



GENETIC ANALYSIS OF CULM STRENGTH AND ITS RELATED TRAITS IN RICE (*Oryza sativa* L.)

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ABSTRACT

The present study was conducted on 48 rice genotypes with the aim to assess the genetic relationship between culm strength and its influencing traits in rice. Culm strength of rice has been used as an index of lodging resistance and it was measured as pushing resistance by prostrate tester. Correlation analysis revealed that culm strength is significantly positively correlated with culm diameter, panicle number and culm wall thickness. Principal component analysis revealed that the first four principal components with Eigen value > 1 explained 79.36% cumulative variance. The maximum variation explained by culm strength and its related traits like culm diameter and culm wall thickness which showed positive contribution in all the four components. Distribution of genotypes into four clusters, based on Ward's minimum variance method, showed that cluster-IV had high mean (261.4 g stem⁻¹) and cluster-II low mean (96.21 g stem⁻¹) values of culm strength. The electron microscopic observations of vascular tissues of lower most elongated internode of four genotypes 'CR 401' and 'Pusa-44' from cluster-IV and 'Swarna' and 'BPT-5204' from cluster-II revealed significant variation in vascular tissue and phloem diameter among the tested genotypes. This indicated significant diversity among rice genotypes which can be useful for identifying the QTLs for culm strength and for releasing new cultivar.

Key words: Cluster analysis, culm strength, principal component analysis, rice genotypes, vascular tissues

INTRODUCTION

Rice (*Oryza sativa* L.) is primary food source for more than half of the world's population. The global rice area (167 million ha) produces 769 million tonnes of rice with a productivity of 3.1 t ha⁻¹ (FAOSTAT, 2018). More than 90% rice is produced and consumed in South and South-East Asia. It is an important food crop for Africa, Latin America and Middle East. India is the 2nd largest country producing rice on an area of 43.78 million ha with annual production of 168.5 million tonnes and productivity of 2.3 t ha⁻¹ (FAOSTAT, 2018). The impressive growth rates witnessed in 1980s is no more and since last 10 years the production growth has declined to less than 1.0% as against the minimum required 1.8% (OECD/FAO, 2017). Meeting the growing targets beyond 2025

is, however, going to be the most challenging task warranting substantial research efforts to tailor the technologies capable of raising progressively the ceiling to genetic yield.

The productivity of rice is directly governed by three key traits viz., grain number, grain weight/size and panicle number and indirectly by many plant architecture related traits such as heading date, plant height, culm strength, etc. Of these, culm strength is an important plant architecture-related trait essential for lodging resistance (Setter *et al.*, 1997). Lodging resistance is a complex trait influenced by many physio-morphological traits like plant height, root thickness, culm diameter, culm strength and weight of upper part of plant (Islam *et al.*, 2007; Hirano *et al.*, 2017). Genotypes having higher lodging resistance possess higher solid pith area, thicker sclerenchyma layer and more number of vascular bundles which contribute to strong culm strength. The major factor that affects crop lodging is weather condition, especially wind and rain (Mobasser *et al.*, 2009), which results in heavy yield loss. Successful rice breeding programme aimed at production of higher yields is mainly dependent on strong culm strength (Wu *et al.*, 2011) and plants with shorter and solid stems (Hasanth *et al.*, 2013). Keeping in view the importance of lodging resistance, the present study was aimed to genetically analyze the culm strength and its related traits in 48 rice genotypes.

MATERIALS AND METHODS

The field experiment was conducted during *kharif*, 2013 at A.N.G. Ranga Agricultural University (ANGRU), Hyderabad, India (78° 16' longitude, 17° 32' latitude with 540 m masl). The experimental material consisted of 48 genotypes viz., 'CN-384', 'CR-401', 'Dular', 'INRC-10192', 'IR-64' and 'Jagannath' procured from CRRI, Cuttack, 'Basmati 370', 'BM-71', 'Pusa-44', 'Pusa Basmati-1' and 'Swarna sub1A' from IARI, New Delhi, 'Acharumati', 'BPT-5204', 'Chittimuthyalu', 'Erramallelu', 'Jaya', 'JGL-11470', 'JGL-11721', 'JGL-17004', 'JGL-1798', 'JGL-3844', 'JGL-3855', 'MTU-1001', 'MTU-1010', 'MTU-1064', 'MTU-1071', 'MTU-3626', 'N22', 'NLR-34449', 'Raasi', 'Ramappa', 'Ravi', 'Swarna', 'Satya', 'TKM-6', 'Varalu', 'WGL-14' and 'WGL-32100' from ANGRAU, 'Selection-1 (S1)', 'Selection-2 (S2)', 'Selection-3 (S3)', 'Selection-4 (S4)', 'Selection-5 (S5)' and 'Selection-6 (S6)' selected lines from the segregating generations and 'SV-1', 'SV-2', 'SV-3' and 'SV-4' local land races. The trial was laid out in a randomized complete block design with three replications. Each entry was grown in two rows of 1.5 m length, at a plant spacing of 25 cm × 10 cm. Standard agronomic and cultural practices were followed to raise a healthy crop during the season (Annon, 2013). At maturity, the data were recorded on culm strength and its related traits and averaged over five plants from each replication of experimental genotypes.

For measuring culm strength, at fully ripening stage, a prostrate tester (Daiki Rika Kogyo Co., Tokyo, Japan) was set perpendicular to the middle of plant from 20 to 25 cm above the ground and the plant was pushed at an angle 45° to measure the pushing resistance (Kashiwagi and Ishimaru, 2004). Culm strength (CS) was then estimated using the following formula given by Yadava *et al.* (2017).

$$\text{Culm strength (g stem}^{-1}\text{)} = (\text{Test reading} \div 40 \times 1000) \div \text{Number of stems}$$

Phenotypic scoring (PS) of culm strength at maturity was done by using 0 to 9 scale of standard evaluation system for rice (IRRI, 2013). The culm diameter and culm wall thickness were measured at the centre of first elongated internode using sliding Vernier caliper (Mitutoyo Digmatic Caliper, Japan). Tiller bearing panicles were counted manually and plant height measured from ground level to the tip of highest panicle. Culm height was measured from the base of stem to the tip of top node of plant. The internodes were counted from top to bottom between nodes and intermodal length was measured using meter scale. Pith diameter was measured according to the equation:

$$\text{Culm diameter} = \text{Culm wall thickness} + \text{Pith diameter}$$

Further, at fully ripening stage, lower most elongated internodes of four contrasting genotypes were subjected to scanning electron microscope (SEM model: JOEL-JSE 5600) at 8 KV and magnification at 1000X and 2500X as per the standard procedure at RUSKA Lab, College of Veterinary Sciences, Sri Venkateswara Veterinary University, Rajendranagar, Hyderabad (India).

The data was subjected to correlation analysis and principal component analysis using STAR (Statistical Tool for Agricultural Research) 2.1.0 software (Gulles *et al.*, 2014). The first two principal components were plotted against each other to find out the pattern of variability among genotypes. Cluster analysis was performed using the same software to construct tree diagram based on Euclidian distances as per Ward's minimum variance method (Szekely *et al.*, 2005)

RESULTS AND DISCUSSION

The analysis of variance revealed considerable variability among genotypes for all the traits measured in present study. The estimations of mean, range, coefficient of variation (CV) and heritability of all the characters were studied to assess the variability pattern among the genotypes. The highest variation was observed for culm strength followed by plant height and culm height (Table 1). Relatively low variation was noticed for culm wall thickness followed by the number of internodes and pith diameter. The observed variability among genotypes can probably be attributed to the inherent genetic differences and the environment in which they were grown. The frequency distribution of traits was approximately continuous and indicated that the traits were typical quantitative traits controlled by a few minor genes.

Pearson's correlation is the measure of strength of linear relationship between the characters. In present study, culm strength was significantly and positively correlated with panicle number, culm diameter and culm wall thickness. Phenotypic scoring of culm strength was also significantly and positively associated with panicle number and culm strength (Table 2). Such strong positive correlations among genotypes suggest that they are heritable and genetically controlled traits which could be simultaneously transferred to the desirable genotypes. Similarly, several workers have reported the correlation of culm strength with culm length, culm diameter and culm wall thickness in rice (Ookawa *et al.*, 2010a; Zhang *et al.*, 2010; Yang *et al.*, 2012; Zhang *et al.*, 2016). Fallah (2012) observed that lodging index in hydroponical rice was reduced with increase in stem diameter. Plant height was not necessarily the most important factor in determining the lodging resistance (Ookawa *et al.*, 1992) but decreasing the culm length is essential to improve lodging resistance in rice (Chuanren *et al.*, 2004) particularly in breeding super rice (Chen *et al.*, 2005). Laza *et al.* (2014) and Ming-liang *et al.* (2012) reported positive correlations between percentage lodging and plant height.

Table 1: Estimation of basic statistics for 10 quantitative traits in 48 rice genotypes

Characters*	CS	PS	CD	PH	CH	PN	NI	IL	CWT	PD
Mean	126.16	6.83	5.60	82.06	62.94	9.18	6.97	8.18	3.08	2.53
Min	12.50	1.00	3.50	45.80	33.00	5.40	4.90	3.00	2.20	0.80
Max	383.99	9.00	7.20	132.00	106.20	15.80	8.80	17.70	4.70	4.40
Range	371.49	8.00	3.70	86.20	73.20	10.40	3.90	14.70	2.50	3.60
SeM _±	17.50	0.59	0.21	2.93	2.66	0.54	0.17	0.81	0.13	0.22
SD	76.88	2.16	0.87	13.63	12.44	2.38	0.69	2.57	0.50	2.53
CV(%)	16.99	10.55	4.66	4.38	5.18	7.19	2.98	12.11	5.26	10.77
Variance	16964.01	12.39	2.17	530.97	443.59	16.32	1.36	18.07	0.73	1.83
Heritability	99.90	54.01	31.77	99.18	98.85	69.42	31.34	59.87	12.19	14.52

*Characters: CS - Culm strength (g stem⁻¹), PS - Phenotypic scoring, CD - Culm diameter (mm), PH - Plant height (cm), CH - Culm height (cm), PN - Panicle number, NI - Number of internodes, IL - Internodal length, CWT - Culm wall thickness, PD - Pith diameter

Table 2: Pearson phenotypic correlation coefficient among 10 quantitative traits in rice genotypes

	PH	CH	NI	PN	CD	IL	CWT	PD	CS
PH	1.000								
CH	0.982**	1.000							
NI	0.198	0.210	1.000						
PN	-0.250	-0.257	0.060	1.000					
CD	0.260	0.220	0.155	-0.064	1.000				
IL	0.456**	0.462**	0.038	-0.124	-0.037	1.000			
CWT	0.355*	0.373**	0.292*	-0.345*	0.428**	0.201	1.000		
PD	0.058	0.003	-0.015	0.149	0.820**	-0.167	-0.166	1.000	
CS	0.283	0.276	0.112	0.55**	0.331*	0.120	0.630**	-0.038	1.000
PS	-0.223	-0.240	-0.225	0.295*	0.033	-0.275	-0.383	0.279	0.456**

*, ** significant at 5% and 1% respectively; For explanation of abbreviations see Table 1

All the other culm strength contributing traits were positively correlated with each other indicating that the selection may be positive direction based on these traits towards crop improvement.

Principal component (PC) analysis was carried out to identify the minimum number of components which can explain maximum variability out of total variability (Sharma, 1998; Fenty, 2004) and also rank genotypes on the basis of PC scores. In this analysis, all the variables were standardized to avoid the influence of scale effects on the composition of derived components due to different units of variables. Scree plot (Fig. 1) explained the percentage of variation associated with each PC obtained by drawing a graph between Eigen values and PC numbers. According to Gatten's lower bound principle Eigen values of <1 should be ignored (Kumar *et al.*, 2011). The principal component Scree plot of rice genotypes depicted that the first four Eigen values (>1) correspond to the whole percentage of variance in the data set. The first four main PC's were extracted from the complicated 10 components, the total cumulative variance of these four principal components (PC1, PC2, PC3 and PC4) accounted for 79.36% of total variation (Table 3). This suggests that after PC4 more principal components do not describe much variation. Thus, only first four PC's were considered. From the graph, it is clear that the maximum variation was observed in PC1. The quantitative traits which contributed more to the first PC accounted for 34.03% of the total variation. The 2nd, 3rd and 4th PC's explained 19.56, 14.86 and 10.9% variation, respectively.

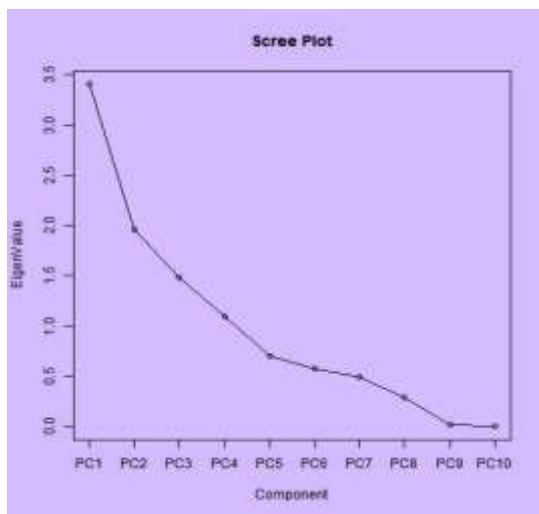


Fig. 1: Scree graph of principal component analysis of 48 genotypes of rice between Eigen values and the number of principal components

Table 3: Eigenvectors, eigenvalues, percentage variance and cumulative variance of ten quantitative traits in rice genotypes

Characters	PC1	PC2	PC3	PC4	% Contribution
CS	0.382	-0.005	0.451	-0.129	0.22
PS	-0.302	0.278	-0.211	-0.204	-35.63
CD	0.213	0.629	0.150	0.005	76.61
PH	0.427	0.071	-0.438	-0.094	17.74
CH	0.429	0.036	-0.437	-0.076	-31.99
PN	-0.290	0.147	-0.324	0.454	28.59
NI	0.179	0.054	-0.011	0.818	2.93
IL	0.268	-0.188	-0.394	-0.107	-5.67
CWT	0.404	0.013	0.295	0.175	59.72
PD	-0.023	0.678	-0.024	-0.105	-12.56
Eigen value	3.403	1.956	1.486	1.090	
Variance (%)	34.03	19.56	14.86	10.900	
Cumulative					
Variance (%)	34.03	53.6	68.46	79.36	

For explanation of abbreviations see Table 1

Table 4: Traits having highest values in 1st four principal components

	PC1	PC2	PC3	PC4
Traits	Culm strength	Phenotypic scoring	Culm strength	Panicle number
	Culm diameter	Culm diameter	Culm diameter	Number of internodes
	Plant height	Panicle number	Culm wall thickness	Culm wall thickness
	Culm height	Pith diameter		
	Number of internodes			
	Intermodal length			
	Culm wall thickness			

Based on PCA score, the culm diameter contributed maximum (76.61%) towards diversity followed by culm wall thickness (59.72%) and panicle number (28.59%). The first PC was related to culm strength, culm diameter, plant height, culm height, number of internodes, intermodal length and culm wall thickness. The second PC was related to phenotypic scoring of culm strength, culm diameter, plant height, culm height, panicle number, number of internodes, culm wall thickness and pith diameter. The third PC was related to culm strength, culm diameter and culm wall thickness and the fourth PC was related to culm diameter, panicle number, number of internodes and culm wall thickness (Table 4). The first and second PCs with a cumulative variance of 53.6% revealed the most variation among genotypes showing a high degree of correlation among the traits studied. Overall, the PCA analysis showed that the phenotypic markers are useful in genotypes of rice and can be used to identify a few key traits that account for the largest variability. These results are in agreement with earlier workers (Yadav *et al.*, 2017; Gour *et al.*, 2017). Distribution of quantitative traits in first two components is shown in biplot (Fig. 2). This plot clearly showed that culm strength, culm diameter, plant height, culm height, number of internodes, intermodal length and culm wall thickness were the traits that contributed towards diversity. The PC scores of three PCs (Table 5) showed that in PC1 the positive scores ranged from 5.716 ('CR 401') to 0.210 ('N22'), in

Table 5: Scoring of 48 genotypes in first three principal components

Genotypes	PC1	PC2	PC3	Genotypes	PC1	PC2	PC3
Acharumati	1.303	1.799	-1.502	N22	0.210	-2.012	-0.762
Basmati 370	2.948	-0.991	-4.260	NLR-34449	-2.050	0.251	0.222
BM-71	0.432	-0.519	-0.013	PUSA- 44	1.766	-2.483	2.346
BPT-5204	-1.029	0.317	-1.202	PUSA BAS	-1.416	-1.216	-0.247
Chittimuthyalu	-1.321	-1.809	-0.869	Raasi	-0.460	-1.692	-0.629
CN-384	-2.886	-3.347	1.101	Ramappa	-0.652	-0.380	0.391
CR-401	5.716	-0.068	0.598	Ravi	-0.626	-0.481	0.805
DULAR	-0.236	2.660	-0.062	Selection 1	-1.585	1.660	-0.070
Erramallelu	-1.966	-0.505	-0.853	Selection 2	-2.531	2.597	-0.788
INRC-10192	3.032	0.445	-3.038	Selection 3	-1.210	2.064	-0.371
IR-64	-0.224	-0.206	-0.231	Selection 4	-0.359	1.477	1.601
Jagannath	-1.593	-1.137	-0.711	Selection 5	-1.654	2.129	-0.527
Jaya	-1.434	-1.128	-1.288	Selection 6	-1.050	1.988	0.824
JGL-11470	0.733	-0.156	-0.084	Satya	-0.019	-1.160	-0.617
JGL-11721	0.547	-1.267	-0.837	SV-1	2.694	1.193	0.912
JGL-17004	-1.146	-0.679	-0.569	SV-2	2.352	-1.198	-0.539
JGL-1798	0.714	-0.995	1.825	SV-3	2.056	2.122	-0.610
JGL-3844	-0.498	1.186	0.389	SV-4	3.658	0.418	1.058
JGL-3855	-0.598	0.299	0.705	Swarna	-3.071	0.856	-0.591
MTU-1001	0.894	0.885	-0.521	Swarna sub1A	-1.269	0.792	1.183
MTU-1010	-0.057	1.632	1.075	TKM-6	0.595	0.492	1.269
MTU-1064	2.621	-0.134	2.403	Varalu	-0.419	-0.957	0.585
MTU-1071	1.830	0.384	1.328	WGL – 14	0.724	-1.523	-0.153
MTU-3626	-2.114	-0.546	1.012	WGL-32100	-1.351	-1.057	0.311

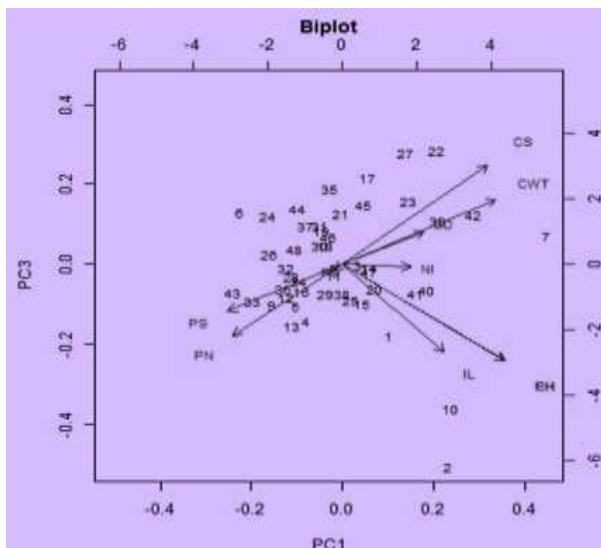


Fig. 2: Biplot of the first two PCAs showing relation among various quantitative traits in rice

PC2 the positive scores ranged from 2.66 ('Dular') to 0.251 ('NLR-34449') and in PC3 the positive values ranged from 2.346 ('Pusa 44') to 0.222 ('NLR-34449'). It was observed that genotype 'CR 401' had highest positive value and 'Swarna' had highest negative value from PC1. So, the selection of genotypes with highest score in PC1 will be desirable for developing strong culm strength varieties in rice. This showed that PCA is a reliable method in identifying few key traits contributing to the largest variation and could be a reliable method in predicting the important traits influencing clustering of different cultivars.

According to Chahal and Gosal (2002) the characters with larger absolute values closer to unity with first PC influence clustering more than those with lower

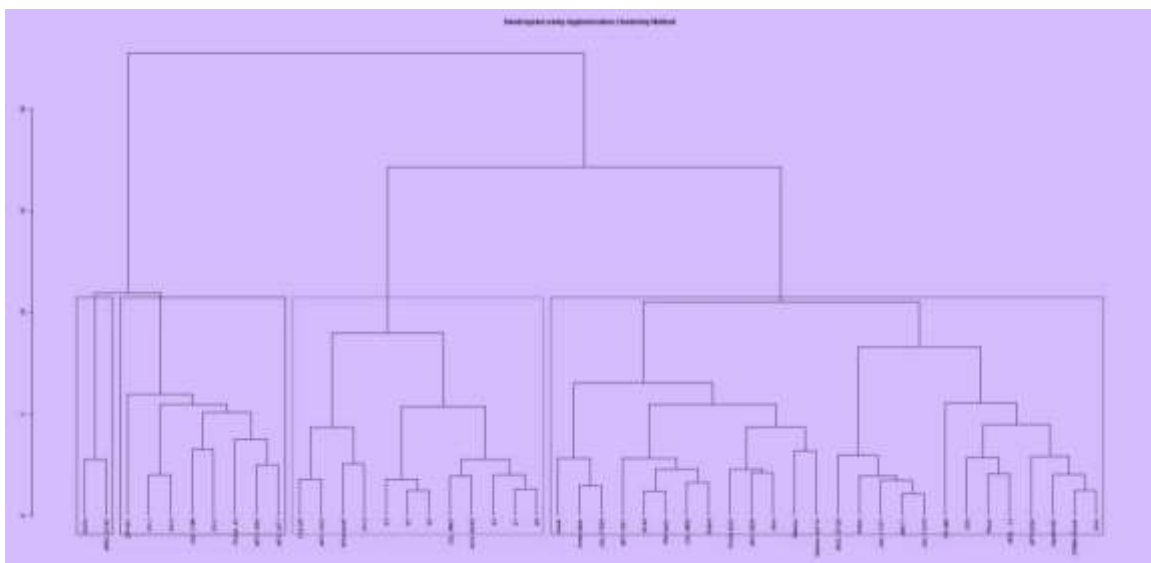


Fig. 3: Dendrogram of rice genotypes resulting from cluster analysis using Ward's method based on standardized data of all the traits

absolute values closer to zero. In present study, the total variability in genotypes seems due to the cumulative effect of many traits rather than the small contribution of each character. Four distinct clusters were observed during cluster analysis of 48 genotypes (Fig. 3). The cluster-III comprised largest number with 26 genotypes, followed by cluster-I with 12 genotypes, cluster-IV with 8 genotypes and cluster-II with 2 genotypes (Table 6). The mean value of culm strength (g stem^{-1}) was highest in cluster-IV (261.4) and lowest in cluster-III (96.21). Cluster-I (103.15) and cluster-II (112.61) showed intermediate mean values for culm strength. The other culm strength related traits viz., phenotypic scoring of culm strength (4.67), culm diameter (6.05), number of internodes (7.27) and culm wall thickness (3.83) were in desirable direction in cluster-IV. The clustering pattern showed that significant genetic variability among the rice genotypes tested which indicated the presence of excellent opportunity to bring about improvement through hybridizing genotypes from different clusters.

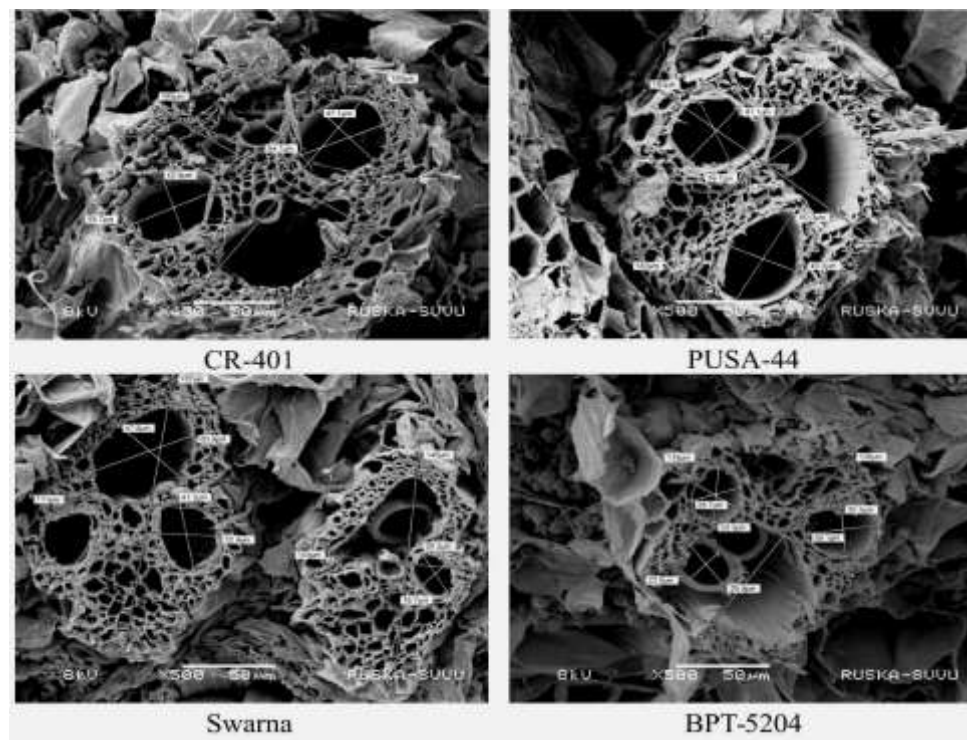


Fig. 4: Scanning electron micrograph of transverse sections of lower most elongated internode of four contrasting rice genotypes

Electron microscopic images of transverse sections were scanned to observe the anatomical structure of lower most elongated internodes of four genotypes representing from the contrasting clusters. Although strong culm strength in genotypes will differ significantly for the number of vascular bundles and width of sclerenchymatous tissue (Wu *et al.*, 2011; Zhang *et al.*, 2016; Wu *et al.*, 2017) and parenchyma cells (Ookawa *et al.*, 2010b; Yano *et al.*, 2015), in present study it was clearly observed that stronger culm strength genotypes ‘CR-401’ and ‘PUSA-44’ exhibited high

Table 6: Mean values of 10 quantitative traits in four clusters of 48 genotypes in rice

Clusters	Genotypes	CS	PS	CD	PH	CH	PN	NI	IL	CWT	PD
I	Acharumati,Dular, JGL-3844, MTU-1010, NLR-34449, S1, S2, S3, S4, S5, S6, SV-3	103.15	8.45	6.03	77.96	58.51	10.52	6.96	7.71	3.08	2.95
II	Basmati 370, INRC-10192	112.61	5.67	5.46	123.67	101.87	10.08	6.69	12.63	2.97	2.49
III	BM-71, BPT-5204,Chittimuthyalu, CN-384,Erramallelu, IR-64, Jaya, Jagannath, JGL-11470, JGL-11721, JGL-17004, JGL-3855, MTU-1001, MTU-3626, N22, Pusabasumati 1,Raasi,Ramappa, Ravi, Satya, Swarna, Swarna sub1A, TKM-6,Varalu, WGL - 14, WGL-32100	96.21	6.85	5.04	78.66	59.94	9.18	6.91	7.72	2.85	2.19
IV	CR-401, JGL-1798, MTU-1064, MTU-1071, PUSA- 44, SV-1, SV-2, SV-4	261.4	4.67	6.05	88.84	69.59	6.96	7.27	9.25	3.83	2.22

*CS - Culm strength, PS - Phenotypic scoring, CD - Culm diameter, PH - Plant height, CH - Culm height, PN - Panicle number, NI - Number of internodes, IL - Internodal length, CWT - Culm wall thickness, PD - Pith diameter

Table 7: Variation in vascular tissue traits among four contrasting genotypes

Genotypes	Culm diameter (mm)	Culm wall thickness (mm)	Pith diameter (mm)	Vascular tissue diameter (mm)	Phloem cell diameter (mm)
CR 401	6.466	0.673	5.793	0.183	0.052
PUSA 44	5.640	0.584	5.056	0.184	0.050
Swarna	3.497	0.350	3.147	0.120	0.034
BPT 5204	5.306	0.378	4.928	0.157	0.030

vascular diameter and phloem cell diameter as compared to the weak culm strength genotypes like 'Swarna' and 'BPT-5204' (Fig. 4; Table 7). In weak culm strength plants, the normal plant canopy structure is destroyed, leading to reduced photosynthetic efficiency (Hitika, 1969). Weak culm strength prevents the transport of water, nutrients and assimilates through the xylem and phloem, resulting in the reduction of assimilates for grain quality (Kashiwagi *et al.*, 2005). This study showed that significant variability exists in vascular tissues which will be useful for selection of strong culm strength genotypes.

Conclusion: In present study the culm strength of rice genotypes and its related quantitative traits revealed genetic diversity during preliminary evaluation. Correlation studies clearly showed that phenotypic scoring of culm strength, culm diameter, panicle number and culm wall thickness were significantly and positively correlated with culm strength. Principal component analysis was found a reliable method in identifying the few key traits like culm diameter and culm wall thickness which favour strong culm strength in rice genotypes. The cluster analysis with four clusters showed the presence of maximum genetic variability among the tested rice genotypes. Electron microscopy of lower most elongated internodes from contrasting genotypes showed the presence of significant diversity in vascular tissues. The present clearly indicates an excellent opportunity to identify the QTLs for culm strength and its influencing traits and their subsequent exploitation for releasing new cultivars in rice.

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