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Full Length Article

Anatomical Observation and Proteomics Analysis of Root Tips of Wild and Cultivated Soybeans

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Abstract

Wild and cultivated soybeans from different areas of China were used as materials for paraffin cross-sections of the juncture between the root elongation and maturation zones of wild and cultivated soybeans during the seedling stage. It was found that the casparian band of the endodermis and duct of metaxylem in the anatomical structure of the cultivated soybean developed faster than those of the wild soybean. Rapid development of these structures sped up transmission and enhanced support capacity. Meanwhile, two-dimensional electrophoresis was adopted to analyze the differences between the wild and cultivated soybean populations in terms of protein expression during the seedling stage. Our results showed that expression levels of 28 proteins from among the wild and cultivated soybean populations were significantly different from one another. In the cultivated population, the protein expression levels were up-regulated in four protein spots and down-regulated in 24 protein spots. Down-regulated proteins were associated with secondary metabolites and metabolism of antibiotics. Decrease in the expression these proteins may speed up nodule formation. These results showed that early growth and development of the cultivated soybean roots occurred at a significantly faster rate than those of the wild soybean. The increased rates of these processes accelerate root shape formation and improve root function. In conclusion, this study systematically explored the anatomical structure and differentially expressed proteins of roots between wild and cultivated soybean, and differentially expressed proteins identified here will be the valuable resource for studying soybean root differences resulting from domestication.

Keywords: Soybeans; Domestication; Root; Anatomical structure; Protein expression difference

Introduction

Cultivated soybean [Glycine max (L.) Merr.] is domesticated from the wild soybean, and this process has caused significant changes to soybean roots. As compared to wild soybeans, modern cultivated soybeans have conspicuous primary roots and decreased lateral roots, and the former has increased root diameter, number of ducts at the secondary xylem, duct diameter, and number of secondary vascular rays (Wang *et al*., 2000; Zhu *et al*., 2003). Differences in root between wild and cultivated soybeans appear at the seedling stage. The extent and depth of root spreading in cultivar are remarkably higher than wild soybean, leading to the improvement on abilities of support and absorption in root (Manavalan *et al*., 2015; Prince *et al*., 2015). Therefore, it is extremely important to identify the differences in the early growth and development of roots between wild and cultivated soybean roots and to explore the mechanism of soybean root evolution in domestication.

Proteomics analysis is an effective method that facilitates the exploration of physiological and biochemical processes (Mathesius *et al*., 2011; Chen *et al*., 2016; Wang *et al.*, 2016). Two-dimensional gel electrophoresis (2-DE) has been used to analyze the differences between wild and cultivated soybean seeds in terms of protein composition (Li *et al*., 2007; Xu *et al*., 2007; Natarajan *et al*., 2012). However, as far as we know, the difference between the proteome of wild soybean roots and that of cultivated soybean roots has not yet been reported.

In this paper, the anatomical structures of the root tips of landraces of wild soybeans were compared to those of cultivated soybeans by examining paraffin sections of the root tips. Meanwhile, 2-DE was used to analyze the proteome of the root tips from the wild and cultivated soybean roots for exploring their differences in regards to their early growth and development. Our findings are not only useful for studying the mechanism of root difference formation between wild and cultivated soybeans, but also helpful for revealing the molecular mechanism of domestication.

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Materials and Methods

Experimental Materials

Soybean [Glycine max (L.) Merr.] seeds were placed on wet absorbent gauze at 27°C in darkness for 20 h to allow germination. The seeds whose radicles slightly penetrated the seed coats were selected and planted in wet autoclaved vermiculites and then grown at 27°C for 27 h. The seedlings that were higher than the vermiculite and had similar heights were selected, and their main roots were cut off for subsequent testing.

For the anatomical structure observations, six wild soybeans and six cultivated soybeans were collected from different areas of China (Table 1). Under a stereoscope, a 0.5 cm section was cut from the bottom of the maturation zone (root hair zone) to the elongation zone. To make the paraffin section, it was fixed in FAA (alcohol 55% 89 mL + glacial acetic acid 6 mL + formalin 5 mL) for 24 h.

For the proteome and quantitative real-time reverse transcription polymerase chain reaction (qRT–PCR) analyses, a 1.5 cm section was cut from the top of the main roots. And we took 40 wild soybeans and 40 cultivars as two samples separately (Table 2) for balanced mixing (D'Ambrosio *et al.*, 2012).

Table 1: Soybean materials for anatomical analysis

Source area	Wild soybeans	Cultivated soybeans
Heilongjiang	ZYD00693	Lin Dian Shuai Yi Ling
Shandong	ZYD03234	Mo Shi Da Dou
Jiangsu	ZYD04111	Liu He Hong Mao Qing Zao
Sichuan	ZYD04318	Wan Xian Bai Dong Dou
Yunnan	ZYD05173	ShuangBaiTuo Dian Hei Pi
Guangxi	ZYD05218	Yu Lin Da Huang Dou

Table 2: Soybean material list

Wild soybeans		Cultivated soybeans	
Name	Source area	Name	Source area
ZYD00693	Heilongjiang	Jian Yang Qiu Da Dou	Fujian
ZYD04429	Zhejiang	Yu Lin Da Huang Dou	Guangxi
ZYD04609	Jiangxi	Wan Xian Bai Dong Dou	Sichuan
ZYD01030	Jilin	Zhen Ba Xiao Bai Huang Dou	Shaanxi
ZYD02213	Liaoning	MiLuo Dou Ban Jin	Hunan
ZYD02726	Beijing	Yu Hang Liu Yue Dou	Zhejiang
ZYD02891	Shanxi	ShuangBaiTuo Dian Hei Pi	Yunnan
ZYD03234	Shandong	Xu Chang BaiHua Cao	Henan
ZYD03296	Gansu	Tong Shan Liu Yue Huang	Hubei
ZYD03387	Henan	Chi Feng Xiao Qing Dou	Neimenggu
ZYD03718	Shaanxi	RenHua Ba Yue Huang	Guangdong
ZYD04111	Jiangsu	Mo Shi Da Dou	Shandong
ZYD04200	Anhui	Su Xian Xiao Hei Dou	Anhui
ZYD04318	Sichuan	Lin Dian Shuai Yi Ling	Heilongjiang
ZYD04390	Hubei	Xiao Bai Mei	Jilin
ZYD04666	Hunan	Liu He Hong Mao Qing Zao	Jiangsu
ZYD04853	Fujian	HengFeng Ma Yi Dou	Jiangxi
ZYD05173	Yunnan	Tong Xian Huang Dou	Beijing
ZYD05190	Guangdong	Yang Yan Jing Dou	Gansu
ZYD05218	Guangxi	Niu Pi Huang	Hebei
ZYD05715	Neimenggu	Xu Yong Xiao BaiShui Dou	Sichuan
ZYD00609	Heilongjiang	BaoLuo Huang	Zhejiang
ZYD03261	Shandong	Da Li Huang	Heilongjiang
ZYD03489	Henan	XuJia Ying Huang Dou	Ningxia
ZYD04008	Shaanxi	Chang Ji Huang Dou No.1	Xinjiang
ZYD04174	Jiangsu	Yang Yan Jing Dou	Shanxi
ZYD04260	Anhui	Da Dou	Shaanxi
ZYD04410	Hubei	Qing Pu Hong Dou	Shanghai
ZYD04541	Zhejiang	Cha Huang Dou	Hubei
ZYD04560	Zhejiang	Sa Dou	Yunnan
ZYD04591	Xizang	Da Huang Dou No.1	Guangdong
ZYD04597	Jiangxi	Da Ke Huang	Guangxi
ZYD04679	Hunan	Huang Dou	Shanxi
ZYD04741	Guizhou	Huang Chi Da Huang Dou	Anhui
ZYD04762	Guizhou	Zhen Jiang Huang Dou	Jiangsu
ZYD04876	Fujian	Wei NingZongZi Dou	Guizhou
ZYD05176	Guangdong	Gui Xi Ma Yi	Jiangxi
ZYD05242	Guangxi	Quan Zhou Shi Tang Wu Yue	Guangxi
ZYD05473	Jilin	She Xian JieChuanHei Dou	Anhui
ZYD06284	Hebei	Yi Xian Hei Dou	Hebei

Production of Paraffin Section

The fixed root segments were stained with Mayer's hematoxylin for 3 days. The loose color on the segments was removed through immersion cleaning with distilled water (six times, 10min each time), and then, the fixed segments were cleaned with running tap water for 12 h for bluing. Then, different concentrations of alcohol (15, 30, 45, 60, 70, 80, 90 and 95% for 15 min; three times with anhydrous alcohol, 20 min each time) were used to dehydrate the segments. Chloroform and ethyl alcohol transparent agents were then applied (1:2, 1:1, 2:1, each for 6 h; three times with absolute chloroform, 3 h each time). Chloroform paraffin (25, 50, 75, 100% wax, each for 3 h) was used to embed the segments. After embedding, the sections were sliced with Leica RM2125RTSat at thickness of 10 µm. The slices were unfolded, affixed, and then dried for 2 h at 45°C. They were subsequently dewaxed with xylene at 35°C and sealed with neutral balsam. The images were obtained under an OLYMPUS CX-41 microscope.

Proteome Analysis

Protein extract and 2-DE were carried out according to the previous study (Xu *et al*., 2007). Bio-Protein Assay Kit (5000202, 5000203) was used to determine the protein concentrations in the samples. Nonlinear strips (17 cm) with pH of 3–10 (Bole Company) were used, and the protein loading amount was 290 µg. Silver staining was performed according to the method of Zhu *et al*. (1999), and a Tif format image was obtained by scanning.

Images of 2-DE were obtained and then analyzed with ImageMaster2D Platinum 6.0 software. The relative gray value (vol%) was selected as the index. According to the ratio value criteria (>2 or <0.5), differentially expressed protein spots were found among the wild and cultivated populations. The relative gray values were analyzed by t-test to determine the candidate protein spots, and the differentially expressed protein spots were finally determined (P <0.05 was considered significant).

Mass spectrometry was performed according to the method of Wang *et al*. (2013). A 4800 MALDI TOF/TOF series time-of-flight mass spectrometer was used to perform peptide mass fingerprinting (PMF) identification. The NCBI database was then searched. Protein functional analysis and clustering were performed in the David, KEGG and Phytozome website.

qRT-PCR

qRT-PCR was performed according to the earlier study Wang *et al*. (2014), and details of the primers used in this assay were listed in Table 3.

Table 3: Primers for fluorescent quantitative PCR

Protein NO	Gene	Primer sequence (5'to3')
1	*Glyma16g08460*	ACTCAAAGGGTTTGATTGTTGG
		CTCACTCCATTCGTAAGCCTC
3	*Glyma08g23860*	TAGTAAGAGCACTCGACAGTGG
		TCTGACAACACCTCAGGAGC
9	*Glyma03g40680*	CAATTAGGAGGGCACATGCTG
		TCCAAGTTCCCTGCAAAGTG
10	*Glyma13g20790*	GGAACTGAAATTGACCCTAGAACC
		CTCTCACAACCTGATGTTCAAGTC
12	*Glyma05g27260*	AAATCTGGGCATGAACTTCTC
		AAGGAGTTCTCAGCAATAGGT
14	*Glyma11g15680*	TCACACTATTGGAGCTGCAC
		ATATTTATCAACGAGAGGGCGG
15	*Glyma10g15910*	CCTCAGTCTCTCACTCGCAG
		TAGCCTCCGAACATCTTGCC
17	*Glyma03g27580*	TGTTGAAGAAGGTGATGCATCTG
		ATGTATGTGTCAGAAGTCGGG
20	*Glyma07g06570*	TGAAACACCAGCAGAAGGAC
		AAATCAAGGTCTCCCAACCC
21	*Glyma14g11711*	GAAGGATCTGGAAGCTGTCG
		TCCAACATCCTTGAGCATCTG
22	*Glyma13g32300*	CTACAGAGACAGGGAGACAAGAC
		AATGTTTCAGGTACCTGCCAC
Reference gene	*GlycineACTIN*	GGCAAGTCCAATCCACAAG
		AGGAAGGGTGTGCTTCTCC

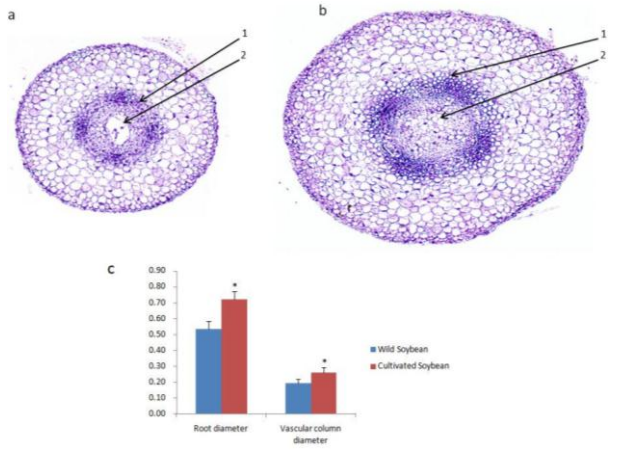

Fig. 1: Comparison between the anatomical structures of the wild and cultivated soybean roots (juncture of elongation and root hair zones)

a. Cross-section of wild soybean roots; b. Cross-section of cultivated soybean roots; c. Comparison between the root and vascular column diameters of the wild and cultivated soybeans, * means the significant difference (*t*-test, $p < 0.05$); 1. Endodermis, the casparian band of the endodermis is forming in cultivated soybean; 2. metaxylem, duct of metaxylem is forming in cultivated soybean

Results

Structures of the Wild and Cultivated Soybean Root Tips

The structures of the juncture between the root elongation and maturation zones of the root tips of the wild and cultivated soybeans were observed. Both soybean accessions had clearly identifiable as epidermis, cortex, and protoxylem as well as contained a tetrarch radial stele. Root structures of the cultivated soybeans developed more rapidly than those of the wild soybeans. Likewise, the endodermis of the cultivated soybeans developed earlier than that of the wild soybeans. In cultivars, some cells had increased cell wall thickness and casparian bands formed. While no such structure was observed in wild soybeans. Furthermore, the metaxylems of the cultivated soybeans developed earlier than those of the wild soybeans. In the cultivated soybeans, cells at the corresponding locations of the metaxylems were regular polygonal and arranged in neat rows, and their cell walls were considerably thicker than those in the wild soybeans. Furthermore, the corresponding location in the wild soybean still had parenchyma cells with weak differentiation (Fig. 1a, b). In addition, the cultivated soybeans had thicker roots than the wild soybeans. Diameters of root and the vascular column in cultivated soybean roots were 0.72±0.05 and 0.26±0.04 mm, respectively, whereas those of the wild soybeans were 0.53±0.05 and 0.19±0.02 mm, respectively. There were significant differences between the soybean groups in terms of root and vascular column diameters (Fig. 1c).

Protein Expression in Root Tips of Wild and Cultivated Soybeans

Approximately 896±52 and 887±43 protein spots were detected in the two-dimensional electrophoretograms of both the populations. A total of 28 differentially expressed protein spots were found between the wild and the cultivated populations. As compared to the wild population, the cultivated population displayed significant up-regulation and down-regulation in 4 and 24 of the protein spots, respectively (Fig. 2). A total of 28 differentially expressed protein spots were identified by PMF performed on ABI 4800 MALDI TOF/TOF. Later on information related to 25 of the differentially expressed protein spots was obtained from the NCBInr database (Table 4).

Functional Analysis of Differentially Expressed Protein

David, KEGG, and Phytozome analyses were used to determine the functions of these proteins (Table 5). We

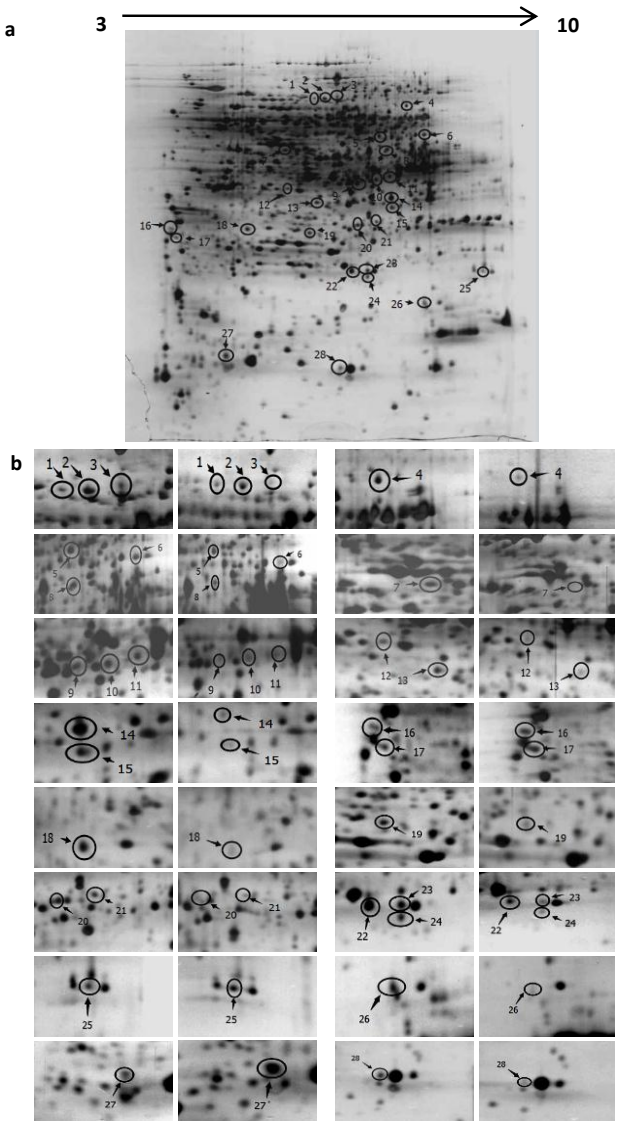

Fig. 2: Differentially expressed protein spots in root tips of wild and cultivated soybean populations

a. Scheme diagram of differentially expressed protein spots in the wild and cultivated soybean root tips; b. Differential protein spots between two soybean populations. Left side is the wild population; right side is the cultivated population

found that down-regulated proteins were enriched on metabolic pathways, carbon metabolism, biosynthesis of secondary metabolites, biosynthesis of antibiotics, pyruvate metabolism, glycolysis/gluconeogenesis (Fig. 3). These proteins were primarily concentrated in two aspects: that involving carbon, sugar and energy metabolism and that involving secondary metabolites and antibiotics metabolism.

Transcriptional Analysis of Differentially Expressed Protein-Encoding Genes

Eleven differentially expressed proteins were randomly

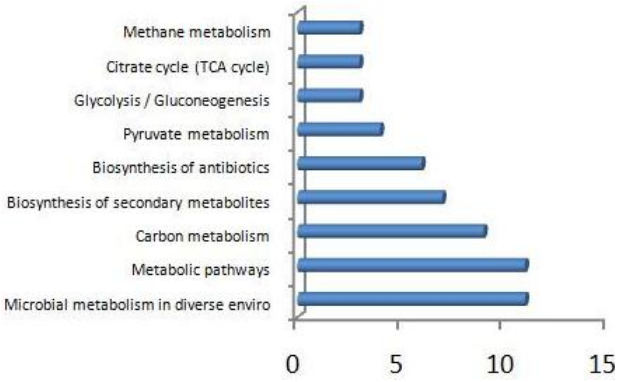

Fig. 3: Functional analyses of differentially expressed proteins in the root tips of wild and cultivated populations

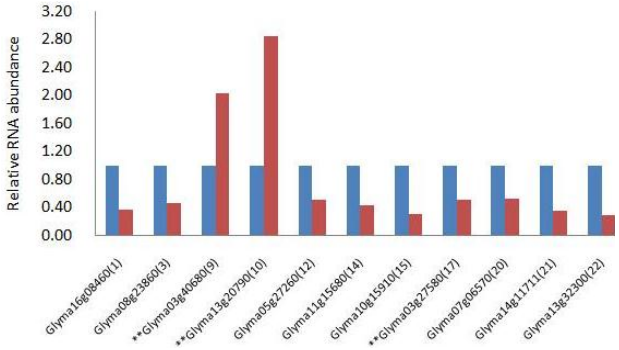

Fig. 4: Transcription levels of the encoding genes of differentially expressed proteins in the root tips of wild and cultivated soybean populations

** *Glyma03g40680* (9): ** In the wild and cultivated soybeans, the genes whose changes of transcription levels are not consistent with the expression level of protein, *Glyma03g40680* gene number, (9) number of differentially expressed protein spots

taken to further check transcriptional levels of these differentially expressed protein-coding genes in the wild and cultivated soybeans. QRT–PCR analysis was performed on the root tip samples from the wild and cultivated soybeans. Our results showed that for eight of the differentially expressed proteins, the possible encoding genes exhibited transcription level changes that were consistent with the protein expression levels; however, those of the other three differentially expressed proteins were not consistent (Fig. 4).

Discussion

Early root growth and development occurred more rapidly in the cultivated soybean roots than in the wild soybean roots. After observing the cross-section of the juncture between the elongation and maturation zones of the wild and cultivated soybeans, it was determined that the casparian band and metaxylem duct in the endodermis developed much more quickly in the cultivated soybeans than in the wild soybeans. This finding is consistent with the

Table 4: Spectrum identification of the differentially expressed proteins in the wild and cultivated root tips

No.	Cultivated/Wild	Protein Name	Accession No.	Protein MW	Protein PI	Protein Score	Protein Score C.I.%	TotalIon Score	Total Ion C.I.%
1	decrease	malic enzyme, putative [*Ricinus communis*]	gi|255546341	65144.60	5.98	302.00	100.00	227.00	100.00
2	decrease	malic enzyme, putative [*R. communis*]	gi|255546341	65144.60	5.98	237.00	100.00	197.00	100.00
3	decrease	unnamed protein product [*Vitis vinifera*]	gi|298204781	46052.30	6.08	118.00	100.00	96.00	100.00
4	decrease	unnamed protein product [*V. vinifera*]	gi|297741775	61468.20	6.23	235.00	100.00	101.00	100.00
5	decrease	unknown [*G. max*]	gi|255635934	25253.00	8.58	263.00	100.00	185.00	100.00
6	decrease	unknown [*G. max*]	gi|255642203	10164.20	9.06	89.00	99.89	52.00	97.05
7	decrease	unknown [*G. max*]	gi|255641490	45811.60	5.95	207.00	100.00	75.00	99.98
8	decrease	unknown [*G. max*]	gi|255640955	40355.50	6.51	227.00	100.00	143.00	100.00
9	decrease	unknown [*G. max*]	gi|255648228	37514.40	6.26	287.00	100.00	117.00	100.00
10	decrease	NAD-dependent isocitrate dehydrogenase alpha subunit [*Brassica napus*]	gi|28974492	36222.60	6.08	195.00	100.00	117.00	100.00
11	decrease	hypothetical protein [*Lotus japonicus*]	gi|244539473	34309.70	6.41	177.00	100.00	94.00	100.00
12	decrease	pyruvate dehydrogenase [*G. max*]	gi|316980596	44097.50	6.15	309.00	100.00	189.00	100.00
13	decrease	Chalconereductase [*Astragalus mongholicus*]	gi|302129635	35506.70	6.19	79.00	98.77		
14	decrease	cytosolicascorbate peroxidase 1 [*G. max*]	gi|37196683	27851.30	5.78	401.00	100.00	195.00	100.00
15	decrease	unknown [*G. max*]	gi|255636647	29806.60	6.65	188.00	100.00	117.00	100.00
16	increase	unknown [*G. max*]	gi|255628877	22115.90	4.43	424.00	100.00	330.00	100.00
17	increase	unknown [*G. max*]	gi|255631888	21386.60	4.34	247.00	100.00	204.00	100.00
18	decrease	N/A							
19	decrease	RecName: Full=Phosphomannomutase; AltName:Full=GmPMM	gi|122194126	28001.20	5.84	497.00	100.00	313.00	100.00
20	decrease	unknown [*G. max*]	gi|255644424	30821.80	6.53	254.00	100.00	95.00	100.00
21	decrease	aspartyl-tRNAsynthetase, putative [*R. communis*]	gi|255564914	60970.20	6.06	124.00	100.00	77.00	100.00
22	decrease	unknown [*G. max*]	gi|255630927	21653.00	6.43	466.00	100.00	327.00	100.00
23	decrease	unknown [*G. max*]	gi|255627623	25881.40	8.68	110.00	100.00	30.00	0.00
24	decrease	N/A							
25	increase	N/A							
26	decrease	Chain A, Crystal structure of *Proglycinin* mutant C88s	gi|42543705	53574.60	5.78	322.00	100.00	226.00	100.00
27	increase	unknown [*G. max*]	gi|255629055	17988.20	5.48	152.00	100.00	97.00	100.00
28	decrease	unknown [*G. max*]	gi|255627117	17750.80	5.96	499.00	100.00	292.00	100.00

morphological differences in roots between cultivated and wild soybeans at the seedling stage (Manavalan *et al*., 2015; Prince *et al*., 2015). Rapid early growth and development of cultivated soybean roots are conducive to root shape formation and its functioning. The formation of the casparian band in the endodermis is associated with the formation of the vascular column, which facilitates the transport of substances in the vascular column. Moreover, the fast development of the metaxylem duct undoubtedly contributes to the transportation of substances, and the thickening of the cell walls of these structures improves the support function of the roots.

Soybean roots contain bacteria in the nodule for nitrogen fixation, which is apart from conduction or support (Munoz *et al*., 2016). In our results, as compared to the wild soybeans, the cultivated soybeans essentially exhibited a decreasing trend in the protein expression levels in their root tips. Interestingly, the decreased protein expression levels were primarily related to secondary metabolites and antibiotics metabolism, which contribute to nodule formation caused by the invasion of nodule bacteria. In addition, it has known that plant stress response has the association with secondary metabolites or antibiotics metabolism. And there is a balance between stress resistance and growth in plant (Deng *et al*., 2017). Therefore, down-regulated genes related to secondary metabolites and antibiotics metabolism may lead to the faster root growth in cultivated soybean.

In regards to the differentially expressed proteins from the root tips of wild and cultivated soybeans, it is likely that useful genes can be identified that can improve the root morphology and function of the modern cultivated soybean. In the domestication process, the artificial selection pressure is oriented. Accordingly, important economic and agronomic traits of crops have been improved as a result of accumulating beneficial genes (Yamasaki *et al*., 2007). The published genomic information of cultivated soybeans differs somewhat from that of wild soybeans (Kim *et al*., 2010; Schmutz *et al*., 2010). In recent years, some researchers have constructed quantitative trait loci (QTLs) related to population exploration and root morphology by using cultivated and wild soybeans as parents (Liang *et al*., 2014; Manavalan *et al*., 2015; Prince *et al*., 2015). However, in this approach, the beneficial target gene is difficult to directly map. Proteomics analysis used here can complement the QTL study, which accelerates the process of functional genes identification through combining two approaches together.

Conclusion

Here we characterized 28 differentially expressed proteins between wild and cultivated soybean populations. Potential functions of these proteins and their correlation with root morphological difference between these soybeans at the seedling stage were studies. Our results are beneficial for understanding the mechanism of root difference directed by domestication and then genes found here are potential valuable for soybean breeding in future.

Table 5: Function analysis of differentially expressed proteins of the root tips of the wild and cultivated populations

Cultivated: Wild	Accession No.	Protein Name	DAVID	KO	KEGG	Gene	Phytozome
decrease	gi|255546341	Malic enzyme, putative [*Ricinus communis*]	NADP-dependent malic enzyme (LOC8268296)	K00029	Microbial metabolism in diverse environments, Metabolic pathways, Carbon metabolism, Pyruvate metabolism, Carbon fixation in photosynthetic organisms	Glyma16g08460	(M=5) PTHR23406:SF26 - NADP-dependent Malic Enzyme 1
decrease	gi|255546341	malic enzyme, putative [*R. communis*]	NADP-dependent malic enzyme (LOC8268296)	K00029	Microbial metabolism in diverse environments, Metabolic pathways, Carbon metabolism, Pyruvate metabolism, Carbon fixation in photosynthetic organisms	Glyma16g08460	(M=5) PTHR23406:SF26 - NADP-dependent Malic Enzyme 1
decrease	gi|298204781	unnamed protein product [*Vitis vinifera*]	D-3-phosphoglycerate dehydrogenase 3, chloroplastic-like (LOC100241666)	K00058	Microbial metabolism in diverse environments, Metabolic pathways, Carbon metabolism, Biosynthesis of antibiotics, Methane metabolism, Biosynthesis of amino acids, Glycine, serine and threonine metabolism	Glyma08g23860	(M=5) 1.1.1.95 - Phosphoglycerate dehydrogenase , Phosphoglyceric acid dehydrogenase
decrease	gi|297741775	unnamed protein product [*V. vinifera*]	pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit beta (LOC100256839)	K00895	Microbial metabolism in diverse environments, Metabolic pathways, Biosynthesis of secondary metabolites, Biosynthesis of antibiotics, Glycolysis , Gluconeogenesis, Fructose and mannose metabolism, Pentose phosphate pathway	Glyma15g11890	pyrophosphate--fructose-6-phosphate 1-phosphotransferase [EC:2.7.1.90]
decrease	gi|255635934	unknown [*Glycine max*]	probable N-succinyldiaminopimelate aminotransferase DapC (LOC100818968)	——		Glyma06g11640	(M=3) PTHR11751,,PTHR11751:SF317 - SUBGROUP I Aminotransferase related
decrease	gi|255642203	unknown [*G. max*]	umaratehydratase 1, mitochondrial (LOC100813552)	K01679	Microbial metabolism in diverse environments, Metabolic pathways, Carbon metabolism, Biosynthesis of secondary metabolites, Biosynthesis of antibiotics, Citrate cycle (TCA cycle), Pyruvate metabolism, Renal cell carcinoma, Pathways in cancer, Carbon fixation pathways in prokaryotes	Glyma02g01930	(M=4) 4.2.1.2 - Fumaratehydratase , Fumarase
decrease	gi|255641490	unknown [*G. max*]	fumarylacetoacetase-like (LOC100817986)	K01555	Microbial metabolism in diverse environments, Metabolic pathways, Tyrosine metabolism, Styrene degradation	Glyma09g01270	(M=2) 3.7.1.2 - Fumarylacetoacetase , Fumarylacetoacetate
decrease	gi|255640955	unknown [*G. max*]	alcohol dehydrogenase class-3-like (LOC100816775)	K00121	Microbial metabolism in diverse environments, Metabolic pathways, Carbon metabolism, Biosynthesis of secondary metabolites, Biosynthesis of antibiotics, Glycolysis , Gluconeogenesis, Methane metabolism, Tyrosine metabolism, Chemical carcinogenesis, Metabolism of xenobiotics by cytochrome P450, Fatty acid degradation, Degradation of aromatic compounds, Naphthalene degradation, Chloroalkane and chloroalkene degradation, Drug metabolism - cytochrome P450, Retinol metabolism	Glyma19g35340	(M=3) 1.1.1.1,,1.1.1.284,,1.2.1.1 - Alcohol dehydrogenase , Aldehyde reductase ,, S-(hydroxymethyl)glutathione dehydrogenase , NAD-linked formaldehyde dehydrogenase（基因）
decrease	gi|255648228	unknown [*G. max*]		——		Glyma03g40680	(M=13) PTHR11732,,PTHR11732:SF253 - ALDO,KETO REDUCTASE
decrease	gi|28974492	NAD-dependent isocitrate dehydrogenase alpha subunit [*Brassica napus*]		K00030	Microbial metabolism in diverse environments, Metabolic pathways, Carbon metabolism, Biosynthesis of secondary metabolites, Biosynthesis of antibiotics, Citrate cycle (TCA cycle), Biosynthesis of amino acids, 2-Oxocarboxylic acid metabolism	Glyma13g20790	(M=6) 1.1.1.41 - Isocitrate dehydrogenase (NAD(+)) , Nicotinamide adenine dinucleotide isocitrate dehydrogenase

Table 5: Continued

Table 5: Continued

decrease	gi|244539473	hypothetical protein [*Lotus japonicus*]		K00344		Glyma12g36990	(M=1) KOG1197 - Predicted quinone oxidoreductase
decrease	gi|316980596	pyruvate dehydrogenase [*G. max*]	pyruvate dehydrogenase E1 component subunit beta-like (LOC100780826)	K00162	Microbial metabolism in diverse environments, Metabolic pathways, Carbon metabolism, Biosynthesis of secondary metabolites, Biosynthesis of antibiotics, Glycolysis , Gluconeogenesis, Citrate cycle (TCA cycle), Pyruvate metabolism, Glucagon signaling pathway, HIF-1 signaling pathway, Central carbon metabolism in cancer	Glyma05g27260	(M=4) PTHR11624:SF76 - PYRUVATE DEHYDROGENASE E1 COMPONENT SUBUNIT BETA-2, CHLOROPLASTIC-RELATED
decrease	gi|302129635	Chalcone reductase [*Astragalus mongholicus*]	socitrate dehydrogenase [NAD] catalytic subunit 5, mitochondrial-like (LOC106403500)	——		Glyma02g47750	(M=4) PTHR11732,,PTHR11732:SF220 - ALDO,KETO REDUCTASE
decrease	gi|37196683	Cytosolic ascorbate peroxidase 1 [*G. max*]	ascorbate peroxidase 1, cytosolic (APX1)	K00434	Ascorbate and aldarate metabolism, Glutathione metabolism	Glyma11g15680	(M=3) PTHR31356:SF10 - L-ASCORBATE PEROXIDASE 2, CYTOSOLIC
decrease	gi|255636647	unknown [*G. max*]		K01070	Microbial metabolism in diverse environments, Carbon metabolism, Methane metabolism,	Glyma10g15910	(M=2) PTHR10061 - S-FORMYLGLUTATHIONE HYDROLASE
increase	gi|255628877	unknown [*G. max*]	uncharacterized LOC100500056 (LOC100500056)	K03626	——	Glyma03g27570	(M=8) PTHR21713 - NASCENT POLYPEPTIDE ASSOCIATED COMPLEX ALPHA SUBUNIT-RELATED
increase	gi|255631888	unknown [*G. max*]	uncharacterized LOC100527254 (LOC100527254)	K03626	——	Glyma03g27580	(M=8) PTHR21713 - NASCENT POLYPEPTIDE ASSOCIATED COMPLEX ALPHA SUBUNIT-RELATED
decrease							
decrease	gi|122194126	Rec Name: Full=Phosphomannomutase; AltName:Full=GmPMM	Phosphomanno-mutase (LOC732605)	K17497	Metabolic pathways, Biosynthesis of secondary metabolites, Fructose and mannose metabolism, Amino sugar and nucleotide sugar metabolism	Glyma18g46390	(M=2) PTHR10466 - PHOSPHOMANNOMUTASE
decrease	gi|255644424	unknown [*G. max*]	glyoxylate,succinic semialdehyde reductase 1-like (LOC100817216)	K18121	Microbial metabolism in diverse environments, Metabolic pathways, Carbon metabolism, Glyoxylate and dicarboxylate metabolism, Butanoate metabolism	Glyma07g06570	(M=3) PTHR22981:SF53 - GLYOXYLATE,SUCCINIC SEMIALDEHYDE REDUCTASE 2, CHLOROPLASTIC
decrease	gi|255564914	aspartyl-tRNAsynthetase, putative [*R. communis*]	aspartate--tRNA ligase 2, cytoplasmic (LOC8282458)	K01876	Aminoacyl-tRNA biosynthesis	Glyma14g11711	(M=4) 6.1.1.12 - Aspartate--tRNA ligase , Aspartyl-tRNAsynthetase
decrease	gi|255630927	unknown [*G. max*]	uncharacterized LOC100500685 (LOC100500685)	K03809	Biosynthesis of secondary metabolites, Ubiquinone and other terpenoid-quinone biosynthesis	Glyma13g32300	(M=30) 1.6.5.2 - NAD(P)H dehydrogenase (quinone) , Vitamin-K reductase
decrease	gi|255627623	unknown [*G. max*]	uncharacterized LOC100306124 (LOC100306124)	——		Glyma20g38260	(M=2) PTHR10302:SF6 - SINGLE-STRANDED DNA-BINDING PROTEIN, MITOCHONDRIAL
decrease	gi|42543705	Chain A, Crystal Structure of *Proglycinin* Mutant C88s		——		Glyma03g32030	(M=6) PTHR31189:SF1 - 12S SEED STORAGE PROTEIN CRA1-RELATED

Table 5: Continued

Table 5: Continued

increase	gi|255629055	unknown [*G. max*]	uncharacterized LOC100306609 (LOC100306609)	——	——	Glyma02g43790	PTHR11306,„PTHR11306:SF13 - NIEMANN PICK TYPE C2 PROTEIN NPC2-RELATED
decrease	gi|255627117	unknown [*G. max*]	uncharacterized LOC100499848 (LOC100499848)	——		Glyma08g24760	(M=39) PF00407 - Pathogenesis-related protein Bet v I family (Bet_v_1)

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