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CROPS AND SOILS RESEARCH PAPER

Characterization of culm morphology, anatomy and chemical composition of foxtail millet cultivars differing in lodging resistance

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SUMMARY

Lodging can be an important factor in limiting yield and quality of summer foxtail millet [*Setaria italica* (L.) P. Beauv.]. Although lodging resistance varies among different genotypes, direct selection for lodging resistance is difficult because of its sporadic occurrence in the field and inconsistency between years. A 2-year-field study was conducted with 35 summer foxtail millet cultivars or advanced breeding lines to determine the association between lodging resistance and culm morphology, anatomy and chemical composition. Path analyses indicated that stem-breaking strength had the most important effect on the lodging coefficient. The breaking strength of stem was associated with specific morphological properties of the culm, such as greater culm diameter and most importantly culm wall thickness. Width of sclerenchyma tissue, and the number and sheath width of the large vascular bundles were the major anatomical properties that influenced stem-breaking strength. The cellulose and lignin compositions of the culm had different effects on stem-breaking strength. Cultivars with smaller lodging coefficients contained higher levels of cellulose, but lower levels of lignin than the cultivars that were more prone to lodging. The findings from the present study provide useful information on lodging-associated traits in the culm that can be used as indicators for the improvement of lodging resistance in foxtail millet.

INTRODUCTION

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is one of the oldest domesticated crops in the world. It has been cultivated as a food and fodder crop for thousands of years in China, as well as in other parts of Asia and in Africa. At present, foxtail millet is usually grown on marginal agricultural land due to its low productivity and inferior palatability as a food crop; however, it has attracted extensive attention as a new model crop for genomic studies (Dwivedi *et al*. 2012; Lata *et al*. 2013). In foxtail millet production, lodging of the stems occurs frequently because it is sown at high density and has a weak culm. In northern China, where summer foxtail millet is grown, heavy rainfall accompanied by strong winds often results in lodging. Stem-based lodging occurs when the stem buckles and it is regarded as an important yield-limiting factor for this cereal crop, as it also results in blighted grain and reduced harvest index, and complicates crop harvest. The impact of stem-based lodging on yield depends on the severity of lodging and the stage of development when lodging occurs (Fischer & Stapper 1987; Tripathi *et al*. 2004). Although there have been no direct estimates of yield reductions due to lodging for foxtail millet, up to 40% yield loss has been reported in wheat (*Triticum aestivum* L.) when severe lodging occurred (Easson *et al*. 1993; Kelbert *et al*. 2004*a*).

Numerous studies have reported variation in lodging resistance among genotypes of cereal crops, such as wheat (Easson *et al*. 1993; Crook & Ennos 1994; Berry *et al*. 2003*a*, *b*; Kelbert *et al*. 2004*a*, *b*), barley (*Hordeum vulgare* L.) (Wang & Du 2001) and rice (*Oryza sativa* L.) (Ookawa & Ishihara 1992; Kashiwagi *et al*. 2010), as well as foxtail millet (Tian *et al*. 2010). In addition to genetic factors, damage

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from insects and/or diseases, together with rain and wind, can also increase the severity of stem-based lodging. Over-application of nitrogenous fertilizers and high seeding rates can result in weak culms, which increases the severity of lodging in wheat (Stapper & Fischer 1990; Easson *et al*. 1993). In vascular crops, the stem not only provides mechanical support for other parts of the plant, but also serves as a storage and transportation organ for the nutrients and water that ensure proper plant development.

Lodging does not occur consistently under natural conditions, so selection for lodging resistance is difficult. Improvement in lodging resistance in crops can be realized by indirect selection for lodging-associated traits. Thus, an understanding of the relationship between lodging and other crop traits is a pre-requisite for genetic improvement of lodging resistance. Lodging in cereal crops has been associated with certain morphological traits, anatomical characteristics and chemical components of the culm (Dunn & Briggs 1989; Kokubo *et al*. 1989; Kelbert *et al*. 2004*b*; Zhu *et al*. 2004; Wang *et al*. 2006; Yao *et al*. 2011; Kong *et al*. 2013). Correlations between lodging resistance and certain anatomical characteristics of the culm, such as the width of sclerenchyma layer (mechanical tissue) and culm walls, have been observed in wheat (Kelbert *et al*. 2004*b*; Kong *et al*. 2013). The unit number of large vascular bundles in a culm had a positive correlation with the mechanical strength of the stem in wheat (Wang *et al*. 2006). Lodging-resistant wheat genotypes had higher levels of carbohydrates (such as, lignin and cellulose) in the cell walls of the culm, but the effects of lignin and cellulose concentrations on lodging resistance appear to be different (Zhu *et al*. 2004; Wang *et al*. 2006). Wang *et al*. (2006) described that the most important anatomical and chemical properties for the selection of lodging-resistant wheat cultivars were the ratio of culm wall thickness to outer radius, the proportion of sclerenchyma tissue in the culm, and the unit number of large vascular bundles.

Most studies on lodging in cereal crops with a soft culm have been carried out in wheat, rice and barley. Little is known about the association between stem-based lodging and the properties of the culm in foxtail millet (Tian 2013). A number of methods have been developed to measure lodging in cereal crops (Baker *et al*. 1998; Berry *et al*. 2003*b*; Sterling *et al*. 2003). Lodging coefficient (LC) proved to be a useful indicator for estimating stem-based lodging in the field, as there was a good correlation between the LC and natural lodging (Wang & Du 2001). Lodging coefficient is determined by plant height (PH), biomass (BM) of above-ground parts, stem failure moment (SFM) and root weight (RW). A cultivar with a low LC is expected to have reduced risk of lodging. The use of a LC to determine variation in stem-based lodging in cultivars and landraces of foxtail millet has been demonstrated: Tian *et al*. (2010) found that stem-based lodging of foxtail millet was associated with stem-breaking strength (SBS), SFM (mechanical strength of stem) and the BM of above-ground parts and the roots. Plant height and internode length were closely related to stem-based lodging in the landraces, but not in the modern improved cultivars. Nevertheless, the association between stem-based lodging and the anatomical parameters and carbohydrate components in the cell walls of the culm has not been determined in foxtail millet. The present study was carried out to understand the association between anatomical and chemical properties of the culm and stem-based lodging in foxtail millet.

MATERIALS AND METHODS

Plant material and experimental design

The study included 35 Chinese commercial foxtail millet cultivars, which have been released during the past three decades and/or used in national yield trials of summer foxtail millet in 2011/12. Lodging coefficients were used to create two groups for analysis of culm anatomical and chemical properties. Group 1 consisted of six cultivars Canggu 3, Yugu 18, Baogu 19, Jigu 19, Jigu 31 and Yugu 19, which had low LC. Group 2 included six cultivars Xiaoxiangmi, Baogu 18, Hangtianlvgu Zheng 07-1, Jigu 18 and 200 475, which had high LC.

During the cropping seasons in 2011 and 2012, field experiments were carried out on a clay loam textured soil at a site in Cangzhou, Hebei province, China (33°13′N and 116°47′E, 6–10 m a.s.l.). The preceding crop in both years was winter wheat. The seeds were sown on 30 June 2011 and 19 June 2012. A compound fertilizer (containing 15% nitrogen, phosphorus and potassium each, w/w) was applied at a rate of 600 kg/ha before sowing to supply the demands of plant growth. A randomized complete block design with three replications was used. Each plot consisted of four rows 5 m long with a row spacing of 40 cm. At the 5-leaf-stage (growth stage

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(GS) 15, Zadoks *et al*. 1974), plants were thinned to 3 cm apart. At the late milk stage (GS 77), ten randomly selected plants were selected to measure the parameters investigated.

Measurements of the traits associated with lodging coefficient

Plant height, leverage force (LF), head weight (HW), RW and BM of the above-ground tissues, including spike, culm, leaves and sheaths were recorded for the ten randomly selected plants in each plot. Roots within the top 40 cm of soil were removed from the soil, washed clean of soil and weighed to determine the BM of roots. Basal internode length (INL) and fresh and dry weights of the internodes were determined for the basal 3–5 internodes. The ratio of root to above-ground tissue weight was determined by dividing the BM of above-ground tissues by RW. To determine SBS, the pull force required to break each 3- to 5-internode segment was determined using a digital pull-push force gauge (model 9500, Aikoh Engineering Co. Ltd., Osaka, Japan). Based on the above traits assessed in the field experiments, SFM and LC were determined using the following equations (Wang & Du 2001; Berry *et al*. 2003*a*):

$$\mathrm{SFM} = \mathrm{SBS}\frac{\mathrm{INL}}{4}$$

$$\mathrm{LC} = \frac{\mathrm{PH} \times \mathrm{BM}}{\mathrm{SFM} \times \mathrm{RW}}$$

Assessments of the anatomical traits of the culm

A determination of anatomical characteristics of the culm was performed on the fourth basal internode of three additional randomly selected plants within each plot at GS 73. The diameter of the culm (the average of the two diameters from the longest and the shortest axis of the culm) was measured with vernier callipers. The middle portions of the fourth internodes were cut with a plant microtome (Model MTH-1, Nippon Medical & Chemical Instruments Co., Ltd., Japan) to produce transverse sections of *c*. 10 μM thick. Under a light microscope, the widths of the culm wall (from the epidermis to the cavity), epidermis, sclerenchyma tissue, large vascular bundle sheath and diameter of small vascular bundles (samples of ten per culm) were measured. The numbers of small and large vascular bundles per unit area within culm tissues were counted.

Analysis of cellulose and lignin

The histochemical localization of cellulose in the cell walls at the middle portion of the fourth basal internodes was examined under a fluorescence microscope (Olympus C41, Tokyo, Japan) after calcofluor staining by immersion of the culm sections (~10 μm in thickness) in a 0·005% aqueous solution of calcofluor (fluorescent brightener 28, Sigma) for 2 min (Li *et al*. 2003). The Wiesner staining reaction was used to histochemically localize lignin (Nakano & Meshitsuka 1992). The culm sections were incubated for 2 min in phloroglucin solution (1% in 95% ethanol, Sigma) on a glass slide, mounted in 35% hydrochloric acid (HCl) and examined under a light microscope.

Cellulose and lignin concentrations in the fourth basal internodes collected 10 days after flowering were determined using methods described previously (Updegraff 1969; Dence 1992). The internode samples (200 mg each) were ground into a fine powder in liquid nitrogen with a mortar and pestle, washed three times with a phosphate buffer (50 mmol/l) and extracted twice with 70% ethanol at 70 °C for 1 h. After drying the cell wall extracts in a vacuum, an anthrone reagent was used to determine cellulose concentrations (Updegraff 1969). Lignin concentration was assayed using the Klason method as described by Dence (1992). The fine powder of culm samples (200 mg) were extracted four times in ethanol, dispersed in 72% sulphuric acid ($H_2S_2O_4$) for 3 h at room temperature and in 1 mol/l $H_2S_2O_4$ and heated at 100 °C for 2·5 h. The insoluble materials were recovered by filtration, washed with hot water (90 °C) to remove the acid and dried at 105 °C overnight. The lignin concentration was measured as the percentage of original weight of cell wall residues.

Statistical analysis

The analysis of variance (ANOVA) was performed on individual experiments and data combined over years for the traits examined using SAS general linear model (Version 8.01; SAS Institute Inc., Cary, NC). An *F*-test was used to determine significance of each source of variation. The comparison of significance between the means of each trait was conducted using Fisher's LSD method. Correlation analysis was performed to

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Table 1. *Summary of ANOVA for LC and associated traits across the 2011 and 2012 cropping seasons*

Trait	Source	D.F.	*F* value	*Pr* > *F*
PH	Genotype	34	13·30	<0·001
	Year	1	214·23	<0·001
	Genotype × year	34	5·00	<0·001
INL	Genotype	34	11·11	<0·001
	Year	1	37·74	<0·001
	Genotype × year	34	5·22	<0·001
BM	Genotype	34	24·13	<0·001
	Year	1	72·12	<0·001
	Genotype × year	34	16·47	<0·001
RW	Genotype	34	65·30	<0·001
	Year	1	1562·92	<0·001
	Genotype × year	34	26·19	<0·001
SBS	Genotype	34	22·01	<0·001
	Year	1	770·92	<0·001
	Genotype × year	34	7·79	<0·001
MS	Genotype	34	21·99	<0·001
	Year	1	441·97	<0·001
	Genotype × year	34	3·87	<0·001
LC	Genotype	34	44·66	<0·001
	Year	1	46·48	<0·001
	Genotype × year	34	1·07	NS

PH, plant height; INL, length of basal 3- to 5-internode; BM, biomass of the above-ground stem and head; RW, root weight; SBS, stem-breaking strength; SFM, stem failure moment of culm; LC, lodging coefficient; NS, not significant.

determine the relationships between the traits investigated. Path analysis, based on multiple regression analysis, can be used to decompose the relationships among complex variables (Wonnacott & Wonnacott 1972). Therefore, path analysis was conducted to evaluate the importance of the traits that contributed to stem-based lodging.

RESULTS

Lodging coefficients and associated traits

The analysis of variance of the LC and associated traits demonstrated that the differences for genotype and year were significant for all the traits examined ($P < 0{\cdot}001$) (Table 1). The effects of genotype × year interactions were significant for all the traits ($P < 0{\cdot}001$), except for the LC. Table 2 shows the means of the lodging traits obtained from 35 foxtail millet cultivars grown in the 2011 and 2012 cropping seasons, as well as their combined means for both years.

Differences in the LC of the 35 cultivars tested were significant in each year and over years ($P < 0{\cdot}05$), demonstrating that there were consistent differences in lodging resistance among these cultivars (Table 2). The mean LC across all the cultivars was 0·27 ± 0·081 with a range from 0·15 to 0·53. Significant differences were also detected in PH, INL, BM of above-ground parts, RW, SBS and SFM ($P < 0{\cdot}05$). Although the mean values of LC-associated traits varied between 2011 and 2012, the mean LCs for 2011 (0·26 ± 0·070) and 2012 (0·28 ± 0·088) were close (Table 2).

Path analysis was performed to examine the effects of the above-mentioned traits on LC (Table 3). Stem-breaking strength of the culm and RW had greater direct and indirect effects on LC than PH, INL and BM of the above-ground parts. This resulted in significant correlations of the breaking strength of the culm and RW with LC ($r = -0{\cdot}83$ and $-0{\cdot}66$, respectively, $P < 0{\cdot}001$). Because both direct and indirect effects of PH, INL and BM of the above-ground parts were small, the correlation coefficients between these traits and LC were weak and not significant ($r = -0{\cdot}18$, $-0{\cdot}24$ and $-0{\cdot}21$, respectively). The results from path analysis indicated that SBS is the major above-ground factor that affects the LC.

Association between stem-breaking strength and plant morphological traits

The results of ANOVA indicated that the genotype × environment interactions for LC, SBS, and all the parameters of plant traits, culm anatomical characteristics and concentrations of culm chemical compositions were not significant for the two groups of cultivars, allowing further analysis with combined data sets (Table 4). The difference in LCs was significant between the two groups of cultivars. The mean for the LC of Group 1 cultivars (0·20 ± 0·022) was significantly smaller than that of the Group 2 cultivars (0·38 ± 0·043, $P < 0{\cdot}001$), indicating that the cultivars in Group 1 were less prone to lodging than those in Group 2. This was supported by the observation that the mean SBS for Group 1 cultivars (3·07 ± 0·469) was significantly greater than that of Group 2 cultivars (2·40 ± 0·299, $P < 0{\cdot}001$). Measurements of the morphological traits of plant, such as PH, basal INL, LF, BM of the above-ground parts (BM) and HW, showed that the two groups of cultivars did not differ for those traits, which indicated that they had different relationships with the LC. Plant height and LF were not

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Table 2. *Lodging coefficients and associated traits in 35 foxtail millet cultivars in the cropping seasons of 2011 and 2012 and their pooled analysis*

Cultivar	Year	PH (cm)	INL (cm)	BM (g)	RW (g)	SBS (kg)	MS	LC
Canggu 3	2011	118·80	23·50	57·75	3·64	2·04	12·02	0·16 (0·15–0·16)
	2012	123·40	19·93	44·90	1·99	3·32	16·53	0·17 (0·16–0·18)
	Combined	121·10	21·71	51·33	2·82	2·69	14·28	0·16 (0·15–0·18)
Cang 615	2011	116·20	24·95	43·05	2·39	2·12	13·21	0·16 (0·15–0·16)
	2012	114·00	20·04	41·98	1·66	3·40	16·93	0·17 (0·16–0·19)
	Combined	115·10	22·50	42·51	2·02	2·76	15·07	0·17 (0·15–0·19)
Yugu 18	2011	105·90	20·80	63·00	2·89	2·79	14·55	0·16 (0·16–0·16)
	2012	115·27	19·73	53·51	1·86	3·69	18·02	0·18 (0·16–0·19)
	Combined	110·58	20·26	58·26	2·38	3·25	16·29	0·17 (0·16–0·20)
Baogu 19	2011	104·30	22·00	53·57	2·30	2·40	13·23	0·18 (0·17–0·20)
	2012	120·18	21·00	53·87	2·35	2·71	14·33	0·20 (0·17–0·22)
	Combined	112·24	21·48	53·72	2·32	2·56	13·78	0·19 (0·17–0·22)
Jigu 19	2011	105·70	18·85	77·98	3·65	2·48	11·69	0·19 (0·19–0·20)
	2012	117·17	20·42	45·43	1·74	3·03	15·47	0·20 (0·18–0·22)
	Combined	111·43	19·63	71·71	2·70	2·76	13·58	0·20 (0·18–0·22)
Jigu 24	2011	119·30	22·60	61·58	3·07	2·18	12·34	0·19 (0·17–0·21)
	2012	122·34	21·19	53·59	1·91	3·23	17·16	0·20 (0·18–0·21)
	Combined	120·82	21·90	57·58	2·49	2·71	14·75	0·20 (0·17–0·21)
Jigu 6	2011	112·70	21·00	44·72	2·90	1·69	8·88	0·20 (0·18–0·21)
	2012	117·25	20·73	47·20	1·83	2·86	14·81	0·20 (0·19–0·22)
	Combined	114·97	20·86	45·96	2·37	2·28	11·85	0·20 (0·18–0·22)
Jigu 31	2011	112·30	22·80	59·98	2·42	2·49	14·17	0·20 (0·19–0·21)
	2012	117·15	22·50	60·77	2·04	2·93	16·40	0·21 (0·21–0·22)
	Combined	114·73	22·65	60·37	2·23	2·71	15·29	0·21 (0·19–0·22)
Yugu 19	2011	117·40	22·70	61·33	2·43	2·58	14·73	0·20 (0·18–0·22)
	2012	119·95	21·24	44·92	1·73	2·71	14·26	0·22 (0·21–0·23)
	Combined	118·67	21·97	53·13	2·08	2·65	14·50	0·21 (0·18–0·23)
Yugu 2	2011	106·00	25·78	34·19	1·55	1·80	11·58	0·20 (0·19–0·22)
	2012	126·56	23·09	51·14	1·79	2·84	16·39	0·22 (0·20–0·25)
	Combined	116·28	24·44	42·67	1·68	2·32	13·99	0·21 (0·19–0·25)
Cang 369	2011	124·10	20·55	75·65	3·98	2·21	11·36	0·21 (0·20–0·21)
	2012	130·25	22·48	46·61	2·08	2·25	12·57	0·23 (0·23–0·23)
	Combined	127·17	21·52	61·13	3·03	2·23	11·96	0·22 (0·20–0·23)
Yugu 7	2011	124·80	26·56	66·28	2·94	2·00	13·25	0·21 (0·20–0·22)
	2012	124·14	21·71	54·24	1·97	2·71	14·70	0·24 (0·20–0·28)
	Combined	124·47	24·14	60·26	2·46	2·35	13·98	0·22 (0·20–0·28)
Canggu 4	2011	106·30	18·60	38·37	1·41	2·75	12·78	0·23 (0·21–0·25)
	2012	109·61	20·13	42·94	1·44	2·76	13·89	0·23 (0·20–0·26)
	Combined	107·95	19·36	40·66	1·43	2·76	13·34	0·23 (0·20–0·26)
Yugu 9	2011	113·50	23·80	58·77	2·40	2·13	12·65	0·22 (0·21–0·23)
	2012	123·23	24·49	47·13	1·29	3·06	18·60	0·24 (0·22–0·27)
	Combined	118·37	24·15	52·95	1·85	2·59	15·62	0·23 (0·21–0·27)
Gufeng 2	2011	114·30	21·60	62·96	3·34	1·81	9·79	0·22 (0·21–0·23)
	2012	113·82	20·20	41·00	1·55	2·46	12·45	0·24 (0·23–0·26)
	Combined	114·06	20·90	51·98	2·45	2·14	11·17	0·24 (0·21–0·27)
Jigu 33	2011	119·60	21·10	59·01	2·46	2·47	13·05	0·22 (0·21–0·23)
	2012	122·94	22·84	42·73	1·63	2·28	12·91	0·25 (0·23–0·27)
	Combined	121·27	21·97	50·87	2·04	2·38	12·98	0·24 (0·21–0·27)
Jigu 32	2011	113·60	18·90	58·23	2·56	2·39	11·33	0·23 (0·22–0·24)

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TABLE 2.
Continued

Cultivar	Year	PH (cm)	INL (cm)	BM (g)	RW (g)	SBS (kg)	MS	LC
	2012	113·29	21·09	43·61	1·26	2·89	15·17	0·26 (0·24–0·30)
	Combined	113·45	19·99	50·92	1·91	2·64	13·25	0·24 (0·22–0·30)
Jigu 15	2011	111·00	22·75	57·70	1·79	2·59	14·73	0·24 (0·23–0·26)
	2012	119·13	20·31	47·95	1·43	3·14	16·11	0·25 (0·24–0·26)
	Combined	115·06	21·53	52·82	1·61	2·87	15·42	0·25 (0·23–0·26)
Jigu 34	2011	115·70	19·90	77·47	3·07	2·31	11·47	0·25 (0·25–0·26)
	2012	132·67	22·42	60·98	2·10	2·67	14·95	0·26 (0·23–0·28)
	Combined	124·18	21·16	69·22	2·59	2·49	13·21	0·25 (0·22–0·28)
Yugu 1	2011	111·00	23·00	50·39	2·22	1·67	9·58	0·26 (0·25–0·28)
	2012	113·60	21·25	41·37	1·40	2·66	14·12	0·24 (0·20–0·28)
	Combined	112·30	22·13	45·88	1·81	2·16	11·85	0·25 (0·20–0·28)
Jingan 7505	2011	109·70	20·60	52·11	1·78	2·34	12·37	0·27 (0·25–0·28)
	2012	130·00	23·09	60·05	1·76	3·00	17·29	0·26 (0·25–0·28)
	Combined	119·83	21·85	56·08	1·77	2·69	14·68	0·26 (0·25–0·28)
Jigu 22	2011	110·10	22·60	43·46	2·01	1·61	9·11	0·26 (0·25–0·28)
	2012	123·77	20·61	51·26	1·51	2·87	14·77	0·28 (0·19–0·22)
	Combined	116·94	21·60	47·36	1·76	2·24	11·94	0·27 (0·25–0·29)
Shi 3839	2011	111·30	26·40	54·56	1·88	1·83	12·08	0·27 (0·26–0·28)
	2012	115·05	22·51	48·54	1·39	2·60	14·58	0·28 (0·27–0·28)
	Combined	113·18	24·46	51·55	1·64	2·22	13·33	0·27 (0·26–0·28)
Cang 372	2011	104·50	22·10	64·30	2·09	2·06	11·36	0·28 (0·28–0·29)
	2012	121·85	22·97	65·95	1·82	2·52	14·49	0·32 (0·22–0·41)
	Combined	113·17	22·54	65·13	1·96	2·29	12·92	0·30 (0·22–0·41)
Yugu 15	2011	109·50	22·00	49·64	1·89	1·87	10·27	0·28 (0·28–0·29)
	2012	110·79	19·08	52·49	1·20	2·98	14·28	0·34 (0·29–0·38)
	Combined	110·14	20·27	51·06	1·55	2·42	12·28	0·31 (0·28–0·38)
Jigu 25	2011	115·20	22·80	64·76	2·51	1·85	10·55	0·28 (0·27–0·30)
	2012	114·77	19·65	58·99	1·44	2·64	12·97	0·36 (0·35–0·39)
	Combined	114·98	21·22	61·87	1·98	2·25	11·76	0·32 (0·27–0·39)
Xiaoxiangmi	2011	115·80	24·22	52·73	2·17	1·49	9·04	0·31 (0·30–0·33)
	2012	124·66	23·88	44·30	1·17	2·23	13·34	0·36 (0·33–0·41)
	Combined	120·23	24·05	48·51	1·67	1·86	11·19	0·34 (0·30–0·41)
A200302	2011	111·40	18·35	64·20	2·28	2·17	9·95	0·32 (0·31–0·33)
	2012	110·13	19·75	44·49	1·23	2·19	10·82	0·37 (0·31–0·44)
	Combined	110·77	19·05	54·34	1·75	2·18	10·38	0·34 (0·31–0·44)
Zheng 07–1	2011	99·40	21·30	26·39	1·73	1·30	6·91	0·30 (0·29–0·31)
	2012	113·31	17·77	51·34	1·27	2·74	12·17	0·38 (0·35–0·43)
	Combined	106·36	19·53	43·87	1·50	2·02	9·54	0·34 (0·29–0·42)
Hangtianlvgu	2011	111·20	20·90	38·94	1·38	1·79	9·36	0·34 (0·32–0·36)
	2012	121·09	23·55	30·36	0·82	2·05	12·12	0·37 (0·33–0·44)
	Combined	116·14	22·23	34·65	1·10	1·92	10·74	0·35 (0·32–0·44)
200 475	2011	122·40	21·50	48·68	2·53	1·39	7·47	0·32 (0·29–0·33)
	2012	138·02	21·87	57·91	1·43	2·58	14·09	0·40 (0·37–0·46)
	Combined	130·21	21·69	53·29	1·98	1·98	10·78	0·36 (0·29–0·46)
Jigu 20	2011	98·70	19·11	37·62	1·28	1·61	7·70	0·38 (0·36–0·39)
	2012	115·15	15·37	40·61	1·24	2·32	8·90	0·43 (0·38–0·51)
	Combined	106·92	17·24	39·11	1·26	1·97	8·30	0·40 (0·36–0·51)
Baogu 18	2011	120·90	24·70	47·55	1·89	1·21	7·46	0·41 (0·40–0·41)
	2012	119·97	21·89	47·27	1·26	2·03	11·12	0·40 (0·37–0·43)
	Combined	120·44	23·29	47·41	1·58	1·62	9·29	0·41 (0·37–0·43)

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Jigu 18	2011	118·90	16·17	53·92	1·49	2·48	5·93	0·39 (0·37–0·40)
	2012	102·60	20·20	38·85	1·74	1·17	10·04	0·43 (0·38–0·50)
	Combined	110·75	18·19	46·38	1·61	1·83	7·98	0·41 (0·37–0·50)
Yugu 11	2011	101·90	22·70	48·57	1·45	1·45	8·20	0·42 (0·39–0·46)
	2012	114·61	19·02	72·02	1·74	2·27	10·78	0·45 (0·36–0·53)
	Combined	108·25	20·86	60·30	1·60	1·86	9·49	0·43 (0·36–0·53)
$LSD_{0·05}$	2011	2·012	0·814	1·490	0·108	0·101	0·755	0·024
	2012	8·590	2·806	9·443	0·302	0·417	2·476	0·064
	Combined	4·373	1·447	4·741	0·159	0·212	1·281	0·034
Average	2011	111·92	22·03	54·41	2·34	2·00	10·97	0·26 (0·15–0·46)
	2012	119·66	20·97	49·87	1·59	2·72	14·42	0·28 (0·16–0·53)
	Combined	115·79	21·50	52·14	1·97	2·36	12·59	0·27 (0·15–0·53)
CV	2011	6·05	9·78	21·20	28·89	22·11	21·51	27·51
	2012	6·53	11·62	18·97	22·76	16·09	18·23	31·22
	Combined	7·14	10·96	20·65	33·48	24·03	23·51	29·97

PH, plant height; INL, length of 3- to 5-internode; BM, biomass of the above-ground stem and head; RW, root weight; SBS, stem-breaking strength; SFM, stem failure moment of culm; LC, lodging coefficient; CV, coefficient of variation. Data in the brackets are the range of lodging coefficients of a cultivar.

Table 3. *Path analysis of LC and its associated traits in foxtail millet cultivars*

Independent variable	*r* (*i*, *y*)	1→*y*	2→*y*	3→*y*	4→*y*	5→*y*	Sum of indirect effects
PH (X1)	−0·18	0·18	0·08	0·15	−0·35	−0·02	−0·14
INL (X2)	−0·24	0·09	0·16	0·06	−0·06	−0·01	0·08
BM (X3)	−0·21	0·06	0·02	0·46	−0·47	0·14	−0·26
RW (X4)	−0·66	0·09	0·01	0·30	−0·72	0·20	0·61
SBS (X5)	−0·83	−0·01	−0·01	0·14	−0·34	0·44	−0·21

PH, plant height; INL, length of basal 3- to 5-internodes; BM, biomass of the above-ground stem and head; RW, root weight; SBS, stem-breaking strength of culm.

correlated with SBS, but INL, BM of the above-ground parts and HW were significantly correlated with SBS ($r = -0·46$, 0·38. and 0·34, respectively, $P < 0·05$ or 0·01) (Table 4). The fresh (INFW) and dry weights (INDW) of the basal internodes, RW, ratio of root to above-ground tissue weight (RC) of Group 1 cultivars were higher than those of Group 2 cultivars ($P < 0·05$). They were significantly correlated with SBS ($r = 0·34$, 0·39 and 0·61, respectively, $P < 0·05$ or 0·001), except for ratio of root to the above-ground tissue weight ($r = 0·06$).

Association between stem-breaking strength and culm anatomical traits

Among the anatomical parameters of the culm examined, only cuticle thickness (CT) and the diameter of the small vascular bundles (DSVB) did not significantly differ between the two groups of cultivars, nor were they correlated with SBS ($r = 0·09$ and −0·01, respectively) (Table 4). The two groups of cultivars displayed significant differences in culm diameter (CD), culm wall thickness (CWT), width of sclerenchyma tissue (WST), numbers of small (NSVB) and large vascular bundles (NLVB), and thickness of the large vascular bundle sheath (TVBS) (Table 4). The mean values of these traits for Group 1 cultivars were greater than those for Group 2 cultivars. These parameters were correlated with SBS, as indicated by their significant correlations ($r = 0·43$ to 0·66, $P \leq 0·01$).

Association between stem-breaking strength and the chemical composition of the culm

The Wiesner staining reaction produced a purple-red colour in lignin-containing structures (Fig. 1(*a*) and (*b*)). The cell walls of sclerenchyma tissues below the epidermis, small vascular bundles, and large vascular bundles within the parenchyma tissues reacted strongly with phloroglucinol–HCl and stained a deep

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Table 4. *Mean values (±S.E.) of different LCs and the correlation coefficients of plant traits, culm anatomical characteristics and lodging associated traits based on all the cultivars across Groups 1 and 2 that differ in LCs*

Trait	Group of cultivars*		Genotype			G × E interaction		Correlation coefficient of plant traits, culm anatomical characteristics and chemical compositions *v.* SBS, CL and CC					
								SBS		CL		CC	
	Group 1	Group 2	*F* value	*P* value	$LSD_{0.05}$	*F* value	*P* value	*R*	*P* value	*R*	*P* value	*r*	*P* value
LC	0·20 ± 0·022	0·38 ± 0·043	471·56	<0·001	0·017	0·71	NS	–	–	–	–	–	–
SBS	3·1 ± 0·47	2·4 ± 0·309	35·58	<0·001	0·267	0·00	NS	–	–	−0·17	NS	0·34	< 0·05
Plant traits													
PH	119 ± 5·0	123 ± 9·6	3·25	NS	5·182	0·55	NS	−0·18	NS	0·14	NS	0·09	NS
LF	60 ± 8·8	62 ± 13·0	1·26	NS	7·527	0·43	NS	−0·12	NS	0·10	NS	−0·30	NS
INL	21 ± 2·0	21 ± 2·9	0·10	NS	1·695	0·11	NS	−0·46	<0·01	−0·18	NS	0·28	NS
BM	51 ± 8·6	48 ± 9·6	1·45	NS	6·154	0·25	NS	0·38	<0·05	0·06	NS	0·29	NS
HW	15 ± 3·9	15 ± 3·3	0·06	NS	2·448	0·47	NS	0·40	<0·05	0·12	NS	−0·05	NS
INFW	8 ± 2·0	6 ± 1·6	12·81	<0·001	1·191	0·87	NS	0·34	<0·05	−0·24	NS	0·30	NS
INDW	2·1 ± 0·26	1·8 ± 0·31	17·04	<0·001	0·192	0·08	NS	0·39	<0·05	−0·42	< 0·05	0·51	< 0·01
RW	2·0 ± 0·25	1·2 ± 0·24	103·23	<0·001	0·168	1·01	NS	0·61	<0·001	−0·06	NS	0·35	< 0·05
RC	0·14 ± 0·057	0·08 ± 0·017	27·23	<0·001	0·028	0·84	NS	0·06	NS	−0·09	NS	0·17	NS
Culm anatomical characteristics													
CD	7·3 ± 0·77	6·5 ± 0·85	14·56	<0·001	0·552	0·37	NS	0·43	<0·01	−0·15	NS	0·20	NS
CWT	2·4 ± 0·44	1·8 ± 0·55	19·01	<0·001	0·336	0·12	NS	0·66	<0·001	−0·19	NS	0·25	NS
CT	75 ± 15·4	79 ± 13·8	0·66	NS	9·909	0·06	NS	0·0908	NS	0·10	NS	0·15	NS
WST	281 ± 44·54	190 ± 33·5	73·45	<0·001	26·697	1·15	NS	0·56	<0·001	−0·30	NS	0·27	NS
DSVB	107 ± 19·2	102 ± 13·9	1·03	NS	11·339	0·26	NS	−0·01	NS	0·03	NS	−0·25	NS
TVBS	67 ± 5·4	41 ± 5·5	342·24	<0·001	3·686	3·59	NS	0·64	<0·001	−0·29	NS	0·40	<0·05
NSVB	7 ± 1·8	5·3 ± 0·97	8·50	<0·01	0·962	0·69	NS	0·44	<0·01	−0·03	NS	0·3163	NS
NLVB	7·3 ± 0·91	5·3 ± 0·75	81·73	<0·001	0·565	0·96	NS	0·50	<0·01	−0·29	NS	0·37	< 0·05
Concentrations of culm chemical compositions													
Lignin	0·27 ± 0·038	0·30 ± 0·047	4·25	<0·05	0·0294	0·44	NS	−0·17	NS	–	–	−0·35	< 0·05
Cellulose	0·52 ± 0·036	0·46 ± 0·030	12·17	<0·01	0·0263	0·17	NS	0·34	<0·05	−0·35	< 0·05	–	–

LC, lodging coefficient; SBS, stem-breaking strength (kg); PH, plant height (cm); LF, leverage force (cm); INL, length of basal 3- to 5-internodes (cm); BM, biomass of the above-ground stem and head (g); HW, head weight (g); INFW, fresh weight of 3- to 5-internodes (g); INDW, dry weight of 3- to 5-internodes (g); RW, root weight (g); RC, ratio of root to canopy; CD, culm diameter (mm); CWT, culm wall thickness (μM); CT, cuticle thickness (μM); WST, width of sclerenchyma tissue (μM); DSVB, diameter of small vascular bundle (μM); TVBS, thickness of large vascular bundle sheath (μM); NSVB, number of small vascular bundle; NLVB, number of large vascular bundles; CL, concentration of lignin (%); CC, concentration of cellulose (%); NS, not significant.

* Group 1: cultivars Canggu 3, Yugu 18, Baogu 19, Jigu 19, Jigu 31 and Yugu 19 with smaller lodging coefficients; Group 2: cultivars Xiaoxiangmi, Baogu 18, Hangtianlvgu, Zheng 07-1, Jigu 18, and 200 475 with greater lodging coefficients.

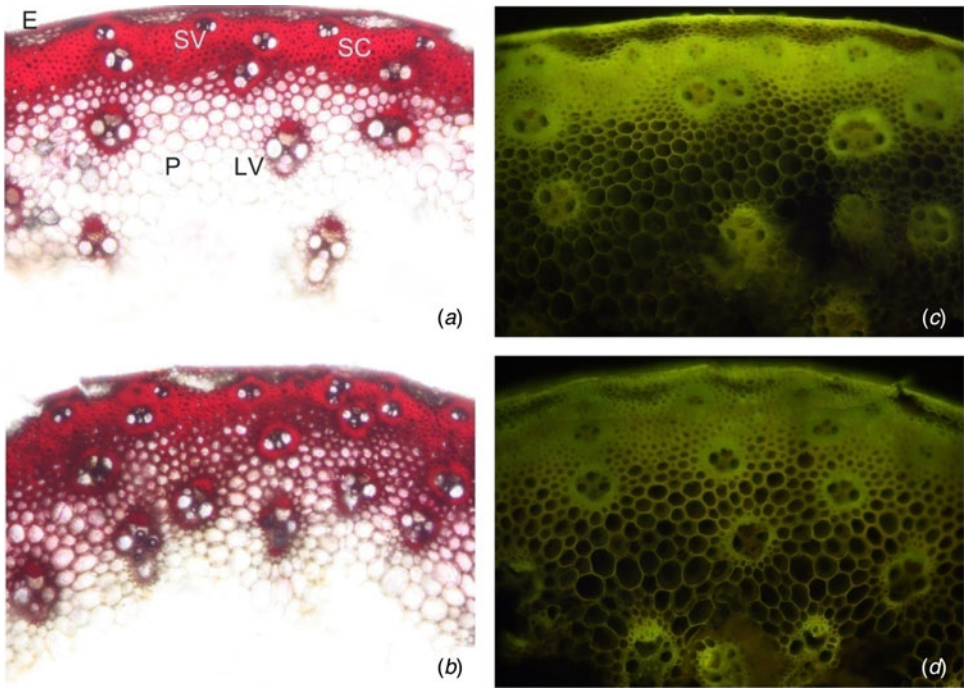

Fig. 1. Wiesner's reaction (A and B) and autofluorescence after calcofluor staining (C and D) showing lignin and cellulose in the foxtail millet cultivars Jigu 19 (LC = 0·20) (A and C) and Xiaoxiangmi (LC = 0·34) (B and D). Lignin in the cell walls of culm tissues were stained purple-red, and cellulose was observed by strong autofluorescent signals. E, epidermis; SC, sclerenchyma; SV, small vascular bundle; LV, large vascular bundle; and P, parenchyma.

purple-red colour, while the parenchyma tissues displayed only a faint purple-red colour. The cultivars with different LCs differed in their responses to the Wiesner reaction for lignin. Group 2 cultivars with greater LCs had stronger reactions to Wiesner reagents than Group 1 cultivars with smaller LCs, as indicated by difference in the intensity and the extent of the areas stained purple-red colour (Fig. 1(*a*) and (*b*)). The fluorescent signals of cellulose were observed in the culm sections after Calcofluor staining. The sclerenchyma layers and large vascular bundles displayed strong fluorescent signals. The intensity of fluorescence for Group 1 cultivars appeared to be stronger than that of Group 2 cultivars (Fig. 1(*c*) and (*d*)).

The mean concentrations of lignin (CL) and cellulose (CC) as determined by the Klason method differed significantly between the two groups of cultivars ($P < 0{\cdot}05$). Group 1 cultivars had a greater mean level of cellulose concentration and a lower mean level of lignin concentration compared to Group 2 cultivars (Table 4). The effects of lignin and cellulose on SBS were different. Lignin concentration was not significantly correlated with SBS ($r = -0{\cdot}17$). In contrast, cellulose concentration was positively correlated with SBS ($r = 0{\cdot}34$, $P < 0{\cdot}05$). The lignin concentration was negatively correlated with the cellulose concentrations ($r = -0{\cdot}35$, $P < 0{\cdot}05$). Both lignin and cellulose concentrations were correlated significantly with the dry weight of internodes, but the direction of the response was reversed ($r = -0{\cdot}42$ and $0{\cdot}51$, $P < 0{\cdot}05$ and $0{\cdot}01$, respectively). Lignin concentration was not correlated with any of the anatomical characteristics of the culm; but the cellulose concentration was correlated with large vascular bundle, in terms of both the thickness of the sheaths and unit number ($r = 0{\cdot}37$ and $0{\cdot}40$, $P < 0{\cdot}05$, respectively).

DISCUSSION

Variation in stem lodging resistance was observed in the foxtail millet cultivars and breeding lines used in the present study, as manifested by their different values for LC, SBS and SFM. Since the LC is determined by SBS, understanding of the relationship between this trait and the morphological, anatomical and carbohydrate compositional properties of the culm will be useful for determining the critical characteristics that are associated with stem-based lodging in foxtail millet. A thorough understanding of lodging in cereal crops has been accumulated from studies on crops such as wheat, rice and barley, but the parameters associated with stem-based lodging of the

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culm in foxtail millet are still poorly understood. Thus, the present study provides the first experimental information on lodging associated traits in this old, domesticated cereal crop.

The association between PH and lodging resistance is clear in wheat (Kelbert *et al*. 2004*b*), although a negative correlation between PH and lodging resistance has been reported in some studies (Crook & Ennos 1994; Navabi *et al*. 2006). The identification and incorporation of dwarfing genes has greatly improved lodging resistance in wheat and rice, leading to the Green Revolution (Khush 2001). In barley, differences in INL have been associated with lodging resistance (Stanca *et al*. 1979; Dunn & Briggs 1989). Reductions in PH might increase lodging resistance by reducing the LF; however some other studies reported that PH was not a dominant factor in lodging resistance (Hua *et al*. 2003; Tripathi *et al*. 2005). Results from both previous and current studies with different genotypes of improved foxtail millet cultivars have demonstrated that PH and INL did not have a major effect on SBS (Tian *et al*. 2010). In the present study, PH, INL and LF of foxtail millet were not correlated with SBS. This indicates that these traits do not directly affect stem-based lodging in improved foxtail millet cultivars. Thus, reducing PH and INL is not a priority for developing lodging-resistant foxtail millet cultivars.

In contrast, the BM of foxtail millet plant, including above-ground BM, HW, fresh and dry weight of basal internodes were significantly correlated with SBS. These BM parameters contribute to the establishment of a stronger plant. Wei *et al*. (2008) and Yao *et al*. (2011) reported that the lodging and SFM in wheat were associated with the dry weight of basal internodes. Similar results were observed in rice (Li *et al*. 2012).

In foxtail millet, the diameter of the basal internodes and CWT were significantly correlated with SBS. This indicates that the cultivars with large culm diameters and thick stem walls may have greater SBS, which in turn, may improve lodging resistance. In other cereal crops such as wheat and rice, the impact of culm diameter and CWT on lodging was inconsistent. A close relationship between culm diameter, as well as CWT, and lodging resistance was reported in wheat and barley (Dunn & Briggs 1989; Zuber *et al*. 1999; Xiao *et al*. 2002), while in other studies, culm diameter was not regarded as an important factor for lodging resistance in rice and barley (Kokubo *et al*. 1989; Kashiwagi & Ishimaru 2004).

Similar to other cereal crops, the structure of the culm of foxtail millet consists of epidermis, sclerenchyma tissues (mechanical tissues), vascular bundles, parenchyma tissues and inner cavities. These structures have different effects on lodging in foxtail millet. The WST, and the number and sheath width of the large vascular bundles played a significant role in improving SBS, whereas the width of epidermis and diameter of the small vascular bundles were not correlated with SBS. In rice, a correlation between vascular bundle number and culm strength was observed (Duan *et al*. 2004). The thickness of sclerenchyma tissue was shown to have a close association with lodging resistance in barley and wheat (Dunn & Briggs 1989; Kelbert *et al*. 2004*b*).

The lignin concentration appears to be less associated with stem-based lodging in foxtail millet, as evidenced by a lack of correlation between lignin concentration and SBS ($r = -0{\cdot}17$). Greater lignin concentration has been associated with more stalk breakage in maize (*Zea mays* L.) (Li, personal communication). The association between the concentrations of lignin and cellulose in culm tissue and lodging resistance was not consistent in wheat. In earlier studies, lignin concentrations and cellulose concentrations were associated with greater lodging resistance in wheat (Zhu *et al*. 2004; Wang *et al*. 2006). When tested in other Chinese wheat genotypes, no significant differences in these chemical components were observed between the genotypes with different lodging resistance (Kong *et al*. 2013).

In conclusion, stem-based lodging in foxtail millet varied among different genotypes. Lodging-resistant cultivars were associated with specific culm characteristics such as more large vascular bundles, wider culm diameter, increased CWT and an increased width of the sclerenchyma ring. These anatomical properties of the culm ensure higher SBS, and in turn, greater resistance to lodging. The concentrations of lignin and cellulose in the cell walls had different effects on SBS in foxtail millet. High levels of cellulose in culm cells were associated with greater SBS. A cultivar with high level of lignin concentration in the culm might still be prone to stem lodging. Stem-breaking strength and greater BM of above- and below-ground tissues were conducive for reducing stem-based lodging. The new findings of the present study on the relationship between the anatomical and chemical properties of the culm and lodging will be useful for the genetic improvement of lodging resistance in foxtail millet.

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