

RESEARCH ARTICLE



Submergence tolerance in rice: A comprehensive screening of 250 Indian landraces for resilience under water stress

Shrirashmi¹, S Rajeswari^{1*}, A John Joel², R Suresh³, Vellaikumar⁴ & S Manju Devi⁴

¹Department of Genetics & Plant Breeding, CPBG, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India ²Department of Plant Molecular Biology & Bioinformatics, CPMB&B, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India ³Department of Rice, CPBG, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

⁴Department of Plant Biotechnology, CPMB&B, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Email: rajeswari.s@tnau.ac.in

ARTICLE HISTORY

Received: 07 October 2024 Accepted: 21 October 2024 Available online Version 1.0 : 24 December 2024

(**I**) Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is

available at https://horizonepublishing.com/ journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/ index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an openaccess article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/ by/4.0/)

CITE THIS ARTICLE

Shrirashmi, Rajeswari S, Joel AJ, Suresh R, Vellaikumar, Devi SM. Submergence tolerance in rice: A comprehensive screening of 250 Indian landraces for resilience under water stress. Plant Science Today.2024;11 (sp4):01-09. https://doi.org/10.14719/pst.5601

Abstract

As erratic rainfall and flooding pose serious threats to rice yields, submergence tolerance is critical for sustainable cultivation. This study screened 250 Indian rice landraces at the seedling stage to identify genotypes capable of surviving submergence stress. Advanced statistical analyses, including trait association, variability, principal component analysis and cluster analysis, revealed the genetic and phenotypic factors influencing submergence stress tolerance. Significant variability was observed among the genotypes for key traits, such as shoot elongation, leaf regeneration and survival percentage. Diversity analysis through cluster analysis indicated that cluster III, particularly IRGC93, IRGC535 and IRGC706, exhibited survival rates exceeding 85%, highlighting their potential as donor lines for submergence tolerance. Further biochemical analysis of selected lines with survival percentages greater than 50% revealed that tolerance is associated with greater chlorophyll and non-structural carbohydrate retention percentages. The findings emphasize the importance of genetic variability and heritability in breeding resilient rice varieties for flash flooding conditions. This research contributes to advancing rice breeding strategies to produce submergence-tolerant varieties, thereby promoting the sustainability and productivity of paddy production systems.

Keywords

cluster analysis; landrace; MGIDI analysis; screening; submergence stress

Introduction

Rice is a staple food for more than half of the global population. Demand for rice crop continues with the rise in global population evoking efforts to increase production (1,2). The increase in production is further hindered by the challenges posed by climate change such as floods, heat, drought and salinity. The changing climate is leading to unprecedented rains and storms leading to frequent floods and heavy loss in production (3). The relationship between submergence stress and climate change is particularly evident in rice cultivation, as the rice crop is highly susceptible to such stress (4). The 49.82 million hectares or 15% of India's arable land, are vulnerable to flooding. Particularly in the Himalayan river basins of the Indus, Ganges, and Brahmaputra, the erratic summer monsoon frequently results in high runoff, which produces severe flooding in lowlands (5). During the rainy season, rice fields are occasionally submerged by heavy rainfall, overflowing rivers and high tides, which have a negative impact on the crop yield (6).

Submergence stress is due to the complete immersion of rice plants for a period ranging from several days to up to two weeks, which usually occurs at the seedling or early vegetative stage. Flash floods, frequent in lowland and rainfed environments, submerge rice plants for 7-14 days, potentially resulting in crop failure (7). Submergence tolerance is defined as the plant's ability to survive up to 10 to 14 days of complete submergence and can continue its growth and developmental process after the flood recedes, with minimum damage to plant morphology (8). Submergence-tolerant rice cultivars exhibit two key strategies for survival, namely, a quiescence and an escape strategy (9). The quiescence strategy occurs during flash floods, where the growth of shoots is minimized to conserve energy for a period of 10-14 days of submergence. This ensures the plant can use the stored carbohydrates once the flood water recedes. The escape strategy involves rapid internode and leaf elongation to rise above the water (9). Both strategies having differing plant growth responses are controlled by the ethylene response factor (ERF) subfamily of transcription factors (10,11).

The submergence-tolerant landraces like FR13A carry the Sub1A gene, which follows a quiescence strategy. This allows plants to adapt by conserving energy, maintaining chlorophyll content, limiting growth during submergence and controlling oxidative stress by managing reactive oxygen species to improve recovery after flooding (7,12). In contrast, high-yielding submergence-sensitive varieties struggle with even short periods of flooding, leading to lower yields due to poor survival, reduced tillering, slow recovery, or death soon after submergence (13).

Traditionally, Indian farmers have cultivated indigenous rice landraces (14). Nevertheless, the recent transition towards high-yielding cultivars, combined with varied cropping systems, reduced genetic diversity, environmental uncertainties and crop failures, has notably influenced the rice germplasm landscape (15). Despite ongoing efforts to develop and promote enhanced varieties, local landraces remain extensively cultivated due to their superior adaptability to a wide range of environmental conditions. Landraces are genetically diverse and act as genetic reservoirs for rice breeding (14,16). Although some traditional landraces show tolerance for flooding, they have less grain yield potential (17). Breeding for submergence tolerance, particularly in response to flash flooding during the vegetative stage, has been a key objective at the International Rice Research Institute (IRRI) Manila, Philippines for over three decades (7,13). To achieve both high yield and strong submergence tolerance, identifying superior pre-breeding donors is crucial. Further, submergence tolerance can be achieved by hybridization of varieties with submergence-tolerant donors (18).

Submergence stress screening can be conducted under controlled conditions in concrete tanks within greenhouses or large pools in the field (4). In this study, screening of rice landraces for submergence tolerance in controlled conditions was studied to identify submergence-tolerant donors. The present study aims to identify rice landraces exhibiting tolerance to complete submergence at the seedling stage, based on their survival and regeneration capacity. Additionally, the study seeks to assess the variation and genetic diversity among the evaluated genotypes.

Materials and Methods

The germplasm used in this study comprised of 250 rice landraces, and four checks, which include FR13A (tolerant), Swarna Sub1(tolerant), IR64 (sensitive) and Jaya (sensitive) (Supplementary Table 1). The rice landraces were obtained from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi. This research was carried out in two phases: preliminary screening and evaluation of landraces in protrays placed within a submergence tank in the greenhouse in 1st phase. Subsequently, the selected germplasm was rescreened for submergence tolerance followed by biochemical and physiological studies in 2nd phase. The submergence screening in the greenhouse was carried out at glasshouse facilities in the Centre of Plant Molecular Biology and Biotechnology at Tamil Nadu Agricultural University (TNAU), Coimbatore.

Experiment 1: Preliminary screening of 250 landraces at seedling stage

The design used in the experiment was the randomized complete block design under two replications. Each replication consists of ten protrays each protray with 216 wells, twentyseven genotypes were sown per protray in such a way that eight wells were assigned to each genotype. Submergence tolerance in landraces was screened using the protocol from the Standard Evaluation System for rice (IRRI 1966) (19). For preliminary screening, 254 genotypes were sown in protrays and maintained for 14 days without submergence stress, followed by placement of protrays in a submergence tank for 14 days to impose submergence stress. Throughout the submergence period, the water level in the tank was kept at 100 cm. After 14 days of the submergence period, water in the submergence tank was removed. The protrays along with seedlings were taken out of the submergence tank and allowed for 14 days to regenerate and during the regeneration period, Yoshida nutrient solution was provided for better recovery. Yoshida media was prepared following the protocol in the Laboratory Manual for Physiological Studies of Rice (20).

Observations were recorded at two stages, 14 Days after sowing (DAS) before submergence and 14 days after desubmergence (DAD). Germination percentage, plant height from the base of the shoot and number of leaves were recorded 14 days after sowing before submergence stress. The observations that were made 14 days after desubmergence (DAD) include plant height from the base of the shoot, number of leaves, shoot elongation, shoot elongation percentage, regeneration percentage of leaves and survival percentage. Scoring of genotypes was done according to Table1.

Table 1. The standard evaluation system of IRRI (1996) (19)

Sl.no	Score	Score description	Survival Percentage
1	1	Erect dark green leaves with very less elongation	91-100%
2	3	Green leaves with less elongation	81-90%
3	5	Droopy and pale green leaves with moderate elongation	51-80%
4	7	Long and pale green colour elongated leaves	11-50%
5	9	Whitish elongated leaves	0-10%

Shoot elongation = Plant height after regeneration - Plant height before the submergence

Plant height after regeneration -Plant height before submergence

Shoot elongation percent	tage =	x 100
	e	
Regeneration percentage	Number of leaves regenerated after desubmergence	v 100
of leaves =	Number of leaves before submergence	X 100
	Number of plants regenerated after desubmergence	v 100
Survival percentage =	Number of plants before submergence	X 100

Experiment 2. Biochemical and Physiological studies in the selected germplasm

The downsized 24 landraces that had a better survival percentage greater than 50% in experiment 1 along with four checks, were further screened in a similar way as experiment 1. In addition to screening the selected germplasm were studied for biochemical and physiological parameters before submergence treatment (14 DAS) and 7 days after desubmergence (DAD). The biochemical traits including total chlorophyll content, total soluble sugars and total starch content were estimated by following the procedure mentioned in experimental protocol of Yoshida (20). The following formula is used to calculate the reduction percentage of chlorophyll content (RPChl).

The reduction percentage of total soluble sugar (TSSRP) and reduction percentage of starch (StaRP) were calculated

using the formulas outlined below.

 (TSS content before submergence - TSS content after regeneration)
 x 100

 TSSRP =
 TSS content before submergence

 (Starch content before submergence - Starch content after regeneration)
 x 100

 StaRP =
 Starch content before submergence

Physiological traits such as fresh weight and dry weight were recorded in 24 landraces. The total fresh weight of seedlings averaged from 5 seedlings, was recorded before submergence (14 DAS) and after desubmergence (7 DAD). Further, seedlings were dried in the drying oven (heated at 70-75°C for 48 h) to measure the dry weight of seedlings.

Statistical analysis

The data was subjected to Analysis of variance (ANOVA) to know the significance followed by calculation of variability parameters such as Phenotypic coefficient of variation (PCV), Genotypic coefficient of variation (GCV), heritability, genetic advance and genetic advance at the percent of mean in the variability package of R software (21). The correlation analysis was done in the metan package of R software. Data were subjected to Multi-trait Genotype-Ideotype Distance Index (MGIDI) analysis in the metan package R software (22). The 254 germplasm were clustered into different groups in cluster analysis through Stats, Scales and fast cluster packages in the R software (23-25). The principal component analysis was conducted using Grapes software (26).

Results

Experiment 1: Preliminary screening of 250 landraces at seedling stage

Significant differences were observed among the 254 genotypes in the analysis of variance for the 9 traits associated with submergence stress tolerance (Supplementary Table 2). These findings underscore the diversity evident across all examined characteristics. Table 2 displays the results of the descriptive statistics computed. This study identified a broad range of responses among the genotypes concerning various traits related to submergence screening.

Table 2. Descriptive statistics for submergence tolerance traits among 254 genotypes

Traits	Minimum	Maximum	Range	Grand Mean	(SEm)	(CD) 5%
GP	46.27	99.83	53.57	84.41	0.59	1.65
PHB (14 DAS)	9.76	35.92	26.16	23.32	0.69	1.93
PHA (14 DAD)	14.19	60.98	46.79	38.42	0.92	2.56
SE	2.06	33.92	31.86	15.10	1.06	2.95
SEP	13.49	259.81	246.31	68.02	6.98	19.45
NLB (14 DAS)	2.00	4.00	2.00	2.54	0.32	0.89
NLA (14 DAD)	0.00	3.00	3.00	0.45	0.22	0.60
RPL	0.00	107.61	107.61	18.07	7.71	21.49
SP	0.00	100.00	100.00	22.12	2.72	7.57

GP, germination percentage; PHB, Plant height before submergence; PHA, plant height after desubmergence; SE, shoot elongation; SEP, shoot elongation percentage; NLB, number of leaves before submergence; NLA, number of leaves after desubmergence; RPL, regeneration percentage of leaves; SP, survival percentage, DAS, days after sowing; DAD, days after desubmergence; Sem, standard error of mean; CD, critical difference.

For 250 landraces, the germination percentage showed a mean value of 84.41%, with values in the range of - 46.27% to 99.83%. The shoot elongation (SE) was observed with a mean value of 15.10 cm, ranging from 2.06 cm to 33.92 cm and shoot elongation percentage (SEP) was observed with a mean value of 68.02%, ranging from 13.49% to 259.81%. The capacity to regenerate leaves significantly contributes in resuming photosynthetic activity, which is crucial for energy generation after submergence stress (27). The regeneration percentage of leaves (RPL) considered a critical trait for better survival of seedlings after submergence stress exhibited a mean of 18.07% with a range of 0% to 107.61% (27). Survival percentage (SP) exhibited a mean value of 22.12%, with values ranging from 0.00% to 100%.

The mean performance, illustrated in Supplementary Table 3, highlighted considerable variability across the rice genotypes under submergence stress. The variability concerning to submergence-tolerant traits is necessary for the selection of submergence-tolerant genotypes (4). The landraces like IRGC227, IRGC803, IRGC773, IRGC560, IRGC295 and IRGC317 exhibited highest germination percentage which was greater than 99%. Whereas in landrace IRGC813 and IRC 873 the lowest germination percentage of 47.05% and 48.5% was observed respectively. The landraces IRGC675, IRGC488, IRGC93, IRGC841, IRGC652 and IRGC793 depicted a shoot elongation percentage of less than 20%. IRGC1032, IRGC1046, IRGC811, IRGC1000 and IRGC623 exhibited shoot elongation greater than 150% with IRGC1032 having the highest shoot elongation percentage of 259.81%. Potential donors for submergence tolerance are cultivars that show the highest survival percentage after giving submergence stress (28). A 100% survival percentage was observed for FR13A, the tolerant check variety used in this study. The highest survival percentage observed in the tolerant check variety highlights its robust adaptive mechanism for submergence stress tolerance, as also mentioned in prior studies (4,13,29) Most of the landraces showed less than 50% survival, falling under the susceptible category, while only a few genotypes exhibited higher survival percentages. 24 landraces exhibited greater than 50% survival percentage. A total of 11 landraces come under the standard evaluation score value of 3 exhibiting a survival percentage of greater than 80%. Among this landrace IRGC93, IRGC535 and IRGC706 exhibited survival percentage greater than 85%.

Trait association analysis

The estimated correlation coefficients are depicted in Fig. 1 for all nine traits under investigation. The results indicated significant relationships among several traits. The trait survival percentage (SP) after 14 DAD displayed a strong positive correlation with the regeneration percentage of leaves (0.88***) and number of leaves after 14 DAD (0.86***) emphasizing the pivotal role of the regeneration percentage of leaves in alleviating submergence stress and promoting successful establishment of seedling after submergence stress(27) In comparison, a negative correlation was noticed with plant height after 14 DAD (-0.36***), shoot elongation (-0.52***) and shoot elongation percentage (-0.47***) after 14 days after desubmergence (DAD). Similarly, Singh et.al, also found a presence of strong negative correlation between survival and shoot elongation (30). The other trait regeneration percentage of leaves showed a strong positive association with the number of leaves at 14 DAD (0.97***) and a nonsignificant association with number of leaves before 14 DAS. Shoot elongation percentage has displayed a strong positive association with shoot elongation (0.78***) moderately positive association with plant height after 14 DAD (0.26***) and a negative association with plant height before submergence at 14 DAS (-0.43***). These results are consistent with previous findings (31).



Fig. 1. Correlation plot for nine submergence tolerance traits of 254 genotypes.

Trait	PV	GV	EV	PCV%	GCV%	ECV%	H2	GA	GAM
GP	113.27	112.57	0.70	12.61	12.57	0.99	0.99	21.79	25.81
PHB(14 DAS)	29.09	28.14	0.96	23.13	22.75	4.19	0.97	10.75	46.08
PHA(14 DAD)	73.09	71.40	1.69	22.25	22.00	3.38	0.98	17.20	44.79
SE	34.50	32.25	2.25	38.90	37.61	9.93	0.93	11.31	74.92
SEP	1072.83	975.25	97.58	48.15	45.91	14.52	0.91	61.34	90.17
NLB(14 DAS)	0.24	0.03	0.20	19.15	7.10	17.78	0.14	0.14	5.42
NLA (14 DAD)	0.40	0.30	0.09	139.29	121.69	67.79	0.76	0.99	218.99
RPL	624.48	505.46	119.02	138.28	124.40	60.37	0.81	41.67	230.56
SP	539.96	525.19	14.77	105.05	103.60	17.37	0.97	46.56	210.48

Table 3. Variability parameters of submergence tolerance-related traits for 254 genotypes

PV, phenotypic variance; GV, Genotypic variance; EV, Environmental variance; PCV%, phenotypic coefficient of variance in percentage; GCV%, Genotypic coefficient of variance in percentage; ECV%, Environmental coefficient of variance in percentage; H2, heritability; GA, Genetic advance; GAM, Genetic advance at percent of the mean.

4

Variability parameters:

Assessment of variability parameter was done for all the 9 traits included in the study (Table 3). For all examined parameters, the phenotypic variance (PV) exceeded the genotypic variance (GV). Notably, the % of PCV surpassed over the % of GCV, which tells the effect of environmental factors (32). Further, traits such as germination percentage, plant height after (14DAD), shoot elongation and survival percentage exhibited comparable PCV and GCV values. Although, high PCV and GCV values were observed for characters like number of leaves after submergence (14DAD) and Regeneration percentage of leaves environmental component variance (ECV) values of 67.79% and 60.37% respectively which suggests that significant environmental influence making them less reliable. The trait such as germination percentage, plant height before submergence, plant height after (14DAD) and shoot elongation have the low environmental coefficient of variance. Whereas survival percentage trait has shown the moderate environmental coefficient of variance.

Heritability plays an important role in breeding programs, as it indicates the degree to which traits are transmitted from one generation to the next generation. Effective and efficient breeding plans consider heritability along with genetic advances and variances (32,33). Every character under study showed strong broad-sense heritability. Germination % had the highest value for broad sense heritability (0.99), while the number leaf before submergence had the lowest (0.14). Survival percentage of seedlings after submergence displayed higher heritability (0.97), followed by the Shoot elongation percentage, which exhibited higher heritability (0.91). The genetic advance as a percentage of the mean ranged from 25.81% for germination (the lowest) to 230.56% for leaf regeneration percentage (the highest). Most of the traits except NLB exhibited high heritability and high GAM which suggests that all of them are governed by additive gene action and the former NLB is highly influenced by environment.

MGIDI Analysis

MGIDI analysis is used in plant breeding for evaluation and ranking genotypes based on several traits simultaneously, by measuring the distance between genotypes and an ideotype (an ideal genotype) (34). To downsize the 250 germplasm and to select only better submergence tolerant lines, 3 traits namely the number of leaves after submergence, regeneration percentage of leaves, and survival percentage were considered with an increasing trend (higher values are desirable). In contrast, three other traits including plant height after submergence, shoot elongation, and shoot elongation percentage were considered with a decreasing trend (lower values are desirable). PCA analysis for 250 germplasm for 6 traits has 6 correlated traits converted into six uncorrelated Principal components out of which two principal components have crossed more than 1 eigenvalue (Supplementary Table 4). Hence, two factors were found in the study. Plant height after submergence, shoot elongation and shoot elongation percentage are grouped under factor 1 and number of leaves after submergence, regeneration percentage of leaves and survival percentage are grouped under factor 2.

At 12% selection intensity following 30 genotypes were selected which include 2 checks Swarna Sub1, FR13A, IRGC93, IRGC 785, IRGC 675, IRGC 170, IRGC 706, IRGC 488, IRGC 841, IRGC 508, IRGC 542, IRGC 535, IRGC 649, IRGC 873, IRGC 94, IRGC 403, IRGC 544, IRGC 625, IRGC 652, IRGC 793, IRGC 361, IRGC 967, IRGC 775, IRGC 557, IRGC 776, IRGC 773, IRGC 437, IRGC 102, IRGC 646, IRGC 527. In the above list of germplasm, 26 genotypes have more than 50% survival percentage. Four landraces IRGC 437, IRGC 102, IRGC 646 and IRGC 527 had less than 50% survival percentage. The selected germplasm showed a 2.27 times higher survival percentage than the overall germplasm under study. The regeneration percentage of leaves was found to show 2.42 times more in selected germplasm in comparison to the overall germplasm under study. The selected germplasm exhibited a 0.55-fold reduction in shoot elongation percentage compared to the overall germplasm (Table 4).

The strength and weakness plot are a unique feature of MGIDI, the radar line being close to the outer circumference indicates the least contribution of the factor to the genotype concerned and the traits included in that factor are best in the performance of the genotype and the radar line close to the center of the circle indicates the high contribution of the factor to the genotype concerned and traits included in the factor are not good in performance of the genotype concerned. On analysing the strength and weakness plot, Swarna Sub1, IRGC488, IRGC 706, IRGC 841, IRGC 542, IRGC 535, IRGC 403, IRGC 544 and IRGC 646 were found to show good performance for the traits included in factor 1. FR13A, IRGC 93, IRGC 785, IRGC 675, IRGC 170, IRGC 508, IRGC 649, IRGC 873, IRGC 94, IRGC 625, IRGC 652, IRGC 793, IRGC 361, IRGC 967, IRGC 775, IRGC 557, IRGC 776, IRGC 773, IRGC 437, IRGC 102 and IRGC 527 were found to show good performance in traits present in factor 2 (Fig. 2).

Table 4. Trait Contributions to MGIDI Index Based on Heritability and Selection Gains in Submergence Tolerance traits

Traits	Factor	Хо	Xs	SD	SD%	H2	SG	SG%	sense	Goal
NLA (14DAD)	FA1	0.45	1.61	1.15	255	0.86	0.999	220	Increase	100
RPL	FA1	18.1	66.9	48.8	270	0.89	43.7	242	Increase	100
SP	FA1	21.2	69.9	48.7	230	0.98	48.0	227	Increase	100
PHA (14DAD)	FA2	38.4	29.0	-9.42	-24.5	0.98	-9.31	-24.2	Decrease	100
SE	FA2	15.1	6.46	-8.63	-57.2	0.96	-8.34	-55.3	Decrease	100
SEP	FA2	68.0	28.8	-39.3	-57.7	0.95	-37.4	-55.0	Decrease	100

Xo, Original mean value for the trait before selection; Xs, Mean value for the trait after selection; SD, Standard deviation; SD%, Standard deviation as a percentage; H2, Broad-sense heritability (H²); SG, Selection gain; SG%, Selection gain as a percentage.



Fig. 2. Strength and weakness plot of MGIDI analysis. *Cluster analysis:*

To group the genotypes into different categories of submergence tolerance i.e., low, moderate and high, the 254 genotypes for 6 major importance traits for submergence tolerant selection were utilized for clustering. The 254 landraces used in this study were divided into three clusters based on six submergence tolerance-related traits namely plant height after submergence (14 DAD), shoot elongation, shoot elongation percentage, number of leaves after submergence (14 DAD), regeneration percentage of leaves and survival percentage. Cluster I, Cluster II and Cluster III have 165, 63 and 26 genotypes respectively (Fig. 3). Cluster III displayed the highest average values for characters. viz., survival percentage (75.62%), regeneration percentage of leaves (78.50%) and lowest value of shoot elongation (5.09 cm) and shoot elongation percentage (23.63%) hence all the genotypes tolerant for submergence stress are grouped under this cluster. Cluster I has the highest number of genotypes with a survival percentage (18.3%), shoot elongation (14.44), shoot elongation percentage (64.22%) and



Fig. 3. Cluster analysis through circular dendrogram for 254 genotypes with 6 submergence tolerant traits.

regeneration percentage of leaves (14.19%). Cluster II has the lowest average survival percentage of 6.02% hence most genotypes in both clusters 1 and 2 are susceptible to submergence stress. Survival percentage is a major criterion for the screening of submergence-tolerant genotypes (4). The 26 genotypes including the 2 tolerant check varieties in cluster III have the highest mean value survival percentage. The genotypes having greater than 50% survival rate includes IRGC93, IRGC94, IRGC170, IRGC361, IRGC403, IRGC488, IRGC508, IRGC535, IRGC542, IRGC544, IRGC557, IRGC625, IRGC649, IRGC652, IRGC675, IRGC706, IRGC773, IRGC775, IRGC776, IRGC785, IRGC793, IRGC841, IRGC873 and IRGC967 belongs to cluster III.

Experiment 2: Biochemical and Physiological studies in the selected germplasm

The 24 landraces having a survival percentage greater than 50% along with 4 check varieties were again screened for submergence tolerance. Significant differences were found across the 28 selected genotypes in the analysis of variance for the 22 characters related to submergence stress tolerance trait except number of leaves before submergence (Table 5). These results depict the variability present across all studied characteristics. Variability analysis for 28 selected lines for 22 traits shows that for all the traits the phenotypic coefficient of variance (PCV) is higher than the genotypic coefficient of variance (GCV) (Supplementary Table 5).

Correlation analysis

Correlation analysis was conducted for all the 22 traits under consideration. The computed correlation coefficient is shown in Fig. 4. The results emphasize the presence of a significant relationship between the various traits. Chlorophyll pigment has a major role in photosynthesis. Previous studies have highlighted that the tolerant check varieties FR13A and Swarna sub1 have the least chlorophyll reduction percentage or higher chlorophyll retention after submergence stress (35-37). Similarly, our result shows that there is a strong negative correlation between the chlorophyll reduction percentage (-0.89***) and the survival percentage of selected tolerant landraces. Further, the survival percentage of seedlings exhibited a strong correlation with total soluble sugars (0.86***) and total starch (0.71***) present after submergence stress. There is a presence of a negative correlation between total starch reduction percentage (-0.85***) and total sugar reduction percentage (-0.96**) with seedling's survival percentage. Our result indicates that non-structural carbohydrates including total soluble sugar and starch which is maintained after submergence have an important role in the seedling survival. This in accordance with the previous findings (35).

Principal component analysis

PCA is used to determine the relative contribution of every trait to overall variability and to guide the selection of traits (38). Biplot principal component analysis of rice landraces for principal component 1 vs. principal component 2. The biplot depicts the distribution patterns of indigenous accessions into four different quadrants based on the traits associated with a submergence tolerance and separates tolerant genotypes from susceptible genotypes (Fig 5). On analysing the 22 traits in 28 traits, the estimated values of the Eigenvectors, the variance Table 5. Analysis of variance through Randomized complete block design for 28 genotypes

Sl. No	Traits	Mean Sum of Squares		
		Genotypes	Replication	
1	Germination Percentage	502.55***	0.12	
2	Plant height before submergence (14 DAS)	40.11***	2.13	
3	Plant height after submergence (14 DAD)	73.64***	4.84	
4	Shoot elongation	21.87***	0.54	
5	Shoot elongation percentage	859.18***	6.75	
6	Number of leaves before submergence (14DAS)	0.23	1.06*	
7	Number of leaves after submergence (14DAD)	1.15***	0.18	
8	Regeneration percentage of leaves	1121.51***	87.36	
9	Survival Percentage	1137.87***	45.54	
10	Chlorophyll content before submergence stress(14DAS)	0.069***	0.0002	
11	Chlorophyll content after submergence (7DAD)	0.621***	0.000	
12	Chlorophyll reduction percentage	855.81***	0.06	
13	Starch content before submergence stress (14DAS)	25.99***	0.0015	
14	Starch content after submergence stress (7DAD)	44.25***	0.000	
15	Starch reduction percentage	629.81***	0.08	
16	Total soluble sugars before submergence stress (14DAS)	31.45***	0.0002	
17	Total soluble sugars after submergence stress (7DAD)	72.765***	0.011	
18	Total soluble sugar reduction percentage	961.20***	0.04	
19	Total fresh weight before submergence (14DAS)	0.127***	0.0068	
20	Total fresh weight after submergence (7 DAD)	0.102***	0.002**	
21	Total dry weight before submergence (14 DAS)	0.0097***	0.0005*	
22	Total dry weight after submergence (7 DAD)	0.0056***	0.0021**	

*,** and ***, refers to significance at p<0.05, 0.01 and 0.001, respectively



Fig. 4 Correlation plot for 22 traits in 28 genotypes.



Fig. 5. PCA- Biplot between Principal components 1 and 2.

was recorded by the first 4 Principal components. PC1, with an eigenvalue of 9.626, made the most substantial contribution to variability, accounting for 43.75%. Characters, viz. shoot elongation percentage (0.86), chlorophyll reduction percentage (0.90), starch reduction percentage (0.88), total soluble sugar reduction percentage (0.93) were positively loaded while number of leaves after submergence (-0.91), regeneration percentage of leaves (-0.97), survival percentage (-0.97) was negatively loaded. The maximum % of variance towards PC1 was contributed by the following traits survival percentage (9.91%), regeneration percentage of leaves (9.85%) and total soluble sugar reduction percentage (9.12%) (Supplementary Tables 7 and 8). The second principal component PC2 with an eigenvalue of 3.72 accounted for 16.92% of the variability. The traits plant height after submergence (0.61), shoot elongation (0.30), and total fresh weight after submergence (0.80) were positively loaded while the number of leaves after submergence (-0.02), chlorophyll reduction percentage (-0.23) was negatively loaded. The maximum % of variance towards PC2 was explained by total fresh weight after submergence (17.51%), total dry weight before submergence (16.51%) and plant height after submergence (10.29%).

percentage and the cumulative percentage are presented in Supplementary Table 6. The first four PCs have crossed the eigen

value greater than 1. Around 78.21% of the cumulative variance

Conclusion

This research focused on assessing the diversity of Indian rice landraces in relation to traits specific to submergence stress tolerance. Detailed statistical analyses emphasize the presence of greater variability between the rice landraces across various traits under study, pointing to potential sources for submergence tolerance. Traits like percentage of leaf regeneration, shoot elongation percentage and survival emerged as critical factors for submergence tolerance. Furthermore, the study highlighted the importance of variability analysis, trait association analysis, MGIDI analysis, diversity analysis, and principal component analysis in revealing the genetic and phenotypic factors governing submergence tolerance. These results emphasize the key role of genetic diversity and heritability in breeding programs aimed at finding the submergence stress-tolerant donor among the landraces for developing rice cultivars resistant to submergence stress, thereby advancing rice production.

Acknowledgements

We acknowledge the support from the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India for funding this network project (Grant number: SAN No. 102/IFD/SAN/4188/2019-20).

Authors' contributions

Shrirashmi carried out screening and biochemical work, did the statistical analysis and prepared the draft copy. Sivagami guided in research work and corrected the draft copy. AJ conceived of the study and participated in the design and coordination of the work. Suresh guided in the research. Vellaikumar guided the research work. Manju participated in screening work.

Compliance with ethical standards

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

Ethical issues: None

References

- Mahajan G, Kumar V, Chauhan BS. Rice Production in India. In: Chauhan BS, Jabran K, Mahajan G, editors. Rice Production Worldwide [Internet]. Cham: Springer International Publishing; 2017;53-91. https://doi.org/10.1007/978-3-319-47516-5_3
- Tian Z, Wang J, Li J, Han B. Designing future crops: challenges and strategies for sustainable agriculture. The Plant Journal. 2021;105 (5):1165-78. https://doi.org/10.1111/tpj.15107
- Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R, et al. Chapter 2 Climate Change Affecting Rice Production. In: Advances in Agronomy [Internet]. Elsevier. 2009;59-122. https:// doi.org/10.1016/S0065-2113(08)00802-X
- Anshori MF, Purwoko BS, Dewi IS, Suwarno WB, Ardie SW. Systematic selection to adaptive doubled haploid rice lines under different environments of submergence screening methods. Journal of Agriculture and Food Research. 2023;14:100775. https:// doi.org/10.1016/j.jafr.2023.100775
- Koppa N, Amarnath G. Geospatial assessment of flood-tolerant rice varieties to guide climate adaptation strategies in India. Climate. 2021;9(10):151. https://doi.org/10.3390/cli9100151
- Schneider P, Asch F. Rice production and food security in Asian Mega deltas-A review on characteristics, vulnerabilities and agricultural adaptation options to cope with climate change. J Agronomy Crop Science. 2020;206(4):491-503. https://doi.org/10.1111/jac.12415
- Bailey-Serres J, Fukao T, Ronald P, Ismail A, Heuer S, Mackill D. Submergence tolerant rice: SUB1'sjourney from landrace to modern cultivar. Rice. 2010;3(2-3):138-47. https://doi.org/10.1007/ s12284-010-9048-5
- 8. Singh A, Septiningsih EM, Balyan HS, Singh NK, Rai V. Genetics, physiological mechanisms and breeding of flood-tolerant rice

(*Oryza sativa* L.). Plant Cell Physiol. 2017;pcw206. https://doi.org/10.1093/pcp/pcw206

- Nakamura M, Noguchi K. Tolerant mechanisms to O₂ deficiency under submergence conditions in plants. J Plant Res. 2020;133 (3):343-71. https://doi.org/10.1007/s10265-020-01176-1
- Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, et al. The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. Nature. 2009;460(7258):1026-30. https://doi.org/10.1038/nature08258
- Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, et al. Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature. 2006;442(7103):705-8. https://doi.org/10.1038/nature04920
- 12. Fukao T, Bailey-Serres J. Submergence tolerance conferred by *Sub1A* is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proc Natl Acad Sci USA. 2008;105(43):16814-9. https://doi.org/10.1073/pnas.0807821105
- Mackill DJ, Ismail AM, Singh US, Labios RV, Paris TR. Development and rapid adoption of submergence-tolerant (Sub1) rice varieties. In: Advances in Agronomy [Internet]. Elsevier. 2012;299-352. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780123942760000068
- Mohammed Ashraf A, Lokanadan S. A Review of Rice Landraces in India and its inherent medicinal values-the nutritive food values for future. Int J Curr Microbiol App Sci. 2017;6(12):348-54. https:// doi.org/10.20546/ijcmas.2017.612.042
- Khoury CK, Brush S, Costich DE, Curry HA, De Haan S, Engels JMM, et al. Crop genetic erosion: understanding and responding to loss of crop diversity. New Phytologist. 2022;233(1):84-118. https:// doi.org/10.1111/nph.17733
- Ahmed F, Rafii MY, Ismail MR, Juraimi AS, Rahim HA, Asfaliza R, et al. Waterlogging tolerance of cops: breeding, mechanism of tolerance, molecular approaches and prospects. BioMed Research International. 2013;2013:1-10. https://doi.org/10.1155/2013/963525
- Ismail AM, Singh US, Singh S, Dar MH, Mackill DJ. The contribution of submergence-tolerant (Sub1) rice varieties to food security in flood-prone rainfed lowland areas in Asia. Field Crops Research. 2013;152:83-93. https://doi.org/10.1016/j.fcr.2013.01.007
- Septiningsih EM, Pamplona AM, Sanchez DL, Neeraja CN, Vergara GV, Heuer S, et al. Development of submergence-tolerant rice cultivars: the Sub1 locus and beyond. Annals of Botany. 2009;103 (2):151-60. https://doi.org/10.1093/aob/mcn206
- 19. IRRI I. Standard Evaluation System for rice 4th edition. 1996.
- Yoshida S, Forno DA, Cock JH. Laboratory manual for physiological studies of rice. 1971.
- Popat R, Patel R, Parmar D. Variability: genetic variability analysis for plant breeding research. R Package Version 01 0. 2020. https:// doi.org/10.32614/CRAN.package.variability
- 22. Olivoto T, Lúcio AD. metan: An R package for multi-environment trial analysis. Jarman S, editor. Methods Ecol Evol. 2020;11(6):783-9. https://doi.org/10.1111/2041-210X.13384
- 23. Müllner D. Fast Hierarchical Clustering Routines for R and Python. R package fast cluster version. 2015; 1:20.
- 24. Team RC, Team MRC, Suggests M, Matrix S. Package stats. The R Stats Package. 2018.
- Wickham H, Wickham MH, RColorBrewer I. Package 'scales.' R package version. 2016;1(0).
- Gopinath P, Parsad R, Joseph B, S. A. grapesAgri1: collection of shiny apps for data analysis in agriculture. JOSS. 2021;6(63):3437. https://doi.org/10.21105/joss.03437
- Zhang R, Zhou N, Zhou L, Ashi R, Feng T, Zhang K, et al. Effects of submergence stress on germination and seedling growth of directseeded rice and evaluation of submergence tolerance. Paddy Water Environ. 2023;21(4):523-38. https://doi.org/10.1007/s10333-023-00945-7

- Singh S, Mackill DJ, Ismail AM. Responses of SUB1 rice introgression lines to submergence in the field: Yield and grain quality. Field Crops Research. 2009;113(1):12-23. https://doi.org/10.1016/j.fcr.2009.04.003
- 29. Panda D, Sarkar RK. Leaf photosynthetic activity and antioxidant defence associated with Sub1 QTL in rice subjected to submergence and subsequent re-aeration. Rice Science. 2012;19 (2):108-16.
- Singh HP, Singh BB, Ram PC. Submergence tolerance of rainfed lowland rice: search for physiological marker traits. Journal of Plant Physiology. 2001;158(7):883-9. https://doi.org/10.1078/0176-1617-00036
- 31. Sarkar RK, Bhattacharjee B. Rice Genotypes with SUB1 QTL differ in submergence tolerance, elongation ability during submergence and re-generation growth at re-emergence. Rice. 2011;5(1):7. https://doi.org/10.1007/s12284-011-9065-z
- 32. Falconer DS. Introduction to quantitative genetics. Pearson Education India; 1996.
- Roy SC, Shil P. Assessment of genetic heritability in rice breeding lines based on morphological traits and caryopsis ultrastructure. Sci Rep. 2020;10(1):7830.

- Olivoto T, Nardino M. MGIDI: toward an effective multivariate selection in biological experiments. Russell S, editor. Bioinformatics. 2021;37(10):1383-9. https://doi.org/10.1093/bioinformatics/btaa981
- Das KK, Sarkar RK, Ismail AM. Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice. Plant Science. 2005;168(1):131-6. https://doi.org/10.1016/ j.plantsci.2004.07.023
- Ella ES, Kawano N, Ito O. Importance of active oxygen-scavenging system in the recovery of rice seedlings after submergence. Plant Science. 2003;165(1):85-93. https://doi.org/10.1016/S0168-9452(03) 00146-8
- Fukao T, Xu K, Ronald PC, Bailey-Serres J. A Variable cluster of ethylene response Factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. The Plant Cell. 2006;18 (8):2021-34. https://doi.org/10.1105/tpc.106.043000
- Reddy CV, Panda S, Dash M, Anandan A, Lenka D, KC S, RK P. Exploring rice genotypes for anaerobic germination: Towards sustainable direct seeding. Plant Science Today. 2024.