Original Research Article

Genetic variability and trait associationstudies ofinternode length and strong culm related traits in inter sub-specific cross derived recombinant inbred lines in rice (*Oryza sativa* L.).

Comment [UMI1]: Culm-related

ABSTRACT

Internode length, plant height and culm thickness are the primary traits affecting the strength of culm in rice.Thepresent investigation was carried out to study genetic variability and trait association of internode length and strong culm related traits in recombinant inbred lines (RILs) in rice.The lodging stress is influenced by intricate interplay of several factors and ~~there are~~ many questions that remain unanswered. To elucidate the complex lodging phenomenon, traits*viz*., the length of uppermost internode (IL1), second internode (IL2), third internode(IL3), fourth internode(IL4), fifth internode (IL5), sixth internode (IL6), number of internodes (INN), plant height (PH), pushing resistance (PR) and culm thickness (CT) were investigated.Analysis of Variance (ANOVA) displayed significant variation in RILs for all the traits studied. The investigation revealed high estimates of PCV and GCV along with high heritability and genetic advance as percent of mean (GAM) for traits under investigation revealing the role of additive gene effect and selection of these attributes will be valuable for rice improvement. Association studies revealed thatstrong culm is reliant on internode length of basal primary internodes and CT.Thus, the direct selection of culm thickness and lower basal internode length can improve the breaking-type lodging resistance in rice. Our conceptual perceptions areexpected to provide valuable information to improve the culm strength in rice.

Keywords: Internode length, inter sub-specific cross, PCV, GCV, heritability, GAM, correlation.

INTRODUCTION

Rice is the main staple food for 40% of the world's population and its production has increased many folds in the past decades, due to green revolution. Extensive cultivation of the developed high yielding semi-dwarf varieties led to increase in grain production, consequently averting lodging and food shortages. This convinced many breeders the use of semidwarfvarieties. However, despite the short stature conferred by *sd1* gene, lodging is now a major concern in rice as rice breeders are developing new varieties with increased plant biomass and harvest index to further increase the grain yield. Recently, several genes associated with grain yield have been identified, *Gn1a*(Ashikari *et al*., 2005), *GS3* (Fan *et al*., 2006), *DEP1* and *WFP* (Huang *et al*., 2009; Miura *et al*., 2010) and are being utilized to develop high yielding varieties. To sustain this breeding objective, there is a need to develop varieties with strong culm, to bear the heavy panicles and abate the damage from cyclones. Lodging can cause severe yield loss and poor grain quality as a result of reduced canopy photosynthesis, increased respiration and reduced translocation of nutrients. Lodging primarily occurs at the maturity and harvesting stage, when the plant's stem is too weak to support the panicle weight and during this vulnerable stage a small wind force can cause lodging. Previous reports have shown that plant height, stem

Comment [UMI2]: Hyphen between them

Comment [UMI3]: Semi dwarf

diameter and thickness are highly correlative to the lodging resistance (Long e*t al.*, 2020; Kashiwagi *et al.*, 2006; Chigira*et al.*, 2023). Green revolution resulted in high yielding varieties with dwarf plant stature but not suitable to cope with the current climate adversities. To avert this problem, we need more robust ~~and strong~~ culm rice varieties.

Comment [UMI4]: High-yielding. Do for all. Please.

Stem lodging results from the interaction and balance of different forces like culm strength and external environmental forces (typhoons and severe cyclones). Stem lodging is of two types*viz.,*stem bending-type and stem breaking-type (Mulsanti*et al.,* 2018). Rice genotypes differ widely in their lodging resistance, which is a complex phenomenon and is determined by many component traits. The stem traits contributing to lodging resistance include basal internode lengths and thickness, plant height, leaf sheath wrapping and pushing resistance. The plant height is determined by internode length, which has an important effect on lodging resistance of the stem.The elongation of the internode is expected to influence the lodging resistance. It is also well known that the biomass production potential of semidwarf varieties is lower than tall varieties because of high leaf area density in the canopy and low CO_2 diffusion efficiency in semidwarf varieties (Nomura *et al.*, 2019). Furthermore, *sd1*negatively affect the biomass and grain weight in rice (Okuno *et al.*, 2014). Therefore, to break the yield ceiling in rice, designing the new plant type (NPT) (Bagudam*et al.,* 2021) with high biomass, long and strong culms that resist the lodging would be an effective strategy. Most of the present-day high yielding *indica* cultivars are short staturedand are prone to lodging, whereas *japonica* tend to have thick culm and high flexibility due to bending stress. A good level of lodging resistance can be achieved through different combinations of strong culm traits.Thus, a viable strategy is to combinenovel germplasm of tropical *japonica* and*indica*and harness large genetic variability and desirable alleles for strong culm.

Comment [UMI5]: Hyphen them

Phenotypic selection based on their performance may not be effective because these genotypes may perform poorly in further segregating generations (Akshay *et al.*, 2022), thus it is very crucial to select the lines based on heritability and genetic advance. Therefore, assessment of variability is essential to design selection criteria for the improvement of culm strength, consequently grain yield. To improve the complex traits like strong culm, it is inevitable to know how attributing traits interact with one another complementing the grain yield.In this context, it is imperative to assess the influence of internode length on lodging resistance. Thus, the present study was conducted to assess Genetic variability and trait interrelationship studies of internode length and strong culm related traits in RILs derived from inter sub-specific crosses in rice.

Comment [UMI6]: Culm-related

Comment [UMI7]: Inter-sub-specific

MATERIAL AND METHODS

A set of 353 RILs and six checks were evaluated during the wet season (WS)2023 at ICAR-IIRR, Hyderabad. The RILs were sown in the raised bed and transplanted into the main field 27 days after sowing with the spacing of 20 x 15 cm in an augmented randomized complete block design (ARCBD). RILs were planted only once while the checks were replicated in all ~~the~~ seven blocks. All the recommended packages of practices were followed for the good establishment of the crop. RILs were evaluated and observations were recorded on random four plants in each line for morphological traits *viz.*, plant height (PH) (cm), number of internodes (INN), length of first internode (IL1), length of second internode (IL2), length of third internode

Comment [UMI8]: An ‘an’ before it

(IL3), length of fourth internode (IL4), length of fifth internode (IL5), length of sixth internode (IL6), pushing resistance (PR)and culm thickness (CT) of fourth internode. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were estimated according to the method elucidated by Burton (1952) and the estimates for variability were treated as per the categorization proposed by Sivasubramanian and Madhavamenon, 1973. The broad sense heritability (h^2) and GAM were calculated as per Lush (1940) and Johnson *et al.* (1955). The degree and direction of association between variables were estimated by using Karl Pearson's Coefficient of correlation (r) utilizing variance and covariance of the variables.

The following traits were determined as per Ookawa*et al.* (2010) and Badri *et al.* (2024)

Culm thickness (CT) = $[(a_1+b_1)/2] – [(a_2+b_2)/2]$

Where a_1 is outer diameter of minor axis, b_1 is outer diameter of major axis, a_2 is the inner diameter of minor axis, b_2 is inner diameter of major axis.

PR is the prostrate tester reading value. The prostrate tester was used to measure the pushing resistance of the culm (Fig 1).

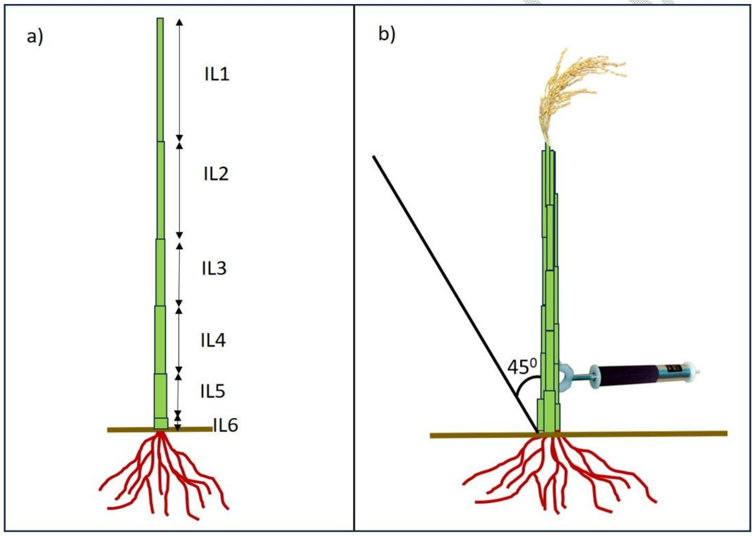

Fig. 1 Graphical representation of some of the measured traits in this study a) Internode elongation and number ; b) Determination of pushing resistance using prostrate tester (DIK-7400, Daiki Rika Kogyo Co. Ltd., Tokyo, Japan)

RESULTS AND DISCUSSION

It is imperative to identify the essential traits that different breeding methods can employ for crop improvement. The detailed knowledge of variability, heritability, magnitude and direction of interaction between strong culm and its attributing traits offers an opportunity to establish an effective selection criterion in crop plants. In the present study, the tropical *japonica* line IRGC 39111, possessing strong culm was used as a donor parent against mega variety swarna to develop RILs. The RILs elicited great phenotypic variability, particularly related to NPT phenology.The Analysis of variance (ANOVA) demonstrated that all the lines

Comment [UMI9]: by

were significantly different for all the traits studied (Table 1). The parents were significantly different for all traits. Swarna was a dwarf with short and weak culm but IRGC 39111 was taller than Swarna with wider and thick culm. The RILs derived from them showed tremendous transgressive segregation. The distribution of traits was continuous and indicated that the traits were controlled by ~~a~~minor genes and are typical quantitative traits (Fig 2).

Comment [UMI10]: thicker

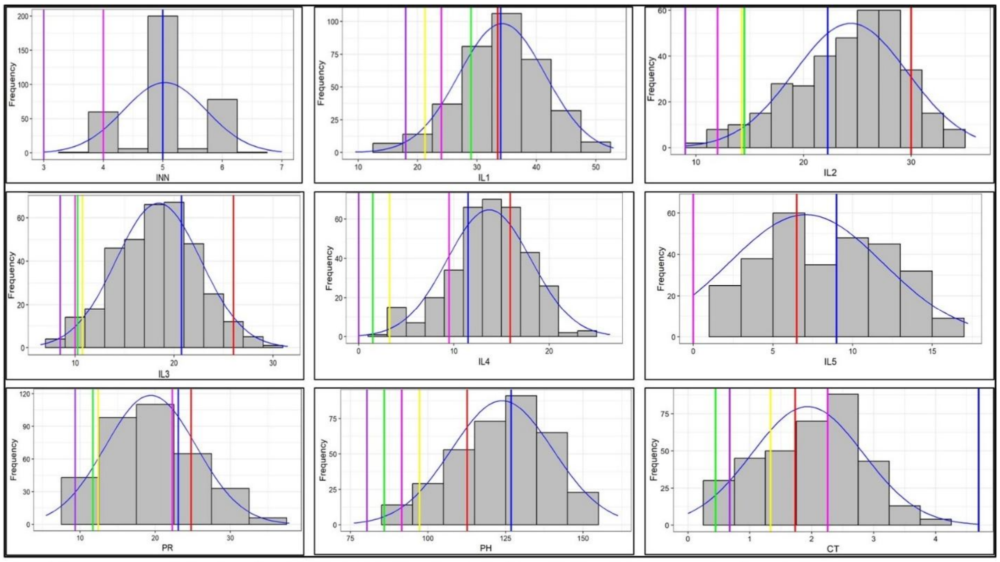

Fig. 2 Frequency distribution of traits INN, IL1, IL2, IL3, IL4, IL5, PR, PH, CT measured in this study

a Green, red , purple, blue, yellow and magenta plotted lines represent the check values of Swarna, IRGC 39111, Samba Mahsuri, Cuba 65, Tellahamsa, RMS 2085 respectively.

The variability analysis revealed that the PCV and GCV values for traits IL1, IL2, IL3 were similar with high heritability and genetic advance as percent of mean connoting no environmental influence on these traits and indicating the role of additive gene action. Thus, direct selection of these traits can be effective in breeding for stem bending-type of lodging, which is often seen in the upper internodes of rice. The PCV was higher than GCV for IL4, PR, PH and CT indicating the apparent influence of the environment apart from genotypes(Table 2). The traits IL5and IL6 also connotated high PCV, GCV, heritability and GAM indicating most likely the heritability is due to additive gene action. The PCV and GCV were higher in the following traits *viz.*,IL1 (21.11, 21.11), IL2 (21.09, 21.09); IL3 (22.85, 22.85); IL4 (31.46, 31.14), IL5(68.2, 68.2), IL6 (229.47, 229.47), INN (13.63, 13.63), PR (32.11, 28.99), PH (12.85,12.07), CT (30.55, 26.33). This result was concomitant with the findings of Zhu *et al.* (2008); Reddy *et al.* (2019), Zhao *et al.* (2021). The observed variability among RILs probably be attributed to the inherent genetic differences and the environment in which they were grown. The mean values and genetic parameters are presented in Table 2.

The heritability for all the characters studied was high (>80%) and it ranged from 81.5 – 100% (Table 2). Heritability coupled with GAM was found to be more useful for selection (Johnson *et al.*, 1955). In the present study, GAM ranged from19.07 (CT) – 473.4 (IL6). The traits IL4, IL5 and IL6 recorded high GAM and high heritability indicating they are governed by additive gene action and these traits were less influenced by the environment. Selection of these traits can be effective to improve stem breaking-type of lodging resistance, which is often seen in lower internodes. These results confirm that there is a possibility of direct selection for these traits, similar results were observed by Zhao *et al.* (2021) and Zhu *et al.* (2008). CT recorded high heritability (90.93 %) and moderate GAM (19.07) indicating the role of both additive and non-additive gene actionsimilar to the findings of Cui *et al*., 2002.

Comment [UMI11]: Stem-breaking-type

Pearson's correlation is the measure of interrelationship between the traits (Reddy *et al*., 2019). All four upper elongated internodes and INN contributed significantly to the plant height. The correlation analysis also disclosed that the PR (measure of culm strength) was positively correlated with CT, INN, IL1, IL2, IL3, PH and negatively correlated with IL4, IL5, IL6 (Table 3). The negative correlation of IL4, IL5, IL6 with culm thickness and pushing resistance depicts the role of these plant attributes for breaking type lodging resistance. This result wasin concomitant with the reports of Rani *etal.*(2018); Zhang *et al.* (2010); Zhao *et al.* (2021). Strong positive correlation between PR, CT and PH may suggest that this might be due to higher leaf sheath wrapping and secondary cell wall components like lignin, cellulose and hemicellulose attributingbending stressin tropical *japonica*(Ookawa*et al.*, 2010).

Comment [UMI12]: A strong

A negative relationship of IL4, IL5 and IL6 with CT and PR has been observed, which suggests that the longer primary internodes may account for low thickness and resistance and result in lodging. The strong association between PR and CT traits revealed that the thickness is strongly associated with the culm strength. Hence selecting the plants with a higher CT and lower basal internodal length strongly improves the culm strength in rice. Similarly, all the internode lengths were significantly and positively correlated with each other indicating that the selection may be positive for these traits. A critical analysis of the character association results revealed a favorable correlation between the basal primary internodes and PR, CT and PH. As a result, choosing ~~for~~ these characteristics may improve the culm strength in rice.

CONCLUSION

From the present study of interrelationships between internode length and strong culm traits, it is evident that basal primary internodes particularly IL4, IL5 and CT were most important for determining the strong culm trait in rice. This study also displayed strong association among PH, PR and CT. This indicated that the tall plant with thick culm and dense leaf sheath wrapping would resist lodging. Thus, selection for lower basal internode length, culm thickness would give a better response for culm strength in the present material. Simultaneous selection of IL4, IL5 and CT might be effective in the improvement of strong culm in rice. This study provides scope for attaining targeted strong culm in rice.

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Table 1 Analysis of variance for plant height and lodging resistance traits in RILs

Source	**Df**	**INN**	**IL1**	**IL2**	**IL3**	**IL4**
Block (ignoring Treatments)	6	0.73**	60.17**	80.17**	89.25**	43.24**
Treatment (eliminating Blocks)	358	0.59**	60.84**	36.41**	22.79**	26.39**
Treatment: Check	5	3.97**	304.46**	419.13**	363.43**	279.32**
Treatment: Test and Test vs. Check	353	0.54**	57.39**	30.99**	17.97**	22.81**
		IL5	**IL6**	**PR**	**PH**	**CT**
		37.14**	25.15**	225.27**	703.75**	0.93**

		26.03**	7.12**	39.56**	334.31**	0.62**
		116.49**	-	325.11**	2151.35**	16.63**
		24.75**	7.22**	35.52**	308.58**	0.39**

*** P <= 0.05; ** P <= 0.01**

Table 2 Summary statistics for plant height and lodging resistance related traits in 353 Swarna/ IRGC 39111 RILs

Traits	Swarna	IRGC 39111	P2-P1	Mean ± SE(RILs)	CV%	PCV	GCV	hBS	GAM
INN	4±0.01	5±0.03	1	4.95±0.03	14.90	13.63	13.63	100	28.12
IL1	29±0.01	34±0.07	5	33.53±0.37	22.36	21.11	21.11	100	43.56
IL2	15±0.12	30±0.19	15	23.7±0.29	24.71	21.09	21.09	100	43.51
IL3	10±0.1	26±0.13	16	18.05±0.23	26.02	22.85	22.85	100	47.15
IL4	1.5±0.01	12.5±0.13	11	13.42±0.23	33.93	31.46	31.14	97.95	63.57
IL5	-	6.5±0.21	6.5	8.62±0.21	43.98	68.2	68.2	100	140.7
IL6	-	-	-	5.42±0.36	60.38	229.47	229.47	100	473.4
PR	15.5±0.01	24.5±0.12	9	19.19±0.31	32.91	32.11	28.99	81.5	53.99
PH	85±0.01	115.25±0.1	30.25	121.64±0.89	14.63	12.85	12.07	88.34	23.41
CT	1.27±0.1	1.71±0.11	0.44	1.92±0.04	48.56	30.55	26.33	90.93	19.07

Table 3 Correlation coefficients between traits related to basal internodes, plant height and lodging resistance traits in Swarna/ IRGC 39111 derived RIL population

Traits	INN	IL1	IL2	IL3	IL4	IL5	IL6	PR	PH	CT
INN	1									
IL1	0.23***	1								
IL2	0.33***	0.70***	1							
IL3	0.34***	0.35***	0.65***	1						
IL4	0.51***	0.21**	0.48***	0.67***	1					
IL5	0.46***	0.05	0.12**	0.30***	0.55***	1				
IL6	0.26***	-0.05	-0.1	0.06	0.19**	0.47***	1			
PR	0.12	0.06	0.12**	0.14**	-0.09	-0.05	-0.07	1		
PH	0.20**	0.17**	0.29**	0.35***	0.33***	0.03	-0.05	0.29***	1	
CT	-0.12	0.09	0.13**	0.16**	-0.10**	-0.06	-0.02	0.18***	0.10	1

*****P <= 0.01, ** P <= 0.05**

[a] Trait abbreviations are number of elongated internodes (INN); Lengths of first internode (IL1), second internode ((IL2), third internode (IL3), fourth Internode (IL4), fifth Internode (IL5), sixth internode (IL6); pushing resistance (PR); plant height (PH); culm thickness (CT).