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Relationship between stem characteristics and lodging resistance of Tartary buckwheat (*Fagopyrum tataricum*)

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ABSTRACT

Stem lodging can limit the grain yield of Tartary buckwheat (*Fagopyrum tataricum*), and creates difficulties in harvesting. A 2-year study was conducted to study the relationships between stem characteristics and lodging resistance in Tartary buckwheat. Six Tartary buckwheat cultivars with three stem types (short-stemmed XQ1 and CQ1; mid-length stemmed YQ1 and QK3; and tall-stemmed DAB and DN1) were used. The stem characteristics differed significantly among the three stem types. Mid-length stems had the thickest stem wall, the greatest numbers of large and small vascular bundles, and the lowest stem diameter/stem wall thickness ratio among the three stem types. Mid-length stems had the highest stem breaking strength (SBS) and stem puncture strength (SPS) among the three types of stem. The lignin content was significantly higher in mid-length stems than in tall and short stems. The activities of phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL), and cinnamyl alcohol dehydrogenase (CAD), but not 4-coumarate: CoA ligase (4CL), differed significantly among the three stem types. In correlation analyses, SBS was significantly positively correlated with stem diameter, stem wall thickness, and the number of large and small vascular bundles, but significantly negatively correlated with stem diameter/stem wall thickness and lodging rate. The lignin content was significantly positively correlated with the activities of lignin-related enzymes and with SBS and SPS, and significantly negatively correlated with lodging rate. The present study provides preliminary insights into the relationship between stem traits and lodging resistance in Tartary buckwheat, laying a foundation for a further understanding of lodging resistance.

Abbreviations: SBS: stem breaking strength; SPS: stem puncture strength; PAL: phenylalanine ammonia-lyase; TAL: tyrosine ammonia-lyase; CAD: cinnamyl alcohol dehydrogenase; 4CL: 4-coumarate: CoA ligase.

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Lodging-resistance; stem characteristics; Tartary buckwheat

1. Introduction

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) is an edible and medicinal crop that is native to southwestern China, northern India, Bhutan, and Nepal. The seeds are widely consumed as buckwheat flour, noodles, bread, tea, and vinegar. Tartary buckwheat is one of the most complete and nutritional foods, being rich in minerals, vitamins, protein, dietary fiber, amino acids, trace elements, and various bioactive phytochemicals (Zhao et al., 2012). Thus, there is increasing interest in its health benefits, and farmers are eager to increase the total yields to meet the increasing market demands and to improve overall returns.

The stable production of Tartary buckwheat is difficult because this crop is prone to lodging; that is, the collapse of the stem when it cannot support its own weight. Lodging is an important constraint limiting the yield and quality of

Tartary buckwheat worldwide (Zhao & Shang, 2009). Severe lodging damages or even kills the plant, leading to reductions in photosynthetic ability, grain yield, grain filling, and harvesting efficiency (Berry & Spink, 2012). Many previous studies have suggested that vascular bundles are damaged during lodging by bending or breaking of the stem. Therefore, enhancing the physical strength of the stem is important for lodging resistance (Kashiwagi, Togawa & Hirotsu, 2008; Setter, Laureles & Mazaredo, 1997). Mechanical properties play an important role in determining the physical strength of the stem, which is determined by its microstructure (Duan, Wang & Wang, 2004). Long-term natural and artificial selection of Tartary buckwheat has resulted in adaptation of the structure of its stem to its function (Hepworth & Vincent, 1999; Kashiwagi, Sasaki & Ishimaru, 2005). However, the ability of the Tartary buckwheat stem to withstand wind, rain, and disease

differs among cultivars, as does the seed setting rate and the grain weight. The weight of the stem and grain is the main load carried by the stem (Duan et al., 2004). Intensive cultivation and the widespread use of chemical fertilizer have increased the yield of Tartary buckwheat. Thus, there is a greater load on the stem, which has increased the need for strong mechanical properties (Skubisz, 1996; Tripathi, Sayre, Kaul & Narang, 2004; Zuber et al., 1999). Tartary buckwheat stems with a thick culm wall or a large cross-sectional area show strong lodging resistance (Kashiwagi et al., 2008; Xiang et al., 2014). Strong, lodging-resistant stems are those with thick-walled mechanically supportive sclerenchyma located near the perimeter of stem cross-sections (Aohara et al., 2009; Yao, Ma, Yao & Yang, 2013).

Previous studies have shown that lodging is a complicated phenomenon, because it is closely related both to the structure and the chemical constituents of the stem (Hondroyianni, Papakosta, Gagianas & Tsatsarelis, 2000; Zuber, Grogan, Michaelson, Gehrke & Monge, 1957). Lignin, which is a major structural component of secondary cell walls in vascular plants, is not only associated with plant growth but also provides the plant body with mechanical strength (Ma, 2009). Lignin plays an important role in improving the strength of plant cell walls and increasing the mechanical strength of the stem (Turner & Somerville, 1997). As reported by Boudet, Kajita, Grima-Pettenati and Goffner (2003), phenylalanine ammonia-lyase (PAL), tyrosine ammonia lyase (TAL), 4-coumarate:CoA ligase (4CL), and cinnamyl alcohol dehydrogenase (CAD) play important roles in lignin biosynthesis. Lignin content and the activities of its related enzymes were shown to be closely related to lodging resistance of the stem (Chen et al., 2011; Peng et al., 2014). A low lignin content was shown to be related to the weak mechanical strength of the stem in wheat and oats (Welton, 1928). The wheat cultivars with high resistance to culm snapping and lodging had higher lignin contents and higher activities of PAL, TAL, 4CL, and CAD than did other cultivars (Chen et al., 2011).

Plant morphology and stem chemical components have been used to evaluate lodging resistance in various crops (Berry & Spink, 2012; Chen et al., 2011; Esechie, Rodriguez & Al-Asmi, 2004; Zhang et al., 2014). However, few studies have focused on the importance of the structure and chemical components of the stem in the lodging resistance of Tartary buckwheat. Therefore, the aim of this study was to investigate the relationship between lodging resistance and various parameters (stem morphology, mechanical properties, lignin content, and activities of enzymes related to lignin synthesis) to explain differences in lodging resistance among three kinds of Tartary buckwheat stems. A greater understanding of the factors that

contribute to lodging resistance can provide a theoretical basis for selecting and breeding high-yielding and lodging-resistant cultivars of Tartary buckwheat.

2. Materials and methods

2.1. Site description and experimental materials

The materials were planted in the 2015 and 2016 growing seasons at the farm of Chengdu University (30°65'N and 104°19'E, 495 m altitude), Sichuan Province, China. The soil in the fields is clay loam in texture and alkaline (pH: 7.82) with 51.0, 23.2, and 32.9 mg kg⁻¹ available N, P, and K, respectively; 0.72, 0.49, and 15.9 g kg⁻¹ total N, P, and K, respectively; and 11.7 g kg⁻¹ organic matter. Soil tests were conducted using samples taken from the upper 20 cm of the soil.

The experiment had a randomized block design with three replications. In each growing season, six typical cultivars of tall-, mid-length-, and short-stemmed Tartary buckwheat were used in the field trials. The six cultivars were as follows: short-stemmed XiQiao No. 1 (XQ1, 71.8 cm) and ChuanQiao No. 1 (CQ1, 73.9 cm); mid-length-stemmed YunQiao No. 1 (YQ1, 95.2 cm) and QianKu No. 3 (QK3, 96.7 cm); and tall-stemmed DaAnBen (DAB, 115.8 cm) and DianNing No. 1 (DN1, 118.2 cm) (Song et al., 2017). These six cultivars were obtained from the National Research and Development Center for Coarse Cereal Processing at Chengdu University, and all are currently grown locally. Healthy Tartary buckwheat seeds were surface-sterilized for 5 min in 0.1% potassium permanganate solution and then rinsed four times for 1-min with deionized H₂O. The seeds were soaked in sterilized water at 25° C for 4 h and subsequently planted (hill distance, 20 cm; row spacing, 25 cm). Seedlings were thinned to the final densities (see above) at 15 days after germination. In this study, insects, diseases, and weeds were intensively controlled to avoid yield losses, and synthetic fertilizer (N:P:K = 15:15:15) was applied as basal fertilizer at the rate of 600 kg ha⁻¹. Other management was based on optimized standards for field production. At the full-bloom stage, the plants were cut at the second internode above the ground. Fifteen individual plants each cultivar were sampled and measured each year.

2.2. Lodging behavior and yield measurement methods

The lodging stage was recorded from the seed formation stage until harvesting. The lodging degree ranged from 0 to 5 on the basis of the angle between the top of the stem and the ground, as defined by

Hagiwara, Izusawa, Inoue and Matano (1999) with some modifications as follows: a score of 0 when the angle is 71°–90°, 1 when it is 61°–70°, 2 when it is 41°–60°, 3 when it is 31°–40°, 4 when it is 16°–30°, and 5 when it is 0°–15°. The number of plants showing lodging (L_1) and the total number (L) of Tartary buckwheat plants in each experiment plot were recorded and then used to calculate the lodging rate ($L_1/L \times 100\%$). Tartary buckwheat plants were harvested at the mature stage (at least 70% of grains mature), and seeds were air-dried for 15 days before measuring yield.

2.3. Morphology measurement methods

At the full-bloom stage, a 0.5-cm-wide portion of the stem was cut from the second internode using a scalpel. The stem section was soaked in water for 2–3 h and then fixed overnight in a solution containing 10% formaldehyde, 50% ethanol, and 5% acetic acid in water. After dehydration using an ethanol series (50%, 70%, 95%, and 100%), the stem sections were cleared twice with xylene for 2 h. The stem sections were infiltrated and subsequently embedded in paraffin wax according to the method of Hu, Wang, Zhan, Liu and Hua (2009) before being observed under a microscope. The stem diameter, wall thickness, and number of the large and small vascular bundles were determined. The internode diameter and rind thickness were measured with a Vernier caliper and a micrometer caliper, respectively. The stem wall thickness was calculated as the average of 10 different measurements from one cross-section.

2.4. Mechanical property measurement methods

Five individual field-grown plants of each cultivar were sampled at each time. Three samples were collected (15 individual plants). The second internode was the position for testing of the stem puncture strength (SPS) and stem breaking strength (SBS) by the method of needling (stressed area 1 mm²) and snapping (stressed area 0.5 cm²), which measure the maximum force instantly according to the pressure sensor. The SPS and SBS of each internode were measured using a Stem Strength Tester (YYD-1; The Zhejiang Top Instrument Co. Ltd., Zhejiang, China). Internode pieces of the same length were used for these measurements.

2.5. Determination of lignin content and activities of lignin biosynthetic enzymes

At the full-bloom stage, 15 standing plants of each cultivar were selected randomly from the experimental plot, avoiding the edge rows. The basal second internode was

harvested, frozen in liquid nitrogen, and stored at –80°C until analysis. The lignin content and the activities of PAL, TAL, 4CL, and CAD were measured as described by Chen et al. (2011).

2.6. Statistical analysis

Microsoft Excel 2010 and Microsoft Office Publisher were used to process data and draw figures, and SPSS Statistics 17.0 was used to conduct analysis of variance. The software packages SigmaPlot 12.0, R2.11, and Cytoscape 2.7.0 were used to conduct correlation network analyses and draw figures. Correlation network analyses were conducted to determine the relationship between lodging behavior and various characteristics (lignin content, enzyme activity) of Tartary buckwheat stems.

3. Results

3.1. Lodging behavior and yield

There was no lodging of the Tartary buckwheat cultivars with mid-length stems (YQ1 and QK3) in both seasons (Table 1). Lodging at the filling stage occurred in the cultivars with tall stems (DAB and DN1), leading to a higher lodging degree and lodging percentage than those of short-stemmed cultivars (XQ1 and CQ1) at the mature stage. Compared with the yield of mid-length-stemmed cultivars, the yields of the short-stemmed and tall-stemmed cultivars were decreased by 16.85% and 25.76% (mean of two cultivars and 2 years), respectively.

3.2. Stem structure

The stem structure differed among the three stem types (Table 2), and the patterns were similar in 2015 and 2016. The stem diameters of mid-length stems (YQ1 and QK3) were in the middle of the range of the six tested cultivars, but these cultivars had the thickest stem wall, and significantly more large and small vascular bundles than the other cultivars. The stem diameter/stem wall thickness ratio was highest in the tall-stemmed cultivars (DAB and DN1) and lowest in the mid-length-stemmed cultivars (Table 2).

3.3. Mechanical properties of stem

As shown in Figure 1, the SBS of mid-length stems (YQ1 and QK3) was significantly higher than that of tall stems (DAB and DN1), but not significantly different from that of short stems (XQ1 and CQ1). The highest SPS was in the mid-length stems (YQ1 and QK3; Figure 2), and was significantly higher than that of tall stems (DAB and

Table 1. Lodging behavior and yields of different Tartary buckwheat cultivars.

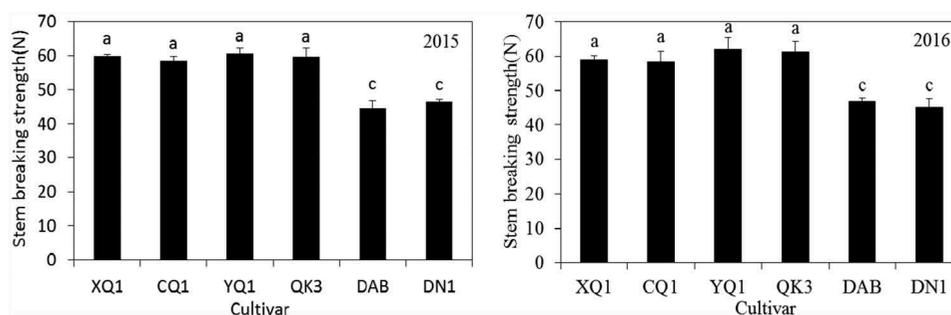
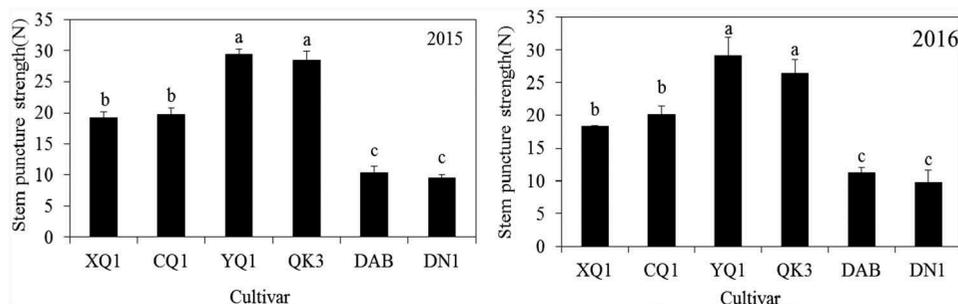
Stem type	Cultivar	Lodging stage		Lodging degree		Lodging rate (%)		Yield (kg/hm ²)	
		2015	2016	2015	2016	2015	2016	2015	2016
Short stem	XQ1	Maturity	Maturity	3	3	9.43 ± 0.57b	9.07 ± 0.61c	1434.4 ± 13.92c	1512.5 ± 12.86c
	CQ1	Maturity	Maturity	3	3	9.80 ± 0.36b	9.50 ± 0.79c	1393.3 ± 12.76d	1413.1 ± 12.29d
Mid-length stem	YQ1	–	–	0	0	0	0	1775.5 ± 19.22a	1790.7 ± 19.98a
	QK3	–	–	0	0	0	0	1669.3 ± 22.77b	1683.2 ± 14.91b
Tall stem	DAB	Filling	Filling	4	4	24.57 ± 2.20a	25.07 ± 1.72a	1261.1 ± 18.39e	1330.4 ± 21.26e
	DN1	Filling	Filling	4	4	23.47 ± 0.80a	23.37 ± 1.12b	1266.6 ± 7.75e	1278.8 ± 12.68f

Data are mean ± SD of three replicates ($n = 3$). Values followed by different letters within a column are significantly different ($p < 0.05$). XQ1: XiQiao No. 1; CQ1: ChuanQiao No. 1; YQ1: YunQiao No. 1; QK3: QianKu No. 3; DAB: DaAnBen; DN1: DianNing No. 1.

Table 2. Stem structure in Tartary buckwheat cultivars.

Year	Item	Cultivar	Stem diameter (mm)	Stem wall thickness	Stem diameter/stem wall thickness	Number of large vascular bundles	Number of small vascular bundles
2015	Short stem	XQ1	5.88 ± 0.03a	3.95 ± 0.06b	1.49 ± 0.02b	27.3 ± 0.78b	38.6 ± 0.97b
		CQ1	5.95 ± 0.23a	3.76 ± 0.30b	1.59 ± 0.06ab	29.1 ± 0.30b	40.7 ± 1.44b
	Mid-length stem	YQ1	5.25 ± 0.14b	4.72 ± 0.19a	1.11 ± 0.05c	34.7 ± 0.97a	46.6 ± 0.95a
		QK3	5.16 ± 0.08b	4.55 ± 0.15a	1.14 ± 0.05c	36.5 ± 0.97a	48.2 ± 1.31a
	Tall stem	DAB	4.49 ± 0.23c	2.75 ± 0.29c	1.64 ± 0.10a	22.3 ± 1.04c	27.6 ± 1.33c
		DN1	4.52 ± 0.19c	2.88 ± 0.05c	1.57 ± 0.06ab	23.4 ± 1.44c	29.4 ± 0.75c
2016	Short stem	XQ1	5.74 ± 0.25a	3.95 ± 0.14ab	1.45 ± 0.06a	29.9 ± 1.27ab	36.2 ± 5.52b
		CQ1	5.85 ± 0.24a	3.52 ± 0.66b	1.69 ± 0.25a	29.5 ± 0.82b	38.3 ± 7.59ab
	Mid-length stem	YQ1	5.17 ± 0.32b	4.58 ± 0.44a	1.13 ± 0.04b	34.3 ± 4.12ab	41.6 ± 4.12ab
		QK3	5.20 ± 0.35b	4.43 ± 0.19a	1.17 ± 0.03b	35.3 ± 4.25a	44.6 ± 3.46a
	Tall stem	DAB	4.27 ± 0.27c	2.69 ± 0.39c	1.60 ± 0.15a	23.3 ± 2.65c	26.0 ± 4.01c
		DN1	4.14 ± 0.13c	2.76 ± 0.22c	1.51 ± 0.09a	23.0 ± 4.20c	28.4 ± 0.66c

Note: Means within a column followed by different letters are significantly different ($p < 0.05$) according to Duncan's multiple range test. ± Standard deviation, $n = 30$. XQ1: XiQiao No. 1; CQ1: ChuanQiao No. 1; YQ1: YunQiao No. 1; QK3: QianKu No. 3; DAB: DaAnBen; DN1: DianNing No. 1.

**Figure 1.** Comparison of stem breaking strength in different Tartary buckwheat cultivars in 2 years.**Figure 2.** Stem puncture strength in different Tartary buckwheat cultivars.

DN1) and short stems (XQ1 and CQ1). Compared with tall-stemmed cultivars (DAB and DN1), the mid-length- and short-stemmed cultivars showed significantly greater SPS (by 178.1% and 89.6% (mean of 2 years), respectively).

3.4. Lignin content and activities of lignin biosynthetic enzymes

As shown in Figure 3, the lignin content of Tartary buckwheat differed significantly among the three kinds of stem, and the patterns were similar in 2015 and 2016. The lignin content was significantly higher in the mid-length stems (YQ1 and QK3) than in the tall stems (DAB and DN1) and short stems (XQ1 and CQ1) (24.81% and 16.39% higher, respectively, in 2015; and 21.90% and 12.25% higher, respectively, in 2016; values averaged over two cultivars in each group). Compared with the tall stems (DAB and DN1), the short stems had significantly higher lignin content (14.27% higher in 2015 and 14.07% higher in 2016). The lignin content of Tartary buckwheat did not differ significantly between the two cultivars of each stem type, and all cultivars showed the same trend in lignin content in both years.

The PAL activity differed significantly among the different stem types, and the patterns were similar in 2015 and 2016 (Table 3). Compared with the tall stems, the mid-length and short stems showed significantly higher PAL

activity (45.7% and 20.0% higher, respectively; mean of two cultivars and 2 years). The PAL activity of mid-length stems (YQ1 and QK3) was significantly higher than that of short stems (18.9% higher in 2015 and 21.2% higher in 2016). The PAL activity did not differ significantly between the two cultivars of each stem type, except for the tall-stemmed cultivars. The highest TAL activity was in mid-length stems (YQ1 and QK3) and was 24.1% and 12.6% higher than that in the tall and short stems, respectively. The lowest TAL activity was in tall stems (DAB and DN1). The TAL activity did not differ significantly between the two cultivars of each stem type, and showed the same trend in both years. The trend in CAD activity among different kinds of stems and cultivars was similar to that of TAL activity. However, 4CL activity did not differ significantly among the three stem types, and showed the same trend in both years.

3.5. Regression analysis

The results of regression analyses indicated that stem diameter, stem wall thickness, number of large vascular bundles, and the number of small vascular bundles were significantly positively correlated with SBS and SPS. The stem diameter/stem wall thickness and lodging rate were significantly negatively correlated with the SBS and SPS (Table 4). The results of these analyses confirmed that these morphological characteristics greatly affect the lodging resistance of Tartary buckwheat.

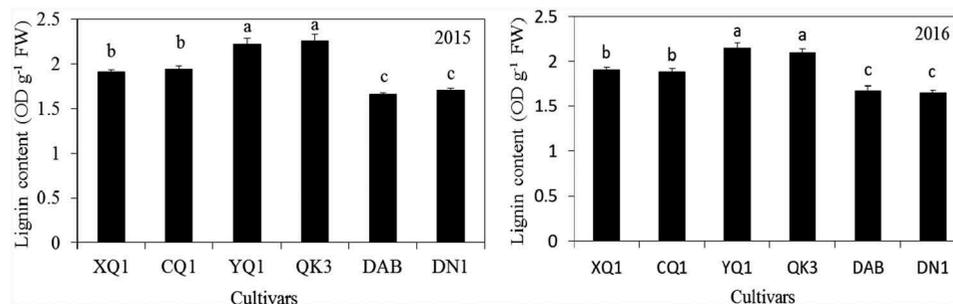


Figure 3. Lignin content in different Tartary buckwheat cultivars.

Table 3. Activities of PAL, TAL, 4CL, and CAD in different Tartary buckwheat cultivars.

Stem type	Cultivar	2015				2016			
		PAL activity	TAL activity	4CL activity	CAD activity	PAL activity	TAL activity	4CL activity	CAD activity
Short stem	XQ1	11.97 ± 0.31b	8.53 ± 0.15b	3.35 ± 0.035a	2.52 ± 0.030b	11.40 ± 0.20b	8.33 ± 0.25b	3.52 ± 0.030a	2.58 ± 0.065b
	CQ1	12.23 ± 0.12b	8.67 ± 0.15b	3.33 ± 0.040a	2.49 ± 0.040b	11.60 ± 0.36b	8.27 ± 0.15b	3.53 ± 0.032a	2.56 ± 0.061b
Mid-length stem	YQ1	14.50 ± 0.30a	9.73 ± 0.21a	3.36 ± 0.045a	3.04 ± 0.066a	14.10 ± 0.20a	9.47 ± 0.21a	3.58 ± 0.030a	3.25 ± 0.040a
	QK3	14.27 ± 0.35a	9.57 ± 0.12a	3.38 ± 0.065a	2.97 ± 0.02a	13.77 ± 0.15a	9.30 ± 0.26a	3.59 ± 0.025a	3.20 ± 0.047a
Tall stem	DAB	9.93 ± 0.15d	8.03 ± 0.21c	3.30 ± 0.032a	2.37 ± 0.051c	9.23 ± 0.15c	7.53 ± 0.31c	3.51 ± 0.075a	2.34 ± 0.035c
	DN1	10.37 ± 0.06c	7.87 ± 0.06c	3.33 ± 0.047a	2.37 ± 0.040c	9.40 ± 0.20c	7.27 ± 0.15c	3.54 ± 0.025a	2.30 ± 0.021c

Data are mean ± SD of three replications ($n = 3$). Values followed by different letters within a column are significantly different ($p < 0.05$). XQ1: XiQiao No. 1; CQ1: ChuanQiao No. 1; YQ1: YunQiao No. 1; QK3: QianKu No. 3; DAB: DaAnBen; DN1: DianNing No. 1. PAL: phenylalanine ammonia-lyase; TAL: tyrosine ammonia-lyase; 4CL: 4-coumarate:CoA ligase; CAD: cinnamyl alcohol dehydrogenase.

Table 4. Regression analyses of mechanical properties and lodging-related traits of Tartary buckwheat.

Item	Stem breaking strength (y_1)	Stem puncture strength (y_2)
Stem diameter	$y_1 = 8.85 + 9.01x_1 (R = 0.8825^{**})$	$y_2 = -14.60 + 6.60x_1 (R = 0.5512^{NS})$
Stem wall thickness	$y_1 = 23.51 + 8.53x_2 (R = 0.9342^{**})$	$y_2 = -16.76 + 9.72x_2 (R = 0.9741^{**})$
Number of large vascular bundles	$y_1 = 20.18 + 1.21x_3 (R = 0.8900^{**})$	$y_2 = -22.65 + 1.44x_3 (R = 0.9768^{**})$
Number of small vascular bundles	$y_1 = 23.90 + 0.84x_4 (R = 0.9104^{**})$	$y_2 = -16.50 + 0.96x_4 (R = 0.9543^{**})$
Stem diameter/stem wall thickness	$y_1 = 83.67 - 20.01x_5 (R = 0.6178^*)$	$y_2 = 61.29 - 29.50x_5 (R = 0.8344^{**})$
Lodging rate	$y_1 = 62.53 - 0.66x_6 (R = 0.9540^{**})$	$y_1 = 27.55 - 0.74x_6 (R = 0.9821^{**})$

* and ** indicate significance at 0.05 and 0.01 probability levels, respectively. NS, nonsignificant at 0.05 probability level.

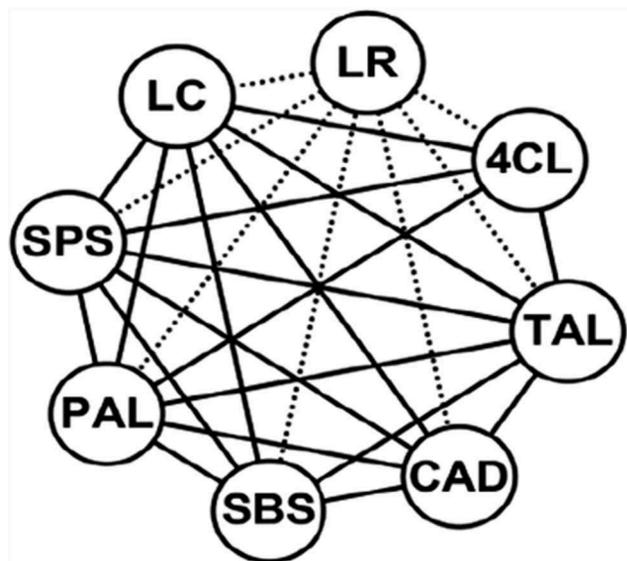


Figure 4. Network of relationships among lignin content, lodging rate, stem mechanical properties, and activities of lignin-related enzymes.

3.6. Correlation network analysis

As shown by the correlation network analysis (Figure 4), strong positive correlations were found between lignin content and the activities of PAL, TAL, 4CL, and CAD. The lodging rate was significantly and negatively correlated with lignin content and the activities of PAL, TAL, 4CL, and CAD. The stem breaking strength was significantly and positively correlated with lignin content and the activities of PAL, TAL, and CAD. The correlation analysis also indicated that SPS was significantly and positively correlated with lignin content and the activities of PAL, TAL, 4CL, and CAD.

4. Discussion

Lodging causes poor grain filling and yield losses, reduces grain quality, and reduces the efficiency of mechanical harvesting (Acreche & Slafer, 2011; Berry & Spink, 2012). Enhancing the physical strength of the basal part of the stem internode is an important target when breeding for lodging resistance. The structure and the chemical

components of stems are very important contributors to the mechanical properties of stems (Ma, 2010; Xiang et al., 2016). In the present study, the Tartary buckwheat cultivars with mid-length stems had the highest lodging resistance and yield (Table 1). Mid-length stems had the thickest stem walls, the greatest number of large and small vascular bundles, and the lowest stem diameter/stem wall thickness ratios among the three kinds of stem (Table 2). A previous study noted that the stem of crop plants includes the internode and burl. The internode is composed of coat, mechanical tissue, thin-walled tissue, and the vascular bundle (Wang et al., 2015a). The large and small vascular bundles are conveyance tissues and also fortify the stem, and the surrounding small vascular bundles protrude outwards to further increase the strength of the stem (Voznesenskaya et al., 2008; Wang et al., 2015a). As shown in Tables 1 and 2, the mid-length stems had a moderate size but the thickest stem wall, resulting in strong lodging resistance.

The stem size, the thickness of the stem wall, the number of vascular bundles, and the structure cannot directly explain the lodging resistance of the stem. Therefore, the SPS and SBS of the three kinds of stems were compared and analyzed. As shown in Table 1 and Figure 1, the SBS and SPS were highest for mid-length stems, and these stems also had the strongest lodging resistance. We detected significant positive correlations between mechanical properties (SPS, SBS) and stem characteristics (stem diameter, stem wall thickness, number of large and small vascular bundles) (Figure 1, 2 and Table 4). On the basis of these results, we concluded that the mechanical properties of Tartary buckwheat stem are mainly decided by stem diameter, stem wall thickness, stem diameter/stem wall thickness, and the number of large and small vascular bundles.

Several studies have reported that lignin is important for the structural integrity of the stem as it provides the plant body with mechanical strength (Berry, Spink, Sterling, Agarwal & Atalla, 2003; Jones, Ennos & Turner, 2001; Ma, 2009). In this study, we found that the lignin content of Tartary buckwheat was positively correlated with the SBS and SPS of stems, suggesting that high lignin accumulation in Tartary buckwheat stems may increase the physical strength of the stem

internode (Figure 4). In this 2-year study, there were significant differences in lignin content among cultivars of Tartary buckwheat with three different stem types (Figure 3). The highest lignin content was in mid-length stems (YQ1 and QK3), and this stem type also showed the minimum lodging rate and the maximum lodging resistance. The correlation analysis revealed that the lignin content of Tartary buckwheat was negatively and significantly correlated with the lodging rate, suggesting that a high lignin content in Tartary buckwheat stem may decrease the lodging rate and increase lodging resistance. Therefore, the lignin content could be used as an index for evaluating the lodging resistance of Tartary buckwheat.

The enzymes PAL, 4CL, CAD, and TAL play key roles in lignin biosynthesis (Campbell & Sederoff, 1996; Khan, Prithiviraj & Smith, 2003; Wajahatullah, Balakrishnan & Donald, 2003). Among them, PAL is a rate-limiting enzyme in the shikimic acid pathway, and it catalyzes the conversion of L-phenylalanine dehydrogenase into *trans* cinnamic acid (Wajahatullah et al., 2003). The conversion of tyrosine to coumaric acid is catalyzed by TAL, and this pathway of lignin biosynthesis only exists in gramineous plants (Chen et al., 2011). The conversion of cinnamic acid into corresponding esters is catalyzed by 4CL, one of the ligases involved in lignin metabolism (Lee, Meyer, Chapple & Douglas, 1997). The final reduction step in lignin synthesis is catalyzed by CAD (Boudet et al., 2003). Our correlation network analysis indicated that the activities of PAL, TAL, 4CL, and CAD were significantly and positively correlated with the lignin content in Tartary buckwheat stems. The lodging rate was significantly and negatively correlated with lignin content and the activities of PAL, TAL, and CAD (Figure 4). Wang et al. (2015a) reported that the lignin content in the buckwheat stem was significantly correlated with the activities of PAL, 4CL, and CAD. However, Chen et al. (2011) found that the lignin content in wheat culm was significantly and positively correlated with the activities of PAL, TAL, and CAD, but not 4CL. High activities of PAL, 4CL, and CAD is the basis for the high lignin content of culm in common buckwheat, and could be responsible for the lodging resistance of common buckwheat (Wang et al., 2015b). Further studies are needed to determine the reason for these differences. Our results suggest that high activities of PAL, TAL, 4CL and CAD resulted in a high lignin content in Tartary buckwheat stem, and ultimately resulted in a lower lodging rate. Lignin biosynthesis is a complex pathway in plant, though it is not yet clear whether a single pathway can explain biosynthesis in all species, all tissues and under varying conditions of environmental stress. Hu et al. (2017) found that the lignin content and PAL, 4CL and CAD activity are closely related to the

lodging resistance of common buckwheat. Tronchet, Balagué, Kroj, Jouanin and Roby (2010) reported that the enzyme involved in the synthesis of lignin was regulated by multigene. The genes *PAL*, *4CL*, *CAD*, *C4H*, *CCoAomt*, *F5H*, *CCR*, *C3H*, and *COMT* were correlated with lignin biosynthesis, and these were different during growth and maturation as well as in different cultivars (Hu et al., 2017). In our research, the TAL was also closely related to the lignin content, which is consistent with previous study with other plants (Rosler, Krekel & Amrhein, 1997). Thus the lodging resistance of Tartary buckwheat could be reflected by the lignin content and the activities of lignin-related enzymes in the stem, but whether other genes were correlated with lignin biosynthesis of Tartary buckwheat needs further investigation.

The previous study revealed that there were significant differences among varieties in lodging resistance (Ghorbannia, Dastan, Mobasser & Ghanbari, 2012). The plant height correlated to lodging severity was previously observed in many cases, reducing plant height will decrease the lodging rate of cultivar, as they diminish the bending stress under unfavorable weather conditions (Tumino et al., 2017). In this study, the cultivars showed different lodging resistance, the type of mid-length stem obtained lower lodging rate and higher yield (Table 1). Hagiwara et al. (1999) also found that the cultivar with long main stem was prior to high-yield and lodging resistance, which should be considered in the breeding programs for improving the productivity of Tartary buckwheat. Berry, Kendall, Rutterford, Orford and Griffiths (2015) found that the reduction of plant height could lead to an increase in grain yield, but further reduction of plant height may negatively affect yield potential. Plant height is a major trait affecting yield potential in rice, higher plant height renders more biomass and yield (Zhang et al., 2017). Therefore, lodging is often a result of the combined effects of inadequate standing power of the crop and adverse weather conditions, such as rain, wind, and/or hail (Tan et al., 2015). Berry et al. (2004) reported that any plants that are initially resistant to lodging may stand erect during favorable conditions, but these plants may fall down when subjected to exceptionally bad weather. Collectively, the mid-length stem cultivars of Tartary buckwheat are easier to obtain high-yield performance and lodging resistance, but the varietal difference of cultivars in lodging resistance needs further validation.

5. Conclusions

The morphological and physiological traits of the stem were found to be closely related to the lodging resistance of Tartary buckwheat. The stem's mechanical properties were mainly decided by its stem diameter,

stem wall thickness, stem diameter/stem wall thickness, the number of large and small vascular bundles, and lignin content. Different Tartary buckwheat cultivars showed a significantly difference in lodging-resistance because of its mechanical properties, especially its lignin content and activities of lignin-related enzymes. Therefore, it may be possible to use these traits of stem to select cultivar that are suitable for lodging-resistance of Tartary buckwheat.

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