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ABSTRACT BOOK

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Research advances on Green, Nutritious, and Super Rice (GNSR) at IRRI

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Rice is the cornerstone of global food security, sustaining over half the world's population. With the population growth and changing climatic conditions, food security is under constant threat, along with constraints on resource inputs. Therefore, advancing rice functional genomics has become imperative to develop and deploy climate-resilient, nutritious, and high-yielding rice varieties. The Green Super Rice (GSR) project helped to release and deploy 75 rice varieties in Asia and Africa over 44 million ha cumulatively. These multiple abiotic and biotic stress-tolerant rice varieties helped to provide stable grain yields under fluctuating climatic conditions, especially for resource-poor farmers. We also developed nutrient-use-efficient rice varieties that could save 20% of the nitrogenous fertilizers without losing grain yields in comparison to recommended fertilizer levels. Currently, IRRI has made significant strides in developing new Green Nutritious Rice varieties to address human health and sustainability, aligning with its mission to improve livelihoods, nutrition, and environmental sustainability in rice-based agri-food systems. In our pursuit to systematically breed NUE rice varieties, we identified highly nutritious rice varieties with high Iron (15ppm) and Zinc (35ppm) combined into a single cultivar. We identified several low glycemic index (50-55) GSR cultivars with low chalkiness and high head rice recovery traits. We released a low-glycemic (51.1) high-yielding irrigated rice variety NSIC Rc514 in 2018. Efforts are underway to develop high-yielding, nutritious red pigmented rice varieties suitable for palatable whole grain rice consumption.

Developing low-carbon footprint rice inbreds and hybrids is a priority. Recently, we identified several low-methane-emitting rice inbreds and hybrids under flooded conditions. Mestiso 120 is a high-yielding, early maturing (114d) two-line rice hybrid with 30% lower methane emissions in comparison to best inbred checks. Further, it gave a 15.5% yield advantage over the best hybrid check Mestiso 99. Both wet and dry direct-seeded rice inbreds and hybrids have direct implications on the greenhouse gas emissions (GHGE). IRRI has systematically incorporated several DSR traits into the inbred and hybrid parental lines. IRRI could identify several high-yielding rice hybrids under wet-DSR, where the yields were relatively higher and maturing 10-12 days earlier than in transplanted conditions.

Pathways to foster global research collaborations in rice functional genomics, especially for the GNSR, need to be charted out and discussed in detail. Through shared knowledge, resources, and visions, participants of IRFGS could help to propel GNSR research toward a more food-secure future, demonstrating the power of global unity in rice science.

Genetic regulatory network underlying rice tiller angle

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Rice (*Oryza sativa*) tiller angle is a key component for achieving ideal plant architecture and higher grain yield. However, the molecular mechanism underlying rice tiller angle remains elusive. Our recent studies have identified multiple novel genes (*LAZY2-5*) regulating tiller angle through distinct pathways. LA2 and LA3 function in a starch-statolith-dependent pathway, regulating starch biosynthesis in gravity-sensing tissues to mediate shoot gravitropism and asymmetric auxin distribution acting upstream of LA1. *LA2* encodes a chloroplastic protein interacting with OspPGM, while LA3 is a chloroplast-localized tryptophan-rich protein associated with starch granules. In contrast, LA4, a nuclear RING E3 ligase, acts starch-statolith-independent pathway, regulating shoot gravitropism and auxin transport through the LA1-dependent pathway. Additionally, LA5, an ABCG transporter, interacts with OsPIN3t to control lateral auxin transport and tiller angle via an LA1-independent mechanism. Notably, LA5 shows strong selection during domestication. Together, these findings establish a comprehensive regulatory network for understanding rice tiller angle and offer targets for high-yield rice breeding through architectural optimization.

Keywords: tiller angle; shoot gravitropism; starch; auxin; rice

Large-scale genomic and phenomic analyses of modern cultivars empower future rice breeding design

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Modern cultivated rice plays a pivotal role in global food security. China accounts for nearly 30% of the world's rice production and has bred numerous cultivated varieties over the last decades that are well adapted to diverse growing regions. However, the genomic bases that underlie the phenotypes of modern cultivars are poorly characterized, limiting access to this vast resource for breeding of specialized, regionally adapted cultivars. In this study, we constructed a comprehensive genetic variation map of modern rice using resequencing datasets from 6044 representative cultivars from five major growing regions in China. Genomic and phenotypic analyses of this diversity panel revealed regional preferences for genomic backgrounds and specific traits, such as heading date, biotic/abiotic stress resistance, and grain shape, associated with adaptation to local growing conditions and consumer preferences. We identified 3131 QTLs associated with 53 phenotypes across 212 datasets under different environmental conditions through genome-wide association studies. Notably, we cloned and functionally verified a novel gene related to grain length, *OsGL3.6*. By integrating multiple datasets, we developed RiceAtlas, a versatile multi-scale toolkit for rice breeding design. We rapidly improved the grain shape of the Suigeng4 cultivar using the RiceAtlas breeding design function. These valuable resources enhance our understanding of the adaptability and breeding requirements of modern rice and can facilitate advances in future rice-breeding initiatives.

Keywords: Modern rice cultivar; Genomic bases; Rice-growing region

SP3 and DEP1 Orchestrate Panicle Architecture by Jointly Regulating *APO2* Expression in Rice

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Panicle architecture is largely determined by meristem activity. Our previous study showed that DNA binding with one finger (Dof) transcription factor Short Panicle 3 (SP3) regulates panicle architecture. However, the molecular mechanisms of SP3 controlling panicle architecture remain largely unknown. Here, SP3 is shown to enhance inflorescence meristem (IM) activity. Histological analysis shows that IM size rather than the timing of the meristem transition significantly reduced in SP3 mutants. Several assays reveal that SP3 interacts with the C-terminal cysteine-rich domain of DENSE AND ERECT PANICLE1 (DEP1), a class C Gγ subunit, thereby regulating its plasma membrane–nucleus shuttle. SP3 directly binds to the cis element (A/T)AAAG located within the -561 to -517 bp region upstream of the ABERRANT PANICLE ORGANIZATION2 (APO2) promoter and activates APO2 expression, a positive regulator of panicle size. Genetic analysis indicates that APO2 functions downstream of SP3 to promote panicle branching. Additionally, loss of function of DEP1 increases APO2 expression and spikelet density. Transcriptional activity assays show that the interaction between DEP1 and SP3 suppresses SP3-mediated activation on APO2. Altogether, this study uncovers a transcriptional regulatory mechanism involving SP3 and DEP1 in controlling APO2 expression, offering new insights into the genetic network underlying rice panicle development.

Keywords: Panicle architecture; meristem activity; Dof transcription factor; protein interaction; APO2 expression

Impact of rice OsUBC12 on low-temperature germination and its application to breeding

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Low-temperature germination (LTG) is an important agronomic trait for rice (*Oryza sativa*). Japonica rice generally has greater capacity for germination at low temperatures than the indica subpopulation. However, the genetic basis and molecular mechanisms underlying this complex trait are poorly understood. Here, we report that *OsUBC12*, encoding an E2 ubiquitin-conjugating enzyme, increases low-temperature germinability in japonica, owing to a transposon insertion in its promoter enhancing its expression. Natural variation analysis reveals that transposon insertion in the *OsUBC12* promoter mainly occurs in the japonica lineage. The variation detected in eight representative two-line male sterile lines suggests the existence of this allele introgression by indica-japonica hybridization breeding, and varieties carrying the japonica *OsUBC12* locus (transposon insertion) have higher low-temperature germinability than varieties without the locus. Further molecular analysis shows that OsUBC12 negatively regulate ABA signaling. OsUBC12-regulated seed germination and ABA signaling mainly depend on a conserved active site required for ubiquitin-conjugating enzyme activity. Furthermore, OsUBC12 directly associates with rice SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE1.1 (OsSnRK1.1), promoting its degradation. OsSnRK1.1 inhibits LTG by enhancing ABA signaling and acts downstream of OsUBC12. These findings shed light on the underlying mechanisms of UBC12 regulating LTG and provide genetic reference points for improving LTG in indica rice.

Keywords: Low-temperature germination; OsUBC12; transposon; ABA signaling

Cytoplasmic RNA granule to regulate the meiosis entry in proper time in rice

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Reproductive processes, including meiosis, are vulnerable to environmental changes such as high and low temperatures, and research on this process will lead to developing new varieties resilient to global climate changes. In sexually reproducing organisms, meiotic cell cycle is strictly regulated, and in most angiosperms, temporal arrest and resumption of meiosis occur during premeiotic interphase. Establisment of synchronized male meiosis among pollen mother cells (PMCs) in the anther also relies on this system.

Rice MEIOSIS ARRESTED AT LEPTOTENE2 (MEL2) is an RNA-binding protein that forms RNA-protein granules in the cytoplasm of male and female spore mother cells just before meiosis. Intriguingly, the MEL2 granule is totally disassembled during premeiosis-to-meiosis transition. In the mel2 knocked-out mutant anther, a synchrony of premeiotic DNA replication and subsequent meiosis are disrupted, and a subset of cells aberrantly underwent additional round of mitosis in place of meiosis, eventually resulting in all PMCs dead during prophase I. These observations clearly indicate the MEL2 roles in meiotic cell cycle control during premeiosis-meiosis transition in rice.

We found that the expression of rice GLUCAN SYNTHASE-LIKE5 (OsGSL5), a callose synthase, was under the control by MEL2. From the analyses of Osgsl5 mutants, we concluded that premeiotic hyper callose deposition is indispensable for faithful male-meiosis progression probably via modulating the frequency of plasmodesmata connecting PMCs and tapetal cells. Here we will propose the importance of MEL2-driven premeiotic arrest/resumption of the meiotic cell cycle, coupled with dynamic reprogramming of the meiocyte-wall composition, in proper premeiosis-meiosis transition in plants.

Keywords: reproduction; meiosis; cell cycle control; sterility; RNA granule

Genetic Basis of Heterosis Unraveled Using Different-Scale F2 Populations Derived from Elite Hybrid Rice Shanyou 63

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The utilization of heterosis has significantly improved the yield of hybrid rice, making a substantial contribution to global food security. Shanyou 63, the most widely cultivated hybrid rice in China, serves as a representative model for studying heterosis in hybrid rice. However, the genetic basis of heterosis involves multi-locus interactions, and its phenotypic regulatory mechanisms are complex. To elucidate the genetic mechanisms underlying heterosis in spikelets per panicle, we conducted a genetic analysis using a small-scale F2 population (156 individuals) derived from Shanyou 63. Quantitative trait locus (QTL) mapping identified eight QTLs associated with spikelets per panicle. Transcriptomic analysis revealed that coordinated regulation of the brassinosteroid (BR) signaling pathway and flowering pathway enhances the spikelets per panicle phenotype in SY63.Subsequently, to better clarify the genetic interaction model for heterosis in spikelets per panicle in Shanyou 63, we established a larger-scale F2 population (5,000 individuals) for genetic interaction analysis. The results revealed that, compared to the small-scale F2 population, more QTLs associated with spikelets per panicle were identified (16 in total). Among these, known genes such as *Ghd7*, *Ghd7.1*, and *FTL1,* as well as a QTL containing an unknown gene (bin1815), not only play critical roles in heterosis for spikelets per panicle but also exhibit more complex genetic interactions. These findings demonstrate that these key heterotic loci are of great significance for rice heterosis and provide valuable insights for future genetic improvement in hybrid rice breeding.

Keywords: heterosis; hybrid rice; large-scale F2 population; quantitative trait locus (QTL); spikelets per panicle

Grain length regulation by BR signaling network in rice

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Grain length influences grain weight and appearance quality. In our previous work, we identified qGL3, a major quantitative trait locus (QTL) controlling grain length in rice (Oryza sativa). Although qGL3 is homologous to Arabidopsis BSU1, which acts as a positive regulator of brassinosteroid (BR) signaling, qGL3 functions as a negative regulator of BR responses in rice. Through biochemical and genetic analyses, we elucidated the qGL3-OsGSK3-OsBZR1 module governing BR signaling and grain length. Phosphoproteomics, transcriptome sequencing, and protein-protein interaction studies further uncovered a sophisticated BR signaling network. Within this network, we demonstrated that a WD40 protein and a 14-3-3 protein modulate the subcellular localization and stability of qGL3 and OsBZR1, respectively. These interactions collectively fine-tune BR signaling to determine grain morphology. To facilitate future research, we established the BR-associated Regulatory Network Database (RiceBRnetwork, http://cbi.njau.edu.cn/RiceBRnetwork), a comprehensive resource for BR signaling components in rice.

Keywords: Grain length; Brassinosteroids; RiceBRnetwork

Genetic Dissection of Inter-subspecies Hybrid Sterility in Rice: Population Specificity and Novel Fertility Variants

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Inter-subspecies hybrids between *Xian* and *Geng* rice have strong heterosis. However, inter-subspecies heterosis has not been widely exploited due to low fertility in hybrids caused by reproduction isolation. The genetic basis of hybrid sterility remains underexplored. In this study, four sets of reciprocal BC_1F_1 populations, each comprising approximately 600 individuals, were constructed using six inbred lines derived from different *Xian*-*Geng* subgroups. High-quality genomes of the six parental lines were assembled, with functional alleles exceeding 59.6% between each pair. Genome-wide analysis revealed segregation distortion regions exceeding 17.6 Mb. A total of 19 QTLs for pollen fertility and 22 QTLs for seed-setting rate were detected, with several known hybrid fertility genes located within confidence intervals. Novel functional variants were identified in fertility-related genes including *pf12*, *Sa*, *Sc*, and *HSA1*. A new seed-setting rate QTL, *SR8*, was stably detected in two populations over two years. Some hybrid sterility QTLs were specially mapped in some populations. QTLs *f5*, *SR8*, and *SR10.3* were mapped in the cross between X1A and TRJ. QTLs *f5*, *pf12*, *SR6.1*, and *SR8* were the main actors in the crosses involving X1A or TEJ. *Sa*, *SR6.1*, and *SR1.1* were the core determinants in X1B and TEJ. Notably, hybrid fertility was mainly governed by *f5*, *DPL1*/*DPL2* and *S9* in the Aus-Basmati hybrid system. Genetic effect evaluation demonstrated that pyramiding three key compatible alleles in the X1A or TEJ cross populations increased the fertility rate by 20% under *S5-n* compatibility. These findings suggest an effective strategy for solving fertility issues in inter-subspecific hybrid.

Keywords: Inter-subspecies hybrid; Backcross populations; Hybrid fertility; seed-setting rate; QTL

Epigenetic Modulation of Gravitropic Response by TAC5 Controls Tiller Angle in Rice

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Tiller angle is a major component of rice plant architecture and affects planting density, photosynthetic efficiency, and ventilation. An extremely narrow or wide tiller angle adversely affects rice yield. Thus, a suitable tiller angle is considered a major factor to achieve ideal plant architecture in rice. In this study, we identified a major quantitative trait locus (QTL) that controls tiller angle and cloned the gene, *TILLER ANGLE CONTROL 5 (TAC5)*, which encodes a NAC domain-containing transcription factor. Epigenetic variants at the CG site in the *TAC5* promoter were stably inherited and associated with *TAC5* mRNA expression. The *TAC5* epiallele with a hypermethylated cytosine in the promoter exhibited an immediate response to gravistimulation with a simultaneous elevation of H_2O_2 levels at the early stage of gravistimulation. Furthermore, *TAC5* affected the expression patterns of transcripts involved in reactive oxygen species (ROS) generation and the response to excessive ROS. Population genetics and evolutionary analyses revealed that *TAC5* alleles for the narrow tiller angle originated from a wild progenitor and were selected independently in temperate japonica and indica subspecies during domestication. Our results provide insight into the genetic mechanism of tiller angle control in rice and suggest potential applications of *TAC5* in developing rice varieties with an ideal plant architecture.

Keywords: Tiller angle; Gravitropism; Quantitative trait loci; Rice; Natural variation; Methylation

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OPTIMIZING SYNTHETIC APOMIXIS IN HYBRID RICE

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Rice engineering for synthetic apomixis enables clonal propagation of F1 hybrids through seed, offering a promising strategy to reach small farmers' fields with enhanced yield level and stability. The most efficient method to date for converting rice hybrids to apomixis combines the induction of the Mitosis instead of Meiosis (MiMe) triple mutation for apomeiosis (production of unrecombined and unreduced gametes) (1) and the egg cell-specific expression of BABYBOOM1 transcription factor to trigger parthenogenesis (2). However, production of clonal seeds at high frequencies (95-100%) can be associated with a reduced grain filling rate (3). To address this bottleneck, we implemented a large-scale imaging screen of ovaries in fully penetrant apomictic hybrids. We observe that most pre-anthesis ovaries contain 16 to 64 cell parthenogenetic embryos. This suggests that, in many of the unfilled grains, polar nuclei fertilization might be hampered by too-early parthenogenesis and that a finer tuning of parthenogenesis induction could improve fertility. We also investigated whether synthetic apomixis can be applied to indica/japonica hybrids, which benefit from even higher heterosis (@30%) than intra-subspecific hybrids, but are generally only partially fertile (4). We show in two indica/japonica F1 hybrids exhibiting an initial average grain filling of 5.5% and 55% that apomeiosis restores near-full and full fertility respectively. Next, combining apomeiosis with a moderate frequency of parthenogenesis resulting in partial synthetic apomixis, we confirm the maintenance of the high level of fertility in clonal diploid progenies. Altogether, these results open new leads for optimizing clonal reproduction through seeds of distant hybrids with high level of heterosis.

Keywords: embryo sac; F1 hybrids; imaging; indica/japonica; parthenogenesis; rice; synthetic apomixis

Role of RALF peptides in rice reproduction

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Pollen tube growth is essential for double fertilization, directly impacting grain yield in crop plants. Rapid Alkalinization Factor (RALF) peptides act as ligands for signal transduction during fertilization. We previously identified 41 RALF peptides and characterized OsRALF17 and OsRALF19, which are specifically expressed in pollen. Double mutants of OsRALF17 and OsRALF19 exhibited defects in pollen hydration, germination, and elongation, resulting in male sterility. Treatment with mature peptides (OsRALF17M and OsRALF19M) on wild-type pollen showed a dose-dependent regulation of pollen tube length, with high concentrations delaying pollen tube rupture. OsRALF17 and OsRALF19 physically interact with OsMTD2, a member of the CrRLK1L family, to initiate ROS signaling. Additionally, RUPO, another CrRLK1L family member, forms a receptor complex with OsMTD2, OsRALF17, and OsRALF19. For receptor binding, secretion of functional RALF peptides should be occurred, and this processing could be mediated by Site-1 Protease (S1P). Further mechanistic studies on RALF peptide processing by S1P are necessary to deepen our understanding of their regulatory roles in rice pollen tube growth and fertilization.

Keywords: RALF peptide; CrRLK1L receptor; Pollen tube growth; Reproduction

Development of the Short-Duration Rice Variety 'Neuldam' with Enhanced Adaptability for Post-Cash-Crop Double Cropping

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"Neuldam" is a short-duration japonica rice cultivar released by the National Institute of Crop Science, Korea, to secure planting windows for double-cropping after garlic or onion. Derived from 'Koshihikari' × 'Haedamssal', the line was advanced by marker-assisted back-crossing and multilocation selection (2017–2024). In late-planting regional trials at Miryang, Sangju and Jeonju (2022–2024) it headed on 22 Aug, two days earlier and matured four days sooner than the check 'Geumo', completing its growth in 96 days. Culm length (67 cm) lowered the lodging index by 28 %. More panicles per hill (+17 %) contributed to a milled-rice yield of 4.46 t ha^{-1}, 3 % above the check. 'Neuldam' resisted all 29 tested races of blast and showed field resistance to bacterial blight K1–K3, while maintaining intermediate cold tolerance. The viviparous germination rate was 20.9 % versus 36.0 % for 'Geumo', raising head-rice recovery to 64.5 %. Grain appearance, amylose (18.2 %), protein (7.5 %) and sensory quality were comparable to premium checks. These traits enable profitable rice production and reliable grain quality under increasingly erratic late-season weather, while preserving the sowing schedule for subsequent cash crops.

Keywords: rice; early maturity; lodging resistance; viviparous germination tolerance; double cropping

Coordinated upregulation of photosynthetic pathways mediates yield advantage in grain-yield-enhanced rice

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Photosynthesis is a fundamental physiological process driving biomass accumulation and grain yield in crops. Enhancing photosynthetic capacity through genetic improvement has emerged as a promising strategy to boost agricultural productivity. Our previous work demonstrated that overexpression of the transcription factor *OsDREB1C*, which coordinately regulates photosynthesis, nitrogen utilization, and flowering, resulted in up to a 30% increase in rice yield with a shortened growth duration. In this study, we investigated previously characterized grain-yield-enhanced (GYE) rice lines, that harbor elite alleles of key regulatory genes such as *IPA1/OsSPL14*, *NRT1.1B*, and *OsGRF4*. While these lines exhibited improved yield performance, the underlying photosynthetic and regulatory mechanisms remained unclear. To elucidate the basis of enhanced yield in GYE rice, we performed comprehensive analyses of photosynthetic physiology, photosynthate production and transport, and transcriptomic profiles in GYE lines and their corresponding controls. Field experiments revealed that GYE lines consistently exhibit higher photosynthetic rates and grain yields. These improvements were associated with increased chlorophyll content, elevated levels of photosynthesis-related proteins, higher stomatal density and chloroplast numbers, and enlarged chloroplast area, indicating enhanced light capture and carbon assimilation. Transcriptomic analysis further revealed coordinated upregulation of genes involved in chlorophyll biosynthesis, electron transport, carbon fixation, stomatal development, sugar metabolism, assimilate transport, and nitrogen utilization. Collectively, these physiological and molecular enhancements contribute to the superior photosynthetic capacity and grain yield observed in GYE rice. Our findings highlight the central role of enhanced photosynthesis in the yield advantage of GYE rice, and provide valuable targets for crop improvement through photosynthetic optimization.

Keywords: Photosynthesis, grain yield, GYE, genetic improvement, rice

Construction of an Indica-Japonica SSSL Platform for Elucidating the Yield-Increasing Mechanism of *qRBG1/OSBZR5* in Rice

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Significant heterosis exists in crosses between *Indica* and *Japonica* rice subspecies. However, hybrid sterility or reduced seed setting poses a bottleneck for further yield improvement through this heterosis. This challenge can be resolved by constructing single-segment substitution lines (SSSLs) with subspecies-specific compatibility. SSSLs has become an ideal genetic breeding material that integrates both functional gene identification and molecular design breeding. We developed two sets of *Indica-Japonica* specific compatibility SSSLs, including 180 *Indica*-compatible *Japonica* SSSLs in the Nipponbare background with substitution segments from *Indica* restorer lines Xihui 18 etc., and 149 *Japonica*-compatible *Indica* SSSLs in Xihui 18 background with substitution segments from *Japonica* cultivar Huhan 3. Based on a SSSL-Z499, a key grain type differentiation gene (*qRBG1/OsBZR5*) was isolated. We report characterization of the *Rice Big Grain 1* (*qRBG1*). Our data show that $qRBG1^{Z}$ is an unselected rare promoter variation that reduces *qRBG1* expression to increase cell number and size, resulting in larger grains, whereas *qRBG1* overexpression causes smaller grains in recipient Nipponbare. We demonstrate that *qRBG1* encodes a non-canonical *BES1*/*BZR1* family member, *OsBZR5*, that regulates grain size upon phosphorylation by OsGSK2 and binding to *D2*and *OFP1* promoters. qRBG1 interacts with OsBZR1 to synergistically repress *D2*, and to antagonistically mediate *OFP1* for grain size. *OsBZR5* is distinct from *OsBZR1-OsBZR4* in rice, which lacks three important conserved domains and negatively regulates rice grain size, while *OsBZR1-OsBZR4* positively regulate rice grain size. Our results reveal regulatory network controlling grain size via *OsGSK2–qRBG1–OsBZR1–D2–OFP1* module, providing a target for improving rice yield.

Keywords: Rice; Grain size: *qRBG1/OsBZR5*; Brassinosteroid; Single segment substitution line

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Polysaccharide Heterogeneity Sustains Xylem Wall Coherence and Grain Yield

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Plant cell functionalities usually depend on the encased walls with complexity and heterogeneity. Plants have evolved a sophisticated conduit system with innovative wall structures to efficiently transport water and nutrients, which is also a key target for crop domestication and trait improvement. The xylem vessel transport of water and nutrients from the roots to the canopy is driven by transpiration, which inevitably creates enormous negative pressure on vessel walls. Vessel pits, the fine three-dimensional (3D) cavity in conduit wall, are key determinants of plant hydraulics and growth plasticity. However, the ultrastructure and formation mechanism of vessel pits remain poorly understood. We recently revealed the nanoscale 3D structure of vessel pits using volume electron microscopy and outlined a molecular pathway that mediates pit shaping, ensuring xylem robustness and promoting grain yield in rice. Through a genome-wide association study, a quantitative trait locus for pit size control, was identified to encode a polysaccharide modifier responsible for regulating pit structure. The elite QTL variant alters xylan modification, promoting binding with cellulose and maintaining wall integrity around the pit boundary in a responsive regulation. The elite haplotypes confer rice varieties with enhanced nitrogen utilization and promoted grain yield under varied nitrogen conditions. Our findings uncover a previously unrecognized “quality control” mechanism for shaping pit nanostructures to boost xylem hydraulics and crop yield, offering a promising strategy for sustainable agriculture.

Keywords: Xylan heterogeneity; Xylem vessel; Pit geometry; Hydraulics; Grain yield; Elite haplotype

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A Poaceae-Conserved RALF peptide, OsRALF4, Controls Rice Seed Development through CrRLK1L Signaling

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The coordinated development of the embryo and endosperm is essential for the seed formation in angiosperms. While CrRLK1L receptor kinases are known to regulate plant reproduction, their peptide ligands involved in seed development remain poorly characterized. Here, we identify OsRALF4, a small secreted peptide, as a key regulator of seed development and grain quality in rice (*Oryza sativa*). OsRALF4 is expressed in the early embryonic tissue and endosperm during grain maturation. It localizes to the cell membrane, cytoplasm, and apoplast. Loss of OsRALF4 function leads to abnormal seed formation and altered grain traits, accompanied by elevated reactive oxygen species, particularly during early embryogenesis. Although *OsRALF4* transcripts were also detected in the pistil prior to fertilization, the absence of pre-fertilization defects in mutants suggests functional redundancy at that stage. In contrast, the strong post-fertilization phenotype indicates that OsRALF4 primarily functions in early zygotic tissues. Co-immunoprecipitation assays revealed that OsRALF4 interacts with six CrRLK1L receptors implicated in seed development, supporting its role in a conserved RALF–CrRLK1L signaling module. OsRALF4 is conserved among Poaceae species, highlighting its potential relevance for cereal crop improvement. Our findings establish OsRALF4 as a secreted peptide acting post-fertilization to regulate seed development and grain quality.

Keywords: RALF; CrRLK1L; Seed development; Rice; Oryza sativa

High-Resolution Spatial Transcriptome Atlas of Rice Seeds during Grain Filling

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Rice (*Oryza sativa*) is one of the most important staple crops worldwide. A comprehensive understanding of the spatial regulatory networks governing endosperm cell development and differentiation is essential for simultaneously improving grain yield and quality. In this study, we used Stereo-seq to construct a spatial transcriptome atlas of rice seeds during the grain-filling stage, identifying 22 cell types, including one newly defined endosperm cell type in which differential expression analysis revealed specific expression of two sucrose transporter genes, and distinguishing five transcriptionally similar cell types corresponding to starchy and peripheral endosperm. The cell-type classifications were validated using multi-omics in situ pairwise sequencing (Mip-seq). Gene Ontology (GO) analysis revealed distinct enrichment patterns such as serine-type endopeptidase inhibitor activity (GO:0004867), sucrose metabolism (GO:0005985) and nutrient reservoir activity (GO:0045735) among endosperm cell types, suggesting coordinated roles in grain development and filling. Based on cell-type-specific gene expression dynamics, we discovered a spatial developmental trajectory from subaleurone layer cells to the starchy endosperm. Furthermore, we identified key genes involved in the biosynthesis and accumulation of starch and protein, the two major storage compounds in rice endosperm, as well as genes participating in phytohormone signaling pathways. In summary, this study not only deepens our understanding of the differentiation and functions of rice endosperm cell types but also provides novel insights for the coordinated improvement of grain yield and quality.

Keywords: rice seed; spatial transcriptome atlas; cell trajectory

Genetic mechanisms governing source-sink coordination for enhanced yield and quality in cereal crops

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Rice endosperm constitutes the world's primary source of human nutrition and animal feed, and starch serves as the dominant storage carbohydrate, comprising the bulk of the endosperm biomass and serving as the key determinant of both grain yield and quality. Generally, cereals yield is determined mainly by sucrose (photoassimilates), sink (the storage of photoassimilates), and flow (photoassimilates allocation from source to sink). Therefore, source-sink coordination is of particular significance for cereal crops yield and quality, which plays a pivotal role in mining yield potential and genetic improvement of crops. Recently, we focus on the genetic regulation of grain filling and photosynthesis in rice, and has identified several important genes, GAF1, OsABCI15/16, GIF10 and etc., are critically responsible for grain filling. Particularly, we revealed that GAF1 plays vital roles in the synergetic regulation of phosphate-limited photosynthesis and grain filling by regulating dynamic phosphate homeostasis thereby functions as a core source-sink coordination factor in rice for the first time. Furthermore, a high-yield and high-quality breeding model with coordinately enhanced high photosynthetic efficiency and grain filling has been successfully raised in crops based on above key source-sink regulators. We found that genetic co-improvement of these genes leads to markedly increased photosynthetic efficiency, optimized carbon partitioning, enhanced grain-filling, and improved sugar metabolism. These synergistic effects contribute to 20%~30% cereal yield increase, accompanied by superior agronomic traits including greater biomass, disease resistance, PUE, and harvest index.

Keywords: Source-sink; coordination; grain filling; photosynthesis; phosphate use efficiency

Integrative Strategies for Functional Rice Flour Development: From Grain Texture to Nutritional Enhancement

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To expand rice utilization, we developed dry-millable rice lines through genetic dissection of endosperm traits. CRISPR/Cas9-based mutants of OsF2KP2 and GLB1 exhibited opaque or altered endosperm with reduced grain hardness, modified starch granules, and improved milling properties. These flour-type traits were achieved without compromising overall plant growth. Building on this platform, we introduced functional traits by activating Nicotianamine synthase genes, increasing nicotianamine and 2′-deoxymugineic acid levels and enhancing iron and zinc accumulation. Additionally, lines with reduced phytic acid and altered endosperm structure showed enhanced bioavailable forms of essential minerals and flour characteristics. This integrative approach offers a promising strategy to develop nutritionally enriched rice flour for health-conscious and diversified food applications.

Keywords: rice flour; nicotianamine; mineral; nutritional enhancement

Toward Breeding Low GI Rice: Genetics & Engineering

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The pursuit of high quality is a vital direction in rice genetic improvement. Developing high-quality, healthy rice varieties with slow digestion and a low glycemic index (GI) is not only an important objective of rice genetic improvement but also crucial for ensuring a healthy diet for the population. In this study, we analyzed the physicochemical properties and digestion characteristics of 562 global rice germplasms and found that the digestion rate of rice is primarily associated with starch-related physicochemical indicators. Based on these results, we conducted a genome-wide association study between key digestion indicators of rice and resequencing data from related germplasms, identifying multiple loci that influence the digestion rate of rice. Among these, the effects of the *Wx* and *RSR1* loci were validated using various genetic materials. Furthermore, this study elucidated the enzymatic mechanisms, structural basis, and functional characteristics underlying the formation of high resistant starch (RS) in rice endosperm through genetic modification of starch biosynthesis. We successively developed three generations of molecular pathways for the creation of high-RS rice and generated a series of new rice germplasms with high RS content, slow digestion, and low GI.

Keywords: Grain quality; Digestion rate; Glycemic index; Resistant starch; Starch biosynthesis

A cooking and eating quality evaluating system for whole grain black rice

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Black rice has a long history of cultivation in Asia especially China. As a whole grain, black rice is rich in diverse nutrients including proteins, vitamins, amino acids, minerals, unsaturated fatty acids, dietary fibers, alkaloids, carotenes, phenolic compounds, and anthocyanins, in addition to starch. Many studies have demonstrated a range of health-promoting effects by black rice, which has greatly attracted the attention of consumers. However, the production and consumption of black rice has been low mostly because of its poor cooking and eating quality. To address this problem, the first is a need for technology to evaluate the cooking and eating quality of black rice. In this study, we investigated the feasibility of using Rice Taste Evaluation System (RTES) as a proxy approach to eating and cooking quality evaluation of whole grain black rice (WGBR). Totally, 775 black rice samples obtained from 363 accessions harvested from field planting were evaluated both with sensory evaluation by panelists and with RTES consisting of a cooked rice taste analyzer and a hardness and stickiness meter, which produced 8 characteristic parameters. We obtained highly significant correlation (R^2=0.867, $P<2.2\times10^{-16}$) between sensory test scores and RTES values by multiple linear regression equation based on the selected variables, which was validated with just as high correlation, indicating that the RTES can provide equivalent results the sensory test. With the efficiency of this equipment, the RTES can provide a convenient and accurate tool for high throughput evaluation of cooking and eating quality of WGBR for breeding and other usages.

Keywords: cooking and eating quality; whole grain black rice; rice taste evaluation system; sensory test; regression equation

Developing Green Nutritious Super Rice for a healthy Anthropocene

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Rice is a mainstay of food security for more than half of the global population. For thousands of years, rice as a staple food has provided mainly calories for the human population, and it has been produced with heavy resources and environmental costs, especially in recent decades. Based on the development of Green Super Rice (GSR), which aims to greatly reduce pesticides, fertilizers, irrigation and other resources in rice production while providing high yield and quality, we propose to modify the deeply rooted conventions by developing Green Nutritious Super Rice (GNSR) to enhance human nutrition and health. In this presentation, we will outline the concept, goals, and potential significance, and suggest a technical strategy for developing GNSR assuming the prototype of whole grain black rice (WGBR). We will also present some of the progress in such development.

Future Directions for Biofortification

Howarth Bouis

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The 20+ years of experience of developing and deploying biofortified crops globally in Low-and-Middle Income Countries (LMICs) through the HarvestPlus program has shown that biofortification can be an efficacious and cost-effective intervention in the fight against mineral and vitamin deficiencies. However, the impact of biofortification has been significantly limited by increasing the density of only one single nutrient per crop using conventional plant breeding techniques. Progress has been slow in increasing the density of these single nutrients.

There are two future strategies which can address the single-nutrient constraint. And these strategies may reinforce one another, multiplying their effects.

The first strategy is to use transgenic and other more advanced crop development techniques. A "three-nutrient-in one" high-yielding transgenic rice has been developed at the International Rice Research in the Philippines, although it is not yet de-regulated in any country. If substituted one-for-one with non-biofortified rice in the Philippines – at no extra cost to consumer food budgets – the vitamin A intakes of the bottom 40% of the income distribution can be doubled. For the whole population on average, zinc intakes will be more than doubled and iron intakes will be increased by 25%. These increases in nutrient intake are based on nutrient densities in milled rice.

The second strategy is to encourage a switch from milled rice to wholegrain rice. Densities of a range of mineral and vitamins tend to be relatively high in the outer bran layers. Indeed, the EAT-Lancet dietary recommendations published by Willet et al., (2019) argues strongly for switching from milled grain to the consumption of wholegrains. However, wholegrain rice in the Philippines is sold for a higher price than milled rice, making it difficult especially for low-income households to make the switch.

De-husking of rice starts a process of oxidation and degradation of unsaturated fatty acids in the bran layer at room temperatures, which results in an unacceptable rancid taste within less than two months after de-husking. Thus, the higher cost of storage, packaging, and marketing is the underlying cause of the higher price of wholegrain rice.

Importantly, there are several cost-saving advantages of processing/marketing unpolished rice over milled rice (lower cost of milling, no broken rice, higher economic value of bran for human consumption than as animal feed). Thus, if rancidity can be controlled through a genetic solution, there is the prospect that unpolished rice can be sold for a significantly lower price

than milled rice. Such a price incentive could drive a switch to wholegrain rice – in particular, by lower income groups who are most price-sensitive and who would benefit most nutritionally from such a switch.

It is critical to explore genetic solutions through plant breeding to control rancidity and so to lengthen the shelf-life of unpolished rice at no extra cost. This is a key step to increasing the feasibility of brown rice consumption. Further, there is a need to ensure that the unpolished rice does not contain harmful levels of heavy metals, as these can accumulate in the bran layer following exposure to degraded soils.

It can be expected that the densities of vitamin A, iron, and zinc in “three-in-one rice” in the bran layer will be exceptionally high.

Rice transcription factor bHLH25 confers resistance to multiple diseases by sensing H_2O_2

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Hydrogen peroxide (H_2O_2) is a ubiquitous signal regulating many biological processes, including innate immunity, in all eukaryotes. However, no transcription factors have been reported to directly sense H_2O_2 in eukaryotes. Here, we report that rice basic/helix-loop-helix transcription factor bHLH25 directly senses H_2O_2 to confer resistance to multiple diseases caused by fungi or bacteria. Upon pathogen attack, rice plants promote production of H_2O_2, which directly oxidizes bHLH25 at methionine 256 in the nucleus. Oxidized bHLH25 represses *miR397b* expression to activate lignin biosynthesis for plant cell wall reinforcement, preventing pathogens from penetrating plant cells. Lignin biosynthesis consumes H_2O_2 causing accumulation of non-oxidized bHLH25. Non-oxidized bHLH25 switches to promote expression of *Copalyl Diphosphate Synthase 2* (*CPS2*), which increases phytoalexin biosynthesis to inhibit expansion of pathogens that have escaped into plants. This oxidization/non-oxidation status change of bHLH25 allows plants to maintain H_2O_2, lignin and phytoalexin at optimized levels to effectively fight against pathogens and prevents these three molecules from over-accumulation that harms plants. Thus, our discovery reveals a novel mechanism for a single protein to promote two independent defense pathways against pathogens. Importantly, the bHLH25 orthologues from available plant genomes all contain a conserved M256-like methionine suggesting the broad existence of this mechanism in the plant kingdom. Moreover, this Met-oxidation mechanism may also be employed by other eukaryotic transcription factors to sense H2O2 to change functions.

Keywords: Hydrogen peroxide, Immunity, Disease resistance, Blast, Rice

Phytic acid with a direct anti-blast fungus effect can be developed into a nanomaterial controlling rice diseases

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Phytic acid (PA) is the main storage form of phosphorus in crop seeds and can function in plant responses to stresses. As a natural molecule, PA is generally recognized as safe, and has been used to inhibit common foodborne bacterial pathogens growth. However, the direct role of plant endogenous PA in restricting crop pathogens growth is ambiguous. Whether PA is suitable for development as an anti-plant pathogens nanomaterial remains unclear. Herein, we found that the rice blast fungus (*Magnaporthe oryzae*) inoculation increased PA level in rice. Knockout of OsITPK6, a key kinase involved in PA biosynthesis, caused reduction of endogenous PA and compromised rice defensive responses to *M. oryzae* infection. Treatment with a relatively high concentration of PA inhibited *M. oryzae* vegetative growth by inducing depolarization of mycelial cells, indicating PA's direct role in anti-rice blast fungus. To increase PA's permeability into *M. oryzae* cells, we developed carbon-dot nanoparticles polymerizing PA (PCDs), which with an average 3 nm diameter are water-soluble and emit autofluorescence. The nanoparticles target M. oryzae F-actin, depolymerize actin filament in vitro and exhibit higher efficiency in depolarizing M. oryzae mycelial cells. PCDs application disrupted M. oryzae appressoria formation and protectively mitigated rice blast disease incidence. Moreover, compared with PA, PCDs also show strong abilities in inhibiting blight bacterium growth by disrupting the cell membrane's integrity and protecting the rice from *Xanthomonas oryzae* pv. *oryzae* invasion. Thus, PCDs efficiently inhibit various rice pathogens and can act as a broad-spectrum protecting agent to control rice diseases.

Keywords: phytic acid; rice resistance; carbon dots; F-actin; cell membrane integrity; diseases control

Identification of SBRR1-R with high potential in rice breeding against sheath blight

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Sheath blight (ShB), caused by necrotrophic fungus Rhizoctonia solani, is one of the most serious rice diseases worldwide. To the best of our knowledge, no genes with high potential for rice ShB resistance breeding have been previously characterized. Here we identify a ShB resistance receptor-like kinase 1 (SBRR1) gene via a genome-wide association study. The SBRR1-R elite allele, containing a 256-bp insertion in its promoter, is preferentially present in indica varieties in geographical regions with highly favorable conditions for ShB development. Introduction of SBRR1-R into a commercial japonica rice variety significantly reduces yield loss under severe ShB disease pressure. Transcription factor bHLH57 specifically binds to the 256-bp sequence and accounts for highly induced expression and stronger resistance of SBRR1-R. Localization of SBRR1 on plasma membrane, aided by SBRR1-interaction-protein 1, and phosphorylation of SBRR1 are required for SBRR1 to rapidly upregulate downstream chitinase genes for resistance. These findings offer mechanistical insights into ShB resistance hidden in natural rice varieties.

Keywords: rice; sheath blight; resistance gene; breeding potential

Perception of viral infections and initiation of antiviral defence in rice

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Crop production faces persistent threats from insect-vector-borne viral diseases[1,2]. Recent advancements have revealed the intricate immune mechanisms that plants deploy against viral pathogens[3–8.] However, the molecular mechanisms through which plant hosts recognize viral infections and initiate antiviral defence at disease onset have not been elucidated. Here, through the natural infection of rice by inoculation with insect vectors carrying the natural forms of viruses, we show that viral coat proteins are perceived by the RING1–IBR–RING2-type ubiquitin ligase (RBRL), initiating the first step of the natural antiviral response in rice. RBRL subsequently targets an adaptor protein of the transcriptional repression complex of the jasmonate pathway, NOVEL INTERACTOR OF JAZ 3 (NINJA3), for degradation through the ubiquitination system, inducing jasmonate signalling and activating downstream antiviral defence. We further show that this phenomenon is a universal molecular mechanism used by rice plants to perceive viral infections and initiate antiviral signalling cascades. This approach is important not only for obtaining a deeper understanding of virus–host interactions but also for further disease resistance breeding.

Keywords: Rice stripe virus; RBR-type ubiquitin ligase; Viral sensor

The selective autophagy in rice blast disease resistance

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Autophagy is an evolutionarily conserved biological process that plays essential roles in plant growth, development, and stress responses. Pathogens secrete various effectors that interfere with the plant immune response during infection; however, their roles in modulating host autophagy remain largely unknown. Here, we found that Magnaporthe oryzae infection induced marked peroxisome degradation via pexophagy, primarily attributable to chitin/OsCERK1-mediated immune activation. Notably, pexophagy contributes to disease resistance against M. oryzae in rice, and OsNBR1, a peroxisome-located protein, likely functions as an autophagy receptor to initiate this process. During M. oryzae infection, OsNBR1 interacted with OsATG8 to target the cargo protein OsPEX5 for autophagic degradation. OsPEX5 acts as a negative regulator of jasmonic acid (JA) biosynthesis. The degradation of OsPEX5 promoted JA accumulation, thereby subsequently enhancing disease resistance to M. oryzae in rice. However, we identified an effector protein, MoHP52, in M. oryzae that hindered rice cell pexophagy by disrupting the interaction between OsNBR1 and OsATG8, thereby subverting the host immune response. Therefore, we demonstrated the critical role of peroxisomes in plant immune responses and propose that peroxisomes may represent a major virulence target of pathogens.

Keywords: Rice; *M. oryzae*; autophage; peroxisome; OsPEX5

Exploring Immune Regulators to Coordinate Growth and Disease Resistance in Rice

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Rice production faces threatens from various devastating diseases such as rice blast and false smut. Utilizing resistant varieties represents the most cost-effective and environmentally friendly way to control these diseases. A successful resistant variety requires overcoming "resistance cost" associated with immune activation and it may suddenly lose resistance after several years of cultivation. Therefore, we aimed to address "why do resistant varieties lose resistance" and "how can effective resistant varieties overcome the resistance cost" and found that co-evolution of rice with *Magnaporthe oryzae* shapes the dynamic interaction between the avirulence genes in *M. oryzae* and the resistance genes in rice varieties, which determine the epidemics of blast disease in a specific rice growing area. Natural variation and the loss of avirulence genes in *M. oryzae* population lead to the loss of disease resistance in rice varieties carrying the corresponding resistance genes. Moreover, a few immune regulators promote disease resistance without yield penalty. Whereas the proteasome maturation factor UMP promotes the activity of 26S proteasome to degrade preferentially reactive oxygen scavenging enzymes and the accumulation of hydrogen peroxide, leading to resistance to multiple diseases, multiple AGO1-associated microRNAs respond to *M. oryzae* and were simultaneously involved in regulating yield traits and immunity. Chitin-triggered immunity and OsRACK1A-mediated defense are crucial for developing resistance to rice false smut disease. In summary, UMP1, OsRACK1A and AGO1 play crucial roles in coordinating growth and disease resistance, overcoming the "resistance cost" and serve as the key gene resources for raising high-yield resistance varieties to control rice diseases.

Keywords: Resistance cost; UMP1; OsRACK1A; AGO1; microRNA; rice blast

Exploring the Mechanism of Ubiquitin E3 Ligase-Mediated Immunity in Rice Against *Magnaporthe oryzae*

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The ubiquitin proteasome system (UPS) plays a crucial role in the interaction between rice and *Magnaporthe oryzae*. However, the function of E3 ubiquitin ligases in rice's immune response to *M. oryzae* is not well understood. In our study, we identified that the *M. oryzae* effector AvrPiz-t targets the rice RING-type E3 ligases APIP6 and APIP10 to suppress rice immunity. Specifically, APIP6 promotes the degradation of OsELF3-2, while APIP10 facilitates the degradation of OsVOZ1/2, both of which negatively regulate Piz-t-mediated resistance. Identifying the substrates of E3 ligases is usually challenging. To efficiently identify these E3 ligases based on hypothetical ubiquitinated proteins, we constructed the rice E3 ligases ORFeome (UbE3) library through PCR amplification and gene synthesis. Utilizing this UbE3 library, we efficiently discovered that members of the phenylalanine ammonia lyase family (OsPALs) are targeted by the F-box type E3 ligase OsFBK16, the transcription factor APIP5 is regulated by the RING E3 ubiquitin ligase OsRING113, and the COP9 signalosome subunit 5 (CSN5) is targeted by the U-box type E3 ligase OsPUB45 in rice immunity. Collectively, these findings highlight the importance of the UbE3 library and reveal novel mechanisms of homeostasis regulation involving the ubiquitin modification of key disease resistance proteins in rice.

Keywords: rice; *Magnaporthe oryzae*; ubiquitination; E3 ubiquitin ligase; UbE3

Integrated GWAS and QTL-seq Reveal Key Genes Associated with Brown Spot Resistance in (*Oryza sativa* L.)

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Brown spot (BS), caused by Cochliobolus miyabeanus, significantly reduces both yield and grain quality in rice (*Oryza sativa* L.), particularly in rainfed and upland production systems. To dissect the genetic basis of BS resistance, we conducted a genome-wide association study (GWAS) using 130 rice cultivars and identified two significant QTLs on chromosome 4. To verify the candidate gene, the indica variety BALA (resistance parent) and the japonica variety Haedamssal (susceptible parent) were selected as parents to generate an F_2 population consisting of 194 individuals. Phenotypic evaluation revealed a near-normal distribution of disease scores: 10 individuals scored 1, 15 scored 2, 36 scored 3, 50 scored 4, 34 scored 5, 30 scored 6, 10 scored 7, 2 scored 8, and 1 scored 9, with an average score was approximately 4.2 ± 1.60. Based on this distribution, two extreme bulks were constructed for QTL-seq analysis: the resistant bulk (R-bulk) comprising individuals with scores of 1 and 2, and the susceptible bulk (S-bulk) comprising individuals with scores of 8 and 9. Both parents and the bulks were subjected to whole-genome resequencing, and QTL-seq analysis is currently underway to refine candidate regions controlling BS resistance. This study is the first to integrate GWAS, gene expression profiling, and functional validation for BS resistance in rice, laying the groundwork for high-resolution mapping through QTL-seq. Our findings provide novel insights into the genetic architecture of BS resistance and offer promising targets for marker-assisted selection and the development of resistant rice varieties.

Keywords: Rice (*Oryza sativa L.*); Brown Spot (BS); Cochliobolus miyabeanus; GWAS; QTL-seq

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Evaluation of Leaf Blast Resistance in Korean Rice Varieties and Regional Analysis of Major Resistance Genes

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Rice is a major staple food for over half of the world's population. Rice blast, caused by Magnaporthe oryzae, occurs throughout all growth stages and typically causes 10–30% yield loss. Cultivating resistant varieties suited to local blast races is key to minimizing damage. This study evaluated leaf blast resistance in 116 rice varieties developed between 2009 and 2024 across major rice-growing regions in Korea: central (Cheorwon, Jinbu, Suwon, Yeoju, Icheon), Honam (Yesan, Jeonju, Iksan, Naju), and Yeongnam (Milyang, Sangju, Yeongdeok), using upland nursery testing. Varieties were further analyzed by subspecies (japonica-type, Tongil-type) and the presence of major resistance genes using molecular markers. The results revealed substantial variation in resistance levels across varieties and regions. 'Dasan' showed the highest resistance with an average lesion index of 1.3 across all regions, while 'Heuknam' had the highest susceptibility (index 7.3). The central region had the greatest number of resistant varieties (57), followed by Honam (48) and Yeongnam (15). Most early-maturing japonica varieties possessed resistance genes and displayed corresponding phenotypic resistance, while all mid-maturing Tongil-types were resistant. However, in mid-to-late maturing japonica varieties, a partial inconsistency was noted between gene presence and observed resistance levels, suggesting potential interactions with environmental or pathogen race factors. Among the tested genes, Piz-5, Piz-t, and Pita consistently conferred strong resistance across all regions. These findings emphasize the importance of considering both genotypic and regional phenotypic data when selecting varieties and can inform future breeding and disease management strategies.

Keywords: Rice; Cultivar; Blast; Resistance gene; Regional adaptation

OsWRKY26 Negatively Regulates Rice Bacterial Blight Resistance by Suppressing *OsXa39* Expression

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Plants face numerous infectious diseases with significant epidemic potential. Among them, rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is the most important disease in both temperate and tropical climates. In this study, we identified and characterized the sucrose-inducible transcription factor *OsWRKY26*, which contributes to plant defense responses triggered by *Xoo* infection. The *OsWRKY26* defective mutant plants showed an enhanced defense response to *Xoo*, suggesting it functions as a negative transcription factor regulating plant defense responses. However, there is no difference between the mutant plants and the wild-type (WT) plants when exposed to the rice blast *Magnaporthe oryzae*. Transcriptomic analysis showed that multiple pathogen resistance genes were upregulated in the mutants compared to WT plants. Among them, *OsXa39* was used for further examination. Transient expressions in rice protoplasts indicated that the luciferase reporter gene driven by the *OsXa39* promoter was suppressed by OsWRKY26. Chromatin immunoprecipitation assay showed that OsWRKY26 directly binds to the promoter region of *OsXa39*. Taken together, OsWRKY26 negatively responds to *Xoo* infection by suppressing OsXa39 and other pathogen-related genes, including *OsXa47*, *OsBBR1*, *OsRSR1*, *OsPR1a*, *OsPR1-11*, *OsPR2*, and *OsPR4c*.

Keywords: OsWRKY26; OsXa39; *Xanthomonas oryzae*; disease; rice.

DECODING LONG-DISTANCE IMMUNE SIGNALING OF CHITIN-INDUCED SYSTEMIC RESISTANCE IN RICE

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Plants employ a two-tier immune system comprising local defense and systemic resistance (SR). SR relies on mobile signals to prime immunity at distal sites and is categorized into Systemic Acquired Resistance (shoot-to-shoot) and Induced Systemic Resistance (ISR) (root-to-shoot). ISR is typically triggered by beneficial root-associated microbes. While ISR has been extensively studied in dicots, direct evidence for its existence and mechanisms in monocots such as rice remains limited, mainly due to their complex vasculature and technical challenges in grafting experiments. In this study, we developed a hydroponic rice culture system and employed chitin treatment to induce Chitin-Induced Systemic Resistance (CISR). Time course transcriptome analysis following chitin treatment to the root revealed the existence of systemic root-to-shoot signaling in rice. Our comprehensive screening identified 46 candidate small secreted peptides (SSPs), that were upregulated with defense-related pathways including Mitogen Activated Protein Kinases and key phytohormones associated with ISR. Interestingly, we found some of the SSPs previously characterized in Arabidopsis for their roles in systemic nutrient signaling. Taken together, our findings provide compelling evidence for the existence of CISR and highlight the roles of SSPs and phytohormones as potential long-distance molecules in rice. Using our established hydroponic culture system, we intent to: i) clarify the molecular dynamics underlying CISR in rice, and ii) investigate the functional roles and mobility of SSPs and phytohormones in CISR.

Keywords: Chitin Induced Systemic Resistance; Long-distance signaling; Small Secreted Peptides

HIJACKED A ORYZA SPECIFIC TRANSCRIPTIONAL MODULE TO NEGATIVELY AND SYNERGENICALLY REGULATE THE BROAD-SPECTRUM RESISTANCE AND HEADING DATE

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Bacterial leaf streak (BLS), caused by *Xanthomonas oryzae* pv. *oryzicola* (Xoc), is an important seed-borne and quarantine disease in China, which is severely danger to the yield and hybrid seeds production. However, it is still limited to know how rice is resistant or susceptible to BLS. Here we identified a bHLH-type gene *OsbHLH81*, which is activated expression by Xoc RS105 dependent on type III secretion effectors (T3SE) and mediated the susceptibility to BLS on rice. The *OsbHLH81*-overexpression (OE) lines enhanced the susceptibility, while both the *OsbHLH81*-knockout (KO) and knock-down (RNAi) lines are remarkably increased the resistance to BLS. In order to further analyze the disease resistance mechanism of OsbHLH81, we mined the target genes for OsbHLH81 by performing a joint analysis of RNA-seq and DAP-seq. And an *Oryza* specific unknown function gene *OsJT1* was identified as one of OsbHLH81 targets via ChIP-qPCR and EMSA experiments. Similar to the *OsbHLH81*, the *OsJT1* confers to Xoc-inducible expression and negatively regulated the resistance to BLS. However, the tissue specific expression pattern of two genes is not correlated in normal condition. Furthermore, the repressed *OsbHLH81-OsJT1* module was identified to broadly resistant to bacterial blight and sheath blight as well as to accelerate early flowering. Importantly, the OsJT1 interacts with and stabilizes the OsJAZ5 to repress the jasmonic acid (JA) signaling for negatively regulating broad-spectrum resistance and heading date in rice.

Keywords: Rice; Bacterial leaf streak; Plant immunity; bHLH; Orphan gene

Extending functionality of *Solanaceae* NLRs into rice confers resistance to bacterial leaf streak

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Plant intracellular nucleotide-binding, leucine-rich repeat (NLR) immune receptors have been used in traditional breeding programs for crops protection. Transfer of NLRs into taxonomically distinct plant species to confer pathogen resistance has proven to be constrained by restricted taxonomic functionality (RTF). Rice production is threatened by many diseases, among which bacterial leaf streak, caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), is one of the most devastating disease. However, no resistance genes encoding NLRs or other proteins have yet been identified in rice germplasm.

Here, we show that co-delivery of pepper *Bs2* gene and its downstream helper NLR partners *NRC* genes from *Nicotiana benthamiana* into rice could extend the functionality of Bs2 to rice. NRCs oligomerize in transgenic rice co-expressing Bs2 and NRCs upon *Xoc* infection and transgenic rice gains resistance against *Xoc* in an AvrBs2-dependent manner, while transfer of Bs2 sensor or NRC helpers alone fail to confer resistance. Importantly, cross-family transfer of the senor and helper NLR stacks doesn't compromise agronomic traits and has no impact on basal resistance and fitness cost in the field. Our results demonstrate that interfamily co-transfer of sensor and helper NLRs would overcome RTF and widen the resistance range of the know NLRs with broad effector recognition capacities, thus serving as a powerful strategy for providing resistance through redeploying these NLRs with their cognate helper NLRs.

How Does Rice Resist Brown Planthopper

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The brown planthopper (*Nilaparvata lugens* Stål, BPH) is the most destructive insect pest of rice (*Oryza sativa*), that can cause leaf wilting and complete drying of whole rice plants. It is a migratory pest and has developed high resistance to pesticide. The increased importance of BPH as a pest has prompted world-wide efforts to identify sources of resistance for breeding resistant varieties. Understanding the molecular mechanism of rice resistance to BPH will help to develop high yield and resistant rice.

We have developed a BPH-resistant rice line derived from a wide-hybridization of wild rice *O. officinalis* and cultivated rice. Then we isolated the BPH resistance gene *Bph14* from the line via the map-based cloning strategy. *Bph14* encodes a CC-NB-LRR receptor. BPH14 forms a homocomplex and enhances WRKY transcription factor activity to confer resistance to BPH. During it feeding on rice plant, BPH secretes saliva, which contains different kinds of proteins, into rice cells. Through yeast two-hybrid screening, we identified a BPH salivary protein, BISP, that interacts with BPH14. In the susceptible rice, BISP interacts with OsRLCK185 and suppresses its phosphorylation activity, thereby inhibiting basal defence and promoting BPH feeding on the rice plant. However, in resistant rice carrying *Bph14*, BISP binds directly to the LRR domain of BPH14 and triggers strong host plant resistance, which suppresses the insect feeding by occlusion of sieve tube.

We observed that continuous activation of Bph14-mediated immunity negatively impacts plant growth and yield. Rice fine-tunes this resistance through autophagy. Upon BISP-BPH14 complex formation, both proteins interact with the selective autophagy receptor OsNBR1, which recruits OsATG8 to deliver BISP into autophagosomes for degradation. This mechanism fine tunes the resistance and allow us to develop high-yield, insect-resistant varieties to control BPH insect.

We have isolated several BPH resistance genes. *Bph14*, *Bph15*, *Bph6*, *Bph9* and *Bph30* are widely employed in rice breeding programs. A number of BPH-resistant rice varieties are developed and released to farmers. The results show that the growing of resistant rice varieties has significantly reduced the field density of BPH insects. The promotion of these new varieties has effectively enhanced pest resistance while reducing pesticides and improving yield.

Keywords: Rice; brown planthopper; NLR receptor; salivary protein; breeding

miR444b.2-HsfA1-*AOC1* module mediates heat priming-enhanced blast resistance in rice

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As global climate change exacerbates extreme heat events, the interplay between heat stress and blast disease resistance in rice remains poorly understood. In this study, through integrated transcriptome profiling and systematic phenotyping of mutants in several thermosensory pathways, we identified HsfA1 as a positive regulator of heat priming-enhanced blast resistance in rice. Systematic analysis of miRNA dynamics, bioinformatics prediction, and RNA pull-down experiments revealed that *miR444b.2*, a temperature-responsive miRNA, directly suppresses the expression of *HsfA1* by targeting the second exon of *HsfA1* mRNA. Genetic analyses demonstrated that heat stress-mediated suppression of *miR444b.2* expression relieves the repression of *HsfA1*, thereby enhancing blast resistance in rice. Furthermore, HsfA1 directly binds to the promoter of *AOC1*, a key jasmonic acid (JA) biosynthesis gene, to activate its expression. Knockout of *HsfA1* or *AOC1* abolishes heat priming-enhanced JA accumulation and blast disease resistance, and the phenotypes are largely restored via *AOC1* overexpression and MeJA treatment. Further identification of *HsfA1* natural variants and generation of the *HsfA1 uORF*-edited lines with improved blast resistance offer potential strategies for breeding disease-resistant rice varieties. This study elucidates the *miR444b.2*-HsfA1-*AOC1* module that linking thermal sensing to JA-mediated blast resistance, providing a molecular blueprint for engineering climate-resilient crops with concurrent biotic-abiotic stress tolerance.

A translational regulator M9 modulates ethylene signaling and plant immunity in rice

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We previously identified a glycinetyrosine-phenylalanine (GYF) domain protein M9, which positively regulates ethylene signaling at translational level in rice. M9 directly binds to the OsEBF1/2 mRNAs for translational inhibition, allowing the accumulation of transcription factor OsEIL1 to activate the downstream signaling [1]. Not only that, we also found that *m9* exhibited a distinct autoimmune phenotype at the heading stage, indicating that M9 might be involved in plant immunity. Through RIP-seq, RNA EMSA assay and *in vitro* translation assays, we demonstrated that M9 can bind to various *R* gene mRNA for translational repression. Furthermore, we also found that MAPK3/6 could interact and phosphorylate M9 at S50 site, and the inhibitory function of M9 in a simulated phosphorylated state on the binding and translation of *R* gene mRNA was significantly eliminated. In conclusion, M9 negatively regulates the disease resistance in rice by inhibiting the translation level of various *R* genes. This inhibitory effect can be modulated by immune-related kinases OsMAPK3/6, thereby enhancing the resistance of rice to the pathogens.

Keywords: rice, GYF domain, translational repression, MAPK3/6

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OsPHR2-OsRNS4 Module-mediated Novel Regulatory Factor for Rice Leaf Senescence Control

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S-like RNases, a subclass of the RNase T2 family, are conserved ribonucleases known to function in senescence, phosphate recycling, and defense responses in dicotyledonous plants. However, the biological roles of S-like RNases in monocotyledons remain largely uncharacterized. In this study, we identified *OsRNS4* as a monocot-specific S-like RNase in rice (*Oryza sativa*). Unlike canonical RNase T2 enzymes, *OsRNS4* has lost most of the conserved amino acid residues in its two catalytic active sites (CAS), which are essential for ribonuclease activity, suggesting it may function through a non-canonical mechanism. In this study, expression analysis revealed that *OsRNS4* is upregulated during dark-induced senescence and in response to abscisic acid (ABA), a key phytohormone that plays a significant role in promoting leaf senescence. Functional analysis using loss-of-function and gain-of-function mutants demonstrated that *OsRNS4* positively regulates leaf senescence. *OsRNS4* knockout mutants exhibited delayed dark-induced senescence, whereas *OsRNS4*-overexpressing (OsRNS4-OX) lines showed accelerated senescence under the same conditions. Alterations in *OsRNS4* transcript levels influence the expression of NAC transcription factors that regulate ABA signaling and leaf senescence during dark-induced senescence. Furthermore, we identified *OsPHR2*, a transcription factor known to senescence, as an upstream regulator that positively controls *OsRNS4* expression. Together, our findings reveal a previously unrecognized role for a monocot-specific S-like RNase in modulating leaf senescence and ABA responses, providing new insights into the functional diversification of RNase T2 family members in monocots.

Keywords: RNase; ABA; Senescence

Natural variations of CTB5 and CTB6 confer cold adaptation in plateau japonica rice

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During cold acclimation in high-latitude and high-altitude regions, japonica rice develops enhanced cold tolerance, but the underlying genetic basis remains unclear. Here, we identify CTB5, a HD-Zip transcription factor, and CTB6, a lipid transfer protein that confer cold tolerance at the booting stage in japonica rice. CTB5 interacts with OsHox12 and targets GA metabolism genes to promote GAs accumulation in anthers and facilitate tapetum development under cold stress. CTB6 interacts with CATs to maintain their stability, thereby scavenging ROS accumulation, and affects the lipid content in anthers to regulate cold tolerance at the booting stage. The CTB5KM and CTB6K alleles are selected during the cold acclimation of japonica rice to plateau habitats in Yunnan Province. Under natural cold habitats, the NILCTB5 and NILCTB6 exhibit significantly higher seed-setting rate and yield per plant compared to Towada, indicating that the CTB5KM and CTB6K alleles can effectively mitigate yield losses by improving cold tolerance. Our findings provide insights into the mechanisms underlying cold adaptation in plateau japonica rice and offer potential targets for breeding cold-tolerant rice varieties.

Keywords: Rice; Cold tolerance; CTB5; CTB6; Molecular mechanism

Genetic basis underlying the domestication of upland rice and its implication in rice breeding

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Upland rice was domesticated in the rain-fed unbounded field and dry-cropping. It is possesses many green traits such as direct seeding, resource-saving (e.g. water, nutrient, labor-force, and etc.) and drought resistant., which are required in rice breeding. However, the origination and evolution of upland rice as well as its green traits remains mysterious. Integrating phenotypic, phylogenomic, and evolutionary analyses, we found that upland rice was domesticated directly from the common wild rice (*Oryza ruffipogen*). Upland rice receives a bi-directional selection during its adaptation to the water limited upland ecosystem due to the genomic trade-offs between drought resistance and productivity. As a result, the balancing selection drives the evolution of drought resistance in upland rice, rather than the directional selection. Interestingly, genetic resources related to nitrogen use efficiency also meet such scenario. It is therefore, abundant genetic resources of drought resistance, as well as other green traits, are remained in upland rice, which makes it an important germplasm in rice breeding. Based on the genetic basis of green traits evolution in upland rice, we have mined many valuable genes associated with drought resistance and NUE. We have also developed the water-saving and drought-resistance rice, which revives the green traits of upland rice in the elite paddy rice.

Keywords: Upland rice; water-saving and drought-resistance rice; nitrogen use efficiency; adaptive evolution; breeding

The NAT1-bHLH110-CER1/CER1L module regulates heat stress tolerance in rice

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Rice production is facing substantial threats from global warming associated with extreme temperatures. Here we report that modifying a heat stress-induced negative regulator, a negative regulator of thermotolerance 1 (NAT1), increases wax deposition and enhances thermotolerance in rice. We demonstrated that the C2H2 family transcription factor NAT1 directly inhibits *bHLH110* expression, and bHLH110 directly promotes the expression of wax biosynthetic genes *CER1/CER1L* under heat stress conditions. In situ hybridization revealed that both *NAT1* and *bHLH110* are predominantly expressed in epidermal layers. By using gene-editing technology, we successfully mutated *NAT1* to eliminate its inhibitory effects on wax biosynthesis and improved thermotolerance without yield penalty under normal temperature conditions. Field trials further confirmed the potential of *NAT1*-edited rice to increase seed-setting rate and grain yield. Therefore, our findings shed light on the regulatory mechanisms governing wax biosynthesis under heat stress conditions in rice and provide a strategy to enhance heat resilience through the modification of NAT1.

Keywords: Rice; Heat stress; Wax

UV-B Photoreceptor OsUVR8 Directly Modulates OsNAC Transcriptional Activity to Drive Differential UV-B-Induced Photomorphogenesis in Japonica and Indica Rice

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Light serves not only as the energy source for photosynthesis but also as a critical environmental cue regulating photomorphogenesis and plant development. In Arabidopsis, UV-B light triggers rapid monomerization of the UVR8 photoreceptor, enabling its interaction with the E3 ubiquitin ligase COP1 and subsequent activation of the UV-B signaling cascade through the stabilization of HY5. UVR8 also modulates transcription by directly interacting with factors such as WRKY36, WRKY70, bZIP18, and bZIP48. However, the core components and mechanisms of UV-B signaling in rice remain largely unexplored. Here, by generating a near-saturation activation-tagged mutant library in the japonica rice cultivar Kitaake, we identify the AP2/ERF transcription factor OsERF112 as a positive regulator of UV-B–induced photomorphogenesis. Loss-of-function mutants (oserf112) exhibit attenuated seedling growth inhibition under UV-B, indicating reduced sensitivity. Transcriptome profiling (RNA-seq) reveals that OsERF112 regulates genes involved in flavonoid biosynthesis, including OsF3′H and OsPAL6. Promoter sequence variation in OsERF112 defines three major haplotypes (Hap1–3), with Hap2 predominant in japonica and Hap1/Hap3 in indica. Hap1/Hap3 confers significantly higher OsERF112 expression and enhanced UV-B responsiveness. One promoter SNP account for major expression differences and are located within cis-elements bound by the NAC transcription factor OsNAC6. Moreover, OsUVR8 promotes the OsNAC6 activity under UV-B. Our findings demonstrate that natural variation in OsERF112 expression underlies differential UV-B sensitivity between rice subspecies, mediated by the OsUVR8-OsNAC6 module. This work uncovers a previously uncharacterized regulatory pathway in UV-B–induced photomorphogenesis and provides new insights into the transcriptional architecture of light signaling in monocots.

Keywords: Photomorphogenesis; UV-B; transcriptional regulation; photoreceptor; rice

PHENOMIC ANALYSIS OF THE 3K INDICA PANEL UNDER DROUGHT FOR PHOTOSYNTHETIC, LEAF STRUCTURAL, AND UAV TRAITS

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The 3000 Rice Genomes Project with its release of genome-wide SNP data and the source genotypes [1, 2] set the stage for detailed analysis of phenomics data on selected germplasm. Here, we present results from analyses of a panel of indica genotypes screened in the field over 2 or 3 seasons under irrigated and managed drought stress conditions. Various phenotypes were measured manually in the field and laboratory: agronomic traits such as biomass, yield, days to flowering, plant height, harvest index, and drought response index (DRI); leaf morphology traits including stomatal density and leaf length, width, and area; physiological traits including leaf water potential and photosynthetic related traits such as photosynthetic rate, stomatal conductance, transpiration, and water-use efficiency; and high throughput (HTP) UAV collected traits such as canopy height, canopy temperature, and NDVI. Genome-wide association studies (GWAS) across the collection of traits identified numerous correlated genomic regions. Exploration of these regions identified candidate genes linked to photosynthesis, stomatal density, water-status regulation, leaf development, and others. UAV-based HTP data effectively tracked plant growth and senescence, which correlated with photosynthetic rate. Changes in leaf architecture such as broader leaves with the capacity to thicken correlated with higher play a role in yield under drought with higher DRI. Co-localization of GWAS QTLs and network analyses of underlying candidate genes suggests multi-trait regulation through common genes.

Keywords: High Throughput Phenotyping; GWAS; stomatal density; leaf morphology; photosynthesis; leaf water potential; agronomic traits

Identification of functional gene for alkaline stress tolerance at rice germination stage

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Alkaline stress as the one of major abiotic stress, it performed serious damage for rice growth, yield and quality. Although there has diverse study for saline stress, but still showed poor understand for alkaline tolerance in rice.

In this study, a set of alkaline tolerance-related traits were measured, including germination energy, germination ratio, relative root length and shoot length. Integrated population were participated, which based on Japonica-Indica core-collection and breeding varieties. Genome-wide association study was performed, based on 1.57 million high-quality single nucleotide polymorphism and 279 varieties. Totally 71 individual or overlap loci were identified in whole panel, Japonica and Indica panel, respectively. Combination with transcriptome profile, gene and GO annotations, significant SNP position, etc. Twelve genes were selected as candidates for alkaline tolerance at rice germination stage. Though qRT-PCR verification, three genes were showed different expression change in resistance and susceptible varieties. Haplotype analysis showed the 3 genes contained diverse haplotype that significant related with alkaline tolerance index. SNP-haplotype analysis results showed that some key SNPs could performe crucial function between Japonica and Indica sub-populations.

Our results identified multiple novel loci and 3 candidate functional genes for the basic research for alkaline tolerance at the rice germination stage, haplotype results implied the pyramid haplotype could be helpful for future variety selection and breeding research.

Keywords: rice; alkaline tolerance; germination stage; GWAS; gene identification

A natural gene on-off system confers field thermotolerance for grain quality and yield in rice

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High temperature severely deteriorates grain quality and reduces grain yield in crops, posing a serious threat to global food security and farmer income. However, how high temperature regulates cereal endosperm development and grain quality and how to achieve synergistic thermotolerance for both quality and yield remains unknown. Here we identified a rice major locus, *QT12*, which negatively controls the thermotolerance for grain quality by disrupting endosperm storage substance homeostasis through exacerbating ER stress under field high temperatures. A natural variation within a *QT12 cis*-element that is recognized by a nuclear factor Y (NF-Y) complex leads to lower *QT12* expression, and the subunits NF-YA8 and NF-YB9/NF-YC10 oppositely regulate the thermotolerance by differentially modulating *QT12* expression, thereby forming a natural gene on-off system that enables stepwise and differential regulation of field thermotolerance. We further demonstrated that high temperature as a switch pusher enhances NF-YA8 transactivation on *QT12* through weakening its interactions with NF-YB9 and NF-YC10, ultimately resulting in higher *QT12* expression and inferior quality. Mutation of *QT12* confers thermotolerance for superior quality and increases yield up to 1.31–1.93 times in elite rice under large-scale field high-temperature trials. Finally, we identified two trait regulatory haplotypes (TRHs) from the co-selected variations of the four genetically unlinked genes of *NF-Ys* and *QT12*, which could generally explain the thermotolerance differences between *indica* and *japonica*. Our work provides mechanistic insights into subspecies thermotolerance differentiation and offers a proof-of-concept breeding strategy to break the stress-growth and yield-quality trade-offs.

Keywords: Rice; field thermotolerance; grain quality; grain yield

WRKY transcription factors: a tool for rice resilience

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Rice is one of the major food sources in the planet. The increase of the human population and the effects of climate change in Agriculture pose a threat to the sustainable production of rice. On top of traditional, novel challenges are faced by geneticists and breeders to develop more resilient crops. WRKY transcription factors are involved in many regulatory responses in the genus Oryza [1]. Our research group has pursued WRKY TFs in rice and relatives in order to find potential candidates for improving rice resilience. *Oryza glumaepatula* is a wild rice species native to South America, possessing a diploid genome (2n=24, AA). This species is well-adapted to South American continent and exhibits key agronomic traits, including drought and heat tolerance [2]. This study aimed to identify cis-regulatory elements (CREs) within WRKY gene promoters in *O. glumaepatul*a, which play critical roles in regulating biological processes. For this analysis, 116 WRKY gene coding sequences (CDS) were retrieved from the PlantTFDB database and used as query sequences in BLASTn to determine genomic locations in EnsemblPlants. The identification of CREs in 2.0-kbp upstream promoter sequences was conducted using the PlantCARE program. A total of 115 CREs were identified from 2.0-kbp upstream of the start codon (ATG). A comparison of WRKY landscape in *O. glumaepatula* and *O. sativa* will help in the understanding gene expression mechanisms and provide new insights for genetic engineering to develop elite genotypes with desirable traits.

Keywords: abiotic stress; biotic stress; climate change; plant responses

Reconstruction of Phosphate Starvation Signaling Networks through Transcriptome Profiling of PHR2, PHO2, and PT1 Regulatory Pathways in Rice (*Oryza sativa* L.)

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Phosphate is an essential macronutrient for plant growth and development. In rice (*Oryza sativa*), phosphate starvation-induced (PSI) signaling is mainly regulated by *OsPHR2*, *OsPHO2*, and *OsPT1*; however, the transcriptional regulatory networks associated with these genes remain poorly understood. In this study, we generated transgenic rice lines overexpressing *OsPHR2* (*OsPHR2-OX*) and *OsPT1* (*OsPT1-OX*) as well as a knockout mutant of *OsPHO2* (*ospho2*), and performed transcriptome analysis to investigate their regulatory roles. We identified 1000, 900, and 976 differentially expressed genes (DEGs) in *OsPHR2-OX*, *ospho2*, and *OsPT1-OX*, respectively, compared to wild type. Gene Ontology (GO) enrichment analysis revealed their distinct roles in the PSI signaling pathway: *OsPHR2-OX* was associated with Pi transport, acid phosphatase activity, and lipid/lignin catabolism; *ospho2* was enriched in cellular responses to Pi starvation and nutrient levels and lipid catabolism; and *OsPT1-OX* was linked to redox homeostasis and epigenetic regulation. Heatmap analysis of known Pi-responsive genes showed upregulation of several *OsPTs*, *OsPAPs*, lipid remodeling genes and RNase genes in *OsPHR2-OX* and *ospho2*. Functional classification indicated that *OsPHR2-OX* broadly regulates transcription factors, hormone-related, and development-associated genes, while *ospho2* enhances expression of Pi recycling and stress-response genes. In contrast, *OsPT1-OX* affected only a subset of development-related genes. These findings reconstruct a transcriptional network linked to Pi homeostasis and adaptation in rice and provide a valuable resource for molecular breeding to improve phosphate use efficiency in crop plants.

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Keywords: rice; Transcriptome; phosphate

A blast-resistant NLR gene confers drought resistance by competitively interacting with an E3 ligase to protect phenylalanine ammonia-lyase in rice

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Drought and diseases represent major challenges to achieve high yield in crops, underscoring the urgent need to explore drought- and disease-resistant genetic resources and breed crop varieties with multi-stress resistance. Here, using a genome-wide association study combined with function analyses, we identified PibH8, a homologous of the blast resistance gene Pib encoding a nucleotide-binding leucine-rich repeat receptor (NLR), which plays a crucial role in rice drought resistance. PibH8 interacts with phenylalanine ammonia-lyase OsPAL1, a rate-limiting enzyme in phenylpropanoids biosynthesis pathway in rice. It protects OsPAL1 from degradation by competitively binding to E3 ubiquitin ligase OsFBK16 which facilitates OsPAL1 degradation. This protective mechanism enhances PAL activity, leading to increased lignin and flavonoids content and improved drought resistance. Genetic evidence indicates that PibH8 acts upstream of OsPAL1 in conferring drought resistance. Furthermore, we identified a causal variation in the PibH8 promoter that is associated with drought resistance. Introgression of a superior haplotype, which exhibits high PibH8 expression, into the elite rice variety Kongyu131 significantly improved drought and blast resistance. This research not only elucidates a regulatory mechanism of NLR protein in drought resistance, but also highlights a promising breeding value of PibH8 for simultaneously improving drought and blast resistance.

Keywords: Rice; Drought tolerance; GWAS; NLR; PAL1; Lignin; Flavonoids

Regulation of Na^+ extrusion and long-distance transport in plant

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To control net sodium (Na^+) uptake, Arabidopsis plants utilize the plasma membrane Na^+/H^+ antiporter SOS1 that catalyzes Na^+ efflux at the root and promotes Na^+ loading into the xylem, and the channel-like HKT1;1 protein that mediates the reverse flux of Na^+ unloading at the xylem. Together, these opposing transport systems govern the partition of Na^+ within the plant, yet they must be finely co-regulated to prevent a futile cycle of xylem loading and unloading. Here I show that the Arabidopsis SOS3 protein acts as the molecular switch governing these Na^+ fluxes by favoring the recruitment of SOS1 to the plasma membrane and its subsequent activation by the SOS2/SOS3 kinase complex under salt stress, while commanding HKT1;1 protein degradation upon acute salt stress. SOS3 achieves this novel role by direct and SOS2-independent binding to previously unrecognized functional domains of SOS1 and HKT1;1. These results evidence that roots first retain moderate amounts of salts to facilitate osmoregulation. When sodicity exceeds the stress set point, activation of SOS3 switches the balance towards Na^+ export out of the root via the xylem. Thus, SOS3 functionally links and co-regulates the two major Na^+ transport systems operating in vascular plants controlling plant tolerance to salinity.

Keywords: sodium loading; sodium transporter; xylem loading; SOS1; HKT1

OsERF66-OsERF15/OsERF99 module regulate salt tolerance in rice

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Rice (*Oryza sativa*) is highly susceptible to salt stress, which significantly compromises yield and grain quality. Understanding the molecular basis of salt tolerance is therefore crucial for developing stress-resistant cultivars. In this study, we identified OsERF15 as a positive regulator of salt tolerance in rice. While OsERF15-overexpressing lines displayed enhanced salt tolerance, OsERF15-knockout mutants exhibited wild-type (Nipponbare, NIP) sensitivity, suggesting potential functional redundancy. Phylogenetic analysis revealed OsERF66 as a close homolog within the ERF-IXc subgroup. Biochemical and molecular characterization demonstrated that OsERF15 physically interacts with OsERF66 and transcriptionally activates its expression. Consistent with this regulatory relationship, OsERF66-overexpressing plants showed markedly improved salt tolerance, whereas knockout lines maintained NIP-like sensitivity. Co-expression network analysis further identified functional redundancy between OsERF15 and OsERF99, as evidenced by the hypersensitive phenotype of OsERF15/OsERF99 double mutants under salt stress. Genome-wide binding analysis (DAP-seq) and intersection studies revealed that both transcription factors co-occupy the promoter region of OsAKT, a key salt-responsive gene. This regulatory interaction was experimentally validated through electrophoretic mobility shift assays (EMSA), chromatin immunoprecipitation (ChIP-qPCR), and dual-luciferase reporter assays. Our findings establish a novel OsERF15-mediated regulatory module, wherein the OsERF66-OsERF15/OsERF99-OsAKT signaling cascade activates downstream genes to confer salt tolerance. This study provides important insights for molecular breeding of salt-tolerant rice varieties.

Keywords: Salt tolerance; rice; Transcriptional regulatory

Lipid gene-related genetic variants underlie rice expansion to northern climates

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Rice (*Oryza sativa*) was originally domesticated in tropical regions but has successfully adapted to higher latitudes due to genetic changes linked to photoperiod sensitivity and cold tolerance. Lipid metabolism, particularly via membrane-associated enzymes, is increasingly recognized as critical to environmental stress responses. In this study, we analysed genomic and geolocation data from 3,000 rice accessions to investigate the role of a lipid-related gene family in high-latitude adaptation. We identified haplotypes across 115 genes and classified them based on single-nucleotide polymorphisms (SNPs) and the mean latitude of occurrence. Haplotypes with average distributions above 35°N were defined as high-latitude haplotypes (HLHs). We selected 10 genes carrying 11 HLHs for further analysis. Haplotype network and protein sequence comparisons revealed a few non-synonymous mutations that may contribute to northern adaptation. Notably, HLHs were primarily found in genes that are highly expressed in roots, indicating a root-mediated adaptation mechanism. Interestingly, only three of 14 known cold tolerance genes carried HLHs, suggesting that latitude adaptation involves genetic components beyond the classical cold-response pathways. Our findings highlight a group of lipids associated genes as previously unrecognized players in rice's ability to adapt to high latitudes, providing new insight into the genetic architecture of environmental resilience in crops.

Keywords: Adaptation; high-latitude haplotype; GDSL-type esterase/lipase; latitude; OsGELP; rice; root

GENOME-WIDE ASSOCIATION AND CANDIDATE GENE ANALYSIS OF ELEMENTAL ACCUMULATION UNDER DIFFERENT SOIL PH CONDITIONS BY USING A RICE MAGIC POPULATION

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Element absorption and accumulation are fundamental physiological functions essential for plant growth. Understanding their genetic basis in rice is key to developing stress-tolerant and nutrient-enriched varieties. In this study, we used a multi-parent advanced generation inter-cross (MAGIC) population [1] to investigate genetic variations in the accumulation of 13 elements (P, K, Ca, Mg, As, Cd, Cr, Cu, Fe, Mo, Mn, Ni, Zn) in straw at flowering and grains at maturity. Genome-wide association studies (GWAS) identified 51 and 53 QTLs in straw and grain, respectively. A total of 104 QTLs were grouped into 18 clusters and 60 independent QTLs. From these, 52 candidate genes related to Ca, Mg, Cd, Cu, Fe, and Mo accumulation were predicted. These candidates included both known isolated genes, such as the transporter gene [2] at the Mo accumulation QTL in grain, and unreported genes, such as the calcium signaling gene at the Ca accumulation QTL in straw [3]. Element absorption profiles also depend on soil conditions. In particular, the details of the interaction between soil pH and genetic factors remain unclear. To clarify this, we evaluated 12 elemental contents in straw and grain of the eight MAGIC founders under acidic, neutral, and alkaline soil paddy field that were newly developed [4]. Furthermore, we applied the MAGIC population to the same field and attempted to detect QTLs common to or specific to each soil condition.

Keywords: GWAS; MAGIC population; QTL; Candidate gene; Element; Soil pH

Pan-transcriptome analysis reveals alternative splicing and transposon insertion polymorphism regulation of cold tolerance in rice

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Plants have evolved complex mechanisms to adapt to environmental stress. Here, we constructed a rice pan-transcriptome and analyzed its regulatory dynamics under cold stress using Iso-Seq and RNA-Seq across 11 cultivars including *Xian* and *Geng* subspecies, and enriched the gene annotation of alternative splicing (AS). We found that AS plays a critical role in cold response, identifying key regulators *OsCATC* and *Os03g0701200*. Notably, serine-arginine-rich splicing factors *OsRS33* and *OsRS2Z38* were central to cold tolerance, with *OsRS2Z38* potentially selected during *Geng* rice domestication for cold adaptation.

Additionally, we explored transposable element (TE) contributions to cold tolerance by analyzing 165 rice accessions, identifying 30,316 polymorphic TE insertions (pTEs). These pTEs showed elevated H3K27me3 marks, suggesting epigenetic regulation of cold-responsive genes. Transcriptome analysis revealed 26,914 cold-induced TEs, implicating them in stress adaptation. Using TIP-GWAS, we discovered novel cold-tolerance genes (*OsCACT* and *OsPTR*), functionally validated via mutants. A web resource enables further exploration of pTEs. Our findings provide insights into AS- and TE-mediated cold adaptation, offering targets for breeding resilient rice varieties.

Keywords: Pan-transcriptome, pangenome, alternative splicing (AS), transposable element insertion polymorphism (TIP), cold tolerance

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The molecular mechanisms of H_2O_2 regulating the salt tolerance in rice

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Hydrogen peroxide (H_2O_2), as the most stable reactive oxygen species, is an important intracellular signaling molecule that is widely involved in plant growth and development as well as stress responses. Catalase (CAT) is an important enzyme for maintaining H_2O_2 homeostasis and plays a critical role in salt stress responses. We previously identified that the kinase STRK1 and the phosphatase PC1 phosphorylates and dephosphorylates CatC to activate and inhibit its activity, respectively, which function as the molecular switch to regulate the salt tolerance in rice (*Oryza sativa* L.). We also found that the zinc finger protein DHHC09 S-acylates STRK1, promoting the transduction of salt stress signals from STRK1 to CatC in the form of phosphorylation. However, the molecular mechanism by which phosphatases activate CAT to participate in salt stress response remains unclear. We identified a PP1-type protein phosphatase OsPP1a from rice and found that it positively regulates salt and oxidative stress tolerance. OsPP1a interacts with CatC in the peroxisome and specifically dephosphorylates CatC at Thr-292, enhancing its activity. The dephosphorylation of Thr-292 reduces the interaction between CatC and the E3 ubiquitin ligase APIP6, thereby inhibiting APIP6-mediated degradation of CatC via the ubiquitin/26S proteasome pathway and increasing CatC stability. *OsPP1a* overexpressing rice lines exhibited enhanced salt and oxidative tolerance with a lower phospho-threonine level of CATs and a higher CAT enzyme activity. Phosphatase activity and seminal root growth assays further indicated that OsPP1a acts as a key regulatory factor upon salt stress to balance salt tolerance and growth and development in rice. Our findings demonstrate that OsPP1a regulates H_2O_2 homeostasis by dephosphorylating and stabilizing CatC, thereby enhancing salt and oxidative tolerance in rice. Moreover, overexpression of *OsPP1a* in rice not only improves salt tolerance at the seedling stage but also markedly mitigates grain yield loss under salt stress. Together, these results shed light on the molecular mechanisms of CAT switch-on and stabilization by phosphatases and H_2O_2 involved in salt tolerant regulation provide a strategy for breeding highly salt-tolerant rice.

Keywords：rice (*Oryza sativa* L.); salt tolerance；hydrogen peroxide（H_2O_2）; catalase (CAT); protein phosphatase

OsCDPK24 and OsCDPK28 phosphorylate heat shock factor OsHSFA4d to orchestrate abiotic and biotic stress responses in rice

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Global warming impacts crop production and increases crop disease. It is commonly known that heat stress (HS) caused by extreme high temperature induces HS responses but suppresses disease resistance in plants. However, the molecular basis of this trade-off remains largely unknown. Here, we report that *OsHsfA4d* gene shows the strongest induction upon HS and pathogen infection among rice heat shock factors (Hsfs). The transcription factor OsHSFA4d enhances thermotolerance by binding to the heat shock element (HSE) in *HSP101* promoter to activate *HSP101* expression. OsHSFA4d also binds to the HSE in first intron of *Cellulose synthase-like F6* (*CslF6*) to promote its expression for suppressing PAMP-triggered ROS bursts and pathogenesis-related gene expression, inhibiting disease resistance. OsCDPK24 and OsCDPK28 interact with OsHSFA4d to form a complex and phosphorylate S146 of OsHSFA4d, thereby enhancing its DNA binding ability. HS induces OsCDPK24/28 kinase activities to increase OsHSFA4d phosphorylation level at S146. Importantly, S146-like residues are conserved in the OsHSFA4d orthologues from other plant species, suggesting that the modules similar to OsHSFA4d phosphorylation by OsCDPK24/28 are broadly utilized to orchestrate abiotic and biotic stress responses in the plant kingdom.

Keywords: Biotic stress; Abiotic stress; Transcription factor; Phosphorylation

High temperature-dependent regulation of chloroplast RNA editing and splicing mediated by a DYW domain-containing protein in rice

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High temperatures compromise chloroplast function, threatening plant survival and crop yield. How plants protect chloroplast gene expression under high temperature remains a key question. Here, we identify OsDYW2, a rice DYW domain-containing protein, as a crucial dual-function regulator of both chloroplast RNA editing and splicing, particularly under high-temperature conditions. In the absence of OsDYW2, rice seedlings are albino and lethal specifically under high temperatures, with defective chloroplast biogenesis and impaired RNA editing and splicing of multiple chloroplast genes. Mechanistically, OsDYW2 forms a temperature-sensitive regulatory complex with core RNA processing factors, including OsMORF proteins. The stability of this complex is directly modulated by high temperature, providing a molecular link between high-temperature response and chloroplast RNA processing. Our work establishes OsDYW2 as a key node integrating environmental cues with post-transcriptional control and provides new insights into chloroplast adaptation to environmental challenges.

Keywords: Rice; Chloroplast; RNA editing; RNA splicing; High temperature; OsDYW2

Evolution of interspecific hybrid sterility gene S13 found in *O. longistaminata*

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Reproductive isolation is a fundamental mechanism which maintains species barrier. In rice, among several types of post-zygotic isolation, hybrid sterility is frequently observed. Hybrid sterility is the phenomenon that hybrid does not produce functional gametes in male or female gametogenesis, causing pollen or seed sterility. Here, we report 1. the identification of the gene for hybrid sterility, S13, observed in African wild rice species, *Oryza longistaminata*. and 2. Possible evolutionally scenario of this hybrid sterility locus. In the S13 locus three genetic alleles, S13s, S13l, and S13n exist. Among the possible allelic combination in hybrids, heterozygotes for S13s and S13l shows hybrid sterility, in particular, the male gametes possessing the S13s allele are preferentially aborted. This preferential abortion causes transmission ratio distortion (TRD) in the later generation. Gene cloning identified that the gene OlCHR, which encodes chromatin remodeling factor, is one of the causal genes of the S13 mediated hybrid sterility (Zin Mar Myint et al. 2024). Interestingly, this gene seems to be identical to HPT originally found in *O. meridianalis* and is causing hybrid sterility in combination with the other gene, HPA (You et al. 2023). HPT and HPA were shown to act as "toxin" and "antidote" factors in the male gametogenesis. By surveying the nucleotide sequences of the AA genome species of rice, we found that several different types of loss-of-functional mutations occurred in Asian rice. This result suggested the presence of genetic load on the hybrid sterility locus in rice.

Keywords: Hybrid sterility; Gametogenesis; *O. longistaminata*

Production of rice zygote in vitro: For investigating basic plant biology and establishing new breeding technology

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As developing zygotes and embryos are deeply embedded in ovular tissues, investigation of the developmental profile and molecular events in early embryogenesis of angiosperm was highly difficult. The establishment of in vitro fertilization system using isolated gametes in rice enables in vitro zygote/embryo culture and that the difficulty in the direct investigation of early zygotes and embryos has been overcome. Employing in vitro fertilization (IVF) system, various cytological and molecular analyses of developing rice zygotes and embryos have been intensively elucidated, including dynamics of reactive oxygen species (ROS) and antioxidant system in developing zygotes and embryos. Electric fusion of isolated gametes also allows fertilization barriers in interspecific and intergeneric breeding to be overcome. Therefore, to broaden genetic diversity in rice species, hybridization of rice with plants from other species and genera is currently under extensive investigation. In addition, fertilization-independent development of isolated egg cells into mature plants induced by cold stress and histone deacetylase inhibitor treatment is also largely understood. Regulatory roles of parthenogenesis-inducing genetic factors, such as Baby Boom 1 gene, in both early zygotic and parthenogenetic development were identified. Through genome duplication, the haploid egg-derived embryos produced by parthenogenesis develops into polyploid rice plants, including diploid and tetraploid, enabling the production of homozygous genome-edited rice plants mediated by CRISPR-Cas9-based mutagenesis and allowing allele fixation in offspring derived from a parent with desirable traits.

Keywords: In vitro fertilization; electrofusion; isolated gametes; ROS; parthenogenesis; interspecific / intergeneric hybrid; genome duplication; OsBBML1; *Oryza sativa*; japonica

Comprehensive Population Genetic Analysis of cBasmati

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cBasmati rice, primarily cultivated on the Indian subcontinent, is a globally celebrated group of aromatic rice varieties. It is highly prized for its distinct fragrance, slender and elongated grains, and soft, fluffy texture upon cooking. This study conducted a systematic analysis of the genetic background, adaptive selection, and gene flow of cBasmati, a world-renowned aromatic rice group. The study adopted a dual-reference genome strategy, using both the Nipponbare and a high-quality cBasmati variety (cB-ARC). At the population genetics level, the cBasmati population was divided into seven subgroups. The genetic structures of cBasmati and its subgroups were revealed through geographical distribution and the construction of a phylogenetic tree. Its genetic diversity was assessed using nucleotide diversity (π), Watterson's θ (θw), and Tajima's D. The genome was scanned for regions with high Fst values to detect areas under strong selection. Candidate genes within these regions were then identified, followed by haplotype analysis, phenotypic comparisons, and evolutionary tracing for a subset of key genes. Furthermore, a Genome-Wide Association Study (GWAS) was performed on some key agronomic traits within the cBasmati population to validate data, cross-reference known loci, and discover novel ones. Finally, the study analyzed gene flow between specific populations using the D-statistic test and a four-population tree model. This comprehensive research provided critical insights into the evolutionary history and genetic foundation of cBasmati.

Keywords: cBasmati rice; Population genetics; Adaptive selection; Population differentiation

New Resources for Hybrid Rice Production Based on Pollen–Pistil Interaction

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Fertilization in flowering plants depends on tightly regulated processes such as pollen hydration, germination, and tube growth. Despite their importance, the molecular mechanisms underlying these events remain poorly understood in rice (*Oryza sativa*). Here, we identify and characterize key regulators of pollen–pistil interaction that contribute to male fertility, offering valuable tools for hybrid rice production. To firstly explore genetic regulators, we screened 107 T-DNA lines targeting 105 pollen-preferential genes. Segregation distortion in 42 lines indicated defects in male gametophyte function. CRISPR validation revealed that some genes caused sterility only when redundant paralogs were also disrupted, highlighting genetic compensation. Transcriptome analysis of sterile mutants revealed a regulatory network to key pollen germination pathways. We then focused on a rice-specific receptor-like kinase gene family (OsPRK1, OsPRK2, OsPRK3) encoding proteins with conserved LRR and kinase domains. CRISPR/Cas9-mediated triple knockout lines (osprk1/2/3) exhibited complete male sterility without affecting vegetative or floral development. Cytological analysis revealed that sterility resulted from impaired pollen hydration and germination, associated with reduced apoplastic ROS accumulation. Exogenous H_2O_2 partially rescued the germination defect. OsPRKs were found to interact with LRR-extension proteins, suggesting a surface receptor complex that mediates ROS signaling during early pollen–pistil interaction. Our results identify essential components of pollen–pistil signaling and establish genetic resources that can be applied to engineer male sterility, advancing hybrid rice breeding strategies.

Keywords: Pollen–pistil interaction; Male sterility; Receptor-like kinase (OsPRK); Reactive oxygen species (ROS); Hybrid rice breeding

Understanding rice inflorescence development: insights from genetic and single-cell perspectives

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Grasses (Poaceae) occupy approximately 40% of Earth's land surface and provide nearly 50% of global dietary energy. Inflorescence architecture—a major determinant of yield governed by axillary meristem activity—is a key focus in developmental biology and crop improvement. In rice, inflorescence architecture comprises multiple orders of branches and spikelets, with diversity shaped by variation in their number and growth patterns. However, the regulatory networks controlling axillary meristem activity and developmental transitions remain poorly understood. This presentation examines the molecular framework through which jasmonic acid (JA) regulates rice inflorescence architecture, emphasizing its spatial distribution and interaction with cytokinin and gibberellin signaling in branch and spikelet development. Additionally, I will explore the molecular basis of the transition from indeterminate inflorescence to determinate spikelet formation using single-cell RNA sequencing.

Keywords: Inflorescence; Hormonal crosstalk; scRNA-seq

Time-ordering japonica/geng genomes analysis indicates the importance of large structural variants in rice breeding

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Temperate japonica/geng (GJ) rice yield has significantly improved due to intensive breeding efforts, dramatically enhancing global food security. However, little is known about the underlying genomic structural variations (SVs) responsible for this improvement. We compared 58 long-read assemblies comprising cultivated and wild rice species, revealing 156,319 SVs. Integrating the SVs and causal genetic variants underlying agronomic traits into the analysis enables the precise identification of breeding signatures resulting from complex breeding histories aimed at stress tolerance, yield potential, and quality improvement. The current study provides genomic resources for retracing the properties of SVs-shaped agronomic traits during previous breeding procedures, which will assist future genetic, genomic, and breeding research on rice.

Keywords: japonica/geng; de novo assembly; structural variations

Identification and breeding utilization of elite natural variations in rice

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Unveiling the genetic diversity within the crop gene pool presents breeders with novel resources that are instrumental in enhancing varietal development. The detailed characterization of intricate structural variations within the rice genome is of paramount importance for propelling advancements in functional genomics research. Leveraging the core collection of rice germplasm, we have constructed a graph-based pan-genome that has enabled us to tackle the challenges associated with the identification and application of complex structural variations. This approach has led to the establishment of a robust gene-identification pipeline, which serves as an innovative platform for rice functional genomics research. Building upon this foundation, we have successfully identified novel genes associated with yield through the analysis of multiallelic and rare genetic variations, such as TGW2, TRGW6, GL11, GW10.2. Furthermore, by integrating multi-omics data, we have efficiently revealed new genes STG5 and OsMADS56 that confer salt tolerance. In addition, through collaborative efforts, we have designed a new salt-tolerant rice cultivar, Lu Yan Dao 13, which has demonstrated a yield increase of approximately 30% compared to prevalent cultivated salt-tolerant varieties. Our results provide a solid foundation for the synergistic improvement of salt-tolerant yields and contribute to the cultivation of new salt-tolerant varieties.

Keywords: Germplasm resources; Pan-genome; Salt-alkaline tolerance; Yield traits; Elite natural variations

Revealing Stage-Specific Transcriptional Signatures during Flag Leaf Senescence in Rice (*Oryza sativa*)

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Leaf senescence in rice coordinates nutrient remobilization to developing grains. To dissect its molecular basis, we compared flag leaf transcriptomes at heading and 5 weeks after heading (5W-AH). GO and MapMan analyses revealed stage-specific signatures: heading-stage genes were enriched in jasmonic acid biosynthesis, oxylipin metabolism, and glutathione metabolism, indicating a primed defense and stress-responsive state. In contrast, 5W-AH genes were enriched in ion transport, carbohydrate metabolism, and plastid reorganization, reflecting a metabolic shift toward nutrient transport and grain filling. A protein–protein interaction (PPI) network was constructed from 213 Leaf Senescence Database proteins and 1,316 5W-AH up-regulated proteins. STRING analysis (score ≥ 0.7) identified interconnected modules linking chlorophyll degradation, nitrogen metabolism, amino acid biosynthesis, and redox regulation. CAO, associated with SGR and HCAR, was connected to "plastid organization," consistent with active chloroplast dismantling. NIA1 acted as a central hub bridging amino acid metabolism, nitrogen assimilation, and redox modules via APRL1, OsPHY2, and OsLASPO.

These findings reveal a coordinated regulatory framework integrating stress signaling, nutrient mobilization, and metabolic reprogramming, offering insights into genetic networks sustaining late-stage senescence and resource allocation in rice. This work provides molecular targets for improving nutrient remobilization efficiency and optimizing senescence timing to enhance rice yield and quality.

Keywords: Flag leaf senescence; Stage-specific gene expression; RNA-seq; Protein–protein interaction (PPI) network; Dark-induced senescence (DIS)

Discovery of a Compact Root-Specific Enhancer Element in rice for Promoter Engineering and Crop Improvement

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Root-specific promoters with strong activity are valuable tools for plant functional studies and agronomic-trait engineering. The Os1-CysPrxB promoter has been previously utilized as a robust root-specific promoter in rice. In this study, we dissected the 1.9 kb promoter region including the first exon and intron and identified a short fragment (pΔ) essential for its root-specific transcriptional activity. Promoter deletion assays using GUS reporters and in-silico cis-element analysis revealed that pΔ lacks known root-related motifs, yet drives strong expression specifically in root tissues. Functional validation using a RUBY reporter fused with a minimal 35S promoter confirmed that pΔ confers high-level, root-specific expression in vivo. This suggests that pΔ functions as a compact and effective root-specific regulatory module. Given its small size and expression strength, pΔ holds potential as a candidate short transcriptional enhancer (STE) for CRISPR-Cas-mediated knock-in strategies. Recent studies have demonstrated that insertion of short STEs enables transgene-free, heritable upregulation of endogenous genes in rice. In this context, pΔ may serve as a root-specific enhancer element for in-locus activation, allowing precise control of gene expression without introducing foreign DNA. We are currently refining the pΔ sequence through fragmentation analysis to identify the minimal functional region responsible for its activity. Our results provide new insights into root-specific gene regulation and offer a valuable genetic resource for future applications in genome editing and plant synthetic biology.

Keywords: root-specific enhancer; promoter engineering; rice; minimal regulatory element

Genetic diversity and evolution of rice centromeres

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Understanding the driving force of centromere dynamics is crucial for deciphering the complexity of eukaryotic evolution and speciation. Here, we assembled 67 rice genomes from the Oryza AA group, and analyzed over 800 nearly complete centromeres. Through de novo annotation of centromeric satellite CEN155 sequences and employing a progressive compression strategy, we quantified the local homogenization and multi-layer structures of rice satellite arrays. Our results indicate that genetic innovations in rice centromeres primarily arise from structural variations and centrophilic retrotransposon insertions. Single-base substitution rate in rice centromeres appears relatively lower relative to chromosome arms. Comparisons of CEN155 arrays, retrotransposons, and functional centromeres highlight their dynamic but correlated interplay. Contrary to the KARMA model for Arabidopsis centromere evolution, we propose a hypothesis that retrotransposon invasion probably contributes to the decline of progenitor centromeric satellite arrays, and promotes centromere repositioning, as evidenced by extended CENH3 ChIP-seq enrichment beyond the native satellite arrays.

Keywords: Rice; Centromeric satellite DNA; Retrotransposon invasion; CENH3 ChIP-seq

Genomic investigation of 18K lines reveals the genetic architecture of rice

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Although many genes have been functionally identified, a global view of genetic architecture, including the number of genes affecting a trait, genetic effects and their interactions with each other, remains lacking in rice. To address this, we established a permanent population of 18,421 rice lines (18K-rice) with reduced population structure. We generated reference-level genome assemblies for the founders, and obtained high-density genotypes of all 18K-rice lines through whole-genome sequencing. In total, we mapped 1,207 quantitative trait loci (QTL) for 16 agronomic traits and developed an integrated genomics method (RiceG2G) to prioritize causal genes. Of 1,207 QTL, 28.0% contained known genes, suggesting that previously identified quantitative trait genes are a small proportion of the total set in rice. For panicle number and heading date, we experimentally validated two newly identified causal genes, OsMADS22 and OsFTL1. Furthermore, we constructed a genetic interactome using 18K-rice, where 170 masking genes were implicated in the cause of genetic background effects. We estimated that additive and epistatic effects of identified QTL collectively explained 49.9% and 2.2% of phenotypic variation, respectively. In contrast, the genomic heritability accounting for additive and epistatic effects was estimated to be 56.2% and 8.8%, respectively. Regarding the overall genetic architecture, additive effects are the main force in shaping rice traits, while the genotype-to-phenotype relationship becomes complex in the presence of numerous genetic interactions. These findings advance our understanding of rice genetics and provide a powerful basis to guide rice improvement.

Keywords: Rice; Quantitative traits; Genetic architecture; Genetic interactions; Epistatic effects; 18K rice lines

Deciphering rice feralization: insights from genomics of weedy rice

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Worldwide feralization of crops into agricultural weeds poses a serious threat to global food security. Weedy rice, a feral form of rice, infests paddies globally and outcompetes cultivated varieties. However, the genomic basis of this transformation remains poorly understood. By analyzing 524 global weedy rice samples from all major rice-growing regions, we found that weed populations have evolved multiple times from cultivated rice. Notably, some aggressive Asian strains trace back to recently discontinued Green Revolution cultivars like “Nanjing11”. Genome scans reveal that feralization mainly involves changes at loci not selected during domestication. Strikingly, independently evolved strains share “de-domestication” genomic regions, including genes linked to seed dormancy and allergenic proteins, suggesting parallel adaptation.

Moreover, we leveraged knowledge of the specific cultivar progenitor “Nanjing11” of a weedy rice population from East China (EastCN-WR) to dissect the process of recent rice feralization. We documented the role of adaptive introgression from local rice landraces like “Hanmadao” in the evolution of these weedy strains, including the Rc gene, which confers the key weed traits of red pericarp and seed dormancy. Importantly, the progenitor cultivar of EastCN-WR is characterized by a low genetic load, which may have made it a “naturally selected” material, conferring strong fitness for weedy rice feralization. Overall, our study provides a more refined genomic perspective on the origin of weedy rice and highlights landrace introgression as a particularly critical factor driving modern cultivar feralization.

Keywords: Weedy Rice; Feralization; Adaptive introgression; De-domestication block

Research on YSL Gene Function in Rice

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YSL are widely present in eukaryotes and have relatively conserved sequences, however, only homologous genes in *Homo sapiens* and *Arabidopsis thaliana* have been characterized. We found a pair of *YSL* homologous genes named as *OsYSL1* and *OsYSL2* in rice. The knockout mutants of *OsYSL1* and *OsYSL2* showed a decrease in plant height and tiller number, suggesting that *OsYSL* may be involved in regulating the elongation and tillering process of rice. Gene expression profiling analysis showed that the expression of *OsYSL* was quite different in various parts and at various stages, and its expression patterns in roots and leaves were different. Furthermore, the expression of the other unknocked gene increased in the knockout mutants, indicating that there may be a synergistic or complementary relationship between *OsYSL1* or *OsYSL2*. OsYSL1 and OsYSL2 are localized in the nucleus and cell membrane. The results of subcellular localization were consistent with YSL in Arabidopsis thaliana, which indicates that YSL is evolutionarily conserved. Phylogenetic analysis showed that only plants of Poaceae widely have two *YSL* homologous genes with low homology, indicating that sub-functionalization or neofunctionalization may occur.

Keywords: *Oryza sativa*; Dwarf; Low-tillering

Haploid genome activation exposes genes with a specific chromatin signature for pollen selection

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Pollen, the male gametophyte, is developed from haploid microspore after meiosis, transcribes its haploid genome and displays independence and phenotype diversity. However, the molecular basis underlining the male germline development and haploid genome activation remains unclear. Here we show that the process of rice male germline development is accompanied by new transcription of cell type-specific genes with chromatin states primarily set up before meiosis. Haploid genome activation occurs shortly after meiosis in the undifferentiated microspore that displays overall enhanced open chromatin with increases of H3K4me3 and decreases of CHH methylation relative to meiocytes. Genes activated from the haploid genome show clear depletion of body CG methylation that is enriched in sperm repressed genes and marks mainly evolutionarily conserved genes. Thus, rice haploid genome activation exposes genes biased with high nonsynonymous mutation rates for haploid selection, which may have implication in reducing inbreeding depression and increasing offspring fitness.

Keywords: Male gametophyte; Meiocyte; Microspore; Sperm; Haploid genome activation; Epigenome; DNA methylation; Chromatin

The International *Oryza* Map Alignment Project: *Oryza* genome evolution through a tetraploid lens

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Oryza is a remarkable genus comprising 27 species and 11 genome types, with ~3.6-fold genome size variation, that possesses a virtually untapped reservoir of genes that can be used for crop improvement and neodomestication. Here we present 11 chromosome-level assemblies (nine tetraploid, two diploid) in the context of ~15 million years of evolution and show that the core *Oryza* (sub)genome is only ~200 Mb and largely syntenic, whereas the remaining nuclear fractions (~80–600 Mb) are intermingled, plastic and rapidly evolving. For the halophyte *Oryza* coarctata, we found that despite detection of gene fractionation in the subgenomes, homoeologous genes were expressed at higher levels in one subgenome over the other in a mosaic form, demonstrating subgenome equivalence. The integration of these 11 new reference genomes with previously published genome datasets provides a nearly complete view of the consequences of evolution for genome diversification across the genus.

Decoding and modeling the rice pan-regulome guides rational trait engineering

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Understanding cis-regulatory elements and their interacting trans-factors is essential for rational crop improvement, yet these remain largely uncharacterized in rice. Here we decode the rice pan-regulome through paired RNA-seq and ATAC-seq analysis of 1-2 mm young panicles from 219 varieties, revealing 151,844 open chromatin regions (OCRs) that mediate genetic effects on gene expression. To systematically identify functional regulatory elements, we developed a computational framework that associates OCR accessibility with gene expression patterns. This approach successfully mapped putative cis-regulatory regions for 73.2% of expressed genes, with 20.5% showing causal relationships. However, determining which variants drive these regulatory effects remained challenging. We addressed this challenge by developing pan-genome-aware deep learning models trained on 40 varieties, each with matched genome sequences and ATAC-seq profiles, to predict variant impacts on chromatin accessibility in different genetic contexts. The models achieved remarkable accuracy and revealed that transcription factor binding motif disruptions constitute the primary mechanism of regulatory variation. Critically, ensemble predictions across multiple varieties outperformed single-variety models, demonstrating the power of population-aware modeling. To validate our computational predictions, we used CRISPR/Cas9 to edit model-identified regulatory elements. Disrupting a predicted repressive element upstream of *APO1* increased grain number per panicle. Similarly, editing regulatory regions of *IPA1* and *PAP2* produced the predicted changes in panicle architecture. Our pan-regulome resource reveals the cis- and trans-regulatory landscape in rice, establishing an AI-driven blueprint for rational trait engineering.

Keywords: pan-regulome, deep learning, chromatin accessibility, rational design

OsbHLH064, an IVb subgroup bHLH transcription factor, regulates iron homeostasis in rice

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Iron (Fe) is an essential micronutrient for plant growth and development. The maintenance of Fe homeostasis relies on sophisticated regulatory networks where bHLH transcription factors play a key role. However, how these factors coordinate to regulate this vital process is not fully understood. Here, molecular, genetic, biochemical and imaging approaches were used to investigate the role of OsbHLH064 in the regulation of Fe homeostasis in rice.It showed that the nuclear accumulation of OsbHLH064 was promoted by its interaction with subgroup IVc bHLHs (*i.e.*, OsPRI1 to 4), that are positive regulators of the Fe deficiency response. It also revealed a negative correlation between Fe availability and OsbHLH064 expression in roots, and that high level of OsbHLH064 expression leads to hypersensitivity to Fe deficiency and Fe overload. We then found that OsbHLH64 directly represses the expression of both regulatory and structural genes involved in the maintenance of Fe homeostasis. This mechanism relying on the binding of OsbHLH64 to cis-regulatory sequences targeted by OsPRIs, allowing counteracting their activity. Altogether, the data presented herein supports that OsbHLH064 function as a central upstream regulator of the Fe homeostasis network, coordinating diverse aspects of Fe uptake, Fe translocation, and Fe signaling.

Keywords: bHLH; Iron homeostasis; Transcriptional regulation

Loss of function of pollen-expressed phospholipase OsPLDα2 triggers haploid induction in rice (*Oryza sativa*)

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Doubled haploid (DH) technology has revolutionized plant breeding by enabling the fixation of complete homozygosity within a single generation. Elucidating the molecular mechanisms underlying haploid induction is therefore a major goal for optimizing DH systems in major crops. Phospholipase D (PLD) is known to hydrolyze phospholipids to produce phosphatidic acid, which plays critical roles in membrane remodeling, intracellular signal transduction, and homeostatic regulation in plants. However, the function of PLD in reproductive processes remains largely uncharacterized. A recent study revealed that ZmPLD3, a pollen-specific PLD in maize, functions as a maternal haploid inducer, suggesting the need for further investigation into the reproductive roles of pollen-specific PLDs. In this study, we aimed to investigate whether *OsPLD$_{α2}$*, the rice homolog of *ZmPLD3*, retains an evolutionarily conserved function and whether it plays a role in rice reproduction, particularly in pollen function and fertilization. Phylogenetic analysis revealed that *OsPLD$_{α2}$* is closely related to *ZmPLD3* and retains conserved features essential for PLD enzymatic activity, including a C2 domain and two HKD motifs. Public expression database showed that *OsPLD$_{α2}$* is highly expressed in mature pollen, and this pattern was confirmed through GUS assay and qRT-PCR analysis. To examine its function, we generated *OsPLD$_{α2}$* knockout mutants using the CRISPR/Cas9 system. The mutants exhibited normal vegetative growth but showed a significant decrease in fertility and induction of haploids. These results demonstrate that *OsPLD$_{α2}$* is involved in male fertility in rice, providing a foundation for future studies on the reproductive functions and haploid induction potential of pollen-specific PLDs.

Keywords: Haploid induction; Phospholipase D; Pollen; Rice fertilization; *Oryza sativa*

miRNA-Mediated Gene Regulation and Agricultural Applications in Rice

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Owing to their precise and efficient modulation of gene expression, miRNAs have emerged as promising tools for fine-tuning complex agronomic traits in crops. Among these, we identified a novel mature miRNA, miR408-5p, in rice and demonstrated that it modulates an alternative auxin signaling pathway through functional mode switching, which provides new insights into the mechanistic diversity and cooperative networks of miRNA-mediated regulation in plants. We also revealed that miR528 and its target UCL23 are key regulators of Cd uptake, tolerance, and accumulation in rice, which offers a promising strategy to reduce Cd accumulation, facilitating the development of low-Cd rice varieties for safer crop production.

Beyond miR408 and miR528, miR397 belongs to a group of Cu-miRNAs that target genes encoding copper-containing proteins in monocots. In rice, 18 out of 28 Laccase (LAC) genes, which encode multicopper oxidases essential for lignification, are predicted targets of miR397, miR408, and miR528. We found that co-overexpression of these three miRNAs (3miR-OE) in transgenic rice lines not only suppresses LAC gene expression but also alters the transcriptional landscape of lignin catabolism and phenylpropanoid metabolism. Notably, 3miR-OE transgenic Japonica and Indica rice exhibited significantly enhanced tolerance to cold and drought stresses compared to wild-type and single-miRNA overexpressors, highlighting a novel strategy for improving crop stress resilience through the coordinated manipulation of multiple miRNAs.

Collectively, our findings reveal that the intricate regulatory networks mediated by miR408, miR528, and miR397 underscore the potential of miRNA-based approaches for rice improvement.

Keywords: Cu-miRNAs; target regulation; Cadmium; tolerance; rice

A greener agriculture governed by understanding the auxin-nitrogen module

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The application of nitrogen (N) fertilizers is crucial for boosting crop production, but excessive use increases production costs and harms the environment. Enhancing crop N-use efficiency (NUE) is therefore vital for sustainable agriculture. Here, we characterize the rice NUE quantitative trait locus DULL NITROGEN RESPONSE1 (qDNR1), which regulates auxin homeostasis and contributes to differences in nitrate uptake, N assimilation, and yield between indica and japonica rice varieties. We further show that REGULATOR OF N-RESPONSIVE ROOT SYSTEM ARCHITECTURE 10 (RNR10) monoubiquitinates and stabilizes DNR1, which suppresses auxin accumulation. This process fine-tunes N-responsive root development and activates auxin response factors on N metabolism genes. The DNR1 regulatory network is further expanded by transcriptional control from OsWRKY23 and MYB-family protein SOD5. Thus, our dissection of this multi-layered DNR1-auxin-NUE regulatory module provides direct targets for breeding high-NUE rice varieties to enhance agricultural sustainability.

Keywords: Auxin; NUE; Grian yield; Rice

A fantasy journey to the secrets of Gγ tails

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Heterotrimeric G-proteins act as molecular switches in signal transduction in response to stimuli in all eukaryotes. However, what specifies G-protein signaling in plants and how the mechanism evolved and diverged remain unsolved. Here, we found that the recently evolved tails of three Gγ subunits, Dense and erect panicle 1 (DEP1), G-protein gamma subunit 2 of type C (GGC2), and Grain size 3 (GS3), determine their distinct functions and specify grain size in rice (Oryza sativa L.). These Gγ subunits originated and expanded by an ancestral σ duplication ~130 million years ago (mya) and a pancereal ρ duplication ~70 mya in monocots, increasing genome complexity and inspiring functional innovations. In particular, through the comprehensive creation of artificial chimeric Gγ proteins, we found that this signaling selectivity is driven by repetitive elements and a link region hidden in plant-specific Gγ tails, allowing crops to switch from positive regulation to negative control. Unlike the tails, the conserved Gγ heads did not bias the signaling specificity; however, the change in the interaction between the mutated Gβ and Gγ affected the subsequent downstream signal transduction and grain size. Manipulating G-protein signaling also affects organ size in maize (Zea mays) and is expected to constitute a general mechanism for crop improvement. Collectively, these findings reveal that plant-specific Gγ tails drive signaling selectivity and serve as valuable targets for optimizing crop traits through G-protein manipulation.

Keywords: G-proteins; Rice; Grain Size

QTL analysis using segregation population of autotetraploid rice

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Tetraploid rice (2n=4x=48) is generated by genome doubling of diploid rice (2n=2x=24) and is known to exhibit increased biomass and enhanced stress tolerance. Despite its potential as a future breeding resource, tetraploid rice shows complex inheritance patterns and reduced fertility, limiting its agricultural use. To establish a foundation for tetraploid rice research, we developed a QTL mapping approach using the GRAS-Di method and performed comparative analyses of QTLs detected in diploid and tetraploid populations. Diploid and autotetraploid lines of 'Senichi' (japonica) and 'Dular' (indica), along with two F_2 populations (80 lines each) derived from reciprocal crosses, were used for genotyping and phenotyping. Genotypes were determined using GRAS-Di, and traits such as heading date, grain size, and seed fertility were analyzed using generalized linear regression models. Significant differences were observed between ploidy levels across many traits. While parental tetraploids showed significantly reduced seed fertility, this was not observed in the tetraploid F_2 populations. We identified 8,897 and 8,882 genome-wide SNPs in the diploid and tetraploid populations, respectively, and their segregation ratios closely matched theoretical expectations. QTL analysis detected significant SNPs near *Hd1, Hd2* (heading date), and *GW5* (grain size) in diploids. In tetraploids, only SNPs near *GW5* were detected. Whole-genome sequencing revealed sequence difference near ploidy-specific QTLs, explaining the difference in QTL detection. This study also reports QTLs associated with seed fertility in tetraploid populations, providing insights into the genetic basis of fertility restoration in tetraploid rice.

Keywords: QTL; Agronomic traits; Ploidy; Tetraploid

During the development of rice anther and seed, cell wall invertases from maternal tissues regulate sucrose flux in apoplastic pathways

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Efficient sucrose transport and metabolism are vital for seed and pollen development in plants. Cell wall invertases (CINs) hydrolyze sucrose into glucose and fructose, maintaining a sucrose gradient in the apoplast of sink tissues. OsCIN1 and OsCIN2, two CIN isoforms in rice, have been identified to be exclusively expressed in the anther but not in pollen. Through genetic crosses and mutant characterization, functional investigations revealed that oscin1/2 double mutants generate shrunken seeds and have a sporophytic male-sterile phenotype. This implies that appropriate pollen development and seed formation in rice depend on CIN activity. The phenotype of oscin1/2 seeds is influenced by the genotype of the mother tissue, according to observations of the progeny genotypes and phenotypes from different genetic crosses. This means that CIN function in the apoplast between the mother and filial tissues is crucial for sucrose transport and metabolism. It was discovered that the CIN activity in wild-type rice's anthers and seeds was much higher than that in the leaves, more than 500 times in the anthers and 5 times in the seeds. This highlights the significance of CIN in promoting the effective unloading of sucrose. These findings indicate that fine-tuning of CIN activity in the apoplast, via tissue-specific expression and CIN isoform control, is critical in directing carbohydrate distribution throughout tissues. Gaining insight into this regulation process may make it possible to control the distribution of carbohydrates to sink organs, which could increase agricultural yields.

Keywords: OsCIN1; OsCIN2; Sucrose transport; Sucrose metabolism; Seed development; Pollen development

Enhanced Accumulation of Iron and Zinc in Rice through Simultaneous Activation of OsNAS2 and OsNAS3

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Rice nicotianamine synthases (OsNASs) have the key roles in biosynthesizing nicotianamine (NA) and 2′-deoxymugineic acid (DMA), which play crucial roles in chelating essential minerals such as iron (Fe) and zinc (Zn) and maintaining metal homeostasis. Here, we developed a transgenic plant (*23D*) by crossing two T-DNA insertional activation-tagged mutants, namely *OsNAS2-D1* (*2D*) and *OsNAS3-D1* (*3D*), resulting in simultaneous activation of *OsNAS2* and *OsNAS3*. The *23D* plants exhibited the highest concentrations of Fe and Zn in shoots and roots, both under normal and also Fe and Zn deficient conditions. Expressions of genes involved in the biosynthesis of mugineic acid family phytosiderophores (MAs) and the uptake of Fe and Zn were enhanced in the roots of *23D* seedlings. Moreover, the *23D* plants demonstrated enhanced growth compared to wild type (WT), *2D* and *3D* in higher pH levels. Notably, the mature brown grains of *23D* plants had significantly higher levels of NA and DMA, being 50.6-fold and 10.0-fold higher than those in the WT. Consequently, the mature brown grains of the *23D* plants contained 4.0- and 3.5-times higher Fe and Zn contents, respectively, compared to WT grains. Moreover, the *23D* plants exhibited superior resistance in the presence of excessive metals. These findings suggest that the concomitant activation of *OsNAS2* and *OsNAS3* can enhance Fe and Zn accumulation in rice grains and also improve plant's tolerance to metal deficiency and metal toxicity.

Keywords: iron; zinc; nicotianamine; biofortification

CTP synthase enzyme activity regulates rice seed yield and quality

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Understanding the molecular processes governing early seed development is essential for improving grain yield and quality in crop plants. The endosperm, abundant in starch and protein, constitutes the main part of the grain and is vital for human nutrition. However, the mechanisms controlling nuclear division during early endosperm development remain largely unclear, which affects seed size determination. In our previous study, we identified the *endospermless 2* (*enl2*) mutant in rice, characterized by the absence of endosperm. This phenotype results from mutations in *OsCTPS1*, encoding cytidine triphosphate synthase (CTPS). OsCTPS1 assembles macromolecular structures in the endosperm at early developmental stages, indicating a key structural role. Overexpression of *OsCTPS1* enhanced seed weight by stimulating endosperm nuclear division. Moreover, increased CTPS activity positively correlates with beneficial agronomic traits such as reduced plant height, earlier heading date, longer panicle length, and increased tiller number. Among six *CTPS* genes in rice, only *OsCTPS1*'s function has been characterized. Comparative amino acid analysis revealed variations at residues critical for enzyme activity regulation, especially at the 36th position. The S36A mutation in OsCTPS1 caused a marked decrease in CTPS activity, whereas replacing the corresponding threonine with a phosphorylatable serine (T36S) in OsCTPS2 increased enzyme activity and seed size. Building on these insights, we are engineering key regulatory sites in CTPS proteins to develop rice varieties with improved agronomic traits, aiming to enhance both yield and quality for sustainable crop production.

Keywords: endosperm development; CTP synthase; grain yield; grain quality; agronomic trait improvement

SUMOylation of OsPSTOL1 is essential for regulating phosphate starvation responses in rice and Arabidopsis

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Recently, in rice it was shown that a specific serine-threonine kinase known as *Phosphorus – starvation tolerance 1* (*PSTOL1*) is important for conferring low phosphate tolerance in rice. Nonetheless, knowledge about the mechanism underpinning PSTOL1 activity in conferring low Pi tolerance is very limited in rice. Post Translation Modifications (PTMs) play an important role in plants in providing a conduit to detect changes in the environment and influence molecular signaling pathways to adapt growth and development. In recent years the PTM SUMOylation has been shown to be critical for plant growth and development. It is known that plants experience hyper SUMOylation of target proteins during phosphate starvation. Here we demonstrate that PSTOL1 is SUMOylated *in planta*, and this affects its phosphorylation activity. Further, we also provide new evidence for the role of SUMOylation in regulating PSTOL1 activity in plant responses to Pi starvation in rice and *Arabidopsis*. Our data indicated that overexpression of the non – SUMOylatable version of OsPSTOL1 negatively impacts total root length and total root surface area of rice grown under low Pi. Interestingly, our data also showed that overexpression of OsPSTOL1 in a non-cereal species, *Arabidopsis* also positively impacts overall plant growth under low Pi by modulating root development. Taken together our data provide new evidence for the role of PSTOL1 SUMOylation in mediating enhanced root development for tolerating phosphate limiting conditions.

Keywords: SUMO, phosphate, signaling, stress

Functional Reprogramming and Epigenetic Regulation of Histone H3K4me1 in Rice

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Monomethylation of histone H3 lysine 4 (H3K4me1) marks enhancers in mammals. However, the function of H3K4me1 in plants remains largely unclear. Here, we analyzed the genome-wide distribution of H3K4me1 in diverse species across evolution, revealing that H3K4me1 displayed a distinctive genome-wide distribution pattern in land plants, especially in rice compared with animals. To explore the function of H3K4me1 in plants, we identified an H3K4me1-specific reader protein, Early heading date 3 (Ehd3) in rice, and solved the structure of Ehd3 in complex with the H3K4me1 peptide, revealing a unique binding module differing from the previously reported PHD finger proteins. We further identified an Ehd3-binding protein, SET domain group 724 (SDG724), and the deletion of either *Ehd3* or *SDG724* caused similar defects in plant phenotype and changes in transcriptome and epigenome profiles. Both Ehd3 and SDG724 are enriched at chromatin regions marked by H3K4me1 but not H3K4me2 or H3K4me3. Ehd3 activates the H3K36 methyltransferase SDG724, and H3K36me2/me3 are colocalized with H3K4me1 in the genomes of rice. Collectively, our results reveal that H3K4me1 directs the establishment of H3K36me2 and H3K36me3 in rice.

Synthetic apomixis with normal seed setting in hybrid rice by genome editing

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The development of synthetic apomixis enables the fixation of heterosis, representing a breakthrough that promises to transform conventional hybrid breeding strategies and drive a new wave of agricultural green revolution. Current engineered synthetic apomixis system relying solely on genome editing exhibits compromised fertility, limiting their practical utility. Here, we demonstrate that loss-of-function mutations in *OsPLDα2*, an endogenous gene specifically expressed in mature pollen, induce haploid formation in rice. By integrating *MiMe* genes with *OsPLDα2* via genome editing, we developed a novel apomixis system, designated *Fix4* (*Fixation of Hybrids 4*). The *Fix4* system generates stable and heritable clonal seed in hybrid rice, and notably displays normal seed-setting rates, offering both theoretical foundations and innovative strategies to accelerate the application of apomixis in hybrid rice breeding.

Keywords: Synthetic apomixis, Phospholipase Dα2, Normal seed-setting, Genome editing, Hybrid rice

BioDeepDiscovery (BDD): An AI Scientist for Accelerated Biological Discovery

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BioDeepDiscovery (BDD) is an AI-powered scientific research assistant designed to accelerate biological discovery by integrating literature mining, hypothesis generation, workflow design, and automated analysis. The platform incorporates over 40 million domain-specific research articles and more than 1,900 bioinformatics tools, enabling it to construct rich biological knowledge graphs and provide comprehensive support across a wide range of biological topics. By leveraging foundation models, BDD helps researchers uncover hidden insights, generate testable hypotheses, and design and run customized computational workflows. It empowers life science research with an open, interoperable computing environment that streamlines the journey from scientific question to actionable insight. Based on BDD, we combine wet experimental approaches with a suite of AI-driven computational methods, including mining for depalmitoylating enzymes, structural modeling with palmitic acid, identification of critical enzymatic site, and high-throughput virtual screening of inhibitory compounds, to elucidate the enzymatic details underlying how palmitoylation, a reversible post-translational lipid modification, regulates viral invasion in plant. We also demonstrate the application of AI-based mining and designing to discover and design novel luciferases that exhibit significantly enhanced potency compared to known luciferases.

Keywords: AI; Scientific research assistant; Agent

Efficient plant genome engineering using a probiotic sourced CRISPR-Cas9 system

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Among CRISPR-Cas genome editing systems, *Streptococcus pyogenes Cas9* (SpCas9), sourced from a human pathogen, is the most widely used. Here, through in silico data mining, we have established an efficient plant genome engineering system using CRISPR-Cas9 from probiotic *Lactobacillus rhamnosus*. We have confirmed the predicted 5'-NGAAA-3' PAM via a bacterial PAM depletion assay and showcased its exceptional editing efficiency in rice, wheat, tomato, and Larix cells, surpassing LbCas12a, SpCas9-NG, and SpRY when targeting the identical sequences. In stable rice lines, LrCas9 facilitates multiplexed gene knockout through coding sequence editing and achieves gene knockdown via targeted promoter deletion, demonstrating high specificity. We have also developed LrCas9-derived cytosine and adenine base editors, expanding base editing capabilities. Finally, by harnessing LrCas9's A/T-rich PAM targeting preference, we have created efficient CRISPR interference and activation systems in plants. Together, our work establishes CRISPR-LrCas9 as an efficient and user-friendly genome engineering tool for diverse applications in crops and beyond.

Keywords: probiotic sourced CRISPR-Cas9; A/T rich PAMs; plant genome editing

carbon dots-mediated transient seed transformation for genomic studies in plants

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Genotype restriction poses a significant bottleneck to stable transformation in the vast majority of plant species, thereby severely impeding advancement in plant bioengineering, particularly for crops. Nanoparticles (NPs) can serve as effective carriers for the transient delivery of nucleic acids, facilitating gene overexpression or silencing in plants in a genotype-independent manner. However, the applications of NP-mediated transient systems in comprehensive genomic studies remained underexplored in plants, especially in crops that face challenges in genetic transformation. Consequently, there is an urgent need for efficient NP-mediated delivery systems capable of generating whole plants or seedlings with uniformly transformed DNA. Here we developed a straightforward and efficient carbon dots mediated transient transformation system for delivering DNA plasmids into the seeds of rice, wheat, and other plant species as well. This system facilitates the generation of whole seedlings that contain the transferred DNA plasmids. Furthermore, our study demonstrates that this system serves as an excellent platform for conducting functional genomic studies, including the validation of gene functions, protein interactions and regulation and genome editing. This advancement significantly enhances functional genomic research for any plants or crops that face challenges in stable transformation.

Keywords: carbon dots; transient seed transformation; genome editing

AI-driven phenotypic prediction modeling for rice breeding programs

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Asian rice (*Oryza sativa*) is a staple crop that feeds nearly half of the global population. To help ensure global food security and precision breeding programs, we developed a set of predictive models for key agricultural traits, including grain length, grain width, grain length-to-width ratio, grain weight, and flowering time. Artificial intelligence (AI) was employed as a new multidisciplinary approach to apply machine learning (ML) and deep learning (DL) techniques in training these models. The genotypes and phenotypes of five traits were collected for more than ten thousand rice accessions, representing the largest training dataset for rice to date. A uniform modeling workflow from genomics to phenotypes was designed, which used four ML models and two DL models. Model performance was evaluated using accuracy, error rates, and training time through baseline modeling, two rounds of five-fold nested cross-validation, and one standard ten-fold cross-validation. Finally, we developed models for phenotype prediction with the highest accuracy compared to any publicly available models to date. Notably, the models could predict phenotypes for five thousand samples within just 300 milliseconds. The external validation using predicted and observed data (field-collected) confirmed strong correlations for grain length and grain width in all five environments and heading date in three out of six environments, based on 710 samples. In summary, our study developed accurate phenotypic prediction models that could be used to accelerate rice breeding and contribute to enhanced food security.

Keywords: Agricultural Traits Prediction Models; Machine Learning; Rice breeding; Food Security

Hyper Cloning: An efficient way to construct CRISPR/Cas9 vectors with multiple targets

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Hyper Cloning is a method that enhances cloning efficiency compared to the Speed Cloning method. Due to genetic redundancy in higher plants, multiple genes often need to be knocked out to reveal their functions. To mutate multiple genes in rice, the CRISPR/Cas9 system with polycistronic tRNA-gRNA (PTG) arrays is widely used. Previously, we reported the Speed Cloning method, which utilizes Gibson assembly to construct CRISPR/Cas9 vectors targeting multiple sites with a tandem-arrayed tRNA-gRNA structure. Although the Speed Cloning method is effective for constructing multi-target CRISPR/Cas9 vectors, we found that it could be further improved by reducing the number of unnecessary fragments. In the Hyper Cloning method, we reused the gRNA from the pRGEB32t vector instead of that from the pGTR vector. As a result, the final vector generated by Speed Cloning contains two gRNAs at the end, whereas the vector generated by Hyper Cloning contains only one. Therefore, Hyper Cloning requires one fewer fragment than Speed Cloning. For example, when designing a CRISPR/Cas9 vector targeting four sites, five fragments (including the enzyme-digested vector) are needed for the Speed Cloning method, but only four fragments are needed for Hyper Cloning. This reduction significantly increases cloning efficiency in constructs with three, four, and five targets. We also experimentally investigated the relationship between overlapping base pairs (20 bp and 24 bp) and cloning efficiency. In vectors targeting four and five sites, 20 bp overlaps resulted in higher cloning efficiency than 24 bp overlaps, whereas in three-target constructs, there was no significant difference.

Keywords: CRISPR/Cas9; Multiple Genome Editing; Cloning Efficiency; polycistronic tRNA–gRNA (PTG)/Cas9 system; Rice

Unifying Rice Diversity: The GrameneOryza 2025 Pan-Genome Platform for Research and Community Engagement

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Gramene Plants (www.gramene.org), a comparative genome browser for plants, was established 21 years ago following the landmark sequencing of the first complete crop genome, *Oryza sativa* Japonica. In response to the growing number of sequenced rice genomes, Gramene Plants launched the GrameneOryza[1] pan-genome portal (oryza.gramene.org) in 2008. Developed in collaboration with AGI, MSU, USDA-ARS, IRRI, RAP-DB, I-OMAP, and PanOryza, the platform supports rice genomics and breeding through integrated tools for comprehensive term-based search, sequence alignment, gene annotation, expression data, gene trees, function projection, synteny mapping, genetic markers, germplasm resources, and community curation.

In the era of pan-genomes, GrameneOryza currently hosts 28 wild and domesticated Oryza genomes, many of which are of the highest assembly quality, including those from the MAGIC16 panel[2]. The platform also aligns with FAIR principles[3], aiming to make data Findable, Accessible, Interoperable, and Reusable. The latest release (R8) incorporates 68 million rsIDs from EVA[4] release 6 into its variation module. SNPs and genotypes from global studies (e.g., 3K-RGP, USDA Mini-Core, World and Japanese Rice Collections) are integrated, with gene consequences predicted. This enables the Gramene Search Interface to identify germplasms carrying loss-of-function alleles and direct users to stock center websites for seed acquisition.

New features, such as Oryza CLIMtools[5], allow users to explore genotype-environment associations across curated germplasm. GrameneOryza continues to support community engagement through data stewardship, training, and feedback coordination. The project is supported by USDA-ARS (8062-21000-051-000D).

Keywords: Genome Database; FAIR principle; Genetic variation and rsID; CLIMtools

Targeted Protein Degradation and Protein-condensate Degradation for Plant Science and Crop Breeding

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Gene expression can be modulated at the DNA, RNA, or protein level, with targeted protein degradation (TPD) representing a well-established and effective strategy for directly manipulating protein function. TPD enables selective elimination of proteins, protein condensates or organelles by co-opting cellular degradation pathways—such as the ubiquitin-proteasome system, autophagy, or endocytosis—via induced proximity mechanisms. While TPD has had transformative impacts in biomedical research over the past two decades, its application in plant science has lagged behind. This gap stems from the sequential dominance of RNA interference and CRISPR technologies, as well as the complexity and cost of implementing chemical, macromolecular, and recombinant degrader platforms in plants. The recent development of genetically encoded chimeric protein degraders (GE-CPDs) offers a timely and promising alternative. These transgene-based systems provide a plant-adaptable, precise, tunable, and conditional means to control endogenous protein levels, opening new avenues for studying dynamic biological processes and engineering complex traits in crops. As genome engineering technologies continue to advance, GE-CPDs are poised to become a versatile and scalable platform for both basic plant biology and agricultural innovation. In this review, we highlight five key opportunities—Selective-Targeting, Co-Targeting, Organelle-Targeting, Conditional-Targeting, and Synthetic-Engineering (SCOCS)—that illustrate the emerging importance of TPD technologies, particularly GE-CPDs, in advancing plant science. We argue that the field is now well-positioned to harness the full potential of TPD for next-generation crop improvement.

Keywords: Targeted protein degradation (TPD); Protein condensate; ubiquitin-proteasome system; autophagy; RNA interference (RNAi); CRISPR

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GeneScientist: A Virtual Plant Biologist for Scalable AI-Driven Functional Genomics in Crops

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Crop breeding aims to optimize agronomic traits through the deliberate modification of crop genomes, with gene function research providing the theoretical foundation. However, gene function research, traditionally driven by human expertise, faces significant challenges. These include difficulties in integrating gene–trait knowledge, limited prediction capabilities for gene–trait associations, and a heavy reliance on expert experience for experimental design, which hinder efficiency and leave many gene functions unexplored, particularly in major crops like rice.

In this study, we developed GeneScientist (Chinese name: 丰登·基因科学家), an AI system designed to automate all stages of crop gene function research. Built on SeedLLM-Rice (Yang et al., 2025), a large language model integrated with the rice biological knowledge graph, GeneScientist consolidates knowledge summarization, gene function prediction, and research strategy planning into a unified platform. It autonomously handles tasks such as hypothesis generation and experimental design, overcoming the scalability limitations of traditional research. GeneScientist consists of three core modules: KnowledgeMiner, which leverages a plant biological knowledge graph to integrate data from millions of publications; GenePredictor, which predicts over previously unexplored gene–trait associations across rice and maize, aiding in gene prioritization; and HypothesisNavigator, which autonomously generates hypotheses and experimental strategies using causal reasoning. We applied GeneScientist to explore unannotated genes in rice and maize, demonstrating its ability to autonomously design and analyze multi-step experiments. This work validates the potential of AI-assisted gene function discovery and establishes GeneScientist as a valuable tool in functional genomics and crop science.

Keywords: Virtual Plant Biologist, AI, Large Language Model, Gene Function Discovery

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Developing novel plant synthetic biology tools for engineering future functional food crops

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The development of functional food crops with diverse and high-density nutrients and special functional active substances is essential for future nutrition and health. Multigene stacking and genome editing are core strategies for this purpose. We have developed a suite of advanced biotechnological tools for plant synthetic biology and biotechnology breeding, including the transgenic stacking (TGS) systems TGSII and TGSII-UNiE, which utilize Cre/loxP irreversible recombination and unique nucleotide-guided nick endonuclease (UNiE)-mediated DNA assembly. We also created efficient plant multiplex genome editing systems and broadly targeted high-efficiency precise base editors (PhieABEs/CBEs/DBEs and TadDBE) for synthetic metabolic engineering and germplasm innovation. Using these platforms, we engineered several novel functional rice germplasms, including anthocyanin-rich Purple Endosperm Rice ('Zijingmi'), Astaxanthin Rice 1.0 ('Chijingmi'), betalain-rich eRUBY crops, theanine-rich TheaRice ('Chami'), and Crocin Rice. Furthermore, using our new dual-base editor, TadDBE, we precisely modulated OsBADH2 protein function in a graded manner, creating germplasms that balance aromatic 2-acetyl-1-pyrroline (2-AP) and beneficial γ-aminobutyric acid (GABA). To accelerate such research, we launched PlantGPT (http://www.plantgpt.icu/), the first AI-powered question-answering system for plant functional genomics. Overall, these advancements provide powerful tools and precedents for engineering complex metabolic pathways, synthesizing vital bioactive compounds, and enhancing crucial multigenic agronomic traits.

HDR-mediated gene insertion and site-specific replacement in rice

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Genome editing based on the homology-direct repair (HDR) pathway enables scar-free and precise genetic manipulations. However, the low frequency of HDR hinders its application in plant genome editing. We engineered the fusion of Cas9 and viral replication protein (Rep) as a molecular bridge to tether donor DNA *in vivo*, which enhances the efficiencies of targeted gene insertion via the HDR pathway. This Rep-bridged knock-in (RBKI) method combines the advantages of rolling cycle replication of viral vectors and *in vivo* enrichment of donor DNA at target site for HDR. Chromatin immunoprecipitation indicated that the Cas9-Rep fusion protein bound up to 66-fold more donor DNA than Cas9 did. We exemplified the RBKI method by inserting small- to middle-sized tags into 3 rice genes. Compared with Cas9, Cas9-Rep fusion increased KI frequencies by 4 – 7.6-fold and up to 72.2% of stable rice transformants carried in-frame knock-in events at the T_0 generation. Further analysis suggested that the RBKI method reduced the number of byproducts from nonhomologous end joining, and, however, the HDR-mediated knock-in tend to accompany microhomology-mediated end joining events. We also found that optimizing the Rep and Cas nucleases can further increase the efficiency of RBKI-mediated knock-in in rice. Together, this study shows that the in vivo tethering of donor DNAs with Cas9-Rep is an effective strategy to increase the efficiency of HDR-mediated genome editing.

Keywords: Rice, Genome editing, HDR, knock-in