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Research article

A comparative study of proteomic differences between pencil and storage roots of sweetpotato (*Ipomoea batatas* (L.) Lam.)

Jeung Joo Lee [a, 1], Yun-Hee Kim [b, 1], Youn-Sig Kwak [a, 1], Jae Young An [a], Pil Joo Kim [c], Byung Hyun Lee [d], Vikranth Kumar [e], Kee Woong Park [f], Eun Sil Chang [g], Jae Cheol Jeong [h], Haeng-Soon Lee [h], Sang-Soo Kwak [h, *]

[a] Department of Applied Biology, IALS, Gyeongsang National University, Jinju 660-701, Republic of Korea
[b] Department of Biology Education, College of Education, IALS, PMBBRC, Gyeongsang National University, Jinju 660-701, Republic of Korea
[c] Division of Applied Life Science (BK21 Program), IALS, College of Agriculture and Life Sciences, Gyeongsang National University, Jinju 660-701, Republic of Korea
[d] Division of Applied Life Science (BK21 Program), IALS, PMBBRC, College of Agriculture and Life Sciences, Gyeongsang National University, Jinju 660-701, Republic of Korea
[e] Division of Applied Life Science (BK21 Program), PMBBRC, Gyeongsang National University, Jinju 660-701, Republic of Korea
[f] Department of Crop Science, Chungnam National University, Daejeon 305-764, Republic of Korea
[g] Gyeongsangnam-do Agricultural Research & Extension Services, Jinju 660-985, Republic of Korea
[h] Plant Systems Engineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-806, Republic of Korea

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ABSTRACT

Fibrous roots of sweetpotato (*Ipomoea batatas* (L.) Lam.) usually develop into both pencil and storage roots. To understand protein function in root development, a proteomic analysis was conducted on the pencil and storage roots of the light orange-fleshed sweetpotato cultivar, Yulmi. Two-dimensional gel electrophoresis showed that expression of 30 protein spots differed between pencil and storage roots: 15 proteins were up-regulated or expressed in pencil roots and 15 in storage roots. Differentially expressed proteins spots were investigated using matrix-assisted laser desorption/ionization time of flight mass spectrometry, and 10 proteins from pencil roots were identified as binding protein isoform A, catechol oxidase, peroxidases, ascorbate peroxidase, endochitinase, flavanone 3-hydroxylase and unknown proteins. Of the proteins up-regulated in, or restricted to, storage roots, 13 proteins were identified as protein disulfide isomerase, anionic peroxidase, putative ripening protein, sporamin B, sporamin A and sporamin A precursor. An analysis of enzyme activity revealed that catechol oxidase and peroxidase as the first and last enzymes of the lignin biosynthesis pathway, and ascorbate peroxidase had higher activities in pencil than in storage roots. The total concentration of phenolic compounds was also far higher in pencil than in storage roots, and lignin accumulated only in pencil roots. These results provide important insight into sweetpotato proteomics, and imply that lignin biosynthesis and stress-related proteins are up-regulated or uniquely expressed in pencil roots. The results indicate that the reduction of carbon flow toward phenylpropanoid biosynthesis and its delivery to carbohydrate metabolism is a major event in storage root formation.

1. Introduction

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is the seventh ranked food crop in annual production across the world. It is energy-rich with high carbohydrate and sugar content, and is a good source of nutrients such as calcium, iron and other minerals, as well as various antioxidant compounds (Yoshinaga et al., 1999; Teow et al., 2007). Storage roots of sweetpotato are used mainly for human consumption, animal feeds and industrial products. Despite its importance, there have been few large-scale systematic studies of sweetpotato genomics, transcriptomics or proteomics.

Sweetpotato yield and quality is dependent upon storage root development during plant growth (Tanaka et al., 2005; Noh et al.,

* Corresponding author.
E-mail address: sskwak@kribb.re.kr (S.-S. Kwak).
[1] These authors contributed equally to this work.

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2010). The root systems of sweetpotato are consists of fibrous roots and storage roots that absorbs nutrients and water, and store photosynthetic products, respectively. Otherwise, as the plant matures, lignified thick pencil roots are produced, whereas storage roots have no lignification (Human, 1992). Recently, transcriptomic analysis of sweetpotato root development revealed up-regulation of starch biosynthesis-related genes and down-regulation of lignin biosynthesis-related genes in developing storage roots (Firon et al., 2013). However, the molecular and biochemical mechanisms underlying development of the different root types remain unclear.

Proteomic research involves the systematic study of the proteome, the entire set of proteins expressed by a genome, and allows qualitative and quantitative analysis of a large number of proteins that directly influence biochemistry at the cellular and tissue levels and in different conditions (Chen and Harmon, 2006). It can provide diverse information related to the function of an organism by allowing an accurate analysis of both the cellular state and of systematic changes during plant growth and development. Proteomic analysis also offers molecular information that assists in the classification of different plant species or cultivars. We have previously reported on the differences in protein expression between the storage roots of the light orange-fleshed and purple-fleshed sweetpotato cultivars (Lee et al., 2012). We identified 35 proteins that were up-regulated or uniquely expressed in either cultivar, and characterized the function of 23 of these. We compared the expression levels and activities of several enzymes in the two cultivars, and investigated the resistance of each cultivar to the root-knot nematode to determine the roles of up-regulated or uniquely expressed proteins in nematode resistance. Increases in protein expression could confer resistance to biotic and/or abiotic stresses on sweetpotato storage roots.

To better understand proteomic regulation of the root developmental mechanisms in sweetpotato plants, we used two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) to identify proteins up-regulated or uniquely expressed in either pencil or storage roots of the light orange-fleshed sweetpotato cultivar, Yulmi. We also investigated the transcript levels of mRNAs of several identified proteins, concentration of glucose, activities of several enzymes, and determined lignin biosynthesis in the two root types. The results will contribute to a better understanding of proteomic differences and the morphological and physiological factors involved in the root development between pencil and storage roots of sweetpotato.

2. Materials and methods

2.1. Plant materials

Shoot cuttings, about 20 cm long and with four leaves, were harvested from the light orange-fleshed sweetpotato (*I. batatas* (L.) Lam.) cultivar Yulmi and planted in plastic pots of 40 × 30 × 20 cm (width × length × height) filled with a mix of clay loam and vermiculite (3 loam: 1 vermiculite). The plants were cultivated according to standard agricultural practices in the greenhouse. After three months, pencil roots (approximately 2 mm in diameter) and storage roots (approximately 2 cm in diameter) were collected from each pot and washed with tap water. Plants were cultivated as six repetitions. The root samples were ground to a fine powder in the presence of liquid nitrogen using a pestle and mortar and stored at −70 °C until further analysis.

2.2. Protein extraction and 2-dimensional electrophoresis

Total proteins were extracted from the pencil and storage roots of sweetpotato according to the modified phenol-based method (Hajduch et al., 2005). The extraction buffer was contained 0.9 M sucrose, 0.1 M Tris–HCl, pH 8.8, 10 mM EDTA, and 0.4% [v/v] 2-mercaptoethanol. Total protein content was measured according to the Bradford method (Bradford, 1976).

Isoelectric focusing (IEF) and 2-dimensional SDS-PAGE (2-DE) were performed, as described previously (Lee et al., 2012). Five hundred micrograms of total protein extract was separated on Bio-Rad 17 cm immobilized pH gradient gel strip (pH 4–7). IEF conditions were as follows: 250 V for a conditioning step of 15 min, followed by a slow ramping step to 10,000 V for 3 h, and finally 10,000 V for 9 h. After IEF, the IPG strips were equilibrated according to the manufacturer's protocol (Bio-Rad). Two-dimensional SDS-PAGE gel (13% total monomer, with 0.8% crosslinker) was electrophoresed using a PROTEAN II xi Cell (Bio-Rad). The 2-DE gel was stained with colloidal Coomassie brilliant blue (Matsui et al., 1999). At least three independent protein samples were extracted from different repetitions of pencil and storage roots and used in 2-DE analysis. Gel images were analyzed using a GS-800 Calibrated Imaging Densitometer (Bio-Rad) and PDQuest software (Version 7.2; Bio-Rad, Hercules, CA, USA). The intensities of the up-regulated or uniquely expressed protein spots in the two root types were normalized to a relative intensity, and the mean values calculated from triplicate data were compared. Only protein spots with a significant and reproducible increase over 1.2 times were considered to be up-regulated or uniquely expressed proteins, and the spots showing a statistical level of $p < 0.05$ by Student's t test were selected for identification.

2.3. MALDI-TOF MS analysis

The differentially expressed protein spots were excised from the Coomassie brilliant blue-stained gels and subjected to in-gel digestion with trypsin, as described previously (Lee et al., 2007). The peptides were extracted twice with a mixed solution of acetonitrile (ACN), water, and trifluoroacetic acid (TFA) (66:33:1, respectively). The combined supernatant was lyophilized and dissolved in 50% ACN, 0.1% TFA solution. The peptide solution was lyophilized again, and then dissolved in a matrix solution containing 50% ACN, 0.5% α-cyano-4-hydroxycinnamic acid (CHCA), and 0.1% TFA (Yan et al., 2005).

MS analysis was carried out using a Voyager-DE STR MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA, USA). Spectra were obtained in the reflection/delayed extraction mode (Kwon et al., 2010). Peptide peaks were selected in the mass range 800–3000 Da. The spectra were internally calibrated using des-Arg1-bradykinin (m/z 904.4681), angiotensin 1 (m/z 1296.6853), neurotensin (m/z 1672.9175), and adrenocorticotropic hormone (m/z 2465.1989) (Sigma–Aldrich). Monoisotopic peptide masses were analyzed with MoverZ (http://www.proteomics). Peptide mass fingerprints (PMFs) were searched for in the NCBInr database using Mascot (http://www.matrixscience.com) and Protein Prospector (http://prospector.ucsf.edu) (Lee et al., 2012). The following parameters were used for database searches: mass tolerance of 50 ppm, one missed cleavage, oxidation of methionine, and alkylation of cysteine by iodoacetamide (Kwon et al., 2010).

2.4. Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from the pencil roots and storage roots of sweetpotato using Trizol reagent (Invitrogen, USA). First-strand cDNA was synthesized from total RNA (1 μg) using the Improm-II reverse transcription kit (Promega, USA), according to the manufacturer's instructions. PCR amplification reactions were initially incubated at 94 °C for 5 min, followed by 28 cycles of 94 °C for 30 s,

60 °C for 30 s, and 72 °C for 45 s. As an internal control, we also analyzed the expression of alpha tubulin. Gene-specific primers were designed from the 3′-UTR or the region near the translation stop codon of each gene. The primers are shown in Table 1.

2.5. Glucose determination

The glucose content of pencil roots and storage roots was determined by the glucose oxidase–peroxidase method using the glucose (GO) assay kit according to the manufacturer's instructions (Sigma, Saint Louis, Missouri, USA). The kit contained glucose oxidase–peroxidase reagent (product code G 3660), O-dianisidine reagent (product code D 2679) and a glucose standard (product code G 3285). Pencil roots and storage roots (250 mg) were extracted with deionized water. A standard curve for glucose within the range 0–80 μg ml^{-1} was prepared. The determination of glucose content were done with four repetitions.

2.6. Enzyme assays

For determination of guaiacol-type peroxidase (POD; EC 1.11.1.7) and ascorbate peroxidase (APX; EC 1.11.1.11) activity (Park et al., 2011), approximately 2 g of the frozen powder of root sample was homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 5 mM EDTA, 0.1 mM ascorbic acid, and 10% insoluble polyvinylpolypyrrolidone (w/w). The homogenate was centrifuged at 12,000 *g* for 20 min at 4 °C, and the supernatant was used for the determination of enzyme activity (Lee et al., 2012). Guaiacol-type POD activity was analyzed by monitoring the increase in absorbance at 470 nm resulting from guaiacol oxidation (E ± 26.6 mM^{-1} cm^{-1}) (Blume and McClure, 1980). The assay mixture comprised 50 mM potassium phosphate buffer (pH ± 7.0), 1.5 mM guaiacol, 0.1 mM H_2O_2, and an aliquot of the crude enzyme. APX activity was determined by monitoring the decrease in absorbance at 290 nm resulting from ascorbate reduction (E ± 2.8 mM^{-1} cm^{-1}) (Nakano and Asada, 1981). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM ascorbate, and an aliquot of crude enzyme. Additionally, crude enzyme for the determination of catechol oxidase (CO; EC 1.10.3.1) activity was extracted from root tissue samples using 20 mM phosphate buffer (pH 6.0). CO activity was assayed by determining the increase in absorbance at 410 nm resulting from catechol oxidation, as described previously (Gherardi et al., 1999). All enzyme assays were done with four repetitions.

2.7. Determination of total phenolic compounds and lignin

To measure contents of phenolic compounds in root tissue, approximately 1 g of the frozen, powdered root sample was homogenized in 80% ice-cold ethanol and centrifuged, and the supernatant was used to determine the phenolic compound content (Singleton and Rossi Jr., 1965). A standard curve was prepared across the range 0–100 μM using gallic acid.

To determinate lignin content in sweetpotato roots, approximately 1 g of the frozen, powdered sample was homogenized with ethanol and dried at room temperature (Bruce and West, 1989). Next, 20 mg of the dried residue was boiled in 2 N HCl and thioglycolic acid. The heated residue was centrifuged and agitated with 2.5 N NaOH. Lignin thioglycolate was precipitated from 500 μl of the supernatant using 2 N HCl and dissolved in 0.5 N NaOH. The lignin content of the sample was determined using a calibration curve of commercial alkali lignin (Sigma–Aldrich, Steinheim, Germany), obtained over the range 0–20 μg (Müsel et al., 1997). Determination of total phenolic compounds and lignin contents were done with four repetitions.

2.8. Histochemical staining for lignin

Fresh pencil roots (approximately 2 mm in diameter) and storage roots (approximately 2 cm in diameter) were collected and washed under running tap water. Root sections were cut using a plant MTH-1 microtome. Sections were immersed in 1% phloroglucinol-HCl solution for one minute (Galavazi, 1965) and blotted with a paper towel to remove excess solution. Photographs were taken through a light microscope (Carl Zeiss Inc.).

3. Results

3.1. 2-DE analysis

The total proteins extracted from pencil and storage roots were electrophoresed using 2-DE. Representative 2-DE gels of high quality are shown in Fig. 2. To investigate protein expression patterns in the two root types, protein spots of all replicate gels were compared and quantified using PDQuest software. In total, 30 protein spots showed reproducible up-regulation (>1.7 times) or unique expression in either pencil or storage roots (Fig. 2). Of these protein spots, eight spots (Fig. 2: spots 1, 2, 8, 9–11, 16 and 27) were uniquely expressed and seven spots (Fig. 2: spots 4, 5, 12, 14, 15, 18 and 26) were up-regulated in pencil roots. Two spots (Fig. 2: spots 23 and 28) were uniquely expressed and 13 spots (Fig. 2: spots 3, 7, 8, 13, 17, 19–22, 24, 25, 29, and 30) were up-regulated in storage roots. The expression levels of the up-regulated and uniquely expressed spots were quantified and the results are shown in Table 1.

3.2. Identification of the up-regulated or uniquely expressed proteins

The excised protein spots were digested with trypsin and identified by their PMF using MALDI-TOF MS. Of the initial 30 spots

Table 1
Primer sequences used for expression analysis of sweetpotato genes in this study.

cDNA	Forward primer	Reverse primer	Product size (bp)
Catechol oxidase (AJ309176.1)	GTCGTCACCGTCGAGAACAT	ACCACATGCTAGGCCAACTC	176
Anionic peroxidase (Z84472.1)	AGATTGCTTGCTTCACCCGA	TTTTTGGAGCATATCATCACATAATTT	199
Flavanone 3-hydroxylase (AB023790.1)	GGAAGGGGACAAGCCGATTA	CAAGTTCTTATCCGCAGCAGC	151
Ascorbate peroxidase (AY206407.1)	TGCCTTCTTTGCTGACTATCCAGA	CGCATACACTTTCAGAGCACAACA	199
Protein disulfide isomerase (AY622222.1)	CTGCAACGGGTAACATAACACC	TCGCCATGCTATTCGACTCC	178
Sporamin A (X15091.1)	CGACCACATGTTGAGGACCA	ACACAACCATACCAGCTCGG	197
Sporamin B (X13510.1)	GGTGTTGAGGTTGCATGCAT	ACACGGGCTCATTCACATAGA	181
Sporamin B precursor (X15094.1)	GGTCAACGACAACCTTAACGC	ATGCATGCAACCTCAACACC	188
α-tubulin (BM878762.1)	CAACTACCAGCCACCAACTGT	CAAGATCCTCACGAGCTTCAC	212

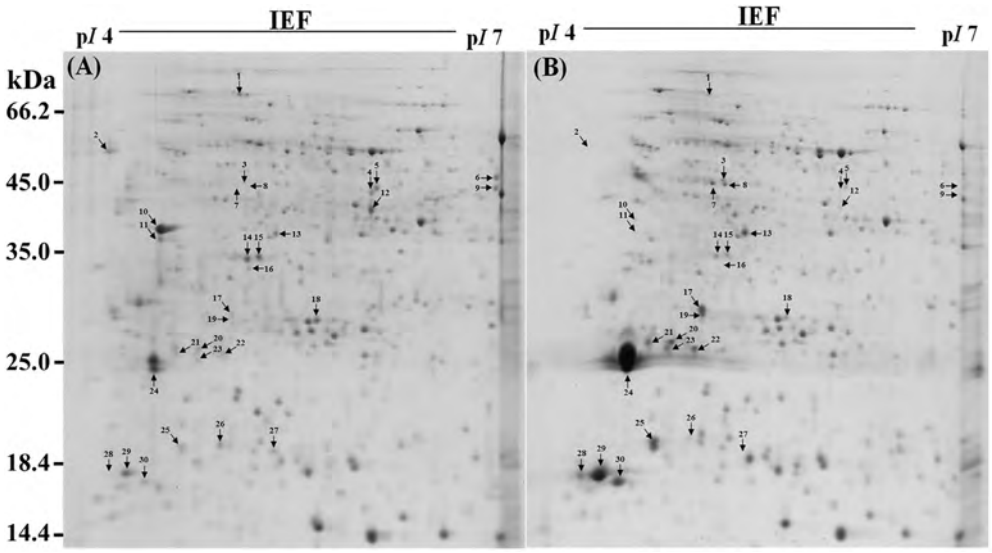

Fig. 1. Representative 2-DE image of total proteins extracted from pencil roots (A) and storage roots (B) of the sweetpotato cultivar, Yulmi. The spot numbers indicate proteins that were increased or uniquely expressed in either pencil or storage roots.

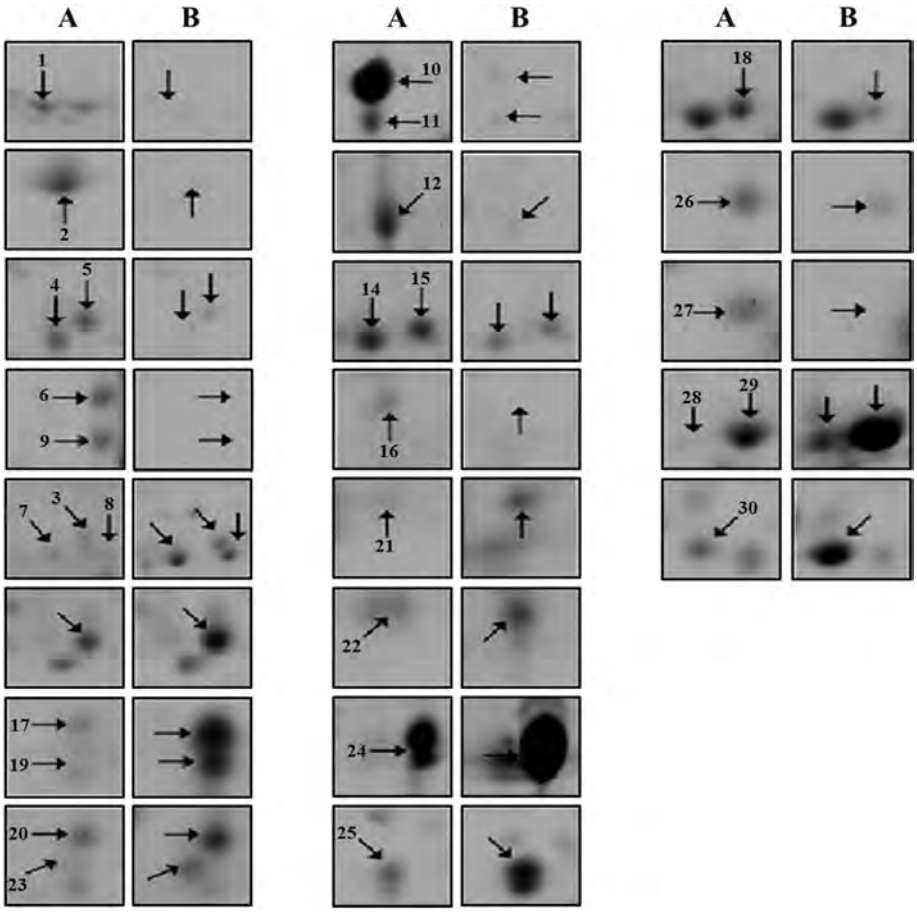

Fig. 2. Enlarged views of 2-DE maps of the up-regulated or uniquely expressed proteins marked in Fig. 1. (A) Pencil roots. (B) Storage roots.

showing differential expression between root types, 23 could be identified (Table 2) but seven spots (Fig. 2: 14–16, 26, 27, 29, and 30) remained unknown. In pencil roots, nine protein spots were identified as binding protein (BiP) isoform A (spots 1), CO (spot 2), anionic POD (spot 4), neutral POD (spots 6 and 9), acidic endochitinase (spots 10 and 11), flavanone 3-hydroxylase (F3H; EC 1.14.11.9) (spot 12), and APX (spot 18). Among the pencil roots expressed proteins, CO, PODs and F3H are phenylpropanoid pathway

Table 2
Identification of proteins up-regulated or uniquely expressed in either pencil or storage roots of the sweetpotato cultivar, Yulmi, by MALDI-TOF MS analysis.

Spot ↓ no[a]	Protein name	NCBI accession no.	Organism	Abundance ratio[b]	M_r[c]/pI[d]		Score	M.P.[e]	S.C. (%)[f]	Tissue[g]
					Theoretical	Observed				
1	Bip isoform A	AAA81956	*Glycine max*	2.14 ± 0.08	73.3/5.1	71.1/5.1	419	3	34	P
2	catechol oxidase	CAC83610	*Ipomoea batatas*	6.00 ± 1.00	56.6/6.0	54.5/4.5	74	5	16	P
3	protein disulfide isomerase	AAT39459	*Ipomoea batatas*	2.76 ± 0.21	60.0/4.8	45.0/5.2	46	4	11	S
4	Anionic peroxidase	O04795	*Ipomoea batatas*	3.71 ± 0.44	38.7/5.6	44.0/6.0	62	6	18	P
5	Unidentified	AAB86473	*Zea mays*	2.98 ± 0.96	45.5/5.6	44.5/6.1	135	6	20	P
6	Neutral peroxidase	O04796	*Ipomoea batatas*	3.12 ± 0.41	37.2/6.9	46.0/7.0	121	7	26	P
7	protein disulfide isomerase	AAT39459	*Ipomoea batatas*	4.81 ± 0.85	60.0/4.8	44.0/5.1	48	3	8	S
8	anionic peroxidase Swpa3	AAF00094	*Ipomoea batatas*	3.88 ± 0.47	37.0/5.0	44.5/5.3	38	3	9	S
9	Neutral peroxidase	O04796	*Ipomoea batatas*	4.52 ± 0.60	37.2/6.9	44.0/7.0	171	–	29	P
10	Acidic endochitinase	P29024	*Vigna angularis*	17.54 ± 3.40	32.1/4.9	38.5/4.8	59	3	6	P
11	Acidic endochitinase	P29024	*Vigna angularis*	4.95 ± 1.03	32.1/4.9	33.0/4.8	63	3	6	P
12	flavanone 3-hydroxylase	BAA75309	*Ipomoea batatas*	6.13 ± 1.52	41.1/5.8	40.5/6.0	72	7	27	P
13	putative ripening protein	BAF46301	*Ipomoea nil*	1.75 ± 0.09	26.0/5.2	33.0/5.4	88	4	27	S
14	Unidentified			2.39 ± 0.54		34.7/5.2				P
15	Unidentified			2.18 ± 0.35		34.8/5.3				P
16	Unidentified			2.78 ± 0.25		33.5/5.2				P
17	Spramin B	P10965	*Ipomoea batatas*	4.48 ± 1.13	23.9/5.4	29.9/5.1	73	7	44	S
18	acorbate penroxidase	AAP42501	*Ipomoea batatas*	3.38 ± 0.95	27.6/5.3	27.4/5.7	80	8	32	P
19	Spramin B	P10965	*Ipomoea batatas*	4.60 ± 1.19	23.9/5.4	27.4/5.1	107	10	52	S
20	Spramin A (Clone PIM0335)	P14715	*Ipomoea batatas*	6.78 ± 0.44	24.0/5.8	26.3/5.0	85	9	46	S
21	Spramin A precursor (Clone PIM0335)	S07465	*Ipomoea batatas*	2.87 ± 0.41	24.0/5.8	25.6/4.9	68	9	46	S
22	Spramin A (Clone PIM0335)	P14715	*Ipomoea batatas*	2.37 ± 0.35	24.0/5.8	25.6/5.1	81	9	46	S
23	Spramin A precursor	ABB97544	*Ipomoea batatas*	2.57 ± 0.25	24.0/5.7	25.3/5.0	89	8	46	S
24	Spramin B	P10965	*Ipomoea batatas*	45.21 ± 7.24	23.9/5.4	24.0/4.8	74	7	48	S
25	Spramin B (Clone PIM0535)	P14716	*Ipomoea batatas*	3.22 ± 0.57	24.0/5.4	19.4/4.9	59	6	29	S
26	Unidentified			2.20 ± 0.10		19.6/5.0				P
27	Unidentified			3.42 ± 0.35		19.2/5.4				P
28	Spramin B	P10965	*Ipomoea batatas*	5.32 ± 2.14	23.9/5.4	17.2/4.5	56	6	38	S
29	Unidentified			25.12 ± 2.45		17.2/4.6				S
30	Unidentified			15.34 ± 2.38		16.4/4.7				S

[a] Spot numbers correspond to the 2-DE gels shown in Figs. 1 and 2.
[b] The abundance ratios of each spot were measured using a densitometer (Bio-Rad)and then compared to pencil roots and storage roots.
[c] Molecular weight.
[d] Isoelectric point.
[e] Number of matched peptide.
[f] Sequence coverage.
[g] P, pencil roots; S, storage.

compound-related proteins. PODs and chitinase are cell wall compound-related proteins. PODs are also antioxidant defense enzymes with APX. Therefore, pencil root expressed proteins are phenylpropanoids, cellwall compound and antioxidant defense related proteins.

In storage roots, 13 protein spots were identified as protein disulfide-isomerase (PDI; EC 5.3.4.1) (spots 3 and 7), anionic POD (spot 8), putative ripening protein (spot 13), sporamin B (spots 17, 19, 24, 25, and 28), sporamin A (spots 20 and 22), and sporamin A precursor (spots 21 and 23). Spot 5 was annotated as an unknown

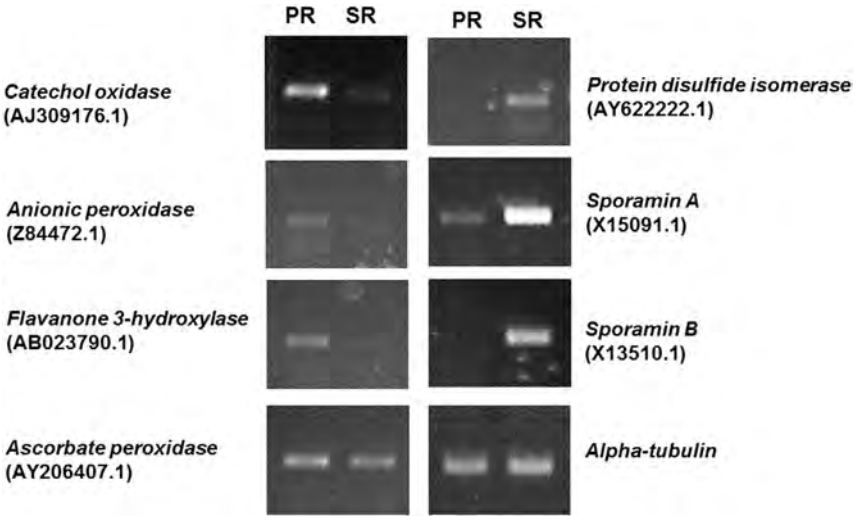

Fig. 3. RT-PCR of genes encoding selected proteins that were differently expressed in pencil roots (PR) and storage roots (SR) of the sweetpotato cultivar, Yulmi. Alpha-tublin served as an internal control.

protein. Sporamins are trypsin inhibitors that protect plants from insects by inhibiting insect digestion. Our results indicate the almost all expressed proteins in storage root are involved in maturity of storage roots or defense proteins against insect attack.

3.3. Correlation between protein and transcript abundance in pencil roots and storage roots of sweetpotato

To determine whether the expression levels of the proteins identified by proteomic approaches correlated with the abundance of their mRNAs, we performed RT-PCR analysis using gene specific primers for several genes encoding proteins up-regulated or induced only in pencil roots or storage roots of sweetpotato (Fig. 3). The expression levels of mRNAs for catechol oxidase (spot 2), anionic peroxidase (spot 4), flavanone 3-hydroxylase (spot 12), and ascorbate peroxidase (spot 18) up-regulated or expressed only in pencil roots were higher in pencil roots than in storage roots. The mRNA levels for protein disulfide isomerase (spot 3), sporamin A (spot 20 and 22) and sporamin B (17, 19, 24, 25, 28) up-regulated or expressed only in storage roots were higher in storage roots than in pencil roots.

3.4. Activities of several enzymes

We determined the activities of several enzymes up-regulated or uniquely expressed in pencil roots, and compared them with the activities in storage roots. CO activity was 1.7 times higher in pencil roots than in storage roots (Fig. 4A). The activity of guaiacol-type POD was 35.2 times higher in pencil roots than in storage roots (Fig. 4B), and APX activity was 1.8 times higher in pencil roots than in storage roots (Fig. 4C).

3.5. Accumulation of glucose, total phenolic compounds, and lignin

3.5.1. Concentration of glucose was determined in pencil and storage roots

The results showed that glucose accumulated to 0.38 mg g^{-1} FW in pencil roots, whereas it accumulated to 7.6 mg g^{-1} FW in storage roots (Fig. 5A). The accumulation of total phenolic compounds and lignin was also investigated in both pencil and storage roots. The level of total phenolic compounds was more than 12 times higher in pencil roots than in storage roots (Fig. 5B). Lignin levels accumulated to more than 2.2 mg g^{-1} FW in pencil roots, whereas lignin could not be detected in storage roots (Fig. 5C). Furthermore, lignin specific staining revealed the accumulation of high levels of lignin around the xylem in pencil roots (Fig. 6).

4. Discussion

Although sweetpotato is one of the most important root crops, study of its genomics and proteomics is still in its early stages. Different metabolic and molecular processes underlie the development of sweetpotato roots into pencil roots and, subsequently, into storage roots. Despite considerable efforts to investigate physiological responses during root development, the molecular and biochemical mechanisms involved remain incompletely understood. In this study, we evaluated differences in proteomic profiles between pencil and storage roots, which resulted in the identification of proteins whose expression varied between the two root types. We also quantified the differential activities of several enzymes that play potential roles in root development.

Three protein spots (4, 12 and 18) displayed up-regulated expression and six protein spots (1, 2, 6, 9, 10 and 11) expressed only in pencil roots were identified as proteins with known physiological roles (Table 2). Spots 1 was identified as the BiP isoform A. BiP, a molecular chaperone, mediates folding of proteins into their correct conformations, and also appears to play an essential role in the recognition of misfolded or malformed proteins in the endoplasmic reticulum (ER) (Kalinski et al., 1995). Stress conditions further increase the need for molecular chaperones to prevent protein aggregation and refold misfolded polypeptides (Sung et al., 2001). Conditions, such as glucose starvation, which inhibit glycosylation of proteins in the ER, are universal inducers of BiP synthesis (Pedrazzini et al., 1994). In this study, we that the glucose content of storage roots was 20-fold higher than the glucose content of pencil roots (Fig. 5A). We suggest this is the reason for the higher expression of BiP in pencil roots, as it would counteract the low glucose level in pencil roots. Spots 2 and 4 were identified as CO and POD, which catalyze the biosynthesis of lignin (Sterjiades et al., 1993; Mandal and Mitra, 2007; Singh et al., 2010), respectively. Spots 6 and 9 were identified as a neutral POD. COs are ubiquitous plant enzymes that catalyze the *o*-hydroxylation of monophenols to *o*-diphenols, and the oxidation of *o*-diphenols to *o*-quinones by dioxygen (Klabunde et al., 1998; Wuyts et al., 2006). PODs catalyze

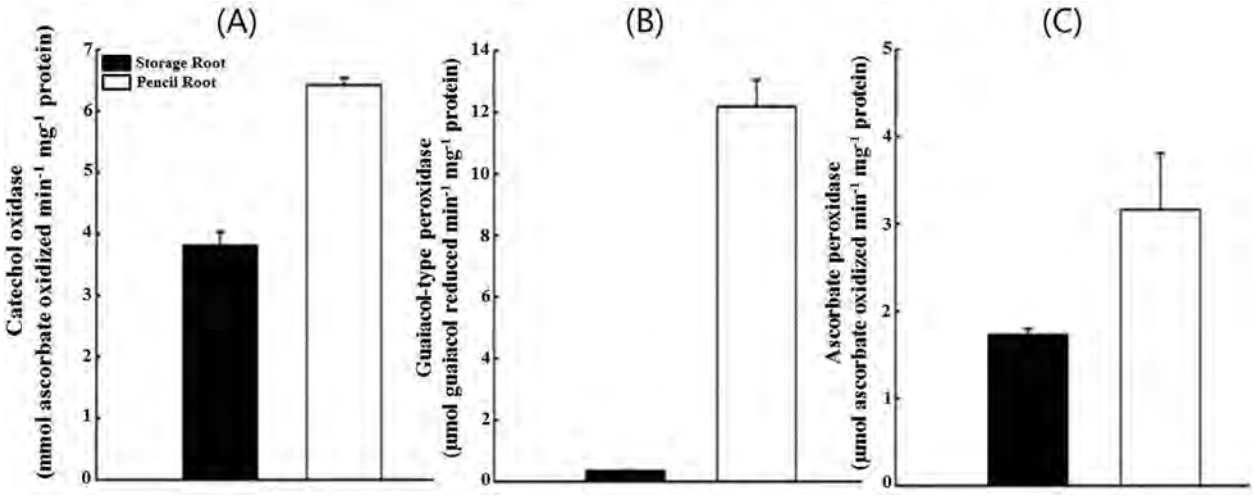

Fig. 4. Activities of several enzymes in pencil and storage roots of the sweetpotato cultivar, Yulmi. (A) Catechol oxidase. (B) Guaiacol-type peroxidase. (C) Ascorbate peroxidase. Data are means ± SE of four independent replicates.

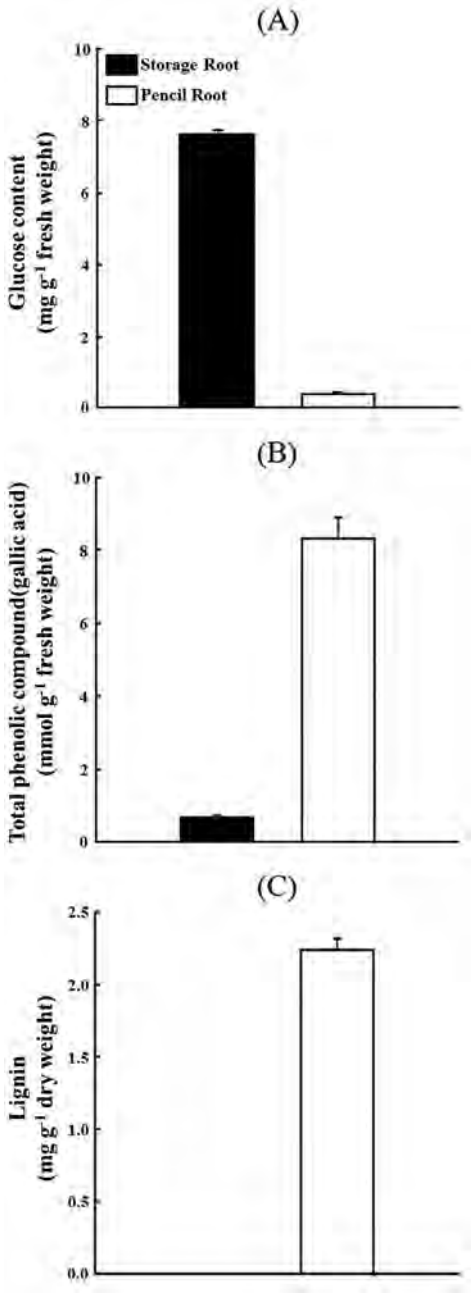

Fig. 5. Concentration of (A) glucose, (B) total phenolic compounds, and (C) lignin in pencil roots and storage roots of the sweetpotato cultivar, Yulmi. Data are means ± SE of four independent replicates.

the reduction of hydrogen peroxide into water using electrons from various donor molecules (Bernards, 2002; Yang et al., 2007). There are a number of POD isoenzymes, which are usually classified as cationic, neutral or anionic, according to their isoelectric points (Yang et al., 2007). Each isoenzyme is thought to serve a different function during plant cell growth and development; however, their actual physiological roles in plants are still unclear (Kim et al., 1999). Spots 10 and 11 were an acidic endochitinase. Chitinases (EC 3.2.1.14) catalyze the hydrolysis of chitin, which is present in the cell walls of a variety of pathogens, including fungi (Santos et al., 2004). Plant chitinases are generally endochitinases, which may be acidic or basic in form (Sahai and Manocha, 1993). Chitinases are induced in higher plants by different types of stress, but they are also expressed constitutively at a low level under normal condition (Christopher et al., 2004). Spot 12 was a F3H, which is the third enzyme of central flavonoid metabolism. In plant cells this enzyme catalyzes the hydroxylation of naringenin to dihydrokaempferol, the common precursor of the major classes of 3-hydroxy flavonoids, flavonols, anthocyanins, and proanthocyanidins (Owens et al., 2008). Diverse flavonoid compounds, including anthocyanins, play important roles in plant defense against a variety of biotic and abiotic stressors, as well as in the coloration of fruits, flowers, and underground organs (Chalker-Scott, 1999; Winkel-Shirley, 2002). Spot 18 was APX, which is a hydrogen peroxide-scavenging antioxidant defense enzyme with a high specificity for ascorbate as the electron acceptor. APX activity in higher plants increases in response to abiotic or biotic stress conditions (Park et al., 2004).

Eleven protein spots (spots 3, 7, 8, 13, 17, 19–22, 24, and 25) up-regulated and two spots (spots 23 and 28) expressed only in storage roots were identified as proteins with known physiological roles (Table 2). Spots 3 and 7 were a protein PDI, which is an essential enzyme for the modification (i.e., formation, isomerization or reduction) of protein disulfide bonds (Huang et al., 2005). Although little is known about the regulation of tuber development in potato (*Solanum tuberosome* L.), this enzyme was also up-regulated during tuber development in that species (Agrawal et al., 2008). Spot 8 was an anionic POD (EC 1.11.1.7) encoded by *swpa3*. Ten POD genes encoding anionic POD *swpa3* have been identified previously in sweetpotato cell cultures (Kim et al., 2007), and *swpa3* expression is induced at a high level in response to various environmental stresses, including chilling, ozone, SO_2, UV and heavy metals (Kim et al., 2007, 1999, 2010). Spot 13 was identified as a putative ripening protein. Expression of fruit-ripening protein is strongly induced during fruit maturation in tomato (*Solanum lycopersicum* L.) (Rocco et al., 2006). It has also been reported that the expression levels of ripening proteins increase under various abiotic stress conditions (Hara et al., 2002). However, little information is available regarding the major functions of these proteins in sweetpotato. Approximately 80% of soluble proteins in storage roots of sweetpotato is stored in the form of sporamins (Maeshima et al., 1985). Sporamins are encoded by a multigene family, which can be grouped into two subfamilies, sporamins A and B (Matsuoka and Nakamura, 1991; Yeh et al., 1997). Several sporamin polypeptides were expressed only in storage roots or were expressed at higher levels in storage roots than in pencil roots. Spots 20 and 22 proved to be proteins of the sporamin A subfamily, and spots 21 and 23 were the sporamin A precursor. Spots 17, 19, 24, 25, and 28 were proteins of the sporamin B subfamily.

Sweetpotato root system obtained by vegetative propagation begins with adventitious roots that develop into primary white fibrous roots and subsequently pigmented storage roots through thick root stage (Human, 1992). The pencil roots are highly lignified central cylinder formed roots from the fibrous or thick roots. It has been reported that, as sweetpotato matures, pencil roots are lignified but storage roots are not (Wilson and Lowe, 1973; Human, 1992). Unfavorable soil conditions for storage root development, such as dehydration or excessive nitrogen, are thought to cause pencil roots. In this study, we found that several lignin biosynthesis and abiotic stress-related proteins were up-regulated or uniquely expressed in pencil roots (Table 2). The higher expression of CO, POD, and APX in pencil than in storage roots was associated with an increase in their enzyme activities (Fig. 5). It has been suggested that CO is primarily responsible for the initial polymerization of monolignols into olignols in the lignin biosynthesis pathway (Sterjiades et al., 1993), whereas POD catalyzes reactions that convert olignols into highly condensed macromolecular lignin (Mandal and Mitra, 2007; Singh et al., 2010). Lignin increases not

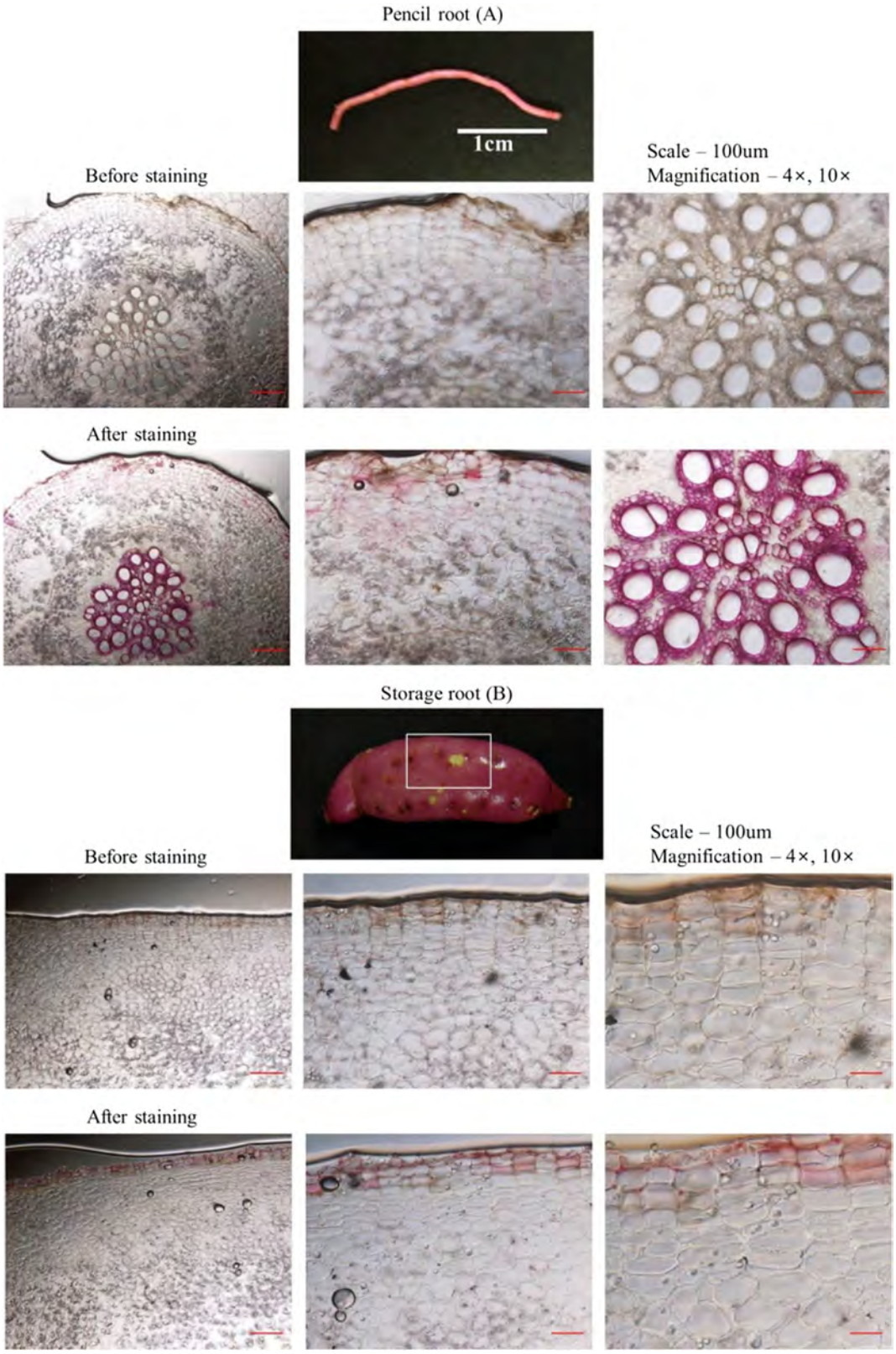

Fig. 6. Phloroglucinol staining of lignified tissue in pencil and storage roots of the sweetpotato cultivar, Yulmi. (A) The central stele of the pencil roots contains several files of lignified xylem vessels. (B) Storage roots contain no lignified parts. Scale bar: 100 μm.

only cell wall rigidity and compressive strength but also cell wall impermeability to water by increasing its hydrophobicity (Whettena and Sederoffa, 1995). The level of total phenolic compounds, the precursor of lignin, was much higher in pencil roots than in storage roots (Fig. 6A), and a consistent accumulation of lignin was observed only in pencil roots (Fig. 6B). These results

indicate that, during sweetpotato root development, physical and biochemical defense mechanisms to environmental stresses are prevalent in pencil roots metabolism. Therefore, these results could contribute to understanding of the physiological and molecular mechanism of pencil and storage root formation in root crops.

5. Conclusions

This study compared the proteomes between the pencil and storage roots of sweetpotato. We found a total of 30 protein spots that showed increased or unique expression in one or other root type, and identified 23 of these spots using MALDI-TOF MS analysis. These proteins were involved in various metabolic processes that characterize their physiological roles in pencil and storage roots. Proteins that were up-regulated or uniquely expressed in pencil roots included resistance-related proteins involved in responses to biotic or abiotic stress conditions, including Bip isoform A, acidic endochitinase, F3H, APX, CO, and POD isoenzymes. Several proteins, including sporamins, PDI, putative ripening protein, and anionic POD, were up-regulated or expressed only in storage roots. Furthermore, an increase of expression of APX, CO, and POD in pencil roots correlated with an increase in the activity of each enzyme; for example, CO and POD, which catalyze the biosynthesis of lignin, showed increased expression and activity, and an accumulation of lignin was observed in pencil roots. It is likely, therefore, that the increased expression of these proteins promotes lignin biosynthesis in pencil roots of sweetpotato.

Further study of the proteins currently annotated as 'unidentified' or 'unknown protein' will provide more information on root-specific differences in protein expression in sweetpotato. This study provides a starting point for future insight into the organ-specific variation of the sweetpotato proteome by delivering new information on the expression and physiological functions of proteins differentially expressed in two sweetpotato root types. Additionally, further investigation will be required to elucidate the exact role sweetpotato proteomes play in the regulation of root formation in sweetpotato under field conditions.

Author contributions

J.J. Lee, Y.-H. Kim, Y.-S. Kwak and S.-S. Kwak conceived and designed the experiments. J. Y. An, P.J. Kim, B. H. Lee, V. Kumar, K.W. Park, E.S. Chang, J.C. Jeong and H.-S. Lee performed the experiments and analyzed the data. J.J. Lee, Y.-H. Kim, Y.-S. Kwak and S.-S. Kwak wrote the paper.

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