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Indian Wild Rice: Diversity, Population Structure, Trait Value and Relation with Cultivated Rice

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Wild relatives of crop plants are rich in mutations for adaptive agronomically important traits, which could be used in crop improvement for sustainable agriculture under global climatic change. However, human developmental activities pose serious threats to the natural habitats leading to erosion of genetic diversity of wild rice populations. The germplasm utilisation strategies involve collection, characterisation and evaluation to understand the underlying morphological and molecular variations for the adaptive agronomic traits. India has a huge wealth of untapped wild rice resources and to explore the variation present in these we made hasty expeditions around diversity hot-spots in nine different eco-geographical regions of the country and collected more than 600 accessions wild rice *Oryza nivara/Oryza rufipogon* representing wide range of ecological niches. Enormous variation was observed among these accessions on evaluation for 46 morphological descriptors. Cluster analysis based on Euclidean distance matrix revealed three major morphological groups but these did not correspond to their geographical origin. Genome-specific *pSINE1* DNA markers revealed that all the accessions belonged to AA genome and these can be easily used for introgression of useful genes in the cultivated rice (*Oryza sativa*) genome. Further characterisation using ecotype-specific *pSINE1* markers classified these accessions into annual (for *O. nivara*), perennial (for *O. rufipogon*), intermediate (mixed type) and unknown types not described earlier. Principal component analysis revealed continuous variation for the morphological traits in each ecotype group.

Genetic diversity analysis using genome wide multi-allelic SSR markers clustered these accessions into three major groups. Analysis of molecular variance (AMOVA) in relation to the eco-geographical origin revealed that 68% of the genetic variation was inherent amongst individuals while only 11% variation was due to geographical regions. Though there was significant correlation between genetic and spatial distances of the accessions. Model based population structure analysis using genome wide unlinked bi-allelic SNP markers revealed three sub-populations which we have designated 'Pro-Indica', 'Pro-Aus' and 'Mid-Gangetic' populations, which showed no correspondence to the ecotypes or *O. nivara*/*O. rufipogon* distinctions. Wide eco-geographical distribution of the Pro-Indica and Pro-Aus sub-populations indicates a more fundamental grouping based on the ancestry. The Pro-Indica and Pro-Aus populations are closely related to 'Indica' and 'Aus' groups of rice cultivar, respectively and hence may represent their ancestral wild progenitors, whereas the Mid-Gangetic population was distinct from major cultivated rice groups.

Soil salinity covers a large part of the arable land of the world and is a major factor for yield losses in salt-sensitive crops, such as rice. Rice productivity is adversely affected by salt stress prevalent in about 30 percent of the cultivated land. For developing salt-tolerant rice varieties through conventional breeding or biotechnological interventions, there is an urgent need to identify natural allelic variations conferring salt tolerance. We screened a large number of these wild rice accessions collected from different agro-climatic regions of India for growth under salt stress. Further, 95 representative accessions were sequenced for members of HKT ion transporter family genes by employing Ion Torrent PGM sequencing platform. Haplotype analysis revealed that specific haplotypes, namely H5 of the *HKT1;5* genes and H1 of the *HKT2;3* were significantly associated with high salinity tolerance. This was the first report of allele mining of eight members of HKT gene family among wild rice germplasm reporting a salt tolerant allele of the *HKT2;3*. The *HKT1;5* gene also showed a salt tolerant allele from wild rice. Phylogenetic analysis based on the nucleotide sequences of these genes showed a different grouping of the HKT family

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**Author for Correspondence: Email- nksingh4@gmail.com* genes as compared to the known protein sequence based classification. Different gene families that respond to salinity have been identified in rice, but limited success has been achieved in developing salt-tolerant cultivars. Therefore, 21 salt stress-responsive candidate genes belonging to different gene families were re-sequenced to analyse their genetic variation and association with salt tolerance. The average single nucleotide polymorphism (SNP) density was 16 SNPs per kbp amongst these genes. The identified nucleotide and haplotype diversity showed comparatively higher genetic variation in the transporter family genes. Linkage disequilibrium (LD) analysis showed significant associations of SNPs in *BADH2, HsfC1B, MIPS1, MIPS2, MYB2, NHX1, NHX2, NHX3, P5CS1, P5CS2, PIP1, SIK1, SOS1, and SOS2* genes with the salt-tolerant phenotype. A combined analysis of SNPs in the 21 candidate genes and eight other HKT transporter genes produced two separate clusters of tolerant genotypes, carrying unique SNPs in the ion transporter and osmotic adjustment related genes. Haplotype network analysis showed that all the major alleles and few minor alleles were distributed over distant geographical regions. Minor haplotypes may be recently evolved alleles and subsequently migrated to distant geographic regions through human intervention, representing recent expansion of Indian wild rice. The analysis of genetic variation in different gene families identified the relationship between adaptive variations and functional significance of the genes. Introgression of the identified alleles from wild relatives will enhance the salt tolerance and consequently rice production in the salinity-affected areas.

Drought is a serious constraint to rice production globally that can be addressed by deployment of drought tolerant genes. *OsDREB1F*, is a potent drought tolerance transcription activator gene. We sequenced the *OsDREB1F* gene from diverse wild rice collections for allele mining and association study in a set of 136 wild rice accessions and four cultivated rice. This analysis led to identification of 22 SNPs with eight haplotypes based on allelic variations in the accessions used. The nucleotide variation based neutrality tests suggested that the *OsDREB1F* gene has been subjected to purifying selection in the studied set of rice germplasm. Six different OsDREB1F protein variants were identified on the basis of translated amino acid residues. Five protein variants were truncated due to deletions in coding region and the genotypes carrying these were found susceptible to drought stress. Association study revealed that three coding SNPs of this gene were significantly associated with drought tolerance. Three-dimensional homology modeling helped understand the functional significance of this potentially useful allele for drought tolerance in rice. The natural allelic variants mined in the *OsDREB1F* gene can be further used through translational genomics for improving the water use efficiency in rice.

Single nucleotide polymorphism (SNP) is the most abundant DNA sequence variation present in plant genomes. We designed and validated a unique genic-SNP genotyping chip for genetic and evolutionary studies and molecular breeding applications in rice. The chip incorporates 50,051 SNPs from 18,980 different genes spanning 12 rice chromosomes, including 3,710 single-copy genes conserved between wheat and rice (CSCWR), 14,959 single-copy genes unique to rice (SCR), 194 agronomically important cloned rice genes (AGCR) and 117 multi-copy rice genes (MCR). Assays with this 50K SNP chip showed high success rate and reproducibility because of the SC gene based array with no sequence redundancy and cross-hybridization problems. The chip was used successfully to study genetic diversity and phylogenetic relationship of rice cultivar groups and *O. nivara/O. rufipogon* wild rice accessions. The origin and domestication of rice has become a subject of considerable debate of late. Rice cultivars have been categorized based on isozymes and DNA markers in to two broad groups, 'Indica' and 'Japonica'. Among other well-known groups of cultivated rice varieties, 'Aus' is closer to Indica and 'Aromatic' including Basmati is closer to Japonica, while deep-water rice varieties belong to both 'Indica' and 'Japonica' cultivar groups. This analysis based on the genome wide high-density genic-SNP haplotype analysis confirmed that the Aus and Indica groups of rice cultivars are nested in their respective wild rice progenitor populations and are more distant from each other than their wild rice progenitor. Similarly, a broad analysis including more wild accessions from the North-Eastern regions of India, South-East Asian countries and Chinese wild rice accessions shows that even the japonica and aromatic groups of rice cultivars have closely related wild rice ancestors in the wild rice accessions from South Asia and may have polyphyletic origin different from Aus and Indica groups of rice cultivars but in the Indian Subcontinent and South-East Asian regions.

We analyzed haplotype networks and phylogenetic relationship in a diverse rice germplasm including a large set of *O. nivara/O. rufipogon* wild rice accessions and representative varieties of different cultivar groups, based on red pericarp, grain size and eight starch synthase genes to study the phylogenetic relationship of these gees in wild and cultivated rice. Our results with *RC*, *GS3*, *GBSSI*, *SSSI*, *SSIIa*, *SSIIb, SSIIIa*, *SSIIIb, SSIVa* and *SSIVb* genes also suggested a polyphyletic origin of the cultivated rice. It further showed that *O. nivara/O. rufipogon* accessions from different eco-geographical regions of India have the ancestral haplotypes of all the ten genes and in most cases the alleles in the rice cultivars were shared by wild rice accessions which could be their probable progenitors. Our study shows a complex pattern of migration of domestication alleles from wild rice to different rice cultivar groups. These findings will be useful in understanding the domestication history of rice and utilisation of wild rice germplasm in genetic improvement of rice cultivars.

In addition to the evaluation for abiotic stress tolerance and analysis of the origin of cultivated rice, the new cultivated Indian wild rice accessions have also been evaluated for resistance to a range of biotic stresses including, bacterial leaf blight, blast and sheath blight as well as productivity related traits such as fertility restoration, hybrid vigour and improved plant types. The analysis has shown that our wild rice collection is tremendously rich source of genes for these important traits. Efforts are now on for the introgression of these novel genes in to cultivated rice for practical utilisation for enhancing rice productivity and production stability.