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Marker assisted breeding of *Sub1* introgressed rice (*Oryza sativa* L.) lines and identification of stable variety MTU 1232 suitable for flood prone ecosystem

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Abstract

The Development of flood-tolerant rice varieties is a prerequisite for climate resilience in flood-prone areas. The present study aimed to develop a stable, high, yielding, and tolerant rice variety against flash floods and stagnant flooding across multiple environments. *Sub1A* was incorporated into a popular rice variety MTU 1075 using Swarna-sub1 as a donor to generate BC_3F_5 families. Sub1BC2 was used as a foreground marker for selection, a proxy for the *Sub1A* gene. RM23865 and RM464 on Chromosome 9 were used as recombinant markers. Backcross families from the BC_3F_2 generation were evaluated under two weeks of flash floods 15 days after transplanting. This was followed by stagnant flooding and survived BC_3F_4 families were used for background selection using a 50K high-density SNP chip. The nine best families identified were included in the field trial evaluation under eight environments. Consequently, MTU Rice 1232 was identified as a high-yielding, flood-tolerant rice variety using Additive Main effects and Multiplicative Interaction stability analysis. The MTU Rice 1232 ranked first by the Best linear unbiased prediction (BLUP) based ranking parameters and 4th based on stability parameters ranking. This flood-tolerant rice variety can tolerate both flash floods and stagnant flooding and possesses an 80% survival rate. It has a yield potential of 3792 Kg ha^{-1} under severe floods and 6000 Kg ha^{-1} under normal conditions.

Keywords

Sub1A ; AMMI stability; flash floods; stagnant floods; rice

Introduction

Rice is widely grown as a staple food crop and is facing extreme events of climate change. Flood-prone ecology in India occupies 15 million ha, resulting in drastic yield loss (1). Submergence of crops at different growth stages is limiting rice productivity. Rice crop experiences both flash floods and stagnant flooding (30-50 cm water depth) under low-lying coastal areas with ill-drained conditions. Most often, cyclonic rains at the maturity stage result in lodging and submergence of crops, leading to huge economic losses in flood-prone areas (2). Flash flood tolerance conferring *Sub1A* (3) was widely incorporated into popular rice varieties for climate resilience with minimal linkage drag using marker-assisted breeding (4-8). Most of the *Sub1A* introgressed lines tolerate flash floods by quiescence strategy but are

vulnerable to stagnant flooding due to semi-dwarf maternal parents that have minimal shoot elongation (9-10). *Sub1A* introgressed lines Swarna-sub1, Samba-sub1, CR1009-sub1 and IR64-sub1 tolerate flash floods, but these varieties were not preferred by farmers under stagnant flooding (11). The availability of rice varieties that can tolerate both flash floods and stagnant flooding that possess lodging resistance is limited. The study of genotype by environment (GXE) interactions is essential to identify the best-performing stable genotype across environments. Identification of stable genotypes in varied flood-prone situations is also a challenging task, as it is influenced by the type of flood, period of submergence, and the stage of the crop.

The presence of G X E Interaction complicates the selection of widely adapted superior genotypes for yield under varied flood situations for making cultivar recommendations in flood-prone areas. Selection of stable genotypes across environments and genotypes suitable for specific environments for genetic gains (12) are possible with some statistical methods such as Additive Main effects and Multiplicative Interaction (AMMI) model (13) and genotype + genotype by environment interaction (GGE) model (14). AMMI model combines ANOVA of the genotype and environment with PCA of GXE interaction. The effectiveness of the AMMI procedure has been clearly demonstrated for stress environments of rice crops (15-17). A multi-location and multi-year evaluation is essential for identifying high-yielding stable genotypes with consistently specific and wider adaptation to agro-climatic zones. Yield evaluation of Near Isogenic Lines (NIL) for submergence tolerance, lodging resistance, and BLB tolerance developed between Swarna (18) and Sub1 incorporated Drought-Tolerant Japonica Rice DT3 (19) helped to identify necessary NIL. Performance of NILs of Sub1 was evaluated under multi-location trials of flood environments (20-21). The present study aimed to develop a stable, high-yielding rice variety that is tolerant against flash floods and stagnant flooding across multiple environments. The variety MTU Rice 1232 was developed by marker-assisted backcrossing breeding in the genetic background of MTU1075 for flash floods and Sub1 introgressed lines at advanced generations, intensively tested for both flash floods and stagnant flooding. The best NILs of Sub1 were evaluated for yield in multi-location trials of varied flood situations to identify a stable high-yielding flood-tolerant rice variety MTU Rice 1232.

Materials and Methods

The popular high-yielding rice variety (MTU1075) was developed by the Regional Agricultural Research Station (RARS), Maruteru of Acharya NG Ranga Agricultural University (ANGRAU) of Andhra Pradesh, India, by pedigree method using MTU 2716 as female and MTU 1010 as male parent. This MTU 1075 was released in 2008 for the states of Andhra Pradesh, Tamil Nadu, Gujarat, and Maharashtra of India. The MTU1075 rice variety is non-lodging with a plant height of 110 cm and is susceptible to floods. It was used as a recurrent parent, and Swarna-sub1 was used as a donor parent for the incorporation of *Sub1A* by adopting marker-assisted backcrossing breeding. Generation advancement was made with three successive back-crosses from 2010 to 2012 (up to BC_3F_1), followed by selfing to generate BC_3F_2 families. Phenotypic evaluation for submergence tolerance was adopted from BC_3F_2 to BC_3F_5 generations.

Methodology for Phenotyping of Submergence Tolerance Progenies

Thirty-day-old seedlings were transplanted at 20 × 15 cm spacing in submergence ponds. Flash floods (FF) were artificially imposed at 15 days after transplanting (DAT) for 15 days. Then, stagnant flooding (SF) of 30-50cm water depth was maintained for one month from 10 days after de-submergence of flash floods. Plant survival % was recorded at 60 DAT. BC_3F_2 to BC_3F_5 families were screened using this procedure. Families exhibiting more than 75% plant survival were genotyped with *Sub1A* gene-specific markers. *Sub1A* plants possessing maximum resemblance to the recurrent parent were selected phenotypically in each generation. Phenotyping for seedling stage flood tolerance was carried out by sowing thirty pre-germinated seeds in plastic cups, which were submerged in a tank 14 days after sowing for 14 days. The survival rate was recorded on the 10^{th} day after de-submergence.

Yield evaluation of NILs of Sub1A

Yield evaluation trials were conducted with 14 entries in 2 replications. The entries are the nine best near-isogenic lines of *Sub1A* along with recurrent parent MTU1075, donor Swarna-sub1, and two other *Sub1A* incorporated lines along with recurrent parent MTU1064. These entries were transplanted at 25 days after sowing by adopting spacing of 20 X 15 cm and fertilizers 90:60:60 NPK kg/ha in eight environments, i.e. artificial submergence pond for flash floods for two weeks at 15 DAT (days after transplanting) followed by stagnant flooding for one month during wet season of 2015 (E1), dry season 2015 (E2), wet season 2016 (E6), normal irrigated condition during dry season 2015 (E3) and wet season 2016 (E5) at RARS , Maruteru; flood prone farmer's field under flash floods (for 10 days at 10 days after transplanting (DAT), for 7 days at 20 DAT, for 5days at 35 DAT) + stagnant flooding (30-50 cm) at 50 DAT for one month at Ramanapalem of West Godavari district during wet season of 2015 (E4) and same field during wet season of 2016 expressed stagnant flooding at 40 DAT for one month (E8), stagnant flooding of 30-50 cm at Ethakota village farmer field of East Godavari district of Andhra Pradesh at tillering stage for 15 days during wet season of 2016 (E7). The stability performance of these *Sub1A* lines was assessed by adopting the AMMI method using PB tools. The best-performing MTU Rice1232 was tested in an adaptive minikit testing along with recurrent parent MTU1075 under flood-prone areas and in normal environments during 2018, 2019, and 2020. At each stage of trialing, the presence of *Sub1A* was confirmed using the *Sub1A* specific-marker Sub1BC2.

Marker Assisted Back Crossing Breeding

Sub1BC2 was used as the foreground marker for the selection of the *Sub1A* gene in every generation. Two SSR markers, RM23865 (position 6.3 Mb) and RM464 (position 6.5 Mb) on chromosome 9, were used for recombinant selection. Plant genomic DNA was extracted from the leaves of 30-day seedlings using a Tissue lyzer (Qiazen) as per the protocol (22). The quality and quantity of DNA were measured using an eight-channel spectrophotometer (Thermo Scientific). Polymerase Chain Reaction (PCR) was set up for a 10 uL mixture comprising of 10X Taq buffer A 1uL, forward and reverse primer each 1uL (Sigma Aldirch), 2.5mm dntp 0.5uL (Genei), one unit of Taq DNA polymerase 1uL (Genei), 25ng of genomic DNA 3uL and sterile distilled water 2.5uL for amplification. PCR was programmed for initial denaturation at 94 ^{0}C for 5 min, followed by 35 cycles of denaturing at 94 ^{0}C for 30 seconds, annealing at 55 ^{0}C for 0.5 min, extension at 72 ^{0}C for1.0 min, and ending up with 7 min at 72 ^{0}C for the final extension using Pro S master cycler (Eppendorf). Electrophoresis was carried out on 3% high-resolution metaphor agarose (Lonza) gels and amplified products were captured by the Syngene gel documentation system.

The background selection of 18 advanced lines at BC_3F_4 was carried out using a 50K chip at National Institute on Plant Biotechnology (NIPB), New Delhi, as per the procedure of the Affymetrix SNP 6.0 protocol (5).

Results

Development of NILs of *Sub1A* in the genetic background of MTU 1075: Marker-assisted backcross breeding resulted in the incorporation of *Sub1A* in the background of MTU1075, and details of the selection procedure adopted are represented in Table 1. The incorporation of Sub1 A for flash flood tolerance using foreground and recombinant markers resulted in the selection of 52 positive plants at BC_1F_1, 51 plants at BC_2F_1, and 52 plants at BC_3F_1. At BC_3F_1 generation, the best 24 plants out of 52 *Sub1A* confirmed plants that have a phenotypic resemblance of recurrent parent MTU 1075 were selfed to study as BC_3F_2 families for phenotyping under flash floods and stagnant flooding in artificial submergence pond. Genotyping of 2668 plants at BC_3F_2 generation resulted in the selection of 34 best plants confirming *Sub1A* positive for foreground and recombinant markers. These plants were studied as 34 BC_3F_3 families with 2550 single plants under flash floods and stagnant flooding during the wet season of 2013. The *Sub1A* confirmed that 57 plants were advanced to BC_3F_4 generation by foreground, recombinant, and phenotypic selection. Gel images of *Sub1A* positive families and recombinant selection were furnished in Fig. 1.

The developed 18 *Sub1A* lines were selected for background selection using 50K SNP genotyping and for yield (Table 2.) and flood tolerance (Fig. 2.). There was a wide variation of 0 to 65% of plant survival at the seedling stage (28 DAS), and 9.43 to 80 % plant survival was observed at 60DAT under FF+SF among 18 NILs. Only NIL 4 expressed 80 plant survival % under FF+SF and 43.33 plant survival % at the seedling stage with a grain yield of 52 g plant^{-1} with the highest SNP genome recovery of 91.88 % of the recurrent parent. This is followed by NIL2 with Plant survival of 73.17 % FF+SF and 65 % at the seedling stage under FF.

Nine best *Sub1A* Near Isogenic Lines (NILs) were identified based on foreground, recombinant, and background selection, plant survival %, grain yield per plant, and genome recovery % of recurrent parent (> 90%) for further yield evaluation under the targeted environment.

Stability analysis of yield evaluation trials under floods and normal conditions

Analysis of variation for eight individual trials (Table 3.) indicated there is significant variation among the genotypes under varied flood and normal environments at the probability of 0.05. The heritability of the experiment above 0.5 indicates trial selection is accurate, and genotype response to the environment can be estimated by pooled ANOVA and GE interactions. Maximum mean grain yield of 4564 Kg ha^{-1} (E5) was observed in normal conditions, followed by E3 (3843 Kg ha^{-1}).

Combined ANOVA and AMMI analysis was performed with yield data of 14 test genotypes under eight environments (6 under floods and two under normal situations; Table 4.). The results revealed that the environments, genotypes, and GEI showed significant variation in grain yield. GEI contributed a large portion of the total variation (39.64 %), followed by environment (30.34 %)

Table 1. Details of the selection of *Sub1A* positive plants in different generations in the background of MTU 1075.

Season and year	Generation	No of plants genotyped	No. of plants selected	Type of selection
Wet season 2011	BC_1F_1	196	52	
Dry season 2011	BC_2F_1	458	51	Foreground and recombinant selection
Wet season 2012	BC_3F_1	169	52	
Dry season 2012	BC_3F_2	2688	34	Foreground and recombinant, phenotypic selection for FF+ SF
Wet season 2013	BC_3F_3	2550	57	
Dry season 2013	BC_3F_4	57	18	Foreground, recombinant, Background selection with 50K SNP chip, Phenotypic selection for FF+SF
Wet season 2014	BC_3F_5	18	18	
Dry season 2014	BC_3F_6	18	9	Panicle rows for seed increase, *Sub1A* gene confirmation, selection of nine best lines for yield trials

FF: Flash floods, **SF**: Stagnant flooding

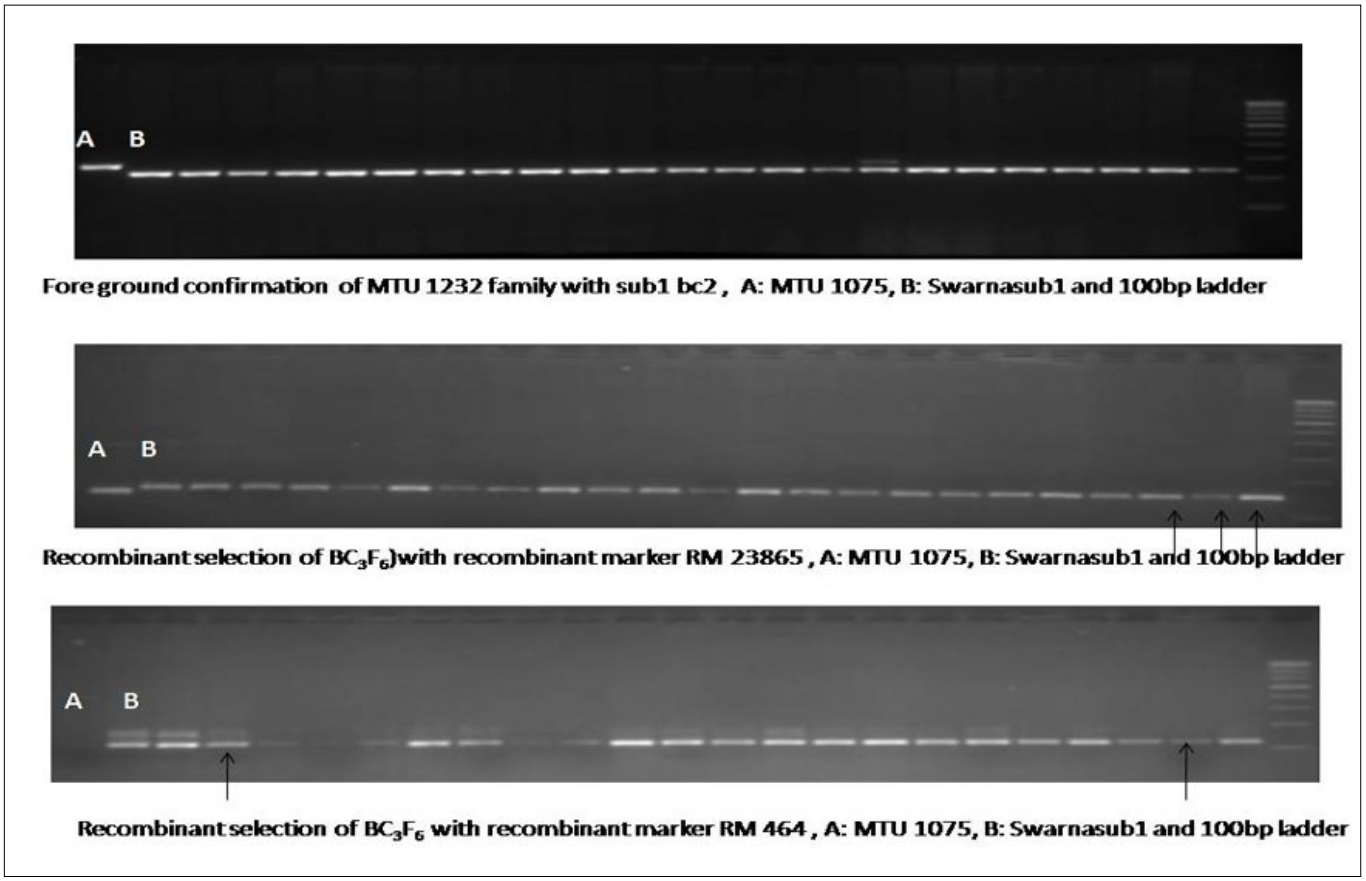

Fig. 1. Foreground and recombinant selection gel images of *Sub1 A* introgressed line of MTU 1075.

Table 2. Background recovery and grain yield of 18 NILs of Sub1A.

Sub1A NILs	Days to 50% flowering	Grain yield/plant (g)	Plant Survival% in plastic cups under FF(28DAS)	Plant survival % FF+SF at 60DAT	recovery% of Recurrent parent
NIL1	119	49	8.00	9.43	90.00
NIL2	123	52	65.00	73.17	91.50
NIL3	119	50	26.67	66.00	91.50
NIL4	119	52	41.67	80.00	91.88
NIL5	121	31	16.00	32.80	90.50
NIL6	121	68	0.00	60.00	91.50
NIL7	125	32	0.00	16.00	91.20
NIL8	117	28	13.04	52.00	91.50
NIL9	120	50	0.00	38.67	91.00
NIL10	119	90	36.67	38.67	91.00
NIL11	119	45	20.00	13.51	90.00
NIL12	125	20	53.33	45.33	91.00
NIL13	122	82	43.33	36.00	90.00
NIL14	120	45	16.67	2.67	90.90
NIL15	119	41	60.00	21.88	91.00
NIL16	119	53	24.00	72.97	91.00
NIL17	117	21	20.00	26.47	90.50
NIL18	119	21	24.00	60.00	91.00
MTU1075 (RP)	125	51	0.00	15.00	
Swarna-sub1	119	48	36.00	29.00	

FF: Flash floods, **SF**: Stagnant flooding **RP**: Recurrent parent

and genotypes (17.92 %). The first five PCs significantly explained 95.3 % variation. GEI was distributed among 7 PCs, and PC1 contributed 51.6 % for variation.

Six genotypes (G1, G4, G5, G6, G7, and G8) in the first and second quadrants of the AMMI biplot (Fig. 3a.) expressed above-average yield across the environments.

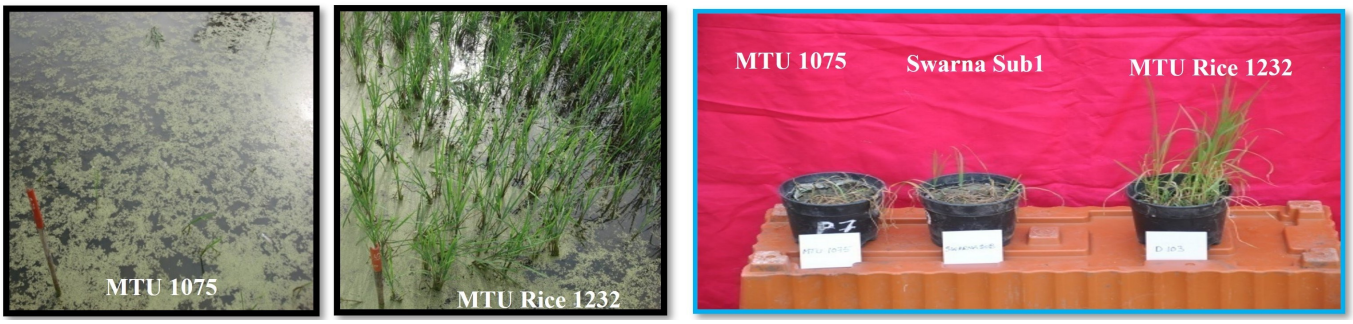

Fig. 2. Plant survival of MTU 1075 recurrent parent vs *Sub1* introgressed line in the field at 60DAT after Flash flood+ Stagnnant flooding and Seedling stage tolerance at 14 days after sowing for 14 days in plastic cups.

Table 3. ANOVA for grain yield (Kg ha^{-1}) for eight multiple environments .

Environment	Mean Grain yield (Kg ha^{-1})	Particulars	DF	MSS	F value	P value	CV (%)	h^2	AS
		Genotype	13	995933.43*	5.80	0.002	10.33	0.83	0.91
E1	4011	Replications	1	138462.89	0.81	0.39			
		Error	13	171624.20					
		Genotype	13	517397.59*	8.23	0.000	10.19	0.88	0.94
E2	2461	Replications	1	2681.29	0.04	0.84			
		Error	13	62831.67					
		Genotype	13	1152546.34*	37.15	0.000	4.58	0.97	0.99
E3	3843	Replications	1	12728.89	0.41	0.53			
		Error	13	31019.97					
		Genotype	13	2159209.60	3.06	0.027	24.20	0.67	0.82
E4	3474	Replications	1	10716131.57	15.16	0.00			
		Error	13	706762.49					
		Genotype	13	287854.49	2.31	0.072	7.74	0.57	0.75
E5	4564	Replications	1	311647.00	2.50	0.14			
		Error	13	124697.62					
		Genotype	13	2012656.50*	39.69	0.000	6.39	0.97	0.99
E6	3525	Replications	1	4862.89	0.10	0.76			
		Error	13	50712.43					
		Genotype	13	1462442.11*	23.62	0.000	8.01	0.96	0.98
E7	3105	Replications	1	130562.29	2.11	0.17			
		Error	13	61916.05					
		Genotype	13	2531285.36*	10.24	0.000	13.62	0.90	0.95
E8	3651	Replications	1	79289.29	0.32	0.58			
		Error	13	247287.13					

DF: Degrees of freedom, **MSS**: Mean sum of squares, **CV**:Coefficient of Variation, **h^2** : Heritability, **AS**: Accuracy of selection

Among them, genotype G4 (MTU 1232) recorded higher mean grain yield of 4593 Kg ha^{-1} followed by G1 (MTU 1231: 3980 Kg ha^{-1}), G7 (3979 Kg ha^{-1}) and G5 (3876 Kg ha^{-1}). Five genotypes, G4, G5, G7, G12, and G13, near to abscissa in the AMMI biplot, were found stable across environments. G4, G5, and G7 contain *Sub1A* and were found stable with a higher yield than recurrent parent MTU 1075 and G12 (3449 Kg ha^{-1}), G13 (3431 Kg ha^{-1}) NILs of MTU 1064 were found to be better than respective recurrent parent. Genotype G1 can be identified for Environment 1, G11, G14 for Environment 7, G10 for Environment 2, G3 for Environment 6, and G2 for Environment 4. Environments E4, E8, E6, E7, and E2 were the farthest from biplot origin showed strong interaction, and E1, E3, E5 are nearest to origin with short spokes and showed weak interaction forces (Fig. 3b.). Flood environments (E4, E8 and E6) exhibited high G X E followed by E7, E1 and least G X E under normal environments (E3, E5). Genotypes G13 and G4 (MTU 1232) were found near PCA origin and can be suitable across environments.

Stable genotype MTU 1232 expressed a higher yield and was found to be an ideal genotype with an inner concentric circle (Fig. 3c.). Next ideal genotypes were G1, G7, and G5, with good yield under floods and normal situations. G8, G6, G12, G13, G2, and G10 have positive scores suitable for favorable environments, and G9, G11, and G14 with negative scores for unfavorable environments (Fig. 3d.). Genotypes G4 (MTU 1232) is found to be stable with high yield and is suitable for all environments except E4 (Fig. 3e.). Genotypes G1, G7, and G5 are found to be best

Table 4. Analysis of variance of yield of *Sub1A*introgressed lines under floods and normal conditions across environments using AMMI.

Source	Degrees of freedom	Mean sum of squares	F value	Proportion %	Accumulated %
Environment	7	10883939*	7.64	30.34	
Replication (Environment)	8	1424546*	7.82	4.53	
Genotype	13	3461697*	19.00	17.92	
Genotype X Environment	91	1093947*	6.00	39.64	
PC1	19	2702022*	14.84	51.60	51.6
PC2	17	895985.7*	4.92	15.30	66.9
PC3	15	939340.4*	5.16	14.20	81.0
PC4	13	624280.1*	3.43	8.20	89.2
PC5	11	554161.7*	3.04	6.10	95.3
PC6	9	372540.6	2.05	3.40	98.7
PC7	7	189229.5	1.04	1.30	100.0
Residuals	104	182106.4			
Total	314	1116635			

performing in normal environments, E3, E5 and flood environments E1 and E2. The details of the stability ranking are presented in Table 5. Genotype G4 (MTU 1232) ranked first as per BLUP (Best linear Unbiased prediction) based HMGV (harmonic mean of genotypic values), RPGV (relative performance of genotypic values), HMPGV (harmonic mean of relative performance of genotypic values) ranking and 4th based on stability parameters ranking of ATAB (AMMI-based stability parameter), ASI (AMMI stability index), ASV (AMMI stability value).

The stable genotype G4 (MTU 1232) is tolerant for 2-week flash floods and stagnant flooding for one month and recorded 11.27 % higher grain yield than the recurrent parent MTU 1075 at the national level in All India Coordinated Rice Improvement Programme (AICRIP) submergence trials across the seven locations. MTU 1232 outyielded MTU 1075 with a 17.10 % yield advantage under

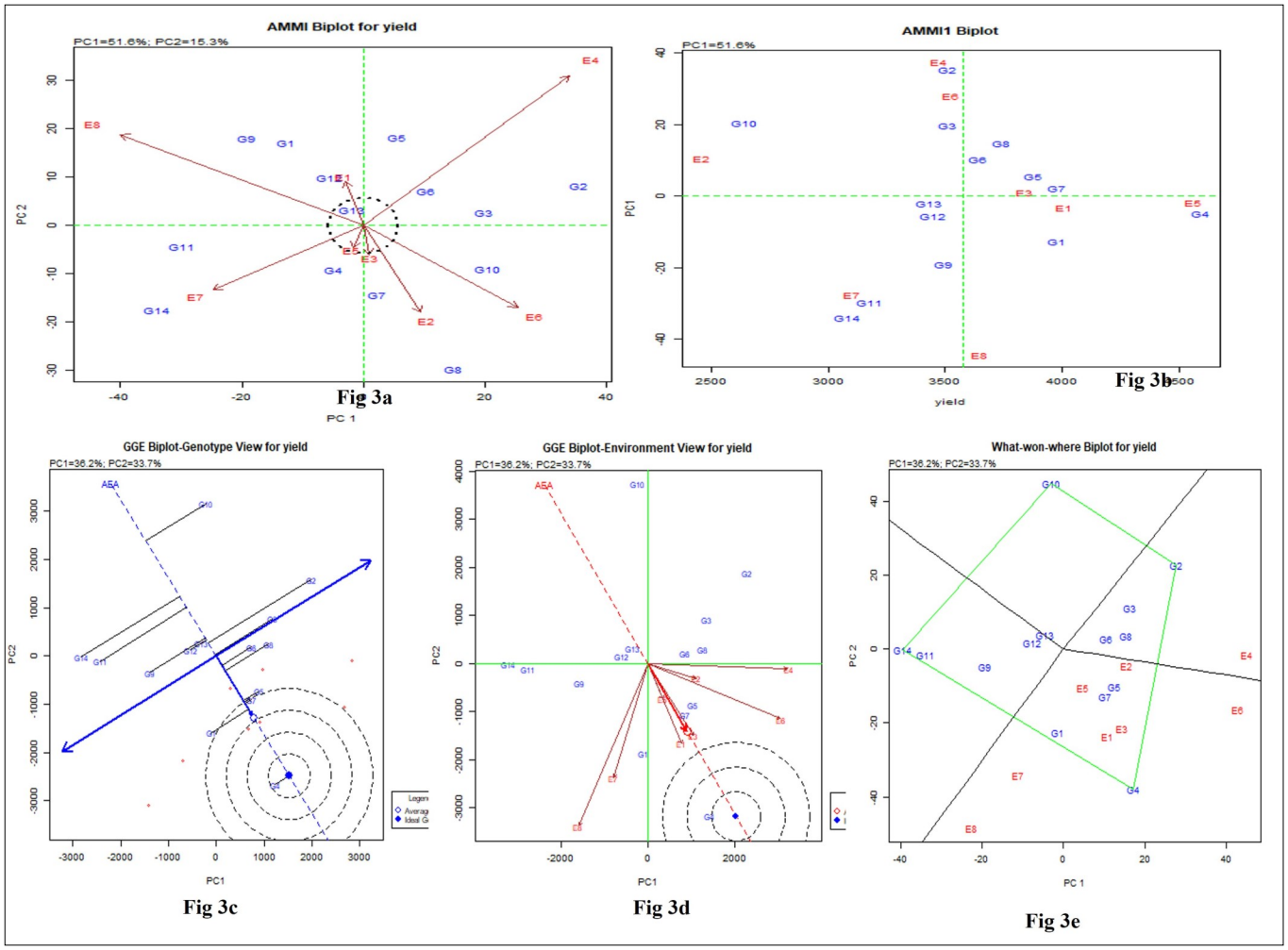

Fig. 3. **3a**. AMMI stability biplot for yield and PC1, **3b** for PC1 and PC2, **3c**. GGE biplot genotypic view for yield and **3d**. environmental view and **3e** what won biplot for 8 environments of NILs of *Sub1A*.

Table 5. Stability-based ranking of genotypes across environments.

Genotype	Designation	Grain yield Kg ha^{-1}	PC1	Stability parameter ranking			BLUP based ranking		
				ASTAB	MASI	MASV	HMGV	RPGV	HMRPGV
G1	MTU 1231	3980	-12.74	8	7	8	3	3	3
G2	MTU2336-70-46-25-37	3510	35.33	5	11	10	8	7	9
G3	MTU2336-70-46-25-42	3513	19.76	11	12	12	9	8	7
G4	**MTU 1232**	4593	-4.84	4	3	4	1	1	1
G5	MTU2336-62-25-38-21	3877	5.44	10	5	7	4	4	4
G6	MTU2336-70-46-25-39	3639	10.28	12	13	13	6	6	6
G7	MTU2336-70-46-28-36	3980	2.23	14	14	14	2	2	2
G8	MTU 2336-70-46-25-48	3742	14.87	6	9	9	5	5	5
G9	MTU 1075	3493	-19.20	2	2	2	11	9	11
G10	Swarna-sub1	2641	20.45	7	4	5	14	14	14
G11	MTU2336-65-83-39-8	3179	-29.89	1	6	3	12	12	12
G12	MTU 2244-119-59-63-40	3449	-5.63	3	1	1	7	10	8
G13	MTU 2244-47-15-6-77	3431	-1.96	9	8	6	10	11	10
G14	MTU 1064	3081	-34.09	13	10	11	13	13	13

HMGV (harmonic mean of genotypic values), **RPGV** (relative performance of genotypic values), **HMPGV**(harmonic mean of relative performance of genotypic values), **ATAB** (AMMI-based stability parameter), **ASI** (AMMI stability index), **ASV** (AMMI stability value).

floods in 118 locations in adaptive minikit testing at farmer's fields of flood-prone areas of Andhra Pradesh (Table 6.).

generation advanced back cross families was observed. A similar trend of variation among the *Sub1A* fixed lines in the background of Ranjit and Bahadur under flash floods for 12 days was found by earlier workers (23).

Table 6. Mean performance of MTU 1232 Vs recurrent parent MTU 1075 in AICRIP and adtive minikit testing under floods.

S.No	Trials	Year of testing	No. of locations	Stress	Grain yield Kg ha^{-1}		% Increase over best check
					MTU 1232	MTU 1075	
1	AICRIP Submergence	2015	7	Flash floods & Stagnant flooding	5132	4612	11.27
2	Adaptive minikits	2018-2020	118	Floods	6153	5255	17.10

The flood-tolerant culture MTU Rice 1232 comes under the medium duration group with 135-140 days duration in the wet season in normal conditions and 140-145 days under submerged conditions suitable for irrigated wetlands in Andhra Pradesh (Table 7.). It is non-lodging, high-yielding, nitrogen-responsive, semi-tall with dark green foliage, one-week seed dormancy, and low grain shattering. MTU Rice 1232 showed head rice recovery of 66.36 % with translucent grains, which is very much desired for marketing.

NIL 4 expressed highest plant survival with the higher grain yield than recurrent parent under FF + SF, followed by NIL1. Expression of *Sub1A* and plant survival under SF is specific to NIL and might be due to constitutive expression of ethylene response genes depending on stress (24). This can be attributed to phenotypic selection being crucial after genotypic confirmation. The best nine NILs were selected for yield evaluation considering background SNP chip recovery of more than 90%. Earlier workers (8) also identified the best NILs of *Sub1A* using background SNP chips by yield

Table 7. Salient features of MTU Rice 1232.

S. No	Item	Details
1	Maturity Group	Medium
2	Duration (Days)	135-140 days (Normal) & 140-145 days (Submergence)
3	Agronomic features	Non lodging, low grain shattering Fertilizer :90:60:60 Kg ha^{-1}
4	Grain Quality	Medium Slender, Straw glume, Head rice recovery 66.32%
5	Grain Yield (Kg ha^{-1})	3792 (floods), 6000 (Normal)
6	Reaction to abiotic stress	Flash floods for 10 days (15DAS- tillering) & Stagnant flooding (30-50cm)
7	Specific Areas of Adaptation	Flood prone ecosystem

Discussion

Wide variation among *Sub1A* positive plants for plant survival under flash floods followed by stagnant flooding from BC_3F_2 evaluation under varied stress environments of submergence and drought. Foreground selection for three backcrosses, background selection at BC_3F_2 generation, and identification of best NILs for agronomic and flood tolerance in the

background of *Japonica* rice DT3 (19). *Sub1A* and drought pyramided lines were evaluated under varied environments for identification of the best NIL with high yield (21).

Stability analysis of NILs for yield revealed that the major contribution of variation is due to GEI followed by environment and genotype. The expression of the yield of NILs of *Sub1A* was highly influenced by GEI due to variations in flood environments. In higher environments, GEI interaction was also reported under water stress conditions among drought-tolerant pyramided lines (15).

Genotype G4 (MTU 1232) was found as a stable genotype across environments with a higher mean grain yield of 4593 Kg ha^{-1} as it is near to abscissa in the AMMI biplot and in inner concentric circles. Stability analysis helped in the identification of suitable genotypes for specific environments. Six genotypes (G8, G6, G12, G13, G2, and G10) with positive scores were found suitable for favorable environments, and three genotypes (G9, G11, G14) with negative scores for unfavorable environments. Higher GEI in flood environments E4, E8, and E6 might be due to variations in the type and duration of floods. Genotype G4 (MTU 1232) is found to be stable with high yield and is suitable for all environments except E4. Ideal and winning genotypes for different stress conditions were identified by earlier workers for salinity (17) and drought (15). Genotype G4 (MTU 1232) ranked as first as per BLUP based on HMGV, RPGV, and HMPGV ranking and 4thbased on stability parameters ranking of ATAB, ASI, and ASV.

Best performing Chierang-Sub1 was identified (20) by evaluating the best NILs of *Sub1A* under varied environments and assessing phenotypic performance for morphological, grain quality and biotic stresses. *Sub1A* tolerant, BLB resistant high, yielding introgressed lines using marker-assisted backcross breeding in the background of Swarna was developed (18). The selection of submergence-tolerant lines by screening in varied environments found that GEI inter-action plays a crucial role in submerged conditions (25).

The stable genotype MTU 1232 was further conferred for a higher yield of 11.27 % than the recurrent parent MTU 1075 at the national level in All India Coordinated Rice Improvement Program (AICRIP) submergence trials and 17.10 % in adaptive minikit testing across 118 locations at farmer's field of flood-prone areas of Andhra Pradesh.

The present study implied that incorporation of *Sub1A* into MTU 1075 with intermediate plant height and subjecting the advanced back cross families from BC_3F_2 generation to both flash floods and stagnant flooding might have helped in the selection of the best flood-tolerant rice variety MTU 1232. Modern cultivars should give stable yields under any type of flood, in flood-prone ecosystems, and also under adverse effects of climate-changed conditions. The constitutive expression of *Sub 1A* under flash floods conserved energy for revival of growth after submergence, and MTU 1232 adopted moderate shoot elongation under stagnant flooding, which might be due to signal cascading of ethylene response genes (24).

Conclusion

Our research on the development and identification of stable high-yielding flood-tolerant rice variety MTU 1232 possessing *Sub1A* that can tolerate both flash floods and stagnant flooding with non-lodging nature could serve as a climate resilient variety. MTU Rice 1232 variety tolerates flash floods for 10-14 days (15 DAS to tillering) and stagnant flooding (30-50cm) for more than one month, both under transplanted and direct seeding methods of rice cultivation.

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Authors' contributions

All authors contributed to the study's conception and design. Study of the work, material preparation, data collection, analysis, and manuscript preparation were performed by M, review and technical guidance of the work by PV, Y, NK , associated in study and manuscript preparation by P, B.N.V.S.R., N. , T. , Y. and assistance in Biotechnology lab for genotyping by K. and T. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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