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Genetic diversity studies in the association mapping panel for sheath blight and attributing traits in rice (*Oryza sativa* L.)

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Abstract
The genetic divergence analysis of the association mapping panel using Mahalanobis D² statistics revealed substantial genetic variability among the 161 rice genotypes, which were categorized into 20 distinct clusters. Cluster III included the highest number of genotypes (41), followed by Cluster II with 34 genotypes. The highest intra-cluster distance was noted in Cluster XII (3408.90), indicating a high level of genetic variability within this group, followed by Clusters VIII, XIV, IV, III, II, and V, with the lowest observed in Cluster I (144.79). The greatest inter-cluster distance was recorded between Clusters XII and XIV (104161.70), followed by Clusters X and XIV (93870.59), emphasizing significant genetic divergence between these groups. The consistently higher inter-cluster distances compared to intra-cluster distances further underscore the wide genetic diversity across clusters. Considerable phenotypic variation was also observed for RLH% at flowering and associated yield traits. Clusters such as XX, I, IV, VIII, V, XIX, III, and XII exhibited lower RLH%, suggesting the presence of potential sheath blight (ShB) tolerant genotypes. Conversely, Cluster XVII recorded the highest RLH%, indicating susceptibility. Substantial differences among clusters were also noted for traits including days to 50% flowering, plant height, panicle number, panicle length, grain number, and test weight. These distinct and contrasting trait patterns across clusters provided valuable resources for breeding programs, QTL discovery, and allele mining aimed at enhancing ShB resistance and yield performance in rice.

Keywords: Genetic divergence, rice, Mahalanobis D², sheath blight, phenotypic variation

Introduction
Rice (*Oryza sativa* L.) serves as a staple food and remains one of the most critical crops for global food security, providing nourishment to over 50% of the world's population (Rathna Priya *et al.*, 2019) [20]. In India, it is the leading food crop in terms of cultivated area, total production, and productivity, positioning the country as the second-largest rice producer and exporter globally. To meet future demands and break existing yield barriers, the development of high-yielding varieties through the use of genetically diverse parental lines in breeding programs is essential. The effectiveness of any crop improvement strategy relies heavily on the extent of genetic variability present among genotypes (Allard, 1960) [2]. Rice, being genetically diverse, offers immense potential for enhancement. Assessing the level of genetic diversity for economically important traits is a crucial preliminary step, as these traits directly impact yield, marketability, and the overall sustainability of rice cultivation. However, Rice productivity is persistently threatened by various abiotic and biotic stresses. Among the most damaging biotic stresses is sheath blight (ShB), caused by the fungal pathogen *Rhizoctonia solani* Kühn, with its teleomorph known as *Thanetophorus cucumeris* (Frank) Donk. This disease can cause yield losses of up to 50% in severe cases and also reduces the quality of straw, affecting its use as livestock feed (Prakasam *et al.*, 2025) [18]. To date, no immune genotype has been identified in either cultivated rice (*O. sativa*) or its wild relatives (Eizenga *et al.*, 2002; Liu *et al.*, 2006; Dey *et al.*, 2016) [10, 15, 9]. Nonetheless, previous studies have documented considerable variation in ShB resistance, with most genotypes exhibiting only moderate levels of resistance (Jia *et al.*, 2007; Dey *et al.*, 2016) [12, 9].

Recently, the association mapping panel used in this study was explored for culm-related traits through GWAS (Badri *et al.*, 2024) [4]. Building on that, the present investigation focused on assessing the genetic divergence among genotypes concerning ShB resistance and key agronomic traits, aiming to support future efforts in molecular mapping and trait improvement.

Materials and Methods

The present investigation was carried out during *Kharif* 2024 at two locations, the ICAR-Indian Institute of Rice Research farm, Rajendranagar, and the ICAR-Indian Institute of Rice Research farm at Ramachandrapuram (ICRISAT campus). The experimental material consisted an association mapping panel comprised of 164 diverse genotypes, obtained from ICAR-Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad, Telangana, were sown in nursery beds and transplanted into the main field in augmented randomized complete block design (ARCBD) across two locations with a spacing of 20 x 15 cm. The local virulent isolate of rice sheath blight pathogen Wgl-12-1, ICAR-IIRR strain obtained from the division of Plant Pathology at ICAR-IIRR, Hyderabad, was utilized for phenotyping and was inoculated with colonized typha pieces at the maximum tillering stage. Plant height (cm) and lesion height (cm) were measured at the flowering stage, and RLH% was calculated using.

$$\text{RLH\%} = \frac{\text{Lesion height}}{\text{Plant height}} \text{x}100$$

The mean RLH values recorded for floering stage were used to categorize the disease reaction of each genotype according to the Standard Evaluation System (SES) developed by IRRI, Philippines (IRRI, 2002), presented in Table 1.

Table 1: Standard Evaluation System (SES) (IRRI, 2014) for sheath blight of rice

Disease score	Disease reaction	Relative Lesion Height (RLH %)
0	Immune	No infection
1	Highly Resistant	Vertical spread of lesion up to 20% of the plant height
3	Resistant	Vertical spread of lesion up to 21-30% of plant height
5	Moderately Resistant/Moderately Susceptible	Vertical spread of lesion up to 31-45% of plant height
7	Susceptible	Vertical spread of lesion up to 46-65% of plant height
9	Highly Susceptible	Vertical spread of lesion up to 66-100% of plant height

Single plant observations were recorded on five plants for characters viz., days to 50 percent flowering (DFF), plant height (cm), panicle length (cm), panicle number (PN), test weight (TW), and number of grains per panicle. However, DFF recorded on a plot basis. Best Linear Unbiased Prediction (BLUP) values were utilized for statistical analysis using INDOSTAT software, and genetic divergence was assessed following Mahalanobis' D^2 statistics (Mahalanobis, 1936) [16].

Results and Discussion

Using Tocher's method based on D^2 statistics (Rao, 1952), the 161 genotypes in the association mapping panel were classified into 20 distinct clusters (Table 2). Cluster III contained the highest number of genotypes, comprising 41 entries. This was followed by clusters II, VIII, IV, XII, V, XIV, and I, with 34, 21, 19, 15, 10, 7, and 2 genotypes, respectively. In contrast, clusters VI, VII, IX, X, XI, XIII, XV, XVI, XVII, XVIII, XIX, and XX each included one genotype. The overall cluster composition underscored high, medium, and low genetic similarity among the genotypes. Clusters III (41 genotypes) and II (34 genotypes) reflected a considerable level of close genetic relationships among their members, indicating a common origin, possibly derived from similar geographic regions. Clusters like VIII (21 genotypes), IV (19 genotypes), XII (15 genotypes), V (10 genotypes), XIV (7 genotypes), and I (2 genotypes) showed moderate levels of diversity. These clusters might have represented distinct sub-groups within the broader gene pool, sharing some traits but also maintaining distinguishable differences. Shahidullah *et al.* (2009) [22] suggested selecting genotypes belonging to moderate diversity in order to exploit the benefits of heterosis. Above all, the selection of genotypes is dependent on the objectives of the breeding programme. Clusters with only one genotype indicated high heterogeneity. These results were in accordance with Karuppaiyan *et al.* (2013) [13], Manohara and Singh (2013) [17], Allam *et al.* (2014) [1], Bhadra and Roy (2014) [6], Beevi and Venkatesan (2015) [5], Kumar *et al.* (2015) [14], Bharathi *et al.* (2016) [7], Chandramohan *et al.* (2016) [8], and Ashok *et al.* (2017) [3].

The average intra-and inter-cluster D^2 values among the 20 clusters were presented in Table 3. Intra-cluster D^2 values were zero in clusters VI, VII, IX, X, XI, XIII, XV, XVI, XVII, XVIII, XIX, and XX, as these clusters were composed of single genotypes, and the genotypes within them were diverse from all others in the panel, presenting an opportunity for identifying novel QTLs or alleles for molecular mapping approaches. The intra-cluster distance observed in 8 distinct clusters, with highest in cluster XII (3408.90), followed by VIII (2191.87), XIV (2126.86), IV (2068.45), III (1902.65), II (1486.83), V (206.91), and least for I (144.79), revealing that some of the genetic divergence still existed among the genotypes of the cluster. Diversity among the genotypes ranges from 290.45 to 104161.70. The highest inter-cluster distance was observed between clusters XII and XIV (104161.70), and the second highest distance with 93870.59 between clusters X and XIV. The greater the distance, the wider the genetic diversity among the genotypes of those clusters (Haque *et al.*, 2014). This finding also implies that higher genetic diversity among the genotypes of those clusters can be potentially exploited for heterosis in cross-breeding. The lowest inter-cluster distance was observed between clusters XVI and XVII (290.45). Additionally, lower intra-cluster distances than inter-cluster distances reflect the presence of considerable allelic variation among the genotypes in different clusters, highlighting the genetic richness within the panel.

Table 2: Clustering pattern among in the panel under study by the Tocher's method

Cluster number	Number of genotypes	Name of genotypes
Cluster I	2	G124, G126
Cluster II	34	G97, G98, G69, G105, G83, G67, G76, G78, G54, G74, G52, G156, G53, G70, G131, G81, G138, G130, G51, G132, G157, G88, G147, G160, G103, G44, G86, G104, G143, G152, G68, G40, G149, G75
Cluster III	41	G118, G125, G119, G129, G87, G115, G102, G111, G123, G64, G36, G42, G90, G91, G148, G27, G93, G49, G79, G140, G96, G77, G62, G101, G7, G50, G66, G3, G60, G154, G146, G155, G5, G24, G73, G59, G58, G89, G43, G141, G41
Cluster IV	19	G19, G29, G34, G32, G1, G57, G10, G65, G26, G150, G31, G151, G48, G46, G63, G55, G39, G161, G13
Cluster V	10	G107, G120, G108, G128, G114, G144, G116, G94, G121, G109
Cluster VI	1	G56
Cluster VII	1	G134
Cluster VIII	21	G137, G145, G37, G18, G25, G16, G21, G30, G28, G12, G61, G9, G22, G15, G33, G23, G11, G47, G113, G84, G4
Cluster IX	1	G99
Cluster X	1	G92
Cluster XI	1	G158
Cluster XII	15	G122, G133, G127, G100, G153, G136, G135, G112, G110, G95, G106, G139, G142, G85, G72
Cluster XIII	1	G71
Cluster XIV	7	G17, G20, G45, G14, G35, G38, G8
Cluster XV	1	G80
Cluster XVI	1	G6
Cluster XVII	1	G82
Cluster XVIII	1	G117
Cluster XIX	1	G159
Cluster XX	1	G2

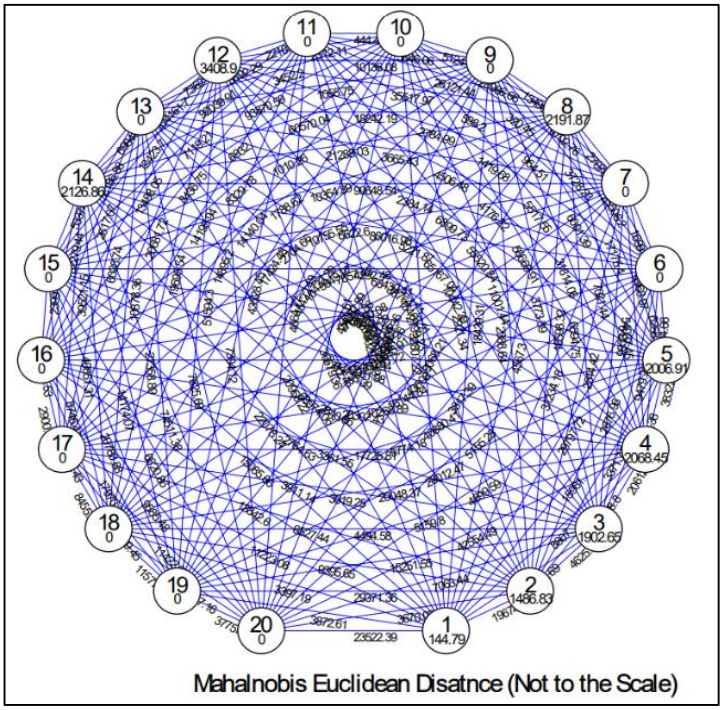

Fig 1: Pairwise genetic divergence (using Mahalanobis Euclidean distances) among clusters formed in the association mapping panel.

The cluster means of RLH% recorded at flowering stage and attributed yield traits were presented in Table 4. Clusters have shown considerable variability for the traits under study. For RLH% at flowering stage, the lowest RLH% values were observed in clusters XX (25.11), I (33.14), IV (36.52), VIII (39.02), V (41.73), XIX (41.88), III (42.51) and XII (43.39), indicating these clusters may harbor ShB-tolerant genotypes and are good candidates for identifying favorable alleles/QTLs to ShB. While, highest RLH% were scored by clusters XVII (70.77), indicating highly susceptible genotypes within the clusters. Similarly, for days to 50% flowering highest cluster mean was observed for cluster VII (117.83) and the lowest for VI (88.17). PH was recorded highest in cluster XVIII (155.01) lowest was recorded in cluster XIII (8.52). PN recorded the highest in cluster XVII (14.67) and lowest in cluster XIX (6.27). Cluster I and XI recorded the highest mean value for PL with 27.58, and the lowest was recorded by cluster IX with 18.78. GN recorded the highest in cluster XIV (385.28) and the lowest in cluster XII (67.87). TW recorded the highest in cluster XX (26.26) and the lowest in cluster IX (15.09). Similarly, Supriya *et al.*, 2017 [24], Sridhar *et al.*, 2016 [23] and Rathod *et al.*, 2017 [21] also reported varied cluster means for yield and related characters in rice genotypes.

Table 3: Intra (diagonal) and inter-cluster distances (D^2 values) of the association mapping panel

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
Cluster I	144.79	19674.77	8721.69	8807.00	15981.00	29797.72	31234.18	4567.3	18439.31	32807.22
Cluster II		1486.83	4625.91	38008.80	3371.75	2676.96	2544.42	16598.32	2088.64	2921.34
Cluster III			1902.65	20612.74	5509.38	9479.36	9933.90	6594.75	3773.39	11001.44
Cluster IV				2068.45	38322.47	51094.66	54037.45	7021.44	31814.06	56669.91
Cluster V					206.91	6256.53	4968.97	17175.40	6001.39	5517.55
Cluster VI						0.00	1699.01	25506.20	3728.23	964.51
Cluster VII							0.00	27079.17	4702.76	320.45
Cluster VIII								2191.87	13490.89	28986.66
Cluster IX									0.00	5124.90
Cluster X										0.00
Cluster XI										
Cluster XII										
Cluster XIII										
Cluster XIV										
Cluster XV										
Cluster XVI										
Cluster XVII										
Cluster XVIII										
Cluster XIX										
Cluster XX										

	Cluster XI	Cluster XII	Cluster XIII	Cluster XIV	Cluster XV	Cluster XVI	Cluster XVII	Cluster XVIII	Cluster XIX	Cluster XX
Cluster I	30202.49	36679.36	20958.22	22078.24	15085.66	13842.60	11223.08	3397.19	3872.61	23522.39
Cluster II	2799.23	6279.21	2112.48	68974.06	2764.63	3911.14	6527.44	9395.65	29371.36	3670.02
Cluster III	10442.39	15468.21	5675.06	44158.39	2840.86	3361.55	3019.25	4494.58	15251.55	7063.44
Cluster IV	55520.84	65167.04	38764.71	6782.96	26961.98	26307.43	17725.81	20048.37	5159.80	42554.49
Cluster V	4176.22	6899.73	5221.87	68439.41	6494.99	6469.94	10268.89	5774.18	28012.47	4699.59
Cluster VI	1419.68	4506.48	2384.14	86016.98	4292.46	7275.89	11261.72	16144.84	40880.41	5155.25
Cluster VII	598.20	2784.99	3665.43	90648.54	6622.60	7657.42	13080.08	16533.91	43608.10	3817.39
Cluster VIII	28121.44	35517.97	18242.19	21288.03	10354.89	10755.55	5949.97	7857.36	6981.26	19068.21
Cluster IX	5646.06	10136.08	1055.75	60570.04	1010.26	1788.62	2704.66	10433.29	24082.89	6609.36
Cluster X	444.40	2512.11	3452.20	93870.59	6932.00	8329.78	14140.04	17424.48	45422.49	4337.61
Cluster XI	0.00	2210.68	3692.29	92039.91	7119.21	8436.75	14195.94	14895.20	43528.45	4664.72
Cluster XII		3408.90	7362.76	104161.70	12423.70	13498.05	20681.72	19695.54	51594.30	7304.12
Cluster XIII			0.00	69848.15	2186.88	2617.07	6028.74	10878.36	27358.89	7625.69
Cluster XIV				2126.86	53374.75	52279.44	39521.15	40851.31	14174.07	74511.38
Cluster XV					0.00	2399.59	2284.53	8746.47	20780.68	6620.86
Cluster XVI						0.00	290.45	7497.43	17970.35	9585.48
Cluster XVII							0.00	8455.94	14059.45	11422.65
Cluster XVIII								0.00	11577.63	12587.16
Cluster XIX									0.00	37757.14
Cluster XX										0.00

Table 4: Cluster means of the panel for sheath blight and its attributing traits

Clusters	RLH% at FS	DFF	PH	PN	PL	GN	TW
Cluster I	33.14	105.00	250.20	8.25	27.58	250.20	21.91
Cluster II	49.63	106.79	127.29	10.82	22.99	127.29	22.02
Cluster III	42.51	109.95	180.34	9.61	23.05	180.30	20.28
Cluster IV	36.52	114.80	316.60	8.13	23.34	316.60	18.41
Cluster V	41.73	109.15	129.71	7.61	26.02	129.71	19.52
Cluster VI	51.97	88.17	97.93	10.03	21.77	97.73	23.16
Cluster VII	47.55	117.83	87.30	7.67	20.98	87.30	19.49
Cluster VIII	39.02	112.43	246.56	8.55	25.34	246.56	19.54
Cluster IX	60.92	104.67	147.37	13.27	18.78	147.37	15.09
Cluster X	49.44	107.67	81.97	6.87	21.78	81.97	22.79
Cluster XI	54.13	105.83	85.10	7.87	27.58	85.10	23.10
Cluster XII	43.39	106.20	67.87	8.70	23.78	67.87	20.97
Cluster XIII	57.70	99.67	130.17	9.87	21.98	130.17	25.09
Cluster XIV	38.68	112.78	385.28	8.19	24.63	385.28	19.74
Cluster XV	50.60	94.67	161.57	9.87	25.38	161.57	24.25
Cluster XVI	62.95	131.60	165.80	8.39	22.26	165.80	16.92
Cluster XVII	70.77	105.67	196.17	14.67	20.38	196.17	17.28
Cluster XVIII	45.64	105.50	194.10	6.55	27.38	194.10	22.49
Cluster XIX	41.88	111.83	288.50	6.27	23.58	288.50	23.27
Cluster XX	25.11	106.83	116.10	9.29	26.89	116.10	26.26

RLH% at flowering, Relative Lesion height at flowering stage; DFF, Days to fifty percent flowering; PH, Plant height; PN, Panicle number; PL, Panicle length; GN, Grain number; TW, Test weight

Cluster-based trait associations revealed key groups of interest for ShB resistance and yield-related traits. Cluster XVII, though it exhibited the highest RLH%, also recorded the highest PN among all clusters. This distinct combination makes it a valuable resource for developing segregating populations to dissect the genetic basis of disease susceptibility and yield traits through trait contrast analysis. Given its consistent performance in disease susceptibility, Cluster XVII could be standardized as a susceptible check in both association mapping studies and field-based phenotypic screenings. In contrast, Clusters XX, I, IV, and VIII, which showed lower RLH% values, are promising candidates for GWAS targeting ShB resistance. These clusters also exhibited favorable performance for key agronomic traits, making them ideal for the simultaneous identification of genomic loci related to resistance and productivity. Additionally, Clusters I and XIV may serve as suitable resources for trait pyramiding strategies that aim to combine resistance and yield potential. Collectively, the diverse and contrasting trait profiles observed across the clusters provide a robust foundation for targeted breeding, QTL mapping, and allele mining to enhance ShB resistance and productivity in rice. These findings, along with prior evaluations of the same panel for culm-related traits (Badri *et al.*, 2024) [4], can be further leveraged to conduct GWAS for sheath blight tolerance.

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