



## Thigmostimulation alters anatomical and biomechanical properties of bioenergy sorghum stems

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### ARTICLE INFO

#### Keywords:

Sorghum  
Stems  
Lodging  
Mechanical stimulation  
Morpho-anatomical properties  
Biomechanical properties

### ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is a tropical grass that can be used as a bioenergy crop but commonly suffers from stem structural failure (lodging) when exposed to mechanical stimuli, such as rain and wind. Mechanical stimulation can trigger adaptive growth in plant stems (thigmomorphogenesis) by activating regulatory networks of hormones, proteins, transcription factors, and targeted genes, which ultimately alters their physiology, morphology, and biomechanical properties. The goals of this study are 1) to investigate differences in the morpho-anatomical-biomechanical properties of internodes from control and mechanically-stimulated plants and 2) to examine whether the changes also depend on the plant developmental stages at the time of stimulation. The sweet sorghum cultivar Della was grown in a greenhouse under two growth conditions: with and without mechanical stimulation. The mechanical stimulation involved periodic bending of the stems in one direction during a seven-week growth period. At maturity, the anatomical traits of the stimulated and non-stimulated stems were characterized, including internode lengths and diameters, and biomechanical properties, including elastic (instantaneous) modulus, flexural stiffness, strength, and time-dependent compliance under bending. The morpho-anatomical and biomechanical characteristics of two internodes of the stems that were at different stages of development at the time of mechanical stimulation were examined. Younger internodes were more responsive and experienced more pronounced changes in length due to the stimulation when compared to the older internodes. Statistical analyses showed differences between the stimulated and non-stimulated stems in terms of both their anatomical and biomechanical properties. Mechanical stimulation produced shorter internodes with slightly larger diameters, as well as softer (more compliant) and stronger stems.

### 1. Introduction

Sorghum is widely harvested for food, biofuel, and feedstock. However, sorghum plants are susceptible to structural failure (lodging) of both their stem and roots, which reduces yield. Root lodging is attributed to the weak bonding between the root and soil. Stem lodging is inherently a material and structural failure that commonly arises from wind-induced drag forces and is strongly correlated with stem mechanical properties (Bashford et al., 1976; Niklas and Spatz, 2012; Robertson et al., 2015; Gomez et al., 2017). Studies on the biomechanical properties of stems of grass plants, such as rice, sorghum, and maize, have focused on characterizing their elastic modulus, bending rigidity, and strength (Robertson et al., 2015; Gomez et al., 2017; Ookawa et al., 2010). By examining the biomechanical properties of

stems, breeders can classify and identify lodging-resistant and lodging-susceptible variants (Wu and Ma, 2016).

The morphological and anatomical traits and constituents of plants are the product of both genetic and environmental factors (responses to mechanical loading, temperature, soil conditions, etc.). For instance, repeated mechanical stimulation during growth (thigmostimulation), due to wind, rain, touch, etc., affects the growth and development of many plants (Hernández, 2016). The extent of the response depends not only on the species but also on the physiological stage of the plant during stimulation (Sparke and Wünsche, 2020). Boyer showed that by gently rubbing *Bryonia dioica* at either the upper or lower internodes, growth was reduced to less than 58% of the control plants (those without stimulation) (Boyer, 1967). Similarly, Meng et al. (2006) concluded that tethering a 10-m lodgepole pine reduced the bending moment by 38%,

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which increased the elongation growth rate and decreased the secondary diameter growth, as compared to control trees. In another study, a gentle rubbing of the stem was found to slow the elongation of many plants, e.g., *Hordeum vulgare*, *Bryonia dioica*, *Cucumis sativus*, *Phaseolus vulgaris*, *Mimosa pudica*, and *Ricinus communis* (Jaffe, 1973). In herbaceous and woody plants, mechanical stimulation typically increases stem diameter and decreases height (Jaffe, 1973; Jaffe et al., 1980; De Jaegher et al., 1985; Kern et al., 2005; Paul-Victor and Rowe, 2011; Lemloh et al., 2014; Brulé et al., 2016).

Many studies on thigmostimulation have focused on the morpho-anatomical features of plants, while relatively few studies have addressed how mechanical stimulation affects the biomechanical properties of plant stems (Kern et al., 2005; Paul-Victor and Rowe, 2011; Lemloh et al., 2014; Niklas, 1998). Changes in stem physical properties (e.g., stiffness, viscosity, etc.) due to mechanical stimulation have been correlated with changes in their microstructural morphologies, e.g., rind thickness, number of vascular bundles in the pith, and the size, shape, and composition of cell walls (De Jaegher et al., 1985; Kern et al., 2005; Paul-Victor and Rowe, 2011; Lemloh et al., 2014; Brulé et al., 2016). Periodic mechanical stimulation from 7 to 14 weeks after planting increased the bending strength of sorghum stems by 12–71% depending on the genotype and internode position, while changes in the elastic modulus varied for different genotypes (Lemloh et al., 2014). Bruchert and Gardiner (Brüchert and Gardiner, 2006) found that Sitka spruce (*Picea sitchensis*) exposed to wind had higher flexibility of the crown with higher flexural stiffness of the stem base compared to the wind-sheltered controls, which ultimately resulted in shorter trees with larger diameters and an overall lower modulus of elasticity. Likewise, Badel et al. (2015) and Telewski (1989) found that thigmostimulation of *Abies fraseri* decreased the elastic modulus of wood materials.

Changes in the plant's biomechanical properties can be attributed to cell wall components. Several studies have suggested that touch-induced morphogenic changes are associated with increases in the production of strengthening tissue, e.g., secondary cell walls with high lignin content, thus improving resistance to damage by lateral forces such as from wind (De Jaegher et al., 1985; Biddington, 1986; Braam, 2005). Badel et al. (2015) reported that the mechanical properties of cell walls depend on the cell wall structure, e.g., the microfibril angle (MFA), as well as on chemical composition, e.g., lignin, cellulose, and hemicellulose. Recently, Wu et al. (2020) have shown that touch increased the cell wall stiffness and decreased cell elongation of Arabidopsis seedling hypocotyls, which was attributed to pectin degradation elicited by touch activation of ethylene signaling. Mechanical stimulation of *Brachypodium distachyon* elevated lignin, increased pectin methylesterase activity, and reduced release of carbohydrates from the cell wall (Gladala-Kostarz et al., 2020). These anatomical and microstructural changes also increased the rigidity of the stems.

Gomez et al. (2018) implemented a three-point bending test for freshly harvested sorghum stems and found that stems with lower flexural stiffness and higher strength were correlated with lodging-resistance. Most studies have considered plant stems as elastic materials, but sorghum stems are known to exhibit pronounced visco-elastic responses (Lee et al., 2020), which will affect their response to wind loading at various speeds and frequencies. Limited information exists regarding the biomechanical properties of sorghum altered by mechanical stimulation. Lemloh et al. (2014) characterized bending strength and tissue distributions (rind thickness, pith area, and vascular bundles) of sorghum internodes from stimulated and control plants grown in a growth chamber. Further investigation of other mechanical properties and anatomical and morphological features of plant stems modified by mechanical stimulation is needed to enhance our understanding on the thigmostimulation effect on plant biomechanics. Furthermore, it is not understood how mechanical stimulation influences the sorghum stems at various developmental stages and thereby impacts the biomechanical and anatomical properties of sorghum internodes.

This study explored the influence of periodic mechanical stimulation on the biomechanical properties and morpho-anatomical traits of internodes at different stages of development in bioenergy sorghum. The overall focus of this study was to provide insight into how mechanical stimulation alters biomechanical properties of sorghum stems with the goal of enhancing our understanding of the role of biomechanics in producing lodging-resistant plants. We hypothesized that mechanical stimulation would result in more pronounced changes in the biomechanical properties of elongating internodes (a younger internode at the time of stimulation) in bioenergy sorghum, e.g., elastic modulus, bending strength, flexural stiffness, and time-dependent compliance, as well as in more pronounced changes in anatomical traits, e.g., internode length and diameter and the morphological features of the rind and pith tissues.

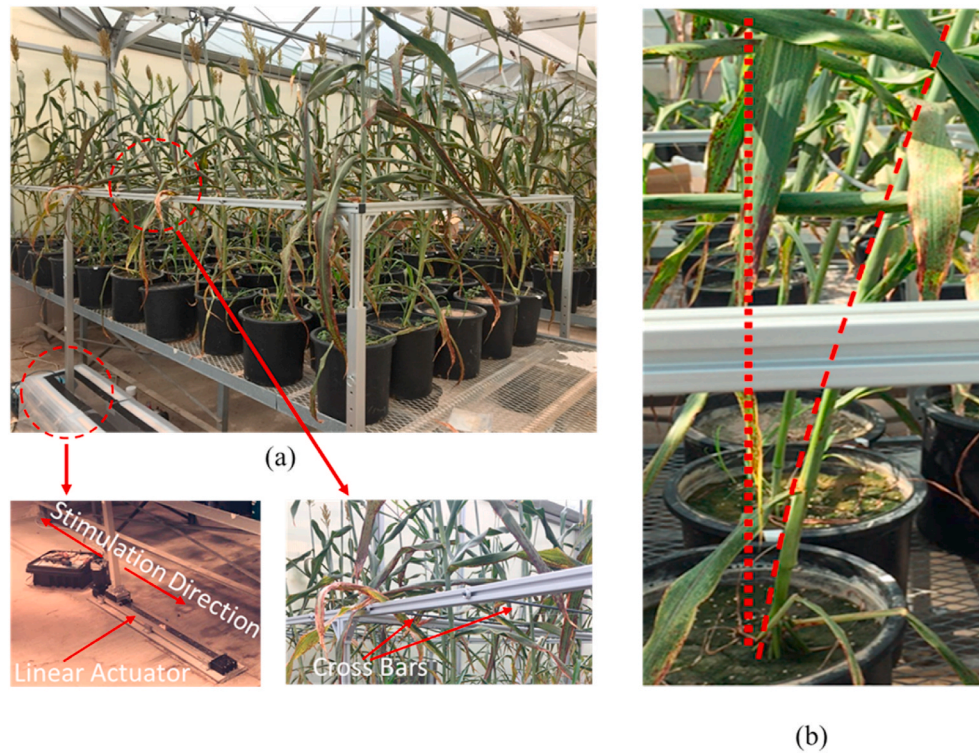
## 2. Materials and methods

### 2.1. Plant material

The sorghum cultivar Della was used for this research. Della is sweet sorghum that is suitable for use as a bioenergy feedstock. Growth of plants began on May 29th, 2020, in a greenhouse at Texas A&M University located at 30.6° N. The temperature was 26–30 °C day/21–26 °C night, and the photoperiod was 14 h day/10 h night, with supplemental light provided by high-pressure sodium lamps (Lee et al., 2020). Plants were grown in pairs in 14.88 L pots (10 7/8 " Dia x 11 1/8 " H) containing a fine sandy loam soil amended with 12.5% potting mix (Jolly Gardener C/20), and adequate water and nutrients (Osmocote 13-13-13 and soluble micronutrients) were provided. At 39 days after planting, half of the plants were mechanically stimulated, and the other half were not (control). Plants were harvested for mechanical testing at grain maturity 13 weeks after planting. Internodes were counted from the base of the plant upwards. The study focused on the response of internodes at different stages of development to mechanical stimulation, including internode 4 (older, around 7 cm, near the end of the elongation phase at the onset of stimulation) and internode 7 (younger, less than 1 cm, early in the elongation phase at the onset of stimulation).

### 2.2. Mechanical stimulation

An experimental apparatus (~6 × 14 feet, see Fig. 1 and the sketch-up of the system in Figure S1 in the Supplemental Document) capable of mechanically stimulating ~100 plants during growth in a greenhouse was constructed. The set-up consisted of a programmable controller/motor and linear actuator. The height of the frame was adjustable in accordance with the height of plants during their growth. The frame was integrated with round 3 mm diameter PVC rods placed parallel to the shorter side of the rectangular frame. The rods came into contact with the plants when the frame moved laterally, thereby inducing bending of the stems. At the beginning of stimulation, the average distance between the bottom of the stem (soil surface) and the rods was set to 43 cm. For most plants, when the stimulation started, the youngest internode was internode 7, which was less than 1 cm, and the total height from the 1st internode to the 7th internode was less than 40 cm at that point in time. When plants started to flower (around 8 weeks after planting), the rods were moved to a height of ~54 cm from the soil surface. The height of the structure was adjusted to ensure that plant stimulation did not cause failure at the internodes, as the top internodes were more flexible than the bottom internodes when the plants grew taller. The mechanical stimulation was applied periodically to induce cyclic bending in one bending axis at a frequency of 3 cycles per minute with a 20 cm amplitude continuously for 2 h, followed by a 6-h resting period (see Fig. 1a and b), before continuing the next stimulation cycle. These conditions were implemented to avoid any potential inertial effects in deforming the stems. Likewise, the relatively slow stimulation reduced the likelihood of damaging the stems during stimulation. Stimulation



**Fig. 1.** (a) Assembled apparatus for mechanical stimulation of plants in the greenhouse. (b) Lateral bending of stems during mechanical stimulation. In the photographs, the plants are at the flowering stage, which is approximately 2 months after planting.

was performed continuously from 39 days after planting to 13 weeks after planting. Non-stimulated (control) plants were grown on an adjacent bench under the same conditions in the same greenhouse.

### 2.3. Sample preparation for four-point bending tests

Plants were harvested 13 weeks after planting by cutting the stems at the soil level between 8 and 10 a.m. before greenhouse temperatures increased. Ten replicate plants were randomly sampled and the leaves were removed to avoid dehydration. Internodes 4 and 7 were selected for mechanical testing. These internodes were chosen to examine the influence of mechanical stimulation on the morpho-anatomical and biomechanical properties of stem tissues at different stages of development. All tests were conducted within 6 h after cutting the internodes. For bending experiments, samples were cut 25 mm past the nodes to produce a sufficiently long test specimen. Before the bending tests, the overall length and diameter of the specimens were measured using calipers. The diameter of the internodes was measured in 4 different positions to produce an averaged value.

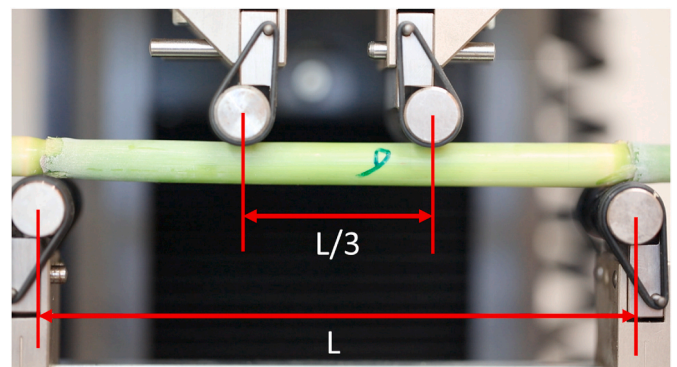
### 2.4. Sample preparation for morphological analysis

Stem cross-sections of stimulated and control plants were cut 4 weeks after anthesis in the middle of the 4th and 7th internodes with a small table saw and fixed overnight in FAA solution (50% ethanol, 5% acetic acid, and 4% formaldehyde). The samples were then paraffin-embedded, sectioned to 20  $\mu\text{m}$ , stained with FASGA solution (Tolivia and Tolivia, 1987), and scanned at 20X using brightfield optics, which was performed in the histology lab of the Texas A&M College of Veterinary Medicine & Biomedical Sciences.

### 2.5. Four-point bending tests

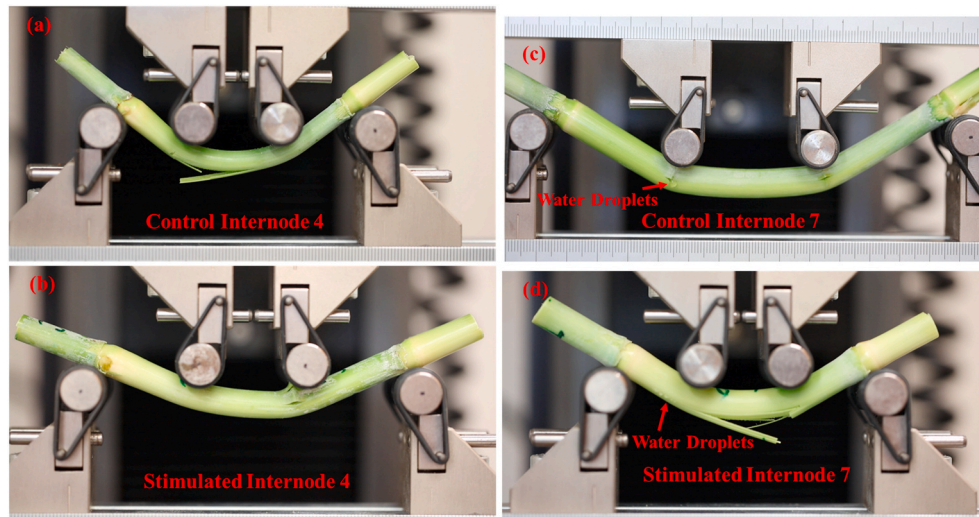
Bending tests were conducted on sorghum internodes (internodes 4 and 7) at room temperature using a four-point bending device fixed to an

Instron 5943 with a 1 kN load cell at a displacement rate of 10 mm  $\text{min}^{-1}$ . Fig. 2 shows images of a four-point bending test. The loading anvils (top) contacted the internode of interest, and the supports (bottom) were placed near the nodes. In all tests, the span lengths between the loading anvils were kept at 1/3 of the internode length (BSI, 2005; Structural round timber - Test methods) (Fig. 2). We used rubber layers on the supports to decrease slippage issues between the supports and the internodes. Failure at the end of the bending tests was classified into two different general modes. In one case, failure arose from the breaking of the rind and subsequent separation from the pith along the length of the stem in the tension region (bottom surface) of the bending test (see Fig. 3 a and d). In the other case, cracks formed in the circumferential direction of the stem, in the compression region, and wrinkling of the rind was also observed; this failure is common in fiber composites because the circumferential direction is often weaker than the axial direction and a thin outer layer can easily buckle under compression (see Fig. 3 b and c). In both cases, water flowed out of the specimens after the initiation of failure (see Fig. 3c and d).



**Fig. 2.** Four-point bending test geometry.





**Fig. 3.** Mechanical failure of the control and stimulated plant stems: (a) control internode 4, (b) stimulated internode 4, (c) control internode 7, and (d) stimulated internode 7.

In the four-point bending tests of the stimulated plants, we implemented two testing scenarios to examine whether the direction of mechanical stimulation (compression vs. tension) impacted the internodes' overall biomechanical properties in a direction-dependent manner. In scenario one, the bending force was applied to induce bending in the same direction that the plant experienced from the mechanical stimulation during growth (i.e., the region that was under compression during the stimulation was also under compression during the four-point bending test, and likewise for the tension region). In scenario two, the bending force was applied to induce bending in the opposite direction of the mechanical stimulation (i.e., the region that was under compression during the stimulation was under tension during the four-point bending test). Since directional differences were not observed, the data were combined to generally represent stimulated internodes.

Additionally, we conducted limited four-point bending creep tests from internodes 4 and 7 of stimulated and control plants to show the significant effect of viscoelastic responses of the stems. The discussion of the creep tests is given in the Supplemental Document. This preliminary creep tests should motivate future study to examine the viscoelastic responses of plant stems.

## 2.6. Modeling of the stem deformations under bending tests

From the observed experimental results, the stems undergo relatively large displacements prior to failure, which is attributed to large rotations, and the overall recorded force-displacement responses indicate nonlinear responses, as will be shown later. The bending responses depend on the geometrical parameters of the stems and the material responses to mechanical loading. A beam bending model was used to simulate the four-point bending tests and extract the biomechanical properties of the stems. Additionally, previous studies have indicated that plant tissues and especially fresh plant stems exhibit pronounced viscoelastic responses (Lee et al., 2020).

In this study, we considered the overall stem bending as exhibiting a nonlinear viscoelastic response and undergoing large deformations attributed to large rotations while the strains are relatively small. From available biomechanical studies (Köhler and Spatz, 2002; Song and Muliana, 2019), plant tissues typically fail at relatively low strains, e.g., less than 10%; as such, modeling the tissues as being under relatively small strains is reasonable. We adopted a material model for the stems that was previously developed for polymers (Muliana et al., 2015; Muliana and Rajagopal, 2012). We also used the Reissner beam theory,

which incorporates large deformation bending with a relatively small strain (Reissner, 1972). An implementation of a nonlinear viscoelastic model to the Reissner beam theory can be found in (Muliana, 2015). A detailed discussion on the large deformation four-point bending analyses with the nonlinear viscoelastic material is given in the Supplemental Document.

The constitutive material model for the nonlinear viscoelastic stem is given as:

$$F(\varepsilon) = \sigma(0) J(t) + \int_0^t J(t-s) \frac{d\sigma(s)}{ds} ds \quad (1)$$

where the left-hand side  $F(\varepsilon)$  is the axial stress measure that depends on the axial strain  $\varepsilon(t)$ ;  $\sigma(t)$  is the axial stress; and  $J(t)$  is the normalized creep function, which is a non-dimensional time-dependent function with  $J(0) = 1$ . In the absence of time-dependent effects,  $F(\varepsilon) = \sigma(0)$ . To incorporate the nonlinear elastic response of the stem tissue, with an assumption that tension and compression behaviors are the same, the following model for the axial stress measure is implemented:

$$F(\varepsilon) = \sigma = \frac{E_0}{\beta} (e^{\beta|\varepsilon|} - 1) \frac{\varepsilon}{|\varepsilon|} \quad (2)$$

where  $E_0$  and  $\beta$  are material parameters that can be determined from experimental data. Linearization of Eq. (2) reduces to a linear elastic response, i.e.  $\sigma = E_0\varepsilon$ , with  $E_0$  being the elastic modulus of the material. The material parameter  $\beta$  characterizes the nonlinear responses of the materials, which is correlated with the maximum stress (strength) of materials. To interpret the nonlinear response, we take a Taylor series expansion of Eq. (2) up to a second order and evaluate it at zero, i.e.,  $\sigma = E_0\varepsilon + \frac{E_0\beta}{2}\varepsilon^2$ . It is seen that increasing  $\beta$  increases the stress at a fixed strain, as will be shown later. A positive or negative value of  $\beta$  indicates a stiffening or softening response, respectively.

In analyzing bending deformations, the axial strain is given as  $\varepsilon(x, y, t) = -y\kappa(x, t)$ , where  $x$  is the longitudinal axis of the beam,  $y$  is the lateral location along the beam cross-section, and  $\kappa(x, t)$  is the beam curvature that varies in space and time. The internode is modeled as a beam with a circular cross-section of a radius  $r$ . Imposing the equilibrium equation  $M = -\int_A y\sigma dA$ , where  $M = M(x, t)$  is an internal bending moment, Eq. (1) can be rewritten as:

$$\int_{A_0} yF(\varepsilon) dA = \int_{A_0} y\sigma(0) J(t) dA + \int_{A_0} \int_0^t J(t-s) \frac{d\sigma(s)}{ds} ds dA$$

$$\int_{-r}^r \frac{E_0}{\beta} (e^{\beta|y\kappa(x,t)|} - 1) \operatorname{sgn}(\varepsilon) (2\sqrt{r^2 - y^2}) dy = M(x, 0) J(t) + \int_0^t J(t-s) \frac{dM(x,s)}{ds} ds$$
(3)

Eq. (3) defines the governing equation of beam bending with nonlinear viscoelastic response.

Finally, the lateral displacement of the beam under bending  $u_y(x, t)$  can be determined as follows:

$$\frac{du_y(x, t)}{dx} = \sin \varphi(x, t); \quad \frac{d\varphi(x, t)}{dx} = \kappa(x, t)$$
(4)

where  $\varphi(x, t)$  is the rotational angle. In simulating the four-point bending tests, the following boundary conditions are considered:  $M(0, t) = u_y(0, t) = \varphi(L/2, t) = 0$  where  $L/2$  is the mid-span of the beam.

From the quasi-static four-point bending test, where the time-dependent part is insignificant due to a relatively fast loading rate, the elastic (instantaneous) modulus and strength of the stem tissue can be calibrated. Fig. 4 shows examples of force-displacement responses from the model and experiment, in which responses from the model were used to determine the mechanical properties of the stems. The strength was determined at the location of the first “sudden drop” in the force-displacement curves, i.e., around 8 mm in Figs. 4a and 6 mm in Fig. 4b, which corresponds to the onset of cracking in the stems. The elastic modulus and nonlinear parameter were calibrated to match the force-displacement response prior to the start of failure. Unpaired t-tests were used to assess the significance of differences between the calibrated parameters of the stimulated and control internodes. The unequal number of samples between treatments arose from plants that were removed from the data set due to significant sliding of the samples on the supports, axial rotation of the samples, and/or movement of the supports during testing, as well as plants that demonstrated stem lodging in the greenhouse. However, in calculating the elastic modulus, more samples could be used as valid tests, as only data from the early loading stage (e.g., before significant slipping was observed in some specimens) were required.

### 2.7. Data analysis

Force-displacement data were acquired through the Instron software (Bluehill® Universal 4.01) and processed in MATLAB® R2016b version 9.1. For force-displacement plots, each force represents the average of all the samples at a specified displacement. When a given plant reached

its strength (maximum stress before failure starts, as defined in the previous section), it was removed from the averaging. For instance, if a plant reached its strength at a displacement of 8 mm, that specific plant was removed from the data set in determining the average force for displacements larger than 8 mm. Likewise, the last point in the plots represents the largest displacement among all the samples. Statistical analysis was conducted using R (version 4.1.1) and RStudio (version 1.4.1717). Data that did not follow a normal distribution were normalized. Two-way analysis of variance (ANOVA) followed by planned contrasts (where appropriate) and unbalanced t-tests were used to identify differences in anatomical traits and biomechanical properties between control and stimulated plants, with the significance level at  $p \leq 0.05$ .

## 3. Results

### 3.1. Comparison of anatomical traits between stimulated and non-stimulated plants

Anatomical traits, including the length and diameter of the internodes, are shown in Fig. 5. The total height of stimulated plants was less than that of control plants, as can be seen in Fig. 6. Mechanical stimulation decreased the lengths of all internodes, except for internodes 2 and 3 (statistical analyses shown in Table S2 in the Supplemental Document). From the two-way ANOVA analysis in Table S2 the total decrease in the lengths of internodes 8 and 9 was significantly greater than that in internodes 6 and 7, which in turn was significantly greater than that in internodes 4 and 5, indicating that internodes that were younger (internodes 6–9) when mechanical stimulation was initiated showed more pronounced changes in length compared to the internodes that were nearly fully elongated (internodes 4 and 5). When the diameter of each pair of internodes were compared independently, there was a slight increase in all stimulated internodes. From the two-way ANOVA result, mechanical stimulation also increased internode diameter, but this effect was not dependent on internode position (Table S3 in the Supplemental Document). When examining stems under bending, the important geometrical parameter is the second moment of area, i.e.  $I = \frac{\pi d^4}{64}$ , where  $d$  is the diameter of the cross-section. Thus, slight changes in

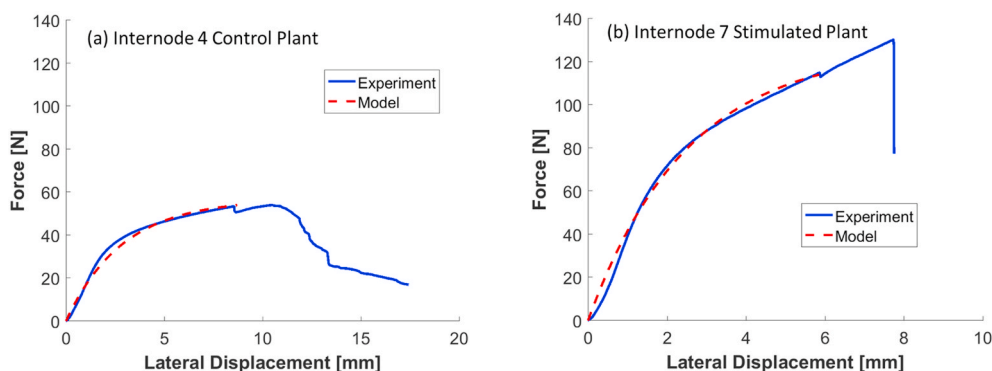


Fig. 4. Experimental data and corresponding model of four-point bending tests to characterize material properties from: (a) internode 4 and (b) internode 7.

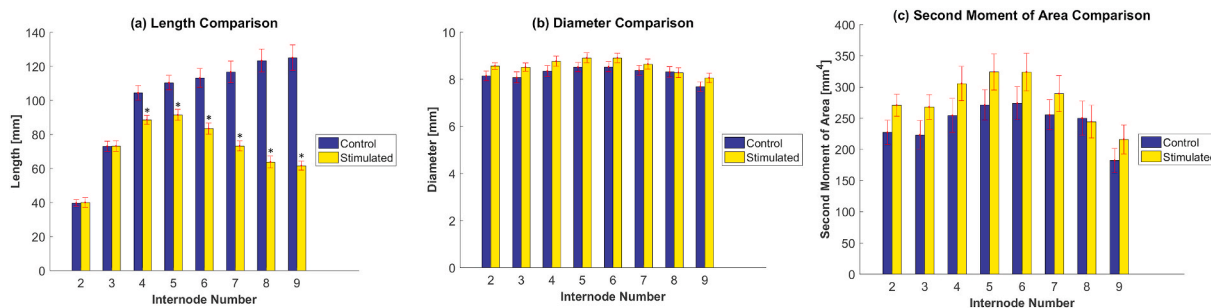


Fig. 5. Comparisons of anatomical traits of the control and stimulated plants: (a) internode length, (b) diameter, and (c) second moment of area; data are means  $\pm$  SE (Standard Error) and Asterisks (\*) indicate a significant difference in internode length between control and stimulated plants at  $\alpha = 0.05$ .

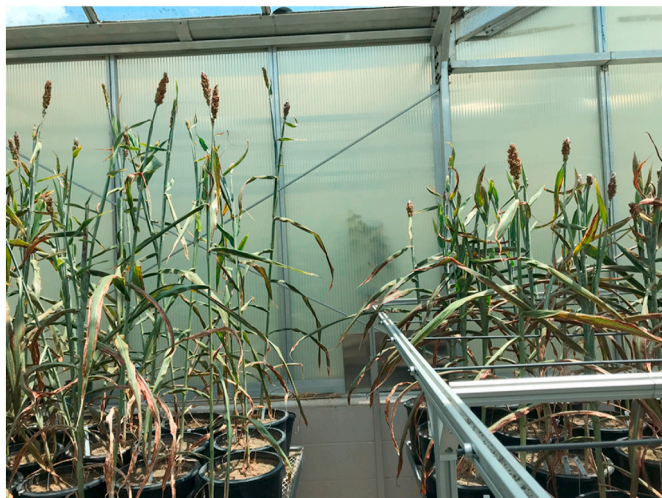


Fig. 6. A comparison between control and stimulated plant in a group (the left group is control, and the right group is stimulated).

the diameter can have a pronounced effect on the bending responses of the stems. Fig. 5 shows the second moment of area, and the corresponding statistical analyses are given in Table S3 in the Supplemental Document. The second moment of area of stimulated plants was greater than that of controls, in agreement with the effects of mechanical stimulation on internode diameter.

### 3.2. Comparison of mechanical properties between stimulated and non-stimulated plants

Fig. 7 shows representative plots from quasi-static four-point bending tests of internodes 4 and 7 with the recorded bending force and lateral deformation at the location of the prescribed load. When the mechanical stimulation during growth was initiated, internode 4 was almost finished elongating while internode 7 was at an early stage of elongation. Clear differences were apparent in the responses of the stimulated and control plants, which are associated with changes in the morpho-anatomical and biomechanical properties of the plants, as will be discussed later. In both internodes 4 and 7, the stimulated stems supported much larger bending forces (about twice as much), which was attributed to changes in the stems' lengths, diameters, moduli, and strengths. The stems' elastic moduli and strength are discussed below and summarized in Fig. 8.

The calibrated biomechanical parameters, i.e., elastic modulus  $E_0$ , strength, flexural stiffness, and nonlinear parameter  $\beta$ , and the corresponding box plots are shown in Fig. 8. In this study, the strength was defined as the axial stress immediately before the first "sudden drop" in the force-displacement curves (see Fig. 4). The sudden drop indicates the

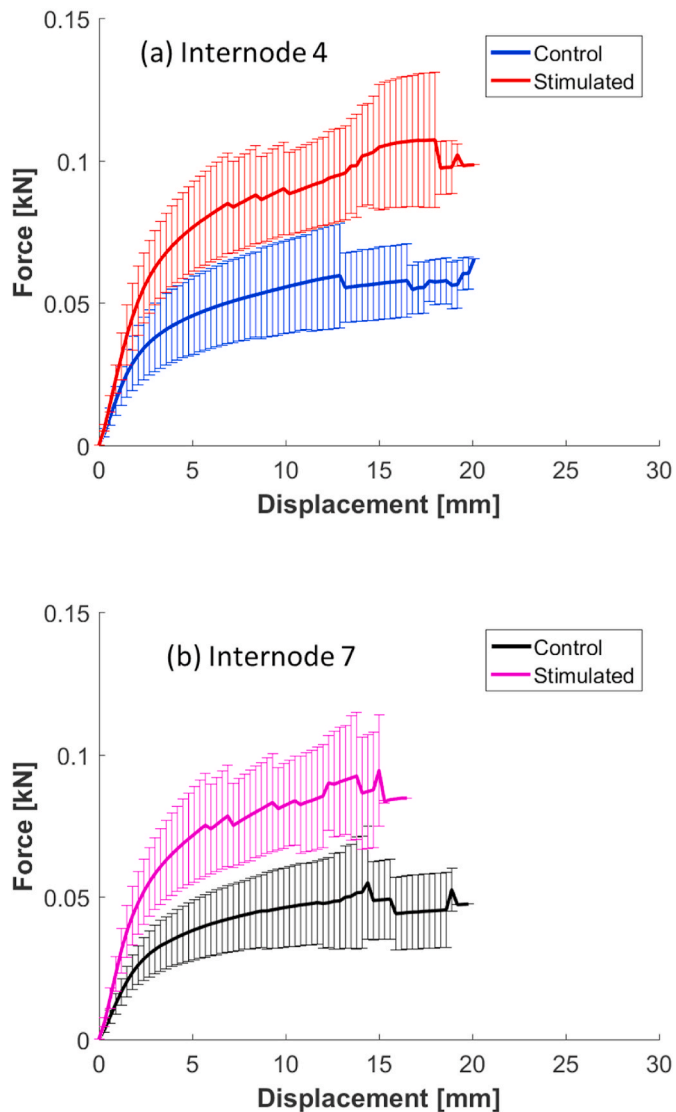
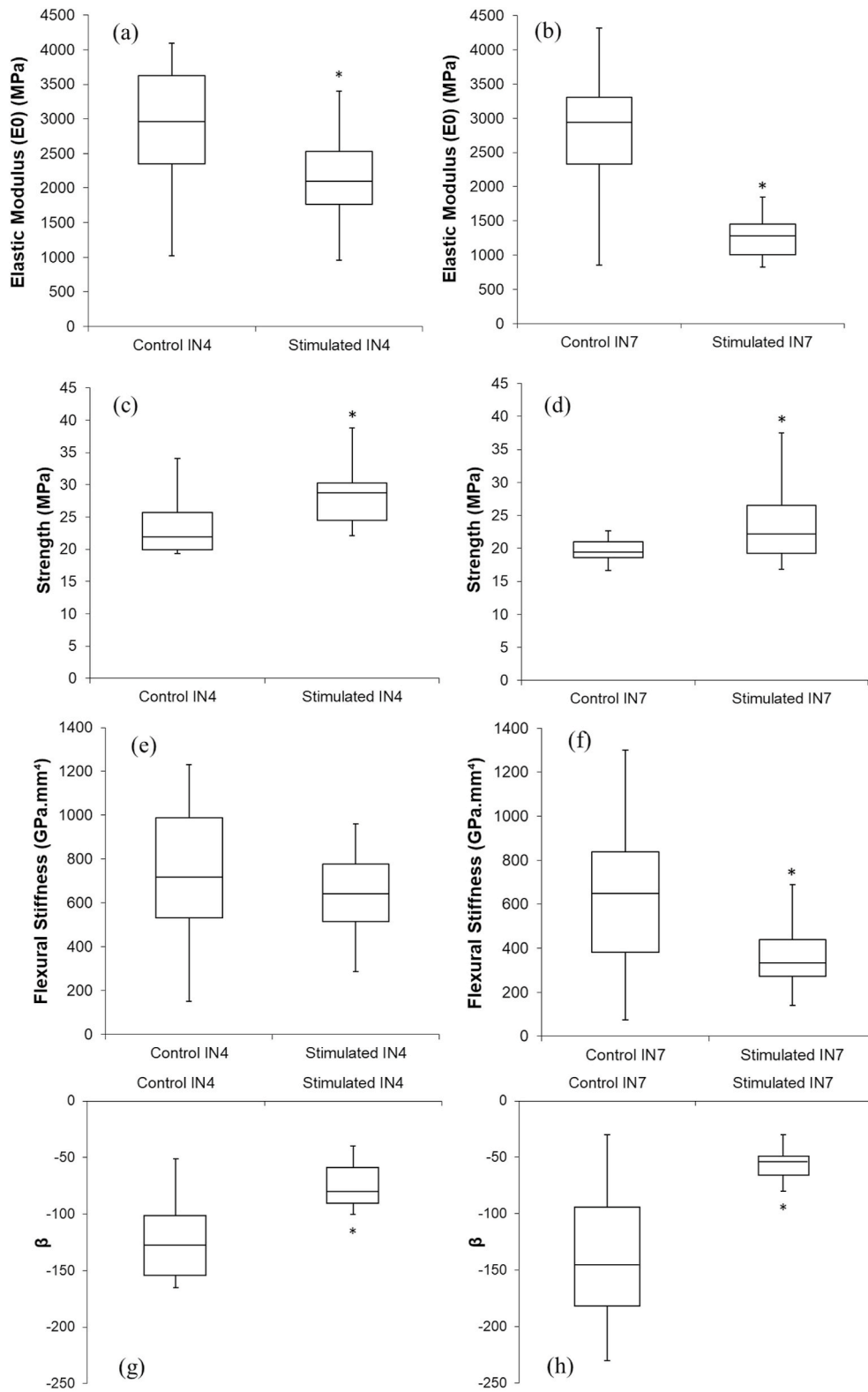


Fig. 7. Force and lateral displacement of sorghum stems during four-point bending of control and stimulated plants: (a) internode 4 and (b) internode 7; data are means  $\pm$  SD (Standard Deviation).

start of failure/damage in the bending test. Overall, the stimulated plants showed significantly smaller values of elastic modulus (softer material behavior) but relatively greater strength in both internodes 4 and 7. Fig. 8 indicated that significant differences existed between stimulated and control plants for both internode 4 and 7 in terms of elastic moduli (Fig. 8a and b) and strength (Fig. 8c and d). Overall, the



**Fig. 8.** Boxplot comparison of control and stimulated plants for internodes 4 and 7 regarding (a–b) elastic modulus, (c–d) strength, (e–f) flexural stiffness, and (g–h)  $\beta$ ; Asterisks (\*) indicate a significant difference between control and stimulated plants at  $\alpha = 0.05$ .

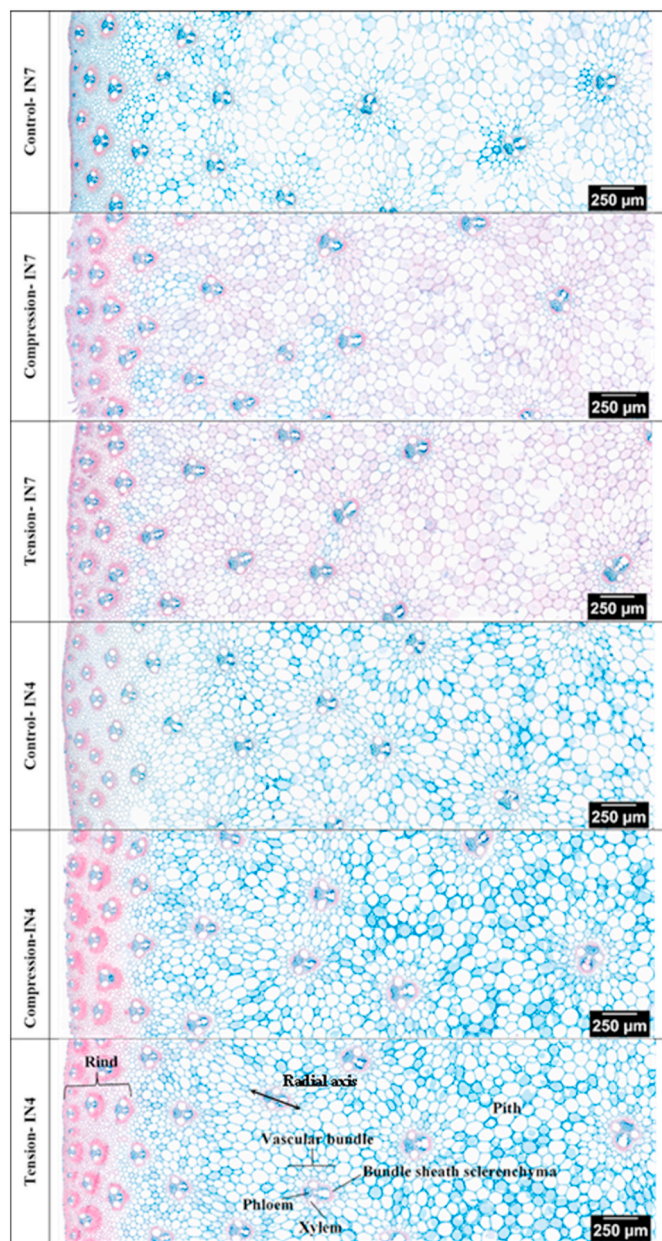
mechanically stimulated plants were softer (more compliant) than the control group. When also accounting for differences in geometry (e.g., incorporating the effects of varying diameters produced through the different growth treatments), internode 7 of stimulated plants still exhibited less flexural stiffness (a product of elastic modulus and second

moment of area of the cross-section) as compared to the control plants; this trend was the same for internode 4, although the flexural stiffness of stimulated internode 4 was not significantly different from control. Fig. 8d shows that there was also a large difference between control and stimulated plants with respect to  $\beta$  (nonlinear material parameter) for



internodes 4 and 7. The coefficient  $\beta$  is defined such that a larger value of  $\beta$  indicates a greater strength. The stimulated plants showed relatively larger (less negative)  $\beta$  values compared to control plants for both internodes 4 and 7. The statistical results are provided in Table S4 in the Supplemental Document.

The direction of the mechanical stimulation applied did not affect internode biomechanical properties when tested in a tension versus compression side specific manner. For the elastic modulus and flexural stiffness, there was little difference in the average values between the same-direction bending and the opposite-direction bending (see Fig. S2 in the Supplemental Document) and no statistically significant differences were identified. (See Table S5 in the Supplemental Document).



**Fig. 9.** Microstructural morphologies of sorghum stem tissues (rind and pith) from control and mechanically stimulated plants (Compression – the side of stem subjected to compression [away from the point of stimulation] and Tension – the side of stem subjected to tension [near the point of stimulation]). The red color in the images indicates lignin accumulation. Young samples (at the time of stimulation) were from internode 7 and more mature samples (at the onset of stimulation) were from internode 4.

### 3.3. Comparison of microstructural morphologies between stimulated and non-stimulated plants

Compared to the control, mechanical stimulation appeared to increase the vascular bundle size and density, especially in the outer layer of rind tissue of the younger internode 7 (Fig. 9). No obvious differences were observed between the compression and tension sides (away from and toward point of stimulation, respectively) of stimulated stems. Increased staining (lignification) of vascular bundles was observed with mechanical stimulation - some of this staining was due to increased development of the sclerenchymatous bundle sheath. Additionally, mechanical stimulation seemed to alter the form of the vascular bundles resulting in greater elongation along the radial axis. Parenchyma cells of the pith, which form the inner part of internodes, also appeared to respond to mechanical stimulation with elevated lignification. Increases in lignin content are often associated with increases in the strength of the tissues (Badel et al., 2015; Özparpucu et al., 2019; Telewski, 2006). Overall, mechanical stimulation seemed to result in more pronounced changes in the morphology of the rind tissue as compared to the pith tissue. This phenomenon may be due to the mechanical stimulation inducing more severe stresses in the rind than in the pith. However, since the images were not quantified, strong conclusions may not be warranted.

## 4. Discussion

Adaptive growth in plant stems due to mechanical stimulation (thigmomorphogenesis) occurs by activating various regulatory networks, i.e., hormones, proteins, transcription factors, and target genes, and their actions may manifest through altering physiology, morphology, and biomechanical properties. (Samad et al., 2017; García-Gómez et al., 2017; Dobránszki, 2021). The present study shows that the internodes that were still actively elongating or had just begun elongating at the beginning of mechanical stimulation (younger internodes, i.e., internodes 6–9) experienced more pronounced adaptive growth effects than internodes that were fully or nearly fully elongated (mature internodes, i.e., internodes 2–5). The decrease in the internode lengths with thigmostimulation was greater for the younger internodes. Mechanical stimulation also increased internode diameter, but this effect was not dependent on internode position. From the microstructural morphologies in Fig. 9, the cell-wall structure reprogramming of the stimulated plants appeared to increase the vascular bundle size and density, especially in the rind tissue of young internodes (internode 7), and to increase lignification of vascular bundles in both pith and rind tissues. These data should be interpreted with caution however, as they remain unquantified. In general, the observations may explain the observed strengthening of the stimulated internodes, as discussed below.

For both internodes 4 and 7, mechanical stimulation increased the force required to cause failure under the four-point bending tests. This result indicates that the stimulated plants can resist higher external loads, which can potentially minimize stem failure due to mechanical loading (e.g., from wind). A similar study by Lemloh et al. (2014) on *Sorghum bicolor* found that mechanical stimulation increased the thickness of rind tissue and the bending strength of mature internodes 2 and 3. To understand the higher load bearing ability of the stimulated internodes under bending, several internode physical parameters, e.g., length, diameter, elastic modulus, and strength, were examined. The length and diameter are geometrical parameters, while the modulus and strength are material (tissue) properties. The stimulated internodes exhibited slightly larger diameters compared to the corresponding control internodes which may enhance the resistance to bending owing to an increase in the second moment of area. For internode 7, the stimulated internodes were much shorter than the control internodes, while only modest differences were observed between the lengths of control and stimulated internode 4. When a fixed magnitude of force is



applied to shorter internodes, it results in smaller maximum internal bending moments due to smaller moment arms and hence leads to smaller axial stresses. Thus, the shorter internodes can withstand larger forces before they reach the strength of the internode, i.e., without causing failure. The observed changes in the geometrical parameters of the stimulated internodes contributed to their higher load bearing capacity. The decrease in the internode length and hence the overall reduced height with mechanical stimulation has been demonstrated in several other plants, e.g., *Bryonia dioica*, *Cucumis sativus*, *Ricinus communis* (Jaffe, 1973), *Brachypodium distachyon* (Gladala-Kostarz et al., 2020) and *Arabidopsis* (Paul-Victor and Rowe, 2011; Zhdanov et al., 2021). Such changes are typically accompanied by an increase of thickness of stems such as in *Phaseolus vulgaris* (Jaffe, 1976) and Sweetgum. However, in some species the diameter of stimulated stems was reduced, such as in *Arabidopsis* (Paul-Victor and Rowe, 2011) and wheat (Hindhau et al., 2021). As discussed below, the varied responses to mechanical stimulation may result from a variety of factors including the experimental methodology employed and the plant species examined. Our study also showed that younger internodes experienced more pronounced changes in length due to mechanical stimulation compared to the older internodes.

In addition to stem geometry, the biomechanical properties of the tissues can also change due to mechanical stimulation during growth. To assess their changes, we examined the elastic modulus and strength (stress at the onset of failure) of the internodes. For both internodes 4 and 7, the stimulated plants exhibited greater tissue strengths. Overall, these observations indicate that the increase in the load bearing ability of the stimulated internodes was not purely from geometrical changes but also from the strengthening of the tissues themselves. The tissue strengthening likely arises from the observed increased lignification of cell walls in both the pith and rind tissues of stimulated internodes. The stimulated internodes demonstrated reduced elastic moduli for both internodes 4 and 7 when compared to the controls, which indicates that the stimulated internodes had softer (more compliant) tissues. A detailed explanation as to why the mechanical stimulation resulted in more compliant stem tissues is currently lacking. However, previous studies (Badel et al., 2015; Telewski, 1989) have indicated that a smaller modulus can result from increases in the cell wall microfibril angle (MFA) of the stimulated plants. An increase in the lignin content of mechanically stimulated plants has also been reported in *Brachypodium distachyon* (Gladala-Kostarz et al., 2020). This study examined two geographically diverse genotypes (Bd21 and ABR6) which were grown in controlled greenhouse conditions and subjected to mechanical stimulation at the same developmental stage (three tillers). In Bd21, mechanical stimulation decreased the area of both inner and outer vascular bundles, while in ABR6 mechanical stimulation increased the area of vascular bundles. The authors also reported that mechanical stimulation increased the elastic modulus of internodes 2 and 3 for fully mature stems, which the authors associated with reduced stem elongation. For monocot plants, one study observed that mechanical stimulation resulted in a reduction in elastic modulus, which was found to be correlated with reduced lodging of the stems (Gladala-Kostarz et al., 2020).

When examining the overall stem behavior in bending, flexural stiffness is often used to assess the stem's bending ability. The flexural stiffness is a product of the elastic modulus, which is a tissue property, and the second moment of area, which is a geometrical property. For internode 4, no apparent differences were observed in the flexural stiffness of the stimulated and control internodes. The stimulated internode 4 had a slightly smaller elastic modulus which compensated for the slightly larger second moment of area. However, internode 7 exhibited more pronounced differences in the flexural stiffness between the control and stimulated stems. The stimulated internode 7 showed much lower flexural stiffness, which allows the internode to bend more easily. Gomez et al. (2018) reported that field grown lodging-resistant sorghum lines had more compliant tissue, lower flexural stiffness, and stronger stems. Thus, lodging-resistant plants readily deform upon

mechanical loading (e.g., from wind) instead of resisting the force. Paul-Victor and Rowe (2011) have reported that mechanical stimulation of *Arabidopsis* decreased the stem flexural stiffness, which the authors attributed to a reduction in pith tissue and lignin content. They also found that mechanical stimulation decreased the density of the lignified cells. Wang et al. (2009) studied the influence of mechanical stimulation on the flexural stiffness and biomass content of *Corispermum mongolicum* plants. They found that mechanical stimulation produced stiffer stems, which was attributed to thicker tissue and higher elastic modulus. The variabilities in flexural stiffness and lignification responses resulting from mechanical stimulation in different species may be due to dissimilar acclimation programs, differences in the tissue composition (i.e., tissues resulting from secondary growth in some dicots which are not present in monocots) and tissue organization, and/or differences in the nature of the mechanical stimulation applied. Future research reconciling these issues would be beneficial.

The responses of mature internodes to bending applied in the same and opposite directions to the mechanical stimulation applied during growth were also assessed. No statistically significant differences were identified for the elastic modulus, strength, or flexural stiffness of the stimulated internodes when bending in the same and opposite directions from the bending stimulation. These results are consistent with the lack of apparent differences in the morphological features of the tissues subjected to tension and compression during stimulation. It is possible that the amplitude of bending applied during the stimulation was too small to cause significant changes in the development of the tissue between the tension and compression regions. Future work may consider increasing the amplitude of bending deformations during mechanical stimulation to further understand the influence of tensile and compressive stimulation.

Mechanical stimulation promotes changes in the abundances/expression of hormones and genes that can strongly influence the morphology and constituent compositions of cell walls and tissues, which impact the anatomical and biomechanical traits of the stems. A future study investigating how mechanical stimulation-induced changes in hormone abundances and transcriptome expression may be linked to the morpho-anatomical and biomechanical properties of stems would be beneficial.

## 5. Conclusions

Mechanical stimulation reduced internode elongation and altered the anatomical features of the sorghum stem. The biomechanical properties were also altered by mechanical stimulation: the stimulated internodes had higher strength and lower elastic modulus compared to the control internodes. Younger internodes (at the onset of the stimulation) demonstrated a more pronounced decrease in length due to mechanical stimulation. The changes in the geometrical, morphological, and tissue biomechanical properties induced by mechanical stimulation contributed to increases in the load bearing ability of the stimulated internodes. The changes in the tissue biomechanical properties, i.e., lower elastic modulus, higher strength, and lower flexural stiffness, that contribute to increases in the load bearing ability of the stimulated internodes are consistent with the biomechanical properties of lodging-resistant sorghum genotypes grown in the field. The results provide a foundation for future exploration of the potential for engineering lodging-resistant genotypes tailored to their specific environments, e.g., regions with strong winds.

## CRedit authorship contribution statement

**Omid Zargar:** Writing – original draft, Validation, Methodology, Investigation. **Qing Li:** Data curation, Experimentation, and Formal analysis. **Chiedu Nwaobi:** Data curation. **Matt Pharr:** Writing – review & editing, Supervision, Investigation, Formal analysis. **Scott A. Finlayson:** Writing – review & editing, Supervision, Resources,

Conceptualization. **Anastasia Muliana:** Investigation, Methodology, Analysis, Supervision, Resources, Writing, Conceptualization.

### Declaration of competing interest

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.

### Acknowledgments

This research is sponsored by the National Science Foundation under grant CMMI-1761015. The authors thank Dr. W. Rooney for providing the Della seed used in the study.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmbbm.2022.105090>.

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