BIOLOGIA PLANTARUM 61 (1): 138-146, 2017 DOI: 10.1007/s10535-016-0685-4

The lignin synthesis related genes and lodging resistance of *Fagopyrum esculentum*

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Abstract

Lignin is closely related to the lodging resistance of common buckwheat (*Fagopyrum esculentum* Moench.). However, the characteristics of lignin synthesis related genes have not yet been reported. We investigated the lignin biosynthesis gene expression, activities of related enzymes, and accumulation of lignin monomers during branching stage, bloom stage, and milky ripe stage by real-time quantitative PCR, UV spectrophotometry, and gas chromatography-mass spectrometry in the 2nd internode of three common buckwheat cultivars with different lodging resistance. The results showed that lignin content and the activity of phenylalanine ammonia lyase (PAL), 4-coumarate: CoA ligase (4CL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD) were closely related to the lodging resistance of common buckwheat. Further, we studied gene expression of cinnamate 4-hydroxylase (C4H), caffeoyl-CoA O-methyltransferase (CCoAOMT), ferulate 5-hydroxylase (F5H), cinnamoyl-CoA reductase (CCR), and caffeic acid O-methyltransferase (COMT). The lignin biosynthesis genes were divided into three classes according to their expression pattern: *1*) expression firstly increasing and then descending (*PAL*, *4CL*, *CAD*, *C4H*, *CCoAOMT, F5H*, and *CCR*), *2*) expression remaining constant during maturation (*C3H*), and *3*) expression decreasing with maturation (*COMT*). The present study provides preliminary insights into the expression of lignin biosynthesis genes in common buckwheat, laying a foundation for further understanding the lignin biosynthesis.

Additional key words: common buckwheat, enzyme activities, gene expression.

Introduction

Common buckwheat (*Fagopyrum esculentum* Moench.), a member of the *Polygonaceae* family, has been a popular grain and forage crop for centuries (Gondola and Papp 2010). It is well-known as healthy food because of high content of proteins, amino acids, fatty acids, vitamin B, flavonoids, microelements, and macroelements (Bonafaccia *et al.* 2003.

Lodging limits crop yields and quality around the world (Berry *et al.* 2003) even if a lot of effort has been done to reduce it in major cereal crops, as wheat and rice (Peng *et al.* 2014, Zhang *et al.* 2014). It was reported that lodging is one of the limiting factors of common buckwheat production (Baniya 1990). There is a strong consensus in published literature that increasing lignin content can be used as main indicators to evaluate the lodging resistance of crops (Chen *et al.* 2011, Peng *et al.* 2014). Lignin is a major structural compound of cell wall in vascular plants, which is associated with plant growth and development. It also confers physical strength on a plant body (Ma 2009). It affords stiffness and strength of the stem of plants, which is related to the lodging resistance. The phenylalanine ammonia lyase (PAL), 4-coumarate:CoA ligase (4CL), cinnamyl alcohol dehydrogenase (CAD), and peroxidase (POD) play an important role in the biosynthetic pathway of lignin (Boudet *et al.* 2003). It has also been found that the lignin content and its related enzyme activities are significantly related to lodging resistance of culm in common

Submitted 13 January 2016, *last revision* 17 April 2016, *accepted* 20 April 2016.
Abbreviations: CAD - cinnamyl alcohol dehydrogenase; CCR - cinnamoyl-CoA reductase; CCoAOMT - caffeoyl-CoA *O*-methyltransferase; C3H - *p*-coumarate 3-hydroxylase; C4H - cinnamate 4-hydroxylase; 4CL - 4-coumarate: CoA ligase; COMT - caffeic acid *O*-methyltransferase; F5H - ferulate 5-hydroxylase; PAL - phenylalanine ammonia lyase; qPCR - quantitative PCR.
Acknowledgements: This study was supported by the Fundamental Research Funds for the Central Universities (No. XDJK2016D023). The first two authors contributed equally to this paper.
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buckwheat (Wang *et al*. 2014a,b).

Lignin as complex of racemic aromatic hetero-polymers is derived mainly from three hydroxycinnamyl alcohols: *p*-coumaryl, coniferyl, and sinapyl alcohols (for more detail see Fig. 1 Suppl.). These compounds generate three monomers of lignin: hydroxyphenyl (H), guaiacyl (G) and syringyl (S) type (Boerjan *et al.* 2003). The amount and composition of lignin differ in different species (Ralph *et al.* 2004). Some types, such as those derived from the monomer sinapyl acetate, can make up to 85 % of all S-type in the polymer (Martínez *et al.* 2008). Whereas lignin biosynthesis has drawn increasing research attention in some plant species, only few studies have been done on common buckwheat. This study intends to fill this gap. There are two aims in this study: *1*) understanding the lignin biosynthesis in common buckwheat culm by analyzing lignin biosynthesis gene expression; *2*) exploring the characteristics of the accumulation of the lignin monomer and the ratio of S/G monomers in common buckwheat culm during different growth periods.

Materials and methods

Three common buckwheat (*Fagopyrum esculentum* Moench.) cultivars were sown on 24th February 2014 at the Xiema experimental station, Southwest University, in Beibei, Chongqing, China (19º51′N, 106º37′E). Youqiao2 (YQ2) is a lodging-resistance cultivar, Xinnong1 (XN1) is a moderate lodging resistance cultivar, and Ukraine daliqiao (UD) is a lodging-susceptible cultivar (Wang *et al.* 2014b). Plant samples were collected from the 2nd internode of culm at branching stage, bloom stage, and milky ripe stage and then were frozen at -80 °C. Part of the samples was oven dried at 60 °C for 2 d. After drying, the stem was ground to flour in a mill pulverizer (100 mesh), then extracted for 24 h with hot acetone in a *Soxhlet* apparatus, and dried in air.

The lignin content was measured according to the method reported by Chen *et al.* (2011). Samples (0.5 g) were homogenized in 4 cm^3 of 95 % (v/v) ethanol and then centrifuged at 2 600 *g* for 5 min. Sediments were extracted twice with 95 % ethanol at 80 °C for 2 h and then twice with ethanol in hexane (1:2, v/v) at 62 °C for 1 h. Sediments were then dried at 50 °C for 2 d, digested in 25 % (v/v) acetyl bromide in acetic acid, and incubated in a water bath at 70 °C. After 30 min, samples were mixed with 2 M NaOH, 6 M acetic acid, and 7.5 M hydroxylamine hydrochloride and the absorbance at 280 nm (A_{280}) was measured by a spectrophotometer (*UV-5800PC*, Shanghai, China). The monomer proportions at different growth stages were analyzed by gas chromatography - mass spectrometry. Five technical replicates were conducted on each sample. Thioacidolysis of 5 μg sample was performed as described by Rolando *et al.* (1992). Approximate elution time under our conditions was: 37.8 - 38.5 min for the H-type monomer, 41.2 - 41.8 min for the G-type monomer, and 43.8 - 44.3 min for the S-type monomer.

The PAL activity was assayed according to Assis *et al.* (2001) using 20 mM 1-phenylalanine as substrate. The absorbance was read at 290 nm and one unit of enzyme activity (U) was defined as a change of 0.01 in absorbance per h. The 4CL activity was measured according to Knobloch and Hahlbrock (1975) using 1 μM coenzyme A (CoA-SH) as substrate and U was defined as a change of 0.01 in A_{333} per h. The CAD activity was determined as reported Morrison *et al.* (1994) using 1 M *trans*-cinnamic acid as substrate and U was defined as a change of 0.01 in A_{340} per h. The POD activity was measured as reported Moerschbacher *et al.* (1989) using 50 mM guaiacol as substrate and U was defined as a change of 0.01 in A_{470} per h.

Total RNA was isolated using the *RNeasy Plant Mini* kit (*Qiagen,* Valencia, CA, USA) according to the manufactorer′s instructions. From 1 μg of total RNA, cDNA was synthesized using the *Prime Script TM RT* reagent kit with *gDNA Eraser* (*TaKaRa*, dalian, China) and an oligo (dT) primer. Gene expression was normalized to that of the *histone H3* gene as a housekeeping gene. Real time - quantitative PCR reaction was performed in triplicate on *Step One Plus* (real time PCR system) with the *SYBR® Premix Ex TaqTM II* kit (*TaKaRa*). The PCR protocol was as follows: pre-denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 34 s (the annealing temperature was adjusted according to the different primers), and elongation at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Degenerate primers were designed by using the cDNA that encoded the amino acid conserved sequence known from other species, then, the PCR products of degenerate primers were subcloned into a *TOPOTA* vector (*Invitrogen*) and sequenced at *Sangon Biotech* (Shanghai, China). The cDNA of *F. esculentum PAL* (HM149783.1), *4CL* (HM149785.1), *C4H* (HM149784.1), and *C3H* (KC404850.1) were isolated previously. Sequence similarities were calculated with the Basic Local Alignment Search Tool (*BLAST*). The sequence data were used to design new primer pairs for the RT-qPCR (Tables 2 and 3 Suppl.).

Analyses of variance were performed with *SPSS v.19.0* (*SPSS Institute*, Chicago, USA), and data from each sampling date were analysed separately. The significance of differences between means were evaluated by least significant difference test at $P < 0.05$. The relative gene expression was calculated by $2^{-\Delta\Delta Ct}$ method (Yang *et al.* 2012).

Results

The cloned genes (*CAD*, *F5H*, *COMT*, *CCOAOMT*, and *CCR*) were aligned and compared with orthologous sequences from other plants. The 443-bp *F. esculentum CAD* showed 76.1 % amino acid sequence identity to *Arabidopsis thaliana*, 81.6 % to *Glycine max*, 80.9 % to *Morus notabilis*, 80.9 % to *Populus tomentosa*, and 79.5 % to *Populus tremuloides*. The 1108-bp *F. esculentum F5H* displayed 62.4 % amino acid sequence identity to *Gossypium raimondii*, 58.9 % to *Leucaena leucocephala*, 52.9 % to *Phoenix dactylifera*, and 59.5 % to *Trapa bicornis*. The 641-bp *F. esculentum COMT* exhibited 53.1 % amino acid sequence identity to *Elaeis guineensis*, 57.2 % to *Hibiscus cannabinus*, 58.1 % to *Prunus mume*, 60.6 % to *Theobroma cacao*, and 57.2 % to *Vitis vinifera*. The 436-bp *CCOAOMT* showed 91.0 % amino acid sequence identity to *Camellia sinensis*, 90.3 % to *Dimocarpus longan*, 91.0 % to *Eucalyptus camaldulensis*, 91.7 % to *G. raimondii*, 91.7 % to *Lonicera japonica*, and 95.1 % to *V. vinifera*. The 199-bp *F. esculentum CCR* showed 51.5 % amino acid sequence identity to *Populus euphratica*, 53.1 % to *P.tomentosa*, 53.1 % to *P. tremuloides*, 50.0 % to *Populus trichocarpa*, 51.5 % to *P. mume*, and 58.7 % to *V. vinifera* (Figs. 2 to 6 Suppl.).

The lignin content progressively increased from branching to milky ripe stage and the increases were 112.0, 129.6, and 109.2 % for cvs. YQ2, XN1, and UD, respectively. The lignin content was significantly different among cultivars in every stage. For example, the lignin content in YQ2 was 1.65, 1.89, and 1.52 times higher than in XN1 and 2.73, 2.52, and 2.75 times higher than in UD during branching stage, bloom stage, and milky ripe stage, respectively (Table 1). Total monomer yields increased gradually from branching stage to milky ripe stage by 38.9, 108.3, and 89.2 % for YQ2, XN1, and UD, respectively. The H-G-S composition of the lignin was different in every stage. The H-type monomer decreased from branching stage to milky ripe stage about 20.5, 24.1, and 23.5 % for YQ2, XN1, and UD, respectively, whereas the S-type and G-type monomers

Table 1. Lignin content and the activities of related enzymes in different buckwheat cultivars at three growth stages. Means ± SEs, $n = 3$. Different lowercase and uppercase letters indicate significant difference at $P < 0.05$ and $P < 0.01$, respectively. YQ2, XN1, and UD represent the cultivars Youqiao2, Xinnong1, and Ukraine Daliqiao, respectively.

Stage	Cultivar	Lignin content [A_{280} g^{-1}(f.m.)]	PAL activity [U mg^{-1}(f.m.)]	4CL activity [U mg^{-1}(f.m.)]	CAD activity [U mg^{-1}(f.m.)]	POD activity [U mg^{-1}(f.m.)]
Branching stage	YQ2	1.24 ± 0.03 aA	6.81 ± 0.11 aA	10.29 ± 0.05 aA	0.85 ± 0.04 aA	13.18 ± 0.07 aA
	XN1	0.75 ± 0.04 bB	5.85 ± 0.07 bB	8.52 ± 0.05 bB	0.69 ± 0.04 bB	10.66 ± 0.08 bB
	UD	0.46 ± 0.03 cC	5.11 ± 0.05 cC	6.72 ± 0.06 cC	0.52 ± 0.03 cC	7.95 ± 0.10 cC
Bloom stage	YQ2	2.20 ± 0.03 aA	8.14 ± 0.08 aA	12.06 ± 0.08 aA	1.31 ± 0.03 aA	16.26 ± 0.12 aA
	XN1	1.16 ± 0.05 cB	6.89 ± 0.07 bB	10.35 ± 0.07 bB	0.90 ± 0.04 bB	12.62 ± 0.12 bB
	UD	0.87 ± 0.04 dC	5.92 ± 0.06 cC	7.90 ± 0.06 cC	0.62 ± 0.04 cC	9.77 ± 0.09 cC
Milky ripe stage	YQ2	2.62 ± 0.03 aA	6.08 ± 0.05 aA	9.75 ± 0.07 aA	0.68 ± 0.04 aA	12.00 ± 0.06 aA
	XN1	1.73 ± 0.07 bB	5.21 ± 0.07 bB	7.30 ± 0.04 bB	0.52 ± 0.04 bB	8.51 ± 0.06 bB
	UD	0.95 ± 0.06 cC	4.58 ± 0.05 cC	6.03 ± 0.06 cC	0.43 ± 0.03 cC	6.94 ± 0.08 cC

Table 2. Proportion of lignin monomers measured by thioacidolysis reaction. H, G, and S represent *p*-hydroxyphenyl, guaiacyl, and syringyl lignin monomers, respectively. Means ± SEs, $n = 5$. YQ2, XN1, and UD represent the cultivars Youqiao2, Xinnong1, and Ukraine Daliqiao, respectively.

Stage	Cultivar	H monomer [μmol g^{-1}(d.m.)]	G monomer [μmol g^{-1}(d.m.)]	S monomer [μmol g^{-1}(d.m.)]	S/G ratio	Total monomers [μmol g^{-1}(d.m.)]
Branching stage	YQ2	2.29 ± 0.25	17.92 ± 2.14	54.17 ± 4.36	3.02	75.38
	XN1	1.45 ± 0.14	8.89 ± 1.23	31.50 ± 3.59	3.54	41.84
	UD	1.32 ± 0.11	10.01 ± 1.04	23.01 ± 3.15	2.30	34.34
Bloom stage	YQ2	2.06 ± 0.25	30.29 ± 3.89	56.84 ± 5.25	1.87	89.19
	XN1	1.36 ± 0.15	22.35 ± 2.74	43.91 ± 3.95	1.96	67.62
	UD	1.21 ± 0.12	13.09 ± 1.08	25.31 ± 2.11	1.93	39.61
Milky ripe stage	YQ2	1.82 ± 0.24	40.55 ± 4.09	62.36 ± 6.54	1.54	104.73
	XN1	1.10 ± 0.13	34.15 ± 4.11	51.91 ± 5.58	1.52	87.16
	UD	1.01 ± 0.09	24.22 ± 2.89	39.74 ± 4.16	1.64	64.97

increased from branching stage to milky ripe stage by 126.3, 284.1, and 142.0 % for the G-type, and 15.1, 64.8, and 72.7 % for the S-type, respectively. The S/G ratios were 3.02, 3.54, and 2.30 during branching stage; 1.88, 1.96, and 1.93 during bloom stage; and 1.54, 1.52, and 1.64 during the milky ripe stage for YQ2, XN1, and UD, respectively. The ratio of S/G had no obvious importance for lodging resistance in these cultivars. In all stages, the S-type monomer was the most abundant and H-type monomer content was very low. The amounts of the S-type, G-type, and H-type monomers were the highest in YQ2, and the lowest in UD (Table 2).

The PAL, 4CL, CAD, and POD activity increased and then decreased from branching to milky ripe stages, especially decreased sharply from bloom to milky ripe stage. These enzyme activities differed significantly among cultivars in all stages, they were significantly higher in the lodging-resistance cv. YQ2 than in the lodging-susceptible cv. UD and moderate lodging resistance cv. XN1. For example, the PAL activity of YQ2 was 1.17 and 1.35 times higher than that of XN1 and UD, respectively; the corresponding ratios were 1.23 and 1.56 for the 4CL activity, 1.35 and 1.81 for the CAD activity, and 1.30 and 1.68 for POD activity (Table 1).

Expressions of nine genes (*PAL, 4CL, C4H, C3H, CAD, CCR, F5H, COMT,* and *CCoAOMT*) were normalized to that of the *histone H3* as a housekeeping gene. The expression of *PAL, 4CL, C4H, CAD, CCoAOMT, F5H*, and *CCR* reached the maximum at the bloom stage. The expression of *COMT* decreased from branching to the milky ripe stage. The *C3H* expression changed with no obvious regularity (Fig. 1*A*,*B*,*C*).

At branching stage, the nine gene expressions were the highest in YQ2 and the lowest in UD except for *C4H*, *C3H*, and *COMT*. The expression of *COMT* was the highest in UD and the lowest in YQ2, and the expression of *C3H* was the highest in XN1. *4CL*, and *C4H* had higher expression than other genes. The expression of *CAD* and *F5H* was lower than the expression of other genes, and the *F5H* expression was the lowest.

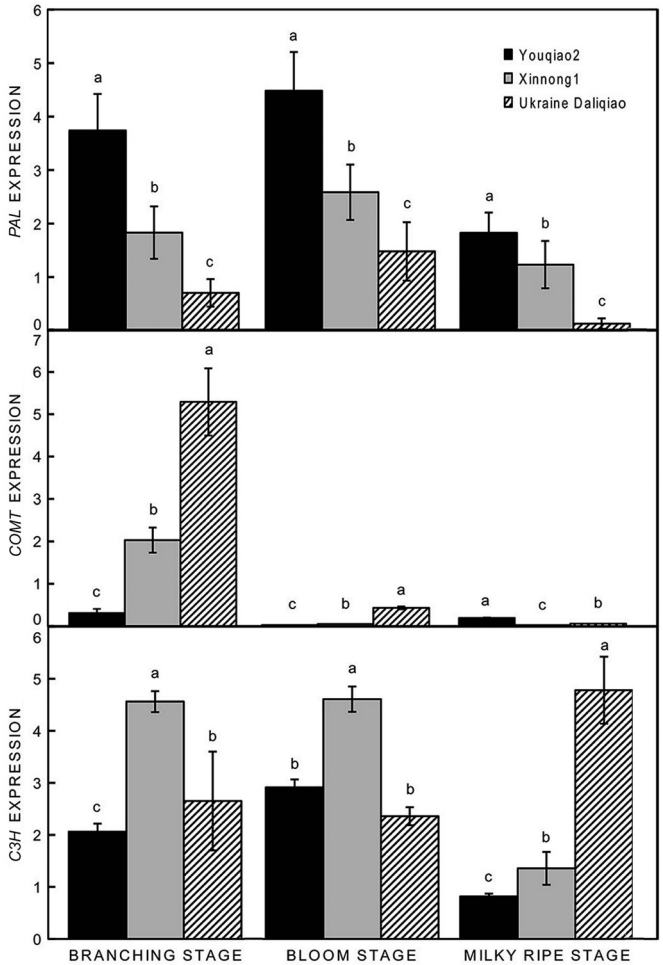

Fig. 1*A*. Expressions of *PAL*, *COMT*, and *C3H* genes related to lignin synthesis in the culm of different common buckwheat cultivars analyzed by real-time quantitative polymerase chain reaction. Means ± SEs (n = 3). Different lowercase letters indicate significant difference at $P < 0.05$.

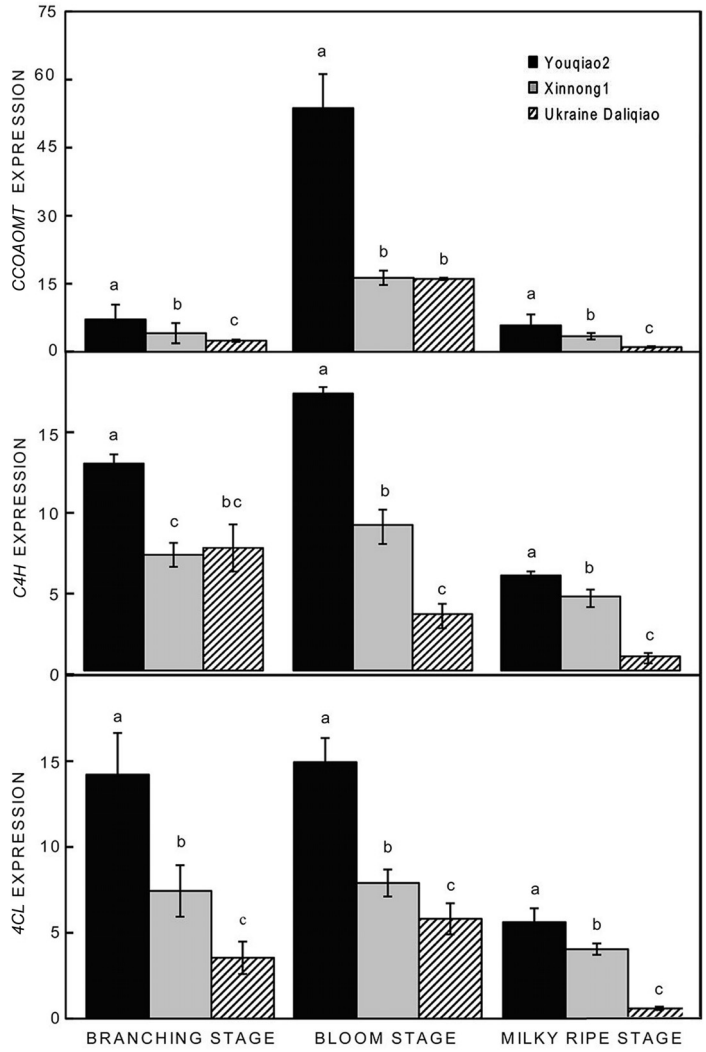

Fig. 1*B*. Expressions of *CCoAOMT*, *C4H*, and *4CL* genes related to lignin synthesis in the culm of different common buckwheat cultivars . Means ± SEs (n = 3). Different lowercase letters indicate significant difference at $P < 0.05$.

At bloom stage, the expressions of *PAL, 4CL, C4H, CAD, CCoAOMT, F5H*, and *CCR* were similar as in branching stage. The expression of *COMT* was the highest in UD and lowest in YQ2 and the expression of *C3H* was highest in XN1. However, the expression of *CCoAOMT* increased more sharply in this growth stage in comparison with other genes in all buckwheat cultivars and especially in YQ2. In contrast, the expression of *COMT* decreased sharply in this growth stage in all buckwheat cultivars, but expression in UD was significantly higher than in other cultivars.

At milky ripe stage, the expressions of all genes were significantly lower than in bloom stage. The expression of *C3H* was the highest in UD and the lowest in YQ2, and the expression level of *COMT* was the highest in YQ2 and the lowest in UD.

Discussion

Lignin plays a key role in improving the strength of plant cell walls, and lignin content has been found to be closely related to the culm rigidity (Turner and Somerville 1997). The occurrence of lodging in wheat, rice, corn, and miscanthus has been proved causing by shortage of lignin (Hondroyianni *et al.* 2000, Kaack *et al.* 2003, Wei *et al.* 2008, Okuno *et al.* 2014). In addition, increase in lignin content significantly improves the mechanical properties of culm, which further increases the lodging resistance (Chen *et al.* 2011, Peng *et al.* 2014). This result shows that the cv. YQ2 with strong lodging-resistance had higher lignin content compared with cv. XN1 with moderate lodging resistance cultivar and lodging-susceptible cv. UD. Thus, the lignin content can be used

as an important indicator to evaluate lodging resistance of common buckwheat, which is consistent with previous studies with other species.

The lignin composition and structure were determined by the availability of monomers and the type of bonds formed during the polymerization. It was reported that the H-type is deposited at first, followed by the G and then S types in *Eucalyptus globulus* (Rencoret *et al.* 2011). In this research, the ratio of S/G and the amount of H-type monomer decreased from branching stage to milky ripe stage in all samples. The result indicates that the H-type is deposited first, followed by the S and then G types. An increase of lignin S/G ratio during plant maturity has also been reported in non-woody fibers (Del Río *et al*. 2010). The wild type poplar exhibits an S/G ratio of 70:30, which is typical for many deciduous trees (Robinson and Mansfield 2009). Besides, the lignin of *Brassica napus* is constituted by S and G types lignin, the proportion of the S-type monomer is small in bud stage, but it increases gradually and becomes the main monomer after flowering stage (Huang 2013). Thus, the decreased S/G ratio during development of common buckwheat is inconsistent with *Brassica napus*. In addition, the cv. YQ2 with strong lodging-resistance had high G and S monomer content compared with less resistant XN1 and UD. This result shows that the G and S monomers also exhibit the positive impact on lodging resistance. It has been reported that the degree of lignin polymer cross-linking is determined by the S/G monomer ratio and an increase in G-type monomer results in a greater degree of cross-linking (Ferrer *et al.* 2008). This has led to speculation that a reduction in S-type monomer (with a relative or actual increase in the G-type monomer) will increase lodging resistance (Angela *et al.* 2010, Eduardo *et al.* 2012). Whether the decrease in S/G ratio will increase the lodging resistance of common buckwheat

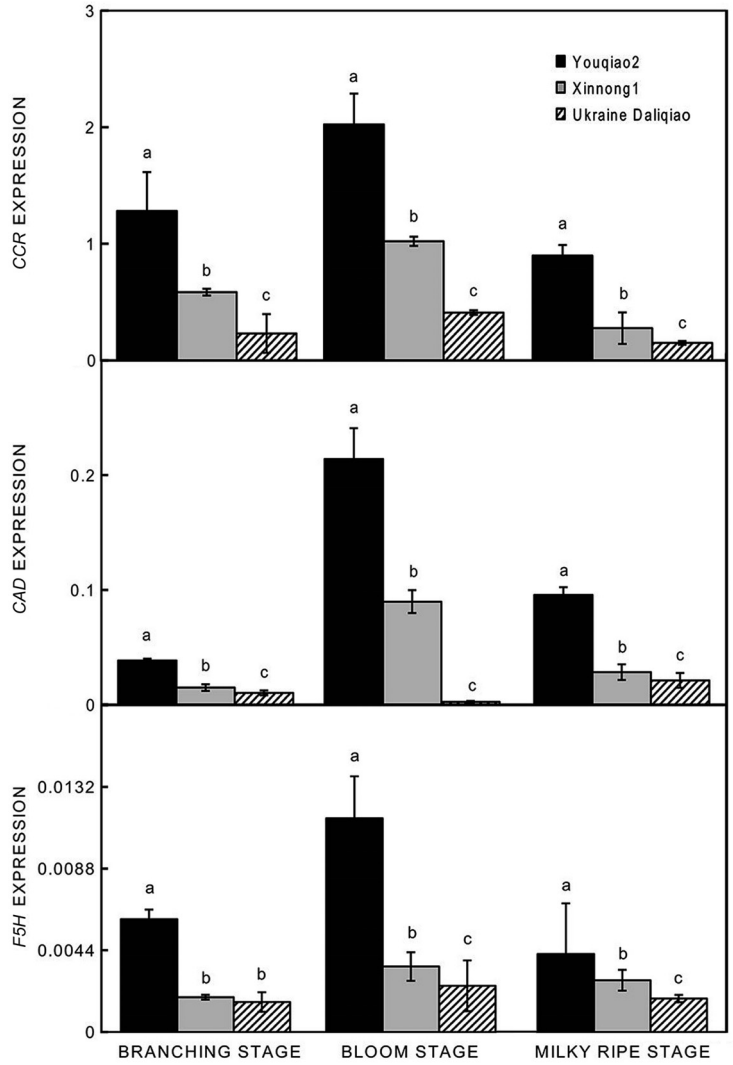

Fig. 1*C*. Expressions of *CCR*, *CAD*, and *F5H* genes related to lignin synthesis in the culm of different common buckwheat cultivars. Means ± SEs ($n = 3$). Different lowercase letters indicate significant difference at $P < 0.05$.

needs further validation.

Lignin biosynthesis is a complex pathway in plants, though it is not yet clear whether a single pathway can explain biosynthesis in all species and all tissues under varying environmental conditions. In the case of lignin biosynthesis, the expression of genes and the composition of lignin monomers are important for the genetic modifications of plants. A general pathway for lignin biosynthesis has been inferred from studies of specific steps in several diverse species. The combination of lignin biosynthesis pathway gene expression with lignin monomer deposition in common buckwheat is not currently available. Nine genes (*PAL, 4CL, C4H, C3H, CAD, CCR, F5H, COMT,* and *CCoAOMT*) were analyzed in this study.

The expression patterns of lignin biosynthesis genes in common buckwheat stem could be divided into three types: *1*) expression offered upgrade from branching to bloom stage and then descending to milky ripe stage (*PAL*, *4CL*, *CAD*, *C4H*, *CCoAOMT*, *F5H*, and *CCR*), *2*) expression remained relatively constant during maturation (*C3H*), and *3*) expression decreased during plant maturation (*COMT*).

The increase in *CCoAOMT* and *CAD* expressions in full bloom stage was mainly observed in YQ2. CCoAOMT was responsible for the 3' methylation of caffeoyl-CoA to produce feruloyl-CoA (Raes *et al.* 2003, Hisano *et al.* 2009), a key step in the production of G-type and S-type lignin monomers. When the expression of *CCoAOMT* in transgenic plants is inhibited, the lignin content and S/G ratio increases and G-type lignin content is reduced (Guo *et al.* 2001, Zhao *et al.* 2004). In antisense *CCoAOMT* transgenic alfalfa, the lignin content and G-type lignin decrease, but S-type lignin biosynthesis is unaffected (Guo *et al.* 2001). We found that the ratio of S/G decreased in full bloom stage in common buckwheat, which indicates that up-regulation of *CCoAOMT* induced an increase of G-type monomer content. Moreover, this confirms that CCoAOMT should be a bottleneck in the pathway to G-type lignin synthesis, which is consistent with previous studies.

The CAD represents the final enzyme in the lignin biosynthesis pathway catalysing the production of precursors monolignols and committing them to H, G, and S lignin monomer synthesis (Halpin 2004, Ferrer *et al.* 2008, Vogt 2010). Contrary to our results, Hua *et al.* (2013) has found that *CAD* expression decreases before stem tissue maturation in *Ginkgo biloba*, whereas Casu *et al.* (2007) has found that *CAD* expression in sugarcane increases with tissue age. In our research, the CAD activity and *CAD* expression was significantly different among cultivars, but extremely low in all of them.

The expression of *PAL*, *4CL*, *C4H*, *F5H*, and *CCR* was significantly different in different cultivars. The PAL is the first rate-limiting enzyme in the shikimic acid pathway. Kolahi *et al.* (2013) has found that *PAL* expression in sugarcane increases with stem maturity. High *PAL* expression and PAL activity in young common buckwheat stems found in this study may represent an initial burst of metabolites of the various phenyl-propanoid pathways including lignin biosynthesis. The 4CL represents an important branch point where metabolites are directed into lignin biosynthesis or alternative phenylpropanoid biosynthesis pathways (Vogt 2010, Weng and Chapple 2010). Its position allows direct metabolite contribution into H-type monomer biosynthesis or redirection of metabolites for G-type or S-type monomer biosyntheses (Vogt 2010). The high *4CL* expression and 4CL activity in young common buckwheat stem may reflect its response to the metabolic flux into the phenylpropanoid pathway initiated by PAL. The C4H catalysed the hydroxylation of the phenyl-propanoid skeleton after its deamination by PAL (Ferrer *et al.* 2008, Weng and Chapple 2010). Papini-Terzi *et al.* (2009) has found no difference in *C4H* expression in young and maturing sugarcane stem, however, the expression of *C4H* decreased in maturing common buckwheat stem. Another hydroxylase F5H is essential for the S-type monomer branch of the lignin biosynthesis pathway and its expression is necessary for the production of S lignin monomers (Weng and Chapple 2010). The expression pattern of *F5H* may ensure continued flow of metabolites for S lignin monomer production. However, in our study, the expression of *F5H* was very low in all cultivars, and further research is needed to prove whether other genes of this family participate in S lignin synthesis in common buckwheat. The CCR functions in the final stages of lignin biosynthesis and is considered to be a key committed step in the production of the H, G, and S lignin monomers (Vogt 2010, Weng and Chapple 2010). To a lesser degree, *CCR* shares the same expression patterns as *PAL* and *4CL*. High CCR expression in young stems of the lodging-resistant cultivar may ensure a high metabolite commitment into lignin biosynthesis, which is fundamentally important for healthy plant development.

The expression of *C3H* showed little change during the growth of common buckwheat. The C3H catalysing the second aromatic hydroxylation reaction in the lignin biosynthesis pathway (Weng and Chapple 2010) is an important point in controlling metabolic flux into G-type and S-type lignin monomer synthesis (Barrière *et al.* 2004). The steady expression of *C3H* in the maturing common buckwheat stems may reflect continued requirement for feruloyl residue production for ongoing cell wall lignification and not just its role in lignin monomer biosynthesis.

The COMT is the last of the two enzymes entirely responsible for the production of the S-type lignin monomer within the lignin biosynthesis pathway (Bonawitz and Chapple 2010, Weng and Chapple 2010). In this study, *COMT* expression decreased with stem maturity, which is in contrast to the findings of Casu

et al. (2004) and Papini-Terzi *et al.* (2009) who reported increased *COMT* expression during sugarcane stem maturation. Simultaneously, the proportion of the S-type monomer decreased, and the lignin content changed little indicating that the expression of *COMT* mainly ensures S-type monomer production in young common buckwheat stems.

Conclusion

The lignin content and PAL, 4CL, CAD, and POD activities were closely related to the lodging resistance of common buckwheat. H-type lignin was deposited firstly, followed by S- and then G-types. G- and S-type monomers were the main monomers, and the amount of S-type monomer was the highest. Thus, the G and S monomers could exert positive impact on lodging resistance. The patterns of the expression of lignin biosynthesis related genes for *PAL*, *4CL*, *CAD*, *C4H*, *CCoAOMT, F5H, CCR, C3H*, and *COMT* differed during growth and maturation as well as in different common buckwheat cultivars. The results of our study are essential for evaluating the plant lodging-resistance. In future, they may facilitate breeding and genetic engineering in common buckwheat.

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