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Genetic analysis and screening of Indian landraces of rice for submergence tolerance in target production environment (TPE)

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Abstract

Complete submergence is one of the most important abiotic stress constraining rice production in India. From the past few decades effect of this stress has intensified and is anticipated to increase in coming years as a result of global climate change. Rice has developed many adaptive mechanisms to grow well in flood ecosystems however, too much water for long time at any stage of plant growth can lead to serious injuries like decaying of plant parts, lodging and reperfusion causing total crop loss. Because of these conditions, farmers usually rely on traditional landraces that can survive through this stress, despite the fact that these tends to be very low yielding. In the present study, 660 rice landraces along with checks were subjected to screening for submergence tolerance at vegetative stage in target production environment. Identification of tolerant genotypes was based on the survival percentage of plants and recovery by which they grown new tillers and leaves. Principle component analysis revealed three principle components accounted for 99.9 per cent of total variation. Regeneration percentage of tillers found to be the most discriminating character and possess additive gene action. Confirmation through cluster analysis grouped the tolerant and susceptible genotypes in the respective clusters. Results of screening showed that, out of 660 accessions, three accessions *viz.*, IC388692, IC377169 and IC205953 have highest tolerance to 14 days of submergence and the survival ability was similar to the tolerant checks FR13A and CO 43-Sub1. Apart from the above three accessions, eight accessions *viz.*, IC216378, IC126210, IC114413, IC386238, IC133584, IC114971, RL-1312 and RL-3055 scored 1 for faster regeneration of tillers denoting the ability to have good tolerance for submergence stress.

Keywords: Indian landraces of rice, target production environment, genetic variability, principle component analysis, correlation

Introduction

Rice is a staple food for the large population of India and is cultivated in almost all states of country. Since long time, majority of the farmers of India have grown traditional landraces of rice crop. Recently, due to the adoption of high yielding varieties, narrow genetic base of the crop, diversified cropping system, environmental fluctuations and crop failures, several challenges have been altered the rice germplasm scenario. Despite attempts to produce and disseminate better varieties, local landraces are still cultivated in the majority of the country's regions because of their high environmental adaptability. Such rice landraces have contributed significantly to local food security and sustainable agricultural development, as well as adding value to the genetic resources for rice varietal improvement (Dikshit *et al.*, 2013) [4]. Among the biotic and abiotic stresses affecting rice production, complete submergence is considered as the third most important constraint for higher productivity in Eastern India. Fifteen percent of the total area in India (approximately 49.82 million hectares) is extremely vulnerable to floods. Moreover, the variable summer-monsoon season in India has often precipitated floods, especially in the river basins like Indus, Ganges and Brahmaputra which cause significant monsoon runoff leading to immense flooding in the plains (Koppa *et al.*, 2021) [7]. Heavy downpour, overflowing rivers or high tides frequently drown the fields during the rainy season, which has a negative impact on crop yield. Breeding efforts for the tolerance to submergence stress caused by flash floods during the vegetative stage has been a top priority at the International Rice Research Institute (IRRI) for more than three decades (Septiningsih *et*

al., 2013) [21].

Submergence can cause complete inundation of the entire plant for several days to two weeks, and usually occurs at the seedling or early vegetative stage (Singh *et al.*, 2014) [22]. Submergence causes fast reduction in gaseous exchange, light intensity and induces underwater leaf senescence and unnecessary stem elongation which results in death either during submergence or shortly after de-submergence (Jackson and Ram 2003) [11]. Submergence tolerant traditional varieties such as FR13A contains *Sub1* gene which is responsible for quiescence strategy in which plants undergo various physiological and biochemical changes such as conserving their energy reserves, maintaining chlorophyll content, controlling reactive oxygen species (produced due to oxidative stress) and adapting limited elongation ability during underwater stress to recover its growth when flood water recedes (Ella *et al.*, 2003) [5].

Submergence intolerant high yielding varieties are very sensitive even for a few days of submergence, obviously resulting in reduced yield because of their low survival rate, less tillering, slow recovery or immediate death of plants after de-submergence (Ismail *et al.*, 2008 [10]; Singh *et al.*, 2014) [22]. Few traditional landraces, meanwhile, are tolerant to submergence stress, and these cultivars are also hampered by having naturally low grain yields (Mackill *et al.*, 1996 [16]; Mackill *et al.*, 2012) [17]. To meet the need for high yield with the best performance in submergence tolerance, elite pre-breeding donors must be found. Earlier studies conducted on submergence tolerance in rice using highly tolerant landrace FR13A, were mostly focused on the survival rate of plants and their observation showed that the speed at which surviving plant recover and regenerate new tillers and leaves is an important aspect for higher grain yield. Therefore, present study was carried out to identify the rice landraces showing tolerance to complete submergence in vegetative stage based on their survival and regeneration ability and also an attempt was made to assess variation and divergence among the genotypes studied.

Material and Methods

The experimental material comprised of six hundred and sixty rice landrace accessions obtained from National Bureau of Plant Genetic Recourses, New Delhi. Submergence tolerance screening for these accessions was carried out along with checks *viz.*, FR13A (tolerant), CO 43-*Sub1* (tolerant), IR64 (Susceptible), IR42 (Susceptible) and Swarna (Susceptible) in target production environment at Tamil Nadu Rice Research Institute, Aduthurai during (October-December) 2021. The tolerant check FR13A selected for the studies was derived from pure-line selection of the landrace “Dhalputtia” originating from Orissa, India, which was identified as the most tolerant cultivar (HilleR is Lambers and Vergara 1982) [8] and subsequently used as a tolerant donor in several breeding programs. Second tolerant check CO 43-*Sub1*, was developed through introgression of *Sub1* gene into CO 43 variety by using marker assisted backcross breeding program at Tamil Nadu Agricultural University, Coimbatore, India (Rahman *et al.*, 2018) [18]. In most of the pervious experiments IR64, IR42 and Swarna have been reported as most sensitive genotypes for the submergence stress, so for the current studies these varieties were selected as susceptible checks. Screening was carried out in well-equipped concrete tank (20m x 10m x 1m) constructed in upland field filled with clay

loam texture soil with slightly alkaline pH (pH: 7.74) and an electrical conductivity of 0.35dSm⁻¹.

Screening for submergence tolerance was carried out by using standard protocol followed at IRRI (SES of IRRI, 1966) [9]. The experiment was performed by sowing the seeds of each accession with a spacing of 20 x 10 cm along with checks in augmented design. Fifteen plants of each genotypes were grown and maintained healthy for 45 days and were submerged for 14 days by maintaining the water level to the depth of 1m. After 14 days the tank was de-submerged and the plants were allowed to regenerate for 14 days. After 14 days of regeneration period, plants were observed for survival percentage, regeneration percentage of tillers and regeneration percentage of leaves.

Survival Percentage =

$$\frac{\text{Number of plants regenerated after submergence}}{\text{Total number of plants planted before submergence}} \times 100$$

Regeneration Percentage of tillers =

$$\frac{\text{Number of tillers regenerated after submergence}}{\text{Total number of tillers before submergence}} \times 100$$

Regeneration Percentage of leaves =

$$\frac{\text{Number of leaves regenerated after submergence}}{\text{Total number of leaves before submergence}} \times 100$$

Evaluation of submergence tolerant genotypes was carried out by using standard evaluation system of IRRI (1996) [9].

Table 1: Standard evaluation system of IRRI (1996) [9]

Score	Score description	Survival Percentage
1	Erect dark green leaves with very less elongation	91-100%
3	Green leaves with less elongation	81-90%
5	Droopy and pale green leaves with moderate elongation	51-80%
7	Long and pale green color elongated leaves	11-50%
9	Whitish elongated leaves	0-10%

Principal component analysis was studied to identify the plant traits that contributed most of the observed genotypic variation and was performed using the software GRAPES version 1.1.0. Genetic variability parameters *viz.*, Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV) and genetic advance as per cent of mean was estimated as per the formula given by Johnson *et al.* (1955). Heritability was calculated based on formula suggested by Lush (1940) [15]. Cluster analysis was carried out for selected tolerant and susceptible genotypes by Ward's method using the software R.

Results and Discussion

The six hundred and sixty rice landrace accessions along with five checks were screened for submergence tolerance and the accessions were categorized into five groups based on their visual score for submergence tolerance as highly tolerant-1, tolerant-3, moderately tolerant-5, susceptible-7, and highly susceptible-9 as given by standard evaluation system of IRRI (1996) [9] and the scale described by Gomosta (2001) [1], (Table 1). After 14 days of de-submergence, accessions were observed for plant injuries, survival percentage and regenerating ability. Tolerant checks FR13A and CO 43-*Sub1* showed less plant injury with 100% survival percentage.

Similarly Rahman *et al.* (2018) ^[18] and Vijayalakshmi (2022) ^[23] observed, the seedlings of FR13A were found to recover better and showed 100% of survival percentage and produced fresh leaves within 10 days of de-submergence. All the seedlings of FR13A were found to remain green as well as CO 43-Sub1 NILs harboring the FR13A allele were found to remain green and healthy and showed 95%-100% survival percentage after de-submergence.

Susceptible checks IR64, IR42 and Swarna showed very less or zero survival percentage with totally decayed and lodged plant parts. These results shown similarity with submergence studies carried out by Kar *et al.* (2017) ^[14] in which survival percentage of susceptible genotypes was reported as 0% for IR64, IR42 and 7.7% for Swarna. Comparing with the checks, all the 660 accessions were categorized according to their score by taking visual observations for greenness of leaves, elongation, standing or lodging status of tillers and the plant ability for regenerating new leaves and tillers.

According to categorization of 660 accessions along with checks based on the submergence tolerance score, group of 13 genotypes showed score 1 (Table. 2). Next group of 11 genotypes showed score 3 (Table. 3) followed by 78

genotypes with score 5 (Table. 4), 162 genotypes with score 7 and 401 genotypes with score 9 (data not shown). These results showed that among 660 accessions around 84% of the genotypes are highly sensitive to complete submergence stress and showed least or no survival after de-submergence. Seventy eight accessions observed to have score 5 which shows moderate tolerance for submergence with 51-80% of survival percentage. The accessions showing score 1 and 3 showed highest survival percentage with fast regeneration capacity of new leaves and tillers which helps plants to restore its growth. Singh *et al.* (2014) ^[22] observed plants exhibiting submergence tolerance and regeneration of number of tillers and leaves assists faster recovery of plants. The accessions showing susceptibility to submergence showed no or very less ability to regenerate new tillers and leaves.

The flow of analysis carried out for rice genotypes on the basis of different traits is given in Fig 1. Principle component analysis was carried out for rice landraces selected on basis of submergence tolerance score (1, 3 and 5), regeneration percentage of tillers (> 20%) and regeneration percentage of leaves (> 20%) along with five checks.

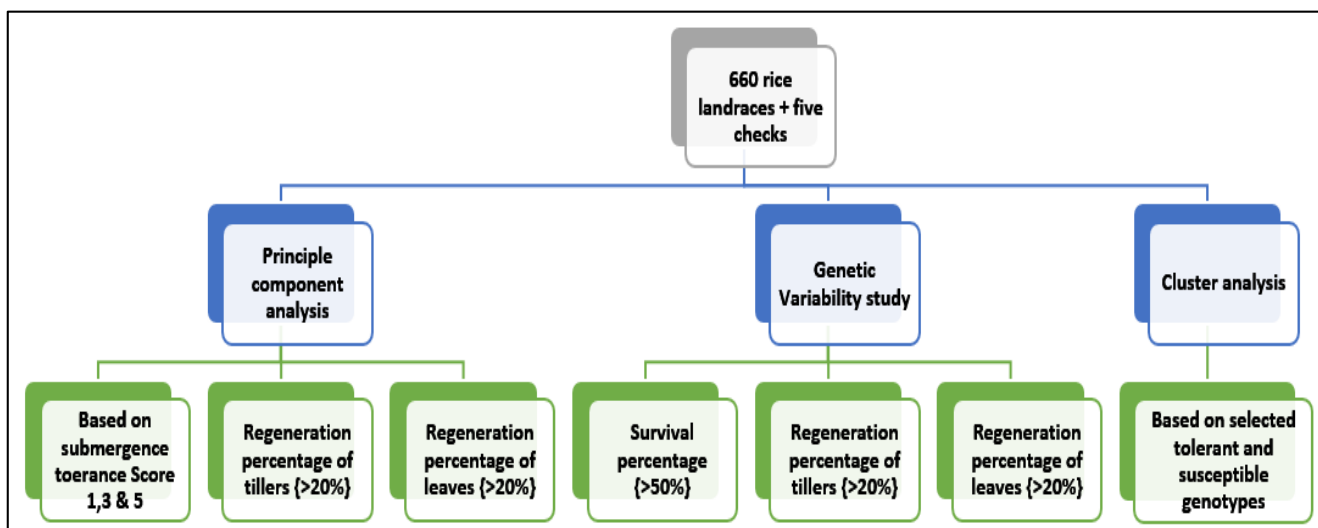


Fig 1: Diagrammatical representation of analysis carried out for 660 rice landraces and five checks

Table 2: Genotypes with submergence tolerance score 1

S. No.	Acc. No.	Scoring	Survival %	ANTRAS	ANLRAS
1	IC216378	1	93.3	5	3
2	IC126210	1	92.3	4	3
3	IC388692	1	100	5	4
4	IC114413	1	93.3	5	3
5	IC377169	1	100	4	4
6	IC386238	1	93.3	5	4
7	IC205953	1	100	7	4
8	IC133584	1	92.3	5	4
9	IC114971	1	92.9	4	3
10	RL- 1312	1	92.9	3	4
11	RL-3055	1	92.9	3	3
12	FR13A	1	100	10	5
13	CO 43-Sub1	1	100	11	4

ANTRAS- Average number of tillers regenerated after submergence. ANLRAS- Average number of leaves regenerated after submergence

Table 3: Genotypes with submergence tolerance score 3

S. No.	Acc. No.	Scoring	Survival %	ANTRAS	ANLRAS
1	IC208155	3	80	5	2
2	IC388900	3	83.3	3	3
3	IC518991	3	81.8	3	3
4	IC114575	3	84.6	4	4
5	IC115775	3	86.7	3	4
6	IC301077	3	85.7	5	4
7	IC218157	3	86.7	5	3
8	RL- 1348	3	85.7	3	3
9	RL- 1757	3	84.6	4	3
10	RL-3860	3	85.7	2	4
11	RL- 7220	3	80	4	4

ANTRAS- Average number of tillers regenerated after submergence. ANLRAS- Average number of leaves regenerated after submergence

Table 4: Genotypes with submergence tolerance score 5

S. No.	Acc. No.	Scoring	Survival %	ANTRAS	ANLRAS
1	IC248059	5	55.6	2	2
2	IC458407	5	75	2	2
3	IC459854X	5	73.3	2	3
4	IC458416	5	77.8	1	2
5	IC114501	5	72.7	2	2
6	IC115726	5	58.3	1	2
7	IC377660	5	60	2	2
8	IC378252	5	70	1	2
9	IC207746	5	54.5	2	2
10	IC378565	5	61.5	3	2
11	IC462995	5	55.6	2	2
12	IC460367X	5	53.3	2	2
13	IC377259	5	58.3	2	2
14	IC459860	5	78.6	3	3
15	IC460497	5	61.5	2	2
16	IC465229	5	77.8	2	2
17	IC134265	5	66.7	2	2
18	IC256483	5	70	2	2
19	IC311821	5	76.9	3	2
20	IC377212	5	75	3	2
21	IC516702	5	76.9	3	2
22	IC463901	5	70	3	2
23	IC450300X	5	77.8	3	2
24	IC252314	5	70	2	2
25	IC518703	5	69.2	3	2
26	IC300474	5	70	2	2
27	IC379489	5	57.1	3	2
28	IC466717	5	64.3	3	2
29	IC213833	5	66.7	3	2
30	IC115230	5	53.8	3	2
31	IC208959	5	58.3	2	2
32	IC218454	5	66.7	2	2
33	IC425999	5	53.8	3	2
34	IC114270	5	66.7	2	2
35	IC114439	5	57.1	3	2
36	IC376497	5	66.7	2	2
37	IC301126	5	71.4	3	3
38	IC378152	5	69.2	3	2
39	IC114477	5	66.7	2	2
40	IC537485	5	77.8	2	2
41	IC216727	5	58.3	2	2
42	IC115551	5	61.5	3	2
43	IC517086	5	71.4	3	2
44	IC115439	5	60	2	3
45	IC458582X	5	64.3	2	3
46	IC218349	5	61.5	2	2

47	IC217810	5	60	2	2
48	IC215139	5	55.6	2	2
49	IC299616	5	70	2	2
50	IC390457	5	76.9	3	2
51	IC116006	5	66.7	2	2
52	IC114969	5	75	3	2
53	IC379667	5	61.5	2	2
54	IC98653	5	78.6	4	3
55	IC386173	5	66.7	3	3
56	IC257587	5	69.2	3	2
57	IC115973	5	77.8	2	2
58	IC519095	5	64.3	3	3
59	IC461318	5	61.5	4	3
60	IC115270	5	60	3	2
61	IC126115	5	75	3	2
62	IC86316	5	61.5	2	3
63	IC218741	5	63.6	3	3
64	RL- 18	5	69.2	3	2
65	RL-43	5	66.7	2	3
66	RL- 53	5	61.5	3	3
67	RL-224	5	70	3	3
68	RL-240	5	60	2	2
69	RL- 737	5	58.3	3	2
70	RL- 1242	5	60	3	2
71	RL- 2308	5	66.7	3	2
72	RL-2730	5	70	3	2
73	RL- 3923	5	70	2	3
74	RL- 3986	5	60	3	3
75	RL-4007	5	70	3	3
76	RL-5374	5	70	2	3
77	RL- 5618	5	55.6	3	3
78	RL- 6636	5	60	2	3

ANTRAS- Average number of tillers regenerated after submergence. ANLRAS- Average number of leaves regenerated after submergence

The results of principle component analysis revealed three principle components accounted for 99.9 per cent of total variation. Among the three PCs, PC1 showed 71.86% of variation with Eigen value 2.15 followed by PC2 and PC3 (17.43% and 10.70%), respectively (Table. 5). The scree plot depicts the percentage of variation between Eigen values and all the principle components (Fig. 2). Submergence tolerance score had the highest significant contribution according to PC1 (35.90) following regeneration percentage of leaves

(34.79) and regeneration percentage of tillers (29.29). Regeneration percentage of tillers showed higher contribution in PC2 (70.03) followed by regeneration percentage of leaves (20.00) and submergence tolerance score (9.95) (Table. 6). Reetisana *et al.* (2022) ^[19] observed effective tillers per plant has positively influenced for contribution of variation. As a result, the regeneration percentage of tillers in the current study revealed the primary basic discriminant character.

Table 5: Eigen values, percentage of variance, cumulative percentage of variance of rice landraces

Principle component	Eigen value	Percentage of variance	Cumulative percentage of variance
PC1	2.15	71.86	71.86
PC2	0.52	17.43	89.29
PC3	0.32	10.70	100

Table 6: Contribution of three principle components of variation in rice landraces

Variables	PC1	PC2	PC3
Score	35.90	9.95	54.13
Regeneration percentage of tillers	29.29	70.03	0.67
Regeneration percentage of leaves	34.79	20.00	45.19

The bio plot (Fig. 3) between PC1 and PC2 depicts the

distribution pattern of genotypes and also the diversity among the genotypes and the traits studied. Fifteen genotypes falling under the III quadrant are assumed to have higher values for regeneration percentage of tillers. Among them IC208155, IC126210, IC205953, IC301077, IC114413, IC216378, IC114971, IC218157, IC115775 and RL-1348 represented the highest mean (3-7 tillers).

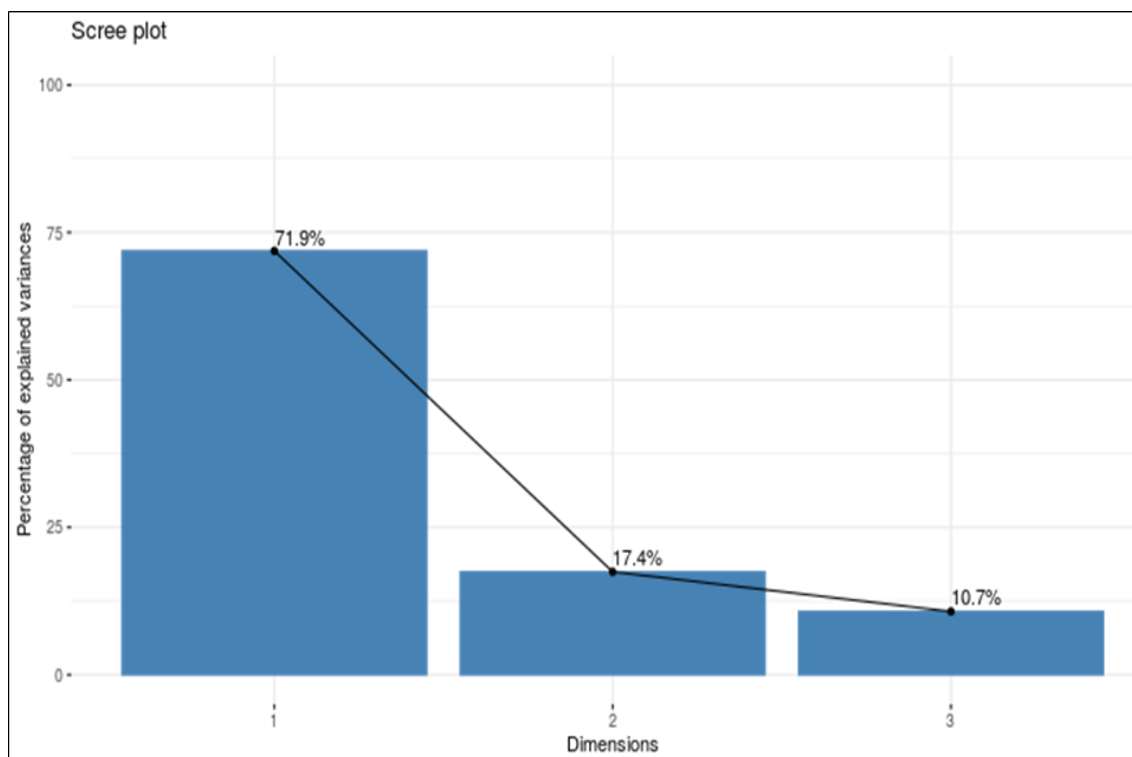


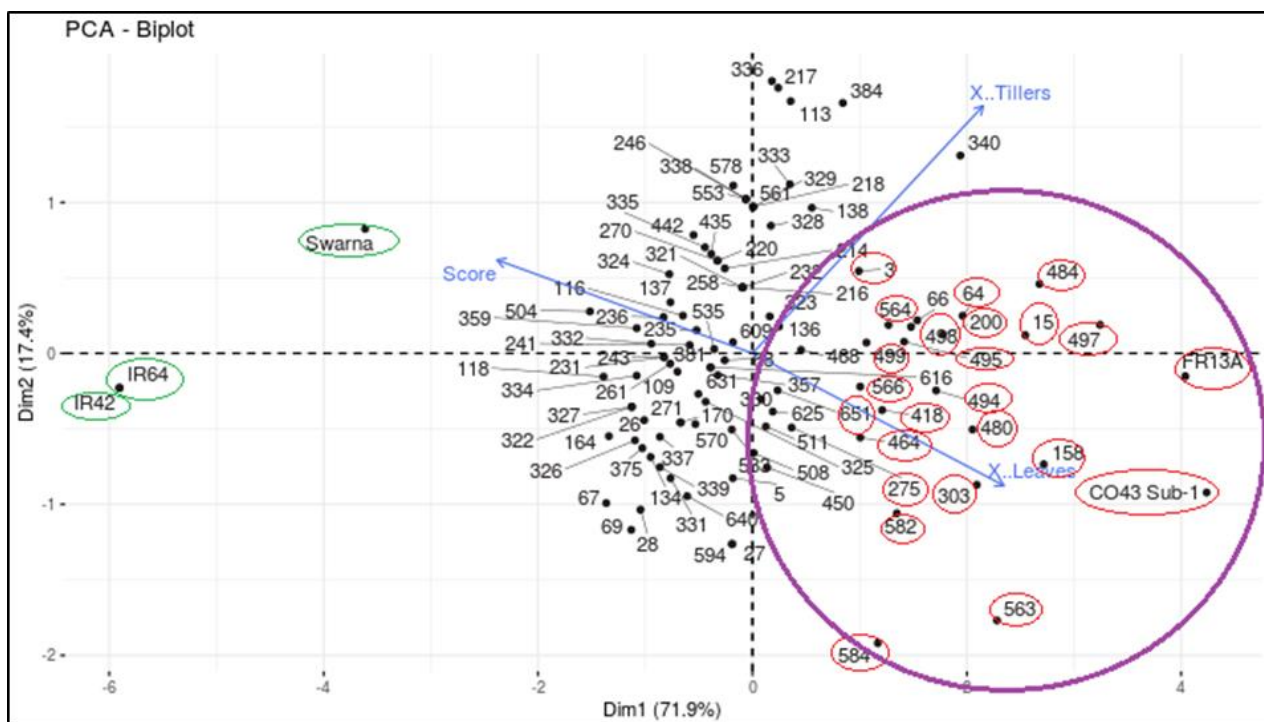
Fig 2: Scree plot diagram constructed using three principle components

In IV quadrant, regeneration percentage of leaves seems to be the most influential character with 20 genotypes. Among them, RL-1757, IC133584, RL-7220, IC518991, IC114575, IC386238, IC388692, IC388900, IC377169, RL-3055, RL1312, RL-3860, FR13A and CO 43-Sub1 have the highest mean (3-5 leaves). The susceptible checks IR64, IR42 and Swarna were observed to present in I and II quadrant denoting lower values for all the characters. This depicts the divergence between the tolerant and susceptible genotypes.

Analysis of variance has revealed significant difference among the genotypes studied (Table 7). Among 660 rice genotypes and five checks, the genotypes showing more than 50% of survival percentage along with > 20% of regeneration percentage of tillers and leaves were selected for genetic variability studies. The analysis of variance showed significant variation among the genotypes, checks and genotypes x checks for all the traits studied which indicates the presence of sufficient variability among the traits. In general phenotypic coefficient of variation was higher than genotypic coefficient of variation indicating that these traits are influenced by environment (Table 8). Phenotypic coefficient of variation was observed to be high in regeneration percentage of tillers (23.02%) whereas, moderate PCV was observed in survival percentage (18.00%). Similar results found by Barik *et al.* (2020) [2] showed higher PCV and lower GCV values for survival percentage in the profiling of rice landraces for submergence tolerance. Low PCV was found in regeneration percentage of leaves (7.91%). Genotypic coefficient of variation was observed as high and

moderate in regeneration percentage of tillers (21.47%) and survival percentage (13.47%) respectively. Difference between GCV and PCV was seen higher in survival percentage (4.53%) followed by regeneration percentage of leaves (2.76%) and regeneration percentage of tillers (1.55%). Heritability defines the traits are least influenced by the environment and have high capacity of the traits to be transmitted from one generation to the subsequent generation. High heritability found in regeneration percentage of tillers (87.05%) subsequently moderate in survival percentage (55.99%) and regeneration percentage of leaves (42.41%). The success of genetic advance under selection depends on the amount of genetic variability present in the base population and the heritability of the trait under study and is expressed in percentage. Genetic advance as percent of mean was found to be high in regeneration percentage of tillers (41.02%) and survival percentage (20.29%). Low genetic advance as percent of mean was low in regeneration percentage of leaves (6.70%). High heritability along with high genetic advance as percent of mean was expressed by regeneration percentage of tillers which signifies additive gene action. Regeneration percentage of tiller has considerable importance, as more number of tillers are likely to produce more number of leaves and panicles which eventually produces higher photosynthetic activity and higher yield per plant respectively.

*Red circled genotypes are the selected ones for further studies as submergence tolerance both by phenotypic and quantitative studies.



*Red circled genotypes are the selected ones for further studies as submergence tolerance both by phenotypic and quantitative studies

Fig 3: Biplot diagram depicting principle components for selected genotypes with high survival percentage

Table 7: Analysis of variance for three traits of rice landraces after de-submergence

Source	DF	Survival percentage	Regeneration percentage of tillers	Regeneration percentage of leaves
Genotypes	24	258.62**	203.68**	43.92**
Checks	1	417.31**	219.02**	43.68**
Total number of genotypes	22	163.22	130.11	7.05
Checks vs genotypes	1	2198.79**	1807.00**	855.44**
Error	8	71.83	16.84	4.06

** Significant at 1 per cent

Table 8: Variability among three traits of rice genotypes after de-submergence

Characters	Mean	Range	PCV %	GCV %	Heritability %	Genetic advance as per cent of mean
Survival percentage	70.96	48.2-96.3	18.00	13.47	55.99	20.29
Regeneration percentage of tillers	49.55	28.1-69.2	23.02	21.47	87.05	41.02
Regeneration percentage of leaves	33.54	27.6-45.9	7.91	5.15	42.41	6.70

Correlation measures the interrelationship between the traits studied which helps to determine the right selection of traits studied. Survival percentage of genotypes found positive and significant intercorrelation with regeneration percentage of tillers (0.68) and regeneration percentage of leaves (0.53). Regeneration percentage of tillers shown positive and significant intercorrelation with regeneration percentage of leaves (0.55) (Table 9) After de-submergence rice genotypes start to recover in normal condition which allows them to utilize the reserved energy to regenerate tillers and subsequently leaves, which together help the plant to recover faster. Farooq *et al.* (2010) [6] observed positive correlation in between the number of tillers and the number of leaves under well-watered conditions while working on rice cultivars of IR64 and four of its NILs.

Cluster analysis was performed for the thirty genotypes selected based on high mean values (regeneration percentage of tillers above 20% and regeneration percentage of leaves above 20%) obtained in third and fourth quadrant of principle

component along with checks and 3 highly susceptible accessions (selected on the basis of most injured plant type) using Ward's method (Table 10). Dendrogram analysis made for the selected 30 genotypes have classified them into five clusters (Fig. 4). Cluster III contains the three most tolerant accessions *viz.*, IC388692, IC377169 and IC205953 and two tolerant checks *viz.*, FR13A and CO43-Sub1 showing highest mean survival percentage and score as one which indicates that these accessions have similar survival rate with the tolerant checks and have good regeneration ability. Next to cluster III, cluster II and cluster I specified the groups containing 8 and 11 accessions respectively, which have tolerant to moderately tolerant genotypes. Cluster IV and V grouped each three accessions in each groups which denotes truly susceptible accessions along with all susceptible checks having lowest survival percentage. Hence, the genotypes grouped under the cluster III can be utilized as potential candidate genotypes for submergence tolerance studies.

Table 9: Correlation between three characters after de-submergence

Traits	Survival percentage	Regeneration percentage of tillers	Regeneration percentage of leaves
Survival percentage	1	0.68**	0.53**
Regeneration percentage of tillers		1	0.55**
Regeneration percentage of leaves			1

Table 10: Cluster composition of highly tolerant and highly susceptible selected rice landraces along with checks

Cluster	Number of genotypes	Name of the genotypes	Mean survival %	Mean Score
I	11	IC208155, IC388900, IC518991, IC114575, IC115775, IC301077, IC218157, RL- 1348, RL- 1757, RL-3860, RL- 7220	84.7	3
II	8	IC216378, IC126210, IC114413, IC386238, IC133584, IC114971, RL- 1312, RL-3055	92.9	1
III	5	IC388692, IC377169, IC205953, FR13A, CO 43 <i>Sub-1</i>	100	1
IV	3	IC135299, IC378553, Swarna	9.37	9
V	3	IC387752, IR64, IR42	0	9

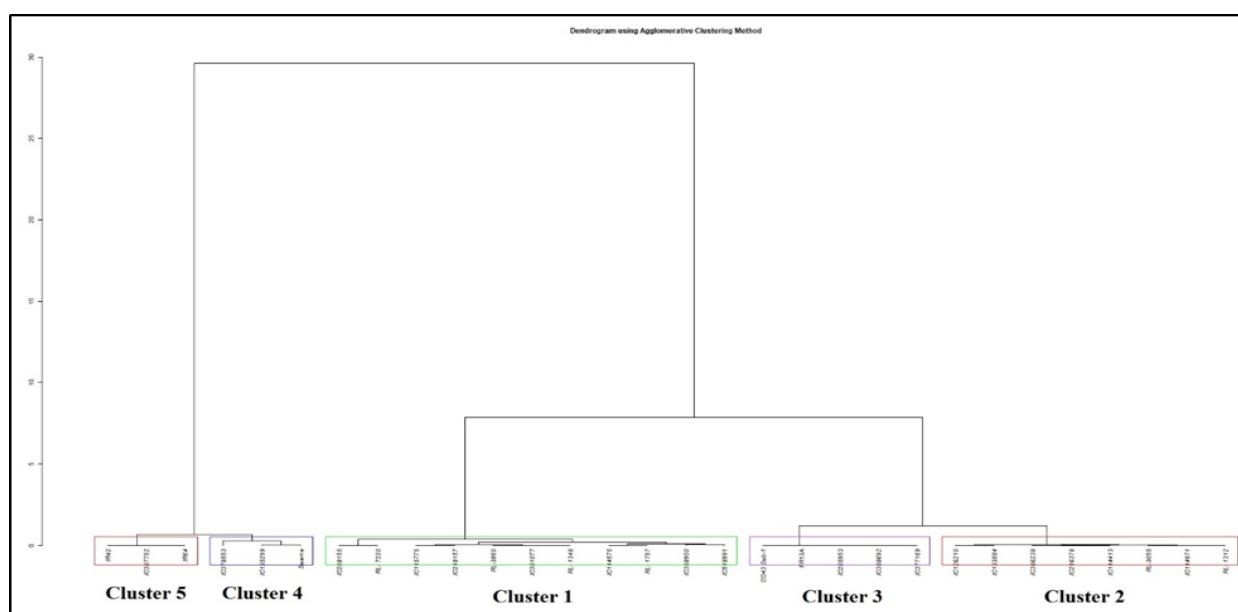


Fig 4: Cluster analysis of highly tolerant and highly susceptible selected rice landraces along with checks

Summary and Conclusion

As complete submergence limits the gaseous exchange, photosynthesis rate, light intensity and reduces the energy reserves it is expected to observe high mortality in plants after going through this stress. Screening was carried out for the submergence tolerance for 660 rice landraces along with checks clearly defines the severances of the submergence stress. Out of 660 accessions approximately 22 accessions viz., IC388692, IC377169, IC205953, IC216378, IC126210, IC114413, IC386238, IC133584, IC114971, RL-1312, RL-3055, IC208155, IC388900, IC518991, IC114575, IC115775, IC301077, IC218157, RL-1348, RL-1757, RL-3860 and RL-7220 showed better performance for tolerance similar to FR13A and CO 43-*Sub1*. Cumulative variance of greater than 70% for the first three PCs showed that the characteristics identified with in axes exhibited a significant influence on the accessions and can be used effectively for selection. Through principle component analysis, submergence tolerance score and regeneration percentage of tillers exhibited high variability which could be highly beneficial to select the suitable tolerant accessions based on these traits. With regard to variability study, additive gene action was expressed by regeneration percentage of tillers and this trait could be

further used in the selection criteria and the genotypes possessing high mean values for this trait could be used as a pre-breeding material and also as a parent in crossing programmer for further improvement in submergence tolerance in rice. Additionally cluster analysis was performed to confirm whether the selected accessions were grouped under respective tolerant and susceptible cluster. This concluded that the selected accessions viz. IC388692, IC377169 and IC205953 performs similar to FR13A and CO 43-*Sub1* were grouped under one cluster. The genotypes viz., IC216378, IC126210, IC114413, IC386238, IC133584, IC114971, RL- 1312, RL-3055 were grouped under separate cluster expressed tolerance to submergence in which these accessions could be further screened and genotyped to use as potential pre-breeding material to be used in submergence tolerance breeding programmer in rice.

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