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MORPHOLOGICAL TRAIT-BASED CLUSTER ANALYSIS REVEALS GENETIC DIVERSITY AND YIELD STABILITY IN SUBMERGENCE-TOLERANT RICE (*ORYZA SATIVA* L.) BREEDING LINES

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ABSTRACT

Submergence stress is a major constraint to rice production in flood-prone ecosystems, particularly in rainfed lowland areas. Identification of genetically diverse and submergence-tolerant breeding lines with stable yield performance is essential for developing resilient rice varieties. The present study evaluated 25 pre-screened submergence-tolerant rice breeding lines along with two checks, FR13A (tolerant) and DRR44 (susceptible), during the *Kharif* seasons of 2021–22. Thirteen agro-morphological and yield-related traits were recorded to assess genetic variability and divergence among genotypes. Significant variation was observed for all studied traits, indicating substantial genetic diversity among the breeding lines. Traits such as moderate plant height, higher seedling vigour, increased number of productive tillers, spikelet number, grain number per panicle and stable spikelet fertility were identified as key contributors to yield performance under submergence conditions. Cluster analysis based on Euclidean distance grouped the genotypes into two major clusters, clearly differentiating submergence-adapted, high-yielding genotypes from poorly performing lines. The tolerant check FR13A clustered with elite breeding lines including IR18A2043, IR18A1567, IR18A1325, IR18A1967 and IR18A1383, indicating their potential utility as donors for submergence tolerance breeding programmes. The study demonstrates the effectiveness of multivariate analysis in identifying promising rice genotypes for improving flood tolerance and yield stability.

***Keywords*:** Submergence tolerance, Rice, Morphological traits, Cluster analysis, Flood stress, Yield performance.

Introduction

Rice (*Oryza sativa* L.) is one of the world's most important staple crops, providing food and livelihood security for billions of people, especially in Asia and Africa (Asma *et al.*, 2023). Despite this, floods and other extreme weather conditions caused by climate change are becoming a greater threat to rice productivity, especially in rainfed lowland habitats (Tellman *et al.*, 2021). Flooding and prolonged submergence impede gas exchange, reduce photosynthesis, restrict aerobic respiration, and alter metabolic homeostasis, resulting in severe growth restriction, which ultimately leads to yield loss. (Oladosu *et al.*, 2020; Wang *et al.*, 2024). Submergence stress occurs during early vegetative stages, including seedling and tillering, which are vulnerable to oxygen deprivation (Wang *et al.*, 2024). These scenarios are common in monsoon-dominated areas of South, Southeast, and East Asia, where rice fields can be fully submerged during heavy rainfall events, resulting in significant yield losses and jeopardising regional food security.

Extensive research over the past decades has identified key genetic and physiological mechanisms underlying rice submergence tolerance. Submergence1A (Sub1A) regulates ethylene-mediated responses that conserve carbohydrate reserves, suppress elongation growth under water, and improve survival and post-stress recovery (Hussain *et al.*, 2023). According to Xu *et al.*, 1996; Fukao *et al.*, 2006, the landrace FR13A is a historical donor of this essential tolerance gene and is frequently employed as a positive control in screening assays because of its high heritability of the trait. Vulnerable lines like IIRR's DRR 44 drought-tolerant variety, which lacks the effective Sub1A-1 allele, die quickly from energy-depleting stem elongation.

Effective breeding programs rely on understanding the genetic diversity and character association among breeding lines (Anumalla *et al.*, 2015; Azam *et al.*, 2023). Morphological parameters such as plant height, days to 50% flowering, panicle length, and spikelet fertility are useful for selection (Raja *et al.*, 2025; Bordoloi *et al.*, 2023). Multivariate statistical approaches, particularly cluster analysis, are frequently employed in rice improvement to classify genotypes based on these variables. This allows breeders to identify genetically diverse accessions for strategic crossing to increase heterosis and improve tolerance (Singh *et al.*, 2024).

The purpose of this study is to analyse morphological diversity and genetic divergence among 25 pre-screened submergence-tolerant rice breeding lines, with FR13A and DRR44 serving as control (Singh *et al.*, 2024). By using cluster analysis on 13 agro-morphological and yield-related variables, we intend to discover superior lines and possible varied donors for future breeding cycles to improve rice production's flood resistance.

Materials and Methods

Experimental site and plant material

The research included 27 rice genotypes, comprising 25 advanced breeding lines previously evaluated for submergence tolerance. Two reference genotypes were included as controls: FR13A served as the submergence-tolerant positive control, and DRR44 served as the susceptible negative control. The experiment was performed in the Crop Physiology Experimental Field, Department of Plant Molecular Biology and Genetic Engineering, Acharya Narendra Deva University of Agriculture and Technology, Kumar Ganj, Ayodhya (U.P.), India, during the *Kharif* seasons from 2020 to 2022.

Morphological Characterization

Data were recorded on 13 morphological traits for each genotype (Tables 1 &2):

- **Vegetative Traits**: Seedling vigour, Plant height (cm), Flag Leaf Area (cm²), Days to 50% Flowering.
- **Yield Components**: No. of spikelets/panicle, No. of grains/panicle, Spikelets fertility (%), Test weight (g), Biological yield/plant (g), Harvest Index (%), Grain yield per plant (g), Panicle length (cm), and Panicles bearing tillers/plant.

Measurements were taken at appropriate growth stages, following standard evaluation procedures outlined by the International Rice Research Institute (IRRI).

Statistical analysis

Analysis of Variance (ANOVA) was performed to identify significant differences among genotypes for all traits. Mean values across three seasons were used for cluster analysis to minimize environmental effect. To group the 25 submergence-tolerant lines, a cluster analysis was conducted using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Euclidean distance. All computations were performed using the R software statistical package and PAST v5.3 (Hammer *et al.*, 2001).

Result and Discussion

Morphological traits analysis

Morphological traits were recorded during the *Kharif* seasons from 2020 to 2022. Days to 50% flowering (DTF) ranged from 78.6 days (IR17A2949) to 118 days (IR 126952:173) reflecting wide variation in crop duration. The tolerant check FR13A flowered at 107.93 days, consistent with its known medium-to-late maturity. Seedling vigour exhibited prominent differences with values ranging from 9.71 (IR15F1869, IR18A1329) to 39.73 (IR 126952:173). High seedling vigour observed in genotypes such as NDR2065, IR17A2949, IR18A1325 and IR18A2043 suggests enhanced early establishment and recovery potential following submergence which is a critical adaptive trait under flash flood conditions (Oliver *et al.*, 2008). Plant height (PH) varied from 71.47 cm (IR 126952:173) to 131.8 cm (IR15F1710). The tolerant donor FR13A recorded a moderate plant height (127.3 cm), supporting the quiescence strategy mediated by Sub1A, which suppresses excessive elongation while maintaining sufficient structure for post-submergence recovery (Das *et al.*, 2005; Sarkar and Bhattacharjee, 2011). Flag leaf area (FLA), a key determinant of photosynthetic capacity ranged between 13.63 cm² (IR18A1567) and 24.85 cm² (IR18A1329). Genotypes such as IR18A2011, IR18A1269, NDR 2065 and IR18A2043 exhibited comparatively larger flag leaf area.

Significant variation was also recorded for yield and yield-contributing characters (Table 2). Panicles bearing tillers per plant (PTP) ranged from 2.95 (IR 126952:173, IR18A1564) to 8.41 (IR18A1967). Higher productive tiller numbers observed in FR13A, IR18A1325, IR18A1967 and IR18A2043 indicating superior sink establishment under stressed conditions. Panicle length (PL) varied from 19.78 cm (IR17A2949) to 32.68 cm (IR17A2906) while, the number of spikelets per panicle (SP) ranged widely with maximum values recorded in IR18A1383 (141.87) and FR13A (111.96). Grain number per panicle (GNP) was highest in IR18A1383 (140.78) and FR13A (136.9), reinforcing the role of effective spikelet retention and fertility under submergence. Spikelet fertility (SF) showed notable variation, ranging from 38.92% (IRRI 148) to 75.59% (IR14T156). FR13A maintained relatively high fertility (69.62%) confirming its stable reproductive performance under submergence stress. Biological yield (BYP) and harvest index (HI) also varied significantly with IR18A2043, IR18A1567, IRRI148 and FR13A recording superior grain yield per plant indicating better source–sink balance (Pradhan *et al.*, 2017).

Table 1: Mean values of vegetative traits in screened submergence breeding line

Genotypes	Days to 50% Flowering	Seedling vigour (SV)	Plant height (PH) (cm)	Flag Leaf Area (FLA)(cm^2)
FR13A	107.93	24.724	127.3	22.013
DRR44	93	15.024	93.13	15.033
IR18A2044	96.6	20.951	90.2	19.013
IR15F1710	90.5	12.651	131.8	15.513
IR18A2011	92.6	17.651	73.8	22.81
IR18A1269	105.9	19.151	74.8	22.11
IR17A3046	90.6	19.151	93.8	19.31
IR18A1126	93.6	19.551	91.8	22.01
IR18A1558	79	11.851	88.8	15.71
IR18A1876	89	14.724	77.13	19.45
IR17A2906	115.5	25.024	93.13	21.35
IR18A2041	81.27	31.224	84.13	17.759
IR18A1383	113.93	13.084	76.8	19.346
IR17A2949	78.6	32.214	78.8	21.349
IRRI148	87.1	14.654	100.3	14.759
IR18A1325	94.6	33.714	81.8	19.749
IR15F1869	101.6	9.714	114.2	21.949
IR18A1281	97.6	22.214	102.8	17.149
IR18A1967	112.3	13.914	122.6	16.649
IR18A1329	95.4	9.714	110	24.849
IR17A2977	91.9	18.127	100.47	15.033
IR18A1567	108.5	18.427	117.47	13.633
IR18A2043	97.2	30.327	102.47	21.533
NDR 2065	101.2	38.197	96.97	22.923
IR14T156	113	10.727	112.47	15.933
IR126952:173	118	39.727	71.47	19.533
IR18A1564	112.5	32.627	90.47	15.033

Table 2: Mean Performance of Yield and Yield-Attributing Traits in screened submergence breeding line.

Genotypes	Panicles bearing tillers/ plant	Panicle length (PL)	No. of spikelet/ panicle	No. of grains/ panicle	Spikelets fertility (SF)	Biological yield/ Plant (BYP)	Harvest Index (HI)	Test weight (TW)	Grain yield per plant (GYP)
FR13A	8.081	28.92	111.96	136.9	69.62	37.6	40.02	24.49	12.26
DRR 44	6.021	21.75	106.42	110	65.25	27.4	25.48	19.46	8.9
IR18A2044	7.361	25.25	99.77	74.1	66.1	32.3	18.69	23.61	6.37
IR15F1710	7.361	27.05	85.84	92.1	69.62	22.4	27.62	15.2	7.86
IR18A2011	5.361	21.75	108.9	67.1	64.25	27.5	23.26	23.04	6.61
IR18A1269	7.361	23.15	106.1	80.1	48.65	40	22.25	15.45	7.99
IR17A3046	6.021	25.15	89.75	73.1	63.35	31.4	21.61	17.98	6.61
IR18A1126	4.361	28.25	83.35	66.1	73.57	22.9	34.56	22.24	8.72
IR18A1558	4.691	26.15	92.56	64.1	61.31	34.9	20.77	22.46	7.48
IR18A1876	6.364	29.78	116.3	79.51	53.45	34.8	25.63	24.07	8.26
IR17A2906	6.364	32.68	101.79	76.51	51.66	18.6	32.1	16.06	6.57
IR18A2041	6.0347	27.68	95.07	127.56	61.9	34.9	26.507	24.72	8.754
IR18A1383	3.8346	27.68	141.87	140.78	44.98	29.2	26.234	20.55	9.344
IR17A2949	7.7513	19.78	120.83	94.46	45.18	21.5	26.227	13.44	6.837
IRRI 148	5.8013	22.56	123.48	78.66	38.92	37.6	29.667	27.89	12.517
IR18A1325	8.0813	27.88	114.33	90.6	42.06	33.7	26.837	15.65	8.437
IR15F1869	5.4113	24.78	69.84	86.54	70.22	15.2	34.117	15.66	6.727
IR18A1281	5.0813	23.68	125.96	84.54	41.28	27.4	25.867	14.35	7.627
IR18A1967	8.4113	26.88	85.22	81.54	53.57	10.4	44.187	14.14	8.137
IR18A1329	6.7513	24.88	71.79	89.54	67.42	16.8	33.097	19.55	6.537
IR17A2977	4.618	22.62	107.18	79.75	57.49	10.2	36.337	18.18	5.034
IR18A1567	4.618	28.92	120.8	86.75	46.04	27	36.447	14.08	12.944
IR18A2043	6.948	27.62	111.96	77.75	42.01	31.8	33.137	24.49	12.264
NDR 2065	6.558	27.36	106.42	63.75	58.41	31.4	28.857	19.46	8.904
IR14T156	6.948	23.72	95.46	90.75	75.59	15.6	33.077	21.81	6.474
IR 126952:173	2.948	22.62	80.66	83.75	71.67	8.3	40.027	26.46	4.854
IR18A1564	2.948	24.72	102.3	86.75	64.01	37.1	25.487	13.57	8.234

Cluster Analysis

Cluster analysis based on 13 agro-morphological and yield traits grouped the 27 genotypes (25 breeding lines and 2 checks) into two major clusters supported by a high bootstrap value (100) at the basal node, reflecting genetic divergence among the screened lines (Figure 1). The clustering broadly separated genotypes with high yield potential and submergence adaptability from those with poor reproductive efficiency and lower recovery ability. The tolerant check FR13A and IR18A1383 form a sub-cluster in Cluster1 with moderate bootstrap support (~50), indicating similar agro-morphological responses associated with submergence tolerance. Moreover, IR18A1383 and FR13A recorded high spikelet and GNP. Additionally, FR13A also sub-clustered with genotypes such as IR18A2043, IR18A1567, IR18A1325 and IR18A1967, which exhibited higher productive tillers, superior GYP, moderate PH and SF. Cluster 1 represented breeding lines combining morphological resilience with yield stability and can serve as a valuable donor group for submergence tolerance breeding.

In contrast, the susceptible check DRR44 grouped with genotypes characterized by lower BYP, reduced HI and inferior GY, confirming its sensitivity to submergence stress due to the absence of the effective Sub1A-1 allele.

Cluster 2 included genotypes such as IR15F1710, IR15F1869, IR17A2977, IR17A2906, NDR 2065 and IR126952:173 that exhibited moderate to low GYP. This cluster was characterized by relatively lower spikelet and grain numbers but higher SF and SV, suggesting that early vigour alone is insufficient for sustained tolerance and yield recovery.

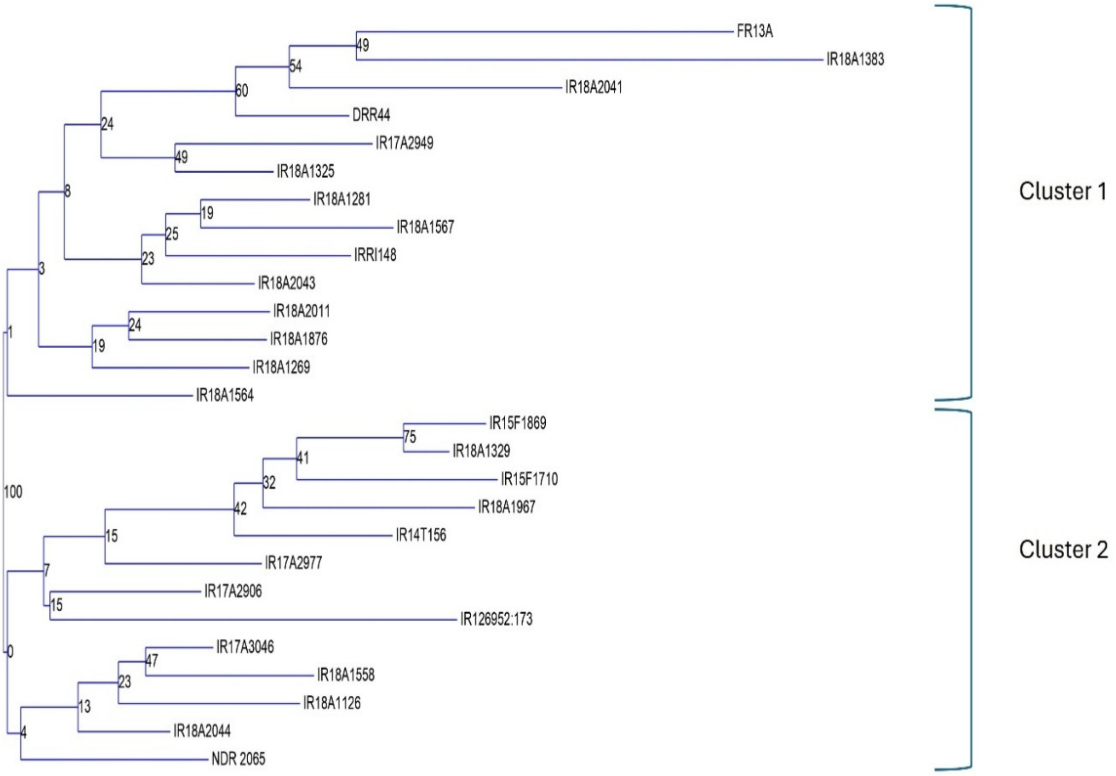

Fig. 1: Cluster analysis of 27 breeding lines based on 13 morphological traits.

Conclusion

The study revealed significant genetic variability among the 25 screened rice breeding lines for key morphological and yield-related traits under submergence conditions. Traits such as moderate PH, higher productive tillers, greater spikelet, GNP and stable SF were critical determinants of yield resilience under stress.

Cluster analysis grouped the genotypes into two major clusters, clearly separating submergence-adapted, high-yielding lines from poorly performing genotypes. The tolerant check FR13A clustered with elite lines including IR18A2043, IR18A1567, IR18A1325, IR18A1967 and IR18A1383, indicating their potential as valuable donors for submergence tolerance breeding. In contrast, DRR44 grouped with low-yielding genotypes confirming its susceptibility due to the absence of the effective Sub1A-1 allele.

Overall, multivariate analysis proved effective in identifying genetically diverse and agronomically superior genotypes that can be exploited in future breeding programs to enhance flood tolerance and yield stability in rice.

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