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Marie-Louise Lemloh*, Anna Pohl, Eva Weber, Marco Zeiger, Petra Bauer, Ingrid M. Weiss, Andreas S. Schneider

Structure-property relationships in mechanically stimulated *Sorghum bicolor* stalks

Abstract: Mechanical properties of plants and underlying structure-property relationships are important for agricultural purposes as well as for biomimetic concepts. In this study, the effect of mechanical stimulation on morphology and bending properties of the stalk was investigated for *Sorghum bicolor* (Poaceae), a widely used drought-tolerant biomass grass. An experimental set-up allowing for defined growth and mechanical perturbation (flexing) during a defined growth period was designed. Mechanical properties of individual internodes of the stalk were determined by three-point bending tests. We found that the three investigated lines showed differences in their general bending strength in the non-stimulated condition. However, similar high range of bending strength values was measured for all plant lines after they underwent the mechanical stimulation procedure. The anatomy of internode cross-sections was examined to evaluate structure-property relationships. An increased thickness of the outer sclerenchymatous tissue was observed for internodes with higher bending strength values. Dried internodes fail under lower strains but showed higher bending strength. These findings show that mechanosensitivity in sorghum is dependent on genetic as well as environmental factors. The experimental system presented here offers new straight-forward possibilities for *S. bicolor* line selection for applications requiring mechanical strength of the stalk.

1 Introduction

Sorghum bicolor (Poaceae) is an important crop plant. This African grass is a close relative of maize and sugar cane which are all high biomass sweet grasses of the tropical regions belonging to the subfamily Panicoideae. In comparison to maize, sorghum is a newly cultivated crop in several regions of the world [1]. However, it was produced for food since 5000 years in Africa, partly because of its drought tolerance [2]. During 1988, more than 61,000,000 mt sorghum grains were produced worldwide on a harvested area of 43,445,000 ha. Of these 61,000,000 mt, around 20,000,000 mt were produced in Africa [1]. Sorghum is mainly grown for food, fibre and fuel requirements [3]. Beside this, it is also used in feed for swine, cattle and poultry [4]. For the production of animal feed and bioenergy, the plant leaves and stalks are valuable products. Another option for the use of sorghum stalks is in paper production because of similarity of sorghum fibres to hardwood fibres [5]. For all these industries, it is inevitable to produce high biomass in a cost-efficient manner per cultivated area. Therefore, it is a prime breeding goal to increase biomass yield and to avoid crop failure. The permanent displacement of crop plants from their natural position because of root- and shoot-failure poses a serious challenge for cultivating biomass grasses [6,7]. One strategy to improve yields and to maintain inflorescences as well as grain maturity is to enhance the mechanical properties of sorghum stalks.

*Corresponding author: Marie-Louise Lemloh: INM - Leibniz Institute for New Materials, Campus D2 2, 66123, Saarbruecken, Germany, E-mail: Marie-Louise.Lemloh@inm-gmbh.de
Anna Pohl, Eva Weber, Ingrid M. Weiss, Marco Zeiger, Andreas S. Schneider: INM - Leibniz Institute for New Materials, Biomineralization Group, Campus D2 2, 66123, Saarbruecken, Germany
Eva Weber: Present address: Department of Materials Engineering and the Russell Berrie Nanotechnology Institute, Technion Israel Institute of Technology, Haifa 32000, Israel
Petra Bauer: Saarland University, Department of Biosciences - Plant Biology, Campus A2 4, 66123 Saarbruecken, Germany
Petra Bauer: Present address: Heinrich Heine University, Institute of Botany, Universitaetsstr. 1, 40225 Duesseldorf, Germany

Plant morphology is evolutionarily optimized regarding natural environmental constraints. Not only the whole plant, but also certain plant cell compartments like the cell wall and vacuole harbour remarkable properties for stabilization. Plant stalks have to stabilize the whole plant and simultaneously transport nutrients from the roots to the leaves. They are hierarchically structured composite materials consisting of different types of tissues. The inside of sorghum stems is composed mainly of ground and vascular tissue. The ground tissue serves filling, metabolic and stabilization functions. It contains either parenchyma cells, which are living cells with a thin cell wall, often taking over starch storage, or sclerenchyma cells that provide strength and support. The latter have thick, lignified secondary cell walls and may be dead cells [8]. Sclerenchyma cells frequently occur in bundles such as around the vascular system or in layers such as below the epidermis. Such sclerenchymatous assemblies usually form fibres in the longitudinal direction in the stem. Vascular tissue includes xylem vessels, important for both water and solute conduction and for mechanical support, as well as phloem elements for transport of organic molecules. Properties that influence plant mechanics include the cell wall of the different tissues, the level of cellulose content and lignification of xylem and sclerenchyma, the arrangement of vascular bundles and the turgor pressure. From detailed studies of mechanical behaviour of plant stems it has been suggested that they can be described as thin-walled cylindrical shell structures with an outer shell of dense material supported by a low density, cellular core [9,10]. On a smaller scale, the cell wall material can be considered as a fibre-reinforced composite material consisting of four main building blocks: cellulose, hemicellulose, pectin and lignin [11].

Since land plants are sessile, they have to respond dynamically to external stimuli by adapting their morphology. Rapid responses can be thigmotropic or thigmonastic based on the direction of the stimulus [12]. Touch-induced morphogenetic changes that occur slowly are defined as thigmomorphogenesis [12,13]. Such mechanic stimuli occur frequently in the field through wind, weather, neighbouring plants or animals. Mechanical stimulation like rubbing or flexing may also result in modified plant growth and development [13,14]. The effect of mechanical stimulation of plant stems was extensively studied in woody plants [15]. Such studies have highlighted the ability of woody plants to adapt their growth in response to mechanical perturbation at different hierarchical levels. Mechanical properties were also studied in some plants belonging to the Poales

(e.g. grasses). Graminaceous plants that are used for construction applications such as structural bamboo were investigated to achieve engineering data from bending and compression tests [16]. *Arundo donax* (giant cane) stems respond to dynamic deflections with damped harmonic bending oscillations [17]. Stems of these plants were also examined regarding bending behaviour and Young`s moduli of the different tissues [18].

Only few studies have been reported so far describing the mechanical properties of sorghum. The use of sorghum stalks as building material motivated the study of its deflection characteristics [19]. Mechanical properties in relation to quasi-static deformation using flat knifes were examined to evaluate harvesting behaviour of sorghum [20] and tensile tests revealed that the middle region of the sorghum rind possesses the highest tensile strength [21].

Due to the increasing interest of using sorghum as a bioenergy plant and the frequent challenges associated with lodging in the field, we selected it as a model system for our biophysical studies. Biomass varieties are available and its genome sequence was determined [3]. Here, we established a mechanical test for describing the stability of sorghum stalks and we show that it can be successfully applied to quantify and collect mechanical data of sorghum lines. It was then possible to establish a system for mechanical stimulation and to study the influence of flexing the plant stalks during growth and to monitor both, morphological and mechanical characteristics of the stalks. We suggest that with our assay it is possible to screen sorghum lines to breed varieties with superior mechanical qualities.

2 Methods

2.1 Plant material and cultivation

Seeds of *Sorghum bicolor* were obtained from the KWS Saat AG, Einbeck, Germany. The lines were named S4, S10 and S11. S10 and S11 are inbred lines, whereas S4 is a hybrid variety. According to information obtained from the supplier, S4 and S10 were classified as "stable lines" (tolerant to loading), while S11 was classified as a "less stable line" (more susceptible to lodging) when grown in field conditions. Seeds were allowed to germinate in substrate-plugs (Jiffy, Moerdijk, Netherlands). Two week-old seedlings were transferred into pots with substrate (Hawita Group GmbH, Vechta, Germany) and were grown for an additional 12 weeks (Figure 1). Cultivation was performed in two different cultivation chambers. Plant

growth chamber AR22L (CLF Plant Climatics, Emersacker, Germany) was used during the germination and plant chamber ATC60-Flex (Conviron, Manitoba, Canada) was utilized during the remaining growth period. The following growth conditions were applied: a light intensity of 150 µmol/sm² (week 1-2) and 300 µmol/sm² (each 16 h light/8 h dark cycle), humidity of 60 – 70 % and temperature of 23 °C. All plants were fertilized with Ferty Mega 1 (Planta, Regenstauf, Germany) biweekly and watered as required. After a growth period of 14 weeks, the second and third internodes from the bottom of the stalk were analysed for morphological and mechanical properties.

2.2 *In vivo* mechanical plant stimulation system

For mechanical stimulation of the plants, a self-built setup was integrated inside the plant chamber, composed of round bars actuated by an electric motor (Figure 1 B). The bars had a diameter of 3 mm and were arranged in parallel with 30 mm space between them. *S. bicolor* plants were allowed to grow between the bars in such a way that damage of the *S. bicolor* stalks was avoided. The load was applied at one point at a height of 420 mm measured from the root/stalk transition marking the beginning of the first internode. A motor with an eccentric disc (30 mm radius) performing 30 cycles per minute was applied to shift the stalks in two directions. Thus, the plants were stimulated from week 7 to 14 in intervals of two hours of stimulation followed by a period of four hours rest four times per day. Control plants were grown under the same conditions in parallel without mechanical stimulation.

2.3 Mechanical analysis of internodes by three-point bending tests

S. bicolor plants were harvested at the age of 14 weeks by cutting with a razor blade (Personna, Cedar Knolls, United States of America) slightly above the roots and in the centre of each node. The leaves were carefully removed. Length and diameter of the internodes were measured right after cutting by using a micrometre calliper. Diameters of the internodes were measured at six positions on each internode sample in order to calculate the average value. The second and the third internodes were examined since the first and fourth internodes were too short for the bending test. To avoid any effects caused by uncontrolled drying, freshly harvested specimens were bent immediately after harvesting. For testing the influence of the moisture content, additional bending tests were performed on dried internodes. In this case, internodes were dissected and prepared as described for the freshly harvested plants, whereby specimens were kept within a drying cabinet at 30 °C for 26 days prior to bending tests. For each *S. bicolor* line (S4, S10, S11) 14 samples per experiment (stimulated/non-stimulated) were analysed.

Three-point bending tests were performed with a universal testing machine Inspect table BLUE with 5 kN capacity (Hegewald & Peschke MTP, Nossen, Germany) (Figure 1 C). A constant displacement rate of 20 mm per minute and a maximal displacement range of 15 mm were used. The latter was limited to 15 mm by the dimensions of the test setup and the thickness of the sample (about 10 mm). The measured internodes displayed a length of at least 60 mm and a diameter of about 10 mm. To compare the different samples, the distance between the supporting blocks was set to 50 mm for all measurements.
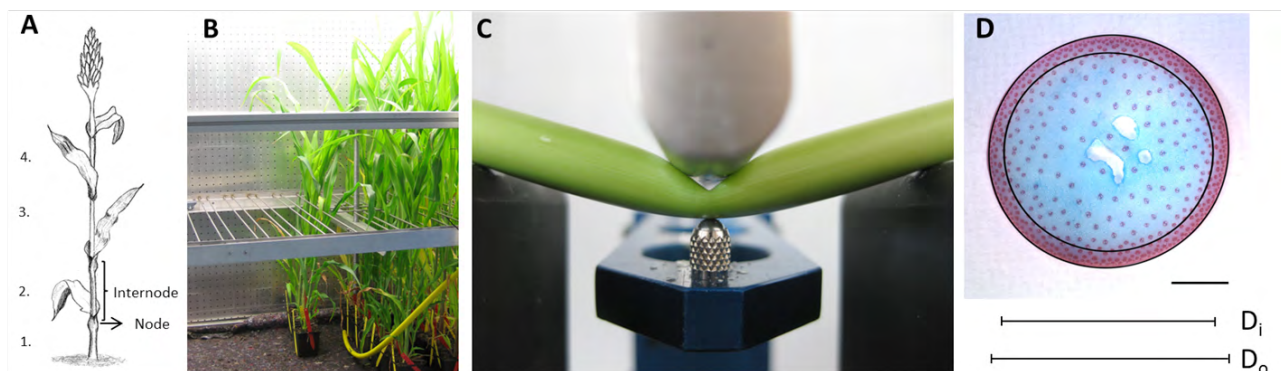

Figure 1: Experimental set-up. **(A)** schematic drawing of *S. bicolor*, **(B)** mechanical stimulation set-up within plant chamber, **(C)** three-point bending test of a *S. bicolor* internode, **(D)** thickness of the outer tissue ring was defined as d: d = D_o-D_i. Scale = 2mm

The required force and deflection were continuously recorded during the bending test. The software LabMaster (Hegewald & Peschke MPT GmbH, Nossen, Germany) was used to convert the force values in the bending stress for a solid cylinder geometry which is given in equation 2.1.

$$\sigma = \frac{8 \cdot F \cdot L}{\pi \cdot D_0^3}$$

2.1

F is the measured force, L the distance between the supporting blocks and D_0 the outer diameter of the sample. The resulting bending strain was calculated using the displacement Δl and the outer diameter D_0 as shown in equation 2.2.

$$\varepsilon = \frac{6 \cdot \Delta l \cdot D_0}{L^2} \cdot 100 \ \%$$

2.2

Within this study, the bending strength is defined as the maximum bending stress. The bending strength values for the second and third internodes were subjected to statistical analyses with respect to the mechanical properties as a function of *S. bicolor* lines, internode segments and mechanical stimulation. A *t*-test (alpha = 0.05) was performed to determine significant correlations using the software Origin 8 G. Due to the limited resolution of the strain measurement device, the bending test used for this study is not suited to measure the Young's modulus with a high accuracy. Nevertheless, we calculated the Young's modulus to determine if it is influenced by the mechanical stimulation of the plant stalks. Values from 0.05 to 0.25% strain were analysed.

2.4 Morphological analysis of stem internodes

The morphology of *S. bicolor* internodes was characterized by light microscopic investigation of thin sections. Thin sections of about 100-200 μm were manually prepared by using a razor blade, immediately washed with distilled water and stained for 5 min with a solution of Astrablue (0.5 g Astrablue in 100 ml of 2% aqueous tartaric acid solution). Afterwards, samples were washed two times with H_2O and a second staining using 1 g safranin in 100 ml deionized H_2O for 5 min was performed. The sections were then washed for 5 min with EtOH 70%, 5 min with EtOH/HCl (0.5 ml conc. HCl in 100 ml 70% EtOH) and additional 2 – 5 min with EtOH/HCl. A diamond wire saw (Well Diamantdrahtsägen GmbH, Mannheim, Germany) without water was used for thin slice preparation of dried internodes, as they could not be dissected with a razor blade due to their hardness. Dried samples were observed unstained. Light microscopy was performed using a stereomicroscope M165C (Leica Microsystems GmbH, Wetzlar, Germany) equipped with a 0.63x PlanApo objective. Photographs of microscope sections were recorded using an integrated camera and the Leica Application Suite V 3.8.0 software (Leica Microsystems GmbH, Wetzlar, Germany).

The geometry of the outer sclerenchymatous tissue ring was quantified using images of whole sections recorded with a Canon EOS 300 D digital camera. For this purpose, images were scaled and processed using the distance calculation tool of the Inkscape software (version 2). The total diameter of the internodes was defined as D_0 (outer diameter). The inner diameter (D_i) marking tissue interface between the intensely stained sclerenchymatous and the weakly stained parenchymatous tissue was determined based on morphology, cell size and colour intensity of staining. Thickness of the outer tissue ring was defined as d: $d = D_0 - D_i$ (see Figure 1D).

3 Results

3.1 Comparison of the mechanical properties of three different sorghum lines grown in an *in vivo* mechanical plant stimulation system and grown under non-stimulation conditions

We analysed whether three genetically distinct sorghum varieties, including two varieties with elevated lodging resistance in the field, namely S4 and S10, and one line with lower lodging resistance, namely S11, showed any differences in the mechanical strength properties of their stalk internodes. We hypothesized that perhaps the more lodging resistant lines S4 and S10 might show high mechanical stem strength, while the less stable line S11 might display lower mechanical stem strength. Towards this end, plants of all three lines were cultivated under controlled conditions in the growth chamber to the age of 14 weeks. This growth was termed non-stimulated condition since any significant mechanical perturbation did not take place inside the growth chamber. To obtain mechanical strength values under mechanical stimulation, one plant group from each line was in addition cultivated in the presence of an *in vivo* mechanical plant stimulation system. In this system, the plants were subjected to regular intervals of lateral movement by an in-built motorized set-up.

We found that non-stimulated plants, exhibited mechanical properties which were different between the three *S. bicolor* lines (S4, S10, S11). Representative

bending stress-strain curves for the second internode are given in Figure 2A. For non-stimulated S11 plants, a strain to failure of about 23 % was measured. Line S4 and line S10 exhibited higher strain to failure values, about 30 % and more than 35 %, respectively (Figure 2A). The bending strength (the maximum bending stress value) was 9.0 MPa for S4, 11.6 MPa for S10 and 6.7 MPa for S11 (Figure 2A,E). Although the bending stresses and strain to failure values differ between the three lines, the plants showed a similar bending behaviour. Very interestingly, it can be deduced from all curves that on small strains the stress increased linearly with strain. In this regime, the internodes most likely deformed elastically. At higher strains, the stress-strain behaviour was no more linear and stress increased only slightly with further increasing strain. It seems that, in this regime, the deformation could not be elastically accommodated and permanent deformation occurred. In addition, some of the bending stress-strain curves showed multiple stress drops prior total failure of the internode (Figure 2A). These stress drops might indicate events where some internal parts (fibres) of the internode broke and afterwards an internal structural rearrangement of fibres took place such that the stalk can sustain further loading.

Next, the bending stress-strain curves from the second internodes were compared between non-stimulated and mechanically stimulated plants. Figures 2B,C and D show stress-strain curves for the second internode of the lines S4, S10 and S11, respectively. Higher stress values were measured for all stimulated internodes in comparison to non-stimulated plants. The average bending strength for the stimulated second internode was 13.5 MPa (S4), 14.0 MPa (S10) and 13.4 MPa (S11). To further find support for the above results, we performed the same analysis for the third internode and compared the bending strength values for all lines and treatments of the second and third internodes (Figure 2E).

Compared to the non-stimulated (control) condition, we observed that the bending strength of the second and third internode of S4 increased significantly upon stimulation by 49.7 % and 71.7 %, respectively. Bending strength increased by 20.1 % (second internode) and 12.6 % (third internode) for S10, this was not found to be a significant increase. S10 internodes displayed a relatively high bending strength also for non-stimulated internodes in contrast to S4 and S11 non-stimulated internodes. Non-stimulated S11 plants had the lowest bending strength value between the three lines, however a similar value as S4 for the third internode, in the non-stimulated condition. Upon stimulation the bending strength increased by 98.6 % to 13.4 MPa for the second internode (significant difference from control). Bending strength of the third internode increased by 36.0 %. The bending strength values and their changes upon mechanical stimulation
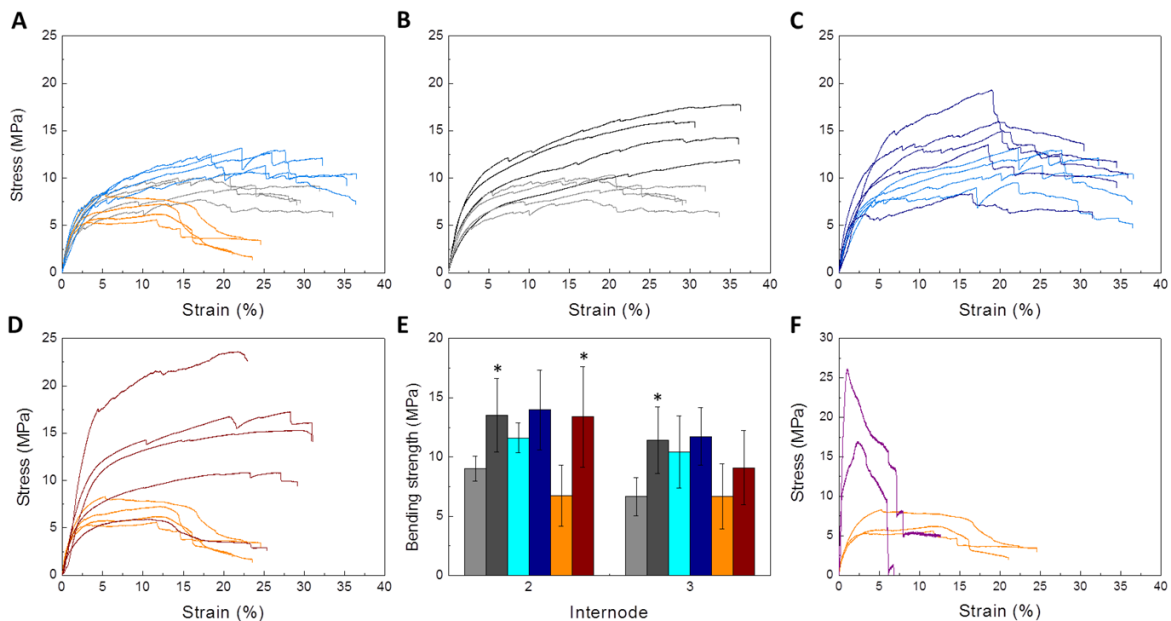

Figure 2: Results of three-point bending tests. **(A)** stress-strain diagram comparing the non-stimulated (control) second internode of S4 (grey), S10 (blue) and S11 (orange), **(B-D)** stress-strain diagrams for second internode of non-stimulated (control) (light colours) and mechanically stimulated (dark colours) S4 **(B)**, S10 **(C)** and S11 **(D)**, **(E)** bending strength (maximum bending stress) for second and third internode for non-stimulated (S4 light grey, S10 light blue, S11 orange) and stimulated (S4 dark grey, S10 dark blue, S11 red) plants, (significant differences are indicated by an asterisk), **(F)** stress-strain diagram for fresh (orange) and dried (purple) second internodes of S11.

were found to be comparable for the second and third internode in the three lines. Moreover, mechanical stimulation resulted either in no significant change or in a significant increase of the bending strength values.

To determine whether the water content of the stems affected the mechanical properties, we compared fresh and dried internodes. We observed that in the dried samples the stress-strain curves exhibited a much higher slope in the elastic regime and much higher maximum stresses were reached (about 55 MPa for S4, 65 MPa for S10 and 25 MPa for S11). However, in contrast to the fresh samples, the dried internodes demonstrated smaller strain to failure values, which were in the range of 5- 10 % for all lines (Figure 2F, shown for S11). The calculated Young's modulus was in the range of 0.35 (± 0.07) GPa for non-stimulated S4 internodes and about 0.44 (± 0.14) GPa for stimulated internodes. S10 internodes exhibited values of 0.47 (± 0.24) GPa (non-stimulated) and 0.45 (±0.18) GPa (stimulated). A Young's modulus of about 0.63 (± 0.17) GPa was calculated for non-stimulated S11 internodes and stimulated internodes displayed lower values, of about 0.46 (±0.26) GPa.

3.2 Comparison of the morphology of stem sections of three different sorghum lines grown in the stimulated and non-stimulated condition

The mechanical behaviour of the internodes must be a consequence of the composition and architecture of the stem. One explanation could be that the mechanical strength might be connected with the morphology of the internode. Hence, we hypothesized that the stem morphology should differ between the lines and between the stimulated versus non-stimulated condition. We therefore investigated the tissue composition of the stems by observing cross-sections from the internodes. Particular emphasis was placed on the distribution of parenchyma and sclerenchyma cells. In general, cross-sections of *S. bicolor* internodes showed closed collateral vascular bundles embedded in a matrix of parenchyma cells surrounded by a ring of sclerenchyma cells (Figure 3, 1D). Vascular bundles were scattered across the whole section whereby the cortex had a higher number of small bundles, while towards the mark of the internode, the vascular bundles increased in size and were spaced further apart. A central medullary cavity was observed in some cross-sections.

We noted that the internode sections differed in the thickness of the outer sclerenchymatous ring which we

designated as $d = D_0\text{-}D_i$ (see Figure 1D). We compared the d and D_0/d values between the different lines in the stimulated and non-stimulated conditions (Table 1). We found that in the non-stimulated condition S4 and S10 did not differ significantly in their d and D_0/d values, which were $d = 0.81$ mm and 0.98 mm and $D_0/d = 10.38$ and 9.7, respectively, while S11 had a lower d value of 0.67 mm, but a similar D_0/d value as the other two lines of 10.24. Upon the stimulated condition, all three lines displayed similar values for d, namely for S4, S10 and S11 $d = 1.03$ mm, 1.01 mm and 0.99 mm, respectively. However very interestingly, these d values were increased in all three lines (27.2 % for S4, 3.1 % for S10) in the stimulated condition versus the non-stimulated condition, and this increase was significant for the line S11 (47.8 %). D_0/d ratio decreased for all stimulated plants, for S4 and S10 a decrease of 12.4 % and 5.1 % was observed. This effect was strongest for S11 with a decrease of about 22.2 % (Table 1).

Cross-sections of the dried internodes showed a reduced diameter, which was expected due to the water loss (Figure 3). Parenchyma cell tissue displayed a stronger shrinkage than the outer sclerenchyma tissue which can be explained by the increased strength of sclerenchyma irrespective of water turgor. Taken together, stem internode morphology differed between the lines and the stimulation condition. Hence we can deduce that, likewise for the mechanical properties, the morphology is under genetic and environmental control. An increased d value and a decreased D_0/d value seem to correlate with increased mechanical strength.

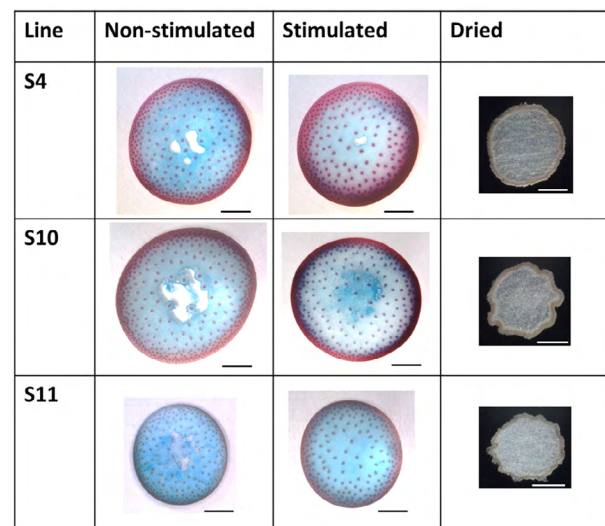| Line	Non-stimulated	Stimulated	Dried
S4			
S10			
S11			

Figure 3: Tissue distribution in cross-sections of *S. bicolor* stalks (second internode). Transmitted light microscope images of thin sections of the internodes of non-stimulated (control), stimulated and dried plants of the lines S4, S10 and S11. Scale = 2 mm

Table 1: Tissue distribution in cross-sections. Estimated values for thickness of the outer tissue ring (d) (d = D_0-D_i) and proportion of outer diameter (D_0) to outer tissue ring (d) (values for second and third internodes are combined).

Line	Non-stimulated (control)		Stimulated	
	Outer tissue ring d (mm)	D_0/d	Outer tissue ring d (mm)	D_0/d
S4	0.81 ± 0.18	10.38 ± 0.27	1.03 ± 0.13	9.09 ± 0.20
S10	0.98 ± 0.19	9.70 ± 0.29	1.01 ± 0.17	9.21± 0.22
S11	0.67 ± 0.13	10.24 ± 0.29	0.99 ± 0.17	7.97 ± 0.27

4 Discussion

In this study we presented an analysis of bending strength properties and morphological characteristics for three genetically distinct *S. bicolor* varieties in response to mechanical stimulation. Our analysis revealed the following points: first, the mechanical and morphological assays we developed here were well suited for quantitative measurements to obtain statistically solid data for comparison studies. Second, the mechanical analysis of internodes showed that relevant differences were found between the three genetically distinct lines and between the stimulated and non-stimulated condition. Thus, the mechanical behaviour of stems is predetermined by genetic factors, but then can be adjusted in response to environmental constraints. Third, the morphological analysis was found to be regulated conform to the mechanical properties. Very importantly, two morphological properties, an increased d value and a decreased D_0/d value were observed for internodes with an increase in mechanical strength.

The more unstable line S11 showed partly lower mechanical strength compared to the stable lines S4 and S10, which was conform to our hypothesis. However, surprisingly, even the unstable S11 line could increase mechanical strength upon stimulation to values similar as S4 and S10. Thus, the mechanical characteristics of the stem are affected by genetic and environmental factors, and these stem properties can be quantified in physical tests. Stress-strain curves revealed an elastic deformation of internodes followed by permanent deformation with increasing strain. Interestingly, the strain to failure values of internodes derived from stimulated plants that showed higher bending strength did not differ from non-stimulated ones. An increase of bending strength for mechanically stimulated plants was observed for all selected plants while this effect was strongest for

S11 whose non-stimulated plants displayed the lowest bending strength. After stimulation, the bending strengths were quite similar for the different plant lines, especially for the second internode (13.4-14.0 MPa). Until now, for *S. bicolor* only deflection characteristics after grain maturity have been described by Mittal *et al.* [19]. They found that the bottom sections of sorghum stalk had higher tensile and compressive strenghts than the upper sections. In agreement with this study, we found slightly higher bending strength values for the second internode compared to the third one.

A highly hierarchical material was analysed. Comparing different methods, the stress-strain curves together with the bending strength (maximum bending stress) have been selected as the best parameters to compare the material properties of plant stalks analyzed by three-point bending test. The Young's modulus would also be a useful measure to compare the different plants. However, the bending test used for this study is - due to the limited resolution of the strain measurement device - not suited to measure the Young's modulus with a high accuracy. The calculated Young's moduli values displayed high standard deviations and did not correlate with bending strength or morphological properties.

In response to a mechanical stimulation, an increase of strengthening and supporting tissue (like sclerenchyma cells) was observed for all selected lines of *S. bicolor*. High bending strengths were especially observed for low D_0/d ratios. This indicates that *S. bicolor* may enhance its bending properties by increasing the thickness of the outer sclerenchyma cell tissue relative to the stalk cross-section. A certain sclerenchymatous tissue thickness was characteristic for certain bending properties, especially after stimulation. This indicates that this is a shared adaptation to the applied mechanical stimulation. The mechanical behaviour of stems is therefore predetermined by genetic factors adjusted in response to environmental

stress. Morphological changes in response to mechanical perturbation (thigmomorphogenesis) were also described for other plants. *Pinus taeda* responds with a reduction of extension growth, a decreased flexibility and an increased elasticity [22]. Hepworth and Vincent measured an increased bending stiffness for tobacco stems with a thicker xylem tissue cylinder after flexural stimulation [23].

Mechanical properties of plants are determined on different hierarchical levels and by different tissues, their interfaces and moisture content. In the present study, dried internodes reached higher bending strengths but exhibited much lower strain to failure values. A similar effect (decreased bending strength and Young's modulus with increase in moisture content) was previously reported for wheat straws [24]. This emphasises the important role of moisture content for bending properties. Water may be important for stretching and sliding at different hierarchical levels. In this study, a solid cylinder sample geometry was selected to give consideration to all tissues of the plant stalk, their interfaces and the impact of moisture content. This is supported by the assumption that the outer shell of the cylinder is supported by a low-density cellular core [9,10]. Assuming that the outer sclerenchymatous tissue ring is mostly responsible for the mechanical properties, a tube-like geometry can be calculated alternatively (see Appendix).

Bending properties, influence of mechanical stimulation, morphological characteristics and moisture content should be considered when plant lines are selected for agricultural and technical requirements. The presented experimental set-up evaluated the influence of mechanical stimulation on bending properties and morphology and is suitable for a systematic approach. Beside the morphological alterations presented here, especially the influence of mechanical stimulation on a genetic level would be worth studying. So far, little is known about mechanosensors and gene expression related to thigmomorphogenesis [14,25]. Since genome information is available [3], it is promising to identify genes that correlate with certain mechanical properties in *S. bicolor*. So far, detailed molecular biology studies have been successfully performed for genes in rice [26].

We suggest that the established laboratory growth system is applicable to screening of plant lines with increased mechanical strength and improved responsiveness to mechanical stress. Hence, our study is a highly valuable contribution to developing new techniques for producing high biomass crops and selecting crops with improved material characteristics. Furthermore, the presented approach will allow further insight into structure-property relationships in plant stalks.

5 Conclusion

The effect of mechanical stimulation on morphology and bending properties of a biological material with a highly hierarchical structure was analysed. Three genetically distinct *S. bicolor* varieties showed differences in their general bending strength in the non-stimulated condition. Similar high range of bending strength values were measured for all plant lines after they underwent the mechanical stimulation procedure. Thus, the mechanical behaviour of stems can be adjusted in response to stimulation. An increased thickness of the outer sclerenchymatous tissue was observed for internodes with higher bending strength values.

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Appendix

Assuming that the outer sclerenchymatous tissue ring is mostly responsible for the mechanical properties, bending stress (σ) for a tube-like geometry can be calculated alternatively by:

$$\sigma = \frac{8 \cdot F \cdot L \cdot a}{\pi \cdot d^3 \cdot (a^4 - (a-1)^4)}$$

1.1

Where F is the measured force, L the distance between the supporting blocks, $d = (D_0 - D_i)$ the diameter of the sclerenchymatous tissue ring and a the ratio D_0/d. The resulting bending strain is calculated using the displacement Δl and the outer diameter D_0 as shown in equation 1.2.

$$\varepsilon = \frac{6 \cdot \Delta l \cdot D_0}{L} \cdot 100\,\%$$

1.2

In this case, bending stress and strength are almost between two and three times higher than the values calculated for a solid cylinder. The mathematical explanation for this difference is given in equation 1.3.

$$\sigma_{tube} = \frac{D_0^4}{D_0^4 - D_i^4} \cdot \sigma_{cylinder}$$

1.3

σ_{tube} is the bending stress for a tube-like geometry and $\sigma_{cylinder}$ for a solid cylinder. The proportionality factor which gives the ratio between both bending stresses can be calculated using the outer diameter D_0 and the inner diameter D_i. For example, the mean value of the proportionality factor for S4 reference is 3.06 ± 0.18 and for stimulated S4 2.75 ± 0.18 (bending stress-strain curve for S4 assuming a tube-like geometry is given as supplemental file S1).

Supplemental data
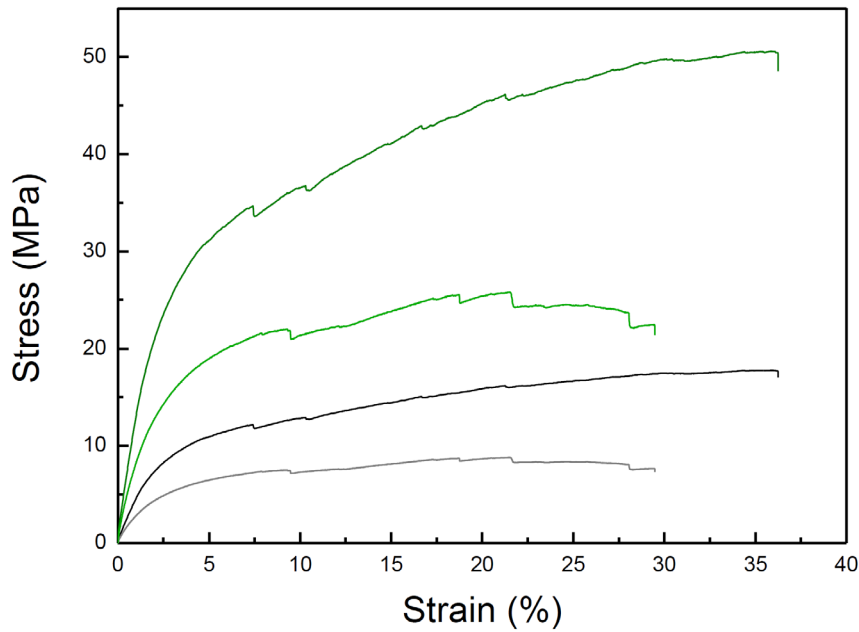
S1: Stress-strain diagram for S4 second internode. Assuming solid cylinder sample geometry: non-stimulated (grey) and stimulated (black); assuming a tube-like geometry: non-stimulated (light green) and stimulated (dark green)