WOOD RESEARCH doi.org/10.37763/wr.1336-4561/66.5.833843
e-ISSN 2729-8906
66(5): 2021 833-843 pp.

COMPARATIVE PROTEOMIC ANALYSIS OF THE THICK-WALLED RAY FORMATION PROCESS OF *HALOXYLON AMMODENDRON* IN THE GURBANTUNGGUT DESERT, CHINA

CHAOBIN ZHOU
ZUNYI NORMAL COLLEGE
CHINA

JUNJIE DING
XINJIANG ACADEMY OF AGRICULTURAL AND RECLAMATION SCIENCE
CHINA

XIAOJING HU
XINJIANG AGRICULTURAL UNIVERSITY
CHINA

WEI GONG
SICHUAN AGRICULTURAL UNIVERSITY
CHINA

(RECEIVED DECEMBER 2020)

ABSTRACT

Thick-walled ray cells of *Haloxylon ammodendron* were first reported by Zhou and Gong in 2017, but their formation mechanism remains unknown. In this study, we performed a proteomic analysis of ray cell wall formation in the xylem. *H. ammodendron* in Shihezi exhibits a thicker ray cell wall than that in Jinghe. During the process of cell wall biosynthesis in the xylem of *H. ammodendron*, the nonspecific lipid-transfer protein and beta expansin EXPB2.1 (*Mirabilis jalapa*) first loosen the cell wall, and this step is followed by extension and expansion. Subsequently, xyloglucan endotransglycosylase/hydrolase 1 cleaves and links the xyloglucan chains. Photosystem I P700 apoprotein A1, reversibly glycosylated polypeptide 1 and GDP-mannose-3′,5′-epimerase are involved in the cellulose, hemicellulose and pectin biosynthesis processes in the cell wall by providing components or energy. Finally, the proteins involved in phenylpropanoid biosynthesis promote lignification of the ray cell wall and complete the biosynthetic process of the cell wall.

KEYWORDS: Xylem special character, isobaric tag for relative and absolute quantitation, differentially expressed proteins, phenylpropanoid biosynthesis, ray cell.

INTRODUCTION

Haloxylon ammodendron is an important afforestation species in the arid desert regions in both Asia and Africa. Previous studies have found that the xylem of *H. ammodendron* has ray tissues, that these tissues are uniseriate or multiseriate with a height of 53.80 μm - 434.85 μm and a width of 7.60 μm - 87.15 μm, that the wall of ray cells is clearly thickened in the xylem of *H. ammodendron*, and that the wall thickness of the ray cell can reach 2.85 μm - 3.08 μm, which is 3 - 6 times the thickness of axial parenchyma, slightly higher than that of fibre (2.64 μm - 2.97 μm) (Zhou and Gong 2017), and markedly higher than that of fibre in *Salix psammophila* (a xerophytic deciduous shrub in Kubuqi Desert, China) (Zhou et al. 2017). In general, ray tissue is composed of parenchyma cells, and the wall thickness of ray cells is markedly thinner than that of fibre cells in most species (Plavcová and Jansen 2015). However, thick-walled ray cells have been found in the parenchyma of some species, such as *Melia azedarach* and *Symbolanthus macranthus* (Carlquist and Grant 2005). The mechanical properties of tissues with thickened walls are enhanced (Alves and Angyalossy-Alfonso 2002, Xi 2018). Therefore, research on the regulatory proteins (genes) involved in the cell wall formation process will be important for both understanding the environmental adaptability of xylem characteristics and improving the wood quality in plantations.

With the development of genomics and molecular genetics, research on plant cell wall formation has achieved good progress in recent years. Studies have mainly focused on *Arabidopsis thaliana* (Taylor et al. 2003), *Populus trichocarpa* (Suzuki et al. 2006), *Picea sitchensis* (Bong.) Carr. (Fernandes et al. 2011), and *Gossypium hirsutum* (Pear et al. 1996). Although many studies have investigated cell wall biosynthesis, the content and structure of the cell wall components vary among species and tissues, which leads to diversity and complexity in the cell wall composition (Burton et al. 2010). Therefore, the biosynthetic pathway of the cell wall in specific tissues of different species remains to be studied.

Thus, the proteomic characteristics of the process of ray cell wall formation in *H. ammodendron* were studied. GO annotation analysis combined with KEGG pathway enrichment and other bioinformatics methods was performed to explore the differentially expressed proteins and metabolic pathways related to ray cell wall formation in this plant.

MATERIALS AND METHODS

Sample location and sampling

The climate and growth characteristics of *H. ammodendron* plantations in the sampling area were described by Zhou and Gong (2017). At the end of June 2017, samples were collected at the Jinghe (82°53′35″E, 44°36′10″N) and Shihezi (86°14′44″E, 45°00′34″N) Desert Research and Experimental Station of Shihezi University in the Gurbantunggut Desert, Xinjiang, China. The identification of *H. ammodendron* was performed according to the morphology using

a website (http://www.iplant.cn/info/Haloxylon%20ammodendron) built by the Institute of Botany, Chinese Academy of Sciences. Perennial branches of *H. ammodendron* with a diameter of approximately 1 cm were collected. The bark, phloem and cambium were scraped from the branches, and a blade sterilized with anhydrous ethanol was used to scrape the xylem. The samples were wrapped in aluminium foil, placed in liquid nitrogen, rapidly cooled, transported to the laboratory and stored in a refrigerator at -80°C.

Observation and measurement of the wall thickness of ray cells

Observations under a light microscope and measurements of the wall thickness of ray cells of *H. ammodendron* were performed as described by Zhou and Gong (2017).

Scanning electron microscopy (SEM)

The xylem of *H. ammodendron* was cut into blocks of 1 x 1 x 1 cm, air dried and polished to a smooth surface. The samples were dehydrated using a graded ethanol series and dried using the liquid CO_2 critical point method. The sample was soaked in 98% H_2SO_4 for 5 min. The sample was attached to conductive tape, metal spraying was performed for 200 s, and the sample was then observed by SEM.

Proteomic analysis

Protein extraction

Samples (1 g-2 g) with 10% polyvinylpolypyrrolidone (PVPP) were ground into powder in liquid nitrogen, and protein was then extracted by adding 5-fold volumes of 10% trichloroacetic acid (TCA)/acetone. Each sample included three biological replicates.

Protein digestion

After determination of the protein concentration using the Bradford assay and SDS-PAGE, the protein solution (100 μg) with 8 M urea was diluted 4-fold with 100 mM triethylammonium bicarbonate (TEAB). Trypsin Gold (Promega, Madison, WI, USA) was used to digest the proteins at a protein : trypsin ratio of 40 : 1 overnight at 37°C.

Peptide labelling

The peptides were dissolved in 30 μL of 0.5 M TEAB with vortexing. The isobaric tag for relative and absolute quantitation (iTRAQ) labelling reagents were brought to ambient temperature, and these reagents were then transferred and mixed with the proper samples. Peptide labelling was performed using the iTRAQ Reagent 8-plex Kit according to the manufacturer's protocol.

Peptide fractionation

The peptides were separated on a Shimadzu LC-20AB HPLC Pump system coupled with a high-pH RP column.

High performance liquid chromatography

Each fraction was resuspended in buffer A (2% acetonitrile (CAN) and 0.1% free folate (FA) in water) and centrifuged at 20000 rpm for 10 min. The supernatant was loaded onto a C18 trap column at 5 $\mu L \cdot min^{-1}$ for 8 min using the autosampler of an LC-20AD nano-HPLC instrument (Shimadzu, Kyoto, Japan).

Mass spectrometry analysis

The peptides separated by nano-HPLC were subjected to tandem mass spectrometry Q EXACTIVE (Thermo Fisher Scientific, San Jose, CA, USA) for data-dependent acquisition (DDA) detection by nanoelectrospray ionization. The parameters for MS analysis are listed as follows: electrospray voltage, 1.6 kV; precursor scan range, 350 $m \cdot z^{-1}$ - 1600 $m \cdot z^{-1}$ at a resolution of 70000 in the Orbitrap; MS/MS fragment scan range, >100 $m \cdot z^{-1}$ at a resolution of 17500 in the HCD mode; normalized collision energy setting, 27%; dynamic exclusion time, 15 s; automatic gain control (AGC) for full MS target and MS2 target, 3E6 and 1E5, respectively; and number of MS/MS scans following one MS scan, 20 most abundant precursor ions above a threshold ion count of 20000.

Tab. 1: Mascot search parameters.

Item	**Value**
Type of search	MS/MS ion search
Enzyme	Trypsin
Fragment mass tolerance	0.05 Da
Mass values	Monoisotopic
Variable modifications	Oxidation (M), iTRAQ8plex (Y)
Fixed modifications	Carbamidomethyl (C), iTRAQ8plex (N-term), iTRAQ8plex (K)
Peptide mass tolerance	20 ppm
False discovery rate (FDR)	< 0.01
Fold change	> 1.2
P value from the significance test	< 0.05

Bioinformatics

For peptide data analysis, raw mass data were processed using Mascot 2.3.02 (Matrix Science, London) against a database. The search parameters are shown in Tab. 1. Blast2GO software was used for the Gene Ontology (GO) analysis of differentially expressed proteins. A database (http: www.genome.jp/kegg/) was used for the KEGG pathway enrichment analysis of the differentially expressed proteins and detected the most significant pathways.

RESULTS

Wall thickness of ray cells in the xylem of *Haloxylon ammodendron*

The ray cell walls in the xylem of *H. ammodendron* showed an obviously thick wall structure (Fig. 1), and the wall thickness of *H. ammodendron* ray cells in Jinghe (2.85 ± 0.42 μm) was significantly lower than that in Shihezi (3.08 ± 0.44 μm) ($p < 0.01$).

Differentially expressed proteins

In total, 6767 peptides and 3076 proteins were identified with an FDR of 1%. Repeat experiments defined differentially expressed proteins with a 1.2-fold change ($P < 0.05$). A total of 795 and 421 proteins were identified as upregulated and downregulated in Shihezi, respectively.

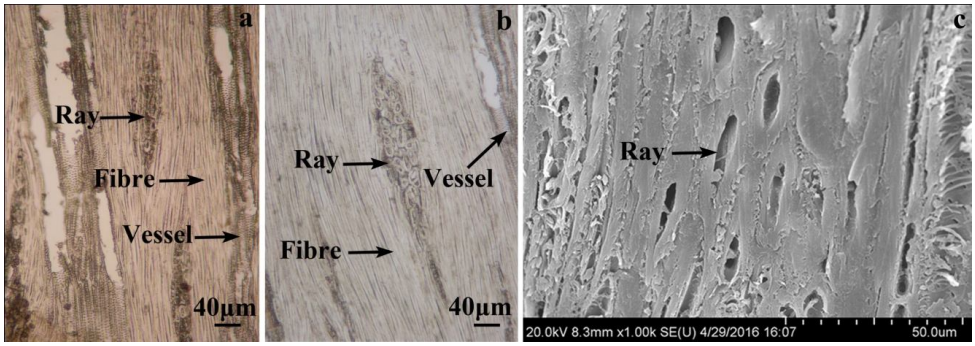

Fig. 1: Anatomical characteristics of Haloxylon ammodendron rays (SEM) in tangential sections: a) Jinghe, b), Shihezi, c) SEM. The arrows show ray cells with thick walls.

Among the upregulated differentially expressed proteins, a total of 55 proteins, including beta expansin EXPB2.1 (*Mirabilis jalapa*), glucan endo-1,3-beta-D-glucosidase (*Beta vulgaris* subsp. *vulgaris*), hypothetical protein JCGZ_24101 (*Jatropha curcas*), pectin acetylesterase family protein (*Theobroma cacao*), and polyphenol oxidase (*Spinacia oleracea*), were related to loosening, cellulose/hemicellulose and pectin in the process of ray cell wall formation in the *H. ammodendron* xylem (Fig. 2).

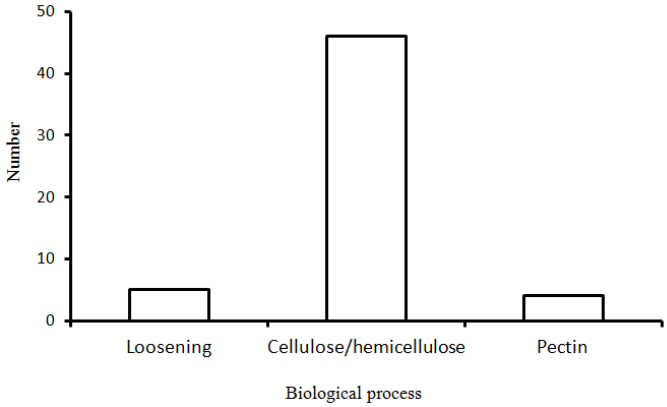

Fig. 2: Differentially expressed proteins related to ray cell wall formation in the Haloxylon ammodendron xylem.

Pathway annotation of the identified proteins

To better understand the pathways involving the differentially expressed proteins during the formation of ray cell walls in the xylem of *H. ammodendron*, we performed pathway annotation analysis of the differentially expressed proteins based on the KEGG database. The results showed that a total of nine pathways were related to ray cell wall synthesis in the xylem of *H.*

ammodendron, such as phenylpropanoid biosynthesis, photosynthesis, glycolysis/gluconeogenesis, carbon metabolism, starch and sucrose metabolism, and metabolic pathways (Tab. 2).

DISCUSSION

The proteome related to ray cell wall formation in the xylem of *H. ammodendron* from the Gurbantunggut Desert was studied. A total of 3,076 proteins were identified, and among these, 795 and 421 proteins were significantly upregulated and significantly downregulated, respectively, in *H. ammodendron* from Shihezi, which harboured ray cells with a greater cell wall thickness than the ray cells of *H. ammodendron* in Jinghe. Among the metabolic pathways involving the differentially expressed proteins, phenylpropanoid biosynthesis, photosynthesis, glycolysis/gluconeogenesis, carbon metabolism, starch and sucrose metabolism, metabolic pathways, plant hormone signal transduction, cysteine and methionine metabolism and amino sugar and nucleotide sugar metabolism were found to be related to ray cell wall synthesis (Tab. 2).

Tab. 2: Significantly enriched pathways related to cell wall formation in the Haloxylon ammodendron xylem.

Number	Pathway	Differentially expressed proteins with pathway annotations (1041)	Pathway ID
1	Photosynthesis	25 (2.4%)	ko00195
2	Starch and sucrose metabolism	36 (3.46%)	ko00500
3	Phenylpropanoid biosynthesis	34 (3.27%)	ko00940
4	Metabolic pathways	378 (36.31%)	ko01100
5	Amino sugar and nucleotide sugar metabolism	29 (2.79%)	ko00520
6	Glycolysis/gluconeogenesis	39 (3.75%)	ko00010
7	Plant hormone signal transduction	7 (0.67%)	ko04075
8	Cysteine and methionine metabolism	18 (1.73%)	ko00270
9	Carbon metabolism	71 (6.82%)	ko01200

Among the metabolic pathways, five differentially expressed proteins were associated with cell wall loosening, the biosynthesis of cellulose and hemicellulose involved 46 differentially expressed proteins, and four differentially expressed proteins were found to be involved in pectin biosynthesis (Fig. 2).

Lipid transfer proteins can transfer lipids between membranes and might be involved in cell wall relaxation (Yeats and Rose 2008). In this study, a protein (protein ID of XP_021763617.1) named probable nonspecific lipid-transfer protein AKCS9-like (*Citrus sinensis*) was upregulated in the ray cell walls of the xylem of *H. ammodendron* in Shihezi (with a higher ray cell wall thickness), and this protein might be involved in the process of cell wall loosening.

Expansin is a protein that loosens the cell walls of plants and participates in the process of cell wall expansion (Zhang et al. 2018). Expansin consists of four subfamilies: α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA) and expansin-like B (EXLB) (Kende et al. 2004). In our study, we found that β-expansin EXPB2.1 was upregulated in the ray cell walls

of *H. ammodendron* in Shihezi, which indicated that this protein is involved in the loosening process of the ray cell wall in the xylem of *H. ammodendron*.

Cell wall expansion depends on xyloglucan degradation or xyloglucan-cellulose hydrolysation, and xyloglucan endotransglycosylase (XET) plays an important role in this process (Darley et al. 2001). XETs were first identified and described by Fry et al. (1992) and Nishitani and Tominaga (1992). The main activity of XETs is transglycosidase activity, which catalyses the cleavage of the xyloglucan chain and moves the half chain to the nonreducing terminal of the second xyloglucan chain (Sulova et al. 1998). The cleavage (degradation) of cell wall xyloglucan promotes cell wall loosening, growth and cellulose accumulation (Park et al. 2004). Previous studies have shown the potential transformation and relaxation ability of XET in cell walls, and the expression and activity of XET are significantly correlated with the expansion area of the cell (Fry et al. 1992, Xu et al. 1996). Xyloglucan endotransglycosylase/hydrolase 1, which belongs to the plant hormone signal transduction pathway, is upregulated in the ray cell walls in the xylem of *H. ammodendron* in Shihezi. Xyloglucan endotransglycosylase/hydrolase 1, somatic embryogenesis receptor kinase 1-like precursor and kinase protein with tetratricopeptide repeat domain isoform 1 catalyse various biochemical processes from brassinosteroid biosynthesis to cell elongation (Tab. 3).

Reversibly glycosylated polypeptide 1 is involved in the transport of sugar into the Golgi cavity and the biosynthesis of noncellulosic polysaccharides (Saxena and Brown 1999). In this study, reversibly glycosylated polypeptide 1 was found to be involved in amino sugar and nucleotide sugar metabolism and was upregulated in Shihezi (ratio of 1.28).

GDP-mannose-3′,5′-epimerase (GME) catalyses the conversion of GDP-D-mannose to GDP-L-galactose (Ma et al. 2014), which is a structural component of agar and cell wall polysaccharides (Siow et al. 2013). GME is involved in ascorbic acid biosynthesis in the Smirnoff-Wheeler pathway (Smirnoff and Wheeler 2000), and the first step is the GME-catalysed formation of GDP-D-mannose (Wolucka and Van Montagu 2003). Ascorbic acid, an antioxidant and cofactor of enzymes, plays an important role in the photosynthesis and biosynthesis of cell wall components (Conklin and Barth 2004). In this study, GME was shown to be involved in metabolic pathways and was upregulated in the ray cell walls of *H. ammodendron* in Shihezi.

Tab. 3: Differentially expressed proteins in the plant hormone signal transduction pathway.

Protein ID	Mass	Mean Ratio	NCBI nr Description
KMT14924.1	24371.44	0.78	Somatic embryogenesis receptor kinase 1-like precursor [*Glycine max*]
KMT01268.1	56957.73	0.7	PREDICTED: probable serine/threonine-protein kinase At4g35230 [*Prunus mume*]
XP_010695172.1	54916.24	0.87	Kinase protein with tetratricopeptide repeat domain isoform 1 [*Theobroma cacao*]
XP_021754164.1	54688.02	1.11	Kinase protein with tetratricopeptide repeat domain isoform 1 [*Theobroma cacao*]
XP_021774662.1	57370.1	1.02	PREDICTED: probable serine/threonine-protein kinase At4g35230 [*Prunus mume*]
KMT19882.1	31419.18	0.98	Hypothetical protein VITISV_036640 [*Vitis vinifera*]
XP_021838893.1	31624.29	1.36	Xyloglucan endotransglycosylase/hydrolase 1

			[*Neolamarckia cadamba*]

Beta-1,4-glucanase, encoded by the KORRIGAN gene, is involved in cellulose biosynthesis (Szyjanowicz et al. 2004). However, little is known about the functions of KORRIGAN EGase (KOR). This study revealed that beta-1,4-glucanase participates in starch and sucrose metabolism and was upregulated in the ray cell walls of *H. ammodendron* in Shihezi.

In conclusion, during the process of ray cell wall biosynthesis in the xylem of *H. ammodendron*, it can be hypothesized that various proteins, including the predicted nonspecific lipid-transfer protein-like protein At5g64080-like (*Vitis vinifera*), the predicted probable nonspecific lipid-transfer protein AKCS9-like (*C. sinensis*) and beta expansin EXPB2.1 (*M. jalapa*), first cause cell wall loosening, extension and expansion, and xyloglucan endotransglycosylase/hydrolase 1 then cleaves and links xyloglucan chains. Subsequently, photosystem I P700 apoprotein A1, reversibly glycosylated polypeptide 1 and GDP-mannose-3′,5′-epimerase are involved in cellulose, hemicellulose and pectin biosynthesis in the cell wall by providing components or energy. Finally, the proteins involved in phenylpropanoid biosynthesis promote the lignification of ray cell walls and complete the biosynthetic process of cell walls.

Notably, this study revealed that the upregulated proteins corresponded to a significantly increased ray cell wall thickness. The regulatory proteins or genes related to thickening of the ray cell walls in *H. ammodendron* can be further explored to determine their functions, and the genes can be applied to the improvement of timber plantations, which is important for both identifying the effect of a specific xylem component on the environment and improving the mechanical properties of wood in timber plantations.

CONCLUSIONS

(1) The wall thicknesses of ray cells of *H. ammodendron* in Jinghe and Shihezi were 2.85 ± 0.42 μm and 3.08 ± 0.44 μm, respectively ($p < 0.01$). *(2)* During the process of ray cell wall biosynthesis in the xylem of *H. ammodendron*, it can be assumed that nonspecific lipid-transfer protein-like proteins and beta expansin EXPB2.1 (*Mirabilis jalapa*) first loosen the cell wall, and this step is followed by extension and expansion. Subsequently, xyloglucan endotransglycosylase/hydrolase 1 cleaves and links the xyloglucan chains, and photosystem I P700 apoprotein A1, reversibly glycosylated polypeptide 1 and GDP- mannose-3′,5′-epimerase are then involved in cellulose, hemicellulose and pectin biosynthesis in the cell wall by providing components or energy. Finally, the proteins involved in phenylpropanoid biosynthesis promote lignification and complete the biosynthetic process of ray cell walls.

ACKNOWLEDGEMENTS

This study was funded by the National Natural Science Foundation of China (grant numbers 31500471 and 31660196).

REFERENCES

1. Alves, E., Angyalossy-Alfonso, V., 2002: Ecological trends in the wood anatomy of some Brazilian species. 2. Axial parenchyma, rays and fibres. IAWA Journal 23: 391–418.
2. Burton, R.A., Gidley, M.J., Fincher, G.B., 2010: Heterogeneity in the chemistry, structure and function of plant cell walls. Nature Chemical Biology 6: 724–732.
3. Carlquist, S., Grant, J.R., 2005: Wood anatomy of gentianaceae, tribe helieae, in relation to ecology, habit, systematics, and sample diameter. Brittonia 57: 276–291.
4. Conklin, P., Barth, C., 2004: Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens and the onset of senescence. Plant Cell and Environment 27: 959–970.
5. Darley, C.P., Forrester, A.M., McQueen-Mason, S.J., 2001: The molecular basis of plant cell wall extension. Plant Molecular Biology 47: 179–195.
6. Fernandes, A.N., Thomas, L.H., Altaner, C.M., Callow, P., Forsyth, V.T., Apperley, D.C., Kennedy, C.J., Jarvis, M.C., 2011: Nanostructure of cellulose microfibrils in spruce wood. Proceedings of the National Academy of Sciences of the United States of America 108: E1195–E1203.
7. Fry, S.C., Smith, R.C., Renwick, K.F., Martin, D.J., Hodge, S.K., Matthews, K.J., 1992: Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochemcal Journal 282: 821–828.
8. Kende, H., Bradford, K.J., Brummell, D.A., Cho, H.T., Cosgrove, D.J., Fleming, A.J., Gehring, C., Lee, Y., McQueen-Mason, S., Rose, J.K.C., Voesenek, A.C.J., 2004: Nomenclature for members of the expansin superfamily of genes and proteins. Plant Molecular Biology 55: 311–314.
9. Ma, L.C., Wang, Y.R., Liu, W.X., Liu, Z.P., 2014: Overexpression of an alfalfa GDP-mannose 3, 5-epimerase gene enhances acid, drought and salt tolerance in transgenic *Arabidopsis* by increasing ascorbate accumulation. Biotechnology Letters 36: 2331–2341.
10. Nishitani, K., Tominaga, R., 1992: Endoxyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule to another xyloglucan molecule. Journal of Biological Chemistry 267: 21058–21064.
11. Park, Y.W., Baba, K., Furuta, Y., Iida, I., Sameshima, K., Arai, M., Hayashi, T., 2004: Enhancement of growth and cellulose accumulation by overexpression of xyloglucanase in poplar. FEBS Letters 564: 183–187.
12. Pear, J.R., Kawagoe, Y., Schreckengost, W.E., Delmer, D.P., Stalker, D.M., 1996: Higher plants contain homologs of the bacterial celA genes encoding the catalytic subunit of cellulose synthase. Proceedings of the National Academy of Sciences of the United States of America 93: 12637–12642.
13. Plavcová, L., Jansen, S., 2015: The role of xylem parenchyma in the storage and utilization of nonstructural carbohydrates (Chapter 8). In: Functional and Ecological Xylem Anatomy (ed Hacke, U.). Pp 209- 234, Cham, Springer.
14. Saxena, I.M., Brown, R.M.Jr., 1999: Are the reversibly glycosylated polypeptides implicated in plant cell wall biosynthesis non-processive β-glycosyltransferases? Trends

Plant Science 4: 6–7.
15. Siow, R-S., Teoh, S., Teo, S-S., Shukor, M.Y. bin Abd, Phang, S-M., Ho, C-L., 2013: Molecular cloning and characterization of GDP-mannose-3′,5′-epimerase from *Gracilaria changii*. Journal of Applied Phycology 25: 1309–1318.
16. Smirnoff, N., Wheeler, G.L., 2000: Ascorbic acid in plants: biosynthesis and function. Critical Reviews in plant Sciences 35: 291–314.
17. Sulova, Z., Takacova, M., Steele, N.M., Fry, S.C., Farkas, V., 1998: Xyloglucan endotransglycosylase: evidence for the existence of a relatively stable glycosyl-enzyme intermediate. Biochemcal Journal 330: 1475–1480.
18. Suzuki, S., Li, L., Sun, Y.H., Chiang, V.L., 2006: The cellulose synthase gene superfamily and biochemical functions of xylem-specific cellulose synthase-like genes in *Populus trichocarpa*. Plant Physiology 142: 1233–1245.
19. Szyjanowicz, P.M.J., McKinnon, I., Taylor, N.G., Gardiner, J., Jarvis, M.C., Turner, S.R., 2004: The irregular xylem 2 mutant is an allele of korrigan that affects the secondary cell wall of *Arabidopsis thaliana*. Plant Journal 37: 730–740.
20. Taylor, N.G., Howells, R.M., Huttly, A.K., Vickers, K., Turner, S.R., 2003: Interactions among three distinct CesA proteins essential for cellulose synthesis. Proceedings of the National Academy of Sciences of the United States of America 100: 1450–1455.
21. Wolucka, B., Van Montagu, M., 2003: GDP-mannose 3', 5'-epimerase forms GDP-L-gulose, a putative intermediate for the de novo biosynthesis of vitamin C in plants. Journal of Biological Chemistry 278: 47483–47490.
22. Xi, E.H., 2018: Dynamic relationship between mechanical properties and chemical composition distribution of wood cell walls. Wood Research 63: 179–192.
23. Xu, W., Campbell, P., Vargheese, A.K., Braam, J., 1996: The Arabidopsis XET-related gene family: environmental and hormonal regulation of expression. Plant Journal 9: 879–889.
24. Yeats, T.H., Rose, J.K.C., 2008: The biochemistry and biology of extracellular plant lipid-transfer proteins (LTPs). Protein Science 17: 191–198.
25. Zhou, C.B., Gong, W., 2017: Effect of provenance and climate on xylem anatomy of *Haloxylon ammodendron* (C.A. Mey) bunge in the Gurbantunggut desert, China. Applied Ecology and Environmental Research 15: 1309–1321.
26. Zhou, X.Q., Wang, Y.R., Wang, L., Lv, J.X., Zhao, R.J., Yao, L.H., Chen, Z.J., 2017: Cell wall structure and mechanical properties of *Salix psammophila*. Wood Research 62: 1–12.

CHAOBIN ZHOU
ZUNYI NORMAL COLLEGE
INSTITUTE OF BIOLOGY AND AGRICULTURE SCIENCE & TECHNOLOGY
PINGAN ROAD, XINPU NEW DEVELOPMENT ZONE, ZUNYI CITY
GUIZHOU PROVICE
563006 CHINA

JUNJIE DING
XINJIANG ACADEMY OF AGRICULTURAL AND RECLAMATION SCIENCE
SHIHEZI CITY, XINJIANG PROVICE
832000 CHINA

XIAOJING HU
XINJIANG AGRICULTURAL UNIVERSITY
URUMQI CITY, XINJIANG PROVICE
830000 CHINA

WEI GONG*
SICHUAN AGRICULTURAL UNIVERSITY
CHENGDU CITY, SICHUAN PROVICE
611130 CHINA
*Corresponding author: gongwei@sicau.edu.cn