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Genetic diversity analysis of *aus* rice, a valuable source of stress tolerance genes

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Abstract

Genetic diversity is one of the most basic prerequisites in any crop improvement programme. It acts as a reservoir for identifying superior alleles influencing key agronomic and qualitative traits through allele mining. The *aus* rice variety group is a prospective source for the development of novel stress-tolerant rice cultivars. The present study was aimed to characterize the genetic variation present in the *aus* type 3K rice germplasm subset, further it can be utilized to identify the genes related to stress tolerant by allele mining. Genetic diversity of this *aus* type 3K rice genome panel subset indicates the presence of admixture in the population. Therefore, it is necessary to generate a good association mapping panel for the stress tolerant crop improvement in rice.

Keywords: Rice, *aus* subgroup, genetic diversity, stress tolerant breeding

Introduction

Rice, being the staple food crop for more than 50% of the world population is cultivated in 162.05 million hectares with the production of 755.43 million tonnes (Sundaramoorthy *et al.*, 2022) [13]. Asia alone produces around 90% of the world's rice requirements. Modern genetics, genomics tools provides insights into understanding the rice domestication, which indicates elite favored traits were retained in cultivated rice to adapt to the local stress environment (Gross *et al.*, 2010, Larson *et al.*, 2014, Meyer and Purugganan 2013) [6, 8, 11]. The *aus* have the characteristics of both indica and japonica and originated in the hill areas of Eastern India, Bangladesh, and Myanmar. The *aus* subgroup of rice has been discovered as a valuable source of stress tolerance genes, having evolved in the stress-prone regions of Bangladesh and Eastern India (Glaszmann, 1987) [4]. Submergence tolerance QTL/gene *SUB1* (Xu *et al.*, 2006) [17] from FR13A, phosphorus-deficiency tolerance QTL *Pup1/PSTOL1* (Gamuyao *et al.*, 2012; Wissuwa *et al.*, 1998, 2001) [5, 15, 16] from Kasalath, and drought-yield QTLs *qDTY2.2* and *qDTY4.1* from Aday Sel were identified. Interestingly, all these three genotypes *viz*, FR13A, Kasalath and Aday Sel were belongs to *aus* type subgroup. The donor parent of the major-effect drought-yield QTL *qDTY1.1* is Nagina-22 (N22), a heat-tolerant variety (Satake & Yoshida, 1978; Jagadish *et al.*, 2010) [12, 7] were also belongs to this *aus* subgroup. Despite their potential for improving rice stress tolerant breeding, only a few large-scale phenotypic and genetic assessments of *aus* accessions for stress tolerance have been attempted (Cal *et al.*, 2019; Chen *et al.*, 2020) [2, 3] due to their large number of *aus* accessions. Therefore, there is an urge to develop a corset/mapping panel of *aus* subgroup for the successful stress tolerant crop improvement in rice. A good collection of *aus* subgroup need to be identified from the available 201 *aus* type rice germplasm present in the 3K rice genome panel. Initially, genetic diversity for this 201 *aus* lines were carried out for the successful creation of association mapping panel for the development of stress tolerant breeding. The current record level production and productivity of rice is due to the availability and utilization of rich genetic diversity existing in rice germplasm. Understanding the genetic/molecular basis of target traits is essential for the accurate genetic manipulation of complex quantitative traits such as yield, resistance to biotic/abiotic stresses.

Materials and Methods

Genomic data information

A set of 201 *aus* type rice germplasm of 3K panel were used for genetic diversity analysis (Table 1).

The core SNP Dataset (v0.7; 404K) was obtained from the 3K filtered SNP set (v0.7) by applying two-step LD pruning procedure *viz* (i) LD pruning with window size 10kb (ii) LD pruning with window size 50 SNPs (https://s3.amazonaws.com/3kricegenome/snpseek-dl/3krg-base-filt-corev0.7/readme_core_v0.7.txt) and this was readily available in the rice SNP-Seek Database (<http://snpseek.irri.org>). This core SNP dataset was further filtered for this 201 *aus* type rice genotypes by adopting the following criteria using TASSEL 5.0 (Bradbury *et al.*, 2007), SNPs with Minor Allele Frequency (MAF) ≥ 0.1 and heterozygous proportion ≤ 0.1 were retained, pruning SNPs with minimum distance of 100000 bp between the filtered SNPs. Finally a set of 3401 SNPs covering all the twelve chromosomes were retained and this was used for genetic diversity analysis.

Genetic diversity

For this set of *aus* type 201 rice germplasm, VCF file of final set which contains 3401 SNPs was downloaded from TASSEL 5.0 after performing all the above mentioned filtering criteria. This VCF file was used for the genetic diversity analysis. Neighbor joining clustering method was performed with 3401 SNPs by VCF2PopTree and the tree was visualized in iTOL. All the 201 accessions were used for the estimation of polymorphic information index (PIC), a genetic diversity parameter by using the program POWERMARKER Ver3.25 (Liu and Muse 2005) [10]. Polymorphic information content (amount of polymorphism within a population) was

estimated based on Botstein *et al.* (1980) [1].

Results and Discussion

All the extracted 201 *aus* type rice germplasm was used for the genetic diversity analysis. Clustering analysis based on neighbor joining clustering method using VCF2PopTree separated the accessions into two major clusters. Out of the two clusters, one cluster showed many admixtures (Fig 1). This indicates that 201 *aus* type 3K subset rice germplasm was not entirely pure lines and it contains many admixtures. PIC value represents the relative informativeness of each SNP marker used in the present study and the average PIC value was found to be 0.292. The *aus* rice variety group have rich genetic diversity with wide variation in abiotic stress resistance. A set of 65 candidate genes which are responsible for seedling stage salt tolerance were identified from the Bengal and Assam *aus* accessions (Chen *et al.*, 2020) [3]. A major QTL for grain yield, *qDTY1.1*, was identified on rice chromosome 1 from the N22, a drought tolerant *aus* cultivar background (Vikram *et al.*, 2011) [14]. Candidate genes conferring tolerance to Zn deficiency was identified marker in a diverse aus panel (Lee *et al.*, 2018) [9]. *Aus* type rice serves as a promising source for the development of novel stress-tolerant rice cultivars. In order to generate the good association mapping panel for the identification of stress tolerant related genes from the *aus* type group, it is necessary to separate the pure lines from the whole *aus* type subset available in the 3K rice genome panel.

Table 1: Details on the 3K subset of 201 *aus* type rice germplasm

S. No	Name	Accessions	Origin	S. No.	Name	Accessions	Origin
1	AUS 171	IRIS 313-11048	Bangladesh	102	ARC 14756	IRIS 313-11272	India
2	AUS 219	IRIS 313-11049	Bangladesh	103	ARC 14901	IRIS 313-11274	India
3	AUS 233	IRIS 313-11050	Bangladesh	104	ARC 15129	IRIS 313-11277	India
4	AUS 242	IRIS 313-11051	Bangladesh	105	ARC 5756	IRIS 313-10845	India
5	AUS 278	IRIS 313-11052	Bangladesh	106	ARC 7001	IRIS 313-10849	India
6	AUS 282	IRIS 313-11053	Bangladesh	107	ARC 7336	IRIS 313-10852	India
7	AUS 295	IRIS 313-11054	Bangladesh	108	ARC 7425	IRIS 313-11291	India
8	AUS 299	IRIS 313-11055	Bangladesh	109	AUS PADDY (RED)	IRIS 313-11348	India
9	AUS 301	IRIS 313-11056	Bangladesh	110	BAK TULSI	IRIS 313-11164	India
10	AUS 308	IRIS 313-11057	Bangladesh	111	BARI SUTAR	IRIS 313-11454	India
11	AUS 329	IRIS 313-11058	Bangladesh	112	BEGUM	IRIS 313-9641	India
12	AUS 344	IRIS 313-11059	Bangladesh	113	BENA JHUPI	IRIS 313-11165	India
13	AUS 359	IRIS 313-11060	Bangladesh	114	BHUT MURI	IRIS 313-11166	India
14	AUS 439	IRIS 313-8641	Bangladesh	115	BUTNAPAR	IRIS 313-11917	India
15	AUS 449	IRIS 313-11061	Bangladesh	116	CHILE BORO	IRIS 313-11353	India
16	AUS 84	IRIS 313-11047	Bangladesh	117	CHUNDI	IRIS 313-11737	India
17	BATHURI	IRIS 313-8963	Bangladesh	118	CIPPI	IRIS 313-11455	India
18	BAZAIL 975	IRIS 313-11123	Bangladesh	119	D 204-1	IRIS 313-10534	India
19	BHAUDIOA 303	IRIS 313-8321	Bangladesh	120	DANGAR	IRIS 313-9610	India
20	BINNAFUL	IRIS 313-11481	Bangladesh	121	DODGUI	IRIS 313-10543	India
21	BONGEZA	IRIS 313-11482	Bangladesh	122	GORA DHAN 2	IRIS 313-11712	India
22	BORA DIGA	IRIS 313-10979	Bangladesh	123	KADA CHOPA	IRIS 313-11168	India
23	BORO 275	IRIS 313-11064	Bangladesh	124	KALIBAJARI	IRIS 313-11742	India
24	BORO 394	IRIS 313-11065	Bangladesh	125	KALIPINCH	IRIS 313-11476	India
25	BORO 70-2	IRIS 313-11063	Bangladesh	126	KALUT T 139	IRIS 313-11489	India
26	BOTESHSHORE	IRIS 313-11483	Bangladesh	127	KANAI BASHI	IRIS 313-11169	India
27	BOTESWAR 2	IRIS 313-11013	Bangladesh	128	KANPURI	IRIS 313-11477	India
28	CHAKILA	IRIS 313-11014	Bangladesh	129	KARAHANI	IRIS 313-11458	India
29	CHANDARHAT	IRIS 313-9368	Bangladesh	130	KARANGI	IRIS 313-11963	India
30	CHUNGUR BALI	IRIS 313-9422	Bangladesh	131	KOLAMBA	IRIS 313-11456	India
31	DB 3	IRIS 313-10587	Bangladesh	132	LAL TAURA	IRIS 313-11170	India
32	DD 126	IRIS 313-10598	Bangladesh	133	LOCAL BHAT	IRIS 313-11452	India
33	DHARIAL	IRIS 313-11154	Bangladesh	134	M 142	IRIS 313-11171	India
34	DJ 29	IRIS 313-10594	Bangladesh	135	MALCHI	IRIS 313-11602	India
35	DJ 47	IRIS 313-10593	Bangladesh	136	N 22	IRIS 313-10150	India

36	DJ 69	IRIS 313-10592	Bangladesh	137	NCS 271 A	IRIS 313-11636	India
37	DL 5	IRIS 313-10595	Bangladesh	138	PANDRI PAREWA	IRIS 313-11603	India
38	DM 49	IRIS 313-10602	Bangladesh	139	R 146	IRIS 313-11232	India
39	DV 110	IRIS 313-10606	Bangladesh	140	RANI BHOG	IRIS 313-11172	India
40	DV 2	IRIS 313-10604	Bangladesh	141	SADA AUS	IRIS 313-11173	India
41	DV 86	IRIS 313-10605	Bangladesh	142	SALSI	IRIS 313-11462	India
42	EJALI	IRIS 313-11484	Bangladesh	143	SATHI	IRIS 313-11371	India
43	GORIA	IRIS 313-11557	Bangladesh	144	SIMUL KHURI	IRIS 313-8771	India
44	HANPA	IRIS 313-11015	Bangladesh	145	SIRHANTI	IRIS 313-11492	India
45	HATEA	IRIS 313-12141	Bangladesh	146	SLO 19	IRIS 313-11174	India
46	HIJOL DIGA	IRIS 313-11112	Bangladesh	147	SONA AUS	IRIS 313-11175	India
47	HOLOI BASH (SOLOI BASH)	IRIS 313-8721	Bangladesh	148	SUGARKAND	IRIS 313-11604	India
48	JABOR SAIL	IRIS 313-8410	Bangladesh	149	T 21	IRIS 313-10608	India
49	JAGLI BORO	IRIS 313-8252	Bangladesh	150	UPRH 166	IRIS 313-11619	India
50	JASURE AUS	IRIS 313-11322	Bangladesh	151	UPRH 197	IRIS 313-11620	India
51	JHORA	IRIS 313-11323	Bangladesh	152	UPRH 33	IRIS 313-11617	India
52	JHUL DIGA	IRIS 313-10981	Bangladesh	153	UPRH 58	IRIS 313-11618	India
53	KACHILON	IRIS 313-11016	Bangladesh	154	W 398	IRIS 313-11374	India
54	KALABOKRI	IRIS 313-11324	Bangladesh	155	MHARAKA	IRIS 313-11809	Kenya
55	KALIA	IRIS 313-9626	Bangladesh	156	DEVARASI	IRIS 313-10734	Nepal
56	KALIBORO 600	IRIS 313-11067	Bangladesh	157	GHAIYA	IRIS 313-10737	Nepal
57	KATAKCHIKON	IRIS 313-10963	Bangladesh	158	KAMULI	IRIS 313-10925	Nepal
58	KHAMA 1183	IRIS 313-11216	Bangladesh	159	LAL SAR	IRIS 313-10735	Nepal
59	KORTIK KAIKA	IRIS 313-11116	Bangladesh	160	LATIJHABAR	IRIS 313-11629	Nepal
60	KOYRA	IRIS 313-8283	Bangladesh	161	MANSARA DHAN	IRIS 313-12139	Nepal
61	LAKHSMI DIGHA	IRIS 313-10976	Bangladesh	162	RERM BILASH	IRIS 313-10736	Nepal
62	LAL MOTI	IRIS 313-12055	Bangladesh	163	SETYA	IRIS 313-12183	Nepal
63	LALSAITA	IRIS 313-8789	Bangladesh	164	SIDALI	IRIS 313-10623	Nepal
64	LARA BORO	IRIS 313-12002	Bangladesh	165	SUGA PANKHA	IRIS 313-10927	Nepal
65	LENJA MURALI	IRIS 313-9661	Bangladesh	166	34	IRIS 313-11036	Pakistan
66	LOROI	IRIS 313-11017	Bangladesh	167	421	IRIS 313-11037	Pakistan
67	MADISA	IRIS 313-11018	Bangladesh	168	BAMLA SUFFAID 320	IRIS 313-11020	Pakistan
68	NARIKEL BADI	IRIS 313-11213	Bangladesh	169	BEGMI 135	IRIS 313-11024	Pakistan
69	NATEL BORO	IRIS 313-11163	Bangladesh	170	DHAN 263	IRIS 313-11025	Pakistan
70	NOROI	IRIS 313-8864	Bangladesh	171	JAMBALI	IRIS 313-9449	Pakistan
71	PANKHIRAJ	IRIS 313-10930	Bangladesh	172	JHONA 101	IRIS 313-11027	Pakistan
72	PORANG	IRIS 313-11019	Bangladesh	173	KANGRI	IRIS 313-11888	Pakistan
73	PORASHI	IRIS 313-11982	Bangladesh	174	KHARSU 80	IRIS 313-8398	Pakistan
74	SADUMONI	IRIS 313-10964	Bangladesh	175	MOTIA	IRIS 313-11028	Pakistan
75	SAITA	IRIS 313-11111	Bangladesh	176	MUSHKAN 340 A	IRIS 313-11029	Pakistan
76	SHITTA AMAN	IRIS 313-11214	Bangladesh	177	NAWAN SATHRA BAGAR 344	IRIS 313-11031	Pakistan
77	STRAW 23-400	IRIS 313-8822	Bangladesh	178	RATUA 81	IRIS 313-10549	Pakistan
78	SUNGA WALA	IRIS 313-10987	Bangladesh	179	SANTHI 206	IRIS 313-11034	Pakistan
79	TILOKCHAN (SONABETHI)	IRIS 313-10965	Bangladesh	180	SOLAY GHAT	IRIS 313-9695	Pakistan
80	UCP 122	IRIS 313-10603	Bangladesh	181	SUFIAID 246	IRIS 313-8390	Pakistan
81	UCP 41	IRIS 313-10600	Bangladesh	182	TAK	IRIS 313-8655	Pakistan
82	ZARBASAIL	IRIS 313-11210	Bangladesh	183	TAK SUFAID	IRIS 313-9283	Pakistan
83	INDIA DULAR (NO ORDEM 7)	IRIS 313-10969	Brazil	184	IR 73690-7-2-1-1-3-2-2-1	IRIS 313-10380	Philippines
84	AMAKOYALI	IRIS 313-11595	India	185	HODARAWALA	IRIS 313-10020	Sri Lanka
85	ARC 10100	IRIS 313-9137	India	186	KARUTHA SEENATI	IRIS 313-10718	Sri Lanka
86	ARC 10145	IRIS 313-10854	India	187	KURKARUPPAN	IRIS 313-9861	Sri Lanka
87	ARC 10843	IRIS 313-10673	India	188	KURULU WEE (WHITE)	IRIS 313-8342	Sri Lanka
88	ARC 11276	IRIS 313-10861	India	189	PODI HEENATI	IRIS 313-9636	Sri Lanka
89	ARC 11751	IRIS 313-10869	India	190	RANRUWAN	IRIS 313-11191	Sri Lanka
90	ARC 11777	IRIS 313-10871	India	191	WIR 1391	IRIS 313-9963	Sri Lanka
91	ARC 11822	IRIS 313-10873	India	192	COLOMBIA XXI	IRIS 313-15908	
92	ARC 11959	IRIS 313-8554	India	193	DAWN CI 9534	IRIS 313-8073	
93	ARC 12021	IRIS 313-10875	India	194	HUHUI 91269	B243	
94	ARC 12067	IRIS 313-10876	India	195	JHONA 349	CX63	
95	ARC 12079	IRIS 313-10877	India	196	KALAR KAR	IRIS 313-11413	
96	ARC 12101	IRIS 313-10878	India	197	KARIA	IRIS 313-10545	
97	ARC 12433	IRIS 313-10882	India	198	KASALATH	CX227	
98	ARC 12867	IRIS 313-10891	India	199	N22	CX368	
99	ARC 12920	IRIS 313-10892	India	200	QINGKE	B164	
100	ARC 13544	IRIS 313-11298	India	201	ZACAODAO 13	B049	
101	ARC 14150	IRIS 313-11265	India				

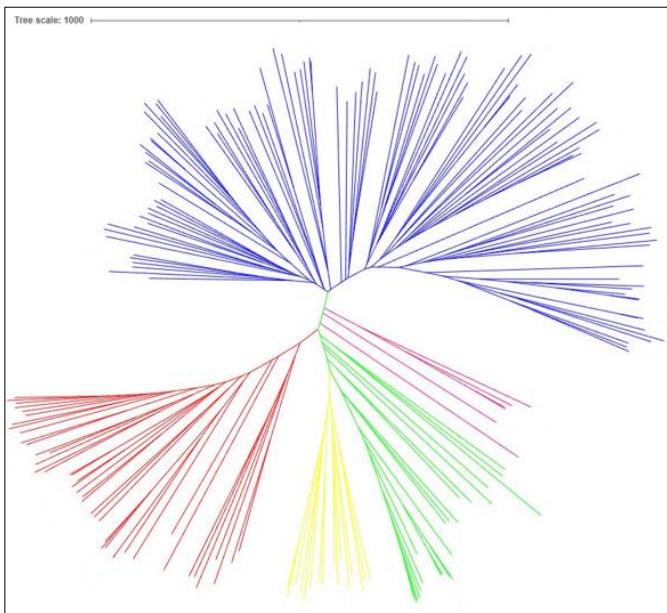


Fig 1: Unrooted neighbour joining tree of 201 *aus* type rice germplasm

Conclusion

Genetic diversity needs to be assessed in order to characterise, use, and conserve germplasm. The present study has identified that the 201 *aus* type rice germplasm available in the 3K rice genome project contains few admixtures. Therefore, there is a need to extract the pure lines from the available subset for the construction of core subset. A strong association mapping panel is required for stress resistant crop improvement in rice. This panel can be used for the identification of novel alleles or genes related to stress tolerant by allelic mining approaches.

In future, this information will be beneficial in the selection of genetically varied parents and in the production of traits employing genotypes in rice breeding programmes.

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