Indian Journal of Experimental Biology
Vol. 57, December 2019, pp. 893-898

Culm anatomy revealed association of vascular bundle number and silicon content with lodging behaviour in Kodo millet (*Paspalum scrobiculatum* L.)

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Received 14 June 2017; *revised 09 June 2018*

In dry lands, small millets such as kodo millet contribute to regional food security to some extent. It is normally harvested using paddy combine harvester due to labour scarcity. However, the current varieties are not amenable for mechanized harvesting as they lodge at grain filling and maturity stages, thus resulting in heavy grain loss in terms of quality and quantity. In this context, we studied the anatomical features of culm and elemental composition in relation to lodging behaviour in kodo millet (*Paspalum scrobiculatum* L.), which is one of the important yield limiting factors in this crop. The strong culm genotype, *Adari* had higher culm thickness, thicker mechanical tissue, more lignin deposition and more number of vascular bundles per cross section when compared to the weaker counterpart, Aamo10. However, not all the genotypes with thicker culm were lodging resistant. Sel21 which recorded the highest culm thickness (1283.4 μm) among the genotypes lodged heavily as higher culm thickness in Sel21 was not supported by an increased number of vascular bundles. Interestingly, TNPsc183 which had a moderate culm thickness of 782.82 μm exhibited a low degree of lodging and had more number of vascular bundles per cross section than Sel21. Hence, 'number of vascular bundles per unit area' appears to be an important trait in contributing lodging resistance in kodo millet. SEM-EDX studies for silicon and potassium contents in culm implicated the role of silicon, but not potassium in imparting culm strength in kodo millet. However, more potassium content in parenchymatous cell wall suggests its role in imparting strength to the non-lignified cells of the culm.

Keywords: EDX, Parenchyma, Potassium, Sclerenchyma, SEM, Silicon

Though rice and wheat form a major share in India's food production, small millets like kodo millet (*Paspalum scrobiculatum* L.) still contribute to the regional food security in dry and marginal lands[1]. In kodo millet growing tracts of Tamil Nadu, India, the crop is. However, kodo millet varieties and land races currently into cultivation are not amenable for mechanized harvesting due to their tendency to lodge at grain filling and maturity stages, thus resulting in heavy grain loss in terms of quality and quantity. Hence, understanding the lodging behaviour in kodo millet is important to identify genotype(s) which can suit to machine harvest.

A previous study with 299 genotypes in kodo millet concluded that morphological traits, such as high culm diameter, plant height and recovery angle after bending as ideal selection criteria to identify genotypes with good culm strength[2]. Among the biochemical traits, lignin content per unit length of the culm contributed to lodging resistance than cellulose or hemicellulose contents[3]. Besides, anatomical features like thickness of mechanical tissue, number of vascular bundles, hollowness of the culm, secondary wall thickness and elemental (silicon, potassium) composition were implicated in culm strength and thus lodging resistance in crops[4-6] like rice, wheat, oats, etc. Thus, a comprehensive understanding of the culm strength and lodging behaviour in kodo millet with respect to anatomical traits is the focus of this investigation.

Since literature related to culm strength and lodging resistance in kodo millet is not available, leads were taken from rice and other cereal members to understand this trait. The anatomical traits implicated in lodging resistance were, thicker band of sclerenchyma at the periphery of the stem[7], more number of vascular bundles, more number of outer vascular bundles fused with sclerenchyma tissue, compact parenchyma cells and a fewer or smaller air space[8,9], width of mechanical tissue[10] and thickened cell walls in peripheral vascular tissue and sclerenchyma cells under the epidermal layer were presumed to provide mechanical strength to the plant body[11].

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However, there were works with contradictory results, as well. For example, some authors did not find a significant correlation between the stem diameter and lodging resistance in wheat[12-16]. In oats and barley, a negative correlation between the stem diameter and culm strength were reported[17,18]. Here, we tried to characterize culm strength and lodging behaviour in kodo millet based on anatomical and elemental composition of the culm.

Materials and Methods

Anatomical features

Four kodo millet genotypes *viz*., *Adari*, Aamo10, Sel21 and TNPsc183 were chosen for this study. These genotypes were selected based on the data on culm weight and lodging behaviour observed from the previous field studies (Table 1). *Adari*, a landrace had high culm diameter and culm weight; Sel21 was a high yielder, with moderate culm weight, culm diameter and exhibited intermediate degree of lodging during maturity; TNPsc183 was a high yielder with moderate culm weight, culm diameter and exhibited low degree of lodging and Aamo10 was the weakest culm line of all and lodged with the onset of flowering. These four genotypes were raised during Jan-May, 2014 at the Department of Millets, Tamil Nadu Agricultural University, in a randomized block design with five replications. Each of the genotypes was raised in plots of size 3×3 m and replicated. Planting was taken up with a spacing of 22.5 cm between rows and 10 cm between plants. Field observations were recorded for plot yield (kg/plot) and degree of lodging (score 3: low degree of lodging; score 5: intermediate degree of lodging; score 7: high degree lodging). Ultrastructure of the culm *via* cross section was observed using SEM (scanning electron microscope) and measurements were recorded for seven traits *viz*., number of vascular bundles, culm thickness, mechanical tissue thickness, air space thickness, sclerenchyma cell wall thickness, parenchyma cell wall thickness and parenchyma cell diameter at physiological maturity of the crop. Observations were recorded from five randomly selected plants for each genotype per replication and subjected to statistical analysis.

Table 1 — Mean Performance of kodo millet genotypes over previous two seasons

Geno-types	Seasons	Culm weight per unit length (mg/cm)[#]	Culm diameter (mm)	Degree of lodging (score)[#]*	Grain yield per plant (g/plant)
Aamo 10	Jan.-May 2013	5.65	3.20	7	6.78
	Aug-Dec 2013	5.62	1.75	7	4.49
Sel 21	Jan.-May 2013	9.50	3.85	5	25.87
	Aug-Dec 2013	9.80	2.68	5	9.23
TNPsc 183	Jan.-May 2013	8.90	4.05	3	18.37
	Aug-Dec 2013	8.62	2.90	3	7.08
Adari	Jan.-May 2013	23.40	4.20	5	6.71
	Aug-Dec 2013	22.50	4.06	3	4.58

[[#]Traits culm weight per unit length (mg/cm) and degree of lodging exhibited minimal variations over seasons. Hence, based on these two traits, the above four genotypes were chosen for this study. *Degree of lodging: score 3, low degree of lodging; score 5, intermediate degree of lodging; score 7, high degree lodging]

Elemental composition

Silicon and potassium contents in percentage from different tissue types (mechanical tissue, sclerenchyma cells around the fiber sheath and parenchyma tissue) constituting the culm of each genotype were measured using SEM-EDX (Scanning Electron Microscopy- Energy Dispersive X-Ray Analysis).

Statistical analysis

The data recorded from the investigations carried out were subjected to analysis of variance (ANOVA). Wherever, the 'F' test was significant, the mean values were compared by Duncan's Multiple Range test ($P \leq 0.05$). A two way ANOVA was also performed to analyze the effect of variations due to genotypes (Factor A) and tissue types (Factor B) on the elemental (Silicon and Potassium) contents.

Result and Discussion

Culm internodes normally consists of different tissues such as pith (pith is absent in kodo millet), parenchyma, vascular bundles, sclerenchyma and epidermis. The structure and composition of cell walls of these tissues are different to suit different functions they perform. For example, parenchyma cells provide the main structural support in growing regions of the plant body and mainly consist of primary cell wall. Sclerenchyma cells, which have both primary walls and thick secondary walls, provide the major mechanical support in non-elongating regions of the plant body[19]. Hence, compactness and composition of different types of tissues may have a direct bearing on culm strength and hence the lodging resistance.

Number of vascular bundles contributed for lodging resistance

Among the seven anatomical traits investigated, only four traits *viz*., number of vascular bundles, culm thickness, mechanical tissue thickness and air space thickness showed significant variation among genotypes (Table 2).

Among the four genotypes, *Adari*, the land race recorded the highest values for number of vascular bundles (55.80 per section) and mechanical tissue thickness (43.37 μm) (Table 3). Moderate degree of lodging was observed in *Adari* (Table 4) which can be attributed to a higher number of vascular bundles and higher mechanical tissue thickness as these two traits were already reported to be correlated with culm strength in crops like foxtail millet[20] and rice[21].

Sel21 recorded the highest values for culm thickness (1283.40 μm) and air space thickness (291.68 μm), while Aamo10 the weak line with heavy lodging recorded least values for all the four traits (Table 3). Though Sel21 had higher culm thickness and air space thickness, it lodged severely during grain filling (Table 4). Hence, it can be presumed that a mere increase in culm thickness and air space thickness need not render the plant resistant to lodging. Aerenchyma was reported to be mechanically weak and imparted little strength to plants[22] and a negative association between air space thickness and culm strength was reported in rice[21]

Table 2 — ANOVA for traits relating to anatomical features in four genotypes of kodo millet

Source	Mean sum of squares		
	Replications	Treatments	Error
Degrees of freedom	4	3	12
Number of vascular bundles	28.075	322.05**	26.508
Culm thickness	331.124	465773.474**	2285.414
Mechanical tissue thickness	18.777	470.622**	15.628
Air space thickness	416.388	36575.033**	318.353
Cell wall thickness in sclerenchyma around vascular bundles	0.227	0.713	0.206
Cell wall thickness in parenchyma	0.098	0.127	0.082
Parenchyma cell diameter	75.555	98.494	83.234

[*significant at 5% level; **significant at 1% level]

However, among these four genotypes, TNPsc183 alone showed low degree of lodging. Sel21, though had a maximum culm thickness (Fig. 1) and mechanical tissue thickness (Fig. 2) lodged severely (Table 4) during grain filling itself. This means that a mere increase in culm thickness might not support the plant to stand erect. In TNPsc183, it is evident that a higher number of vascular bundles per cross section rendered the plant to stand erect. Though the culm thickness was lower in TNPsc183, the number of vascular bundles per cross section was comparable with that of Sel21 (Table 3), which means that vascular bundles per unit area were higher in TNPsc183 than Sel21 and this could be the reason for its better lodging resistance. Chaturvedi *et al.*[9] also reported that lodging tolerant varieties of rice had more vascular bundles, than that of the susceptible genotypes. Another study in wheat[16] indicated that, number of vascular bundles was an important factor affecting mechanical strength of the culm. The additional culm thickness found in Sel21 was not supported by an increased number of vascular bundles. This suggests that rather than ‘culm thickness’, the ‘number of vascular bundles’ is associated with lodging resistance in kodo millet. Thus the vascular bundles could be supporting the plant to stand erect. Hence, more the number of vascular bundle, more will be the lodging resistance.

Silicon an important player in strengthening the culm

The ANOVA for silicon and potassium contents showed significant differences between genotypes as well as across different tissues (Table 5). Silicon content (%) estimated using SEM-EDX revealed that silicon content was more in genotypes with higher culm diameter, *viz*., Sel21, TNPsc183 and *Adari*. But it was lower in weak culm genotype Aamo10 (Table 6. This could be one of the reasons for the severe lodging observed in Aamo10. Unlike other

Table 3 — Mean performance of kodo millet genotypes for anatomical traits observed through SEM

Genotypes	Number of vascular bundles (count)	Culm thickness (μm)	Mechanical tissue thickness (μm)	Air space thickness (μm)	Cell wall thickness in sclerenchyma around vascular bundles (μm)	Cell wall thickness in parenchyma (μm)	Parenchyma cell diameter (μm)
Adari	55.80a	958.30b	43.37a	245.20b	2.07	2.39	56.76
TNPsc183	44.40b	782.82c	23.74b	138.40c	2.15	2.21	55.92
Sel21	48.60b	1283.40a	39.02a	291.68a	2.69	2.33	47.63
Aamo10	36.60c	560.30d	25.73b	111.95d	1.79	2.59	50.14
Mean	46.35	896.21	32.97	196.81	2.17	2.38	52.61
SE	3.26	30.24	2.5	11.28	0.29	0.18	5.77
CD (0.05%)	7.1	65.88	5.45	24.59	NA	NA	NA

[NA, Not applicable since treatments are insignificant. a-d: Mean values followed by same letters do not differ significantly at $P \leq 0.05$ as per Duncan’s multiple range test]

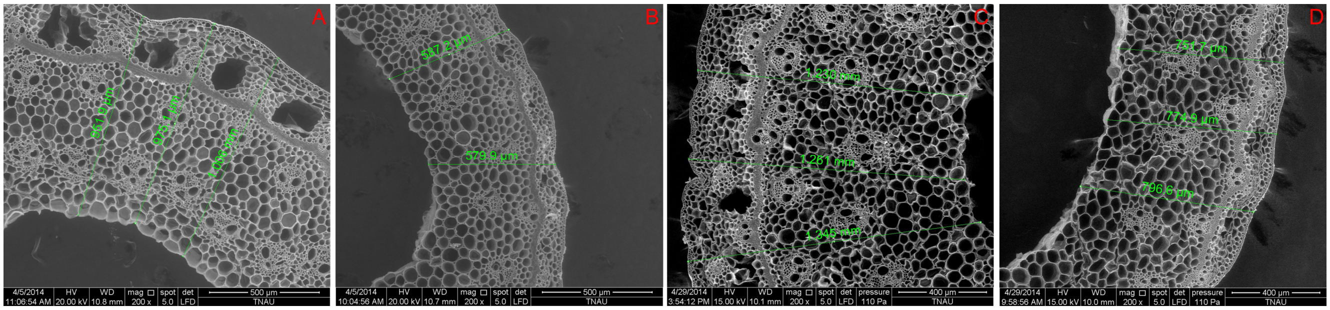

Fig. 1 — Variations in culm thickness in kodo millet genotypes. (A) Adari; (B) Aamo 10; (C) Sel 21; and (D) TNPsc 183. [Sel 21 exhibited the highest culm thickness of all. Aamo 10 with least thickness (200X magnification)]

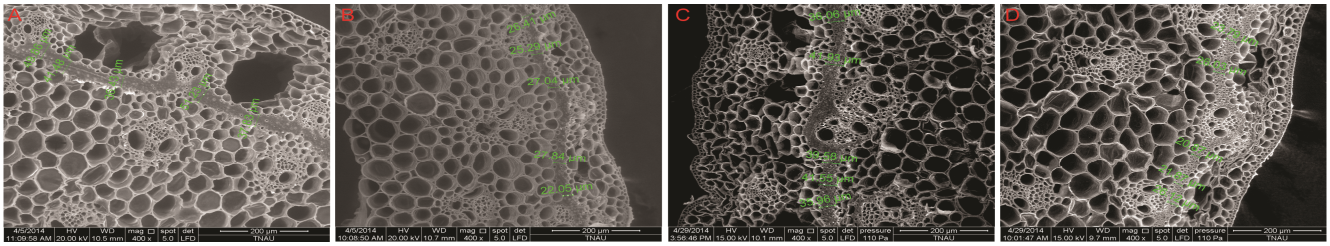

Fig. 2 — Variations in mechanical tissue thickness kodo millet culm. (A) *Adari*; (B) Aamo 10; (C) Sel 21; and (D). TNPsc 183. [*Adari* and Sel 21 had significantly thicker mechanical tissue than that in Aamo 10 and TNPsc 183 (400X magnification)]

Table 4 — Mean performance for yield per plot and degree of lodging in four genotypes of kodo millet (Jan.-May, 2014)

Genotypes	Yield (kg/plot)	Degree of lodging (score#)	Remarks
TNPsc 183	2.585*	3	Low incidence of lodging in two out of five plots towards maturity
Sel 21	2.853*	7	Lodged severely during grain filling
Adari (Check)	1.434	5	Moderately lodged during grain filling
Aamo 10 (weak culm line)	2.129	7	Lodged severely after heading
Mean ± SE	2.250±0.141		
CD (0.05%)	0.301		

[#3, Low degree of lodging; 5, Intermediate degree of lodging, 7: High degree of lodging]

Table 5 — ANOVA for elemental composition (%) using SEM-EDX in kodo millet genotypes

Source of variation	Degrees of freedom	Mean sum of squares	
		Silicon	Potassium
Replications	4	0.005	0.633
Treatments	11	0.087**	4.726**
Factor A (Genotypes)	3	0.163**	10.803**
Factor B (Tissues)	2	0.061*	2.798*
A x B	6	0.058**	2.33*
Error	44	0.017	0.803

[*significant at 5% level; **significant at 1% level]

lodging genotypes, which showed lodging only during grain filling, Aamo10 showed severe lodging even at the onset of flowering (Table 4). Hence, it can be inferred that silicon content also played an important role in strengthening the culm. Similar reports of association of silicon content with mechanical strength were reported in rice[23-25] and wheat[26].

Potassium content in culms did not differentiate the lodging and non-lodging lines, as it was higher in *Adari*, the strong culm line and Aamo10, the weak culm line (Table 6). Hence, it can be assumed that potassium content was not associated with lodging resistance at least in these kodo millet genotypes. This corroborated with the findings in maize[27] in which the correlation between potassium content and culm related traits such as crushing strength, ring thickness and weight of 2-inch section were negative and statistically not significant.

Potassium accumulation in parenchyma

Silicon content (%) across different tissue types of the culm (mechanical tissue, sclerenchyma cells around vascular bundles and parenchyma tissue) did not differ significantly in all the genotypes studied (Table 6). However, potassium content was higher in prenchyma cells and lesser in sclerenchyma cells in mechanical tissue and in sclerenchyma cells around vascular bundles. Similar report of high potassium accumulation in axial parenchyma was reported in black heartwood (*Cryptomeria japonica*) using SEM-EDX[28].

Table 6 — (A) Silicon; and (B) Potassium content (%) in different tissues and genotypes of kodo millet

Genotypes	Sclerenchyma in mechanical tissue	Parenchyma cell	Sclerenchyma cell around vascular bundles	Mean (genotypes)
(A) Silicon content				SE=0.05 CD (0.05%) = 0.095
Adari	0.158	0.32	0.204	0.227^{ab}
TNPsc183	0.438	0.32	0.052	0.270^{a}
Sel21	0.28	0.336	0.298	0.305^{a}
Aamo10	0.052	0.082	0.072	0.069^{b}
Mean (tissue) SE=0.03; CD (0.05%) = 0.082	0.232^{a}	0.265^{a}	0.157^{a}	
(B) Potassium content				SE=0.69 CD (0.05%) = 0.659
Adari	1.082	3.914	3.368	2.092^{a}
TNPsc183	1.32	1.35	0.52	1.063^{b}
Sel21	0.622	0.85	0.948	0.807^{b}
Aamo10	1.968	5.982	3.134	2.604^{a}
Mean (tissue) SE=0.51. CD (0.05%) = 0.571	1.248^{b}	3.024^{a}	1.993^{ab}	

Though association of potassium content with lodging resistance is reported in rice[29], it is not evident through this study. However, elevated potassium accumulation in non-lignified cells like parenchyma need to be explored further as it could be an alternate mechanism to provide strength in the absence of lignin, a component proved to be associated with lodging resistance in kodo millet[3].

Conclusion

Through anatomical study using SEM-EDX, it was found that the number of vascular bundles (per cross section) rather than culm thickness is important in imparting lodging resistance in kodo millet. Hence, breeding for genotypes with more number of vascular bundles may be promising in developing lodging resistant lines. Silicon content was found to be associated with non-lodging genotypes. However, potassium content was higher in parenchyma cells compared to sclerenchyma suggesting that it may have a role in providing strength in non-lignified cells.

Acknowledgement

Funding by University Grants Commission (UGC), New Delhi, Govt. of India for the above research is gratefully acknowledged.

Conflict of interest

The authors declare no conflict of Interest.

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