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Genetic Diversity for Lodging Resistance in Rice Genotypes

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Abstract Lodging of the rice crop at reproductive stage limits the rice productivity in coastal areas. Genetic diversity among 51 elite rice lines for lodging resistance was determined using 13 molecular markers linked to *SCM2* and estimated magnitude of genetic diversity for 14 phenotypic characters using D^2 statistics. Polymorphic information content (PIC) of the markers ranged from 0.38 to 0.77 with average of 0.55. Cluster analysis of marker data grouped 51 genotypes into two major clusters at 18% similarity level. While, 51 rice line were grouped into 8 clusters by employing Tocher's method. Maximum genetic diversity to total divergence was majority contributed by per cent of lodging (46.12%), followed by days to 50% flowering (16.39%) and test weight (9.57%). Present study revealed that choice of parents for traits of interest can be successfully practiced based on molecular diversity besides genetic diversity of Mahalonobis D^2 analysis to foster the breeding programs.

Keywords Lodging resistance, Rice, Molecular markers, D^2 analysis, Genetic diversity.

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Introduction

Rice crop in coastal areas of south India is most frequently subjected to cyclones particularly in Andhra Pradesh at reproductive phase limiting the rice productivity. This makes most of the rice crop to lodge at grain filling to harvesting stage resulting in drastic yield reduction. Lodging of rice crop not only reduces the yield, quality and impede mechanical harvesting.

Assessment of genetic diversity is the prerequisite for framing breeding strategy for the development of lodging resistant rice varieties. Lodging resistance depends on morphological, biochemical and environmental parameters. Complexity of lodging resistance is herculean task for breeding non lodging rice varieties. Detection of genetic diversity using molecular markers linked to lodging resistant loci would help in selection of diverse parents for lodging resistance for hybridization. Magnitude genetic diversity can be assessed using Mahalonobis D^2 analysis. Genotypes with heavy panicles and tall nature are more prone to lodging. Effective quantitative trait loci (QTL) for culm diameter, *STRONG CULM2* (*SCM2*) on chromosome 6 with flanking markers RM20546–RM20562 and cloned QTL conferred lodging resistance in the near isogenic lines developed in cross between Habataki and the *Japonica* variety Sasanishiki [1]. Present study aimed to assess genetic diversity among elite rice lines using lodging resistant linked molecular markers (*SCM2*) and D^2 statistics.

Materials and Methods

Genetic diversity for lodging resistance using molecular markers

Fifty one elite lines were screened with molecular markers linked to *SCM2* (Strong Culm 2) on chromosome 6 conferring lodging resistance. PCR reaction (10ul) consists of 10X Taq Buffer A with 15mM MgC12, lul of forward and reverse primers (5uM), 0.5ul of dNTPs (2.5mM), 2.5 ul of genomic DNA and 3ul of sterilized millipure water. PCR reactions was performed at thermoprofile of 94°C for 5 minutes followed by 35 cycles of denaturing at 94°C for 0.5 min, annealing temperature adjusted with marker at 55°C to 62°C for 0.5 min, extension at 72°C for 1.0 min and ending up with 7 min at 72°C for the final extension. The PCR products were electrophoresed with high resolution agarose stained with ethidium bromide (10 mg / ml) at 100 volts for 2 h in 1X TBE buffer. A 100 bp ladder (Banglore Genei) was used for appropriate sizing of the products. The gel image was visualized under UV light using Ingenius gel doc system.

Gel pictures were scored as 1 for presence or 0 for absence of bands. The data was subjected to NTSYS for analysis.

Genetic diversity was assessed using phenotypic characters

Fifty one elite rice lines were studied in two replications during *kharif* of 2012. Each line was transplanted with 3m row length in 4 rows with a spacing of 20 × 15 cm. Data was collected on eight yield traits such as days to 50% flowering, plant height, ear bearing tillers per plant, panicle length, number of filled grains per panicle, spikelet fertility and grain yield per plant. Data pertaining to lodging related traits culm diameter and culm thickness was measured on 5 randomly selected plants using vernier calipers and 4th internodal length at 20 days after heading. Data on culm strength, per cent of lodging and bending strength was collected at harvesting stage. Culm strength and per cent of lodging was measured as per the IRRI standard evaluation systems, 2002 and bending strength was measured using diaki prostrate tester.

Table 1. Polymorphic information content (PIC) of molecular markers used for genetic diversity.

Marker	Major allele frequency	Allele No.	Polymorphic information content (PIC)
RM 20557	0.55	6.00	0.77
RM 20546	0.84	2.00	0.46
Indel 8	0.69	3.00	0.63
Indel 9	0.87	2.00	0.38
Indel 2	0.83	2.00	0.45
Indel 12	0.59	2.00	0.43
RM 20562	0.61	3.00	0.62
RM 1370	0.57	4.00	0.72
RM 5509	0.70	3.00	0.61
RM 6274	0.63	2.00	0.53
RM 6395	0.76	2.00	0.52
RM 7269	0.73	2.00	0.50
RM 20564	0.37	4.00	0.48
Mean	0.67	2.85	0.55

Data pertaining to 14 characters was analyzed using Mahalonobis D^2 analysis.

Results and Discussion

Genetic diversity for lodging resistance with molecular markers

Number of alleles ranged from 2 to 6 with a mean of 2.82. Rice microsatellite marker RM 20557 exhibited maximum of 6 alleles followed by 4 (RM 1370 and RM 20564), 3 (RM 5509, RM 20562, Indel 8) and 2 for the rest of the markers. Polymorphic information content (PIC) of these markers ranged from 0.38 to 0.77 with average of 0.55 indicated potentiality of these markers for assessing genetic diversity. Markers such as RM 20557 (0.77), Indel 8 (0.63), RM 20562 (0.62), RM 5509 (0.61) showed higher polymorphic content among 51 rice lines would help in marker assisted selection for lodging resistance (Table 1). Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus.

Cluster analysis grouped 51 genotypes into two

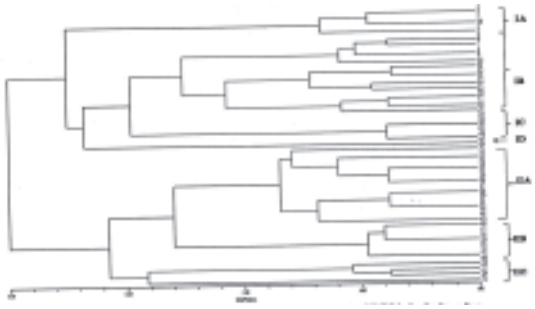

Fig. 1. Dendogram showing genetic diversity among 51 elite rice lines using *SCM2* linked molecular markers.

major clusters at 18% similarity level (Fig. 1). Cluster I comprises of 26 genotypes sub divided into 4 clusters. Sub cluster IA with 6 genotypes, IB with 14 genotypes consist most of the widely cultivated varieties. Sub cluster IC has 5 genotypes and cluster 1D is monogenic with MTU 1081.

Most of the elite breeding lines were grouped into Cluster II with 25 genotypes. It was sub grouped into 3 clusters. Fourteen genotypes grouped into sub Cluster IIA, 7 into IIB and 4 into IIC. Hybridization between distantly related genotypes of Cluster IA and IB with Cluster IIB and IIC would realize in good combiners for lodging resistance. *SCM2* alleles in indica rice varieties were investigated using molecular markers [2].

Genetic diversity for lodging resistance using phenotypic characters

Based on relative magnitude of D^2 values, 51 rice line were grouped into 8 clusters by employing Tocher's method of *D*2 analysis. Highest number of genotypes (24) were grouped into Cluster I followed by Cluster II with 10 genotypes. Cluster III and IV consist of 5 genotypes in each group. Three genotypes were grouped in cluster V and Cluster VII comprises of 2 genotypes. Cluster VI and VIII were mongenic. Mixed pattern of distribution of released rice varieties and elite rice lines in each cluster was observed. Genetic diversity of these genotypes contributed by parents used in hybridization, selection criteria considered and environmental influence paricularly for the percent of lodging.

Inter and Intra cluster distances

The intra and inter cluster distance average values computed among 8 clusters were presented in the Table 2. All the inter-cluster distances were higher than the intra-cluster distance indicating presence of wider diversity among genotypes of distance groups. Highest inter cluster distance value of 633.11 was found between Cluster III and V, followed by Cluster IV and VI (505.53), Cluster V and VIII (504.44), VIII and IV (438.24), II and III (404.59) and II and VIII (375.71). Lowest inter cluster values were observed between I and III (140.77). Genotypes in the Cluster V were found to be distantly related to genotypes of Cluster III and Cluster VIII and also genotypes of Cluster II were diverge with Cluster III and VIII with inter cluster values of 404.59 and 375.71 respectively. These results revealed that hybridization between parents of diverse clusters would realize in heterotic combinations and further selection of lodging resistant genotypes.

Table 2. Inter and intra cluster D^2 values of eight clusters during *kharif* of 2012-13.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	70.47	240.37	140.77	340.32	380.97	125.60	189.91	193.23
Cluster II		55.29	404.59	152.03	175.98	307.67	194.55	375.71
Cluster III			61.99	394.41	633.11	278.46	174.69	265.29
Cluster IV				130.74	366.42	505.53	199.66	438.20
Cluster V					155.79	305.81	335.96	504.44
Cluster VI						0.00	258.36	215.50
Cluster VII							68.02	369.17
Cluster VIII								0.00

Table 3. Cluster means for yield and lodging related traits pertaining to 51 elite rice lines during *kharif* of 2012-13.

Cluster/trait	Days to 50% flowering (1)	Plant height (cm) (2)	No. of ear bearing tillers plant^{-1} (3)	Panicle length (cm) (4)	No. of filled grains panicle^{-1} (5)	Spikelet fertility (%) (6)	Test weight (g) (7)
Cluster I	113.75	138.26	6.71	24.84	181.10	75.33	18.81
Cluster II	112.55	112.57	7.07	24.88	212.47	80.38	19.28
Cluster III	104.00	159.29	6.85	24.89	203.86	81.16	20.27
Cluster IV	99.70	103.03	6.75	21.67	176.56	85.63	19.70
Cluster V	125.00	121.18	6.98	22.99	202.95	76.46	14.58
Cluster VI	126.00	166.23	7.15	25.46	246.70	87.35	14.17
Cluster VII	106.00	168.30	6.10	24.65	227.58	67.86	17.57
Cluster VIII	111.50	111.72	5.90	24.03	200.70	82.34	18.30

Table 3. Continued.

Cluster/trait	Grain yield plant^{-1} (g) (8)	Culm diameter (mm) (9)	Culm thickness (mm) (10)	Culm strength (11)	4th Inter nodal length (cm) (12)	Bending strength (g stem^{-1}) (13)	Lodging (%) (14)
Cluster I	13.63	5.95	1.26	7.29	10.09	103.87	83.90
Cluster II	18.25	6.02	1.35	5.50	8.21	104.74	8.50
Cluster III	16.12	7.48	2.58	7.20	11.97	94.05	90.00
Cluster IV	19.52	5.71	1.74	3.80	10.19	106.19	1.70
Cluster V	17.18	6.87	1.38	2.33	9.24	107.23	20.42
Cluster VI	8.85	5.78	1.10	7.00	14.71	88.57	92.50
Cluster VII	14.26	7.98	2.99	3.50	15.00	119.59	37.50
Cluster VIII	16.50	4.68	0.98	9.00	5.69	44.49	95.50

Cluster mean values for different characters

Wide range of cluster means were exhibited for the 14 characters studied (Table 3). Cluster mean value of days to 50% flowering ranged from 99.70 (Cluster IV) to 126.00 (Cluster VI). Cluster VI exhibited highest mean values for the traits days to 50% flowering (126.00), ear bearing tillers per plant (7.15), panicle length (25.46 cm), number of filled grains per panicle (246.70), spikelet fertility (87.35%). Cluster III for test weight (20.27 gms), cluster IV for grain yield per plant (19.52 g). Cluster VII exhibited genotypes with wider (7.98 mm) and thicker (2.99 mm) culms coupled with highest bending strength (119.5 9 g stem^{-1}). Cluster V possess genotypes with strong culm with minimum culm strength score of 2.33 and percent of lodging 1.7 indicating lodging resistant genotypes in that cluster. Highest cluster mean value of 15.00 cm for 4th inter nodal length was observed for Cluster VII and lowest value of 5.69 cm for Cluster VIII.

Maximum per cent of lodging was observed in the cluster VIII where susceptible rice variety Swarna (MTU 7029) present. Next to the Cluster VIII, Cluster VI (92.50), Cluster III (90.0) and Cluster I (83.90) showed higher mean value of per cent of lodging. Lodging resistant genotypes were grouped in Cluster IV (1.70%), Cluster II (8.50%) followed by Cluster V (20.42%) with minimal per cent of lodging.

Maximum genetic diversity to total divergence

Table 4. Contribution of yield and lodging resistance traits towards genetic divergence among 51 rice lines during *kharif* of 2012-13.

Sl. No.	Source	Times ranked 1st	Contribution %	Rank
1	Days to 50% flowering	209	16.39	2
2	Plant height (cm)	99	7.76	4
3	Number of ear bearing tillers plant^{-1}	0	0.00	–
4	Panicle length (cm)	6	0.47	11
5	Number of filled grains panicle^{-1}	3	0.24	12
6	Spikelet fertility (%)	2	0.16	13
7	Test weight (g)	122	9.57	3
8	Grain yield plant^{-1} (g)	27	2.12	8
9	Culm diameter (mm)	7	0.55	10
10	Culm thickness (mm)	58	4.55	7
11	Culm strength	85	6.67	5
12	4th Inter nodal length (cm)	10	0.78	9
13	Bending strength g stem^{-1}	59	4.63	6
14	Per cent of lodging	588	46.12	1

was contributed by per cent of lodging (46.12%), followed by days to 50% flowering (16.39%), test weight (9.57%), plant height (7.76%), culm strength (6.67%), bending strength (4.63%), culm thickness (4.55%), and grain yield per plant (2.12%) (Table 4). Out of the 14 characters, these eight characters together contributes contribute 97.81% of total divergence indicating importance of these characters in breeding for lodging resistant rice genotypes.

Varietal difference for percent of lodging incidence and culm strength among African rice genotypes [3] and genetic diversity for culm diameter was observed [4]. Contributions of yield and its related traits such as days to 50% flowering, test weight and plant height towards maximum diversity was also reported by earlier workers [5, 6]. Maximum amount of heterosis will be manifested in cross combinations involving the parents belonging to most divergent clusters. Development of high yielding rice genotypes with lodging resistance is necessary to get sustainable rice yields even under adverse climatic conditions.

When we compare genetic diversity using molecular markers and D^2 statistics, susceptible rice varieties for lodging viz., MTU 7029, MTU 1061, MTU 1001 and MTU 1010 were found to be distantly related with resistant lines PS140–1, II 110-9-1-1-1-1, BPT 2270 and MTU 1121. These susceptible rice varieties and resistant lines for lodging showed co-segregation banding pattern for *SCM2* linked markers conferring lodging resistance with minimum similarity index values. While lodging susceptible parents Swarna (MTU 7029) was grouped in Cluster VIII, Indra (MTU 1061) in Cluster I, MTU 1001 in Cluster III and MTU 1010 in Cluster IV in Tocher's method of clustering. These lines exhibited higher inter cluster distances with selected testers from Cluster V (II 110-9-1-1-1-1 and BPT 2270), Cluster VII (PS 140-1) and cluster II (MTU 1121).

Per se performance of the selected lines for the lodging related characters studied were found to be diverse with the testers. Identification of traits (per cent of lodging, days to 50% flowering, test weight, plant height, culm strength, bending strength, culm thickness and grain yield per plant) contributed to the divergence for lodging resistance would help in use of these traits in selection of parents for hybridization and also to advance best transgressive segregants in the crop improvement programs of lodging resistance. Present study revealed that choice of parents can be successfully practiced based on molecular diversity of trait linked markers and polymorphic co-segregation pattern of susceptible parents with resistant donors besides genetic diversity of

Mahalonobis D^2 analysis to hastenup the breeding programs.

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