment, we have observed several interesting phenotypes putatively related to abiotic stress tolerance such as erect leaves, long lateral roots or heat-tolerance at the germination stage, and identified several putative targets (such as heat-shock proteins) which are being validated (BiFC, degradation assays, etc.). Taken together, this work will enhance our comprehension of the molecular and physiological mechanisms underlying the function of these E3-ubiquitin ligases to develop stress tolerant crops.

OP.21

Type: Oral Presentation

Topic: Plant-environment interactions: Abiotic stress

Climate smart at the molecular level: WRKY Transcription Factors and chilling stress regulation in rice

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The planet is being affected by our actions and we have seen effects of biotic and abiotic stress factors in populations of animals and plants. Regarding to plants, agriculture is expected to shift from current locations pushed by population growth and climate change. The understanding of plant response mechanisms is key to the development of stress resilient crops. WRKY transcription factors (TFs) are responsible for the regulation of genes responsive to many growth and development cues, and are involved in biotic and abiotic stress responses. Our group has been studying WRKY TFs under a pleiad of different stresses. One of these responses, to chilling, is important to let farmers to widen the cultivation window in regions where there is a transition between tropical and temperate climates. Chilling stress was applied at germination in two rice genotypes (tolerant and sensitive to chilling). Leaves at S3 stage were collected for RNA-seq to identify *OsWRKY*, *OsMAPKs* and *OsVQs* expression. The association in the expression of MAPKs, WRKYs and VQs was predicted through correlation analysis. *OsWRKY* transcriptional regulation was predicted by *in silico* analysis of *cis*-regulatory elements. 39 *OsWRKYs* were differentially expressed and divided in positive and negative regulators of chilling tolerance. 12 *OsMAPKs* were differentially expressed. *OsMAPKs* were downregulated and negatively correlated with the upregulated *OsWRKYs* in the tolerant genotype. 19 *OsVQs* were differentially expressed, 3 and 6 *OsVQs* were positively correlated with *OsWRKYs* in the tolerant and sensitive genotype, respectively. 7 differentially expressed *OsWRKYs* presented cold responsive elements in its promoters and 5 upregulated *OsWRKYs* in the tolerant genotype showed W-box motif. Chilling stress causes changes in *OsWRKY*, *OsMAPK* and *OsVQ* gene expression at germination. Although, further investigation is needed, *OsWRKYs* may act with VQs to regulate chilling tolerance.

OP.22

Type: Oral Presentation

Topic: Plant-environment interactions: Abiotic stress

GWAS on source: sink ratio reveal the genetic basis for low radiation tolerance during grain filling in rice

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In rice fields the continuously cloudy weather or rainfall, especially during the grain filling phase, induce a reduction of grain yield up to 50% in Asia (Sekhar *et al.*, 2019) (Ren *et al.* 16) and Latin America (Delerce *et al.* 2016). In rice, only traits related to source activity were suggested as tolerance mechanisms for low radiation. However, during grain filling low radiation had higher significant impact on carbon sink growth rather than carbon source supply (Shao *et al.* 2020). Therefore, our hypothesis is that studying the genetic basis for traits related to the plant capacity to adjust its source: sink ratio could reveal candidate genes conferring tolerance to low radiation conditions during grain filling.

A panel of 300 Indica accessions was evaluated under field conditions during the dry and wet season at CIAT, Colombia. At flowering, low radiation plots were covered until maturity with a polyethylene mesh. A genotypic matrix with 83,789 SNPs was used in TASSEL for the association mapping of grain yield (GY), yield components, phenology, leaf dimensions, aerial biomass and a proxy trait for carbon source sink balance (SSR) (Fabre *et al.*, 2020).

Low radiation significantly reduced GY, spikelet sterility and grain weight for both seasons. SSR significantly increased under low radiation and was significantly and negative related to GY, suggesting a higher impact of low radiation in sink organs. We found OsGRAS19 and PhyA genes within three QTLs associated to SSR under low radiation for both seasons. Previous studies showed that light availability regulate the expression of these candidate genes and inhibit the undesirable shade avoidance mechanism in rice plants. Proxy traits as SSR could help to identify tolerant rice varieties in the field. Once validated, GRAS19 and PhyA could be used for marker-assisted selection in rice breeding programs.

OP.23**Type: Oral Presentation****Topic: Plant-environment interactions: Abiotic stress**

Filtering the jasmonate-dependent component of the salt stress response by comparative transcriptomic approach

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Plant growth and survival in their continuously changing environment relies on the functions of different hormonal pathways which orchestrate adaptative responses throughout their life cycle. Phytohormones were long thought to exert unique functions but accumulating evidence shows that most of them have versatile activities. Jasmonates (JAs), initially discovered as central hormonal coordinators of defense responses against biotic aggressors, are also associated to many developmental processes including reproduction and growth control. Their spectrum of action, more recently, extended to the regulation of different abiotic stress responses where they mostly have positive effects. However, the impact of JAs on plant response to salinity stress, a major threat in rice cultivation, is still debated. Overexpression of the *AOC* JA biosynthetic gene enhances salinity tolerance in wheat while JA deficiency in rice and maize correlates with less salt induced damages than in wild type plants. Furthermore, natural or engineered rice lines with increased catabolism of JA-Ile, the bioactive JA, display better salt tolerance. This knowledge argues for negative impact of JA signaling on the salt response. Unfortunately, among the massive responses triggered under salt stress, the specific target genes of JA signaling and the underlying mechanisms affecting tolerance are poorly known. In the present study, we setup a kinetic salt stress assay where a JA deficient rice *aoc* mutant line displays weaker leaf damage than the wild type. We determined JAs dynamics and transcriptional profiles to filter out the JA-dependent component within the complexity of the rice salt response. This JA component was analyzed in root tissues sensing first the applied stress and also in shoots where contrasted symptoms are recorded. Differentially expressed genes were associated with distinct biological processes that could impact the management of salt-associated stress.

OP.24**Type: Oral Presentation****Topic: Plant-environment interactions: Abiotic stress**

Tuning of H₂O₂ accumulation and redox regulatory mechanisms is central for a specific intra-species tolerance in different rice cultivars under salt stress.

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Plants have continuously to cope with changing environmental conditions. In this situation, adaptive strategies emerge increasing biodiversity. Therefore, the identification of intra-species variability in terms of molecular responses activated toward oncoming adverse situations, represents a research goal for increasing crop resilience under climate change scenario. In this context, Baldo (B) and Vialone Nano (VN), two rice cultivars grown in the North of Italy, have been identified for showing different susceptibility to soil salinization. In particular, VN plants showed more marked growth reduction and earlier suffering symptoms than B ones under NaCl exposure. Here we present the effects of salt stress on root phenotype and metabolism, being this organ primarily involved in sensing this stress. Different root growth inhibition was reflected in different alteration of cell cycle progression and cellular viability. Upon perception of stress, ROS, as molecular warning signals, were rapidly modulated to trigger plant defence mechanisms. The higher capability to face a constraint condition by plants also involves a more efficient anti-oxidative system and a higher capacity to promptly modulate ROS after stress exposure. Consistently, the salt tolerant B shows a different H₂O₂ signature compared to VN. Studies on root redox state, metabolite concentrations, expression and activity of the enzymes involved in glutathione biosynthesis and metabolism suggest a fine cross-talk between GSH metabolic network and the modulation of growth pathways differently exerted in tolerant versus sensitive cultivars.

Taken together our results contribute to highlight the role of ROS and anti-oxidative pathways as a part of a complex signalling network working in plant responses against salt stress. A better knowledge of the mechanisms acting in tolerant varieties as B, will also allow the identification of effective strategies aimed at promoting crop resilience toward environmental stress.

Plant-environment interactions: Biotic stress

OP.25

Type: Oral Presentation

Topic: Plant-environment interactions: Biotic stress

Search for potassium transport systems involved in arbuscular mycorrhiza-rice symbiotic interactions.

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Arbuscular mycorrhizal fungi (AMF) develop interdependent connections with roots of about 90% of plant species. These interactions increase availability as well as translocation of nutrients (especially N and P), and thereby improve plant nutrition and growth. Moreover, resistance to a variety of stresses, among which salt stress, has been shown to be improved by AMF-plant interactions, for example in rice. Intense research to explain the molecular mechanisms of AMF-plant beneficial interactions led to the identification of phosphate and ammonium transporters involved in nutrient exchanges from AMF to the plant, in several plant species. In spite of the importance of potassium (K⁺) for plant physiology, the contribution of AMF symbiosis to plant K⁺ nutrition has been little documented. Over-expression of plant K⁺ transporters has been described in *Lotus japonicus* and tomato in condition of AMF symbiosis. Furthermore, K⁺ transport systems in the AMF *Rhizophagus irregularis* have been identified *in silico*. Here, K⁺ nutrition in rice colonized by *R. irregularis* has been analyzed at molecular and physiological levels. Surprisingly, major K⁺ transport systems in rice were down-regulated upon AMF interactions, suggesting strong increase in K⁺ availability for uptake by root cells in symbiotic conditions. Role of K⁺ in the relationships between rice and *R. irregularis* will also be discussed.

OP.26

Type: Oral Presentation

Topic: Plant-environment interactions: Biotic stress

Induced resistance through CINNAMIC ACID-4-HYDROXYLASE inhibition in rice involves diterpenoid phytoalexins

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Induced resistance (IR) is a promising crop protection strategy based on stimulating plant innate immunity to confer partial but broad-spectrum resistance to a wide range of pests and pathogens. Previous research by our group showed that transient chemical inhibition of the key phenylpropanoid pathway enzyme CINNAMATE-4-HYDROXYLASE (C4H) induces IR in the dicot crop tomato. We were able to show that C4H inhibition also induces IR in the monocot crop rice, as evidenced by significantly reduced susceptibility to the rice root-knot nematode *Meloidogyne graminicola* and the rice blast pathogen *Magnaporthe oryzae*. This suggests that C4H inhibition-IR is a conserved phenomenon in higher plants, and we wanted to investigate whether the molecular mechanisms underlying the phenotype were similarly conserved.

Using high-throughput transcriptome and metabolome analysis (via mRNA-sequencing and UPLC-MS/MS respectively), infection experiments in mutants, and biochemical assays, we were able to show substantial similarities in the upstream signaling leading to C4Hi-IR in tomato and rice. In both species, establishment of C4Hi-IR is associated with reprogramming of plant phenylpropanoid metabolism and an oxidative burst, and is largely independent of phytohormone signaling. However, the downstream defense responses exhibited during C4Hi-IR differ substantially between both species.

In particular, we found that C4Hi-IR against *M. graminicola* in rice involves increased biosynthesis of diterpenoid phytoalexins (DPs), a class of metabolites not found in tomato – where CH4i-IR instead mostly involves phenolic phytoalexins. Genetic impairment in DP biosynthesis significantly increased rice susceptibility to *M. graminicola*, and reduced the effectiveness of IR stimuli. DPs in rice roots are primarily exuded to the rhizosphere, and our data show that exuded rice DPs are nematostatic towards *M. graminicola*. These results highlight the crucial but somewhat underappreciated role of root exudates in interactions between plants and soilborne pests and pathogens.

OP.27**Type: Oral Presentation****Topic: Plant-environment interactions: Biotic stress**Rice microRNAs coordinate yield traits and immunity against *Magnaporthe oryzae*Yan Li¹, He Wang¹, Zhi-Xue Zhao¹, Yan-Yan Huang¹, Jing Fan¹, Wen-Ming Wang¹

1) Sichuan Agricultural University

Disease resistance activated by immunity often penalizes growth, which often leads to fitness cost in crops. Immune-regulators are emerging to be identified to coordinate defense-growth tradeoffs that can benefit crop improvement. One type of such immune-regulators come from *MicroRNA (MIR)* genes. Transcripts from a *MIR* gene are sequentially processed into pre-miRNA and miRNA-5p/3p duplex by Dicer-like proteins (DCLs). Mature miRNAs are in 19-24 nucleotides that are loaded into Argonaute proteins (AGOs), forming miRNA-induced gene silencing complexes that mediate the suppression of target genes in a sequence-complementary manner. By screening of miRNAs differentially responsive to *Magnaporthe oryzae*, the causative agent of rice blast disease, we identified a set of miRNAs that are involved in rice blast disease resistance. We then functional characterized these miRNAs in defense against *M. oryzae* and yield traits development via over-expressing the precursors of the miRNAs and their target mimics. Our data indicate that some miRNAs act positively, including miR159, miR160, miR162, miR171, miR398, miR1320, and some miRNAs act negatively in rice blast defense, including miR156, miR164, miR167, miR168, miR169, miR396, miR439, miR530, miR535, miR1873, miR444b.2. Particularly, alteration of miR168-AGO1 module leads to changes in the expression of a subset of miRNAs that act in growth, development, and rice immunity against *M. oryzae*. Intriguingly, knock-down of *MIR168* could simultaneously improve blast disease resistance, flowering time and yield, which may be attributable to a subset of miRNAs. Therefore, defense-growth tradeoffs can be coordinated via miRNAs.

OP.28**Type: Oral Presentation****Topic: Plant-environment interactions: Biotic stress**

Xanthomonas oryzae-Triggered Production of Atypical Rice Small RNAs During Infection

Ganna Reshetnyak¹, Jonathan Jacobs¹, Florence Auguy¹, Coline Sciallano¹, Lisa Claude¹, Clemence Medina¹, Alvaro Perez-Quintero¹, Aurore Comte¹, Adam Bogdanove², Ralf Koebnik¹, Boris Szurek¹, Anne Dievart³, Christophe Brugidou¹, Severine Lacombe¹, Sebastien Cunnac¹

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Eukaryotic regulatory small RNAs (sRNA) range from 20 to 24 nucleotides and are ubiquitous in plants. As mediators of transcriptional and post-transcriptional gene silencing, they contribute to the orchestration of growth, development and stress responses. In the plant cell, sRNAs are mostly endogenous but can derive from viral sequences or be delivered by interacting organisms. sRNA biogenesis begins with the cleavage of double-stranded RNA precursors by a dicer-like enzyme (DCL). The resulting sRNA duplexes are then protected from degradation by HEN1-mediated methylation, and the mature guide sRNA strand is incorporated into the RNA-induced silencing complex (RISC). RISC targets complementary nucleotides to dampen gene expression. Early insight on sRNAs in plants revealed their role in antiviral defense and they are now extensively studied in response to diverse pathogens. Here we describe a novel class of sRNA in rice (*Oryza sativa*) associated with foliar diseases caused by *Xanthomonas oryzae* pathovars. Analysis of high-throughput sRNA sequencing data suggests that Xanthomonas-induced small RNAs (xisRNAs) possess features of regulatory sRNA and may target genes involved in plant immune signaling or sRNA production. xisRNAs biogenesis is still enigmatic but we showed that it depends on canonical sRNA pathways components. In addition, transcription of protein-coding loci overlapping the genomic sequences of xisRNAs is required for their accumulation. Our results further indicate that *X. oryzae* uses its virulence arsenal of type III effectors for maximal xisRNA accumulation. Overall, our results identify a group of unusual sRNAs with a potential role in plant-microbe interactions.

OP.29**Type: Oral Presentation****Topic: Plant-environment interactions: Biotic stress**

The role of iron in rice innate immunity

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1) CRAG

Iron is an essential micronutrient required for plant growth and development. Although the impact of iron in plant-pathogen interactions is well recognized, the molecular basis underlying the effect of the iron nutritional status on the immune response of rice plants to pathogen infection is poorly understood. Here, we investigated the impact of treatment with high iron in rice immunity at the cellular and molecular level. Treatment with high iron confers resistance to infection by the rice blast fungus *Magnaporthe oryzae*. Histochemical staining of *M. oryzae*-infected leaves revealed that iron and Reactive Oxygen Species (ROS) accumulate in cells in the vicinity of the infection site. During pathogen infection, a stronger induction of defense-related genes occurs in leaves of iron-treated plants (e.g. *Pathogenesis-Related* genes). Notably, a superinduction of phytoalexin biosynthetic genes, both diterpene phytoalexins and sakuranetin, is observed in iron-treated plants during pathogen infection. As a consequence, upon pathogen challenge, phytoalexin accumulation is higher in iron-treated plants compared with control plants. *Transcriptional* alterations of iron homeostasis-related *genes* and a reduction in apoplastic iron content are observed in leaves of iron-treated rice plants. These results illustrate that the iron nutritional status plays a key role in the response of rice plants to infection by the rice blast fungus, while reinforcing the notion that iron signaling and defense signaling must operate in a coordinated manner in controlling disease resistance in plants. This information provides a basis to better understand the molecular mechanisms involved in rice immunity.

OP.30**Type: Oral Presentation****Topic: Plant-environment interactions: Biotic stress**

SUMO code in Rice: Deciphering the language of an emerging protein modification system in crops

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SUMO (Small Ubiquitin-like Modifier) conjugation onto target proteins has emerged as an influential class of protein modification systems in plants. SUMO1/2 double mutant plants are nonviable, underlining the importance of SUMO conjugation to plant survival. Once covalently bound, SUMO can alter a conjugated protein's stability and/or function. SUMO conjugation is a highly dynamic process that can be rapidly reversed by the action of SUMO proteases. The balance between the conjugated/deconjugated forms is a major determinant in the modulation of SUMO-target function. Despite the ever-increasing importance of SUMO in model plants, there is little information on the function of the SUMO system in any crop plant. In this talk, we map the SUMO system in rice (*Oryza sativa*) and demonstrate a critical role for OsOTS1 SUMO protease in salt stress. OsOTS-RNAi rice plants accumulate high levels of SUMO-conjugated proteins during salt stress and are highly salt sensitive; however, in non-salt conditions, they are developmentally indistinguishable from wild-type plants. Transgenic rice plants overexpressing OsOTS1 have increased salt tolerance and a concomitant reduction in the levels of SUMOylated proteins. We demonstrate that OsOTS1 confers salt tolerance in rice by increasing root biomass. High salinity triggers OsOTS1 degradation, indicating that increased SUMO conjugation in rice plants during salt stress is in part achieved by down-regulation of OTS1/2 activity. OsOTS1 is nuclear localized indicating a direct requirement of OsOTS1-dependent deSUMOylation activity in rice nuclei for salt tolerance. OsOTS1 depleted transgenic rice plants accumulate more ABA and exhibit more productive agronomic traits during drought while OsOTS1 overexpressing lines are drought sensitive but ABA insensitive. We reveal that OsOTS1 SUMO protease directly targets the ABA and drought responsive transcription factor OsbZIP23 for de-SUMOylation affecting its stability. OsOTS-RNAi lines show increased abundance of OsbZIP23 and increased drought responsive gene expression while OsOTS1 overexpressing lines show reduced levels of OsbZIP23 leading to suppressed drought responsive gene expression. Our data reveal a mechanism in which rice plants govern ABA-dependant drought responsive gene expression by controlling the stability of OsbZIP23 by SUMO conjugation through manipulating specific SUMO protease levels.

Rice development and phenomics

OP.31

Type: Oral Presentation

Topic: Rice development and phenomics

A common set of genes regulated by florigenic and photoperiodic induction at the shoot apical meristem of rice

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Rice is a staple crop which, like other higher plants, evolved to flower at the right season in order to maximize seed yield, and the chances of species survival. Although several key players in this process have been intensively studied and characterized, many others remain to be identified. Central to the flowering process are the two florigens Hd3a (Heading Date 3a), active in short day (SD) conditions, and RFT1 (Rice Flowering locus T 1), mostly active in long day (LD) conditions. The florigens are mobile signals which are expressed in the leaf blades and are then transported to the SAM through the phloem.

We used a transcriptomic approach to discover new genes involved in the flowering process. To this end, we produced and compared three independent RNA-seq datasets obtained from shoot apical meristems (SAM). Two datasets contain lists of genes differentially expressed upon induction of SAMs by either Hd3a or RFT1, exploiting a dexamethasone-inducible system to selectively express only one florigen at a time. The third dataset was obtained comparing the transcriptomes of SAMs exposed to photoperiodic inductive short-day conditions, to that of SAMs grown under long day.

Using stringent parameters, we identified sixteen genes as strongly regulated by all three conditions. Some of these genes were already well-known regulators of the reproductive transition, including *OsMADS14*, *15*, *18* and *34*, and *PREMATURE INTERNODE ELONGATION 1*. We proceeded to confirm the datasets through qRT-PCR, comparing the expression in wild type plants and in florigen knock-out plants, both single and double mutants. Expression of four genes in the SAM was also assessed by *in situ* hybridization. We then started editing of genes using the CRISPR-Cas9 technique and identified an F-BOX protein controlling tillering. This study identified a core set of genes integrating florigen response at the apical meristem.

OP.32

Type: Oral Presentation

Topic: Rice development and phenomics

A triple florigen system is essential for flowering and panicle architecture in rice

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The transition from a vegetative phase to a reproductive one has always been deeply studied in plants and in particular in the cereals due to the relevance of this trait for seed yield. The transition occurs upon the arrival of a mobile flowering signal, the florigen, at the shoot apical meristem (SAM).

In rice (*Oryza sativa*), there are two different florigens: *Heading Date 3a (Hd3a)*, that promotes flowering in short day (SD) conditions and *Rice Flowering Locus T 1 (RFT1)* that promotes flowering in both short and long-days (LD). Florigens are produced in leaves once environmental conditions are optimal, and then transported through the phloem to the SAM where they activate the expression of inflorescence identity genes.

A transcriptional screen at the SAM identified another florigen-like gene, *FLOWERING LOCUS T-LIKE 1 (FT-L1)*, whose expression is strongly induced at the apex upon exposure to SDs.

Here we show that *FT-L1* expression is activated by the florigens during floral commitment. Expression data from qRT-PCR, in situ hybridization and transcriptional marker lines indicated that its mRNA is mainly expressed in the SAM rather than in the leaves and persists during all developmental stages of inflorescence development. Misexpression of *FT-L1* from tissue-specific promoters indicates that only when expressed in the SAM, *FT-L1* has florigenic activity.

Isolation and analysis of EMS and CRISPR mutant alleles, led to conclude that *FT-L1* has dual roles during reproductive development, promoting transition to flowering under different day-length conditions and suppressing secondary branch development.

These data unravel a novel model for the transition to flowering in rice, where a triple florigen system integrates environmental and molecular signals to initiate the panicle and determine its architecture.

OP.33**Type: Oral Presentation****Topic: Rice development and phenomics**

Laboratory phenomics predicts field performance and identifies superior haplotypes for early seedling vigour

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Seedling vigour is an important agronomic trait and is gaining attention in Asian rice (*Oryza sativa*) as cultivation practices shift from transplanting to forms of direct seeding. To understand the genetic control of rice seedling vigour in dry direct seeded (aerobic) conditions we measured multiple seedling traits in 684 accessions from the 3000 Rice Genomes (3K-RG) population in both the laboratory and field at three planting depths. Our data show that phenotyping of mesocotyl length in laboratory conditions is a good predictor of field performance. By performing a genome wide association study, we found that the main QTL for mesocotyl length, percentage seedling emergence and shoot biomass are co-located on the short arm of chromosome 7. We show that haplotypes in the indica subgroup from this region can be used to predict the seedling vigour of 3K-RG accessions. The selected accessions may serve as potential donors in genomics-assisted breeding programs.

OP.34

Type: Oral Presentation

Topic: Rice development and phenomics

SMALL REPRODUCTIVE ORGANS, a SUPERMAN-like transcription factor, regulates stamen and pistil growth in rice

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Organ size is mainly determined by cell division and cell expansion. Several genetic factors regulating development of plant lateral organs have been characterised, but those involved in determining reproductive organ size and separation in rice (*Oryza sativa*) remain unknown. Previous research on rice reproductive development has focussed mainly on the determination of floral organ identity or the regulation of fertility.

We have isolated the rice gene *small reproductive organs* (*sro*) encoding a nucleus-localised C₂H₂ zinc finger protein. Combined developmental, genetic, histological and transcriptomic analyses were used to determine the function of *SRO* in regulating reproductive organ size. *SRO* affects genes involved in cell division, cell expansion and phytohormone signalling in the rice flower. *SRO* is specifically expressed in the first stages of stamen filament development to regulate their correct formation and separation, showing a novel regulatory mechanism for reproductive organ development in rice. In addition, *SRO* non-cell-autonomously regulates the size and functionality of male and female reproductive organs. The B-class MADS-box gene *OsMADS16/SPW1* is epistatic to *SRO*, while *SRO* regulates reproductive organ specification and floral meristem determinacy synergistically with C-class genes *OsMADS3* and *OsMADS58*.

Furthermore, we demonstrate that *SRO* is the rice orthologue of *Arabidopsis SUP*, a pivotal gene regulating the male/female boundary that has been proposed as an essential element in the evolution of whorled flowers. The functional comparison of orthologues from far distant model plants can provide insights into the evolution and robustness of floral development models. Our findings show how an evolutionarily conserved transcription factor has a pivotal role in reproductive organ development in core eudicots and monocots, through partially conserved expression, function, and regulatory network.

Key Words: C₂H₂ zinc finger, cell division and expansion, evo-devo, organ separation, *Oryza sativa* (rice), plant reproductive development, *Small Reproductive Organs* (*SRO*), *SUPERMAN* (*SUP*), stamen filamen

OP.35**Type: Oral Presentation****Topic: Rice development and phenomics**

An Ankyrin gene cluster is associated with panicle branching diversity in rice

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The number of grains per panicle is an important yield-related trait in cereals which depends in part on panicle branching complexity. One key component of this complexity and its diversity is the number of secondary branches per panicle. Using GWAS in conjunction with a specific landrace population, we identified a genomic region co-associated with secondary branch and spikelet numbers per panicle in rice. Combining gene capture, bi-parental genetic population analysis, expression profiling and transgenic approaches, we investigated the functional significance of a cluster of 6 Ankyrin (ANK) and Ankyrin-Tetratricopeptide (ANK-TPR) repeat domain-encoding genes within the identified QTL. Four of the ANK and ANK-TPR genes display a differential expression pattern associated with panicle secondary branch number in morphologically contrasted accessions. The observed differential expression patterns correlate in the different alleles of these genes with specific deletions of potential cis-regulatory sequences in their promoters. Two of the genes were confirmed through functional analysis as playing a role in the control of panicle architecture. Our findings indicate that secondary branching diversity in the rice panicle is governed in part by differentially expressed genes within this cluster encoding ANK and ANK-TPR domain proteins that may act as positive or negative regulators of panicle meristem identity transition from the indeterminate to the determinate state.

OP.36

Type: Oral Presentation

Topic: Rice development and phenomics

Functional study of PINE1 as a link between flowering and stem elongation

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Environmental factors, mainly photoperiod, are responsible for flowering in rice. Upon the perception of the correct daylength, florigenic signals are sent from leaves to the meristem, leading to transition from a vegetative meristem to a reproductive one. Other changes in plant architecture occur during transition to flowering. In particular, internode elongation is critical for heading of the panicle, thus flowering and internode elongation have to be temporally coordinated. A molecular link between the two processes is provided by *PREMATURE INTERNODE ELONGATION 1 (PINE1)*. *PINE1* is a transcription factor responsible for repressing internode elongation during vegetative growth. Since its transcription is repressed by florigenic signals, its reduction during panicle development permits internode elongation. *pine1 knockout* mutants display a strong phenotype of internode elongation during vegetative growth, caused by increased sensitivity of stems to gibberellins. The molecular mechanisms with which *PINE1* exert its role are still unknown.

Using Yeast two-hybrid and BiFC, we identified *TOPLESS* proteins as *PINE1* interactors and demonstrated that the interaction is dependent on *PINE1* EAR-motifs. Through FRET/FLIM we also found an interaction between *TOPLESS* and an histone deacetylase, suggesting the existence of a complex acting as a transcriptional repressor by compacting chromatin regions. The function of EAR-motives has also been evaluated *in vivo*, by complementation of the *pine1 knockout* mutant with clones of *PINE1* lacking these motives. The results showed that EAR motives are essential for *PINE1* function.

To identify and study genes downstream of *PINE1* and potentially being subject to chromatin remodeling, an RNA-sequencing experiment was performed. Several genes were found to be deregulated in the mutant. Taking advantage of CRISPR/Cas9 mutagenesis, we are interrogating the function of some of the resulting genes.

OP.37**Type: Oral Presentation****Topic: Rice development and phenomics**

Identification and characterization of genes controlling rice inflorescence development

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Plant inflorescence architecture is an important determinant of the reproductive success of a plant but also of agronomical interest, because it determines yield in many crops. Inflorescence development is a complex process and in the plant kingdom many variations in the developmental program have been observed.

In rice the inflorescence meristem, (rachis meristem) starts to develop primary branches. On these primary branches secondary branches are often formed which develop spikelet meristems that develop the florets (flowers). The result is a determinate panicle inflorescence structure. The timing of spikelet meristem development determines the number of branches and seeds that will develop. Our lab is interested in the molecular mechanisms that determine the identity of the different reproductive meristems since this stands at the base of the final architecture. Recently, we have laser micro-dissected the rice reproductive meristems and used this material for RNAseq analysis (Harrop et al., 2016). This resulted in the identification of candidate genes involved in inflorescence development and panicle branching. Using CRISPR-Cas technology we are targeting several of these genes and we are analyzing mutants for inflorescence architecture phenotypes. Approaches and recent results will be discussed.

Rice genome research and evolution

OP.38

Type: Oral Presentation

Topic: Rice genome research and evolution

Pan-genome study reveals evolutionary insights into the subpopulation structure of 16 Asian rice

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Rice (*Oryza sativa*) is a key commodity that is needed to support global food security in a world that will reach 10-billion by 2050. Breeders are doing everything possible to create rice varieties that are highly nutritious, productive and are climate resilient, e.g. green super rice (GSR). Exploiting the genetic diversity that exists in germplasm banks across the globe is of paramount importance to accomplish that task. To meet this demand, we studied the genetic diversity of the 3K-RGP and selected 16 accessions representative of each of 15 subpopulations and the largest mixed subpopulation, which includes *O. sativa* vg. Nipponbare. During the past five years, we generate Platinum Standard Reference Sequences (PSRefSeqs) from a representative of each of the 15 rice subpopulations. Genome annotation for each genome was performed using Maker-P and independent Iso-seq and RNA-seq data. The gene prediction identified an average of 36,347 genes per genome. We then studied large structural variants (SVs) across the 16 RefSeqs and found preliminary evidence of SVs possibly affecting genome function and evolution. For instance, genes' expression, genome recombination, and LD blocks were affected by inversions. Finally, the 16 RefSeqs were used as a template to detect standing natural variation using the 3k-RGP data set. To accelerate this process, we compiled the GATK pipeline for high-performance computing (HPC) platforms, which can complete variant calling for 3,000 accessions in 4 days per genome. By doing so, we generated a huge variant dataset including 416 Million InDels and SNPs. This comprehensive dataset combined with phenotype data will enable pan-genome wide association studies (p-GWAS), which in turn could facilitate traditional gene/QTL cloning.

OP.39**Type: Oral Presentation****Topic: Rice genome research and evolution**

KitBase: a whole-genome sequenced fast-neutron-induced mutant population in the model rice cultivar KitaakeX

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Fast neutron mutagenesis is an effective approach to generate plant mutants. We have generated a fast-neutron mutagenized population in the model rice cultivar KitaakeX, a Kitaake plant carrying the rice XA21 immune receptor. Kitaake is an early flowering, short-statured, short life cycle rice that is easy to transform, compared with other *Japonica* and *Indica* rice varieties. In collaboration with Joint Genome Institute and Hudson Alpha, we have sequenced ~3,200 mutagenized plants. Genome analysis of an additional 237 mutant plants is now underway. Detailed analysis of 2,871 mutant lines reveals mutations in ~40,000 genes. For a population size of 3,108, researchers will have a 78% probability of finding a mutation in a gene of interest. The average number of genes affected per line is 45. Mutation types include single base substitutions, deletions, insertions, inversions, translocations, and tandem duplications. Single base substitutions predominate, but deletions affect the greatest number of genes. Because phenotypic data is useful for researchers studying particular traits, we have recorded germination rate, days to heading, tiller number, and grain weight traits for ~400 lines. We have created KitBase (<https://kitbase.ucdavis.edu/>) to make these analyses publicly available. KitBase integrates JBrowse and BLAST tools to facilitate identification of mutations and database searches.

OP.40**Type: Oral Presentation****Topic: Rice genome research and evolution**

An inferred functional impact map of genetic variants in rice

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Interpreting the functional impacts of genetic variants (GVs) is an important challenge for functional genomic studies in crops and next-generation breeding. Previous studies in rice (*Oryza sativa*) have focused mainly on the identification of GV, whereas systematic functional annotation of GV has not yet been performed. Here, we present a functional impact map of GV in rice. We curated haplotype information for 17 397 026 GV from sequencing data of 4726 rice accessions. We quantitatively evaluated the effects of missense mutations in coding regions in each haplotype based on the conservation of amino acid residues and obtained the effects of 918 848 non-redundant missense GV. Furthermore, we generated high-quality chromatin accessibility (CA) data from six representative rice tissues and used these data to train deep convolutional neural network models to predict the impacts of 5 067 405 GV for CA in regulatory regions. We characterized the functional properties and tissue specificity of the GV effects and found that large-effect GV in coding and regulatory regions may be subject to selection in different directions. Finally, we demonstrated how the functional impact map could be used to prioritize causal variants in mapping populations. This impact map will be a useful resource for accelerating gene cloning and functional studies in rice, and can be freely queried in RiceVarMap V2.0 (<http://ricevarmap.ncpgr.cn>).

OP.41

Type: Oral Presentation

Topic: Rice genome research and evolution

PanOryza: Globally coordinated genomes, proteomes and pathways for rice

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The first rice genome was sequenced in 2002 for the Asian domestic rice *O. sativa* Japonica Nipponbare. The gene models for this subspecies of rice have been predominantly used in rice research, although gene models for other varieties (e.g. Indica and some landraces) have also been generated. The Japonica gene models were generated independently from two research teams: the Rice Annotation Project (RAP) and the Michigan State University (MSU) Rice Genome Annotation Project. International sequencing projects are now generating 16 "platinum standard" reference sequence (PSRefSeq) genomes for Asian rice, which represent the genetic diversity of *O. sativa*. This presents a great resource for crop research but also a challenge as there is potential for inconsistencies in gene model sets i.e. two different annotations for Nipponbare, plus 16 new gene model sets, meaning it is increasingly difficult to compare gene or protein sequences across varieties, and no consistent gene identifier nomenclature has been adopted.

Our teams at U. Liverpool, EBI, Oregon State, Arizona Genomics Institute and IRRI (Philippines) have been funded by BBRSC/NSF (2020-2023) to achieve consistent gene models, proteins and pathways for 16 high-quality rice genome assemblies in the "PanOryza" project. In PanOryza, we are developing an approach for aligning all reference genomes, generating a pan gene set, and creating a voting system to determine the best gene model within the pan gene set, which will then be used to call the same locus consistently in all assemblies. We are also generating a pan gene identification system. This update presents an opportunity to inform the rice research community on our work in progress, gain wider input onto our planned methods and build a bigger community to assist in gene model annotation, for example through future annotation workshops.

OP.42

Type: Oral Presentation

Topic: Rice genome research and evolution

Linear pangenome reference of Asian rice constructed from genome graph

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To compare multiple genomes, a linear reference genome was often used as a coordination system to describe genes, variations and other functional annotations across individuals. However, this single reference was shown not to be sufficient to grasp every existing genomic variation such as copy number variations (CNV), presence/absence variations (PAV) or more general structural variations (SV). To overcome this limitation, the concept pangenome composing a core-genome and a dispensable genome was applied to investigate a group of genomes. Graph-based data model generated by incrementally incorporating genome-to-graph alignment information was one of the novel approaches to represent pangenome information. Nevertheless, there are still limitations in tools and packages used to analyze genome graph. Here, we propose a method to extract the most representative path from a genome graph to work as the pangenome references and its application on Asian rice genomes.

Considering the pangenome graph as a bidirected graph with non-repetitive DNA sequences as vertices and the linkage between two sequences as edges, the longest representative path is defined as the path containing sequences appearing in the greatest number of individuals. Our developed Julia package BioGraph helped to extract the longest representative path contained in a genome graph of a population by solving a linear program model. This longest path will be later incorporated with annotation information to act as a synthetic linear reference genome of the population.

We used minigraph to generate a pangenome graph of 12 near-gap-free reference genomes sequences 12 subpopulations of cultivated Asian rice and the common reference *Oryza sativa Nipponbare* acting as the first genome. The longest representative path was extracted from the graph using BioGraph. The size of the longest path is approximately 100 MBs more than the size of *Oryza sativa Nipponbare* reference.

OP.43**Type: Oral Presentation****Topic: Rice genome research and evolution**

Hd1 regulates the expression of genes involved in phenylpropanoids biosynthesis in rice

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The *Oryza sativa* gene *Hd1*, orthologous of Arabidopsis *CONSTANS* (*CO*), encodes for a zinc finger transcription factor that is known as one of the master regulators of flowering time in rice.

Hd1 regulates the reproductive transition under both short days (SD), promoting it, and long days (LD), delaying it. This functional switch is a major feature of Hd1, but its impact on global gene expression remains unexplored. In this work, we performed a global analysis of gene expression and identified all the genes whose transcription depends upon Hd1 either under SD or LD. We thus detected the main regulatory pathways under Hd1 control.

RNA-Sequencing identified 451 and 9288 genes differentially expressed under SD and LD, respectively. Interestingly 360 genes were differentially expressed in opposite directions depending upon the photoperiod. Among them, we identified *PHENYLALANINE AMMONIA LYASE 1* (*PAL1*), catalysing the first committed step in secondary metabolites biosynthesis. *De novo* motif discovery revealed enrichment for the 5'-*TGTG*-3' motif in the promoters of genes differentially regulated, similar to the published 5'-*TGTGG*-3' motif bound by both *CO* and Hd1. ChIP assays showed the physical interaction of Hd1 on this motif present in the promoters of *PAL1* and *Hd3a*, the latter being a known target of Hd1. This work shows that Hd1 has additional roles, other than photoperiodic flowering control.

OP.44**Type: Oral Presentation****Topic: Rice genome research and evolution**

Meiotic recombination in rice is modified by structural genomic variation

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Meiotic recombination preferentially occurs in certain genomic regions. This phenomenon has been investigated in animal, human and plant species. Genetic and epigenetic features have been identified as factors that can modify the frequency of crossovers. In breeding programs, the ability to predict recombination would allow to choose progenitors in function of their expected local recombination around important genes or quantitative trait loci. This would (1) make linkage drag elimination easier and (2) provide means to choose optimal marker density. Usually, only genomic data are available to breeders. We investigate how precisely crossover occurrence can be predicted in an F_1 hybrid, knowing *only* its parental genomes. For this, we use an *Oryza sativa* [indica x japonica] cross as a model of study. Platinum-grade parental genomes are available, and the progeny of the cross was sequenced at low depth using the Illumina technology. A new algorithm for data imputation was developed, which allowed precise mapping of recombination breakpoints. Structural variation among the parental genomes was investigated using different bioinformatics techniques. A mathematical model for recombination prediction was developed. Depending the chromosome, the correlation between genome “resemblance” and crossover frequency ranged from 0.77 to 0.88.

OP.45**Type: Oral Presentation****Topic: Rice genome research and evolution**

Impact of Transposable Element dynamics on rice trait variation

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Transposable elements (TEs) are a rich source of genetic variability. Among TEs, LTR-retrotransposons and MITEs are the most prominent and active in plants. In this communication we will present the analysis of 103,109 Transposon Insertion Polymorphisms (TIPs) in 738 *O. sativa* genomes representing the main rice population groups. Our data shows that LTR-retrotransposons and MITEs have a different dynamics in rice. While both seem to have been active in rice recent evolution, LTR-retrotransposons seem to have a high turnover whereas a fraction of MITE insertions seem to have been fixed in rice concomitantly with its domestication. We used both LTR-retrotransposon and MITE TIPs to perform TIP-GWAS and study the impact of these elements on agronomically important traits. Both LTR-retrotransposons and MITE TIPs uncover more associations with rice traits than SNPs. Our data shows the importance of TEs as drivers of variability used in rice domestication and breeding and highlights the interest of the using TIPs in GWAS approaches.

OP.46**Type: Oral Presentation****Topic: Rice genome research and evolution**

Construction and analysis of a platinum standard reference genome panel for all species of the genus *Oryza*

Rod A. Wing, King Abdullah University of Science & Technology, Center for Desert Agriculture, Kingdom of Saudi Arabia & University of Arizona, Arizona Genomics Institute, USA

The International *Oryza* Map Alignment Project (I-OMAP) is devoted to the understanding and application of standing natural variation in the genus *Oryza* to address both basic and applied research questions. One aspect of this work has been to establish a panel of publically available ultra-high-quality reference genomes (a.k.a. Platinum Standard Reference Sequences) and annotations that represent at least one accession of each of the 27 species of the genus *Oryza* (i.e. 11 extant genome types [6 diploid and 5 polyploid] and a 3.6-fold genome size variation [ranging from ~350 to 1283 Mb]) as well as the population structure of cultivated Asian rice ($k=15$). In my talk I will summarize our work to complete the I-OMAP PSRefSeq Panel and some recent analyses conducted by several postdocs and graduate students in my group. A more detailed description of these analyses can be found from the talks and posters presented at the meeting.

Epigenomics

OP.47

Type: Oral Presentation

Topic: Epigenomics

Dissecting the Dynamic Changes of Methylome During Rice Transformation

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In rice transformation, tissue culture often carries epigenetic and genetic changes underlying somaclonal variations leading to unpredictable phenotypes. The components of transformation procedure, such as tissue culture, culture medium, Ti-plasmid, selection medium and transformation techniques, may individually and jointly contribute to the somaclonal variations, though their specific impacts on the rice epigenomes that have never been examined. To improve the control over the somaclonal variations and transformation outcomes, we examined the changes of methylomes and transcriptomes associated with each of these components throughout the rice transformation in leaf samples. We observed that tissue culture and transformation, resulted in a global reduction of DNA methylation, and a large number of differentially methylated regions (DMR). Tissue culture induced hypo-methylated DMRs in all genomic contexts by targeting different genomic regions, whereas the transformation induced hypo-methylated DMRs specifically at CHH sites of promoters. Intriguingly we found that these hypo-methylated promoters were positively associated with gene expression enriched in functions of stress response and oxidation reduction. Altogether our results may imply a potential epigenomic response during rice transformation. Lastly we identified specific DMR from successful transformation occurred consistently and concordantly between calli and leaves, as potential biomarkers of successful transformation. Our study elucidated for the first time that the effect of each transformation component on DNA methylation to guide the improvement of transformation system.

OP.48**Type: Oral Presentation****Topic: Epigenomics**

Emerging Rainbow Rice: Insight into genetic variation in cryptic leaf pigmentation in rice

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The colorful rainbow rice varieties were produced from the crossbreeding between the white striped mutant and the purple rice varieties. These rainbow rice varieties exhibit variations in leaf color patterns, ranging from cherry-red to white-green, making the rainbow rice potential attractive landscape plant, novel sources of nutrition for human consumption, and excellent materials for studying leaf coloration. To understand molecular mechanisms leading to the differential leaf coloration in these rainbow rice varieties, the leaf blade transcriptome of three different varieties, including cherry-red (P), pink-white (PW), and white (W), was analyzed. P vs. W exhibited the highest number of differentially expressed genes (DEGs), followed by P vs. PW, and then PW vs. W. Many of the DEGs were identified as candidate genes in the flavonoid metabolism such as *4-coumarate-CoA ligase like 6*, *chalcone synthase1*, *anthocyanidin reductase*, *O-methyltransferase*, *UDP-glucuronosyl/UDP-glucosyltransferases*, and *OsmATE1*. These genes could be classified as structural genes, decorating genes, and the transporter gene. Most of them were up-regulated in the white variety and some of them in the pink-white variety compared to the cherry-red. The putative functions of some of these genes are involved in the conversion of anthocyanidins into flavan-3-ols, associated with the biosynthesis of flavone derivatives, and the transport of flavonol. These results point to the difference in the flavonoid biosynthesis pathways among three varieties of rainbow rice. Moreover, changes in the expression of genes involved in chloroplast development and chlorophyll metabolism were detected, implying that there are probably chlorophyll and chloroplast degradation in the white variety and low level of chlorophyll biosynthesis in the white and the pink-white varieties. Taken together, differences in the expression of genes in flavonoid biosynthesis, chlorophyll metabolism, and chloroplast development among rainbow rice varieties may contribute to variation in leaf pigmentation in these varieties. This study provides better understanding about leaf coloration and potential candidate genes for developing molecular marker in breeding program.

Posters

Advances in rice biotechnology and genome editing

P.01

Type: Poster

Topic: Advances in rice biotechnology and genome editing

Engineering rice plants with SARS-CoV-2 receptor binding domain (RBD) proteins for candidate vaccines

Andrea Saba-Mayoral¹, Xin Huang¹, Guillermo Sobrino-Mengual¹, Derry Álvarez¹, Wenshu He¹, Can Baysal¹, Pedro Cerda-Bennasser¹, Ashwin Vargheese¹, Carla Morea¹, Teresa Capell¹, Paul Christou^{1,2}, Victoria Armario-Najera¹

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel coronavirus responsible for an ongoing human pandemic (COVID-19) that has infected more than 230.1 million people and caused more than 4 million deaths¹. The rapid spread of COVID-19 makes necessary the development of diverse technology platforms for the production of vaccines and protein reagents for diagnosis or therapy. We engineered the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein (S) in rice plants. The construct was driven by the strong constitutive maize ubiquitin 1 promoter with its first intron. We also compared a novel tag for recombinant protein identification and purification (C-tag) with the more commonly used 6xHis-tag. Our preliminary results demonstrated that crude extracts from rice callus expressing RBD were able to bind to recombinant ACE-2 produced in HEK-cells, confirming that the rice-expressed protein was soluble and correctly folded.

P.02

Type: Poster

Topic: Advances in rice biotechnology and genome editing

CRISPR/Cas9-Mediated Knock Out of *OsSBEIIb* - Transcriptional and Metabolome Reprogramming in Rice Endosperm

Wenshu He¹, Can Baysal¹, Margit Drapal², Gemma Villorbina³, Vicente Medina¹, Andrea Saba Mayoral¹, Teresa Capell¹, Gurdev S. Khush⁴, Changfu Zhu¹, Paul D. Fraser², Paul Christou^{1,5}

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Resistant starch (RS) has a high content of amylose, which is more difficult to digest. RS inhibits insulin release and thus lowers the risk of diabetes, obesity, and heart disease and acts as a prebiotic to support a healthy colon microbiome. The content of RS in rice endosperm is typically < 2%. *OsSBEIIb* transfers short chains to the crystalline lamellae of amylopectin thus extending its branching. We used CRISPR/Cas9 to knock out *OsSBEIIb*. RS and amylose content in mutant endosperm increased from 0.2 and 19.6 to 17.2 and 27.4%, respectively. We investigated the effect of the mutation on the structure and function of the *OsSBEIIb* protein, on the morphology of starch granules in the mutant endosperm, and on the properties of the extracted starch. We analyzed expression of all major starch biosynthetic pathway genes in the endosperm and the leaves of the mutant plants and we also measured the abundance of diverse primary and secondary metabolites in the endosperm. Our results provide insights into the broader implications of perturbing starch metabolism in rice endosperm and its impact at the whole plant level.

P.03

Type: Poster

Topic: Advances in rice biotechnology and genome editing

Functional characterization of the nitrogenase cofactor maturase NifB from engineered rice

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Nitrogenase is an enzyme complex that catalyzes the reduction of molecular nitrogen into ammonia in the nitrogen-fixation process of certain prokaryotes, known as diazotrophs. The biosynthesis of the iron-molybdenum cofactor (FeMo-co) located at the nitrogenase active site relies on NifB. The NifB product (NifB-co) constitutes the [8Fe-9S-C] core of FeMo-co. The production of NifB in plants is challenging because this protein is often insoluble when expressed in heterologous eukaryotic cells, and its Fe-S clusters are extremely unstable and sensitive to O₂. We generated transgenic rice plants expressing NifB from thermophilic prokaryotes. The recombinant proteins were targeted to the mitochondria, thus limiting exposure to O₂ and providing essential Fe-S clusters required for NifB-co biosynthesis. NifB accumulated in soluble form and the purified protein was functional in an *in vitro* FeMo-co biosynthesis assay. This represents an important step toward the expression of a complete functional complex as required for independent biological nitrogen fixation in important crop plants.

P.04

Type: Poster

Topic: Advances in rice biotechnology and genome editing

An ectopic cytosolic MEP pathway in rice endosperm induces profound metabolite and transcriptome changes

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In nature, there are 2 main biosynthetic routes of isoprenoid: mevalonate pathway (MVA), mostly present in most eukaryotes and some bacteria and the non-mevalonate pathway (MEP), which is present in bacteria, plants, and apicomplexan protozoa.

We expressed the *E. coli* MEP pathway in the cytosol of rice endosperm to ascertain whether this might increase the pool of isoprenoid precursors. We have recovered a transgenic rice callus combinatorial population which is now being characterized at the DNA level to determine the precise transgene complement in each line. This population will be subjected to transcriptomic and metabolomic profiling to understand the mechanistic basis of these genetic interventions. Independent lines will be regenerated to intact plants to determine whether metabolic changes in callus and seed endosperm are congruent

P.05

Type: Poster

Topic: Advances in rice biotechnology and genome editing

Enhancing photosynthetic efficiency of rice by incorporating an algal Carbon Concentrating Mechanism

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Increases in population growth, decreasing agricultural land and depletion of natural resources require substantial increases in crop productivity, in particular in developing countries. Considerable efforts have been made towards enhancing the photosynthetic efficiency of crops to achieve higher yields through conventional breeding¹. However, gains have been modest, in part due to limited genetic variation in photosynthetic efficiency within breeding germplasm². The photosynthetic efficiency of C3 plants is far from its theoretical maximum in comparison to C4 crops. The slow catalytic rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), photorespiration, and low specificity of RuBisCO for CO₂ are some of the major bottlenecks that need to be overcome to enhance photosynthetic efficiency in C3 crops such as rice and potato. The limited availability of CO₂ in the mesophyll cells, and the inability of leaves to intercept adequate amounts of solar radiation pose additional challenges³. We describe ongoing experiments aiming to limit photorespiration, by incorporating an algal CO₂ Concentrating Mechanism (CCM) into rice^{4,5}. The algal carbonic anhydrase genes (CAH1 and CAH3) and bicarbonate transporters (LCIA and LCIB) were rice codon-optimized and cloned into pUC57 vectors under the control of the phosphoenolpyruvate carboxylase (PEPC) or the maize ubiquitin 1 promoter. They were then introduced into rice by direct DNA transfer. We present preliminary results of our experiments within the H2020 EU funded project PhotoBoost.

P.06

Type: Poster

Topic: Advances in rice biotechnology and genome editing

Carotenoid profile in rice endosperm using early endogenous pathway biosynthetic genes

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The endosperm of cereal grains serves as a major food staple world-wide, even though it is deficient in nutritionally essential carotenoids. Carotenoids are hydrophobic compounds that play important roles in plant photosynthesis, photoprotection and in the synthesis of phytohormones (Farré et al., 2014). Importantly, they constitute the major source of provitamin A in the human diet. Vitamin A deficiency causes many serious health disorders (Farré et al., 2011). Rice, an important food security crop, similarly to the other major cereals does not accumulate carotenoids in the endosperm and consequently rice-based diets in developing countries are associated with vitamin A deficiency which affects ca: 250 million children in such countries. Phytoene synthase 1 (*PSY1*) and phytoene desaturases and isomerases in the early part of the carotenoid biosynthetic pathway are recognized as rate limiting steps (Bai et al., 2016). The precise nature and number of plant (phytoene) desaturases and/or (zeta-carotene) isomerases required to recapitulate the pathway leading to beta carotene are currently unknown. Our work focuses on the role of the latter two classes of plant enzymes in recapitulating a rice-only functional carotenoid pathway leading to the accumulation of beta carotene in rice endosperm.

P.07

Type: Poster

Topic: Advances in rice biotechnology and genome editing

Interactomics for Rice Flowering: a Proximity Labelling Approach

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Variability in flowering-controlling genes has been fundamental over history for adapting rice cultivation to different latitudes. The molecular network controlling floral induction in rice is highly complex and it is the result of a stratification of interactions: transcriptional, epigenetic and post-translational. A relatively new technique called Proximity Labelling (PL) allows a high-throughput identification of protein interactors by molecular engineering, and has never been tested in rice. The goal of this research is to implement PL in rice, establishing efficient vectors and protocols while applying it to the identification of interactors of OsFTL1 and Hd1. The former is a novel protein, involved in floral commitment, homologous to the Arabidopsis florigen FT and whose interactors are still mostly unknown. The latter is a master regulator of floral induction, homologous to Arabidopsis CONSTANS, which promotes or represses flowering depending on photoperiod. Hd1 functional switch relies on the interaction with other flowering regulators and on the formation of NF-Y complexes. However, the centrality of Hd1 in the photoperiodic flowering pathway suggests that the number of real interactors could be higher than we already know. The application of PL to OsFTL1 and Hd1 will deliver a list of the proteins laying in their contiguity. The method exploits a biotin-ligase fused to the two proteins of interest. Exposure of tissues to biotin will lead to biotinylation of OsFTL1- and Hd1-interacting proteins. Biotin tags will allow for selective precipitation of the proximal proteome followed by mass spectroscopy analysis. As a result, a list of presumed interactors will be provided, potentially unveiling undisclosed aspects of flowering regulation in rice based on posttranslational control. The findings of this research would be extremely precious to the scientific community and could represent a useful means for rice breeding. Indeed, flowering is a key event in rice development and, since it is strictly linked to productivity, represents a pivot for yield improvement and acclimation to challenging environmental changes.

Epigenomics

P.08

Type: Poster

Topic: Epigenomics

Parental variation in CHG methylation is associated with allelic-specific expression in elite hybrid rice

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Heterosis refers to the superior performance of hybrid lines over inbred parental lines. Besides genetic variation, epigenetic differences between parental lines are suggested to contribute to heterosis. However, the precise nature and extent of differences between the parental epigenomes and the reprogramming in hybrids that govern heterotic gene expression remain unclear. In this work, we analyzed DNA methylomes and transcriptomes of the widely cultivated and genetically studied elite hybrid rice (*Oryza sativa*) SY63, the reciprocal hybrid, and the parental varieties ZS97 and MH63, for which high-quality reference genomic sequences are available. We showed that the parental varieties displayed substantial variation in genic methylation at CG and CHG (H = A, C, or T) sequences. Compared with their parents, the hybrids displayed dynamic methylation variation during development. However, many parental differentially methylated regions (DMRs) at CG and CHG sites were maintained in the hybrid. Only a small fraction of the DMRs displayed non-additive DNA methylation variation, which, however, showed no overall correlation relationship with gene expression variation. In contrast, most of the allelic-specific expression (ASE) genes in the hybrid were associated with DNA methylation, and the ASE negatively associated with allelic-specific methylation (ASM) at CHG. These results revealed a specific DNA methylation reprogramming pattern in the hybrid rice and pointed to a role for parental CHG methylation divergence in ASE, which is associated with phenotype variation and hybrid vigor in several plant species.

Future prospects: Food security and nutrient enhancement

P.09

Type: Poster

Topic: Future prospects: Food security and nutrient enhancement

Creating a DNA Fingerprint Panel for Southern US Rice Varieties

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Extreme weather resulting from the rapid effects of climate change is threatening global rice productivity at an ever-increasing rate. These effects will be felt most by tropical countries. Consequently, US rice exports to these countries will need to increase to fill this drop in production. As each quality preferences differ among consumers, specific varieties are desired by different countries. To ensure identity preservation of varieties through the supply chain, there has been a desire to establish a core set of DNA markers for distinguishing US rice varieties to ensure international buyers of varietal purity in export shipments. In this study we set out to create a Fingerprint Panel of molecular markers for 382 Southern US Varieties. We started with a set of 2200 robust markers known to work across KASP and AgriPlex platforms. Historical data was used to select a set of 80 markers that optimized the number of polymorphisms between varieties and the distance between markers. This set was tested on all lines using a KASP system and missing data offset with historical data were possible. Lines were then filtered on missing data resulting in a final set of 358 lines. Next, an R script was used to further optimize the set to 60 markers. Of the 63,903 unique pairwise line combinations this panel distinguishes all but 56. The combinations not distinguished are between lines of low relevance and will likely not need to be distinguished between in practical applications. Additionally, 99.9% of distinguished combinations are by at least two markers and the average separation of all combinations is 19 markers. The set is dispersed throughout all twelve chromosomes with an average distance of 9 Mb between markers. Hence, we propose this panel as a valid method for quick and reliable varietal identification in Southern US Germplasm.

P.10

Type: Poster

Topic: Future prospects: Food security and nutrient enhancement

Physiological investigation of nitrogen use efficiency in Bengal and Assam Aus Panel of rice populations

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Rice, an agricultural crop supplies calories for more than 50% of the population in many countries. In the future, the global demand for rice will have increased by 85% between 2013 and 2050 with increasing population. To achieve high productivity farmers have relied on large scale use of nitrogen (N) applications in rice fields. But rice crop only takes up 40% of the N applied while the rest causes environmental problems like increase of greenhouse gas and eutrophication in water bodies. So, there is need of increase the nitrogen use efficiency (NUE) in rice crop plants. The aim of this study was to find the response of different cultivars of rice under different treatments of nitrogen. In this context, we have conducted green-house experiments on Bengal and Assam Aus Panel (BAAP) rice genotypes to check traits (plant height, leaf nitrogen content, nitrogen balance index (NBI) and plant biomass) related to the NUE of rice under different N applications. There was a significant impact of treatments on genotypes and the leaf nitrogen content was significantly correlated with NBI across rice genotypes. The experiment was extended to 230 cultivars of the BAAP to find the difference in physiological efficiency (plant height, NBI, tiller number, and shoot biomass) at N-0% and N-100% (recommended dose of N) among rice genotypes. A genetic mapping will be done on the data sets using Genome Wide Association Studies (GWAS) to trace efficient QTLs/genes for NUE. The research will be helpful in enabling lower nitrogen input in rice fields by increasing the NUE in plants.

Integrative physiology and functional genomics

P.11

Type: Poster

Topic: Integrative physiology and functional genomics

PyRice: a Python package to facilitate post-GWAS and gene functional analyses

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Nowadays, Rice Research is producing a huge amount of data spread out on various online databases and web portals. For the scientists, the challenge remains in collecting gene information quickly from multiple loci or large gene lists and combining it logically, to facilitate their research. We developed PyRice to facilitate this process. PyRice supports several rice databases to query gene information. Currently, PyRice covers search on genomic information, gene ontology annotation, transcription factor interactions, gene expression, orthology and co-citation interactions. Moreover, its design is modular and implements a smart query system which fits the computing resources to optimize in parallel the query speed. A typical search with PyRice could start with a genomic locus or a gene list. Users can select the databases to query or leave all by default, then configure the type of outputs. The outputs are two kinds. First PyRice creates a spreadsheet where all genes are listed along with a summarised information such as annotation, gene ontology, keywords, names and synonyms, etc. Then, it creates a document for each gene to aggregate all information. This document is linked with the spreadsheet. As a result, PyRice is easy to use and produces intuitive results.

Molecular breeding under a changing climate

P.12

Type: Poster

Topic: Molecular breeding under a changing climate

Integrative approach for precise genotyping and transcriptomics of a salt tolerant introgression line in rice

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Rice is the most salt sensitive cereal crop and its cultivation is particularly threatened by salt stress, which is currently worsened due to climate change. This study reports the development of salt tolerant introgression lines (ILs) derived from crosses between the salt tolerant *indica* rice variety FL478, which harbours the *Saltol* QTL, and the salt-sensitive *japonica* elite cultivar OLESA. Genotyping-by-sequencing (GBS) and Kompetitive allele specific PCR (KASP) genotyping, in combination with step-wise phenotypic selection in hydroponic culture, were used for the identification of salt-tolerant ILs. Transcriptome-based genotyping allowed the fine mapping of *indica* genetic introgressions in the best performing IL (IL22). A total of 1,595 genes were identified in *indica* regions of IL22, which mainly located in large introgressions at Chromosomes 1 and 3. In addition to *OsHKT1;5*, an important number of genes were identified in the introgressed *indica* segments of IL22 whose expression was confirmed (e.g. genes involved in ion transport, callose synthesis, transcriptional regulation of gene expression, hormone signalling and ROS accumulation). These genes might well contribute to salt stress tolerance in IL22 plants. Furthermore, comparative transcript profiling revealed that *indica* introgressions caused important alterations in the background gene expression of IL22 plants (*japonica* cultivar) compared with its salt-sensitive parent, both under non-stress and salt-stress conditions. In response to salt treatment, only 8.6% of the salt-responsive genes were found to be commonly up- or down-regulated in IL22 and OLESA plants, supporting massive transcriptional reprogramming of gene expression caused by *indica* introgressions into the recipient genome. Interactions among *indica* and *japonica* genes might provide novel regulatory networks contributing to salt stress tolerance in introgression rice lines. Collectively, this study illustrates the usefulness of transcriptomics in the characterization of new rice lines obtained in breeding programmes in rice.

P.13

Type: Poster

Topic: Molecular breeding under a changing climate

TOWARD THE FUNCTIONAL CHARACTERIZATION OF ARF8 and 18 IN FEMALE GAMETOPHYTE DEVELOPMENT IN ORYZA SATIVA

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The plant hormone auxin regulates a variety of physiological and morphological processes including gynoecium and fruit development. Auxin signal transduction and signalling are processes largely performed by AUXIN RESPONSE FACTORS (ARFs) transcription factors and the auxin/indole acetic acid protein family.

Recent studies suggest that a deregulation of auxin signalling can switch the developmental program of ovules from sexuality to agamic (asexual) reproduction. Asexual reproduction is a highly desirable trait in agriculture because it allows the perpetuation of superior hybrid genotypes. In *Paspalum notatum* (a forage grass), miR160 and its target transcript *ARF10* showed consistent differential representation in both reproductive types (Ortiz et al. 2017, 2019; Podio et al. 2021). Very interestingly, in rice ovules before fertilization, miR160 was reported to be down regulated while *OsARF10*, *OsARF18* and *OsARF22* were up regulated (Wu et al., 2017). These data strongly suggest a key role of *ARFs* and miR160 on the female gametophyte development in different species, with a potential role on the regulation of asexual reproduction.

To test this hypothesis, we performed a phylogenetic analysis of ARF family proteins from *Arabidopsis* and rice and selected *OsARF8* (LOC_Os02g41800) and *OsARF18* (LOC_Os06g47150) as putative rice orthologs of *Arabidopsis* *ARF10*. Using *Agrobacterium*-mediated transformation protocol on rice embryogenic calli, we simultaneously induced CRISPR/Cas9-mediated genome modifications on both genes. After plant regeneration, we confirmed the establishment of loss-of-function double mutant lines. Currently, we are evaluating the *osarf8 osarf18* phenotype, especially focusing on reproductive development characterization, to provide insights on its potential use for agronomic breeding programs in the future.

Plant-environment interactions: Abiotic stress

P.14

Type: Poster

Topic: Plant-environment interactions: Abiotic stress

Genotypic Variation in Heat Tolerance in Rice During the Vegetative Stage

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Rice is the third most important agricultural commodity worldwide. Increases in global temperatures have a negative impact on rice productivity. Chlorophyll fluorescence can provide useful information about a plant's ability to tolerate stress and the extent to which environmental stress has damaged the photosynthetic apparatus and caused photoinhibition. The aim of this study was to develop a screening system for heat tolerance which could be used to rapidly phenotype large populations of rice. The specific objective was to conduct experiments to establish protocol for testing heat tolerance during the vegetative stage using a Qubit FluorPen.

To investigate genotypic differences in heat tolerance during the vegetative stage, fifteen different genotypes replicated six times were used to measure Fv/Fm. Fv/Fm indicates the photosystem II quantum yield (QY) in dark-adapted leaves. To do this, leaf section of 3 cm were placed in plastic zip-seal bags and place in the dark for 30 minutes. The leaves were then placed in a water bath set at 47°C for 60 minutes and the FV/Fm measured every 10 minutes. The results demonstrated that for all genotypes the Qy significantly ($p < 0.000$) decreased overtime. However, the degree that the Qy decreased over time was different between genotypes ($p < 0.001$). For example; the Qy for Black Gora was 0.79 prior to the heat treatment, but decreased to 0.21 after 60 minutes, while Tepaboro 508 had an initial Qy of 0.78, but only decreased to 0.48 after 60 minutes.

Using this technique it will be possible to screen large numbers of genotypes for heat tolerance. This technique will now be used to compare vegetative heat tolerance to heat tolerance at flowering and to screen a genetic mapping population for heat tolerance with the aim to identify quantitative trait loci for heat tolerance in rice.

P.15

Type: Poster

Topic: Plant-environment interactions: Abiotic stress

UAV phenotyping of dynamic drought response in rice

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- Accurate and high-throughput phenotyping of the dynamic response of a large rice population to drought stress in the field is a bottleneck for genetic dissection and breeding of drought resistance.
- Here, high-efficiency and high-frequent image acquisition by an unmanned aerial vehicle (UAV) was utilized to quantify the dynamic drought response of a rice population under field conditions. Deep convolutional neural networks (DCNN) and canopy height models were applied to extract highly correlated phenotypic traits including leaf-rolling score (LRS_uav), plant water content (PWC_uav), and a new composite trait, drought resistance index by UAV (DRI_uav).
- The DCNN achieved high accuracy (correlation coefficient $R=0.84$ for modeling set and $R=0.86$ for test set) to replace manual leaf-rolling rating. PWC_uav were precisely estimated (correlation coefficient $R=0.88$) and DRI_uav were modeled to dynamically and comprehensively monitor the drought resistance of rice accessions. A total of 111 significantly associated loci were detected by genome-wide association study for the three dynamic traits, and 30.6% of them were not detected in previous mapping studies using non-dynamic drought response traits.
- UAV and deep learning are confirmed effective phenotyping techniques for more complete genetic dissection of rice dynamic responses to drought and exploration of valuable alleles for drought resistance improvement.

P.16

Type: Poster

Topic: Plant-environment interactions: Abiotic stress

Putative Role of Jasmonic Acid Signalling Pathway in Coleoptile Elongation Under Submergence

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As semi-aquatic plant, rice (*Oryza sativa* L.) has a certain ability to germinate and survive under submergence. Hypoxia stress response is triggered, mainly driven by perception of phytohormones levels. In Asian countries, rice cultivation methods are shifting to direct-seeding in flooded fields, where the capacity to rapidly germinate and elongate the coleoptile are desirable traits. Previous lab work showed that rice HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE1 (*OsHOS1*) is involved in root curling through modulation of jasmonic acid (JA) signalling. Two transcription factors (TFs), identified as binding to *OsHOS1* promotor, belong to the group VII of the ethylene response factor family, which is highly correlated with hypoxia stress response. One of those TFs, *SUB1B*, belongs to the *SUBMERGENCE1* locus that contains the *SUB1A-I* allele, considered responsible for the strategy adopted by submergence-tolerant varieties. In preliminary analyses, RNAi::*OsHOS1* mutant plants exhibited shorter coleoptiles under submergence. In our work, RNAi::*OsHOS1* lines appeared to have less sensitivity to JA, when incubated with exogenous JA. Given this, we raised the possibility that JA signalling pathway is related to coleoptile elongation and hypoxia response under submergence. We studied the variability of coleoptile length among 10 rice genotypes submitted to aerobic versus submergence conditions for 4 and 8 days. Five genotypes were selected for further characterization by real-time quantitative polymerase chain reaction to analyse the expression of candidate genes (e.g. involved in JA biosynthesis and signalling). Interestingly, higher transcription levels of *SUB1B* correlated with shorter coleoptiles under submergence. Our results point for an involvement of *OsHOS1* in the JA signalling pathway in submerged coleoptiles, as well as to a putative role of *SUB1B* on coleoptile elongation, which had not been previously described. Further studies on this subject are still necessary and important for the development of new thriving varieties, especially targeting submerged environments.

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Type: Poster

Topic: Plant-environment interactions: Abiotic stress

Salt and alkaline tolerant cyanobacteria from Ebro Delta paddies for biofertilization of salt-stressed rice

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Among the diverse cyanobacteria several species have been explored as plant growth promoting biofertilizers to improve nutrient supply and tolerance to biotic and abiotic stress. Under the current and future climate change conditions, paddy fields in the Mediterranean area are at risk of progressive salinization. Here we isolated different cyanobacteria from paddy fields in the Ebro Delta to characterize their tolerance to saline-alkaline conditions and to make an initial evaluation of a selected strain as biofertilizer for rice grown under saline conditions. Soil samples were collected from paddy fields at the Institute of Agrifood Research and Technology and La Marquesa (Deltebre). For cyanobacteria isolation, soil samples were inoculated into sterilized TSA plates and mixed with BG-11 medium with or without combined nitrogen (pH 7.8). Cyanobacterial colonies were visible after 10-15 days incubation at $28\pm 1^\circ\text{C}$ with constant illumination of $100\ \mu\text{mol photon m}^{-2}\ \text{s}^{-1}$. Axenic populations were obtained by serial dilution. Thirty axenic genera of cyanobacteria were isolated. To our best knowledge, *Nostoc paludosum*, *Nostoc linckia*, *Nostoc muscorum*, and *Cylindrospermum tropicum* were recorded for the first time in this region. Previously undescribed *Nostoc* strain UAB206 was used for further polyphasic characterization. Exposure to pH ranging from 7 to 11 in combination with different salinity conditions (17, 80, 160, 240 and 320 mM NaCl) allowed to establish *Nostoc* strain UAB 206 as highly tolerant to alkaline-saline conditions. First assays of the influence of this strain on performance of rice cultivars grown in hydroponics under saline conditions (80, 50 or 100 mM NaCl) revealed a positive influence of *Nostoc* strain 206 on photosynthesis of cultivar Copsemar. Further studies to characterize the mechanisms underlying these positive effects are under progress

Supported by the Spanish MICINN project PID2019-104000RB-I00. We thank Mar Català (IRTA Deltebre) for her help in sampling site selection

P.18

Type: Poster

Topic: Plant-environment interactions: Abiotic stress

OsRIP1, a cytoplasmic ribosome-inactivating protein, is involved in the jasmonate signaling pathway?

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Ribosome-inactivating proteins (RIPs) are known as toxins, which are capable of catalytically inactivating prokaryotic and/or eukaryotic ribosomes with a highly specific rRNA *N*-glycosidase activity (EC 3.2.2.22). RIPs can remove an adenine residue from the large rRNA, leading to the complete arrest of translation and the inhibition of protein synthesis. Most plant RIPs are synthesized with an N-terminal signal sequence and follow the secretory pathway. The mature proteins end up in the vacuole or in the extracellular space. It is widely accepted that many RIPs are involved in plant defense responses. In addition, transgenic plants over-expressing certain RIPs have been reported to be less susceptible to different types of abiotic and biotic stresses.

Recently, OsRIP1, a cytoplasmic RIP lacking a signal peptide was identified as one of 38 RIPs in the *Oryza sativa* (ssp. japonica) genome. *In silico* analysis revealed that OsRIP1 transcript levels were responsive to jasmonate (JA) treatment. This is in line with experimental results showing that OsRIP1 was transcriptionally upregulated in wild type (WT) plants after methyl jasmonate (MeJA) treatment. The phytohormone JA is known to play an essential role in a variety of environmental adaptations and plant development. To understand the importance of OsRIP1 in JA-related processes, transgenic lines overexpressing OsRIP1 were subjected to MeJA treatment. After seed germination, WT plants and OsRIP1-overexpressing plants were hydroponically grown in Hoagland solution prior to MeJA administration. The performance of transgenic plants was investigated, and it was found that plants overexpressing OsRIP1 grew better than WT plants in the presence of MeJA. Additionally, mRNA sequencing was performed and showed that GO terms related to protein metabolic process and translation were suppressed in OsRIP1-overexpressing plants subjected to MeJA compared to WT plants, while GO terms for photosynthesis and lignin metabolism were enriched.

Plant-environment interactions: Biotic stress

P.19

Type: Poster

Topic: Plant-environment interactions: Biotic stress

Transcriptional response during immune cell death in rice

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Cereals represent the biggest and most widely spread group of cultivated plants across the globe, for which their yield is in constantly threatened by new disease outbreaks. Yet so far, most information on host-pathogen recognition and immune signaling pathways comes from studies on the model plant *Arabidopsis thaliana*. However, the relevance of the *A. thaliana* immunity network for cereal crops is unclear. Therefore, fundamental knowledge of the monocot plant immune system is still needed.

Plant immune receptors, mostly cytosolic nucleotide-binding/leucine-rich repeat proteins (NLRs), perceive pathogen virulence factors (effectors) and trigger a strong defense response, effector triggered immunity (ETI), which is often accompanied by immune cell death at the infection site. However, it is not known how these early signaling events lead to cell death and how this affects resistance of the surrounding tissues to the invading pathogen.

Here we aim to identify which are the genes involved in the early transcriptional reprogramming leading immune cell death in infected tissues in a cereal crop, rice, and compare them to recently identified markers of immune cell death in *A. thaliana*. For this we employ Kitaake rice lines, which contain the NLR pair RGA4-RGA5 and therefore recognize the effector proteins AVR1-CO39 and AVR-Pia from *Magnaporthe oryzae* to trigger ETI. *M. oryzae* is the causal agent of the blast disease of cereals. The fungus is one of the most devastating for agriculture worldwide, as well as an important model system, in which host-pathogen interactions are well characterized. By making use of dexamethasone-inducible Kitaake lines we will express either AVR1-CO39 to trigger ETI or the Beta-glucuronidases gene (GUS) and follow changes in gene expression in tissues which will later undergo immune cell death. Our results will help gain a better understanding of immune responses in monocots and determine the degree of conservation of immune networks.

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Type: Poster

Topic: Plant-environment interactions: Biotic stress

Mechanism underlying phosphate-induced susceptibility to the rice blast fungus *Magnaporthe oryzae*

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Rice blast caused by the fungus *Magnaporthe oryzae* is the most devastating fungal disease of rice worldwide. To reduce losses due to *M. oryzae* infection, and to obtain maximum yields, high amounts of pesticides and fertilizers continue to be applied in rice cultivation causing an adverse impact on human health and environment. Ironically, excess of fertilization might have a negative impact on resistance to infection by the rice blast fungus *M. oryzae* (e.g. nitrogen supply). In this study, we investigated the effect of phosphate (Pi) supply on disease severity caused by *M. oryzae* in rice. A *M. oryzae* strain co-expressing two genes encoding fluorescently-labeled effectors (*PWL2* and *BAS4*) was used to monitor the infection process *in vivo*. Confocal microscopy revealed early induction of *M. oryzae* effector genes in rice plants that had been grown under high Pi supply compared with plants grown under a normal Pi regime. High fertilization was also accompanied by a weaker induction of defense-related genes during *M. oryzae* infection. Altogether, these observations are in agreement with the phenotype of blast susceptibility that is observed in rice plants grown under a high Pi regime. These results support that Pi, when in excess, has an effect on the two partners, by stimulating fungal pathogenicity and undermining host defense programmes. Therefore, from the perspective of the rice plant, the two signalling pathways, Pi signalling and defense signalling, must operate in a coordinated manner in controlling Pi-induced susceptibility to the rice blast fungus. This information provides a basis to understand why phosphate fertilization increases the impact of the blast disease, an aspect that should be considered in management of the rice blast disease.

Resources for systems biology in rice

P.21

Type: Poster

Topic: Resources for systems biology in rice

The Rice Genome Hub, a portal for multi scale analysis of rice genomic resources

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With the advent of long read sequencing, the number of high quality genome sequences of rice has greatly improved. Here we introduce the Rice Genome Hub (<https://rice-genome-hub.southgreen.fr/>), a genomic resource portal that integrates numerous high quality rice genome sequences, a wide range of experimental data, as well as a large number of bioinformatic tools. This information system includes twenty genomes : 11 from *O.sativa* ssp. *indica*, 5 from *O.sativa* ssp. *japonica*, 1 *O. glaberrima* and 3 others.

All of these genomes are provided with a high quality annotation, de-novo performed specially for the hub purpose when not available with the genome release. These annotations have been enriched with gene ontology, InterPro terms and information extracted from different databases (e.g. related publications), and can be easily queried by keywords. A large array of tools has been deployed around these genomes, like Blast on the genome or on the annotation, primer designer tools and primer specificity check, along with tools using the data that may have been generated based on these genomes, like RNA-seq, or SNP list. Some public data, such as the variants from the 3000 genomes projects, or data from the IRD/CIRAD research centers have been integrated to the hub, with detailed information that can be accessed on specific pages. Integrated RNA-seq dataset can be interactively visualised with ease and data can be explored for differentially expressed genes or expression clusters using DIANE or iDEP. Genomic variations can be explored, filtered and analyzed with Gigwa, as well as visualized using the genomes browsers (Jbrowse) which have been setup for all genomes and allow visual access to much of the data. The hub will be enriched with new data in the future, either from major rice related publications or from the institutes and their partners.

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Type: Poster

Topic: Resources for systems biology in rice

Identification of SUMOylation machinery in rice (*Oryza sativa*)

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SUMOylation is a key post translation modification (PTM) in plants. This process has been reported as a critical regulator in plant development and response to abiotic and biotic stresses. The mechanisms of the SUMOylation are best known in the model organisms, *Saccharomyces cerevisiae* and *Arabidopsis thaliana*. The SUMOylation pathway consist of 5 main steps, maturation of SUMO proteins, activation of mature SUMOs by E1 ligase (e.g., SAE1a), conjugation of SUMO-E1 complex by E2 ligase (e.g., SCE1), ligation of SUMO complex with a substrate (target proteins) by E3 ligase (e.g., HYP2 and SIZ1), polySUMOylation (alternative) by E4 ligase (e.g., PIAL), and deSUMOylation by protease enzymes (e.g., ULP and Desi). To translate the information from model species to crop plants (i.e. *Oryza sativa*), we identified SUMOylation machinery genes in rice by querying the protein sequences of *A. thaliana* against *O. sativa* genome (TBLASTN). In this preliminary work, we have uncovered six SUMO modifiers and seven (E1-E4) ligase encoded genes in rice genome. Work is on-going to profile the SUMO proteases and their evolution. The number of genes in rice are not comparable to genes in *Arabidopsis*, suggesting an expansion/reduction of SUMOylation genes and potentially leading to evolution of some distinct mechanism of rice throughout the evolution. In addition, the ambiguous gene models are being revised by integrating transcriptomic data and implementing bioinformatic tools such as Apollo. The identified and improved gene models could be the starting point and resources for the study of the SUMOylation mechanism in rice and potentially be a target for gene editing in crop improvement.

Rice development and phenomics

P.23

Type: Poster

Topic: Rice development and phenomics

Opposite polarity programs regulate asymmetric subsidiary cell division in grasses

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Stomata are epidermal valves on plant leaves that efficiently balance gas exchange and evaporation. Grass stomata acquired the unique graminoid morphology consisting of two dumbbell-shaped guard cells (GCs) flanked by two subsidiary cells (SCs), which enable stomata to open and close rapidly. The transcription factor *MUTE* specifies subsidiary mother cells (SMC), which subsequently divide asymmetrically to generate SCs. Here, we used the *Brachypodium distachyon* stomatal complex as a model to investigate SMC polarization and division in grasses. Mutations in the novel factor *BdJIXING* (*BdJIX*) induced defects in the polarization of SMCs and ~24% of SCs formed aberrantly. Surprisingly, *BdJIX* localized in the SMC periphery while being mostly absent proximal to guard mother cells (GMCs). This is in contrast to well-established SMC polarity factors like the leucine-rich repeat–receptor-like kinase *PANGLOSS1* (*BdPAN1*), which specifically accumulated at the proximal GMC/SMC interface. *Bdpan1* mutants showed similar defects during SC divisions as *bdjix* yet with a higher frequency (~44%). The double mutant *bdjix;bdpan1*, however, has a synergistic phenotype with a remarkably increased frequency (~82%) of aberrant SCs, which strongly affects stomatal opening and closing kinetics. We used quantitative imaging of pre-mitotic nuclear migration in SMCs and SMC division behavior in different mutant backgrounds, and found that *BdPAN1* seemed to primarily guide nuclear migration. *BdJIX*, on the other hand, was rather associated with specifying SMC division plane orientation and/or division potential. Taken together, our work suggested that *BdPAN1* and *BdJIX* are two distinct polarity modules with opposing polarity domains that synergistically promote SMC polarization and division, respectively, and ultimately facilitate the formation of SCs in grasses.

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Type: Poster

Topic: Rice development and phenomics

Genetic control of inflorescence architecture in rice by a specific inflorescence meristematic gene

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In rice, panicle size and architecture are considered critical key targets for the breeding programs to improve grain yield potential of the plant. The inflorescence architecture depends on the activity of many genes acting in different reproductive meristem types. Especially primary and secondary branch meristem activities directly affect the complexity of branching, and therefore the number of seeds set that will develop on the panicle (Ikeda et al., 2004; 2013). Here, we report an initial study of an uncharacterized gene previously identified by a rice reproductive meristems specific transcriptome analysis using laser microdissection microscopy (Harrop et al., 2016). Interestingly, our initial studies reveal that this gene is present only in *Oryza* species and encoding a protein of just 100 amino acids. To address the biological role of this protein, we successfully generated homozygous CRISPR-Cas9 mutant plants and performed preliminary phenotypic analysis on the panicle structure. Our findings show that loss of function of the gene affects panicle architecture suggesting that this *Oryza* specific gene may have evolved to determine rice specific panicle characteristics.

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Type: Poster

Topic: Rice development and phenomics

The ALOG family members OsG1L1 and OsG1L2 regulate inflorescence branching in rice

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Human population growth makes of primary importance to find new ways to improve agricultural crop yield and meet the increasing food demand. In this context, inflorescence architecture is one of the key agronomical traits which determines grain yield; thus, it has been a major target for crop domestication and improvement.

In *Oryza sativa*, inflorescence architecture is established at early stages of reproductive development. During vegetative phase, the Shoot Apical Meristem (SAM) produces leaves. When a rice plant undergoes floral transition, the SAM differentiates into Inflorescence Meristem (IM), which in turn gives rise to Primary Branch Meristems (pBMs). The pBMs can produce Spikelet Meristems (SMs) or form a more complex architecture with Secondary Branches Meristems (sBMs), that will in turn produce SMs. When the SM differentiation occurs, the meristem loses its indeterminate state to become determinate, stopping all sorts of branching potential. This leads to the development of Floral Meristems (FMs), that differentiate into floral organs.

The *ALOG* gene *TAWAWA1* (*TAW1*) has been shown to be a regulator of meristem activity: it promotes IM activity and the suppression of the phase change to SM identity. Combining laser microdissection of rice inflorescence meristems with RNA-seq, we observed that other two members of the *ALOG* gene family, *OsG1-like 1* (*OsG1L1*) and *OsG1L2*, present an expression profile similar to *TAW1*. Furthermore, the loss-of-function CRISPR mutants *osg1/1* and *osg1/2* present a phenotype similar to the *taw1* mutant, suggesting that these three genes may act in related pathways controlling inflorescence development.

A transcriptome analysis was performed on *osg1/2* mutant, suggesting the interaction of *OsG1L2* with other genes known to control inflorescence architecture. The obtained dataset was also used to generate a gene regulatory network (GRN).

The analysis of the loss-of-function CRISPR mutant of the homeodomain-leucine zipper transcription factor gene *OsHOX14* suggests that the proposed GRN can be useful to identify players involved in inflorescence development in rice.

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Type: Poster

Topic: Rice development and phenomics

Effects of *O. glaberrima* introgression on panicle branching in *O. sativa* x *O. glaberrima* CSSLs

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The inflorescence (panicle) architecture of rice is one of the key components of yield potential and displays high inter- and intra-specific variability. The architecture of the rice inflorescence is mainly determined by the length and number of primary branches (PBL, PBN) and secondary branches (SBN) which can influence spikelet number (SpN). The mechanisms underlying the diversity of panicle complexity are still poorly understood. The *Oryza* genus features two different crop species: the Asian rice (*Oryza sativa* L) and the independently domesticated African rice (*O. glaberrima* Steud). One of the main morphological differences between the two domesticated species relates to panicle structure (or complexity), *O. sativa* bearing a highly branched panicle carrying a larger number of seeds compared to *O. glaberrima*.

In order to identify genetic factors linked to the diversity of panicle architecture between these two crops species, we used a population corresponding to a set of 60 Chromosome Segment Substitution Lines (CSSLs) (BC₃DH) carrying contiguous chromosomal segments of *O. glaberrima* (cv. MG12) in the genetic background of *O. sativa* ssp. tropical japonica (cv. Caiapó). Phenotypic data were collected for Rachis Length (RL), Primary Branch Length (PBL), Primary Branch Number (PBN), Secondary Branch Number (SBN), Tertiary Branch Number (TBN) and Spikelet Number (SpN). A total of 16 QTLs were localised on chromosomes 1, 2, 3, 7, 11 and 12. Recombinant BC₄F_{3/4} lines were produced to validate the effects of QTLs relative to a decreased SBN and an increased TBN. The CSSLs harbouring qTBN7-1 display a higher number of tertiary and secondary branches and a decreased primary branch number compared to the *O. sativa* parent. The analysis of this genomic region is under investigation with the aim of identifying candidate genes likely to be involved in the regulation of axillary meristem production during panicle differentiation.

P.27

Type: Poster

Topic: Rice development and phenomics

CLIMBER - Confronting CLIMate change impacts in BarLEy and Rice

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The CLIMBER project exploits Rice and Barley, important genetic models for the grass family and among the most significant crops worldwide to explore conserved and species-specific responses to abiotic stresses such as heat and drought which cause substantial crop loss worldwide threatening food security particularly in the context of climate change. We are generating and characterizing rice and barley plants expressing specific hormone biosensors to explore the role of auxin and cytokinin signaling and identify tissue domains in the developing inflorescence where hormone levels change under stress conditions.

In parallel, transcriptomic analyses of developing rice and barley inflorescences under control and heat-stress conditions are underway. The resulting data will be analyzed with innovative approaches to identify molecular mechanisms that are either shared, or distinct between the relatively distantly related species in question, and generate testable hypotheses regarding the role and mechanism of action of phytohormones in developmental responses to stress.

Acknowledgements

CLIMBER is funded by the University of Milan under the SEED 2019 call.

P.28

Type: Poster

Topic: Rice development and phenomics

Natural allelic variants associated with panicle architecture variation in *Oryza glaberrima*

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The Asian rice *Oryza sativa* and the African rice *Oryza glaberrima* are the two cultivated rice species in the world. They were independently domesticated in Asia and Africa and are both characterized by an intra-specific diversity of panicle architecture complexity and grain number. Compared to *O. sativa*, little is known about genes governing panicle architecture in relation to haplotype combinations in *O. glaberrima*. In this study, the allelic and structural diversity of 40 genes associated with panicle architecture in *O. sativa* was studied in *O. glaberrima* using the CG14 reference genome OMAP v2 in conjunction with a set of 160 *O. glaberrima* accessions. The results indicated that 18 of the targeted genes registered more than 20 haplotypes, while 16 genes showed fewer than 10 haplotypes. The genes *OsPLT10*, *OsPLT1*, *OsPLT8*, *DEP1*, *SP1*, *NAL3* showed a single haplotype present in more than 10 accessions. In contrast, the genes *OPB*, *LP/EP3*, *AP2/EREBP22*, *OsPLT9*, *IDS1*, *APO2*, *MOC1*, *SNB*, *ASP*, *Ghd8* and *NAL2* showed 4 haplotypes in more than 10 accessions. Furthermore, neutrality tests indicated positive values for nucleotide diversity and for Fu and Li's D and F for all tested genes, indicating genetic conservation for these genes in the population. Haplotype-based association analysis was also conducted for panicle morphological traits. As an example, the *LAX1* gene region showed 20 haplotypes with significant differences between hap1 and hap14, hap1 and hap17, and hap14 and hap17 for secondary branch number, whereas hap14 was associated with higher spikelet number compared to other haplotypes. Overall, these findings demonstrate the possibility of characterizing functional gene alleles related to panicle architecture in *O. glaberrima* based on previously characterized genes from *O. sativa*. Identified alleles that are significantly associated with panicle-related traits provide novel insights into the genetic basis of panicle architecture diversity, thereby laying a foundation for improving grain yield in rice.

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Type: Poster

Topic: Rice development and phenomics

OsPIL15 and OsPIL16 are key regulators of the etiolated coleoptile growth

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Light is a crucial environmental cue for rice seedling emergence and development and is a major regulator of coleoptile elongation. While light represses coleoptile growth and allows leaves to grow and expand, dark promotes rice coleoptile elongation. Nevertheless, the regulatory mechanisms underlying this process remains largely unknown. In the plant model *Arabidopsis thaliana*, phytochromes, the red/far-red photoreceptors, are activated by light and thus induce the degradation or sequestration of Phytochrome-Interacting Factors (PIFs), which are bHLH transcription factors (TFs). PIFs are more stable under dark, being more abundant and freer to promote plant growth and development. In rice, seven PIF-like (OsPIL) were identified so far, and single knockout mutants for each OsPIL were produced in our lab using the CRISPR/Cas9 technology. When seedlings of the OsPILs mutants were grown under dark, we observed that *ospil15* and *ospil16* mutants show shorter coleoptiles and longer leaves as compared to WT, resembling a light-grown seedling phenotype. Under white, red, or far-red light, no significant difference in the coleoptile growth was observed between *ospil15* and *ospil16* mutants and WT, suggesting that both OsPILs are inactivated/degraded by light, possibly by interaction with phytochromes. In order to unveil the molecular mechanisms underlying OsPIL15 and OsPIL16 regulation of rice seedling development, we are analysing the interactions of OsPIL15 and OsPIL16 with the phytochromes. We are also assessing the ability of OsPIL15 and OsPIL16 to form homo- and heterodimers to understand if they have a cooperative role in coleoptile elongation. These data will be further shown and discussed. Overall, the results obtained suggest that both OsPIL15 and OsPIL16 are key TFs in the rice seedling development, inducing coleoptile elongation under dark, and that light triggers OsPIL15 and OsPIL16 inactivation through interaction with phytochromes. Our work will contribute for the identification of possible targets for future improvement of rice seedling establishment.

Rice genome research and evolution

P.30

Type: Poster

Topic: Rice genome research and evolution

PlantDeepSEA, a deep learning-based web service to predict the regulatory effects of genomic variant in plants

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Characterizing regulatory effects of genomic variants in plants remains a challenge. Although several tools based on deep-learning models and large-scale chromatin-profiling data have been available to predict regulatory elements and variant effects, no dedicated tools or web services have been reported in plants. Here, we present PlantDeepSEA as a deep learning-based web service to predict regulatory effects of genomic variants in multiple tissues of six plant species (including four crops). PlantDeepSEA provides two main functions. One is called Variant Effector, which aims to predict the effects of sequence variants on chromatin accessibility. Another is Sequence Profiler, a utility that performs ‘in silico saturated mutagenesis’ analysis to discover high-impact sites (e.g., cis-regulatory elements) within a sequence. When validated on independent test sets, the area under receiver operating characteristic curve of deep learning models in PlantDeepSEA ranges from 0.93 to 0.99. We demonstrate the usability of the web service with two examples. PlantDeepSEA could help to prioritize regulatory causal variants and might improve our understanding of their mechanisms of action in different tissues in plants. PlantDeepSEA is available at <http://plantdeepsea.ncpgr.cn/>.

Citation: Zhao, H., Tu, Z., Liu, Y., Zong, Z., Li, J., Liu, H., Xiong, F., Zhan, J., Hu, X. and Xie, W. (2021) PlantDeepSEA, a deep learning-based web service to predict the regulatory effects of genomic variants in plants. *Nucleic Acids Res.*, 49, W523-W529.

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Type: Poster

Topic: Rice genome research and evolution

Platinum Standard de novo assembly of the CCDD rice wild relatives *Oryza grandiglumis* and *Oryza latifolia*

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One of the highest-demand crops globally is rice as a primary dietary resource for more than half of the world's population. Rice and its wild relatives are part of the genus *Oryza*. Studying *Oryza* species in a comparative genomics framework is a practical approach to provide a wealth of knowledge that can significantly improve valuable agronomic traits. The *Oryza* genus includes 27 species, with 11 different genome types identified by genetic and cytogenetic analyses. Six species, including the domestic rice *O. sativa*, are diploid, and the remaining 5 are tetraploids. Three tetraploid species contain the CCDD genome types (*O. grandiglumis*, *O. latifolia*, and *O. alta*), which arose less than 2 million years ago. Polyploidization is one of the significant contributors to the evolutionary development of wild species by advancing valuable traits. However, the sequencing and analysis of polyploid genomes are challenging due to the sequences' repetitiveness and complexity. Thus, having a high-quality genome assembly for polyploids like the CCDD genome species would improve our understanding of *Oryza* genome evolution and help identify traits that may be useful for crop improvement. This project generated two high-quality genomes assemblies for *O. grandiglumis* and *O. latifolia* using PacBio long-read sequencing technology in three genome assemblers (i.e., Canu/2.0, Mecat2, and Flye/2.5) and followed by sequence polishing with both Arrow and Pilon/1.23. Notably, the assemblies were arranged into pseudomolecules and homologous chromosomes assigned to their specific genome type (i.e., CC or DD). Then, the assemblies were edited using Genome Puzzle Master, a pipeline that assisted in the closing of gaps using contigs from the three assemblies and validated using Bionano optical maps. Both assemblies were analyzed for genome annotation, transposable elements, and structural variant content present in homoeologous chromosomes; This work will enable us to study the evolutionary divergence of those two genomes and explore the possibility of neo-domesticating in either species.

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Type: Poster

Topic: Rice genome research and evolution

Platinum Standard de novo assembly of rice wild relative *Oryza ridleyi* Hook

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With the expanding world population, addressing the challenge of securing a sustainable food supply under changing climate is key. The wild relatives (WRs) of the genus *Oryza* can significantly contribute to solving this challenge. The untapped genetic pool of the *Oryza* WRs provides advantageous adaptive traits such as abiotic/biotic resistances, which can be utilized for improving cultivated rice. One such WRs is the allopolyploid *Oryza ridleyi* Hook (2n=48, HHJJ), with the largest genome size in the *Oryza* genus (~1,283 Mbp). *O. ridleyi* has useful abiotic/biotic traits of interest such as resistances to blast, bacterial blight, stemborer; low radiation/flooding tolerances as well as moisture plasticity. Since *O. ridleyi* is an understudied species, the first step towards establishing a high-quality genomic resource was to create a Platinum Standard (i.e. contiguous near-gap free) reference genome. For the reference assembly, PacBio reads were assembled using five genome assemblers (Canu, Mecat, Flye, wtgb2, Falcon) with two steps of polishing with long and short reads using Arrow/Pilon software. The primary contigs were sorted into two subgenomes using HakMer and GenomePuzzleMaster was used to close the gaps. The assembly was validated using Bionano optical maps. The resulting assembly size is 1,203 Mbp with 25 contigs in pseudo-molecules (scaffold N50=53.8 Mbp) and a single gap. The assigned HH and JJ subgenomes were estimated to be 728.35 Mbp and 474.45 Mbp, respectively, with subgenome HH 1.5-fold larger than JJ. Preliminary analysis revealed that the subgenome size difference is mainly due to Transposable Element (TE) -related sequences of Long Terminal Repeat retroelements. Thus, for HH out of 728.35 Mbp TE-related sequence is 506.89 Mbp whereas for JJ it is 286.42 Mbp out of 474.45 Mbp. Analysis of *O. ridleyi* genome is ongoing and the finalized genome assembly and annotations will be a useful community resource for the study of *Oryza* WRs.

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Type: Poster

Topic: Rice genome research and evolution

Genetic structure and demographic history of the halophyte wild relative of rice *Oryza coarctata*

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Oryza coarctata is an allotetraploid wild relative of rice ($2n=4x=48$) and is found along coastal estuaries from Pakistan to Myanmar. This species is the only halophyte of the *Oryza* genus, making it a valuable resource for the study of salinity stress tolerance traits. Given its high cross-incompatibility with *O. sativa*, our team is developing the molecular genetics resources to neo-domesticate *O. coarctata*. Neo-domestication is an alternative approach to conventional trait introgression into a domesticated species, by targeting orthologs of known domestication genes from rice in *O. coarctata* to produce a high-yielding ideotype that is adapted to salinity stress.

Our aim is to analyse genetic diversity in a population of *O. coarctata* samples collected in different coastal regions of Bangladesh. Samples were whole-genome re-sequenced, and SNPs were called using a high-quality reference genome assembly. Population structure analysis revealed stratification into three subpopulations. We wanted to explore whether gene flow occurred within the population. The occurrence of allopolyploidy poses an interesting biological problem in this context. While the presence of non-recombining, divergent genomes can bring detrimental effects, the establishment of polyploidy can increase genome diversity and plasticity, contributing to the adaptation to new environments. For these reasons, each sub-genome was analysed separately. We employed a coalescent-based simulation framework to infer the timing and directionality of gene flow among the subpopulations. We tested three alternative topologies of consecutive lineage divergence among subpopulations based on the global structure. We added migration to the models by testing the two opposite scenarios of asymmetrical migration and absolute isolation post-divergence. Composite likelihood of the observed data under each model was calculated using the site-frequency spectrum.

Salinity stress contributes the total crop losses worldwide. Insights into the genetic diversity of this wild relative of rice may help to uncover its domestication potential, and to exploit its adaptive traits.

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Topic: Rice genome research and evolution

A preliminary survey of Transposable Elements and Structural Variant content in two *Oryza alta* accessions

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Transposable Elements (TEs) and Structural Variants (SVs) are important sources of natural variation which have been often overlooked primarily due to the lack of ultra-high-quality, near-gap-free genome assemblies. We took advantage of the availability of high quality genome assemblies for two accessions (IRGC105145 and IRGC105685) of the CCDD polyploid *Oryza alta* to look into TE and SV abundance and distribution.

We produced a TE library including 6,788 non-redundant entries representative of all major TE classes. It masks 54.8% and 57.8% of the IRGC105145 and IRGC105685, respectively. The phylogenetic analysis of Long Terminal Repeat Retrotransposons (LTR-RTs) identified a large clade consisting of paralogous copies of the Ty3-gypsy element *Atlantys* that mostly amplified after polyploidization and that altogether represents 11.62% of the genome.

To search for SVs, a total of ~ 123 Gbp of PacBio reads from IRGC105685 were mapped onto the IRGC105145 genome assembly and parsed using the tool *SVIM* to identify insertions, deletions and duplications. For IRGC105145 we identified 45,258 insertions (plus 1,711 tandemly duplicated regions) for a total amount of 117,496,821 bp out of 883,586,035 Mbp (13.3% of the entire genome sequence). The frequency of insertions is 51.25 per Mbp. In IRGC 105685 43,845 insertions were identified with a frequency of 49.25 per Mbp. Altogether the vast majority of SV sequence (> 87%) can be associated to TEs. This amount is significantly greater than the average TE content in the entire genome. The length distribution of SVs shows two peaks, a large one at ~ 500 bp corresponding to MITEs, and a second at ~13,800 bp corresponding to CACTA DNA TEs. Phylogenetic analyses of TEs in SVs identified TE groups and families that seemed to be more active than others in generating insertional polymorphisms, in particular were the Ty3-gypsy elements *RIRE2* and *Atlantys*, and a CACTA element.

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Topic: Rice genome research and evolution

The Genomics of Three Underutilized Wild Rice Species

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Rice (*Oryza sativa*) is a staple grain crop for over 50% of the world's population. However, due to the reduction of genetic diversity during breeding, breeding is at a genetic disadvantage when it comes to rice's ability to adapt to a changing environment. To better understand how rice and its wild relatives can thrive under different environmental conditions, and apply this knowledge to improve cultivated rice, the International *Oryza* Map Alignment Project (I-OMAP) initiated an investigation of all 11 genome types of the genus *Oryza*. To accomplish the mission, I-OMAP created a set of platinum standard reference sequences (PSRefSeq) for all known rice species in the *Oryza* genus. Here we present data for three PacBio RSII sequenced and assembled high-quality genomes for three underutilized wild relatives of rice: *O. ridleyi* (HHJJ), *O. schlechteri* (HHKK), and *O. coarctata* (KKLL).

The assembled genomes, with coverage of 50-160x of PacBio RSII sequences, have 1, 9, and 16 gaps and scaffold N50s of 52, 21, and 28Mbp for *O. ridleyi*, *O. coarctata* and *O. schlechteri*, respectively. These high-quality genomes were further annotated using *de novo* predicted and in-house refseq for transposable elements (TE) and illumina short and PacBio long RNA reads for protein-coding gene annotations. *De novo* annotated TE and repeat content are 41, 43 and 67% and average of 63, 59 and 53k protein-coding genes of *O. coarctata*, *O. schlechteri* and *O. ridleyi* genomes, respectively.

These wild polyploid genome assemblies and annotations offer new opportunities to study how the same sub-genome types behave in different polyploid contexts. For example, the KK subgenome is shared with both *O.schlechteri* and *O.coarctatai*, while the HH subgenome is shared by *O.ridleyi* and *O. schlechteri*, thereby creating a resource for evolutionary studies on the effect of polyploidization on genome evolution and natural trait plasticity. These three species can now be further utilized to create a genus-wide pan-genome for the *Oryza* genus.