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Proteomic Analysis of the Inflorescence Stem Mechanical Strength Difference in Herbaceous Peonies (*Paeonia lactiflora* Pall.)

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Cite This: *ACS Omega* 2022, 7, 34801−34809

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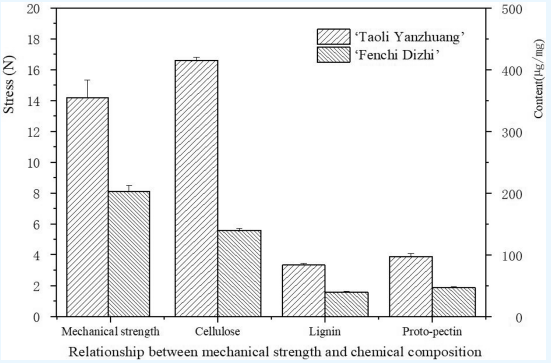
ABSTRACT: The herbaceous peony (*Paeonia lactiflora* Pall.) is a traditional rare flower in China, and production of its cut flowers has developed gradually in many places of the world. However, the inflorescence stems of some *P. lactiflora* cultivars have such low mechanical strength that the cut flower production was severely restricted. To better understand the causes of this problem from a protein expression level, two *P. lactiflora* cultivars with different inflorescence stem mechanical strengths were analyzed by two-dimensional electrophoresis and MALDI-TOF/MS. More than 1700 clear protein spots were detected, 53 of which varied significantly. Moreover, 23 of the differentially expressed proteins were identified and confirmed and are involved in various biological processes such as metabolism, protein biosynthesis and transport, signal transduction, and defensive response. Especially, cinnamyl alcohol dehydrogenase (CAD) and xyloglucan endotransglucosylase/hydrolase (XTH) were strongly connected to the inflorescence stem mechanical strength in *P. lactiflora*.



INTRODUCTION

The herbaceous peony (*Paeonia lactiflora* Pall.) is both a traditional rare flower and an herb in China that can be used in flower bed arrangements, in courtyards, and also as a potted plant. In addition to these uses, cut flower production of *P. lactiflora* has been developing gradually in many places throughout the world. However, the inflorescence stems of some *P. lactiflora* cultivars have such low mechanical strength that their cut flower production was severely restricted.[1] So, selecting *P. lactiflora* cultivars that have high mechanical strength stems together with further research in the related high mechanical strength mechanism of the stems would allow progress to be made toward solving this problem; then, the peony cut flower production losses could be avoided.

The main functions of the plant stem are conduction and mechanical support, and the stem mechanical strength can be used as direct evidence of its flexural capacity.[2] A large number of studies have shown that a plant stem's mechanical strength has a close relationship with the stem length, roughness, and internode length, and stem secondary cell wall material content such as cellulose, hemicellulose, pectin, lignin, and so on; the stem secondary cell wall material plays the leading role in stem mechanical strength.[3−5] Cellulose biosynthesis mainly depends on cellulose synthase gene (CesA) family.[6] Lignin biosynthetic pathways related enzymes and genes such as phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), caffeoyl-CoA-O-methyltransferase (CCoAOMT), cinnamyl alcohol dehydrogenase (CAD), etc. have been cloned, and the functional analyses have been validated. The synthetase activity change of one of the hemicellulose main ingredients, xylan, can be used as one of the indicators of the secondary wall formation.[7−9] Glycosyltransferase 8D genes in *Populus trichocarpa* caused reduced mechanical strength and xylan content in wood, which shows that hemicelluloses have more correlation with stem mechanical strength than lignin. Pectin is one of the main components of the cell wall and mainly exists in the intercellular layer;[10] it makes the cells bond together and has a hardening effect,[11] inolving some biosynthesis enzymes and genes such as xyloglucan endotransglucosylase/hydrolase (XTH), UDP-glucuronate-4-epimerase (UGlcAE),[12,13] and so on. In addition, the transcription factors, such as MYB, NAC, and SND1, play significant role in the biosynthesis of the secondary wall.[14−17]

For *P. lactiflora*, studies on the mechanical strength of the inflorescence stem were most focused on the relationship between the plant development and the physiological characteristics of the stem.[18−20] However, little is known

Received: May 3, 2022
Accepted: September 6, 2022
Published: September 26, 2022

Figure 1. Morphological expression of the inflorescence stem of two *P. lactiflora* cultivars, (a) "Taoli Yanzhuang" and (b) "Fenchi Dizhi".

about the proteins that are directly responsible for the functions as well as the phenotypes of the cells. In this study, a proteomic approach was applied to examine the proteome changes in different inflorescence stem mechanical strengths, and the results complement previous genomic data and highlight the importance of molecular and functional characterization at the protein level.

MATERIALS AND METHODS

Test Materials. From April to May of 2021, two *P. lactiflora* cultivars, namely, "Taoli Yanzhuang" and "Fenchi Dizhi", with stems of clearly different mechanical strengths, were selected from the *P. lactiflora* resource nursery of the Horticulture and Plant Protection College of Yangzhou University. Their inflorescence stems were cut and taken to the laboratory during the full-blossom period. After photographing and determining the mechanical strength, they were frozen with liquid nitrogen and stored in a cryogenic refrigerator at −80 °C.

Compositional Analysis and Protein Extraction. The cell wall materials were isolated following the method of Li et al.[18] The cellulose and lignin as well as proto-pectin contents were measured with the method described by Zhao et al.[19] The experiments were performed in triplicate, and the results were expressed as means. Analysis of variance (ANOVA) was performed with Tukey's test ($P < 0.01$) for locating significant differences between mean values using SPSS 16.0 software (SPSS Inc., Chicago, IL, U.S.A.). Total soluble proteins were isolated from 300 mg of the inflorescence stem of *P. lactiflora*. Proteins were precipitated and purified following the procedures described by Yu et al.[21] In brief, proteins were precipitated with 10% (w/v) trichloroacetic acid (TCA)−acetone and recovered by centrifugation. The protein pellet was washed three times with 100% acetone and then dissolved in the protein solubilization buffer. The insoluble fraction was removed via centrifugation at 12 000*g* for 45 min, and a 0.5% (v/v) IPG buffer (pH 4−7) from GE Healthcare (Piscataway, NJ, U.S.A.) was added into the soluble protein fraction. Protein concentrations were quantified according to the Bradford method.[22]

2-DE Gel Electrophoresis. Isoelectric focusing (IEF) was carried out on Immobiline DryStrip gel (IPG) strips (17 cm, nonlinear, GE Healthcare, Piscataway, NJ, U.S.A.). Prior to the second dimension of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the focused IPG strips were equilibrated in equilibration buffers for 15 min. The equilibrated IPG strips were transferred onto 12.5% SDS acrylamide gels by use of an Ettan DALT Six Electrophoresis Unit (GE Healthcare, Piscataway, NJ, U.S.A.). Protein spots were visualized by staining with Coomassie Brilliant Blue dye (CBB-G250).[23] Gels were scanned, and protein spot profiles were analyzed by PDQuestTM7.0 (Bio-Rad Laboratory). Protein spots were considered differentially expressed if the intensity changed more than one and a half times in different stems. All the 2-DE experiments were repeated three times. MS/MS analysis was conducted using a MALDI-TOF/TOF mass spectrometer 4800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, U.S.A.). MS spectra were

https://doi.org/10.1021/acsomega.2c02749
ACS Omega 2022, 7, 34801−34809

Table 1. Mechanical Strengths and Chemical Compositions of the Inflorescence Stems of Two *P. lactiflora* Cultivars[a]

cultivars	mechanical strength (N)	cellulose (μg/mg)	lignin (μg/mg)	proto-pectin (μg/mg)
"Taoli Yanzhuang"	14.2 ± 1.15a	415.7 ± 4.50a	83.6 ± 2.5a	97.5 ± 4.6a
"Fenchi Dizhi"	8.1 ± 0.42b	139.8 ± 2.8b	39.2 ± 2.3b	47.2 ± 1.9b

[a]Data are expressed as $\bar{x}$ ± SD (n = 3). Mean values followed by different letters in the same column are significantly different (p < 0.01).

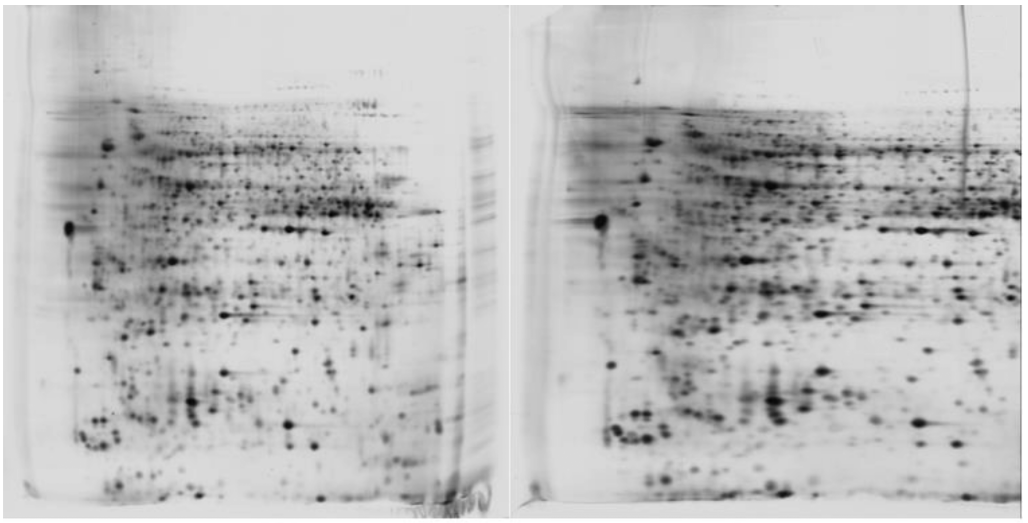


Figure 2. Representative 2-DE images of total protein for the *P. lactiflora* inflorescence stem from (a) "Taoli Yanzhuang" and (b) "Fenchi Dizhi". Proteins prepared from the inflorescence stems, which have different mechanical strengths, were separated by 2-DE and stained by Coomassie Brilliant Blue.

acquired in positive reflector mode. Combined MS and MS/MS spectra were submitted to MASCOT (V2.2, Matrix Science, London, U.K.) by GPS Explore software (V3.6, Applied Biosystems, Framingham, MA, U.S.A.), and the intracellular localization of the identified protein was analyzed through WOLF PSORT (http://wolfpsort.seq.cbrc.jp), Swiss-Port, NCBI, and so on.

RESULTS

Mechanical Strength and Chemical Composition Differences of the Inflorescence Stems. During the full-blossom period, the inflorescence stem morphologic expressions of two *P. lactiflora* cultivars had obvious differences (Figure 1), and the mechanical strength showed the same trend (Table 1). The inflorescence stem of "Taoli Yanzhuang" is straight, whereas the stem of "Fenchi Dizhi" is seriously bent. The mechanical strength of "Taoli Yanzhuang" is 14.2 N, whereas that of "Fenchi Dizhi" is 8.1 N, which is 57.04% of that of "Taoli Yanzhuang". The difference between the mechanical strengths of these two cultivars is significant (P < 0.01). Chemical compositions of the "Taoli Yanzhuang" inflorescence stem cell wall had significantly higher amounts cellulose, lignin, and proto-pectin (415.7, 83.6, 97.5 μg/mg respectively) than that of "Fenchi Dizhi" (139.8, 39.2, and 47.2 μg/mg, respectively). Additionally, there is an obvious positive correlation between the mechanical strength of the inflorescence stem and the contents of cellulose, lignin, and proto-pectin. The higher the content of cellulose, lignin, and proto-pectin, the greater the mechanical strength. This is especially true for the content of cellulose.

Protein Electrophoresis Pattern Analysis. 2-DE electrophoretic techniques are used to separate the total protein of two kinds of *P. lactiflora* inflorescence stems with different mechanical strengths, and more than 1700 protein spots can be detected repeatedly (Figure 2). After automatic matching of the protein spots in the gel, there is a difference of 53 points (Figure 3). The expression of 35 proteins in the inflorescence stem of "Taoli Yanzhuang" with high mechanical strength is higher than that of "Fenchi Dizhi", which has low mechanical strength, while the expression of 18 proteins in the stem of "Taoli Yanzhuang" is lower than that of "Fenchi Dizhi".

Identification and Functional Classification of the Proteins Differentially Expressed in the Inflorescence Stems. MALDI-TOF/MS analysis is made for 40 differential proteins with high differential separated from pH 3−10 IPG glue tape to obtain peptide mass fingerprinting (PMF). The NCBInr eukaryon biological protein database is retrieved through the ion search mode of MASCOT, and 23 protein spots are identified successfully (score > 95) (Table 2). The success rate of the identification is approximately 57.5%. Two groups of protein spots, namely, Spots 721 and 876 and Spots 397 and 392, are the same protein after identification. For the rest of the protein spots, the effective peak cannot be detected or the peak value is excessively low for obtaining the PMF because of an excessively low abundance or high complication of the protein spots or some unknown factors that influenced the sample preparation. PMF is obtained for 23 differential protein spots after MALDI-TOF-MS analysis. In this test, the two groups of protein spots, namely, Spots 721 and 876 and Spots 397 and 392, are the same proteins after identification. They are probably the same protein with different types and different posttranslational modifications. After identification, the remaining protein spots are the sole protein direction.

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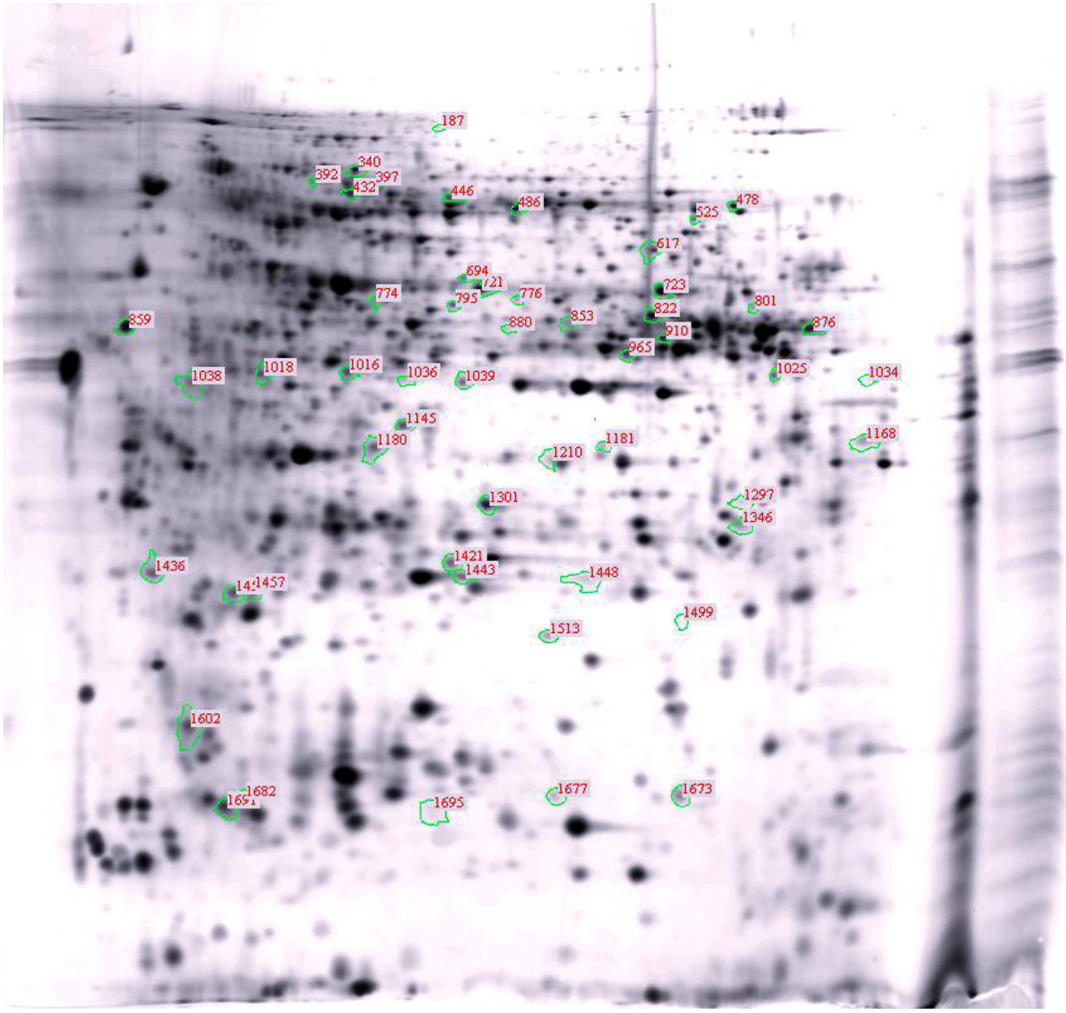

Figure 3. Representative 2-DE gel electrophoresis image showing spot identification and localization of proteins from *P. lactiflora* inflorescence stem. Proteins were separated using 17 cm IPG PH 3−10 strips and 12.5% SDS-PAGE.

Therefore, the effective segregation of the proteins is the precondition for identification in the mass spectrum.

The function of the aforementioned protein is classified through the retrieval in the MIPS database (http://mips.gsf.de/projects/funcat) and the data analysis for the identified protein (Figure 4). These successfully identified proteins participate in the carbohydrate and energy metabolism, protein anabolic metabolism and modification process, signal transduction, and defense and emergency response. Among the successfully identified 21 proteins, 5 proteins are related to carbohydrate metabolism, a share of 24%; 3 proteins are related to energy metabolism, a share of 9%; 5 proteins are related to the protein synthesis, metabolism, and modification process, a share of 24%; 3 proteins are related to defense and emergency response, a share of 14%; and 1 other protein remains, a share of approximately 5%.

The intracellular localization of the identified protein is analyzed through WOLF PSORT, SwissPort, NCBI, and a literature report to understand further the function of the differential expressed proteins in the *P. lactiflora* inflorescence stem and to specify the intracellular localization of the identified protein (Figure 5). Four proteins are located in the cytoplasm, a share of approximately 15%. Seven proteins are located in the chloroplast, with a share of approximately 27%. Two proteins are located in mitochondria, a share of approximately 8%. One protein is located in the Golgi apparatus, a share of approximately 4%. Three proteins are located in the plasma membrane, a share of approximately 12%. Three proteins are located in the cell nucleus, a share of approximately 14.3%. Three proteins are located in the cell wall, a share of approximately 11%. The remaining three protein spots, a share of 12%, cannot be located, and this needs further research. The statistical result indicates that most of the identified proteins are located inside the cell, whereas parts of the proteins are located in multiple cell organelles. For example, UDP-glucuronate decarboxylase 1 is located in the Golgi membrane, vacuole, and plasmalemma of a cell. The subunit of the ATP synthesis enzyme CF1α is located in the mitochondria and chloroplast membrane. The proteasome β subunit 1 is distributed in the chloroplast, cytoplasm, cell nucleus, and plasma membrane. Heat shock protein-70 (HSP-70) is mainly distributed in the chloroplast, cell wall, and plasma membrane.

Function Identification and Abundance Change Analysis of the Differential Expressed Protein. After the database retrieval and query and analysis in the relevant literature for the identified protein, 21 proteins and 4 categories are sorted on the basis of their functions and are illustrated by the trend of the change in protein abundance (Figure 6).

https://doi.org/10.1021/acsomega.2c02749
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Table 2. Identification of the Differentially Expressed Proteins of the Inflorescence Stems of *P. lactiflora* "Taoli Yanzhuang" and "Fenchi Dizhi"

no.	protein name	species	accession no.	Pi	MW	MASCOT score
1	isoflavone reductase homologue	*Pyrus communis*	gil3243234	6.02	33801.8	100/160
2	23 kDa polypeptide of the oxygen evolving complex of photosystem II	*Sonneratia ovata*	gil146454490	5.85	25033.6	100/160
3	udp-glucuronic acid decarboxylase 1-like	*Ostreococcus tauri*	gil116787327	6.54	45916.5	97.55/46
4	12-oxophytodienoate reductase 2	*Medicago truncatula*	gil302783961	5.95	40564.4	99.99/48
5	cinnamyl alcohol dehydrogenase 1	*Nicotiana attenuata*	gil399515847	5.43	39356.6	99.99/84
6	enolase	*Vitis vinifera*	gil225455555	6.17	48307.9	100/388
7	enoyl-acp reductase	*Glycine max*	gil356566218	8.83	57864.7	100/83
8	alanine−glyoxylate aminotransferase 2	*Zea mays*	gil226490867	6.6	51422.1	100/119
9	xyloglucan endotransglucosylase/hydrolase	*Vitis vinifera*	gil147771556	5.98	33501.3	151/100
10	peroxidase 12-like	*Nicotiana tabacum*	gil14031049	5.99	39494.7	97.1/59
11	ubiquitin-like protein smt3	*Arabidopsis thaliana*	gil297803412	5.33	11760.7	100/82
12	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase-like	*Glycine max*	gil356563928	7.53	53844.6	100/185
13	atp synthase cf1 alpha subunit	*Platanus occidentalis*	gil114329731	5.26	55406	100/185
14	cytochrome c oxidase subunit	*Oryza sativa Indica Group*	gil218192966	4.46	19482	100/118
15	chalcone isomerase	*Paeonia lactiflora*	gil340396634	4.93	gil340396634	100/193
16	small ubiquitin-related modifier 2-like	*Populus trichocarpa*	gil224132216	4.95	11035.3	100/120
17	histidine-containing phosphotransfer protein 1-like	*Glycine max*	gil356504072	5.02	17372.7	99/80
18	proteasome subunit beta type-1-like	*Glycine max*	gil388505884	6.2	24822.2	100/128
19	xylem cysteine proteinase 2-like	*Medicago truncatula*	gil357467173	5.64	39901.6	100/152
20	heat shock protein 70	*Ostreococcus tauri*	gil308813361	8.98	72694.4	99.4/51
21	patatin group a-3-like	*Vitis vinifera*	gil296084715	5.5	42515.6	100/86

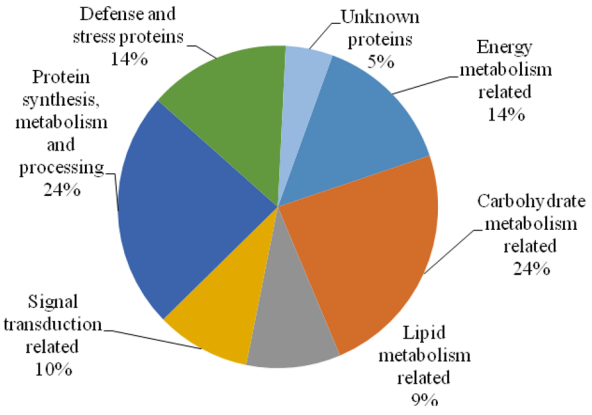

Figure 4. Functional classfication of the differentially expressed proteins identified in the *P. lactiflora* inflorescence stem.

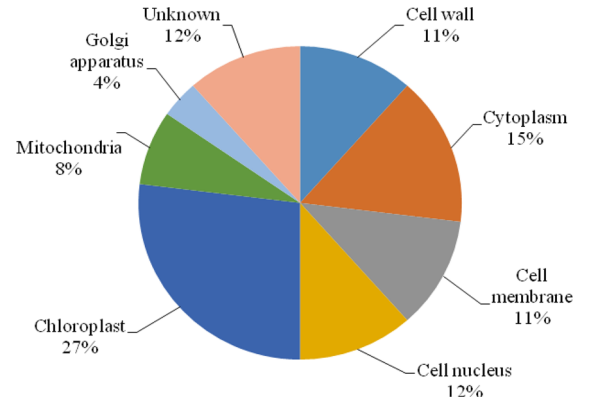

Figure 5. Subcellular localizations of the differentially expressed proteins identified in the *P. lactiflora* inflorescence stem.

Proteins Related to the Saccharides and Energy. *Proteins Related to Carbohydrate Metabolism (Five Proteins).* For UDP-glucuronate decarboxylase 1 (Spot 801), cinnamyl alcohol dehydrogenase 1 (Spots 721 and 876), enolase (Spot 486), and 3-phosphoglyceraldehyde dehydrogenase (Spot 478), their expressed abundances in the two groups of samples are higher in "Taoli Yanzhuang" than that in "Fenchi Dizhi". However, the expressed abundance of xyloglucan endotransglycosylase/hydrolytic enzyme (Spot 1210) in the two groups of samples is lower in "Taoli Yanzhuang" than in "Fenchi Dizhi".

Proteins Related to Energy Metabolism (Three Proteins). For the photosystem III (Spot 1448) and ATP synthesis enzyme CF1α subunit (Spots 397 and 392) that are increased by oxygen evolution, the expressed abundance is lower in "Taoli Yanzhuang" than that in "Fenchi Dizhi" in the two groups of samples. For the cytochrome C oxidase subunit (Spot 859), the expressed abundance is higher in "Taoli Yanzhuang" than in "Fenchi Dizhi".

Proteins Related to Lipid Metabolism (Two Proteins). For ACP reductase (Spot 1018) and patatin a3-like protein (Spot 822), the expressed abundance is higher in "Taoli Yanzhuang" than in "Fenchi Dizhi".

Proteins Related to Protein Synthesis, Metabolism, and Processing and Modification (Five Proteins). For alanine-glyoxylate transaminase 2 (Spot 617), ubiquitin-like protein SMT3 (Spot 1691), small ubiquitin-related modifier (Spot 1682), proteasome β subunit (Spot 1443), and histidine-contained phosphate transport protein 1 (Spot 1695), the expressed abundances in the two sample groups are higher in "Taoli Yanzhuang" than in "Fenchi Dizhi".

Protein Related to Signal Transduction and Defense Stress Response. *Proteins Related to Signal Transduction (Two Proteins).* For the homologous protein of isoflavone reductase (Spot 1036), the expressed abundance is higher in "Taoli Yanzhuang" than in "Fenchi Dizhi". For cysteine

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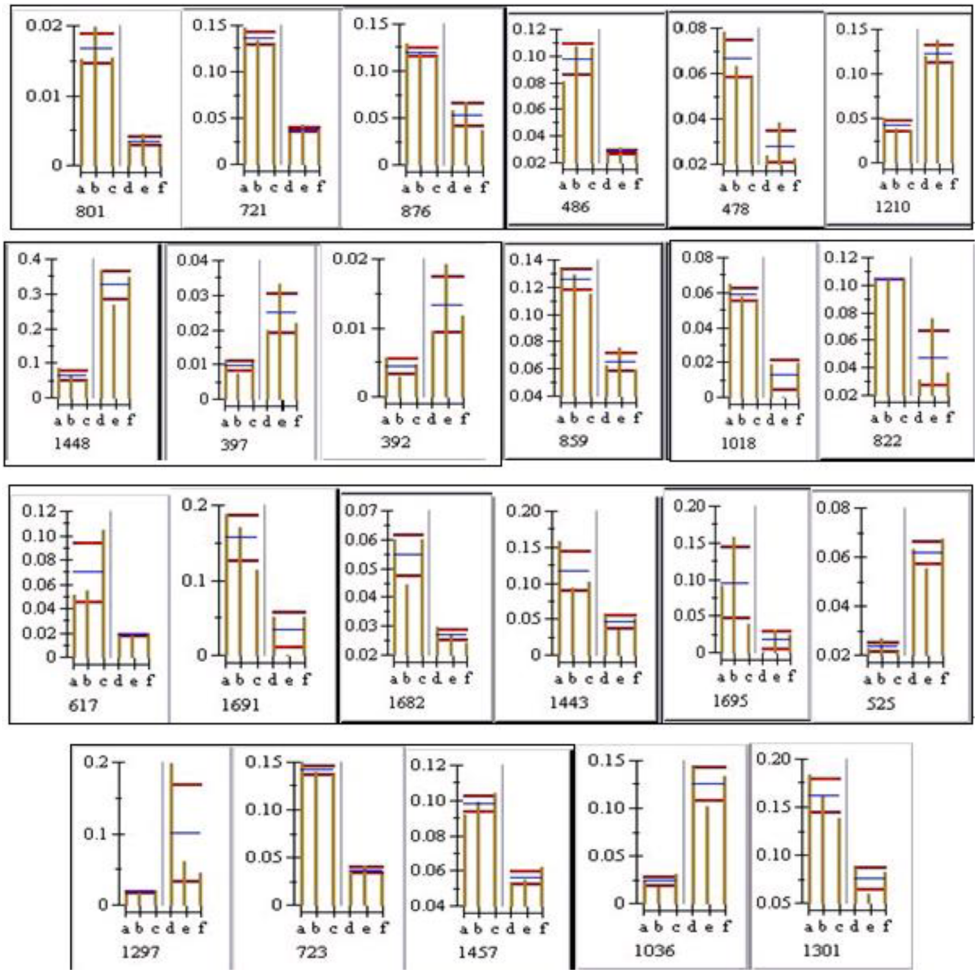

Figure 6. Changes in expression abundance of inflorescence stem protein spots between two *P. lactiflora* cutivars: (a−c, the abundance of triplicate) "Taoli Yanzhuang" and (d−f, the abundance of triplicate) "Fenchi Dizhi".

protease 2 (Spot 1301), the expressed abundance in the two samples is the opposite; namely, it is higher in "Taoli Yanzhuang" than in "Fenchi Dizhi".

Proteins Related to Defense Stress Response (Three Proteins). For peroxidase 12 (Spot 525) and HSP-70 (Spot 1297), the expressed abundance is higher in "Taoli Yanzhuang" than that in "Fenchi Dizhi". For 12-oxide-OPDA reductase (Spot 723), the expressed abundance is higher in "Taoli Yanzhuang" than in "Fenchi Dizhi".

Other Proteins (One Protein). For chalcone isomerase (Spot 1457), the expressed abundance is higher in "Taoli Yanzhuang" than that in "Fenchi Dizhi" in the samples.

DISCUSSION

Differential Expression of the Proteins Related to Carbohydrate and Energy Metabolism. First, the expression abundance of UDP-glucuronate decarboxylase and cinnamyl alcohol dehydrogenase in this study researching the proteins related to the carbohydrate metabolism is large in "Taoli Yanzhuang" with high mechanical strength, which implies that the level of carbohydrate metabolism is high in "Taoli Yanzhuang".[24] The irreversible UDP-glucuronate in UXS produces UDP-xylose, which is the nucleotide sugar necessary for araboxylan, arabinogalactan proteins, and pectic polysaccharide in the cell wall.[25] CAD is one of the key enzymes during the synthesis of lignin and can catalyze the precursor to generate a lignin monomer, such as coumaric aldehyde, sinaldehyde, or coniferyl aldehyde.[26] Polysaccharide is the main chemical component of the cell wall and can provide mechanical support for the plant stem. Therefore, the high level of carbohydrate metabolism in "Taoli Yanzhuang" is a guarantee to maintain the high mechanical strength of its inflorescence stem.[27] Second, enolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) have large expression abundance as a key enzyme during the glycolysis. Enolase can catalyze the transfer from PGA to PEP in the process of glycolysis during cell energy metabolism; upregulation may promote the glycolysis, and ATP may be produced.[28] However, GAPDH can catalyze glyceraldehyde-3-phosphate to transfer to 1,3-diphosphoglyceraldehyde and to produce NADH with the hydrogen acceptor NAD^+, thereby promoting the production of the first ATP during glycolysis.[29] Studies have shown that GAPDH is highly expressed in the scape of the oncidium during the flowering stage.[30] Therefore, an energy guarantee is needed to maintain high mechanical strength and to ensure the straightness of the inflorescence stem for "Taoli Yanzhuang". Xyloglucan endotransglucosylase/hydrolase (XTH) is a key enzyme during the reconstruction of the cell wall. XTH can also modify the network structure of hemicellulose and cellulose and is related to the inflation of protein in the cell wall and to the loosening and extension of the cell wall. XTH can also promote the growth of plants and

https://doi.org/10.1021/acsomega.2c02749
ACS Omega 2022, 7, 34801−34809

mellowing of fruits, and carbohydrate release can be accelerated. In this research, the expression abundance of XTH in the "Taoli Yanzhuang" stem with high mechanical strength is considerably less than that in the "Fenchi Dizhi" inflorescence stem with low mechanical strength.[31] This finding shows that the cell in the low mechanical strength scape than that in the high mechanical strength scape, which is one of the major reasons for the mechanical strength difference in the scapes.

The proteins that increase oxygen evolution in the 23 kDa photosystem II and ATP synthetase CF1α subunit related to the energy metabolism have a large expression abundance in "Fenchi Dizhi", whereas the cytochrome C oxidase subunits have a large expression abundance in "Taoli Yanzhuang." As a peripheral protein of photosystem II, the protein that increases oxygen evolution in the 23 kDa photosystem II, which can promote the combination of Ca^{2+} and Cl^{-} to maintain the oxygen evolving activity, is combined with the side of the cyst cavity.[32] Plants could strengthen the capability of oxygen evolution when they are forced to by the external environment. Other studies have also shown that *Chlamydomonas reinhardtii*, which lacks the 23 kDa protein, is sensitive to light.[33] Further research is needed because of the high expression abundance of the oxygen evolution increasing protein in the 23 kDa photosystem II in the scape of "Fenchi Dizhi" with low mechanical strength. As a protein related to the synthetase of ATP, the CF1α subunit of the synthetase of ATP has the function of organization during the assembly of many subunits of the synthetase of ATP.[34] Further research is needed because of the high expression abundance of this protein in the scape with low mechanical strength. The cytochrome C oxidase subunit is the terminal enzyme in the respiratory chain of mitochondria intima, and its function is directly related to ATP synthesis.[35] In this research, the cytochrome C oxidase subunit in the "Taoli Yanzhuang" scape has a higher expression abundance than that in "Fenchi Dizhi". This finding shows that, compared with that of the "Fenchi Dizhi" scape, the formation of the "Taoli Yanzhuang" scape may need increased ATP.

In the lipid metabolism, ACP reductase and the patatin group a-3-like protein have a high expression abundance in "Taoli Yanzhuang" with high mechanical strength. The enoyl ACP reductase catalyzes the transfer from fatty alcohol to anti-2-enoyl ACP in the last rate-limiting step of the synthesis and circular response of the fatty acid II. Studies have shown that ACP reductase plays an important role in the development[36] and formation of the extine of the plant pollen.[37] The patatin group a-3-like protein is a kind of protein that stores the sugar particularly in the tuber. It has lipid acyl hydrolase (LAH) activity,[38] and it plays a part in the signal transduction of the plant.[39] In this research, patatin group a-3-like protein has a higher expression abundance in "Taoli Yanzhuang" than in "Fenchi Dizhi". The protein can supposedly promote the lipolysis of the tuber in the Chinese peony. Compared with the low mechanical strength scape, the high mechanical strength scape may need increased LAH activity.

Differential Expression of the Protein Related to Protein Synthesis, Metabolism, and Processing and Modification. In the plant alanine-glyoxylate, transaminase is an important enzyme that participates in photorespiration. Its two kinds of isozymes are identified in the peroxisome. These transaminases are used widely in the overlapped areas of amino acids as a donor. After combination, the acceptor acts in various metabolic pathways, including the photorespiration glyoxylate circulation route and common amino acid metabolism. Ubiquitin and its pathway play an important part[40] in the formation of eukaryocyte ribosomes, the regulation of the cell cycle, and the stress response. In the process of ubiquitination, ubiquitin guarantees that the cell has a proper immune response by recognizing some marked immune cell membrane acceptors. The proteasome β subunit has ATP-dependent protease activity. It is mainly used to degrade the proteins not necessary for the cell or the damaged proteins; it is also a major mechanism for cells in regulating certain proteins and in removing misfolding proteins, which can in particular be a defense for cells in the adverse environment.[41] The phosphate transport protein plays an important role in absorbing phosphorus for plants. The studies have shown that the mitogen can combine with the acceptor *Arabidopsis* histidine kinases to make phosphorylate, and the phosphate group is transferred to *Arabidopsis* histidine-phosphotransfer proteins (AHPs) in cytoplasm. The phosphorylated AHPs enter the nucleus and transfer the phosphate group to *Arabidopsis* response regulators, and then cytokinin promotes plant growth by regulating cell division.[42] The present research has shown that the expression abundances of alanine-glyoxylate transaminase 2, ubiquitin-like protein SMT 3, the small ubiquitin-related modifying factor, the proteasome β subunit, and histidine-contained phosphate transport protein 1 are higher in "Taoli Yanzhuang" than in "Fenchi Dizhi". The cell division is supposedly active in the high mechanical strength scape during the flowering period, and the scape grows vigorously. Scapes are bent because of gravity with regard to scape growth and flower weight. At this time, the proteasome β subunit combined with the ubiquitin to degrade the proteins unnecessary for cells or the damaged proteins, thereby regulating certain proteins and retaining the high mechanical strength of the scapes. Further research is needed to discover the details.

Differential Expression of the Protein Related to Signal Transduction and the Defense Stress Response. For the expression abundance of the homologous protein of isoflavone reductase, "Taoli Yanzhuang" is higher than "Fenchi Dizhi", whereas for cysteine protease 2, "Taoli Yanzhuang" is lower than "Fenchi Dizhi". Isoflavone reductase is a key enzyme to synthesize lignin and isoflavone. The cysteine protease is related to the program cell death.

For the expression abundance of peroxidase and HSP-70, "Taoli Yanzhuang" is lower than "Fenchi Dizhi", whereas for the expression of OPDA reductase, "Taoli Yanzhuang" is higher than "Fenchi Dizhi". Peroxidase is common in plants. It also has various isozymes. As a kind of new peroxidase, 2-cysperoxiredoxin can catalyze H_2O_2 or various alkyl peroxides to reduce to water and corresponding alcohol under a hydrogen donor. Therefore, this enzyme can supposedly prevent the biotic stress or abiotic stress from damaging the chloroplast protein. For these specific reasons, further research is needed. The expression abundance of peroxidase is large in "Fenchi Dizhi". The reason is that the chloroplast protein of this kind is supposedly damaged during the full-blossom period. For these detailed reasons, further research is needed. HSP70 also plays an important part in the growth of a plant except in cases of abiotic stress or an invading pathogen.[43] Studies have shown that HSP70 influences the early development of plants through chloroplast protein transport.[44] The overexpression of HSP70-1 causes the reduction of roots of

https://doi.org/10.1021/acsomega.2c02749
ACS Omega 2022, 7, 34801−34809

Arabidopsis and the reduction of root meristem mitotic activity, thereby causing the undersize growth of expressed plants.[45] Some studies have shown that HSP70 can be combined with the hydrophobic region on the surface of a misfolding protein under shock conditions, and E3 ubiquitin ligase (such as CHIP) can be guided to cause proteasome to degrade them by marking the misfolding protein with ubiquitin.[46] OPDA reductase is a key enzyme to merge the octadecenoic acid of jasmonic acid from linolenic acid, and the last step of the composition of JA is controlled. That is to say, this phenomenon is the natural precursor of jasmonates. Studies have shown that jasmonates play an important role in the regulation and defense response of plant growth.[47] For example, methyl jasmonate can induce the activity expression of CHS and peroxidase, thereby improving lignin content.[48]

Differential Expression of Other Proteins. Chalcone isomerase is widely found in various plants and is a rate-limiting enzyme for the biosynthesis of flavonoid compounds. Chalcone isomerase plays an important role in the antistress activity of plants, cell development and differentiation, and pigment accumulation.[49] For our studies, the expression abundance of chalcone isomerase is large in "Taoli Yanzhuang", and further research is needed to discover the detailed reasons.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was financially supported by the Science and Technology Program of Jiangsu Agri-animal Husbandry Vocational College (NSF2021ZR04), the Science and Technology Program of the Taizhou Municipal Government (Project No. TN202005), the Taizhou Municipal 311 Personnel Training Program (2017-1-42), and the Jiangsu Provincial Qing Lan Project [(2020)10]. We thank Jennifer Smith, Ph.D., from Liwen Bianji (Edanz) (https://www.liwenbianji.cn) for editing the language of a draft of this manuscript.

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https://doi.org/10.1021/acsomega.2c02749
ACS Omega 2022, 7, 34801−34809