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ORIGINAL RESEARCH

Survey of wheat straw stem characteristics for enhanced resistance to lodging

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Abstract Lodging is one of the major constraints that threaten crop productivity. Although the relationship between cell walls and straw strength has been well recognized, little relevant research has been done in wheat, particularly on the monomer composition and structural characteristics of cell wall polymers and the arrangement of vascular bundles. In this study, we systematically investigated cell wall- and straw-related traits in a range of wheat germplasm resources and culm mutants using a high-throughput platform for cell wall analysis. We found that varieties with higher breaking force exhibited higher levels of crystalline cellulose but fewer hemicellulose components than other varieties. The lignin content was not consistent with the breaking force; instead, the lignin monomer constitution might be important because a significantly higher proportion of *p*-hydroxyphenyl (H) and guaiacyl (G) but a lower proportion of syringyl (S) monomers of lignin was found in the higher breaking force group. The crystallinity detected by X-ray diffraction was positively correlated with breaking force, indicating that the physical/chemical properties of polysaccharides also deserve attention. In terms of anatomical characteristics, the varieties with higher breaking force had a lower number and area of smaller vascular bundles in the peripheral sclerenchyma than other varieties. These results, together with the finding of a highly significant correlation between stem breaking force and straw fresh weight, 2nd internode width, flag leaf width and SiO_2 content, should provide systematic information for breeding for lodging resistance.

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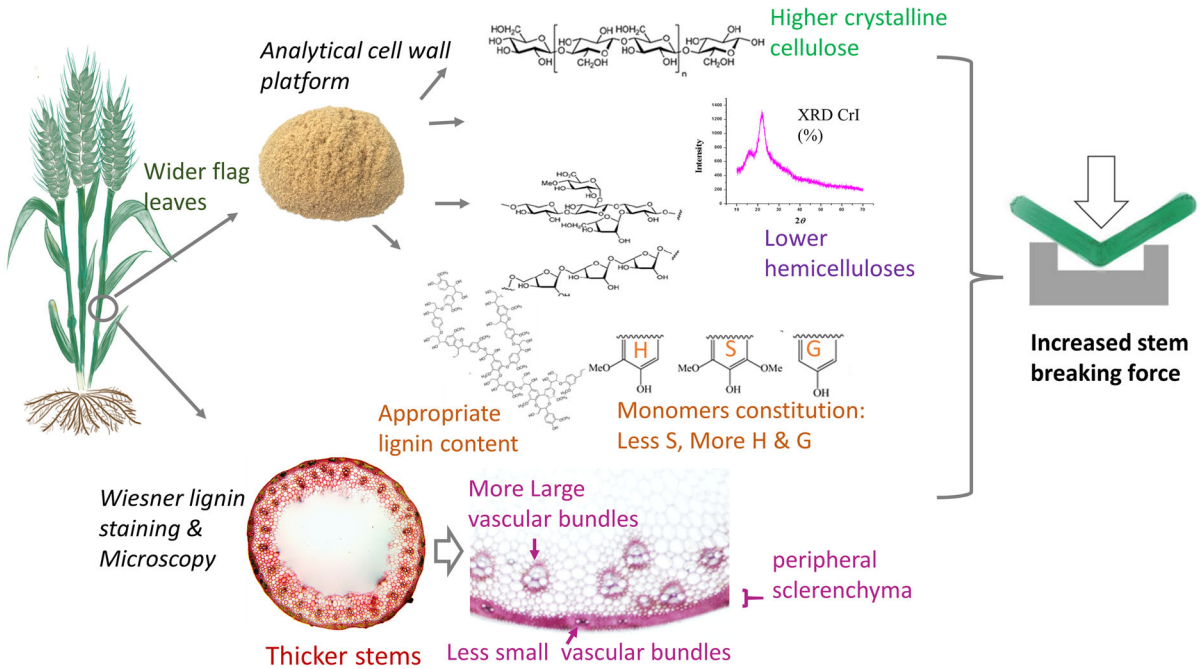
Graphic abstract



Keywords Wheat · Lodging · Cell wall · Cellulose · Hemicellulose · Lignin

Introduction

Lodging is defined as the permanent displacement of plant shoots from an upright position due to internal and external factors (Berry et al. 2007). Crop lodging reduces grain yield and deteriorates grain quality (Berry and Spink 2012; Rutto et al. 2013; Setter et al. 1997). Wheat (*Triticum aestivum* L) is a major staple food crop worldwide, accounting for 20% of the caloric intake by humans (Zhang et al. 2018). In wheat, up to 80% yield loss was reported due to lodging in the most serious cases (Acreche and Slafer 2011; Berry et al. 2007). The severity of lodging depends upon both plant characteristics and many environmental factors. Genetic improvement of the stem or root properties provides one promising strategy for lodging resistance (Hai et al. 2005; Huang et al. 2006; Kashiwagi and Ishimaru 2004). However, lodging in crops is a complex event and is typically inherited quantitatively. The first problem confronted by researchers is to determine which trait or which group of traits are reliable as an index of lodging resistance during genetic studies or breeding selection. In other words, it is important to effectively assess the risks of lodging and the association between morphological characteristics, stem features, and stem strength and lodging resistance.

In the field, lodging was measured by the percent decrease in plant or canopy height relative to those of erect plants and the degree of lodging is also measured by the angle of inclination. This method is intuitive, simple and direct, but it is not very accurate and is not suitable for large-scale screening (Chauhan et al. 2020). Another direct parameter is the pushing force, which is the stem strength of plants measured using an instrument such as a prostrate tester when a plant has been bent at a certain angle (Hai et al. 2005; Kashiwagi and Ishimaru 2004). The instrument is easy to operate and can thus be used for a large population. However, this is a complex trait affected by many factors and this method has relatively high rates of operating errors, which necessitates conjoint analysis with other lodging-associated traits to obtain a comprehensive understanding of lodging resistance with genetic studies (Hai et al. 2005; Huang et al. 2006; Kashiwagi and Ishimaru 2004).

Since lodging is also considered to result from the imbalance between the weight of the upper plant parts and the sturdiness of the basal parts, a lodging index (LI) was proposed to measure the lodging susceptibility of the lower culm internodes (Crook and Ennos 1994; Fan et al. 2017; Islam et al. 2007; Li et al. 2017). The LI has been defined by calculating the moment of bending divided by the breaking resistance (calculated as plant height × plant weight × 100/breaking resistance) according to Amano et al. (1993), among several proposed formulas. The LI combined the effects of major internal forces of plants and was widely used. However, the LI also represents a complex phenotype determined by many traits. In addition, the three components in the formula are not always independent of each other. As a result, we often observed that long stemmed varieties did not tend to lodge because they have very thickened cell walls. On the other hand, higher stem weight is not always a negative factor, as many studies have validated that plants with higher stem weight are often associated with increased stem/internode diameters and thicker stem walls (Dong et al. 2003; Xiao et al. 2002).

Many studies have indicated that stem-related traits, such as basal internode length and thickness, stem wall thickness, leaf sheath covering and thickness, contribute to culm strength and lodging resistance (Islam et al. 2007; Zhu et al. 2008). Consequently, efforts to understand the mechanism of lodging and to improve stem strength and its related components should be an important focus in future wheat breeding for lodging resistance. Moreover, stem strength is a complex trait including the mechanical elasticity and rigidity of the stem and is closely associated with stem morphological, chemical and anatomical traits (Kong et al. 2013; Yao et al. 2011). Plant cell wall polymers have significant impacts on stem strength and rigidity, hence regulating stalk leaning (Li et al. 2014a; Ma 2009; McFarlane et al. 2014). Cellulose microfibrils, formed by hydrogen bonding of β-1,4-linked glucan chains, provide the main source of tensile strength to the cell wall, contributing markedly to stem physical strength, and are critical for plant growth (McFarlane et al. 2014). Lignin has also been considered the main factor responsible for the varietal differences in bending stress related to lodging resistance. Several studies found a positive association between the lignin content and breaking strength of stems in several wheat varieties (Kong et al. 2013; Peng et al. 2014). It was also substantiated that the improved accretion of hemicellulose and lignin contents together affected the stem breaking resistance (Berry et al. 2004).

Although the plant cell wall composition and features were proposed to affect plant mechanical strength in rice, little is known about the impacts of these parameters on lodging resistance in wheat. Furthermore, despite the potential role of these cell wall components on stem strength, the mechanisms of their interaction have not been fully elucidated. Although plants have similar levels of wall polymers, the polymer features such as cellulose crystallinity, hemicellulosic monosaccharides, and lignin monomer constitution can have major effects on the properties of the lignocellulosic biomass (Park et al. 2010; Wu et al. 2013; Zhang et al. 2013). However, to our knowledge, few studies have investigated the effects of the structural features or constitution of polymers on stem strength.

This study aimed to improve the understanding of the morphological, cell wall composition, lignocellulose and anatomical features in wheat varieties influencing their stem breaking resistance. With a high-throughput platform for cell wall analysis and the X-ray diffraction (XRD) method, the monomer constitution and the internal structural features of cell wall polymers were also investigated in this study. In addition, one goal of this study was the exploratory testing of cell wall-related traits and thus we examined possible key parameters for the study of lodging resistance.

Materials and methods

Plant materials

A total of 31 wheat germplasm accessions from the collection at the Hubei Agricultural Science Institute in Hubei Province, China, which represent a wheat gene pool adapted to central China and the Yangzi River region, were planted at the experimental farm at Hanchuan (HC) and Wuhan (WH) in the growing seasons of 2013 and 2014, respectively. Twenty individuals from each variety (line) were grown in two rows with a distance of 15 cm between plants in each row and 20 cm between rows. Field management essentially followed normal local wheat cropping

practices. The lines were harvested individually at maturity to prevent seed contamination among lines. Six wheat homozygous mutants were also planted together with two corresponding wild-type cultivars Zhengmai 9023 and E'mai 596 in the fields. The six mutants were selected from a project with a large-scale screening of culm mutants using the mutagenesis pools of genome-wide chemical EMS inductions with more than 50,000 individual wheat lines and then underwent homozygosis with several generations of multiplications.

The measurement of agronomic traits, stem-related traits and breaking force

We evaluated wheat germplasm collections for important agronomic and stem-related traits, including plant height, culm fresh weight, internode length, internode diameter, flag leaf length, flag leaf width, spike length, spike weight, tillers per plant and thousand kernel weight, in addition to mechanical traits and the breaking force, across 2013 and 2014 (Table 1). The length from the base of the culm to the apex of the panicle represented the plant height. The breaking force (BF) was measured using a prostrate tester (DIK 7400, Japan) with the distance between the two fulcra set to 5 cm. The breaking site was arranged at the center of the second internode and the middle point between the two fulcra. The lodging resistance of the second internode was measured as described previously (Islam et al. 2007).

Powder sampling and cell wall extraction

The mature stem tissues were collected and dried at 50 °C after inactivation at 105 °C for 10 min. Approximately 60–70 mg of dried plant material was ground in a 2 ml screw cap tube using a grinding and dispensing robot (Santoro et al. 2010). After grinding and dispensing, 1.5 ml of 70% aqueous ethanol was added, and the samples were vortexed. The suspension was centrifuged at 10,000 rpm for 10 min to pellet the alcohol-insoluble residue. Chloroform/methanol (1.5 ml of 1:1, v/v) was used to resuspend the pellet. The suspension was centrifuged at 10,000 rpm for 10 min, and the supernatant was aspirated. The pellet was resuspended in 500 µl of acetone, and then the solvent was evaporated with a stream of air at 35 °C to dry the sample. The dry pellet

Table 1 Stem-related traits of wheat genotypes and their correlation with the breaking force

Trait	Year 2013					Year 2014				
	Mean	Maximum	Minimum	CV %	Correlation with the breaking force	Mean	Maximum	Minimum	CV %	Correlation with the breaking force
Plant height (cm)	80.67 ± 11.75	112.96	59.52	14.57	0.165	79.18 ± 12.02	114.03	61.03	15.18	0.043
Fresh weight (g/culm)	14.11 ± 2.35	20.48	10.16	16.65	0.437*	16.00 ± 3.52	26.53	10.63	22.00	0.409*
2nd internode length (cm)	7.04 ± 1.70	11.78	4.48	24.15	0.074	6.31 ± 1.4	9.87	4.07	22.19	0.143
2nd internode width (cm)	4.49 ± 0.46	5.77	3.43	10.24	0.374*	4.60 ± 0.5	5.86	3.81	10.87	.587**
Flag leaf length (cm)	24.27 ± 3.18	30.24	18.62	13.10	0.09	21.75 ± 3.4	28.03	14.50	15.63	0.326
Flag leaf width (cm)	1.99 ± 0.42	3.93	1.51	21.11	0.445*	2.08 ± 0.23	2.93	1.67	11.06	0.422*
Spike length (cm)	10.36 ± 0.99	12.03	8.35	9.56	0.289	10.69 ± 1.24	13.63	8.63	11.60	0.198
Spike weight (g)	2.61 ± 0.55	3.59	1.63	21.03	0.314	2.60 ± 0.43	3.38	1.83	16.36	− 0.210
Tillers per plant	11.56 ± 0.79	13.00	9.60	6.87	− 0.182	12.06 ± 1.29	14.33	8.89	10.66	− 0.257
Thousand kernel weight (g)	42.18 ± 3.48	48.75	32.64	8.26	0.239	44.88 ± 2.81	51.73	38.35	6.27	− 0.130
Breaking force (N)	14.04 ± 2.86	20.01	9.47	20.37		15.65 ± 3.28	24.83	9.80	20.96	

*,** indicate significant correlations at $p < 0.05$ and 0.01, respectively

was resuspended in 1.5 ml of 0.1 M sodium acetate buffer (pH 5.0) and the supernatant was heated for 20 min at 80 °C in a heating block and cooled on ice. After cooling, 35 µl of 0.01% sodium azide (NaN_3), 35 µl of amylase (50 µg/mL in H_2O; from *Bacillus* species, Sigma) and 17 µl of pullulanase (17.8 units from *Bacillus acidopullulyticus*, Sigma) were added to remove starch. The suspension was incubated for 16 h at 37 °C in a shaker. After incubation, the suspension was heated at 100 °C for 10 min in a heating block to terminate the digestion. The mixture was centrifuged (10,000 rpm, 10 min), and the supernatant containing the solubilized starch was discarded. The remaining pellet was washed three times with 1.5 ml of water by centrifugation. The pellet was resuspended in acetone, and then the solvent was evaporated with a stream of air at 35 °C until dry. The dried material represents isolated cell walls.

Crystalline cellulose content assay

The crystalline cellulose content assay was performed as described by (Foster et al. 2010b) on the basis of the Updegraff (1969) method. First, 1 mL of Updegraff reagent [acetic acid: nitric acid: water (8:1:2, v/v/v)] was added to dissolve the TFA pellet. Then, the samples were heated at 100 °C for 30 min, cooled to room temperature, and centrifuged at 10,000 × g for 15 min. The supernatant was discarded, and the pellet was washed once with 1.5 mL of water and 3 additional times using 1.5 ml of acetone, and then air-dried. Then, 175 µl of 72% sulfuric acid was added, and the sample was incubated at room temperature for 30 min to hydrolyze the crystalline cellulose. Finally, the glucose content was quantified using the colorimetric anthrone assay as follows: 10 µl of each sample was added to a 96-well polystyrene microtiter plate with 90 µl of water and 200 µl of anthrone reagent (2 mg anthrone mL^{-1} concentrated H_2SO_4). A standard curve for glucose (0 µg, 2 µg, 4 µg, 6 µg, 8 µg, and 10 µg) was also added to each plate. The plate was heated at 80 °C for 30 min and allowed to cool down, and the absorption was read at 620 nm using a Tecan Sunrise microplate. The glucose content was calculated based on the absorbance compared to the standard curve on the same plate.

Matrix polysaccharide composition assay for hemicellulosic monosaccharides

Cell wall material (2 mg) was mixed with 20 µl of inositol solution (5 mg/ml) as an internal standard in a 2 ml Starstedt tube. After adding 250 µl of 2 M trifluoroacetic acid (TFA), the tube was incubated for 90 min at 121 °C for weak acid hydrolysis and cooled to room temperature. After that, the sample was set on ice and then centrifuged at 10,000 rpm for 10 min. Then, 100 µl of the acidic supernatant containing the matrix polysaccharide-derived monosaccharide was transferred to a glass screw-capped vial. The pellet was used for the crystalline cellulose assay below. The TFA was evaporated gently in an evaporation device. The pellet was rinsed with 300 µl of isopropanol three times. Then, 50 µl of acetic anhydride and 50 µl of pyridine were added to reduce the monosaccharides to their corresponding alditols. Then, 500 µl of ethyl acetate and 2 ml of water were added to extract the alditol acetates. A clear ethyl acetate layer (top layer) was obtained. Fifty microliters of the ethyl acetate layer was pipetted into GC/MS vials with inserts and diluted by adding 100 µl of acetone. The samples were injected into a GC equipped with a quadrupole MS. Peaks were identified by their mass profiles and/or the retention times of standards. Monosaccharides were quantified based on standard curves.

Acetyl bromide lignin assay

The acetyl bromide lignin (AcBrL) content was quantified using the Foster et al. (2010a) acetyl bromide method, based on the method reported by (Fukushima and Hatfield 2001). A volume of 1–1.5 mg of prepared cell wall material was transferred into a 2 ml volumetric flask, leaving one tube empty as a blank. The cell wall material was concentrated to the bottom of the tube by rinsing tube walls with 250 µl of acetone, and the acetone was evaporated under very gentle airflow. Then, 100 µl of freshly made acetyl bromide solution (25% v/v acetyl bromide in glacial acetic acid) was added carefully. Samples were incubated at 50 °C for 2 h. After incubation, the suspension was heated for an additional hour with vortexing every 15 min and then cooled on ice to room temperature. Then, 400 µl of 2 M sodium hydroxide and 70 µl of freshly prepared 0.5 M hydroxylamine hydrochloride were added to a

volumetric flask and vortexed. The volumetric flask was filled with 2.0 ml of glacial acetic acid and then capped and inverted several times to mix these solutions. Two hundred microliters of the solution was pipetted into a UV-specific 96-well plate, and the absorption was read using a Shimadzu UV-1800 spectrophotometer at 280 nm. The percentage of acetyl bromide soluble lignin (% ABSL) was determined using the following formula: (*Absorbance* × *total volume* × 100%) ÷ (*coefficient* × *path length* × *weight*). For grasses, a coefficient of 17.75 is used.

Lignin composition assay

The cell wall material (2 mg) was transferred into a screw-capped glass tube for thioacidolysis. First, 175 µl of dioxane, 20 µl of 10% ethanethiol (EtSH) and 5 µl of 2.5% boron trifluoride diethyl etherate (BF_3) were added to each sample, the vial headspace was purged with nitrogen gas, and the vials were capped immediately. The suspension was heated at 100 °C for 4 h with gentle mixing every hour, followed by cooling on ice for 5 min. Sodium bicarbonate (150 µl of 0.4 M) was added to the sample and vortexed. One ml of water and 0.5 ml of ethyl acetate were added and vortexed. The top (ethyl acetate) layer (150 µl) was transferred to a 2 ml tube. The solvent was evaporated by a concentrator with air. Then, 200 µl acetone was added and evaporated twice. For the TMS derivatization, 500 µl of ethyl acetate, 20 µl of pyridine, and 100 µl of N, O-bis (trimethylsilyl) acetamide were added together, and the mixture was incubated for 2 h at 25 °C. One hundred microliters of the reaction was transferred to a GC/MS vial, and 100 µl of acetone was added. The sample was analyzed by GC equipped with a quadrupole mass spectrometer or flame ionization detector in addition to an Agilent HP-5 MS column (30 mm × 0.25 mm × 0.25 µm film thickness). The following temperature gradient was used with a 30 min solvent delay and a 1.1 ml/min flow rate: the temperature was initially held at 130 °C for 3 min, a 3 °C/min ramp up was made to a 250 °C, which was held for 1 min, and equilibration back to the initial temperature of 130 °C was allowed. Peaks were identified by characteristic mass spectrum ions of 299 m/z, 269 m/z, and 239 m/z for S, G, and H monomers, respectively. The composition of the lignin components was quantified by setting the total peak area to 100%.

Detection of cellulose crystallinity (CrI)

CrI was detected using the X-ray diffraction (XRD) method (Rigaku-D/MAX instrument, Uitima III, Japan) as described by (Segal et al. 1959). The raw biomass powder of randomly selected 25 wheat genotypes (Table S1 in the supporting documents) was laid on a glass sample holder (35 × 50 × 5 mm) and detected under plateau conditions. Ni-filtered Cu Kα radiation (λ = 0.154056 nm) was generated at a voltage of 40 kV and a current of 18 mA and scanned at a speed of 0.0197°/s from 10° to 45°. The crystallinity index (CrI) was estimated from the X-ray diffraction patterns using the intensity of the 200 peak (I_{200}, $\theta = 22.5°$) and the intensity at the minimum between the 200 and 110 peaks (I_{am}, $\theta = 18.5°$) as follows: $CrI = 100 \times (I_{200} - I_{am})/I_{200}$ (Figure S1 in the supporting documents). I_{200} represents both crystalline and amorphous materials, while I_{am} represents amorphous material. The standard error of the CrI method was detected at $\pm$ 0.05 $\sim$ 0.15 using five representative samples in triplicate.

Silica assay

Silica content was measured as described by (Sun et al. 2017; Zhu and Lin 1990).The dried biomass tissues were ground through 80 mesh screens, and digested for 30 min in a mixture of NaClO (AR) and 2 mol L^{-1} NaOH. The samples were then mixed with 1 mol L^{-1} H_2SO_4, 5% ammonium molybdate, 5% oxalic acid, and 0.5% ascorbic acid, and silicon content was photometrically measured under reading at a wavelength of 810 nm. All experiments were carried out in triplicate.

Microscopy of anatomical features

For analysis of anatomical features, the stems were collected at the heading stage. Basal stem transverse Sects. (100 µm thick) were obtained using a vibratome (Leica Microsystems Inc., Buffalo Grove, IL). For Wiesner lignin staining, sections were incubated for 3 min in phloroglucinol-HCl and rinsed with water before being observed with a microscope (Olympus BX-61).

Statistical analysis

Analysis of variance (ANOVA) was performed for all traits using Statistical Product and Service Solution 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The figures were drawn using Origin software. The significance of each source was determined by *t* test. Correlative analysis was performed using Spearman's rank correlation analysis at the two-sided 0.05 level of significance (*$p < 0.05$, **$p < 0.01$). These analyses used the average values calculated from all original determinations for a given trait pair.

Results

Correlation analysis of stem-related traits in wheat germplasms

In the present study, we evaluated wheat germplasm collections for important agronomic traits, including plant height, culm fresh weight, internode length, internode diameter, flag leaf length, flag leaf width, spike length, spike weight, tillers per plant and thousand kernel weight. In addition to these traits, the mechanical traits and breaking force of wheat straw across 2 years were also evaluated (Table 1). Consequently, the two-year data (Hanchuan (HC) 2013 and Wuhan (WH) 2014) revealed conflicting results for all stem-related traits except the internode diameter at $p < 0.01$.

Significant phenotypic variation was detected in the stem-related traits among accessions with coefficients of variation (CVs) ranging from 6.87 to 24.15% in 2013 in HC and 6.27 to 22.19% in 2014 in WH (Table 1).

Plant height showed a strong positive correlation with 2nd internode length (r = 0.640**) and spike length (r = 0.462**), whereas culm fresh weight had no or a less significant correlation with the above length-related traits but a significant correlation with 2nd internode width (r = 0.602**) and flag leaf width (r = 0.613**). Interestingly, 2nd internode width was positively correlated with flag leaf width (r = 0.613**), which are two different but closely related traits. Both the width and length of the flag leaf affect the length and weight of the spike, as indicated by the correlation coefficients among these traits (r = 0.241 to 0.467**). Furthermore, the thousand kernel weights were significantly increased with the flag leaf width (r = 0.404*), spike length (r = 0.403*) and spike weight (0.415*), while tillers per plant had a strong negative correlation with flag leaf width (− 0.521**) and spike length (− 0.444**). It should be noted that culm fresh weight, culm width, flag leaf length, flag leaf width, spike length and spike weight were all positively correlated (Table S2 in the supporting documents).

The physical strength of the wheat stem and its correlation with stem-related traits

In addition to being used for greater yield and better future breeding programs, improving the physical strength of the wheat stem is a good means by which to reduce lodging. The breaking force is considered an important physical property to evaluate stalk strength, whereas the second basal internode is thought to play a critical role in the physical strength of wheat as it relates to the incidence of lodging. Substantial genotypic differences were observed for the breaking force of wheat stems in both seasons. The breaking force ranged from 9.47 to 20.01 N in 2013 in HC and 9.80 to 24.83 N in 2014 in WH with CVs of 20.37% and 20.96%, respectively (Table 1). It is clear from the breaking force results that the selected materials possessed a high genetic diversity for the studied traits.

The relationship of mechanical properties with multiple stem-related traits is of great interest. Here, it was found that breaking force was positively associated with fresh weight, 2nd internode width, and flag leaf width but non-significantly correlated with plant height, 2nd internode length and spike length in both years (Table 1). Because culm fresh weight and flag leaf width contribute largely to the wheat grain yield and biomass yield, the results of the correlation analysis suggested that mechanical properties can potentially be improved without impaired grain and biomass yields. Moreover, these results indicated that fresh weight, culm thickness, and flag leaf width may be potential indicators for both high yield and lodging resistance breeding in wheat.

The relationship between breaking force and cell wall composition

The chemical structure of the stem plays an important role in stem strength. To disentangle the effects of

stem chemical components from those of morphological traits, two divergent groups, group L (low breaking force) and H (high breaking force), were formed based on their different breaking strengths. Both groups differed greatly in terms of breaking force, showing diversity in the groups, with a mean breaking force of 11.73 N and 11.07 N for 2013 in HC and 2014 in WH in group L, and 17.01 N and 18.09 N for group H, respectively (Fig. 1a). The relationship between stem mechanical properties and the structural components of the stem was also resolved by the correlation analysis (Table S3 in the supporting documents).

The effects of lignin content and H, S, G constitution on breaking force

The lignin content of group L ranged from 22.79 to 24.81%, with a mean value of 23.69%, while that of group H varied from 23.35 to 24.66%, with a mean value of 24.16%. Group H exceeded group L by 1.98% in total lignin content, although the difference was not statistically significant (Table S4 in the supporting documents). Interestingly, the levels of lignin monomers (H, S, and G) were dramatically different. The average amounts of H, S, and G in group L were 2.08, 41.65, and 23.47 μg/mg, respectively. A marked reduction of 11.51 μg/mg (by 27.64%) in the average

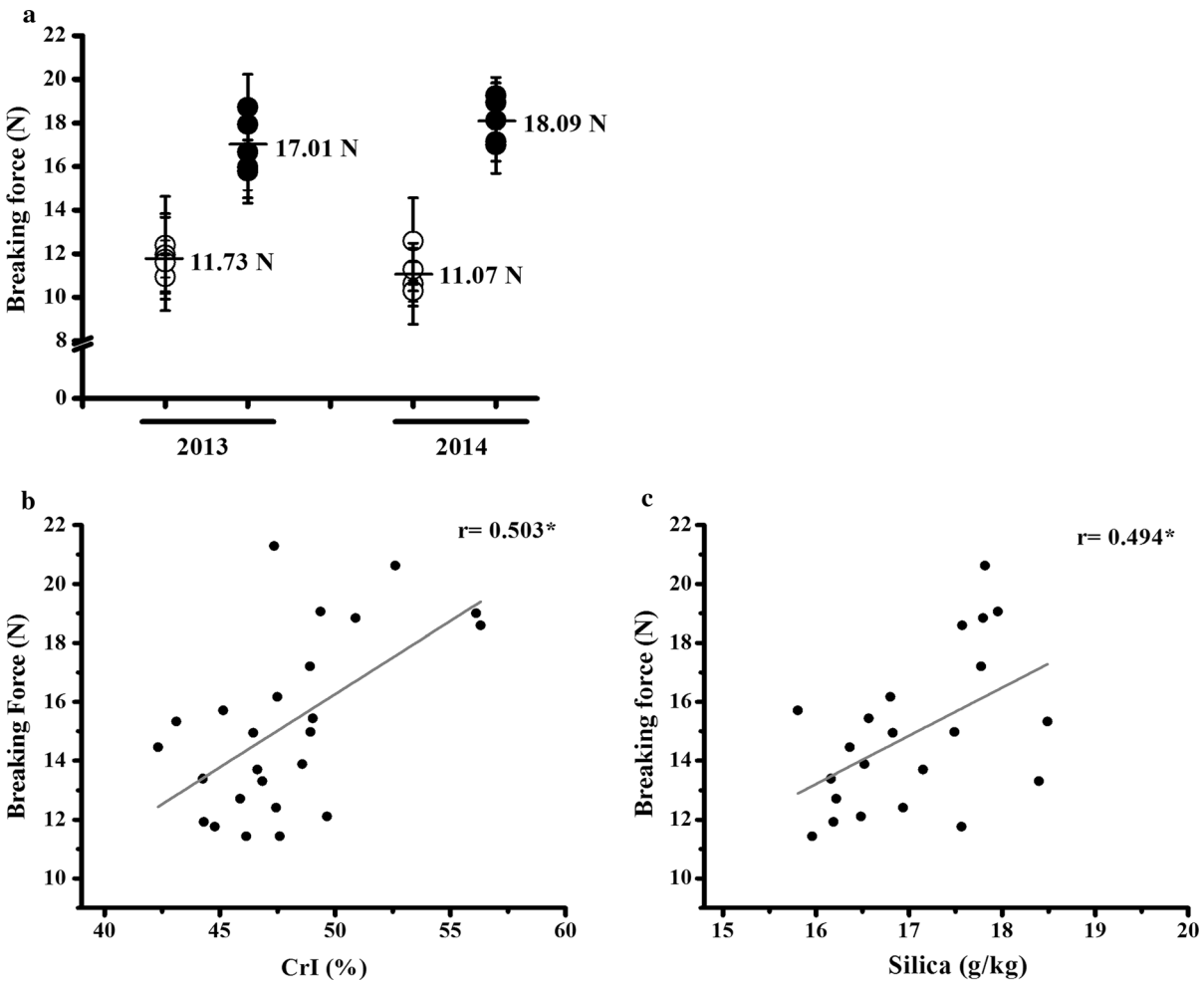


Fig. 1 Comparative study of breaking force (N). **a** Evaluation of two groups of wheat genotypes based on breaking force. Hollow circles represent group 'L', and solid circles represent group 'H'. The lines within the dots indicate the mean values of all data; the bars indicate the standard deviations of replicated data (n = 5). **b**, **c** Correlation analysis of CrI and silica with breaking force, respectively. * indicates significant correlation at $p < 0.05$

S monomers of group H was recorded at the $p < 0.01$ level of significance (Table S4 in the supporting documents). While the relative amount of the lignin monomers, H, S, and G in group L were 3.09%, 61.93% and 34.97%, respectively. Compared with group L, the relative amount of S was significantly reduced by 7.56% and conversely H and G were substantially increased by 19.74% and 11.67% in group H at $p < 0.05$ (Fig. 2, Table S4 in the supporting documents), resulting in a dramatic decrease in the S/G ratio by 16.85% in group H. In conclusion, the fewer S monomers, the greater amount of G monomers and the lower S/G ratio may play important roles in the determination of the breaking force.

The effects of cellulose, hemicellulose, CrI and SiO_2 on the breaking force

The cellulose contents of group H (451.61 μg/mg) were significantly higher than those of group L (428.8 μg/mg). For the hemicellulosic monosaccharides, in group H, the xylose (Xyl) content of hemicellulose was significantly decreased (218.42 μg/mg) at $p < 0.01$, while the fucose (Fuc) content (0.14 μg/mg) was significantly increased compared to that of group L (0.12 μg/mg) at $p < 0.05$ (Table 2). However, slighter variations for the other monosaccharides were present in the two groups but were not statistically significant (Table 2). On the whole, we can conclude that the higher cellulose content and lower hemicellulose content might benefit the stem strength. Moreover, the positive correlation between crystallinity index (CrI) and breaking force was detected (Fig. 1b). Accordingly, it was shown that the varieties in Group H (50.68%) have higher CrI of raw materials than those in the Group L (47.18%) with the exception of variety H4. Since the silica is considered as "beneficial plant nutrient" in plant growth and development (Naeem et al. (2018), this study also investigated the silica content of raw material and found that it positively correlated with breaking force (Fig. 1c), with a mean value of 17.31 g/kg in group H and 16.53 g/kg in group L (Table 2). Our result was consistent with some previous studies which indicated the positive effects of silica on plant development, mechanical strength and grain yield (Adrees et al. 2015; Murozuka et al. 2015; Zhang et al. 2015). However, it should be mentioned that although the CrI and silica content are contributors to increased breaking force, they are most likely indicative but not decisive.

Anatomical structure and the breaking strength of the stem

The wheat stalk is a hollow tubular structure formed by the organization of different wall tissues, including parenchyma tissues, vascular bundles (VB), sclerenchyma tissues and epidermal cells. The improved strength by higher deposition of lignin and cellulose contents in the mechanical tissue layer signifies the importance of a wider layer of mechanical tissues (Kong et al. 2013; Zhang et al. 2016). Our study analyzed the differences in anatomical features between two groups (Fig. 3). Group H exhibited an increased thickness of the mechanical tissue layer, with a mean thickness of 106.51 μm, and an increased average number and area of large vascular bundles (9.67 and 30.08 10^3 μm^2, respectively), but significantly decreased small vascular bundles per area

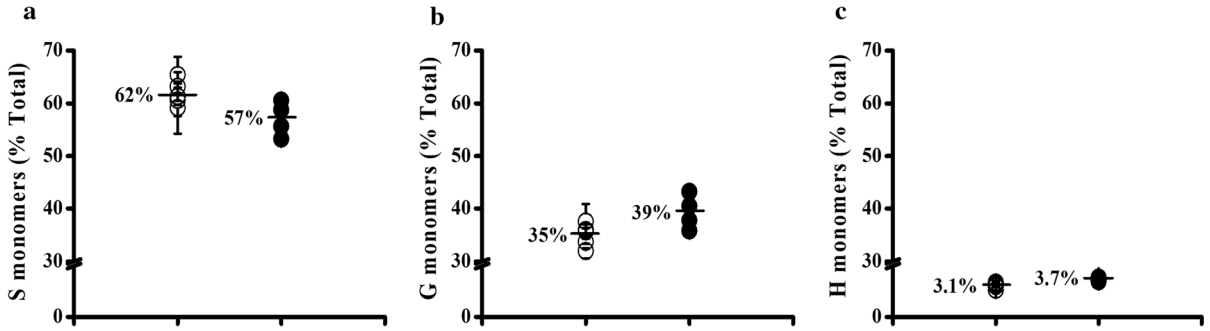


Fig. 2 Lignin monomer composition (% of total **a** S, **b** G, **c** H) of two selected groups. Hollow circles represent group 'L', and solid circles represent group 'H'. The lines within the dots indicate the mean values of all data; the bars indicate the standard deviations of replicated data (n = 5). Abbreviations: S—syringyl, G—guaicyl, H—*p*-hydroxyphenyl

(5.13**), with a mean proportion of 34.64% small vascular bundles (small vascular number × 100/the number of total vascular bundles) (Table 3). Conclusively, the increased breaking force of group H was mainly due to the higher number and greater area of large vascular bundles.

Validation of wall polymer effects on breaking force of culm mutants

In this study, a total of six wheat homozygous culm mutants with significantly lower breaking force in the basal stem than that of the wild type varieties (E'mai 596 and Zhengmai 9023) were selected for cell wall analysis. The four mutants from Zhengmai 9023 showed decreases of 22.3% (wbc4) to 46.9% (wbc2) in breaking force, while the two mutants from E'mai 596 showed decreases of 35.9% (wbc5) and 41.5% (wbc6) (Fig. 4a). All mutants had lower significant crystalline cellulose content, higher hemicellulose content, and higher or equal lignin content compared to wild types. For instance, in the mutants from Zhengmai 9023, crystalline cellulose levels varied from 17.65% (wbc3) to 23.39% (wbc4), hemicellulose levels from 27.2% (wbc4) to 28.7% (wbc1 and wbc2) and lignin levels from 22.11% (wbc1) to 23.83% (wbc3) on a dry matter basis (Fig. 4b). More interestingly, the alteration level of the polysaccharides roughly correlated with the reduced value of the breaking force. Altogether, these results from the mutants further strengthen the fact that a higher breaking force was observed in response to higher levels of crystalline cellulose and reduced hemicellulose content in the germplasm resources.

Discussion

Lodging is an integrated trait associated with many plant characteristics. In addition to plant height and weight, stem strength plays a significant role in stem lodging risk (Xiao et al. 2002; Berry et al. 2004, 2007). Like most complex traits, stem strength and its related traits are quantitatively inherited and remain poorly understood in wheat, which has limited further improvement of lodging resistance through breeding. Although the importance of cell wall components in determining stem mechanical properties has been recognized (Tanaka et al. 2003; Zhang and Zhou 2011), few studies have been reported in wheat due to the complicated structures of the cell wall and the lack of suitable methods for analysis on a large scale. Some

Table 2 Cell wall composition of two groups of wheat genotypes

Groups	Materials	Cellulose μg/mg	Silica (g/kg)	CrI (%)	Hemicellulosic monosaccharides (μg/mg)							Total hemicelluloses (μg/mg)
					Rha	Fuc	Ara	Xyl	Man	Gal	Glc	
L	L1	418.74	16.94	47.43	0.89	0.12	24.83	231.41	1.53	3.41	29.05	291.24
	L2	436.68	17.56	44.78	0.89	0.12	25	227.98	1.77	3.86	30.65	290.27
	L3	429.78	16.80	47.50	1	0.12	22.91	223.46	1.88	3.91	35.25	288.53
	L4	427.66	16.52	48.57	0.91	0.13	23.1	222.46	1.83	3.59	36.95	288.97
	L5	431.16	14.85	47.60	0.93	0.13	24.92	226.83	1.87	3.75	31.08	289.51
	Mean	428.8	16.53	47.18	0.93	0.12	24.15	226.43	1.78	3.7	32.6	289.71
H	H1	450.5	17.57	56.31	0.82	0.13	23.15	213.37	1.62	3.15	31.28	273.52
	H2	460.26	16.48	49.64	0.92	0.15	24.92	219.18	1.89	3.63	30.96	281.65
	H3	437.01	17.80	50.89	1.02	0.14	24.02	221.27	1.75	3.46	30.91	282.57
	H4	458.86	16.22	45.88	0.84	0.13	22.13	217.81	1.39	3.05	28.57	273.92
	H5	451.46	18.49	49.37	0.88	0.13	23.44	220.48	1.95	3.8	28.97	279.65
	Mean	451.61**	17.31	50.68	0.9	0.14*	23.53	218.42**	1.72	3.42	30.14	278.27
p values		0.00	0.24	0.13	0.49	0.02	0.37	0.01	0.64	0.13	0.16	

CrI crystallinity index, *Rha* rhamnose, *Fuc* Fucose, *Ara* Arabinose, *Xyl* Xylose, *Man* mannose, *Gal* galactose, *Glc* glucose

*,** indicate significant differences between the mean values at $p < 0.05$ and 0.01 by *t* test, respectively

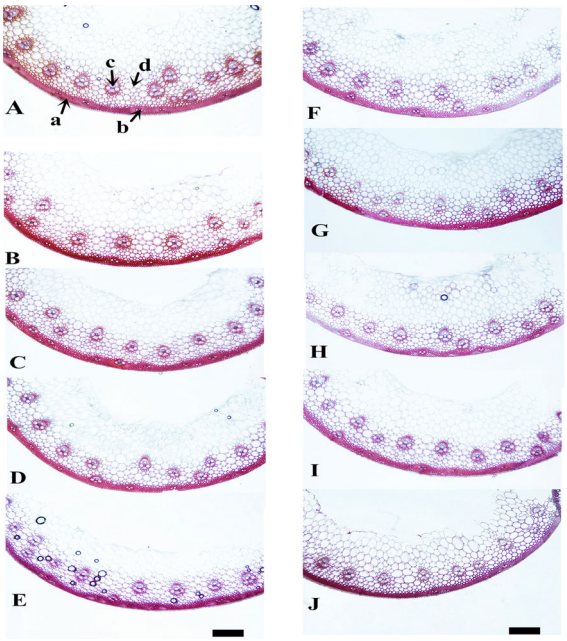


Fig. 3 Microscopic observation of the anatomical structure of the basal second internode of two groups of wheat genotypes. Group L, low breaking force (L): **A** L1; **B** L2; **C** L3; **D** L4; **E** L5. Group H, high breaking force (H): **F** H1; **G** H2; **H** H3; **I** H4; **J** H5. a—mechanical tissue; b—small vascular bundle; c—large vascular bundle; d—parenchyma cells. Ruler: 400 μm

studies have investigated lignin accumulation and the activity of enzymes in the lignin biosynthesis pathway and their relation to lodging resistance (Ma 2009; Nguyen et al. 2016; Peng et al. 2014). Several previous studies have focused on the anatomical features associated with lodging resistance in wheat (Kelbert et al. 2004; Kong et al. 2013). However, the analysis of a single or several components of the cell wall in a few varieties is not sufficient. The mechanical strength of wheat straw is determined by complex cell wall characteristics arising from cell wall components, including their single concentrations, ratios to each other and arrangements in the structure, as well as by anatomical features. In the present study, we performed comparative and correlative analyses among the contents of the wall polymers, the constitution of hemicellulose and lignin monomers, the lignocellulose features characterized by crystallinity, and the breaking force using both a range of germplasm resources and six mutant cultivars in wheat with a high-throughput platform for cell wall analysis at the Great Lakes Bioenergy Research Center (GLBRC) of Michigan State University and the X-ray diffraction (XRD) method. This allowed us to conduct a

Table 3 Morphological and anatomical features between two groups of wheat genotypes

Groups	Sample	Large vascular bundle area (10^3 μm^2)	Mechanical tissue thickness (μm)	Large vascular bundle	Small vascular bundle	Small vascular bundle ratio (%)
L	L1	32.97 ± 2.67	88.31 ± 3.99	10.00 ± 1.00	6.67 ± 0.58	40.02 ± 1.15
	L2	30.99 ± 2.82	107.63 ± 2.38	9.00 ± 0.00	8.33 ± 0.58	48.04 ± 1.7
	L3	26.61 ± 2.8	109.92 ± 7.53	9.00 ± 0.00	7.00 ± 1.00	43.6 ± 3.53
	L4	26.67 ± 0.04	102.25 ± 7.76	8.50 ± 0.71	8.00 ± 1.41	48.34 ± 2.35
	L5	27.43 ± 1.81	108.52 ± 7.76	8.50 ± 0.71	7.50 ± 0.71	46.87 ± 0.28
Mean		28.93	103.3	9.00	7.5	45.37
H	H1	35.27 ± 1.56	106.98 ± 8.33	8.67 ± 0.58	6.00 ± 0.00	40.95 ± 1.65
	H2	29.26 ± 3.84	107.28 ± 15.91	10.33 ± 0.58	4.33 ± 0.58	29.52 ± 3.43
	H3	29.01 ± 2.24	104.91 ± 0.5	10.33 ± 0.58	4.33 ± 0.58	29.52 ± 3.43
	H4	31.84 ± 1.81	117.4 ± 7.13	10.00 ± 1.00	5.33 ± 1.53	34.33 ± 4.15
	H5	25.01 ± 0.74	96.00 ± 15.65	9.00 ± 1.73	5.67 ± 0.58	38.87 ± 3.8
Mean		30.80	106.51	9.67	5.13**	34.64**
Increment(%)					31.60%	23.64%
p values		0.606	0.56	0.171	0.001	0.005

** significant difference between the mean values at $p < 0.01$ by *t* test

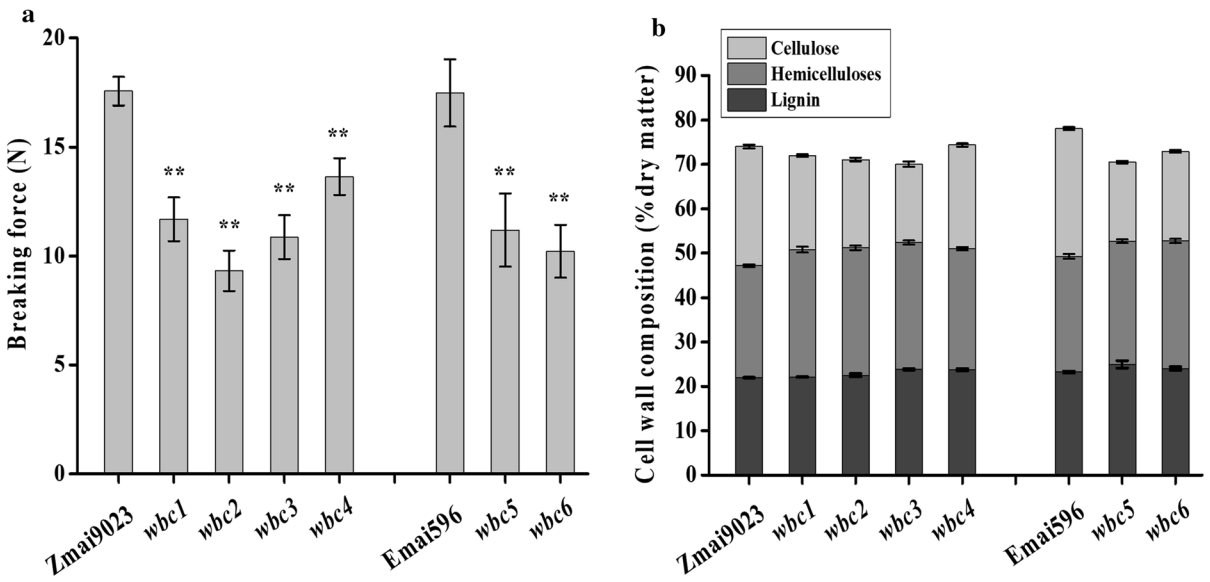


Fig. 4 Comparative study of wild types and their corresponding mutants. **a** Culm breaking force of wild types and mutants. ** indicates a significant difference between wild types and their corresponding mutants at $p < 0.01$ by t test. **b** Cell wall composition of wild types and mutants (% dry matter). The bars indicate standard deviations (n = 3). Abbreviations: Zmai—Zhengmai, Wbc—wheat brittle culm

systematic analysis and determine the key factors that affect the breaking force of wheat stems.

Basal internode weight, culm thickness and flag leaf width contribute to stronger stems

It was found that breaking force was positively associated with fresh weight, 2nd internode culm thickness, and flag leaf width in both years (Table 1). The results are in accordance with those of Yadav et al. (2017) and Islam et al. (2007), which corroborated the positive association of breaking strength with the diameter of basal internode and stem weight. The number of tillers per plant was negatively correlated with flag leaf width, which is consistent with the results obtained by Jia et al. (2013), revealing the determination of these traits during the growth stage. This may be because an increased number of tillers would reduce space for the plant to produce wider leaves. The flag leaf is considered an important contributor to wheat yield due to its key contribution in capturing light for photosynthesis. Our data clearly indicated that the flag leaf length and width were positively correlated to the stem strength. Some previous studies provided insights that plants with thicker culms and larger flag leaves would show increased yield by the reduction of lodging (Kamran et al. 2018; Xiao et al. 2002). It is reasonable that strong stems could transport photosynthetic products and mineral nutrients effectively and thus be associated with a high grain yield. Thus, we suggest that fresh weight, culm thickness, and flag leaf width together are the key indicators for both high yield and lodging resistance in wheat breeding.

The contrary effects of cellulose and hemicelluloses on the mechanical properties of stems

The stem strength of wheat is a complex trait composed of two characteristics, i.e., stem mechanical elasticity and rigidity (Hai et al. 2005). The microfibrils of cellulose embedded in a hemicellulose and lignin matrix are the main factors in cell wall tensile strength and are essential for plant development and stature (Ambavaram et al. 2011; McFarlane et al. 2014). In this study, we found that group H had a higher content of crystalline cellulose but a lower level of almost all of the hemicellulosic monosaccharides than the other groups. Furthermore, these results were further verified by the six culm mutants with lower breaking force that exhibited lower levels of

crystalline cellulose and higher hemicellulose contents than their corresponding wild types. Moreover, generally the positive correlation of CrI with the breaking force indicating that the physical/chemical properties of polysaccharides also deserve attention. Our result was consistent with some previous studies which indicated that the cellulose crystallinity was the key factor to prevent the degradation of straw biomass (Zhang et al. 2013), whereas hemicelluloses can negatively affect the crystallinity for higher biomass digestibility (Li et al. 2013).

Appropriate lignin levels and monomer constituents are important for the mechanical properties of stems

Lignin is closely associated with cellulose and hemicellulose, whereas its deposition in the walls of specialized cells imparts stem rigidity. Moreover, it is optimal for plants such as trees to have higher contents of cellulose and lignin (Sannigrahi et al. 2009); however, in grass species, the contents of three major cell wall polymers cannot simultaneously reach high levels. Previous studies reported the controversial roles of lignin in lodging resistance (Esechie et al. 2004; Hai et al. 2005; Hondroyianni et al. 2000; Ke et al. 2019; Liu et al. 2018). In this study, the lignin content was not found to be associated with the breaking force. Despite a higher lignin content being better in some circumstances, a higher lignin content might also limit the cellulose content in many germplasm accessions of grass species. We found that all six wheat culm mutants had higher levels of hemicellulose and lignin but significantly lower levels of crystalline cellulose, resulting in decreased breaking force, which was consistent with findings in rice (Li et al. 2015).

In addition, lignin has three basic monomers, p-coumaryl alcohol (H), coniferyl alcohol (G) and sinapyl alcohol (S), which are cross-linked by ether-, ester, and C–C bonds to form a stable and waterproof lignin complex (Li et al. 2014b). Therefore, the lignin monomers represent the lignin constituents and determine their linkage style with polysaccharides, and these monomers are thus referred to as key factors in straw biomass digestibility in wheat and rice (Li et al. 2015; Wu et al. 2014). For the first time, this study reported that a lower ratio of S and a relatively higher H and G monomers was better for increasing the breaking force in wheat. Some previous studies also stated the positive effect of G monomers on lodging resistance presumably by the interaction of hemicellulosic monosaccharide arabinose in rice (Li et al. 2015; Wei et al. 2017; Wu et al. 2014). The mechanism underlying this correlation remains to be elucidated in the future.

Distinct effects of two kinds of vascular bundles on stem mechanical properties

Little is known about the relationship of stem anatomical features with stem lodging in wheat. Previously, it has been reported that strong lodging resistance is obtained when thick-walled mechanically supportive sclerenchyma tissues are located near the perimeter of stem cross sections (Ookawa et al. 2014; Zhang et al. 2017). Similarly, the association of a higher number of vascular bundles and a wider sclerenchyma ring with improved stem rigidity are also in corroboration with previous studies (Kong et al. 2013; Zhang et al. 2016). Our study signified the importance of anatomical structure on stem breaking force. Interestingly, we found that the effects of the two kinds of vascular bundles on the breaking force were contrasting, possibly due to their locational effects. The large vascular bundles were located in the inner layer of the culm surrounded by thinner cell wall parenchymal cells, and their mechanical strength was enhanced by thick lignified secondary walls. On the other hand, the small vascular bundles could be considered a hollow cavity and were embedded in peripheral sclerenchyma cells, which have the thickest secondary cell wall. Thus, the smaller number and area of the small vascular bundles would increase the density of peripheral sclerenchyma cell, leading to higher stem density.

Implications for breeding for lodging resistance in wheat

One of the key strategies of high yield breeding is the selection of thicker and stronger stems, which are promising for lodging resistance in crops (Zhang et al. 2016). Developing a suitable approach to determine the stem strength of wheat is pivotal. However, genes and QTLs associated with culm strength have yet to be identified and isolated in wheat. Studying the association of important characteristics and lodging

resistance can serve as an important criterion, as explained by the correlation analysis and their coefficients. Our results encourage the use of integrated stem strength and related traits as a selection index for lodging resistance (Hyles et al. 2017). The cell wall composition and the ratios of cell wall constituents play an important role in the mechanical properties of stems, and it is unlikely that efforts to increase the mechanical stem strength should focus on a single component of the cell wall. As we have found in this study, the mechanical strength of wheat stems is affected by a series of factors at different levels. The mechanical strength of the cell wall and consequently of wheat straw is determined by the complex characteristics of the cell wall components, i.e., their single concentrations, monomer proportions, and interactions/arrangements in the structure. Regarding the wheat types used in this study, we proposed that the improvement of lodging resistance should focus on increasing the cellulose content and crystallinity while maintaining an appropriate level of total lignin because the lignin content is sufficiently high in current wheat varieties compared with rice. In addition, the fine modification of the lignin monomer ratios and the allocation of two different vascular bundles should also be emphasized in breeding programs. Conclusively, further investigations, particularly focusing on the detailed mechanisms of the chemical components of the cell wall and their altered chemical structure, are essential to understanding the function of these fine structures in stem sturdiness. Thus, a good level of lodging resistance can be achieved through different combinations of lodging resistance component traits.

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