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Research Article

STUDIES ON GENETICS AND VALIDATION OF MOLECULAR MARKERS LINKED TO LODGING RESISTANCE *LOCI* IN ELITE RICE LINES

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Abstract- Stem lodging risk is the major limiting factor for rice productivity in coastal areas. Selection of lodging resistant lines in early generation is a herculean task as it is influenced by structural and weather parameters. Present study aimed to study genetics and to validate markers linked to lodging resistant *loci* using F_2 and F_3 lines of highly susceptible mega rice variety Swarna (MTU 7029) and elite lodging resistant lines II 110-9-1-1-1-1 and MTU 1121. Studies on genetics of lodging resistance indicated modified epistatic dihybrid ratio of 9:7 in both crosses for lodging susceptible and resistant lines revealing complementary epistatic interactions of lodging resistant *loci*. RM 20557 and RM 5509 were found to be associated with percent of lodging, culm strength and culm diameter in F_2 of MTU 7029/II 110-9-1-1-1-1. While in another cross MTU 7029/MTU 1121, RM 6933 was found to be linked with culm strength and culm diameter. Identified markers linked to lodging resistant traits were confirmed by genotyping and phenotyping of F_3 families, back crosses and markers RM 20557 and RM 5509 were found to be associated with lodging resistance related traits even in other genetic back grounds. Adoption of marker assisted selection would help in fixing favorable alleles of lodging resistant *loci* as epistatic gene interactions were involved in both the crosses.
Key words- Bulked segregant analysis, lodging resistance, Genetics, Rice

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Introduction

Rice is the staple food of most of the people of South East Asia. India stands second in position in production after China contributing to 26 % of global rice production. Rice crop in coastal areas of South India is occasionally subjected to unpredicted cyclones at reproductive phase limiting the rice productivity. It was reported that lodging reduced canopy photosynthesis by 60–80%, and every 2% of lodging caused a decrease of 1% in grain yield [1]. Rice genotypes differ widely in their lodging resistance, which is a complex phenotype determined by morphological and biochemical components. Lodging resistance with low heritability is highly affected by the environment and difficult to estimate in the early stages of breeding. Understanding genetics and identification of molecular markers linked to lodging related traits would help in precise selection of lodging resistant lines. Studies on genetics of lodging are limited and duplicate type of epistatis was observed for second internode breaking strength in wheat [2]. Identification of molecular markers associated with lodging resistance related characteristics would hasten the selection of lodging resistant lines. Markers linked to QTLs associated with lodging resistance related traits *viz*., basal culm thickness, culm length and culm strength [3,4], pushing resistance [5], STRONG CULM2 (SCM2) on chromosome 6 for culm diameter [6], SCM3 and SCM4 conferring culm strength on chromosome 2 [7], thick and stiff culm traits, with low lignin concentrations on chromosome 2 [8] would help in marker assisted selection for lodging resistance. QTLs for prevention of culm strength deterioration after grain filling [9], cortical fibre tissue thickness [10], culm diameter and culm strength [11] also helps development of lodging resistant lines. Present study aimed to study genetics and to validate lodging resistant *loci* using bulked segregant analysis in F_2 derived population between susceptible parent MTU 7029 and resistant elite lines II 110-9-1-1-1-1 and MTU 1121. Identified molecular markers linked to lodging resistant *loci* are further confirmed in F_3 population, back crosses and other genetic back grounds.

Materials and Methods

Phenotypic screening: Crossing programme was initiated during 2012-13 between lodging susceptible parent MTU 7029 and resistant elite lines II 110-9-1-1-1-1 and MTU 1121 and generated F1s were selfed to study F_2 generation. F_2 individual plants from two crosses MTU 7029/II 110-9-1-1-1-1 (180) and MTU 7029/MTU 1121 (160) were randomly selected for phenotyping and genotyping out of 4000 F_2 single plants studied during wet season of 2013-14 and these plants were advanced to study F_3 generation during dry season 2013-14. Phenotyping of two F_2 populations derived from MTU 7029/ II 110-9-1-1-1-1 and MTU 7029/ MTU 1121 was carried out for lodging related traits culm diameter, culm strength and percent of lodging. Culm diameter was measured at 4th internodal length from the top using vernier calipers at 20 days after heading, culm strength and percent of lodging was measured as per the IRRI standard evaluation systems [12]. The reaction to lodging was confirmed in F_2 derived F_3 families, where families exhibiting 0 to 25 percent of lodging were treated as resistant families and families showing percent of lodging more than 25 percent were designated as lodging susceptible. Generated advanced back crosses using lodging susceptible parent MTU 7029 as recurring parent using donors II 110-9-1-1-1-1 and another susceptible parents MTU 1061 and resistant line BPT 2270 for validation in the years 2014 and 2015.

Genotypic screening with molecular markers: The total genomic DNA was extracted from the leaf samples collected at 45 days [13] using Qiagne Tissue lyzer for tissue grinding with DNA extraction buffer (50 mM Tris HCl, pH 8.0; 25 mM EDTA; 300 mM NaCl and 2% CTAB).

Table-1 Segregation ratios for lodging résistance in two crosses

Cross	F_1	F_2		x^2 at 0.05 probability 9:7 ratio	F_3		x^2 at 0.05 probability
		Susceptible	Resistant		Susceptible	Resistant	
MTU7029 / II110-9-1-1-1-1	Resistant	114	66	3.66 ns	113	67	9:7 ratio 3.11 ns
MTU7029 / MTU1121	Resistant	100	60	2.53 ns	10	150	1:15 ratio 0.0000ns

Table-2 Single marker analysis of lodging resistant *loci*

Trait	Marker	F_2		F_3	
MTU 7029/II110-9-1-1-1-1					
		F static	Pr(F)	F static	Pr(F)
Percent of lodging	RM20557	0.11	0.74	2.73	0.10
	RM5509	15.04	0.00**	5.82	0.02*
Culm strength	RM 20557	0.01	0.98	0.02	0.80
	RM5509	3.83	0.05	10.53	0.00**
Culm diameter	RM20557	2.3	0.13	6.34	0.56
	RM5509	1.17	0.28	4.16	0.04*
MTU 7029/MTU 1121					
Percent of lodging	RM6933	1.31	0.25	1.87	0.17
	RM216	0.45	0.51	1.85	0.18
Culm strength	RM6933	1.66	0.20	4.82	0.03*
	RM216	3.19	0.08	0.42	0.52
Culm diameter	RM6933	3.40	0.07	4.46	0.03*
	RM216	0.04	0.84	4.27	0.04*

** Significant at 5% level ,** Significant at 1% level*

Parental polymorphism survey was performed between susceptible parent and elite resistant lines using 585 SSR markers covering 12 chromosomes. The purity and concentration of the isolated genomic DNA samples were estimated by Eight channel spectrophotometer (Nanodrop Technologies, U.S.A). The PCR was carried out using thermocycler (Eppendorf, Germany). Template DNA of 2.5 µl (20 ng) was pipetted into each of the PCR tubes after proper labelling and 7.5 µl of master mix comprising 1 µl of 10 pmol primer (both forward and reverse primer), 0.5 µl of 2.5 mM deoxy ribonucleotides (dNTPS), 1 µl of Genei® 10 X assay buffer (10 mM TrisHCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin) and 1 µl of 1 U/µl Taq DNA polymerase of Genei® and 3 µl of sterile distilled water was added to make up the volume to 10 µl. Thermal profile of initial denaturation at 94ºC for 5 minutes, denaturation at 94oC for 30 seconds, annealing at 58°C for 30 seconds for 35 cycles and elongation at 72°C for 1 minute was followed fro amplification. The PCR products were analyzed by electrophoresis using a 3 % high resolution agarose (Lonza) gel using a gel Electrophoresis Unit (Genaxy). Later the gel was transferred to Electrophoresis unit containing 1 X TAE buffer. 100 bp ladder was added in one well to determine the size of amplified fragments. The DNA fragments were then visualized under UV-transilluminator and documented using gel documentation system (SYNGENE Gene flash U.K.). Selected ten lodging resistant individual plants with wider (>7mm culm diameter) and strong culm with culm strength score of 1 in each F_2 individual plants of the crosses MTU 7029/ II 110-9-1-1-1-1 and MTU 7029/MTU 1121 were bulked as resistant bulk and ten highly susceptible plants for lodging with narrow (<4mm) and weak culm (9) as susceptible bulk separately for respective cross. Bulked segregant analysis was adapted with polymorphic markers in each cross to identify lodging resistant *loci* in new donors [14]. Marker trait association was identified by single marker analysis using Map Manager and Win QTL cartographer [15].

Results and Discussion

Genetics of lodging resistance: Phenotypic segregation pattern of F_2 individual plants and F_3 families of MTU 7029/ II 110-9-1-1-1-1 and MTU 7029/ MTU 1121 towards lodging résistance were furnished in the [Table-1]. Lodging susceptible and resistant parents segregated in modified dihybrid ratio of 9:7 of epistatic interaction indicating digenic interaction is involved among the lodging resistant *loci* in 180 F_2 derived individual plants of MTU 7029/II 110-9-1-1-1-1. And also Modified dihybrid ratio of 9:7 susceptible and resistant was observed among 160 F_2 individual plants of MTU 7029/ MTU 1121 cross combination. Chi square test results of observed ratio showed non-significance with expected ratio of modified dihybrid ratios of respective crosses indicating epistatic gene interactions in the expression of lodging resistance. In both crosses, selfing of lodging resistant F_1 plants resulted in F_2 ratio of 9: 7 lodging susceptible to resistant individual plants and this ratio occurs due to expression of susceptibility when dominant alleles of both genes present together and resistance was exhibited with the presence of homozygous recessive condition at either of genes. Expression of lodging is on individual plant basis in F_2 generation was confirmed in F_3 on family basis for percent of lodging. F_3 families ratio of 9:7 was observed in MTU 7029/ II 110-9-1-1-1-1 and 1:15 for susceptible and resistant families in case of MTU 7029/MTU 1121. Deviation in expression of lodging resistance in F_3 families compared to F_2 in MTU 7029/ MTU 1121 is due to fixation of alleles more towards resistant *loci* in F3 families through duplicate dominance epistatic interactions. This result can be attributed as phenotypic expression of individual plants for lodging is more dependent on weather parameter like wind, rains and surrounding plant canopy and actual realization of percent of lodging was occurred on family basis of progenies. Out of 585 SSR markers screened, 105 SSR markers found to be polymorphic between MTU 7029 and II 110-9-1-1-1-1 and 90 between MTU 7029 and MTU 1121 indicating specific polymorphic banding pattern between susceptible parent and corresponding resistant parents for lodging. Two markers, RM 20557 and RM 5509 showed clear co-segregation banding pattern of susceptible bulk and resistant bulk with respective susceptible (MTU 7029) and resistant (II 110-9-1-1-1-1) parents for lodging. Whereas RM 6933 on chromosome 2 and RM 216 on chromosome 10 co-segregated with susceptible and resistant bulks in accordance with respective susceptible parent MTU 7029 and resistant parent MTU 1121. The polymorphic markers linked to *loci* conferring lodging resistance segregated between resistant and susceptible bulks while unlinked markers will show co-dominance pattern. Individual F_2 plants and F_3 families of both crosses were screened with respective co-segregated markers and adopted single marker analysis to know the marker association with traits *viz*., percent of lodging, culm diameter and strength [Table-2]. Results of single marker analysis revealed that RM 5509, found to be linked to percent of lodging, culm strength and culm diameter in F_2 derived F_3 families but in F_2 individual plants it exhibited association with percent of lodging. The results revealed that clarity in phenotypic expression of the complex traits involving multiple genes such as lodging resistance can be assessed on family basis rather than individual single plant in F_2 population. Lodging resistant parent, II 110-9-1-1-1-1, contributed favourable alleles of lodging resistant *loci* for culm diameter, culm strength and percent of lodging. Identified markers, RM 20557 and RM 5509, were linked to lodging resistance *loci* from the donor II 110-9-1-1-1-1 found to be associated with previously reported QTL region of strong culm 2 (SCM2) on chromosome 6 [6] which confers lodging resistance with pleiotropic effect on APO (Apparent panicle organization). In another elite line (MTU 1121) derived population, RM 6933 was found to be linked with culm strength and culm diameter and RM 216 was-

associated with culm diameter. Earlier scientists [3] also reported RM 216 was associated with culm strength and identified RM 6933 marker is in the vicinity of reported QTLs of for basal elongating internodes [4] and QTL SCM4 [7] which confers culm strength present on chromosome 2. Identified markers in both crosses were validated in F_2 plants of another cross MTU 1061/BPT2270 and advanced back crosses of MTU 7029/II 110-9-1-1-1 and MTU 1061/BPT 2270 in the years 2014 and 2015 and RM 20557 and RM 5509 were found be associated with lodging resistance in these population indicating these markers are useful for use in marker assisted selection. In the present investigation, lodging resistant *loci* were identified on chromosome 6 in the donor II 110-9-1-1-1-1 and on chromosome 2 in the donor MTU 1121. It indicates the contribution of alleles for lodging resistance is highly genotypic specific. In view of complexity in inheritance pattern, development of lodging resistant genotypes without yield penalty is quite challenging so understanding gene interactions would help in planning suitable breeding strategy. Variation in gene interactions in both crosses for percent of lodging on family basis is one of the reasons for different lodging resistant *loci* linked markers in the two populations derived from two elite lodging resistant lines (II 110-9-1-1-1-1 and MTU 1121) with same susceptible parent MTU 7029. Identified markers, RM 20557 and RM 5509, associated with lodging resistant *loci* SCM 2 in donor, II 110-9-1-1-1-1, conferring lodging resistance with wider and strong culm using bulked segregant analysis. The donor, MTU 1121, possessed favorable alleles of the markers, RM 6933, associated with culm strength on chromosome 2 and RM 216 on chromosome 10 indicating involvement of multiple lodging resistant *loci* in expression of lodging resistance. The present study revealed that identification of suitable donor and associated marker are necessary for recombining favorable alleles for lodging resistant *loci* to evolve lodging resistant genotypes with high yield. Identification of lodging resistant genotypes in early generation using marker assisted selection would help in selection of high yielding genotypes with lodging resistance as this trait is controlled by epistatic gene interactions.

Application of research: Identification of associated molecular markers for lodging resistant *loci* and complementary epistatic gene interaction for percent of lodging in the population developed using elite lines II 110-9-1-1-1-1 and MTU1121 indicated that adoption of marker assisted breeding is one of the best strategies for development of lodging resistant rice lines. These resistant donors can be used as genetic stocks for future breeding programmes of lodging resistance in rice.

Research Category: Plant breeding

Abbreviations: QTL- Quantitative triat *Loci*, SCM- Strong Culm, MTU- Maruteru

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