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Haplotype diversity and association analysis of *SNAC1* gene in wild rice germplasm

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Abstract

Effect of *SNAC1* gene variants on drought tolerance was investigated in wild rice accessions. A total of 46 INDELs and 44 SNPs were identified including 17 SNPs in the coding region of the gene across the 164 Indian wild rice accessions and four cultivated rice genotypes. Based on the sequence variation, 25 haplotypes were identified including four specific to drought tolerant accessions. Translation of different allele sequences showed existence of five different SNAC1 protein variants. Evolutionary study on the basis of nucleotide sequence variation in the *SNAC1* gene with different neutrality test suggested purifying selection in the wild rice accessions. Association analysis revealed that four SNPs were showing significant association with drought tolerance. The significantly associated SNPs and their associated haplotypes can further be used for the development of drought tolerant rice cultivars.

Key words: Association analysis, haplotype diversity, single nucleotide polymorphism, *SNAC1* gene, wild rice

Introduction

Drought is the most serious abiotic stress that limits crop production under rainfed conditions. Rice (*Oryza sativa* L.) is generally grown in flooded conditions and it is susceptible to drought stress due to its shallow root distribution and limited capacity to absorb water from deep soil (Kondo et al. 2003). The global warming that has occurred in recent years has caused serious drought damage in rice-growing areas that rely on rainwater and lacking source for irrigation. So, the enhancement of drought tolerance in rice is becoming an important way to stabilize rice production in areas with rainfed agriculture. Some drought stress related parameters in crop species have been investigated as criteria for improving water deficit tolerance through breeding programmes. Assessment of drought tolerance in rice can be easily evaluated using physiological parameters like canopy temperature, chlorophyll content, leaf rolling and relative water content (RWC). First response to drought stress is the reduction of transpiration which increases canopy temperature due to less transpiration cooling (Rizhsky et al. 2004) and it can be measured using infrared (IR) thermometry (Carrity and Toole 1995). Drought stress produces reactive oxygen species (ROS) which lead to lipid per-oxidation and consequently, results in chlorophyll destruction (Fotovat et al. 2007). Leaf rolling occurs due to the inability of leaves to sustain the transpiration demand of the plant (Blum 1988) and can successfully used to assess drought tolerance (Chang et al. 1974). Plants showing leaf rolling at early stage of stress appear to have poor drought tolerance (Abd Allah 2009). Stress intensity also affects leaf RWC and tolerant genotypes have higher rate of RWC than susceptible genotypes (Alizade 2002; Ganji Arjenaki 2012).

Drought tolerance is a complex quantitative trait, and in rice, tremendous efforts have been made to dissect drought tolerance through QTL mapping (Yu et al. 2006). The loci controlling drought tolerance are widely distributed across the rice genome but only few major QTLs are mapped for a specific population and environment. Drought tolerance leads to induction

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of several stress-responsive and stress-tolerance genes and in plants, most of the early abiotic stress-responsive genes are transcription factors (TFs). Several previous studies have investigated the role of specific TFs families in abiotic stress responses (Puranik et al. 2012). The NAC family constitutes one of the largest plant-specific TF families with approximately 117 genes in *Arabidopsis* and 151 genes in rice (Puranik et al. 2012). NAC was originally derived from the names of the first three proteins containing NAM (no apical meristem), ATAF1-2 (*Arabidopsis* transcription activation factor) and CUC2 (cup-shaped cotyledon), that contain a similar DNA-binding domain. *SNAC1* gene is a member of NAC plant-specific gene family which encodes an NAC transcription factor and predominantly induced in leaf guard cells under drought stress in rice. Over expressing *SNAC1* significantly enhanced drought tolerance in transgenic rice (22-34% higher seed setting rate than the control) at the reproductive stage in the field under severe drought stress conditions without showing any phenotypic changes or yield penalty (Hu et al. 2006). Detection of allelic differences and variations in *SNAC1* gene across wild and cultivated germplasm in rice is an important approach which can be used to isolate valuable alleles and haplotypes contributing for drought stress tolerance and can provide foundation for plant breeding and translational genomic approaches. Although phenotypic variations associated with different physiological processes are the consequences of allelic diversity in plants, information on allelic variations of abiotic stress-responsive genes is scanty. Allele mining plays an important role in this respect and is able to identify naturally occurring allelic variants at loci of those genes that affect crop characteristics and performance. The success of allele mining operations is dependent on the availability of diverse germplasm collections (Kumar et al. 2010). Targeted sequencing of the candidate genes from a large number of accessions can also be used to study phylogenetic relationships of crop plants, their evolution, domestication and ecological adaptation. Several genes were mined to detect their allelic isoforms associated with a particular abiotic stress tolerance trait such as ABA stress, ripening (ASR) (Philippe et al. 2010), *OsDREB1F* allele for drought tolerance among *O. sativa*, *O. rufipogon* and *O. nivara* accessions (Singh et al. 2015) and *sub1* gene of *O. sativa* for submergence tolerance (Fukao et al. 2009).

Several candidate genes underlying a particular biotic and abiotic stress are yet to be mined in search of superior allelic isoforms employing a diverse set of germplasm including wild relatives which can be used in the breeding program for development of agronomically superior cultivars. By relying on the ability of allele mining and association mapping, this study was implemented to re-sequence *SNAC1* gene across Indian wild rice accessions to find out allelic variations, and to establish association of these variants with drought related traits. The allelic variations and haplotype information were also used for evolutionary study and identification of the wild rice accessions possessing the effective alleles for drought tolerance.

Materials and methods

Plant material

For present study, 121 wild rice accessions were collected from Uttar Pradesh (UP) and Bihar states of India along with their passport data (73 from UP and 48 from Bihar), 43 wild rice accessions from NBPGR gene bank (8 *O. rufipogon* and 35 *O. nivara*) and four check varieties *i.e.* two drought tolerant (Nagina-22 and APO) two susceptible (IR64 and Swarna). All the collected wild rice accessions were further characterized based on p-SINE markers. The p-SINE markers are commonly used to classify the genus *Oryza*. Most annual wild rice accessions had this retrotransposon, while most perennial types lacked this element (Yamakanta et al. 2003). The details of these wild rice accessions and cultivars are given in supplementary Table 1 and our wild rice database (http://nksingh.nationalprof.in/).

Phenotypic evaluation

All the accessions were planted in rain out shelter facility following augmented block design where four checks replications *i.e.* two drought tolerant (Nagina-22 and Apo) and two susceptible (IR64 and Swarna) rice genotypes were used as control to check the performance of wild rice accessions under drought stress at vegetative stage. The seedlings were transplanted in 10 X 35m plot where plant to plant and row to row distances were maintained as 20 and 30 cm, respectively. After 26 days of transplanting, drought stress was imposed by draining-off irrigated water and phenotypic data for leaf rolling (LR), canopy temperature, chlorophyll content and relative water content (RWC) was recorded. LR data was observed using 0-9 scale based on IRRI Standard Evaluation System adopted for rice (1996). Plant canopy temperature was recorded by IR thermometer with five

readings per plant. The SPAD-502 chlorophyll meter was used to estimate leaf chlorophyll content (Chelah et al. 2011). The upper-most collared leaf was used to measure chlorophyll content and five readings per leaf were taken from each plant. Fresh healthy leaves were collected from three plants of each row for the RWC estimation. The RWC was calculated according to Jahan et al. (2013).

Targeted resequencing of SNAC1 gene

DNA from fresh leaf tissue of each wild rice accessions was extracted by CTAB method (Murray and Thompson 1980). Amplicons resequencing approach was used for the allele mining and association analysis. Nucleotide sequence of the *SNAC1* gene (DQ394702.1/LOC_Os03g60080) was retrieved from NCBI/MSU-TIGR database, and flanking region was searched in NCBI data base to find any variation in the regulatory elements. A total of 2102 bp sequence (530 bp 5' flanking, 474 bp CDSf, 130 bp intron, 477 bp CDSl and 491bp 3' flanking region) of this gene was resequenced by Ion Torrent sequencer. Four overlapping oligos were designed using Primer 3 software to amplify 2102 bp region of this gene (Table 1). The PCR reactions were carried out in a 20 µl volume including 10 µl of PrimeSTAR HS Premix (Takara Bioscience's), 1.0 µl of each primer (10 pmol each), 2.5 µl (90 ng) of DNA template and 5.5 µl of miliQ water for each sample. The PCR cycles were as follow, initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 98°C for 20s, annealing at 53-57°C for 30s, and extension at 72°C for 1.0 min followed by final extension at 72°C for 10 min. The PCR amplicons were analyzed by electrophoresis in 2 % agarose gel in 1X TBE buffer. For sequencing, PCR amplicons were pooled in equimolar ratio and purified by Agincourt AMPure XP reagent according Ion torrent sequencing manual. Library preparation was done with the Ion Fragment Library Kit (Life Technologies, Invitrogen) according to the protocol. Size selection was done with E-gel Size Select 2% agarose (Invitrogen). Template preparation was carried out with Ion Xpress Template Kit according to Ion Xpress^{TM} Template Kit user guide with a modified protocol for Ion Sphere recovery. Emulsified Ion Sphere particles were collected by centrifugation. The Ion Sequencing Kit (Life Technologies) was used in the Ion Torrent Sequencer as described in Ion Sequencing Kit manual. The complete sample was loaded on an Ion 316 chip and sequenced using sequencer for 65 cycles. SAMtools mpileup was used for consensus sequence generation and were aligned by Bioedit software for SNPs and INDEL detection.

Sequence data analysis

The sequencing coverage of *SNAC1* gene was 250X and >20 phred score (Q) was used for base calling accuracy. Assembled sequences were aligned with the reference sequence of *SNAC1* gene using Clustal W in BioEdit software. Nucleotide polymorphisms were analyzed in this gene using the DnaSP software version 5.10 (Rozas et al. 2003). Levels of silent-site nucleotide diversity per site were estimated as π (Nei 1987) and population mutation parameter as θ. Average rates of non-synonymous (ka) and synonymous (ks) substitutions and divergence (Pisyn and Pinon) were calculated to examine the selection force acting on the *SNAC1* gene. Software DnaSP version 5.10 was used for combined analysis of inter specific comparisons for estimating the ka/ks ratio and for determining deviations from neutral evolution. Nucleotide polymorphism across the *SNAC1* gene in all accessions was analyzed using Sliding window analysis in DnaSP software. Statistical tests of neutrality such as Tajima's D (Tazima 1989), Fu and Li's D and F were calculated to examine the evolutionary force at this gene. A haplotype network was constructed for comparison of genealogical relationships among *SNAC1* haplotypes using Network software v4.6. The nucleotide sequences were translated into amino acid sequences and SNAC1 protein variants were identified and compared with the

Table 1. Details of gene-specific primers designed for the PCR amplification of SNAC1 gene

S.No.	Primer ID	Forward primer sequence	Reverse primer sequence	Annealing temperature (^{o}C)	Product size (bp)
1	NKS_nac1	CCTAATCAACTTGCAACCTAAG	TGACCCATTAGGATACTTGC	55	818
2	NKS_nac2	AGGTGGATCTCTACAAGTTCG	ATATCGTCGTAGCTCAGGTC	57	799
3	NKS_nac3	TGTACAACAAGAAGAACGAGTG	GATCGGCAGTAGTAAGGTAGAC	56	742
4	NKS_nac4	GGGGCATTCTTTAGTTTTTC	TCTCGAAGGATTCATCTAGTCT	53	651

already reported SNAC1 protein. Population structure was estimated using 36-plex SNP assay in sequenom (Singh et al. 2007). Structure 2.3.4 program was run by assuming populations (k) from 2 to 7 with 50,000 MCMC replicates after a burn-in of 50,000 (Pritchard et al. 2000). The result file of structure program was extracted through structure harvester online program where mean LnP(K) and Evanno plot inferred K=3, hence Q-metrix was generated at K=3. The association between phenotypic performance of the accessions and nucleotide variation (SNPs) was evaluated using mixed linear model (MLM) in TASSEL software (Bradbury et al. 2007).

Results and discussion

The majority of allelic variation at any given locus is predicted to occur in the wild relatives of a crop, and not the crop itself, due to the inevitable loss of variation at the domestication bottleneck (Zhu et al. 2007). Thus allele mining efforts increasingly focus on wild material to identify useful new alleles not already present in the crop gene pool (Johal et al. 2008). So, in present study, wild rice accessions were collected to explore the allelic variation in the *SNAC1* gene.

Phenotyping of the wild rice accessions under drought stress

The current set of rice accessions was evaluated for drought stress employing four parameters *i.e.* canopy temperature, chlorophyll content, leaf rolling and relative water content. Plant phenotypic performance under drought stress categorized 29 accessions as drought tolerant, 95 as susceptible and 40 accessions as moderate to drought stress. A statistical correlation among all the traits was estimated and a significant but negative correlation was observed for leaf rolling with chlorophyll content and RWC while a significant positive correlation was observed with canopy temperature. RWC was significantly positively correlated with chlorophyll content under drought stress (Table 2).

Table 2. Correlation coefficients among phenotypic traits under drought stress

	Canopy temp	Leaf rolling	RWC
Leaf rolling	.210**		
RWC	-.174*	-.876**	
Chlorophyll content	-0.094	-.602**	.618**

Nucleotide diversity at the SNAC1 locus

Nucleotide diversity at the *SNAC1* locus in Indian wild rice was analyzed by amplification and resequencing of this gene among 164 wild rice accessions (Fig. 1). Sequence information of all the *SNAC1* alleles has been submitted in the NCBI nucleotide database from accession number KM265193 to KM265360. Overall nucleotide diversity at the *SNAC1* locus was 0.001 nucleotide variations per site (π). Nucleotide variations were high in the coding region (531-1004bp CDSf and1134-1610 bp CDSl) of this gene among the wild rice accessions (Fig. 2A). The structure of the *SNAC1* gene is shown in the Fig. 2B. A total of 32 polymorphic sites were found in the gene including nineteen in the coding region. In addition to SNPs, 46 INDELs sites were also observed in the gene.

Four statistical parameters *viz*., $\theta\omega$ (Watterson's estimator), D statistics (Tajima's D test), D* and F* statistics (Fu and Li's test) were estimated across the three populations for the evolutionary analysis of *SNAC1* gene. All the above statistics revealed deviation from neutrality test (Table 3). Average pairwise values for nucleotide diversity (π) and Watterson's nucleotide diversity estimator ($\theta\omega$) of all the alleles were assessed and found to be 0.001 and 0.00281, respectively (Table 3). Among the *SNAC1* alleles of different populations, the nucleotide diversity was maximum in the alleles of *O. rufipogon* (π =

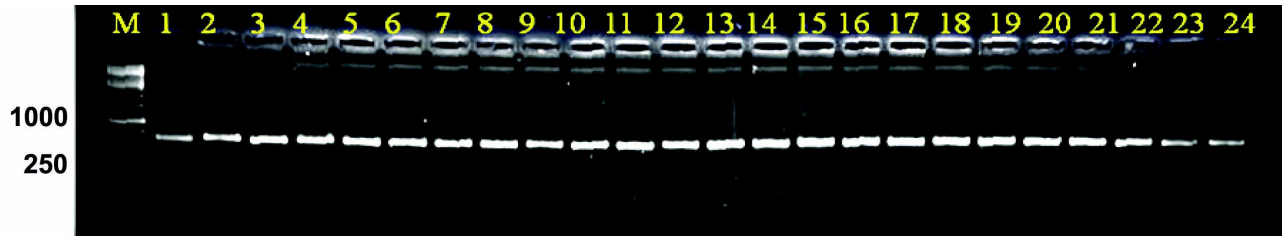


Fig. 1. A 2% agarose gel showing amplification of *SNAC1* gene fragments using NKS_nac4 primer having product size 651 bp. M= 1kb DNA marker, 1-24 wild rice accession

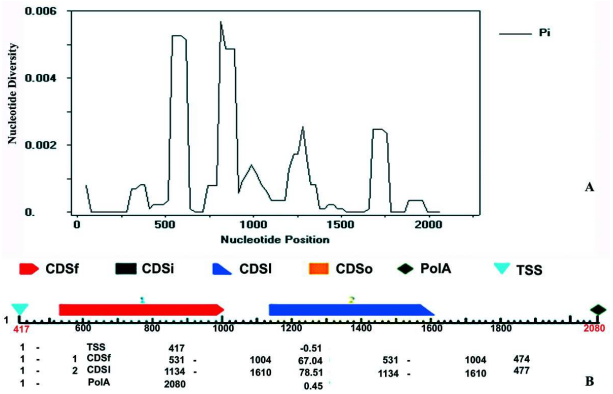


Fig. 2. Graph of nucleotide variation at *SNAC1* locus A) nucleotide diversity represented in the whole gene including promoter region of *SNAC1* gene among 168 wild rice accessions and B) gene structure of *SNAC1* is shown at the bottom. TSS: transcription start site, CDSf: first coding sequence, CDSl: last coding sequence, PolA: polyadenylation sequence

0.00332) and minimum in *O. nivara* (0.00087). While analyzing each population individually, *O. nivara* acquired highest (26) segregating sites (S) within *SNAC1* locus followed by *O. rufipogon* (23) (Table 3). There was very less polymorphism in the coding region of *O. sativa* population (0.00053) (Table 3). Further the statistical parameters showed negative values of Tajima's D test for all the populations except *O. sativa* (0.16766). Among all populations, highest but negative D test value was obtained in population of *O. nivara* (–1.8035), while it was found minimum in case of *O. rufipogon* (–0.5491). The D* and F* tests values were observed positive in *O. rufipogon* and *O. sativa* populations while these values were negative in entire populations.

The synonymous and non-synonymous substitution was found in all populations except *O. sativa*. The values for both the ratios, Pinon/Pisyn and Ka/Ks (JC), were 0.0443686 and 0.04399 in all the three populations. Both the ratios Pinon/Pisyn and Ka/Ks(JC) were obtained less than one (Table 4). Maximum divergent population for *SNAC1* coding region was *O. nivara* (Hd = 0.605).

The negative values observed in Tajima's D test suggest that the *SNAC1* gene has been subjected to purifying selection in all wild rice species of India, especially in *O. nivara* accessions and is still, trying to stabilize the most potential allele of *SNAC1* in the rice populations against drought stress. However, the positive values of D* and F* tests in *O. rufipogon* and

Table 3. Overall statistics of neutrality tests for *SNAC1* alleles

Population	Gene segment	S	π	θω	D	D*	F*
O. nivara	Coding	15	0.00137	0.00281	-1.3486	-4.279	-3.8048
	Noncoding	11	0.00044	0.00192	-1.9385	-4.6029	-4.3343
	Entire	26	0.00087	0.00233	-1.8035	-5.6221	-4.9097
O. rufipogon	Coding	11	0.00375	0.00395	-0.2199	1.1069	0.87174
	Noncoding	12	0.00297	0.00363	-0.7966	0.16597	-0.0885
	Entire	23	0.00332	0.00378	-0.5491	0.66049	0.39841
O. sativa	Coding	1	0.00053	0.00057	-0.6124	-0.6124	-0.4787
	Noncoding	2	0.00101	0.00095	0.59158	0.59158	0.50356
	Entire	3	0.00079	0.00078	0.16766	0.16766	0.14992
All	Coding	19	0.0016	0.0035	-1.4651	-0.8451	-1.3033
	Noncoding	13	0.00048	0.00222	-2.0095	-3.4358	-3.4747
	Entire	32	0.001	0.00281	-1.875	-2.5338	-2.7185

Table 4. Nucleotide diversity (synonymous and non-synonymous) at coding region of *SNAC1* gene

Population	Pisyn	Pinon	Pinon/Pisyn	Ks(JC)	Ka(JC)	Ka/Ks(JC)
O. nivara	0.00508	0.00019	0.03740157	0.00511	0.00019	0.03718
O. rufipogon	0.012	0.00113	0.09416667	0.01225	0.00114	0.09306
O. sativa	0.00219	0.0000	0.0000	0.00219	0.0000	0.0000
All	0.00586	0.00026	0.0443686	0.00591	0.00026	0.04399

O. sativa (entire region) suggested directional selection on *SNAC1* alleles. But this selection has no effect on the entire set of populations and overall selection mode is only purifying selection due to negative value of Tajima's D test. It was also supported by other neutrality tests (D* and F*), because the results of these tests were in negative values for the combined dataset of populations. High non-synonymous to synonymous ratio (<1) (Ka/Ks) along with its Juke and Contour correction values (<1) indicates purifying selection on the whole data set for the *SNAC1* alleles.

Across all the wild rice accessions, a total of 25 haplotypes were estimated including reference sequence of *SNAC1* gene excluding INDELs of 168 alleles (Fig. 3). Maximum (12) connecting haplotypes were missing from H6 to H24 haplotypes. Haplotype H1 was consisted of reference *SNAC1* gene sequence. Two haplotypes connecting junctions mv1 and mv2 were also found. Junction mv1 connected H2 haplotype to H4 along with H10 haplotype and mv2 connected haplotype H6 to H24 and H9. Largest H5 haplotype consisted of 61 accessions belonged to 53 *O. nivara,* six *O. rufipogon* and two *O. sativa*. H3 haplotype consisted 16 accessions of *O. nivara,* Six from UP and 10 from Bihar. Second largest haplotype H4 was made up of 58 accessions, of which 57 were *O. nivara* and one was *O. rufipogon* from UP and Bihar states of India. Haplotype H17 consisted of 5 accessions of *O. nivara,* out of which four were from West Bengal and one from Orissa. Haplotype H2, H8, H10 and H14 consisted of 3 accessions each while each minor haplotype consist of only one accession. Four H9, H12, H19 and H20 haplotypes were consisted of the drought tolerance accessions. Details of these haplotypes along with their origin are available online as (Supplementary Table 1). The drought tolerance

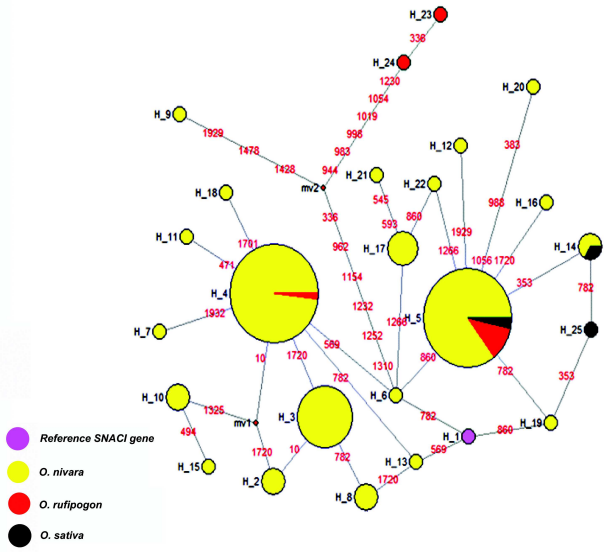


Fig. 3. A haplotype network based on nucleotide polymorphisms of the *SNAC1* gene in 168 rice accessions. Each haplotype is shown as a solid circle. Each branch represents mutational events. Different sizes of circles represent the frequency of that haplotypes

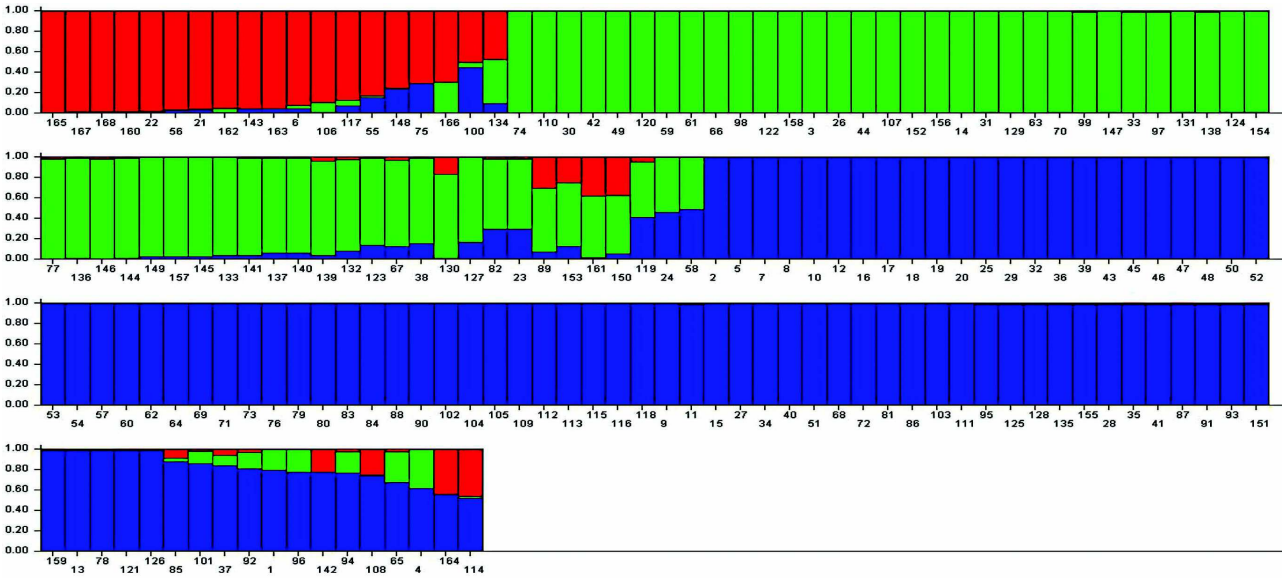
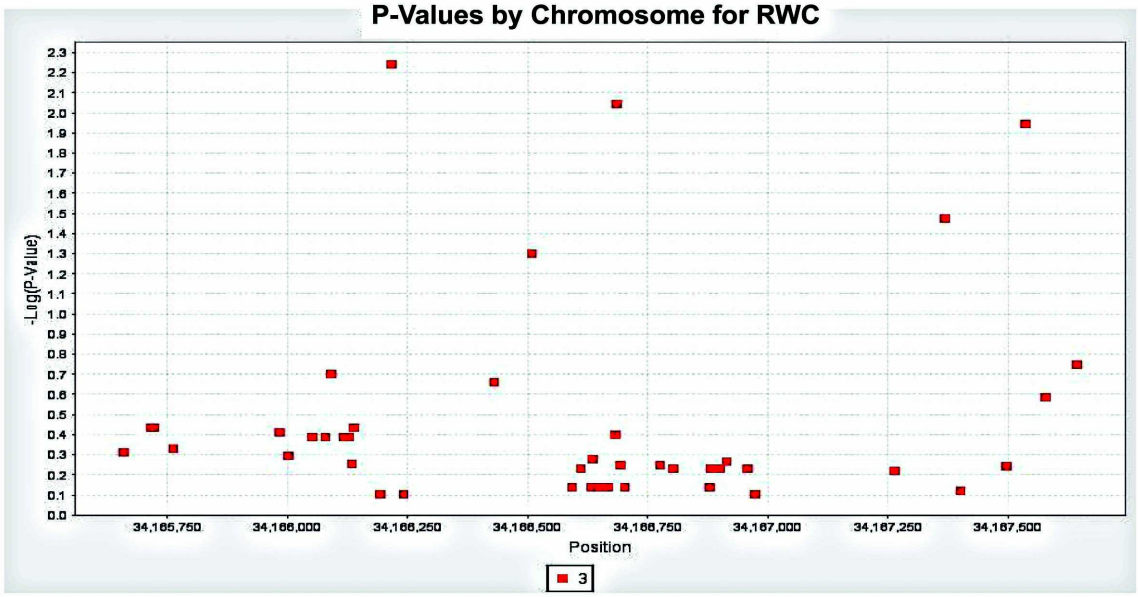
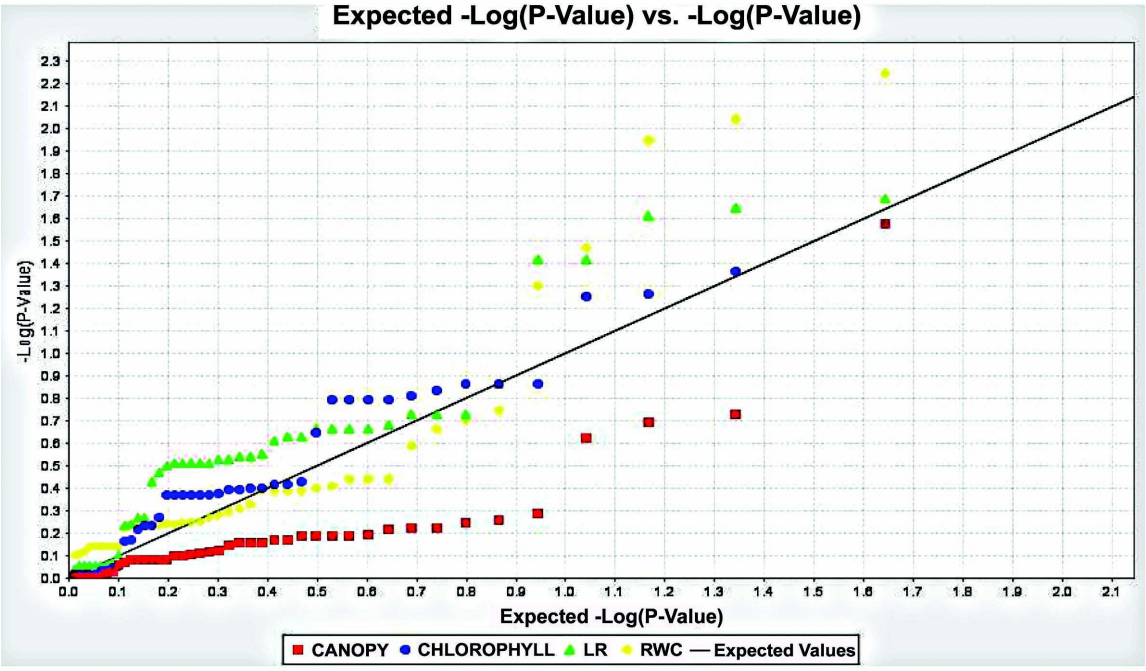


Fig. 4. Population structure of 168 rice genotypes depicting three sub-populations. Genotypic admixture can also be viewed from the graph



A



B

Fig. 5. Distribution of SNP-trait association in A) Manhattan plot and B) Quantile-Quantile (QQ) plots. Significant associations were observed for RWC, LR and chlorophyll content

mechanism in H20 haplotype might be due to a single point mutation *i.e.* A/G_{458} transition in this gene, leading to conversion of lysine to arginine while in haplotype H9 there were two non-synonymous SNPs in the coding region A/G_{594} and A/G_{771} which changed histidine to arginine and thrionine to alanine amino acids, respectively.

Protein diversity of SNAC1 alleles

One hundred and sixty eight protein sequences translated from the *SNAC1* alleles were used for the amino acid diversification analysis. All the amino acid sequences were aligned and compared with the reference SNAC1 protein. In MSU database nearest

Table 5. Association of *SNAC1* gene based SNPs with drought related traits

Trait	SNP name	Major/minor SNP allele	SNP annotation	Physical position(bp)	p-value	R^2
RWC	SNP15	G/A	CDS1	34166217	0.0057	0.073
RWC	SNP26	A/G	Intron	34166684	0.00905	0.06626
RWC	SNP42	T/A	3' UTR	34167535	0.01128	0.06483
LR	SNP42	T/A	3' UTR	34167535	0.02052	0.03419
LR	SNP43	A/T	3' UTR	34167577	0.02257	0.04621
Chlorophyll	SNP43	A/T	3' UTR	34167577	0.02664	0.04301

LR: Leaf rolling; R^2: Phenotypic variance explained by corresponding SNP

locus-ID is LOC_Os03g60080 for *SNAC1*. *SNAC1* gene is located in rice chromosome 3 having two CDS of 474 and 477 bp and one intron with 130 bp in length with a protein length of 316 aa. SNAC1 protein contains a conserved DNA-binding domain, putative nuclear localization signal (NLS) sequences (located at the regions of 77-89 aa and 114-130 aa, respectively), and a non conserved C-terminal region. NLS region of this protein was highly conserved among all the accessions and there was no variation in the amino acid sequence. Most variable region of SNAC1 protein was C-terminal region in the studied set of accessions. Six coding SNPs were responsible for causing non synonymous substitutions in this set of germplasm.

Association of SNPs in the SNAC1 gene with drought tolerance

For association mapping, MLM approach was used, where q-matrix generated from structure program was used as fixed effect while kinship matrix was used as random effects. The q- and kinship-matrices were generated using a 36plex SNP assay containing 3 SNPs from each of the rice chromosome. Using structure harvester, 3 sub-populations were estimated within the current germplasm set and used for the association study (Fig. 4). The structure-based (Fst) categorization of the current set of wild rice accessions has been described and available online as (Supplementary Table 2). In association analysis, out of the four phenotypic traits phenotyped for drought tolerance, significant association was found for three traits. The Manhattan plot and Q-Q plot showing the significance association of SNPs for RWC trait are shown in (Fig. 5A & B). When SNPs called from the *SNAC1* gene were used for the linkage disequilibrium (LD) and association study, four SNPs with high LD were found significant at 0.02 *p*-value for drought related parameters. On an average, ~5.5 % phenotypic variation was explained by all the significant SNPs. Maximum 7% phenotypic variation was explained by SNP15 (G/A) for RWC (Table 5). Among all the drought related traits, maximum SNPs (3) were found to be associated with RWC followed by LR (2). Out of three significant SNPs for RWC, one SNP26 (A/G) was located in the intron of *SNAC1* gene. The mechanism of drought tolerance for intron SNP might be due to its influence on splicing process leads to different form of protein. Also the alteration in intron of *SNAC1* gene may be linked with potential major loci or genes affecting drought tolerance (Wu et al. 2012, Ban et al. 2012). Two SNPs (SNP42 & SNP43) from 3' UTR region of *SNAC1* gene were also found significant for drought tolerance. These SNPs create or disrupt binding sites for RNA polymerase and regulatory elements and ultimately leads to altered expression of the gene (Jin and Lee 2013). For chlorophyll content, single SNP (SNP43) was found significant (*p*-value 0.02). SNP43 from 3' UTR A/T was also associated with H9 and H12 drought tolerant haplotypes.

Conclusively, the current germplasm set presented sufficient variability for *SNAC1* gene showing highly significant association of SNPs with drought tolerance. The significantly associated SNPs with the carrying genotypes can be deployed after functional characterization for improving drought tolerance in rice through molecular breeding.

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Supplementary Table 1. Details of 164 wild and four cultivated rice genotypes and SNP haplotypes of *SNAC1* gene

S.No. (1)	Accession No. (2)	Species (3)	Source (4)	Origin (5)	SNP Haplotype (6)
1	APO	*O. sativa*	CRRI Cuttack	India	H14
2	IC330621	*O. nivara*	NBPGR New Delhi	Birbhum, West Bengal	H22
3	IC330630	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H5
4	IC330631	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H5
5	IC330639	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H5
6	IC330641	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H17
7	IC330642	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H5
8	IC330644	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H5
9	IC330646	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H5
10	IC330647	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H5
11	IC330648	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H5
12	IC330649	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H17
13	IC330650	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H17
14	IC330651	*O. nivara*	NBPGR New Delhi	24 Parganas, West Bengal	H17
15	IC330654	*O. nivara*	NBPGR New Delhi	Dakshin dinajpur, West Bengal	H5
16	IC336676	*O. nivara*	NBPGR New Delhi	Khurda, Orissa	H21
17	IC336679	*O. rufipogon*	NBPGR New Delhi	Bauda, Orissa	H5
18	IC336680	*O. nivara*	NBPGR New Delhi	Bauda, Orissa	H5
19	IC336681	*O. nivara*	NBPGR New Delhi	Bauda, Orissa	H5
20	IC336682	*O. nivara*	NBPGR New Delhi	Bauda, Orissa	H5
21	IC336683	*O. nivara*	NBPGR New Delhi	Bauda, Orissa	H20
22	IC336684	*O. nivara*	NBPGR New Delhi	Bauda, Orissa	H5
23	IC336685	*O. nivara*	NBPGR New Delhi	Sonepur, Orissa	H5
24	IC336687	*O. nivara*	NBPGR New Delhi	Balangir, Orissa	H5
25	IC336689	*O. nivara*	NBPGR New Delhi	Balangir, Orissa	H17
26	IC336693	*O. nivara*	NBPGR New Delhi	Balangir, Orissa	H5
27	IC336694	*O. nivara*	NBPGR New Delhi	Kalahandi, Orissa	H5
28	IC336697	*O. nivara*	NBPGR New Delhi	Kalahandi, Orissa	H18
29	IC336698	*O. nivara*	NBPGR New Delhi	Kalahandi, Orissa	H5
30	IC336699	*O. nivara*	NBPGR New Delhi	Kalahandi, Orissa	H19
31	IC336700	*O. nivara*	NBPGR New Delhi	Kalahandi, Orissa	H5
32	IC336705	*O. nivara*	NBPGR New Delhi	Kalahandi, Orissa	H5
33	IC336706	*O. nivara*	NBPGR New Delhi	Raigad, Maharashtra	H5
34	IC336707	*O. rufipogon*	NBPGR New Delhi	Raigad, Maharashtra	H5
35	IC336708	*O. nivara*	NBPGR New Delhi	Raigad, Maharashtra	H5
36	IC336713	*O. nivara*	NBPGR New Delhi	Ganjam, Andhra Pradesh	H5
37	IC336715	*O. nivara*	NBPGR New Delhi	Ganjam, Andhra Pradesh	H5
38	IC336716	*O. nivara*	NBPGR New Delhi	Ganjam, Andhra Pradesh	H5
39	IC336721	*O. rufipogon*	NBPGR New Delhi	Ganjam, Andhra Pradesh	H23
40	IC336723	*O. rufipogon*	NBPGR New Delhi	Ganjam, Andhra Pradesh	H5

(1)	(2)	(3)	(4)	(5)	(6)
41	IC336724	*O. nivara*	NBPGR New Delhi	Ganjam, Andhra Pradesh	H5
42	IC336726	*O. nivara*	NBPGR New Delhi	Khurda, Orissa	H5
43	IC336727	*O. rufipogon*	NBPGR New Delhi	Khurda, Orissa	H24
44	IC336728	*O. rufipogon*	NBPGR New Delhi	Khurda, Orissa	H5
45	IR64	*O. sativa*	CRRI Cuttack	India	H25
46	N22	*O. sativa*	CRRI Cuttack	India	H5
47	NKSWR10	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H5
48	NKSWR100	*O. nivara*	New Collection	Ghazipur, Uttar Pradesh	H5
49	NKSWR103	*O. nivara*	New Collection	Varanashi, Uttar Pradesh	H2
50	NKSWR104	*O. nivara*	New Collection	Kaimpur, Bihar	H3
51	NKSWR105	*O. nivara*	New Collection	Bhabhua, Bihar	H4
52	NKSWR107	*O. nivara*	New Collection	Kaimur, Bihar	H4
53	NKSWR109	*O. nivara*	New Collection	Kaimur, Bihar	H3
54	NKSWR11	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H13
55	NKSWR110	*O. rufipogon*	New Collection	Kaimur, Bihar	H5
56	NKSWR111	*O. nivara*	New Collection	Kaimpur, Bihar	H4
57	NKSWR113	*O. nivara*	New Collection	Kaimur, Bihar	H4
58	NKSWR114	*O. nivara*	New Collection	Rohtas, Bihar	H4
59	NKSWR115	*O. nivara*	New Collection	Rohtas, Bihar	H5
60	NKSWR116	*O. nivara*	New Collection	Rohtas, Bihar	H4
61	NKSWR117	*O. nivara*	New Collection	Rohtas, Bihar	H5
62	NKSWR118	*O. nivara*	New Collection	Rohtas, Bihar	H3
63	NKSWR119	*O. nivara*	New Collection	Buxar, Bihar	H5
64	NKSWR120	*O. nivara*	New Collection	Buxar, Bihar	H3
65	NKSWR121	*O. nivara*	New Collection	Buxar, Bihar	H3
66	NKSWR122	*O. nivara*	New Collection	Bhojpur, Bihar	H3
67	NKSWR123	*O. nivara*	New Collection	Bhojpur, Bihar	H4
68	NKSWR124	*O. nivara*	New Collection	Bhojpur, Bihar	H5
69	NKSWR125	*O. nivara*	New Collection	Bhojpur, Bihar	H5
70	NKSWR126	*O. nivara*	New Collection	Bhojpur, Bihar	H5
71	NKSWR127	*O. nivara*	New Collection	Rohtas, Bihar	H4
72	NKSWR129	*O. nivara*	New Collection	Rohtas, Bihar	H3
73	NKSWR13	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H4
74	NKSWR130	*O. nivara*	New Collection	Rohtas, Bihar	H3
75	NKSWR131	*O. nivara*	New Collection	Rohtas, Bihar	H4
76	NKSWR132	*O. nivara*	New Collection	Rohtas, Bihar	H5
77	NKSWR134	*O. nivara*	New Collection	Aurangabad, Bihar	H10
78	NKSWR135	*O. nivara*	New Collection	Aurangabad, Bihar	H5
79	NKSWR136	*O. nivara*	New Collection	Aurangabad, Bihar	H4
80	NKSWR137	*O. nivara*	New Collection	Aurangabad, Bihar	H5
81	NKSWR138	*O. nivara*	New Collection	Aurangabad, Bihar	H4
82	NKSWR139	*O. nivara*	New Collection	Aurangabad, Bihar	H9

(1)	(2)	(3)	(4)	(5)	(6)
83	NKSWR14	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H4
84	NKSWR140	*O. nivara*	New Collection	Aurangabad, Bihar	H4
85	NKSWR141	*O. nivara*	New Collection	Aurangabad, Bihar	H4
86	NKSWR142	*O. nivara*	New Collection	Gaya, Bihar	H3
87	NKSWR143	*O. nivara*	New Collection	Gaya, Bihar	H3
88	NKSWR144	*O. nivara*	New Collection	Gaya, Bihar	H4
89	NKSWR145	*O. nivara*	New Collection	Gaya, Bihar	H4
90	NKSWR146	*O. nivara*	New Collection	Gaya, Bihar	H6
91	NKSWR148	*O. nivara*	New Collection	Jehanabad, Bihar	H4
92	NKSWR149	*O. nivara*	New Collection	Patna, Bihar	H4
93	NKSWR15	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H4
94	NKSWR150	*O. nivara*	New Collection	Patna, Bihar	H8
95	NKSWR151	*O. nivara*	New Collection	Patna, Bihar	H5
96	NKSWR152	*O. nivara*	New Collection	Patna, Bihar	H4
97	NKSWR153	*O. nivara*	New Collection	Patna, Bihar	H4
98	NKSWR156	*O. nivara*	New Collection	Bhojpur, Bihar	H7
99	NKSWR16	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H4
100	NKSWR160	*O. nivara*	New Collection	Buxar, Bihar	H8
101	NKSWR17	*O. nivara*	New Collection	Sonbhadra, Uttar Pradesh	H5
102	NKSWR18	*O. nivara*	New Collection	Sonbhadra, Uttar Pradesh	H4
103	NKSWR19	*O. nivara*	New Collection	Sonbhadra, Uttar Pradesh	H4
104	NKSWR2	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H5
105	NKSWR21	*O. rufipogon*	New Collection	Sonbhadra, Uttar Pradesh	H4
106	NKSWR22	*O. nivara*	New Collection	Sonbhadra, Uttar Pradesh	H4
107	NKSWR23	*O. nivara*	New Collection	Sonbhadra, Uttar Pradesh	H10
108	NKSWR24	*O. nivara*	New Collection	Sonbhadra, Uttar Pradesh	H4
109	NKSWR25	*O. nivara*	New Collection	Chandauli, Uttar Pradesh	H4
110	NKSWR26	*O. nivara*	New Collection	Chandauli, Uttar Pradesh	H3
111	NKSWR28	*O. nivara*	New Collection	Chandauli, Uttar Pradesh	H5
112	NKSWR3	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H12
113	NKSWR31	*O. nivara*	New Collection	Chandauli, Uttar Pradesh	H4
114	NKSWR33	*O. nivara*	New Collection	Chandauli, Uttar Pradesh	H10
115	NKSWR34	*O. nivara*	New Collection	Chandauli, Uttar Pradesh	H4
116	NKSWR35	*O. nivara*	New Collection	Ghazipur, Uttar Pradesh	H4
117	NKSWR36	*O. nivara*	New Collection	Ghazipur, Uttar Pradesh	H4
118	NKSWR37	*O. nivara*	New Collection	Ghazipur, Uttar Pradesh	H4
119	NKSWR38	*O. nivara*	New Collection	Ghazipur, Uttar Pradesh	H4
120	NKSWR4	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H3
121	NKSWR40	*O. nivara*	New Collection	Ghazipur, Uttar Pradesh	H5
122	NKSWR41	*O. nivara*	New Collection	Ghazipur, Uttar Pradesh	H4
123	NKSWR43	*O. nivara*	New Collection	Buxar, Bihar	H11
124	NKSWR44	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H4

(1)	(2)	(3)	(4)	(5)	(6)
125	NKSWR45	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H4
126	NKSWR46	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H4
127	NKSWR47	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H4
128	NKSWR49	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H16
129	NKSWR5	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H4
130	NKSWR50	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H4
131	NKSWR51	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H4
132	NKSWR53	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H5
133	NKSWR57	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H5
134	NKSWR58	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H5
135	NKSWR59	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H3
136	NKSWR6	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H4
137	NKSWR60	*O. nivara*	New Collection	Ballia, Uttar Pradesh	H4
138	NKSWR61	*O. nivara*	New Collection	Rampur, Uttar Pradesh	H4
139	NKSWR62	*O. nivara*	New Collection	Rampur, Uttar Pradesh	H4
140	NKSWR64	*O. nivara*	New Collection	Mau, Uttar Pradesh	H4
141	NKSWR66	*O. nivara*	New Collection	Mau, Uttar Pradesh	H4
142	NKSWR67	*O. rufipogon*	New Collection	Mau, Uttar Pradesh	H5
143	NKSWR68	*O. nivara*	New Collection	Mau, Uttar Pradesh	H2
144	NKSWR69	*O. nivara*	New Collection	Mau, Uttar Pradesh	H4
145	NKSWR70	*O. nivara*	New Collection	Mau, Uttar Pradesh	H14
146	NKSWR71	*O. nivara*	New Collection	Mau, Uttar Pradesh	H5
147	NKSWR73	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H4
148	NKSWR74	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H3
149	NKSWR78	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H5
150	NKSWR79	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H4
151	NKSWR8	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H15
152	NKSWR80	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H5
153	NKSWR83	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H3
154	NKSWR84	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H4
155	NKSWR85	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H5
156	NKSWR86	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H4
157	NKSWR87	*O. nivara*	New Collection	Azamgarh, Uttar Pradesh	H4
158	NKSWR88	*O. nivara*	New Collection	Mau, Uttar Pradesh	H14
159	NKSWR9	*O. nivara*	New Collection	Mirzapur, Uttar Pradesh	H3
160	NKSWR91	*O. nivara*	New Collection	Ghajipur, Uttar Pradesh	H5
161	NKSWR92	*O. nivara*	New Collection	Ghajipur, Uttar Pradesh	H4
162	NKSWR94	*O. nivara*	New Collection	Jaunpur, Uttar Pradesh	H4
163	NKSWR95	*O. nivara*	New Collection	Varanashi, Uttar Pradesh	H5
164	NKSWR96	*O. nivara*	New Collection	Varanashi, Uttar Pradesh	H8
165	NKSWR97	*O. nivara*	New Collection	Jaunpur, Uttar Pradesh	H5
166	NKSWR98	*O. nivara*	New Collection	Jaunpur, Uttar Pradesh	H4
167	NKSWR99	*O. nivara*	New Collection	Varanshi, Uttar Pradesh	H2
168	SWARNA	*O. sativa*	CRRI Cuttack	India	H5

Supplementary Table 2. Inferred ancestry details of wild rice accessions with 36 SNP markers

S.No.	Wild Rice	Fst1	Fst2	Fst3	S.No.	Wild Rice	Fst1	Fst2	Fst3
1	NKSWR103	0.003	0.203	0.794	45	NKSWR140	0.003	0.003	0.994
2	NKSWR104	0.003	0.003	0.994	46	NKSWR148	0.003	0.003	0.994
3	NKSWR105	0.005	0.991	0.003	47	NKSWR149	0.003	0.003	0.994
4	NKSWR107	0.006	0.379	0.615	48	NKSWR43	0.003	0.003	0.994
5	NKSWR109	0.004	0.003	0.994	49	NKSWR3	0.002	0.994	0.004
6	NKSWR110	0.925	0.025	0.05	50	NKSWR4	0.003	0.003	0.994
7	NKSWR113	0.003	0.003	0.994	51	NKSWR5	0.004	0.003	0.993
8	NKSWR114	0.003	0.003	0.994	52	NKSWR6	0.003	0.003	0.994
9	NKSWR116	0.003	0.003	0.993	53	NKSWR61	0.003	0.003	0.994
10	NKSWR121	0.003	0.003	0.994	54	NKSWR66	0.003	0.003	0.994
11	NKSWR122	0.004	0.004	0.993	55	NKSWR67	0.833	0.012	0.155
12	NKSWR125	0.003	0.003	0.994	56	NKSWR80	0.965	0.012	0.023
13	NKSWR131	0.004	0.008	0.988	57	NKSWR83	0.003	0.003	0.994
14	NKSWR135	0.007	0.989	0.004	58	NKSWR87	0.005	0.505	0.49
15	NKSWR136	0.004	0.003	0.993	59	NKSWR91	0.002	0.993	0.004
16	NKSWR138	0.003	0.003	0.994	60	NKSWR94	0.003	0.003	0.994
17	NKSWR141	0.003	0.003	0.994	61	NKSWR95	0.003	0.993	0.004
18	NKSWR143	0.003	0.003	0.994	62	NKSWR96	0.003	0.003	0.994
19	NKSWR144	0.003	0.003	0.994	63	NKSWR97	0.006	0.988	0.005
20	NKSWR145	0.003	0.003	0.994	64	NKSWR98	0.003	0.003	0.994
21	NKSWR146	0.96	0.006	0.034	65	NKSWR99	0.022	0.301	0.677
22	NKSWR151	0.984	0.004	0.011	66	NKSWR100	0.002	0.992	0.006
23	NKSWR152	0.015	0.685	0.3	67	NKSWR10	0.03	0.846	0.124
24	NKSWR153	0.004	0.532	0.464	68	NKSWR11	0.003	0.003	0.993
25	NKSWR156	0.003	0.003	0.994	69	NKSWR13	0.003	0.003	0.994
26	NKSWR160	0.003	0.991	0.006	70	NKSWR17	0.004	0.988	0.008
27	NKSWR120	0.004	0.003	0.993	71	NKSWR19	0.003	0.003	0.994
28	NKSWR142	0.003	0.007	0.989	72	NKSWR64	0.004	0.003	0.993
29	NKSWR150	0.003	0.003	0.994	73	NKSWR86	0.003	0.003	0.994
30	NKSWR115	0.002	0.994	0.004	74	NKSWR92	0.003	0.995	0.003
31	NKSWR139	0.003	0.989	0.008	75	NKSWR70	0.71	0.004	0.286
32	NKSWR111	0.003	0.003	0.994	76	NKSWR21	0.003	0.003	0.994
33	NKSWR117	0.009	0.987	0.004	77	NKSWR2	0.016	0.981	0.003
34	NKSWR118	0.003	0.004	0.993	78	NKSWR8	0.004	0.008	0.988
35	NKSWR119	0.009	0.003	0.989	79	NKSWR9	0.003	0.003	0.994
36	NKSWR123	0.003	0.003	0.994	80	NKSWR14	0.004	0.003	0.994
37	NKSWR124	0.061	0.099	0.84	81	NKSWR15	0.003	0.003	0.993
38	NKSWR126	0.012	0.832	0.156	82	NKSWR16	0.021	0.686	0.294
39	NKSWR127	0.003	0.003	0.994	83	NKSWR18	0.003	0.003	0.994
40	NKSWR129	0.004	0.003	0.993	84	NKSWR22	0.003	0.003	0.994
41	NKSWR130	0.003	0.008	0.989	85	NKSWR23	0.086	0.03	0.884

S.No.	Wild Rice	Fst1	Fst2	Fst3
42	NKSWR132	0.002	0.994	0.004
43	NKSWR134	0.003	0.003	0.994
44	NKSWR137	0.006	0.991	0.003
89	NKSWR28	0.301	0.626	0.074
90	NKSWR31	0.003	0.003	0.994
91	NKSWR33	0.003	0.008	0.989
92	NKSWR34	0.035	0.156	0.809
93	NKSWR35	0.004	0.007	0.989
94	NKSWR36	0.025	0.207	0.769
95	NKSWR37	0.003	0.007	0.99
96	NKSWR38	0.004	0.218	0.778
97	NKSWR40	0.01	0.987	0.002
98	NKSWR41	0.003	0.992	0.005
99	NKSWR44	0.008	0.988	0.004
100	NKSWR45	0.502	0.051	0.448
101	NKSWR46	0.019	0.121	0.86
102	NKSWR47	0.003	0.003	0.994
103	NKSWR49	0.004	0.003	0.993
104	NKSWR50	0.004	0.003	0.994
105	NKSWR51	0.004	0.003	0.994
106	NKSWR53	0.893	0.095	0.013
107	NKSWR57	0.003	0.99	0.007
108	NKSWR58	0.251	0.01	0.739
109	NKSWR59	0.003	0.003	0.994
110	NKSWR60	0.002	0.995	0.003
111	NKSWR62	0.004	0.003	0.993
112	NKSWR68	0.003	0.003	0.994
113	NKSWR69	0.003	0.003	0.994
114	NKSWR71	0.462	0.011	0.527
115	NKSWR73	0.003	0.003	0.994
116	NKSWR74	0.004	0.003	0.994
117	NKSWR77	0.878	0.049	0.073
118	NKSWR79	0.003	0.003	0.994
119	NKSWR84	0.048	0.543	0.408
120	NKSWR85	0.003	0.994	0.003
121	NKSWR88	0.009	0.003	0.988
122	O.niv336726	0.004	0.992	0.003
123	O.niv330630	0.014	0.849	0.137
124	O.niv330648	0.003	0.984	0.012
125	O.niv330649	0.007	0.003	0.99
126	O.niv330651	0.009	0.003	0.988
127	O.niv330654	0.007	0.823	0.17
128	O.niv336697	0.007	0.003	0.99
129	O.niv336699	0.004	0.989	0.008
130	O.niv330631	0.166	0.824	0.01
131	O.niv330639	0.004	0.985	0.011
132	O.niv330642	0.023	0.895	0.082

S.No.	Wild Rice	Fst1	Fst2	Fst3
86	NKSWR24	0.004	0.003	0.993
87	NKSWR25	0.008	0.003	0.989
88	NKSWR26	0.003	0.003	0.994
133	O.niv336680	0.005	0.958	0.037
134	O.niv336683	0.474	0.427	0.1
135	O.niv336689	0.007	0.003	0.99
136	O.niv336693	0.01	0.981	0.009
137	O.niv336698	0.012	0.924	0.065
138	O.niv336700	0.008	0.985	0.008
139	O.niv336705	0.036	0.923	0.041
140	O.niv336713	0.013	0.924	0.064
141	O.niv336724	0.008	0.95	0.042
142	O.niv336676	0.225	0.003	0.772
143	O.niv336681	0.952	0.007	0.041
144	O.niv336682	0.01	0.976	0.014
145	O.niv336684	0.006	0.968	0.026
146	O.niv336685	0.018	0.978	0.004
147	O.niv336694	0.004	0.988	0.008
148	O.niv336706	0.757	0.006	0.237
149	O.niv336715	0.005	0.97	0.025
150	O.niv336716	0.374	0.572	0.054
151	O.niv330641	0.008	0.003	0.989
152	O.niv330644	0.007	0.99	0.004
153	O.niv330646	0.252	0.621	0.126
154	O.niv330647	0.004	0.983	0.013
155	O.niv330650	0.007	0.003	0.99
156	O.niv330621	0.003	0.99	0.007
157	O.ruf336708	0.005	0.969	0.026
158	O.ruf336721	0.005	0.992	0.003
159	O.ruf336728	0.008	0.003	0.989
160	O.ruf336727	0.989	0.004	0.007
161	O.ruf336679	0.382	0.6	0.017
162	O.ruf336687	0.955	0.035	0.01
163	O.ruf336707	0.951	0.004	0.045
164	O.ruf336723	0.44	0.005	0.555
165	IR64	0.993	0.004	0.003
166	N22	0.698	0.298	0.004
167	APO	0.991	0.006	0.003
168	Swarna	0.991	0.005	0.004