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Genetic diversity studies and identification of donors for lodging resistance in rice (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) stands as the world's primary food crop, and caters to the dietary requirements of countless individuals each day. Modernization of rice cultivation warrants the development of non-lodging rice cultivars which facilitate mechanical harvesting without loss of grains in the field. Lodging of rice is influenced by anatomical and chemical characteristics of the stem cell wall. In the present study, 54 rice genotypes were evaluated for morphological traits including plant characters determining stem strength like stem girth (SG), stem wall thickness (ST), and plant height. Diversity analysis based on quantitative traits categorized the 54 genotypes into nine clusters. The highest inter cluster distance was shown between cluster IV and cluster IX (12025.89) and the maximum intra-cluster distance was shown by cluster V. The Principal Component Analysis identified four PCs explaining 71.85% of the total variation. Histochemical staining for lignin deposition revealed that the genotype Rajamudi had dark red staining pertaining to more lignin content which is an indicator of lodging resistance. The germplasm was further analyzed using two SSR markers linked to *Strong Culm Locus* responsible for culm strength, the results confirmed that the following genotypes *viz., Salem Channa, Vellai Chithiraikar, Vellai Kuruvai* and *Rajamudi* might harbour the genes for strong culm. Besides molecular basis, morphological and histochemical studies also confirmed the lodging tolerance of these genotypes and can be employed as efficient donors to develop lodging-resistant varieties with high yield.

Keywords: Genetic diversity, lodging resistance, SCM 2 locus, lignin, stem characters

INTRODUCTION

Rice stands as a prominent food crop on a global scale and in India, it is cultivated over an extensive area of approximately 46.4 million hectares. India is the world's second-largest rice producer with a production of 195.4 million metric tons. The success of breeding program depends upon the quantum of genetic variability available for exploitation and the extent to which the desirable characters are heritable. Selection is also effective when there is a significant amount of genetic variability among the individuals in breeding materials (Sumanth *et al.*, 2017). The genetic distance estimated from genetic divergence analysis provides a contribution of traits for the total divergence (Kumari *et al.*, 2018). The traits contributing most to the observed variation are estimated by the statistical tool Principal Component Analysis (Sheela *et al.*, 2019). The advent of the Green Revolution introduced semi-dwarf varieties with the *semidwarf 1* (*sd1*) gene and these varieties exhibit reduced internode length, and enhanced resistance to lodging. Nonetheless, the reduction in the upper internode's length could potentially hinder the proper positioning of the panicle and negatively impact the culm diameter, resulting in the development of weakened stems that make it susceptible to lodging (Shah *et al.*, 2019). Lodging refers to the shifting of plant stems from their upright orientation. Lodging has the potential to decrease

the yield due to diminished canopy photosynthesis, high respiration, restricted movement of nutrients and carbon for grain development, and increased vulnerability to pest attacks (Hitaka et al., 1969). Consequently, delving into the mechanisms governing the lodging resistance of rice and understanding their capabilities holds immense importance. The key factors contributing to lodging encompass both morphological and anatomical attributes, as well as the stem's chemical composition (Shah et al., 2019). The strength of the stem is intricately linked to its anatomical traits, including stem diameter, the thickness of stem wall tissues, the quantity of large vascular bundles, and sheath width. Lignin content represents a pivotal constituent within the cell wall, playing a crucial role in maintaining plant robustness and resilience against both biotic and abiotic challenges. such as lodging. The presence of lignin contributes significantly to enhancing the mechanical fortitude and firmness of the stem, thereby upholding the stability of various cell wall elements (Li et al., 2022). Through GWAS (Genome-Wide Association Studies), (Ookawa et al, 2010) unearthed novel Quantitative Trait Loci (QTL) linked to traits associated with robust culms. These advantageous genetic variants were found to be present in traditional landraces. This highlights the potential of leveraging strong culm-related genes from landraces, previously assumed to lack lodging resistance due to their long stems, to enhance the lodging resistance of modern breeding rice varieties. The current study focuses on the genetic studies in 54 rice germplasms and identification of donors for lodging resistance based on morphological, biochemical traits and molecular markers studies (presence of linked markers RM1130 and RM3430) based on the presence of Strong Culm Loci (SCM2).

MATERIALS AND METHODS

The experimental material comprised of 54 rice germplasm lines. The seeds of these genotypes were collected and sown at Department of Rice, CPBG, Tamil Nadu Agricultural University, Coimbatore. The genotypes were sown in nursery and one month old seedlings were transplanted by adopting randomized block design with three replications. The observations were recorded on days to 50 % flowering (DFF), plant height (PH), number of productive tillers (NPTP), length of flag leaf (FLL), flag leaf width (FLW), length of panicle (PL), 100 grain weight (GW) and single plant yield (SPY). Observations on stem girth (mm) and stem wall thickness (mm) were recorded at the stage of 20 days after heading. Basal stem part above 20cm from the ground level was taken for measuring stem girth using vernier caliper. Stem thickness was measured by cutting thin sections of stem part and visualizing in stereo microscope with image analyzer. Statistical analysis for genetic diversity and PCA were done using 'Biotools' packages of R software (ver. 4.2.1) and GRAPES software respectively.

Histochemical Staining: Eight genotypes (four lodging tolerant and four lodging susceptible) were chosen for histochemical staining of the stem cuttings. Weisner reaction was carried out to histochemically locate the lignin according to the standard protocol. Fresh hand-cut sections (~20 μ m) of culms of the genotypes *viz.*, Rajamudi, Salem Channa, Vellai Gundu and Salem 3 were incubated in phloroglucinol-HCL solution (2 volume 3% in ethanol:1 volume concentrated HCI) for 2 min. The sections were rinsed with distilled water 3-4 times until the solution was clear. These sections were then mounted on a glass slide and observed under a bright-field microscope and viewed under magnification of 10X and 40X for vivid visualization of lignin distribution across the stem.

Molecular evaluation for *Strong culm loci* (*SCM2*) linked markers: *SCM2* locus confers thick culm which ultimately results in lodging resistance. Among the 54 germplasms, 20 genotypes were selected based on the highest and the lowest stem girth and stem wall thickness. Seeds of these entries were grown in green house condition. Fresh leaf samples were taken to isolate genomic DNA using CTAB method. The quality of DNA was checked by Nanodrop method and diluted for PCR amplification. Two SSR markers defined to *SCM2 viz.*, *RM1130* and *RM3430* locus were used to identify the lodging resistant donors.

Marker	Sequence	Вр
RM1130	F-5' AGATCGGATTGGGATGGC 3' R-5'ACCCAACCAATTAGTGCCAC 3'	129
RM3430	F-5' ACGACGACGATCAAGAAC 3' R-5' CGAGAGCCACCTAATCTTG 3'	214

RESULTS AND DISCUSSION

Characterization and evaluation of germplasm is prerequisite to screen out the desired genetic materials for the use in breeding program (Begna and Begna 2021). The mean values for the traits are given in Table 1. Analysis of variance of 54 genotypes for the 10 characters is furnished in Table 2. All genotypes showed significant variation for all the traits including stem girth and stem wall thickness. These results agree with the findings of Keerthiraj et al, 2020. The result showed significant difference among all the genotypes for all other traits. Among the genotypes, maximum stem girth was recorded for Vellai Chithiraikar (6.60mm) followed by Rajamudi (6.3mm) and Salem channa (6.47) and the lowest was recorded for Kitchalli Samba (2.53mm). For stem wall thickness, the highest reading was recorded for IC205953 (1.04mm) and the lowest was recorded for Manavi selection (0.26mm). The maximum grain yield was recorded by Salem Channa (37g) and minimum grain yield was recorded for IC218157 (14.21g). The maximum plant height was recorded by IC205953 (159.57cm) and the minimum plant height was recorded by Muttakur (60.2cm). Plant height is one of the most important morphological traits that plays a significant role in determining whether

Table 1. List of rice genotypes and their mean values

S.No.	Entry	DFF	PH	NPTP	FLL	FLW	SG	ST	PL	GW	SPY
1	Aruvatham Kugava	56.00	79.50	23.00	22.46	0.57	3.77	0.38	22.22	2.06	25.90
2	Arupatham Vellai	104.00	114.20	8.67	46.59	1.42	4.40	0.65	21.59	2.26	26.65
3	Ayyan samba	106.00	118.50	12.67	41.34	1.80	4.47	0.69	27.13	2.24	31.60
4	Channangi	77.00	113.60	24.00	34.37	1.26	4.57	0.52	18.45	2.59	35.27
5	Ghandhasala	98.00	107.30	9.33	39.64	1.65	4.27	0.60	24.07	2.23	24.96
6	Arupatham samba	90.00	107.20	12.00	40.70	1.05	4.63	0.45	27.24	1.53	20.41
7	Karappa	100.00	155.20	5.66	39.65	1.39	5.80	0.48	26.22	2.39	23.95
8	Karthigai Samba	101.00	133.60	10.30	31.71	0.93	4.97	0.77	25.99	1.74	26.30
9	Kattu Ponni	94.00	101.40	11.67	32.22	0.88	5.10	0.68	19.77	2.81	16.97
10	Katta Samba	88.00	118.00	10.00	42.44	1.44	5.83	0.70	20.59	2.31	22.85
11	Katta samba (S)	88.00	112.00	12.67	37.06	1.69	5.67	0.70	28.44	2.04	22.36
12	Kaviya Samba	100.00	137.90	15.00	37.91	1.37	5.30	0.66	24.47	2.16	24.91
13	Kitchalli Samba	99.00	107.00	12.33	32.82	1.65	2.53	0.30	20.76	1.82	20.98
14	Athur Kichali Samba	112.00	100.00	16.00	28.82	1.74	4.10	0.54	20.71	1.60	22.48
15	Kallunkar	87.00	134.20	9.00	32.60	0.88	4.27	0.66	22.28	2.14	22.79
16	Konanga Samba	109.00	116.50	12.00	36.85	1.60	3.20	0.48	24.38	1.78	31.04
17	Koolavalai	112.00	147.70	9.33	42.06	1.54	5.73	0.82	25.61	2.22	24.70
18	Malayatham samba	100.00	96.73	10.33	33.53	1.62	5.20	0.66	20.79	1.73	17.35
19	Manavi election	85.00	135.10	9.33	33.37	1.99	3.73	0.26	23.21	2.10	24.28
20	Mangam Samba	112.00	122.90	8.33	30.64	1.86	4.60	0.59	22.06	1.88	19.66
21	Mappillai Samba	96.00	125.60	11.33	42.67	1.56	6.10	0.86	28.46	2.82	29.28
22	Muttakur	82.00	60.20	10.67	30.17	0.97	3.47	0.40	19.37	2.23	16.94
23	Nellor samba	111.00	113.60	11.33	38.88	1.69	5.50	0.52	24.84	1.78	30.56
24	Pamani Samba	96.00	128.70	8.67	32.15	1.15	4.67	0.60	20.79	2.77	24.62
25	Panamara Samba	87.00	93.40	17.33	35.10	1.65	4.40	0.48	24.68	1.78	29.93
26	Poongar	77.00	99.65	15.33	42.63	0.85	4.33	0.65	20.74	2.56	23.91
27	Purple Puttu	89.00	101.20 101.10	6.8.0 9.90	41.07	1.04	4.50	0.59 0.55	25.02	2.48 2.19	22.83 25.68
28 29	Puthupatty Samba Rajamudi	110.00 113.00	142.70	9.90 18.00	47.90 42.79	1.49 1.52	4.32 6.53	0.55	21.96 23.48	2.19	25.08 31.09
30	Salem 3	129.00	142.70	8.33	37.53	1.68	3.50	0.92	23.40	2.31	20.71
31	Salem Channa	129.00	103.10	10.67	31.98	1.81	6.47	0.42	25.18	2.25	37.00
32	Sadai Samba	88.00	104.50	9.67	46.90	1.02	3.93	0.49	22.96	2.29	26.61
33	Milagu samba	117.00	100.90	10.00	32.40	1.52	4.40	0.54	20.13	1.96	24.62
34	Thattan samba	104.00	130.90	10.00	34.93	1.56	5.67	0.63	22.43	2.52	25.81
35	Thillainayagam	112.00	135.90	15.67	37.50	0.99	4.60	0.45	22.45	2.30	19.36
36	Uppumolagi Selection	99.00	125.30	6.80	40.83	1.36	5.23	0.55	27.30	2.20	24.61
37	Vadakathi Samba	95.00	112.50	7.30	41.23	1.14	4.73	0.49	23.97	2.87	27.87
38	Varalli samba	114.00	98.70	14.00	30.23	1.68	4.50	0.52	20.68	1.60	24.79
39	Varakkal	85.00	106.00	9.33	45.30	1.16	4.46	0.51	22.29	2.99	21.30
40	Vellai Chithiraikar	112.00	154.70	8.33	37.68	1.70	6.60	0.78	29.06	1.67	33.00
41	Vellai Gundu	87.00	105.30	11.00	45.53	1.03	4.46	0.72	24.04	2.90	31.34
42	Vellai Kuruvai	103.00	116.80	14.00	43.93	1.55	5.28	0.74	23.18	1.58	31.55
43	Vella ottu	101.00	144.60	11.33	36.97	0.95	5.73	0.57	25.49	2.89	30.54
44	White paddy	101.00	141.80	9.67	31.00	1.28	5.67	0.79	23.14	2.07	29.94
45	IC 114971	105.00	131.50	22.00	39.50	1.24	3.59	0.63	30.93	1.62	25.03
46	IC 133384	100.00	141.50	29.00	48.34	1.24	4.41	0.65	27.63	2.05	15.41

S.No.	Entry	DFF	PH	NPTP	FLL	FLW	SG	ST	PL	GW	SPY
47	IC205953	107.00	159.50	21.33	60.54	1.31	5.73	1.04	24.30	2.10	21.86
48	IC 301077	108.00	147.10	28.00	38.70	1.13	5.90	0.64	21.23	2.73	24.89
49	IC 218157	100.00	155.10	24.33	74.09	1.21	6.22	0.84	21.77	2.38	14.21
50	IC 115775	99.00	140.50	13.00	64.98	1.49	6.40	0.80	30.40	3.00	14.36
51	IC 388692	102.00	134.40	29.33	31.41	1.57	5.29	0.63	26.97	2.65	23.53
52	IC 12610	111.00	138.10	26.67	48.17	1.22	4.99	0.58	27.13	2.15	21.68
53	IC 114575	104.00	129.70	27.33	38.00	1.20	4.64	0.61	29.53	1.56	23.07
54	IC 386238	109.00	134.20	23.67	41.08	1.21	4.55	0.56	32.67	2.30	23.25
	Max	129.00	159.50	29.33	74.09	1.99	6.60	1.04	32.67	3.00	37.00
	Min	56.00	60.20	5.66	22.46	0.57	2.53	0.26	18.45	1.53	14.21

Note: Stem girth(SG), Stem Wall Thickness(ST), Flag leaf length(FLL),Flag leaf width(FLW),Plant height(PH),Number of productive tillers per plant(NPTP),Panicle length(PL),100 grain weight(GW),Days to 50% flowering(DFF),Single plant yield(SPY).

Source of variation	Genotype	Replication	Error
Degrees of Freedom	53.00	2.00	106.00
SG (mm)	2.27***	0.03	0.01
ST (mm)	0.066***	0.00	0.00
FLL (cm)	227.35***	0.32	0.35
FLW (cm)	0.301***	0.13	0.05
PH (cm)	1253.44***	2.58	3.86
NPTP	122.68***	0.56	2.31
PL (cm)	30.45***	0.20	0.35
GW (g)	0.48***	0.01	0.00
DFF	456.75***	1.17	3.20
SPY (g)	88.19***	0.52	1.55

Table 2. ANOVA for recorded traits in germplasm

Note:**significant at p<0.01; ***significant at p<0.001

plant will be lodged or not. Nonetheless, some researchers reported that plant height is not primary factor for lodging; stem diameter and stem wall thickness are the key traits determining the susceptibility of plant towards lodging. It has been found that higher stem dry weight, stem diameter, and stem wall thickness increased bending strength (Shah *et al.*, 2017).

Diversity study: In plant breeding, genetic diversity plays a very important role as it helps in selecting the suitable parents for hybridization programme resulting in superior hybrids and desirable recombinants (Rathi *et al*, 2011). From the D² values, 54 genotypes were grouped into nine clusters (**Table 3**). Cluster I consisted of 33 genotypes making it the largest cluster followed by cluster II and cluster III containing six and seven genotypes respectively. Cluster IV and V had two genotypes each and rest of the clusters had each one genotype. From overall clustering result, it was observed that most of the landraces collected from Tamil Nadu were grouped together in one cluster and the other genotypes were distributed in different clusters. D² statistics measures the degree of diversification and relative proportion of each component characters to total divergence. Inter and intra cluster distance is given in **Table 4**. The inter cluster distances were higher than intra cluster distances. The highest inter cluster distance was between cluster IV and cluster IX (12025.89) followed by cluster VI and cluster IX (9425.48) and between cluster VI and cluster IX (9425.48) and between cluster distance was shown by cluster V. Higher inter and intra cluster distance indicates more variability between the genotypes. The genotypes with maximum stem girth *viz., Vellai chithiraikar* (cluster VII), *Rajamudi* (cluster III) and *Salem Channa* (cluster VII) can be used as parents for hybridization for improving lodging resistance in rice.

Principal Component Analysis: Principal component analysis is a dimension reduction method. It converts large data variables into smaller variables without compromising the information from the large data set. In order to achieve these goals, PCA computes new variables called principal components which are obtained as linear combinations of

Cluster	Number of genotypes	Genotypes
1	33	Athur Kichali Samba, Varalli samba, Ghandhasala, Kattu Ponni, Malayatham samba, Mangam Samba, Panamara Samba, Milagu samba, Ayyan samba, Arupatham samba, Kallunkar, Pamani Samba, Purple Puttu, Thillainayagam, Uppumolagi Selection, Vadakathi Samba, Arupatham Vellai, Karthigai Samba, Katta samba (S), Kaviya Samba, Nellor samba, Thattan samba, Vellai Kuruvai, IC 388692, IC 12610, Channangi, Katta Samba, Poongar, Puthupatty Samba, Vellai Gundu, Vella ottu, White paddy and IC 386238
II	6	Konanga Samba, Manavi selection, Salem 3, Sadai Samba, Varakkal and IC 114971
III	7	Karappa, Koolavalai, Mappillai Samba, Rajamudi, IC 133384, IC 301077 and IC 12610
IV	2	Arupatham Kugava and Muttakur
V	2	IC205953 and IC115775
VI	1	Kitchalli Samba
VII	1	Salem Channa
VIII	1	Vellai Chithraikar
IX	1	IC218157

Table 4. Inter and intra cluster distance between clusters

Cluster 1	Cluster 2	Cluster3	Cluster4	Cluster 5	Cluster 6	Cluster 7	Cluster8	Cluster9
686.185	943.596	1104.64	2407.48	3190.06	1717.68	1383.83	1885.45	5753.12
943.596	766.253	1698.43	2153.29	3763.68	907.693	2618.29	3084.96	6161.3
1104.64	1698.43	649.339	4673.5	1680.61	3394.19	1503.14	943.607	3619.8
2407.48	2153.29	4673.5	631.636	8486.46	1219.44	3923.60	6455.94	12025.8
3190.06	3763.68	1680.61	8486.46	837.885	6574.24	3928.43	2476.44	910.42
1717.68	907.693	3394.19	1219.44	6574.24	0	4217.87	5289.41	9425.48
1383.83	2618.29	1503.14	3923.60	3928.43	4217.87	0	1078.72	7198.54
1885.45	3084.96	943.607	6455.94	2476.44	5289.41	1078.72	0	4961.85
5735.12	6161.30	3619.80	12025.8	910.420	9425.48	7198.54	4916.85	0
	686.185 943.596 1104.64 2407.48 3190.06 1717.68 1383.83 1885.45	686.185943.596943.596766.2531104.641698.432407.482153.293190.063763.681717.68907.6931383.832618.291885.453084.96	686.185 943.596 1104.64 943.596 766.253 1698.43 1104.64 1698.43 649.339 2407.48 2153.29 4673.5 3190.06 3763.68 1680.61 1717.68 907.693 3394.19 1383.83 2618.29 1503.14 1885.45 3084.96 943.607	686.185943.5961104.642407.48943.596766.2531698.432153.291104.641698.43649.3394673.52407.482153.294673.5631.6363190.063763.681680.618486.461717.68907.6933394.191219.441383.832618.291503.143923.601885.453084.96943.6076455.94	686.185943.5961104.642407.483190.06943.596766.2531698.432153.293763.681104.641698.43649.3394673.51680.612407.482153.294673.5631.6368486.463190.063763.681680.618486.46837.8851717.68907.6933394.191219.446574.241383.832618.291503.143923.603928.431885.453084.96943.6076455.942476.44	686.185 943.596 1104.64 2407.48 3190.06 1717.68 943.596 766.253 1698.43 2153.29 3763.68 907.693 1104.64 1698.43 649.339 4673.5 1680.61 3394.19 2407.48 2153.29 4673.5 631.636 8486.46 1219.44 3190.06 3763.68 1680.61 8486.46 837.885 6574.24 1717.68 907.693 3394.19 1219.44 6574.24 0 1383.83 2618.29 1503.14 3923.60 3928.43 4217.87 1885.45 3084.96 943.607 6455.94 2476.44 5289.41	686.185 943.596 1104.64 2407.48 3190.06 1717.68 1383.83 943.596 766.253 1698.43 2153.29 3763.68 907.693 2618.29 1104.64 1698.43 649.339 4673.5 1680.61 3394.19 1503.14 2407.48 2153.29 4673.5 631.636 8486.46 1219.44 3923.60 3190.06 3763.68 1680.61 8486.46 837.885 6574.24 3928.43 1717.68 907.693 3394.19 1219.44 6574.24 0 4217.87 1383.83 2618.29 1503.14 3923.60 3928.43 4217.87 0 1885.45 3084.96 943.607 6455.94 2476.44 5289.41 1078.72	686.185943.5961104.642407.483190.061717.681383.831885.45943.596766.2531698.432153.293763.68907.6932618.293084.961104.641698.43649.3394673.51680.613394.191503.14943.6072407.482153.294673.5631.6368486.461219.443923.606455.943190.063763.681680.618486.46837.8856574.243928.432476.441717.68907.6933394.191219.446574.2404217.875289.411383.832618.291503.143923.603928.434217.8701078.721885.453084.96943.6076455.942476.445289.411078.720

the original variables (Abdi et al., 2013). Characters with high variability are expected to provide high level of gene transfer during breeding programs (Gana et al, 2013). The eigen values and cumulative variance contributed by each principal component are depicted in Table 5. The four Principal Components had eigen values of more than 1 i.e., PC1, PC2, PC3 and PC4 had eigen values of 2.95, 1.85, 1.35 and 1.03 respectively. Altogether, the first four PCs explained 71.85% of total variance in genotypes. First principal component accounted for 29.5% of total variance contributed by stem girth, stem wall thickness, flag leaf length, plant height, panicle length. Second principal component accounted for 18.5% of total variance and has the contribution of flag leaf width, days to 50% flowering and single plant yield. Stem girth, stem thickness, 100 grain weight and single plant yield contributed to third principal component which accounted for 13.48% of total variance. Fourth principal component had the share of 10.31% to total variance and has the contribution of flag leaf length, flag leaf width, 100 grain weight and days to 50% flowering. In PC1, the traits viz., stem girth (0.47), stem wall thickness (0.46), flag leaf length (0.36), plant height (0.47) and panicle length (0.31) had high positive eigen values. In PC2, the traits flag leaf width (0.59), days to fifty percent flowering (0.46) and single plant yield (0.29) had positive eigen values and the remaining traits had negative eigen values. In PC3, the positive eigen values were observed for stem girth (0.33), stem wall thickness (0.23), 100 grain weight (0.41), single plant yield (0.50) and flag leaf width (0.03). The traits with positive eigen values in PC4 were flag leaf length (0.34), flag leaf width (0.24), 100 grain weight (0.18) and days to fifty percent flowering (0.32). The PCA biplot between PCA1 and PCA2 depicted the interaction among traits contributing to variation. In Fig. 1, the vector lengths of each trait depicted that the longest vector was shown for flag leaf width followed by stem girth, plant height, stem wall thickness and days to fifty percent flowering indicating their contribution to the total variation. The correlation between the traits is indicated by the angle between them. They are positively correlated if they are less than 90° apart (acute), negatively correlated if they are more than 90° apart (obtuse) and no correlation if they are at right angle (90°) (Christina et al., 2021). The

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
SG	0.47	-0.04	0.33	-0.04	0.17	-0.12	-0.22	0.42	-0.06	-0.63
ST	0.46	-0.05	0.23	-0.06	0.34	-0.41	-0.10	-0.09	0.27	0.59
FLL	0.36	-0.28	-0.15	0.34	-0.14	-0.30	0.53	-0.42	-0.16	-0.25
FLW	0.11	0.59	0.03	0.24	-0.03	-0.03	0.50	0.49	-0.19	0.22
PH	0.47	0.00	-0.13	-0.01	-0.08	0.46	-0.29	-0.13	-0.62	0.25
NPTP	0.14	-0.21	-0.53	-0.43	0.49	0.22	0.35	0.21	0.10	-0.04
PL	0.31	0.07	-0.28	-0.40	-0.73	-0.14	-0.05	0.15	0.30	0.03
GW	0.10	-0.47	0.41	0.18	-0.20	0.53	0.27	0.22	0.31	0.17
DFF	0.27	0.46	-0.17	0.32	0.14	0.38	-0.12	-0.31	0.53	-0.19
SPY	0.03	0.29	0.50	-0.59	0.00	0.15	0.34	-0.41	-0.08	-0.11
Eigen values	2.95	1.85	1.35	1.03	0.72	0.61	0.53	0.43	0.34	0.19
% Var.	29.51	18.54	13.49	10.31	7.19	6.11	5.31	4.26	3.41	1.88

Table 5. Eigen values and percentage of variation contributed by each Principal Component



Fig. 1. Percentage contribution of different traits

acute angle (<90%) between stem girth and stem wall thickness indicated positive correlation. Other traits in positive association with stem girth are plant height, days to fifty percent flowering, flag leaf length, panicle length and number of productive tillers per plant. Based on the PCA biplot (**Fig. 2**), it is observed that maximum stem girth genotypes *viz.*, *Vellai chithiraikar*, *Rajamudi* and *Salem Channa* were grouped in same quadrant.

Histochemical analysis: When it comes to lodging resistance, variation in lignin content of culm region is the principal cause of differences among varieties in bending and lodging resistance (Bisht *et al*, 2022). Lignin is primarily deposited in the walls of cells that have secondary wall thickening, such as tracheary elements and fibers. Lignin gives mechanical strength

in the walls of these sclerenchyma cells. Histochemical staining of lignin by Weisner stain (phloroglucinol-HCL) was done for non-lodged genotypes such as Rajamudi, Salem Channa and lodged genotypes Vellai Gundu, Salem 3 and Kitchalli Samba having difference between stem girth and stem wall thickness. Difference in colour was observed between Rajamudi and Kitchalli Samba for region below epidermis and vascular bundle. The parenchyma tissue appeared pink slowly from inside to outside in Kitchalli Samba. A noticeable dark red colour staining appeared in Rajamudi (Fig. 3). Dark red colour indicates more deposition of lignin in the sclerenchyma tissues which gives more rigidity and thus gives lodging resistance and light pink colour indicates less lignin deposition (Bisht et al., 2022)



Fig. 2. PCA biplot of germplasm lines



Fig. 3. Histochemical studies of Kitchalli Samba and Rajamudi

Molecular evaluation for *SCM2* gene resistant to lodging: Identification and pyramiding of quantitative traitloci (QTLs) associated with strong culms constitute an important approach in breeding rice varieties with high breaking strength. Ookawa *et al.*, 2010 reported that superior alleles of *SCM2* locus increased the culm thickness in Habataki cultivar of rice. Among 54 germplasm lines, 20 genotypes selected based on stem girth and stem wall thickness were checked for the presence of two linked markers *RM1130* and *RM3430*. Scoring for genotypes is given in **Table 6 and Fig 4**. From the results it was concluded that genotypes *viz.*, *Rajamudi, Salem Channa, White paddy, Vellai Chithiraikar, Vellai Kuruvai* and *Koolavalai* had the *SCM2* locus, hence these genotypes may have the lodging resistance. Among these *Salem Channa* recorded the highest grain yield followed by *Vellai Chithiraikar, Vellai Kuruvai* and *Rajamudi*. These genotypes can be employed as efficient donors to develop lodging resistant varieties coupled with high yield.

In the context of changing environmental conditions and associated unforeseen events, genetic diversity may serve as the reservoir of many novel traits. The current study of genetic and phenotypic analysis of rice germplasm has provided important information on diversity and lodging resistance among the rice genotypes. The landraces

Table 6. Genotypes and their score	ring for RM1130 and RM3430
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Entry	RM1130	Rm3410
Rajamudi	1	1
Salem Channa	1	1
White paddy	0	1
Vellai Chitharaikar	0	1
Vellai Gundu	1	0
Kitchalli Samba	0	0
Karthigai Samba	1	1
Salem 3	0	0
Vellai Kuruvai	1	1
Pamani Samba	1	1
Thillainayagam	1	1
Channangi	1	1
IC301077	1	1
IC205953	1	1
Koolavalai	0	1

Note: 1-Present ; 0 - Absent





RM3430

Fig. 4. Molecular analysis of selected germplasm lines

viz., Salem Channa, Vellai Chithraikar and Rajamudi were identified as lodging tolerant genotypes based on molecular, morphological and histochemical analysis. These genotypes can be used as potential donor parents for the development of high yielding non lodging rice varieties.

REFERENCES

Abdi, H., Williams, L. J. and Valentin, D. 2013. Multiple factor analysis: principal component analysis for multitable and multiblock data sets. *Wiley Interdisciplinary*

https://doi.org/10.37992/2023.1403.142

reviews: computational statistics, **5**(2): 149-179. [Cross Ref]

- Begna, T. and Begna, T. 2021. Role and economic importance of crop genetic diversity in food security. International Journal of Agricultural Science and Food Technology, 7(1): 164-169. [Cross Ref]
- Bisht, D., Kumar, N., Singh, Y., Malik, R., Djalovic, I., Dhaka, N. S. and Kumar, S. 2022. Effect of stem structural characteristics and cell wall components related to stem lodging resistance in a newly identified mutant of hexaploid wheat (*Triticum aestivum* L.). *Frontiers in Plant Science*, **13**: 1067063. [Cross Ref]
- Christina, G. R., Thirumurugan, T., Jeyaprakash, P. and Rajanbabu, V. 2021. Principal component analysis of yield and yield related traits in rice (*Oryza sativa* L.) landraces. *Electronic Journal of Plant Breeding*, **12**(3): 907-11. [Cross Ref]
- Gana, A. S., Shaba, S. Z. and Tsado, E. K. 2013. Principal component analysis of morphological traits in thirtynine accessions of rice (*Oryza sativa* L.) grown in a rainfed lowland ecology of Nigeria. *Journal of plant breeding and crop science*, 5(10): 120-126. [Cross Ref]
- Hitaka, H. 1969. Studies on the lodging of rice plants. *Japanese Agric. Res.*, **4**(3): 1-6.
- Kumari, S., Singh, P., Bisen, P., Loitongbam, B., Rai, V. and Sinha,B. 2018. Genetic diversity analysis of rice (*Oryza sativa* L.) germplasm through morphological markers. *International journal of agriculture, environment and biotechnology*:953-957
- Li, Q., Fu, C., Liang, C., Ni, X., Zhao, X., Chen, M. and Ou, L. 2022. Crop lodging and the roles of lignin, cellulose, and hemicellulose in lodging resistance. Agronomy, **12**(8): 1795. [Cross Ref]
- Ookawa, T., Hobo, T., Yano, M., Murata, K., Ando,T., Miura, H., Asano, K., Ochiai,Y., Ikeda, M. and Nishitani, R. 2010. New approach for rice improvement using a pleiotropic QTL gene for lodging resistance and yield. *Nature communications*, **1** (1):132. [Cross Ref]
- Rathi, S., Kumar, R., Munshi, A. D. and Verma, M. 2011. Breeding potential of brinjal genotypes using D2 analysis. *Indian Journal of Horticulture*, **68**(3): 328-331.
- Shah, A.N., Tanveer, M., Rehman, A.U., Anjum, S.A, Iqbal,J. and Ahmad, R. 2017. Lodging stress in cereal—effects and management: an overview. *Environmental Science and Pollution Research*, 24:5222-5237. [Cross Ref]

- Shah, L., Yahya, M., Shah, S.M.A., Nadeem, M., Ali, A., Wang,J., Riaz, M.W., Rehman, S. and Wu, W. 2019. Improving lodging resistance: using wheat and rice as classical examples. *International journal* of molecular sciences, **20** (17):4211. [Cross Ref]
- Sheela, K. S., Robin, S. and Manonmani, S. 2020. Principal component analysis for grain quality characters in rice germplasm. *Electronic Journal of Plant Breeding*, **11**(01):127-31. [Cross Ref]
- Sumanth, V., Suresh, B. G., Ram, B. J. and Srujana, G. 2017. Estimation of genetic variability, heritability and genetic advance for grain yield components in rice (*Oryza sativa* L.). *Journal of Pharmacognosy* and Phytochemistry, 6(4): 1437-1439.