
ISRFG 2023
20th International Symposium on
Rice Functional Genomics
Rice Research for
Food and Nutritional Security
November 3-5
University of Agricultural Sciences
Bengaluru, India
UNIVERSITY OF AGRICULTURAL SCIENCES, BANGALORE
कृषितो नास्ति दुर्भिक्षं
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ISRFG 2023

Bengaluru, India

20th International Symposium on Rice Functional Genomics

"RICE RESEARCH FOR SUSTAINABLE FOOD AND NUTRITION SECURITY"

3rd to 5th NOVEMBER 2023
Bengaluru, India

PROCEEDING BOOK

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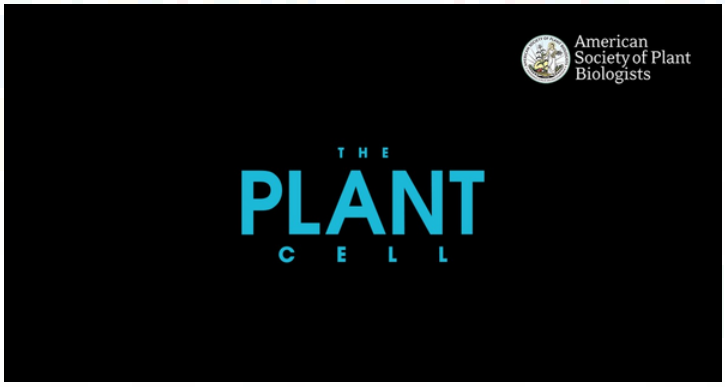

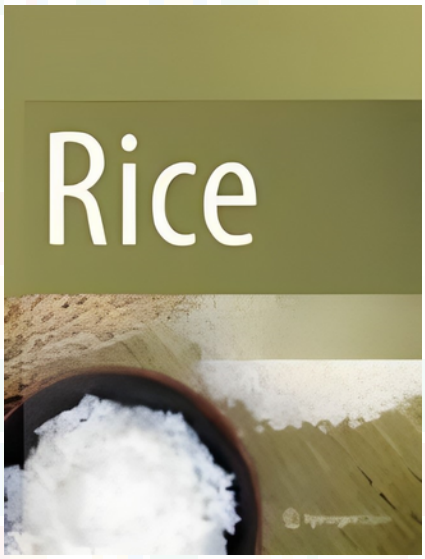

PROGRAM OVERVIEW

1 November 3rd

Dr. Babu Rajendra Prasad International Convention Centre, UAS, Bengaluru: Main Lobby

Room 1: ORYZA

08:00 - 09:30 Registration and Kit distribution: Main Lobby

09:30 - 11:00 Inaugural Session: Room 1: ORYZA

09:30 - 09:40 Welcome Address, **MS Sheshshayee,** Convener ISRFG2023, UAS, Bangalore, India

09:40 - 09:45 Lighting of lamp and Inaugration of ISRFG2023

09:45 - 09:55 Address by **SV Suresha,** Vice Chancellor, University of Agriculture Sciences, Bangalore, India

09:55 - 10:05 Indian efforts on rice genomics and involvement with ISRFG, **Akhilesh K. Tyagi,** University of Delhi, India

10:05 - 10:50 Inaugural Keynote lecture by **Rod Wing,** KAUST, Saudi Arabia and University of Arizona, USA The international Oryza map alignment project: What gap-free genomes can tell about the evolution of the genus Oryza

10:50 - 11:00 Vote of thanks, **Jitender Giri,** Co-Convener ISRFG2023, National Institute of Plant Genome Research, New Delhi, India

11:00 - 11:30 - COFFEE/TEA BREAK

MS Swaminathan Plenary Session: Room 1: ORYZA

Chair: LS Shashidhara, India & MS Sheshshayee, India

11:30 - 12:00 **Usha Vijayraghavan,** Indian Institute of Science, India Genomic studies of rice floret meristem and organ development transcription factors unravel functional diversification of conserved regulators

12:00 - 12:30 **Bin Han,** National Center for Gene Research, China Genomic approach to fully address the genetic basis and mechanism of heterosis in rice

12:30 - 13:00 **Akhilesh K. Tyagi,** University of Delhi, India Analysis of rice gene function during development

13:00 - 13:10 - Group Photo

13:10 - 14:00 - LUNCH

PROGRAM OVERVIEW

14:00 - 17:50 Concurrent Sessions

Room 1: AKKI

Genomics and epigenomics
Chairs: Pao-Yang Chen & Sanjay Kapoor, India

14:00 - 14:30

Mathias Lorieux, IRD, France Lead Lecture: New insights on the influence of genomic structural variation on crossover occurrence

14:30 - 14:50

Pao-Yang Chen, Academia Sinica, Taiwan Estimating genome-wide DNA methylation heterogeneity in rice

14:50 - 15:10

Mukesh Jain, JNU, India Unveiling genomic and epigenomic signatures associated with drought stress response/tolerance in rice

15:10 - 15:30

PV Shivaprasad, NCBS, India A Histone H4 variant predisposes H4 Lysine5 acetylation marks to modulate salt stress

Room 2: ORYZA

Pan-Genomics: Finding hidden genomic diversity
Chairs: Kenneth McNally, Philippines & Saurabh Raghuvanshi, India

14:00 - 14:30

Kenneth McNally, IRRI, Philippines Lead Lecture: SNP-Seek and other tools for uncovering rice diversity

14:30 - 14:50

Andrew Jones, University of Liverpool, UK PanOryza – public access to pan genes and pan proteomes for Asian rice

14:50 - 15:10

Gopal Misra, KAUST, Saudi Arabia Establishment of reference genomic resources for the wild relative of rice: Towards the neo-domestication of salttolerant rice: Oryza coarctata "

15:10 - 15:30

Antonio Costa de Oliveira, Federal University of Pelotas, Brazil Strategies for mutation breeding in Brazilian rice

Room 3: BHATTA

Plant development: Vegetative and reproductive
Chairs: Manoj Majee, India & Takeshi Izawa, Japan

14:00 - 14:30

Manoj Majee, NIPGR, India Lead Lecture: Rice seed vigor and viability: Role of protein repairing enzymes

14:30 - 14:50

Imtiyaz Khanday, UC Davis, USA Rice embryogenesis magic: Unlocking clonal seeds for hybrid vigor preservation

14:50 - 15:10

Satendra K. Mangrauthia, IIRR, Hyderabad, India Novel allele of OsCKX2 created through CRISPR/Cas12a confers yield superiority, stronger culm and earliness in indica rice cv. Samba Mahsuri

Discussions

15:30 - 16:00 - COFFEE / TEA BREAK

PROGRAM OVERVIEW

Concurrent Sessions

Room 1: AKKI

Genomics and epigenomics

16:00 - 16:30

Sanjay Kapoor, University of Delhi, India Lead Lecture: Understanding role of OsMADS29 in early seed development and manipulating its expression domain to reduce grain chalkiness

16:30 - 16:50

Takeshi Fukao, Fukui Prefectural University, Japan SUB1A coordinates distinct acclimation responses to submergence in sink and source leaves of rice

16:50 - 17:10

Saurabh Raghuvanshi, University of Delhi, India Pivotal role of miRNA genes in orchestrating drought stress response in rice

17:10 - 17:30

Raja M,
Genotypic, India
First telomere to telomere Indian rice genome

17:30 - 17:50

Tapan K. Mondal, NIPB, India Functional characterization of salt tolerant genes from Oryza coarctata : an triploid wild species of rice

Room 2: ORYZA

Pan-Genomics: Finding hidden genomic diversity
Chair: E. Guiderdoni, France & Nelson Saibo, Portugal

16:00 - 16:30

Nese Sreenivasulu, IIRI, Philippines Lead Lecture: Metabolomics and machine learning techniques unravel multi-nutritional properties of pigmented rice in germinated sprouts

16:30 - 16:50

Jong-Seong Jeon, Kyung Hee University, Korea Crucial role of PPi-dependent metabolic pathways in rice endosperm

16:50 - 17:10

Nelson Saibo, ITQB NOVA, Portugal Different strategies to improve rice photosynthesis

17:10 - 17:30

Apichart Vanavichit,
Kasetsart University, Thailand Pyramiding-by-design: Nutrient-dense and climate-resiliency toward organic pigmented rice

17:30 - 17:50

Haritha Bollinedi, IARI, India Molecular and biochemical characterization of gamma-oryzanol and its components in rice

Room 3: BHATTA

Plant development: Vegetative and reproductive

16:00 - 16:30

Toru Fujiwara, University of Tokyo, Japan Lead Lecture: Genetic independency of rice tillers revealed through chemical mutagenesis

16:30 - 16:50

Shri Ram Yadav, IIT Roorkee, India Species-specific functional innovations of conserved regulators during tissue transdifferentiation and branching

16:50 - 17:10

Pinky Agarwal, NIPGR, New Delhi, India A C2H2 zinc finger transcription factor regulates rice grain traits

17:10 - 17:30

Ranjan Swarup, University of Nottingham, UK Mechanistic insight into the role of AXR4 in regulating trafficking of auxin influx transporters AUX1 and LAX2

17:30 - 17:50

Aashish Ranjan, NIPGR, India Harnessing natural variation to integrate leaf development and photosynthesis in rice

18:00 - 19:30 Poster Session 1

19:30 Onwards - DINNER

PROGRAM OVERVIEW

November 4th

Day 2: November 04, 2023 | Saturday

Dr. Babu Rajendra Prasad International Convention Centre, UAS, Bengaluru: Main Lobby

Room 2: ORYZA

08:00 - 09:00 Registrations

09:00 - 13:00 **Plenary Session: Room 2: ORYZA**

Chairs: Martin Kater, Italy & Julia Bailey-Serres, USA

09:00 - 09:30 **Martin Kater,** University of Milan, Italy Characterisation of ALOG genes controlling rice inflorescence development

09:30 - 10:00 **Ramesh V. Sonti,** ICGEB, India A complex interplay of multiple Xanthomonas effectors in suppression of rice immune responses

10:00 - 10:30 **Alok K. Sinha**, NIPGR, New Delhi, India Probable role of Mitogen-Activated Protein Kinases in the regulation of the cell cycle in rice

10:30 - 11:00 **MS Sheshshayee,** UAS, India Improving water productivity of rice for aerobic cultivation: An approach through combining "constitutive" and "acquired" traits

11:00 - 11:30 - COFFEE / TEA BREAK

Plenary Session: Room 2: ORYZA

Chairs: Ramesh V. Sonti, India & Ari Sadanandom, UK

11:30 - 12:00 **A.K. Singh,** IARI, New Delhi Molecular breeding for biotic stress tolerance in Basmati rice

12:00 - 12:30 **Tuan-hua David Ho,** IPMB, Taiwan Role of intrinsically disordered proteins in conferring abiotic stress tolerance in rice

12:30 - 13:00 **Motoaki Seki,** RIKEN Japan Ethanol-mediated novel survival strategy against drought, heat and high-salinity stresses in plants

13:00 - 14:00 - LUNCH

PROGRAM OVERVIEW

14:00 - 17:10 Concurrent Sessions

Room 1: AKKI

Climate resilience: Plant biotic interactions
Chairs: Subhra Chakraborty, India & Apichart Vanavichit, Thailand

14:00 - 14:30

Siwaret arikit, Kasetsart University, Thailand Lead Lecture: Unveiling essential genes for enhancing rice resistance to BPH amidst climate change challenges

14:30 - 14:50

Kamal Kumar Malukani, TIGS, India Understanding the molecular intricacies of Rice-Xanthomonas interaction

14:50 - 15:10

Gokulan CG, CCMB, India QTL-seq and transcriptome analyses provide mechanistic insights into Yellow Stem Borer tolerance in rice

15:10 - 15:30

Anindita Seal, University of Calcutta, India Rhodotorula mucilaginosa JGTA-S1 - a reservoir of endobacteria improves nitrogen nutrition of rice plants.

Room 2: ORYZA

Climate resilience: Abiotic stresses
Chairs: Motoaki Seki, Japan & Julie Gray, UK

14:00 - 14:30

Rahul Bhosale, University of Nottingham, UK Lead Lecture: Adaptive responses of rice roots to high temperature stress

14:30 - 14:50

Pallavi Singh, University of Essex, UK Understanding the dynamic correlation between rice roots, shoots, and water efficiency in order to increase productivity

14:50 - 15:10

Eswarayya Ramireddy, IISER, Tirupati Genes to Field: Deciphering the genetic basis of traits suitable for direct-seeded rice (DSR)

15:10 - 15:30

Annapurna Devi Allu, IISER, Tirupati Using insult to overcome injury: Mechanisms to cope heat stress in rice

Room 3: BHATTA

Workshop: Gene-editing
Chairs: Viswanathan Chinnusamy, India & Jitender Giri, India

14:00 - 14:30

Bing Yang, University of Missouri USA Lead Lecture: Genome editing enables rice to resist bacterial blight

14:30- 15:00

Emmanuel Guiderdoni, CIRAD, France Bringing apomictic hybrids to the rice fields

15:00 - 15:30

Viswanathan Chinnusamy, IARI, New Delhi Genome editing for improving yield and abiotic stress tolerance of rice,

15:30 - 16:00 - COFFEE/TEA BREAK

PROGRAM OVERVIEW

14:00 - 17:50 Concurrent Sessions

Room 1: AKKI

16:00 - 16:30

Gopala Krishnan S, IARI, New Delhi Lead Lecture: Mapping QTLs governing resistance to sheath blight of rice

16:30 - 16:50

AP Padmakumari, IIRR, India Rice gene differentials – a tool for monitoring field virulence in Asian rice gall midge, Orseolia oryzae

16:50 - 17:10

Asif Bashir Shikari, SKAUST, India Mapping QTLs for resistance to panicle blast in a recombinant inbred line population of temperate Japonica rice (Oryza sativa L .)

Room 2: ORYZA

16:00 - 16:30

Tiago Filipe Lourenco, ITQB NOVA, Portugal An E3-Ubiquitin ligase mediating rice drought response: Insights into brassinosteroids and ABA signaling interactions

16:30 - 16:50

Ramu V, RCB, India Ribosomal RNA biogenesis and translation ability regulate drought tolerance of plants

16:50 - 17:10

B. Mohan Raju, UAS, India Morphological and physiological markers to identify haploids in rice at an early stage

Room 3: BHATTA

16:00 - 16:30

Ajay Gupta, University of Missouri, USA Prime genome editing for disease resistance in rice

16:50 - 17:10

Discussions

17:10 - 18:30 Poster Session 2

18:30 - 19:30 Cultural Program: Room 2: ORYZA

19:30 Onwards - DINNER

November 5th

Day 3: November 05, 2023 | Sunday

Dr. Babu Rajendra Prasad International Convention Centre, UAS, Bengaluru: Main Lobby

Room 2: ORYZA

09:00 - 13:00 **Plenary Session: Room 2: ORYZA Chairs: NK Singh, India & Tuan-hua David Ho, Taiwan**

09:00 - 09:30 **Blake Meyers,** Donald Danforth Plant Science Center, USA Secondary siRNA pathways as key regulators of male reproductive development in plants

09:30 - 10:00 **Julia Bailey-Serres,** UC Riverside, USA Shaping root traits for wet and dry soil

10:00 - 10:30 **Matthias Wissuwa,** JIRCAS, Japan A genomic prediction based approach to identify best donors for abiotic stress tolerance and nutrient-dense rice

10:30 - 11:00 **Subhra Chakraborty,** NIPGR, India Commuting to Fight: Organellar crosstalk and post-translational control shaping plant immunity

11:00 - 11:30 - COFFEE/TEA BREAK

Plenary Session: Room 2: ORYZA
Chairs: Blake Meyers, USA & Ashwani Pareek, India

11:30 - 12:00 **NK Singh,** National Institute of Plant Biology, India Genomics-assisted breeding of climate-resilient rice varieties

12:00 - 12:30 **Julie Gray,** University of Sheffield, UK Manipulating stomata to enhance rice stress tolerance

12:30 - 13:00 **Takeshi Izawa,** The University of Tokyo, Japan Fertilization controls tiller numbers via transcriptional regulation of a MAX1-like gene in rice cultivation

13:00 - 14:00 - LUNCH

PROGRAM OVERVIEW

14:00 - 17:30 Concurrent Sessions

Room 1: AKKI

Translational genomics: Molecular and classical breeding
Chairs: Raman M. Sundaram, India & Mukesh Jain, India

14:00 - 14:30

Raman M. Sundaram, IIRR, India Lead Lecture: Making samba mahsuri climate resilient through molecular breeding

14:30 - 14:50

Subhas Chandra Roy, University of North Bengal, India New model for origin of black rice from wild rice of India: Based on genetic evidence of interspecific hybridization (O. sativa x O. rufipogon) and genome analysis

14:50 - 15:10

Saurabh Badoni, IRRI, Philippines Unveil novel epistatic targets among major effect loci impacting rice grain chalkiness utilizing genome-wide association-coupled epistatic interaction studies

15:10 - 15:30

Jauhar Ali, IRRI, Philippines Genomics assisted breeding of climateresilient and nutritious rice varieties

Room 2: ORYZA

Plant nutrition and sustainable rice production
Chairs: Mathias Wissuwas, Germany & Jitender Giri, India

14:00 - 14:30

Ki-Hong Jung, KHU, South Korea Lead Lecture: Strategy to enhance phosphate use efficiency and grain yield through modulation of RNA decay pathway in rice

14:30 - 14:50

Jitender Giri, NIPGR, India A citrate efflux transporter important for manganese distribution and phosphate uptake in rice

14:50 - 15:10

Thi Mai Huong To, UST, Hanoi, VietNam A novel glycerophosphodiester phosphodiesterase is involved in the phosphate starvation response in rice root

15:10 - 15:30

CN Neeraja, IIRR, India Functional genomics of grain zinc content in rice

Room 3: BHATTA

Workshop on plant phenotyping and GWAS in rice
Chairs: Blanca S. Segundo, Spain & Siwaret Arikit, Thailand

14:00 - 14:30

Ramegowda HV, UAS, Bengaluru, India Lead Lecture: Importance of plant phenotypingfor crop improvement: The novel drought simulator phenomics facility

14:30- 15:00

Swarup K Parida, NIPGR, India Pangenome based GWAS for accelerated crop improvement in rice

15:00 - 16:00

Field phenotyping facility visit MS Sheshshayee

15:30 - 16:00 - COFFEE/ TEA BREAK

PROGRAM OVERVIEW

November 5th

Day 3: November 05, 2023 | Sunday

Dr. Babu Rajendra Prasad International Convention Centre, UAS, Bengaluru: Main Lobby

Room 2: ORYZA

16:00 - 17:40 **Young Scientists Session I Room 2: ORYZA I Chairs: Alok Sinha; Bing Yang; Pallavi singh**

16:00 - 16:10 **Ajit Pal Singh,** National Agri-Food Biotechnology Institute, Mohali, India
Positive aspects of jasmonates signaling for improving agronomic traits

16:10 - 16:20 **Akanksha Bhatnagar,** University of Delhi, New Delhi, India
HY5 genes in rice: The regulators of light-mediated development

16:20 - 16:30 **Eshan Sharma,** University of Liverpool, UK
Gene discovery for drought-resilient rice in an era of big data science and analytics

16:30 - 16:40 **Harshita Singh,** University Heidelberg, Germany
Functional genomics of hormonal regulation of crown root development in rice

16:40 - 16:50 **Hasthi Ram,** National Institute of Plant Genome Research, New Delhi, India
Developing genome-edited population in Indica rice using CRISPR-Cas9 pool library approach

16:50 - 17:00 **Jyotirmaya Mathan,** University of Essex, UK
Variation in photosynthesis and photoassimilate partitioning across cultivated and wild rice and the underlying attributes

17:00 - 17:10 **Lokesh Verma,** National Institute of Plant Genome Research, New Delhi, India
Deciphering the role of membrane lipid remodeling genes in combating phosphate deficiency

17:10 - 17:20 **Nitin Kamble,** John Innes Center, UK
Protein L-isoaspartyl Methyltransferase (PIMT): A key player for controlling agronomically important seed traits

17:20 - 17:30 **Preethi Vijayaraghavareddy,** University of Agricultural Sciences, Bengaluru, India
Unravelling the mysteries of nocturnal transpiration: Insights across crop species, seasons, and genotypes

17:30 - 17:40 **Yong Zhou,** King Abdullah University of Science and Technology, Saudi Arabia
Pan-genome studies of asian rice population reference panel (RPRP, O. Sativa)

18:00 - 18:30 **Panel Discussion I Chair:** Trilochan Mohapatra **I Panelists:** Rod Wing; Akhilesh K. Tyagi; Martin Kater; Blake Meyers; Usha Vijayraghavan

18:30 - 19:00 **Awards Distribution I Room 2: ORYZA**

19:00 - 19:30 **Announcement of ISRFG2024 and Concluding Remarks**

19:30 onwards - FAREWELL DINNER

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Keynote & Plenary Sessions

Keynote Lecture

THE INTERNATIONAL *ORYZA* MAP ALIGNMENT PROJECT: HARVESTING 15 MILLION YEARS OF EVOLUTIONARY HISTORY TO HELP SOLVE THE 10-BILLION PEOPLE QUESTION

Rod A. Wing

Center for Desert Agriculture, King Abdullah University of Science & Technology, Kingdom of Saudi Arabia, Rod.Wing@KAUST.edu.sa
Arizona Genomics Institute, University of Arizona, Tucson, AZ, USA, rwing@Arizona.edu

The genus *Oryza* is composed of 27 species and includes one of the world's most important food crops – rice. As our population approaches 10-billion, and in the face of climate change, plant biologists and breeders much do all that is possible to develop climate resilient crops that are not only high yielding and nutritious but have also have less of and environmental footprint. In my lecture I will discuss how we are interrogating the genus *Oryza* to unlock 15 million years of evolutionary history to meet the challenge of our lifetime – i.e. How can feed our world without destroying our planet!

Day 1 Plenary Session

GENOMICS STUDIES OF RICE FLORET MERISTEM AND ORGAN DEVELOPMENT TRANSCRIPTION FACTORS UNRAVELS FUNCTIONAL DIVERSIFICATION OF CONSERVED REGULATORS

Usha Vijayraghavan

Department of Microbiology and Cell Biology
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Cereals have evolved varied and complex inflorescence architectures that share a short lateral branch called the spikelet as a common unit. The rice inflorescence (panicle) forms two orders of branch meristems (primary and secondary) before generating spikeletseach with a single determinate floret meristem. The cumulative action of several regulators direct the progressive change in meristem identity from branch, to spikelet to incipient floret meristem to determinate floret meristem with organ primordia. As compared to eudicot flowers, rice and cereal florets have functionally and morphologically distinct outer organs. These are a pair of large bract-like organs called the lemma and palea that fully enclose all inner organs and a pair of asymmetrically positioned small, fleshy lodicules that aid in controlled opening and closing of the floret for pollination and fertility. These organs with distinctive development and differentiation programs are excellent models to uncover how new organ morphologies and function can be driven by functional variation of conserved developmental regulators. Organ identity in flowers is determined the combinatorial activity of Class A, B, C, D, and E genes most of which encode MADS-domain transcription factors.Our functional genomic studies on rice *OsMADS1*, a member of the *LOFSEP* subclade of Class E *SEP* genes, and the rice Class B *PISTILLATA*-like paralogous genes *OsMADS2* and *OsMADS4* will be presented. These studies expand our understanding of neo-functionalization and sub-functionalization of evolutionarily conserved floral patterning factors and reflect innovations that underlie species-specific differences.

Day 1 Plenary Session

QUANTIFICATION AND GENOMIC PREDICTION OF HETEROSIS FROM HYBRID RICE GENOMES

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Heterosis or hybrid vigor refers to better performance of the progeny than its parental lines. Exploitation of heterosis in crop hybrid breeding is one of the most important innovations in agriculture, and is still a crucial way to increase agricultural production for global food security. However, modern hybrid rice breeding, which is mainly relying on random crosses between diverse varieties and comprehensive phenotypic selection, is still labor-intensive and time-consuming. We have developed an integrated genomic and forward genetic approach to construct a genome map for elite hybrid rice varieties and their inbred parental lines. We identified that the accumulation of numerous rare superior alleles with positive dominance is an important contributor to the heterotic phenomena. Furthermore, we quantitatively characterized genetic effects of heterotic loci regarding grain yield. We found that hybrid rice breeding has constantly pyramided superior alleles by searching for optimal heterotic combinations and purging inferior alleles in both parental lines simultaneously, and applied flowering time genes to balance productivity and environmental adaptation. Additionally, we demonstrated that widespread genetic complementary in indica-japonica hybrids mainly contributed to interspecific heterosis. Accordingly, a genomic model featured with the customized selection index for diverse rice varieties is developed and optimized to predict the performance of hybrid combinations by using the genomic and phenotypic data from rice hybrids and segregation individuals derived from hybrids. Our data offer a valuable resource for advancing the understanding of rice heterosis. This study reveals novel insights regarding rice heterosis and optimal hybrid combinations.

Day 1 Plenary Session

ANALYSIS OF RICE GENE FUNCTION DURING DEVELOPMENT

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After decoding rice genome sequence, significant progress has been made in transcriptomics (the messages generated from genes) and gene discovery by way of genetic mapping, overexpression or knock-down/out of genes in transgenics and phenotypic analysis. This has potential to fill the gap between the genome and the phenotype and help practice genomics-assisted molecular breeding. One of the primary aims of ongoing investigations in our group is to understand phylogeny, regulatory networks and gene function. By analysing molecular model-based relationship of encoded proteins by members of several gene families, we have identified new genes and clades in rice genome. Further, highly significant number of genes is differentially expressed in different organs at different time of development, which provides useful input for selecting target genes/promoters for functional genomics. We have undertaken study of anther and seed related genes to identify genes involved in male sterility and seed development. Some of these genes have pleiotropic effects. Results of these effects and analysis of their molecular basis of action will be presented. The knowledge generated is expected to reap the benefit of genomics research for plant improvement.

Day 2 Plenary Session

CHARACTERIZATION OF *ALOG* GENES CONTROLLING RICE INFLORESCENCE DEVELOPMENT

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Plant inflorescence architecture is an important determinant of the reproductive success of a plant but also of great agronomical interest since it determines yield in many crops. Especially, considering the fast increase in world population and the need to prevent further expansion of use of natural habitats for agriculture practice, yield increase per hectare is of enormous importance. Rice yield increase is central to this issue since world population growth is highest in countries where rice is the main food source.

In rice the inflorescence meristem, (rachis meristem) starts to develop primary branches. On these primary branches secondary branches are often formed which develop spikelet meristems that develop the florets (flowers). The result is a determinate panicle type inflorescence structure. The timing of the determinate spikelet meristem development determines the number of branches and seeds that will develop. Our lab is interested in the molecular mechanisms that determine the identity of the different reproductive meristems since these stands at the base of the final architecture. Recently, we have laser micro-dissected the rice reproductive meristems and used this material for RNAseq analysis (Harrop et al., 2016). This resulted in the identification of *ALOG* genes putatively involved in inflorescence development and panicle branching. Using CRISPR-Cas technology we havemutated*OsG1L1*, *OsG1L2 and (TAW1) OsG1L5* and studied their roles in inflorescence development and the regulatory networks controlled by these transcription factors (Beretta et al., 2023). The latest results will be presented.

Keywords: Inflorescence architecture, reproductive meristem, transcriptional regulation

Day 2 Plenary Session

A COMPLEX INTERPLAY OF MULTIPLE XANTHOMONAS EFFECTORS IN SUPPRESSION OF RICE IMMUNE RESPONSES

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As part of its virulence repertoire, Xanthomonas oryzaepv. oryzae (Xoo) secretes a battery of cell wall degrading enzymes (CWDEs) that target different components of the rice cell wall. However, the CWDEs are double edged swords as the damage that they cause serves as a mark of infection and results in induction of Damage Triggered Immune responses that are part of pathogen triggered immunity (PTI). Xoo suppresses these defense responses using four different effector proteins (Xanthomonas Outer Protein N [XopN], XopQ, XopX and XopZ) that it secretes into rice cells via a type 3 secretion system (T3SS). Mutational analysis indicates that these four proteins function redundantly in suppression of PTI. Three of these T3SS effectors (XopQ, XopX and XopZ) have 14-3-3 protein binding motifs. Each of these T3SS effectors interacts with different rice 14-3-3 proteins and this interaction is necessary for the ability of the effectors to suppress PTI. XopQ and XopX proteins also interact with each other. This leads to an alteration in the intracellular localization of XopQ and triggering of what can be referred to as Effector Triggered Immunity (ETI). Xoo is able to suppress XopQ+XopX triggered immune responses using five other T3SS effectors (XopG, XopP, XopU, XopV and AvrBs2) that can each individually suppress these responses. At least for XopG, the suppression of ETI appears to be due to physical interaction with XopQ and XopX and possibly their sequestration in the cytoplasm. These results suggest a complex interplay of Xoo T3SS effectors in suppression of both PTI and ETI to promote virulence on rice.

Day 2 Plenary Session

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

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Due to the immobile nature of Pi, plants have developed complex molecular signalling pathways that allows them to discern changes of Pi concentrations in the environment and adapt their growth and development. 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 signalling 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 hyperSUMOylation 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.

Day 2 Plenary Session

ADVANCING DROUGHT TOLERANCE IN RICE: A FORWARD APPROACH

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In light of the growing global population and the increasingly unpredictable impacts of climate change, the demand for resilient, high-yielding crops capable of withstanding multiple stresses is on the rise. Rice, a staple food crop for nearly half of the world's population, faces a substantial challenge in the form of drought, which significantly hampers its production. To address this challenge, our laboratory is actively involved in the development of new plant types using genetic modification techniques. Simultaneously, mutational breeding has proven to be a successful strategy for creating stress-tolerant, high-yield crop genotypes worldwide.One of our major breakthroughs involved the creation of a gamma-induced mutant population of rice, within the genetic framework of the elite rice cultivar IR64. We meticulously screened these mutants for their ability to produce high grain yields under drought conditions, leading to the identification of ten mutants. These mutants displayed variations at morpho-physiological, biochemical, and molecular levels, warranting a comprehensive analysis under both managed drought conditions and open field environments to evaluate the relevance of their traits compared to the wild-type IR64. To pinpoint the specific genetic changes responsible for these improvements, we employed the MutMap+ approach, identifying genomic regions with high specificity. In another study, we have also developed a novel gamma-irradiated IR64 rice mutant with multi-stress tolerance, boasting high grain quality and quantity, but with the added attributes of early maturity, senescence, and a shorter life span compared to the wild-type. Detailed information on these mutants will be presented during the forthcoming discussion.

Day 2 Plenary Session

BREEDING PREMIUM QUALITY BASMATI RICE –PROGRESS AND PROSPECTS

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Basmati rice is a specialty rice popular across the world for its appealing aroma and exquisite quality parameters. Annually, ~8.0 million tons of Basmati rice is grown from an area of ~2.0 million hectares across the states of Punjab, Haryana, Delhi, Uttarakhand, J & K, Himachal Pradesh and western Uttar Pradesh earmarked as GI (Geographical Indications) area for Basmati cultivation. Basmati rice improvement at ICAR-Indian Agricultural Research Institute, New Delhi has significantly improved the yield as well as quality parameters leading to development of several varieties such as Pusa Basmati 1, Pusa Basmati 1121, Pusa Basmati 6, Pusa Basmati 1509, Pusa Basmati 1692. Quantum jump in grain yield has been made wherein the productivity has increased from a mere 2.0 t/ha in Basmati 370 to 8.0 t/ha in Pusa Basmati 1692. Significant improvement has been made for cooked kernel length from 12.00 mm to 22.00 mm in Pusa Basmati 1121. The improvement in grain quality accompanied by higher grain yield created a synergy among the various stakeholders, which led to surge in Indian Basmati rice exports to the tune of 4.8mt worth US$ 4.78 billion in 2022-23. An equal volume is consumed in the domestic market within India. These varieties have improved livelihood of millions of farmers, millers, exporters and all the stakeholders in the Indo-gangetic plains of India. There has been an increasing concern on the potential risk of pesticide residues in Basmati rice grains, which is being actively addressed through host plant resistance. Effective integration of molecular breeding in Basmati rice improvement has helped in introgression of genetic resistance to major biotic stresses such as bacterial blight, blast, bakanae and brown plant hopper resistance, which led to development of Basmati varieties with resistance to bacterial blight, blast resistance, the latest among which are Pusa Basmati 1885, Pusa Basmati 1886 and Pusa Basmati 1847 with combined resistance to both bacterial blight and blast diseases. These improved varieties are gaining popularity among the stakeholders, which will help in sustaining our Basmati

exports. Limitations in water and labour availability, is necessitating the shift from irrigated-transplanted production to Direct Seeded Rice. Weeds are a major issue under DSR, and to address this issue, a mutant allele of AHAS expressing tolerance to a broad-spectrum herbicide Imazethapyr was identified and incorporated into popular Basmati rice varieties to develop Imazethapyr herbicide tolerant Basmati rice varieties, Pusa Basmati 1979 and Pusa Basmati 1985, respectively. To address water usage, a Basmati variety, Pusa Basmati 1882, incorporated with a QTL, *qDTY1.1* governing tolerance to reproductive stage drought tolerance has been developed and released. Nutritional quality improvement of Basmati rice is being made through introgression of QTLs for high endosperm Zn content. Rice bran oil quality and storability are being enhanced through introgression of non-functional *LOX3* allele in the premium quality Basmati rice varieties. Efforts are also underway to develop varieties suited for dry-DSR conditions through incorporation of traits such as tolerance to anaerobic germination and lodging, resistance to root knot nematodes, improved root system architecture, tolerance to biotic and abiotic stresses, which will help in sustaining the Basmati rice production.

Day 2 Plenary Session

MULTIFACETED ROLES OF RICE ABA/STRESS-INDUCED INTRINSICALLY DISORDERED PROTEINS IN AUGMENTING DROUGHT RESISTANCE

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Besides the late embryogenesis abundant (LEA) proteins, two groups of intrinsically disordered proteins, i.e., Repetitive Proline-Rich Proteins (RePRP) and Rice Big Grain protein (RBG), have been discovered to be involved in conferring tolerance to abiotic stresses in rice. RePRP reduces water loss by decreasing stomata conductance in shoot. In addition, RePRP enhances the level of extracellular water barriers such as lignin and suberin, primarily in the root vascular bundle. Several groups of genes involved in lignin biosynthesis, especially the wall-bound peroxidase responsible for the final assembly of the lignin network, are induced by RePRP. Furthermore, overexpression of RePRP leads to lowered root osmotic potential, and the protein levels of two aquaporins important for drought stress tolerance are elevated. Hence, ABA/stress-induced RePRP expression leads to several beneficial traits of drought resistance, including lower water loss rate upon dehydration and higher root water use efficiency under drought conditions. Rice Big Grain 1 (RBG1) regulates grain and organ development, as well as abiotic stress tolerance. Ectopic expression of RBG1 leads to significant increases in the size not only of grains but also other major organs such as roots, shoots and panicles. Increased grain size is primarily due to elevated cell numbers rather than cell enlargement. RBG1 is preferentially expressed in meristematic and proliferating tissues. Ectopic expression of RBG1 also increases auxin accumulation and sensitivity, which facilitates root development, particularly crown roots. Ectopic expression of RBG1 regulated by a specific constitutive promoter, GOS2, enhances harvest index and grain yield in rice. It appears that RBG1 regulates two distinct and important traits in rice, namely grain yield and stress tolerance, via its effects on cell division, auxin biosynthesis/signaling and stress protein induction.

Day 2 Plenary Session

ETHANOL-MEDIATED NOVEL SURVIVAL STRATEGY AGAINST DROUGHT, HEAT AND HIGH-SALINITY STRESSES IN PLANTS

Seki, M.1,2,3, Todaka, D.1, Bashir, K.1,4, Utsumi, Y.1, Tanaka, M.1 and Sako, K.1,5

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Water scarcity, high temperature, and salt accumulation on lands are serious agricultural problems causing significant losses to crop yield. Developing environmentally friendly strategies to mitigate these agricultural problems could boost agricultural production in unfavorable conditions and/or uncultivated lands. We have found that pretreatment with ethanol, a cheap and environmentally friendly chemical, enhances drought, heat, and high-salinity stresses in various plants, such as *Arabidopsis*, rice, wheat, maize, lettuce and cassava, and analyzed the molecular mechanisms of the ethanol-mediated stress tolerance. Transcriptome and metabolome analysis showed that the expression of several stress tolerance-related genes and the accumulation of its related metabolites were increased. Ethanol pretreatment enhances high-salinity- and high-light- stress tolerance by detoxifying ROS[1),2)], and induces heat tolerance through stimulation of the endoplasmic reticulum stress response[3)]. Furthermore, ethanol pretreatment induces stomatal closure, resulting in a reduced transpiration rate and higher water content in the leaves during drought stress treatment[4),5)]. In the meeting, the current status for the analyses of the molecular mechanisms of ethanol-mediated stress tolerance and its application will be presented.

Day 3 Plenary Session

PHASED, SECONDARY SIRNAS IN PLANT REPRODUCTION AND OTHER PATHWAYS

Blake Meyers

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In plants, 21 or 22-nt miRNAs or siRNAs typically negatively regulate target genes through mRNA cleavage or translational inhibition. Heterochromatic or Pol IV are 24-nt and function to maintain heterochromatin and silence transposons. Phased "secondary" siRNAs (phasiRNAs) are generated from mRNAs targeted by a typically 22-nt "trigger" miRNA, and are produced as either 21- or 24-mers via distinct pathways. Our prior work in maize and rice demonstrated the temporal and spatial distribution of two sets of "reproductive phasiRNAs", which are extraordinarily enriched in the male germline of the grasses. These two sets are the 21-nt (pre-meiotic) and 24-nt (meiotic) siRNAs. Both classes are produced from long, non-coding RNAs, generated by hundreds to thousands of loci, depending on the species. These phased siRNAs show striking similarity to mammalian piRNAs in terms of their abundance, distribution, distinctive staging, and timing of accumulation, but they have independent evolutionary origins. The functions for these small RNAs in plants remain poorly characterized. I will describe our recent work investigating the functions of plant phasiRNAs and their roles in modulating traits of agronomic importance in plants, including male fertility.

Day 3 Plenary Session

SHAPING ROOT TRAITS FOR WET AND DRY SOILS

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Since their emergence over 400 million years ago, vascular land plants (tracheophytes) have acquired traits that enablefitness in dynamic and extreme environments. Existent diversity in Oryza including *Oryza sativa* demonstrate adaptations associated with growth in waterlogged or dry soils. These include plastictraits -conditionally activated by environment – often lost through selective breeding in specific environments (i.e., shallow or deep roots). Root systems are challenging to monitor in thefield, let alone the specific cells and molecular processes that determine anatomical or morphological plasticity. We developedtools and methods to decipher gene regulatory pathways that determine root plasticity towater extremes (1). By profiling regions of open chromatin and translated mRNAs incell populations, we resolve similarities and distinctions across conditions innetworksassociated with cell division, cell differentiation, hormone signaling, and more. The data allow prediction of transcription factor hierarchies and hormonal interplay that determine advantageous traits for water extremes, such as ABA-induced formation of suberin lamellae on theepidermal side of the root exodermis. This lipid barrier restricts radial oxygenloss during waterlogging andmoisture loss under water deficit but may have trade-offs. These findings illuminate conservation and distinctions in conditional gene regulatory networks that maybe manipulated toimprove climate resiliency of rice.

(1) Reynoso et al. (2022)Dev Cell.10.1016/j.devcel.2022.04.013; Funded by US NSF IOS-211980, DBI-1922642 and USDA 1026447.soils esilience to water extremes Developmental plasticity and water extreme resilience

Day 3 Plenary Session

A GENOMIC PREDICTION BASED APPROACH TO IDENTIFY BEST DONORS FOR ABIOTIC STRESS TOLERANCE AND NUTRIENT-DENSE RICE

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Rice accessions held in gene banks are a reservoir of novel and potentially useful alleles waiting to be tapped in breeding for stress tolerance or nutritionally important traits. How to efficiently utilize these genetic resources remains a challenge. We explored options for the SNP-Seek set of accessions publicly available at IRRI. Of the 3000 available accessions, a subset of 10% (300) were imported to Madagascar and grown at two locations in farmers' fields under low-input paddy conditions. Traits assessed were plant height, straw weight, days to heading, grain yield and grain zinc (Zn) concentrations. In a first step Genome-Wide Association Studies (GWAS) were employed to identify influential loci controlling grain yield and Zn concentrations. In a second step Genomic Prediction (GP) models were built and used to predict the performance of the untested 90% (2700) of accessions. Best predicted accessions were then imported again to Madagascar to confirm predictions and select possible donors.

GWAS identified two influential loci for low soil fertility tolerance (LFT) on chromosomes 5 (*qLFT-5*) and 11 (*qLFT-11*). For both loci a rare allele (MAF = 0.1) increased total panicle weight per plant by about 30%. Genomic prediction based on the GBLUP model identified potentially high-yielding accession for confirmation. Simultaneous prediction of maturity and plant height allowed for selection of a set with acceptable maturity and plant height for Madagascar. Prediction accuracies ranged from 0.10 - 0.30 for grain yield to 0.71 for heading date. One favorably predicted accession, IRIS 313–11949, carried positive alleles at *qLFT-5* and *qLFT-11* and combined high grain yield with early maturity. It is being considered for a short-track variety release and breeding populations derived from it are in the advanced variety testing stage.

For grain Zn concentrations GWAS identified several minor loci, confirming results of others that this trait is highly polygenic and not amenable to marker assisted selection.

Day 3 Plenary Session

Predicted concentrations ranged from 17.1 to 40.2 ppm with a prediction accuracy of 0.51. An independent confirmation with 61 gene bank seed samples provided high correlations (r = 0.74) between measured and predicted values. GP highlighted that variation within the *indica*genepool is insufficient to reach Zn biofortification targets of 30 ppm Zn.Highest grain Zn concentrations were predicted for accessions from the *aus* sub-species and these were confirmed in the field in Madagascar, where at least 7 accessions surpassed 35 ppm Zn. We have now used two *aus* donors in our Zn-biofortifications breeding and one high-Zn *aus*-derived breeding line is already being considered for variety release. These results demonstrate the utility of GP, as phenotyping merely 10% of available accessions and predicting the performance of the remaining 90% is a highly efficient way of exploring gene bank resources for breeding purposes.

Genomic prediction, genomic selection, GWAS, grain zinc, grain yield, SNP-Seek

Day 3 Plenary Session

COMMUTING TO FIGHT: ORGANELLAR CROSSTALK AND POST-TRANSLATIONAL CONTROL SHAPING PLANT IMMUNITY

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Impending changes in the global climate coupled with mortality associated with fungal infections have resulted in challenges related to food and nutrition. Blast is one of the most destructive diseases of rice, causing considerable productivity loss. Cell compartmentalization into different subcellular organelles is an attribute conserved in eukaryotes, including plants. Evidences suggest role of these organelles in defense response. However, the dynamic role of their intrinsic crosstalk during pathogen infection remains largely unknown. Moreover, often these cross-talks are regulated by post-translational modifications of organellar proteins, including lysine acetylation. To explore the numerous molecular interactions that occur between organelles during rice blast, we performed mass-spectrometry based quantitative proteomic, phospho-proteomic, metabolomic, and acetylome analyses. Integrative multiomics data of two different organelles, extracellular matrix (ECM) and nucleus, uncovered convergence and divergence of defense signalling. Dynamic changes in protein, phosphoprotein, metabolites, and acetylated proteins revealed a unique signature characterizing each stage of infection. Altogether, our data highlighted five major signalling cascades, calcium, oxylipin, eATP, hormone, and kinase signalling operating between ECM and nucleus. The multilevel regulatory network generated in this study sets the foundation for in-depth mechanistic dissection of the inter-organellar crosstalk in immunity. Furthermore, we identified and functionally characterized a nucleotide-binding multi-functional protein with differential acetylation status upon infection, OsIRF6, as a key regulator of immunity that functions downstream of the oxylipin signalling pathway. This study provides high resolution cellular map and valuable insight into understanding the intricate molecular mechanism that governs immunity against blast pathogen and biotechnological strategies for fungal disease resistance in crops.

Keywords: Rice, Blast disease, Organellar cross-talk, Post-translational modifications, Immunity

Day 3 Plenary Session

GENOMICS-ASSISTED BREEDING OF CLIMATE-RESILIENT RICE VARIETIES

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One of the greatest challenges of present day Agriculture is to produce sufficientnutritious food for the growing human population from diminishing acreage, deteriorating soil health and environmental stresses resulting from global climate change. It is necessary to develop high-yielding rice varieties with tolerance to different abiotic stresses. Rice became the first crop with high-quality reference genome available in the public domain. Rice breeders at International Rice Research Institute, Philippines and national agricultural research system institutions in the rice growing countries have developed thousands of rice varieties over the years,some of which aree.g. 'IR64' and 'Swarna' are highly popular among the farmers even after decades of their release. These are cultivated in millions of hectares for their superior quality and yield stability. These are called mega varieties (MVs) that represent the best available assortments of superior alleles of agronomically important genes through recombination breeding. The MVs provide an ideal base for further improvement by infusion of validated genes for climate resilience by marker-assisted accelerated breeding.Green revolution (GR) high-yielding varieties (HYV) carrying a gene for semi-dwarf plant height have rapidly replaced the traditional climate-resilient but low yielding tall rice varieties in most part of the world. The GR-HIV were selected primarily for yield under high input conditions and therefore are sensitive to climaticadversities. Knowledge of rice genome and causal genes for tolerance to different abiotic stresses likeheat, drought, flood and soil salinity has provided opportunity to transfer the favourable alleles of these genes into MVs bymarker-assisted backcross breeding (MABB) through a multi-institutional network. We have transferred six genes/QTLs for grain yield under drought; viz.*qDTY1.1*, *qDTY2.1*, *qDTY2.2*, *qDTY3.1*, *qDTY3.2* and *qDTY12.1* into flood-tolerant versions of Swarna, Samba Mahsuri and IR 64 to develop two-in-one drought-flood tolerant HYV of

rice. To address the problem of flash flooding,*SUB1*gene for submergence tolerance has been transferred into nine regional MVs of rice, viz. ADT 46, Bahadur, HUR 105, MTU 1075, Pooja, Pratikshya, Ranjit, Rajendra Mahsuri and Sarjoo 52. Further, *qSALTOL1*gene for seedling stage salt tolerance and *qSSISFH8.1*genes for reproductive stage salt tolerance have been transferred intofive MVs, viz. ADT 45, Gayatri, MTU 1010,Pusa 44 and Sarjoo 52.

We used foreground selection markers for the presence of desired gene, recombinant selection markers to reduce the linkage drag around the target gene and high-density background selection using a 50K SNP chip. Finally, MV-NILs with more than 95% similarity to the recipient parent genome have been released for commercial cultivation. These climate-smart rice varieties are gaining popularity and will provide yield stability in the adverse climatic conditions. So far more than 20such varietiesof rice developed through marker-assisted breeding have been released for commercial cultivation by farmers inIndiae.g. Pusa Basmati 1, Improved Samba Mahsuri, Pusa 1637, Ranjit-Sub1, DRR Dhan 50, Pusa 1847. MABB is the most promising and technically feasible option for introgression of useful genes in the background of MVs cultivated in millions of hectares but are sensitive to one or more of the climate-change induced stresses. Correction of these susceptibilities by introgression of small genomic segments promises high impact on rice production stability. A major limitation of molecular breeding is the availability of validated markers. Availability of affordable genotyping services within reach is another limitation that requires establishment of genotyping service centres.

Further, we are using wild rice germplasm collected from different parts of India to identifynovel genes for climate resilience. Crop wild relatives are adapted to wide geographical and climatic conditions and hence are a rich source of genes that can be harnessed for developing climate-resilient varieties. Therefore exploration, conservation, evaluation and utilization of fast depleting crop wild relatives gene pool is the need of the day. Efforts are also underway to combine genes for disease and pestresistancein these MVs without losing theiryield and quality attributes crucial for consumer acceptance.In the near future, genome editing tools for allele replacement (SDN-2) are likely to substitute the present backcross breeding approach for high speed precision breeding of climate-resilient varieties of rice and other crops.

Day 3 Plenary Session

MANIPULATING STOMATA TO ENHANCE RICE STRESS TOLERANCE

Julie Gray

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Stomata underpin crop productivity by allowing carbon dioxide to enter leaves and water vapour to exit. They open in the light for photosynthesis, and close in the dark or on dehydration. Studies of a signalling peptide that regulates stomatal development in Arabidopsis have allowed the identification of rice epidermal patterning factor orthologues and their manipulation. Transgenic rice plants have been produced with abnormally low stomatal densities and reduced stomatal conductance. These modified crops have substantially lower levels of water loss and show enhanced drought tolerance. They require less water to grow, and yet maintain seed yields. For example, rice seedlings with approximately half the usual number of stomata, use only 60% of the normal amount of water, are better able to survive drought, and still yield well. Optimisation and adoption of this technology could enhance yields under stressful conditions, reduce agricultural water requirements, help to mitigate the impacts of climate change on food security, and reduce future GHG emissions.

Keywords:stomata, development, water, carbon dioxide

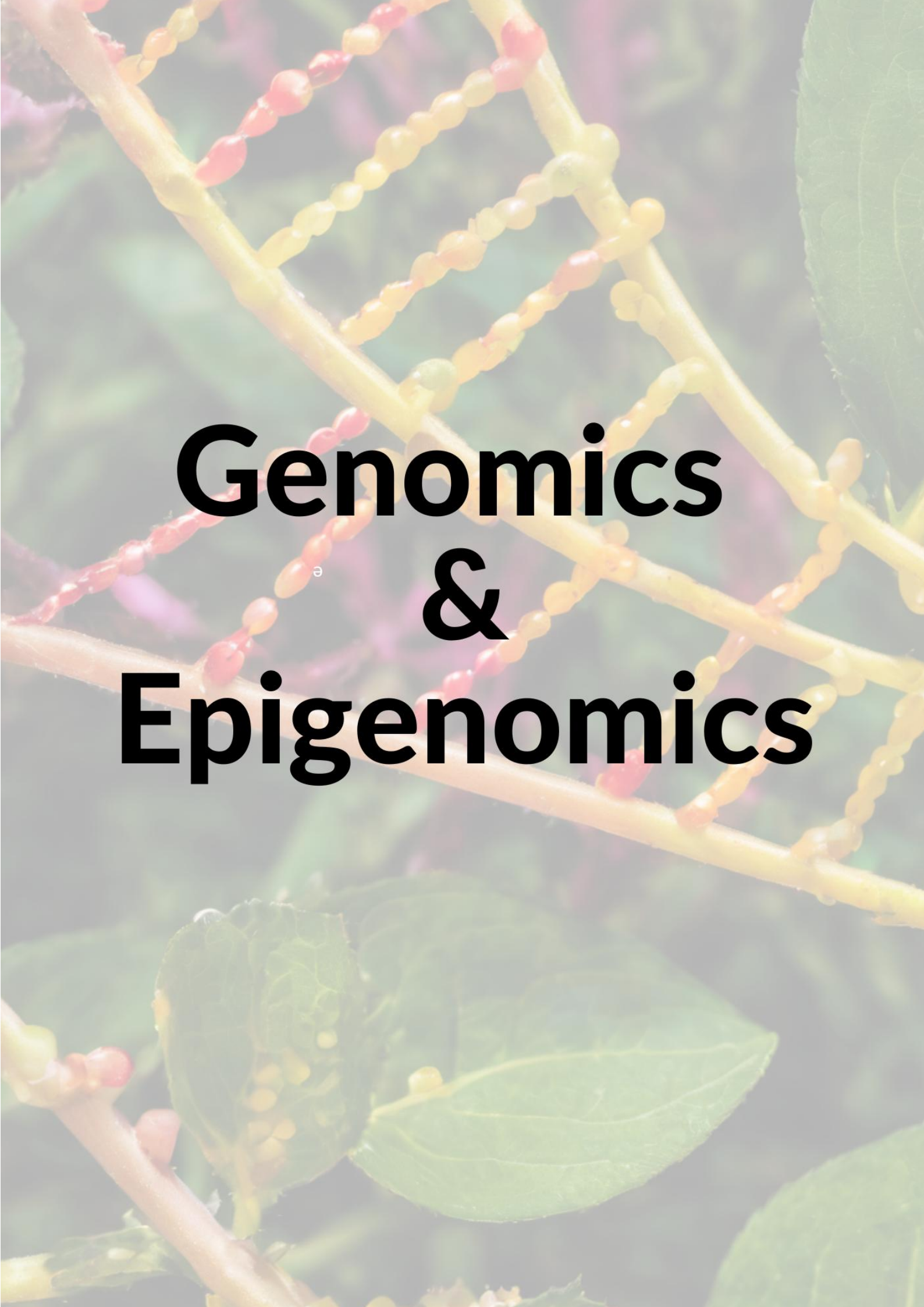
Day 3 Plenary Session

FERTILIZATION CONTROLS TILLER NUMBERS VIA TRANSCRIPTIONAL REGULATION OF A *MAX1*-LIKE GENE IN RICE CULTIVATION

*Authors: Jinying Cui, N. Nishide, K. Mashiguchi, K. Kuroha, M. Miya, K. Sugimoto, J.-I. Itoh, S. Yamaguchi & *Takeshi Izawa*

Fertilization controls various aspects of cereal growth such as tiller number, leaf size, and panicle size. However, despite such benefits, global chemical fertilizer use must be reduced to achieve sustainable agriculture. Here, based on field transcriptome data from leaf samples collected during rice cultivation, we reliably identify 107 fertilizer responsive genes and in this work focus on one of them,*Os1900*, a gene orthologous to *Arabidopsis thaliana MAX1*, which is involved in strigolactone biosynthesis. Elaborate genetic and biochemical analyses using CRISPR/Cas9 mutants reveal that *Os1900* together with another *MAX1-like* gene, *Os5100*, play a critical role in controlling the conversion of carlactone into carlactonoic acid during strigolactone biosynthesis and tillering in rice. Detailed analyses of a series of *Os1900* promoter deletion mutations suggest that fertilization controls tiller number in rice through transcriptional regulation of *Os1900*, and that a few promoter mutations alone can increase tiller numbers and grain yields even under minor-fertilizer conditions, whereas a single defective *os1900* mutation does not increase tillers under normal fertilizer condition. Such *Os1900* promoter mutations have potential use in breeding programs for sustainable rice production.

Session Lectures



Genomics & Epigenomics

Lead Lecture

NEW INSIGHTS ON THE INFLUENCE OF GENOMIC STRUCTURAL VARIATION ON CROSSOVER OCCURRENCE

Mathias Lorieux, IRD, France

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In Eucaryotes, meiotic crossovers are generally not distributed evenly across chromosomes. We examine how crossover occurrence is related with different genomic and epigenomic features. We evaluate the recombination landscape by generating a highly saturated genetic linkage map of rice (Oryza sativa L.), obtained from 2,000 F2 recombinants sequenced by short reads at 2-3x. We compare genomic features from the parental genomes sequenced by long reads.

Lead Lecture

UNDERSTANDING ROLE OF OSMADS29 IN EARLY SEED DEVELOPMENT AND MANIPULATING ITS EXPRESSION DOMAIN OF TO REDUCE GRAIN CHALKINESS"

Sanjay Kapoor, Vibha Vermaand Meenu Kapoor[1]*

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*Presenting Author

OsMADS29 (M29) is a seed-specific transcription factor with a pivotal role in governing diverse facets of rice seed development. It influences endosperm and embryo development, impacting grain filling and seed viability. Prior investigations conducted within our research group have revealed M29's ability to modulate hormone (Auxin/cytokinin) homeostasis infavor ofcytokinins, thereby affecting divisions and differentiation of cells in target organs.The analysis of the upstream regulatory region (URR) of *M29*revealed the presence of auxin-responsive and seed-specific elements. Analyzing the nuclear transport of M29, we have found that the Ca^{++} sensor Calmodulin (CaM) sequesters M29 in the cytoplasm, probably at the surface of the endoplasmic reticulum and regulates the rate of its nuclear entry. Furthermore, conditions of low Ca^{++} favor M29 sequestration by CaM and downregulate its nuclear import.Considering M29's effects on downstream starch biosynthesis and plastid biogenesis, we overexpressed M29 under the control of two seed/endosperm-specific promoters. The resultant transgenic rice plants show reduced grain chalkiness by up to 90% and increased grain width, length, and grain weight by up to 6%, 16%, and 23%, respectively.These findings underscore the potential utility of M29's direct impact on various facets of seed development as a valuable tool for enhancing grain quality in rice.

Lead Lecture

ESTIMATING GENOME-WIDE DNA METHYLATION HETEROGENEITY IN RICE AND PLANTS

Pei-Yu Lin[1], Ya-Ting Chang[1], Jenny Lee[1], Yu-Chun Huang[1] and Pao-Yang Chen[1]*

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In a heterogeneous population of cells, individual cells can behave differently and respond variably to the environment. This cellular diversity can be assessed by measuring DNA methylation patterns. The loci with variable methylation patterns are informative of cellular heterogeneity and may serve as biomarkers of diseases and developmental progression. Cell-to-cell methylation heterogeneity can be evaluated through single-cell methylomes or computational techniques for pooled cells. However, the feasibility and performance of these approaches to precisely estimate methylation heterogeneity require further assessment. Here, we proposed model-based methods adopted from a mathematical framework originally from biodiversity, to estimate genome-wide DNA methylation heterogeneity. We evaluated the performance of our models and the existing methods with feature comparison, and tested on both synthetic datasets and real data. Overall, our methods have demonstrated advantages over others because of their better correlation with the actual heterogeneity. We also demonstrated that methylation heterogeneity offers an additional layer of biological information distinct from the conventional methylation level. In the case studies, we showed that distinct profiles of methylation heterogeneity in CG and non-CG methylation can predict the regulatory roles between genomic elements in rice and Arabidopsis. We also observed an inverse pattern of heterogeneity associating with transcription between genebody and transposons in rice, that all together may imply a unique link between methylation heterogeneity and transcription. This opens up a new direction for plant epigenomics. Our methods, namely MeH, have been implemented, evaluated with existing methods, and are open to the research community.

Keywords: DNA Methylation pattern, Methylation heterogeneity, DNA methylation, Mathematical modelling, Next Generation Sequencing, Epigenetics, Plants, Rice

UNVEILING GENOMIC AND EPIGENOMIC SIGNATURES ASSOCIATED WITH DROUGHT STRESS RESPONSE/TOLERANCE IN RICE

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Abstract:

Drought stress is one of the major factors that limits crop productivity globally. The production of climate-smart crop plants is an utmost requirement of today, which may be achieved via implementation of cutting-edge innovative technologies. I shall demonstrate the use of integrated genomics approaches to generate essential knowledge helpful for production of drought-tolerant rice plants. Rice is an important crop accounting for food security of over half the world population. Water-deficit is a major abiotic factor that affects rice productivity worldwide. Rice germplasm exhibit high variability in their response to drought stress, which may be attributed to genetic and epigenetic variations. We employed various genomics interventions and performed whole-genome, transcriptome and bisulphite sequencing of rice cultivars with contrasting responses to drought stress. RNA-seq analysis identified several novel stress-responsive transcripts/isoforms and pathways. We discovered extensive DNA methylation at single-base resolution in rice cultivars. Numerous differentially methylated regions (DMRs) among different cultivars were identified and many of them were found associated with differential gene expression. DNA polymorphisms among the rice cultivars locatedwithin the differentially expressed genes present in the known quantitative trait loci were also identified. The genome-wide discovery of open chromatin regions was found correlated with differential expression of drought-responsive genes. The mining of these global datasets provided insights into the regulation of drought stress response via a candidate homeobox gene. Overall, these integrated data analyses led to the identification of molecular signatures and regulatory mechanisms, which can be used for engineering drought stress tolerance in rice.

HISTONE VARIANT H4.V: A GATEKEEPER TO H4K5AC MARKS AND SALT STRESS TRANSCRIPTOME

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Abstract:

Paralogous variants of canonical histones guide accessibility to DNA and function as additional layers of genome regulation. Across eukaryotes, mechanism of action and functional significance of several variants of core histones are well-known except that of histone H4. We identified a novel variant of H4 (H4.V) expressing tissue-specifically among *Oryza* members. This variant mediated specific epigenetic changes contributing to salt tolerance. H4.V was incorporated to specific chromosomal locations where it blocked deposition of active histone marks. Stress dependent re-distribution of H4.V enabled incorporation of active H4 Lysine5 Acetylation (H4K5Ac) marks. Mis-expression of H4.V led to defects in reproductive tissues and in mounting stress responses. H4.V mediated these alterations by condensing chromatin as seen with cryo-EM structures of reconstituted nucleosomes. These results not only uncovered the presence of a H4 variant among plants, but also of a novel chromatin regulation that might have contributed to the adaptation of semi-aquatic *Oryza* members.

SUB1A COORDINATES DISTINCT ACCLIMATION RESPONSES TO SUBMERGENCE IN SINK AND SOURCE LEAVES OF RICE.

Takeshi Fukao

Department of Bioscience and Biotechnology, Fukui Prefectural University

SUB1A, the ethylene-responsive transcription factor gene, confers robust tolerance to submergence in rice. A typical adaptation response regulated by *SUB1A* is the restricted elongation of growing leaves under submergence, a quiescence strategy that allows plants to ensure complete submergence for 14-16 days. However, functional characterization of *SUB1A* has been performed using entire shoot tissues, most of which are mature leaves that do not elongate under submergence. Mature leaves serve as source tissues that produce and provide energy reserves, whereas growing leaves are sink tissues that receive and consume energy reserves. The cooperative relationship between source and sink leaves is more critical under submergence because optimal growth and energy management is key to the survival of inundation. Here, we identified leaf-type-specific and overlapping adaptations to submergence coordinated by *SUB1A* using time-course analysis of RNA-Seq and hormone profiling. This study has revealed that *SUB1A* confers optimal adaptations to submergence in two functionally distinct leaves. For example, *SUB1A* repressed pathways associated with carbohydrate and nitrogen metabolism in both leaf types, with more severe restriction in sink leaves that have a greater energy demand if *SUB1A* is absent. In sink leaves, *SUB1A* suppressed the accumulation of mRNAs involved in cell division and elongation under submergence, but this trend was not observed in source leaves. Consistent with this result, accumulation of and responsiveness to growth-regulating hormones were properly modulated by *SUB1A* only in sink leaves. These data suggest that *SUB1A*, the ethylene-responsive transcription factor, directly or indirectly regulates the expression of distinct downstream target genes in sink and source leaves, enabling the coordination of optimal adaptations in the two functionally distinct leaves under submergence.

Key words: flooding, hormones, leaf maturity, *Oryza sativa*, submergence, transcriptome.

PIVOTAL ROLE OF MIRNA GENES IN ORCHESTRATING DROUGHT STRESS RESPONSE IN RICE

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Adverse environmental conditions such as drought invoke a multi-faceted global response at both molecular and biochemical level. Small regulatory RNAs play a pivotal role in orchestrion of the of these response. Our studies clearly indicate that miRNA genes actively respond to drought stress conditions in a most unique fashion. The global miRNA response has evolved uniquely in drought tolerant and sensitive rice cultivars indicating an adaptive selective pressure. Since, most miRNA genes target several genic transcripts, they act a natural regulatory 'hubs'. Comparative miRNome analysis in drought tolerant and sensitive rice cultivars identified several such regulatory hubs that behave in a contrasting manner under similar drought stress conditions. This indicated that the regulation of miRNA genes itself is quite dynamic. It was found to be dynamically regulated by the modulation of the cytosolic Ca^{2+}levels via the internal and external calcium channel. Direct involvement of calmodulin and CAMTA TFs could also be demonstrated. Functional characterization of several drought regulatory miRNAs such as miR528 and miR408 in transgenic system clearly indicated their pivotal role in coordinating several molecular processes such as copper homeostasis, ROS production and other developmental process in response to drought stress conditions in rice.

First Telomere-to-Telomere Indian Rice Genome

Dr. Raja Mugasimangalam

Genotypic Technology Bangalore and QTLomics Technologies, Bangalore, India

Abstract:

"Basmati" is a long-grain aromatic rice that has been grown for centuries in the Himalayan foothills of the Indian subcontinent. We have sequenced the genome of the well-known and cultivated and exported Punjab Basmati PB3 using long-read Nanopore technology. The rice genome was sequenced at 350X coverage using Nanopore Promethion P24 long reads with the aim of achieving telomere-to-telomere whole-genome sequencing and covering the missing parts of the genome references constructed using short-read technologies. High-accuracy base-called sequences were assembled, scaffolded, and annotated. The total scaffold size is 373.5873 Mb and mapped to the reference rice genome at 99.99% horizontal coverage.

We will present the telomere-to-telomere genome of the Basmati rice genome, particularly significant single nucleotide variants (SNVs) and insertions and deletions (Indels) and long and short genome translocations/rearrangements, the telomeres sequences, and mitochondrial and chloroplast sequences. To our knowledge, this is the first time that telomere-to-telomere sequencing of an Indian plant has been reported. This work is a three-way collaboration with Srivignesh Lab, Department of Horticulture, School of Life Sciences, Central University of Tamil Nadu (CUTN), Genotypic Technology Bangalore, and QTLomics Technologies, Bangalore, India.

FUNCTIONAL CHARACTERIZATION OF SALT TOLERANT GENES FROM *ORYZA COARCTATA*: AN TRIPLOID WILD SPECIES OF RICE

*Swati Mishra, Soni Chowrasia, Jyoti Nishad, Nitasana Rajkumari, Hukum C. Rawal, Tapan Kumar Mondal**

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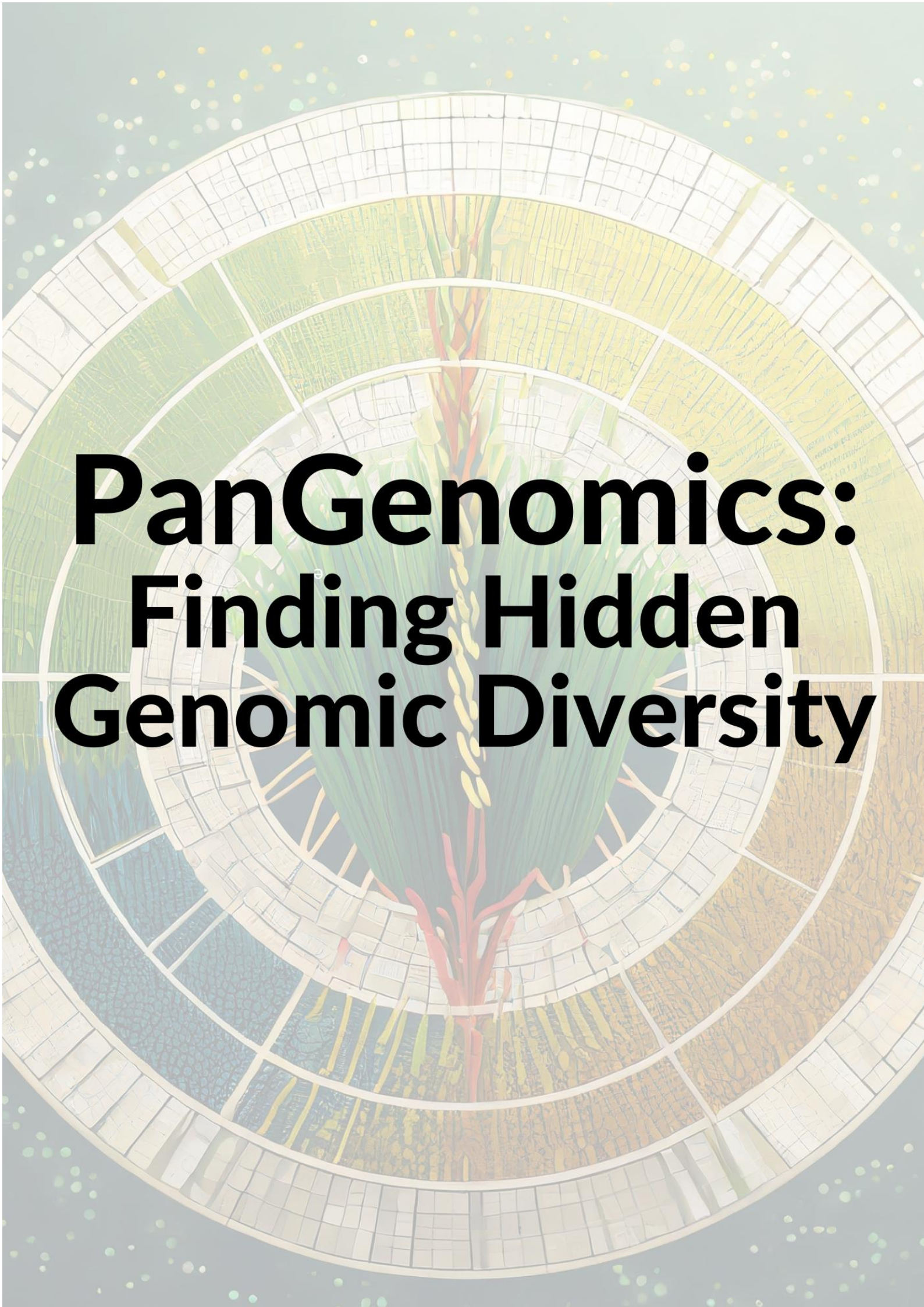
Oryza coarctata posses' very distinct anatomical, morphological and physiological characteristics which help it to thrive well under high salinity conditions. Since ever we decoded the chromosome level genome sequence of *O. coarctata*, deciphering genes responsible in regulation of salt has become easy. There are specific cellular and metabolic pathways responsible to govern salinity tolerance to this species which in turn involves multiple genes. This study focuses on identification of novel salt tolerance genes under salinity stress. The cDNA libraries from the salt treated root tissues of *O. coarctata*was prepared for generation of overexpressed transgenic Arabidopsis line through *Agrobacterium* mediated transformation. The screening and functional validation of the developed transgenic lines was performed under salinity stress condition which indicate that allantoin biosynthesis pathways are active and render salinity tolerance to this species. Therefore, this work opens new possibilities to get insight into the salt tolerance mechanisms of this species.



PanGenomics: Finding Hidden Genomic Diversity

Lead Lecture

SNP-Seek and Other Tools for Uncovering Rice Diversity

Kenneth L. McNally

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3000 Rice Genomes analyses relative to the high quality (often gap-free) reference genomes has enabled the use of pan-genome analyses to explore rice diversity. The first versions of the SNP-Seek database (https://snp-seek.irri.org) were limited to variant calls relative to Nipponbare. Now, we are extending SNP-Seek V3 to include additional reference genomes (initially Minghui 63) and its 3K RG variant calls. We have also been developing other tools to support various aspects of digital breeding: 1) Haplotool for extended haplotype analysis; 2) Crop Galaxy (http://cropgalaxy.excellenceinbreeding.org) with GS, ML, GWAS, among others; Rice ImageBreed for UAV data curation and analysis; 3) a Streamlit G2P prediction engine for LM and ML methods from UAV data; 4) Rice-pilaf for post-GWAS analyses to provide supporting evidence for candidate genes. Together, the new genomics resources and bioinformatics tools will allow in silico modeling of rice, and accelerate rice improvement through allele mining and digital breeding. We hope that SNP-Seek V3 will revitalize the International Rice Informatics Consortium (IRIC) for further support of its development and the curation of datasets for the public to access, analyze, and use for discovery. For future development of SNP-Seek (V4), we are exploring incorporation of Tripal and use of plink2 for variant data storage in place of HDF5.

Keywords: SNP-Seek, genomic diversity, pan-genome

PANORYZA – PUBLIC ACCESS TO PAN GENES AND PAN PROTEOMES FOR ASIAN RICE

Pankaj Jaiswal[1], Parul Gupta[1],Justin Elser[1], Guy Naamati[2], Shradha Saraf[2], Dmytro Chebotarov[3], Kapeel Chougule[4], Zhenyuan Lu[4], Sharon Wei[4], Yong Zhou[5], Jianwei Zhang[6], Zhichao Yu[6], Eshan Sharma[7], BrunoContreras Moreira[8], Ken McNally[3], Doreen Ware[4,9], Rod Wing[3,5,10], Dario Copetti[10],Maria Martin[2], Sarah Dyer[2], Andrew R Jones[7]

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10. Arizona Genomics Institute, School of Plant Sciences, University of Arizona, 24 Tucson, Arizona 85721, USA

The rice genome was sequenced in 2005 for *O. sativa* Japonica Nipponbare andindependently annotated by several groups. International sequencing projects have nowalso generated 16 "platinum standard" reference sequence genomes for Asian rice (the “MAGIC-16” set), which are available in public databases Ensembl Plants and Gramene. This presents a great resource but also some difficulties in interpretation, since genes predicted in one cultivar are not linked to their orthologs in other cultivars or in the reference genome. Our team has built several resources to assist with biological interpretation and provide added value.

First, we have developed the GET_PANGENES pipeline, which creates pan-gene clusters – linking assumed true orthologs across aligned genomes. We have a proposal for a stable identifier system and implementation, to assist the rice researchers in referencing pan-genes, for which we seek community feedback.

Second, we have processed large volumes of public proteomics data, mapped to the MAGIC-16 data set, providing independent evidence that predicted coding sequences

produce observable proteins under different conditions. We are using this evidence, as well as transcriptomic and protein domain data, to rank different genes models at each locus in the pan-genome.

Third, we have produced an atlas of the rice phosphoproteome, from all publicly available phospho-enriched data sets, mapped onto the reference genome, and the 3K genome variation data set. We are able to demonstrate phosphosites that are fully conserved in all varieties, and those that are lost in particular cultivars or families. We believe these resources will assist rice researchers in prioritising searches for genes/proteins of interest across the pan-genome.

ESTABLISHMENT OF REFERENCE GENOMIC RESOURCES FOR THE WILD RELATIVE OF RICE - ORYZA COARCTATA: TOWARDS THE NEO-DOMESTICATION OF SALT-TOLERANT RICE

Gopal Misra

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Rice (Oryza sativa) is one of the world's most important staple crops and consumes about one-third of the world's freshwater resources for irrigation. As fresh water supplies dwindle and become more saline, breeders are tasked with the development of both drought resistant and salt tolerant varieties that will also maintain their yield and quality. Despite considerable efforts to breed salt-tolerant rice, most cultivated rice still remains highly salt sensitiveIn contrast to rice, wild relative of rice, Oryza coarctata is the only halophytic species in the genus Oryza, which are found across the coastal regions of South Asia from Myanmar to Pakistan. Understanding the genetic diversity and dissecting the genomic regions associated with this species' salt tolerant mechanisms could help us to develop salt-tolerant rice cultivars via neo-domestication. Establishing a reference genome is prerequisite step in this direction. The Wing lab at KAUST, Saudi Arabia, has developed a high-quality reference genome of Oryza coarctata. The next goal will be to create a germplasm bank of around 400s of accessions of O.coarctata collected along the Indian coastline to understand its genetic diversity. Our first germplasm collection was launched in the Sundarban area of India, known as the hub for this wild rice species. Other different sites on the Indian coast will be covered soon. My talk will summarize the current status of the generation of reference genomic resources, a germplasm collection, and our future course of action to neodomesticate the O. coarctata.

MUTATION BREEDING FOR STRESS RESILIENCE IN RICE

*Eduardo Venske, Raymond Joseph, Vivian Viana, Viviane Kopp da Luz, Luiz Chairez-Tejeda, Latoia Eduarda Maltzahn, Allisson Ramirez, Luciano Carlos da Maia, Camila Pegoraro and Antonio Costa de Oliveira**

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Rice is among the three major cereals produced and an important source of energy to more than a third of the world's population. Climate change and population growth are pushing agriculture to situations of increasing biotic and abiotic stresses. The understanding of plant response mechanisms to stresses is key to the development of stress resilient crops. Rice is an important crop in Brazil, being the State of Rio Grande do Sul responsible for ca 70% of its production. Several abiotic stresses are identified in the rice crop, such as chilling, iron toxicity, salinity and more recently drought starts to become a problem. Mutants at advanced generation and reverse genetics strategies are targeting stress resilient ideotypes. Recently, a chilling tolerant line FAEML 140 was registered in the Ministry of Agriculture of Brazil and may help to improve chilling tolerant genotypes. Mutants resilient to drought were also characterized morphological and molecularly.

Plant Development: Vegetative and Reproductive

Lead Lecture

TITLE: PROBABLE ROLE OF MITOGEN-ACTIVATED PROTEIN KINASES IN THE REGULATION OF THE CELL CYCLE IN RICE

Dr. Alok Krishna Sinha

National Institute of Plant Genome Research, Aruna Asaf Ali Road, New Delhi 110067

Abstract:

Mitogen-Activated Protein Kinase (MAPK), a group of Ser/Thr protein kinase are known to regulate multidisciplinary pathways in all studied eukaryotic system. The evidence of regulation of the cell cycle by MAP kinases is initiated from the identification of the Ras-cRaf-MEK-ERK pathway which phosphorylates cMYC, JUN transcription factor, which are involved in initiation of the cell cycle in the mammalian system. In later studies, MAP kinase was found to regulate cell cycle by influencing the function of several other components like Retinoblastoma (Rb), $p53^{kip2}$, SIC1, $p27^{kip1}$, WEE1 etc, in Yeast and animal systems. However, the role of MAP kinases in regulating plant cell cycle is scanty. We tried to elucidate the role of MAP kinase cascade components in the regulation of plant cell cycle in rice. Initial investigation complying cell cycle inhibitors as well as MAP kinase pathway inhibitors have demonstrated co-regulation of multiple MAP Kinase and cell cycle gene expression. Further, interaction studies with MAP kinase and cell cycle regulating proteins have indicated the involvement of MAP kinase in regulating cell cycle from G1 to S phase. *In-vivo* studies with mutants, as well as over-expression plants of cell cycle and MAP Kinase genes, have shown significant influence in plant architecture and yield parameter traits. Our study indicates the importance of MAP kinase in regulating plant architecture and yield by influencing the cell cycle.

Keywords: MAP Kinase, Cell Cycle, protein-protein Interaction, Gene editing, Phosphorylation

Lead Lecture

GENETIC INDEPENDENCY OF RICE TILLERS REVEALED THROUGH CHEMICAL MUTAGENESIS

Kiyoshi Yamazaki[1]*, *Naoyuki Sotta*[1], *Toru Fujiwara*

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1 Equal contribution

Generation of mutant populations with high genetic diversity is key for mutant screening and crop breeding. The single-seed descent method, in which one mutant line is established from a single mutagenized seed, is commonly used to assure independency of the mutant lines. This method, however, limit the size of the mutant population to the number of fertile M_1 plants. The rice mutant population size can be increased if a single mutagenized plant produces genetically independent siblings. However, to our knowledge, the cell lineage that gives rise to tillers in rice is not clear. I the process of chemical mutagenesis of rice, we examined independency of tillers using genomic resequencing. Patterns of mutations of seedlings (M_2) derived from independent tillers were examined from a plant derived from a single ethyl methanesulfonate (EMS)-mutagenized seed (M_1). We used three independent M_1 plants and five tillers were selected from each plant. A single M_2 seed was selected from each tiller, and the distributions of mutations induced by EMS were compared. Surprisingly, in most pairwise combinations of M_2 siblings from the same parent, ≥85.2%–97.9% of all mutations detected were not shared between the siblings. This high percentage of independency suggests that the M_2 siblings were derived from different cells of the M_1 embryo. It also indicates that several genetically independent lines can be obtained from a single M_1 plant. This approach should allow a large reduction in the number of M_0 seeds needed to obtain a mutant population of a certain size in rice.

Reference

Yamazaki*, K., Sotta*, N. Fujiwara T. M_2 plants derived from different tillers of a chemically mutagenized rice M_1 plant carry independent sets of mutations. (2023) Plant J in press doi.org/10.1111/tpj.1639

TITLE: RICE EMBRYOGENESIS MAGIC: UNLOCKING CLONAL SEEDS FOR HYBRID VIGOR PRESERVATION

Imtiyaz Khanday

Department of Plant Sciences, University of California, Davis, USA.

Fertilization of an egg cell by a sperm cell initiates zygote development. The molecular pathways that govern embryo initiation and prevent its occurrence in the absence of fertilization, remain poorly understood. Our studies in rice have identified AP2-family *BABY BOOM* (*BBM*) transcription factors as the key genes responsible for embryo initiation post-fertilization. Four *BBM* genes (*BBM1-4*) redundantly regulate embryogenesis and their male allele-specific expression in zygotes is essential for embryo initiation. Egg cell-specific expression of *BBM* genes leads to parthenogenesis, resulting in haploid progeny. Thus, the expression of these sperm cell-expressed transcription factors in the egg cell is sufficient to overcome the fertilization block and initiate embryogenesis. Our recent analysis reveals female-expressed auxin biosynthesis *YUCCA* genes as direct targets of BBM1, implying male and female genomes collaborate for zygotic development with paternal BBMs activating maternal *YUCCA* genes. Hybrids exhibit enhanced vigor compared to their inbred parents, significantly boosting yields. However, high-yielding hybrids cannot be maintained with normal seed propagation due to genetic segregation. This limits their utilization, including in crops like rice, a vital global staple. Previously, using our understanding of embryo initiation, we developed a clonal seed formation method with 30-35% efficiency, demonstrating the preservation of parental heterozygosity in the progeny. Recently, our collaborative efforts have yielded remarkable results, achieving a clonal seed efficiency exceeding 95% in a commercial rice hybrid. Furthermore, new findings on alternative methods to enhance parthenogenesis efficiency will be presented.Thus, these breakthrough studies establish the feasibility of hybrid vigor fixation in rice and possibly in other crops.

Further reading:

Khanday et al., 2023, Somatic embryo initiation by rice BABY BOOM1 involves activation of zygote-expressed auxin biosynthesis genes. *New Phytologist* 238:673–687

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Khanday et al., 2019, A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature* 565:91–95

A NOVEL ALLELE OF *OsCKX2* CREATED THROUGH CRISPR/CAS12A CONFERS YIELD SUPERIORITY, STRONGER CULM AND EARLINESS IN INDICA RICE CV. SAMBA MAHSURI

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Cytokinin is a major plant hormone that regulates cell division, leaf development and senescence, shoot growth, source-sink relationship, and several other processes involved in plant growth and development. In coordination with other phytohormones, cytokinins play a crucial role in gametophyte development, root growth, vascular development, nutrients uptake, and stress response. Cytokinin oxidases (CKX) cleave the cytokinins and inactivate them irreversibly. The rice genome encodes eleven CKXs, among them *OsCKX2* has been identified as negative regulator of grain yield. *Grain number 1a* (*Gn1a*) allele of *OsCKX2* (containing 16 bp deletion in 5'UTR and 6 bp deletion in exon-1) was associated with the high grain number/panicle, while its null allele (with 11 bp deletion in exon-3) improved lodging resistance through increasing the culm diameter along with high grain number per panicle. In this study, we utilized CRISPR/Cas12a multiplex genome editing method to edit two different exons of *OsCKX2* in a popular rice cultivar Samba Mahsuri. Several variants of *OsCKX2* were generated and transgene free homozygous edited mutant lines were obtained. One such mutant named as *KAMALA* showed significant increase in grain number per panicle and overall yield advantage of >35% over the wild type Samba Mahsuri. In addition, the mutant exhibited stronger culm, earliness and broader leaves. Increased grain yield of Samba Mahsuri without any alteration in grain and cooking quality can pave way for enhancing the yield of several other low yielding cultivars through the deployment of novel variant of *OsCKX2*.

TITLE: RICE SEED VIGOR AND VIABILITY: ROLE OF PROTEIN REPAIRING ENZYMES

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Reduction of seed germination vigor and viability during seed storage is one of the main concerns in agriculture, since about 25% annual loss of total harvested crop occur due to seed deterioration upon storage. Our study reveals that Protein Repairing Enzymes (PRE) play a crucial role in preserving seed vigor and viability in rice seeds. We have identified protein repairing enzymes PROTEIN L-ISOASPARTYL O-METHYLTRANSFERASE (PIMT) and Methionine Sulfoxide Reductase (MSR), which catalyse the conversion of spontaneously modified isoAsp to Asp, and MetSO to Met, respectivelyin proteins, play vital roles in preserving seed vigor and viability. Our analyses of Oryza species with contrasting seed desiccation tolerance further reveals that PIMT also acts as a key player that govern seed desiccation tolerance to orthodox seeds but ineffective in recalcitrant seeds. We showed that unlike orthodox seed *Oryza sativa*, desiccation intolerance and poor seed vigor of recalcitrant seeds of *Oryza coarctata* are associated to reduced PIMT activity and increased isoaspartyl accumulation due to the lack of coordinated action of ABA and ABI transcription factors (TF) to upregulate PIMT during maturation. We showed that suppression of PIMT reduces, while its overexpression increases seed desiccation tolerance, seed vigor and longevity in *O. sativa*. Our study further reveals those various proteins, including ABI–TFs, undergoes isoAsp formations that affect their functional competence; however PIMT interacts, repairs isoAsp and facilitate their functions and improves seed desiccation tolerance, vigor and seed storage life of rice.

Collectively, our study illustrates that PIMT mediated protein repair mechanism plays a key role in preserving seed vigor and viability by protecting functionality of proteins from isoAsp mediated protein damage.

TITLE: SPECIES-SPECIFIC FUNCTIONAL INNOVATIONS OF CONSERVED REGULATORS DURING TISSUE TRANS-DIFFERENTIATIONAND BRANCHING

ShriRamYadav

Department of Biosciences and Bioengineering, Indian Institute of Technology Roorkee, Uttarakhand, India

Direct organogenesis provides tremendous plasticity during plant growth and development undervarious conditions. Highly derived fibrous root system in grasses is primarily determined byadventitious/crownroots.Unlikeroot-bornelateralroots,thecrownrootsoriginatefromtheshoottissuesbyshoot-to-roottissuetrans-differentiation,thusinvolveslineagereprogramming.Weusethisasamodelsystemtoinvestigatethemechanismunderlyingthetissue-trans-differentiation/lineage reprogramming. Using laser-capture microdissection coupled with RNAsequencing, we generated high-resolution genome-wide landscape of transcriptional signaturesandin-depthspatio-temporalexpressionpatternsofpotentialepigeneticmodifiersandtranscription factors during priming and outgrowth of rice crown root primordia. Functionalanalysesofricecellfatedeterminantsfrom*WUSCHEL-RELATEDHOMEOBOX*and*PLETHORA*genefamiliesrevealtheirnon-redundant,andspecies-specificrolesindeterminingrootarchitecture and shoot branching. Auxin signaling has been considered as the earliest regulator ofdirect root organogenesis. We identified an upstream direct regulator of local auxin biosynthesisgenes to promote crown root development. Interestingly, by complementing Arabidopsis mutantdefectsbyricefactors,weuncoveredtheirconservedroleinrootprimordiaoutgrowthirrespectiveoftheirdevelopmentalorigin.Together,ourfindingsunveilamolecularframeworkoftissuetrans-differentiation during root primordia establishment, leading to culmination of robust fibrous rootarchitecture. This also suggests that conserved factors have evolved their transcription regulationtoacquire species- and organ-specificfunction.

A C2H2 ZINC FINGER TRANSCRIPTION FACTOR EXHIBITS PLEIOTROPIC EFFECTS ON RICE GRAIN

Pinky Agarwal, *Priya Jaiswal, Arunima Mahto, Ankur Vichitra*

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REP1 is a C2H2 zinc finger transcription factor (ZF TF), which has two characteristic ZF domains and a DLN repressor motif. It expresses at high levels during the stages of rice seed development, specifically in the endosperm. It is a transcriptional repressor, which localizes to the nucleus and nucleolus. Rice plants over expressing this gene show an extreme phenotype, imparting plantlet lethality. Rice plants harboring the knock down (KD) construct of *REP1* and CRISPR mutants (KO) of *REP1* show decreased plant height, leaf width and number of panicles per plant. The leaf length is increased in these plants. The overall yield per plant decreases in knock down plants, but is unaffected in CRISPR mutants. Seeds on these plants have decreased grain length but increased grain width and the grain weight is lesser than control. The cell number in the grain husk decreases in both longitudinal and transverse directions. The grain width increases because the husk cells are wider than longer. The KD and KO of *REP1* affects starch morphology and the expression of related genes. These grains have a chalky endosperm, with loosely packed spherical starch granules. The total starch and amylose content decreases in KD and KO grains. The total protein content also decreases because of differential expression of related genes. This is because REP1 binds to the promoter of a related gene, and alters its expression. REP1 interacts with histone deacetylases to act as a repressor. Hence, REP2 affects multiple rice grain traits, which are of commercial importance.

MECHANISTIC INSIGHT INTO THE ROLE OF AXR4 IN REGULATING TRAFFICKING OF *AUXIN INFLUX TRANSPORTERS* AUX1 AND LAX2

Ranjan Swarup

University of Nottingham, UK

Plant hormone auxin plays crucial roles in almost all aspect of plant growth and development. Auxin transport is carrier mediated and facilitated by auxin influx and efflux transporters. In *Arabidopsis*, auxin influx carriers are encoded by a small gene family within the Auxin-amino acid permease superfamily comprising four members *AUX1, LAX1, LAX2 and LAX3*. AUX1/LAX proteins are multi membrane spanning plasma membrane proteins. Sorting controls are crucial for the regulation of targeting of the plasma membrane (PM) proteins and thus they define various key processes including directional transport of auxin. Despite the importance of auxin transport in plant development, in contrast to the PIN auxin efflux carriers, sorting and PM targeting of their counterparts auxin influx carriers AUX1/LAX proteins is not well understood. In *Arabidopsis*, ER (endoplasmic reticulum) protein AXR4 has been shown to regulate the trafficking of AUX1 and LAX2. In the absence of AXR4, AUX1 and LAX2 are predominantly localised in the ER (Tidy *et al* 2023, *Plant Physiology*, In Press).

A rice homolog of *AtAXR4* has been identified but it only shares a 30% similarity with *AtAXR4* at the protein level and is predicted to localise to the chloroplasts. Using a functional complementation approach, we now show that OsAXR4 is localised to the ER in Arabidopsis and is able to restore Arabidopsis *axr4* mutant phenotypes as well as can correct the targeting defect of LAX2 to the PM. A CRISPR-Cas9 mutant of OsAXR4 has been obtained that show auxin related developmental defect including defect in root gravitropism and root hair elongation under low Phosphate besides several other novel phenotypic growth defects not known before in Ataxr4. The presentation will also provide our recent understanding of the mechanistic aspects of AXR4 function. AXR4 is a plant-specific protein and contains a weakly conserved α/β hydrolase fold domain that is found in several classes of lipid hydrolases and transferases. We have previously proposed that AXR4 may either act as 1) a post translational modifying enzyme through its α/β hydrolase fold domain or 2) an ER accessory protein, which is a special class of ER protein that regulates targeting of their cognate partner proteins. We now have provided evidence that AXR4 is unlikely to act as a post-translational modifying enzyme as mutations in several highly conserved amino acids in the α/β hydrolase fold domain can be tolerated and active site residues are missing. Our results suggest that AXR4 acts as an ER accessory protein (Tidy *et al* 2023, *Plant Physiology*, In Press).

HARNESSING NATURAL VARIATION TO INTEGRATE LEAF DEVELOPMENT AND PHOTOSYNTHESIS IN RICE

Aashish Ranjan

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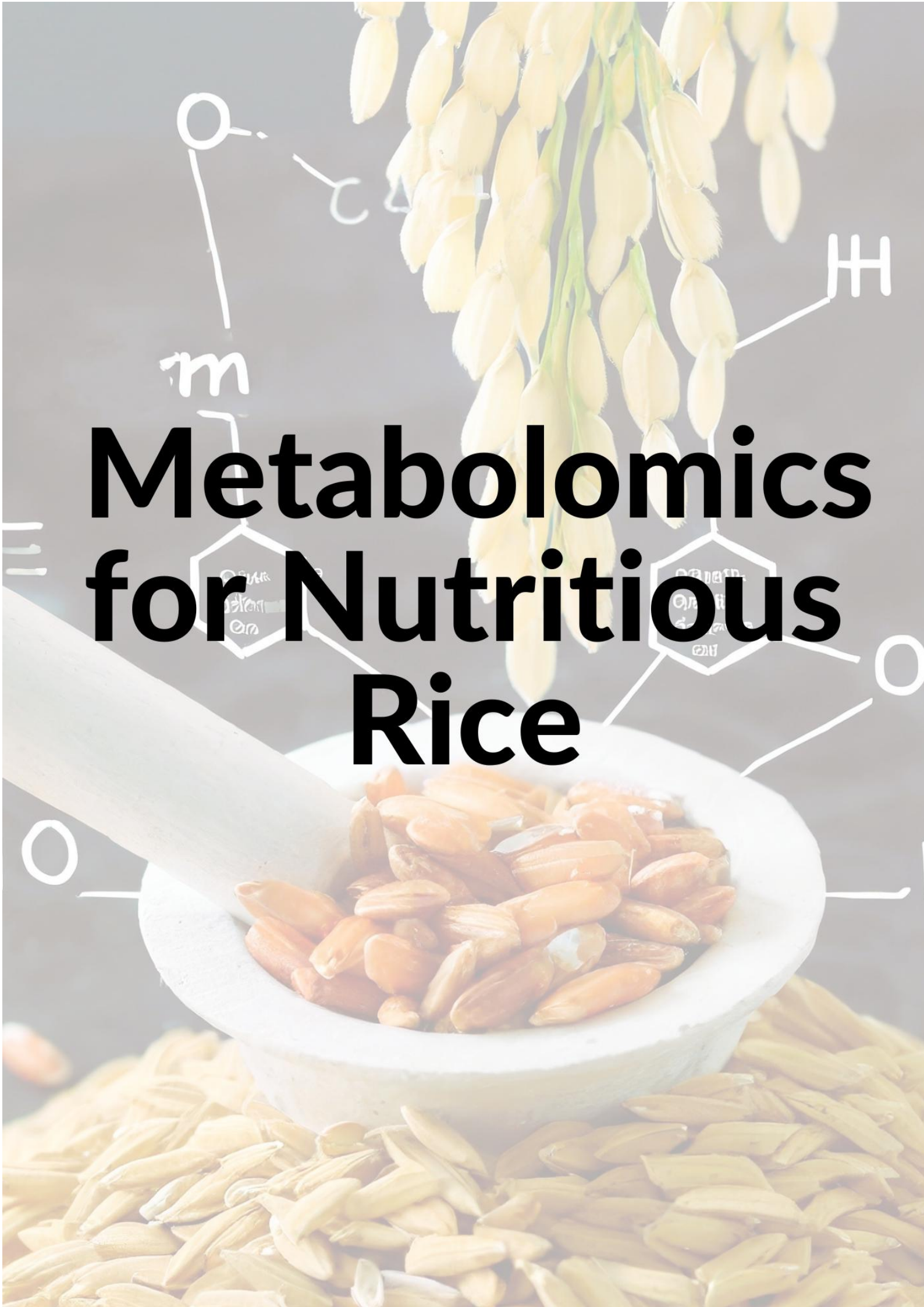
The importance of increasing photosynthetic efficiency for sustainable crop yield increases is well recognized. The natural genetic variation in leaf photosynthesis and its underlying developmental and biochemical basis is an overlooked and untapped resource. The genus *Oryza*, including cultivated rice and wild relatives, offers tremendous genetic variability to explore photosynthetic differences and underlying developmental and biochemical attributes. Investigation of the variations in photosynthesis across multiple wild and cultivated rice accessions revealed that several wild relatives of rice had higher net photosynthesis per unit leaf area compared to cultivated rice varieties. Leaf photosynthesis in cultivated rice varieties IR 64 and Nipponbare was limited due to Rubisco activity and electron transport rate compared with photosynthetically efficient wild rice accessions *Oryza australiensis* and *Oryza latifolia*. Leaf morphological traits, such as wider and thicker leaves, and anatomical features, such as mesophyll features and chloroplast surface area exposed to intercellular space, contribute to higher photosynthetic efficiency in wild rice accessions. Moreover, large-scale field phenotyping exhibited remarkable variation in leaf photosynthesis and related leaf physiological and developmental traits among cultivated Indian rice accessions. GWAS with cultivated landraces and comparative transcriptomics involving cultivated and wild rice accessions are being used to identify the genetic loci/genes regulating the desirable leaf developmental and physiological features that could be targeted for increasing the photosynthetic efficiency of cultivated rice varieties.



Metabolomics for Nutritious Rice

Lead Lecture

METABOLOMICS AND MACHINE LEARNING TECHNIQUE UNRAVELS MULTI-NUTRITIONAL PROPERTIES OF PIGMENTED RICE IN GERMINATED SPROUTS

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ABSTRACT:

Enhancing the dietary properties of rice is crucial to contribute to alleviating hidden hunger and non-communicable diseases in rice-consuming countries. Germination is a bioprocessing approach to increase the bioavailability of nutrients in rice. However, there is a scarce information on how germination impacts the overall nutritional profile of pigmented rice sprouts (PRS). Herein, we demonstrated that germination could increase certain dietary compounds, such as free phenolics and micronutrients (Ca, Na, Fe, Zn, riboflavin, and biotin). Metabolomic analysis revealed the preferential accumulation of dipeptides, GABA, and flavonoids in the germination process. Genome-wide association studies of the PRS revealed the activation of specific genes such as *CHS1* and *UGT* genes responsible for increasing certain flavonoid compounds. Haplotype analyses showed a significant difference ($P < 0.05$) between alleles associated with these genes. Genetic markers associated with these flavonoids were incorporated into the random forest model, improving the accuracy of prediction of multi-nutritional properties from 89.7% to 97.7%. Deploying this knowledge to breed rice with multi-nutritional properties will be timely to address double burden nutritional challenges. Further, increased levels of carotenoids, triacylglycerols (TAGs), and diacylglycerols (DAGs) were particularly prominent in pigmented rice sprouts (PRS)

Lead Lecture

compared to non-pigmented rice samples contributed to enhanced antioxidant capacity. We also explored potential interactions between lipid molecules (lyso-counterparts) and starch components, which may contribute to the presence of resistant starch. Genome-wide association studies (GWAS) combined with metabolome based lipidome profiling (mGWAS), gene-set analysis, and targeted association analysis revealed 72 candidate genes involved in the regulation of these accumulating lipids, with a particular emphasis on lipase-related genes. This study provides valuable insights into the variations in the lipidome between non-germinated and germinated rice, the genetic basis of the genome-lipidome relationship, and the potential health benefits of germination as a valuable dietary source for human health.

CRUCIAL ROLE OF PPi-DEPENDENT METABOLIC PATHWAYS IN RICE ENDOSPERM

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The endosperm is a primary constituent of mature seeds in rice as well as in other cereal crops, serving as the major storage reserve of starch. Observations indicate that the central part of the endosperm is subject to hypoxic conditions, which require a switch of energy metabolism owing to limited mitochondrial respiration. Uniquely, this endosperm generates a large source of inorganic pyrophosphate (PPi) as a byproduct of the reaction of ADP glucose pyrophosphorylase in the cytosol. Recent results derived from examination of the mutants of cereal crops, especially rice, for PPi-utilizing enzymes clearly suggest an important role of PPi as an alternative energy currency for integrating carbon metabolism from sucrose breakdown to starch synthesis in the endosperm. We will present our results on the interlaced PPi-dependent metabolic pathways, which are critical for starch synthesis in the endosperm in terms of energy metabolism in rice.

DIFFERENT STRATEGIES TOWARDS IMPROVING PHOTOSYNTHESIS IN RICE

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Rice production can be enhanced by increasing photosynthetic efficiency and this can be achieved by either implementing C_4 metabolism in rice or improving its C_3 photosynthesis. To engineer C_4 metabolism into a C_3 plant such as rice, it is essential to understand how C_4 genes, such as *phosphoenolpyruvate carboxylase* (*PEPC1),* are expressed at high levels and in a cell-specific manner. The *PEPC1* promoter from several C_4 grasses (e.g. *Zea mays* and*Setariaviridis*) has been shown to drive cell-specific gene expression in rice, but the transcriptional regulators are largely unknown. We have identified and characterized the function of several transcription factors regulating C_4*PEPC1* promoters in rice and this will be presented and discussed. In addition, it has been shown that different strategies can be used to improve C_3 photosynthesis, but the combination of these strategies has never been tested. For instance, it is known that accelerating the relaxation of non-photochemical quenching (NPQ) can greatly improve plant yield. Another process that limits photosynthesis is the oxygenase activity of Rubisco, which leads to photorespiration. Different strategies are known to reduce photorespiration, thus improving photosynthetic efficiency. In order to improve photosynthesis in rice, we have combined the over-expression of different enzymes involved in the NPQ dynamics with the over-expression of enzymes predicted to promote oxygen scavenging. We have observed that simultaneous integration of both strategies in rice is possible, improves NPQ dynamics, and enhances the photosynthetic efficiency.

PYRAMIDING-BY-DESIGN: NUTRIENT-DENSE AND CLIMATE-RESILIENCY TOWARD ORGANIC PIGMENTED RICE

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When plant breeding aims to integrate multiple traits into a favourable rice variety, marker-assisted backcrossing (MAB) is the most popular method. The shortcoming of MAB has been a low turnaround time when a large number of markers are involved. We routinely employed the marker-assisted Pseudo-backcrossing (MAPB) method to shorten the cycle in large-scale gene pyramiding schemes. PinK+4, a high-yielding, low glycemic index rice, was the first successful case of MAPB. Following PinK+4, we demonstrated long-term efforts to improve highly nutritious rice for climate resiliency by introgressing multiple resistances to biotic and abiotic stresses using MAPB. We regularly pyramided "+4" (Flooding, BB, BL, and Bph) onto seven popular irrigated cultivars and two most popular rainfed-lowland rice cultivars, including Thai Jasmine rice and RD6. For biotic stresses, 12 functional markers tagging resistant genes for bacterial leaf blight, leaf blast, and brown planthopper were utilized for gene pyramiding schemes. Additional ten tightly linked markers were also used for introgressing stress tolerance caused by flash flooding, Fe toxicity, salinity, reproductive heat, and reduced stomata density. We successfully developed Riceberry+3 for purple rice, providing the first high-antioxidant purple rice with bacterial leaf blight, leaf blast, and brown planthopper to improve productivity in organic farms. To enhance resiliency to climate change, we have isolated multiple functional mutants from a large-stabilized mutant population by a fast-neutron bombardment. We also successfully isolated functional mutants showing tolerance to Fe toxicity, heat, salinity, and cold stresses during the critical reproductive stage. Recently, we isolated four low-density stomatal mutants, saving irrigation water and significantly improving water use efficiency. These eminent mutants were integrated into PinK+4 to generate multiplex low-glycemic, nutrient-dense and climate-smart purple and white rice varieties.

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FROM GENES TO GRAINS: UNVEILING THE MOLECULAR AND BIOCHEMICAL ASPECTS SHAPING NUTRITIONAL QUALITY IN RICE FOR MULTI-NUTRIENT VARIETAL IMPROVEMENT

Haritha Bollinedi[1], Neha Singh[1], Neha Sajwan[1], Meena Adikari[1], Rakesh Bharadwaj[2], Gopala Krishnan S[1], Prolay K Bhowmick[1], Nagarajan M[3], Ranjith K Ellur[1], Vinod KK[1], Singh AK[1]*

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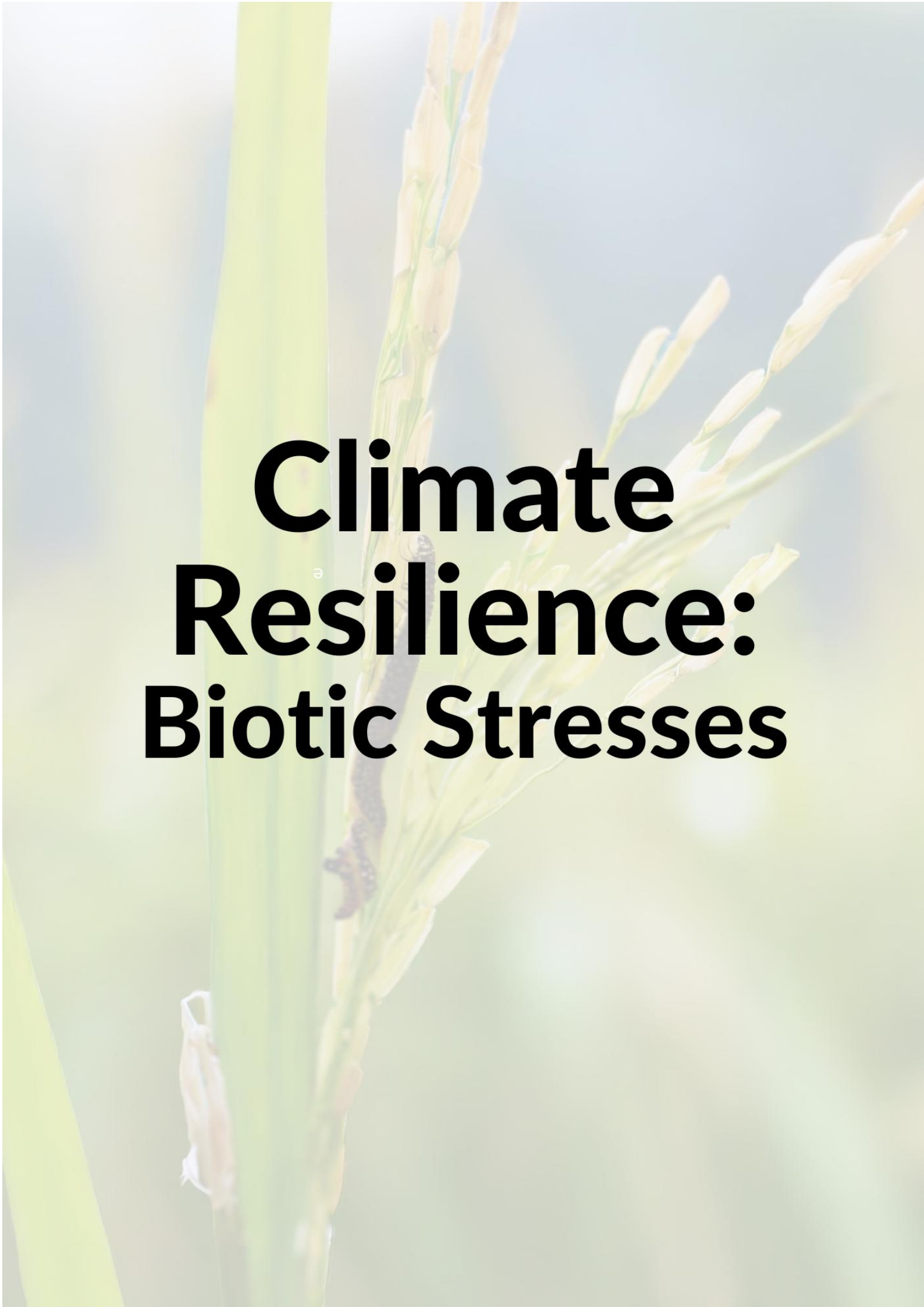
The increasing global population's demand for nutritious food necessitates innovative approaches to enhance crop productivity and nutritional value. Rice, a staple for many worldwide, plays a pivotal role in addressing food security and nutritional deficiencies. However, rice often lacks vital nutrients, especially in resource-limited regions. Development of micro-nutrient-rich rice varieties necessitates identification of stable donors with high trait value. The landrace accession Karuppunel showed consistently higher endosperm Zn content of 40.8 ppm across multiple locations. The QTLs governing high Zn content in Karuppunel have been identified using various mapping approaches like GWAS, bi-parental QTL mapping and QTLseq analysis. Promising QTLs are currently being transferred into the background of popular Basmati and non-Basmati varieties. Parallelly, the reduction of anti-nutritional factor phytic acid is also under progress in order to enhance the micronutrient bioavailability. An EMS Pusa LPA Mutant 11 (PLM11) and a germplasm accession Shah Pasand were identified as donors for LPA trait and QTLs associated with LPA in these accessions have been identified. Further, novel allele of OsLOX3 gene associated with rice bran degradation have been identified and efforts are underway to develop varieties with better bran quality and storability. Furthermore, considering the alarming rise of non-communicable diseases (NCDs), development of tailor-made rice varieties suiting diverse consumer needs is crucial. In this direction, the association among various starch parameters, viscosity traits, and grain composition, and their influence on Glycaemic Index has been analysed and significant marker-trait associations were identified through GWAS.



Climate Resilience: Biotic Stresses

Lead Lecture

UNVEILING ESSENTIAL GENES FOR ENHANCING RICE RESISTANCE TO BPH AMIDST CLIMATE CHANGE CHALLENGES

Siwaret Arikit

Brown planthopper (BPH; *Nilaparvatalugens* Stål) poses a significant threat to rice cultivation in many Asian countries. Severe pest outbreaks, including BPH, have become more common in direct-seeded rice fields, possibly due to wide-spread insecticide use and climate change. While numerous BPH resistance genes have been previously characterized, limited information exists regarding genes specifically associated with the resistance at early seedling stage, a crucial stage for direct-seeding cultivation. Currently, two resistance genes, *Bph3* and *Bph32*, have been successfully used in Thailand to control BPH infestation at later seedling stage and vegetative stage. However, neither gene was able to control the BPH infestation at the early seedling stage. Based on BPH resistance screening, RathuHeenati (RH) showed remarkable resistance to BPH at different stages, including the early seedling stage. A QTL-seq analysis was then conducted on an F2 population derived from a cross between RH and HCS-1$^{(Bph3+Bph32)}$ to unveil QTLs and genes linked to BPH resistance during the critical early seedling stage. An important QTL was successfully pinpointed on chromosome 3, and further investigation identified *Bph14* as a potential candidate gene. Differential gene expression and sequence variations were observed between the two parental lines. The functional Bph14 from RH being prevalent in all resistant F2 plants. Intriguingly, the susceptible F2 plants and HCS-1 exhibited a notable 2703 bp deletion in the Bph14 gene. The findings underscore the significance of the functional Bph14 gene from RH in conferring BPH resistance during the early seedling stage of rice. The functional Bph14 gene of RH appears to be important for BPH resistance at the early seedling stage, offering a targeted solution for enhancing resistance to BPH at both early seedling and subsequent growth stages when combined with other BPH resistance genes. This research contributes crucial insights for sustainable rice cultivation practices, particularly in the context of evolving pest challenges.

Keywords:*Oryza sativa* (Rice); RathuHeenati; brown planthopper; QTL-Seq; resistance; marker-assisted breeding; Bulk-segregant analysis; BPH; *Nilaparvatalugens* Stål

Lead Lecture

MAPPING QTLS GOVERNING RESISTANCE TO SHEATH BLIGHT OF RICE

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Sheath blight of rice caused by *Rhizoctonia solani* Kühn is an important fungal disease-causing yield reduction up to 60 % on severity. The pathogen infects a wide range of crops. A total of 35 *R. solani* isolates infecting different hosts from diverse locations were collected and characterized for their morphology, pathogenicity as well as diversity, out of which two isolates were found to be highly virulent. Two hundred and eighteen accessions of *Oryzanivara* and *O. rufipogon* were evaluated for three years based on which two *O. rufipogon* accessions, namely, IC336719 and IC336721 were identified as resistant. In order to utilize it in rice improvement, there is a need to understand the basis of resistance and map the genomic regions governing sheath blight resistance. A sheath blight resistant *O. rufipogon*-derived introgression line, Pusa 1908-13-12-5 was developed using IC336719. Mapping of the genomic regions governing resistance to sheath blight was carried out using recombinant inbred line (RIL) population developed by crossing it with a highly susceptible variety, Pusa Basmati 1. The RILs were phenotyped for sheath blight disease for two years and genotyped with RPGA array. ICIM with an additive gene model identified six QTLs governing relative lesion height and relative single plant yield loss. Four QTLs could explain phenotypic variation of more than 10, of which five were novel. Three major QTLs were colocalised within 1.4Mb region of chromosome 1, wherein 12 candidate genes were identified including bZIP transcription factor, Cytokinin glycosyltransferase, Peroxidase and Oligopeptidase with potential role in sheath blight resistance. One SNP linked to a QTL have been validated in an independent backcross population explaining 6.6 % PV, which is being utilized in breeding rice genotypes with sheath blight resistance.

Lead Lecture

BIOFORTIFICATION OF RICE USING MUTATION BREEDING

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Abstract:

Rice is a staple food across the world. Polished white rice is the major form of rice consumed globally. But white rice is severely deficient in most nutritional parameters including micronutrients. Additionally, most of white rice is amylopectin-rich starch, so it digests fast leading to the quick release of glucose upon consumption. So, it is also not suitable for diabetic patients. To develop nutrition-rich rice, we are generating a mutant population of rice in the background of Improved Samba Mahsuri, a bacterial blight tolerant, low glycaemic index (GI) rice variety. Polished grains from the M2 plants will be screened for nutritional traits such as high iron, zinc, protein, or low GI.

Parallelly we also screen some advanced-stage high-yielding mutants in the same background for high iron and zinc in the polished rice. We have identified some rice lines that show high Zn in the endosperm in three successive generations. We are trying to get pure lines with high zinc content in polished rice.

While doing this analysis we also observed that polishing of rice leads to a severe loss of iron content and a significant loss of zinc content. We tested if normal cooking practices also affect Fe and Zn in rice. We observed washing rice before cooking or soaking it in water for long hours also leads to a significant loss of iron and zinc.

Research impact: During my Ph.D. with Dr. Ramesh V. Sonti at CSIR-CCMB, I worked on rice-Xanthomonas interaction with a major focus on cell wall damage-triggered immunity. The results were published in Plant Physiology. I was also involved in a rice mutagenesis project with a major focus on sheath-blight tolerant rice lines where we identified genes and markers associated with sheath blight tolerance. I was also involved in other projects

including a large-scale transcriptome meta-analysis of rice, proteomic analysis of rice, database development, and the role of vitamins in plant-pathogen interactions. All these manuscripts are either under preparation or in communication. Currently, at TIGS I have identified rice mutant lines that have a low glycaemic index, high yield as well and higher zinc content in the edible part i.e. endosperm. We have also observed how various processing and cooking practices such as polishing, washing, and soaking have negative effects on micronutrient content in white rice.

Future plan: In the course of domestication, we have moved to white rice, particularly polished rice. Various studies including our observations have confirmed that polished rice is a very poor source of nutrition and is also not very favourable for diabetic patients. Rice being the most consumed food in India, its poor nutritional qualities are pushing us towards hidden hunger. I'm trying to develop rice lines that show higher nutritional content in the endosperm and are also diabetic-friendly. To achieve this, we have developed a rice mutant population that is being screened for added nutritional parameters such as higher iron, zinc, protein, or lower glycaemic index (GI). Additionally, by screening a small subset of the old mutant population, I have identified some low GI, high-yielding, and high Zn-containing lines. Once we get these pure lines, we will try to identify associated genomic loci/markers and also apply for the national trials.

Keywords: Biofortification, Rice, Nutrition, Mutation breeding

QTL-SEQ AND TRANSCRIPTOME ANALYSES PROVIDE MECHANISTIC INSIGHTS INTO YELLOW STEM BORER TOLERANCE IN RICE

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Yellow stem borer (YSB), *Scirpophagaincertulas* (Walker) (Lepidoptera: Crambidae), is a major pest of rice in India, that can lead to 20-60% loss in rice production. Current management practices majorly include chemical insecticides that poses threat to the environment while triggering the evolution of resistant insect biotypes. Sustainable management of YSB infestation is challenged by the non-availability of adequate source of resistance and poor understanding of resistance mechanisms, thus necessitating studies for generating resources to breed YSB resistant rice and to understand rice-YSB interaction. Here, we used bulk-segregant analysis in combination with next-generation sequencing to map the Quantitative Trait Loci (QTL) intervals in five rice chromosomes that could be associated with YSB tolerance at vegetative phase in a highly tolerant rice line named SM92. Further, multiple SNP markers that showed significant association with YSB tolerance in rice chromosomes 1, 5, 10, and 12 were developed. RNA-sequencing of the susceptible and tolerant lines revealed multiple genes present in the candidate QTL intervals to be differentially regulated upon YSB infestation. Comparative transcriptome analysis revealed a putative candidate gene that was predicted to encode an alpha-amylase inhibitor. Analysis of the transcriptome profiles further revealed a possible link between phenylpropanoid metabolism and YSB tolerance. Taken together, this study provides insights into rice-YSB interaction at genomic, and transcriptomic level, thereby facilitating the understanding of tolerance mechanism. Importantly, a promising breeding line and markers for YSB tolerance have been developed that can potentially aid in marker-assisted breeding of YSB resistance among elite rice cultivars.

RHODOTORULAMUCILAGINOSA JGTA-S1 - A RESERVOIR OF ENDOBACTERIA IMPROVES NITROGEN NUTRITION OF RICE PLANTS

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Nitrogen fixation, the conversion of atmospheric nitrogen into a form that plants can use, is typically performed by certain prokaryotes called diazotrophs. Some of these diazotrophs can form associations with a monophyletic clade of plants, leading to the formation of root nodules where nitrogen fixation occurs. Most agriculturally important crop plants cannot initiate the developmental program that leads to nodule organogenesis and therefore cannot fix nitrogen efficiently. *Rhodotorulamucilaginosa* JGTA-S1: is a basidiomycetous yeast that was isolated from the internal tissues of a wetland plant called *Typha angustifolia* that was collected from a uranium mine. This yeast, JGTA-S1 colonizes rice as a surrogate host and improves the growth and nitrogen content of rice plants. We found that JGTA-S1 fixes nitrogen in a free-living state and inside the plants. Our studies show that JGTA-S1 contains several microbes as endosymbionts including diazotrophs which probably help it to fix nitrogen and supply nitrogen to treated rice plants. The yeast changes from yeast to filamentous form when associated with host plants. A strain of *Pseudomonas stutzeri*, was observed to actively invade the filaments of JGTA-S1. The study suggests that a continuous process of endosymbiosis takes place in JGTA-S1. However, not all its endosymbionts are culturable. These findings highlight an intricate three kingdom relationship between the yeast *Rhodotorulamucilaginosa* JGTA-S1, its endobacteria, and rice plants.

Rhodotorulamucilaginosa, Endosymbiosis, nitrogen nutrition

RICE GENE DIFFERENTIALS – A TOOL FOR MONITORING FIELD VIRULENCE IN ASIAN RICE GALL MIDGE, *ORSEOLIAORYZAE*

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Asian rice gall midge, *Orseoliaoryzae*(Diptera: Cecidomyiidae) is predominantly a vegetative phase pest of rice crop. Host plant resistance is one of the important tools and in endemic areas, resistant varieties are deployed for the management of this pest. Resistance is governed by a single gene and till date, 12 genes have been identified as conferring resistance to Asian rice gall midge of which 10 are dominant and 2 are recessive in nature. Field reaction of the local gall midge populations to a set of 11 gene differentials along with a susceptible check and two pyramided lines which are categorized into 5 groups was monitored continuously in multi-location testing under AICRPR trials. Based on the reaction, plants with <10% plant damage or 1 % silver shoot are considered as resistant. The results suggested that donors with *Gm8* and *Gm1* gene were conferring resistance across locations and across years. Insect virulence was monitored through the single female progeny testing. Adult female insects collected from light traps were released into pots @ one female per pot in a choice test where 3 gene differentials with susceptible check were raised per pot. Variation in virulence was observed at different locations. In the current scenario of climate change, virulence monitoring can help in assessing the status of the insect as a pest. Both the trials help in the choice of genes for their utilization in the breeding program and their deployment for effective management of the rice gall midge.

Keywords: Rice ; Asian rice gall midge; *Orseoliaoryzae*; gene differentials: virulence monitoring

MAPPING QTLS FOR RESISTANCE TO PANICLE BLAST IN A RECOMBINANT INBRED LINE POPULATION OF TEMPERATE JAPONICA RICE (*ORYZA SATIVA* L.)

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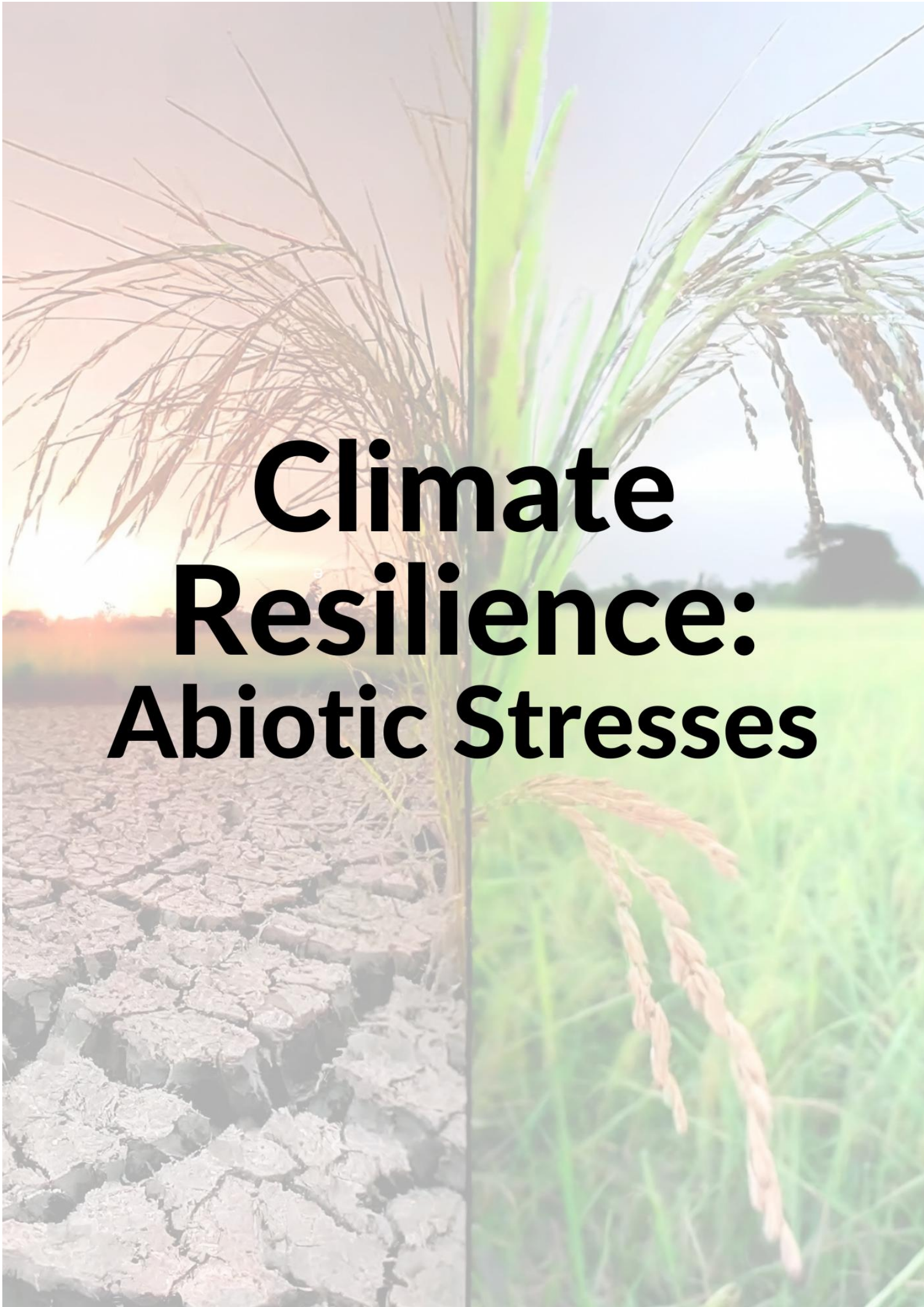
Abstract:

Rice (*Oryza sativa* L.) is affected by 114 pathogens across the world of which *Magnaportheoryza* causing rice blast mostly appears on panicle base (neck blast), rachis, leaf and node. The disease assumes high severity under uplands and cold regions causing 50 to 90 per cent yield loss and deteriorates grain quality. The study outlined a comprehensive investigation into identifying genetic markers associated with resistance to rice blast disease in the rice varieties K-332 and GS-88. Through the use of a recombinant inbred line (RIL) population and advanced molecular techniques, the research pinpointed specific regions on the rice genome, particularly on chromosome 11, where genes linked to resistance against the *Magnaportheoryzae* pathogen were located. One notable finding was the identification of the qPB11.1 QTL, situated at 22.79 Mb positions, displaying significant resistance with a LoD score of 6.49 and explaining 46% of the phenotypic variance. The study's overarching objective is to unravel the genetic foundations of rice blast resistance, paving the way for the development of blast-resistant rice varieties through marker-assisted selection (MAS). By transferring the identified resistance genes, particularly those within the qPB11.1 QTL, to elite rice cultivars; the research aims to enhance their resistance against rice blast. This approach holds immense promise for improving crop yield and quality, especially in regions vulnerable to blast disease.

Keywords: Rice; Panicle blast; KASP markers; RIL; QTL; mapping



Climate Resilience: Abiotic Stresses

Lead Lecture

RICE ROOT ANATOMY CHANGES IN RESPONSE TO HIGH TEMPERATURE STRESS

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Rice, a tropical crop well-suited to high temperatures, faces the challenge of rising temperatures from climate change that may surpass its tolerance limits. With increasing interest in cultivating rice in non-paddy systems, the root system is likely to be exposed significantly to higher temperatures due to the absence of the cooling effect from submergence. Our research aims to understand the changes in rice root system architecture and anatomy under elevated temperatures and the mechanisms driving these changes. Our results indicate that temperature stress induces various architectural and anatomical changes in different rice (sub)species, with the most consistent change being a reduction in root diameter. This reduction in root diameter can recover depending on the duration of the heat stress. Reducing root diameter may enable plants to optimize resource allocation and maintain optimal root-to-shoot ratios, enhancing the balance between water uptake and transpiration in high-temperature environments. Therefore, understanding the molecular mechanisms controlling this response can facilitate the development of temperature-tolerant rice varieties better suited for upland cultivation methods.

Lead Lecture

IMPROVING WATER PRODUCTIVITY OF RICE FOR AEROBIC CULTIVATION: AN APPROACH THROUGH COMBINING "CONSTITUTIVE" AND "ACQUIRED" TRAITS

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Increasing water productivity and saving water in rice cultivation has become an apparent goal globally. The adoption of semi-irrigated aerobic cultivation though saved water, a concomitant reduction in yield was often encountered because of periodic water limitation. While selection of higher absolute yields under water limitation provided the initial impetus, the reduction in yield is still a concern. This necessitated the identification and introgression of specific drought adaptive morpho-physiological traits. Several traits associated with water acquisition, WUEand water conservation such as root system architecture, photosynthetic mechanisms, epicuticular wax etc have been shown to have significant relevance. However, soil dries at a much slower rate between episodes of irrigation in field. This gradual drying induces the upregulation of certain specific traits that are associated several cellular processes including maintenance of membrane permeability, metabolism and preventing oxidative damage through scavenging of reactive oxygen species. These traits are collectively referred to as Acquired Tolerance Traits (ATT). We hypothesized that when the constitutive traits such as WUE and roots are combined under the background of reasonably high ATT, the productivity under water limited conditions was significantly high. We developed a high throughput phenotyping facility for capturing the genetic variability in ATT. A thorough phenotyping of a panel of 90 rice germplasm led to the identification of specific QTL for these traits through GWAS. Transcriptomic and Metabolomic analysis of the contrasts led to the identification of specific mechanisms associated with polyamine biosynthesis and ethylene metabolism to be the reason for the variations in ATT.

Keywords: Acquired tolerance traits, Aerobic cultivation, GWAS, Metabolomics, Rice

UNDERSTANDING THE DYNAMIC CORRELATION BETWEEN RICE ROOTS, SHOOTS, AND WATER EFFICIENCY IN ORDER TO INCREASE PRODUCTIVITY

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Water Use Efficiency (WUE) is a complex trait and is defined as the amount of carbon assimilated as biomass or yield per unit of water used by the crop. WUE represents the ability of crop to utilise water efficiently while maintaining optimal growth and productivity. The intricate interplay of anatomical determinants such as stomatal density and regulation, leaf anatomy, and root architecture, with physiological traits such as transpiration rate, photosynthetic capacity, and water transport between the root-shoot continuum affect WUE. Talk specifies multidisciplinary innovative approach to fully comprehend and exploit the genetic architecture of WUE traits to develop strategies for enhanced rice WUE, contributing to sustainable agriculture in the face of increasing water scarcity and climate change.

GENES TO FIELD: DECIPHERING THE GENETIC BASIS OF TRAITS SUITABLE FOR DIRECT-SEEDED RICE (DSR)

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Climate change led to water scarcity looming over the production and productivity of water-intensive crops like rice. Direct-seeded rice (DSR) is regarded as one of the sustainable methods for mitigating the impact of water scarcity on rice production while ensuring sustainable and efficient use of water resources in agriculture. Early seedling vigour(ESV) traits, including germination index, seedling vigour index I and II (SVI-I and SVI-II), root and shoot length, and biomass, are crucial for DSR because they determine seedling stand density and weed competitiveness. A genome-wide association mapping (GWAS) study was carried out in three environments for ESV-related traits by employing a diverse set of rice germplasms. We quantified 15 ESV-related traits on the 7th, 14th, and 21st days under laboratory conditions and Wet-DSR and Dry-DSR in field conditions. Our analysis identified several quantitative trait loci (QTL) and markers associated with ESV-related traits. In all three environments, 40 and 35 QTLs were identified under wet-DSR, compared to 68 and 67 QTLs under dry-DSR for SVI-I and SVI-II, respectively.Further, our study also identified common QTLs across the environment for different time periods. For example, we identified three common QTLs under wet and dry-DSR conditions. Among them, one is significant for SVI-I on the 14th day and is located on Chromosome 10 (at 5.10 Mbp). The remaining two QTLs show significance for SVI-II on the 7th day, with one situated on Chromosome 04 (23.3 Mbp) and the other on Chromosome 12 (8.88 Mbp). The identification of QTLs specific to each environment and time point indicates the complex nature of ESV traits. The discovery of candidate genes within the common QTLs provides a starting point for further investigations into the molecular mechanisms underlying ESV-

related traits. This knowledge can be used to develop targeted breeding programs and advance genetic studies related to DSR.

USING INSULT TO OVERCOME INJURY: MECHANISMS TO COPE HEAT STRESS IN RICE

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Cultivation of rice (*Oryza sativa*), an important staple food crop, is under increasing threat due to the escalating frequency and intensity of heat stress events associated with climate change. In response to heat stress, plants evoke a range of adaptive mechanisms, such as the induction of heat shock proteins, antioxidant systems, and osmoprotectants. However, repeated stress responses in biological systems comes with a potential energy trade-off, impacting the overall energy budget of the organism.This study explores the concept of stress memory-based priming in rice, where exposure to mild or non-lethal stress events equips plants to better confront subsequent, more severe stressors. We find that rice seedlings primed by mild heat stress exhibit enhanced tolerance to subsequent intense heat stress, both during the seedling stage and later in the reproductive phase. This increased resilience can be attributed to the establishment of an efficient reactive oxygen species (ROS)-antioxidant homeostasis and a strategic balance between growth and defense mechanisms. We also unravel howpriming facilitates the rewiring of transcriptional regulatory networks to orchestrate a more organized stress response.Through field experiments, we investigate how pre-exposure to mild stress events can enhance crop resilience and yield. We posit that employing priming (an insult)could avoid the damaging effects of intense heat stress (injury), in the face of changing environmental conditions and therefore aids in sustainable agricultural practices aimed at ensuring food security.

RIBOSOMAL RNA BIOGENESIS AND TRANSLATION ABILITY REGULATE DROUGHT TOLERANCE OF PLANTS.

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Abstract:

Drought affects protein synthesis;ribosome is mother of everything in plant cell that makes proteins. Ribosome biogenesis is a complex process involving several ribosomal proteins, ribosomal RNA (rRNA) processing factors (RPFs), small nucleolar proteins etc. We identified more than 400 copies of rRNAs are arranged in tandem repeats. The analysis of 5S rRNA repeats in Indica rice identified SNP variations, different length sequences. The 5SrRNA intergenic regions also showed variations and different transcription factor binding sites were identified. The role of rRNA processing factor (RPF2) in processing of 25SrRNA and 5SrRNA has been explored. RPF2 interact with plant specific SOC1 encoding MADS box transcription factor known to regulate flowering in rice. RPF2 also interact with an important ribosomal protein large subunit L10 (RPL10) which regulate translation. Mutations in *rpl10* affects expression of several translation associated genes. Further, the translation ability in contrasting rice genotypes APO and IR64 at anthesis stage drought stress was analyzed using polysome profiling and polysome bound mRNA sequencing. APO maintained higher polysomes with 60S-to-40S and polysome-to-monosome ratio which resulted in higher protein levels compared to IR64 under stress. APO hadhigher levels of N^6-Methyladenosine (m^6A) mRNA modifications which contributed for sustained translation. The photosynthetic machinery associated proteins are more stable in APO. The protein stability and ribosome biogenesis mechanisms favoured improved translation in APO. Reduced water potential under drought stress in IR64 affected transcription and translation machinery under stress.

Keywords: Drought; Ribosome; protein synthesis; rRNA; translation

AN E3-UBIQUITIN LIGASE MEDIATING RICE DROUGHT RESPONSE: INSIGHTS INTO BRASSINOSTEROIDS AND ABA SIGNALING INTERACTIONS

Tiago F Lourenço[1], Joan Sàrria[1,2], Vanessa Duarte[1], Nelson JM Saibo[1], M Margarida Oliveira[1]*

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Rice is one of the most important crops worldwide, being the primary source of calories for more than half of the world population and a species very sensitive to water deficit. The characterization of important molecular mechanisms modulating drought response, like the Ubiquitin-Proteasome system (UPS), will play a key role towards a more sustainable rice production (Melo et al., 2021).

Our focus has been the E3-ubiquitin ligases since they confer specificity to protein targets. We selected and validated by RT-qPCR, 16 E3-ubiquitin ligasegenes showing differential expression under stress.One of those genes, named RiP4g, is up-regulated by drought and we developedbothknock-out (CRISPR/cas9 gene edition) and overexpression transgenic rice lines to functionally characterize its function. These lines showed an interesting contrasting phenotype with OX-RiP4g showing a darker green and erect leafs (2nd leaf and flag-leaf), and shorter seeds, while the opposite was seen in KO-RiP4g. This phenotype can be correlated with Brassinosteroids (BR) signaling. To confirm if RiP4g is also involved in BR-signaling, we have been performing Leaf Inclination assays and molecular analysis of BR-marker genes under Brassinolide treatment. Interestingly, RiP4g is also responsive to Abscisic acid (ABA), a known hormone involved in drought response and antagonist of BR signaling. To investigate its role in ABA signaling, we performed morpho-physiological assays and shown that RiP4g OX confers hypersensitivity while KO is hyposensitive to ABA. Taken together, our resultssuggest that RiP4g mediates the antagonist crosstalk between ABA and BR signaling to modulate rice response to drought.

Acknowledgments:

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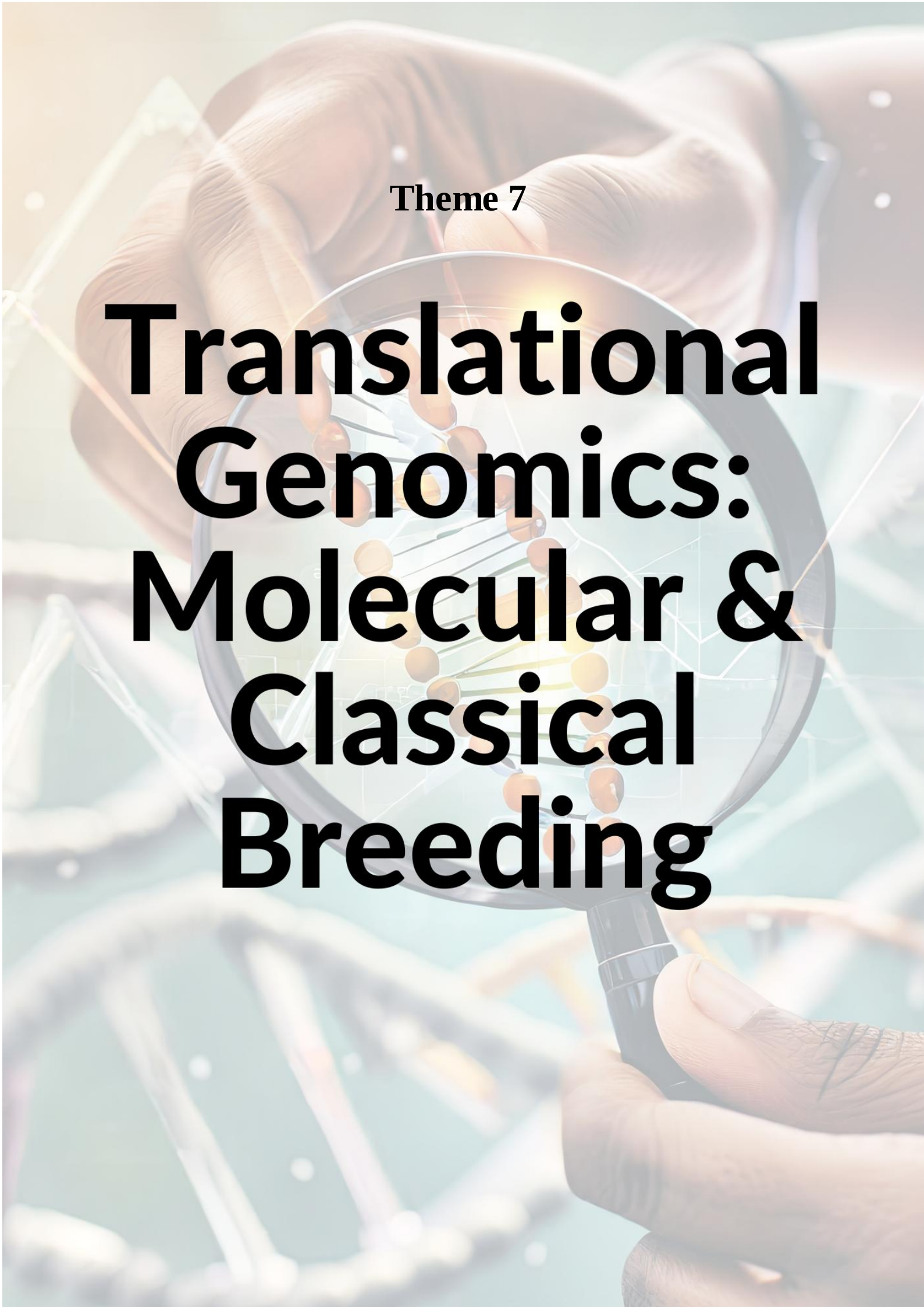
Ref: Melo FM, Oliveira MM, Saibo NJM, Lourenço TF (2021), Frontiers in Plant Science. DOI:10.3389/fpls.2021.640193

Keywords: Oryza sativa, Proteostasis, water-scarcity, hormonal interplay



Theme 7

Translational Genomics: Molecular & Classical Breeding

Lead Lecture

MAKING SAMBA MAHSURI CLIMATE RESILIENT THROUGH MOLECULAR BREEDING

Sundaram RM, Laha GS, Fiyaz RA, Senguttuvel P, Jyothi B, Suneetha K, Mangrauthia SK, Neeraja CN, Kalyani KB, Papa Rao V, Rekha G, Kousik MBVN, Anila M, Dilip Kumar T, Punniakoti E, Hajira Sk, MasthaniSk, Sinha P, Gokulan CG, Patel HK and Sonti RV

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A rapidly changing climate is posing serious risks to sustainable rice production in the future. Biotechnological tools like molecular markers and genomics can add precision to breeding and accelerate breeding efforts towards development of climate change resilient rice varieties. Samba Mahsuri is a mega-variety of rice developed and released at Agricultural Research Station, Baptla, Andhra Pradesh Agricultural University. It is being cultivated in more than 4 Mha across the country, principally due to its high yield, excellent grain and cooking quality along with desirable plant height. However, Samba Mahsuri is susceptible to most of the pests and diseases including the deadly disease of bacterial blight. Towards this objective, our research team at ICAR-IIRR, in collaboration with CSIR-CCMB has applied molecular breeding for improvement of multiple traits in Samba Mahsuri like resistance/tolerance against three major diseases, viz., bacterial blight (BB), blast and sheaths blight, resistance against the two insect pests, viz., gall midge and brown planthopper, (BPH) and tolerance to four abiotic stresses, viz., salinity, low soil phosphorus, drought and submergence along with improvement of yield related traits in order to develop climate resilient breeding lines/varieties in the genetic background of Samba Mahsuri. Through marker-assisted backcross breeding (MABB), we introgressed three major BB resistance genes (viz., *Xa21, xa13* and *xa5*) into Samba Mahsuri, resulting in the development and release of a high-yielding, bacterial blight resistant rice variety possessing fine-grain type and low glycemic index (GI), named Improved Samba Mahsuri (ISM). Two novel BB resistance genes, *Xa33* and *Xa38* have been identified from the wild rice *Oryza*

Lead Lecture

nivara along with another novel gene from distant wild species of rice, *Oryza officinalis*, *Xa48* and transferred to Samba Mahsuri. Through these efforts, a breeding line of ISM possessing *Xa38* and having 10 % more yield as compared to Samba Mahsuri along with broad-spectrum resistance against bacterial blight has been released as a new variety, called DRR Dhan 53,has been recently released along with a few other breeding lines of ISM having *Xa33* and *Xa48* in the pipeline for release. Additionally, gene-pyramid lines of ISM, possessing resistance against blast and tolerance to salinity and low soil P tolerance have also been developed in the genetic background of ISM and released as new varieties under the names, DRR Dhan 62, DRR Dhan 58 and DRR Dhan 60, respectively. We are also pyramiding major QTLs associated with tolerance to drought and submergence along yield enhancing genes and improved breeding lines of ISM possessing multiple traits are being evaluated in multi-location trials of All India Coordinated Research Project on Rice (AICRPR) and few such lines are in final year of testing.

Lead Lecture

NEW MODEL FOR ORIGIN OF BLACK RICE FROM WILD RICE OF INDIA: BASED ON GENETIC EVIDENCE OF INTERSPECIFIC HYBRIDIZATION (O. SATIVA X O. RUFIPOGON) AND GENOME ANALYSIS

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Abstract:

Rice (*Oryza sativa* L.) is an important staple food grain because more than half of the world's population depends on it for their livelihood but its origin is still debatable. It hypothesized that cultivated rice (*Oryza sativa* L.) has been originated through domestication from one of its progenitor wild rice species, *O. rufipogon*about ~9000 yr ago and considered as reservoirs of many important genes (biotic/abiotic stress tolerance). It was proposed that different subspecies of rice (japonica, indica, aus) had originated from distinct subpopulations of progenitor wild rice *O. rufipogon*, considered asmultigeographic origin.

Origin of cultivated rice is still a somewhat complex and controversial subject matter. Most studies indicate that japonica originated in the Yangtze River valley China, whereas indica originated in the Ganges plains of India. Similarly, origin of black rice is a debatable issue. Wild rice of which geographic region is the main source of black rice origin, although all the wild rice accessions (*O. rufipogon*) of the world are red in pericarp colour.

No black grain in *Oryza rufipogon*, indicates that black rice pericarp phenotypeis a newly acquired trait and incorporated during domestication. Three genes are responsible for black pericarp formation (Kala1, Kala3, and Kala4), but Kala4 gene (bHLH-TF) is the decisive one. Kala4 promoter had acquired a neo-functionalization trait through LINE1 transposon insertional mutation in chromosome 4 and gave birth to black rice. Again controversy arises that whether black pericarp originated first in which subspecies of rice japonica, indica or in

aus. Previous study proposed that it was first originated in tropical japonica and then transferred to indica rice, without any concrete confirmation.

In the present study, we report for the first time in the history of rice breeding that aromatic black rice lines developed through interspecific hybridization (*O. sativa* x *O. rufipogon*) in the genetic background of cultivarsBadshabhog, Chenga and Ranjit. Possible reason may be the rearrangement and insertional mutation (LINE1) at the promoter region of Kala4 allele through chromosomal recombination leading to ectopic expression of anthocyanin genes ultimately gave birth of black rice in the breeding lines.

Gene specific PCR was carried out to detect the presence of Kala4 locus among the breeding lines. Based on experimental evidences we propose a new model of black rice origin. Black rice (mainly indica type) of Indian subcontinent originated independently through natural out crossing, gene-flow and artificial selection in the course of domestication from the wild rice of India. We also support the view of multi-regional independent origin of cultivated rice, that means indica and japonica subspecies originated from distinct genetic resources of *O. rufipogon*. Genetic base has been enhanced (widen) in the breeding lines by transferring untapped genes from wild rice *O. rufipogon*. Many improved high yielding breeding lines have been developed in this interspecific hybridization program (Badshabhog x *O. rufipogon*; Chenga x *O. rufipogon;* Ranjit x *O. rufipogon*) with high grain number per panicle (>450), and early maturity trait (125 days). High yield potentiality with good nutritional quality in the breeding lines will boost up the food and nutritional security of the world by 2050.

Keywords: New model of Black rice origin, wild rice O. rufipogon germplasm, new trait black pericarp discovery, Kala4 gene, neo-functionalization.

UNVEIL NOVEL EPISTATIC TARGETS AMONG MAJOR EFFECT LOCI IMPACTING RICE GRAIN CHALKINESS UTILIZING GENOME-WIDE ASSOCIATION-COUPLED EPISTATIC INTERACTION STUDIES

Saurabh Badoni[1,†,#], *Gopal Misra*[1,†], *Sabiha Parween*[1], *Rakesh Kumar Singh*[1,‡], *Hei Leung*[1], *OluFunmilayo Ladejobi*[2], *Richard Mott*[2] *and Nese Sreenivasulu*[1]

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†Joint contribution

Abstract:

Rice varieties demonstrating excellent grain quality show a very lower percent grain chalkiness (PGC) of two per cent and below together with higher head rice yield upon milling, leading to higher economic returns for farmers. In current study, a combined panel of *indica* and *japonica* rice varieties was employed for a genome-wide association study (GWAS), which led us to identify a total of 746 single nucleotide polymorphisms (SNPs) spanning 78 Quantitative Trait Loci (QTL) that were strongly associated with the chalk phenotype. 21 were identified as high-value QTLs among them explaining > 10 % of the phenotypic variance for PGC. Furthermore, a combined epistasis analysis and GWAS resulted in dissecting the genetics of the complex chalkiness trait, and its regulatory cascades were validated using gene regulatory networks. Promising novel epistatic interactions narrowed down between chromosomes 6 (*PGC6.1*) and 7 (*PGC7.8*) loci contributed to lower PGC. Interestingly, a few modern rice varieties exhibiting lower chalkiness carried several PGC QTLs as revealed by haplotype mining. The importance of *PGC6.1* was validated through multi-parent advanced generation intercrosses (MAGIC) and several low-chalk MAGIC lines possessing superior haplotypes were identified. The results of this investigation have deciphered the underlying genetic networks that can reduce PGC below 2% and will thus support future breeding pipelines to improve the grain quality of elite genetic material with high-yielding potentials.

Keywords: Grain chalkiness; rice; appearance quality; GWAS; Epistatic analysis; gene regulatory networks; Quantitative Trait Loci

GENOMIC-ASSISTED BREEDING OF CLIMATE-RESILIENT AND NUTRITIOUS RICE VARIETIES

Jauhar Ali

Rice Breeding Innovation Department, International Rice Research Institute (IRRI), Los Banos, Philippines

Climate-change and human nutrition are two important challenges for sustaining rice production in the coming decades. Rice is consumed by 56% of the human population and, therefore, needs to be secured and be more nutritious. IRRI has been developing and deploying climate-resilient rice varieties in Asia and Africa with considerable socioeconomic impacts. We used genomic-assisted breeding strategy to breed multiple abiotic and biotic stress-tolerant rice varieties. It helped in the release of seventy-three rice varieties to farmers across the Green Super Rice Project target countries in Asia (62) and Africa (11). These varieties have been successfully deployed over a cumulative area of 33.7 million ha in Asia and Africa, reaching to over 28.6m farm households. The nutritional values recorded in the newly developed nutrient-use efficient GSR inbreds were touching 15 ppm of Iron and 35 ppm of Zinc. Our recent efforts to biofortify climate-resilient rice varieties with Iron and Zinc elements will be highly helpful in mitigating the hidden hunger. IRRI is in the forefront to promote low-GI rice varieties as a healthier option and developed molecular markers to identify low-GI rice from breeding materials. Interestingly, we identified several multi-stress tolerant varieties to possess low GI based on the SNP molecular markers. However, it needs to be validated, and in the future, it will be an excellent tool for rice breeders to develop low-GI rice varieties. These multi-stress tolerant and nutritious inbred materials will be used as parents in developing climate-resilient and nutritious rice hybrids (CRRH) to accelerate the global rice production. We are currently using genomic prediction of heterosis and AI & ML to identify the superior combinations and thereby significantly reduce the number of unwanted crosses. The current hybrid rice program at IRRI is fully integrated with the OneRice Breeding Strategy to exploit the outputs from different breeding pipelines using genomic selection to address different market needs. Efforts to deploy climate-resilient and nutritious rice varieties in Asia and Africa will help to maximize the socioeconomic impacts.

Plant Nutrition & Sustainable Rice Production

STRATEGY TO ENHANCE PHOSPHATE USE EFFICIENCY AND GRAIN YIELD THROUGH MODULATION OF RNA DECAY PATHWAY IN RICE

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Root development is a fundamental process that supports plant survival and crop productivity. One of the essential factors to consider when developing biotechnology crops is the selection of a promoter that can optimize the spatial–temporal expression of introduced genes. However, there are insufficient cases of suitable promoters in crop plants, including rice. *osrns1* mutants had defects in root development based on T-DNA insertional mutant screening and *CRISPR* technology. To optimize the function of *OsRNS1*, we generated *OsRNS1*-overexpression plants under two different promoters: a whole-plant expression promoter and a novel root-preferred expression promoter. Root growth, yield-related agronomic traits, RNA-seq, and reactive oxygen species (ROS) accumulation were analyzed for comparison. *OsRNS1* was found to be involved in root development through T-DNA insertional mutant analysis and gene editing mutant analysis. To understand the gain of function of *OsRNS1*, *pUbi1::OsRNS1* was generated for the whole-plant expression, and both root growth defects and overall growth defects were found. To overcome this problem, a root-preferential overexpression line using *Os1-CysPrxB* promoter (*Per*) was generated and showed an increase in root length, plant height, and grain yield compared to wild-type (WT). RNA-seq analysis revealed that the response to oxidative stress-related genes was significantly up-regulated in both overexpression lines but was more obvious in *pPer::OsRNS1*. Furthermore, ROS levels in the roots were drastically decreased in *pPer::OsRNS1* but were increased in the *osrns1* mutants compared to WT. The results demonstrated that using a root-preferred promoter effectively optimizes the function of OsRNS1 and is a useful strategy for improving root-related agronomic traits and ROS regulation.

Lead Lecture

A CITRATE EFFLUX TRANSPORTER IMPORTANT FOR MANGANESE DISTRIBUTION AND PHOSPHORUS UPTAKE IN RICE

Poonam Panchal[1], Chitra Bhatia[1], Yi Chen[2], Meenakshi[1], Jyoti Bhadouria[1], Lokesh Verma[1]Kanika Maurya[1], Anthony J. Miller[2], Jitender Giri[1]*

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The plant citrate transporters, functional in mineral nutrients uptake and homeostasis, usually belong to the multidrug and toxic compound extrusion transporter family. We identified and functionally characterized a rice (*Oryza sativa*) citrate transporter, OsCT1, which differs from known plant citrate transporters and is structurally close to rice silicon transporters. Domain analysis depicted that OsCT1 carries a bacterial citrate-metal transporter domain, CitMHS. OsCT1 showed citrate efflux activity when expressed in *Xenopus laevis* oocytes and is localized to the cell plasma membrane. It is highly expressed in the shoot and reproductive tissues of rice, and its promoter activity was visible in cells surrounding the vasculature. The *OsCT1* knockout (KO) lines showed a reduced citrate content in the shoots and in the root exudates, whereas overexpression (OE) line showed higher citrate exudation from their roots. Further, the knockout and overexpression lines showed variations in the manganese (Mn) distribution leading to changes in their agronomical traits. Under deficient conditions (Mn sufficient conditions followed by 8 days of 0 µM $MnCl_2.4H_2O$ treatment), the supply of manganese towards the newer leaf was found to be obstructed in the knockout line. There were no significant differences in phosphorus (P) distribution; however, P uptake was reduced in the KO and increased in OE lines at the vegetative stage. Further, experiments in *Xenopus* oocytes revealed that OsCT1 could efflux citrate with Mn. In this way, we provide insights into a mechanism of citrate-metal transport in plants and its role in minerals homeostasis, which remains conserved with their bacterial counterparts.

A NOVEL *GLYCEROPHOSPHODIESTER PHOSPHODIESTERASE* IS INVOLVED IN THE PHOSPHATE STARVATION RESPONSE IN RICE ROOT

Linh Thi Nguyen[1,2], Duong Thi Thuy Nguyen[1,2*], Tuan Anh Tran[1], Tam Thi Thanh Tran[1], Nhue Phuong Nguyen[2], Phat Tien Do[2], Huong Thi Mai To[1]*

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Phosphorus (P) is one of the most vital macronutrients that plays a determinant role in plant development and productivity. However, the inorganic phosphate (Pi), the only form that can be assimilated by plants, is low availability in soil, coupled with the fact that plants have a significantly low P–use efficiency (PUE) resulting in the excessive application of P fertilizers. Therefore, there is an urgent need to develop P-efficient crops. Plants have adopted various specialized strategies related to morphological, physiological, and biochemical adaptation during Pi deficiency. GLYCEROPHOSPHODIESTER PHOSPHODIESTERASE (GDPDs) are phospholipid remodeling proteins that have been suggested to play important roles in maintaining phosphate homeostasis. Genome-wide association studies (GWAS) in a Vietnamese rice panel have discovered a robust QTL, which was co-localized to the promising gene *OsGDPDx* in Pi-starvation responses. In this study, we focused on studying the function of the candidate gene *OsGDPDx* by theloss function study. Two potential *osgdpdx* mutant lines were selected to characterize the phenotypic plasticity under Pi starvation conditions. Our results indicated that *gdpdx* loss-of-function mutants fail to inhibit the primary root growth as well as accelerate the number of crown roots and lateral roots thereby limiting Pi uptake. This proposes the specific function of the *OsGDPDx* gene on root growth under P deficiency. These findings will provide valuable information contributing to the development of crop plants with higher PUE.

Keywords: rice, CRISPR/Cas9,*OsGDPDx*gene, phosphate starvation, phosphate use efficiency

FUNCTIONAL GENOMICS OF GRAIN ZINC OF RICE (*ORYZA SATIVA* L.) TOWARDS BIOFORTIFICATION

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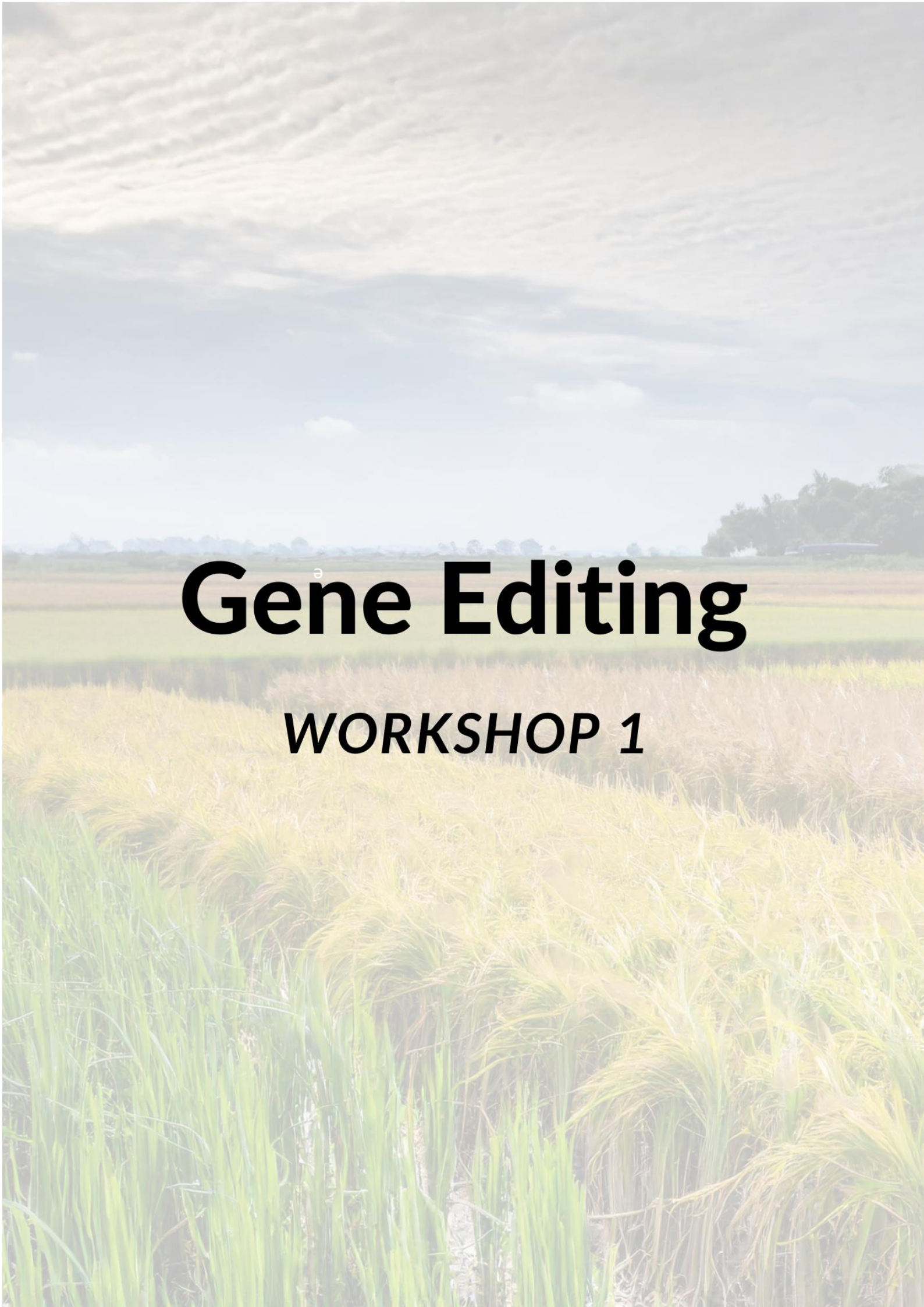
Rice is one of major staple food crops feeding more than 50% of the world's population. Biofortification is one of the sustainable and effective ways to address the micronutrient malnutrition (hidden hunger) is to increase mineral content, particularly Zinc (Zn) in polished rice grain. Deciphering the molecular mechanisms of metabolism leading to high grain zinc (Zn) and identification of candidate genes associated with high grain Zn would accelerate the rice biofortification programs. Evaluation of Zn content in the vegetative tissues of rice genotypes in our experiments indicated the high grain Zn is not always correlated with high Zn of vegetative tissues suggesting the importance of translocation of the Zn during the grain filling stage. Therefore, we studied transcriptome of two contrasting genotypes *viz.*,Karuppunel (landrace with 38ppm grain Zn) and MTU1010 (mega variety with 18ppm grain Zn) using RNA-Seq analysis. Six tissues *viz.*, internode (IN), node I (ND1), flag sheath (FS), flag leaf (FL), rachis (RCH), middle of the panicle (MG) were analyzed during the grain filling stage. Only three upregulated differentially expressed genes (DEGs) and six down regulated DEGs were found to be common in six tissues. High number of significantly upregulated DEGs (5505) were observed in FL (1574), followed by MG (1178), FS (1105), RCH (864), ND1 (685) and IN (99). Significantly down regulated DEGs (5873) were observed in MG (1384), followed by FL (1347), FS (1220), ND1 (963), RCH (939) and IN (20). In Karuppunel, DEGs of 295 transporters, 68 phytohormones, 14 transferases, 537 transcription factors, 909 kinases were upregulated. The role of these DEGS in the Zn uptake, movement, transfer, accumulation, sequestration, and detoxification processes across the cells and tissues of rice plants is being further elucidated.Simultaneously, expression profiling of 57 reported Zn homeostasis genes were investigated in flag leaf of four genotypes with contrasting grain Zn. 10 genes belonging to *NRAMP, MATE, IMP, HMA, OsIRT, ENA, YSL1* and *MDH* exhibited upregulation and were found to be significantly correlated with the Zn content in brown and polished rice. By deploying the knowledge of identified genes, superior alleles can be identified for facilitating efficient, targeted, and focused development of Zn biofortified rice varieties.



Gene Editing

WORKSHOP 1

Lead Lecture

GENOME EDITING ENABLES RICE TO RESIST BACTERIAL BLIGHT

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CRISPR (clustered regularly interspaced short palindromic repeats) systems have been engineered into potent biotechnological tools for both basic and applied research. The most promising utilization of CRISPR technologies is for targeted genome editing, leading to precise genetic alterations within any genome of interest, as demonstrated in a plethora of organisms including several important crop plants. Bacterial blight (BB) of rice is one of devastating diseases to cause yield loss in Asia and Africa. The causal agent *Xanthomonas oryzae*pv. *oryzae*(*Xoo*) uses secreted TAL effectors (TALes) to ectopically activate host disease susceptibility (S) genes including those encoding SWEET sucrose transporters, conditioning a state of disease susceptibility. *Xoo* uses a limited set of TALes to target promoters, so called effector binding element (EBEs) of S genes. We used CRISPR/Cas9 and its variants (base editor, prime editor) to engineer rice lines that carry EBE variants and become highly resistant to BB. Our results demonstrate a few routes to realize the promising potential of genome editing and basic understanding of crop disease biology in agriculture.

BRINGING APOMICTIC HYBRIDS TO THE RICE FIELDS

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The transfer from wild species to cultivated plants of the asexual mode of reproduction by clonal seed, known as apomixis and naturally found in more than 120 genera of angiosperms, has long been a major objective for developmental biologists, geneticists and breeders. This mode of reproduction indeed enables heterozygous genetic structures to be multiplied identically by seed, thereby benefiting from their higher value that can be associated to hybrid vigor. However, although knowledge of the determinism of apomixis has advanced considerably in the last decades, the conversion of cultivated species to apomixis has yet to be achieved. The recent ability to obtain high-frequency clonal seeds by engineered synthetic apomixis in a crop, rice, (Vernet et al., 2022) has paved the way for its application in crop improvement. Here, after presenting our last advances, we will analyze the remaining challenges and future implications for routine application of synthetic apomixis in rice breeding programs.

Vernet et al., 2022, Nat Commun. 13(1):7963.

Keywords: Apomixis, F1 hybrids, Plant Breeding, Rice

GENOME EDITING FOR IMPROVING YIELD AND ABIOTIC STRESS TOLERANCE OF RICE

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Rice crop contributes to about 40% of the food grain production of India. India is also the world's largest rice exporter of rice with an export of 17.8 million tonnes of non-basmati rice worth Rs. 51,088.72 Crores/USD 6,355.74 Million during the year 2022-23. In India Rice is cultivated in an area of 47 m ha with an average productivity of about 2.8 t/ha. Rice uses more than 50% of the irrigation water is it has a low WUE. Rice grown in rainfed ecosystems (38% of the total rice grown area) suffers from drought stress. Dwindling fresh water scarcity and global climate change also threaten rice production. Therefore, for future food, nutrition, livelihood and environmental security, it is imperative to develop crop precision crop management technologies and genetically improved rice varieties with high WUE, NUE, abiotic and biotic stress tolerance and yield. Our lab is working on understanding and improving yield and climate resilience of rice by using genetic engineering and CRISPR-Cas genome editing. Genome editing of 1) *DROUGHT AND SALT TOLERANCE* (*DST*), RRS1 and *STOMAGEN* (*EPFL9*) genes for improvement of yield, WUE and stress tolerance; 2) *Protein Phosphatase 2C* (*PP2C*s) Clade A group, *MIR169*, *Farnesyl Transferase* (*FTA*), *Robust Root System 1* (*RRS1*), and Phytomelatonin Receptor (*PMTR*) genes for improvement of stress tolerance; 3) Carbon Assimilation Rate 8 (*CAR8*), *HEXOKINASE1* (*HXK1*) and *DREB1C* for yield improvement in rice. Mutations in *MIR169*, *FTA* and *PMTR* showed pleiotropic effect on plant development. Mutants of *PP2Cs* showed enhanced abiotic stress tolerance. We developed four different mutant alleles of *dst* gene and identified two lines free of introduced exogenous DNA. These *dst* mutants exhibited reduced stomatal density, at least, in part due to downregulation of stomatal developmental genes. The *dst* mutants exhibited tolerance to osmotic and salt stress in seedling stage in hydroponics study and adult plant stage pot culture studies. The *dst*mutants use about 25% less water per unit leaf area as compared with WT MTU1010 cultivar. Further, these mutants

showed >20% yield enhancement over wild type plants under field conditions in a transgenic net house in kharif 2021 and 2022. Mutants with high yield and stress tolerance developed in this study will be useful to release as variety and as a genetic stock for introgression of *dst*mutations in other indica varieties for genetic improvement in yield and climate resilience.

Keywords: ABA signalling; CRISPR-Cas; Transcriptome; Stomatal development; WUE.

PRIME GENOME EDITING FOR CROP IMPROVEMENT

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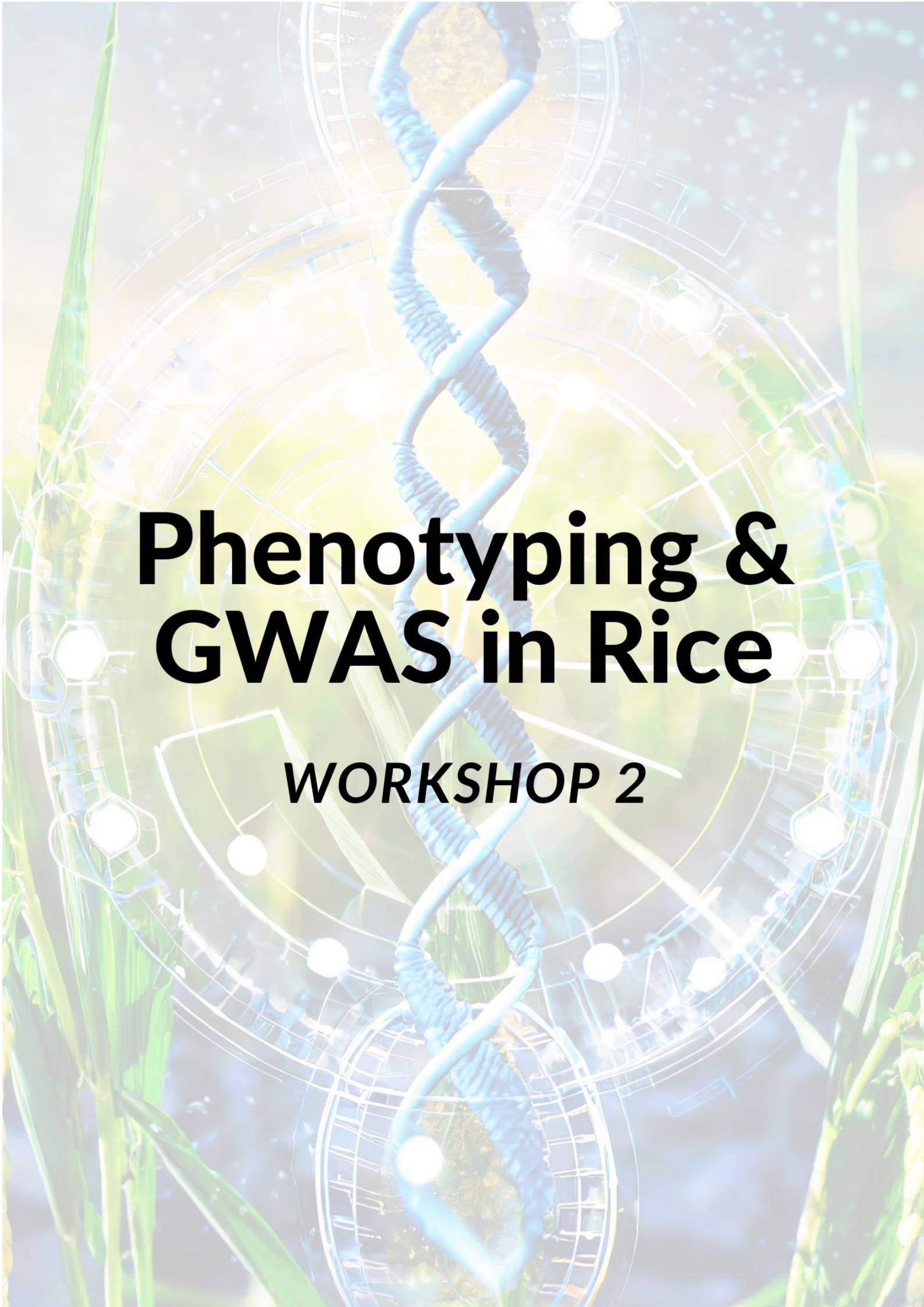
Prime editing (PE) is the latest revolution in the field of genome editing. With the power to precisely target and modify a genome, PE is making strong impacts in fields such as medicine, animal science, and plant science. PE has huge potential for crop improvement in the face of climate change, increasing population, and decreasing agricultural land. Rice is one of the most important cereal crops at a global scale and it is also the first crop to demonstrate the potential of PE in crops. In this study, we used prime editing to engineer rice with strong and broad resistance against bacterial blight of rice. Using genetic resistance against bacterial blight (BB) caused by *Xanthomonas oryzaepv. oryzae* (*Xoo*) is a major objective in rice breeding programs. We used PE to create novel alleles and allelic combinations that are highly resistant against multiple *Xoo* strains.

We achieved knock-in of Transcription Activator-Like (TAL) effector binding elements (EBEs) derived from the BB-susceptible gene *SWEET14* into the promoter of a dysfunctional executor *R* gene allele, *xa23*, with an editing efficiency of 73.5% and a biallelic editing rate of 18% in the T_0 generation. This editing enabled an inducible TAL effector-dependent BB resistance. Additionally, we edited the transcription factor TFIIA gamma subunit gene, *TFIIAγ5*, which is required for TAL effector-dependent BB susceptibility. This recapitulated the resistance of *xa5* at an editing efficiency of 88.5% with a biallelic editing rate of 30% in the T_0 generation

In a multiplexed approach, we generated both alleles at an editing efficiency of 46% in the T_0 generation. These edits were successfully inherited by the T_1 generation and provided strong broad-spectrum resistance against multiple *Xoo* strains. This novel approach enhances rice's adaptation against fast-emerging *Xoo* bacteria and represents a major step in fighting endemics that threaten global rice production.

Prime editing; rice; Xanthomonas; BLB; Genome editing; CRISPR-Cas9



Phenotyping & GWAS in Rice

WORKSHOP 2

Lead Lecture

PANGENOME GENOTYPING ARRAY FOR ACCELERATED CROP IMPROVEMENT IN RICE

Anurag Daware[1], Ankit Malik[2], Ranjith K. Ellur[2], Ashok K. Singh[2], Akhilesh K. Tyagi[1,3], Swarup K. Parida[1]

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Conventionally, a single reference genome is considered sufficient to get a complete insight into the genetic blueprint of a species. However, the recent availability of multiple reference genomes suggest the existence of previously unexplored genomic variations between different individuals of the same species. These novel genomic variations exist predominantly in the form of structural variants (SVs) which cannot be represented with a single reference genome. In such a scenario, a pan-genome which represents the entire gene content for a species provides a viable alternative to single reference genome for a wide range of genomics-assisted breeding applications. Pan-genome based genetic mapping approach is emerging as an attractive strategy to identify trait-associated genetic variants that are missing from the traditional reference genome. However, pan-genome based genotyping relies heavily on high coverage whole-genome sequencing which makes it extremely resource-intensive. Therefore, there is a need for a cost-efficient and user-friendly pan-genome based genotyping assay which can help leverage pan-genome for diverse genetic studies. In this perspective, we developed a novel SNP array, “Rice Pan-genome Genotyping Array (RPGA)” which provides efficient solution for performing rapid, cost-effective and user-friendly pan-genome based genotyping solution in rice. Global rice germplasm with rich trait diversity harbors extensive genetic variations that cannot be optimally captured with conventional SNP arrays based on a single reference genome for their eventual utilization in genetic enhancement of rice. To overcome this, a first-ever pan-

Lead Lecture

genome based SNP genotyping assay, “RPGA”is developedwhich includes more than 80,000 genome-wide informative SNPs, selected from millions of pan-genome based sequence variants, identified using thousands of diverse global rice accessions. It enables researchers to target novel trait-associated genomic variations that are missing from the traditional reference genome. Therefore, this array provides higher genomic coverage and captures almost entire genomic variations existing within global germplasm accessions compared to other genotyping arrays available for rice. This makes the array ideal SNP genotyping solution for pan-genome based genetic studies including complex trait dissection and diverse genomics-assisted breeding applications including GWAS, QTL mapping, marker-assisted selection and genomic selection, etc. to drive genetic improvement of rice.

Keywords: GWAS, Pangenome, QTL mapping, SNP array

Lead Lecture

IMPORTANCE OF PLANT PHENOTYPING FOR CROP IMPROVEMENT: THE NOVEL DROUGHT SIMULATOR PHENOMICS FACILITY

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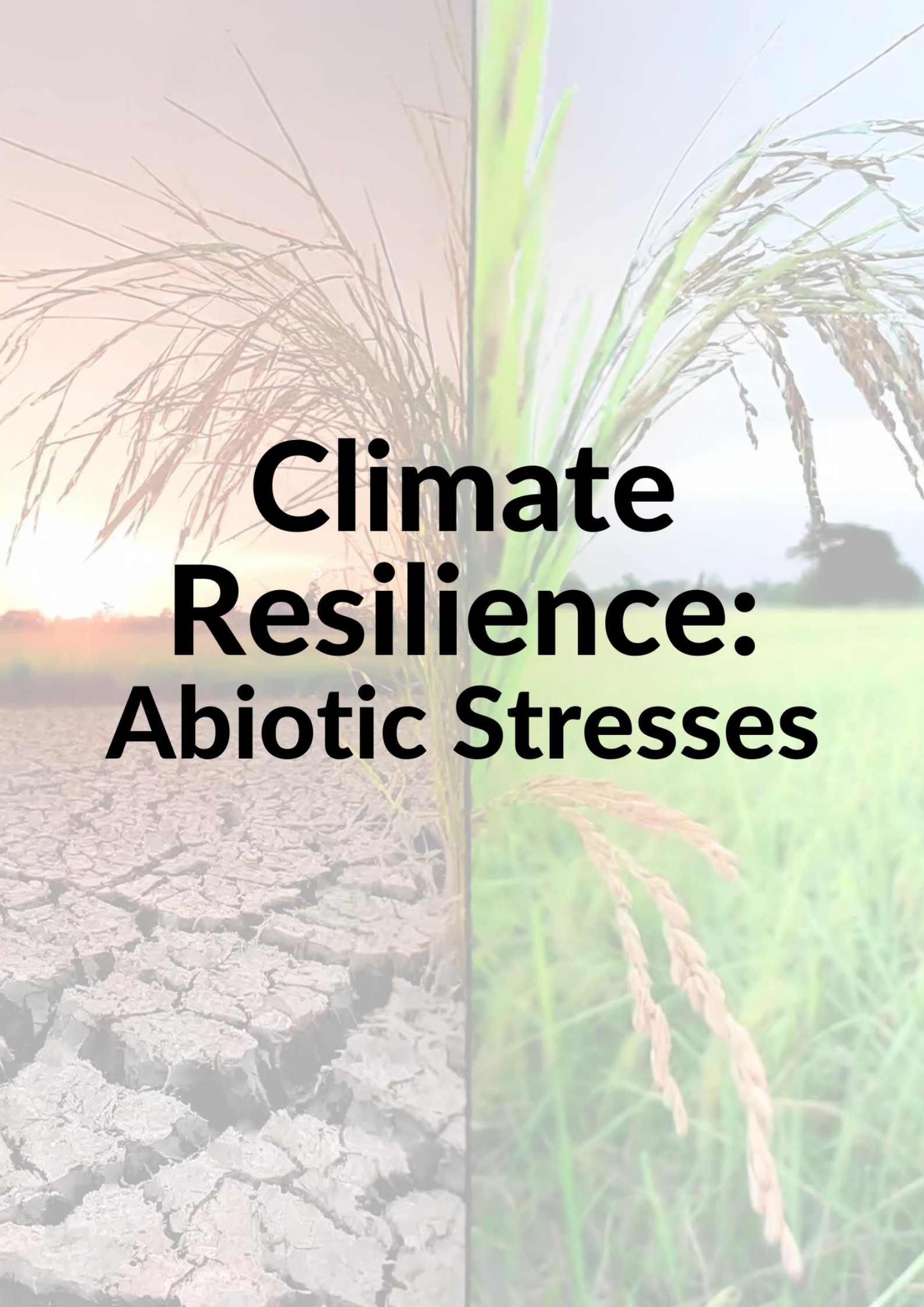
Abstract:

Anticipated population growth and highly variable environmental factors will continue to threaten global food security. Sustaining crop yields under these challenging circumstances is critical in the coming years. Last two decades have witnessed a big leap in sequencing and molecular technologies which hastened the genotyping part of the crop improvement programs. However, the efforts in associating plant genomics to trait of interest did not keep pace. Precise phenotyping bottlenecks have limited our ability to dissect the genetics of quantitative traits such as drought tolerance. The major requirement for such studies is the accurate imposition and continuous maintenance of specific water regimes in the soil as well as in the crop canopy. Gravimetry is by far the most widely adopted method to create precise moisture stress regimes and phenotype plants for drought tolerance. Despite continued efforts to make gravimetry a high throughput phenotyping method, there are several disadvantages associated with platforms developed through such efforts. The novel drought simulator phenomics facility indigenously developed at our organization will overcome several of these disadvantages. The unique features and key applications of the facility including its ability to create user defined drought stress cycles at any stage of crop growth simulating the field soil drying, precise maintenance of soil moisture across genotypes despite differences in root traits and transpiration rates and hence capturing acquired tolerance traits, real-time monitoring of water use, measurement of nocturnal transpiration, monitoring stomatal conductance in response to changing VPD, etc will be discussed.

Key words: Phenotyping, gravimetry, drought simulator phenomics facility, acquired tolerance traits.

Posters



Climate Resilience: Abiotic Stresses

QTL ANALYSIS OF NOVEL GENOMIC REGIONS ASSOCIATED WITH SODICITY TOLERANCE AT REPRODUCTIVE STAGE IN RICE (ORYZA SATIVA L.)

S. L. Krishnamurthy, B. M. Lokeshkumar, Ashish, Suman Rathor, A S Warraich, P. C. Sharma

Sodicity is one of the major abiotic stresses that limits rice production globally, especially in modern high-yielding rice varieties that are highly sensitive to sodic stress. While there are salt-tolerant landraces and traditional rice varieties at reproductive stage, information on genomic regions (QTLs) and genes responsible for their tolerance is limited. To address this issue, we used recombinant inbred lines (RILs) from MTU1001/Kalarata to map QTLs for sodicity tolerance at reproductive stage. We constructed high-density linkage groups of 1451.67 cM length using 6020 non-redundant-filtered SNP markers, with an average distance of 0.24 cM between markers. We identified 12 quantitative trait loci (QTLs) among them, two on chromosome 1 (*qDF1.1* and *qSIS1.1*), three on chromosome 2 (*qPH2.1, qPH2.2* and *qBW2.1*), two on chromosome 3 (*qPH3.1* and *qTT3.1*), one on chromosome 4 (*qGY4.1*), one on chromosome 8 (*qDF8.1*), and three QTLs on chromosome 9 (*qSIS9.1, qPL9.1* and *qGY9.1*). We used the mapped QTLs, to identify fifty-two promising candidate genes responsible for sodicity tolerance, including *OsHKT2:3 (High-affinity* K^+ *transporter)*, *OsOSCA1.1 (Hyperosmolality-induced* $[Ca^{2+}]$ *cyt increase), OsCAX1a* $(Ca^{2+})/(H^+)$ *exchangers), OsBBX11 (B-BOX protein), OsPIP2:3 (Plasma membrane receptor-like kinase)* and *OsPEX11-4* (*Peroxisomal Biogenesis Factor 11*). We identified novel quantitative trait loci (QTLs) related to salt tolerance and candidate genes for salt tolerance at reproductive stage in rice. This information could be utilized in development of new breeding strategies aimed at improving salt tolerance in rice varieties, as well as further investigations into the mechanisms underlying this tolerance.

PHENOTYPIC EVALUATION OF RECOMBINANT INBRED LINES OF BURARATA × PUSA44 FOR SALINITY TOLERANCE AT REPRODUCTIVE STAGE IN RICE

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Salinity is the main issue facing the entire world. It affects the production of important crops like rice. Rice (*Oryza sativa* L.), which is grown on 160 million hectares of land worldwide and produced in 493 million tonnes (Mt), is a staple food for around half of the world's population. The goal of the current study was to assess the recombinant inbred lines (RILs) resulting from the cross between Burarata (Tolerant) and Pusa 44 (Sensitive).During Kharif 2022, 190 RILs and their parents were involved in replicated Simple Lattice Designs in two different environments: normal (EC 1.0 dS/m) and saline (EC 8.0 dS/m). In non-stress conditions, the grain yield (kg/hectare) ranged from 405 (RIL172) to 10080 (RIL123). In terms of physiological characteristics, the range for Na content (mg/g dry wt) was 0.10 (RIL147) to 2.205 (RIL57), while the range for K content (mg/g dry wt) was 5.96 (RIL166) to 29.92 (RIL106). Grain yield (Kg/ha) ranged from 6.67 (RIL1141) to 5041 (RIL 93) in conditions of salinity stress, while spikelet fertility (%) ranged from 05 (RIL141) to 91.33 (RIL93). Na content ranged from 2.755 (RIL42) to 27.91 (RIL104), while k content ranged from 0.13 (RIL88) to 1.56 (RIL181), according to the physiological characteristics. The rice line RIL 93 (5041kg/ha) showed the highest grain yield followed by RIL164 (4883 kg/ha), RIL161 (3633 kg/ha), RIL163 (3281 kg/ha), RIL83 (2955 kg/ha), RIL114 (2788 kg/ha), RIL26 (2491 kg/ha), RIL123 (2336 kg/ha), RIL46 (2311 kg/ha) and RIL176 (2301 kg/ha) under saline stress. Based on grain yield and spikelet fertility under saline stress, the top ten tolerant and bottom ten susceptible RILs were determined. These RILs can be employed in bulk segregant analysis (BSA) to locate the region associated with reproductive stage salinity tolerance. This RIL population can also be utilized to find QTL for salt tolerance during the reproductive stage.

PROPHYLACTIC TREATMENT OF SEEDS WITH AQUEOUS EXTRACT OF AGRICULTURAL WEED *AMARANTHUS VIRIDIS* RESCUES ARSENIC TOXICITY AND REDUCES ARSENIC ACCUMULATION IN RICE PLANTS

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The grievous heavy metalloid Arsenic (As) shows substantial bioaccumulation in rice plants that not only affects the rice productivity by hampering the germination rate and deformed morpho-anatomical structures, but also causes serious health hazard to rice consuming human population. This study aims to determine the potency of seed priming with agricultural weed *Amaranthus viridis* aqueous extract (AvE) to alleviate As-induced adverse effects, and reduce As uptake by the rice seedlings. Along with increased germination rate (70-73%), AvE-primed seedlings demonstrated significantly improved morpho-physiological parameters under As stress. Additionally, AvE-primed seedlings displayed enhanced level of antioxidant polyphenols (upto 2.3 folds) along with diminished contents of As-induced stress markers i.e. H_2O_2 (21-38%), malondialdehyde (14-24%), and proline (19-44%). The reduced oxidative damage in the AvE-primed rice seedlings was further validated by light microscopy and SEM studies, which exhibited considerably amended anatomical organization under As stress. Further, ICP-OES analysis indicated AVE-priming to play crucial roles in limiting the uptake of As (9-75%), and its subsequent root to shoot translocation (34-58%). Interestingly, qRT-PCR analysis also revealed the potential of AvE-priming to regulate the expression of a few critical morphological (*OsRBOHC* and *OsSHR2*) and stress (*OsABCC1, OsARM1,* and *OsCLT1*) marker genes in the root of primed seedling under As stress. Taken together, this report for the first time depicts the potential of rice seed priming with AvE to ameliorate the As-induced stress. Furthermore, the study also establishes AvE as an As stress reducing agent that can be used for sustainable low As-rice production in As-contaminated areas.

Keywords: Abiotic stress resilience; Arsenic stress amelioration; Low Arsenic rice; Seed priming; Sustainable rice production

GENETICS AND PHYSIOLOGY OF SUBMERGENCE TOLERANCE IN RICE-SUB1A, SK AND BEYOND

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Keywords: Rice. Submergence, *Sub1a*, *SK*, combinatorial gene expression

Though the individual roles of *Sub1a* and *SK* are well documented, their way of coordination for providing survival backup under mixed submergence is yet to be established. Considering this lacuna, an attempt was made to study the expression profile of the major genes involved in submergence response among two core collections of rice lines (having differential presence of *Sub1a* and *SK*) popularly grown in South Bengal and Assam-the two major rice-producing states of Eastern India. From the detailed expression profiling of the submergence responsive genes including *Sub1a* and *SK* in rice lines possessing genes of both loci, it can be concluded that the conventional concept stating the sole responsibility of each loci modulating quiescence and elongation respectively is not exclusive and straightforward, rather the existence of both is pre-requisite and functioned coordinately to provide the tolerance to mixed flood incidence. *Osa-miR6245* targeting *Sub1a* loci was identified and validated in an indigenous rice line (var. Kaliray) showing quiescence growth and two other miRNAs (*osa-MIR1319b* and *osa-MIR1439*) were identified as two effective miRNAs targeting *SK1* and *SK2* involved in escaping mode of adaptation. This study carried out in the present work is unique, as all the published work stated their exclusive role for individual growth patterns (either quiescence or elongation), but in this work both the loci were considered at the same time and experimented on such rice lines carrying both the loci simultaneously. Parallelly, both genes' expression patterns and associated responsive genes were studied in combination.

REGULATION OF SK1- AN ADAPTIVE MECHANISM FOR UNDERWATER GROWTH IN RICE

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Despite the availability of flood-tolerant varieties with *Sub1a*-mediated elongation, recently *SK1* has been depicted as the primary gene responsible for elongation under submergence. However, the combined role of both genes is yet to be revealed. In the present work dual nature of both genes with their combinatorial expression showed differential activity under flash flood followed by stagnant flood. This work showed that both genes are pre-requisite for differential tolerance (under varying depth and duration) as on the onset of flood, *Sub1a* is switched on in both the var. Ganga Sali (elongating line) and, FR13A (following quiescence strategy) but as the flood prevails for a longer period, expression of *Sub1a* is masked by *SK1* which assists the plant to elongate out of the water. On the contrary negative check, IR42 activates all the necessary genes required for its survival under flooding conditions but as time proceeds the sustainability of the genes becomes less effective and the plant shows stunted growth. In FR13A both the gene expresses simultaneously, but as the water lodging sustains over 20 days, the expression of both the genes is reduced and as a substitute, they moderately express *adh1*, *pdc1,* and *susy1* genes to survive underwater for a prolonged period. The uniqueness of this work is the simultaneous consideration of the expression of both the loci against a varying degree of depth and duration of flood regime.

Gene annotation and enrichment analysis of the polymorphic markers linked to high temperature tolerance in rice.

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Keywords: Sugar signaling pathway; Gene expression; Bulked Segregant Analysis

High temperature is an important abiotic stress affecting the pollination and fertilization in rice and causes spikelet sterility. Sugar signaling is an important pathway affecting the assimilate partitioning between the source and sink organs in rice. We tried to understand the expression of the genes involved in sugar signaling mainly hexokinases2 (*OsHXK2*), Sucrose-nonfermentation1-related protein kinase1 (*OsSnRK1*), Trehalose-6-phosphate synthase1 (*OsTPS1*) and Target of Rapamycin (*OsTOR*) under high temperature stress was examined in heat tolerant NERICA– L 44 (NL-44) and susceptible rice variety Uma (MO-16). In the vegetative phase, the expression of *OsTOR* seems to be the difference between NL-44 and Uma for their differed heat stress tolerance whereas, in the grain-filling phase, the difference between the varieties lay in the regulation of *OsHXK2*. The comparative changes in the expression levels between the genes under the varying conditions indicate the sugar status in the source and sink organs that are available for translocation or remobilization. Bulked Segregant Analysis of the F_3 population of NL-44 X Uma identified ten SSR markers associated with high temperature tolerance in rice. Gene annotation and enrichment analysis of these associated markers revealed the functions of genes associated with polymorphic markers. Result revealed that the sequence- specific site of that chromosome was mostly enriched with biological processes like metabolic pathways, molecular mechanisms, and subcellular function. Among those was RM337, a newly reported marker for heat tolerance. Expression analysis of two genes corresponding to RM337 revealed that *LOP1* (LOC_Os08g01330) was linked to high temperature tolerance in rice. The results demonstrate that BSA using SSR markers is useful in identifying genomic regions that contribute to thermo-tolerance.

MOLECULAR PROFILING OF *PUP*1 INTROGRESSED BACKCROSS INBRED LINES OF RICE (*ORYZA SATIVA* L.) FOR PHOSPHORUS STARVATION TOLERANCE

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Keywords: Rice, Phosphorus deficiency tolerance, *Pup*1 QTL

Rice (*Oryza sativa* L.) is the staple food crop consumed by the majority of the world's population. The introduction of essential genes plays a crucial role in bolstering resistance to a range of environmental and biological stresses, ultimately safeguarding food security. The management of nutrients faces a new hurdle due to climate change, impacting crop yields. Phosphorus is a vital nutrient necessary for numerous physiological and biochemical processes within plants that is influenced by factors such as temperature fluctuations, pH levels, drought, and increased CO_2 levels which affect the availability, absorption, and movement of phosphorus. A major quantitative trait locus known as *Phosphorus uptake* 1 (*Pup*1) located on rice chromosome 12 is found to be linked to phosphorus deficiency tolerance in soil. The study aims to introduce P deficiency tolerance QTL into the rice variety CR 1009 *Sub* 1 through Marker marker-assisted backcross breeding. Pup1- introgressed backcross inbred lines of CR 1009 *Sub* 1 derived from the cross between CR 1009 *Sub* 1 and Samba Mahsuri *Pup*1 were assessed using gene-specific markers (K29-3 and K46) of *Pup*1 and genome-wide markers for foreground and background selection respectively. Our findings demonstrate the successful incorporation of the *Pup*1 QTL while minimizing the inclusion of unwanted genetic traits from Samba Mahsuri *Pup*1. This dual selection approach offers a robust framework to select 18 inbreds from the BC_3F_4 and BC_1F_5 generations. This helps in the development of CR 1009 *Sub* 1 rice varieties with improved low phosphorus tolerance and adaptability to various agricultural conditions.

WOUND INDUCED SMALL-PEPTIDE MEDIATED SIGNALING CASCADE REGULATED BY OSPSKR, DICTATES BALANCE BETWEEN GROWTH AND DEFENSE IN RICE

Wounding is a general stress in plants that results from various pest and pathogenic infections in addition to environment induced mechanical damages. Plants have sophisticated molecular mechanisms to recognize and respond to pests and pathogens. Several molecules such as phytohormones, peptides and receptors have been attributed to wound responses in dicots and monocots might have distinct molecular mechanisms. Here, we show the involvement of two distinct categories of temporally separated, endogenously derived peptides, namely, plant elicitor peptides (PEPs) and phytosulfokine (PSK), that mediate wound responses in rice. These peptides trigger a dynamic signal relay in which a receptor kinase named OsPSKR played a major role. OsPSKR perceived PSK ligand, acting in association with a co-receptor OsSERK1, to activate downstream responses in a kinase activity-dependent manner. Perturbation of OsPSKR expression in rice led to compromised development and constitutive autoimmune phenotypes. These results suggested that OsPSKR maintains the trade-off between growth and exaggerated defense responses, both during homeostasis and wounding. Collectively, these findings indicate the presence of a stepwise peptide-mediated signal relay that regulates the transition from defense to growth upon wounding in monocots.

DEVELOPMENT OF MULTIPLE STRESS TOLERANT RICE BY OVEREXPRESSING DUAL ACTIVITY *PUTRANJIVA ROXBURGHII PURINE NUCLEOSIDE* PHOSPHORYLASE (*PRPNP*)

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Keywords: Climate change, PRpnp, resilient varieties, overexpression, transgenic rice.

Climate change is a global problem that is affecting mankind. In 2023, the effect of climate change can be seen from the report of the hottest February and the dried August. These unpredictable climate conditions pose a grave threat to food production. Apart from these climatic conditions, several biotic components of the ecosystem also cause damage to food crops and, hence, reduce food productivity. Plants, being sessile, cannot escape from stressful environmental conditions. Biotic and abiotic stresses are the primary factors limiting the production. Climate change has worsened the number of stressful environmental conditions a plant experiences; thus, the development of stress-tolerant crop varieties is crucial. *Rice is the staple food for half of the world's population, so efforts are needed to develop rice crop varieties that are resilient to stressful environmental conditions. Putranjiva roxburghii* purine nucleoside phosphorylase (*PRpnp*), a dual activity *pnp* having phosphorylase and trypsin inhibitor activity, is reported as a factor promoting biotic and abiotic stress tolerance in transgenic *Citrus. Thus, taking cues from the previous study, PRpnp is overexpressed in rice to develop resilient varieties that will be resilient to biotic and abiotic stresses.*

RICE DUAL SPECIFICITY PHOSPHATASE *OsPP117* DIFFERENTIALLY REGULATES OXIDATIVE AND OSMOTIC STRESS RESPONSES IN PLANTS

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The interplay of kinases and phosphatases regulate reversible phosphorylation and is required to maintain normal functioning of cellular processes. Among phosphatases, which target both serine/threonine and tyrosine residues are known as DSPs (Dual Specificity Phosphatases). DSPs are known to participate in various physiological, developmental and biochemical responses in plants. Previous reports have discussed the involvement of phosphatases under abiotic stress responses via direct or indirect regulation of ROS generation and scavenging. Our study has also demonstrated the potential role of rice phosphatase *OsPP117* under oxidative and osmotic stress responses. The result was validated by qRT-PCR analysis in which upregulation of *OsPP117* under methyl-viologen (oxidative stress) and mannitol (osmotic stress) stress could be seen. Moreover, ectopic expression of 2XCaMV35S::*OsPP117* transgenic lines impart tolerance to oxidative stress in both light and dark condition in Arabidopsis. In comparison to wild type (*Col-0*), the enhanced level of proline accumulation and reduced level of MDA content was observed which further supports tolerant behaviour of transgenic lines under oxidative stress. Additionally, the transgenic lines show sensitive response in terms of poor root and shoot growth as compared to wild type under osmotic stress condition in Arabidopsis. This could be possibly because of enhanced ROS content in transgenic lines which was corroborated by superoxide assay and hydrogen peroxide content analysis. Together these reports suggest the rice *OsPP117* act as a positive regulator under oxidative stress and negative regulator under osmotic stress responses in plants.

Keywords: Dual Specificity Phosphatases, Rice, Transgenic, Osmotic stress, Oxidative stress, Methyl viologen, Mannitol, Reactive oxygen species.

EXPRESSION ANALYSIS AND DEVELOPMENT OF MIRNA-LINKED MOLECULAR MARKERS IN RICE FOR SALT TOLERANCE.

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In this present study, a total number of six rice landraces were taken for detailed profiling for their salt tolerance and IR 64 with Nonabokra were taken as negative and positive checks respectively. Physiological and biochemical screening was performed under varying concentrations of salt solution to select the potential genotypes showing salt tolerance. This experiment was performed to develop miRNA-linked SSRs (Simple sequence repeats) based molecular markers linked with salt tolerance in rice and study the expression of the miRNA parallelly with their target genes. A list of five miRNAs associated with salt tolerance in rice was selected from the miRNA database. A standard bioinformatics pipeline was standardized for the development of miRNA-linked SSRs markers. The genomic DNA of the studied rice line was isolated and used for preparing genotypic profiling based on bioinformatically designed miRNA-linked SSR-based molecular marker. The total RNA and miRNA were isolated from the stressed and control sample of the highest salt-tolerant variety, positive and negative checks to study the expression of miRNA and its target through qPCR analysis. The amplified products were resolved and the different amplified bands were detected. Among five miRNA-linked SSR markers osa-mir396a and osa-mir172b showed a significant difference in molecular weight of PCR produced band and these two markers were able to differentiate the tolerant and susceptible genotypes. This result indicates that miRNA-linked molecular markers can be used for varietal identification as well as their functional identity and can be considered as a functional marker for different valuable agronomic traits.

THE REGULATORY ROLE OF RICE DUAL SPECIFICITY PHOSPHATASE, *OsPP42* IN ROOT DEVELOPMENT THROUGH MODULATION OF REACTIVE OXYGEN SPECIES HOMEOSTASIS

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The maintenance of reactive oxygen species (ROS) homeostasis is crucial for plant root development. To cope up with stress and maintain ROS homeostasis, plants regulate numerous ion-permeable channels triggered by kinases/phosphatases which serve as a signaling mechanism in developmental processes in root cells. Previous investigations have shown that *SEX4* (*Starch Excess4*), a dual specificity phosphatase (DSP), regulates Arabidopsis starch metabolism. However, there is a limited understanding of the mechanism through which *SEX4* affects ROS homeostasis. In this study, we found that *OsSEX4*, also known as *OsPP42*, regulates root growth by modulating ROS homeostasis in response to oxidative and osmotic stress. Our functional investigation showed that Arabidopsis overexpressing *OsPP42* was more tolerant to methyl viologen (MV)-induced oxidative stress in both light and dark conditions in contrast to mannitol-induced osmotic stress, where root length decreased significantly. Under oxidative stress, *OsPP42* overexpression (OX) lines had enhanced proline and decreased MDA, superoxide generation rate, hydrogen peroxide, H_2DCFDA and DAB staining. Conversely, OX lines had lower proline levels and higher MDA and ROS levels than wild-type, following osmotic stress. These results demonstrate the hitherto unidentified role of *OsPP42* in regulating root development by modulating ROS homeostasis in Arabidopsis under oxidative and osmotic stress. By elucidating the intricate functions of *OsPP42* in Arabidopsis, we have uncovered potential avenues for enhancing stress tolerance in rice. The ongoing process of functionally validating abiotic stress tolerance in *OsPP42* OX and RNAi lines in rice through leaf disc tolerance and phenotypic analysis will contribute to the development of resilient and productive agricultural systems.

Keywords: Dual specificity phosphatase (DSP); kinase; osmotic stress; oxidative stress; abiotic stress; reactive oxygen species (ROS)

RICE PHOSPHATE STARVATION-INDUCED RIBONUCLEASE MIGHT BE REGULATED PI STRESS ADAPTATION BY MAINTAINING INTRACELLULAR PI HOMEOSTASIS.

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Root development is a fundamental process that supports plant survival and crop productivity. One of the essential factors to consider when developing biotechnology crops is the selection of a promoter that can optimize the spatial-temporal expression of introduced genes. However, there are insufficient cases of suitable promoters in crop plants, including rice. In this study, we identified *OsRNS1* as a root-preferred expression. To enhance the function of *OsRNS1* gene, which functions primarily in the root, two constructs were generated the *OsRNS1*-overexpression lines through expressing coding sequence of the *OsRNS1* with either a *ubiquitin 1* promoter (*Ubi1*) or *Os1-CysPrxB (Per)* promoter with root-preferred expression. Compared with the WT, the p*Per::OsRNS1* showed an increase in root length, plant height, and grain yield compared to wild-type (WT). The p*Per::OsRNS1* significantly enhanced tolerance to Pi starvation, whereas the p*Ubi1::OsRNS1* showed overall growth defect with or without Pi. Taken together, our results suggest that this strategy would be a great way to optimize the function of root-preferred genes and is hoped to help develop rice that improves Pi use of efficiency and yield.

GENETIC DISSECTION OF SALINITY AND HIGH TEMPERATURE STRESS TOLERANCE TOWARDS CLIMATE RESILIENT RICE

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Key words: Abiotic stress, rice, salinity, heat, climate resilience

Abiotic stresses such as drought, salinity, temperature variations are complex and limit rice production. Genetic improvement is the most economical, environmentally friendly approach. Identification of promising tolerant sources for incorporation into high yielding genetic background reduce the yield loss. Diverse germplasm of 500 lines screened for heat tolerance over three years during rabi season and in controlled conditions and for salinity in multilocation hot spots. In field based staggered experiments, the days to 50% flowering coincided with high temperature (35℃ to 42.5℃) indicated the heat stress. Among the 500 lines screened over the years, where Spikelet fertility varied from 15.5 % to 95.5 % among the genotypes. we identified 114 lines with spikelet fertility varying between 60% to >95% which were further validated along with other abiotic stresses namely salinity, drought tolerant lines and released varieties in three different staggered plantings. AMMI and GGE analysis revealed stable and reliable heat tolerant genotypes namely Rasi, Giza 178, IR 50, Khao Daw Tai, IRGC 126084, IRGC 127227, IRGC 127663, Nerica-L44, HIRA, IRGC 128335, IRGC 72918-1, IRGC 117280, IRGC 127424 and IRGC 128373 with spikelet sterility ranged from 5 to 10%. The saline stress level varied from EC6 $dSm^{-1}$to EC14 dSm^{-1}across locations. At high stress of EC 14 dSm^{-1} tolerant genotypes IRGC 128372, IRGC 125873, IRGC 128085, IRGC 125944, IRGC 125876, IRGC 125882, Sadri identified. Interestingly, the salinity tolerant genotypes were found to be heat sensitive and *vice versa*. Association mapping revealed genomic regions associated with tolerance with insights for the genetic improvement.

UNDERSTANDING THE ROLE AND REGULATION OF METHIONINE SULFOXIDE REDUCTASE (MSR) IN *ORYZA SATIVA*

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Keywords: Methionine sulfoxide reductase (MSR), reactive oxygen species, rice, protein repairing enzyme

Proteins are involved in each and every molecular process that influences the structure and function of the cell. Cell often generates Reactive Oxygen Species (ROS) particularly under stressful environments, and excess accumulation of ROS causes cumulative oxidative damage to the cell. Interestingly, ROS can oxidize methionine (Met) residue to methionine sulfoxide (MetSO) in proteins or peptides, and presence of such MetSO in proteins adversely affects protein's structure and function. To repair such protein damage, organisms including plants possess Methionine Sulfoxide Reductase (MSR) enzymes that reduces the methionine sulfoxide back to methionine. This repair process helps to re-establish the native structure and function of the proteins. Essentially, oxidation of methionine leads to the formation of two diastereomers, methionine S-sulfoxide (Met-S-SO) and methionine R-sulfoxide (Met-R-SO) which can be reverted back by two specific types of Methionine Sulfoxide reductase (MSR), MSRA and MSRB, respectively. These MSR proteins are ubiquitously distributed among organisms and shown to play important functions in stress tolerance, prevention of aging-associated diseases, and enhancing lifespan in bacterial, yeast and mammalian cells. However, such functional studies on MSR in plants are rather limited.

Rice (*Oryza sativa*) possesses three members of MSRA and MSRB and both types of MSR are implicated in biotic and abiotic stress tolerance to plants. Recently, we have shown that OsMSRB5 is involved in seed vigor and longevity by protecting various proteins from MetSO modification which in turn maintain ROS homeostasis in seeds. We have shown that these OsMSR isoforms are differentially expressed and regulated in plants. Interestingly, OsMSRB3 gene produces two possible isoforms (OsMSRB3 and OsMSRB3.1) through alternative splicing. We have bacterially expressed these recombinant protein isoforms and purified to homogeny and subsequently biochemically characterized. Further, accumulation pattern of these two transcript variants in different tissues and during development has also been analyzed. Further, to understand the role of OsMSRB3 isoforms, various constructs are presently being made to generate overexpression and genome edited lines of OsMSRB3.

EMPLOYING EMS MUTAGENESIS TO ENHANCE THE MULTIPLE ABIOTIC STRESS TOLERANCE ABILITY OF RICE CULTIVAR NAGINA22

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Keywords: *Oryza sativa*, Abiotic stress, EMS mutagenesis, Nagina22, Yield

Nagina22 (N22), a rice cultivar known for its ability to withstand heat and drought, has been extensively utilized in genomic studies to identify and characterize rice genes linked to tolerance against various abiotic stresses. In this particular study, we conducted screenings on N22 mutants induced by EMS to determine their enhanced tolerance to high temperature (HT) and low phosphorus (P) stress during both the seedling and reproductive stages. Four heat-tolerant N22 mutants (NH363, NH663, NH733, and NH669) exhibited superior grain yield and spikelet fertility than N22 under HT stress. Notably, NH733 displayed superior pollen fertility, stigma receptivity, spikelet fertility, photosynthetic rate, SPAD value, shoot length, root length, total dry weight, and antioxidant activity compared to N22 under high HT and low-P stress conditions. We performed whole-genome RNA sequencing of NH733 mutant under heat, drought, and low-P stress. Transcriptome analysis of NH733 revealed the commonly induced genes under three stress environments as well as the exclusive genes associated with specific stress. Several novel genes associated with above tested abiotic stresses were identified. These results will be discussed in the presentation. Taken together, the mutant NH733 might serve as valuable genetic resources for the discovery of novel genes associated with multiple stress tolerance in rice.

DIGGING DEEPER FOR UNRAVELLING THE HIDDEN HALF IN RICE: ASSOCIATED GENOMIC REGIONS, GENES, SNPS, AND MOLECULAR MECHANISMS UNDER AEROBIC CONDITION.

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The root system architecture critically influences water uptake, especially under water-limiting condition as in the aerobic system of cultivation with dry direct seed establishment in rice. Understanding the useful root-related traits such as root length, root volume, root dry weight, early seedling vigor, mesocotyl length, coleoptile length is essential for current scenario of water scarcity and climate change. Mapping the genomic regions, QTLs, markers associated with yield and yield-related traits under aerobic conditions is crucial for transferring traits in elite rice backgrounds in molecular breeding programs. A root phenotyping method has been developed and roots are scanned at panicle initiation stage. The molecular mechanism of aerobic adaptation through RNA-seq analysis of root and shoot at panicle initiation stage in cultivars adapted to aerobic-CR Dhan 202 and traditional transplanted anaerobic BPT 5204 conditions. A possible mechanism of aerobic adaptation was proposed that partially mimics drought tolerance mechanism - root tissue sensing water limiting condition initiate expression of transcripts related to sensor molecules like MAPK, CBP, from roots to shoots by increasing expression of hormonal signaling including ethylene, abscisic acid and brassinosteroid, that leads to expression of abscisic acid-responsive transcripts viz. RAB21, RAB16B, RAB16C which might be responsible for regulating expression of MADS family TFs viz. MADS4, MADS5, MADS6, MADS7, putative WRKY 6. In order to map the genomic regions of seedling vigor index, root and yield-related traits, a panel of 118 rice lines consisting of North-Eastern landraces, popularly cultivated varieties, aerobic released varieties, basmati rice, aromatic short grain lines, advanced breeding lines, introgression lines, soft rice lines, ethyl methanesulfonate (EMS) mutants of BPT-5204, Nagina 22 (N22), wild introgression lines, tropical japonica accessions, Oryza glaberrima accessions were phenotyped under aerobic conditions at multi-locations, control aerobic

condition, genotyped using polymorphic SSR markers. Significant correlations were recorded for root length, root dry weight with SVI, root volume at the PI stage, number of grains per panicle with grain yield per plant. The panel was divided into three sub-groups and Genome-wide association studies GWAS displayed marker-trait associations using GLM and MLM models. Significant MTA for grain yield per plant (RM22961, RM1146), root traits at PI stage (RM2584, RM80, RM410, RM1146, RM18472). Functionally relevant genes near MTAs through in-silico expression in root viz., HBF2 bZIP transcription factor, WD40 repeat-like domain, OsPILS6a auxin efflux carrier, WRKY108, OsSCP42, OsMADS80, nodulin-like domain-containing protein, amino acid transporter were identified. The identified MTAs and rice lines having high SVI traits (Langphou, TI-128, Mouli, TI-124, JBB-631-1), high yield under aerobic (Phouren, NPK-43, JBB-684, Ratnamudi, TI-112), robust root traits like root length (MoirangPhou-Angouba, Wangoo-Phou, JBB-661, Dissi, NPK-45), root volume (Ratnachudi, KJ-221, Mow, Heimang-Phou, PUP-229) can be employed for targeted environments aimed at improving seedling vigour, yield-related traits under aerobic condition as adaptability to water-saving technology. Promising lines with yield/ robust root traits have been deployed in developing mapping populations. Genomic regions have been mapped, SNPs associated with root length and volume have been identified in a F2 of population derived from TI-128*BPT-5204. One line TI-3 has been registered with NBPGR, with INGR 22103.

CATALASE ASSOCIATED WITH ANTAGONISTIC CHANGES OF ABA AND GA IS INVOLVED IN EUGENOL-INHIBITED PREHARVEST SPROUTING IN RICE

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Keywords and Abbreviations: ABA, Catalase, Eugenol, GA, Preharvest sprouting, Rice.

The natural monoterpene eugenol has been reported to inhibit preharvest sprouting in rice. However, the inhibitory mechanism remains obscure. In this study, simultaneous monitoring of GA and ABA responses by the *in vivo* GA and ABA-responsive dual-luciferase reporter system showed that eugenol strongly inhibited the GA response after 6 h of imbibition, whereas eugenol significantly enhanced the ABA response after 12 h of imbibition. Gene expression analysis revealed that eugenol significantly induced the ABA biosynthetic genes *OsNCED2*, *OsNCED3*, and *OsNCED5*, but notably suppressed the ABA catabolic genes *OsABA8ox1* and *OsABA8ox2*. Conversely, eugenol inhibited the GA biosynthetic genes *OsGA3ox2* and *OsGA20ox4* but significantly induced the GA catabolic genes *OsGA2ox1* and *OsGA2ox3* during imbibition. *OsABI4,* the key signaling regulator of ABA and GA antagonism, was notably induced before 12 h and suppressed after 24 h by eugenol. Moreover, Eugenol markedly reduced the accumulation of H_2O_2 in seeds after 36 h of imbibition. Further analysis showed that eugenol strongly induced catalase activity, protein accumulation, and the expression of three catalase genes. Most importantly, mitigation of eugenol-inhibited seed germination was found in the *catc* mutant. These findings indicate that catalase associated with antagonistic changes of ABA and GA is involved in the sequential regulation of eugenol-inhibited seed germination in rice.

EFFECT OF *SALTOL1* INTROGRESSED LINES OF KMR3R FOR SEEDLING STAGE TOLERANCE AND THEIR DERIVED HYBRID PERFORMANCE IN AICRIP

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Keywords: rice, salinity, hybrids, Saltol, RNA Expression

Salinity stress is a critical factor affecting rice cultivation in regions characterized by saline soils and irrigation water. The seedling stage of rice is pivotal, as it significantly influences crop yield. This abstract highlights a breeding strategy aimed at enhancing the salt tolerance of the widely used parental line of rice, KMR3R, by incorporating the *Saltol1* gene from the salt-tolerant rice cultivar FL478. Backcross breeding was employed to introduce the Saltol1 gene, a well-known determinant of salinity tolerance, into KMR3R. Seven BC_2F_5 BILs resulting from this breeding process were rigorously assessed for their ability to withstand salt stress during the seedling stage, employing hydroponics and a modified Yoshida solution as the nutrient medium. Two different NaCl stress levels (60mM and 120mM) were applied over a 14-day period, alongside a control group, maintaining strict pH control throughout the experiment. Evaluation criteria included visual scoring based on the IRRI SES 2014 guidelines and the assessment of various morpho-physiological traits. BILs exhibiting desirable characteristics, such as a low Na^+/K^+ ratio, high relative water content (RWC), improved root and shoot growth, and enhanced chlorophyll fluorescence, were identified as salt-tolerant. The top-performing BIL, RP-6341-VTCP-56, was selected for development of hybrid and nominated for testing in AICRIP trials, focusing for Alkaline and Inland Saline Tolerant Varietal Testing (AL&ISTVT) and in final year of testing. Additionally, the study conducted RNA expression analysis of the *OsHKT1;5*gene, a key player in salt tolerance, during the seedling stage. Expression patterns revealed that *OsHKT1;5* reached maximum expression levels after 24 hours of salt stress exposure, highlighting its role in responding to salinity stress. The successful integration of Saltol1 from FL478 into KMR3R, exemplified by the promising BIL RP-6341-VTCP-56, holds potential for the development of high-yielding hybrid with enhanced salt tolerance, particularly suitable for coastal regions prone to salinity stress.

COMPARATIVE ROOT TRANSCRIPTOME ANALYSIS REVEALS THE INVOLVEMENT OF GLUTATHIONE HOMEOSTASIS IN TWO RICE CULTIVARS DIFFERING IN COPPER STRESS TOLERANCE

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Copper (Cu) plays a crucial role as a micronutrient essential for the healthy growth and development of plants. However, an excessive concentration of Cu can be extremely harmful to plants, leading to severe growth and development inhibition. We conducted a comparative transcriptome analysis of root responses to excess Cu treatment in a Cu-tolerant (TNG67) and a Cu-sensitive (TN1) rice cultivar. We identified 135, 218, and 228 differentially expressed genes that exhibited up-regulation in TN1 but down-regulation in TNG67 after 6, 24, and 48 hours of copper treatment, respectively. These genes, which display diverse expression patterns between TN1 and TNG67, are significantly enriched in the GO categories related to glutathione transferase activity and glutathione metabolic processes. The pathway analysis by the MapMan software also reveals consistent findings within the glutathione-S-transferase (GST) gene family. The expression patterns of four genes, namely, *OsGSTU5*, *OsGSTU19*, *OsGSTU30*, and *OsGSTU37*, were also confirmed in the results displayed in the RNA-seq through qRT-PCR. The glutathione (GSH) levels in the roots were assessed, and they exhibited a significant increase in TNG67, whereas no significant change was observed in TN1. *Glutaredoxin 2*, a gene potentially linked to de-glutathionylation, displayed substantial upregulation in TNG67, whereas its expression showed a modest increase in TN1 in the transcriptome. These findings suggest that GSTs likely play a role in maintaining GSH homeostasis, which affects global protein glutathionylation levels, thereby influencing the variation in copper tolerance between TN1 and TNG67.

ASSESSING THE RELEVANCE OF DROUGHT-ADAPTIVE TRAITS IN DOUBLED HAPLOID RICE LINES PYRAMIDED WITH RELEVANT TRAITS UNDER CONDITIONS OF WATER LIMITATION

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Keywords: Doubled haploids, Rice, Root, Water use efficiency, Cellular level tolerance, Wax

With rising population, urbanization and limited resources, boosting rice productivity per unit area is inevitable to meet the increasing food demand. However, with depleting water resources, increasing the rice productivity is a big challenge. In this scenario, it has been suggested that, pyramiding relevant drought adaptive traits would improve drought adaptation besides sustaining productivity. Pyramiding traits through conventional breeding takes longer time while, the doubled haploids (DH) technology shortens the breeding cycle. In this regard, previously the doubled haploid rice lines were developed using anthers of F1 plants of a cross between trait donor lines (epicuticular waxes, WUE, root characteristics, CLT) and characterized for the traits and identified the DH lines with pyramided traits. In the present study, presence of the pyramided drought adaptive traits and their relevance was examined by growing them under stress condition. The data highlights considerable variability in stress tolerance among the DH lines. Notably, the DH lines with all the three pyramided traits displayed superior drought tolerance, maintaining their yield even under stress conditions. Conversely, DH lines with no pyramided traits experienced a more significant reduction in yield under stress compared to those with one/two and three traits. Furthermore, the three-traits pyramided DH lines exhibited enhanced root characteristics, including greater root length, volume, and weight. Molecular characterization with root and WUE markers confirmed the presence of both WUE and root traits in the traits pyramided DH lines. In conclusion, this study underscores the significance of DH technology for hastening the breeding process to pyramid the traits of interest and also signifies the relevance of the drought adaptive traits under conditions of water limitation.

ROLE OF PANICLE TRANSPIRATIONAL COOLING EFFECTS IN AVOIDING THE HEAT STRESS AT REPRODUCTIVE STAGE

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Keywords: Rice, Abiotic stress, Heat stress, Reproductive stage

Rice is extensively grown over a range of ecosystems with varied environments. Climate change and global warming have negatively affected rice production. By 2050, the global mean temperature is expected to increase by 1.5–2 ºC. Rice crop is vulnerable to heat stress at different developmental stages, especially at the reproductive stage. Therefore, it is essential to study the physiological aspects of heat stress tolerance at the reproductive stage. Transpirational cooling lowers the canopy temperature maintaining the ambient temperature in the normal temperature range. However, at the reproductive stage, the panicle emerges out of the leafy canopy and grows above it. There is wide variability among rice genotypes for panicle length (7-23 cm) and peduncle length (0-16 cm), that will grow the panicle at the height of 6 cm to 25 cm above the canopy. Therefore, it is pertinent to investigate the cooling effect of canopy transpiration on panicle cooling. Further, the panicles also bear stomata on palea, lemma, rachis, and awns. Therefore, it is pertinent, to investigate the cooling effects of panicle transpiration in order to avoid heat stress. We measured the physiological parameters such as photosynthesis rate, transpiration rate, CTD, stomatal density, RWC, leaf area, stomatal conductance, etc. The results indicate that the genotypes vary in the above-mentioned physiological parameters.

MKKK32, A RAF-LIKE MAPKKK WORKS UPSTREAM OF THE MPK3-SUB1A1 MODULE DURING SUBMERGENCE STRESS IN RICE

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Keywords: MAPK, phosphorylation, Submergence tolerance, SUB1A-1, MPK3, MKKK32, MKK4

To address the impending issue of global rice demand potentially exceeding supply due to the ever-expanding population, it is crucial to tackle the current challenges tied to low rice productivity caused by environmental factors. A significant portion of reduced rice yield results from annual crop losses due to flooding. The Sub1A gene from the FR13A variety plays a pivotal role in granting rice its ability to endure complete submersion for up to two weeks and then resume normal growth when floodwaters recede. Prior research in our lab has established a direct physical interaction between Sub1A1 and a component of the mitogen-activated protein kinase (MAPK) cascade, MPK3. This interaction forms a MAPK-Sub1A module responsible for rice's submergence tolerance. The MAP kinase cascade comprises three successive protein kinases that transmit cellular signals through a sequence of phosphorylation events. Our work has confirmed MKKK32's position upstream of the MPK3-SUB1A1 module. Intriguingly, we've also revealed that MKKK32 directly interacts with the final component of the cascade, MPK3, and interacts with and phosphorylates MKK4. Furthermore, by creating transgenic rice lines overexpressing a phosphomimic form of Sub1A1, we've demonstrated that increased Sub1A1 phosphorylation enhances the quiescence response, thus improving submergence tolerance. In summary, our findings provide compelling evidence that the submergence response pathway in rice is subject to multiple levels of regulation. These discoveries open new avenues for research into MAPK cascades and the response to submergence stress in rice, all while safeguarding against a future of inadequate rice supply amidst growing global demand.

DEVELOPING EARLY MORNING FLOWERING VERSION OF RICE VARIETY CO 51 TO MITIGATE HEAT-INDUCED YIELD LOSS

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Key words: Rice; heat avoidance; *qEMF3;* marker assisted breeding

Rice production has to reach 852 million tonnes by 2035 from about 676 million tonnes which requires 0.6-0.9% growth annually. Global climatic predictions indicate increased frequency of heat spikes and warmer nights posing challenges towards achieving higher rice yields. High temperatures have become a severe limitation to global agriculture practices and food security, particularly for major food crops such as rice. Every 1˚C increase in the global mean temperature is expected to reduce rice yields across the globe by 3.2%. The development of heat-resilient rice cultivars has been slow due to the lack of relevant donors for heat tolerance traits and limited information regarding the genetic basis of the component traits. The early morning flowering (*qEMF3*) trait, contributing to heat escape by promoting flowering/anthesis during cooler hours in the morning is demonstrated to offer protection against high-temperature-induced failure of pollination and fertilization. Attempts through marker-assisted backcross breeding led to development of advanced backcross progenies (NILs) of CO 51, harboring *qEMF3*. Evaluation of 88 BC_3F_2 progenies identified 19 progenies harboring *qEMF3* under homozygous conditions. Evaluation of NILs of CO 51 harboring *qEMF3* during summer revealed that the NILs exhibited early onset of anthesis, thereby recorded reduced spikelet sterility than their recurrent parent, CO 51. The current study demonstrated the efficacy of early morning flowering in the mitigation of yield losses under high-temperature conditions in a popular rice variety.

EVALUATING THE SIGNIFICANCE OF ROOT SYSTEM IN INFLUENCING WATER USE EFFICIENCY THROUGH GENOME-WIDE ASSOCIATION STUDIES IN RICE

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Keywords: Genome wide association studies, Phenomics, Rice, Root traits, Water use efficiency.

As a primary food source, rice crop faces challenges related to drought stress. Conventional rice cultivation consumes large amount of water, making it challenging to reduce water usage. To address this issue, a novel approach called the Aerobic rice system was introduced. Aerobic cultivation involves growing rice in non-puddled, non-flooded, and unsaturated soil conditions, reducing the reliance on excessive irrigation. However, this method can lead to decreased yields due to intermittent occurrence of drought between episodes of surface irrigation. Therefore, improving water use efficiency (WUE) in rice is crucial. Enhancing WUE can be achieved by adopting screening techniques to improve important physiological traits, especially root characteristics, as a robust root system is essential for rice to withstand and manage drought stress effectively. Understanding the genetics of root structure, stress resilience and grain yield can be enhanced in rice breeding. Here, we screened 195 rice genotypes from the IRRI-3K rice panel and conducted genome-wide association study (GWAS) on root-related traits including root biomass and root to shoot ratio. A total of 8 significant single nucleotide polymorphisms (SNPs) associated with these root traits were identified. Additionally, ten genes were found to directly influence root growth. Haplotype analysis helped identify germplasm lines with improved root traits. To assess the significance of the root system in determining WUE, the same set was grown in phenomics platform and found that there was a significant correlation between WUE and root traits indicating importance of introgressing root traits to improve WUE.

ESTIMATION OF POTENTIAL AND ACTUAL EVAPORATION IN RICE: A COMPARATIVE ANALYSIS USING EVAPORIMETER AND DROUGHT SIMULATION PHENOMICS PLATFORM

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Keywords: Climate change, precision irrigation, evapotranspiration, evaporimeter, rice.

Rice is traditionally cultivated in semi-aquatic flooded condition, and it is known to utilize a significant portion of the world's freshwater resources, accounting for approximately 50% of total freshwater consumption. The diminishing water resources and prevalent climate change challenges demand to implementation of precision irrigation techniques based on crop evapotranspiration rate. Predicting such a slow process, evapotranspiration rates become pivotal in estimating crop water demands and optimizing irrigation schedules. In our study, we introduce a novel approach to rapidly measure plant evapotranspiration rates using an evaporimeter developed by IISc, Bengaluru, which measures evapotranspiration for every 2.5 to 6 minutes. We validated this novel instrument by comparing its measurements of potential evapotranspiration rates with the actual transpiration data obtained from the drought simulation phenomics platform, which provides real-time transpiration measurements. To validate the instrument's performance, we used Dhaksha rice genotype. Our findings revealed a significant positive correlation between the data collected from the evaporimeter and the actual transpiration over both a 24-hour period and a 12-hour daylight period. However, during the night period, the correlation was not statistically significant. These results strongly suggest that the evaporimeter accurately reflects the evaporation occurring from the surface of the rice leaves. Therefore, this instrument holds promise for calculating crop coefficients and optimizing irrigation strategies to enhance water productivity.

JASMONATE ACTS AS A PIVOTAL PLAYER IN REGULATING BOTH ADVANTAGEOUS AND DETRIMENTAL FACTORS ASSOCIATED WITH SALT STRESS RESILIENCE IN RICE

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In response to salt exposure, plants undergo substantial modifications in their hormonal pathways, orchestrating physiological changes to enhance their tolerance. While Jasmonate (JA) hormones are well-known for their role in defending against both biotic and abiotic stressors, their specific involvement in salt tolerance has remained ambiguous. In this study, we elucidate the dynamics of JA metabolism and signaling in the root and leaf tissues of rice, a plant species highly susceptible to salt stress. We observed that roots swiftly activate the JA pathway, while the second leaf exhibits a biphasic JA response, with peaks occurring at 1 hour and 3 days post-exposure. To better understand the processes governed by JA, we compared a rice mutant deficient in JA (*aoc*), which showed higher salt-tolerance, to wild-type rice, using kinetic transcriptome and physiological analyses. Notably, significant genotype-specific differences emerged that could explain the observed phenotypic variations. The *aoc* mutant showed impaired levels of Abscisic acid (ABA) and compromised ABA-dependent responses to water deprivation in its shoots. Additionally, *aoc* plants accumulated more sodium (Na^+) in their roots and less in their leaves, with reduced ion translocation correlated with the upregulation of a sodium transporter gene in the roots. Furthermore, *aoc* leaves exhibited stronger activity of distinct reactive oxygen species scavengers, along with reduced indicators of senescence and chlorophyll breakdown. In summary, our findings shed light on the distinct contributions of JA signaling to different aspects of the salt stress response in rice.

CHARACTERIZATION OF RICE (*ORYZA SATIVA* L.) GENOTYPES FOR SALINE TOLERANCE SUITABLE FOR EAST COAST REGIONS OF TAMIL NADU

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Keywords: Rice, salinity, morphology, variability, correlation, *in vitro*, tolerant, SSR, diversity, polymorphism

The devastation caused by the tsunami (2004) and the influx of coastal saline water has left the fields of east coast regions of Tamil Nadu inundated with salt slush and salt cakes, rendering it extremely difficult for plant breeders to meet the state's rice production needs. It is thus imperative to concentrate on the development of saline-tolerant and high-yielding rice cultivars. In this context, the present study was conducted to evaluate 40 rice genotypes for their resilience to salinity under field and *in vitro* conditions by evaluating 10 quantitative traits to understand the morphological diversity and employing 35 molecular markers to assess genetic diversity to identify new saline-tolerant cultivars. The experimental results of the evaluation of morphological traits under naturally salt-affected soil conditions (EC: 3.0 dsm^{-1}) laid in randomized block design with three replications at the Department of Genetics and Plant Breeding farm, Annamalai University revealed that the genotypes viz., PS -336, Paiyur-1, AC-39014, PS-226 were found to be superior in per se performance for almost all the traits studied. High to moderate PCV and GCV along with high heritability and genetic advance were recorded for all the morphological traits under study suggesting that all the characters were controlled by additive gene action. The characters such as panicle length, number of tillers, number of productive tillers, number of grains per panicle, seed length, seed breadth and seed weight exhibited positive and significant associations with grain yield per plant and hence selection pressure could be applied for such traits to improve yield under saline condition. The morphological observation of genotypes subjected to cluster analysis for finding the similarities among the genotypes delineated that the genotypes viz., AC39040, PS

336, and Paiyur-1 clustered along with the resistant check Pokkali. The results of in vitro phenotypic screening of rice at the seedling stage undertaken under hydroponics at two electrical conductivity levels (6 dsm^{-1} and 12 dsm^{-1}) based on the scores of the modified standard evaluation system unveiled that the genotypes viz., PS -336, Paiyur-1, AC-39014 and AC39040 were highly tolerant. For studying molecular diversity, 35 SSR markers were utilized among which 26 of them were polymorphic and thus utilized to discover the diversity of various genotypes. The number of alleles ranged from 2 to 8 with an average of 4.26 alleles per locus, indicative of the population's richness. The marker RM 6283 reported the maximum number of eight alleles. The polymorphic information content (PIC) value of the markers ranged from 0.314 (RM 13) to 0.824 (RM 6283) with an average of 0.633 and 22 markers explained a PIC value of above 0.5, designating that the markers used in this study were highly informative for genetic diversity studies. The expected heterozygosity among the SSR markers was 0.690, reflecting the heterozygous nature of the population. The cluster analysis based on the molecular data's dissimilarity coefficients divided the total genotypes into two groups, one featuring genotypes alongside resistant checks and the other comprising genotypes in conjunction with susceptible checks. Thus combining the results of in vitro screening, morphological and molecular diversity, the genotypes viz., PS -336, Paiyur-1 and AC-39014 could be considered as potential saline tolerant genotypes and thus may be further explored to find the novel QTLs for saline tolerance.

Rice Tonoplast Intrinsic Protein Member OsTIP1;2 Confers Tolerance to Arsenite Stress and Reduce Root-to-Shoot Translocation of Arsenic

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Keywords: Tonoplast intrinsic proteins; Rice; Aquaporins; Arsenic; Translocation factor

Aquaporins (AQPs) are involved in the transport of various solutes and metals/metalloids, including arsenic. Arsenic is a group I carcinogen and also significantly reduces crop yield. Arsenic accumulation in the rice grain may cause health hazards to the consumers. The role of TIP AQPs in the arsenic stress response is largely undefined. We cloned five *TIP* genes from the IR64 rice and did functional characterization of *OsTIP1;2* under the As[III] stress. The *OsTIP1;2* expression was enhanced in roots upon As[III] treatment. The expression of OsTIP1;2 in *S. cerevisiae Δycf1* mutant complemented it's As[III] transport function. The *ycf1* mutant expressing OsTIP1;2 accumulated more arsenic than wild type (W303-1A) and *ycf1* mutant yeast carrying pYES2.1 vector. OsTIP1;2 activity was partially inhibited in the presence of the aquaporin inhibitors. The subcellular localization studies confirmed that OsTIP1;2 is localized to the tonoplast. The overexpression of OsTIP1;2 in PB1 rice resulted in enhanced shoot and root growth accompanied by increased antioxidant activity, suggesting a potential role in mitigating oxidative stress induced by As[III]. Moreover, seed priming with *ycf1* mutant expressing *OsTIP1;2* further increased As[III] stress tolerance. The tissue-specific localization showed *OsTIP1;2* promotor PRTIP1;2 activity in root and root hair, indicating its possible root-specific function. The arsenic translocation factor (TF) for WT was around 0.8, while that of OE lines was around 0.2. Notably, the reduced TF in OE lines implies lower arsenic transport to the shoots, indicating the potential of OsTIP1;2 to reduce arsenic content in rice grains and minimize associated health risks.

CHARACTERIZATION OF RICE TRANSGENICS FOR COMBINED DROUGHT AND HEAT STRESS RESPONSE

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Keywords: Rice; Drought; Heat; Combined stress; IR64 transgenics.

In the present climate change scenario, plants are frequently exposed to drought and heat stresses, which frequently co-occur under field conditions. Among field crops, rice is extremely sensitive to drought, heat and their combination, particularly at reproductive stage. Hence, identification and characterization of genes involved in combined drought and heat stress tolerance is critical towards sustaining the rice yields under changing climate. Meta-analysis of public transcriptome datasets from individual stresses in rice has identified 36 genes common to both drought and heat stress. Revalidation of their expression under individual and combined stress conditions in AVT2-5315 rice genotype and based on preliminary studies using *Arabidopsis* knockout mutants, four genes were selected and overexpressed in the background of one of the leading varieties, IR64. These genes are *Hypothetical protein* (*HP1), Chlorophyll a/b binding protein domain containing protein (Chla/b), Copper Chaperone homolog (CCH)* and *Small GTP-binding protein* (*Rab11).* The stress response of transgenic plants was studied under individual as well as combined stress at seedling, vegetative and reproductive stages. Results showed that *HP1* and *CCH* are positive and negative regulators of individual and combined stress responses, respectively. The *CCH* overexpressing plants showed 91.1-85.9% spikelet sterility which corroborated with 83.8-86.6% reduction in yield. On the other hand, the *HP1* overexpressing plants showed lower spikelet sterility with enhanced yield compared to wild-type plants. Further, empirical studies will help in understanding the mechanisms of altered stress response in these transgenic plants and their use as potential genes towards improving combined drought and heat stress tolerance of rice.

INVESTIGATING THE CONNECTION BETWEEN LEAF WAX CONTENT AND CANOPY TEMPERATURE AS INDICATORS OF WATER USE EFFICIENCY

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Keywords: Canopy temperature; Drought stress; Infrared thermography; Leaf wax content; Mini-lysimeter; Rice; Water use efficiency.

The efficient utilization of water resources in rice production is of paramount importance to address the challenges posed by increasing global water scarcity and climate change. Water use efficiency (WUE) plays a pivotal role in determining their ability to thrive under varying environmental conditions. This research delves into the intricate interplay between leaf wax content, canopy temperature, and their collective impact on WUE. Leaf wax, primarily composed of hydrophobic lipids, acts as a protective barrier against water loss through transpiration. Canopy temperature, on the other hand, is a dynamic indicator of the physiological state of plants and reflects their water stress level. In this experiment, leaf wax content was quantified spectrophotometrically, while plant-level WUE was estimated through automated mini-lysimeters. Concurrently, canopy temperature was assessed using infrared thermography. The acquired data was subjected to rigorous statistical analyses to elucidate the correlation between leaf wax content, canopy temperature, and WUE across 200 rice germplasm lines. The promising trait donor lines for improving rice wax content in the elite variety were identified. The outcomes of this research experiment hold promise for sustainable rice production in the face of escalating water scarcity based on a holistic understanding of plant-water relationships. Overall, this investigation underscores the significance of considering leaf wax content as a potential determinant of plant response to water stress, opening avenues for novel approaches to enhance water use efficiency in rice eco-systems.

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

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Although it is one of the main source of calories for most of the world, nearly 60% of rice is grown in soils which is low in phosphorus especially in Asia and Africa. Given the limitations of bioavailable inorganic Phosphate (Pi) in soils, it is important to develop crops tolerant to low phosphate in order to boost food security. Due to the immobile nature of Pi, plants have developed complex molecular signalling pathways that allows them to discern changes of Pi concentrations in the environment and adapt their growth and development. 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 signalling 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 hyperSUMOylation 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.

NON INTRINSIC ATP BINDING CASSETTE (ABC) PROTEIN INVOLVED IN RIBOSOME FUNCTIONS IN RICE

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Keywords: Ribosome; ABC proteins; Drought; Translation; Gene editing

ATP binding cassette proteins play important role in growth, development and transport activities in multicellular organisms. Many ABC proteins have transmembrane domains, however, in rice - 20 ABC proteins lacking transmembrane domain involved in biogenesis of cytochrome b6/f complex, ferredoxin and photosystem I, transport of phosphatidic acid (PA) from ER to chloroplast and Aluminium stress tolerance etc. An AtABCI20 in Arabidopsis found to be cytokinin induced and overexpression plants showed improved root growth. AtABCI20 found in endoplasmic reticulum and a multiprotein component. We report OsABCI15 an homolog of AtABCI20 interacting with OsRPL10 an ribosomal protein. Rice having five different RPL10s which play important role in growth and development. RPL10 involved in 60S large subunit biogenesis, export and assembly of both subunits of ribosomes. OsABCI15 interacts with different copies of RPL10 in yeast two hybrid assays. OsABCI15 found to localize ER and the interactions with RPL10s were found in Nucleus as well as ER. The CRISPR -Cas9 targeted OsABCI15 mutants showed altered growth, development and photobleaching phenotype. The role of OsABCI15 in ribosome translation mechanism are being explored.

UNRAVELING THE DYNAMIC INTERACTIONS BETWEEN PHOSPHORUS TOLERANCE AND NITROGEN USE EFFICIENCY IN RICE THROUGH PHYSIOLOGICAL RESPONSES

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Keywords: Nitrogen Use Efficiency (NUE), Phosphorus Tolerance, Genotypes, Nutrient Conditions, Physiological response

Effective nutrient management in rice is essential for sustainable crop production, with a critical focus on understanding the interplay between nitrogen use efficiency (NUE) and low phosphorus (P) tolerance. In rice, many genes related to nitrogen (N) and P sensing, signaling, uptake, assimilation, regulation, and remobilization, including epigenetic regulation, have been identified and functionally confirmed. The *PUP1* (Phosphorous Uptake-1) locus has been associated with the ability of rice plants to withstand low-phosphorus soils. To explore interactions between NUE and low P tolerance across six rice genotypes: IET31135, Swarna, Rasi, Varadhan and IET30240 along with Improved Samba Mahsuri (ISM) as susceptible check for low N and P. 15-day-old seedlings of these genotypes were exposed to four nutrient conditions viz., Yoshida media with full-strength nutrients (control), low N (LN), low P (LP), and low N and P (LN & LP) under hydroponics. After 21-days of treatment, in terms of dry root weight and shoot length, it was observed that Rasi genotype performed well under low N, low N and P as well as under control condition and Varadhan showed best performance under low P. Rasi turned out to be the best performer for the dry shoot weight in all 4 treatments. Improved Samba Mahsuri has the highest root length than all other genotypes. From WinRHIZO analysis, IET31135 has shown highest root surface area, root length and root length per volume in low N conditions. In conclusion, Rasi and IET31135 have good performance and selected the best donors for nitrogen and phosphorous stress.

MODULATING LEVEL OF RCAB ENHANCES ABIOTIC STRESS TOLERANCE AND PHOTOSYNTHETIC EFFICIENCY IN TRANSGENIC RICE

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Global climate change, specifically high temperature and drought stress, possesses detrimental threat to global croup productivity and food security. Ribulose-1, 5-bisphosphate carboxylase/oxygenase activase (RCA) is a nuclear gene encoding chloroplast protein catalyzing the first step of both CO_2 assimilation and photorespiratory pathway. Rubisco activase (RCA) catalyses the activation of Rubisco, thereby maintaining photosynthesis under non-stress and stress environment. Upon exposure to severe stresses, inactivation of the enzyme Rubisco activase (RCA) impairs photosynthesis. Present study aims to analyse the role of RCA gene in response to abiotic stresses and to improve the photosynthetic efficiency of plants. In this realm, a thermal stable RCAβ ortholog from *O. australiensis* was cloned under the transcriptional control of stress inducible *AtRD29A* promoter. Agrobacterium mediated genetic transformation was used to develop RCA overexpressing transgenic rice cv. MTU1010. Putative transgenic lines were confirmed through T-DNA specific primer combinations and southern hybridization exhibited higher transcript abundance of RCA gene in overexpression lines compared to non-transformed WT plants. Photosynthetic rate at different photosynthetic photon flux density and internal carbon dioxide concentrations were higher in P_{RD29A}*::RCAβ* overexpressing transgenic plants as compared with WT plants. Further, the higher photosynthetic rate also resulted in production of higher biomass and yield attributes in P_{RD29A}*::RCAβ* overexpressing transgenic plants as compared with WT plants. Thus, transgenic lines overexpressing P_{RD29A}*::OaRCAβ* showed enhanced tolerance to high temperature and drought stress through maintenance of photosynthetic cellular machinery. The results suggest that RCA genes play an important role in development and environmental responses. These results provide a basis for modulating RCA gene expression to improve the photosynthetic rate and plant growth in rice.

ALLEVIATION OF SALT STRESS IN RICE IR64 BY THE ENDOPHYTE *FUSARIUM* SP. ISOLATED FROM THE COLD DESERTS OF HIMALAYA

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Keywords: rice (*Oryza sativa* L.), salt stress, endophyte

Rice stands as one of the world's most vital staple crops, yet its production is frequently hindered by soil salinity, particularly in regions characterized by high salinity levels. Fungal endophytes, known for their stress tolerance, offer an alternative route to enhance salt tolerance in this critical crop. We demonstrate that an endophyte isolated from the cold deserts of Himalaya, a *Fusarium* sp. colonizes and enhances the growth of salt-sensitive rice IR64 under salt stress conditions. During the initial establishment of rice seedlings, endophyte-treated seedlings maintained a significantly higher shoot and root growth under both normal and salt stress conditions (150 mM NaCl). In the pot experiment, endophyte inoculation led to a significant improvement in the growth attributes of rice, including root and shoot length, total biomass, photosynthetic pigments and relative water content (RWC), under both normal and stressed conditions (6 dS m^{-1} salt solution). The biochemical analysis revealed that *Fusarium* sp. considerably increased proline content, and modulated ion uptake, particularly reducing sodium (Na^+) while increasing potassium (K^+) uptake. Furthermore, a significant reduction of the lipid peroxidation products malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) by *Fusarium* sp. under salt stress was observed, validating the effectiveness of endophyte in enhancing rice growth under salt stress conditions. Collectively, these findings emphasize the Himalayan region's potential as a rich source for discovering novel endophytes capable of imparting salinity tolerance to agriculturally significant yet salt-sensitive rice varieties.

HEAT STRESS-INDUCED REGULATORY NETWORKS IN RICE SEEDLINGS

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Rice is a crucial staple food crop that provides sustenance for more than two thirds of the global population. However, the recent change in climate conditions and associated steady rise in temperature is posing a significant threat to rice productivity. To address this issue, several studies have contributed to our understanding of the plant heat stress response mechanism. Nevertheless, there is limited knowledge regarding the transcriptional network that governs the trade-off between growth and stress response under heat stress at the seedling stage. In this study, we analyzed the impact of heat stress on the growth and survival of rice at the morphological, biochemical, and molecular level. Transcriptome analysis of the shoot and root under heat stress revealed gene regulatory networks that are involved in the heat stress response. We observed that the heat stress had a considerable impact on the growth and survival of the rice plants. Our results showed that there is a hub regulatory network containing genes that play a crucial role in the regulation of plant growth and development. These genes may act as potential candidates for modulating the growth-defence trade-off in heat stress in rice. In conclusion, our study provides insights into the transcriptional network that governs the trade-off between growth and stress response under heat stress in rice. The identified genes and pathways may represent potential targets for enhancing the resilience of rice plants to heat stress. These findings have significant implications for ensuring food security in the face of the ongoing climate change.

Adaptive Rice Cultivation for a Changing Climate (Ad-RiCCE): An Overview

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Keywords: Crop modelling, Genomics, Phenotyping, Rice, Social Sciences

Rice is an intense user of water and highly susceptible to the adverse effects of drought and heat stress. This research programme aims to sustain rice productivity while conserving scarce irrigation water. The programme will develop and use integrative research methods that include accurate phenotyping, molecular characterization and crop modelling as the basis of robust scaling from cell to canopy. The overarching aim is to provide insight into genotype × environment interactions as part of climate-proofing our agricultural practices in the face of future droughts and heat waves. We employ a cutting-edge phenomics platform to accurately mimic field conditions to determine whole-plant drought adaptive traits for diverse genotypes under comparable soil water status. The programme will leverage the data produced by this platform in whole-genome SNP sequencing and for Genome-Wide Association Studies (GWAS). Combined with metabolomics and transcriptomic profiling, this programme will ultimately lead to the identification of QTLs related to drought and heat tolerance traits that can be used in breeding programs. Crop modelling will be used throughout the project to guide the selection of target traits and will play a pivotal role in designing ideotypes for future climates that would be predominantly resource limited by testing trait influence on yield under different environments. In addition to these scientific aspects, the program recognizes the importance of social science integration that will play a pivotal role in providing baseline estimates of available resources for rice cultivation and understanding societal attitudes towards technology adoption. This will generate valuable feedback for water management and rice crop improvement processes. In conclusion, this programme employs an integrated approach encompassing advanced technology, genomics, crop modelling and social sciences to develop climate-smart solutions for rice cultivation.

Climate Resilience: Biotic Stresses

MOLECULAR DIVERSITY AND DNA BARCODING OF RICE YELLOW STEM BORER, *SCIRPOPHAGA INCERTULAS* IN COASTAL ANDHRA PRADESH, INDIA

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Keywords: Yellow Stem borer, Haplotype diversity, Nucleotide diversity, Phylogenetic analysis, DnaSP, Genetic diversity, Mt COI, Oryza sativa

Yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) is the most predominant and economically important pest of rice ecosystem in Andhra Pradesh. This pest attacks rice crop at all stages from Nursey to harvest, symptoms manifest as dead hearts and white ears and directly results in crop losses. The study of genetic variability of pest populations enables us to interpret the ecological investigations correctly and also enables us to understand the pest behaviour in relation to dissimilar response of pest management tactics. The present study was undertaken to evaluate the genetic diversity in YSB populations collected from 4 districts of Andhra Pradesh. Molecular characterisation studies were carried out using Mt CO1 sequences and fifteen YSB sequences were submitted to NCBI and BOLD databases. DNA barcodes were generated using BOLD systems. Six Indian YSB sequences (Odisha, Jharkhand and Telangana) and Fourteen sequences of different south Asian countries like Indonesia, Thailand, Philippines, China and Pakistan mined from NCBI were used for these studies. Haplotype analysis of 35 YSB sequences resulted in twenty haplotypes out of which six haplotypes were found in Andhra Pradesh. The YSB populations with low nucleotide diversity (π =0.01917) and high haplotype diversity (Hd = 0.92437) indicating demographic expansion following a population bottleneck and retention of new mutations (Grant and Bowen, 1998). Neutrality tests (Fu & Li's D*, F* and Tajima's D) returned significant negative values -3.39969 (P<0.02), -3.50282 (P<0.02), -4.588 and -2.08400 (P <0.05) for populations of AP and India suggesting the possibility of expansion of *S. incertulas* whereas populations outside

India were nonsignificant. Phylogenetic analysis of *S. incertulas* sequences revealed that the grouping of Indian populations together with Pakistan and Indonesia in a single clad and were distinctly separated from YSB populations of China, Malaysia, Philippines and Thailand and out grouped with *Scirpophaga innotata*. This is the first time the DNA barcodes were generated for *S. incertulas* populations of Andhra Pradesh and the attempt was made to understand the haplotype diversity of YSB populations.

OsBGAL9, A B-GALACTOSIDASE GENE RESPONSE TO STRESS CONDITIONS UNDER THE CONTROL OF OsSPL7 IN RICE

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β-Galactosidases (Bgals) are found in bacteria, fungi, animals, and plants with diverse functions. Despite numerous investigations on the evolutionary aspects of BGALs in plants, their functions remain unclear. In this study, we identified a specific rice (*Oryza sativa*) *β-galactosidase*, known as *OsBGAL9*, which is directly regulated by the heat stress-induced transcription factor SPOTTED-LEAF7 (OsSPL7). This was demonstrated through various experimental techniques such as protoplast transactivation analysis, yeast one-hybrid assays, and electrophoretic mobility shift assays. Knockout plants lacking *OsBGAL9 (Osbgal9)* exhibited stunted growth and retardation. Utilizing histochemical ß-glucuronidase (GUS) analysis of transgenic lines containing an OsBGAL9pro:GUS reporter construct, we observed that *OsBGAL9* is predominantly expressed in mature internodes. Under normal conditions, *OsBGAL9* expression was barely detectable in seedlings; however, it increased in response to biotic and abiotic stresses. Ectopic expression of *OsBGAL9* conferred enhanced resistance to the rice pathogens *Magnaporthe oryzae* and *Xanthomonas oryzae pv. oryzae*, as well as improved tolerance to cold and heat stress. Conversely, mutant plants lacking OsBGAL9 displayed opposite phenotypes. Furthermore, OsBGAL9 was found to localize to the cell wall, indicating that OsBGAL9 and its putative orthologs in plants have likely evolved distinct functions compared to their closely related animal counterparts. Enzyme activity assays and analysis of cell wall composition in *OsBGAL9* overexpression and mutant plants revealed that OsBGAL9 specifically acts on galactose residues of arabinogalactan proteins (AGPs). Our study provides clear evidence for the involvement of a BGAL member in AGP processing during plant development and responses to stress.

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PIMT PROTEIN IMPARTS SHEATH BLIGHT DISEASE TOLERANCE IN RICE

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Proteins accumulate spontaneous damage due to oxidations or covalent modifications, which often leads to the loss of protein functions and thereby affects several intracellular pathways. Protein repair system comprises protein repairing enzymes (PREs), and one such repair enzyme PROTEIN L-ISOASPARTYL METHYLTRANSFERASES (PIMT) reversibly repairs the damaged proteins by conversion of L-isoaspartyl residues to normal L-aspartyl residues. Previous reports from our lab have shown that PIMT reduces the age induced isoAsp mediated damage in protein and helps in preserving seed vigor and longevity in plant species. PIMT plays a key role in ROS homeostasis by repairing antioxidative enzymes from isoAsp mediated damage under abiotic stress conditions. Apart from abiotic stress, ROS correlates with successful disease resistance response in plants. Therefore, possible role of PIMT mediated ROS homeostasis during biotic stress in plants is speculated. In present study, we investigate the role of PIMT in tolerance against rice sheath blight disease caused by *Rhizoctonia solani*. We have shown that upon infection of *Rhizoctonia solani* in rice, increased ROS accumulation was observed, which causes detrimental isoAsp accumulation in proteins. Further, to reduce such isoAsp accumulation, PIMT activity was also increased upon *R. solani* infection. Subsequently, to establish the role of PIMT in imparting sheath blight disease tolerance, overexpression and CRISPR/Cas9 mediated genome edited lines for OsPIMTs were generated. Constitutively overexpressed *OsPIMT1* and *OsPIMT2* transgenic lines exhibited increased tolerance to *R. solani* infection while genome-edited plants of OsPIMTs lines were more susceptible compared to WT plants. Further analyses revealed that such increased tolerances to R. *solani* infection of these overexpressed transgenic lines were associated with reduced ROS and isoAsp accumulation, while susceptibility to *R. solani* infection of genome edited lines were associated with reduced PIMT activity and increased ROS and isoAsp accumulation. Overall. our study provides evidence regarding the role of PIMT in imparting sheath blight disease tolerance in rice.

INDUCTION OF RICE IMMUNITY BY CELL WALL DAMAGE IS DIFFERENTIALLY REGULATED AT TRANSCRIPT AND PROTEIN LEVEL

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Xantho*monas oryzae* pv. *oryzae* (*Xoo*), like many phytopathogenic bacteria, secrete different cell wall degrading enzymes (CWDEs) into the plant milieu via their Type 2 secretion system. These CWDEs are known benefactors of virulence. The pathogens deploy these enzymes to sever different plant cell wall components. However, plants have evolved the ability to sense this cell wall damage by sensing cell wall degradation products called damage-associated molecular patterns (DAMPs). This recognition leads to a potent plant innate immune response. LipA, a lipase/esterase, is one such *Xoo*-secreted CWDE and is an important virulence factor of *Xoo*. Treatment of plant tissue with LipA leads to activation of plant immune responses. However, presence of exiguous information on the Pattern-/DAMP-triggered immunity (PTI/DTI) propelled us to probe further into the actions of LipA on rice leaves. In order to understand how LipA induces rice immune responses we have used proteomics as well as transcriptomics approaches to identify differentially expressed proteins (DEPs) and genes (DEGs) in rice following LipA treatment after 16 hours post infiltration (hpi). Our analyses yielded 266 upregulated and 128 downregulated DEPs; and 192 upregulated and 91 downregulated DEGs. Enrichment analysis revealed a significant upregulation in the signaling cascade-related genes and primary metabolism-related proteins. Interestingly, we did not observe any correlation between the DEPs and DEGs or the pathways altered at the protein or transcript level. This possibly indicates a distinct functional bifurcation amidst cellular transcription and translation processes upon induction of DTI in rice. Future studies directed toward time-resolved proteomics, transcriptomics, and active translation analysis could shed light on the transcriptional and translational regulation of LipA-induced immunity in rice.

DECIPHERING SHEATH BLIGHT TOLERANCE MECHANISMS IN A RICE MUTANT USING TRANSCRIPTOMICS APPROACH

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Sheath Blight (ShB) is one of the devastating fungal diseases of rice, causing yield loss of up to 50% in favorable condition. The disease is caused by a necrotrophic pathogen *Rhizoctonia solani* AG1-1A (*R. solani*). A major disadvantage associated with ShB is that there is no source of complete resistance in the rice germplasm that can be used as a donor for breeding, because of which it becomes difficult to tackle ShB. So far only few genes have been identified which upon overexpression provides tolerance to ShB. Hence, it is important to develop a variety which can be used for breeding to overcome this disease. We have developed an EMS mutagenized population in the background of the elite rice variety Samba Mahsuri (SM) and identified a mutant rice line (Ti87) that shows enhanced tolerance to ShB. To understand the mechanisms of tolerance in Ti87 we have performed RNA sequencing analyses in treated and untreated conditions of SM and Ti87. The analyses revealed the upregulation of known resistance genes for ShB upon infection in Ti87. We also observed the upregulation of specific defense related pathways in the mutant. The study revealed some interesting genes/pathways to be common but contrastingly regulated between SM and Ti87 which will give insight into the resistance/susceptibility factors associated with ShB. The understanding about the mechanisms of ShB disease tolerance obtained from this study and future investigations will aid in the generation of ShB tolerant lines for breeding purpose.

TEMPORAL TRANSCRIPTOMIC ANALYSIS REVEALS NOVEL CANDIDATES FOR RICE SUSCEPTIBILITY TO *XANTHOMONAS ORYZAE* PV. *ORYZAE*

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Plant-pathogen interaction is governed by multi-layered functions from both the host and the pathogen side. Plants possess an active basal immunity as well as pathogen-induced immunity that works to keep pathogens at bay. On the other hand, pathogens use various virulence factors for their pathogenesis. For successful pathogenicity, pathogens rely on manipulation of various host susceptibility factors. Here, we hypothesize that the expression of such susceptibility factors will be dynamically regulated in a spatio-temporal manner. To address this, we performed a temporal profiling of rice transcriptome upon infection with the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (Xoo). Our results indicate a progressive change in the expression levels of various known susceptibility genes. Additionally, a clustering analysis reveals similar changes in the expression pattern of various genes and pathways that are hitherto unreported to play role in rice-Xoo interaction. We further aim to validate the roles of the candidate genes in rice susceptibility to Xoo using a multiplexed CRISPR-Cas9 genome editing approach.

LANDRACES OF RICE (*ORYZA SATIVA* . L) REVEAL TRAITS POINTING TOWARDS OSMOTIC STRESS TOLERANCE ON SCREENING AT SEEDLING STAGE.

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As a water loving crop the availability of water is a major constraint for rice cultivation making drought a major abiotic stress for rice. About 75% of the total area under rice cultivation depends on monsoons for its survival. Keeping in mind the recent climate shifts and global warming there is a necessity for developing drought tolerant and resilient lines. Landraces, the bridge between the wild rice and present-day high yielding varieties were selected as the experimental material as they contain several biotic and abiotic resistant traits. The present investigation was carried out at the Department of Genetics and Plant Breeding, NAI (SHUATS), Prayagraj during *Kharif* 2023. Twenty-six landraces of rice were screened for osmotic stress tolerance *invitro* by using external osmotic stress induced by Polyethylene Glycol (PEG). Morphological traits like root length, shoot length and total seedling length, seedling vigour index, and germination percentage were recorded and statistically analysed. Among the 26 Landraces screened, three landraces showed traits for osmotic stress tolerance at seedling stage with high germination percentage, root length, shoot length, total seedling length and seed vigour index. A basic screening at seedling stage on the basis of these traits can help in selection of possible candidates for drought tolerance and for further detailed study. The different concentrations of PEG affected the morphological traits with significant variation. Based on the overall mean performance including all the concentrations of PEG stress the best performing landraces in terms of germination percentage are *Koshankari, Dular* and *Poongar*. In terms of root length high rate of root elongation was found in *Koshankar*i, *Black Rice* and *Poongar*. The highest rate of shoot elongation was observed in *Poongar, Albela* and *Chittimuthyalu Black*. In case of total seedling length, the highest performing landraces are *Poongar, Koshankari and Black Rice*. Coming to the seed vigour index the best performance was shown by *Poongar, Koshankari* and *Dular*. Some of the landraces like Sathi,

although were not the best performing, but they can be utilised for their traits like earliness combined by the moderate tolerance as seen in the present study. In conclusion, these landraces can be used for future breeding programmes. The use of external osmotic stress inducers like PEG for initial stages of screening can prove beneficial as it would greatly reduce cost and time.

Genomics & Epigenomics

UPSTREAM REGULATOR OF GENOMIC IMPRINTING IN RICE ENDOSPERM IS A SMALL RNA-ASSOCIATED CHROMATIN REMODELER

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Genomic imprinting is observed in endosperm, a placenta-like seed tissue, where transposable elements (TEs) and repeat-derived small(s)RNAs mediate epigenetic changes in plants. In imprinting, uniparental gene expression arises due to parent-specific epigenetic marks on one allele but not on the other. The importance of sRNAs and their regulation in endosperm development or in imprinting is poorly understood in crops. Here we show that a previously uncharacterized CLASSY (CLSY)-family chromatin remodeler named *OsCLSY3* is essential for rice endosperm development and imprinting, acting as an upstream player in sRNA pathway. Comparative transcriptome and genetic analysis indicated its endosperm-preferred expression and its paternally imprinted nature. These important features were modulated by RNA-directed DNA methylation (RdDM) of tandemly arranged TEs in its promoter. Upon perturbation of *OsCLSY3* in transgenic lines we observed defects in endosperm development and loss of around 70% of all sRNAs. Interestingly, well-conserved endosperm-specific sRNAs (siren) that are vital for reproductive fitness in angiosperms were dependent on *OsCLSY3*. We also observed many imprinted genes and seed development-associated genes under the control of CLSY3- dependent RdDM. These results support an essential role of *OsCLSY3* in rice endosperm development and imprinting, and propose similar regulatory strategies involving *CLSY3* homologs among other cereals.

CONCURRENT EFFECT OF DROUGHT AND HEAT STRESS ON RICE PLANT- A PHYSIO-BIOCHEMICAL AND MOLECULAR APPROACH

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Keywords: Global warming; Genotypes; Combined stress; Quantum yield; Spikelets sterility; Yield

Global warming and climate change are two major issues for drought and heat waves affect on growth, development and yield of rice plants. The present study investigated the morpho-physiological, biochemical and molecular responses of two rice genotypes: one improved well-known drought and heat-tolerant line (var. N22) and another one an upland drought-tolerant rice landrace (var. Noichi) from rarh Bengal of Eastern India under individual and combined drought and heat followed by their effects on yield attributes. The stresses were imposed before the onset of panicle initiation for 5 days. It was shown that drought, heat and their combined effect cause oxidative damage through the over-production of ROS (like H_2O_2) and enhanced malondialdehyde contents, which leads to reduced chlorophyll content and photosynthetic efficiencies like quantum yield (Fv/Fm). The antagonistic stomatal activity was found under the combined stress conditions, stomata were open under control and heat stress conditions, conversely, stomata remained closed under drought and combined drought heat stress conditions. Pollen viability was lost under all stress levels but severe loss was found under combined stress, which resulted in spikelet sterility and thus caused yield losses for both the studied genotypes. Different drought and heat-responsive genes like, *DREB*, *LEA3*, *WRKY* and *HSP* showed unique expression under the combined stress which is totally different from their individual. In conclusion, the concurrent occurrence of drought and heat stress was more severe for rice for growth and yield parameters than the single effect.

INTERPLAY OF COPPER-RESPONSIVE MIRNAS, MIR408 AND MIR528 IN DROUGHT STRESS RESPONSE OF CONTRASTING RICE CULTIVARS

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miRNAs are a critical component of regulatory mechanisms involved in plant growth, development, and stress response including phytohormone action. Comparative miRNome and transcriptome analysis identified a group of copper responsive miRNAs "Cultivar-specific drought responsive" (CSDR)-miRNAs (osa-miR159f, osa-miR1871, osa-miR398b, osa-miR408-3p, osa-miR2878-5p, osa-miR528-5p and osa-miR397a) exhibiting upregulation in drought tolerant cultivars and downregulation in sensitive ones in response to drought. Most prominently, *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE-9* (OsSPL9) regulated miR408 and miR528 collectively downregulate transcripts of different families of copper requiring proteins (Plantacyanins, laccases and Copper/Zinc superoxide dismutases) along with several other genes in tissue-mediated drought responsive manner. The above response is governed by the differential copper accumulation in contrasting cultivars due to the drought-mediated downregulation of copper transporters in tolerant cultivars. Functional characterization of conserved miR408 and monocot specific miR528 in rice delineated the interplay of these two in rice growth, development, yield and stress response via targeting canonical to rice-specific target pool involved in different pathways in addition to copper homeostasis and phytohormone signalling. miR408 lead massive enhancement of vegetative growth in rice with improved ETR and Y(II) and enhanced dehydration stress tolerance presenting it as an potential candidate for engineering drought tolerance in rice. While miR528 preferentially targets *Dwarf3* (OsD3), which is a critical component of the rice SL signalling pathway as resulted from the comparative analysis of miRNA-transcriptome in different tissues under drought and miR528 overexpression. miR528 overexpression plants exhibited similar traits as reported for *Osd3* mutants including high tiller number, short plant height, early heading, root architecture under low nitrogen conditions, and moderate insensitivity to exogenous GR24 levels. Furthermore, SL (GR24) negatively impacts the miR528 transcription. The impact of miR528:D3 module is also reflected in the D53-mediated downstream signalling involving Ideal Plant Architecture 1 (IPA1) regulon. Here we report

that miR528 impacts the SL signalling via the post-transcriptional regulation of OsD3. We suggest that manipulating the expression of miR408 and miR528 could be a useful strategy for improving rice productivity and stress adaptation.

Impact and future prospects:

The above research presents a complex example of how drought tolerant rice cultivars cope with drought conditions by regulating intricate stress-responsive molecular networks. One of the key mechanisms involves the transcriptional deregulation of copper transporters, which creates a low copper pool in response to drought. This triggers the activation of the copper-master regulator, OsSPL9, which switches on the transcription of copper-responsive miRNAs, namely the evolutionarily conserved miR408 and the monocot specific miR528. These miRNAs then mediate the transcriptional downregulation of several copper-dependent genes, such as PLANTACYANINS, laccases, and Cu-Zn SODs, to save copper for the photosynthetic electron transport chain via PLASTOCYANIN and to increase the ROS levels to induce stomatal closure. In addition to these shared targets, miR408 and miR528 also have several unique high confidence targets. For instance, overexpression of miR408 in a sensitive background enhances vegetative vigour and drought response. On the other hand, miR528 regulates rice-specific OsD3 expression, which affects SL-signalling by modulating the D53/IPA1 regulon. The above research reveals a new regulatory module that integrates copper and SL signalling in rice, mediated by miR408 and miR528. These miRNAs have been implicated in copper homeostasis, drought response and plant development. What makes this module interesting is that miR528 is a monocot-specific and variety-specific regulator of SL signalling under drought conditions. The evolutionary significance and functional implications of such a specific regulation need to be further explored. In future, the new high confidence targets identified in this study can be manipulated to uncover the hidden roles of these miRNAs. Moreover, the results of this study suggest that these miRNAs can be promising candidates for engineering drought tolerant rice with high yield and biomass.

ENGINEERING BLAST DISEASE RESISTANCE IN RICE (*ORYZA SATIVA* L.) BY GENE EDITING OF DISEASE SUSCEPTIBILITY GENES

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Keywords: *Magnaporthe oryza,* rice, resistance, susceptibility, tolerant variety

Rice cultivation is influenced by both biotic and abiotic factors. Among the 70 different types of rice diseases reported, blast disease by *Magnaporthe oryzae*, a hemi-biotrophic filamentous ascomycete fungus causes the most severe constraints on rice production worldwide. The application of fungicides is no longer considered an efficient strategy as it causes environmental pollution and health hazards. Thus, an efficient strategy to control this devastating pathogen is to utilize the host's immune response or by manipulating the aspects that cause vulnerability of the host to the pathogen. Although resistance gene-mediated strategies are significant, large-scale applications of resistance genes are limited because of the risk of losing resistance due to the rapid evolution of *M. oryzae*. Thus, the best strategy to develop tolerant rice varieties is to modify susceptibility genes that facilitate infection. We performed a meta-analysis of 16 publicly available rice transcriptome datasets in response to Magnaporthe infection. Our analysis identified differentially expressed genes that are commonly regulated, irrespective of the host genotype or *Magnaporthe* strain. We prioritized a few genes from our RT-qPCR validations based on their known functions in plants. Some of the candidate genes are suspected to resist fungal infection, while others are found to help the fungus sustain and multiply in the system. Proteins encoded by some candidate genes have effector binding sites that can interact with effectors secreted by *M. oryzae*. Further, we wish to validate this hypothesis using experimental methods at the protein level. These candidates will be used for the functional characterization by gene editing and development of rice genotypes tolerant to blast disease.

CRISPR/Cas12a multiplex genome editing of popular indica rice cultivar Samba Mahsuri for yield improvement

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Keywords - Samba Mahsuri; CRISPR/Cas12a; OsCKX2; Yield

Samba Mahsuri (BPT5204), an elite mega-rice cultivar of 145-150 days duration, has expanded to several states of India occupying more than 4 million-hectare area under cultivation, and is considered as one of the 'mega' rice varieties. Despite its moderate yield potential of ~5.0-6.0 tons/ha, the cultivar is preferred by farmers and consumers due to its premium grain quality and superior market price. In this study, we utilized CRISPR/Cas technology to edit the rice cytokinin oxidase (*OsCKX2*) gene to increase the yield of Samba Mahsuri and improve culm strength. Guide RNAs for two different exons of *OsCKX2* were designed and a multiplex CRISPR/Cas12a construct was developed to produce guide RNAs and Cas protein. Forty transformed lines showing clear presence of Cas12a, hygromycin phosphotransferase, and CaMV35S promoter sequences were obtained. The sequencing of forty lines showed edits in target region with a range of 1bp-77bp deletion. The editing efficiency was 100% with 98% biallelic mutations. All the lines showed heterozygous mutations in T0 generation. The genome edited lines in T0 showed 200-496 grains/panicle in comparison to 150 grain/panicle in wild type Samba Mahsuri under biosafety glasshouse conditions. In addition, the genome edited lines showed stronger culm. The seeds from all the T0 lines were collected and grown in biosafety screenhouse for further evaluation.

RAPID MAPPING OF QUANTITATIVE TRAIT LOCI FOR GRAIN NUMBER EMPLOYING NGS-BASED WHOLE GENOME RESEQUENCING IN RICE (*ORYZA SATIVA* L.)

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Keywords: QTL Seq, grain number, bulked segregant analysis, SNP, NGS, grain yield

The grain number per panicle is a key yield attributing trait in rice. Elucidation of the inheritance of grain number is of paramount importance for raising the yield thresholds in rice. Grain number per panicle is highly variable and depends on the panicle architecture including the number of primary and secondary branches, panicle length, and percentage of filled grains. Multiple genes are involved in regulating the inheritance of the grain number trait that shows continuous variation in the segregating populations. To identify quantitative trait loci associated with grain number, we employed Next-Generation Sequencing based QTL-Seq approach in the 612 $F_{2:3}$ population derived from the cross between 'DRR Dhan 48' and 'Moudamani'. 'DRR Dhan 48' is a biofortified elite fine grain medium slender grain type cultivar with high zinc (22 ppm in polished rice) and low glycemic index (51.1). It has resistance to bacterial blight with the incorporation of *xa5, xa13* and *Xa21* in the background of improved samba Mahsuri. 'Moudamani' is a high yielding cultivar with high grain number and short bold grain type. Analysis of variance revealed highly significant variation for the studied traits. High genotypic coefficient of variation, broad sense heritability and genetic advance were observed for the primary rachis, secondary rachis, grain number on primary rachis, grain number on secondary rachis and total grain number. Grain number in F_2 population ranged from 29 to 333 grain with a mean of 162.72 whereas in F_3, its varied from 49 to 368 with a mean of 184.17. Extreme phenotypic values for grain number in both F_2 and F_3 generations indicated the presence of transgressive segregation. Correlation analysis revealed significant correlation among the studied traits. QTL-Seq which combines bulked segregant analysis and whole genome

resequencing is one of the rapid approaches to uncover novel QTLs controlling complex traits. 10 Transgressive segregants each on either side of the phenotypic extremes were selected for constituting high and low bulks. We performed whole genome resequencing of parents and bulks and the short reads obtained were aligned on the reference genome *Oryza sativa* japonica IRGSP1.0 for variant calling. A total of 22,25,532 variants including 18,33,285 SNPs and 3,92,27 indels obtained were used in the calculation of SNP-index of bulks and ΔSNP index between the bulks. Graphical representation of the relationships between SNP-index and SNP position in the genome and lines depicting their mean in SNP index plots and ΔSNP index plots revealed the genomic regions harbouring QTLs that contributed to the difference in the grain number between the two bulks. SNP-indices of these regions for 'Highest' and 'Lowest' bulks appeared as mirror images with respect to the line of SNP-index = 0.5. Genomic region on chromosome 7 between 24135761 to 24988611 bp was found associated with grain number. This candidate genomic region could be a potential target for map-based cloning and marker-assisted transfer to enhance the grain number in rice.

LONG NON-CODING RNAS: DIFFERENTIAL EXPRESSION IN THE ROOTS OF CONTRASTING GENOTYPES UNDER AEROBIC CONDITIONS

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Keywords: rice, long non-coding RNA, robust root system architecture, aerobic condition

The long non-coding RNAs (lncRNAs) are transcripts of > 200 bp nucleotides in length without any coding potential and play crucial roles by affecting various steps of gene expression at a particular time in a specific tissue. Roots have a critical function of nutrient uptake, especially under aerobic i.e. water limiting condition in rice. To understand the role of lncRNAs in roots under aerobic condition (maintained in polyhouse, grown in polythene bags), transcriptome analysis of roots in genotypes having robust root system architecture viz. TI-128 (LT), aerobic adapted CR Dhan 202 (LC), flooding adapted mega variety BPT-5204 (LB) at the panicle initiation stage (three biological replicates) was executed on the Illumina NovaSeq 6000 platform. Approximately 7 Gb cleaned data was obtained for each sample and aligned with the reference indica genome. The lncRNAs were searched with existing databases for known lncRNAs and also were predicted based on sequences for unknown novel lncRNAs. Differential expression of lncRNAs in roots of LB, LC, and LT emanated in a total of 89 and 129 up-regulated lncRNAs shared between LC_LB vs LT_LB and LT_LC vs LT_LB respectively. A total of 107 and 94 downregulated lncRNAs were shared between LC_LB vs LT_ LB and LT_LB vs LT_LC respectively. The consistent root lncRNAs upregulated in the robust root genotypes TI-128 and CR Dhan 202 viz. XLOC_049896, XLOC_051416, XLOC_054216, XLOC_075495 suggests their possible role in roots under aerobic condition. The potential lncRNAs need to be further characterized along their target genes for a complete understanding of their regulation.

RICE CROWN ROOT DEVELOPMENT: AN INTERPLAY BETWEEN REGULATORY FACTORS

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Rice (Oryza sativa) is a model organism recently being explored in the field of epigenetics. It develops post-embryonic adventitious roots known as, the shoot-borne crown roots (CR) to survive after the degeneration of the ephemeral embryonic seminal root. Gene expression profile from laser-capture micro-dissected crown root primordia revealed diverse families of transcription factors, the AP2/ERF, Homeodomain, ARFs and epigenetic factors from SDG, PHD, GNAT, JmjC families being involved at the preliminary stages of crown root development. Upon chemically interfering with epigenetic modifications, DNA methylation and histone de-acetylation, we observed altered root architecture showing changes in the CR numbers in both conditions. Furthermore, effect of these chemicals on the expression of transcription factors showed an alteration in expression profiles of some transcription factors indicating an early activation of certain epigenetic modifiers at the time of CR Development. Moreover, in our phospho-proteome analysis, it was observed that apart from the abundance of various proteins upon auxin induction, chromatin-associated histone H3 protein levels were high during crown root primordia development, suggesting a role of auxin signaling in regulating the dynamic chromatin conformational changes with DNA replication and gene expression required for cell cycle re-activation during crown root primordia establishment. Also, methylation marks on promoter regions of chosen transcription factors revealed a potential action of methyltransferases during CR development. Furthermore, in-vitro assay showed no possible interactions among these regulatory factors, In-planta needs to be checked. Taken together, our findings point towards a probable interplay between auxin-signaling, transcription factors and epigenetic factors at CR establishment.

Abbreviations: CR: Crown roots; ARFs: Auxin Responsive Factors; SDG: SET-domain group; PHD: Plant Homeodomain; GNAT: GCN5-related N-acetyltransferase; JmjC: JumonjiC domain containing proteins; AP2/ERF: APETALA2/Ethylene-responsive factor

$[Ca^{2+}]_{CYT}$ AND EPIGENETIC REGULATORS OF DROUGHT INDUCED miRNOME IN RICE

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miRNAs have been regarded as critical regulators of many vital processes in plants such as phase transition from juvenile to adult and subsequently flowering, flowering time, seed development and moreover various kinds of stress responses including biotic and abiotic. Hence it is imperative to study their own regulation under the developmental and environmental cues. Investigations into the same reveal that miRNA genes are under the influence of $[Ca^{2+}]_{cyt}$ signaling in rice. The influence appears to be on the transcription of miRNA genes since only a subset of miRNAs are regulated and not all. Furthermore, the response of certain miRNAs towards drought and ABA are also mediated via $[Ca^{2+}]_{cyt}$. Apart from the secondary messenger, we also identify epigenetic factors such as cytosine methylation and histone modification to occupy the miRNA precursors and promoter regions. The marks behave differentially under control and drought stress and might play a role in regulating their transcription. All these three regulators studied here appear to act in a variety specific manner since in contrasting varieties a different subset of miRNAs appear under the specific regulation. Hence the study sheds light upon some of the molecular mechanisms by which these master regulators are regulated by developmental and abiotic signals. It also shows that miRNA genes encoded by the genome and transcribed by RNAPol II do undergo similar regulatory pathways that are applied to protein-coding genes at the level of transcription.

THE COMPARATIVE ANALYSIS OF THE EXTANT ORYZA RIDLEYI GENOME AND ITS MINIMALLY INFERRED ANCESTRAL HH AND JJ SUBGENOMES

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Keywords: Oryza ridleyi, polyploid, Ancestral genome, Transposable elements

To address the challenge posed by global population increase under changing climate conditions, the utilization of rice and its wild relatives presents a promising solution due to their untapped genetic reservoir of adaptive traits. The wild rice O. ridleyi Hook (2n=48, HHJJ) has the largest genome among Oryza species, estimated at 1,283 Mbp, and exhibits resilience to both abiotic and biotic stressors, including resistance to blast and flooding.

We recently provided a near gap free high-quality Platinum Standard Reference Genome (PSRefSeq) for O. ridleyi, comprising 25 pseudo-molecule contigs with an N50 of 53.8 Mbp and only a single gap.

Notably, the HH subgenome (728.35 Mbp) is approximately 1.5 times larger than the JJ subgenome (474.45 Mbp). This disparity arises primarily by the differential accumulation of Transposable Element (TE)-related sequence, with 505.10 Mbp in HH and 284.87 Mbp in JJ. Further analysis reveals that TE accumulation, particularly LTR retroelements, rather than TE removal, drives subgenome expansion, with HH exhibiting a higher prevalence of TE nesting compared to JJ.

Gene annotation identifies 88,313 putative genes, among which 73% are retained between the homeologous subgenomes, while 16% and 11% are fractionated in HH and JJ subgenomes, respectively.

Using the software Cactus we reconstructed the ancestral genome of the HH and JJ subgenome donors. Albeit heavily fragmented, they show a remarkable completeness when genes are taken into account with BUSCO scores of 93.9% and 94.5% for HH and JJ respectively. The gene annotation reveals comparable gene numbers and lengths among ancestral genomes and progeny subgenomes.

Next, our investigation examines the conservation of TEs in both extant and ancestral subgenomes, exploring the potential impact of conserved fixed TEs on gene transcription. We pinpointed the ancestral TE remnants located less than 3 Kbp upstream of the closest ancestral gene in HHJJ genomes. These remnants have been characterized through gene ontology enrichment analysis. Currently, we are in the process of identifying potentially functional ancient TE remnants by evaluating the selective pressures acting upon them.

Furhermore retrocopied genes were investigated genome wide on the extant O. ridleyi genome: 25,358 protential events affecting 2,210 genes were identified and are currently under investigation.

This O. ridleyi PSRefSeq, coupled with TE and gene annotations, ancestral genome reconstructions, and the database of retroposed genes, represents a pivotal resource for advancing research in Oryza genome evolution, organization, crop development, and potential neo domestication.

GENOME-WIDE ASSOCIATION STUDY (GWAS) FOR IDENTIFICATION OF GENOMIC REGIONS ASSOCIATED WITH COMPONENT TRAITS OF YIELD IN RICE.

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Rice is a primary staple for many around the world. To meet the increasing global food demands due to a growing population, it's crucial to tap into rice's genetic diversity, in terms of 19 physiological and agronomic characteristics. Accordingly, a genome-wide association study (GWAS) was undertaken on an association mapping panel genotyped with a 50K SNP array. The panel was phenotyped for four consecutive *Kharif* seasons at New Delhi. Four multi-locus GWAS models were used *viz*., BLINK (Bayesian-information and Linkage Disequilibrium Iteratively Nested Keyway), FarmCPU (Fixed and Random Model Circulating Probability Unification), SUPER (Settlement of Mixed Linear Models Under Progressively Exclusive Relationship), and MLMM (Multiple Locus Mixed Linear Model). Study was aimed to uncovering significant associations between SNP markers and the observed traits. The population structure accounted by first three principal components and kinship matrix were used as covariates in the mixed model. While the false discovery was controlled by choosing a threshold p-value of < 0.0001. To determine the most suitable model for our analysis, we compared quantile-quantile (Q-Q) plots for each model, juxtaposing observed and expected -$\log_{10}$(P) values. In our investigation, we also spotlighted significant SNPs that appeared consistently across different environments. Manhattan plots were charted at a p-value threshold of <0.0001. 165 marker-trait associations (MTAs) were identified, with crucial SNPs related to yield components, some of which coincided with previously identified genes or QTLs, out of which 10 MTAs were found common across seasonal combinations. The findings, including donor sources and molecular markers, will guide future rice genetic enhancement efforts and shed light on the intricate genetic structures driving these agronomic features.

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TRANSGENERATIONAL MAINTENANCE OF *FUSARIUM EQUISETI* INDUCED SALT STRESS TOLERANCE IN RICE

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Keywords: Salt stress, Fusarium equiseti, Suaeda salsa, IR-64

Rice production is severely affected by salinity stress across the world. Locally occurring high- yielding rice varieties coupled with salinity stress tolerance remains elusive. We demonstrate that a salt-tolerant endophyte isolated from salt-adapted *Suaeda salsa*, a *F.* equiseti, colonizes salt-sensitive rice variety IR-64 and confers salinity stress tolerance. Physiological parameters, such as carbon assimilation rate and chlorophyll stability index were significantly higher in the colonized plants. Interestingly, endophyte-enriched plants had a significantly lower amount of ROS production such as H_2O_2 and $O_2^{\bullet-}$, indicating a reduced amount of oxidative stress. On the other hand, malondialdehyde (lipid peroxidation marker) content of the tissue decreased significantly in endophyte-enriched plants. Further, endophyte-enriched plants had significantly less amount of Na^+/K^+ ratio. The decrease in ratio appears to be primarily driven by a reduced level of tissue Na^+ content in plants colonized by the endophyte compared to those uncolonized. Compared with the non-enriched plants, endophyte-enriched plants, the grain yield content was increased by 25 to 30 %. Mature seeds were collected from greenhouse-grown plants and *F. equiseti* endophyte was recovered from seeds, and from subsequent sporophytic tissue. We observed the vertical transmission of *F. equiseti* for three seedling generations of salt-sensitive IR-64 plants. Comparative transcriptome analysis revealed pathways encoding starch-sucrose metabolism, hormone signaling and phenylpropanoid pathway genes upregulated in plants colonized by the endophyte. In summary, our study suggests that *F.* equiseti could be developed as an effective crop treatment in salt sensitive paddy and may have the potential to increase crop yield in salinity stress conditions.

COST-EFFECTIVE SEQUENCING METHOD(S) FOR SINGLE AMPLICON SEQUENCING (E.G.,16S/18S/ITS) AND GENOTYPING BY SEQUENCING ON ILLUMINA SEQUENCING PLATFORM

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Keywords: Single amplicon sequencing, 'N' (0-10) spacer-linked primer pool, Illumina, Genotyping by sequencing (GBS).

Illumina sequencing platform requires base diversity in the initial 11 cycles for efficient cluster identification and colour matrix estimation. This limitation yields low-quality data for libraries (e.g., 16S/18S/ITS) having homogeneous base composition. Spike-in of PhiX library ensures base diversity but reduces the overall number of sequencing reads for data analysis. With amplicon sequencing and genotyping by sequencing being used for analysing the genetic variants in specific genomic regions as well as for exploring plant genetic diversity on a genome-wide scale.

To overcome such low diversity issues during single amplicon sequencing on Illumina platforms, we developed high throughput sequencing method by introducing spacers in target gene specific primers that are pooled for simple handling. We evaluated the efficiency of 'N' (0-10) spacer-linked primers by targeting bacterial 16S V3-V4 region. The addition of 'N' (0-10) spacers causes sequencing frame shift at every base that leads to base diversity and produces heterogeneous high-quality reads within a single amplicon library. We further demonstrated the accuracy of this method by comparative mock community analysis (ZymoBIOMICS^{TM}) with standard illumina V3-V4 primer method and observed no difference between the communities represented by both the methods.

We have also improved the challenges faced in Genotyping by sequencing (GBS) method by introducing a custom primer for read1 and read2 sequencing and thus shifting the sequencing read frame from restriction digestion site to the insert start site and thus tackling the base diversity issue.

The above discussed methods eliminate the need for PhiX spike-in for sequencing libraries having homogeneous base composition on Illumina platforms and allows for sequencing of more number of samples in a run, greatly reducing the overall cost and yields improved sequence quality. Further, these strategies can be simply adopted for generating high-quality sequences for any libraries with low base diversity in a high throughput manner on Illumina platform.

OsAP2/ERF-40, KEY REGULATOR DURING ADVENTITIOUS ROOT FORMATION

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Keywords: Crown root, IAA (auxin) treatment; BAP (cytokinin) treatment;PAT (Polar auxin transport); aerial roots

In dicots, the tap root system is the major root system and contains primary root (PR) along with its lateral roots (LR). However, monocots are no longer dependent on their PR after seedling stage, and they develop adventitious roots (ARs)/crown roots (CRs) with LRs for their root functions. Rice is a cereal crop that feeds two-thirds of the world population and is also a good monocot model plant species for plant developmental studies. In rice (*Oryza sativa*), the CRs are formed from the innermost ground meristem cells at stem base. In our study, we have found that Auxin, a phytohormone, promotes CR formation in rice, but the other, called Cytokinin, inhibits it. Therefore, we first treated rice seedlings with hormones (IAA and BAP) and then collected stem base samples for RNA-seq analysis. In our transcriptomic analysis, we have identified the gene regulated by auxin and cytokinin. Among them, we have selected *OsAP2/ERF-40* for its functional characterization. For loss of function studies, we have generated RNA-interference-based knock-down lines for *OsAP2/ERF-40.* Partial downregulation of *OsAP2/ERF-40* showed that it is required for proper development of the rice root system. Furthermore, Constitutive ectopic overexpression of *OsAP2/ERF-40* has increased the internode elongation and induced CR formation on aerial nodes in rice. Interestingly, these aerial roots penetrate the soil to support plant growth and development. Since the root formation is promoted in these lines, we have checked the synergistic role of *OsAP2/ERF-40* with polar auxin transport (PAT) machinery. By Inferring with PAT machinery, we have identified that *OsAP2/ERF-40* displayed synergistic role with PAT machinery during CR formation in rice. Next, we checked the expression levels of reported key root developmental regulators in the transgenic lines. It is well known that *OsWOX11-OsERF3-OsRR2* regulatory module plays a crucial role in proper CR development in rice. In

conclusion, we found that this regulatory module is altered in the transgenic lines and supported that *OsAP2/ERF-40* is sufficient to promote adventitious root development in rice, a critical trait for plant breeders to improve crop yield.

UNRAVELLING MECHANISMS UNDERLYING PHOSPHATE-INDUCED SUSCEPTIBILITY TO RICE BLAST.

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Keywords: Defense response, Effectors, *Magnaporthe oryzae,* Phosphate Transporters, Phosphite

In rice agroecosystems, the rice plants are simultaneously exposed to a combination of biotic and abiotic stresses. However, most studies to determine the effects of environmental stress have been performed on plants exposed to an individual stress. One of the most important pathogens affecting rice production is the fungus *Magnaporthe oryzae*, the causal agent of the rice blast disease. On the other hand, phosphorus is a main macronutrient in plants and although the overall content of phosphorus in soil is generally high, its low bioavailability represents a limiting factor for plant growth. As a consequence, phosphate fertilizers are commonly used in rice farming, leading to a scenario of phosphate excess in rice fields. We previously reported that excess phosphate fertilization increases susceptibility to rice blast. Here, we investigated the effect of phosphate supply to rice plants on the two interacting partners: host and pathogen. On the plant side, treatment with high Pi is accompanied by weaker induction of defense responses during *M. oryzae* infection. On the pathogen side, Pi accumulation alters the pathogen's infection strategy by fostering the expression of *M. oryzae* effectors, and genes involved in the control of host cell death. Then, the indiscriminate use of Pi fertilizers might have adverse effects on the rice plant by increasing the likelihood of blast disease. However, the use of phosphite, in combination with phosphate, counteracts negative effects of phosphate on blast resistance. These findings provide a basis to understand interconnected regulations between phosphate signaling and immune signaling in rice.

VISUALIZING COMPARATIVE GENOMICS OF THE *ORYZA* GENOMES USING PERSEPHONE

Manjula Thimma, Alice Fornasiero, Luis Rivera Serna, Yong Zhou, Maxim Troukhan, Rod A. Wing

The *Oryza* Map Alignment Project (*O*MAP) initiated in 2003 has provided comprehensive genomic resources for comparative, evolutionary, and functional characterization of the wild relatives of rice, facilitating the cloning of more than 600 rice genes, including those for grain width (GW5) and submergence tolerance (SUB1A). Two decades later, the 'I*O*MAP: the Americas' project focuses on the present and historic genetic diversity of the wild rice species endemic to the North, Central and South America – i.e., *O. alta*, *O. grandiglumis* and *O. latifolia* – for which high-quality genome assemblies have been produced in the Wing Lab. These species lack domestication traits, but present a wide range of biotic and abiotic resistance traits that can be used to generate more sustainable crops. The recently accomplished neodomestication of *O. alta via* editing of key domestication genes opens the door to the potential neodomestication of the other wild rice in the Americas. First step is the identification of homologous of the domestication genes in *O. sativa*, such as Shattering1 (SH1) and GW5, in the wild *Oryza* species.

Here, we present Persephone, a stable, powerful and versatile genome browser for the visualisation of comparative genomic data. Persephone is capable of rapidly showing large data sets due to unique compression algorithms, optimized data transfer, and a fast-rendering engine that engages some cutting-edge technologies borrowed from the gaming industry. This tool is used to visualize SNPs, INDELs, transcripts, gene orthology, sequence collinearity, etc., and to facilitate fast navigation in the ever-expanding world of genomic information. We used Persephone to compare sequences at the nucleotide and amino-acid level and to visualize orthology of domestication genes in *O. sativa* and the wild rice species of the Americas to look for potential for the neodomestication in the latter species.

Genome-wide Analysis of Expression Signatures Driven by Genetic Regulators Identify Key Pathways in Rice Floral Meristem Establishment and its Determinate Development

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Rice inflorescence architecture is a major determinant of yield. The development of the apical inflorescence/panicle meristem is characterized by the generation of a series of primary and secondary branch meristems that terminate in spikelet meristem, each with a single floret meristem. The combinatorial action of the ABCDE class of floral homeotic transcription factors controls the identity of floral organs in the floral meristem. Interestingly, functional characterization of genes that are homologs in rice and maize that are involved in determining floral organ identity is documented with instances of sub-functionalization or neo-functionalization. To decipher the global developmental transcriptome during floret meristem establishment and organ development, we have generated several NGS-based transcriptome datasets from wild-type panicles binned to represent three broad development pools (floral meristems, floral organ primordia establishment, organ differentiation). In these three developmental pools, co-expressed genes were identified to decipher how their coordinated action could contribute to floral meristem specification and maintenance vs. termination and organ specification. We identified sets of co-expressed genes belonging to specific biological processes that are characteristic to the respective development pools. The regulation of various transcription factors on these co-expressed genes during these stages of panicle development was also examined. For the latter, we determined and analysed the transcriptome in mutant panicles from *osmads1 knockout*, which is observed to lose floral meristem determinacy and its role in flower organ differentiation. Meta-analysis is performed over publicly available

transcriptome and microarray data sets of respective mutant transcription factors or genes that are known to play role in organ identity and development. Subsequently, we observed *osmads34* transcriptome dataset, where mutants are delayed for branch meristem to spikelet transition had positive correlation of regulation with our *osmads1* transcriptome in the early inflorescence developmental pool. It is also observed that class E transcription factors (OsM1 and OsM34) regulate the meristem homeostasis genes OSH1 and OSH15 dynamically across the developmental transition. This supports our hypothesis of a temporal switch in regulatory interactions between the E-class and downstream stem cell homeotic factors during the transition of growing incipient floral meristem to a determinate meristem undergoing floral organ patterning. We propose this network motif and its temporal switch of regulation in the dynamical gene regulatory network involved in the development of an adequately patterned and fertile rice floret.

NEW PLATINUM-STANDARD GENOME ASSEMBLIES OF *ORYZA AUSTRALIENSIS* AND *O. MEYERIANA* AND STRUCTURAL VARIANT ANALYSIS UNCOVER FRESH INSIGHTS INTO GENOME OBESITY IN THE *ORYZA* GENUS

Leonardo Fabbian

Utilizing third-generation sequencing techniques, we achieved platinum-standard genome assemblies for two wild rice relatives, *Oryza australiensis* and *O. meyeriana*, which provide new insights into the 'genome obesity' observed in these two species.

Our research underscores the significant role of a high concentration of long terminal repeat (LTR) elements — comprising 58% of the genome in *O. australiensis* and 57% in *O. meyeriana* in contributing to 'genome obesity', updating current knowledge. This phenomenon is characterized by the substantial expansion of the genome, driven by the proliferation of repetitive elements. In this case, significant contributions come from LTR families such as the Atlantys family, previously unquantified in *O. australiensis*, which covers more than 10% of the entire genome. In contrast, *Gypsy* elements RIRE1, Kangourou, and Wallabi appear to contribute around 22% of the genome, rather than the 60% predicted from previous analysis.

"The structural variant analysis, drawing upon both the new assemblies and previously published data, has provided a comprehensive understanding of the current genomic dynamics of these species. We identified over 20,000 structural variants (SVs) in the new *O. australiensis* genome compared to its predecessor. Notably, 85% of these variants are associated with LTR elements, a figure that rises to over 90% in the O. meyeriana study. This research not only identifies active transposing elements but also tracks those being eliminated, offering insights into ongoing evolutionary processes and changes over time."

An important element of this study was the creation and validation of a portable framework for structural variant analysis, a toolkit useful for deepening our comprehension of the ongoing genomic dynamics within the *Oryza* genus. This tool holds potential for broader applications in rice studies, enhancing our understanding of the role of LTRs in the genomic evolution of the *Oryza* genus.

DIFFERENTIALLY EXPRESSED RICE TRANSCRIPTION FACTOR PROFILES ASSOCIATED WITH EARLY INTERACTION WITH NITROGEN-FIXING BACTERIA

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The mechanism by which plants differentiate between microorganisms and interact to establish a beneficial relationship or activate restrictive responses is one of the most intriguing aspects of plant-microbe interactions. Identifying the molecular processes underlying the differential response of plants is crucial for harnessing beneficial microorganisms for sustainable agriculture. Plant responses to microbes are controlled at the transcriptional level by transcription factors (TF). In this study, the effect of inoculation of rice variety BPT5204 with nitrogen-fixing *Gluconacetobacter diazotrophicus* (GD) and *Bradyrhizobium japonicum* (BJ) on transcription factors was studied through transcriptome profiling at 72 hours post-inoculation under hydroponic conditions. Plant transcriptome factors were identified using PlantTFDB 4.0. The results show that a total of 25 and 22 differentially expressed TFs were observed in BPT5204 during interaction with GD and BJ. TF factor upregulated by both the bacteria was heat shock factor OsHsfB2b, while the MYB-related TF's were uniquely upregulated in GD-inoculated BPT5204. Of the upregulated TF in rice BJ interaction, the ethylene-responsive ERF family was predominantly and uniquely represented (LOC_Os03g08460, LOC_Os08g31580, LOC_Os09g11480, LOC_Os09g11460). The rice TF, OsWRKY22 (LOC_Os01g60490) of the WRKY family with an active role in response to various stresses was highly downregulated in the presence of both bacteria. The other commonly downregulated TF's in the presence of both the bacteria belonged to bHLH, MYB, DBB, WRKY and C2H2 families. Four WRKY family genes (OsWRKY47, OsWRKY76, OsWRKY62 and OsWRKY21) were specifically downregulated in response to GD inoculation while OsMYC2 which is known for mediating defence-related transcriptional changes via jasmonic acid signalling in rice is highly and uniquely downregulated in the rice -BD interaction. This study thus provides fundamental information for understanding the microbe responsive differential expression of TF, which can later be harnessed for improving association of rice with beneficial bacteria.

RICE ROOT ANATOMY SHOWS ADAPTIVE RESPONSE(S) TO HIGH TEMPERATURE STRESS

Aneesh Lale

Rice is a tropical crop adapted to high temperatures throughout its life cycle. However, recent trends suggest that rising temperatures from climate change may exceed the crop's tolerance limits. With a growing interest in cultivating rice in non-paddy systems, the root system would be exposed to significantly higher temperatures due to the absence of the cooling effect caused by submergence. Our research aims to understand the changes in rice root system architecture and anatomy under elevated temperatures. Our findings indicate that certain root anatomical features, such as root diameter, decrease with prolonged exposure to heat stress but recover when the stress is alleviated. Phytohormones, like ABA and Auxin, play a crucial role in these observed phenotypic changes, as mutants exhibit significant differences compared to the wild type. We are currently exploring the molecular mechanisms governing these responses using temporal resolution transcriptomics studies. These insights have the potential to facilitate the development of temperature-tolerant rice varieties better suited for upland cultivation methods.

HISTONE DEACETYLASE PROTEINS REGULATE HEAT STRESS INDUCED HAPLOID EMBRYOGENESIS

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Keywords: haploid embryogenesis; histone deacetylases ; cell pluripotency

Haploid embryogenesis refers to the ability of male or female reproductive cells to regenerate in vitro into a complete plant via embryogenesis. How these cells initiate embryogenic growth in response to inducing signals is poorly understood. We have shown that histone deacetylases (HDAC), chromatin modifying proteins that epigenetically control gene expression through histone deacetylation, repress heat-stressed-induced in vitro haploid embryo development from immature pollen in Brassica napus, a process termed as microspore embryogenesis. Through comparative RNA-seq and ChIP-seq and ATAC-seq analysis of embryogenic samples vs pollen samples we identified important embryogenic as well as stress related genes that are regulated by histone acetylation during microspore embryogenesis. Also, our work revealed that mutation of selected HDAC genes can enhance the rate of microspore embryogenesis and bypass the requirement of heat stress for this process. Our work holds strong potential to induce haploid embryogenesis in other important crops that are recalcitrant for haploid embryogenesis and thus can assist in speeding up the breeding program.

COMPARATIVE ANALYSIS OF SALINITY RESPONSE TRANSCRIPTOMES IN SALT-TOLERANT POKKALI AND SUSCEPTIBLE IR29 RICE

Samuel Fox

Rice is a major cereal crop responsible for feeding the world's population. To improve grain yield and quality, meet growing demand, and face the challenges posed by abiotic and biotic stress, it is imperative to explore genetic diversity in rice for candidate genes and loci that may contribute to stress tolerance. High salinity abiotic stress in the rice growth environment affects growth, yield, and quality. Therefore, we conducted a salt stress-responsive RNA-Seq-based transcriptome study of two rice (Oryza sativa) varieties, the salt-tolerant Pokkali and the salt-sensitive breeding line IR29. To identify early and late salinity response genes, we collected samples from the treated and untreated plants in this study at 1, 2, 5, 10, and 24 hours after treatment with 300 mM NaCl solution. We identified 7,209 and 6,595 salt-induced differentially expressed transcripts from Pokkali and IR29, respectively, over all time points. We identified ~190,000 single nucleotide polymorphism (SNP) sites and ~40,000 simple sequence repeat (SSR) sites, allowing analysis of their consequences on genetic diversity, transcript structure, gene function, and differential expression. We identified and validated the polymorphic SSRs in the differentially expressed salt-responsive genes Respiratory Burst Oxidase Homolog B (RBOHB) and Rice Salt Sensitive 1 (RSS1) that underlie nearby salt tolerance QTLs. This study provides insight into transcriptional programming during salt stress, evidence for improving Oryza genome annotations, and reveals SNP and SSR sites associated with differential gene expression and potential gene function.

IDENTIFICATION AND EXPRESSION ANALYSIS OF *CKX* GENE FAMILY IN *ORYZA SATIVA* L.

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Keywords: *CKX* gene family; phylogenetic analysis; collinearity analysis; expression analysis; *Oryza sativa* L.

Cytokinin oxidase/dehydrogenase (CKX) enzyme catalyzes the catabolism of cytokinin and reduces the level of this key plant hormone in the cell. Cytokinin mediates plant growth, development, yield and stress tolerance. In this study, we carried out genome-wide analysis of *CKX* gene family members in *Oryza sativa* L. We identified 11 *OsCKX* homologous genes with typical CK binding and FAD-binding domains and they were renamed as *CKX1* to *CKX11* based on their different properties. Additionally, we analyzed phylogenetic relationship, conservative motif, gene structure, cis-acting element of promoter, and expression pattern of *CKX* gene family. Our analysis revealed that the number of amino acid ranged from 505 to 621aa, the molecular weight ranged 52.58 to 66.98 kDa, whereas the theoretical isoelectric point ranged from 6.05 to 12.12 among 11 OsCKX proteins. The phylogenetic tree analysis can group family members into subgroups for better understanding the relation. The genes were more conserved in their structure form. In the upstream region of the promoter, we observed several cis-acting elements, including plant growth and development, biotic and abiotic stress response. Combined with transcriptome data, we have validated the *CKX* gene expression at varying developmental stages in *DROUGHT AND SALT TOLERANCE* (*DST*) gene mutants and WT MTU1010 plants. Taken together, these findings provide new insights into the functions of *CKXs* genes in rice growth and may provide the foundations for future studies aimed at improving rice yield.

Germplasm Characterisation & Trait Discovery

CHROMOSOME SEGMENT SUBSTITUTION LINES FROM UNTAPPED RICE GENETIC RESOURCES FOR THE CLIMATE RESILIENT AGRICULTURE

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Rice being a major crop nourishing half of the global population and there are several varieties developed and cultivated in wide ecologies across the globe. Large scale domestication and green revolution focusing on few donor genotypes leads to narrowing the genetic diversity in cultivated germplasm. However, much of the genetic diversity remains underutilized as wild accessions and landraces contain a wealth of genetic traits that can be harnessed to improve modern cultivars. Prebreeding using these untapped genetic resources in the genomics era hold significant promise for enhancing rice production, improving crop resilience, and addressing food security challenges. Chromosome segment substitution lines are a very useful genetic resource for mapping QTLs/gene for complex traits for accelerating the gene discovery. Development of CSSLs using elite x wild crosses was carried out to serve as a national resource at ICAR IIRR using popular mega varieties like MTU1010 and Swarna as recurrent parents and wild accession of *Oryza rufipogon* and *O. nivara* with high photosynthetic efficiency as donor parents. These CSSLs were employed in QTL mapping for both yield and stress tolerance. Advanced genotyping and molecular tools helps in rapid identification of novel genes associated with desirable traits and transferring them effectively to the cultivar background. This also helps in bridging the gap between available genetic resources and crop improvement programs and broadening the genetic base. Successful prebreeding efforts can lead to the development of novel rice varieties contributing to global food security and addressing the challenges of a changing climate scenario.

GENETIC DIVERSITY FOR EARLY SEEDLING VIGOUR IN *AUS* RICE (*ORYZA SATIVA* L.)

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Key words: rice, seedling vigour, GWAS, aus rice, population structure, QTLs

Rice (*Oryza sativa* L.) is a vital global staple food. Direct seeded rice (DSR) is emerging as a more advantageous method compared to traditional transplanting, despite challenges like weed growth and uneven crop establishment. Seedling vigour, including rapid germination, robust growth, and effective weed competition, plays a crucial role in DSR. This study focuses on unravelling the genetic variability of seedling vigour in *aus* rice by characterizing 181 accessions from the 3,000 Rice Genome Panel for seven seedling vigour traits, including grain yield, to understand natural genetic variations. GWAS was conducted using 918,863 SNPs to identify marker-trait associations for four seedling vigour traits. Results revealed significant variations in seedling vigour, growth rate, height, and dry weight. Positive correlations were observed between yield, seedling vigour, chlorophyll content, and growth rate. Population structure analysis using 399,115 SNP markers identified six distinct *aus* sub-groups. Genomic analysis showed a linkage disequilibrium decay of approximately 140 Kbp. GWAS identified 25 significant loci associated with seedling vigour traits, and post-GWAS analysis revealed well-established QTLs governing various aspects of seedling vigour, including germination, coleoptile length, low-temperature germinability, shoot dry weight, height, and chlorophyll content. The study also found overlaps between identified SNPs and key QTLs/genes for early vigour. Notably, an SNP on Chr3 associated with vigour overlapped with a trait related to abscisic acid sensitivity during germination and seedling stages. This study advances our knowledge of the genetic framework governing seedling vigour, promising improvements in rice productivity and quality. Further validation of identified marker-trait associations is essential.

ALLELE MINING FOR SEEDLING STAGE SUBMERGENCE TOLERANCE IN RICE (*ORYZA SATIVA* L.)

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Keywords: Allele Mining, Seedling Submergence, SUB1A, Submergence Recovery

Rice (*Oryza sativa* L.) is one of the major staple food crops belonging to the family Poaceae. Submergence is one of the major abiotic stresses in rice prevalent in rainfed growing situation of India and South East Asia. Developing rice cultivars with tolerance to submergence is the best strategy to circumvent the situation. Rice is grown in vast majority of areas with diverse rice growing ecology in Eastern India under rainfed condition. Sudden rainfall with higher intensity and continuous rainfall at onset of monsoon in this region causes complete submergence of rice seed bed. Screening rice genotypes under submergence stressed condition at seedling stage by observing morphological traits in response to submergence combined with molecular marker approach helps to identify the genotypes having innate capacity to survive under flooding condition more precisely than either of the approach alone. This study was planned to identify the landraces and genotypes having the presence of desired alleles so that gene of interest can be transferred in high yielding genetic background for new breeding line development. Morphological and molecular dissection of Fifty rice (*Oryza sativa* L.) genotypes for submergence tolerance using both morphological and molecular characterization in the field and laboratory respectively. Different stress indices, namely Elongation%, Survival%, Mortality%, Relative Growth Index, Dry Mass%, and Recovery Rate, were examined in the field seven days and fourteen days after submergence. The genotypes were screened with SSR markers to identify the presence of the desired gene. A Significant variation was found among the genotypes both at morphological and molecular level. Submergence related SSR markers used for molecular dissection showed high polymorphism among the genotypes. Based on morphological and molecular screening, out of all rice genotypes, Brahmabalak, Jaladhi 1-2, Jaldubi, Kerala Sundary, China IRRI, Sonachur, Pravat, Pankaj, Ranjit SUB1, and Bahadur SUB1 showed higher levels of tolerance to submergence than the tolerant control Swarna SUB1. Sequencing of SUB1A-1 specific PCR products resulted identification of unique allele (OQ317922) linked to tolerant genotype. Validation and characterization of G/A haplotype identified through sequencing in AP domain containing protein will be done using SNP based marker in segregating generations. They have one or more loci different from the SUB1 locus, which can be used to create improved submergence-tolerant rice varieties with high yield for rainfed lowland agroecosystem.

CHARACTERIZATION AND MOLECULAR MAPPING FOR STEM-STURDINESS IN RICE

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Keywords: GWAS, CSM, stem-sturdiness, MTAs, elasticity

To study stem-sturdiness in rice, a set of 280 *indica* rice germplasm lines were screened for mechanical strength, elasticity, and anatomical traits. There were 65 and 35 genotypes superior to that of positive check IET28438 (*SCM2*) for bending force (BF) and cross section modulus (CSM), respectively, indicating opportunity to explore germplasm for breeding varieties with sturdier stem. CSM along with BF can be used as a valuable indicator of stem sturdiness for phenotypic selection. K-means clustering categorized the genotypes into three distinct clusters wherein, cluster-1 comprised of 91 lines with low stem-sturdiness; cluster-2 comprised of 118 lines with moderate sturdiness; and cluster-3 with 68 lines possessing high stem-sturdiness. Notably, most varieties tested resided in cluster-1, while MTU1010 and PB1847 stood out in cluster-2. Significant positive correlation was observed between anatomical and stem-sturdiness parameters; as well as between BF and CSM. The top five novel sources identified were Daharnagra, ARC13373, Dadkhani, ARC15743, and ARC18092, which can form potential donors for breeding programs. We identified 49 significant MTAs which explained the phenotypic variation ranging from 2.4% to 31.8%. These MTAs co-localized with important genes such as *IPA1, FC1, OsGAE1*, *4CL* etc. Haplotype analysis revealed no influence of *sd1* on stem sturdiness, while seven significant haplotype groups for *SCM2* were identified. Furthermore, three significant haplotypes were identified for *4CL1* which was associated with BF and CSM. The integration of haplotypes into breeding programs could yield varieties with enhanced stem sturdiness, paving the way for robust and productive rice crop.

INSIGHTS ON THE AGRO-MORPHOLOGICAL VARIATIONS IN *AUS* RICE GERMPLASM

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Keywords: rice, aus rice, genetic diversity, GWAS, agronomic traits

The *aus* subgroup within *Oryza sativa* encompasses both aus and boro ecotypes, and exhibits unique stress tolerance traits, making it valuable for breeding. The extent diversity for agro-morphological traits in *aus* rice is poorly understood. Elucidation of the genetic control of yield and agronomic traits is crucial due to the global demand for high crop yields. In this study, genetic diversity analysis of 181 *aus* accessions from 3K Rice Genome Panel using high-density SNP markers revealed six subpopulations with strong geographical structuring. Subpopulation-specific differences has been noted for phenotypic traits.

Principal component analysis (PCA) using 11 agronomic traits revealed first three principal components (PCs) explaining 55% of total variation. PC1 provided most information on panicle traits, plant height and heading date. PC2 represented panicle weight, grain yield and harvest index, while PC3 explained grain yield. A genome-wide association study (GWAS) using PCs identified many significant associations. Notably, the *GLT1* (*LOC_Os01g48960,* annotated as glutamate synthase) gene, responsible for panicle architecture, grain yield and nitrogen-carbon metabolism, was identified based on GWAS using PC2 as a dependent variable.

Haplotype analysis of the *GLT1* gene showed significant differences in PC2 scores along with grain yield and other agronomic traits among three gene haplotypes. Accessions with Hap-2

exhibited higher PC2 scores, yield, and 1000-grain weight, while Hap-3 accessions displayed longer days to heading and taller plants. The distribution of *GLT1* haplotypes in a larger rice germplasm panel revealed *indica*-, *aus* + *aromatic*-, and *japonica*-specific haplotypes.

In summary, this comprehensive study provides insights into the genetic structure and phenotypic diversity of *aus* rice accessions. It identifies significant loci associated with important agronomic traits and highlights the potential of the *GLT1* gene as a key player in determining yield and other important characteristics.

TACKLING RICE FRAUDULENT VARIETY CLAIMS: IDENTIFICATION OF MOLECULAR MARKERS WITH DISCRIMINATING POTENTIAL

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Keywords: whole-genome sequencing, InDel markers, food fraud, varietal identification

Rice (Oryza sativa) is the staple food for over half of the global population. It has a large genetic pool resulting in a vast number of certified varieties, that range in economic value and quality. Rice is highly prone to adulteration, particularly fraudulent variety claims. Developing methods for varietal authentication is, therefore, of utmost importance. Methods based on molecular markers and polymerase chain reactions (PCR) are considered efficient and relatively inexpensive. Europe is a rice-producing region, especially in the Mediterranean countries, thus, this work corresponds to the first steps towards developing a DNA-based method for the discrimination of rice varieties circulating the Mediterranean market. Within the European project TRACE-RICE framework, a panel of 20 economically valuable varieties was selected. The whole genome of each selected variety was sequenced, and, together with the previously published sequences of two additional genotypes, they were mapped and used for short variants calling. The proposed GATK workflow was followed and an array of single nucleotide polymorphisms (SNPs) and insertions and deletions (InDels) was obtained. InDel markers longer than five bps were selected, obtaining a final number of 97576 InDels. For DNA fingerprinting, the Conditional-Random-Selecting (CRS) method, previously described for selecting SNPs with discriminating potential was applied. A group of six InDels was identified as the minimal number of markers necessary to identify each of the 22 varieties. Work is ongoing on their experimental validation, with the ultimate aim of applying multiplex-PCR and validating it for discrimination and identification of a broader panel of varieties.

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NUCLEOTIDE DIVERSITY ANALYSIS OF BACTERIAL BLIGHT RESISTANCE GENES IN WILD RICE SPECIES: INSIGHTS FROM *XA27* AND *XA23* ALLELES

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Key words: Nucleotide diversity, single nucleotide polymorphisms, haplotypes, Tajima's D, Fu and Li's D.

The wild rice accessions containing variant alleles for the resistant genes can be used for developing new lines for disease resistance. Nucleotide diversity analysis helps to study the diverse function of the genes and their role in disease resistance enhancement. To support crop resilience, genetic diversity of wild rice relatives has explored for two rice bacterial blight resistance gene i.e *Xa27* and *Xa23*. Focusing on these two major executor R genes, 114 wild *Oryza* species were screened through allele mining approach. This analysis revealed 20 types of *Xa27* and 5 types of *Xa23* homologous accessions. Through different analysis like polymorphic sites, indels, haplotypes, single nucleotide polymorphisms, and statistical tests such as Tajima's D, Fu and Li's D, valuable insights of the notable candidate alleles were identified. *Xa27* alleles from rice accessions CG86, CG105, CG180, and CG02 exhibited substantial substitutions, and potentially provides strength to disease resistance from the disease-scoring assays. Surprisingly, the CG12 accession showed an abundance of nucleotide substitutions, hinting at a novel, unrelated allele and providing disease resistance response both from the genotype as well as phenotype study. Expression analysis was performed on wild rice accessions (*O. minuta* and *O. rufipogon*) and near isogenic lines (IRBB27, IRBB23) and IR24 highlighting varying expression levels of *Xa27* and *Xa23* genes. These insights are crucial for improving disease resistance in rice breeding programs. By using the genetic diversity of wild rice, it revealed specific major alleles to secure crop improvement trial and ensuring food security among the evolving *Xanthomonas* strains.

EXPLOITING THE ALLELIC VARIATION AND SUPERIOR HAPLOTYPES FOR *OsGW7* GOVERNING GRAIN WIDTH IN RICE

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Keywords - *Oryza sativa*; Grain yield; Grain quality; *OsGW7*; Novel alleles; Haplotype breeding.

Grain size is an important trait that directly influences rice yield. The projected demand for rice by 2050 is estimated to be 197.40 MT for a population of 1.65 billion almost 80% higher than the current demand. Genetic diversity is one of the most basic prerequisites in any crop improvement programme acting as a reservoir for identifying superior alleles through allele mining strategies. To increase rice yield and improve grain quality, it is required to find superior alleles influencing yield component traits. *GW7* regulates control grain length and width in rice, resulting in improved grain quality and increased grain yield. In the present study, allelic variations for the gene *OsGW7* governing grain width were examined in a subset 280 rice germplasm from 3K panel. Haplotype analysis with four non-synonymous SNPs formed two haplotype groups (H1&H2) contributed by two significant SNPs with allelic combination TG and GA respectively. 254 accessions were found be H1 haplogroup while 22 accessions belongs to H2 type. Grain width was associated with these two haplotypes and found to have significant difference between these haplotype explaining the effect of the allelic combinations. Upon trait association H2 allelic combination is superior to H1 allelic combination. The understanding of the role *GW7* allelic combinations within the 280 accessions may aid to elucidate novel alleles that regulate grain development in rice, and contribute to grain quality improvement and increased rice yield through haplotype breeding.

CHARACTERIZATION OF *ORYZA SATIVA*. L VAR *SATHI*: FROM SCRIPTURES TO LABORATORY.

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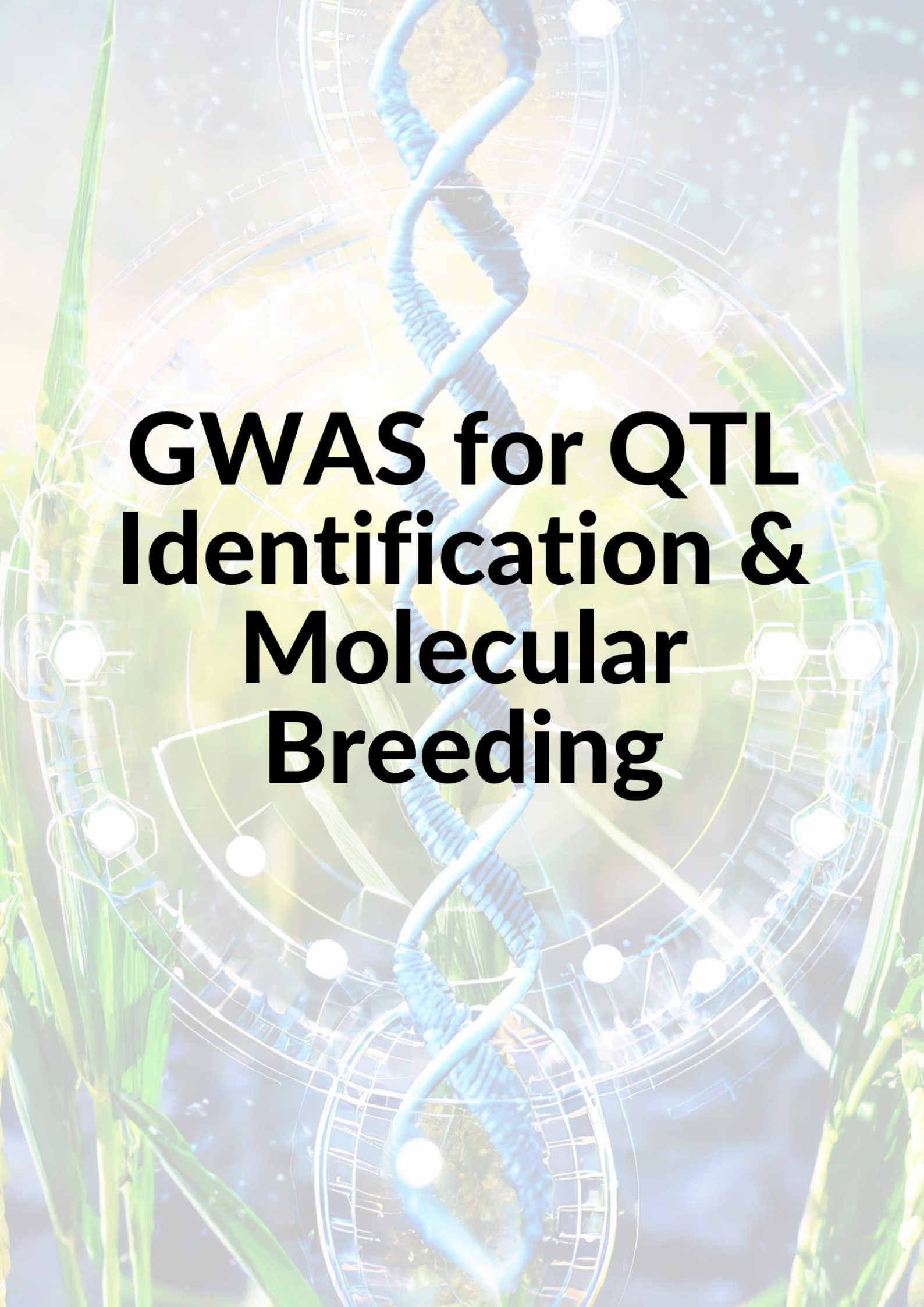
Ancient Indian literature is full of references to germplasm especially that of rice which has been classified based on their nutritional values and also based on agronomic practices. One of the most common rice mentioned in *Ayurveda* is *Sathi* (*Shastika* in Sanskrit), commonly grown in the Terai of the Himalayas in Nepal and India, which derives its name from the fact that it matures in sixty days. It falls under the category of *Bhadai* rices, the ones which mature in *Bhadrapada* (August or September). *Sathi* has a number of unique characters, one being its sheathed panicle which is an advantage against heat stress. Nutritionally, *Sathi* is considered superior and it was found to be moderately rich in Zn and Fe and also was good in crude fibre. Also, indigenous knowledge and studies show that *Sathi* has allelopathic effects. In our study *Sathi* shows allelopathic effects on the common rice field weed, the barnyard grass. The study revealed that co-cultivation of barnyard grass with *Sathi* when compared to normal cultivars showed improper growth of the barnyard seedlings. To investigate the mode of action, extracts of *Sathi* plants were applied to barnyard seedlings which showed a clear cell death pattern. The cell viability test clearly showed that the plants treated with *Sathi* extracts had higher number of dead cells. A landrace with so many phenotypes, *Sathi,* offers scope for studies related to mining genes for earliness, stress tolerance, allelopathy and biofortification which could be utilised for future breeding programs.



GWAS for QTL Identification & Molecular Breeding

DECIPHERING GENOMIC REGIONS AND CANDIDATE GENES ASSOCIATED WITH SALINE STRESS TOLERANCE IN RICE USING GENE BASED GWAS APPROACH

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One of the main abiotic stresses that hinders the growth and productivity of crop plants is salinity. Rice is sensitive to saline stress during seedling and reproductive stages. Saline affected soil with ECe of more than 4 ds/m might lead to 50% to 100% decrease in grain yield, depending on the stage and severity of stress across the crop growth phase. Although multiple genes have been identified as being involved in salt tolerance, the mechanism by which saline stress tolerance is still unclear due to the intricacy of the process. In this study, seedlings of 214 rice genotypes from the 3000 Rice Genome Project (3K-RG) were evaluated in hydroponic culture solution with ECe of 10.0 dS/m for identification of genomic regions and candidate genes genetic loci that govern the rice salt tolerance at seedling stage. Out of 214 genotypes, 12 were tolerant (SES-3), 18 were moderately tolerant (SES-5), 59 were susceptible (SES-7) and remaining was showing highly susceptible (SES-9) to salinity. A genome-wide association study (GWAS) results in the 5 SNP strongly associated with salt tolerant traits vigor score and shoot length with LOD score of >4.5, distributed on chromosomes 1, 3, 8 and 11. Two QTLs explains 8% phenotypic variations for vigor score and 15% phenotypic variation for shoot length present on chromosome 1 and 11, respectively. The sequence information salt tolerant related genes from public databases like QTARO and funricegenes were used for identification of candidate gene based GWAS analysis. Results indicated that the genes encoding for ion transporter, Na+/K+ symport (Os06t0701700-01); similar to Cation transporter HKT1. (Os06t0701700-02) and Os01g0678500 encoding for voltage-gated Ca^{2+} channel protein, elicitor-induced defense responses, and hypersensitive cell death, an activator of MAPK cascade were significantly associated with vigor score. The identified tolerant lines could be used in breeding programme to enhance salinity tolerance in rice. After the validation, identified QTLs and candidate genes could be used in rice improvement for salinity tolerance through marker assisted selection (MAS).

IDENTIFICATION OF MARKERS ASSOCIATED WITH YIELD AND PHYSIOLOGICAL TRAITS AT REPRODUCTIVE STAGE TOLERANCE IN RICE

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Keywords: Rice; Salinity; Reproductive stage; Association mapping; Salt tolerance

Rice under saline stress incorporates several defense mechanisms, which involves different set of genes working collaboratively to mitigate the effect of salinity. To identify these QTLs/ genes, a GWAS analysis was conducted using 180 diverse rice genotypes at reproductive stage under saline stress (E.C.-10dSm^{-1}). Marker trait associations (MTAs) were identified for physiological (Na^+, K^+, Ca^{2+}, Mg^{2+}) and morphological (grain yield and SES score) traits on different chromosomes of rice using GWAS analysis by incorporating mixed linear model. A total of 28 MTAs were identified from the analysis with phenotypic variance ranging from 5.12-13.37%. Comparative genomic analysis of MTAs revealed several candidate genes in these regions. These genes play active function of transcription factor, signal transducer and membrane transporters and few of these genes have shown active role in salt tolerance. Further, 20 salt tolerant genotypes were identified based on salt injury score and grain yield with each genotype accumulating low to moderate Na^+ uptake, while very few genotypes exhibited tissue tolerance. CSR 50 recorded the highest grain yield (10.07 g) under salt stress. While, Na^+ uptake and Na^+/K^+ homoeostasis in leaf was recorded lowest in CSR 54 and K^+ content was recorded highest in CARI Dhan 4 (57.2 ppm). The salt tolerant genotypes identified in the study panel exhibited good allelic variation with respect to one or more traits which encourage the use of these genotypes as donor parent for future breeding programs. Finally, the functional characterization of identified genes and their possible role in other genetic backgrounds would confirm their role in salt tolerance at reproductive stage in rice.

DECIPHERING NOVEL MECHANISMS FOR PHOSPHORUS DEFICIENCY TOLERANCE IN RICE

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Keywords: Phosphorus, QTL, epistasis, root architecture

Phosphorus (P) is one of the essential macronutrients for the growth and development of the plant. Soil P deficiency is a common problem in many rice ecosystems, particularly in areas with high soil P fixation, reducing available Pi and thus resulting in severe yield losses. Complex physiological modulations constitute low P response; hence, the tolerance is affected by various physiological and morphological traits. To identify these complex modulations and QTLs, we screened two bi-parental populations generated by crossing tolerant parents (Rasi and Wazuophek) with sensitive variety improved samba mahsuri (ISM) for various morphological, morpho-physiological, and root architectural traits. We infer the contribution of different root architectural and morphological traits for P content to identify traits crucial for P uptake. QTL mapping was performed for both populations, and various major and novel QTLs were identified for the traits. Also, many significant epistatic interactions were identified for both populations. The sensitive parent contributes a significant number of QTLs in the Rasi X ISM population, as the population showed transgressive segregation for nearly all the traits. In comparison, the tolerant parent contributed many QTLs for the Wazuophek x ISM population. We also found a trade-off between average diameter and branching traits in P deficiency and identified QTL for this trade-off. Interestingly, this QTL is only present in P deficiency and absent from the sufficient P treatment, suggesting a crucial role of this trade-off during P deficiency. In conclusion, our study highlights the importance of transgressive segregation and trade-offs for traits during P deficiency.

GENOME-WIDE ASSOCIATION ANALYSIS FOR ALKALINE TOLERANCE AT THE GERMINATION STAGE IN RICE

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Saline-alkaline stress is one of the major detrimental factors affecting plant growth and yield in rice. Developing alkalinity tolerant rice varieties is challenging due to the complex nature of alkaline stress tolerance. Therefore, the functional loci or gene resources contributing to alkaline tolerance in rice still need to be identified. In the present study, we conducted a GWAS for alkaline tolerance with four alkaline-related traits in germination stage. A total of 71 significant loci were found by GWAS using the GLM model at the germination stage, and candidate genes were filtered by the RNA-seq profile, GO annotation, functional similarity of homologs, and qRT-PCR experiments. Finally, three candidate genes were identified for alkaline tolerance at the germination stage. These results will provide novel gene resources, valuable variety information, and diverse haplotype variation resources for the molecular breeding of alkaline tolerance varieties.

Keywords: alkaline tolerance, abiotic stress, germination stage, genome-wide association study

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MULTI-TRAIT GENOME-WIDE ASSOCIATION STUDY AMONG LEAF ROLLING INDEX AND YIELD COMPONENTS IN RICE AT HEADING STAGE

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Leaf shape is one of the main factors determining the plant architecture. In the case of V-shaped leaf rolling, it increases canopy photosynthesis by enhancing CO_2 penetration and improving light capture, as it reduces the shadow between leaves. Therefore, it is thought that moderate leaf rolling contributes to a higher grain yield per unit area compared to flat leaf. In this study, the adaxial leaf rolling index (LRI) was investigated using 296 accessions at the stage when heading started. SNPs with a minor allele frequency of less than 0.05 were removed and ultimately, 845,723 high-quality SNPs were used for multi-trait GWAS. In addition, the Bonferroni correction method was used to correct errors in the multiple comparisons problem and the significant threshold was set at $-\log10(P) > 7.228$. In the results, we detected six lead SNPs considering the LD block of 250kbp and compared the multi-trait QTLs for yield components and the QTLs detected from the single-trait GWAS of each yield component.

Keywords: leaf rolling index, heading stage, yield components, genome-wide association study, multi-trait

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EVALUATION OF *BAKANAE* DISEASE RESISTANCE-RELATED MARKERS USING THE GERMPLASMS SELECTED FROM KOREAN RICE LANDRACES AND CORE COLLECTION

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Bakanae disease is increasingly important in several rice-growing countries, resulting in incremental production losses. In our previous study, a total of 395 rice accessions selected from Korean landrace and core collection were screened for *bakanae* resistance, enabling the identification of accessions exhibiting high-to-moderate levels of resistance to *bakanae*. Among them, 90 accessions (top 45 accessions each from the susceptible and resistant group) were selected to evaluate the *bakanae* disease-related markers. These markers consisted of InDel, SSR, and KASP markers and were designed based on the reported QTLs that are highly associated with phenotypic variation in response to *bakanae* infection. The applicability of the markers was verified in 90 accessions of Korean landrace and core collection. The markers evaluated in this study will be effectively used for genotyping the *bakanae* resistance lines, thereby facilitating their utilization in marker-assisted selection (MAS) for the rice breeding program.

Keywords: *bakanae,* resistance, molecular markers, marker-assisted selection, landrace, core collection

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MAPPING GENOMIC REGIONS ASSOCIATED WITH ROOT ARCHITECTURAL AND YIELD RELATED TRAIT UNDER AEROBIC CONDITIONS IN RICE (*ORYZA SATIVA* L.)

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The dry direct-seeded aerobic system of rice cultivation requires significantly less water than the traditional flooded systems and has emerged as a promising water-saving technology. The identification of Quantitative trait loci (QTLs) conferring root and yield-related traits under aerobic adaptation is essential in the present scenario and has the potential to facilitate the development of high-yielding aerobic rice varieties. The present investigation was carried out during dry season 2022 at two locations ICAR-IIRR, Hyderabad, and AHRS Kathalagere, Shivamogga to map QTLs for yield and yield-related traits and root architectural traits in polyhouse at panicle initiation stage under aerobic conditions at ICAR-IIRR using 150 Recombinant Inbred Lines (F_7) along with parents (TI-128×BPT-5204) and checks (Rasi, GNV1109, DRR Dhan-41, CR Dhan 202, MTU 1010). A positive significant correlation of grain yield per plant with root length, root volume and shoot length was recorded indicating the role of root traits in improving yield traits through improved water/nutrient uptake. Genotyping was executed using 1k-Rice Custom Amplicon (1k-RiCA) SNP markers and QTLs were mapped using IciM 4.2. A total of 43 QTLs for 14 traits were identified for yield and root-related traits. Four major QTLs were identified associated with grain yield per plant *qYPP-5.1* (PVE 12.87%), number of grains per panicle *qNGPP-1.1* (PVE 14.65%), test weight *qTW-5.1* (PVE 10.06%), number of productive tillers *qPT-1.1* (PVE 10.43%), Root length *qRL-9.1* (15.48%) and Root volume *qRV-12.1* (13.80%). The identified QTLs may be used in marker-assisted breeding to develop novel high-yielding aerobic rice varieties.

Key words: Aerobic rice, QTL, Root architecture, 1k-RICA

GENOME WIDE ASSOCIATION STUDIES FOR CULM MORPHOLOGY TRAITS IN INDICA AND TROPICAL JAPONICA POPULATIONS REVEAL NOVEL GENOMIC REGIONS FOR STRONG CULM IN RICE

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Lodging remains a serious concern in high yielding rice varieties, despite the short stature conferred by the *sd1* gene. Reducing the plant's height also reduces photosynthetic capacity and total biomass production, restricting the plant's potential for further yield increase. The attempts to increase the sink capacity will be successful only when the source efficiency is enhanced which is the basis of new plant type concept. Although the improved source-sink relationship is essential to maximize harvest index, it also promotes biomass via increased stem and leaf elongation with an overall increase in the plant height leading to lodging. To avert the discernable negative effects of weak culms on plant type, we evaluated various culm morphological and physical strength traits. Our comprehensive understanding of various parameters concomitant with culm strength provided useful insights of culm morphological traits. Phenotypic evaluation of culm morphological traits and culm physical strength traits was carried out for two seasons where culm wall diameter ranged from 2.68 to 7.29 mm. High heritability (85%) and genetic advance over mean (27%) observed for the outer and inner culm diameter both on major and minor axes merited genetic dissection of the culm diameter trait. Histograms for culm diameter revealed normal distribution in the population that indicated quantitative inheritance of the trait. Further, highly significant positive correlations of the culm diameter were observed with other components of culm physical strength traits, viz., pushing resistance and breaking resistance of the culm. GWAS was conducted in an association mapping panel comprising tropical japonica accessions, indica cultivars, landraces and breeding lines derived from indica and tropical japonica. The panel was essentially constituted to capture maximum phenotypic diversity for culm strength traits. Kinship algorithm and genome association and prediction integrated tool (GAPIT) using multi-locus model of BLINK employing GBS derived 6,822 filtered SNPs revealed presence of two sub-

groups in the panel and indicated less relatedness among the genotypes. We identified two highly significant major effect QTLs on chromosomes 2 and 12 and several minor effect loci across the chromosomes for culm diameter. Marker trait association (MTA), *qIDMa12.1* on chromosome 12 at *pos* 8010637 bp for inner diameter on major axis (IDMa) explained a phenotypic variance of 26.15 % at *P* value of 1.76×10^{-9} while MTA, *qODMi2.1* on chromosome 12 at *pos* 37491093 for outer diameter on minor axis (ODMi) explained a phenotypic variance of 19.66 % at *P* value of 2.15×10^{-7}. These loci were further analyzed *in silico* for the presence of putative candidate genes previously reported for lodging resistance and were found to be novel regions. We identified five strong culm donors with wider culms (6.78- 7.29 mm) both in indica and tropical japonica sub species groups. The elite strong culm donors in the indica background with beneficial alleles for the novel QTLs could be a valuable resource with greater significance in practical plant breeding focusing on indica plant type improvement. This is the first report on GWAS for culm strength traits in the Indian sub-continent.

Keywords: Plant type; strong culm; culm diameter; lodging resistance; GWAS; MTAs; phenotypic variance; association mapping

IDENTIFICATION OF MARKER TRAIT ASSOCIATIONS FOR GRAIN NUMBER ACROSS INTER SUB-SPECIFIC POPULATIONS IN RICE

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Rice grain yield is a complex trait, mainly influenced by number of panicles per plant, number of grains per panicle and grain weight per panicle. New Plant Type (NPT) concept aims to increase the yield of modern *indica* cultivars by using genetic material from tropical *japonica* accessions which are characterized by large panicles, large leaves, a vigorous root system, thick stems and few unproductive tillers. Grain number per panicle, determined by the panicle architecture or panicle related traits is an important agronomic trait directly associated with grain yield. The transition from less grain number per panicle to a greater number per panicle is one of the most important hallmark events in rice domestication. The most effective way to improve rice grain yield is to increase grain number per panicle through breeding practice. Grain number is a typical quantitative trait affected by several genetic and environmental factors and understanding the mechanisms controlling grain number has become an important research field in rice biotechnology and breeding. To genetically dissect and elicit the valuable NPT traits, we conducted GWAS in an association panel composed of elite *indica* cultivars, tropical *japonica* accessions, breeding lines derived from *indica*/tropical *japonica* crosses and *indica* breeding lines. The panel was phenotypically evaluated in two cropping seasons in the dry seasons of 2022 and 2023. Highly significant phenotypic variation for the trait, grain number was observed which varied from 80 to 450 with the mean of 154 grains. Highest GCV (30.95), PCV (31.66) coupled with high heritability (95.54) and genetic advance over mean (62.42) was observed which indicated genetic control and merited dissection. Two season phenotypic data and GBS generated genotypic data of 35,331 filtered single nucleotide polymorphism (SNP) markers was subjected to GWAS using genome association and prediction integrated tool (GAPIT) in R studio. Efficient mixed model association (EMMA) coupled mixed liner model identified eight highly significant marker trait associations at *P*

value of 6.56 X 10^{-8} on chromosomes 1, 3, 4, 8 and 9 which explained the phenotypic variance of 3.18 to 14.19 %. One major effect QTL, *qGN8.1* on chromosome 8 at *pos*5608969 explained a phenotypic variance of 14.19%. We identified three *indica*/tropical *japonica* breeding lines (JBB 6315, JBB 4914 and JBB 5952) and one tropical *japonica* accession (IRGC 74607) with high grain number (more than 350). Notably, the three *indica*/tropical *japonica* breeding lines (JBB 6315, JBB 4914 and JBB 5952) recorded high grain yield of 8415 to 9801 kg/ha in our replicated yield trials and possess high biomass and strong culm which are characteristic features of NPT cultivars. The use of these high yielding NPT lines associated with beneficial alleles for high grain number in rice breeding could improve plant architecture without changing grain quality or growth period, which are important for regional adaptability.

Key words: New plant type; plant architecture; GWAS; Genotyping by sequencing; grain yield; QTL; SNP; mapping

NOCTURNAL TRANSPIRATION: A GENOMIC INSIGHT ON RICE'S HIDDEN THIRST

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The escalating global temperatures and the increasing vapor pressure deficit during nighttime have brought to the forefront the significance of understanding a phenomenon known as "Nocturnal Transpiration". Nocturnal transpiration, is the substantial loss of water by plants during night without resulting uptake of carbon, accounting for 8-30% of daytime transpiration. In context of evolving climatic challenges, the need to comprehend nocturnal transpiration, particularly for optimizing crop water use is of paramount importance. Rice, a staple crop essential for global food security, in the spotlight for this investigation. To delve into this intriguing area, we conducted a comprehensive analysis involving 200 diverse rice germplasm lines. Utilizing an automated mini lysimeter-based drought simulator phenomics facility, hourly transpiration rates throughout the entire crop growth cycle were recorded. Our findings unveiled a wide range of transpiration rates. Notably, a significant pre-dawn surge in nocturnal transpiration was observed in several genotypes, suggesting genotypic variability in water loss dynamics. Subsequently, a Genome-Wide Association Study was performed, revealing specific single nucleotide polymorphisms associated with nocturnal transpiration at distinct nighttime intervals. These markers shed light on genetic architecture and the underlying mechanisms governing nocturnal transpiration in rice and holds the potential to possibly enhance water use efficiency in rice cultivation.

Keywords: Genome wide association studies, Nocturnal Transpiration, Phenomics, Predawn transpiration, Rice.

IDENTIFICATION OF STABLE GENOTYPES THROUGH G×E ANALYSIS AND SNP ALLELES THROUGH GBS FOR GRAIN ZINC CONTENT IN POLISHED RICE (*ORYZA SATIVA* L.)

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Polished rice is one of the most consumed staple foods across the world and it is known to be a poor source of micronutrients. Biofortification of rice for high grain zinc (Zn) is proving to be a promising strategy for addressing micronutrient malnutrition. To identify stable genotypes and genomic regions for high grain Zn, a total of 88 rice genotypes consisting landraces, breeding lines and varieties were evaluated in six environments at four research farms during three wet season for nine phenotype traits including grain Zn in brown (BZn) and polished rice (PZn). Based on genotype and genotype x environment interaction (GGE) analyses across six environments, four stable genotypes (Karuppanel, Pandari-Gurmatiya, EC739834 and Surabhi) were identified for high grain Zn (>30ppm). The genotypes were subjected to double-digest restriction site associated DNA (dd-RAD) sequencing resulting in 88,811 polymorphic single nucleotide polymorphisms (SNPs). Genome wide association study has shown eight significant SNPs with PZn and 98 significant SNPs with BZn. The SNPs of PZn were found to be associated with SNPs of BZn and five SNPs were found in four putative candidate genes (*LOC_Os01g16260*-antiporter-gene), *LOC_Os02g06660*-P21-Rho-binding-gene), *LOC_Os02g54500*-WD40-gene) and *LOC_Os12g16080*-expressed protein) and other were found in *intergenic* regions. The genomic region of *LOC_Os01g16260* and *LOC_Os02g54500* were co-localized with reported candidate gene regions. Characterization of germplasm in terms of stability for their grain Zn in polished and yield identified promising donors and recipients along with genes/genomic regions in the present study to be deployed for rice Zn biofortification along with yield breeding program.

Keywords: Grain Zinc in polished rice, stable genotypes, GWAS, SNPs and Candidate genes

GENOME WIDE ASSOCIATION STUDIES FOR YIELD COMPONENTS TRAITS IN AN ELITE RICE BREEDING PANEL

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To characterizing a panel of elite rice genotypes for yield components and to identify MTAs, a set of 190 elite breeding lines were evaluated in three locations during *Kharif* 2021. Lines were genotyped with 80K rice pan genome array and phenotyped for traits plant height (PH), panicle length (PL), days to fifty percent flowering (DFF), tiller number (TN), grain number (GN), thousand grain weight (TGW) and plot yield. Across the locations, significant variation among the genotypes was observed for all the traits, DFF was positively correlated with GN and negatively correlated with TGW and GN had a positive correlation with yield. The structure analysis identified two sub-populations with Fst values of 0.12 and 0.28, two main clusters were observed by kinship analysis and LD block size at half LD decay was 304kb. Association analysis using BLINK model for the studied traits in multiple locations and their cBLUP values identified 61 MTAs. Four stable MTAs each were identified for PH and PL, qDFF8.1 was a stable QTN identified for DFF and was also pleotropic with PL, two and one stable MTAs were identified for GN and TGW respectively. Insilco analysis was done to identifying putative candidate genes in 300kb regions across the 11 stable MTAs. Few previously reported genes such as *HD5* and *TBP1*(*OsBAK1*) regulating PL and DFF, *OsSPL18* regulating GN, *sd1* regulating plant height were in the vicinity of identified MTAs. Characterization of elite breeding genotypes will help in developing the elite core panel, deciding elite x elite crosses for closed recurrent breeding programme and MTAs identified can be utilized for early generation selections and informing genomic prediction models for increasing prediction accuracy.

Keywords: Rice, GWAS, MTAs

GENETIC ANALYSIS OF EARLY SEEDLING VIGOUR IN DIRECT SEEDED RICE CONDITION

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Direct-seeded rice (DSR) is an old but re-emerging rice cultivation practice in water scarce areas, allowing a high-water-use-efficiency. It is renowned for its labour and water saving attributes by enabling mechanization. In the fast global climate change crisis, the development of high-yielding, climate-smart rice with improved nutritional quality and low glycemic index is must. Genome Aided Breeding (GAB) and Genome-wide Association Study (GWAS) approaches have significantly impacted crop breeding efficiency and delivered superior varieties by addressing complex polygenic traits and population improvement. But, the efficacy of these approaches in larger germplasm is rare. Comprehensive haplotype analysis aids GWAS in identifying genomic variants linked to a particular trait. The availability of sequence data for 3K RG Panel in Rice SNP-Seek Database offers an advantage for cost effective GWAS and extends provision for effective phenotyping. A total of 200 accessions were screened for early seedling vigour (ESV) traits in field condition during *Rabi* 2022 and *Kharif* 2023. Observations attributing to ESV were recorded at 15 and 30 days after sowing, respectively. A descriptive statistical analysis displayed the existence of potential variability and correlation among the considered traits. Statistical software R studio v. 4.3.1 and TASSEL v. 5.2 were used to identify the QTLs. Seven and 14 significant QTLs were obtained with minimum LOD of 6 and R^2 of 10 for ESV I and ESV II, respectively. Our comprehensive outcomes display that, study of candidate genes and identifying haplotype variants for ESV would be a prospective for breeding of direct seeded rice.

Keywords: Direct Seeded Rice, Early seedling Vigour, QTLs, GWAS, Haplotypes

EVALUATION OF GENETIC BASIS OF SEEDLING VIGOUR TRAITS IN *O. GLABERRIMA* DERIVED BACKCROSS POPULATION UNDER DRY DIRECT SEEDED CONDITION

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Direct seeded rice (DSR) is a sustainable method of rice cultivation, particularly in the era of climate change. Early seedling vigor (ESV) is an essential trait for weed competitiveness in this system. Traits contributing to ESV, along with vigour indices, SV I and SV II, can be assessed genetically to develop varieties with ESV. However, before genetic perusal, any trait must be evaluated to ensure sufficient variation in the population. This study investigated variability of morphological traits contributing to ESV in the backcross population of IR64*1/*O. glaberrima*. The BC_1F_7 generation was evaluated at ICAR-Indian Institute of Rice Research during *Rabi* 2021, with 184 lines grown under weed-free and weedy conditions. Augmented design was employed to evaluate the lines, parents, and five checks. Traits like germination percentage (37.55% and 24.67%), shoot dry weight (39.34% and 56.49%), SV I SV I (35.13% and 35.69%), and SV II (40.51% and 63.69%) exhibited high estimates of genotypic coefficient of variation under weed-free and weedy conditions, except for seedling length. Similarly, phenotypic coefficient of variation was found to be high under weed-free and weedy conditions for all the traits. However, both the coefficients were only moderate (20.64% PCV and 16.47% GCV) for seedling length under weed-free condition. Whereas, it was moderate PCV (17.10%) and low GCV (9.12%) under weedy conditions. Hence, all traits had sufficient GCV (moderate to high), indicating significant genetic variation. Further genetic analysis may help identify high vigor lines and QTLs and genes affecting early seedling vigor under DSR conditions.

Keywords: Direct seeded rice, seedling vigour traits, *O. glaberrima,* variability

Metabolomics for Nutritious Rice

A Comparative Metabolomic Analysis Reveals the Nutritional and Therapeutic Potential of Grains of the Traditional Rice Variety Mappillai Samba

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Rice (*Oryza sativa* L.) is the staple food of the majority of the population, particularly in Asia and Africa. Enriching rice with nutritional and therapeutic contents can improve its benefits for patients with lifestyle disorders. This study aimed to profile the phytochemical contents of the therapeutically known traditional rice Mappillai Samba against white rice CBMAS 14065 using non-targeted gas chromatography–mass spectrometry (GC-MS/MS). An analysis of the data using a mass spectrometry–data independent analysis (MS-DIAL) and MetaboAnalyst identified 113 metabolites belonging to 21 different classes of metabolites. A partial least square-discriminant analysis (PLS-DA) revealed 43 variable importance in projection (VIP) metabolites. This study identified therapeutically important metabolites, including phenylpropanoids, phytosterols, flavonoids, and polyamines, in the grains of Mappillai Samba. Three significant metabolic pathways, viz., phenylpropanoid biosynthesis, ubiquinone and other terpenoid-quinone biosynthesis, and steroid biosynthesis, were responsible for the grain metabolome variation between CBMAS 14065 and Mappillai Samba. Overall, the results of this study unravelled the biochemical complexity of Mappillai Samba, paving the way for the genetic mapping of the therapeutic compound accumulation in rice and the development of similar therapeutic rice varieties through molecular breeding.

Keywords: Traditional rice; Mappillai Samba; metabolomics; therapeutic compounds; phytosterols; antioxidants

UNLOCKING THE POTENTIAL OF *OSRIBA1* FOR RIBOFLAVIN ENHANCEMENT IN RICE

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The UN Sustainable Development Goal-2 aims to eradicate hunger by 2030, yet over 2 billion people still suffer from hidden hunger. Rice, the primary calorie source in developing nations, lacks essential vitamins, particularly in its most consumed form, white rice. To address this, biofortification through metabolic engineering presents a compelling solution, only hampered by limited knowledge of target biosynthetic pathways. Our research focuses on rice vitamin B1 and B2 pathways aiming to build knowledge and tools to assist in their biofortification. For B1, we identified the key biofortification genes (*OsTH1*, *OsTHIC*, *OsTHI1*) and extensively characterized *OsTH1*. We also explored B1 binding proteins, vital for B1 accumulation in the endosperm.

While we have achieved significant strides in B1, here we focus on vitamin B2. We constructed a comprehensive mathematical model of the B2 biosynthesis pathway in rice, pinpointing RibA (*GTPCHII*/*DHBPS*) as the rate-limiting enzyme. *OsRibA*, previously unknown in rice, was identified through genomic analysis, gene expression data, and phylogenetic studies. Heterologous expression experiments showcased *OsRibA1* ability to rescue the growth of yeast mutants lacking GTPCHII and DHBPS activities. *In vitro* assays confirmed *OsRibA1* as a bifunctional enzyme and provided valuable insights into its biochemistry. To bridge the gap between fundamental knowledge and practical applications, we overexpressed *OsRibA1* in rice callus, yielding a substantial increase in riboflavin production.

In conclusion, our work characterized *OsRibA1* and proposed a strategy for enhancing rice's micronutrient content. This research contributes to the global effort to combat hidden hunger and aligns with the UN's sustainable nutrition goals.

Keywords: Multibiofortification, DHBPS, GTPCHII, Kinetic modelling, Limiting-step, RibA, Rice, Riboflavin

STRATEGIC UNDERSTANDING OF MECHANISMS THAT RESTRICT GRAIN YIELD, GRAIN PROTEIN CONTENT AND QUALITY IN RICE THROUGH AN INTERFACE BETWEEN CLINICAL NUTRITION AND AGRICULTURE RESEARCH: AN OVERVIEW

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Rice stands as a cornerstone of global food security, providing sustenance for a substantial portion of the world's population. Nonetheless, achieving optimal grain yield while simultaneously increasing grain protein content present a challenge due to the inverse correlation between grain yield and grain protein content. Therefore, we formulated a hypothesis suggesting that this trade off may arise from competition for reductants during carbon and nitrogen assimilation processes. In pursuit of understanding and unravelling this trade off, our focus was on studying 40 varieties that encompass both high yield and high grain protein content types. Further analysis will involve an assessment of amino acid composition, sensory attributes, and non-targeted metabolomics profile, deciphering the intricate interplay between grain protein content, quality, and yield. Finally, to bridge the gap between laboratory findings and real-world impact, protein digestibility will be scrutinized in humans using the dual stable isotope approach. In summary, this study underscores the significance of merging clinical nutrition and agriculture research to unravel the intricate mechanisms that hinder grain yield, protein content, and quality in rice. By embracing this interdisciplinary framework, novel pathways for sustainable rice production and enhanced nutritional outcomes can be charted, thus paving the way for a more food-secure and nutritionally enriched global future.

Keywords: Carbon assimilation, grain protein content, nitrogen assimilation, protein digestibility.

GENETIC EVALUATION OF BLACK PERICARP BASMATI RICE GENOTYPES

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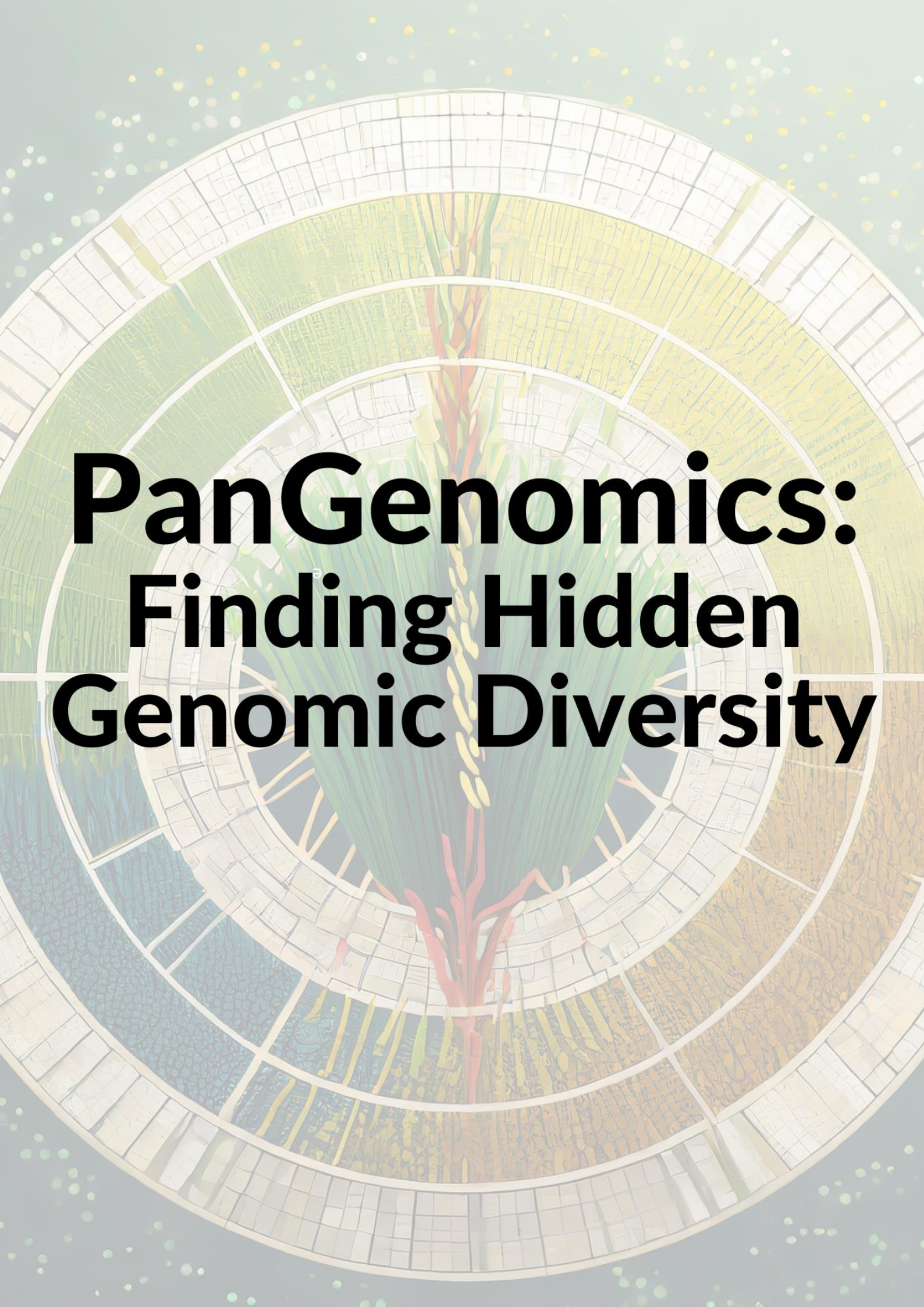
Basmati rice is highly popular in the international market due to its unique grain, cooking and eating qualities along with its pleasing aroma. Modern day lifestyle associated diseases such as diabetes in rice consuming population has necessitated enhancement of nutritional quality in addition to improving the productivity. With the raising concern about the health, coloured rice have been considered valuable for their health benefits. Chakhao Poireiton, an aromatic black rice from Manipur and Kavuni from Tamil Nadu are popular for its high antioxidant property. The present study was carried out on characterizing the black pericarp Basmati rice genotypes developed through introgression of high anthocyanin trait sourced from black rice with the desirable grain, cooking and eating qualities of Basmati rice varieties aimed at creating the functional foods with improved nutritional content. Sixty-nine black pericarp Basmati rice genotypes developed by transferring anthocyanin content from Manipur black rice, Chakhao Poireiton and Kavuni, to the genetic background of Basmati rice. Significant differences in agro-morphological traits were observed among the genotypes, including plant height, panicle length, tiller number, days to flowering (DFF), and yield. As many as 23 black pericarp rice genotypes was found to yield on par with the popular Basmati varietal checks namely, Pusa Basmati 1121 and Pusa Basmati 1509, with two genotypes namely Pusa 3215-16-115-6-1-5-1, Pusa 3214-16-30-13-2-2-1 significantly superior to the checks. Further the black pericarp genotypes were classified into 17 black, 32 dark brown, 19 light brown and 1 white coloured pericarp classes based on the Hunter-Lab colorimeter values of the dehusked rice. Anthocyanin estimation from the dehusked rice samples of the 69 black pericarp rice genotypes revealed that the total anthocyanin content ranged from 3.50 to 169.07 mg/100g DW, and the specific components like C3G and P3G ranged from 167.88 to 2.91 mg/100g DW and 0.59 to 40.11 mg/100g DW, respectively. The standardization of the milling duration

carried out in Chakhao Poireiton revealed that 15 seconds milling was the best for achieving desirable cooking quality as well as retention of the anthocyanin contents. As many as 5 genotypes showed higher anthocyanin content retention with 15 seconds milling than the check, Chakhao Poireiton. Although, seven black pericarp rice genotypes could qualify the minimum Basmati grain and cooking quality parameters, their total anthocyanin content was found to be less than 30mg/100g DW. Molecular characterization of the 69 black pericarp rice genotypes using gene specific markers governing resistance to bacterial blight (BB) and blast diseases revealed that 13 genotypes possess the resistance alleles of at least three genes for blast and BB genes in two combinations namely *xa13+Pi2+Pi54* or *xa13+Xa21+Pi2,* while 16 genotypes possessed at least one gene each for resistance to BB and Blast. The research demonstrates the feasibility of combining the desirable attributes of both aromatic and Basmati rice varieties to produce grains that possess not only exquisite aroma and cooking qualities but also improved anthocyanin content for added health benefits. While some genotypes exhibited promising qualities for agronomic performance, anthocyanin content, and disease resistance, further rounds of improvement are necessary to achieve desirable combination of key traits. Overall, these developed improved Basmati genotypes with black pericarp provide base materials for further improvement of the nutritional quality, so that it can be tested for their suitability to meet the Basmati qualities along with enhanced anthocyanin content and resistance to both the BB and blast diseases.

Keywords: Anthocyanin, Antioxidant, Agro-morphological performance, Basmati rice, Chakhao Poireiton



PanGenomics: Finding Hidden Genomic Diversity

IDENTIFICATION OF SUMOYLATION MACHINERY IN RICE (ORYZA SATIVA): AN EXPANSION OF SUMO PROTEASES IN RICE

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SUMOylation is a key post translation modification (PTM) across eukaryotes. This process has been reported as a critical regulator in plant development and response to abiotic and biotic stresses. The mechanisms of the SUMOylation are best known in the model organisms, *Saccharomyces cerevisiae* and *Arabidopsis thaliana*. The SUMOylation pathway consists of five main steps, maturation of SUMO proteins, activation of mature SUMOs by an E1 ligase (e.g., SAE1a), conjugation of SUMO-E1 complex by an E2 ligase (e.g., SCE1), ligation of SUMO complex with a substrate (target proteins) by an E3 ligase (e.g., HYP2 and SIZ1), polySUMOylation (alternative) by an E4 ligase (e.g., PIAL), and deSUMOylation by protease enzymes (e.g., ULP and Desi). To translate the information from model species to crop plants (i.e. *Oryza sativa*), we identified SUMOylation machinery genes in rice by querying the protein sequences of *S. cerevisiae* and *A. thaliana* against *O. sativa* genome (TBLASTN/BLASTP), searching for specific protein domains, and exploring transcriptomics data. Interestingly, the number of SUMOylation components, especially SUMO proteases are not comparable to genes in *Arabidopsis*, suggesting an expansion of this gene family in rice. Furthermore, we compared the number of SUMO proteases between wild and domesticated rice, with an apparent expansion of the family in domesticated rice, especially MAGIC16 rice (*O. sativa*). The identified and improved gene models could be the starting point and resources for the study of the SUMOylation mechanism in rice and potentially be a target for gene editing in crop improvement.

Keywords: SUMOylation; post translation modification; Oryza sativa; MAGIC16 rice

GENOME-WIDE ASSOCIATION STUDIES OF RICE GRAIN SIZE PARAMETERS IN THE *INDICA* SUBSET PANEL OF 3K ACCESSIONS

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Rice grain size-related traits are one of the vital traits that determine marketability and consumer preference. Deciphering the genomic variants governing grain size traits aids in breeding high-yielding cultivars with preferred grain types, resulting in increased yield and marketability. Genome-wide Association Studies (GWAS) were carried out in 273 indica rice accessions of 3K panel for grain size traits using a compressed mixed linear model (CMLM) with a total of 42729 SNPs. The analysis has generated 26 significant marker-trait associations (MTAs), of which 22 were novel, and rest were earlier reported MTAs. Two MTAs S11_19445868 (qGL11.1, qGP11.1- PVE-16.93%) and S11_28768409 (qGL11.2- PVE-17.08% and qGP11.2-PVE- 15.52%) governed both grain length and grain perimeter. Certain MTAs were located in close proximity governing multiple traits forming QTL clusters. The MTAs S2_24540552 (qGW2- PVE-24.72%) & S2_24882438 (qGA2.1- PVE-18.88%) associated with grain width and area; S11_19445868 & S11_19603576 (qGL11.1-PVE-16.93%, qGP11.1-PVE-15.53%) associated with grain length and perimeter. Candidate gene analysis of the aforesaid MTAs has identified *OsFBX421, zinc finger DHHC protein, ZOS11-09, BR11, OsGT1, Pentatricopeptide protein, OsMPS, MADS-box* genes, transcription factors, zinc finger family proteins. Further marker analysis and functional cloning are required to understand the association of genes with the grain shape and length.

Keywords: Rice, Grain size traits, Association mapping, Marker trait associations (MTAs), Candidate gene.

IDENTIFICATION AND EXPRESSION ANALYSIS OF *CKX* GENE FAMILY IN *ORYZA SATIVA* L.

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Cytokinin oxidase/dehydrogenase (CKX) enzyme catalyzes the catabolism of cytokinin and reduces the level of this key plant hormone in the cell. Cytokinin mediates plant growth, development, yield and stress tolerance. In this study, we carried out genome-wide analysis of *CKX* gene family members in *Oryza sativa* L. We identified 11 *OsCKX* homologous genes with typical CK binding and FAD-binding domains and they were renamed as *CKX1* to *CKX11* based on their different properties. Additionally, we analyzed phylogenetic relationship, conservative motif, gene structure, cis-acting element of promoter, and expression pattern of *CKX* gene family. Our analysis revealed that the number of amino acid ranged from 505 to 621aa, the molecular weight ranged 52.58 to 66.98 kDa, whereas the theoretical isoelectric point ranged from 6.05 to 12.12 among 11 OsCKX proteins. The phylogenetic tree analysis can group family members into subgroups for better understanding the relation. The genes were more conserved in their structure form. In the upstream region of the promoter, we observed several cis-acting elements, including plant growth and development, biotic and abiotic stress response. Combined with transcriptome data, we have validated the *CKX* gene expression at varying developmental stages in *DROUGHT AND SALT TOLERANCE* (*DST*) gene mutants and WT MTU1010 plants. Taken together, these findings provide new insights into the functions of *CKXs* genes in rice growth and may provide the foundations for future studies aimed at improving rice yield.

Keywords: *CKX* gene family; phylogenetic analysis; collinearity analysis; expression analysis; *Oryza sativa* L.

STRUCTURAL VARIATIONS ALTER THE TRANSCRIPTOME LANDSCAPE UNDER DROUGHT STRESS IN *ORYZA SATIVA*

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Transposable elements (TE) are an important source of genome variability Although it has been demonstrated that their actions– i.e. insertions/deletions/inversions etc. can be triggered by a wide array of stresses in plants, very little is known about their role in stress response regulatory networks, and how their movement is influenced by selection during local adaptation, speciation, domestication and breeding. Here, we carried out the transcriptome landscape of a global rice diversity panel under three different irrigation regimes during vegetative-stage and identified the interplay between the structural variations (SVs) and stress responses in plants. To achieve this goal, we long-read resequenced 180 domestic rice accessions representing the 15 major subpopulations of *O. sativa*, maximizing both genetic diversity and the tolerance variation to drought stress. Comparison of each accession to the *O. sativa* vg. *japonica* cv. Nipponbare reference genome led to the identification of ten of thousand SVs that totaled to an average of 33.70 Mb per genome. We used the SVs as genetic markers in a combined forward genetic approach to pinpoint candidate genes involved in drought stress resistance Traits collected over two dry seasons (2022 and 2023) were also associated with Structural Variants in a genome-wide association (GWA) study. Then traits were correlated with gene expression levels to identify transcript-trait correlations. We performed an expression quantitative trait locus mapping approach to associate SVs with gene-expression levels across the three treatments. Our data highlight the role of TEs plant-environmental adaptation showing that TE are associated with changes in gene expression and phenotypic variability. Our results tackle the complexity of drought-resistance that may provide potential genomic targets for precision breeding of crops with greater stress tolerance.

Keywords: Structural Variations (SV); Trasposable Elements (TE); drought resistance; long-read sequencing; eQTL mapping; pangenomics; transcriptomics; GWAS; environmental stress; diversity panel.

Plant Development: Vegetative and Reproductive

CALLOSE, AN UNSUNG POLYSACCHARIDE FOR MALE MEIOSIS INITIATION AND PROGRESSION IN RICE (ORYZA SATIVA. L)

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Mitosis-meiosis transition in flowering plants entails drastic remodelling of the shape, size, volume and composition of spore mother cells. In anthers, prior to meiosis onset, the pollen mother cells' (PMCs) walls are composed of cellulose, a plant β-1,4-glucan polysaccheride. As the PMCs exit mitosis/enter meiosis, the cellulose is gradually replaced by callose, an another plant polysaccharide made up of β-1,3-glucans. This hyper callose accumulation during meiosis onset is known as a histological hallmark of PMCs entering meiosis. However, the biological significance of such a dramatic switch from cellulose-to-callose for meiosis initiation and progression was largely unknown until now. To explore the impact of callose on meiosis process, I focused on rice GSL5, a callose synthase highly expressed in anthers and two independent mutant lines was generated by CRISPR-Cas9. Our findings reveal that GSL5 is mainly responsible for callose deposition in premeiotic and meiotic anther locules, which is required for timely meiosis initiation and progression and for proper meiotic mode of chromosome condensation and behaviour. Electron microscopy results revealed that callose is required for the stability of the “plasmodesmata”, the cytoplasmic bridges connecting two neighbouring plant cells, aiding in symplastic communication. Further, we also found that callose deposition in anther locules is required for appropriate space maintenance among the anther locular cells during mitosis-meiosis transition. This study answered the longstanding question of the biological meaning of the germ cell-wall remodelling that usually happens during the meiosis onset in majority of flowering plants.

RIBOSOMAL RNA PROCESSING FACTOR (RPF2) REGULATE RIBOSOME BIOGENESIS AND FLOWERING IN RICE.

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Ribosome biogenesis is a complex process involving several ribosomal proteins, ribosomal RNA (rRNA) processing factors (RPFs), small nucleolar proteins etc. More than 400 copies of rRNAs are arranged in tandem repeats. The analysis of 5S rRNA repeats in Indica rice identified SNP variations, different length sequences. The 5SrRNA intergenic regions (promoter) also showed variations and promoter analysis found different cis-element including stress specific transcription factor binding sites. The rRNA processing factor (RPF2) is known to regulate 25SrRNA and 5SrRNA processing in yeast and Arabidopsis, differentially regulated in rice at different stages. AtRPF2 overexpression or downregulation showed dwarf, mottled phenotype. RPF2 found to interact with plant specific SOC1 encoding MADS box transcription factor known to regulate flowering in rice. The cis-element of MADS box proteins were found in 5SrRNA promoter and in ITS2 region of 45SrDNA. The Arabidopsis RPF2 overexpression plants showed early flowering and mutants delayed. The OsRPF2 localized to nucleus and nucleolar region and OsMADS50/56 found only in nucleus. The interactions of rice RPF2 with OsMADS50 and 56 were confirmed by Y2H and Bi-Fc assays. Interactions in nuclear fractions suggesting their role in transcription and in rRNA processing. The MADS50/56 targeting RFT and 5SrRNA promoters were cloned upstream to Luciferase. The RPF2 having sigma motif also binds to 5SrRNA promoter. The transactivation assay with *RFT:Luc* and *5SP:Luc* containing RPF2 and MADS50/56 coregulate expression of Luc. The genetic manipulation studies to understand their role in rice development and flowering is in progress.

Keywords: Flower, ribosomal RNA, MADS, Transcription factors, promoter

INVESTIGATION OF THE ROLE OF MITOGEN ACTIVATED PROTEIN KINASE CASCADE IN REGULATING PHOTOSYNTHESIS IN RICE

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Post-translational modification enables the rapid response of plants towards changing environmental conditions. Phosphorylation is known to play a crucial role in the regulation of photosynthesis. However, Mitogen-Activated Protein Kinase (MAPK) cascade is known to regulate a variety of processes (such as cytokinesis, ovule development, stomatal patterning, cell differentiation during early embryo development, grain size and weight, submergence tolerance, tolerance to abiotic as well as biotic stress), does not have any direct reported role in the regulation of photosynthesis. To elucidate the regulatory role of MAPKs in photosynthesis we investigated the changes in net photosynthesis rate and related parameters in DEX inducible over-expressing (OE) lines of two members of the MAPK gene family namely OsMPK3 and OsMPK6 in rice. Interestingly, significant changes were found in net photosynthesis rate and related parameters in OsMPK3 and OsMPK6-OE lines compared to its wild-type relatives. Additionally, we examined the impact of OsMPK3 and OsMPK6 over-expression on the expression of nuclear encoded genes related to photosynthesis. Given that MAPKs are predominantly localized within the nucleus and cytoplasm, we conducted an exploration to identify other cytoplasmic proteins regulated by MAPKs that might be implicated in the regulation of photosynthesis. Interestingly, our investigation unveiled a dual specificity cytoplasmic protein kinase as a substrate of MPK3 and MPK6, responsible for phosphorylating nuclear-encoded photosynthesis related proteins. This study presents evidence of a novel MAPK signaling cascade that potentially plays a role in the regulation of photosynthesis.

Keywords: MAPK, Phosphorylation, Photosynthesis

IMT AND HSF CASCADE REGULATE SEED VIGOR AND SEED SIZE IN RICE

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Preservation of seed vigor and viability during seed storage (longevity) are essential concerns in agriculture, especially under subtropical climates where seeds of most crop species including rice show rapid seed deterioration and reduced seed viability during storage. Reduction of seed vigor and longevity is often associated with damage to nucleic acids, proteins, and lipids during storage. Previously, our laboratory reported that PROTEIN L-ISOASPERTYL METHYLTRANSFERASE (PIMT, EC 2.1.1.77), a protein-repairing enzyme plays an essential role in preserving seed vigor and viability for prolonged periods. In the present study, while resolving the detailed molecular riddles regarding the role of OsPIMTs in seeds, we found OsHSF as a molecular partner of OsPIMTs in aged rice seeds through MS/MS analyses. The initial co-expression analyses of OsPIMTs and OsHSFs were performed during seed development, maturation, and aging to explore their functional significance. We found high co-expression of selected OsHSFs and PIMT during the late maturation stage, while gradually decreased expression during aging. We observed that OsHSF proteins undergo isoAsp modification during stress conditions that adversely affect their function, while OsPIMT physically interacts and repairs these isoAsp modifications and helps to reestablish their functions. Further, our genetic studies clearly revealed that the PIMT-HSF cascade regulates seed vigor and seed size in rice seeds. For instance, the CRISPR-Cas9 mediated knockout line of both OsHSF and OsPIMTs shows a significant decrease in seed vigor along with seed size to single knockout plant. Overall, our study provides detailed mechanisms of how OsPIMT positively regulates OsHSF function to improve seed vigor and seed size in rice.

Keywords- Protein Repairing Enzyme, PIMT, HSF, seed vigor, seed size

INVESTIGATION OF THE ROLE OF MAPK KINASE IN REGULATING PLANT DEVELOPMENT AND YIELD

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Mitogen-activated protein kinase is an evolutionarily conserved signaling module in the eukaryotes, comprised of MAPKKKs, MAPKKs and MAPKs which transduces signals from the upstream receptor to the downstream targets (mostly transcription factors) via a phosphorelay system. MAPK kinase regulates many developmental processes related to the yield of the plants. In addition, many phytohormones like auxin, cytokinin, gibberellin etc. cross-talk with each other to regulate the yield of plants. Among them, ethylene is an important phytohormone that can regulate many development processes like yield. We wanted to investigate the role of MAPK kinase in regulating plant development and yield. We screened several transcription factors with the MAPKs. We report here interaction and phosphorylation of one of the ethylene-responsive factors (ERF) by MPKs (MPK3/MPK6) by in-vitro phosphorylation, yeast-2-hybrid and Bimolecular fluorescence complementation assays. We also mapped the MAPK phosphorylation site on the selected (ERF) by changing the putative Serine/threonine residues to alanine. In this study, the role of MPK3/6 along with its downstream target ethylene-responsive factors gene that regulates plant developmental processes will be discussed.

Keywords: Ethylene Responsive Factor (ERF); MAP kinase; Rice, Yield,

THE *PLETHORA* GENES ORCHESTRATE POST-EMBRYONIC ROOT SYSTEM DEVELOPMENT IN RICE

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The root system in plants is fundamentally important as it facilitates water and mineral uptake along with structural support. In dicots, the taproot system consists of an embryonic primary root (PR) also called radicle, and the post-embryonic root-borne lateral roots (LRs). Together with PR and LRs, monocots exhibit a highly diversified fibrous root system, which contains post-embryonic adventitious roots (ARs). Rice is not only an important staple cereal food crop, but also a good monocot model plant species to explore fundamental questions about shoot-borne ARs/crown roots (CRs) development, and its morphological diversity associated with evolution. In rice, CRs transdifferentiate from cells adjacent to vascular tissues after cell reprogramming, which is associated with increased transcriptional activity. In our study, we have identified key determinants during rice crown root primordium (CRP) early developmental stages by laser-captured microdissection (LCM)-coupled RNA sequencing (RNA-seq). The subsequent high-resolution map of temporal gene expression provided that the expression of six *PLETHORA* (*OsPLT1-6*) family genes was sharply activated during these stages. *In-situ* hybridization and functional characterization analysis of *OsPLT1* demonstrated that it positively regulates both shoot-borne CRs and root-borne LRs formation in rice. However, a similar analysis of *OsPLT2* revealed that, although it is expressed during CR development, it positively and specifically regulates root-borne LRs formation in rice. Further, *OsPLT1* directly activates *YUCCA1* (*OsYUC1*) and *OsYUC3*, two auxin biosynthetic genes, to induce auxin biosynthesis during shoot-borne CRs formation in rice. *OsPLT1* expression was altered upon pharmacological interference of epigenetic processes, suggesting that *OsPLT1* expression is epigenetically regulated during CRs formation. Expressing *OsPLT1* and *OsPLT2* in *Arabidopsis* lateral root primordium (LRP) transcriptional domain rescued LRP outgrowth defect in *Arabidopsis plt3;plt5-2;plt7* triple mutants. Therefore, our study showed that rice *PLT* genes have acquired species-specific function in regulating

development of shoot-borne CRs while retaining their conserved role in root-borne LRs formation.

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MYSTERIOUS 100 (MYS100) A SMALL PROTEIN CONTROLLING INFLORESCENCE ARCHITECTURE AND TILLER DEVELOPMENT IN RICE

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Rice (*Oryza sativa*) is an economically and nutritionally important crop because of its fundamental role in feeding a large part of the world population. Rice is also used as a model crop to study important agronomical traits including, high yield potential, resistance to abiotic and biotic stresses. Concomitantly, in rice plant architecture depends on the commotion of numerous genes acting in different reproductive meristem types (Gaarslev et al., 2021). Recent studies revealed that stable tiller number is the key determinant to establish panicle number and consequently controls grain yield (Ikeda et al., 2004; 2013).

We are studying the function of *MYS100*, encoding a short disorganized protein of 100 amino acids, expressed during inflorescence development (Harrop et al., 2016). Interestingly, so-far our studies indicate that the *MYS100* gene is exclusively present in the Poaceae (grass) family. Rice loss-of-function mutants revealed its function in inflorescence and tiller development, resulting in a substantial reduction of the tiller number, grain yield and plant size. On the contrary, over-expression of *MYS100* drastically enhanced tiller and seed numbers.

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DECIPHERING THE ROLE OF THE AUXIN RESPONSE FACTORS IN *ORYZA SATIVA* OVULE DEVELOPMENT

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Ovule formation is a key event in the life cycle of flowering-plants, whose main purpose is to form the female gametophyte and develop into seed upon fertilization which guarantee the plant's offspring and ultimately determine yield in seed-crop plants. Therefore, understanding ovule formation in rice is of great importance for potential biotechnology applications to increase crop yield. It has been shown that the phytohormone auxin influencing ovule initiation and pattern definition in other species (Cucinotta et al., 2021).

We have analyzed a rice ovule transcriptome at different developmental stages, OVR1 (ovule at megaspore mother cell process), OVR2 (ovule at megaspore mitosis), OVR3 (ovule containing mature embryo sac) and OVR4 (ovule/seed at two days after fertilization) (Wu et al., 2015). In this dataset we identified several *AUXIN RESPONSE FACTOR* (*ARF*) genes expressed in ovules during the different developmental stages. We decided to focus our attention on three *OsARF* genes based on their expression pattern. We are generating mutants with CRISPR Cas9 technique. Moreover, through *in-situ* hybridization we have analyzed the expression pattern of these genes during ovule and seed development.

We aim to elucidate genetic and molecular mechanisms regulating rice ovule development, focusing on the cross-talk between hormones and genes activity.

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TRANSCRIPTION FACTOR- TF46 MODULATES RICE GRAIN SIZE AND QUALITY

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Being the second most important cereal crop, rice (*Oryza sativa* L.) caters to more than half of the world's population. To compensate the exponential rate of increase in the global population, increasing rice productivity and nutritional quality has been one of the major objectives in the breeding programs over the years. A complete molecular mechanism of seed development has not been deduced till date, though various investigations have laid the role of several signaling pathways including G protein signaling, the mitogen-activated protein kinase (MAPK) signaling pathway, the ubiquitin–proteasome pathway, phytohormone signaling, and transcriptional regulatory factors in ascertaining seed characters. Various transcriptional factors (TF) take part in the different pathways of grain development in combinatorial manner. In this study, TF46, a TF having seed-preferential expression has been studied for its role in seed development. Its transcriptional properties such as activation/repression and localization have been determined. Rice over expression transgenic lines of TF46 have been raised. Phenotypic analysis of vegetative and reproductive traits of these transgenic plants has been done. Grain length and starch production have been found affected by over expression of TF46. Interactions of TF46 with other TFs having a significant role in rice seed development have also been determined. This suggested the importance of TF46 in rice grain development which could be positively used further to enhance the yield potential and quality in rice.

Key words: grain development, rice, transcription factor, starch

Investigating the Regulatory Role of DNA Binding with One Finger Transcription Factor on Seed Vigor in Rice

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Seed vigour represents an intricate yet essential physiological trait in crop plants, as they are pivotal for ensuring the successful establishment of seedlings and exert a significant influence on crop productivity. Transcriptional regulation plays a pivotal role in governing seed development, maturation, and desiccation tolerance, which are important attributes of seed vigour and longevity. In this study, we have investigated the regulatory role of a seed-specific DNA-binding transcription factor called RPBF (Rice P-box Binding Factor) in influencing seed vigour. Our investigation revealed that RPBF plays an important role in modulating the expression of galactinol synthase, thereby governing seed vigour and longevity. Additionally, we observed an elevation in the expression levels of both RPBF and *OsGolS* during seed development. Moreover, we provided evidence of direct interactions between RPBF and the *OsGolS* promoter using multiple approaches, including yeast one-hybrid assays, in-planta promoter-GUS assays, and in-silico molecular docking studies. To functionally assess the role of RPBF, we conducted Agrobacterium-mediated genetic transformations in rice to generate RNAi lines with reduced RPBF expression. In these RNAi lines, we observed a decrease in the levels of galactinol, which is known to contribute to seed vigour. Subsequently, we evaluated the vigour and viability of these transgenic seeds by using controlled deterioration test (CDT) and scoring their germination potential. The results demonstrated that the RNAi seeds exhibited sensitivity to CDT compared to their control counterparts. In conclusion, our study provides compelling evidence that the RPBF plays a crucial role in regulating seed vigour through the transcriptional modulation of galactinol synthase.

Keywords: Seed vigour, Transcriptional regulation, Galactinol, Artificial aging, *Oryza sativa,* Promoter analysis

IDENTIFICATION AND CHARACTERIZATION OF GENETIC DETERMINANTS FOR RICE LEAF DEVELOPMENT AND PHOTOSYNTHESIS

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The leaves play a vital role in photo-assimilation of CO_2 during photosynthesis, which directly impacts biomass and yield. Therefore, leaf developmental attributes, morphological and anatomical, play a significant role in determining crop photosynthetic efficiency. Manipulation of leaf developmental attributes have been shown to enhance and optimize the photosynthetic efficiency in staple crop rice. The natural genetic diversity for leaf developmental attributes and underlying genetic mechanisms offers a valuable resource for optimizing photosynthesis rate. We aimed to identify strong genetic regulators of leaf morphological traits and photosynthetic efficiency employing GWAS. To this end, we performed large scale phenotyping of leaf morphological and photosynthetic parameters for a subset of rice 3K panel. We observed remarkable variation in the observed traits, such as leaf photosynthesis rate ranging from 2.17 to 31.16 µmol m^{-2} s^{-1} and leaf area from 28.59 to 151.81 cm^2. GWAS coupled with haplotype block analysis using a comprehensive set of single nucleotide polymorphisms (SNPs) identified numerous strongly associated SNPs and linked genes for leaf attributes. These included known leaf developmental regulators, such GRFs and GIFs, along with novel regulators of rice leaf development.

Leaf anatomical features, particularly mesophyll cell attributes, in addition to morphological features are strong determinants of leaf photosynthesis rate. We aimed to investigate the genetic basis of mesophyll cell differentiation using transcriptomics approach. Cellular phenotyping revealed the developmental gradient for mesophyll differentiation from rice leaf base to tip. Transcriptomic comparisons of the different zone with varying degree or mesophyll differentiation identified key genetic regulators and gene network modules that operate along the basipetal gradient determining the mesophyll cell features. Together, we identified strong genetic regulators of leaf developmental attributes using GWAS and transcriptomics that can be used to optimize rice photosynthetic efficiency.

A ZINC FINGER TRANSCRIPTION FACTOR-miRNA MODULE CONTROLLING RICE GRAIN SIZE

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According to United Nations and the Food and Agricultural Policy Research Institute (FAPRI) global rice demand is expected to increase to 555 million tons in 2035. Various agronomic traits such as the number of panicles per plant, number of grains per panicle, grain weight, and size are vital factors affecting rice yield. These agronomic traits are regulated by several regulatory molecules such as transcription factors (TFs), small non-coding RNAs, various epigenetic factors etc. TFs are said to be master regulators of any process. One such TF, called TF217, was selected for our study. To functionally validate TF217, transgenic lines overexpressing *TF217* in a seed-preferential manner were generated. Transgenic lines overexpressing *TF217* showed increased grain length and width as compared to WT, revealing its essential role in seed development. Knockout of *TF217* through CRISPR/Cas9 approach led to a seed-lethal phenotype. TF217 knockout plants showed severely reduced pollen viability, resulting in complete loss of seed setting. We identified and validated that TF217 is under the regulation of miR007n at post-transcriptional level. Transgenic lines over expressing miR007n showed similar phenotype as that of TF217 knockout lines. TF217 localised to the nucleus and endoplasmic reticulum, possessed both activation and repression properties and showed homo-dimerization property. Interaction studies demonstrated TF217 to be interacting with one major seed development regulating protein and a histone deacetylase. Taken together, we propose that miR007n-TF217 module regulates rice pollen and seed development, involving interaction of TF217 with a histone deacetylase and an essential rice TF.

Key words: grain, rice, transcription factor, yield

DISSECTING HORMONAL CROSSTALK ORCHESTRATING EARLY STEPS IN NITROGEN MEDIATED AXILLARY BUD OUTGROWTH IN RICE

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Tillers, or panicle-bearing branches, are a key architectural trait that affects rice yield. Tillers grow from the leaf axils of the main culm in a two-stage process; axillary bud (AB) formation and its subsequent outgrowth. The outgrowth of an AB is heavily influenced by external as well as internal factors and is governed by complex molecular circuitry. Availability of nutrients like nitrogen, phosphorus and iron is important for tillering, but the molecular occurrences triggering early stages of AB outgrowth in response to nutrients remain to be addressed. We manipulated nitrogen supply to synchronize AB outgrowth at a precise time point in Indica rice (IR64) seedlings. We observed that ammonium (Am) induces AB outgrowth more efficiently than nitrate (Ni). Comparative tissue-specific RNA sequencing at specific time points revealed that Am triggers early transcriptomic changes compared to Ni, and cytokinin signaling is one of the biological processes to exhibit significant early changes. Pharmacological studies confirmed that cytokinin can induce AB outgrowth and cell division independently of nitrogen supply. Several genes corresponding to auxin signaling and transport also exhibited significant changes in response to Am, Ni and cytokinin. Together, our results suggest that involvement of auxin-cytokinin nexus in response to nitrogen supply fine-tunes AB outgrowth. Efforts are being made to further link phytohormone signaling components and precisely define the molecular footprint lying underneath AB outgrowth in rice. These molecular modules should provide a footing for the manipulation of tillering in context with nitrogen availability in a translational way.

Keywords: Tillering; Nitrogen; Axillary bud; Cytokinin; Cell Division

FUNCTIONAL CHARACTERIZATION OF A WRKY MEMBER IN REGULATING FLOWERING IN RICE

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The WRKY transcription factor is specific to plant kingdom and are known to provide defense against biotic and abiotic stressors. Nonetheless, new discoveries have identified a large variety of WRKYs that influence plant growth and development processes. Mitogen Activated Protein Kinase (MAPK) cascade on the other hand, is an evolutionarily conserved signaling pathway that consists of three canonical steps. A MAPK is activated through phosphorylation by a MAPK kinase (MAPKK) which itself is phosphorylated by a MAPKK Kinase (MAPKKK). The environmental and developmental signals are detected and perceived by specific receptor kinases, which then phosphorylate and transfer the signal downstream MAP kinase cascade. The last component of the cascade, MAPK phosphorylates cytosolic or nuclear proteins, resulting in a specific response. As the WRKY TFs family members are crucial for plant growth and development, we selected two WRKYs for our study. We found that these two WRKY interacts with OsMPK3, OsMPK4, and OsMPK6 and also gets phosphorylated. To get a better insight on the functional role of these WRKYs, we have generated knock-out lines using CRISPR/Cas9 genome editing tool. The knock-out lines exhibit interesting flowering phenotypes. Further, to decipher the possible biological relevance of the phosphorylation, we are developing phosphodead and phosphomimic lines of these WRKYs in rice under the knock-out background.

Keywords: WRKY, MAP kinase, Rice, Development, Yield

AUXIN-RESPONSIVE, DYNAMICALLY EXPRESSED ENZYMATIC ANTIOXIDANT GENES IN RICE CROWN ROOT PRIMORDIA MORPHOGENESIS

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The fibrous root system architecture (RSA) in rice is formed, primarily, of adventitious/crown roots (CRs) originating from the shoot base as a result of tissue trans-differentiation. RSA determines the ability of the plant to endure challenges posed by mechanical, physical, chemical and biological stressors. Reactive oxygen species (ROS), the potent chemical stressors, ensure homeostasis maintenance in a state of chemical equilibrium. In defense against ROS imbalance, the widely reported enzymatic antioxidant defense system (ADS) members including peroxidases, glutathione s-transferases (GSTs), glutathione reductases (GR) and thioredoxin play a pivotal role especially, for maintaining the cellular redox homeostasis and RSA. We probed the NGS transcriptomics data generated by our group, to ascertain the aforementioned enzymes responsive to auxin hormone impetus as well as showing dynamic expression across CR primordia (CRP) establishment and progression. By quantifying the levels of gene expression upon auxin stimulation by RT-qPCR, the auxin-mediated differential transcriptome data were confirmed. Hereon, RNA in situ hybridization reveals a set of peroxidases, GSTs, a GR and a thioredoxin posing spatially restricted expression. Conclusively, our findings showcase multifaceted investigation into dynamically expressing, auxin (IAA) responsive, ROS metabolizing enzymatic candidates partaking in the CR primordia establishment regime.

Crown roots (CRs); CRP morphogenesis; Dynamic expression; Reactive oxygen species (ROS); Enzymatic antioxidants

AUXIN-*OSWOX10* REGULATORY MODULE ENSURES DE NOVO ROOT ORGANOGENESIS

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Plant growth regulatory/hormonal dynamics plays crucial role in every developmental stage of the plants. Auxin is known to be major contributor of organ development and patterning. Fibrous root system (rice) is comprised of ephemeral short-lived primary root and shoot-borne adventitious/crown roots (AR/CR), along with root-borne lateral roots (long L-type and short S-type). Our histochemical analyses revealed the overlap of auxin response with CR primordia development programme. The expression of auxin biosynthesis genes and Auxin Response Factors are also activated during crown root initiation and outgrowth. Our detailed functional characterisation of *OsWOX10* revealed its requirement in timely control of CR development. LCM-Seq performed on captured primordia showed sharp increase in *OsWOX10* expression during CRP initiation but gets decreased in outgrowth stage. Exogenous auxin application induces *OsWOX10* sharply and significantly w.r.t mock. Occasionally, we observed precocious rooting upon overexpressing *OsWOX10* ectopically. It promoted de-novo root organogenesis. Overexpressed Os*WOX10* induced rooting in even shoot induction media. While *OsWOX10* downregulation significantly decreased the ARs from regenerated plantlets. Root induction in-vitro showed same results. We also studied this gene for its evolutionary conservancy. Constitutively overexpressed *OsWOX10* in *Arabidopsis* induced adventitious roots from hypocotyls. Together, these findings suggest common Auxin-*OsWOX10* regulatory module for adventitious root organogenesis across plant species.

Keywords: Adventitious/Crown roots, *OsWOX10*, Auxin, Auxin Response Factors, de-novo root organogenesis.

AUXIN SIGNALLING AND ITS SPATIAL RESTRICTION DURING CROWN ROOT PRIMORDIA DEVELOPMENT IN RICE

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Vascular plants are made up of overlapping and amalgamating parts, which largely form a continuum. This continuum often results in instances when one organ develops from another. In rice, this is evident in the formation of crown roots which arise from stem tissue, making it an excellent model for studying tissue trans-differentiation. Auxins, a group of low-molecular weight compounds, have been attributed to a number of facets related to root development. This includes cell fate acquisition to the initiation of meristem, followed by the emergence and elongation of the roots. Many evidences have suggested the occurrence of auxin maxima for root primordium formation. In order to study the spatio-temporal activation of auxin signalling during crown root primordium (CRP) development, DR5rev (a synthetic auxin-responsive promoter) was used in conjugation with yellow fluorescent protein (YFP). Using RNA *in situ* hybridization and immunohistochemistry against YFP mRNA and protein respectively, a strong auxin response was observed during the initiation of CRP. Eventually, this auxin response got restrained to the tip of the outgrowing primordia. DR5rev:*GUS* lines were also used to study this phenomenon. Overall, this data suggests the onset of auxin response during CRP initiation and its ensuing restriction in specific domains during CRP development.

Keywords: Crown root primordium, auxin response, in situ hybridization, DR5, GUS assay

PLETHORAS: MASTER REGULATORS OF RICE ROOT SYSTEM ARCHITECTURE

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Roots, the invisible part of plant, in spite of being embedded in soil, is very important in terms of nutrient and water absorption for plant growth. Rice root system is majorly composed of shoot-borne adventitious roots known as crown roots (CR) which develop from the base of stem and is an excellent model system for studying trans-differentiation process. Phytohormones, particularly, Auxins and the regulated transcription factors are major determinants at various stages of crown root development and are involved in maintaining stem cell identities. One of our studies involving genome wide identification of genes regulated by auxin and Laser Capture Microdissection -Sequencing (LCM-Seq) performed on CR primordia at different stages revealed various Transcription factors (TFs) involved in meristem establishment in rice. *PLETHORA*(*PLT*) genes belonging to AP2/ERF family of transcription factors involved in tissue differentiation, are highly induced upon auxin treatment. RNA in-situ hybridization revealed that *PLT*s are highly expressed in crown root tissues. Genetic redundancy is also observed in rice *PLT*s as downregulation of two of them causes embryo lethality and at later stages affects root system architecture, including both crown roots and lateral roots suggesting role of *PLT*s in CR formation. Further studies will help in unravelling the regulatory modules of the *PLT*s involved in acquiring pluripotency and cell fate conversion during adventitious root formation in rice.

Keywords: Adventitious/ Crown roots; Tissue trans-differentiation; Pluripotency; LCM Seq; *PLT;* AP2/ERF.

THE SMALL RNA BIOGENESIS IS REGULATED BY MAP KINASE-MEDIATED PHOSPHORYLATION OF OSCDKD

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Cell division and proliferation play an important role in determining the growth of living organisms. Cyclin-dependent kinases (CDKs) serve as the primary regulators of cell division, with their activity being controlled by regulatory subunits known as cyclins and phosphorylation by CDK-activating kinases (CAKs). The RNAPII CTD phosphorylation by CDKD and CDKF regulates small RNA biogenesis in plants. In this study, we identified that MAP kinases co-express with CDKs. Specifically, OsMPK3, OsMPK4, and OsMPK6 physically interact with OsCDKD and its regulatory subunit, OsCYCH. Furthermore, we have identified OsCDKD and OsCYCH as phosphorylation targets of MAP kinase. We also mapped the MAP kinases phosphorylation sites on OsCDKD. We found that the WT OsCDKD and phosphomimic OsCDKD phosphorylated the RNAPII CTD while phosphodead OsCDKD was unable to phosphorylate the RNAPII CTD. Next, we found the accumulation of small RNA precursor transcript in the phosphodead OsCDKD over-expression line as compared to the phosphomimic and over-expression line of the WT OsCDKD. Additionally, over-expression of WT OsCDKD and phosphomimic OsCDKD leads to enhanced plant growth as compared to the wild type while the phosphodead and kinase-dead OsCDKD are similar or comparable to the WT plant. Thus, our findings indicate that MAP kinases phosphorylate and activate OsCDKD thereby controlling small RNA biogenesis and plant growth.

FUNCTIONAL INNOVATIONS OF OSPLT2 IN ROOT BRANCHING IN PLANTS

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The root system architecture (RSA) in monocots have a fibrous root system known as adventitious root system or crown root (CR) besides the primary root (PR) and lateral root (LR); which later forms a compact network of roots adjacent to the soil surface and absorbs mineral nutrients. Crown roots are shoot-born roots in origin and are examples of the transition from differentiated to newly dedifferentiated cell identity. In Arabidopsis, PLETHORAs (PLTs) are essential for stem cell specification and maintenance in the RAM, but the functions of its homologous genes in monocot rice remain unknown. Rice *OsPLT2* is one of the closest homologs of Arabidopsis *AtPLT5*, which functions redundantly with *AtPLT3* and *AtPLT7* in LR formation and is also expressed during CRP initiation and outgrowth in CRP as cited in LCM-seq data. We investigated the two AP2 domains containing transcription factor *OsPLT2* as a unique and redundant function with *OsPLT5* in crown root development. Single mutant *osplt2* showed less LR originating from PR and CR while double mutant *osplt2osplt5* exhibited short root (both PR and CR) with less LR as an additive phenotype. Whereas, *OsPLT2* overexpression leads to high-density lengthy LR formation from both PR and CRs. Importantly, the CR number was not significantly affected. *OsPLT2* expression under the Arabidopsis *PLT5* promoter rescued LR outgrowth in the triple mutant *plt3;plt5-2;plt7*. These indicate that *OsPLT2* has a conserved function in regulating root-borne LRs and also shows redundancy with *OsPLT5* in CR formation.

Keywords: Root system architecture (RSA), crown root (CR), lateral root (LR), PLETHORAs **(PLTs)**, conserved function, genetic redundancy

UNEQUAL GENETIC REDUNDANCY AMONG THE RICE TRANSCRIPTION FACTORS OSMADS2 AND OSMADS4 REVEALS DISTINCT ROLES IN FLORET ORGAN DEVELOPMENT

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Functional diversification of transcription factors and of their downstream targets can contribute to emergence of new organ morphologies, for example rice lodicules. These small fleshy rice petal analogues aid floret opening, thus facilitate pollination and fertility. To better understand mechanisms underlying organ specification, we investigated functions of *OsMADS2* and *OsMADS4*, the rice *PISTILLATA* (*PI*) paralogs. Phenotypes in *osmads2* null mutants reiterated its nonredundant role in lodicule development seen in prior studies with *osmads2kd* lines. Furthermore, null mutants revealed new roles for OsMADS2 in flowering time, floral meristem size, floral organ number, cell wall metabolism, and sterile lemma development. Although downregulation of *OsMADS4* did not affect floral organs, doubly perturbed *osmads2*$^{d8/d8}$ *osmads4kd* florets exhibited severe organ abnormalities and initiated parthenocarpy. Remarkably, ubiquitous overexpression of *OsMADS4* in *osmads2* rescued the abnormalities of *osmads2* though impaired anther development and reduced seed setting. This finding suggests that increased ubiquitous *OsMADS4* activity can be detrimental to anther development. To uncover genes whose (in)direct regulation may contribute to *osmads2* floral phenotypes, we combined the genome-wide identification of OsMADS2 binding regions (ChIP-Seq) with transcriptome profiling (RNA-Seq) of panicles. Several OsMADS2 target genes that are implicated in lodicule and stamen development and floral organ number control were uncovered. Altogether, our results provide insights on the underlying molecular mechanisms of rice *PI* paralogs in floral organ specification, expand our understanding of their conserved functions and their diversification and reveal species-specific differences.

Keywords: Rice, *OsMADS2*, *OsMADS4*, *PISTILLATA*, lodicule, petal, stamen, floral organ number

INVESTIGATING THE ROLE OF POST-TRANSCRIPTIONAL MECHANISMS IN COORDINATING THE REGULATION OF JASMONATE SIGNALING DURING STRESS CONDITIONS

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Rice is a staple food worldwide; however, it is exposed simultaneously to various stresses. Plant hormones like jasmonic acid (JA) act as key signaling compounds in plant stress responses and development. Despite its importance, exaggerated JA signaling would trigger an overactivation of the defense response which in turn comes at the expense of plant growth. Alternative splicing (AS) is a crucial post-transcriptional mechanism to reprogram gene expression profiles and expand the transcriptome and proteome diversity. Interestingly, there is a conservation of an intron in the JAS motif of JAZ genes in evolutionary diverse plant species. In rice, intron retention (IR) event was detected in eight JAZ through Reverse transcription (RT-PCR). IR introduces a premature stop codon in the transcript resulting in the formation of truncated OsΔPYJAZ isoforms. Our qPCR data suggests that JA treatment quickly upregulates both OsJAZ and OsΔPYJAZ isoforms, highlighting the importance of elimination of the Jas domain for proper regulation of JAZ function. Next, we used qPCR to measure the spliced form/non-spliced form ratio of transcripts in the root and shoot tissues of rice seedlings exposed to drought conditions. Interestingly, we found that drought induced the OsΔPYJAZ3 isoform suggesting an increase in the repressive form of OsJAZ3. Our yeast-two hybrid analysis suggests that ΔPYJAZ isoforms of few JAZ genes have reduced capacity to form complex with OsCOI1b and as a result these isoforms might show stability. Thus, together these results suggest that AS of JAZ genes might contribute towards desensitization of the JA pathway.

Keywords: Jasmonate; drought; alternative splicing.

NOVEL MORPHOLOGICAL AND PHYSIOLOGICAL MARKERS TO IDENTIFY ANDROGENICALLY DERIVED HAPLOIDS IN RICE (*ORYZA SATIVA, L*) AT EARLY GROWTH STAGES

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In rice, a globally important staple food crop, doubled haploid production through androgenesis is increasingly being employed in breeding programs. Amongst the androgenic rice lines, doubled haploids are formed spontaneously at about 50-60%, while the remaining 40-50% of plants remain as haploids. As haploids cannot be easily identified, it is routine to grow all the rice androgenic lines till maturity and harvest the seeds from the fertile doubled haploids. In this regard, any methods which help to identify haploids easily at an early developmental stage would enable to subject them for colchicine treatment to increase doubled haploid production efficiency besides eliminating the operational cost for maintaining the haploids till maturity. Towards this, we discovered physiological and morphological markers that would help identifying haploids and doubled haploids in rice. The haploids invariably have acute leaf apex, which is easily distinguishable from the doubled haploids that attenuated leaf apex shape. Further, the haploids were found to be noticeably different from doubled haploids in photosynthetic rate, transpiration rate, stomatal conductance and morphology of lodicules, stigma and style, features which have not been reported before. Most importantly, very high per cent accuracy in the prediction of ploidy level was observed when haploids were identified at an early developmental stage based on the leaf apex shape and the results verified with flow cytometry perfectly matched with the leaf apex shape. The study therefore establishes 'acute leaf apex shape' as an accurate visual marker to rapidly identify haploid rice lines at an early developmental stage cost-effectively.

Keywords: Anther culture, Acute leaf apex, Attenuate leaf apex, Haploids, Doubled haploids, Markers

INVESTIGATING THE ROLE OF RICE TRANSCRIPTION FACTOR RFL IN MERISTEM FATE: FUNCTIONS IN VEGETATIVE, REPRODUCTIVE INFLORESCENCE AND FLORAL MERISTEMS:

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Rice is an annual crop plant wherein flowering time, body architecture, and growth rate affects the yield. *RFL* (ortholog of Arabidopsis *LEAFY*) in rice has been shown to be involved in regulating plant architecture (vegetative and reproductive) and flowering time. Whereas, in *Arabidopsis thaliana*, *LEAFY*, a key transcriptional regulator, triggers floral development in emerging lateral meristems. Using CRISPR-Cas9 we have raised *rfl* mutant lines with weak and strong alleles (one codon deletion and one base pair insertion respectively), showing delay in reproductive transition, reduced plant height and panicle branching, altered floral morphology, and compromised grain yield. Transcriptome analyses using the stable segregating mutant lines with one amino acid deletion in the DNA-binding domain, has enabled us to identify novel pathways regulated by *RFL*. To investigate the direct targets of *RFL* in rice, we have performed ChIP-seq from young vegetative shoot meristems with incipient axillary meristems and young inflorescence meristem using antigen affinity-purified anti-RFL antibody. The study reveals that *RFL* acts by directly and indirectly regulating genes involved in controlling flowering time, activating the panicle branching genes, and regulating the floral organ identity genes, plausibly by directly targeting epigenetic modifier, as compared to *AtLFY* where it acts by directly activating floral organ patterning genes. Additionally, *RFL* regulates expression levels of auxin, brassinosteroid, GA, strigolactone metabolism and signaling pathway genes to control the plant architecture. Altogether our work gives insights into the genetic network that elucidates the functional divergence of RFL in rice.

Keywords: Rice, CRISPR-Cas9, *RFL*, *LEAFY*, plant architecture, flowering time, floral morphology

A NOVEL METHOD TO PREDICT CALLUSOGENESIS IN RICE ANTHER CULTURE

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Anther culture technique stands out as the most efficient means of developing haploids and homozygous doubled haploid plants in a short window. However, the practical application of this technology in rice improvement is still limited. While anther culture is successfully done in Japonica rice, its effectiveness in Indica rice is very much limited due to its recalcitrant nature. Rice anther culture is highly genotype specific and therefore, standardization of culture conditions is a prerequisite for successful anther culture. After inoculation, it takes nearly one month for callus emergence and not all inoculated anthers proceed to form callus, emphasizing the need for a method enabling early callus detection. Such a technique would not only be advantageous but also economize resources and time. While culturing rice anthers in-vitro, we observed the presence of small water droplets on the anther surface across all culture plates forming shortly after inoculation. Although the significance of these droplets remains unknown, we speculate that they may function as a mechanism for anther desiccation, similar to the process facilitating pollen shedding through locular wall splitting. An intriguing observation was the correlation between callus formation and the presence of water droplets, suggesting a potential link. The presence of water droplets therefore could be marked as an indicator for early detection of callus development in rice, offering a valuable insight into the efficiency of the anther culture process.

OVER-EXPRESSION OF *OSMIRNA397B* AFFECTS YIELD TRAITS BY DOWN-REGULATING ITS TARGET *OSLAC* GENES IN BLACK RICE (*ORYZA SATIVA*, CV. CHAKHAO AMUBI)

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Black rice is a pre-eminent crop in North-eastern India. Chakhao cultivars are also called purple rice due to the presence of anthocyanin pigment. Black rice has high nutrition, and medicinal values since it is a source of high protein, iron, vitamins and antioxidants. Despite of high demands and nutrition values, yields of these cultivars are very poor, as they are highly susceptible to diseases and environmental stresses. Past studies reveal that role of miRNAs in controlling rice yields and suggested that miRNA397 and miRNA408 positively regulate rice yields in Japonica cultivars. Therefore, we studied the role of *OsmiRNA397* in Chakhao amubi cultivar, and we showed that the expression of *OsmiRNA397b* is higher than *OsmiRNA397a* in all the different developmental stages of Chakhao amubi. *OsmiR397* is highly expressed in pistil followed by anther, seedlings, leaf, flag leaf, young panicles, root and stem. To elucidate its functional significance, the over-expressing transgenic lines were generated and analyzed. Phenotypic evaluation of these transgenics showed differences in flowering time, panicle architecture and in grain size as compared to wild-type plants. Panicles heading was found to be 8-10 days earlier in the wild-type as compared to transgenic lines resulting in delayed flowering. The increase in the number of primary and secondary branches led to the increased spikelet number. SEM analysis of stem revealed that the striking difference in the increase in number of vascular bundles and pith diameter in transgenic lines as compared to wild-type. *OsmiRNA397b* down-regulated its target Laccase family genes and increases its sensitivity to brassinoisteroids in all the transgenic lines. Collectively, our work shows that the over-expression of *OsmiR397b* causes delayed flowering and it positively regulates grain yields by increasing the panicles branches, number of vascular bundles and influences yield traits in Chakhao cultivars.

Key words: Black rice, miRNA397, Chakhao amubi, Yield

RICE LEAF TRANSCRIPTOME DYNAMICS REVEAL NOVEL GENE REGULATORS GOVERNING LEAF DEVELOPMENTAL AND PHYSIOLOGICAL PROCESSES

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Leaves are the prime photosynthetic organs of plants and leaf developmental features strongly influence overall crop performance. Therefore, understanding the detailed genetic mechanisms regulating leaf development is of utmost importance for desirable optimization of crop performance. Leaf developmental features have undergone significant changes during the course of evolution and domestication. For example, the leaf morphological and anatomical features of cultivated/domesticated rice varieties are remarkably different from their wild relatives. These differences in wild and cultivated rice allow a systematic comparative study of leaf growth and developmental differences and underlying genetic basis. We performed comparative transcriptomics of the selected cultivated and wild rice accessions at four different leaf developmental stages (SAM + Pi, P3, P4 and P5), capturing initiating leaf primordia to photosynthetically active mature leaves. Differential gene expression analysis followed by clustering of genes delineated the gradient of gene expression and the relevant biological processes across the leaf developmental stages for each accession. Genes regulating cell cycle and growth and core leaf developmental traits were expressed at higher abundance in SAM + Pi and P3 development stages. In contrast, genes associated with chloroplast development and photosynthesis-related processes were highly expressed at the P4 and P5 stages. The gene co-expression networks identified the potential conserved and novel regulators of developmental and photosynthetic processes at different leaf developmental stages of the contrasting rice accessions. The detailed functional studies of the potential gene regulators could be instrumental for optimizing the leaf growth and development for improved photosynthetic and physiological performance.

Keywords: Cultivated and wild rice, Leaf development, Transcriptomics, Gene regulatory networks

IN SILICO AND MOLECULAR CHARACTERIZATION OF *GA2OX* GENES INVOLVED IN RICE TILLERING

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The development of semi-dwarf rice varieties significantly boosted food production by identifying the *sd-1* gene, which governs the GA20ox enzyme involved in gibberellin biosynthesis. Loss of *sd-1* function led to shorter plants with more tillers, improving yields and lodging resistance. An alternative approach is to target *GA2ox* genes which is responsible for gibberellin breakdown and overexpression of this gene resulted in shorter, high tillering semi-dwarf varieties. Earlier, *OsGA2ox* gene family was reported as a candidate gene for tiller number (TN) among 1052 QTLs reported in Meta-QTL analysis. In this study, three genes i.e., *OsGA2ox5, OsGAox6, OsGA2ox7* were investigated for in silico and molecular characterization. The sequence data on the three selected genes was retrieved from Rice SNP Seek database. Tiller number (TN) data on a set of 199 genotypes from 3k rice panel grown across two seasons in lowland acidic soil conditions in Meghalaya was used for marker-trait association studies. Further, statistical analyses (chi square and *t*-test) revealed significant SNPs and InDels in *OsGA2ox5* and *OsGA2ox7*, while *OsGA2ox6* had none. InDels in the 5' UTR of *OsGA2ox5* were assessed for binding sites of cis-acting regulatory elements (CRE) using NewPLACE database and it revealed their significant roles in auxin/salicylic acid and gibberellin pathways. Three haplotypes (based on combination of Indels present in 5′ UTR) showed significant association with TN. The specific SNPs in *OsGA2ox5* and *OsGA2ox7* were validated using ARMS-PCR to aid in selecting genotypes with high tiller numbers. This study emphasises upon the identification of significant SNPs in the genes putatively associated to tiller number in rice.

Keywords: ARMS-PCR primers, Cis-acting regulatory elements, *GA2ox*, Haplotypes, InDel, SNP.

Genome-wide Analysis of Expression Signatures Driven by Genetic Regulators Identify Key Pathways in Rice Floral Meristem Establishment and its Determinate Development

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Rice inflorescence architecture is a major determinant of yield. The apical inflorescence/panicle meristem develops by generating a sequence of main and secondary branch meristems that finish in spikelet meristems, each with a single floret meristem. The combinatorial action of the ABCDE class of floral homeotic transcription factors defines floral organ identity in floral meristems. Previous studies from our lab have established the roles of a few key rice transcription regulators that control floral meristem and organ identity using functional genetics tools such as knockdown, overexpression, and knockout transgenic rice lines. To decipher the global developmental transcriptome during floret meristem establishment and organ development, we have generated several NGS-based transcriptome datasets from wild-type panicles binned to represent three broad development pools (floral meristems, floral organ primordia establishment, organ differentiation). Co-expressed genes were identified to decipher how their coordinated action could contribute to floral meristem specification and maintenance vs. termination and organ specification. We identified sets of co-expressed genes belonging to specific biological processes that characterize the development pool. Meta-analysis was performed over publicly available mutant transcriptome and microarray data sets of key MADS-box genes including OsM1, OsM34, OsM6, OsM32, and other class genes crucial in floral meristem maintenance, organ boundary, and organ identity. These analyses support a hypothesis of a dynamic switch in regulatory interactions between the rice LOFSEP factors and meristem stem cell homeostasis factors OSH1 and OSH15 during the floral transition. We propose this timely switch that ensures a determinate rice floret.

Keywords: Floret meristem, MADS-box, LOFSEP, homeostasis

Plant Nutrition & Sustainable Rice Production

THE ROLE OF RHODOTORULA MUCILAGINOSA JGTA-S1 IN SHAPING RICE MICROBIOTA TO IMPROVE RICE GROWTH AND DEVELOPMENT

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Rhodotorula mucilaginosa JGTA-S1 is a basidiomycetous endophytic yeast isolated from *Typha angustifolia*. This yeast colonizes rice plants as a surrogate host and improves its growth and nitrogen nutrition. According to a recently performed whole genome shotgun (WGS) sequencing data JGTA-S1 harbors several endobacteria. These endofungal bacteria which includes diazotrophs potentially play important roles in growth-promotion of rice plants. Our previous results showed that certain bacteria are able to invade the yeast cell at a given phase of its lifecycle. Our recent study indicates that the yeast modulates the rice endophytic microflora. This could occur either by the release of JGTA-S1 endobacteria into the rice endosphere or by selective enrichment of endophytes endogenous to the rice plants. To distinguish between the two possibilities, we isolated endophytes from JGTA-S1 treated rice plants and compared them with those isolated from untreated plants. The bacteria obtained from only the JGTA-treated plants were searched within the JGTA-S1 metagenomic reads to identify potential bacteria of yeast origin. Given that, all endosymbionts and plant endophytes may not be culturable, a WGS metagenome sequencing from JGTA-S1 treated vs. untreated plants will be carried out to understand the reason behind the selection of bacteria inside the rice endosphere.

Keywords: Rice plants, yeast, endosymbionts, nitrogen nutrition

IDENTIFICATION OF A NOVEL MIRNA WITH POTENTIAL ROLE IN RICE SEED

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Micro-RNAs (miRNAs) are 21-24 bases long non-coding RNA molecules which play vital role in mRNA regulation at the post-transcriptional level and regulate multiple biological processes, including grain yield in rice. Rice grain development and yield are complex traits which are regulated by multiple genetic factors, and it has been observed that miRNAs also play important role. This study is aimed to identify novel miRNAs, which play important role in the rice seed. For this purpose, firstly we have identified a novel miRNA which has very high expression in seed in multiple publicly available RNA-Seq data. We have checked the expression of this miRNA in different tissues through stem-loop Q-PCR, and found that this is highly expressed in the shoot as well as at late stages of seed development. Furthermore, we identified five potential target genes of this miRNA through predicting complementarity by publicly available bioinformatics tools. To validate the miRNA targets, Dual Luciferase assays in nicotiana leaves were performed and it revealed that indeed all of the five target genes are targeted by this miRNA. Gene expression analysis of these target genes and this miRNA in different tissues highlighted inverse correlation in expression of the miRNA and three of the target genes. Thus, all these results suggest potential role of this miRNA in seed development/ maturation/nutrient filling. We have started experiments to over-express and knock-down this miRNA through CRISPR-Cas9 approach to further explore its role in seed. Results from this study will help to understand the seed development/grain filling and develop improved rice varieties.

NON-DESTRUCTIVE ASYMPTOMATIC DETECTION OF BACTERIAL BLIGHT DISEASE IN RICE USING SMART SENSOR

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Bacterial blight disease (BBD) is the most devastating disease in rice caused by *Xanthomonas oryzae pv. oryzae (Xoo)*. It results in approximately 30-40% reduction in rice yields throughout the world. Manual detection of BBD at an asymptomatic stage is difficult since it takes four to eight days for symptoms to appear on leaves after infection. Once the disease is detected based on symptoms, it is too late to control bacterial propagules from spreading the disease. An efficient and non-destructive detection method is imperative for detecting BBD at the earliest stages and limiting their spread. Light-based sensing technology is used in this study to detect BBD at the early asymptomatic stage. The amount of transmitted light is found to be inversely proportional to the bacterial population growing inside the leaf tissue Photoresistor(s) were used to measure transmitted light after monochromatic LED light was applied to healthy and infected leaves. Based on the symptoms on the leaves, the amount of transmitted light was classified as healthy, diseased (asymptomatic), and diseased (symptomatic). Using the transmitted light data, multivariate, and machine learning classification models were constructed using partial least squares discrimination analyses (PLS-DA), random forests (RF), back propagation neural networks (BPNN), and gradient-boosting decision trees to identify diseased leaves at an early stage of disease (without having disease symptoms). The accuracy of the BBD prediction was externally validated, and ~85% accuracy was observed for early detection. In this way, light-based sensors can be effectively used to detect rice bacterial blight disease at an early stage without causing any damage. BBD can be prevented and controlled with this technology to prevent rice yield reductions.

Keywords: Rice, Bacterial blight, *Xanthomonas,* Biotic stress, Disease management, Smart sensor.

DIGALACTOSYLDIACYLGLYCEROL SYNTHASE 1 IS CRUCIAL FOR LIPID ALTERATIONS AND PHYSIOLOGICAL ADAPTATIONS UNDER PI-STARVATION IN RICE

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Plants need phosphate (Pi) for proper growth and development, but it is often insufficient in soil. Under Pi-starvation conditions, membrane lipid remodeling is reported to be an important strategy to utilize the membrane-bound Pi. Under this process, phospholipids are replaced by non-Pi-containing galactolipids (MGDG, DGDG) and sulfolipids (SQDG). Among these lipids, DGDG are major lipids that replace the phospholipids in extra-plastidic membranes. In rice, there are five DGDG synthase genes; *OsDGD1-5*. Gene expression analysis revealed *OsDGD1* as the highly upregulated gene under Pi-starvation. We found that the expression of *OsDGD1* is regulated by Pi status of plants and OsPHR2 transcription factor. To study the role of *OsDGD1,* knock-out (KO) lines were generated using CRISPR/Cas9 system, and marker-free gene edited lines were obtained. Phenotypic analysis of *OsDGD1* KO lines showed a significant reduction in shoot/root length, biomass, and contrasting alteration in root system architecture (RSA). Pi content was also found to be altered in KO lines, leading to a change in Physiological Pi-use efficiency (PPUE) as well as Pi acquisition efficiency (PAE). Lipidome analysis revealed reduced DGDG levels leading to reduced Pi remodeling, thus affecting PPUE and PAE. We also found an increased MGDG: DGDG ratio in OsDGD1 KO lines. As an increase in MGDG: DGDG ratio influences endogenous JA levels; therefore, we performed JA insensitivity assays and expression analysis, which revealed a connection between JA and the resulting altered phenotype. Our study thus shows the role of *OsDGD1* in controlling plant growth and development by influencing lipids and JA signaling in rice.

FIELD SCREENING OF RICE GENOTYPES FOR ENHANCED GROWTH, PHYSIOLOGICAL TRAITS AND GRAIN YIELD UNDER GRADED N APPLICATION.

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Rice is a major food crop and a key dietary source of energy and proteins. Nitrogen (N) is an essential macronutrient and one of the most critical limiting factors for rice production. Only 30%-50% of applied N is taken up and the unused N is lost to the environment, and is assessed that around 60% of N applied is in excess for rice. Improving the utilization efficiency of N is essential to achieve sustainable agriculture. In this study, 95 rice genotypes were evaluated under four graded levels of N fertilizer application viz., N150 (150 kg N ha^{-1}), N100 (100 kg N ha^{-1}), N50 (50 kg N ha^{-1}) and N0 (0 kg N ha^{-1}) at ICAR-IIRR farm during Kharif-2022 (wet season). Flag leaf traits, SCMR values and chlorophyll fluorescence traits were measured during 50% flowering stage. Plant height and number of tillers $plant^{-1}$ recorded at physiological maturity. Significant increase in plant height, number of tillers $plant^{-1}$, SCMR values and total dry matter was noticed with increased N application. Increased application of N significantly delayed mean days to 50% flowering from 109 days at N0 to 115 days at N150. Flag leaf length, width, area and dry weight increased significantly with increased application of N. Maximum quantum yield of PSII (F_v/F_m), actual quantum yield of PSII (ϕPSII), Electron transport rate (ETR) and coefficient of photochemical quenching (qP) enhanced significantly while coefficient of non-photochemical quenching (qN) significantly reduced with increased application of N. In comparison with N100, mean grain yield reduced by 45.16% with N0, 21.43% with N50 and enhanced by 22.32% with N150. MTU-1010 (9.99%), Vasumati (10.51%), DRR Dhan-58 (10.59%), Varadhan (10.68%), Phalguna (10.83%) and DRR Dhan-56 (10.95%) exhibited minimum reduction in grain yield with N50, compared to N100. The better-performing genotypes can be further studied in-depth for nitrogen use efficiency (NUE) components and can be used as donors in breeding programmes for NUE.

Key words: Rice, Nitrogen, F_v/F_m, NUE and Grain Yield

IDENTIFICATION AND CHARACTERIZATION OF RICE CONTRASTS FOR GRAIN MICRONUTRIENTS LOADING

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Rice being an important cereal food crop for half of the world's population lacks essential micronutrients, particularly Zinc (Zn) and Iron (Fe). Zn homeostasis limits grain Zn content at different stages, includes root uptake from the soil, xylem supply to shoot and grain loading. In this study, 135 whole genome sequenced Indica rice accessions along with 21 improved varieties were used to assess the micronutrient levels (Zn and Fe) in leaves and mature grain. Results showed significant genotypic variability in both leaf and grain micronutrient content. Leaf Zn ranged from 1.5 to 10.6 mg/100g dry weight (DW) with a mean of 3.8 mg/100g DW. The grain Zn ranged from 1.6 to 15 mg/100g DW with a mean of 4.6 mg/100g DW. The genotypes with high leaf Zn but contrasting for grain Zn content were selected for understanding the genetic basis of differential grain Zn content among rice accessions. Towards this, expression of genes associated with Zn homeostasis and haplotype analysis for these genes were performed. qPCR analysis showed higher expression of OsZIP4, OsZIP5, OsZIP9, OsYSL9, OsYSL15, OsVIT1 and OsHMA2 genes in high leaf-high grain genotypes GEN_RIC 273, GEN RIC_373 and GEN RIC_259 and lower expression in high leaf-low grain genotypes GEN_RIC 250, GEN_RIC 236 and GEN_RIC 421. By haplo-pheno analysis, two haplotypes viz., *OsZIP3* and *OsZIP9* for leaf Zn content and *OsNRAMP3* for grain Zn content were identified. More significant difference between the haplogroups were seen for *OsNRAMP3*. These haplogroups have to validated in other genotypes with differential Zn content.

Keywords: Rice; zinc; iron; micronutrients; genotypic variation; homeostasis; gene expression; haplotype

RACKING THE YIELD-GPC CONUNDRUM: THE TPU LIMITATION STRATEGY IN CONTRASTING RICE CULTIVARS

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Protein is vital for physical health and cognitive function. Digestibility and absorption of rice protein stands out as it is more efficiently processed by the human body compared to pulse proteins. Rice, being the primary food source for over half of the global population, plays a vital role in providing essential nutrients for maintaining a healthy lifestyle. Therefore, enhancing the protein content in rice grains (GPC) and improving its digestibility is a significant goal. However, the challenge is optimizing rice grain protein content (GPC) and its digestibility without compromising grain yield. This study contributes valuable insights into the delicate balance between carbon and nitrogen assimilation in different rice cultivars with varying GPC. We compared rice varieties with high GPC (Navara and CR Dhan 311) and low GPC (IR-30864 and Dhaksha) in terms of their photosynthetic efficiency, protein accumulation during various stages of grain development, and amino acid composition. The low GPC varieties exhibited superior carboxylation efficiency, PSII activity, and more efficient utilization of Triose Phosphate when compared to the high GPC varieties. High GPC varieties showed a higher accumulation of protein during grain development. For instance, in IR-30864, protein accumulated up to 15 days after anthesis before reaching saturation, while in Dhaksha, protein accumulation was moderate and continued up to 20 DAA. Additionally, Navara demonstrated elevated levels of essential amino acids.

Key words: Amino acids, Grain protein content, Grain yield, Photosynthetic efficiency, Rice.

MODULATING FEEDBACK INHIBITION OF DIHYDRODIPICOLINATE SYNTHASE LEADS TO LYSINE BIOFORTIFICATION AND ABIOTIC STRESS TOLERANCE IN RICE

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Rice is a major energy and nutrition source for humans and livestock, however, it is an inadequate source of some essential amino acids, particularly lysine. Lysine biofortification is essential in preventing malnutrition, particularly in developing countries where staple grains such as rice constitute the primary dietary component. In rice, the rate-limiting step in lysine biosynthesis pathway is catalyzed by dihydrodipicolinate synthase (DHDPS), which is extremely sensitive to feedback inhibition caused by lysine. Therefore, in the present study, we incorporated important mutations at the probable lysine binding sites of DHDPS protein of rice to make it lysine feedback insensitive. The feedback insensitivity of mutated DHDPS (mOsDHDPS) was further validated through functional complementation assay. We developed an overexpression construct by employing the feedback-insensitive mOsDHDPS, under the control of endosperm-specific promoter (GltD1), and subsequently generated transgenic rice plants. These transgenic plants showed a 21% increase in grain lysine content. Moreover, the overexpression lines exhibited reduced yield penalty and higher tolerance to abiotic stresses than the WT plants by possessing better antioxidant machinery, and reduced levels of hydrogen peroxide. This study lays a solid foundation for improving the nutritional quality of rice and other cereal grains, thereby reducing the necessity for dietary supplementation.

Keywords: - Biofortification; Lysine; Rice; Abiotic stress; Yield

NOVEL DONORS FOR NUTRITIONAL ENHANCEMENT FROM WILD SPECIES IN THE BACKGROUND OF POPULAR CV. SWARNA

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Malnourishment has become a major concern and remains a persistent challenge in the growing world population which causes detrimental effects on human health due to inadequate dietary intake of vitamins and minerals. Breeding for biofortified staple food crops holds great promise for addressing and sustainable eradication of hidden hunger (malnutrition). Wild species are significant donors for novel alleles that directly contribute to genetic enhancement in rice, which have the potential for synergistic breeding of biofortified varieties. In the present study, a set of 130 backcross introgression lines (BILs) developed from crosses between Swarna and *O. rufipogon* accession were studied for nutritional (Fe and Zn content) and yield related traits. The grain iron and zinc content were estimated using an energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument. A significant amount of variation was found among Fe, Zn content and yield traits studied in this population. The unpolished grain zinc content ranged between 17.6 (R-52) to 29.6 ppm (R-185) with a mean of 22.0 ppm and iron content ranged from 4.4 (R-12) to 8.1 ppm (Swarna) with a mean of 5.5 ppm. Correlation studies revealed that panicle length, panicle weight, filled number of grains per panicle, and total number of grains per panicle showed a positively significant correlation with single plant yield, while filled grains per panicle showed a highly significant positive correlation with panicle weight and total number of grains per panicle. Iron content showed a positive correlation with zinc content, whereas a negative correlation was observed between nutritional traits (Fe and Zn content) with single yield plant. The promising entries can be used as donors in future breeding programs and the mapping population for identification of novel QTLs/ genes for nutritional enhancement.

Keywords: Biofortification, Variability, Correlation, Backcross Introgression lines (BILs), Iron and Zinc content.

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VACUOLAR PHOSPHATE TRANSPORTER OSVPE2 PLAYS A ROLE IN IRON EXCESS TOLERANT MECHANISM IN RICE

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Iron (Fe) is an essential element for virtually all living organisms. However, excess Fe can cause cellular damage, bronzing on leaves, and impaired plant growth, leading to crop failure in flooded or rainfed rice cultivation in acid soils. Under Fe excess, phosphorous deficiency also occurs because phosphate (PO_4^{3-}) ions are precipitated with ferric ions in the form of $FePO_4$. However, the complex mechanism and regulating network of the plants in response to excess iron in relation to phosphorous remain largely unknown. In our previous microarray analyses, we found that the vacuolar inorganic phosphate transporter, *OsVPE2*, was highly induced in various rice tissues especially roots and old leaves under different Fe excess levels. Thus, in this study, we investigated the role of *OsVPE2* in Fe excess response. *OsVPE2* T-DNA inserted mutant rice lines were hydroponically grown for 5 weeks under excess iron (×70 Fe) compared to normal condition (×1 Fe). As a result, the mutant lines showed inferior growth, severe leaf bronzing, and lower dry matter weight than the wild type, indicating that the mutant is susceptible to Fe excess. Its Fe concentration was increased in old leaves but decreased in the roots during excess Fe. Compared to normal conditions, the inorganic phosphate concentration was decreased in old leaves of wild-type plants grown in Fe excess, and it was decreased more in the leaves of the mutants. Therefore, OsVPE2 may play an important role in iron excess tolerance in rice by supplying phosphorous stored in vacuoles.

Keywords: Iron excess, iron toxicity, P deficiency, OsVPE2, vacuolar phosphate transporter, rice, tolerant mechanism

Enhancing Grain Protein Content in Rice: Strategies to Mitigate Malnutrition in India

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Malnutrition poses a significant challenge to socio-economic development in India. Rice, the most widely consumed cereal in the country, serves as the primary source of dietary protein. However, rice grains typically contain lower protein content (6-8%) compared to other cereals. Despite this, rice protein is highly digestible and absorbable, making it a valuable nutritional resource. Addressing malnutrition in India necessitates an increase in rice grain protein content (GPC). However, one of the major obstacles in achieving this is the trade-off between protein content and biomass accumulation, leading to reduced yields. To overcome this challenge, a comprehensive understanding of the underlying physiological mechanisms is essential. Proteins are synthesized from nitrogen absorbed from the soil. Nitrate (NO_3^-) and nitrite (NO_2^-) are reduced to ammonium (NH_4^+) by utilizing electrons obtained from photochemical reactions. Ammonium is then utilized in the production of various amino acids. In this study, we assessed various physiological parameters in rice germplasm lines with varying GPC, including photosynthetic rate, quantum efficiency, light saturation, and nitrite reductase activity. Our findings revealed that the high protein genotypes exhibited significantly higher values for these parameters when compared to the low protein genotypes. Further, the detailed study in contrasting genotypes for GPC revealed that the high protein genotype moderated the carbon assimilation through regulating triose phosphate utilization to contribute the reductants for increased protein synthesis. This research sheds light on the physiological intricacies of grain protein synthesis and offers insights into potential strategies to enhance protein content in rice without compromising the yield.

Keywords: Electrons, Grain Protein Content, Nitrite reductase activity, Photosynthesis, Yield.

BIOLOGICAL MITIGATION OF N_2O EMISSION THROUGH APPLICATION OF PLANT BASED NITRIFICATION INHIBITORS IN TROPICAL SUMMER RICE FIELD.

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Studies were conducted on tropical rice paddies to evaluate the efficacy of three different plant residues, fresh neem (*Azadirachta indica*) leaves (NL), green manure (SA), and used tea leaves on nitrification inhibition rate (NI), grain productivity, plant growth, and N_2O emission rate, which are crucial for evaluating the long-term potential of plant residue amendment in croplands to mitigate GHG emission while sustaining crop productivity. Plant residues were added to the soil together with the essential amount of chemical fertilizers (NPK). The potential to limit nitrification of plant residues was compared to addition of calcium carbide (CC), a well-known nitrification inhibitor. Combining NL and SA with NPK application enhanced agronomic yield and significantly decreased N_2O emission over control by 16% and 20% ($p < 0.05$) over TL, CC, and NPK respectively. Throughout the study, several soil and plant characteristics were assessed, including soil organic carbon, nitrogen content, plant photosynthetic rate, and grain productivity and their relationship with yield parameters and N_2O emissions were recorded. Significantly low heterotrophic populations of NH^{4+} and NO^{3-} oxidizers (CFUs/gm soil) were also recorded in the treatments which supports the outcomes of effective grain production and emission reduction in NL and SA. The use of plant residues in conjunction with conventional fertilizer (NPK) demonstrated that plant-based materials can aid in reducing N_2O emission from rice agriculture without harming the crop's agronomic productivity and resulting in sustainable rice production.

Key words: Tropical rice, Nitrous Oxide, Mitigation, Plant residues, Agronomic productivity

LANDRACES OF RICE (*ORYZA SATIVA* . L) REVEAL NUTRITIONAL TRAITS POINTING TOWARDS SOLUTIONS FOR HUNGER AND MALNUTRITION.

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Rice (Oryza sativa) being the staple food for almost two thirds of the population plays a pivotal role in Indian economy. Also, rice brings to our mind delicacies like the biryani, rice pudding (kheer) or the plain rice eaten with curry, in India. Recent study which shows that rice domestication happened some 6000 BC suggests to the anthropological aspect of rice and the 8000 years it must have taken for us to get the rice delicacies in their present forms. One of the ways to understand this is to study the traditional varieties and the landraces. An understanding of the cooking qualities and the nutritional aspect of such rices will help understand the reasons for their preference in culinary terms. Another aspect which goes together with feeding habits is the nutritional balance. Many landraces are known to be rich in micronutrients like Zn and Fe. This study investigates cooking traits such as amylose content and starch elongation ratio, which significantly influence the texture and palatability of cooked rice. Through a systematic analysis of diverse rice landraces, employing advanced analytical techniques, this study aims to uncover correlations between genetic diversity, nutritional content, and cooking attributes. The findings not only shed light on the intrinsic qualities of these landraces but also hold immense potential for enhancing global food diversity, nutritional fortification, and culinary preferences. Additionally this study aimed to estimate the genetic variability and screen for the micronutrient content, specifically Iron (Fe) and Zinc (Zn), in traditional rice (*Oryza sativa* L.) landraces. The present investigation was carried out at the Department of Genetics and Plant Breeding, NAINI (SHUATS), Prayagraj during *Kharif* 2023. Twenty-six landraces of rice were studied for cooking traits, and nutritional aspects like

starch, Fe and Zn content. The study on cooking traits demonstrated that rice landraces exhibit a wide range of water absorption and high elongation ratios. The study on physical characters showed that landraces are having lower head rice recovery compared to the varieties. The

study on biochemical properties suggest that in general landraces can be expected to provide a health benefit for people that have prediabetes, diabetes and/or obesity. The genotypes Sathi and Kalamallifool stood out in the current investigation, each with a 20 ppm iron content. Njvara and Tharun Bhog sale had 15 ppm, Albella 16 ppm, and Shyamala 17 ppm. In case of Zn content, Zinco rice- MS stands out with the highest concentration at 33 ppm while amongst the landraces Sathi, Albella, and Njvara also show commendably high zinc content. This study paves the way for the creation of rice varieties that are nutritionally enhanced to meet the dietary requirements of people that are prone to micronutrient shortages. The findings not only shed light on the intrinsic qualities of these landraces but also hold immense potential for enhancing global food diversity, nutritional fortification, and culinary preferences. This research contributes valuable insights into the sustainable utilization of rice genetic resources, paving the way for informed agricultural decisions and improved food systems worldwide.

Land race, malnutrition, nutritional traits

Translational Genomics: Molecular & Classical Breeding

GENOMICS AND MOLECULAR BREEDING OF ENVIRONMENT FRIENDLY READY-TO-EAT RICE

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Indian soft rice is an environment friendly popular ethnic Ready-to-eat food product, prepared from parboiled low amylose-containing glutinous Bora rice with poor yield attributes. This magic rice doesn't require cooking for consumption but has a possible risk of high glycemic response (GR). Present explorative study was done with typical soft rice (Var. Vogali Bora) with respect to normal improved rice (Var. IR36) where the special focus was given to physicochemical basis, whole genome dissection, global transcriptome analysis associated with target trait followed by molecular breeding in HY background with low GR to develop ready-to-eat rice with low GR. Low amylose to amylopectin ratio, low viscosity, pinholes in kernel and less resistant starch possibly made them soft rice with high GR. In respect to the rice reference genome (IRGSP-1.0) soft rice showed less number of SNPs and down regulation of the cascade of starch synthesis related loci during grain filling indicated *japonica* origin of soft rice but a higher number of SNPs within starch synthesis related loci indicated that this rice is specially evolved for RTE trait during the course of evolution. Amylose content and softness linked marker (RM190) showed heterozygous band in hybrid line with RTE trait but despite zygosity for RM190, RILs showed soft trait but less than 20% amylose is the prerequisite for RTE trait. During segregation, anthocyanin deposition in immature spikelets of RILs maintains the monohybrid cross-ratio and is also tightly associated with softness. RILs showed the early flowering trait (a desirable trait) with improved yield attributes and due to improved amylose and resistant starch content in $F_{2:3}$ seeds of RILs showed better GR in the *in vivo* mice model.

PRECISE GENOME MODIFICATION OF RICE BY CRISPR/CAS9 MEDIATED EDITING OF *OsMADS26* GENE FOR DROUGHT TOLERANCE

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Rice (*Oryza sativa* L.) is the most widely consumed staple food for human population belonging to Asia and Africa. Being a semi-aquatic annual plant, rice is highly prone to losses due to various environmental stresses especially drought. Development of drought tolerance in rice is extremely important especially in the changing scenario of climate change. Studies have identified *OsMADS26* transcription factor as a negative regulator of drought tolerance in rice. In the current study, *OsMADS26* was edited using CRISPR/Cas9 system with the objective to develop drought tolerance. The guide RNAs were designed using CRISPR-P and cloned in to pRGEB32 vector. Agrobacterium mediated genetic transformation of rice cv. Nipponbare was carried out. A total of 13 edited lines were identified. Most of the mutations were found to be CT deletions. *In silico* analysis of translated protein sequence of *OsMADS26* mutated gene showed that the modified protein was truncated compared to the wild type protein. No off-target editing was observed in the edited lines for the predicted, tested off-target sites. Comparison of mutations observed between T0 and T1 generation were made, which showed that mutations were stably inherited in the T1 generation. Selected edited lines were also used for gene expression analysis using Real-time qRT-PCR. In the edited lines, *OsMADS26* gene expression was found to be down-regulated compared to the wild type. Expression of *OsMADS26* downstream genes like, *SALT, RAB21*, *OsWRKY28* etc, involved in various stress responses was also found to be altered.

REARCHITECTING THE PROMOTER OF *PHO1;2* AFFECTS PHOSPHATE ACQUISITION IN RICE

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Phosphate (Pi) is an essential macronutrient for plant growth and development. PHO1, a phosphate transporter majorly expressed in root stellar tissue is characterized for its Pi export from root to shoot. Similar to Arabidopsis, rice *ospho1;2* mutant shows higher Pi accumulation in root with reduced Pi transport to shoot. AtWRKY6 has been shown to inhibit PHO1 expression by binding the AtPHO1 promoter. The promoter of *OsPHO1;2* has a W-box which is a putative binding site for the WRKY transcriptional factors that could influence its expression. In this study, we used CRISPR/Cas9 technology to re-architect the promoter of *OsPHO1;2*. We used two gRNAs to remove W-box present in the promoter of *OsPHO1*. Out of 12 W-box edited lines obtained, three marker-free lines were selected for further experimentations. W-box edited plants show enhanced expression of *OsPHO1*;2 in roots and better growth with a higher Pi in root and shoot tissues compared to wild type. ^{33}P uptake assay suggests that removal of W-box increases the Pi uptake in roots and more ^{33}P transfer to the shoot. Interestingly, more Pi in the root is accompanied by the increased activity of PHT transporters suggesting the possible positive role of OsPHO1;2 in driving PHTs expression. Also, at the reproductive stage, W-box edited lines perform better under different Pi regimes. Overall, our study reveals a CRISPR/Cas9 technology application where precise removal of the putative binding site for a transcriptional inhibitor from *OsPHO1;2* promoter improves the Pi uptake in plants and eventually the overall growth.

UNLOCKING RICE HAPLOID INDUCTION: INVESTIGATING THE EFFECT OF MAIZE *PLD3* ORTHOLOGUE

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Doubled haploid breeding possesses immense potential in rice breeding by providing multifarious benefits. However, its full utilization in rice can be realized with the availability of an efficient haploid induction system. Interestingly, maize is naturally gifted with a haploid inducer stock and its genetic mechanism has been unveiled. Recently, *ZmPLD3* gene has been reported to play a key role in the haploid induction ability of maize. In this study, we have identified putative orthologue(s) of the maize *ZmPLD3* gene based on comprehensive phylogenetic and *in-silico expression* analysis in rice. It was observed that *OsPLDα2* (*LOC_Os05g07880*) was most closely associated with *ZmPLD3*. Besides this, it also showed 85% identity at the protein level with *ZmPLD3* along with the highest anther-specific expression. CRISPR/Cas-based genome editing was used for its functional validation with the objective of developing haploid inducer stock in rice. *Agrobacterium* strain LBA4404 harboring a multiplexed pRGEB32 construct designed to target two specific sites in *OsPLDα2,* was employed for the transformation of embryogenic calli obtained from Taipei 309. Subsequently, the transformed calli were subjected to selection (Hygromycin, 50 mg/l), regeneration, and rooting to develop the plants. To identify the transformed events, regenerated plants were screened with Cas9 primers using PCR-based analysis. Among twenty-nine plants, nine plants were found positive for Cas9. From these Cas9-positive plants, *the OsPLDα2* gene was amplified spanning the sgRNA region, and outsourced for sequencing to confirm the desired edits. Based on sequence analysis in T0 plants, no sequence differences were observed in Cas9 positive transformants vs wild types, indicating that editing has not taken place in T_0 plants. Later on, T0 plants were then raised to maturity and showed > 60 % grain sterility at panicle bearing stage indicating the possibility of getting *OsPLDα2* gene edited knock out plants. The seeds of T_1 plants are now being raised and validated through different methods.

Keywords: Doubled Haploid, Haploid Inducer Stock, Orthologue(s), Rice Breeding

TOWARDS DEVELOPING NON-GM GOLDEN RICE

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Rice is an essential crop consumed by billions of people worldwide. The endosperm of white rice lacks carotenoids, hence Pro-Vitamin A deficiency is a major problem among the rice-eating population. Our study identified a traditional pigmented rice cultivar Kavuni which accumulates high levels of lutein and traceable levels of beta-carotene in its endosperm. A comparative transcriptome analysis between maturing panicles of pigmented rice Kavuni and white rice ASD16 elucidated the molecular mechanisms underlying high carotenoid accumulating phenotypes of Kavuni. Carotenoid precursor pathway genes (*OsDXS*, *OsGGPS*) and key carotenoid biosynthetic genes (*OsPSY1*, *OsZ-ISO*) were upregulated in Kavuni grains. We identified high expression of *OsLYC-E*, *OsCYP97A,* and *OsCYP97C* transcripts of alpha-carotenoid pathway leading to high lutein accumulation in Kavuni grains. Similarly, Kavuni grains showed upregulation of positive carotenoid modulators (*Orange*, *OsDjB7,* and *OsSET29*), and down-regulation of carotenoid negative regulators (AP2 and HY5), thus creating a favorable molecular framework for carotenoid accumulation. Our study has identified *OsLYC-E* as a valuable gene control point in carotenoid biosynthesis, hence we planned to knock-out the *OsLYC-E* gene to channelize carotenoid pathway towards accumulation of beta-carotene in Kavuni grains. Earlier studies demonstrated that silencing/knocking-out *LYC-E* gene has significantly enhanced β-carotene content in brassica, potato, and banana. In the present study, we developed 138 putative genome-edited plants of Kavuni harboring mutations in *OsLYCE* gene through CRISPR/Cas9 genome editing. Sequence analysis of targeted loci identified four frameshift mutant alleles (1bp deletion, 1bp insertion, 7bp deletion, 8bp deletion) in homozygous conditions resulting in premature protein truncation. Metabolic Profiling of rice grains of all four edited plants showed nearly two-fold increase in beta-carotene content and complete absence of lutein branch carotenoids compared to non-edited plants.

Keywords: Carotenoids; Lutein; Beta-carotene; Traditional rice; Kavuni; Transcriptomics; CRISPR/Cas9; Knock-out

DEVELOPMENT AND VALIDATION OF HETEROTIC POOLS IN BASMATI RICE

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The global demand for Basmati rice is increasing due to its exquisite grain quality, necessitating the improvement in production to meet the consumer demand. Harnessing heterosis in Basmati rice is a potential strategy to enhance its productivity, as there is no scope for expanding the area beyond the Basmati GI area. Towards this, a panel of improved putative parental lines (PPL) including 107 restorers and 59 maintainers were developed and characterized at Division of Genetics, ICAR-IARI New Delhi. The genome-wide diversity and population structure of these improved Basmati rice panel was assessed using the 80K rice pan genome array, which could clearly demarcate the PPLs into two populations namely, Pop1 and Pop2. Simultaneously, testcross hybrids were generated in Line x Tester fashion by crossing the PPLs with two testers (PRR78 and Pusa 6) and evaluated for their yield performance in three locations across the Basmati GI area. The yield data of Pusa 6 and PRR78-based test cross hybrids from individual locations, could validate the hypothesis that hybrids developed using the tester from a subpopulation crossed with individuals of the different subpopulations yields (Mean SPY of 30.40g) higher than the crosses with individuals belonging to same population ((Pop1/Pop1 (16.64g) and Pop2/Pop2 (13.90g)). Genotypes from both the populations were randomly selected for generating inter and intra-population, which could cross-validate the existence of two heterotic pools in the improved Basmati rice panel. The study laid the strong foundation for Basmati rice hybrid breeding through establishing heterotic groups with the assistance of genomic data as well as hybrid performance. The PPLs can also serves as founders for next cycle of improvement of the Basmati rice parental lines.

GENOME EDITING OF RICE CULTIVARS TO DEVELOP INSECT PEST RESISTANCE

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Rice (*Oryza sativa L.*) is a primary staple food crop for billions of people worldwide. To ensure global food security to support increasing population growth, it is vital to control insect pests that damage rice production. Amongst various rice insect pests, the brown planthopper (BPH) and striped stem borer (SSB) are the two most serious pests affecting rice production. Both BPH and SSB are difficult to control using chemical pesticides, BPH damages rice directly through feeding and by transmitting two viruses. Under favorable conditions, up to 60% yield loss is common in susceptible rice cultivars attacked by BPH. Striped stem borer (*Chilo suppressalis*), which is a chewing insect, feeds on newly formed tillers and stems, causing 'dead hearts' and 'white heads', resulting in significant yield losses. So far, no SSB resistance germplasm source and resistance genes have been identified in rice. The development of insect-resistant rice varieties is seen as a viable and ecologically sustainable approach for controlling these devastating insect pests. In this study, we use CRISPR/Cas9 mediated genome editing technology to knockout the CYP gene that shows increased resistance to BPH and SSB. Using seed derived callus method, we have transformed the gene edit constructs and in the process of generating genome edited events.

Key words: Rice, Brown planthopper (BPH), Striped stem borer (SSB) and CRISPR/Cas9.

GENOME-WIDE IDENTIFICATION OF GENOTYPE-SPECIFIC RNA SPLICING FOR SALT STRESS RESPONSE IN RICE

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Pre-mRNA splicing is an essential step for the regulation of gene expression. In order to specifically capture splicing variants in plants for genome-wide association studies (GWAS), we developed a software tool to quantify and visualize Variations of Splicing in Population (VaSP). VaSP can quantify splicing variants from short-read RNA-seq datasets and discover genotype-specific splicing (GSS) events, which can be used to prioritize casual pre-mRNA splicing events in GWAS. We applied our methods to an RNA-seq dataset from a rice diversity panel exposed to optimal and saline growing conditions. Significant GSS events were used as markers for a GWAS with the shoot Na^+ accumulation, which identified six GSS events in five genes significantly associated with the shoot Na^+ content. Two of these genes, *OsNUC1* and *OsRAD23* emerged as top candidate genes with splice variants that exhibited significant divergence between the variations for shoot growth under salt stress conditions. To investigate the role of *OsRAD23*, two sets of mutant lines targeting different domains within *OsRAD23* were generated using CRISPR-Cas9 systems. One set of mutant lines showed significant reduction in shoot growth as compared to wildtype under salt stress conditions while another set of mutant lines exhibited increased shoot biomass under optimal conditions. These findings improve our understanding of rice plant mechanisms under salinity stress.

Key words: genotype-specific RNA splicing; salt stress; gene-editing; OsRAD23

FUNCTIONAL VALIDATION OF A NOVEL YIELD GENE *AN-1* THROUGH CRISPR/CAS9 MUTAGENESIS

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Rice, a fundamental staple food crop, witnessed substantial yield advancements in the 1960s by enhancing the harvest index through the introduction of semi-dwarf trait. A subsequent leap occurred in the 1980s with the introduction of hybrids. However, since then, rice yield has plateaued, showing no significant increase over the past decade. Considering the significance of doubling rice production by 2050 due to growing global population, there is a need to enhance the genetic potential for yield in rice through breeding programs. This increment can be achieved either by enhancing photosynthetic traits, such as C4 rice, or by exploiting yield genes from the wild gene pool or by creation of unique genetic variations *via* precise mutagenesis techniques. The primary objective of this research was to perform functional validation of *An-1* utilizing the CRISPR/Cas9 targeted mutagenesis. Two sgRNAs were designed, targeting first and second exon of *An-1* and cloned into the binary vector pRGEB31. *Agrobacterium*-mediated transformation of immature embryos from the rice cultivar ASD 16, lead to the successful regeneration of approximately 312 potential genome-edited plants, which was initially screened with vector-specific primers. Sequencing of target regions in the T_0 generation revealed that 29 progenies carried various mutations, including deletions ranging from 2 to 24 base pairs, insertions from 1 to 10 base pairs and single base pair substitutions. Eleven of these promising lines were advanced to the T_1 generation. Among the 46 T_1 progenies, four progenies had homozygous mutations, 21 had multi-allelic mutations, 12 had bi-allelic mutations, one had mono-allelic heterozygous mutations, and eight had no mutations, reverting back to wild type. Subsequent generation advancement and molecular characterization are required to identify progenies with homozygous mutations for phenotyping. An in-depth analysis will facilitate the identification of superior *An-1* alleles with the potential to enhance rice yield through functional validation.

Keywords: *An-1,* rice, yield, CRISPR/Cas9, grain number, targeted mutation.

CRISPR/Cas-mediated multiplex genome editing of disease and herbicide tolerance traits in rice for improved performance under aerobic and irrigated conditions

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Rice meets over half of the global dietary requirement and more than 80% of Asians, heavily relies on freshwater, accounting for over half of global agricultural water usage. Thus, water saving agronomy like direct seeded rice and aerobic rice are ecologically sustainable. Incidence of major diseases especially bacterial leaf blight, blast, and weed infestation threaten rice productivity in such environments. Significant progress has independently been made to understand the mechanisms associated with disease resistance and herbicide tolerance through identification and validation of novel genes. However, not many attempts have been made yet to combine yield, water saving and biotic stress tolerance mechanisms in rice. We aim to enhance already developed drought-adaptive trait introgressed rice varieties, such as Dhaksha, and a disease and herbicide-tolerant mega-variety, MTU1010, through targeted gene editing. Improving disease resistance and herbicide tolerance in aerobic worthy cultivars of rice would significantly enhance their efficacy in harnessing the water-saving advantage of aerobic cultivation practice. Similarly, enhanced resistance to diseases and herbicides will significantly contribute to improved yield under irrigated conditions. Genetic enhancement of specific traits through breeding is undoubtedly the most widely adopted strategy, combining this with editing appropriate alleles can further hasten the process of generating crop cultivars for the target environments. In this study, our proposed approach involves the precise editing of genes associated with bacterial leaf blight (SWEET11, SWEET13, SWEET14), blast (Bsr-d1 and Pi21), and herbicide tolerance (EPSPS and ALS) within the genetic background of aerobic-worthy rice cultivars, including Dhaksha and the mega-variety MTU1010.

Key Words: Biotic & abiotic stress, Genome editing, herbicide tolerance, Rice.

CREATION OF NOVEL ALLELES OF OSEPF1 GENE FOR MANIPULATING PHOTOSYNTHETIC TRAITS THROUGH GENE EDITING

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Stomatal density is closely related with physiological traits and growth characteristics of plants. EPIDERMAL PATTERNING FACTOR-LIKE family genes encode for small secretory mobile peptides and regulate stomatal development and patterning in plants. Climate-smart plants can be developed by altering the genes involved in stomata using CRISPR/Cas9 method. In current study, CRISPR/Cas9 mediated knock out of the gene *OsEPF1* in rice variety ASD 16 was attempted to develop mutants with altered stomatal traits for enhanced photosynthetic efficiency. Seventeen T_0 plants representing two independent transgenic events were regenerated and CRISPR/Cas9 target showed 100% mutagenic efficiency with seven multiallelic, seven biallelic and three monoallelic mutations. The stomatal density was increased by 3.7–44.3% in T_0 mutant lines. In T_1 generation, the three homozygous T_1 progenies (# E1-1-4, # E1-1-9 and # E1-1-11) with 1 bp insertion were identified. These mutants showed increase in the stomatal density (54-95%) and corresponding reduction in the stomatal size. and the transpiration rate by 58-62% compared to the non-transgenic ASD 16. The homozygous T_1 progenies showed an increase in the photosynthetic rate by 14-31%, 60-65% of stomatal conductance. The clustered stomata with change in stomatal file density was observed in the *OsEPF1* mutants compared to non transformed control. In T_2 generation, homozygous condition was maintained in the target region and the seeds will be used to evaluate the mutants under heat stress condition. The above results demonstrate that creation of variations in *OsEPF1* gene leads to changes in stomatal conductance and photo-synthetic efficiency in rice.

Key words: Epidermal patterning factor, Stomatal density, CRISPR-Cas9, abiotic stress

ENHANCEMENT OF GRAIN YIELD-RELATED TRAITS IN BLACK RICE THROUGH CRISPR/CAS9 MEDIATED GENOME EDITING

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Cereal landraces, are vital for sustainable agriculture and achieving a malnutrition-free world, have faced declining relevance due to their historically low yields. Nevertheless, they remain invaluable for their resilience against environmental stresses and their high nutritional value. To address the need for increased agricultural productivity and resilience in the face of climate change, harnessing the potential of rice landraces, particularly the traditional black rice, *Chakhao* in northeast India, through genetic improvement is a promising avenue. Our strategy involves utilizing multiplexed CRISPR/Cas9 genome editing techniques to enhance the *Chakhao amubi* black rice landrace. We aim to edit three key genes associated with plant height and grain yield in rice: OsGn1a, OsDEP1, and OsSD1. These genes, which respectively regulate cytokinin oxidase, panicle density, and gibberellin biosynthesis, have significant impacts on grain production, lateral branching, and plant height in rice. We designed gene-specific guide RNAs using widely recognized gRNA design tools like CRISPR-Plant and CHOP-CHOP. These designed guide RNAs were incorporated into the psgR-Cas9 vector. Subsequently, we integrated the gRNA expression cassette alongside the Cas9 expression cassette into the binary vector pCAMBIA1300. Further, generation of transgenic black rice lines were generated through *agrobacterium* transformation and confirmation of T-DNA integration was conducted using Cas9 and HPTIII primers. Positive events will undergo rigorous evaluation to identify mutations. We anticipate that a triple mutant of these genes will yield plants with increased tiller numbers, enhanced grain production per plant, and reduced overall height. Developing such high-yielding black rice cultivars promises to promote sustainable, less resource-intensive agriculture, while bolstering global nutritional security.

Keywords: Black rice, genome editing, vector, yield

OVER EXPRESSION OF *PI68* IMPARTING RESISTANCE TO RICE BLAST (*M. ORYZAE*) IN SAMBA MAHSURI

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Rice (*Oryza sativa* L.) is one of the most important food crops cultivated worldwide. In the current scenario of climate change and shirking resources, to sustain the rice production, development of stress resistance genotypes is continue to be a priority area. Rice blast (*M. oryzae*), a major disease, severely affects leaf and panicle leading to a drastic decrease in grain yield of rice. A *Pi68* (*Os03g0281466*), a novel candidate gene encoding to malectin-serine threonine kinase, identified and cloned from INGR15002, an introgression line derived from the cross of PR114/*O. glumaepatula* through fine mapping. For the functional validation of *Pi68*, we have cloned the R allele in to a binary vector pCAMBIA1300 and transferred it to blast susceptible cultivar, BPT 5204 (Samba Mahsuri) via *Agrobacterium* transformation using strain EHA 105 by in planta method. Out of the 114 putative T_0 plants, nine were confirmed using hygromycin phospho-transferase (hpt) marker. Further, T_1 seeds of nine plants were raised in transgenic greenhouse and confirmed by hpt marker. Out of nine T_1 progenies, six behaved Mendelian segregation pattern indicating single copy integration which was advanced to the next generation. Seeds from positive T_2 plants collected and screened in uniform blast nursery (UBN) for disease reaction, which showed a moderate level of resistance to rice blast. Further, to elucidate the molecular mechanism, we are exploring the site of integration of *Pi68* by whole genome sequencing/TAIL PCR using highly resistant blast transgenic line.

Keywords: Rice, Pi68, Samba Mahsuri, Transformation, Rice blast, Agrobacterium, In planta

FUNCTIONAL CHARACTERIZATION OF CRISPR-CAS9 MEDIATED GENOME EDITED MUTANTS OF *DROUGHT ANDSALT TOLERANCE* (*OsDST*) GENE FOR IMPROVING ABIOTIC STRESS TOLERANCE AND YIELD IN MEGA RICE CV. MTU1010

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Rice is the important food crop of the world and its production needs to be doubled by 2050 to meet the global food demand. Rice uses more than 50% of the fresh water used by field crops, and is highly sensitive to abiotic stresses. Hence it is necessary to improve productivity, WUE and abiotic stress tolerance of rice. Here, we selected *DROUGHT AND SALT TOLERANCE* (*DST*) gene for genome editing in a mega rice variety MTU1010 by using CRISPR-Cas9 SDN1 approach. To edit *OsDST* gene, two SgRNAs were designed to mutate *DST* at two different target sites. These sgRNAs were cloned under the transcriptional control of *OsU3* promoter, and a modified *SpCas9* under *OsUbiquitin* promoter in a pCAMBIA1300 plant transformation vector. Mature embryogenic calli derived from MTU1010 was transformed with *Agrobacterium* mediated genetic transformation. Hygromycin resistant T_0 transgenic lines were confirmed by PCR using Cas9 specific primers. The target region of *DST* gene was PCR amplified from T_0 plants, sequenced and sequences with degenerate chromatograms were analysed by using DSDecode. In this study, Homozygous mutants of five different alleles of *DST* gene were obtained in T1 generation. We also obtained transgene free *dst* mutant plants at T_2 generation. These homozygous *dst* mutants were analysed for drought and salt tolerance at vegetative stage in pot culture under greenhouse conditions revealed that these mutants exhibited tolerance to drought and salt tolerance and also enhanced yield. Further, these mutants showed >20% yield enhancement over wildtype plants under field conditions in a transgenic net house. The genome edited foreign gene free mutants of *DST* developed in this study will be useful to release as variety and as a genetic stock for introgression of *dst* mutations in other indica varieties for genetic improvement in yield and climate resilience.

Keywords: Abiotic stress, CRISPR-Cas9, Agrobacterium, Drought and salt tolerance, mutant, Indica rice

EXPRESSION ANALYSIS OF ZINC ASSOCIATED GENES IN FLAG LEAF OF RICE (*ORYZA SATIVA* L.) GENOTYPES

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Metal homeostasis is an important physiological phenomenon regulating grain mineral content. Thorough elucidation of the mechanism is needed to overcome the widespread malnutrition problem by increasing grain mineral content such as Iron (Fe) and Zinc (Zn) in staple food crops such as rice. To understand the role of Zn-associated genes in mineral uptake, transport and remobilization in rice, qRT expression profiles of Zn homeostasis gene *OsNRAMP5* was analyzed in flag leaf tissue at ten days after flowering stage. The study included eight genotypes, comprising landraces, high yielding cultivars and biofortified varieties with contrasting grain Zn content in brown and polished rice. Zn content in the brown rice among eight genotypes ranged from 17.5 ppm in Chittimuthyalu to 42.9 ppm in Karuppunel, with an average of 30.2 ppm. Zn content in the polished rice ranged from 13.9 ppm in MTU1010 to 33.0 ppm in Karuppunel, with an average of 23.45 ppm. The relative expression analysis showed higher gene expression in landrace Karupunnel followed by CGZR2, *Edavankudi* Pokkali, RNR15048, Vandana, Chittimuthyalu and Protozin. Highly significant positive correlation was also observed for grain Zn content with relative expression of the *OsNRAMP5* gene. *OsNRAMP5* was earlier reported to be associated with grain manganese and cadmium, the present observation of its association with zinc needs further validation to provide better insight for understanding the role of *OsNRAMP5* gene in Zn uptake and its homeostasis in grain.

Keywords: Rice, Grain zinc content, *OsNRAMP5*, Expression, Correlation

ENHANCING BETA-CAROTENE CONTENT IN RICE BY OVER-EXPRESSION AND CRISPR/CAS9 EDITING OF OR GENE

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Vitamin A deficiency is one of the major public health problems affecting up to 50% of the world's population. Rice and wheat are among the most important food crops for humans and the main staple food for more than half of the world population, however, they lack many essential micronutrients such as vitamin A. In developing countries, majority of population endure monotonous, cereal-rich diets, leading to risks of malnutrition and hidden hunger. In India, the prevalence of subclinical VAD (Vitamin A Deficiency) still exists as one of the highest in the world. Biofortification of cereal crops with β-carotene (provitamin A) as a target for genetic engineering is a potential solution to overcome vitamin A deficiency. The Orange (OR) protein is involved in the regulation of carotenoid accumulation and previous studies demonstrated high level of carotenoid accumulation due to single-nucleotide polymorphism (SNP) from Arg to His in OR protein. *TaOr* gene was cloned from leaves of wheat (*Triticum aestivum* L. cv Bobwhite) and site-directed mutagenesis was done for substitution of a single amino acid at position 110 (Arg to His) of wild-type wheat *TaOr* (referred to as $TaOr^{His110}$). To increase the level of β-carotene in the endosperm, we generated transgenic rice plants overexpressing $TaOr^{His110}$ under the control of the seed-specific promoter *Glu-1D1* via *Agrobacterium*-mediated transformation. HPLC analysis revealed that rice grains from $TaOr^{His110}$ overexpressing plants exhibited increased β-carotene levels of up to 8-fold in case of TP309 (japonica) cultivar and up to 13-fold in case of IET10364 (indica) cultivar as compared to their respective wild-type seeds. Additionally, most of the carotenoid biosynthetic pathway genes were found to be upregulated in $TaOr^{His110}$overexpressing seeds of TP309 and IET10364, including *OsPSY, OsPDS, OsZDS, OsCRTISO, OsLCY, OsβOH, OsVDE* and *OsZE*.

Further, rice *or* gene (*OsOr*), was modified by CRISPR/Cas9 genome editing to increase β-carotene in callus of IET10364 rice cultivar by Agrobacterium-mediated transformation. Both orange and white colored proliferating callus were obtained by transformation. Orange callus showed approximately 3-fold increase in β-carotene content as compared to white callus, as

revealed by HPLC. Sequence analysis also showed insertion of a single base in orange-colored callus to genome editing between junction of third intron and third exon. The above result indicated that the orange color of callus was due to the mutation caused by single base insertion that lead to increase in β-carotene content. This study provided insight into biofortification of rice with application of *Or* gene in combination of seed-specific promoter to increase β-carotene concentration in rice endosperm. The results also indicate the application of genome editing of *or* orthologs to other cereal crops for increased β-carotene levels without insertion of any carotenoid biosynthetic transgenes as an alternative approach to conventional transgenic approaches.

IDENTIFICATION OF QTL'S FOR EARLY FLOWERING/MATURATION TRAIT IN RICE

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Rice (*Oryza sativa* L.) is a major staple food crop worldwide, feeding more than half of the world's population. In rice, early flowering (EF) is a vital agronomical determinant for current varieties of cultivated rice to adapt to specific cultivation regions and cropping seasons. Therefore, control of EF is one of the important objectives in rice breeding. This study was conducted to identify quantitative trait loci (QTL) for the EF using 60 days (EF, 60 to 65 days) rice variety showing extreme EF compared to other regular cultivar in rice. The 60 days rice variety was crossed with a popular rice variety Samba Mahsuri (SM) to assess the inheritance mode of EF and identify major QTL(s) conferring EF, respectively. A set of 250 F2 plants were developed through cross between SM and 60 days rice. QTLseq analysis for the two extreme bulks for early flowering and late flowering containing 15 plants each was done using QTLseq pipeline. Nipponbare was used as the reference genome for the QTLseq analysis. In this study, we identified two major QTL's named, qEF3.1 and qEF7.1 associated with EF on chromosome 3 and 7. Both the QTL's (qEF3.1 and qEF7.1) regions were overlapping with previously reported QTL intervals that are associated with flowering- related traits. qEF3.1 was overlapping with previously reported QTL's HD9, qDEF-3, qFDN-3 and qHDD3-2. qEF7.1 was overlapping with hd7a and qDTH-7 respectively. Furthermore, marker-trait analysis could be performed to determine how QTL regions contribute to the phenotype, which may be a useful source for the control of early flowering in future rice-breeding programs.

Young Scientist

POSITIVE ASPECTS OF JASMONATES SIGNALING FOR IMPROVING AGRONOMIC TRAITS

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Subtheme: Climate resilience: Abiotic stresses

Jasmonates consists of a group of oxylipin derived phytohormones regulating a wide array of plant responses against biotic and abiotic stresses. Unfortunately, these phytohormones are known for their plant growth inhibitory action especially on roots. Our research with jasmonates against different abiotic stresses has established jasmonates as the critical factor fine-tuning plants development promoting its survival in the adverse environmental conditions.

The research outlines the inevitable involvement of jasmonates in nutrient deficiency response, drought stress response and regulation of root growth inhibition during adverse rhizospheric pH conditions. Connecting these dots using multiple plant systems including rice, chickpea and Arabidopsis we proved the highly specific nature of jasmonates in terms of stress, molecular mechanism and tissues under analysis. Briefly, utilising transgenic approach in rice, we report *OsJAZ9* and *OsJAZ11* specifically regulating Potassium (K^+) and Phosphate (Pi) deficiency, respectively. Secondly, our experimentation suggested JA perception as the extra-nuclear phenomenon by a JA receptor from chickpea. Third, we report JA regulating auxin maxima at root tip and thereby root growth inhibition against a range of rhizospheric pH expanding its role in the environmental stimuli sensing.

In conclusion, our research demostrate JAs as the regulator of plant development in response to different abiotic stresses though the severity of the stress may have certain drastic consequences. Therefore, keeping their important roles, they can better be utilised for improving agricultural traits specifically during stress conditions.

Keywords:

Jasmonates; Potassium deficiency; Phosphate deficiency; Rhizospheric pH; Drought stress

HY5 GENES IN RICE: THE REGULATORS OF LIGHT-MEDIATED DEVELOPMENT

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Light acts as a stimulus initiating different developmental pathways in plants enabling them to adapt to the changing environmental conditions. The mechanism by which light brings about the desired phenotypic changes in plants is complex. However, elucidation of these mechanisms will help in improving crop yield and generating climate smart rice crops able to adapt to changing environmental conditions in the future. My research focusses on functional characterization of two bZIP transcription factors which are predicted to be AtHY5 orthologs in rice. HY5 is a master regulator of photomorphogenesis in *Arabidopsis*. In rice, its orthologs have undergone neofunctionalization. They are involved in regulation of plant height, leaf length, seedling development and fertility. They are suitable candidate genes for improvement of crop yield by alleviating lodging in rice. While one of them is developmentally regulated, the other undergoes light regulation. Alternative splicing and post-translational modifications like phosphorylation play a key role in regulation of both the orthologs. My research work has helped in deciphering the complex networks and mechanisms regulating plant responses to light and has enhanced our understanding of how light modulates phenotypic plasticity in plants. In future, I would like to focus on how post-translational modifications in plants respond to environmental cues like light and how light regulates alternative splicing of genes to tailor plant growth. The significance of alternative splicing in the HY5 orthologs will be explored further and how it has increased the repertoire of its functions will be studied.

GENE DISCOVERY FOR DROUGHT-RESILIENT RICE IN AN ERA OF BIG DATA SCIENCE AND ANALYTICS

Eshan Sharma

Institute of Systems, Molecular and Integrative Biology,University of Liverpool, United Kingdom

Global climate change has been severely impacting our agricultural production and is likely to only intensify in coming years. Water deficit conditions due to inadequate rainfall or drought adversely impact the yield in rice. For my work in the area of rice functional genomics, I took an approach of data-driven experimental biology to explore genomic variations existing within the indigenous rice accessions like Dhagaddeshi, Nagina22, Annapurna to identify candidate genes and their associated metabolic and signalling pathways under drought and heat stress conditions. Our work explored ways to identify gene groups that are quantitatively coordinated in their expression under drought stress in rice. We demonstrated the role of *OsFBX257,* a stress induced F-box protein coding gene in drought stress adaptations like root architecture and maintenance of grain yield under drought stress conditions. OsFBX257 works in a molecular network involving OsCDPK1-GF14c and OsSAPK2-OsPP2C08 signalling pathways to influence drought stress and developmental responses in rice. Conserved among land plants, *OsFBX257* shows allelic variations in rice accessions that have been identified for their resistance towards singular or multiple abiotic stresses. Our research works have expanded the current understanding of drought resistance in rice and has furthered the efforts to develop drought-resilient rice cultivars.In the future, data driven approaches that combine information from sequenced genomes with multi-omics like metabolomics, meta-genomics, precision transcriptomics with AI and phenotyping can be used as powerful tools. This would aid selection of right genes and find viable long-term solutions to develop 'robust' crop plants tolerant to adverse climatic conditions. Understanding and rightfully annotating genes and their natural variations in rice still remains a huge task. Thus, it shall be equally important to translate the advances made with the enormous data being generated towards development of useful tools for rice researchers and breeders.

Keywords: Rice, Drought, pathways, F-box protein, alleles, N22

FUNCTIONAL GENOMICS OF HORMONAL REGULATION OF CROWN ROOT DEVELOPMENT IN RICE

Harshita Singh

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The Oryza sativa (rice) root system is primarily composed of shoot-borne adventitious/crownroots (ARs/CRs) that develop from the coleoptile base, and therefore, it is an excellent modelsystem for studying the shoot-to-root differentiation process. In rice, ARs arise constitutively from thebasal coleoptile/stem nodes in a circular pattern, also known as nodal or crown roots (CRs).These post-embryonic shoot-borne CRs dominate the mature root system of the rice plant.Auxin is a key regulator of root organogenesis in plants,and an auxin maximum is a prerequisite for CRP initiation.In previous work, we performed genome-wide identification and characterization of genesregulated by auxin and cytokinin in rice crown tissues. The study provided a list of global genesand transcription factors (TFs), which are commonly and specifically regulated by auxin andcytokinin. The data provide a rich source of fore-mining novel gene functions.

However, there is no linear correlation between transcript and protein abundance. We revealglobal changes in protein and protein phosphorylation in response to an auxin stimulus duringCRdevelopment. Using a label-free, quantitative (phospho)proteomics-based approach, wehaveidentified proteins whose abundance is altered and phosphorylated upon auxin treatment.Gene ontology enrichment analysis of global proteome data uncovered the biological processes associated with chromatin conformational change, gene expression and cell cycle that were regulated by auxin signaling. Spatial gene expression pattern analysis of differentially abundant proteins disclosed their stage-specific dynamic expression pattern during CRP development. Further, our tempo-spatial gene expression and functional analyses revealed that auxin creates a regulatory module during CRP development and activates ethylene biosynthesis exclusively during CRP initiation. Further, the phosphoproteome analysis identified 8,220 phosphosites, which could be mapped to 1,594 phosphoproteins and of which 66 phosphosites were differentially phosphorylated upon auxin treatment. Importantly, we observed differential phosphorylation of the cyclin-dependent kinase G-2 (OsCDKG;2) and cell wall proteins, in response to auxin signaling, suggesting that auxin-dependent phosphorylation may be required for cell cycle activation and cell wall synthesis during root organogenesis. Thus, our study provides evidence for the translational and post-translational regulation during CR development downstream of the auxin signaling pathway.Thus, our study provides evidencefor the translational and posttranslationalregulation during CR development downstream of the auxin signaling pathway.CR meristem provides an excellent system to study the factors and mechanisms operating toregulate pluripotency, cellular memory, and cell-fate transition in plants.

DEVELOPING GENOME-EDITED POPULATION IN INDICA RICE USING CRISPR-CAS9 POOL LIBRARY APPROACH

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High-throughput mutagenesis approaches such as EMS and T-DNA insertion have played vital role in functional genomics studies in plants. However, due to nature of these approaches, the induced mutations are at random locations in the genome. Nevertheless, the advent of the CRISPR-Cas9 system has enabled modification of DNA at precise and desired locations, and researchers around the globe have started to use the CRISPR-Cas system for high-throughput mutagenesis in different crops. To develop such a mutant population in Indica rice, we have generated a CRISPR-Cas9 pool plasmid library for 12000 rice genes. Analysis of this library using Sanger sequencing and Next Generation Sequencing (NGS) approaches revealed 83% accuracy and coverage of 99% gRNAs. The library has been further tested for its functionality and mutation efficiency by its transformation in Indian mega rice variety MTU-1010 through tissue culture approach. Our results shows an 80% editing efficiency of this library in T0 generation of the regenerated plants. To further develop large number of edited plants, a collaborative effort is required. The developed population can be screened for multiple traits as well under different environmental conditions. This developed resource would be very useful not only for identifying designer high-yielding and highly nutritious rice varieties but also for functional genomics studies by rice researchers.

Key words: CRISPR-Cas9; Mutant population; Indica rice, Sequencing

VARIATION IN PHOTOSYNTHESIS AND PHOTOASSIMILATE PARTITIONING ACROSS CULTIVATED AND WILD RICE AND THE UNDERLYING ATTRIBUTES

*Jyotirmaya Mathan[1,2], Anuradha Singh[1], Vikram Jathar[1], Aashish Ranjan[1]**

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Photosynthesis and photoassimilate partitioning are pivotal processes profoundly influencing plant performance, biomass, and yield. Leaf is the prime site for carbon fixation, hence, different developmental, photochemical, and biochemical traits of a leaf affect photosynthesis. Photoassimilate partitioning, in the form of sucrose, depends on the source and sink strength, phloem loading and unloading, and activity of sucrose transporters. We identified wild rice species showing a higher net leaf photosynthesis rate (P_N) compared to cultivated rice varieties. The differences in the leaf photosynthesis rate were associated with distinctive leaf traits, such as mesophyll cell size, lobbing, and vein dimensions, along with efficient photochemical and biochemical features. Consistent with higher photosynthesis, the wild relatives accumulated high non-structural carbohydrates (NSCs) in leaves. ^{14}C lebelled sucrose loading experiment, quantification of sucrose in phloem sap, and expression analysis of *SWEET* genes encoding sucrose transporters suggested efficient phloem loading in the leaves of wild species. However, sucrose transport was not adequate to the grains of the wild species, likely due to abnormal vascular bundles as well as reduced expression of key *SWEET*s and *SUT*s genes at the panicle base. Interestingly, a large portion of sucrose in the wild relatives of rice stem was converted to structural carbohydrates, such as cellulose and hemicellulose, likely due to higher cleavage activity of OsSUS and higher expression of cellulose synthase. Furthermore, our study elucidated the effects of drought and salinity stresses on sucrose distribution and transport in indica rice varieties. We identified key SWEET transporters and their regulatory roles for maintaining sucrose homeostasis under abiotic stress.

Keywords: Rubisco, Mesophyll cell, Sucrose, Sucrose transporters, Sucrose synthase, Cellulose, Abiotic stress

DECIPHERING THE ROLE OF MEMBRANE LIPID REMODELING GENES IN COMBATING PHOSPHATE DEFICIENCY

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*****Presenting author

Phosphate (P) deficiency is a major constraint for crop productivity. To combat P deficiency, plants undergo several adaptions that largely focus on enhanced P acquisition from the soil and efficient use of acquired P. Membrane lipid remodeling is one such adaption that plays a key role in the utilization of P stored in the membrane phospholipids. During membrane lipid remodeling, phospholipids are degraded to release P for essential cellular function, and P-free lipids, especially galactolipids, are synthesized to fill the phospholipids position for the maintenance of membrane function. In our study, we targeted the *Monogalactosyldiacylglycerol-synthase-3* (*OsMGD3*) gene which was found to be the highly upregulated under P deficiency. Our study showed that *OsMGD3* plays a key role in P acquisition under P-sufficient conditions by affecting the Phosphatidic Acid level that regulates the expression of P transporters genes. Under P deficiency, *OsMGD3* majorly plays a role in membrane lipid remodeling that affects P-use efficiency. We also explored the role of Glycerophosphodiester-phosphodiesterase (GDPD) enzymes that participate in the degradation of intermediate products of phospholipid degradation pathways under P deficiency. Our study on the *OsGDPD2* gene revealed its role in root growth and carbohydrate metabolism that affects P deficiency tolerance. To further explore the role of GDPD in root growth, we targeted*OsGDPD5* which is highly expressed in root tissue. OsGDPD5 KO lines have reduced root length and P acquisition. Our study on OsGDPD5 reveals its novel role in regulating root length by affecting soluble sugar levels. Thus, our study suggests crucial role of membrane lipid remodeling genes in combating P deficiency.

PROTEIN L-ISOASPARTYLMETHYLTRANSFERASE (PIMT): A KEY PLAYER FOR CONTROLLING AGRONOMICALLY IMPORTANT SEED TRAITS.

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Protein L-isoaspartyl methyltransferase (PIMT) is a widely distributed and evolutionarily conserved protein repair enzyme (PRE), known for its role in maintaining the native structure and function of proteins by catalyzing the repair of L-isoaspartyl residues back to L-aspartyl residues. Our previous research has established PIMT's significance in enhancing seed vigor and longevity in orthodox rice seeds.

In this study, we investigate the necessity of the PIMT enzyme in recalcitrant seeds. Through a comparative analysis of orthodox (*Oryza sativa*) and recalcitrant (*Oryza coarctata*) rice seeds, we unveil differential regulation of PIMT by ABA and ABI transcription factors during seed maturation. This differential regulation contributes to desiccation tolerance and subsequent longevity, as PIMT repairs ABI-TF in orthodox seeds but not in recalcitrant seeds.

Furthermore, our research delves into PIMT's role in other agronomic seed traitssuch as seed length and weight. Utilizing over-expression and RNAi lines of PIMT in *Oryza sativa*, we demonstrate its influence on these essential seed characteristics. We also identify enolase as a novel protein that interacts with PIMT and validate the conservation of this interaction in various plant species. By employing MS/MS, we identify the amino acid residues in ENO2 susceptible to isoAsp formation. Importantly, we establish that these isoAsp modifications impact enolase activity both *in vivo* and *in vitro*. Remarkably, PIMT, as a protein repairing enzyme (PRE), physically interacts with ENO2, repairing detrimental isoAsp residues.

This comprehensive study sheds light on the multifaceted role of PIMT in seed biology, uncovering its differential regulation in different seed types, and its interaction with ABI-TF and enolase as a mechanism for ensuring its influence on agronomically important seed traits.

UNRAVELLING THE MYSTERIES OF NOCTURNAL TRANSPIRATION: INSIGHTS ACROSS CROP SPECIES, SEASONS, AND GENOTYPES

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Nocturnal transpiration, characterized by increased transpiration rates before dawn in plants, remains a complex and debated phenomenon with implications for plant growth and development. We investigated the predawn rise in nocturnal transpiration across diverse crop species, genotypes, and environmental conditions using phenomics facility. The research uncovers significant disparities in predawn rise patterns, with the Kharif season exhibiting a more pronounced increase compared to Summer, notably influenced by soil moisture levels and stress conditions. Furthermore, the analysis encompasses various C_3 and C_4 plant species, shedding light on variations in the timing of predawn rise. Furthermore, the initiation of predawn rise correlates with the maximum tillering stage, with a decrease in Vapor Pressure Deficit (VPD). Intriguingly, the timing of predawn rise in transpiration varies with the number of sunshine hours and the maximum temperature of the preceding day. Reduced sunshine hours or lower temperatures lead to an earlier predawn rise, affecting dawn transpiration rates. Intriguingly, on days with less sunshine, nocturnal transpiration becomes independent of VPD for both genotypes, highlighting the impact of preceding-day weather conditions. Moreover, the screening of 200 genotypes reveals substantial variability in predawn transpiration, emphasizing its importance. GWAS reveals a genetic switch occurring at predawn, marked by four significant markers. These genes exhibit notable expression differences among contrasting genotypes. This study enriches our grasp of predawn transpiration, emphasizing its role in optimizing plant water use efficiency.

Key words: Nocturnal transpiration; phenomics; predawn transpiration; water use efficiency

PAN-GENOME STUDIES OF ASIAN RICE POPULATION REFERENCE PANEL (RPRP, *O. SATIVA*)

Yong Zhou[1,2], Zhichao Yu[1,3], Dmytro Chebotarov[4], Kapeel Chougule[5], Luis F. Rivera[1], Nagarajan Kathiresan[1], Ramil Mauleon[4], Andrea Zuccolo[1], Doreen Ware[5,], Kenneth McNally[4,*], Jianwei Zhang[3,*], Rod A Wing[1,2,*]*

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Rice (*Oryza Sativa*) plays a critical role in food security and thus, understanding and exploiting genetic diversity that exists in germplasm banks across the globe is required. To meet this demand, we assembled a pan-genome Rice Population Reference Panel (RPRP) that represents the K=15 subpopulation structure of Asian rice, plus two outgroup species, *i.e.*, *O. rufipogon* [AA] and *O. punctata* [BB]. The RPRP consists of 16 platinum standard reference sequences (PSRefSeqs) with an average ContigN50 size, gap number, and BUSCO score of 23 Mb, 18 gaps, and 98.7%, respectively. Using this pan-genome, we identified 71,529 large structural variations (SVs, >50 bp) and generated a pan-genome inversion index (PGII) with 1,769 large inversions (>100 bp), which revealed evolutionary insights into the subpopulation structure of Asian rice. Gene annotation of the RPRP was used to develop a Rice Gene Index (v1.0), where we detected 112,658 Ortholog Gene Indices (OGI). Lastly, the RPRP was used as a template to map resequencing data from the 3k-RGP resequencing data set to identify virtually all standing natural variation that exists in the pan-genome of cultivated Asian rice. Results showed an average of ~27.3 million SNPs per genome, ~2.1 M of which were novel. Using the submergence-tolerant gene *Sub-1A* as a test case to investigate genetic variation across the 3k-RGP, we discovered 180 accessions that possess the submergence-tolerant allele, which may be useful for accelerated breeding programs and functional genomics studies.

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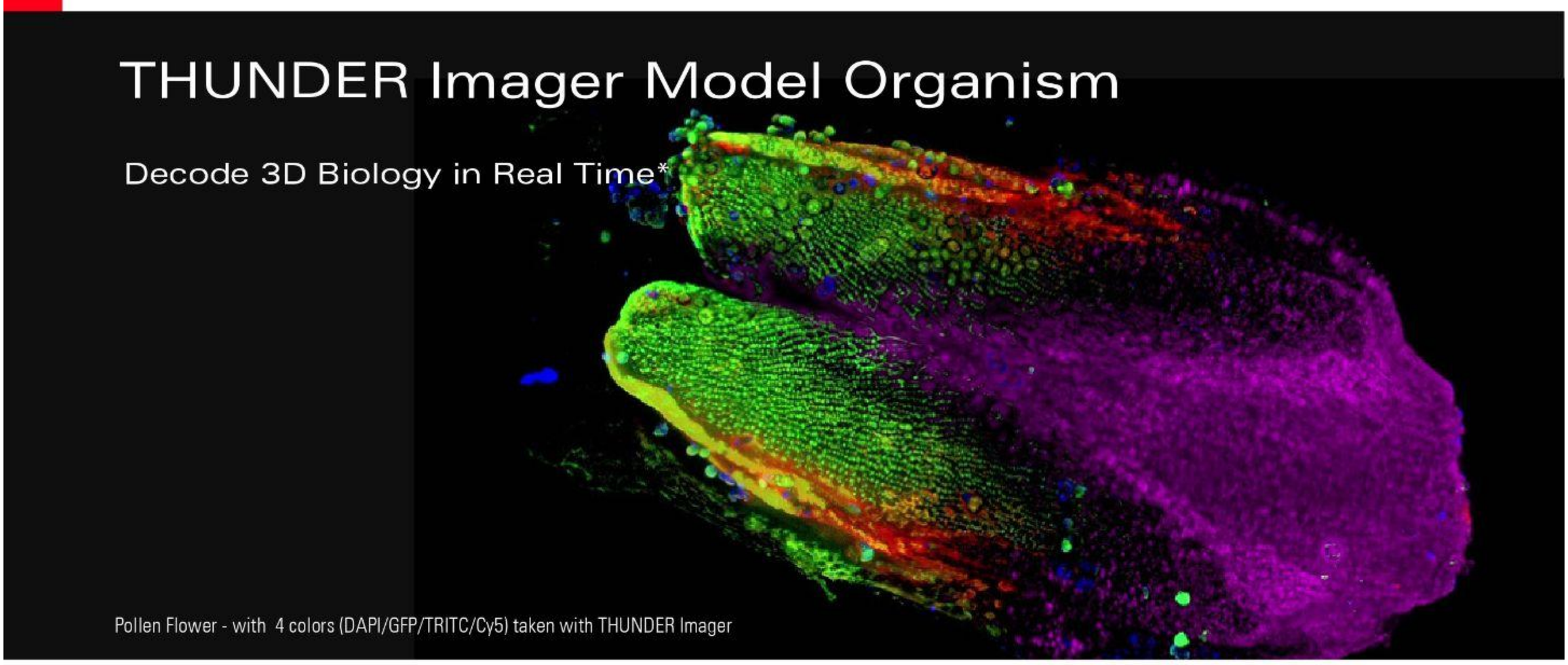

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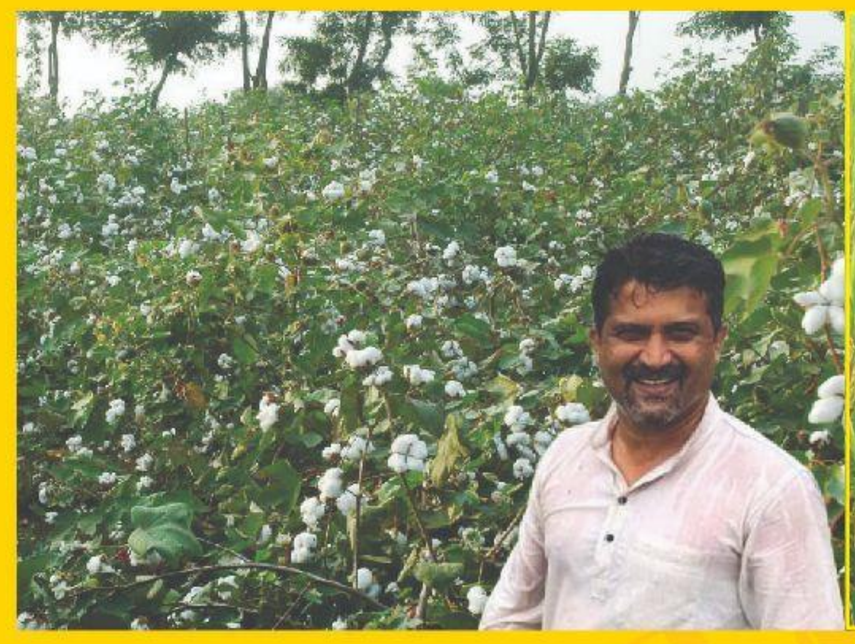

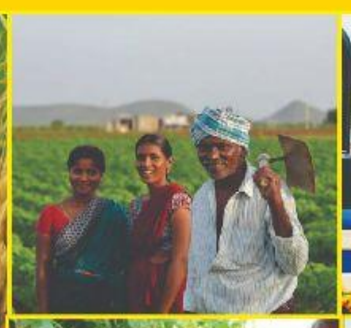

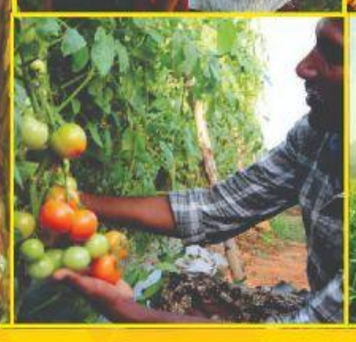

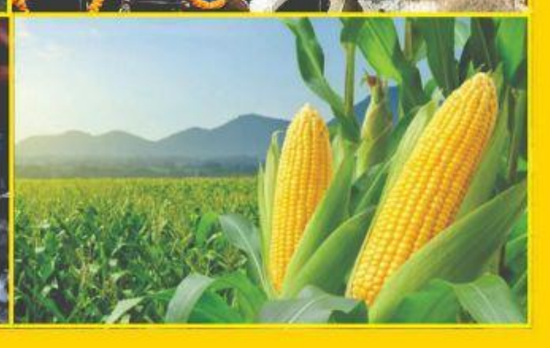

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