Abstracts and Posters

ABSTRACTS

Integrating transcriptome profiling, re-sequencing, high throughput SNP genotyping, and chemically induced mutants for functional genomics of rice

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Rice is a major staple food of Asia, where 92% of world rice is produced and consumed. As the demand for rice grows by the ever increasing population, meeting this demand would be highly challenging in the face of declining availability of cultivable land and water resources. Rice is genetically structured to survive, grow, successfully reproduce and adequately yield in diverse ecosystems. In India, rice genotypes are available that are capable of growing below the sea level in Kerala and on the hill slopes of the north-eastern India. Rice genotypes suitable for upland ecology have retained the capacity to yield better under managed irrigated/lowland ecologies, while the lowland/irrigated ecosystem genotypes do not perform well in upland situation. Although the upland rice is adapted to low moisture conditions, its productivity is however much lower than that is obtained under irrigated ecology. Such ecologically adaptive differentiation observed in rice provides opportunities to understand the process with a long-term objective of appropriate genetic modification so that it becomes possible to tailor rice genotypes for optimum performance with limited use of water. The trait under consideration is genetically complex due to quantitative inheritance with involvement of many genes having varying degree of effect on the trait coupled with interactions among themselves as well as with environment. Several efforts in the past have been made to map the underlying factors named quantitative trait loci, which have been mapped on all the rice chromosomes. Besides, gene expression analysis using microarrays have provided a huge information resource on hundreds of drought responsive genes which function in many different pathways. We have chosen the upland rice genotype Nagina 22 to identify drought responsive genes including those encoding miRNAs at vegetative and reproductive stages. Hundreds of such genes have been identified and validated for their differential expression. Additionally, the genome of this genotype was partially re-sequenced along with seven others belonging to different ecologies to identify single nucleotide polymorphisms (SNPs) by employing Illumina and SOLiD next generation sequencing platforms. Several millions of SNPs in pair-wise comparisons were discovered particularly in the genic regions of the genome. A SNP chip was designed based on the stress responsive genes and validated for use in large-scale genotyping of rice using the Illumina GoldenGate/Infenium assays. The inferred ancestry of 16% among rice genotypes was derived from admix population with maximum between upland and wild *Oryza* species suggesting likely contribution of greater adaptiveness from the wild to the upland genotypes. Non-synonymous SNP loci in nine important abiotic stress-responsive rice genes (WRKY and MYB family transcription factors, protein kinase and heat shock protein) showed differentiation between drought-tolerant upland and susceptible lowland *indica* genotype groups for mis-sense mutation and introduction of premature termination codons. Treating Nagina22 with the chemical mutagen EMS, more than 20,000 lines have been generated and phenotyped for many traits. Identification of drought tolerant mutants and their use will help functional analysis of this complex trait.

Wild species: Novel gene pool for breeding disease and insect resistant rice varieties

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Rice is attacked by a large number of insects. Among these insect pests, planthoppers, stem borers, and gall midges are the most serious pests of rice. Six kinds of planthoppers attack rice plants. In resistance breeding several questions need to be answered: should we give priority to use genes for pest resistance which are

inducible or genes for pest resistance which are inducible or constitutive? Classical genetics and molecular biology research on resistance to leaf blast has made tremendous progress; however, neck blast, which is quite damaging, has not been well studied. Would it be worthwhile to accumulate QTLs with small effect to enhance level of resistance to sheath blight and stem borer? Are we at a stage to develop multi-lines to reduce the pest pressure for sustainable rice production? The genetic variability for some traits such as resistance to sheath blight, tungro and yellow stem borer, and tolerance to salinity and acid sulfate conditions is limited in the cultivated rice germplasm. Wild species are an important reservoir of useful genes. However, several incompatibility barriers such as low crossability and limited recombination between chromosomes of wild and cultivated species limit the transfer of useful genes. Recent advances in tissue culture have enabled production of wide hybrids and molecular marker technology and in-situ hybridization techniques have enabled to precisely detect the introgression of chromosome segments from wild into cultivated species. The first example of transfer of a useful gene from wild species is the introgression of a gene for grassy stunt virus resistance from *O. nivara* to cultivated rice varieties and *Xa-21* for BB resistance was transferred from *O. longistaminata*. Wild species of *Oryza* include *O. ridleyi, O. officinalis, O. minuta, O. alta, O. brachyanta, O. longistaminata* and *O. rufipogon*. These are grass like plants-phenotypically inferior but valuable genetic resource. They can provide useful traits for insect resistance, disease resistance, tolerance to abiotic stresses, yield QTLs, C4-ness, BNF endophytes and apomixes. Some examples on genes identified for insect and disease resistance are: For brown planthopper (BPH) - *Bph1, bph2, Bph3, bph4, bph5, Bph6, bph7, bph8, Bph9, Bph10, bph11, bph12, Bph13, Bph14, Bph15, Bph16, Bph17, Bph18, bph19, Bph20(t) Bph21(t) Bph22, Bph30, Bph20(t), Bph21(t), Bph22…Bph30.* For white backed planthopper (WBPH) - *Wbph1, Wbph2, Wbph3, wbph4, Wbph5, Wbph6, Wbph7(t), Wbph8(t).* For green leafhopper (GLH) - *Glh1, Glh2, Glh3, glh4, Glh5, Glh6, Glh7, glh8, Glh9, glh10, Glh12(t), Glh13(t), Glh14(t).* For gall mididge (GM) - *Gm1, Gm2, gm3, Gm4, Gm5, Gm6, Gm7 … Gm111.* For bacterial leaf blight (BB) (30 genes) - *Xa1, Xa2, Xa3, Xa4, xa5, Xa6, Xa7, xa8, Xa9, Xa10, Xa11, Xa12, xa13, Xa14, xa15, Xa16, Xa17, Xa18, xa19, xa20, Xa21, Xa22, Xa23, xa24, Xa25, Xa26, Xa27, xa28, Xa29, Xa30(t), Xa31(t), Xa32(t), Xa26, Xa27, xa28, Xa29, Xa30(t), Xa31(t), Xa32(t), Xa33 (t), Xa34 (t)*. For blast (40 genes) - *Pi9, Pi18* (some unnamed genes). Cloned genes include *Xa1, xa5, xa13, Xa12, Xa26, Xa27, Pib, Pita, Pikh (Pi54), Pi9 – 17.* (Genes in underlined have been introgressed into rice from wild species).

Inter-specific hybrids have been produced between *O. sativa* and the tetraploid wild species *O. minuta* (2n=48, BBCC). Following backcrossing and embryo rescue, advanced lines have been produced from the cross of *O. sativa* (IR31917-45-3-2) and *O. minuta* (Acc 101141). To understand the molecular basis of broad spectrum resistance to rice blast, fine-scale mapping of the two blast resistance (R) genes, *Pi9(t)* and *Pi2(t),* was conducted. These two genes were introgressed from deferent resistance donors, previously reported to confer resistance to many blast isolates in the Philippines, and were mapped to an approximately 10-cM interval on chromosome 6. To further test their resistance spectrum, 43 blast isolates collected from 13 countries were used to inoculate the *Pi2(t)* and *Pi9(t)* plants. *Pi9(t)*-bearing lines were highly resistant to all isolates tested, and lines carrying *Pi2(t)* were resistant to 36 isolates confirming the broad-spectrum resistance of these two genes to diverse blast isolates. MAS products with *Xa21* gene from wild species released as varieties by national agricultural research systems: NSICRc 142 (Tubigan 7) with *Xa4* and *Xa21* by PhilRice Philippines; NSICRc 154 (Tubigan 11) with *Xa4* and *Xa21* by Philippines; Improved Pusa Basmati 1 with *xa5, xa13* and *Xa21* by India; Xieyou 218 and Zhongyou 218 with *Xa21* by China; Guodao 1, Guodao 3 and Neizyou with *Xa4, xa5, xa13* and *Xa21* by China; lyou 218 and ZhongbaiYou 1with *Xa21* by China; and Amni with *Bph18* by Korea. BPH resistant lines from *O. sativa* x *O officinalis* released as varieties in Vietnam include breeding lines IR 54751-2-44-15-243 (MTL), IR 54751-2-34-10-6-1 (MTL 103), IR 54751-2-41-10-5-1 (MTL 105), and IR 54751-2-44-15-2-2 (MTL114).

Summary of genes transferred from wild species into rice: Grassy stunt resistance - *O. nivara (AA);* BB resistance - *O. rufipogon (AA), O. longistaminata (AA), O. minuta (BBCC) O officinalis (CC) O latifoli (CCDD), O. australiensis (EE), O.brachyantha (FF);* blast resistance - *O. glaberrima (AA), O. minuta (BBCC), O. australiensis (EE);* BPH resistance - *O. glaberrima (AA), O. rufipogon (AA), O.minuta (BBCC), O. officinalis (CC), O. latifolia (CCDD), O. australiensis (EE)(EE);* WBPH resistance - *O. officinalis (CC);* tungro tolerance - *O. rufipogon (AA).*

Use CSSL to fine map and clone novel genes/QTLs wild species. Search for genes controlling homoeologous pairing to transfer genes from distant genomes. With the new advances in molecular biology or genomics the potential to introduce well defined target genes/QTLs for pest resistance can lead to the production of pest resistance can lead to the production of environmentally-friendly designer rice resistant to pests in the future. Future research should focus on establishing high-throughput screening protocols for field resistance, identifying new genes for resistance from diverse sources. Furthermore, there is a need to

develop gene-based markers, particularly single nucleotide polymorphism (SNP) markers to accelerate the transfer of genes into different genetic backgrounds and for breeding varieties resistant to hoppers. Characterization of insect populations/biotypes in different geographical regions is emphasized for the deployment of different genes for resistance to planthoppers.

Generating new resistance sources using forward and reverse genetics in rice

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Marker assisted selection (MAS) is used for rice improvement when the variation for the trait of interest (resistance against a biotic stress) is already present in the gene pool of rice. Transgenic technology is will be used when source of resistance is not available within the gene pool of rice or the available source of resistance is broken down. To increase the extent of variation in the gene pool of rice and tag the variation with molecular markers such that MAS methodologies can be used to provide resistance. Mutagenized rice populations are screened for resistant variants. The isolated lines are sequenced using next generation sequencing (NGS) methodologies and bioinformatics analysis is used to identify candidate mutations. A segregating population is used to assess co-segregation of the mutation with the resistance trait. The DNA marker is used to transfer validated mutations into other genetic backgrounds. Reverse genetics using TILLING (targeting induced local lesions in genomes): Rice genes whose overexpression or underexpression leads to enhanced resistance are identified using transgenic methodologies, VIGS or Agroinfection, etc. An allelic series of mutations is generated in these genes and their regulatory regions using TILLING. These TILLED lines are screened for resistance. Again a segregating population is used to assess co-segregation of the mutation with the resistance trait. The DNA marker is used to transfer validated mutations into other genetic backgrounds. Advantages of these approaches are that these lines are not transgenics. Therefore, expensive and time consuming regulatory steps are minimized. The disadvantages of these approaches are that increased chances of failure. In the forward genetics approach, it may not be possible to isolate any mutant line that has the resistance that is sought. Even if a resistant line is identified, it may be difficult to identify the causative mutation due to limitations of the NGS technologies. The disadvantage of the TILLING approach is that it would be difficult to generate gain of function mutations.

POSTES

Transgenic rice and biosafety

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Transgenic rice was first produced in 1988. Since then transgenic rice has been produced for herbicide resistance, bacterial blight, virus, sheath blight resistance, stem borer resistance, and high iron content. Unfortunately, there has been no commercial release of transgenic rice as it poses biosafety concerns. Uniformity and stability of transgene integration and gene expression level in transgenic plants is a pre-requisite for commercialization purposes. Characterization and evaluation of transgenics based on agronomic and molecular characteristics is essential for commercialization. Different aspects are considered before a plant qualifies for a transgenic. Phenotypic analysis includes field performance, agronomic trait characterization, and yield. Molecular analysis includes genetic stability, identification of insertion site of transgene, structure and inheritance of transgene loci, copy number estimation and expression analysis of transgene, vector backbone contamination and spacial and temporal analysis of transformed plants. Various molecular and cytogenetics techniques applied for transgenic research include Restriction analysis, Southern and Northern hybridization, dot blot, inverse PCR, genome walking- anchor PCR, competitive PCR, real time PCR for absolute and relative quantifications, micro arrays, and FISH, and transgenic protein analysis by Western blot analysis and ELISA. Segregation analysis is essential to study the inheritance of transgene loci. Rapid advances in the development and commercialization of transgenic crops have led to considerable apprehensions and concerns about the safety of GM products for human, animal health and environment safety. Biosafety of transgenic rice includes two major components, food and health, and environmental safety. Transgenic rice should be assessed for toxicity, allergenicity and nutritional imbalances. Effect of GM rice has to be investigated on beneficial insects, soil-microorganisms, non-target organisms, gene flow and pest resistance. Once the biosafety of transgenic rice is established, the transgenic rice can gain regulatory and market acceptance.

Pyramiding insect and disease resistance genes in elite *indica* rice cultivar ASD16

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An elite *indica* rice cultivar ASD16 was transformed with genes expressing a serine-threonine kinase (*Xa21*) conferring bacterial blight (BB) resistance, a thaumatin-like protein (*tlp*) conferring resistance to sheath blight (ShB) pathogen and a *Galanthus nivalis* agglutinin (*gna*) conferring brown planthopper (BPH) resistance, through *Agrobacterium* or particle bombardment. Molecular and biochemical analyses of putative transgenic (T_0) lines revealed stable integration and expression of the transgenes. Further, transgenic homozygous ASD16 lines showing stable expression of *Xa21* or *tlp* or *gna* and significant protection against BB, ShB and BPH were selected through progeny analysis and bioassays. Involving the transgenic homozygous ASD16 lines as source of breeding, resistance against BB, ShB and BPH was developed by gene pyramiding through sexual crossing. Sexual hybridization was carried out in three different gene combinations viz, *Xa21* x *gna*, *tlp* x *Xa21* and *tlp x gna*. In F_1 generation, a line harbouring both the genes in each cross-combination was selected (through PCR and Western blotting analyses) and forwarded to F_2 generation for further studies. F_2 progeny of the different cross-combinations were analysed for the inheritance of transgenes through PCR and Western blotting analyses. Southern blotting analysis was carried out in the selected F_2 progenies along with their respective parental lines with a view to confirming the genomic integration of desired genes. The F_2 progenies of different cross-combinations were screened for BB, ShB and BPH resistance, following the standard bioassay protocols. The F_2 lines harbouring *Xa21* and *gna* exhibited enhanced resistance against BB and moderate resistance against BPH. Similarly the F_2 lines carrying *tlp* and *Xa21* showed enhanced resistance against ShB and BB. The F_2 lines of another combination *tlp* and *gna* were observed with enhanced resistance against ShB and moderate resistance against BPH. In this study, we have developed excellent genetic sources for use in future breeding programs which are aimed at enhancing BB, ShB and BPH resistance in elite *indica* rice cultivars.

Improvement of popular rice variety, Tellahamsa for biotic stress resistance using marker assisted breeding

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The present research was aimed to introgress two BLB resistance genes (*xa 13* and *Xa21*) and gall midge resistance gene (*Gm4*) from a donor line GPP2 (B95-1 x Abhaya) into the genetic background of Tellahamsa, which is a popular rice variety of ANGRAU, Rajendranagar, Hyderabad possessing excellent grain quality and cold tolerance. Three gene linked SSR markers viz, *xa13* promoter, PTA248 (STS marker) and RM547 were used for foreground selection to identify the plants carrying target genes *xa13*, *Xa21* and *Gm4*, respectively. A total of 160 HRM primers were used for polymorphism survey between donor and recipient parent and 40 primer pairs were identified for background selection. Marker assisted backcross breeding method was followed to introgress three genes into Tellahamsa. The donor parent GPP2 was validated for the presence of three genes. Polymorphism between the parents was assessed for all three target genes. Cross was made between Tellahamsa x GPP2 during kharif 2010. F_1 progeny (104) was raised during rabi 2010 and foreground selection was carried out using gene linked primer pairs and found fifteen plants with all three target genes in heterozygous condition. Single F_1 plant was used as male parent and crossed with recurrent parent, Tellahamsa and BC_1F_1 seeds were obtained. The BC_1F_1 progeny (215) was raised during kharif 2011. Foreground selection was carried out and 20 heterozygous plants carrying all three genes were identified. Three-gene positive plants were evaluated by using background primers to identify the plants with maximum gene recovery. Five plants out of twenty BC_1F_1 plants with recurrent parent recovery of above 70% were used for making BC_2F_1 crosses. BC_2F_1 progeny (162) was raised during rabi 2011. Foreground as well as background selection was taken up and eight plants possessing all three genes in heterozygous condition with genome recovery more than 80% were selected and harvested in order to take up BC_3F_1

production during kharif, 2012. The plants with two gene combinations (*xa13* and *Gm4*; *xa13* and *Xa21*; and *Xa21* and *Gm4*) were also obtained.

Molecular basis of rice-gall midge interactions

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The genetics of rice-gall midge interactions are well characterized but the molecular basis of gall midge-rice interaction has not been investigated. Present study revealed the differences in transcriptome profiling in resistance and susceptible rice varieties attacked by different biotypes of the Asian rice gall midge. We have followed whole genome based and map based approaches to indentify the putative candidate genes involved in rice-gall midge interactions. The target genes in whole genome based approach are the well characterized and reported genes are involved in plant–pathogen or plant–insect interaction irrespective of their chromosomal location. The marker delimited region of the nine gall midge resistant genes and search for putative candidates in vicinity is the basis for map based approach. In lieu of whole genome based approach, we have conducted whole genome transcriptome profiling to search putative candidate genes for HR+ type resistance in the cultivar Suraksha rice against GMB4 using suppressive subtractive hybridization (SSH) cDNA library and candidate genes for HR- type resistance in Kavya rice, by microarray analysis using rice affymatix gene chip. Compatible interactions in different rice genotypes against different virulent gall midge biotypes were studied using both the approaches. The shortlisted candidate genes for the different gall midge interactions were further validated using real-time PCR. The present study identified the defense mechanism of HR+ type resistance in Suraksha to be similar to that of plant-pathogen interaction. Involvement of at least three genes viz, NBS-LRR, phenylalanine ammonia lyase and *OsPR10α*, phenylpropanoid pathway appeared to be the underling route of resistance mechanism in Suraksha against the gall midge. However, the HR- type of resistance in Kavya showed downregulation of phenylpropanoid pathway, salicylic acid signalling and other defense related genes. Our results indicated a novel mechanism of resistance in Kavya against the gall midge that needs further characterization. The compatible interaction of rice-gall midge revealed a common mechanism in different varieties and gall midge biotypes including suppression of plant defense and secondary metabolism related genes to circumvent plant surveillance system and increased primary metabolism and nutrient transport to provide insect growth and shelter. Further, the transcriptional reprogramming in compatible interaction of rice- gall midge featured here suggested that susceptibility is not a default phenotype but is the result of coherent interplay of a large number of genes. Manipulating any of the key genes would result in resistance against the pest.

Molecular engineering of ascorbate-glutathione pathway into rice for improving its oxidative stress tolerance

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Environmental stresses lead to generation of ROS or oxidative stress in cells posing as a major limiting factor in plant productivity. ROS is ubiquitously produced in cells at low levels during normal growth conditions. In stress, metabolic disturbances are accompanied by high ROS generation and the inherent anti-oxidative machinery is incompetent in rapid ROS removal. Excess ROS results in peroxidation of membrane lipids, membrane disintegration and eventually cell death. In plants, crucial ROI-scavenging enzymes of ascorbate-glutathione pathway majorly contribute in combating oxidative stress by deactivation of ROIs released during multiple redox reactions. Therefore, to eliminate or alleviate ROS production during adverse stress conditions, we propose to overexpress the vital enzymes of ascorbate-glutathione pathway such as superoxide dismutase (SOD) ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR) driven by a stress-inducible promoter (rd29A). T_7RNA-polymerase gene based system has been utilized for high expression levels of all transgenes in rice crop. All the gene cassettes have been pyramided in a single plant transformation vector by site-specific homologous recombination. Marker free strategy was employed for agro-mediated transformation. Ten putative transgenic rice lines were analyzed and transgene integration in different chromosomes was confirmed. Expression of transgenes in these transgenic lines was confirmed by RT-PCR. Leaf disc assay with methyl viologen (100 μM) revealed that these transgenic

lines were tolerant to oxidative stress without showing any morphological anomaly.

Identification of new genetic resources for durable blast resistance in India

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Rice blast caused by *Magnoporthe griesea* is one of the major diseases in rice and accounts for nearly 50% yield loss per annum. Till date many resistant genes have been identified for the blast resistance but most of them are not truly broad spectrum and durable. In this context, exploring the potential of wild germplasm for the identification of new blast disease resistance genes will be very advantageous. The wild species of *Oryza* have rarely been used for blast resistance gene discovery except for two genes i.e., *Pi 9* and *Pi 40*. The availability of introgression lines with various wild species enable us to evaluate the level and spectrum of resistance and to identify the introgression line(s) which have the broad spectrum blast resistance for major Indian isolates. In the current study, 326 introgression lines (ILs) derived from the cross of various accessions of six different wild relatives viz. *O. nivara, O. glumaepatula, O. rufipogon, O. glaberrima, O. longistaminata* and *O. barthii* in the genetic background of PR114 and Pusa 44 were used. Stringent screening of these ILs in the uniform blast nursery (UBN) screening in three seasons with highly virulent isolate of Andhra Pradesh resulted in the identification of 50 resistant ILs. Among the resistant ILs majority were derived from *O. glaberrima* and *O. longistaminata* followed by *O. nivara, O. glumaepatula O. rufipogan* and *O. barthii.* Genotyping of these 50 introgression lines with tightly linked markers for major blast resistance genes led to the identification of ILs that have multiple known genes or the novel genes for the resistance. Nine extremely resistant ILs which have shown the absence of known blast resistance genes were also tested and showed promising resistance at various endemic regions of the blast. Such novel ILs are presently being used for mapping and tagging of new blast resistance genes. Hence the ILs that have been identified in the present study could serve as viable genetic resources for the identification of novel genes and superior alleles for blast resistance.

Transgenic rice against rice tungro disease: designing, construction and testing of transgenic rice lines for resistance

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Rice tungro disease is a major biotic stress affecting rice in south and south-east Asia. Two viruses, *Rice tungro bacilliform virus* (RTBV), a DNA virus and *Rice tungro spherical virus* (RTSV), an RNA virus are found in infected tissues. In the absence of well-defined genetic sources of resistance against RTBV and RTSV, a transgenic approach, based on RNA-interference (RNAi), using RTBV and RTSV genes, is being used to obtain resistance in rice. RNAi is an inherent defence mechanism in plants, which is activated by the presence of RNA having strong secondary structures, such as hairpin or as double-stranded, and which brings about sequence-specific degradation of homologous mRNA, resulting in silencing of the gene expression. Viruses are targets of RNAi response in plants, which can generally overcome the defence response by viral suppressor proteins, resulting in disease. Constitutive expression of viral RNAi-inducing DNA constructs strengthens the anti-viral RNAi response and results in viral resistance, as demonstrated in a number of cases. The same principle was applied to develop resistance against RTBV and RTSV in rice. DNA fragments representing approximately 300 bp of RTBV DNA and RTSV cDNA were used to make constructs capable of giving rise to hairpin viral RNA (representing fused RTBV and RTSV genes) under strong constitutive promoters in a binary vector. These vectors contained the hygromycin phosphotransferase gene, capable of imparting resistance to plant tissue against 50 mg/l hygromycin, which can act as the selection marker during transformation of rice tissue; the marker genes having flanking *loxP* sites, capable of being excised to remove the gene upon crossing with a rice line carrying the *cre* gene. This incorporates the well-known Cre-Lox system of marker gene removal for obtaining marker-free rice lines. Transgenic rice lines were generated using two different combinations of RTBV and RTSV genes carried in the above constructs, utilizing *Agrobacterium*-mediated transformation. Transgenic plants, selected for resistance to hygromycin were obtained and grown till maturity. The seeds were sown to obtain T_1 lines, which were analyzed for the expression of the viral genes. Resistance to RTBV and RTSV is under evaluation.

Universal *Agrobacterium* - mediated transformation protocol through direct multiplication of in vitro grown mesocotyl explants in rice

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For development of transgenic varieties of interest in rice, we have developed a simple, efficient and universal *Agrobacterium* mediated transformation protocol. Mature seeds of two *indica* (IR64 and Jaya), one each from *japonica* (AC41039) and aromatic (Basmati 370) varieties were used as explants in the present study. *Agrobacterium tumefaciens* strain EHA 105 carrying Ti plasmid (pBI121) with the selectable marker npt^{II} along with the reporter gene *uidA* encoding β-glucuronidase (GUS) was successfully integrated with rice genome without use of acetosyringone. Sterile distilled water washing in place of cefotaxime in the elimination process has been used to control excess growth of *Agrobacterium*. All the material after transformation germinated in 2 to 3 days of co-cultivation on MS basal medium. Germinated seeds transferred to the selection medium i.e. plain MS medium with 50 mg/l of kanamycin, produced two to three primary tillers within 2 weeks. Mesocotyls from 2 week old in vitro grown plants were taken and cultured in the multiplication medium (MS supplemented with 0.5 mg/l BA and 50 mg/l Kanamycin) where within 6–8 days they produced 3–4 secondary tillers. All the five to six tiller shoots so produced in the process developed roots on plain MS and grew well when transferred to pots containing autoclaved soil and vermicompost in the proportion of 4:1. Transient expression of GUS was observed in all the tissues of recipient plants. Integration of the transgene was confirmed by employing Southern blotting and real-time PCR technique for relative quantification by comparing C_T value with an endogenous reference gene coding for sucrose phosphate synthase (SPS). T2 generation plants also gave positive response for GUS genes. Four genotypes representing three major subspecies of rice did not show any significant difference in tissue culture response suggesting that the transformation protocol developed can be efficiently used across the two major subspecies/ecotypes of the Asian rice cultivar *Oryza sativa*. In this method mature seeds are found as best explants for development of improved transformation mediated regeneration protocol for efficient gene transfer usable across a variety of genotypes through in vitro clonal propagation or shoot bud multiplication from mesocotyls avoiding the widely dependent on callus-embryogenic route which is controlled by many physiological and genetic factors like nitrate reductase gene activity in transgenic research. The protocol outlined offers a potential strategy for 100% transformation and regeneration efficiency in rice which can be a best method for large scale propagation of rice –a high food value cereal.

Genetic engineering of Swarna rice for triple herbicide tolerance

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Rice is cultivated under water logged condition mainly to suppress the weed growth in low rice cultivation. Even in upland rice cultivation control of weeds during early stages of crop growth is crucial for capturing yield potential. Manual weed control over large areas is not feasible from the point of labor supply and monetary costs. Some weeds are difficult to distinguish from crop plants at early stages and pose challenge for manual weeding. Under these situations, chemical weed control is relevant for realizing higher productivity and sustainable farming. Currently about 10,000 tonnes of more harmful herbicides are used annually on conventional crops in Indian agriculture. Most of them are non-selective herbicides applied only before sowing and/or pre-emergence of crop plants. Because of limited flexibility in the schedule of application and their residual effect on the subsequent crop plants restrict the herbicide based weed management in Indian agriculture. Genetic engineering of herbicide resistant crop plants allow more choice in choosing environmentally safe herbicides with more flexibility to apply any time during the entire crop growing season for effective control of weeds. In this contest we are developing triple herbicide tolerant rice plants with different modes of action; two systemic herbicides glyphosate (for spraying) and sulphonylurea (for broadcast) and one contact herbicide Glufosinate (for spray). The genes encoding for EPSP-synthase and acetolactate synthase (ALS), the target enzymes for binding herbicide glyphosate and sulphonylurea respectively, were isolated from rice and mutated to herbicide tolerant by site directed mutation. The mutated EPSP synthase and acetolactate synthase along with BAR genes cloned together by in vitro gene pyramiding into a single plant transformation vector for rice transformation. The in

vitro gene pyramiding will help in preventing the subsequent segregation of these 3 herbicide tolerant genes from each other during subsequent generations or move together during the rice-breeding program. The triple herbicide tolerant rice plant is a powerful tool in the integrated weed management of rice cultivation to control large number of weed species, their highly variable life cycles and survival mechanisms by spraying any one or combination of the broad-spectrum non-selective herbicides. Systematic rotation of these broad-spectrum non-selective herbicides, whose mode of action is entirely different from each other, in the rice cultivation areas, can prevent the development of herbicide tolerant weeds effectively.

Galactinol synthase across evolutionary diverse taxa: Functional preference for higher plants for abiotic stress

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Galactinol synthase (GolS), a GT8 family glycosyl transferase, synthesizes galactinol and raffinose series of oligosaccharides (RFOs). We have identified and analyzed the conserved domains in GTs among evolutionarily diverse taxa. The results indicated that GolS is a subfamily of GT8 class of enzymes, present only in plants. Structure prediction by homology modeling and determination of substrate binding pocket showed that the structure of the protein is highly flexible, allowing a broad window for evolution by introduction of point mutations or other subtle alterations, and, thus a high number of isoforms are present in plant kingdom probably linked to the flexibility of the protein. Phylogenetic analyses of GolS sequences establish presence of functional GolS predominantly in higher plants, fungi having the closest possible ancestral sequences. It has also been known that expression of galactinol synthase gene is stress-regulated. Evolutionary preference for a functional GolS expression in higher plants might have arisen in response to the need for galactinol and RFO synthesis to combat abiotic stress, in contrast to other organisms lacking functional GolS for such functions. Emerging evidence for stress signaling and ROS-homeostasis roles for galactinol and raffinose sugars necessitate exploring this pathway for use as a potent target of genetic engineering.

Engeneering inositol metabolism for improved plant survival under abiotic stress

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Agricultural biotechnology aims to improve tolerance of crop plants to salinity, drought and cold-mediated dehydration, and other abiotic stresses among other objectives. Inositol being a molecule of central importance in plant life is connected to numerous life processes. The multifunctional nature of inositol makes it a perfect tool to understand the metabolic networks from the perspective of the biology of the system. The exploration of such pathways indicates that inositol itself and many of its derivatives can impart abiotic stress tolerance (against salinity, dehydration, chilling or oxidative stress, known to date) to plants when overexpressed. *Porteresia coarctata* Roxb. (Tateoka), a halophytic wild rice serves as a model system to study salt-stress biology and the importance of inositol metabolizing pathway in relation to abiotoc stress. Inositol is synthesized from an evolutionary conserved two step pathway where MIPS (coded by *INO1*), the key regulating enzyme converts glucose 6-phosphate to *myo*-inositol 1-phosphate which is further dephosphorylated by L-*myo*-inositol 1-phosphate phosphatase to form free inositol. Hence, an inositol overexpressing system is an excellent system to study the entire network associated with this central molecule rendering abiotic stress tolerance *in planta* in one way and a potential tool with repect to stress-tolerance and productivity on the other side. *Oryza sativa* introgressed with salt-tolerant genes like *PcINO1*(*INO1* from *P. coarctata*) can serve as an inositol overexpressing system to trace the pathway of inositol utilization in comparison to the salt tolerant system *P. coarctata* at transcriptomic, proteomic and metabolomic levels. Keeping this in mind, an investigation was designed to analyse different inositol -1P synthase transgenic lines for their salt tolerance property. The transgenic lines (*PcINO1* and *OsINO1*, O*ryza sativa INO1*) were screened by PCR, GUS assay and Southern blotting, maintained through the reproductive phase and selfed through two consecutive generations; T2 and T3. The salt tolerance property of the *PcINO1* lines was verified by growing in presence of salt and measuring photosynthetic efficiency against the salt sensitive *OsINO1* lines. The lines were photosynthetically

uncompromised, with the desired insertion of the *PcINO1*/*OsINO1* genes under cAMV35S promoter. It can be concluded that the transgenic lines follow 3:1 Mendelian monogenic segregation pattern as expected from a dominant gene and the quality of the rice grains in terms of productivity and grain weight has not been compromised.

Functional validation of a small GTP-binding protein PgRab7 in rice for abiotic stress tolerance

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Small GTP-binding proteins are ubiquitous among eukaryotes. In plants Rab proteins form the largest family of the small GTPases. They are involved in a wide variety of cellular processes in eukaryotic cells including, signal transduction, cell proliferation, vesicular transport, and cytoskeletal organization. Very little information exists on the role of intracellular vesicle trafficking intolerance with reference to environmental stresses particularly abiotic stress, in plants. The present study was therefore undertaken to functionally characterize the role of PgRab7 a small GTP-binding protein from *Pennisetum glaucum*, a relatively drought-stress tolerant food grain crop, in different abiotic stresses with particular reference to salinity and drought stress. The amino acid sequence of OsRab7 and PgRab7 proteins showed 92.3 % identity and the protein structures are also highly similar. Rice has four orthologs of Rab7 distributed on only 2 chromosomes. The transgenic rice plant overexpressing PgRab7 were developed and confirmed by PCR and Western blot analysis. Measurement of different photosynthetic parameters of wild type (WT) and transgenic plants under control conditions suggest that transgenic plants have better photosynthesis efficiency than WT. Leaf disc senescence assay and T2 seed germination assay under NaCl stress showed that transgenic plants have better tolerance towards salinity stress than WT. Transgenic lines growing throughout in 200 mM salinity stress and two months old transgenic plants provided with 200 mM NaCl stress up to seed harvest showed that transgenic plants have better tolerance capacity at vegetative stage as compared to WT but showed yield penalty as tested by seed weights. The better tolerance at vegetative stage may be due to high chlorophyll level and better photosynthetic efficiency. The mechanism of tolerance of transgenic lines to salinity stress may be due to increase in vacuolar volume, intact chloroplast, less oxidative stress, less Na+ penetration to root cell and high K+/Na+ ratio. Under drought stress, transgenic lines showed better tolerance at vegetative as well as at the flowering stage leading to better seed yield. Sortin1 and Brefeldin experiments suggest that Rab7 follows similar trafficking pathways in both WT and transgenic lines. Microarray data showed significant effects of stress condition on gene expression in both transgenic lines and WT plants.

Over-expression of rice endochitinase (*chi11*) gene in rice confers enhanced resistance to fungal pathogens

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Rice serves as the staple food crop for more than two thirds of the world's population. For ensuring stable production and adequate supply of rice, it is imperative to mitigate the losses caused by different pathogens. Genetic engineering of crop plants for disease resistance has become a valuable tool owing to its eco-friendly nature. Exploitation of pathogenesis related (PR) genes for disease resistance, through genetic engineering, has great potential as it leads to durable and broad-based resistance against fungal pathogens. We deployed rice endochitinase (*chi11*) for development of transgenic rice resistant to multiple fungal pathogens. Expression unit of *chi11* was cloned into the pSB11bar intermediate vector, and was mobilized into *Agrobacterium* LBA4404 strain. Leading rice cultivar Swarna was employed for development of fungal resistant transgenic lines. Putative transformed calli were selected on 6 mg/l and 8 mg/l phosphinothricin containing MS medium, and selected calli were regenerated on MS medium supplemented with BAP and NAA. The putative transgenic plants were established in the glass house and tested with 0.25% BASTA for confirmation of bar gene expression. Different transformants exhibited varied levels of tolerance to the herbicide BASTA. Integration and expression of transgenes in rice were confirmed by PCR, Southern and Northern analyses. T1 progenies of selected transformants showed monogenic segregation (3 tolerant:1 susceptible) against BASTA and fungal pathogens. Homozygous T2-transgenic lines harbouring *chi11* exhibited high-level resistance to rice blast (*Magnaporthe grisea*), as well as for rice sheath blight (*Rhizoctonia solani*). These transgenic lines, endowed with multiple disease resistance, hold promise as a potential genetic resource for development of rice cultivars with durable resistance against major pathogens.

Generation of gain-in function mutants in selected rice genotypes by an *in planta* transformation strategy

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Rice (*Oryza sativa* L.), one of the most important crop, is a staple food for almost half of the world's population. It consumes large amounts of water for its growth and productivity. To grow rice under aerobic conditions of non-puddled situation and save 40-60% of irrigation water, attempts have been made to identify rice genotypes with desirable traits like high water mining, water use efficiency, low spikelet sterility and tolerance to moisture stress. Some of the genotypes like JBT 36/14; JBT 38/19 etc. are adapted to aerobic conditions. These genotypes have superior root characteristics and reasonably good productivity, butare photosensitive with poor cellular level tolerance. Activation tagging has emerged out as one of the powerful tool for gene discovery. It involves the upregulation of endogenous genes through the insertion of a strong enhancer (4X element of CaMV 35S promoter). The method can also be used to generate variants with agronomically important traits for crop improvement. The scientific strategy of our study was to generate gain-in function mutants in JBT 36/14 and identify mutants with less photosensitivity, improved growth rates and tolerance to moisture stress. In the present study, activation tagging vector pMN20 with modified EPSPS gene (selectable marker for herbicide (glyphosate tolerance) was developed to generate large numbers of T-DNA tagged transformants through *in planta* transformation technique. The *in planta* transformation strategy is a tissue culture-independent transformation strategy that targets the *Agrobacterium* to the apical meristem and the differentiating cells. Since it involves the generation of chimeric T0 plants and the transformants have to be identified in the T1 generation, a stringent high throughput screening based on the tolerance to glyphosate was developed. Based on the screening, 12,200 putative tagged lines were developed. Integration was confirmed by PCR and in some lines by RT-PCR. Mutants showed significant phenotype variation for a range of traits like, lodging, flowering, plant height, total no. of tillers, no. of productive tillers, no. of chaffy spikelet, total biomass, total seed weight yield, 100 grain wt. and flag leaf breadth. Some of the lines showed significant improvement in growth rate, stress adaptation and cellular level tolerance (as analyzed by temperature induction response). Molecular characterization of the variants will result in the identification of the genes responsible for the variation.

Identification of markers and QTL for diverse drought adaptive traits through an association mapping strategy

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In the background of the looming water crisis, saving water from rice fields has become imperative. Among several cultivation practices, growing rice under semi-irrigated aerobic conditions can save up to 60% of water. However, a concomitant reduction in yield up to 40% must be avoided. For a comprehensive improvement in plant performance under aerobic conditions, several diverse drought adoptive traits such as root traits, water use efficiency (WUE), water conservation traits and cellular level tolerance (CLT) needs to be introgressed. Pyramiding such complex physiological traits can be effectively achieved through the adoption of a focused molecular breeding approach. Further, simultaneous discovery of QTL is possible only through a linkage disequilibrium (LD) based association mapping strategy. In this investigation we assembled a panel of 200 diverse germplasm accessions that showed significant molecular diversity as well as trait variation. The population was screened with 125 SSR markers spanning the whole genome. These markers revealed significant polymorphism with an allelic diversity ranging from 2 to 19 alleles per marker locus with an average PIC value of 0.64. The population structure and kinship among the accessions were assessed using software like STRUCTURE and SPAGeDi. The population segregated into six sub groups. The 125 SSR markers revealed a total of 1138 alleles which were used for determining LD decay, and it was seen that LD decayed around 25cM among the *Indica* rice accessions. The panel was extensively phenotyped for drought adoptive traits in two seasons to observe a significant genetic variability in root traits, WUE assessed by $\Delta^{13}C$, CLT and epicuticular wax accumulation. Marker trait association was carried out with K and Q matrix information using TASSEL software. A number of QTLs were identified and *in silico* validated. Markers RM224 and RM163 were strongly associated with root biomass while RM263 and RM223 significantly

explained the variability in $\Delta^{13}C$. The advantage of association mapping approach in identifying trait contrasts besides discovering markers/QTL simultaneously to diverse traits is discussed.

Improving stress adaptation by enhancing cellular level tolerance through transgenic approach by expressing validated upstream regulatory genes

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Rice is widely cultivated under irrigated condition and its water requirement is very high. Due to dwindling water resources, to save irrigation water growing rice under semi-irrigated aerobic condition has phenomenal relevance. In this ecosystem the crop experiences decreased water availability, high VPD which affects growth and productivity. From this context it is important to improve adaptation of rice under aerobic condition by improving water relations and cellular level tolerance mechanisms. Stress adaptation at cellular level involves activation of stress responsive genes which are regulated by transcription factors. Multiple transcription factors are required for transcriptome reprogramming under stress to increase cellular level tolerance. Three stress responsive TFs from different families i.e EcNAC1, EcMYC57, EcbZIP60 were cloned from fingermillet stress cDNA library and validated their relevance in stress tolerance in model system tobacco. Subsequently rice transgenics were developed coexpressing EcNAC1, EcMYC57 and EcbZIP60 in genotype AC39020 having superior water relations traits. Desirable transformants were identified based on dessication response. Molecular characterization of transformants showed the integration and expression of all three genes. The transgenics expressing these TFs showed improved tolerance to NaCl, mannitol and methyl viologen induced stress. Under drought stress, many promising transgenic lines showed reduced spikelet sterility and higher yield compared to wild type plants. Superior events identified showed improved cellular level tolerance besides being productive under contained field stress situations. The study provides proof of concept that coexpression of a few TFs improved cellular level tolerance and transgenics is a potential option to combine the relevant traits and improve field level tolerance.

Molecular profiling of major Indian rice cultivars using eight hypervariable microsatellite markers

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India bred high yielding rice varieties have enriched to a great extent the global rice germplasm since the mid sixties. Systematic research efforts for development of cultivar-specific DNA fingerprints of major Indian rice cultivars, however, have not received due attention. The present investigation was aimed at development of DNA fingerprints for 90 high yielding varieties employing eight hypervariable microsatellite markers (hvRM) viz, RM11313, RM13584, RM15004, RM5844, RM22250, RM22565, RM24260 and RM8207 covering nearly the entire genome of rice. The set of 8 hvRM markers could, however, be found useful in a wide array of applications such as assisting of rice breeders in choosing of parents for breeding programs, distinguishing traditional Basmati varieties from non-Basmati types, and DUS testing. Thus we suggest that this set of 8 loci be used as standard for DNA fingerprinting of Indian rice cultivars.

Development of phenotyping strategies to assess drought adaptive traits and identification of rice genotypes with contrasting traits

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Accurate phenotyping of large number of germplasm and/or mapping populations is the most essential aspect that determines the success of QTL discovery and subsequent molecular breeding. Research experience over the past two decades at our center has indicated that maintenance of positive turgor and superior metabolism under low turgor need to be achieved for improvement in crop productivity under water limited

conditions. We have shown that while traits associated with water mining, water use efficiency, water conservation would help maintain positive turgor, superior cellular level tolerance (CLT) is useful in supporting a better metabolism under low turgor conditions. Therefore, the major emphasis has been to develop accurate and high throughput techniques to phenotype for relevant drought tolerance traits. For root traits, a novel strategy of raising plants in specially constructed root structures was standardized besides oxygen enrichment technique. A surrogate approach of carbon isotope discrimination ($\Delta^{13}C$) technique has been adapted to assess genetic variability for WUE in plants besides gravimetry. Further, towards assessing the cellular level tolerance, a novel protocol referred to as the temperature induction response technique has been adapted. As stomatal water conservation becomes an important trait, a spectrometry based quantification has been standardized and being adapted to assess the variation in epicuticular waxes in plants. Following the high throughput phenotyping strategies, rice germplasm lines were phenotyped for relevant drought tolerance traits. The entire germplasm consisting of over 200 accessions were raised in specially designed root structures to assess the genetic variability in root traits. Carbon isotope discrimination, a well proven surrogate for WUE was adopted to determine the genetic variability in these traits. The root weight ranged between 2 g/plant to as high as 16 g/plant, representing a significant genetic variability. Similarly, $\Delta^{13}C$ ranged between 16 and 21‰ suggesting a significant variation in WUE. The germplasm accessions also showed significant variability in CLT and non-stomatal water loss through the accumulation of varied amount of epicuticular waxes. Promising trait donor lines and contrasts identified for diverse drought adaptive traits can be utilized in crop improvement either through conventional or molecular breeding approaches.

Development of salinity tolerant lines by expressing transporters to enhance sequestration of sodium

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Salt stress affects plant growth and development in many different ways. To maintain growth and productivity plants must adapt to stress conditions and exercise specific tolerance mechanisms. One such mechanism is sequestration of excess cytosolic Na^+ into the vacuole. In plants vacuolar H^+-PPase (pyrophosphatase) proton pumps which drive the activity of Na^+/H^+ antiporters are localized on vacuolar membranes, actively generating proton gradient which in turn drives Na^+/H^+ antiporters which sequesters Na^+ into the vacuoles for maintenance of a low cytosolic Na^+ concentration. In the present study, *AVP1*, a vacuolar H^+-PPase gene from *Arabidopsis thaliana* and *NHX1,* a vacuolar Na^+/H^+ transporter gene from *Pennisitum glauccum* were overexpressed in rice (var-Vikas) by *Agrobacterium* mediated *in planta* transformation technique to improve salinity tolerance by enhancing sodium sequestration. To screen putative T_1 plants for salt tolerance, stringent salt screening test was followed and root and shoot growth of T_1 putative transformants was used as a selection criterion. Some of the transgenics showed significantly higher root and shoot growth compared to wild type. To confirm the integration of the transgene in putative T_1 transgenic plants, PCR and RT-PCR analysis were performed using genomic DNA. The results showed that 100% of the selected seedlings from the stringent salt screening test were PCR positives. Physiological studies such as chlorophyll estimation, membrane integrity, cell viability tests were also conducted to assess their levels of tolerance at T_1 generation. Some of the T_1 transformants showed lower percent reduction in chlorophyll content, higher cell viability after NaCl treatment compared to wild type. These results clearly demonstrate that single transgenic rice plants overexpressing *AVP1* and *NHX1* seperately have better salt-tolerance. Current research in our lab is focusing on co-expressing both genes in rice to substantially enhance salt tolerance of rice plants over and above which was obtained in single gene transgenics. In this regard both genes have already been cloned in a single vector construct. This was then used to transform rice using *in planta* transformation technique. Putative double gene transformants are currently being analyzed for physiological tolerance in addition to molecular analysis.

Designing tiller dynamics of rice suitable for high grain yield

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Tiller dynamics is one of the principal traits suitable for manipulation to achieve improved yield potential of rice plant. Traditional tall rice cultivars with strong apical dominance have less tillers and reduced grain yield whereas high tillering is encouraged in IR parented rice

cultivars for production of more number of panicles and to obtain larger numbers of grains. But all the tillers especially those formed late are not productive in a profusely tillering rice cultivar and production of excess late tillers is an investment loss on the part of the plant. In view of this, it is highly essential to maintain the rice hill with a suitable tiller number having strong apical dominance and to produce bigger panicles with large numbers of high density grains. However, the physiological nature of relationship between the tillers or inter-tiller competition within a rice hill has not yet been studied extensively. The present work assessed the nature of distribution of assimilates to different type of tillers in different tillering groups of rice with variation in their growth durations and growth habitats. It was concluded that environmental factors such as growth habitats in combination with hormonal factors are more important for maintenance of suitable tiller dynamics to achieve increased yield potential of rice and genetic limitations are marginal.

Confirmation and fine mapping of major QTL for grain size in Basmati rice

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Basmati rice is a unique varietal group that has gained wider acceptance as speciality rice all over the world by virtue of its unique grain quality traits. Grain size plays a crucial role in determining the quality in Basmati rice. Genetic control of Basmati grain size is quite complex, but breeding of new varieties having Basmati grain quality characters can be greatly facilitated by the use of molecular markers tightly linked to these traits. Hence, in the present investigation, 155 recombination inbred lines (F_6) were used for the confirmation and fine mapping of major QTLs for grain size traits earlier identified in the F_2 population of a cross between Basmati 370 and Jaya employing microsatellite and indel markers from the QTL region of the chromosome 5. A minor QTL for grain length *qGL5* and major QTLs *qGB5.1* for grain breadth and *qGLB5.1* for length-breadth ratio were identified. In addition, the QTL cluster region was narrowed down from 26.5cM to 15.6cM and the physical distance also has come down to 685 kb. The microsatellite marker, RM18582 showed close association with the grain size QTLs. This marker has potential to be used in marker-assisted improvement of the grain size in Basmati rice. Further identification of candidate genes underlying the major QTL for grain size is in progress.

Comparative antioxidative responses of rice genotypes to bacterial blight stress

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Bacterial blight (BB) is one of the most important biotic constraints for crop productivity limiting plant growth and development. Crop plants can respond and adapt to BB by altering their cellular metabolism and evoking various defense mechanisms. Production of cytotoxic reactive oxygen species (ROS) like super oxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^-) can seriously disrupt normal metabolism during BB stress through chlorophyll loss, membrane lipid peroxidation, protein carbonylation and inactivating the –SH containing enzymes. A highly efficient antioxidant defense system is present in the plant cells for ROS detoxification including either the non-enzymatic constituents or the enzymatic constituents. Rice exhibits variation in its sensitivity to BB stress. We compared the physiological responses of the two rice genotypes PB-1 (classified as BB susceptible) and *O. longistaminata* (as BB tolerant) in response to BB stress. The chlorophyll degradation, membrane lipid peroxidation and protein carbonylation, along with the changes in the amounts of non-enzymatic antioxidants like phenolic and flavonoids, as well as the activities of the enzymatic constituents, including catalase (CAT) and glutathione peroxidase (GPx) were studied. The reducing power and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were also compared in the leaves of the two rice genotypes exposed to BB stress. Chlorophyll content decreased significantly in PB-1 while it was constant in *O. longistaminata* throughout the study. At the initial stages of infection, the higher production of non-enzymatic antioxidants such as phenolics and flavonoids was observed intolerant genotype *O. longistaminata* compared to susceptible PB-1. Maximum increase intotal antioxidant was in treated *O. longistaminata* (16.19±0.02 mg ascorbic acid equivalent, AAE/g fw) compare to control (9.48 ±.02 mg AAE/g fw). *O. longistaminata* was found to be more active as compared to PB-1 by showing the maximum increase in the ferric-reducing

antioxidant power, FRAP value on 5th DAI (10.09±0.02 mg AAE/g fw). *O. longistaminata* has also showed higher induction of CAT and GPx activity. Comparison of physiological responses, as undertaken in this study, can be useful in future to understand the mechanism of biotic stress management and selection or development of rice genotype resistant to BB stress.

Allele mining for phosphorous deficiency tolerance in rice

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Phosphorous (P) is a major constraint for crop productivity and plants have developed several mechanisms to adapt to low P availability. Being a macronutrient essential for plant growth and development, understanding the uptake and utilization of P is crucial for attaining better P use efficiency. Crop productivity in North Eastern and Eastern region of India is severely hampered due to soil acidity. Due to low pH the phosphorus availability becomes a limiting factor, hampering plant growth and development. In rice, *Phosphorous Uptake 1* (*Pup1*) locus and a transcription factor PTF1 (Pi starvation induced transcription factor 1) for low phosphorous tolerance is known to be involved in efficient P uptake. Markers specific to PUP1 locus have already been developed. We tested a set of 6 markers on 60 diverse rice genotypes adapted to acidic soils of North Eastern and Eastern part of India. Five genotypes, adapted to North Eastern and Eastern part of India have been identified as potential donors for future breeding programs. Using marker assisted selection (MAS), identification of rice genotypes carrying the *PUP1* locus and already adapted to acidic soils will pave way for effective breeding programmes suited to agro-climatic needs to this region of India. Furthermore, in an attempt to understand the alleles for transcription factor PTF1, PCR based markers were designed. The sequencing of 3361bp across the PTF1 gene led to identification of a total of 46 SNPs. CAPS (cleaved amplified polymorphic sequence) and allele-specific markers targeting a set of 6 genic SNPs have been designed and validated across a panel of 16 diverse rice genotypes. These PCR based markers can be used by molecular biologists as well as rice breeders to distinguish different allelic states of this important transcription factor across different rice genotypes. Furthermore, understanding the impact of combination of different allelic states across these two genic regions will lead to improving our knowledge of the important macronutrient affecting rice productivity.

Evaluation of resistance spectrum and field performance of pyramided rice restorer line against bacterial leaf blight

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Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the oldest and the most destructive diseases of rice worldwide. It has become more prominent after the release of high yielding varieties. It causes yield losses of 20-30% and as high as up to 80% under severe conditions. Hybrid rice has made a considerable impact on the sustainability of food security in the developing countries like India. Its yield has reduced to a considerable rate due to the susceptibility of parental lines to the bacterial blight pathogen. This makes it necessary to insulate the parental lines against the pathogen. Using host plant resistance is the most effective, economical and environmentally safe method to control the disease. Pathogenic studies revealed the use of four BB resistance genes (*Xa*4, *xa*5, *xa*13 and *Xa*21) combination as the most effective against the different races of the pathogen. Marker assisted selection (MAS) is a highly efficient breeding method for rapid and precise selection of multiple resistance genes. The present study was carried out with the objective to evaluate the performance of a pyramided popular restorer line, under field conditions with the artificial inoculation of the highly virulent strain of *Xoo* collected from the hotspot, Maruteru, Andhra Pradesh, India. The restorer line was pyramided with *Xa*4, *xa*5, *xa*13 and *Xa*21 genes using MAS for foreground selection and phenotypic traits for background selection. All the nine pyramids at BC_3F_6 generation showed resistance to the pathogen and retained the phenotype of recipient parent. Under disease free condition there was no significant difference in yield between the parent and pyramided line and also among the nine pyramids. Under BB infection there was a significant difference in yield and yield components between parent and pyramid. The parent showed ~ 25.8% reduction in yield and in other yield related components also showed significant reduction in traits like spikelet fertility (26%), filled grains per panicle (24.9%), 1000 grain weight (25.8), biomass (14%) and harvest index (13.5%). The pyramided lines showed no reduction in any of these characters, thereby indicating that the four gene

combination can successfully insulate against the yield loss and other yield related characters against bacterial leaf blight. The resistance gene pyramids can be used as parents in hybrid production. This work illustrates the successful deployment of MAS for multiple genes pyramiding into popular hybrid rice restorer line.

Molecular mapping of the chromosomal regions associated with high iron and zinc content in rice grains using SSR markers

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Biofortification of staple food crops has been considered a sustainable strategy to overcome the problem of micronutrient deficiencies prevalent in rice. The objective was to map chromosomal regions associated with high iron and zinc content. In the F_2 mapping population derived from the cross between Samba Mahsuri and Chittimuthyalu, the markers studied (and the genetic distances from their corresponding candidate genes) for iron content were SC120 (13.6cM), SC123 (12.8cM), SC126 (12.7cM), SC435 (6.2cM), SC448 (13.4cM) based on *YSl transporter* gene and SC 129 (15.6cM) based on *ZIP* gene and SC103 (6.7cM), SC116 (13.5cM) based on *ZT* gene. The markers studied and the genetic distances from their corresponding candidate genes for zinc content were SC120 (21.5cM), SC123 (8.7cM), SC126 (22.3cM), SC435 (13.4cM), SC448 (11.6cM) based on *YSl transporter* gene, SC 129 (19.6cM) based on *ZIP* gene and SC103 (26.2cM), SC116 (15.3cM) based on *ZT* gene. In the F_2 mapping population derived from the cross between Samba Mahsuri and Ranbir Basmati, the markers studied and the genetic distances from their corresponding candidate genes for zinc content were SC129 (9.8cM), SC135 (10.5cM), SC428 (15.9cM), SC 430 (6.4cM) based on *ZIP* gene, SC425 (9.8cM) based on *ZT* gene, SC434 (8.5cM) based on *YSl* transporter gene and SC418 (14.5cM) based on *NRAMP* gene. The markers studied and the genetic distances from their corresponding candidate genes for iron content were SC129 (12.5 cM), SC135 (13.4cM), SC428 (8.8 cM) and SC 430 (10.5cM) based on *ZIP* gene, SC425 (16.5cM) based on *ZT* gene, SC434 (13.9cM) based on *YSl transporter* gene and SC418 (21.6cM) based on *NRAMP* gene. Most of the markers studied in the mapping experiment have shown a clear association with the trait despite the less numbers of F_2 samples analyzed. Thus the mapping results in the present study suggest for the identification of microsatellite markers in the vicinity of candidate genes involved in the cation metabolism and their use in mapping to be very appropriate.

Iron nutrition in relation to aconitase activity and ferritin accumulation in tropical *indica* rice cultivars having contrasting grain iron content

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Rice cultivation is practiced over a wide range of ecologies. Both drought and flood-prone environments influence Fe absorption from the soil and thus affects productivity. Under such situations the plant is expected to maintain Fe homeostasis by modulating the function of its key players such as ferritin. Effect of Fe nutrition on Fe acquisition, aconitase enzyme activity and assimilation of the element in ferritin protein was studied in two *indica* rice cultivars viz, Sharbati and Lalat having contrasting grain Fe concentration. Young rice seedlings were grown in hydroponics with different levels of Fe. For comparison, the two cultivars were also grown in the field under natural conditions of rice culture. Iron accumulation, aconitase activity and ferritin level were higher in the high Fe containing cultivar, Sharbati than the low Fe containing cultivar, Lalat. While aconitase activity increased consistently with the increase in concentration of Fe in the growing medium, the same was not found to be true for accumulation of ferritin protein. Ferritin level in the leaves increased up to an optimum level of Fe in the growing medium and declined thereafter. Optimum levels of Fe in the culture medium for maximum ferritin synthesis were found be different in the two rice cultivars. In both the cultivars, aconitase activity attained maximum level after 20 days of panicle emergence (heading). Pattern of Fe accumulation in the leaves in response to increasing Fe level in the nutrient solution coincided with that of the aconitase activity. It was concluded that accumulation of both ferritin and aconitase enzyme is influenced by internal as well as Fe status of the plant but they didn't appear to be linked.

Identification of drought tolerant hybrid parental lines for enhanced yield under moisture-stress conditions in rice

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Because of the global warming and changes in climate occurring in the present situations, the rainfall pattern has become more irregular in the cropping season, causing widespread drought, which results in severe yield losses. The progress in breeding for drought resistance is rather slow in rice due to the complexity of the trait and poor understanding of the genetic basis and mechanism of drought resistance in real field condition. As reports on exploiting heterosis for drought tolerance are rare, the objective was to identify drought tolerant parental (both maintainer and restorer) lines and estimate heterosis for root traits and grain yield so that, the unexplored heterosis for drought tolerance can be utilized in hybrid breeding programs. A set of male sterile /maintainer lines and putative restorer lines from IRRI, Philippines, were evaluated for root morphological traits and yield related traits under aerobic and irrigated conditions. From that we identified drought tolerant male sterile/maintainer and restorer lines with ideal root parameters and yield under aerobic and irrigated conditions. Identified parental line is used to generate hybrids to estimate heterosis for root morphological traits and yield under moisture stress.

Development of doubled haploid lines towards generating trait specific mapping populations and trait introgression lines in rice

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The semi-irrigated aerobic cultivation is an appropriate method that can save up to 60% of irrigation water. The major constraint in trait pyramiding is the time required to generate such trait introgressed lines and the accuracy in selecting the right progeny. While DNA markers flanking QTL region can enhance the accuracy of selection of trait introgressed lines, doubled haploids (DH) technology is expected to significantly reduce the time required for the generation of trait or QTL introgressed lines. We standardized the anther culture strategy to develop DH lines in of hybrid KRH2 using early uninucleate stage to early binucleate stage of microspores in anthers for callus induction, regeneration of green plantlets and induction of DH by colchicine treatment. A high root and water use efficiency (WUE) donor line, AC39020 was crossed with IR64 with an objective of introgressing WUE and root traits. The anthers of the resultant F_1 from this cross were used to regenerate DH lines using the standardized reproducible protocol to introgress relevant traits. Around 120 DH lines were developed out of the 210 haploid plants and characterized morphologically and cytologically by karyotyping. DH lines are used to identify introgressed lines with superior water relations, yield potential and as a trait specific biparental mapping population.

Development of glyphosate tolerant rice (cv. IR64)

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Herbicide-resistant (HR) rice has the potential to improve the efficiency of weed management and facilitate adoption of resource conservation technologies. Weed control is a serious challenge in non-puddled direct-seeded rice because the initial flush of weeds is no longer controlled by flooding. Herbicide-resistance in rice may overcome the problem of weed management. Hence, the popular Indian rice cultivar, IR64, was transformed with codon optimized synthetic CP4 *EPSPS* gene via *Agrobacterium*-mediated genetic transformation. The *EPSPS* gene, with chloroplast targeting signal peptide and under transcriptional control of *Zea mays* ubiquitin promoter, was delivered into IR64 using binary vector pCAMBIA 1301. Transgenic plants were successfully developed which carry one to two copies of transgene. Q-RT PCR analysis showed high levels of transgene expression. T_0 and T_1 lines tested by herbicide bioassay confirmed that the transgenic rice can tolerate up to 1% commercial weedicide Roundup at the dose used to kill weeds in rice fields..

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