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Research article

Proteomic analysis of amino acid metabolism differences between wild and cultivated *Panax ginseng*

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ABSTRACT

Background: The present study aimed to compare the relative abundance of proteins and amino acid metabolites to explore the mechanisms underlying the difference between wild and cultivated ginseng (*Panax ginseng* Meyer) at the amino acid level.
Methods: Two-dimensional polyacrylamide gel electrophoresis and isobaric tags for relative and absolute quantitation were used to identify the differential abundance of proteins between wild and cultivated ginseng. Total amino acids in wild and cultivated ginseng were compared using an automated amino acid analyzer. The activities of amino acid metabolism-related enzymes and the contents of intermediate metabolites between wild and cultivated ginseng were measured using enzyme-linked immunosorbent assay and spectrophotometric methods.
Results: Our results showed that the contents of 14 types of amino acids were higher in wild ginseng compared with cultivated ginseng. The amino acid metabolism-related enzymes and their derivatives, such as glutamate decarboxylase and S-adenosylmethionine, all had high levels of accumulation in wild ginseng. The accumulation of sulfur amino acid synthesis-related proteins, such as methionine synthase, was also higher in wild ginseng. In addition, glycolysis and tricarboxylic acid cycle-related enzymes as well as their intermediates had high levels of accumulation in wild ginseng.
Conclusion: This study elucidates the differences in amino acids between wild and cultivated ginseng. These results will provide a reference for further studies on the medicinal functions of wild ginseng.

1. Introduction

Ginseng (*Panax ginseng* Meyer), which belongs to the genus *Panax* in the *Araliaceae* family, has long been used as a traditional herbal medicine [1–3]. Wild ginseng is grown in wild environments without artificial intervention, while the growth conditions of cultivated ginseng are artificially controlled. The medicinal components of ginseng reach stable levels only when the ginseng has matured. Because of their different genotypes and growth environments, wild ginseng and cultivated ginseng have different ages of maturity. Wild ginseng takes a long time to mature (> 30 yrs), and cultivated ginseng only needs 5–6 yrs to mature [4]. Thus, cultivated ginseng has been widely employed to meet the market demand for wild ginseng. Ginseng has a wide range of pharmacological activities, including stress reduction, homeostasis, immunomodulation, antifatigue, antiaging, and anticancer effects [5–9]. However, there are some significant differences in the medicinal functions between wild and cultivated ginseng. The biologically active components of ginseng mainly include ginsenosides, polysaccharides, fatty acids, and amino acids [10]. A recent study showed that the amino acids of ginseng are candidate therapeutic agents with antidepressant, blood pressure reduction,

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immunity strengthening, and myocardium- and liver-protective activities. Previous studies have indicated that the total and essential amino acid contents of wild ginseng are 2.4- and 1.9-fold higher compared with cultivated ginseng. Thus, there are notable differences between wild ginseng and cultivated ginseng at the amino acid level. However, the mechanism of these differences and their effect on the medical functions of ginseng are not well understood.

Proteomics can directly address many biological questions by revealing the abundance of specific proteins within organisms. Traditionally, two-dimensional polyacrylamide gel electrophoresis (2DE) has been the gold standard for proteomic analysis. However, this platform is limited by protein identification and quantification capabilities [11]. Isotope tags for relative and absolute quantification (iTRAQ) reagent coupled with matrix-assisted laser desorption/ionization-time of flight/time of flight (MALDI-TOF/TOF) MS analysis can identify proteins that 2DE fails to separate, such as membrane proteins and low abundance proteins [12]. Thus, iTRAQ could be a good complement to 2DE. Recently, proteomic analysis has been performed to reveal the regulatory mechanism of plant amino acid metabolism. The 2DE approach has been used to study amino acid metabolism between different genotypes of *Arabidopsis* [13]. Amino acid metabolism has been found to play an important role in the protein synthesis, photosynthesis, and development of *Arabidopsis*. Moreover, proteomic analysis of the differential molecular responses of rice and wheat coleoptiles to anoxia has revealed the potential role of amino acid biosynthesis in cellular anoxia tolerance [14]. Thus, a proteomic approach could be used to examine the mechanism underlying the difference in amino acid metabolism between wild ginseng and cultivated ginseng.

In the present study, a proteomic approach involving 2DE and iTRAQ was used to investigate the mechanism underlying the difference in amino acid metabolism between wild and cultivated ginseng. In addition, the contents of amino acids and their derivatives, intermediates, and metabolites related to glycolysis and several enzyme activities were compared between the two cultivars. Our findings could be helpful in revealing the mechanism underlying the difference in the medical effects between wild and cultivated ginseng.

2. Materials and methods

2.1. Plant materials

Because mature ginseng has medicinal uses and the ages of mature wild and cultivated ginseng are 30 yrs and 6 yrs, respectively, we used 30 yr old wild ginseng and 6 yr old cultivated ginseng ($n = 10$) as our plant materials. Wild ginseng was collected from Wujie wild ginseng base, Jilin Province, China (127°N, 42°E; 500 m above sea level). Cultivated ginseng was collected from Jilin Province, China (in the same area as the wild ginseng). The ginseng roots were frozen in liquid nitrogen, ground thoroughly to obtain a fine powder and stored at −80°C.

2.2. Amino acids and γ-aminobutyrate analyses

Ginseng samples were hydrolyzed in HCl (hydochlric acid) (6 M), and the pH was adjusted to 2.2 for all amino acids except tryptophan. Tryptophan samples were hydrolyzed in sodium hydroxide solution, and the pH was adjusted to 5.2. Individual amino acids were determined by comparison using an automated amino acid analyzer (Hitachi, Tokyo, Japan). γ-Aminobutyrate (GABA) was extracted as previously described by Baum et al [15] with modifications. The ground samples (200 mg) were thawed in 800 μL of a mixture of methanol: chloroform: water 12:5:3 (v/v/v). The mixture was vortexed and then centrifuged at 12,000*g* for 15 min. The supernatant was collected, and 200 μL chloroform and 400 μL water were added to the pellet. The resulting mixture was vortexed and centrifuged for 15 min at 12,000*g*. The supernatant was collected and combined with the first supernatant and recentrifuged to obtain the upper phase. The collected samples were dried in a freeze-dryer and dissolved in water. The resulting samples contained GABA and other amino acids. Each sample was passed through a 0.45 μL filter and analyzed using an automated amino acid analyzer after 6-aminoquioyl-N-hydroxysuccinimidyl carbonate derivation.

2.3. Metabolite content

Starch was extracted and quantified as described elsewhere [16]. Total soluble sugar from the roots (200 mg) was extracted in boiling water for 30 min, and the sugar levels were determined using anthrone reagent with glucose as a standard. The absorbance was read at 630 nm, and the sugar concentration was determined using a glucose standard curve [17]. The pyruvate content in the sample was determined as described by Lin et al [18]. Protein was removed from the samples using tricarboxylic acid (TCA) precipitation, and in the resulting sample, pyruvate was reacted with 2,4-nitrophenylhydrazine. The product was converted to a red color in the presence of an alkali solution, and the intensity of the color change was measured using a spectrophotometer at 520 nm. A standard calibration curve was obtained using sodium pyruvate as a reagent with a gradient of pyruvate concentrations. For glutathione (GSH), roots were ground in liquid nitrogen and homogenized in 1 mL 5% (w/v) m-phosphoric acid containing 1mM diethylene triamine pentaacetic acid and 6.7% (w/v) sulfosalicylic acid. Root extracts were centrifuged at 12,000*g* for 15 min at 4°C. GSH contents were determined according to the methods of Kortt and Liu [19] and Ellman [20] with some modifications. The S-adenosylmethionine (SAM) and indoleacetic acid (IAA) contents were quantified using an indirect competitive enzyme-linked immunosorbent assay.

2.4. Malate dehydrogenase and fumarase assay

Malate dehydrogenase activity was examined as described by Husted and Schjoerring [21] with some modifications. In this experiment, 10 μL samples were added to a 3 mL reaction mixture containing 0.17mM oxaloacetic acid and 0.094mM β-hydroxylamine reductase disodium salt in 0.1M Tris buffer, pH 7.5. The reaction was measured by a decrease in spectrophotometric absorbance at 340 nm (Hitachi U-2001) for 180 s. The same reaction system with only the addition of sample buffer was used as a blank. Fumarase was assayed spectrophotometrically at 240 nm by following the first order conversion of malate to fumarate. The mixture contained 10mM Tris-HCl, pH 7.8, 4mM dithioerythritol, and 38mM malate in a total volume of 3 mL. A molar absorption coefficient of 2.6×10^3 for fumarate [22] was used for the calculations.

2.5. Protein extraction

The proteins from wild and cultivated ginseng roots were extracted using a phenol procedure with modifications [23]. Ground tissue was precipitated with cold acetone and 0.07% β-mercaptoethanol (at least 3 times). Residual acetone was allowed to evaporate at room temperature. The dry powder was resuspended in 4 volumes of lysis buffer [7M urea, 2M thiourea, 2% (w/v) CHAPS, 1% (w/v) plant protease inhibitor]. Next, an equal volume of Tris-saturated phenol was added, the mixture was shaken at 4°C for 30 min and centrifuged at 12,000*g* at 4°C for 15 min, and the water

phase was discarded. Methanol containing 0.1M ammonium acetate was added to the phenol phase at −20°C overnight and then washed twice with methanol containing 0.1M ammonium acetate and three times with methanol containing acetone to eliminate contaminants. After the complete evaporation of acetate, the proteins were dissolved in the appropriate volume of rehydration solution [5M urea, 2M thiourea, 2% (w/v) CHPAS, 2% (w/v) N-decyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate] [24]. The protein concentrations were measured using Bradford's method [25].

2.6. 2DE

The protein samples were first separated with isoelectric focusing using linear precast immobilized pH gradient (IPG) strips (24 cm, 3–10 liner pH gradients, GE Healthcare, London, UK). IPG strips with 1.2 mg of proteins were rehydrated for 12 h and focused on 72,000 Vhs, as described previously [26]. First-dimension strips were equilibrated immediately or stored at −80°C. The first equilibration was performed in 10 mL *sodium dodecyl sulfate* (SDS) equilibration solution (75mM Tris-HCl, pH 8.8, 6M urea, 2M thiourea, 30% glycerol, 2% SDS, 0.002% bromophenol blue) with 100 mg dithiothreitol for 15 min. The second equilibration was performed with 250 mg iodoacetamide for 15 min in the same volume. Second-dimension SDS–polyacrylamide gel electrophoresis was performed using 12.5% polyacrylamide gels at 2 W per gel for 30 min and 15 W per gel for 5–6 h in six EttanDalt systems (GE Healthcare). Finally, the gels were stained using Coomassie Brilliant Blue (CBB) R-250 (Invitrogen, Carlsbad, CA, USA).

2.7. Image and data analysis

The stained gels were scanned using an Image Scanner (GE Healthcare) at 600 dpi. All spots were matched by gel-to-gel comparison using Image Master 2D Platinum software version 6.0 (GE Healthcare). Volumes of every detected spot were normalized. After normalization, the spots with statistically significant and reproducible changes in abundance were considered to be differentially expressed protein spots. Only those spots with reproducible changes (quantitative changes $>$ 1.5-fold in abundance) were considered for subsequent analyses.

2.8. Protein identification

Protein spots were manually excised from the preparative gels, digested with trypsin and analyzed using MALDI-TOF/TOF MS with a 4700 Proteomics Analyzer (Applied Biosystems, Foster City, CA, USA) as previously described [27]. The peptide mass fingerprint was analyzed with GPS (Applied Biosystems) MASCOT (Matrix Science, London, UK). The identified proteins were named according to the corresponding annotations in the National Center for Biotechnology (NCBI, U.S. National Library of Medicine, Bethesda, MD, USA). For proteins without functional annotations in the databases, homologs of these proteins were searched against the NCBI nonredundant protein database with BLASTP (http://blast.ncbi.nlm.nih.gov/) to annotate these identities. The experimental molecular mass of each protein spot was estimated by comparison with molecular weight standards, whereas the experimental pI was determined by the migration of protein spots on linear IPG strips.

2.9. Isobaric tags for relative and absolute quantitation analysis

Total protein (100 μg) was reduced by the addition of dithiothreitol to a final concentration of 10mM and incubated for 1 h at room temperature. Subsequently, iodoacetamide was added to a final concentration of 40mM, and the mixture was incubated for 1 h at room temperature in the dark. Next, dithiothreitol (10mM) was added to the mixture for 1 h at room temperature in the dark to consume any free iodoacetamide. Proteins were then diluted with 50mM triethylammonium bicarbonate and 1mM calcium chloride to reduce the urea concentration to $<$ 0.6M and the proteins were digested with 40 mg of modified trypsin at 37°C overnight. The resulting peptide solution was acidified with 10% trifluoroacetic acid and desalted on a C18 solid-phase extraction cartridge.

Desalted peptides were then labeled with *isobaric tags for relative and absolute quantitation* (iTRAQ) reagents (Applied Biosystems) according to the manufacturer's instructions. Samples from wild ginseng were labeled with reagent 114, and samples from cultivated ginseng were labeled with reagent 115. Two independent biological experiments with three technical repeats each were performed. The reaction was incubated for 1 h at room temperature. Next, Nano-HPLC-MALDI-TOF-TOF was used for protein quantification and identification. To be identified as being significantly differentially accumulated, a protein must contain at least two unique high scoring peptides at a confidence $>$ 95%, an error factor of $<$ 2 and a rate fold-change $>$ 1.4 and $<$ 0.6 in both technical replicates. These limits were selected on the basis of a previous report with some modifications.

2.10. Statistical analysis

Values in the figures and tables are expressed as the mean ± standard deviation. Statistical analysis was carried out with three biological replicates for proteomic and biochemical analyses. The results of the spot intensities and physiological data were statistically analyzed by one-way analysis of variance and the Duncan's new multiple range test to determine the significant difference between group means. A p value $<$ 0.05 was considered statistically significant (SPSS for Windows, version 12.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Differences in amino acids between wild and cultivated ginseng

Amino acids have important roles in the growth and development of organisms. Thus, we compared 18 amino acids between wild and cultivated ginseng using an automatic amino acid analyzer instrument (Table 1). We found that 14 amino acids were highly accumulated in wild ginseng. Among these amino acids, the levels of methionine, serine, glycine, threonine, alanine, and lysine were increased the most. Branched chain amino acids (leucine and isoleucine) exhibit functions in muscular synthesis and were highly accumulated in wild ginseng. However, interestingly, the contents of arginine and glutamate in cultivated ginseng were higher compared to wild ginseng. However, the biological significance of this phenomenon remains unclear.

3.2. Proteomic analysis of wild and cultivated ginseng

Comparative proteomics analysis based on 2DE and iTRAQ was performed to investigate the differences in the protein profiles between wild and cultivated ginseng. Extracted proteins were separated using 2DE and stained with CBB to evaluate their abundance levels by analyzing the relative intensities of all protein spots from three independent biological replicates using imaging software. Among the 113 differentially abundant protein spots on the 2DE gels (Fig. 1), 52 spots were successfully identified using MALDI-TOF-MS/MS. Based on the information found in the NCBI, Uniprot, and GO protein databases, 14 differentially accumulated protein spots were identified as amino acid metabolism-related proteins (Table 2).

Table 1
Differences in amino acids between wild and cultivated ginseng. Data are mean ± standard deviation from three biological replicates

Amino acids	Wild ginseng (mg/g)	Cultivated ginseng (mg/g)
Gly	4.54 ± 0.16	3.29 ± 0.15**
Met	0.71 ± 0.05	0.55 ± 0.03*
His	1.22 ± 0.15	0.94 ± 0.09
Ile	1.82 ± 0.15	1.41 ± 0.07
Lys	2.70 ± 0.14	2.13 ± 0.07
Ser	2.59 ± 0.05	2.13 ± 0.03**
Phe	1.85 ± 0.13	1.48 ± 0.03*
Ala	3.33 ± 0.10	2.66 ± 0.09**
Thr	2.34 ± 0.10	1.88 ± 0.16*
Leu	3.32 ± 0.21	2.68 ± 0.02
Asp	6.40 ± 0.22	5.24 ± 0.17**
Val	2.34 ± 0.19	1.92 ± 0.10
Cys	0.24 ± 0.05	0.21 ± 0.06*
Trp	0.46 ± 0.04	0.43 ± 0.04*
Tyr	1.32 ± 0.26	1.34 ± 0.10
Pro	2.10 ± 0.18	2.15 ± 0.11
Glu	7.19 ± 0.25	8.09 ± 0.22**
Arg	8.98 ± 0.35	10.87 ± 0.37*
Total amino acids	53.45 ± 1.64	49.31 ± 1.40*
Essential amino acid	15.55 ± 1.65	12.47 ± 0.76*

*$p < 0.05$, **$p < 0.01$.
Ala, alanine; Arg, aginine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenyylaline; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine

These proteins all had high levels of accumulation in wild ginseng. The precise quantification of differentially accumulated proteins has proven to be difficult using gel-based approaches. Thus, we explored the difference in the proteome between wild and cultivated ginseng using the iTRAQ system. This approach enables the simultaneous identification and quantitative comparison of peptides by measuring the peak intensities of reporter ions in the tandem mass spectrometry spectra. A total of 159 distinct proteins with > 95% confidence were identified in our iTRAQ analysis. Among these proteins, nine were related to amino acid metabolism (Table 3), including six highly abundant proteins in wild ginseng, and three highly abundant proteins in cultivated ginseng.

4. Discussion

Intracellular metabolic pathways of sugar, such as glycolysis and TCA cycle, provide material and energy for the synthesis of other substances, including amino acids. Thus, the content of sugars and their metabolic pathways are important for amino acid synthesis. We demonstrated that the contents of starch and polysaccharide in wild ginseng were lower compared to cultivated ginseng (0.41- and 0.80-fold, respectively, Fig. 2A, 2B). Conversely, some proteins involved in sugar catabolism had high levels of relative abundance in wild ginseng, including sucrose synthase (Q3 in iTRAQ results), amylase (Spot 10 in 2DE results), ADP-glucose pyrophosphorylase large subunit 1 (Spot 13 in 2DE results) and some glycolysis related proteins, such as phosphofructokinase (Spot 4 and 5 in 2DE results), aldolase (Spot 11 in 2DE results), glyceraldehyde-3-phosphate dehydrogenase (Spot 3 in 2DE results), phosphoglyceromutase (Spot 9 in 2DE results), and enolase (Spot 1 and 2 in 2DE results). Thus, it can be deduced that in wild ginseng, most of the starch and other polysaccharides were degraded into other intermediate metabolites that provide material and energy for subsequent glycolysis and other metabolic signaling processes.

Glycolysis is a catabolic anaerobic pathway that oxidizes hexoses to generate adenosine triphosphate (ATP), a reductant, and pyruvate and produces building blocks for anabolism [28]. It has been suggested that enhancing the expression of glycolysis-related enzymes could increase the synthesis of some amino acids, such as glutamate, threonine, glycine, and cysteine [29]. Thus, the upregulation of glycolysis-related proteins could promote the generation of amino acids in wild ginseng. These findings were consistent with those obtained from our amino acid analysis results (Table 1).

In plants, pyruvate is one of the most critical metabolites of glycolysis. It is also the starting material for alcohol fermentation (glycolysis branch). In this study, we found a higher accumulation of pyruvate in cultivated ginseng compared to wild ginseng (up to 2.78-fold, Fig. 2C). However, short-chain alcohol dehydrogenase (Q9 in iTRAQ results), which catalyzes the synthesis of alcohol, was upregulated (4.12-fold compared to wild ginseng as assessed using iTRAQ) in cultivated ginseng using 2DE and iTRAQ analysis. These findings could indicate that the metabolic signaling pathway of alcohol fermentation is more active in cultivated ginseng compared to wild ginseng. Most of the pyruvate could be used to produce ATP and other substances via the TCA cycle in wild ginseng.

With regard to the canonical metabolic pathways, the TCA cycle is extremely important in oxidizing acetyl-CoA into CO_2 to produce hydroxylamine reductase, flavin adenine dinucleotide, and ATP and carbon skeletons for use in several other metabolic processes, such as amino acid metabolism [30,31]. Fumarase is a TCA enzyme, and the activity of this enzyme in wild ginseng is higher compared to cultivated ginseng (3.51 times, Fig. 2D). Malate dehydrogenase, which converts malate into oxaloacetate (via nicotinamide adenine dinucleotide) and provides precursors for the synthesis of aspartate and alanine, was upregulated in wild ginseng (Fig. 2E). Thus, these findings could indicate that a higher level of glycolysis and the TCA

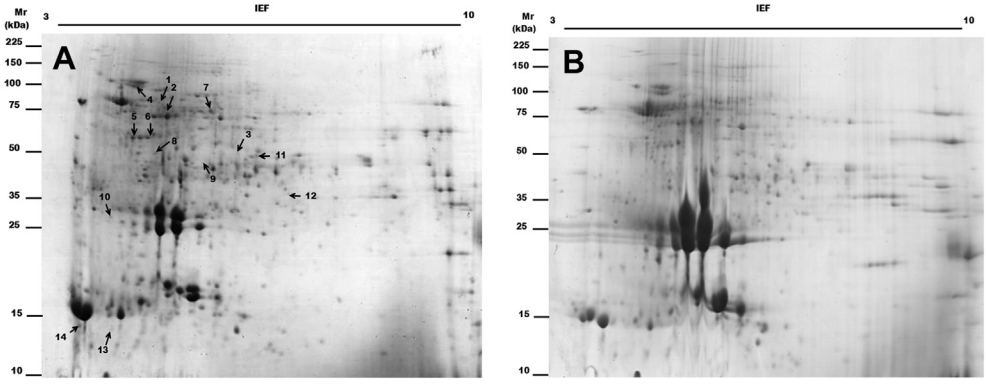

Fig. 1. Comparison of protein profiles of wild and cultivated ginseng. (A) Wild ginseng; (B) cultivated ginseng. The image displays two (of 6) representative Coomassie Brilliant Blue-stained gels. Proteins (1.2 mg) were resolved in 24 cm linear, immobilized pH (3–10) gradient gels and then separated by 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Spot numbers indicated on the gel were subjected to matrix-assisted laser desorption/ionization-time of flight/time of flight mass spectrometry.

Table 2
Identified differentially accumulated protein spots between wild and cultivated ginseng

Spot no.	Protein	Accession	Organism	Theor. Mr(kDa)/pI	Exp. Mr(kDa)/p	Pep. count	PS	Change
1	enolase 1	gi|14423688	*Hevea brasiliensis*	47801/5.57	95.455/5.63	8	256	Up
2	enolase	gi|34597330	*Brassica rapa* subsp. *campestris*	47346/5.46	74.826/5.69	12	219	Up
3	glyceraldehyde-3-phosphate dehydrogenase	gi|262235239	*Panax ginseng*	23276.1/5.67	50.000/6.45	11	157	Up
4	contains similarity to phosphofructokinases	gi|3377841	*Arabidopsis thaliana*	64171.6/5.57	72.222/5.12	9	187	Up
5	contains similarity to phosphofructokinases	gi|3377841	*A. thaliana*	64171.6/5.57	57.692/5.08	16	206	Up
6	cobalamin-independent methionine synthase	gi|47600741	*A. thaliana*	84261.4/6.09	57.692/5.30	10	273	Up
7	glutamate decarboxylase	gi|284192454	*P. ginseng*	56020.8/5.69	74.156/6.19	14	350	Up
8	cobalamin-independent methionine synthase	gi|47600741	*A. thaliana*	84261.4/6.09	49.063/5.44	8	259	Up
9	cofactor-independent phosphoglyceromutase	gi|6706331	*Apium graveolens*	60896.7/5.26	46.250/6.09	9	125	Up
10	beta-amylase	gi|217940	*Ipomoea batatas*	56014.8/5.18	29.000/4.65	13	98	Up
11	cytoplasmic aldolase	gi|218157	*Japonica Group*	39151/6.56	48.125/6.90	5	116	Up
12	malate dehydrogenase	gi|306755938	*Pseudotsuga menziesii*	2519.92/4.43	35.938/7.41	8	165	Up
13	ADP-glucose pyrophosphorylase large subunit 1	gi|2642638	*Lanatus* subsp. *Vulgaris*	58338.6/7.55	13.500/4.70	16	123	Up
14	calmodulin	gi|16225	*A. thaliana*	15648/4.20	14.250/4.02	3	297	Up

Exp. Mr(kDa)/pI, experimental molecular mass and isoelectric point; Pep. count, number of matched peptides; PS, protein score; Spot no., spot numbers corresponding with two-dimensional gel electrophoresis gel as shown in Fig. 1; Theor. Mr(kDa)/pI, theoretical molecular mass and isoelectric point.

Table 3
Isobaric tags for relative and absolute quantitation identification results of the differentially accumulated proteins between wild and cultivated ginseng

Protein on	Protein name	Accession no.	Organism	Theor. Mr/pI	Reliability	Fold change wild ginseng/cultivated ginseng
Q1	Glutamate decarboxylase	D3JX88	*Panax ginseng*	61352.53125/5.69	62.7	1.44
Q2	Calcium-dependent protein kinase	D1MEN6	*P. ginseng*	67492.07813/6.19	84.2	1.43
Q3	Sucrose synthase	B9GSC7	*Populus trichocarpa*	99652.20313/5.9	64.96	2.41
Q4	Tryptophan synthase	F2DVQ0	*Hordeum vulgare* var.	37733.30859/8.39	88.77	2.16
Q5	Asparagine synthetase	F2DJ31	*H. vulgare* var.	71832.40625/6.11	97.85	1.54
Q6	Glutamine synthetase	B7T058	*Musa ABB Group*	50888.17578/9.33	86.77	0.44
Q7	Aldehyde oxidase	Q1MX17	*Brassica campestris*	161099.2031/6.04	79.46	0.5
Q8	Cysteine synthase	A9Y098	Sesamum indicum	34329.65/5.62	75.37	1.63
Q9	Short-chain alcohol dehydrogenase	B8YDG5	*P. ginseng*	32019.21094/6.85	80.1	0.24

cycle in wild ginseng could provide material and energy for the synthesis of amino acids. These findings could underlie the difference in the medical effects between wild and cultivated ginseng.

Unlike cultivated ginseng, wild ginseng generally suffers from various biotic and abiotic stresses during growth and development. Evidence has emerged that several nonprotein and protein thiols, together with a network of sulfur-containing molecules and related compounds, fundamentally contribute to plant stress tolerance [32,33]. A growing number of studies have demonstrated various protective mechanisms of sulfur-

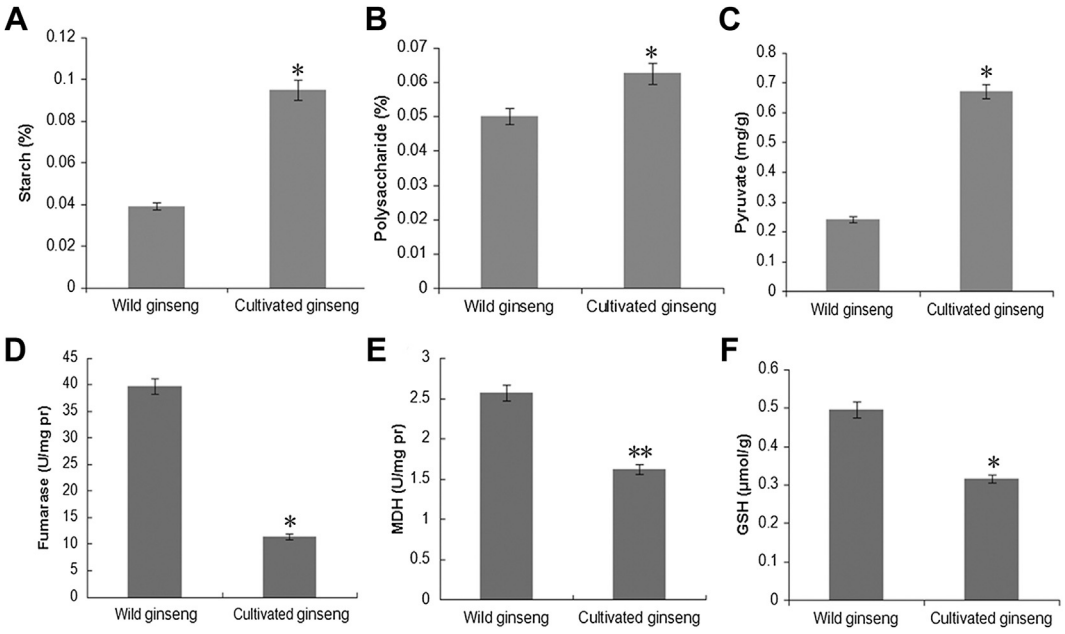

Fig. 2. Activities and contents of relative enzyme and metabolites in wild and cultivated ginseng. (A) Starch content; (B) polysaccharide content; (C) pyruvate content; (D) fumarase activity; (E) malate dehydrogenase activity; and (F) glutathione content. Data are mean ± standard deviation from three biological replicates. $^{*}p < 0.05$, $^{**}p < 0.01$. GSH, glutathione; MDH, malate dehydrogenase.

containing amino acids, such as cysteine (Cys) and methionine (Met) [34–37]. Cys, the by-product of a cysteine synthase-catalyzed reaction, is a precursor for glutathione synthesis, which in turn is a key water-soluble antioxidant and plays a central part in reactive oxygen species scavenging via the GSH-ascorbate cycle and as an electron donor to glutathione peroxidase [38]. We demonstrated that the content of Cys (Table 1) and the accumulation of cysteine synthase (Q8 in iTRAQ results) in wild ginseng are higher compared to cultivated ginseng. In most studies, these increases were reported together with increased GSH. We also demonstrated that the GSH content was consistent with previous reports. The content of GSH in wild ginseng was 1.6-fold greater compared to cultivated ginseng (Fig. 2F). Furthermore, free Cys is often irreversibly oxidized to different by-products [39], such as cysteine (CySS). The redox potential of the CySS/2Cys complex is regarded as an important biochemical marker for early stages of various human diseases [40] and as an important antioxidant system and regulator of the redox state in parasites [41]. Moreover, the CySS/2Cys redox state could also have an important role in the plant stress response [42]. Thus, the difference in Cys metabolism between wild and cultivated ginseng could be one reason for the difference in medicinal functions between wild and cultivated ginseng.

Similar to Cys, Met can undergo ROS-mediated oxidation to Met sulfoxide, which can result in changes in protein conformation and activity [43]. In our results, the Met content in wild ginseng is higher compared to cultivated ginseng (1.3 times, Table 1). Met is a substrate for the synthesis of various polyamines with important roles in stress tolerance [44]. This biosynthetic pathway involves the intermediate SAM as a primary methyl donor. SAM is also a source for ethylene synthesis [45], which reinforces the pivotal role of Met in the plant stress response. The content of SAM in wild ginseng was higher compared to cultivated ginseng (1.23 times, Fig. 3A). These results could indicate that Met metabolism is more active in wild ginseng compared to cultivated ginseng, which could be the result of the different growth conditions. Considering that Met is also an essential amino acid for humans, Met metabolism of ginseng during growth and development could play a partial role in its medicinal functions.

In plants, aspartate provides carbon skeletons for purine and pyrimidine synthesis. The content of this amino acid in wild ginseng was higher compared to cultivated ginseng (1.22-fold, Table 1). Moreover, asparagine synthetase (Q5 in iTRAQ results), an important enzyme in aspartate synthesis, was upregulated in wild ginseng as assessed using iTRAQ. In humans, aspartate has been proposed to have functions in liver- and cardiac muscle-protective functions. Thus, these observations suggest that a higher level of aspartate synthesis could play a partial role in the medicinal functions of wild ginseng.

Our iTRAQ results revealed a higher accumulation of tryptophan synthetase (Q4 in iTRAQ results) in wild ginseng compared to cultivated ginseng. This result was consistent with a high level of tryptophan in wild ginseng (Table 1). Tryptophan has the capacity to serve as a precursor to auxin, IAA [46]. The phytohormone auxin plays a central role in plant growth and development as a regulator of numerous biological processes, ranging from cell division, elongation, and differentiation to tropic responses, fruit development, and senescence [47]. The content of IAA in wild ginseng was higher compared to cultivated ginseng (Fig. 3B). Importantly, similar to methionine, tryptophan is one of the essential amino acids for the human body, and it is the precursor of serotonin synthesis. Serotonin is an important neurotransmitter in the cerebrum, and perturbations in this neurotransmitter can produce humoral and behavioral disorders. Thus, different levels of tryptophan between wild and cultivated ginseng could be one of the reasons underlying the more beneficial medicinal effects of wild ginseng compared to cultivated ginseng.

In plants, glutamate decarboxylase (Q1 in iTRAQ results) catalyzes the synthesis of GABA in the presence of calmodulin (Spot 14 in 2DE results) [15]. We observed that the accumulation of these two proteins in wild ginseng was higher compared to cultivated ginseng. In addition, further studies revealed that GABA had a higher level of accumulation in wild ginseng (2.04-fold greater compared to cultivated ginseng, Fig. 3C). As a four-carbon nonprotein amino acid, GABA is present at high levels in plants. It is also involved in several physiological processes, such as nitrogen metabolism, cytosolic pH regulation, and carbon flux into the TCA cycle [48]. The basic effects of GABA have been characterized as reducing blood pressure and protecting the liver [49]. Thus, a high level of GABA could contribute to the enhanced medicinal functions of wild ginseng compared to cultivated ginseng.

5. Conclusion

The mechanisms underlying the difference in amino acid metabolism between wild and cultivated ginseng were revealed in this study using proteomic techniques. Based on the results of this study, the following conclusions could be drawn (Fig. 4): in wild ginseng, the contents of medicinal amino acids, such as sulfur-containing amino acids (methionine and cysteine) and tryptophan, and their derivatives were higher compared with those of cultivated ginseng. In addition, the expression and contents of enzymes and intermediate products related to glycolysis and TCA, which support amino acid biosynthesis with material and energy, were higher in wild ginseng compared to cultivated ginseng. This

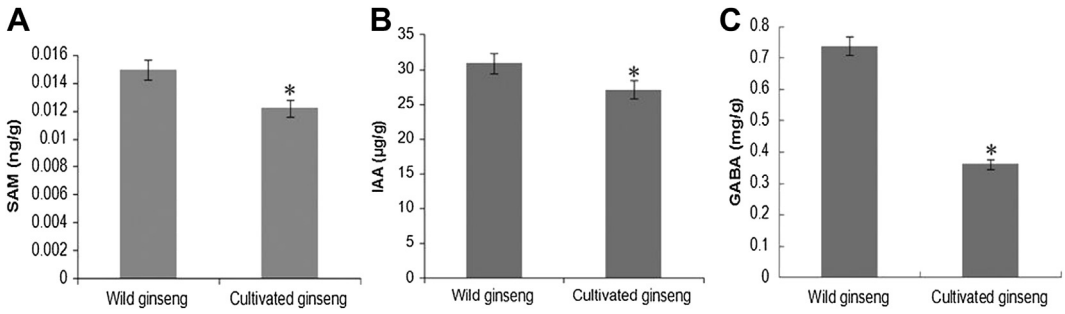

Fig. 3. Contents of SAM, IAA and GABA in wild and cultivated ginseng. (A) SAM; (B) IAA; and (C) GABA. SAM and IAA contents were quantified using enzyme-linked immunosorbent assay, and the GABA content was measured using an automated amino acid analyzer as described in the "Materials and methods" section. Data are means ± standard deviation from three biological replicates. $^{*}p < 0.05$. GABA, γ-aminobutyric acid; SAM, S-adenosylmethionine.

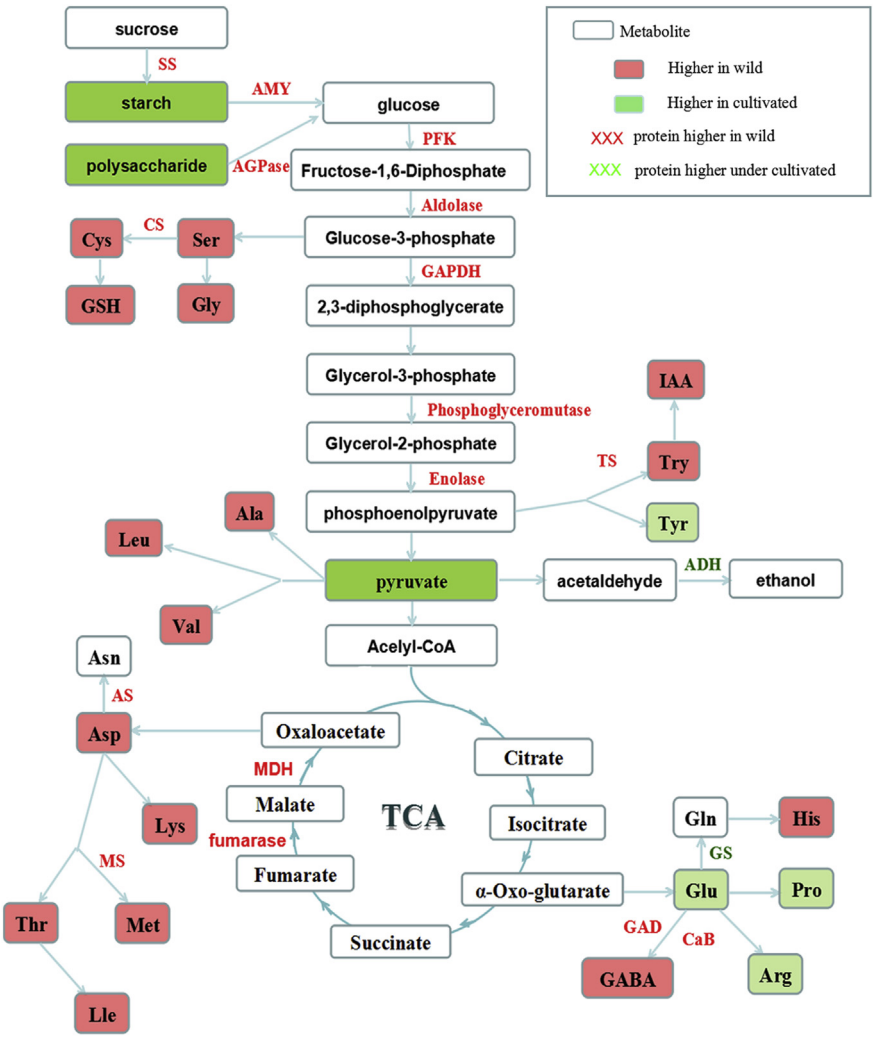

Fig. 4. Carbohydrate metabolism and amino acid metabolism differences between wild and cultivated ginseng. Ala, alanine; Arg, aginine; Asn, *Asparagine;* Asp, aspartic acid; Cys, cysteine; GABA, γ-aminobutyric acid; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenyylaline; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

study elucidated the differences in amino acids between wild and cultivated ginseng. Our results provide a reference for further studies on the medicinal functions of wild ginseng.

Conflicts of interest

The authors declare that thy have no conflict of interest.

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