

# Rapid assessment of rind hardness in sugarcane and its association with biochemical traits

## Abstract

Rind hardness in sugarcane plays a major role in lodging resistance, and internode borer resistance, screening of sugarcane for rind hardness is essential for reducing yield loss. In order to identify the suitability of soil penetrometer for rapid rind hardness measurement, the rind hardness testing was carried out in two sugarcane clones of different rind hardness variability viz., Co 13003 (hard rind type) and Co 14002 (soft rind type). The investigation was carried out by three methods viz., pendulum type impact test rig, texture analyzer, and soil penetrometer and the results revealed that the hard rind type Co 13003 sugarcane clone was observed with significantly greater hardness. Significant correlation between the three methods for the rind hardness trait and among the three methods the soil penetrometer method of determination of rind hardness is easy and rapid. Two-way hierarchical cluster analysis of studied biochemical traits has revealed a better classification of hard-rinded and soft-rind sugarcane clones. The Co 13003 was recorded as least susceptible to borers with high rind hardness (>200 psi), along with better fibre. The NG 77 a hard rind type clone was also observed with high lignin compared to soft rind Gungera.

Key word: Rapid assessment , rind hardness , sugarcane , soil penetrometer , carbohydrates

## 1. Introduction

Sugarcane is a highly economically important C<sub>4</sub> photosynthesis crop with high biomass and it is well known for its potential commercial value of sugar, bioethanol, and other by-products i.e jaggery, molasses etc. The current taxonomy classifies sugarcane into six species viz., *Saccharum officinarum*; *Saccharum spontaneum*; *Saccharum sinense*; *Saccharum barberi*; *Saccharum robustum*; *Saccharum edule*, and among these the cultivated sugarcane are observed as a complex interspecific hybrid primarily between *Saccharum officinarum*, and *Saccharum spontaneum*, with contributions from *S. robustum*, *S. sinense*, *S. barberi*, and allied grass genera such as *Miscanthus*, *Narenga*, and *Erianthus* [1]. It is being grown in approximately 27 million ha by more than 90 countries across the globe where 80% of sugar comes from sugarcane (tropical region) and the rest from sugar beet (temperate region) (<https://www.fao.org/>). India is the second largest sugarcane-growing country after Brazil and both countries together contribute more than 50% of global acreage. In India, it is being

grown nearly 5.11 Mha area with average productivity of 82.0 (t/ha) resulting in 33.0 MT sugar production with the direct involvement of the farming community (average cane growers of 5 crores) and 762 sugar mills where lakhs of stakeholders are benefitted and benefitting common mass. Recently sugar consumption in India has been reported as 19 kilograms per capita per year compared with a global average of 23 kilograms (<https://economictimes.indiatimes.com>).

Rind hardness in sugarcane is been considered an important trait for lodging resistance, internode borer resistance, and also for optimum fibre content which is essential for juice extraction in sugar mills. For achieving higher productivity in sugarcane increasing biomass is one of the major objectives for the sugarcane scientific community, and biomass and lodging tolerance are often found negatively correlated with each other, and moreover identification of high biomass with better source-sink relation along with lodging tolerance is essential for sustaining sugarcane production. Non-lodging varieties are reported to have significant benefits by reducing the yield loss during the cane harvest, and also non-lodging clones are often possessed with better rind hardness and fibre content. Screening for the rind hardness in sugarcane clones is reported by many researchers. Puri and Venkatraman [13] also found a decrease in rind hardness from the bottom to the top of cane stalk and a similar result was reported by Ueno [14]. However, the rapid screening and validation for rind hardness through a hand-held soil penetrometer with the other methodology are not reported. Among the crop plants, sugarcane is the highest biomass-producing plant due to its C<sub>4</sub> nature of photosynthesis and is contemplated as a feedstock for ethanol and sugar production. After sugarcane is milled for juice extraction, bagasse is obtained as a residue, which corresponds to about 25% of the total weight and contains 60 - 80% of carbohydrates. Lignin (acid soluble, acid insoluble) and the structural carbohydrates *viz.*, Cellulose, hemicellulose, and fibre content play a major role in determining the rind hardness in sugarcane. Metabolic constituents of stalk cell walls including lignin, cellulose, and hemicellulose are considered to be imperative for imparting mechanical strength, and their association with stalk lodging is reported [2-6].

Worldwide, moth borers are among the most damaging pests of sugarcane (*Saccharum* spp. hybrids) with about 50 species, mostly belonging to the family Crambidae, reported damaging sugarcane stalks [7]. In India, more than nine species of moth borers are reported to attack sugarcane in both tropical and sub-tropical belts. Among these, the internode borer *Chilosacchariphagus indicus* (Kapur) (Lepidoptera: Crambidae), a subspecies of *C.*

*sacchariphagus* [8], is the most destructive borer pest in peninsular India and generally attacks the crop from internode formation to harvest causing estimated yield losses of 10-35% [9]. Top borer occurs as a major and regular pest in the subtropical region and causes yield loss and sugar recovery. The ICAR-SBI, Coimbatore, India has more than 3500 clones, and the relevance of the biochemical parameters contributing to the rind hardness is important to be studied for the benefit of lodging and insect (borer) tolerance also the rapid screening for rind hardness is essential to accelerate the breeding programme.

Therefore the present investigation was carried out (i) to determine and compare rind hardness through three different methods *viz.*, rind hardness using cutting energy by simple pendulum, texture analyzer, and handheld soil penetrometer, and to identify the rapid method to differentiate the sugarcane clones based on rind hardness and (ii) to identify the variability in lignin and structural carbohydrates content in sugarcane clone in relation to rind hardness for classifying hard/soft types (iii) and to investigate the relevance of rind hardness to insect damage.

## **2. Materials and method**

Two contrasting sugarcane clones *viz.*, Co 13003 (hard rind) and Co 14002 (soft rind) of ten-month-old were collected from the experimental farm field ICAR-SBI, located at Coimbatore, India. The sugarcane clones were grown by following the standard recommendation practices.

### **2.1 Methods of determining rind hardness**

Rind hardness was determined in five random samples collected in each studied sugarcane clone by simple pendulum method, texture analyzer, and soil penetrometer.

#### **2.1.1 Simple pendulum method**

An impact-type pendulum test rig was used [10] to determine the impact energy required for cutting sugarcane (Figure 1). It consists of a frame made of mild steel angle sections, a swinging pendulum from the top of the frame (on which the cutting blades are fixed), an angle indicator, a holder for cutting blade, a specimen holding vice, and a stopper mechanism for pendulum. The overall height of the frame was 1400 mm and the width was 400 mm and pendulum was freely suspended from a shaft mounted on the top of the frame with ball bearings at both ends. The swinging arm of the pendulum was made of rectangular tube sections of 50 x 10 x 3 mm size and having a length of 1178 mm. A holding vice was fixed at

the bottom of the frame to hold the test specimen vertically below the pivot shaft of pendulum. A stopper and release setup for the pendulum was made on one side of the frame so that the pendulum could be raised and stopped at an angle of  $60^{\circ}$  from the vertical and during the test, the pendulum was smoothly released. Facility for altering the approach angle and bevel angle were provided on the blade mount. An angle indicator with a graduated angular scale and pointer fixed on the pendulum pivot shaft showed the angular deflection of the pendulum. A stopper needle was also fixed to display the maximum swinging angle of the pendulum. The weight of the pendulum was 12.54 kg and an additional weight of 5 kg was added to increase the momentum [10]. The samples of uniform measurement were tested for each combination of blade bevel angle and approach angle. The various approach angle of the blade selected were  $0^{\circ}$ ,  $10^{\circ}$ ,  $20^{\circ}$ ,  $30^{\circ}$  and  $40^{\circ}$ . The edge bevel angle of the cutting edge of the blade was kept at  $15^{\circ}$ ,  $25^{\circ}$ ,  $35^{\circ}$  and  $45^{\circ}$ . The energy expenditure was determined as:

$$E = W \times r \times (\cos\theta_1 - \cos\theta_2)$$

Where, E= Cutting energy Nm, W=weight of the pendulum, N, r=distance to the CG pendulum from the pivot.

### **2.1.2 Texture analyzer method**

A stable microsystem texture analyzer was used for the texture measurement system which moves in either an up or down direction and compresses the sugarcane sample. The traveling arm is fitted with a load cell and records the force response of the sample to the deformation that is imposed on it. Force, Distance, and time data are collected and usually presented as a curve on a graph which, when analyzed, indicates the texture of the sample (<https://www.stablemicrosystems.com/>). Sugarcane rind hardness was measured using Texture Analyser (TA-XT2i; Stable Micro Systems Ltd, Godalming, UK) with P/2 mm cylindrical probe with a 500 kg load cell. To obtain a good estimation of rind hardness, measurements were made in four places on each sugarcane from each replicate, and three replicates were carried out. Rind hardness is the peak force (Figure.2) during penetration, which is related to the strength of the rind under penetration.

### **2.1.3 Soil penetrometer method**

The rind hardness of sugarcane was measured using a soil penetrometer (Spectrum technologies) which has a long rod of 76cm length (11.18 mm) along with a sharp pointed tip (Figure. 3 A-D) (2.5cm) in the bottom region and a gauge in the head region (Figure.4) with

three different scaling along with colors i.e 0-200 (pound per square inch) psi green area, 201-300 psi yellow area and > 300 psi red area. The pointed tip of the soil penetrometer was kept over the sugarcane setts and a gentle push allows the tip to insert the rind and the corresponding resistance was noted on the gauge in psi. Higher resistance denotes hard rind, while the opposite is vice versa. The individual sugarcane nodes (starting from the bottom as the number one node and further the numbering ascending towards the top) were tested for rind hardness and the readings are recorded.

## **2.2 Biochemical traits**

The total lignin content of sugarcane biomass was determined according to the NREL-technical report standardized protocol [25] for sugarcane biomass, particularly with the amount of the sample and the duration of digestibility in sulphuric acid for the extraction of the lignin. Matured sugarcane was shredded using a cane shredder and juice was squeezed out. The remaining bagasse was dried at 60°C in an oven for one week. Dried bagasse was powdered in mixer and used for the determination of cellulose and lignin content. Two different methods for the determination of cellulose and lignin have been optimized for sugarcane bagasse. Cellulose content was analyzed using the acetic/nitric reagent method as described by Updegroff [11] with some modifications whereas lignin content was analyzed using National Renewable Energy Laboratory (NREL) method [12].

## **2.3 Insect damage assessment**

Incidence levels of the borer were assessed as per equation (1&2) on 25 canes from each replication at harvest in the cultivar Co 13003 (Hard rind) and Co 14002 (soft rind) in two study years (2021-22) on the basis of internode damage and dead hearts. The total number of canes, canes with internode damage and/or dead hearts, the total number of internodes, and the number of attacked internodes from infested canes were recorded.

Percent incidence = (Number of damaged canes / Total number of canes) x 100 -----(1)

Percent intensity = (Number of damaged internodes / Total number of internodes) x 100 -----(2)

Sugarcane clones were grouped into three categories viz., < 20.0% -least susceptible, 20-40%-moderately susceptible, and > 40.0% highly susceptible based on percent incidence.

## **2.4 Statistical analysis**

Data from each experiment were analyzed using one-way ANOVA ("analysis of variance") through which the means of two or more independent groups are compared in order to determine whether there is statistical evidence that the associated population means are

significantly different. Two-way cluster analysis using the ward method was done to separate the group of sugarcane clones based on biochemical constituents *viz.*, cellulose total solids, oven dry weight, acid-soluble lignin, acid insoluble lignin, total lignin, and ash (%). The strength of the linear relationship between two variables was determined through Pearson correlation and it has a correlation coefficient value ( $r$ ) between -1 to 1, with a value of -1 meaning a total negative linear correlation, 0 being no correlation, and + 1 sense a total positive correlation. All the statistical analysis was done through JMP 9.2 version software. A correlation diagram displaying the correlation between the studied parameters along with the P-value was done through R-studio Software. In order to accomplish that the rind hardness observations obtained by different persons are statistically the same/different a separate rind hardness analysis was performed by two different persons and the results recorded were analyzed through an independent two-sample t-test using the “R” Program, and to confirm whether the samples are from different populations “F” test was conducted to compare the variances.

### **3. Results and Discussion**

#### **3.1. Differences in rind hardness from base to top of the node in sugarcane by various methods**

The results of the rind hardness (Cutting energy) obtained through a simple pendulum method are shown in **figure 6a**. The rind hardness data revealed that the cutting energy required to cut the canes of different internodes differed significantly in both the hard and soft rinded types and the node of the lower region of sugarcane was found to be hard rinded compared to the top portion of the cane. The cutting energy (4<sup>th</sup> node) for Co 13003 and Co 14002 was 89337 and 46040 (j/m<sup>2</sup>) respectively. As the node ascends from the bottom to the top the rind hardness was observed in a declining trend in both the Co 13003 (Hard rinded) and Co 14002 (Soft rinded). Puri and Venkatraman [13] also found a decrease in rind hardness from the bottom to the top of cane stalk and a similar result was reported by Ueno [14]. Due to age of maturity, i.e (lower nodes contain more fibre, lignin, and hemicellulose) the cane hardness was higher in the lower nodes compared to the nodes at the top.

The results of the rind hardness obtained through the texture analyzer method are shown in **figure 2 and 6c**. The rind hardness data revealed that the energy required to puncture the canes of different internodes differed significantly in both the hard and soft rinded types and the node of the lower region of sugarcane was found to be hard rinded compared to the top

portion of the cane. The energy required to puncture (4<sup>th</sup> node) for Co 13003 and Co 14002 was 191 and 152 N (Newtons) respectively. As the node ascends from the bottom to the top the rind hardness was observed in a declining trend in both the Co 13003 (Hard rind) and Co 14002 (Soft rind). These results are in accordance with the [15] report on puncturing resistance in maize stalks.

The results of the rind hardness obtained through the simple hand-held soil penetrometer method are shown in the figure 6b. The rind hardness data revealed that the energy required to puncture/pierce the canes of different internodes were differing significantly in both the hard and soft rinded types and the node of the lower region of sugarcane was found to be hard rinded compared to the top portion of the cane. The energy required to puncture (4<sup>th</sup> node) for Co 13003 and Co 14002 was 180 and 105 Psi respectively. As the node ascends from the bottom to the top the rind hardness was observed in a declining trend in both the Co 13003 (Hard rinded) and Co 14002 (Soft rinded). Pedersen and Toy [16] also reported similar results on sorghum Stalk strength using the electronic rind penetrometer.

### 3.2 Varietal differences in rind hardness

The results showed that the Co 13003 has hard rind compared to the Co 14002 and the Co 86032 was recorded as moderately hard compared to both of the sugarcane (Figure 5, 6b, 7). Also, the NG-77-76 a hard type of sugarcane was observed with a high degree of hardness (10b). Varietal differences have been reported by several investigators [13,14,15,16].

### 3.3 Comparison between sampling for rind hardness through soil penetrometer

In order to conclude that the rind hardness observations obtained by different persons are statistically the same a separate rind hardness analysis was performed by two different persons and the results (Table.1) recorded were analyzed through an independent two-sample t-test using the “R” Program, and to confirm whether the samples are from different populations “F” test was conducted to compare the variances. The results of the analysis revealed that the P value (0.000037) is lesser than the  $\alpha$  value (@ 1%) thus it rejects the null hypothesis ( $H_0: \sigma^2_1 = \sigma^2_2$ ), and accepts the alternate hypothesis ( $H_1: \sigma^2_1 \neq \sigma^2_2$ ), and finally it signifies that both samples are from different populations which is essential for running an independent “t” test. The independent t-test (Welch two sample t-test) between the two samples was carried out, and the results indicated that the P (0.8042) is  $>\alpha$  (5%) resulting in acceptance of null hypothesis ( $H_0: \mu_1 = \mu_2$ ) or rejecting the alternate hypothesis ( $H_1: \mu_1 \neq \mu_2$ ) or the  $t_{\text{calculated}} \leq t_{\text{tabulated}}$  leads to acceptance of  $H_0$ .

### **3.4 Correlation between the simple pendulum method, soil penetrometer, and texture analyzer in relevance to rind hardness of sugarcane**

The association between the simple pendulum method, soil penetrometer, and texture analyzer in relevance to rind hardness of sugarcane is shown in the figure.8. The results revealed that there exist a better correlation among the three different methods for the rind hardness of sugarcane ( $r= 0.83^{**}$  simple pendulum method vs soil penetrometer), ( $r= 0.71^*$  texture analyzer vs rind hardness).

### **3.5 Lignin and structural carbohydrates variability in sugarcane clones**

The method for lignin determination was standardized for sugarcane bagasse and total solids, oven dry weight, acid-soluble lignin, acid-insoluble lignin, total lignin, and ash content was estimated in the selected biomass types (Table 2). The highest lignin content was recorded in the genotype EC11010 (26.87%) which is a hybrid of Co cane and *Saccharum spontaneum*. Similarly, the clones EC11003, EC11007, EC11004, 07-1610, and Thiruvella recorded lignin content of 25.42%, 25.85%, 24.31%, 25.67% and 25.26% respectively. Lignin determination in these biomass types revealed that the energy canes developed for high biomass showed high lignin content followed by pure *Erianthus* and *Saccharum robustum* clones. In general, *Saccharum officinarum* clones which were known to have soft and juicy types recorded lowest lignin content as compared to the rest of the clones.

### **3.6 Cluster analysis**

Two-way cluster analysis showing the grouping of sugarcane clones with the studied parameter is shown in figure 9. The results revealed three distinct clusters: Cluster I: EC 11003, EC 11004, EC 11007, EC 11010, Co 86032, SSCO-2160, 07-1496, 07-1610, IJ 76-384, IJ 76-494, IJ 76-552, IJ 76-556, IJ 76-545, IK 76-81, IK 76-91, IK 76-99, NG 77-73, NG 77-76, NG 77-78, Thiruvella, and Cluster II:, Fiji-B, Gungera, Badilla, and Java. Among the two clusters, cluster I was recorded as relatively better cellulose (42.81%), acid-insoluble lignin (18.44%), and total lignin (23.57%), while Cluster II was observed with better acid-soluble lignin (5.59%) and ash content (2.13%). Additionally, our results confirm the clones in cluster I as hard rinded, while cluster II as soft rinded clones respectively.

### **3.7 Rind hardness and biochemical constituent**

Significantly better rind hardness and fibre content were observed in hard rind Co 13003 and moderately hard rind Co 86032 sugarcane clones compared to the soft rind Co 14002 (Figure

10a). Also, the NG77-76 (hard rind) was recorded with better cellulose, acid-soluble lignin, acid-insoluble lignin, total lignin compared to the Gungera (Soft rind) sugarcane clone (Figure 10b). Buzacott [17] found a positive correlation between rind hardness and fibre content, and Davidson [2] reported that rind hardness measurements would be useful for screening large numbers of varieties for fibre content. It has been demonstrated in pumpkin and squash that higher degrees of lignification were correlated with seed coat hardness [19-21].

### 3.8 Insect damage and Rind hardness

The Co 13003 was recorded as least susceptible to both internode borer and borer with high rind hardness (>200 psi), while the highly susceptible cultivar Co 14002 (Figure 11) was recorded <200 psi rind hardness (soft rind). It is evident that the rind hardness has created variability to insect damage. Charpentier and Mathes [21] suggested that rind hardness may confer resistance to sugarcane borers due to its negative effects on the survival of neonate larva. This was followed up by studies that confirmed the benefits of rind hardness in reducing the prevalence of *D. saccharalis* infestations with the use of a penetrometer [22]. White et al. [23] reported that rind hardness and fiber content were more closely associated with resistance to sugarcane borer, *Diatraea saccharalis* (F.) than pith. Selection for high rind hardness would indirectly help in developing non-lodging canes, tolerance to internode borer, and canes for mechanical harvesting. In sugarcane, rind hardness is an important factor in reducing sugarcane borer damage. The first instar larvae are primarily prevented from entering into the cane by two ways: either by the tightness of leaf sheaths or by the hardness of the rind. The first internode in all the varieties is tightly enveloped by the leaf sheath and the rind is very hard. In resistant varieties, the leaf sheaths at the collar region fit so tightly as to prevent most of the early instar larvae from reaching the inside where they feed on the sheath and tender internode, before boring into the cane stalk [24].

## 4. Conclusion

Based on the results it can be concluded that there exists a genotypic variation in rind hardness and also the rind hardness decreases in ascending order of the sugarcane node from base to the top of the node. Significant correlation among the different methods for rind hardness demonstrated that the rind hardness determined through the soil penetrometer confirms its usefulness for screening sugarcane clones for rind hardness easily. The Co 13003 also exhibited a significant correlation with biochemical traits, stalk borer resistance, and

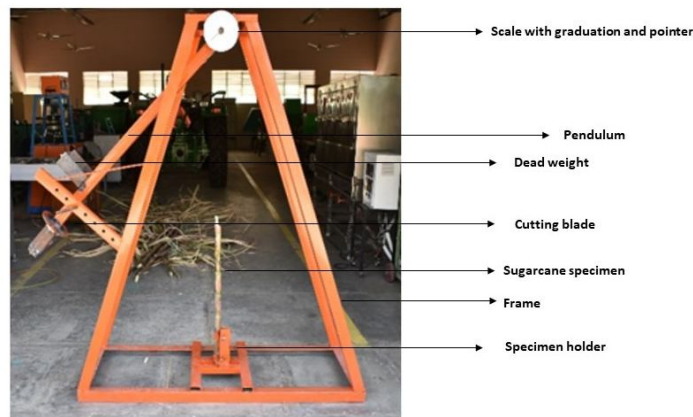
lodging resistance. The Co 13003 was also recorded with high rind hardness (>200 psi), while the highly susceptible cultivar Co 14002 was recorded <200 psi rind hardness (soft rind). It is evident that the rind hardness has created variability in insect damage, and this method may be useful for screening a large number of sugarcane clones for rind hardness. Significantly better rind hardness and fibre content were observed in hard rind Co 13003 and moderately hard rind Co 86032 sugarcane clones compared to the soft rind Co 14002. The NG77-76 (hard rind) was recorded with better cellulose, acid-soluble lignin, acid-insoluble lignin, total lignin compared to the Gungera (Soft rind) sugarcane clone. These findings will be useful to sugarcane scientific researchers for rapid screening of a large number of sugarcane clones for rind hardness which has a direct association with lodging tolerance, and internode borer resistance.

#### Reference:

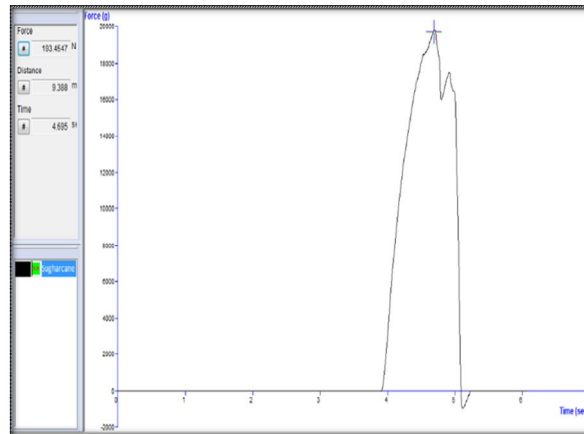
1. Moore PH, Paterson AH, Tew T. Sugarcane: the crop, the plant, and domestication. Sugarcane: physiology, biochemistry, and functional biology. 2013;6:1-7.
2. Davidson J, Phillips M. Lignin as a possible factor in lodging of cereals. Science. 1930;72:401-2.doi: 10.1126/science.72.1868.401
3. Appenzeller L, Doblin M, Barreiro R, Wang H, Niu X, Kollipara K, Carrigan L, Tomes D, Chapman M, Dhugga KS. Cellulose synthesis in maize: isolation and expression analysis of the cellulose synthase (CesA) gene family. Cellulose. 2004;11:287-99.doi: 10.1023/B:CELL.0000046417.84715.27.
4. Ching A, Dhugga KS, Appenzeller L, Meeley R, Bourett TM, Howard RJ, Rafalski A. Brittle stalk 2 encodes a putative glycosylphosphatidylinositol-anchored protein that affects mechanical strength of maize tissues by altering the composition and structure of secondary cell walls. Planta. 2006;224:1174-84.doi:10.1007/s00425-006-0299-8.
5. Kaur S, Dhugga KS, Gill K, Singh J. Novel structural and functional motifs in cellulose synthase (CesA) genes of bread wheat (*Triticum aestivum*, L.). PLoS One. 2016;15:11(1):e0147046.doi: 10.1371/journal.pone.0147046.
6. Kaur S, Zhang X, Mohan A, Dong H, Vikram P, Singh S, Zhang Z, Gill KS, Dhugga KS, Singh J. Genome-wide association study reveals novel genes associated with culm cellulose content in bread wheat (*Triticum aestivum*, L.). Frontiers in Plant Science. Front. Plant Sci. 8:1913.doi: 10.3389/fpls.2017.01913.
7. Long WH, Hensley SD. Insect pests of sugar cane. Annual Review of Entomology. 1972;17(1):149-76.doi: 10.1146/annurev.en.17.010172.001053.

8. David, H., 1986, The internode borer, *Chilosacchariphagus indicus* (Kapur), Breeding Institute, Coimbatore, pp. 121-134.
9. Mahesh P, Srikanth J, Chandran K, Singaravelu B. Resistance of *saccharum* spp. against *chilosacchariphagus indicus* (kapur)(lepidoptera: crambidae) in India. *Experimental Agriculture*. 2018;54(1):83-95.doi: 10.1017/S0014479716000697.
10. Sureshkumar PK, Jesudas DM. Physico-mechanical properties of sugar cane stalks related to mechanical harvesting. *Journal of Tropical Agriculture*. 2015;53(1):48-55.
11. Updegraff DM. Semimicro determination of cellulose in biological materials. *Analytical biochemistry*. 1969;32(3):420-4.doi: 10.1016/s0003-2697(69)80009-6.
12. Sluiter J, Sluiter A. Summative mass closure. NREL, NREL/TP-510-48087; 2010. p. 1-10.
13. Puri AN, Venkatraman TS. An apparatus for testing rind hardness in sugarcanes. *Proc.Surg Technol*. 1929:15-9.
14. Ueno T. Relation of rind hardness to the internal structure of sugarcane. Rept. Govt. Sug. Expt. Sta. Tainan, Formosa. 1938;5:21-9. (Japanese with English summary).
15. Cook DD, Meehan K, Asatiani L, Robertson DJ. The effect of probe geometry on rind puncture resistance testing of maize stalks. *Plant Methods*. 2020;16(1):1-1. doi: 10.1186/s13007-020-00610-8.
16. Pedersen JF, Toy JJ. Measurement of sorghum stalk strength using the Missouri-modified electronic rind penetrometer. *Maydica*. 1999;44:155-158.
17. Buzacott JH. Rind hardness determinations as an aid in beetle borer control. *Proceedings of the Queensl soc surg cane tech*. 1937;8:143-52.
18. Bezold TN, Loy JB, Minocha SC. Changes in the cellular content of polyamines in different tissues of seed and fruit of a normal and a hull-less seed variety of pumpkin during development. *Plant Science*. 2003;164(5):743-52.
19. Li X, Ge Y, Gui M, Cui CS, Qu SP. Histological Analysis of Hulled and Hull-less Squash Seed Coat. *International Journal of Agriculture & Biology*. 2013;15:1047-1055.
20. Konan JA, Koffi KK, Zoro AI. Lignin biosynthesis rate is responsible for varietal difference in fruit rind and seed coat hardness in the bottle gourd *Lagenaria siceraria* (Molina) Standley. *South African Journal of Botany*. 2018;117:276-281.doi: 10.1016/j.sajb.2018.05.025.
21. Charpentier LJ, Mathes R. Cultural practices in relation to stalk moth borer infestations in sugar cane. In *Pests of Sugar Cane*; Williams JR, Metcalfe JR,

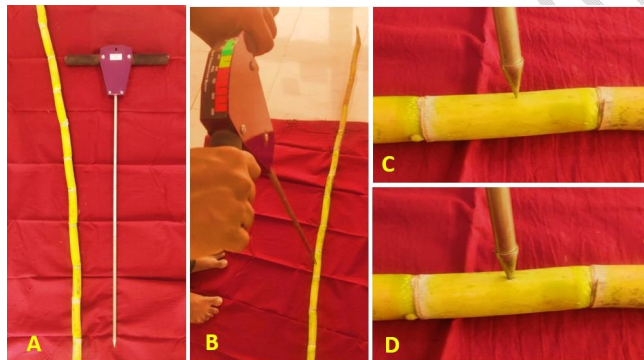
- Mungomery RW, Mathes R, Eds.; Elsevier: Amsterdam, The Netherlands, 1969:163–164.
22. Martin FA, Richard CA, Hensley SD. Host resistance to *Diatraeasaccharalis* (F.): relationship of sugarcane internode hardness to larval damage. *Environmental Entomology*. 1975;4(5):687-688.doi: 10.1093/ee/4.5.687.
  23. White WH, Tew TL, Richard Jr EP. Association of sugarcane pith, rind hardness, and fibre with resistance to the sugarcane borer. *Journal-American Society of Sugar Cane Technologists*. 2006;26:87-100.
  24. Easwaramoorthy S, Nandagopal V. Life tables of internode borer, *Chilosacchariphagus indicus* (K.), on resistant and susceptible varieties of sugarcane. *International Journal of Pest Management*. 1986;32(3):221-8. doi: 10.1080/09670878609371067.
  25. Suresha GS, K.Lashmi, SC Divya, S.Parthiban, P.Govindaraj and K Hari. Profiling high biomass sugarcane clones for cellulose and lignin content. In.Symposium on bioenergy for sustainable development - The potential role of sugar crops. 23-25 June 2014 Coimbatore India. Proceedings eds. R.viswanathan, A.bhaskaran, G.Hemaprabha, T.Ramasubramanian, N.V.Nair. 130-132.



**Figure 1.** Pendulum-type impact test rig



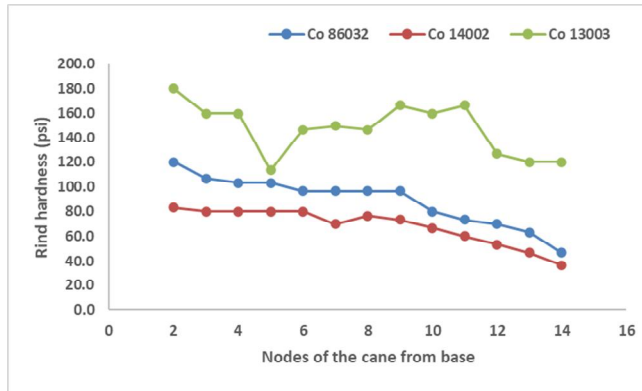
**Figure 2.** Instrumental texture profile analysis using texture analyzer showing a typical graph showing firmness of sugarcane



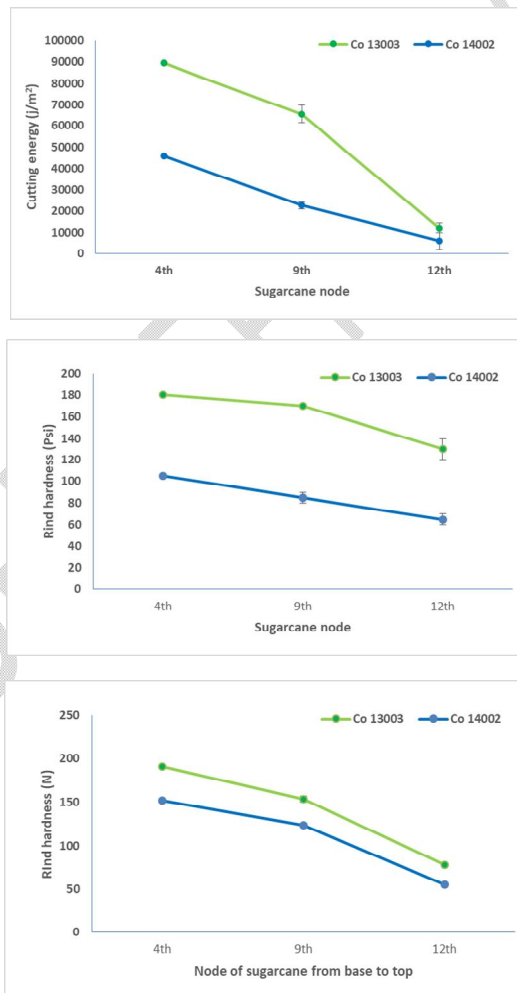
**Figure 3.** Soil penetrometer with an analog displaying the hardness or compaction in bars **A.** Sugarcane and soil penetrometer, **B.** In the initial step for the rind hardness test the sugarcane is kept on flat ground and the penetrometer is placed vertical position, **C.** Pointed tip of the penetrometer before piercing the sugarcane **D.** Pointed tip piercing the sugarcane.



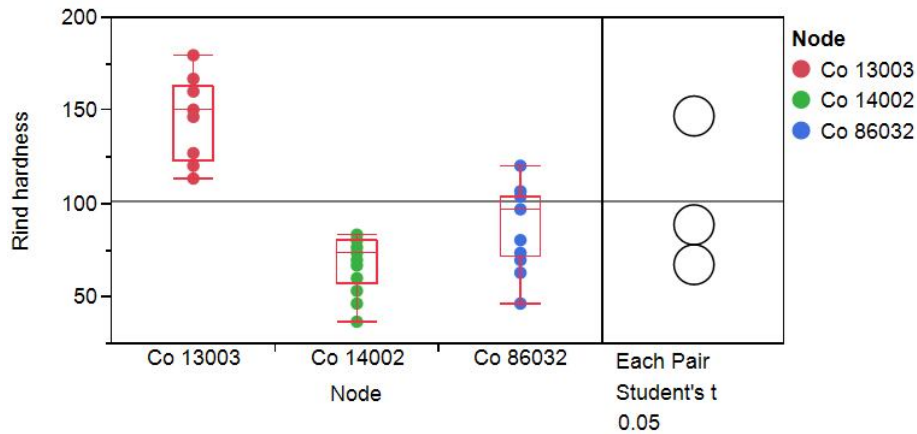
**Figure 4.** Analog of soil penetrometer displaying the hardness or compaction in bars (PSI: Pounds per square inch)



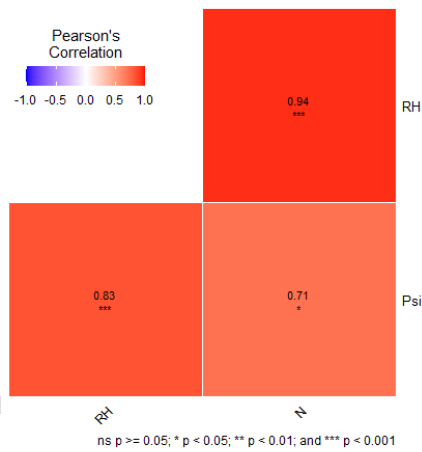
**Figure 5:** Comparison of rind hardness of sugarcane clones based on the soil penetrometer



**Figure. 6 a, b, c:** Comparison of rind hardness of sugarcane clones based on the pendulum type rig, soil penetrometer, and texture analyzer



**Figure 7:** Comparison of rind hardness in sugarcane clones based through soil penetrometer through one-way ANOVA with paired ‘t’ test..



**Figure 8:** Correlation between rind hardness of sugarcane clones based on the soil penetrometer (psi), simple pendulum method (RH), and texture analyzer method (N).\*\* denotes significant at 1%, \* denotes significant at 5%, and ns denotes non-significant. The intensity of the colour indicates the strength of the correlation.

**Table: 1.** Comparison between sampling for rind hardness through soil penetrometer (psi\*) by two different person

Base to top of node	Sampling by Person1	Sampling by Person2
1	140	150
2	150	130
3	155	150
4	150	150
5	150	150
6	150	150
7	140	200
8	140	200
9	130	200

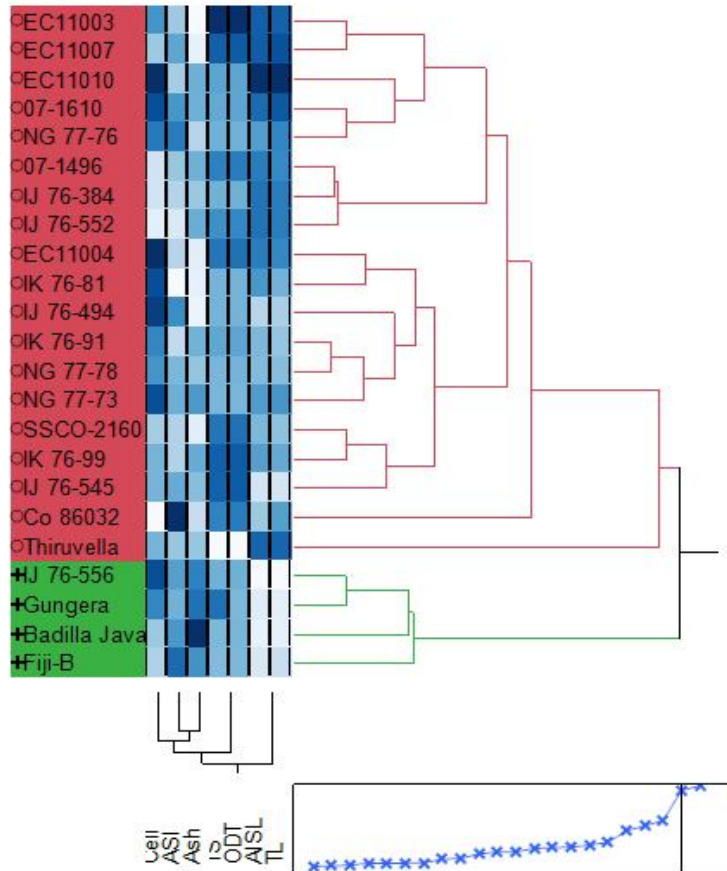
10	140	200
11	140	130
12	130	130
13	150	110
14	130	100
15	130	90
16	130	80
17	100	80
<b>Mean</b>	<b>138.53</b>	<b>141.18</b>
T:-0.251; P value=0.8042		
F: 0.101; P value=0.000037		

T: is the t value and F: is the f value based on the independent two-sample t-test.

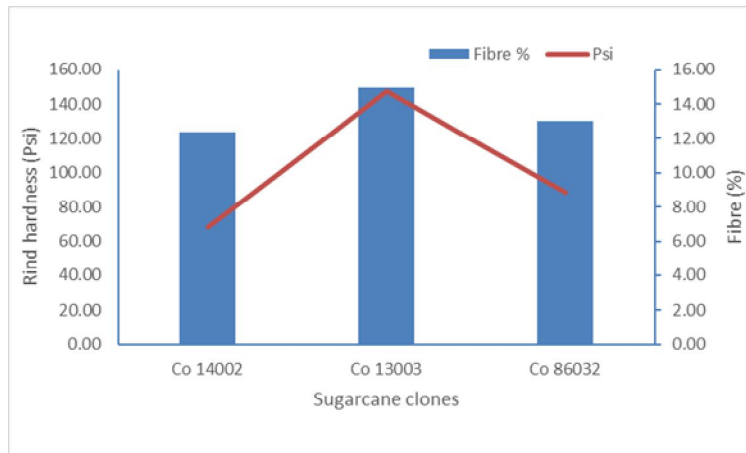
Psi\* : Pound per square inch required to pierce the sugarcane sample

**Table 2.** Cellulose and lignin content in different sugarcane biomass types

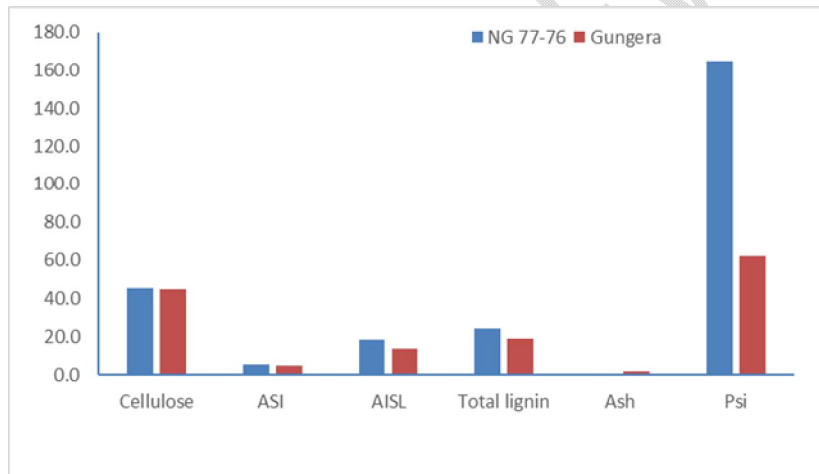
S.NO	NAME OF THE GENOTYPE	CELLULOSE (%)	TOTAL SOLIDS (%)	OVEN DRY WEIGHT (g)	ACID SOLUBLE LIGNIN (%)	ACID INSOLUBLE LIGNIN (%)	TOTAL LIGNIN (%)	ASH (%)
1	EC11003	44.00	97.60	0.488	4.93	20.49	25.42	0.122
2	EC11004	48.80	96.34	0.4817	4.78	19.53	24.31	0.269
3	EC11007	40.00	96.76	0.4838	5.31	20.54	25.85	0.227
4	EC11010	48.80	95.48	0.4774	4.88	21.99	26.87	0.837
5	Co 86032	32.00	96.14	0.4807	6.88	16.66	23.54	0.457
6	SSCO-2160	39.33	96.36	0.4818	4.80	17.45	22.25	0.269
7	07-1496	36.00	96.16	0.4808	4.96	19.53	24.24	0.831
8	07-1610	47.33	95.38	0.4769	5.54	20.13	25.67	0.838
9	IJ 76-384	36.00	95.00	0.4750	4.79	19.83	24.62	0.673
10	IJ 76-494	48.00	94.86	0.4743	5.66	15.85	21.51	0.189
11	IJ 76-552	34.00	95.90	0.4795	4.47	19.83	24.62	0.855
12	IJ 76-556	47.33	95.08	0.4754	5.39	13.04	18.43	1.680
13	IJ 76-545	42.00	96.72	0.4836	5.21	14.784	19.994	0.744
14	IK 76-81	47.33	94.82	0.4741	4.19	18.688	22.878	0.232
15	IK 76-91	44.66	95.44	0.4772	4.70	17.392	22.092	0.817
16	IK 76-99	42.00	96.76	0.4838	4.81	18.375	23.185	0.888
17	NG 77-73	47.33	94.68	0.4734	5.17	18.525	23.878	1.260
18	NG 77-76	45.33	95.08	0.4754	5.90	18.48	24.38	0.546
19	NG 77-78	44.00	94.74	0.4737	5.10	17.31	22.41	0.696
20	Thiruvella	42.00	86.96	0.4348	4.96	20.308	25.268	0.689
21	Fiji-B	38.66	94.36	0.4718	6.11	14.44	20.55	1.390
22	Gungera	44.66	96.44	0.4722	5.15	14.12	19.27	1.940
23	Badilla Java	40.00	94.52	0.4726	5.51	13.71	19.22	3.060



**Figure 9.** Two-way cluster analysis of the biochemical parameters displaying the classification of sugarcane into hard types (Cluster I: EC 11003, EC 11004, EC 11007, EC 11010, Co 86032, SSCO-2160, 07-1496, 07-1610, IJ 76-384, IJ 76-494, IJ 76-552, IJ 76-556, IJ 76-545, IK 76-81, IK 76-91, IK 76-99, NG 77-73, NG 77-76, NG 77-78, Thiruvella), and soft types (Cluster II: Fiji-B, Gungera, Badilla, and Java).



**Figure 10a.** Comparison of rind hardness and fibre % in sugarcane clones



**Figure 10b.** Comparison of rind hardness and biochemical traits (Cellulose, acid-insoluble lignin, acid-soluble lignin, total lignin, and Ash) in NG 77-76 (Hard rind type) and Gungera (soft rind type) sugarcane clones



**Figure 11.** Insect damage in soft-rinded sugarcane

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