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RESEARCH ARTICLE

Evaluation of rice germplasm for tolerance to multiple abiotic stresses using multivariate techniques and molecular screening

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Abstract

Due to climate change, rice productivity and sustainability in rainfed areas have become increasingly threatened by both drought and flooding. Identifying new germplasm resources with tolerance to high and low extremes of precipitation is required to meet the impelling demand of climate-resilient varieties. To identify promising accessions with tolerance to both drought and submergence, 38 gora rice landraces along with 12 traditional cultivars and check varieties from the rainfed ecologies in eastern India were evaluated under drought and submergence stresses, both at the seedling and germination stages of the crop. The marker-based survey of nine DTY QTLs for grain yield under drought revealed that 17 accessions potentially carried multiple DTY QTLs. Most (82%) of the genotypes scored positive for *Sub1A* locus by SNP-based functional marker AEX1. However, their survival rate under 14 days of complete submergence varied widely (0.58–92.4%). By multivariate analysis using important traits of abiotic stress conditions, Dular and Kalakeri genotypes were identified as the most promising genotypes along with a few gora cultivars such as IC640869 and IC0640884. The promising genotypes identified in this study can be utilized as potential donors for multiple abiotic stress tolerance as well as for understanding the molecular basis of adaptation to multiple abiotic stresses.

Keywords: Abiotic stress, multiple stress tolerance, drought, submergence, anaerobic germination, rice.

Introduction

Rice (*Oryza sativa* L.) is the most important staple food with a production of 512.8 million tons from an area of about 165 million hectares globally (https://www.fao.org/). Increasing rice production by about 60% is expected to meet global food needs in the future. This objective is heavily challenged by increasing abiotic stresses such as drought and flooding under recent extreme weather disasters due to climate change (Lesk et al. 2016). Drought and flood are the most important abiotic stresses reducing rice yield significantly in rainfed lowland areas in South and Southeast Asia, around one-third of the total rice production area in the world (Dilley et al. 2005). Drought and flooding stresses coexist in rainfed ecologies (Bin Rahman and Zhang 2022). In the eastern parts of India, heavy rains during July-August cause flash flooding at the vegetative stage, while prolonged dry spells during October month, due to early withdrawal of monsoon or terminal drought stress at the reproductive stage lead to considerable yield loss (Widawsky and O'Toole 1990; Sarkar et al. 2009). Popular high-yielding varieties were introduced to safeguard rice yield with major effect drought QTLs (DTY QTLs) along with the Sub1 QTL to combine drought and submergence tolerance (Sandhu et al. 2019).

Drought tolerance in crop plants involves an interplay between morpho-physiological and biochemical traits (Kamoshita et al. 2008; Beena et al. 2021). In the past decade, intensive research at the International Rice Research Institute (IRRI), Philippines and different countries in Africa and South Asia including India have led to the identification of stable DTY QTLs from diverse donors for grain yield under drought stress (Kumar et al. 2014). Identifying rice accessions with genes or QTLs that contribute to stress tolerance and locating such genetic factors on molecular linkage maps is

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essential to develop stress-tolerant varieties (Sandhu and Kumar 2017). Gautam et al. (2022) evaluated a set of selected 35 BC_2-F_3 lines for genome recovery of *Sub 1* gene in the recipient variety with respect to 62 morpho-physiological traits and recorded substantial improvement in the selected lines. Concurrently, combining BB resistance genes, other rice varieties have been developed for blast resistance, submergence tolerance, salinity, drought and low phosphorus tolerance. A major QTL – *Sub1* was transferred into mega rice cultivar, Swarna, through MABB for improving flood tolerance (Neeraja et al. 2007), resulting in the development of a flood tolerant cultivar, Swarna – *Sub1*. Subsequently, some varieties have been improved for drought tolerance and submergence incorporating different Qtls e.g., *qSub1, DTY1.1, DTY2.1, DTY3.1 qDTY2.2, DTY4.1* (Chauhan et al. 2022).

Submergence tolerance is an important trait,in areas where short-term flash flooding completely inundates rice fields. A breakthrough in developing submergence tolerant varieties was facilitative by the identification of the underlying QTL *Submergence 1* (*Sub1*) from the rice landraces FR13A (Xu and Mackill 1996). The FR13A*Sub1* locus encodes a variable cluster of up to three *ethylene-responsive factors* (ERF): *Sub1A-1*, *Sub1B-1* and *Sub1C-1* (Xu et al. 2006). Germplasm screening revealed that all tolerant genotypes possessed the tolerant *Sub1* haplotype (*Sub1A-1/Sub1C-1*), whereas all accessions without the *Sub1A* gene were intolerant (Singh et al. 2010).

Submergence during seed germination imposes severe hypoxic stress, also known as germination stage oxygen deficiency (GSOD) (Ray et al. 2016). The mode of overcoming hypoxic stress during germination differs from seedling stage submergence stress (Vijayan et al. 2018). Rice can germinate and extend its coleoptile underwater in the absence of oxygen, known as anaerobic germination (AG) (Magneschi and Perata 2009). The anaerobic germination potential (AGP) varies widely within rice germplasm (Angaji et al. 2010; Kretzschmar et al. 2015; Pucciariello 2020). It is an important trait for direct seeded rice (DSR) cultivation. Direct seeding in rainfed lowland ecologies in South and Southeast Asia makes the crop vulnerable to submergence. Unpredicted heavy rain immediately after the seeding often call upon flash floods that could lead to continuous water stagnation for several days, depending on the land situation. This can cause hypoxic stress situations by restricting the free diffusion of oxygen from the air to germinating seeds (Narsai et al. 2015).

Crop genetic resources are the basic raw materials for any crop improvement program.The adaptive traits in germplasm accessions evolved due to both natural and artificial selection. The upland rice landraces prevailing in the Chotanagpur plateau region, covering the state of Jharkhand, in eastern India have evolved under typical rainfed situations and recurrently selected by the farmers for suitability to stress-prone agro-ecological conditions. These landraces may possess superior fitness alleles for better adaptation to stress-prone environments (Redoña and Mackill 1996; Shrestha et al. 2014). Upland gora rice landraces were earlier characterized for morpho-genetic diversity and found that most accessions were genetically *aus* type (Roy et al. 2021a). We assume that tolerance to multiple abiotic stress conditions may coexist in these cultivars. Considering this, the present study was undertaken to evaluate drought, submergence, and germination stage oxygen deficiency (GSOD) tolerance in a set of upland rice landraces of Jharkhand to identify promising genotypes with tolerance to multiple abiotic stress conditions, and to generate information on the presence of nine DTY QTLs along with the *Sub1*locus using molecular markers.

Materials and methods

Plant materials and experimental details

Fifty rice accessionswere used for screening in drought, submergence, and germination under anaerobic conditions (Table 1). This panel included 38 gora rice accessions, 12 traditional cultivars, and check varieties. The gora accessions could be further categorized into white gora (8), brown gora (10), black gora (4), and other gora (16) based on husk colour and farmers' classification (Roy et al. 2021a). Trials for drought tolerance under managed drought screening facility were conducted at Central Rainfed Upland Rice Research Station (CRURRS), Hazaribag in 2019 and 2020 (Supplementary Fig. S1A-B), and for submergence tolerance and anaerobic germination potential were carried out at ICAR-NRRI, Cuttack, Odisha (Supplementary Fig. S1C-D).

Drought phenotyping

The rice genotypes were grown in rainout shelters during the wet season (June-November) in 2019 and 2020 following the phenotyping protocol described elsewhere (Verulkar et al. 2010). In brief, the experiment was laid out in a randomized complete block design with three replications and the genotypes were dry-seeded in a single row plot of 2 m in length with a row-to-row distance of 20 cm. Inorganic fertilizer dose of 60:30:30 (N:P_2O_5:K_2O) was applied. Pre-emergence herbicide butachlor @1.5 kg a.i.ha^{-1} was applied at 3 days after sowing (DAS). Two hand weeding was additionally done to keep the experimental plot weed-free. The crop was grown under well-watered conditions up to 45 DAS. At 46 DAS irrigation was stopped, and the experimental area was covered to protect it from rainfall. Genotypes were scored at the peak stress period around two weeks after the irrigation withdrawal. Re-watering was done about 16 days after the withdrawal of irrigation and the plot was left for the next cycle of drying. At the time of harvesting, data on plant height and grain yield were recorded.The data on physiological parameters such as leaf rolling score,

Table 1. Rice germplasm used in the study

S. no.	Genotype name	Accession number	S. no.	Genotype name	Accession number	S. no.	Genotype name	Accession number
1	White Gora	IC-0640859	18	Gora	IC-0640876	35	AUS257	NA
2	Brown gora	IC-0640860	19	Dani gora	IC-0640877	36	White gora	IC-0640892
3	Brown gora	IC-0640861	20	Barka sariagora	NA	37	Brown gora	IC-0640893
4	Black gora	IC-0640862	21	White gora	IC-0640878	38	Gora	IC-0640894
5	Brown gora	IC-0640863	22	Brown gora	IC-0640879	39	AUS196	NA
6	Gora	IC-0640864	23	Black gora	IC-0640880	40	White gora	IC-0640895
7	Dani gora	IC-0640865	24	Gora	IC-0640881	41	Brown gora	IC-0640896
8	Alsanga gora	IC-0640866	25	Lalmati	IC-0640882	42	Gora	IC-0640897
9	Chakra gora	IC-0640867	26	Kalakeri	IC-0640883	43	Brown gora	IC-0640898
10	Tikragora	IC-0640868	27	White gora	IC-0640884	44	Gora	IC-0640899
11	Brown gora	IC-0640869	28	Brown gora	IC-0640885	45	Black gora	IC-0640900
12	white gora	IC-0640870	29	Black gora	IC-0640886	46	Widdjong	NA
13	Gora	IC-0640871	30	Gora	IC-0640887	47	N22	NA
14	Dani gora	IC-0640872	31	Jonga	IC-0640888	48	Apo	NA
15	Saria gora	IC-0640873	32	White gora	IC-0640889	49	Vandana	NA
16	White gora	IC-0640874	33	Gora	IC-0640890	50	Dular	NA
17	Brown gora	IC-0640875	34	Karhani	IC-0640891			

NA=Accession number not available.

drying score, and other yield related parameters was done according to the Standard evaluation system (SES) for rice (IRRI 2013). The relative leaf water content was estimated following the procedure described in (Turner 1981).

Screening for submergence tolerance

Fifty rice accessions were grown along with three checks viz., FR13A and Swarna-Sub1 as tolerant checks and Swarna as a sensitive check. Seeds were sown in a field tank (l×b×h = 40 m ×8 m ×1.2 m) @ 4-5 seeds hill^{-1} with a spacing of 20 cm (line-to-line) and 15 cm (hill-to-hill) following an augmented block design. Fertilizers were added as basal @ N:P_2O_5:K_2O = 20:20:20 kg ha^{-1}. Single plant per hill was maintained after about 10 days of sowing and one hand weeding was carried out. Twenty-five-day-old seedlings were completely inundated under 90 ± 5 cm of water for 14 days. Around 60 cm of water depth was maintained above the plant height at the initial stage of submergence. After 14 days of submergence, water was removed, and post-submergence data was recorded. The survival rate (recorded after 7 days of de-submergence) and elongation ability of the plants after imposition of submergence stress were calculated as described in (Chakraborty et al. 2021). Elongation ability was expressed as the percent increase in plant height during the period of stress as [(plant height after stress–plant height before stress)/plant height before stress]×100. The number of surviving hills per genotype was counted at 7 days after de-submergence, and the percentage survival was calculated as (number of hills surviving/number of hills before submergence)×100.

Screening for anaerobic germination potential

The hypoxic stress experiment was conducted following Vijayan et al. (2018). Thirty seeds/genotype of 50 rice accessions along with tolerant (AC39416A) and sensitive (Naveen) checks were sown in a polypropylene tray (l×b×h = 37.5 cm×35.0 cm×15.0cm) containing sundried well pulverized sandy loam soil up to 4 cm thickness. Seeds were covered with 1 cm of soil, submerged immediately with tap water up to 10 cm depth, and kept in such condition for 21 days. A control set was also maintained along with the treatment set where the soil surface was properly moistened at regular intervals instead of standing water. The number of seeds germinated was recorded on every 3rd day in both treatment and control sets. Seeds were scored as germinated if the tip of the plumule was visible above the soil surface. The epicotyl length (cm) was measured in the treatment set on 7th, 14th, and 21st days. The final root length was also measured at 21 days after imposing stress. Data were analyzed as per a completely randomized design. From the germination count, traits such as (a) germination % (G_p) which is the percentage of seeds that complete the germination process, (b) the mean germination time (G_t) which denotes the average length of time required for maximum germination of a seed lot, and (c) the mean

germination rate (G_r) denoting the average number of seeds that germinate over a particular periodwere calculated (Chakraborty et al. 2019). The Seedling vigor index (SVI) was calculated as SVI = (root length + shoot length at 21 days)×G_p(ISTA 2003). The anaerobic germination index (AGI) was calculated (AGI = $G_{pGSOD}/G_{pcontrol}$) to find the actual germinability under GSOD.

Statistical analysis

Two years of phenotyping data from drought experiments were analyzed using PBTools v1.4 (http://bbi.irri.org/products). The variance analysis of phenotyping data of the submergence experiment was carried out using 'augmented RCBD' package in the R environment of statistical computing (Aravind et al. 2021). The GSOD phenotyping data generated from CRD experimental design was analyzed using the R package 'agricolae version 1.3-5'. The germination variables such as G_r, G_t, and G_r were calculated using the R package 'GemninaR' (Lozano-Isla et al. 2019). Principal component analysis (PCA) was performed using the standardized data and the number of principal components was selected based on parallel analysis using GraphPad Prism version 9.0.2.

Genotyping for drought and submergence QTLs

Twenty-four SSR markers representing the peak and flanking markers of nine DTY QTLs were utilized for identifying drought-tolerant lines. For PCR analysis of the *Sub1* locus, three markers AEX1, SC3, and Sub1BC2, were selected. Positive checks/donors for QTLs are included in all the assays (Supplementary Fig. S1).

Total genomic DNA was extracted using Qiagen DNeasy Plant Mini Kit (Qiagen, India). The PCR reaction was done using 50 ng of template DNA, 0.5 µM of each forward and reverse primer, 0.2 mM of dNTPs, 1X PCR buffer with 20 mM $MgCl_2$, and 1 U of *Taq* DNA polymerase in a total volume of 25 µL. The PCR consisted of an initial denaturation for 5 minutes at 94°C; 35 cycles of 30 seconds denaturation at 94°C, annealing at primer-specific temperature for 30 s and extension at 72°C for 30 seconds, and final extension for 7 minutes at 72°C. The amplified products were separated by 2–3% agarose gel electrophoresis and visualized using a gel doc (eGel imager, Invitrogen). In case of DTY QTL-linked SSR markers, the amplicon with the lowest molecular weight was assigned allele number '1' and the progressively heavier bands were assigned numbers incrementally.

Results and discussion

Genetic variation for drought tolerance

ANOVA conducted on the nine morpho-physiological traits recorded under drought revealed highly significant differences ($p< 0.01$) among the cultivars. Leaf rolling, leaf drying, and spikelet fertility varied widely under water stress (CV = 50.7, 25.4 and 41.4%, respectively). Eleven check varieties showed the average lowest leaf drying score (LDS) of 3.7, while White gora cultivars recorded the highest (LDS = 4.8) (Table 2). Within each cultivar group LDS varied significantly except in Black gora. The average spikelet sterility under drought was highest in Brown gora cultivars and the lowest in checks. Plant water status in terms of relative water content (RWC) showed very low variation (CV = 3.9%). The highest RWC was recorded in Dular (82.7%) and the lowest in IC-0640873 (68.5%). The check genotypes showed the highest mean grain yield under drought, while the lowest was recorded in Brown gora cultivars (Fig. 1A). Under drought highest grain yield was recorded in Dular followed by N22. When the top fifteen cultivars ranked best on grain yield (Supplementary Table S2), it was observed that cultivars with higher grain yield predominantly showed lower leaf rolling and drying, in addition to lower spikelet sterility and higher RWC of the leaf. Spearman's rank correlation coefficients indicated a significant negative association between leaf drying and grain yield (Fig. 1B). Reduced RWC resulted in increased spikelet sterility. The genotype with higher leaf rolling also recorded higher leaf drying. Characterization of rice cultivars adapted to Southern India revealed that traits like chlorophyll stability index, leaf rolling, days to 50% flowering, chlorophyll content, and root biomass were the most important predictors of grain yield under drought (Beena et al. 2021).

Screening of DTY QTLs

The banding pattern of DTY QTL-linked SSR markers in each test genotype was compared to the respective *qDTY* donors to identify potential qDTY harbouring cultivars. An accessions was noted as a *qDTY*-positive cultivar when it showed amplicons similar to the original donor for all linked markers for a particular DTY QTL. For $qDTY_{1.2}$, Kali *aus* type alleles were amplified only in Kalakeri and Dular (Table 3). Similarly, for $qDTY_{2.1}$, White gora (IC0640892) and Gora (IC0640899) showed similar alleles to the positive check Apo. Overall, $qDTY_{2.2}$ was identified in four, $qDTY_{2.3}$ in four, $qDTY_{3.1}$ in one, $qDTY_{3.2}$ in four, $qDTY_{6.1}$ in two and $qDTY_{12.1}$ in four genotypes. Eight out of 15 top-yielding cultivars had shown the presence of one or multiple DTY QTLs.

Identification of new QTL donors with a desirable background using molecular markers tightly linked to DTY QTLs could be appealing. Recently, a few reports have been published in this direction (Anupam et al. 2017; Mukherjee et al. 2018; Roy et al. 2021b). Although such studies can only predict the existence of a QTL in new germplasm, these have some importance for the identification of novel tolerant QTLs by involving the germplasm lines without any of the reported DTY QTLs. In the present study, 17 accessions were identified to potentially carry DTY QTLs. Kalakeri possessed five DTY QTLs ($qDTY_{1.2}$, $qDTY_{2.2}$, $qDTY_{2.3}$, $qDTY_{3.2}$ and $qDTY_{6.1}$). A similar finding was reported Roy et al. (2021b). A Brown gora accession, IC-0640861, recorded presence of $qDTY_{3.1}$ and $qDTY_{12.1}$. The known drought tolerant cultivars such

Table 2. Morpho-physiological traits under different abiotic stresses for 49 rice cultivars classified into five cultivar groups

Group	Black gora	Brown gora	White gora	Other gora	Checks	Overall	CV%
Drought							
n	3	10	8	17	11	49	
LRS	2.8 ± 1.0	2.5 ± 1.4	3.7 ± 2.0	3.1 ± 1.7	3.2 ± 1.3	3.1 ± 1.6	50.7
LDS	3.9 ± 0.4	4.1 ± 0.8	4.8 ± 1.3	3.8 ± 0.9	3.7 ± 1.1	4 ± 1.0	25.4
DF	71.6 ± 0.7	70.4 ± 5.6	64.4 ± 7.1	68 ± 5.1	65.2 ± 5.3	67.5 ± 5.8	8.6
Ht	115.4 ± 7.6	117.7 ± 5.4	115.6 ± 6.2	119.4 ± 7.0	118.7 ± 5.0	118 ± 6.1	5.2
PnL	15.7 ± 2.0	17.7 ± 0.7	18.7 ± 1.5	17.6 ± 1.7	19.1 ± 1.6	18 ± 1.7	9.2
GnP	73.4 ± 11.5	74.6 ± 8.3	84.9 ± 15.6	77 ± 12.9	79.1 ± 9.8	78.1 ± 11.9	15.3
SpkStr	42.6 ± 0.5	49.9 ± 4.9	43.5 ± 11.5	43.6 ± 8.8	41.7 ± 8.0	44.4 ± 8.5	19.1
RWC	73.8 ± 3.8	78 ± 1.9	76.6 ± 2.4	76.1 ± 3.3	77.4 ± 3.4	76.7 ± 3.0	3.9
Yld	159.5 ± 33.9	130.2 ± 56.4	133.1 ± 55.7	160.5 ± 63	172.6 ± 78.7	152.5 ± 63.2	41.4
Submergence							
n	4	10	8	17	14	53	
SR	55.9 ± 31.1	43.5 ± 33.5	36.3 ± 24.9	27.9 ± 21.8	57.6 ± 27.9	42.1 ± 28.7	68.2
EA	115.0 ± 32.2	111.9 ± 10.8	116.9 ± 31.3	93.7 ± 20.7	96.1 ± 35.0	102.9 ± 27.3	26.5
GSOD							
n	4	10	8	17	13	52	
Gp	60.3 ± 22.4	48.8 ± 16.5	28.9 ± 14.9	57.6 ± 17.1	47.8 ± 21.9	52.1 ± 18.8	35.9
AGI	0.61 ± 0.23	0.50 ± 0.17	0.48 ± 0.15	0.58 ± 0.17	0.49 ± 0.22	0.53 ± 0.19	36.4
Gt	10.1 ± 0.32	9.3 ± 1.15	9.4 ± 0.69	9.5 ± 0.53	9.4 ± 0.72	9.5 ± 0.83	8.7
Gr	0.10 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	7.7
SVI	22.4 ± 8.5	18.4 ± 5.6	19.0 ± 6.8	22.9 ± 7.1	18.6 ± 8.6	20.3 ± 7.6	37.5

LRS = Leaf rolling score; LDS = Leaf drying score; DF = Days to 50% flowering; PH = Plant height (cm); PL = Panicle length (cm); GP = Grain number panicle^{-1}; SS = Spikelet sterility (%); RWC = Relative water content (%); GYP = Grain yield plot^{-1} (g); SR = Survival rate (%); EA = Elongation ability; G_p = Germination (%); AGI = Anaerobic germination index; G_t= Mean germination time under GSOD; G_r= Mean germination rate under GSOD and SVI = Seedling vigour index.

Table 3. Rice germplasm identified for different DTY QTLs

DTY QTL	Linked marker	Positive check/donor	Genotypes identified with similar alleles as donor
qDTY$_{1.1}$	RM431, RM11943, RM12091	N22	-
qDTY$_{1.2}$	RM259, RM315	Kali aus	Kalakeri (IC-0640883), Dular
qDTY$_{2.1}$	RM324, RM327, RM324	Apo	White gora (IC-0640892), Gora (IC-0640899)
qDTY$_{2.2}$	RM279, RM211, RM263, RM324, RM555	Kali aus	Dani gora (IC-0640877), Black gora (IC-0640880), Kalakeri (IC-0640883), Vandana
qDTY$_{2.3}$	RM573, RM3212, RM250	Kali aus	Alsanga gora (IC-0640866), White gora (IC-0640874), Kalakeri (IC-0640883), White gora (IC-0640895)
qDTY$_{3.1}$	RM520, RM416	Apo	Brown gora (IC-0640861)
qDTY$_{3.2}$	RM60, RM22, RM545	Moroberekon	White gora (IC-0640895), Kalakeri (IC-0640883), N22, Vandana
qDTY$_{6.1}$	RM204, RM589	Vandana	Gora (IC0640876), Kalakeri (IC-0640883),
qDTY$_{12.1}$	RM28166, RM28040, RM28199	Way Rarem	Brown gora (IC-0640861, IC-0640869, IC-0640885, IC-0640893)

as, N22, the donor of *qDTY*$_{1.1,}$ was found to carry *qDTY*$_{3.2}$, while Dular possessed *qDTY*$_{1.2}$. The higher level of drought tolerance supported by the presence of DTY QTLs indicates that these genotypes could be utilized further in drought breeding programmes.

Genetic variation for submergence tolerance and screening of Sub1 locus

The rice genotypes showed varied responses to submergence in terms of plant survival and under water elongation (Table 2, Fig. 1C). Among the gora cultivar

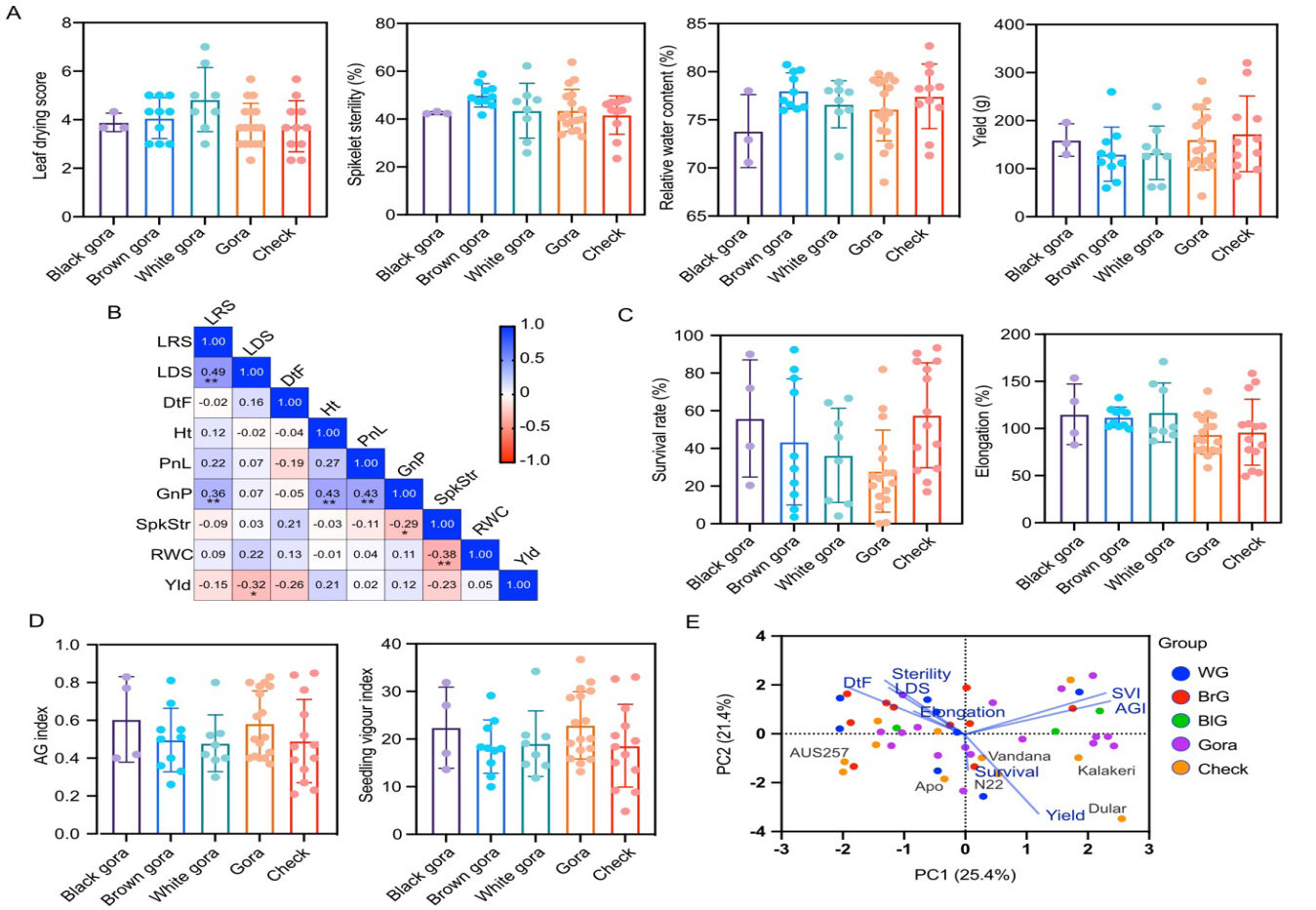

Fig. 1. Variability of stress tolerance traits in five cultivar groups. (A) Important traits recorded under drought stress; (B) Spearman correlation coefficients among drought traits measured on 49 rice cultivars [('*','**' = Significant correlations) ($P < 0.05$ and $P < 0.01$)]; (C) and (D) Traits observed for submergence stress at seedling and vegetative stage, respectively; (E) Genotype-by-trait-biplot analysis of rice genotypes for two principal components with important traits measured under drought, submergence and GSOD

groups, Black gora registered the highest average survival (55.9%). Nine accessions including Black gora (IC-0640862), Brown gora (IC-0640869), Kalakeri, Vandana, and Dular were highly tolerant with>80% survival. The elongation ability varied between 49.2–170.9%. Overall, 28 rice genotypes exhibited elongation percentages >100% (Fig. 1C). The lowest elongation was observed in Apo and the highest in White gora (IC-0640884). All the submergence tolerant lines showed low to moderate internode elongation suggesting they might have employed a quiescence strategy for survival (Colmer and Voesenek 2009). Implementing quiescence is a crucial survival strategy as continued elongation during the period of submergence leads to lodging of plants immediately after de-submergence (Vergara et al. 2014).

The AEX1 marker, detecting functional SNP for *Sub1A* specific for submergence tolerance, generated a 231 bp amplicon in 41 test genotypes having submergence survival of 0.58–92.4% (Fig. 2), while, the SC3 marker closely linked with the *Sub1A* amplified FR13A type allele in 44 tested genotypes. Five accessions including IC-0640866, IC-0640881, IC-0640895, IC-0640896, and IC-0640898, despite being negative for AEX1, showed tolerant allele for SC3. Among these, two accessions, IC-0640896 and IC-0640898 recorded >70% survival. The *Sub1* BC_2 primer covering the 38 bp insertion region between *Sub1B* and *Sub1C* amplified the FR13A type allele in 40 rice accessions indicating that most of the genotypes carry FR13A type

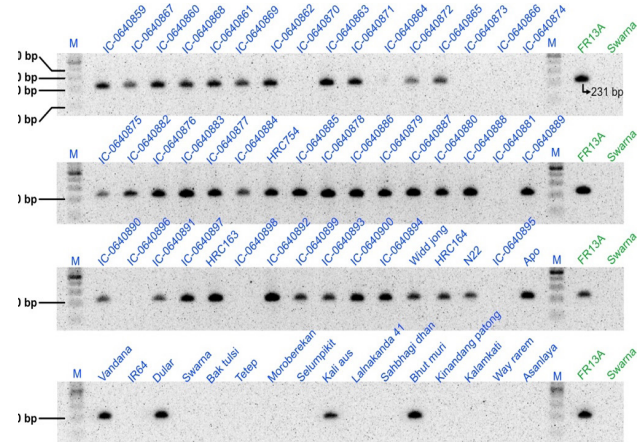

Fig. 2. Amplification of *SUB1A* tolerant allele in the rice germplasm

Table 4. Rice genotypes identified for tolerance to different abiotic stresses

Stress	Promising genotypes identified
Drought	Dular, N22, IC-0640867, IC-0640869, IC-0640897, IC-0640859, IC-0640877, IC-0640871, IC-0640883, IC-0640866, Vandana, IC-0640864, IC-0640880, IC-0640872, IC-0640889
Submergence	Kalakeri, Vandana, Dular, IC-0640897, IC-0640898, IC-0640880
Germination stage oxygen deficiency (GSOD)	IC-0640882, IC-0640880, IC-0640868, IC-0640885, IC-0640865, IC-0640884, IC-0640881, IC-0640867, IC-0640883, IC-0640871, IC-0640862, Dular
Drought + GSOD	Dular,Kalakeri, IC-0640864, IC-0640862, IC-0640880, IC-O640884, IC-0640885, IC-O640881, IC-0640871
Drought + Submergence + GSOD	Dular, Kalakeri, IC0640869, IC-0640884, IC-0640896, IC-0640897, Vandana

haplotype in the *Sub1* locus.

Only a small portion of the present rice genotypes showed a high level of tolerance to 14 days of complete submergence, indicating that the genotypes are adapted to the rainfed upland ecology of eastern India where these are rarely challenged by prolonged submergence during the crop growth period. Being predominantly *aus* genotypes (Roy et al. 2021a), the present cultivars carry the *Sub1* QTL. Molecular screening of *Sub1* QTL did not correlate well with the phenotypic data for submergence tolerance. It is assumed that *Sub1A-1*is under-expressed in most of the present genotypes. Further analysis of the expression of *Sub1A* along with the transcription factors (such as ethylene response factors, ERFs) in these genotypes will shed light on the genotypic response to the submergence. It has been reported that submergence-induced expression of both ERF66 and ERF67 in FR13A shoots, while only the expression of *ERF67* was up-regulated in another *Sub1A-1* positive but submergence-sensitive rice cultivar Baisbish (Oe et al. 2022).

Genetic variation for tolerance to GSOD

Germination under anaerobic conditions revealed significant variation for germination percentage (G_p) as well as seedling vigour index (SVI) (Table 1). The anaerobic germination index (AGI) ranged from 0.21 to 0.84. Lalmati, IC-0640868, IC-0640880, IC-0640885, IC-0640865, IC-0640871, and IC-0640884 registered high AGI of >0.80. Besides, nine moderately tolerant accessions with AGI = 0.60-0.79 were also identified in different gora cultivar groups (Fig. 1D). In total, 14 test genotypes recorded SVI values at par with tolerant check AC39416A (32.63 ± 1.98). Genotypes with a higher SVI were IC-0640868 (36.68 ± 2.83), IC-0640884 (34.22 ± 3.05), and IC-0640882 (33.03 ± 4.72). The germination rate (G_r) showed a significant reduction under GSOD (Fig. 1D). Mean G_r under the control condition was 0.14, while it was reduced to 0.11 under GSOD. In general, genotypes with higher phenotypic values of G_r and G_p also registered higher SVI values. Earlier studies have reported results with diverse sets of rice genotypes for tolerance to GSOD (Barik et al. 2019; Ismail et al. 2009; Miro et al. 2017). In general, GSOD-tolerant genotypes possess better adaptive traits such as faster germination and coleoptile elongation (Miro and Ismail 2013).

Selection of promising multiple stress-tolerant genotypes and implications in rice breeding

PCA performed with eight traits (drought: leaf drying, spikelet sterility, days to 50% flowering, grain yield under drought; submergence: survival rate and elongation ability; and GSOD: anaerobic germination index and seedling vigour index) classified 49 rice genotypes based on the trait loadings (Fig. 1E). The PCA selected two principal components (PCs) capturing 47.0% of the total variance. Both AGI and SVI were highly correlated (r^2>90.0) with PC1 along with grain yield under drought (r^2=47.0). On PC2, Spikelet sterility, DF, LDS and SVI showed positive correlation (r^2>40.0). Therefore, the accessions grouped in the lower right quadrant of the biplot expected to have a higher grain yield under drought, high submergence survival rate, and high anaerobic germination potential. Considering the results of trait variability and PCA, promising accessions for individual as well as multiple stress conditions are listed in Table 4.

Overall, substantial genetic variation that can be exploited to develop climate-resilient rice varieties was identified in the present study. Potential donors for important abiotic stresses in rainfed ecologies appear to be present in both the upland gora cultivars of Jharkhand and some known upland cultivars. Despite significant advancements in developing drought- and submergence-tolerant rice varieties through marker-assisted breeding (MAB), there is a need for further improvement of rice for tolerance to multiple combined stresses. The most promising genotypes identified in this study need to eventually be utilized by the breeders to develop climate-resilient rice. Among these genotypes, Dular (INGR22107) and Kalakeri (INGR21179) have been registered by the Plant Germplasm Registration Committee of Indian Council of Agricultural Research for greater utilization. In MAB for improving drought tolerance, DTY QTLs from the identified donors can be introgressed using peak as well as flanking markers. For submergence tolerance, both AEX1 and SC3 markers need to be used for selecting *Sub1A* gene. Furthermore, system biology studies will be conducted to understand the molecular basis of adaptation of these unique accessions to multiple abiotic stresses.

Supplementary material

Supplementary Tables S1, S2 and Supplementary Fig. S1 are provided and can be accessed online www.isgpb.org.

Author contributions

Conceptualization of research (SR, KC); Designing of the experiments (SR, NPM, KC); Contribution of experimental materials (NPM, SR); Execution of field/lab experiments and data collection (SR, KC, JK, AB, PS, PM, BCV); Analysis of data and interpretation (SR, PS, KC); Preparation of the manuscript (SR, NPM, PM, KC).

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References

Angaji S. A., Septiningsih E. M., Mackill D. J. and Ismail A. M. 2010. QTLs associated with tolerance of flooding during germination in rice (*Oryza sativa* L.). Euphytica, **172**: 159–168. doi: 10.1007/s10681-009-0014-5

Anupam A., Imam J., Quatadah S. M., Siddaiah A., Das A. P., Variar M. and Mandal, N. 2017. Genetic diversity analysis of rice germplasm in Tripura state of Northeast India using drought and blast linked markers. Rice Sci., **24**: 10–20. doi: 10.1016/j.rsci.2016.07.003

Aravind J., Mukesh Sankar S. and Wankhede D. P. K. V. 2021. Augmented RCBD: Analysis of Augmented Randomised Complete Block Designs. R package version 0.1.5.9000.

Barik J., Kumar V., Lenka S. K. and Panda D. 2019. Genetic potentiality of lowland indigenous indica rice (*Oryza sativa* L.) landraces to anaerobic germination potential. Plant Physiol. Rep., **24**: 249–261. doi: 10.1007/s40502-019-00441-3

Beena R., Kirubakaran S., Nithya N., Manickavelu A., Sah R.P., Abida P.S., Sreekumar J., Jaslam P.M., Rejeth R., Jayalekshmy V.G. and Roy S. 2021. Association mapping of drought tolerance and agronomic traits in rice (*Oryza sativa* L.) landraces. BMC Plant Biol., **21**: 1–21. doi: 10.1186/s12870-021-03272-3

Bernier J., Kumar A., Ramaiah V., Spaner D. and Atlin G. 2007. A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Sci., **47:** 507-516. doi: 10.2135/cropsci2006.07.0495

Bin Rahman A.N.M. and Zhang J. 2022. The Coexistence of Flood and Drought Tolerance: An Opinion on the Development of Climate-Smart Rice. Front. Plant Sci., **13**: 8-11. doi: 10.3389/fpls.2022.860802

Chakraborty K., Bishi S.K., Goswami N., Singh A.L., Bhaduri D. and Zala P.V. 2019. Salinity-induced changes in seed germination and the expression profile of antioxidant enzymes in peanut as early and late responses in emerging radicles. Acta Physiol. Plant., **41**: 134.

Chauhan J. S., Choudhury P. R., Singh K. H. and Thakur A. K. 2022. Recent trends in crop breeding, the varietal induction in seed chain and impact on food grain production in India. Indian J. Genet. Plant Breed., 82(3): 259-279. doi: 10.31742/ISGPB.82.3.1

Colmer T.D. and Voesenek L.A.C.J. 2009. Flooding tolerance: suites of plant traits in variable environments. Funct. Plant Biol., **36(8)**: 665-681.

Dilley M., 2005. Natural disaster hotspots: a global risk analysis. Vol. 5. World Bank Publications.

Gautam A., Chetia S.K., Sharma V., Modi M.K., Ahmed T. and Phukon M. 2022. Morpho-molecular investigation of recurrent parent genome recovery of Sub1 introgressed advance lines of rice (*Oryza sativa* L.). Indian J. Genet. Plant Breed., 82(4): 411-418. doi: 10.31742/ISGPB.82.4.4

IRRI. 2013. SES: Standard Evaluation System for Rice, 5th edn. International Rice Research Institute, Manila, Philippines.

Ismail A.M., Ella E.S., Vergara G.V. and Mackill D.J., 2009. Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). Ann. Bot., **103**: 197–209. doi: 10.1093/aob/mcn211

ISTA. 2003. ISTA handbook on seedling evaluation. 3rd ed. International Seed Testing Association.

Kamoshita A., Babu R.C., Boopathi N.M. and Fukai S. 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. Field Crops Res., **109**: 1–23. doi: 10.1016/j.fcr.2008.06.010

Kretzschmar T., Pelayo M.A.F., Trijatmiko K.R., Gabunada L.F.M., Alam R., Jimenez R., Mendioro M.S., Slamet-Loedin I.H., Sreenivasulu N., Bailey-Serres J. and Ismail A.M. 2015. A trehalose-6-phosphate phosphatase enhances anaerobic germination tolerance in rice. Nat. Plants, **1**: 1-5. doi: 10.1038/nplants.2015.124

Kumar A., Dixit S., Ram T., Yadaw R.B., Mishra K.K. and Mandal N.P. 2014. Breeding high-yielding drought-tolerant rice: genetic variations and conventional and molecular approaches. J. Exp. Bot., **65:** 6265-6278.

Lesk C., Rowhani P. and Ramankutty N. 2016. Influence of extreme weather disasters on global crop production. Nature, **529**. 84-87.

Lozano Isla F., Benites A.O.E. and Pompelli M.F. 2019. GerminaR: An R package for germination analysis with the interactive web application "GerminaQuant for R" Ecol. Res., **34**: 339–346. doi: 10.1111/1440-1703.1275

Mach K.J., Kraan C.M., Adger W.N., Buhaug H., Burke M., Fearon J.D., Field C.B., Hendrix C.S., Maystadt J.F., O'Loughlin J. and Roessler P. 2019. Climate as a risk factor for armed conflict. Nature, **571:**193-197.

Magneschi L. and Perata P. 2009. Rice germination and seedling growth in the absence of oxygen. Ann. Bot., **107**: 49–63. doi: 10.1093/aob/mcq212

Miro B. and Ismail A.M. 2013. Tolerance of anaerobic conditions caused by flooding during germination and early growth in rice (*Oryza sativa* L.). Front. Plant Sci., **4**: 1–18. doi: 10.3389/fpls.2013.00269

Miro B., Longkumer T., Entila F.D., Kohli A. and Ismail A.M. 2017. Rice seed germination underwater: morpho-physiological responses and the bases of differential expression of alcoholic fermentation enzymes. Front. Plant Sci., **8**: 1857. doi: 10.3389/fpls.2017.01857

Mukherjee M., Padhy B., Srinivasan B., Mahadani P., Baksh S.Y., Donde R., Singh O.N., Behera L., Swain P. and Dash S.K. 2018. Revealing genetic relationship and prospecting of novel donors among upland rice genotypes using qDTY-linked SSR

markers. Rice Sci., **25**: 308–319. doi: 10.1016/j.rsci.2018.10.001

Narsai R., Edwards J.M., Roberts T.H., Whelan J., Joss G.H. and Atwell B.J. 2015. Mechanisms of growth and patterns of gene expression in oxygen-deprived rice coleoptiles. Plant J., **82**: 25–40. doi: 10.1111/tpj.12786

Neeraja C. N., Maghirang-Rodriguze R., Pamplona A., Heur S., Collard B.C.Y., Septiningsig E. M., Vergara G., Sanchez D., Xu K., Ismai A. M. and Mackill D. J. 2007. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. Theor. Appl. Genet., 115: 767–76.

Oe S., Sasayama D., Luo Q., Fukayama H., Hatanaka T. and Azuma T. 2022. Growth responses of seedlings under complete submergence in rice cultivars carrying both the submergence-tolerance gene *Sub1A-1* and the floating genes SNORKELs. Plant Prod. Sci., **25**: 70–77. doi:10.1080/1343943X.2021.1943465

Palanog A.D., Swamy B.M., Shamsudin N.A.A., Dixit S., Hernandez J.E., Boromeo T.H., Cruz P.C.S. and Kumar, A. 2014. Grain yield QTLs with consistent-effect under reproductive-stage drought stress in rice. Field Crops Res., **161**:46-54.

Pucciariello C. 2020. Molecular mechanisms supporting rice germination and coleoptile elongation under low oxygen. Plants, **9**: 1–11. doi: 10.3390/plants9081037

Ray S., Vijayan J. and Sarkar R.K. 2016. Germination stage oxygen deficiency (GSOD): an emerging stress in the era of changing trends in climate and rice cultivation practice. Front. Plant Sci., **7**: 671.

Redona E.D. and Mackill D.J. 1996. Genetic variation for seedling vigor traits in rice. Crop Sci., **36**: 285-290.

Roy S., Banerjee A., Kumar J., Verma B. C., and Mandal N. P. 2021. Genetic Diversity of Gora Rice (*Oryza sativa* L.). Landraces of Chotanagpur Plateau Region in Eastern India. Indian J. Plant Genet. Resour., **34**: 196–205. doi: 10.5958/0976-1926.2021.00018.8

Roy S., Verma B.C., Banerjee A., Kumar J., Ray U.S. and Mandal, N.P. 2021. Genetic diversity for drought and low-phosphorus tolerance in rice (*Oryza sativa* L.) varieties and donors adapted to rainfed drought-prone ecologies. Sci. Rep., **11**: 1–9. doi: 10.1038/s41598-021-93325-2

Sandhu N. and Kumar A. 2017. Bridging the rice yield gaps under drought: QTLs, genes, and their use in breeding programs. Agronomy, **7**:27. doi: 10.3390/agronomy7020027

Sandhu N., Dixit S., Swamy B.P.M., Raman A., Kumar S., Singh S.P., Yadaw R.B., Singh O.N., Reddy J.N., Anandan A. and Yadav S. 2019. Marker assisted breeding to develop multiple stress tolerant varieties for flood and drought prone areas. Rice, **12**: 8. doi: 10.1186/s12284-019-0269-y

Sandhu N., Singh A., Dixit S., Sta Cruz M.T., Maturan P.C., Jain R.K. and Kumar A. 2014. Identification and mapping of stable QTL with main and epistasis effect on rice grain yield under upland drought stress. BMC Genet., **15**: 1-15.

Sarkar R.K., Panda D., Reddy J.N., Patnaik S.S.C., Mackill D.J. and Ismail A.M. 2009. Performance of submergence tolerant rice (*Oryza sativa* L.) genotypes carrying the Sub1 quantitative trait locus under stressed and non-stressed natural field conditions. Ind. J. agric. Sci., **79**: 876–883.

Shrestha R., Al-Shugeairy Z., Al-Ogaidi F., Munasinghe M., Radermacher M., Vandenhirtz J. and Price A.H. 2014. Comparing simple root phenotyping methods on a core set of rice genotypes. Plant Biol., **16**: 632-642.

Singh N., Dang T., Vergara G.V., Pandey D.M., Sanchez D., Neeraja C.N., Septiningsih E.M., Mendioro M., Tecson-Mendoza E.M., Ismail A.M. and Mackill D.J. 2010. Molecular marker survey and expression analyses of the rice submergence-tolerance gene *Sub1A*. Theor. Appl. Genet., **121**: 1441-1453.

Turner N.C. 1981. Techniques and experimental approaches for the measurement of plant water status. Plant Soil, **58**: 339–366.

Venuprasad R., Bool M.E., Quiatchon L., Sta Cruz M.T., Amante M. and Atlin G.N. 2012. A large-effect QTL for rice grain yield under upland drought stress on chromosome 1. Mol. Breed., **30**: 535-547. doi: 10.1007/s11032-011-9642-2

Venuprasad R., Dalid C.O., Del Valle M., Zhao D., Espiritu M., Sta Cruz M.T., Amante M., Kumar A. and Atlin G.N. 2009. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. Theor. Appl. Genet., **120**: 177-190.

Verulkar S.B., Mandal N.P., Dwivedi J.L., Singh B.N., Sinha P.K., Mahato R.N., Dongre P., Singh O.N., Bose L.K., Swain P. and Robin S. 2010. Breeding resilient and productive genotypes adapted to drought-prone rainfed ecosystem of India. Field Crops Res., **117**:197-208. doi: 10.1016/j.fcr.2010.03.005

Vijayan J., Senapati S., Ray S., Chakraborty K., Molla K.A., Basak N., Pradhan B., Yeasmin L., Chattopadhyay K. and Sarkar R.K. 2018. Transcriptomic and physiological studies identify cues for germination stage oxygen deficiency tolerance in rice. Environ. Exp. Bot., **147**: 234–248. doi: 10.1016/j.envexpbot.2017.12.013

Vikram P., Swamy B.P., Dixit S., Ahmed H.U., Teresa Sta Cruz M., Singh A.K. and Kumar A. 2011. qDTY1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. BMC Genet., **12**:1-15.

Widawsky D.A. and O'toole J.C. 1996. Prioritizing the rice research agenda for eastern India. Rice research in asia: progress and priorities, CAB International, Wallingford, Oxon, UK,109–130.

Xu K. and Mackill D.J. 1996. A major locus for submergence tolerance mapped on rice chromosome 9. Mol. Breed., **2**: 219-224.

Xu K., Xu X., Fukao T., Canlas P., Maghirang-Rodriguez R., Heuer S., Ismail A.M., Bailey-Serres J., Ronald P.C. and Mackill D.J. 2006. *Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature, **442**: 705-708.

Vergara G.V., Nugraha Y., Esguerra M.Q., Mackill D.J. and Ismail A.M. 2014. Variation in tolerance of rice to long-term stagnant flooding that submerges most of the shoot will aid in breeding tolerant cultivars. AoB Plants**, 6**: plu055.

Supplementary Table S1. Drought and submergence QTL linked markers used in the study

QTL/gene	Chromosome	Linked marker(s)	Donor/Positive check	Reference
DTY QTLs				
$qDTY_{1.1}$	1	RM11943, RM431, RM12091	N22	(Vikram et al. 2011)
$qDTY_{1.2}$	1	RM259, RM315	Kali aus	(Sandhu et al. 2014)
$qDTY_{2.1}$	2	RM327, RM262, RM324	Apo	(Ramaiah Venuprasad et al. 2009)
$qDTY_{2.2}$	2	RM263, RM324, RM555, RM279,	Kali aus	(Sandhu et al. 2014)
$qDTY_{2.3}$	2	RM573, RM3212, RM250	Kali aus	(Palanog et al. 2014; Sandhu et al. 2014)
$qDTY_{3.1}$	3	RM520, RM416	Apo	(Ramaiah Venuprasad et al. 2009)
$qDTY_{3.2}$	3	RM60, RM22, RM545	N22	(Vikram et al. 2011)
$qDTY_{6.1}$	6	RM204, RM589	Vandana	(R. Venuprasad et al. 2012)
$qDTY_{12.1}$	12	RM28048, RM28199, RM28166	Way rarem	(Bernier et al. 2007)
Sub1 locus				
Sub1A	9	AEX1	FR13A	Septiningsih et al. 2009
Sub1A		SC3	FR13A	Neeraja et al. 2007
SUB1B + *SUB1C*	9	Sub1BC2	FR13A	Septiningsih et al. 2009

Supplementary Table S2. Highest ranked rice cultivars for grain yield under drought

S. No.	Accession no.	DF	Ht	LDS	SpkStr	RWC	Yld
1	Dular	58	113.77	2.33	30.13	82.67	320.15
2	N22	56.67	109	3.67	46.17	72.37	300.22
3	Chakra gora (IC-0640867)	66	112.87	3	50.17	73.87	282.12
4	Brown gora (IC-0640869)	71	124.07	3	52.72	76.13	259.67
5	Gora (IC-0640897)	55	123.23	5	39.87	78.33	241.01
6	White gora (IC-0640859)	54.33	111.43	4.33	25.92	78.07	229.17
7	Dani gora (IC-0640877)	67.33	123.43	3.67	49.41	75.53	228.58
8	Gora (IC-0640871)	67.67	121.87	4.33	40.58	79.6	228.01
9	Kalakeri (IC-0640883)	67.67	122.47	5	23.53	78.3	206.88
10	Alsangagora (IC-0640866)	64.33	100.03	3	43.88	73.77	206.08
11	Vandana	72	117	3.67	43.11	80.73	203.86
12	Gora (IC-0640864)	68	124.43	3.67	46.67	75.73	197.39
13	Black gora (IC-0640880)	71.33	114.9	3.67	43.18	72.93	196.23
14	Dani gora (IC-0640872)	70.67	127.53	3	32.65	79.07	189.02
15	White gora (IC-0640889)	60	120.43	4.33	30.33	75.83	179.39
	CD (5%)	1.1	4.6	1.4	11.1	3.1	19.6

DF = Days to 50% flowering; Ht = Plant height (cm); LDS = Leaf drying score; SpkStr = Spikelet sterility (%); RWC = Relative water content (%); Yld = Grain yield plot-1

Supplementary Fig. S1. Screening of rice germplasm for abiotic stress tolerance. (A) and (B) drought screening under rainout shelters at CRURRS, Hazaribag; (C) and (D) screening for submergence tolerance at NRRI, Cuttack