A high-throughput method for precise phenotyping sugarcane stalk mechanical strength using near-infrared spectroscopy

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Abstract

Background: Sugarcane (*Saccharum officinarum L.*) is the core crop for sugar and bioethanol production over the world. A major problem in sugarcane production is stalk lodging due to weak mechanical strength. Since there are no efficient methods for determining stalk mechanical strength in sugarcane, genetic approaches for improving stalk lodging resistance are largely limited. This study was designed to use near-infrared spectroscopy (NIRS) calibration assay to accurately assess mechanical strength on a high-throughput basis for the first time.

Results: Hundreds of sugarcane germplasms were harvested at the mature stage in the year of 2019 and 2020. In terms of determining rind penetrometer resistance (RPR) and breaking force, large variations of mechanical strength were found in the sugarcane stalk internodes, based on well-established laboratory measurements. Through partial least square regression analysis, two online NIRS models were established with a high coefficient of determination (R^2) and the ratio of prediction to deviation (RPD) values during calibration, internal cross-validation, and external validation. Remarkably, the equation for RPR exhibited R^2 and RPD values as high as 1.00 and 17.7, as well as showing relatively low root mean square error values at 0.44 N mm^{-2} during global modeling, demonstrating excellent predictive performance.

Conclusions: This study delivered a successful attempt for rapid and precise prediction of mechanical strength in sugarcane stalk by NIRS assay. By using these established models, genetic improvements could be made to phenotyping jobs for large-scale sugarcane germplasm.

Background

Sugarcane (*Saccharum officinarum L.*) is a perennial C4 crop well adapted to subtropical and tropical regions for sugar and bioethanol production [1–3]. Since sugar and biofuel demand has increased, emphasis has been put on maximizing per-area production and standardizing agronomic practices to achieve optimal yields [4, 5]. Stalk lodging (breakage or bend of stalks before harvest) is one of the main factors that largely restricts sugarcane production, resulting in an estimation of 10–20% yield lost annually [6–7]. In the past few years, studies have explored the lodging resistance of plants from the view of field management practices, plant architecture [8], and plant biomechanics [9]. Efforts also have been made to improve lodging resistance through genetic improvement [10, 11], but the results have been limited due to the complex and multifactorial nature of lodging traits and the low efficiency of accurate characterization.

Generally, the stem lodging resistance of cereal crops can be evaluated by mechanical strength according to measuring two types of indicators (rind penetrometer resistance and breaking force) values [12–15]. Breaking force is normally applied in small cereal crops with hollow stems such as wheat and rice [16–18], while rind penetrometer resistance (RPR) seems more suitable for large cereal crops such as corn and sorghum [19, 20]. Different from the crops described above, sugarcane stem failure can be divided into two types: greensnap and stalk lodging. Greensnap refers to stalk breakage at the young stem internode in the face of external force, whereas stalk lodging refers to stem internode buckling at the mature stem internode when the stalk could not support its weight or face of external force. From a plant breeding or phenotyping perspective, both two types of stem failure should be distinct. As such, in our recent study, breaking force and rind penetrometer resistance (RPR) were successfully applied for characterizing these two types of stem failure [21]. However, laboratory-based mechanical phenotyping jobs require a considerable amount of time, making it difficult for an individual to apply for high-throughput phenotyping jobs.

Near-infrared spectroscopy (NIRS) is a speed, simplicity, and high-efficiency analytical technique that integrates measurement, data collection, and analysis altogether to predict the properties of samples [22]. The regression model between the spectrum and the measured value is established by chemometric calibration, to realize qualitative and quantitative analysis [23–26]. In recent years, NIRS has been widely used in the agriculture, food, petrochemical, and pharmaceutical fields [27]. Such as high-throughput screening of plant biomass samples [28], quick and large-scale screen the target traits of crops [29], and analysis of multiple traits in crop breeding [30–32]. In our previous study, NIRS has been successfully applied for sugarcane stalk quality assessment in terms of moisture, soluble sugar, insoluble residue, and the corresponding fundamental ratios [33]. Besides, cell wall features calibration models have also been set up for related genetic development [34]. These proposed methods are showing positive permits for large-scale screening of optimal sugarcane germplasm for sugarcane breeding. However, so far little study has attempted to detect stalk mechanical properties by NIRS in sugarcane or any other crops.

In this work, hundreds of samples were collected from various sugarcane genotypes. Based on the precise methods for mechanical strength and near-infrared spectroscopy analysis, an efficient NIRS assay was developed for the prediction of the rind penetrometer resistance (RPR) and breaking force in sugarcane stalks. Thus, this study provided a precise and high-throughput method for large-scale screening and selection of optimal lodging-resistant sugarcane, which could be integrated as a system project with our previous studies for multipurpose precision breeding.

Results

Precise mechanical strength determination in sugarcane stalks

The mechanical strength of a stalk plays an important role in the growth and development of crops [9]. In this study, the Instron Universal Testing Machine was used to determine the rind penetrometer resistance (RPR) of the sugarcane stalk (Fig. 1B). As sugarcane stalks have multiple internodes (Fig. 1A), we compared the RPR of sugarcane stalks from the different internodes of selected genotypes that had contrasted higher and lower RPR. It was observed that RPR increased dramatically from the 3rd internode to the 5th internode (Fig. 1C). It is noteworthy, however, that no significant change was observed from the 7th internode to the 23rd internode (Fig. 1C). Additionally, the internode RPR showed similar variation patterns between genotypes (Fig. 1C). Therefore, we calculated the differences in RPR between genotypes within the same internode. From the 5th to the 23rd internode, stable differences were observed between materials with high and low RPR (Annex 1A). Furthermore, the results of the multiple comparison analysis of RPR between different internodes revealed that none of them showed significant differences, with the exception of the third internode (Annex 1B). It was suggested that any internode except the third and fourth one can be used as a representative internode for measuring RPR. As a means of verification, we measured the RPR of representative materials at the 12th internode in 2019 and 2020, respectively. Notably, significant differences were detected stably between high and low RPR materials, and no detectable difference was observed within high or low RPR materials between the two years (Fig. 1D). Ultimately, these results validated the reliability of this approach, confirming that the method could be effectively used to analyze RPR in sugarcane stalks in an accurate and suitable manner.

Greensnap is another important problem in multi-internode agriculture crop, influencing the net production [35]. Based on phenotypic observations in the field, we observed that greensnap occurred only in the younger node (the 3rd node) (Annex 2). To examine this phenomenon in more detail, we determined the breaking force across a large number of sugarcane genotypes (Fig. 1E). Several sugarcane varieties with high and low breaking forces were selected in order to determine their breaking force in different environments. Accordingly, there was no significant difference in the breaking force of a given variety in different environments, but there was a significant difference between those materials with a higher and a lower breaking force (Fig. 1F). Considering these results, it was concluded that breaking force is under strong genetic control, hence selection against this trait is possible.

Diverse mechanical strength in collected sugarcane samples

In the present study, RPR data were recorded on 270 and 256 sugarcane genotypes in 2019 and 2020, respectively (Fig. 2A). For comparison, RPR data were recorded on the same 46 genotypes in both 2019 and 2020. Further, the breaking force was measured on 440 sugarcane genotypes, of which 245 samples were common to the RPR and breaking force datasets (Fig. 2A). As a result, we observed a wide variation in RPR in different sugarcane genotypes in 2019 and 2020 (Fig. 2B). In detail, the RPR was ranged from 23.5 to 79.7 N mm^{-2} in 2019 while in 2020 it ranged from 22.8 to 59.7 N mm^{-2} (Annex 3). Besides, the RPR of samples collected in 2019 and 2020 showed an excellent normal distribution (Fig. 2B). Similarly, large variations were observed for breaking force in sugarcane genotypes as well, which presented a normal distribution of breaking forces ranging between 6.6 N and 32.8 N (Fig. 2C; Annex 3). Furthermore, a correlation analysis of common samples of RPR in two years revealed a significant correlation, indicating that RPR is an inherited characteristic (Fig. 2D). Remarkably, these two types of force trait (RPR and breaking force) exhibited a significant correlation coefficient of 0.338 at $p < 0.01$ level, indicating an important relationship between them (Fig. 2E).

NIRS data characterization in collected sugarcane stalks

The collected population of sugarcane with various genotypes was used for near-infrared spectroscopy modeling. In order to perform an online NIRS assay, samples were crashed and a near-infrared spectrum for each genotype was acquired within one minute. During the shredding process, none of the sugarcane stalk components were lost, and the moisture in the shredded bagasse was retained. For NIRS calibration, the OPUS software automatically averaged the collected near-infrared spectral reflectance values. As a result, the near-infrared reflectance values of all samples fluctuated within the normal range, indicating that sugarcane samples exhibit a wide range of characteristics (Fig. 3A&D). In near-infrared spectral data analysis, principal component analysis (PCA) has some advantages, such as characterization of spectral structure of populations [36]. A two-dimensional observation of the sample distribution was conducted using the first three principal components. Despite the fact that samples were collected from different years (2019 and 2020) for RPR determination, no significant discrimination were observed between the spectra (Fig. 3B&C). Observations of the spectra of these common samples revealed a smaller global distance (GH), suggesting a high level of similarity between them (Fig. 3B&C). In the case of these samples used for breaking force measurements, the first three principal components accounted for 98.6% of the total and displayed a continuous distribution (Fig. 3E&F), suggesting that these samples can be incorporated into a global calibration population for NIRS.

Determination of calibration and external validation sets

A calibration equation is typically evaluated by means of calibration and external validation. For RPR modeling, a total of 68 samples were randomly divided into external validation sets, and the remaining 458 samples formed the calibration set (Table 1). In the case of NIRS modeling of breaking force, 440 samples were used: 90 samples for external validation and 350 samples for calibration (Table 1). An analysis of descriptive statistics was conducted in order to compare the calibration and external validation sets. It is important to note that the minimum and maximum values at both ends of the external validation set were included in the calibration set to ensure that the model is both accurate and practical (Table 1). Additionally, RPR and breaking force displayed normal distributions for both calibration and external validation sets (Annex 4). All statistical distributions across calibration and external validation were comparable, suggesting that it is feasible to obtain accurate predictive equations.

Stalk mechanical strength modeling and evaluation

Using the OPUS software, we performed a linear fitting analysis on RPR and near-infrared spectral reflectance values based on the partial least squares (PLS) method. During PLS analysis, multiple parameters are combined based on the wavelength range and the pretreated spectrum to derive calibration equations [37]. Following this, the performance of the calibration equation was evaluated using the cross-validation and external validation parameters.

We applied NIRS modeling independently to two different types of mechanical strength indicators (RPR and breaking force) of sugarcane stalks. According to RPR calibration, we observed that the R^2 was reaching 1.0, the RPD value was reaching 19.60, as well as a relatively low RMSEC value at 0.40 N

(Table 2). In terms of NIRS calibration for breaking force, although the modeling parameters were not as good as for RPR, they still demonstrated excellent fitting with R^2, RPD and RMSEC values of 0.88, 2.88 and 2.15 N, respectively (Table 2). These results indicated that based on the calibration results, both the RPR equation and the breaking force equation exhibited excellent application potential.

Further, internal cross validation was conducted to assess these obtained equations. During internal cross validation, the samples were divided into various groups, some of which were chosen at random from the calibration sets for cross-validation, which provides the root mean square error of cross validation (RMSECV) and coefficient determination (R^2cv), respectively, for equation evaluation. According to our results, a high R^2cv (0.99), RPD (10.30) value and a relatively low RMSECV (0.74 N) were observed for the equation of RPR prediction. Likewise, the R^2cv value was 0.83, RPD was 2.42, and RMSECV was 2.51 N for the equation of RPR prediction (Table 2). In this case, the RPR model showed better predictive performance than the breaking force model, which was consistent with the calibration results.

Additionally, the equations were subjected to an external validation as an independent test to assess their performance. In a similar manner, for equation evaluation, root mean square error of external validation (RMSEP), coefficient determination (R^2ev) and ratio of prediction to deviation (RPD) were calculated. It was found that, in this context, all equations for RPR and breaking force showed R^2ev values of above 0.85 and RPD values well above 2.5 (Table 2). A notable feature of the equation for RPR was that the coefficient of determination and ratio of prediction to deviation remained constant at 0.99 and 10.20, respectively (Table 2), in accordance with the excellent performance observed during calibration and internal cross validation, suggesting their excellent prediction performance.

Global modeling of the stalk mechanical strength

We then combined the external validation set with the calibration set to form an integrated calibration set to perform an integrative calibration analysis to gain higher performance model predictions. The results showed that the parameters of the new RPR equation did not significantly improve, but the prediction performance remained extremely high (Fig. 4A&B; Annex 5). A slightly improved R^2cv (0.84) and RPD (2.51) values were found for the breaking force equation (Table 2; Fig. 4C&D). Despite the high correlation between the true value and the fit (predicted) value (Fig. 4C&D), it is evident that the obtained breaking force equation can provide reliable predictions. Overall, all these newly generated equations for two kinds of force traits performed excellent in terms of R^2, R^2cv, and RPD, as well as relatively low RMSEC and RMSECV values, suggesting their ability to provide precise and consistent predictions.

Discussion

Lodging due to weak mechanical strength is one of the major problems that affect growth and potential yield in agricultural crops [16]. In particular, sugarcane is a large crop that is highly susceptible to stalk lodging, which results in approximately ten percent to twenty percent of sugarcane yield being lost annually [38]. Generally, crop lodging resistance can be improved by either reducing plant height or increasing stalk mechanical strength [39]. For instance, by breeding dwarf varieties, the first green revolution greatly reduced main grain crop failure [40]. However, due to the stalk-harvesting nature of sugarcane, this strategy was not feasible. The efficiency way to increase its resistance to stalk lodging is to enhance its mechanical strength [41]. In general, the RPR and breaking force of the stalk are reliable indicators of the mechanical strength of the stalk [42]. In crop breeding, RPR and breaking force have been used to indirectly screen and develop lodging-resistant varieties [43–45]. Unfortunately, due to the lack of an efficient method for accurately characterizing RPR or breaking force, lodging-resistant breeding in sugarcane has largely been limited.

The objective of this study was to develop a method for the rapid and precise prediction of mechanical strength in sugarcane stalks via NIRS modeling. In the present study, a precise laboratory analytical method was successfully established for determining the RPR and breaking force in sugarcane firstly (Fig. 1). Accordingly, substantial variations in RPR and breaking force were observed in collected sugarcane populations (Fig. 2B&C), which was the crucial element for accurate NIRS modeling in this study. Besides, high significant correlations were observed between breaking force and RPR (Fig. 2E), indicating there would be a certain internal relationship between the RPR of the middle internode and the breaking force of the tip. It would be the evident that there is a sugarcane genotype with a higher RPR in mature internodes, primarily in young nodes with strong breaking forces. As a result of this closely linked relationship, sugarcane breeding programs aimed at increasing the mechanical strength of their plants were supported.

Due to a wide range of genetic variation in collected sugarcane genotypes, a continuous distribution of NIR spectra were obtained (Fig. 3), which provides a well-founded basis for NIRS modeling. As we expected, high-performance of NIRS models for RPR and breaking force determination were obtained based on a PLS calibration analysis (Table 2). Particularly, the parameters of the prediction equation for RPR were much higher than those of the prediction equation for breaking force (Table 2). A possible explanation for the relatively low R^2 and RPD values observed in the breaking force prediction equation could be that only one year of data has been used for the modeling process (Table 2; Fig. 2), thus resulting in some limitations. In spite of this, the prediction equation for breaking force remains relevant, and the prediction performance can be further improved by adding more samples with a variety of features.

Generally, equations having RPD > 2.5 were classified as “Fair”, which were considered effective for screening applications [46]. In this study, all the obtained equations displayed RPD values over 2.5, along with highly correlated true and predicted values in the calibration, internal cross validation, and external validation (Table 2; Fig. 4), suggesting their sufficient prediction capability [29, 47, 48]. Particularly, the equation for RPR characterization displayed the RPD values as high as 19.60 during calibration process, with the R^2 value of 1.0, indicating excellent application performance (Table 2; Fig. 4). It was unexpected that the performance of the RPR prediction equation failed to improve when samples were added from an external validation set. This may be due to the inclusion of some outlier samples in the external validation set. Despite this, the final calibration results showed that all the equations performed exceptionally well in their respective applications. By comparing the results of all equations generated in this study, the newly integrated calibration model appears to offer good potential for high-throughput screening of excellent germplasm from large-scale sugarcane samples.

Conclusions

This study developed a high-throughput analysis method based on NIRS to estimate sugarcane stalk mechanical strength for the first time. Calibration for NIRS was conducted by measuring the rind penetrometer resistance (RPR) and breaking force of 721 samples from several sugarcane populations. Most of the equations exhibited perfect prediction abilities with high values of R^2, R^2_{cv}, $R^2_{ev,}$ and RPD, particularly the equation for RPR characterization displayed the R^2 value as high as 1.0, suggesting excellent application performance. It was demonstrated that the calibration equations obtained could be used for large-scale screening of sugarcane germplasm on the basis of mechanical strength. Consequently, the findings of this study provided reliable technical support and solutions for high-throughput screening of sugarcane breeding and other research areas.

Materials And Methods

Sample processing

Hundreds of sugarcane germplasms were collected and planted in the Fusui experimental field of Guangxi University, China (107°47'17.66" E, 22°31'5.85" N). A total of 721 sugarcane samples were harvested at the mature stage in the year 2019 and 2020. In detail, 270 and 256 samples were collected in 2019 and 2020, respectively, for the determination of RPR. Similarly, a total of 440 sugarcane samples were used for breaking force determination including 245 samples which has been already used in 2019 for RPR determination. Six stalks were randomly selected from each genotype for mechanical strength determination and further NIRS analysis after removing leaves but keeping young tips.

Mechanical strength determination in sugarcane stalks

RPR determination: The Instron Universal Testing Machine (YYD-1) equipped with a circular puncture probe (section 1 mm^2) was used to determine the RPR of sugarcane stalks. In detail, five independent positions in the middle of the 12th and 15th internode were detected by puncture probe in 2019 and 2020, respectively, and the peak value of each position was recorded. The average RPR value of each internode was calculated on these collected data after eliminating the maximum and minimum values. Six biological replications were performed for each sample.

Breaking force determination: The Instron Universal Testing Machine (YYD-1) configured with an arc probe was applied for breaking force measurement. Briefly, the sugarcane with a young tip was fixed flatting on the loading platform, where the 3rd internode was extended out for breaking force detection.

The arc probe was kept perpendicular to push the fourth internode until it broke, and the peak force value was recorded. Six biological replications were performed for each sample.

Online near-infrared spectral data source

After mechanical properties measurement, the collected six stalks of each genotype were used for near-infrared spectral detecting. Sample pretreatment and data collection followed a standard pipeline as described in our previous studies [33]. In brief, sugarcane stalks were shredded using DM540 (IRBI Machines & Equipment Ltd, Brazil), and the shredded fresh sample was immediately transported to the CPS system (Cane presentation system, Bruker Optik GmbH, Germany) by a conveyor belt, where the near-infrared spectral data of samples were online collected by MATRIX-F (Bruker Optik GmbH, Germany) system. The obtained continuous near-infrared spectral reflectance values were then averaged for further analysis.

NIRS data processing and calibration

The OPUS spectroscopy software (version 7.8, Bruker Optik GmbH, Germany) was used for data processing and NIRS calibration. As described by Wang et al. [33], pretreatment and the wavelength range selection of the raw spectral data were performed before calibration to solve the problems associated with the overlapping peaks and baseline correction. Briefly, several spectral pretreatment methods were used in OPUS software, namely constant offset elimination (COE), straight-line subtraction (SSL), standard normal variate (SNV), Min−Max normalization (MMN), multiplicative scattering correction (MSC), first derivative (FD), second derivative (SED), a combination of the first derivative and straight-line subtraction (FD + SSL), a combination of the first derivative and standard normal variate (FD + SNV), and a combination of the first derivative and multiplicative scattering correction (FD + MSC). A combination in terms of wavelength range selection and spectrum pretreatment was made to obtain calibration equations in PLS analysis. Internal cross-validation and external validation were carried out to test the performance of the generated equations. The best equations were selected according to the high coefficient of determination of the calibration/internal cross-validation/external validation ($R^2/R^2_{cv}/R^2_{ev}$), low root mean square error of calibration/internal cross-validation/external validation (RMSEC/RMSECV/RMSEP), and high ratio of prediction to deviation (RPD) values.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

YS, MA and FM completed the major experiment, analyzed the data, and completed the first draft of manuscript. LK participated in RPR and breaking force determination. MW, FJ and QH participated in sugarcane samples preparation and NIRS data collection. WY revised the manuscript. YZ participated sample collection and manuscript revision. JH and MZ designed the project, supervised the experiments, interpreted the data, and finalized the manuscript. All authors read and approved the final manuscript.

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Tables

Tables 1 and 2 are available in the Supplementary Files section.

Figures

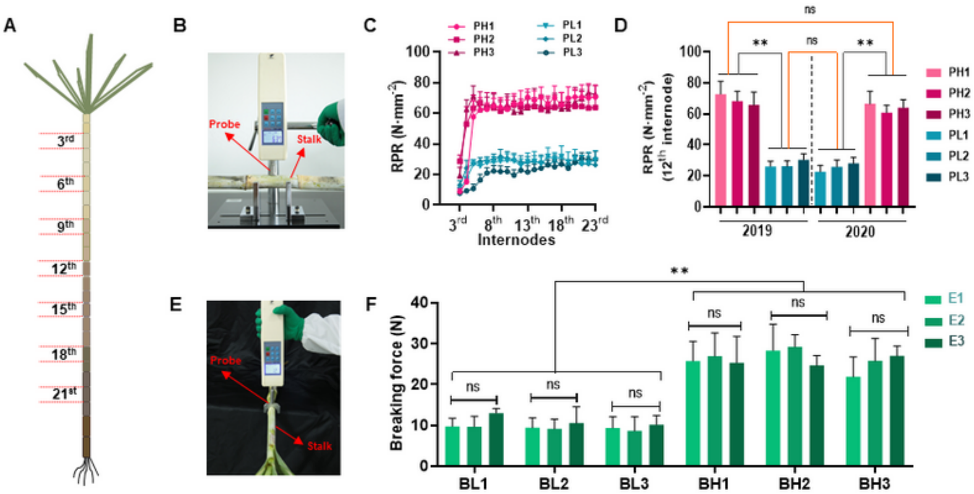

Figure 1

High-performance laboratory analytical methods for measuring mechanical strengths in sugarcane stalk. **(A)** Schematic diagram of multi-internodes of sugarcane. **(B)** RPR measurement. **(C-D)** Distribution of RPR value in different internodes of sugarcane samples. PH1-PH3/PL1-PL3: materials with high/low RPR. **(E)** Breaking force measurement. **(F)** Comparison of breaking force of sugarcane in different environments. BH1-BH3/BL1-BL3: materials with high/low breaking force; E1-E3: plants grew in three different environments. RPR: rind penetrometer resistance. ** indicated statistically significant different at $p < 0.01$ level.

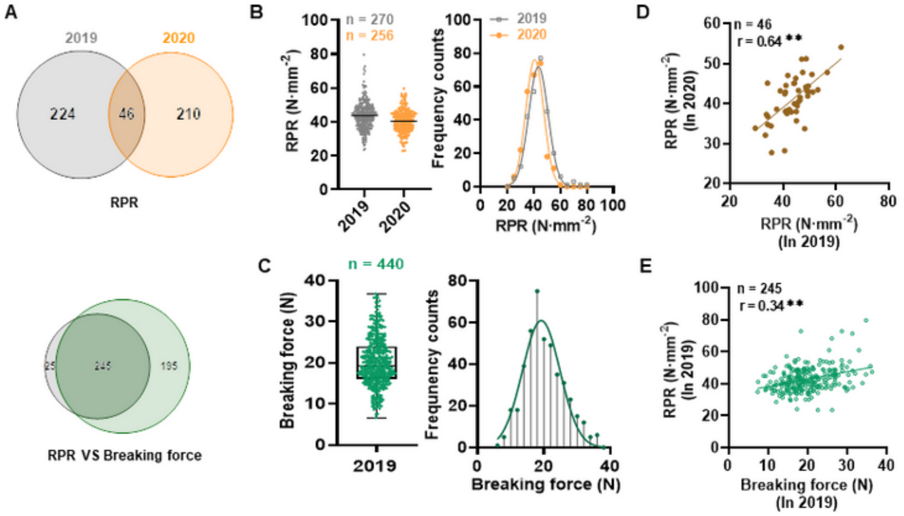

Figure 2

Variation of stalk mechanical strength in sugarcane population. **(A)** Venn diagram representing the number of sugarcane genotypes used for mechanical strengths measurement. **(B, C)** Variated distribution of RPR (B) and breaking force (C) in sugarcane stalks. **(D)** Correlation analysis of sugarcane stalk RPR in 2019 and 2020. **(E)** Correlation analysis between RPR and breaking force in sugarcane genotypes. RPR: rind penetrometer resistance. ** indicated statistically significant correlation at $p < 0.01$ level.

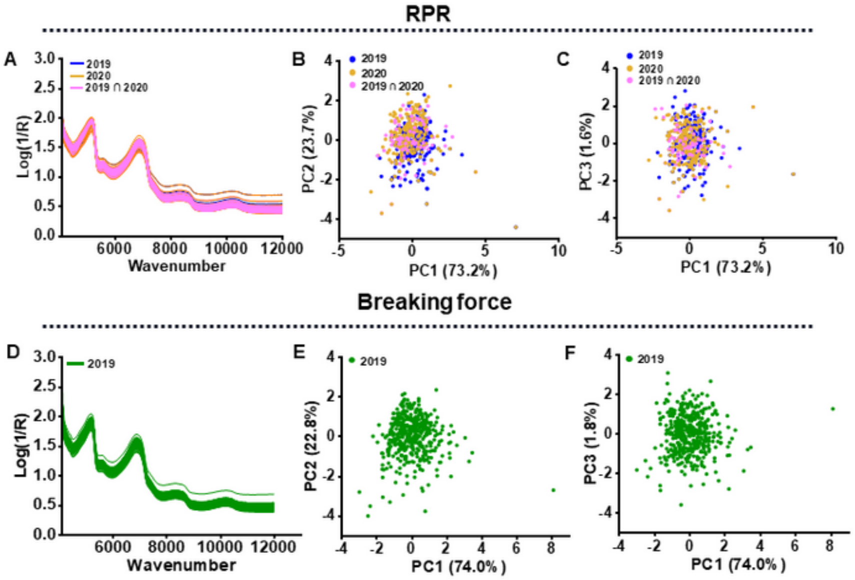

Figure 3

Near-infrared spectral characterizations in sugarcane population. **(A, D)** Original spectral of the samples used for RPR (A) and breaking force (D). **(B-C, E-F)** 2D view of the collected sugarcane samples via PCA. RPR: rind penetrometer resistance.

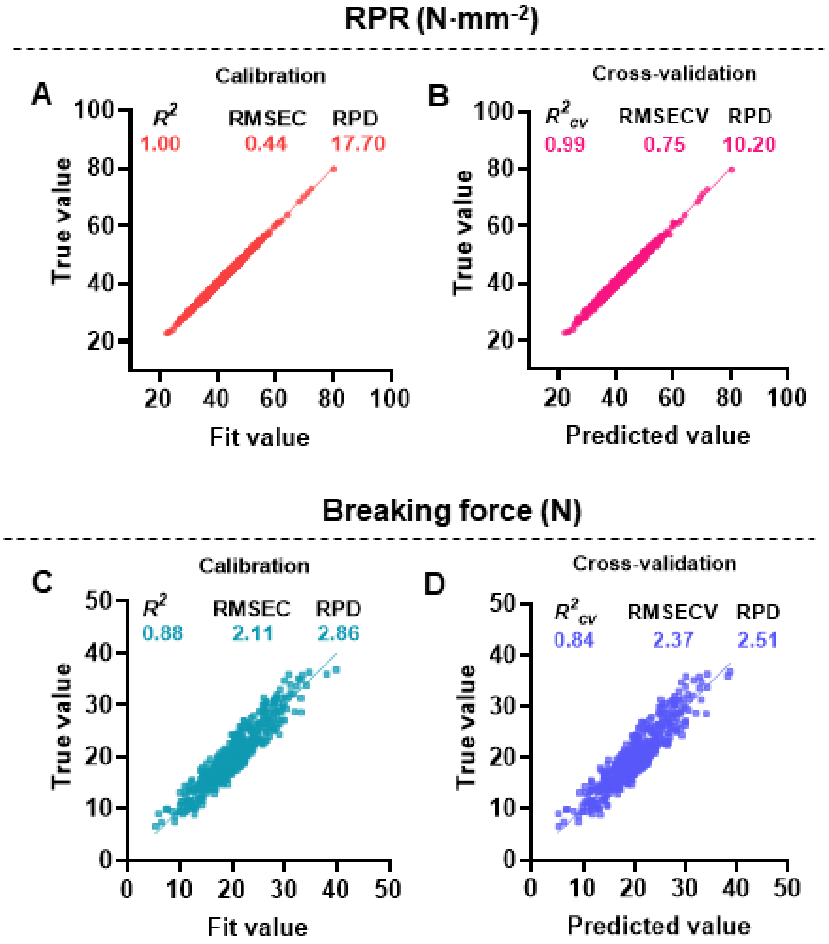

Figure 4

Correlation between the fit (predicted) value and true value for stalk mechanical strengths in sugarcane. **(A, C)** Calibration for RPR (A) and breaking force (C); **(B, D)** Cross-validation for RPR (B) and breaking force (D). RPR: rind penetrometer resistance; R^2, determination coefficient of calibration; R^2cv, determination coefficient of cross-validation; RMSEC, root mean square error of calibration; RMSECV, root mean square error of cross validation; RPD, ratio of prediction to deviation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pptx
- Table2.pptx
- Sup.pptx