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PROTEIN ISOLATION AND IDENTIFICATION OF TRICHOSANTHES KIRILOWII SEED FROM DIFFERENT PLACES OF PRODUCTION

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Abstract

In this paper, 12 samples of *Trichosanthes kirilowii* seed from different places of production were studied. The total proteins of the 12 samples were extracted by Tris-phenol method. Twelve samples of proteins were isolated by SDS-PAGE. SDS-PAGE results showed that the protein bands in sample No.8 are different from those of other samples. And those protein bands were identified by mass spectrometry. At least 10 differentially expressed proteins were identified, including rRNA N-glycosidase, Acetyl-CoA acyltransferase, Citrate synthase, Phosphoglycerate kinase, Isocitrate dehydrogenase, Fructose-bisphosphate aldolase, Ferritin, Urease, Hhydroxyacyl-ACP-dehydratase, Glutathione peroxidase. From the result of protein identification, it can be inferred that the seed of No. 8 sample was superior to other samples during germination.

Key words: *Trichosanthes kirilowii* seed, Protien, Isolation and Identification.

Introduction

Trichosanthes kirilowii seed is the *Trichosanthes kirilowii* (*Trichosanthes rosthornii* Harms) of mature seeds. *Trichosanthes kirilowii* seeds have been commonly used in oriental traditional medicine and in Chinese medicine for the treatment of cough, inflammation, diabetes, and obstipation. Modern pharmacological studies have shown that it has the expansion of coronary artery, increase coronary flow, protection of ischemic myocardium, anti-inflammatory, anti-tumor and diarrhea, antithrombotic and hardening of the arteries to regulate blood sugar, and so on, with strong anti-platelet aggregation function (Wagner *et al*., 2016).

Trichosanthes kirilowii seeds have main effective components including fat (Yan *et al*., 2008), flavonoids (Liu *et al.*, 2013), and organic acids, sterols and terpenoids *(Cheng et al.*, 2005), protein amino acids and trace elements and minerals. So, *Trichosanthes kirilowii* seed has high nutritional value (Xiu *et al.*, 2005).

Trichosanthes kirilowii seed contains almost the entire genomes of *trichosanthes kirilowii*. There is no Complete Genomic Sequence of *Trichosanthes kirilowii* seed. For the study of the subprotein of trichodium, Casellas, P (Casellas *et al.*, 1988) were extracted from the tricho-trichokirin, which had a strong libosomal inactivation. Falasca, a.i. (Falasca *et al*., 1989) extracted the lectin which was belongs to the family Cucurbitaceae from *Trichosanthes kirilowii* seeds. The lactin has molecular weight of 57 kD, consists of two subunits with apparent molecular masses of 37 and 25 kD. (Lee-Huang *et al.*, 1991) extracted TAP 29: an anti-human immunodeficiency virus protein from *Trichosanthes kirilowii* seeds, and the molecular weight was 29kD, and it would be possible to replace the anti-virus protein in AIDS treatment. (Dong *et al*., 1994) also isolated a new ribosomal-inactivating protein beta-kirilowin from the seeds of *Trichosanthes kirilowii*. The protein showed strong abortifacient activity in pregnant mice. (Wong *et al.*, 1996) were able to extract alpha-kirilowin from the *Trichosanthes kirilowii* seeds, which was slightly larger than another previously characterized ribosome-inactivating protein, beta-kirilowin. (Ozaki *et al*., 1996) carried out to elucidate the anti-inflammatory effect of 50% ethanol extract obtained from the fruit of *Trichosanthes kirilowii* Maxm, and its effective parts. (Tai *et al*., 2000), etc., are extracted A new small ribosome-inactivating protein named S-trichokirin from the seeds of *Trichosanthes kirilowii.* (Li *et al*., 2003) and (Yang *et al*., 2005) not only extracted a new peptide trichokirin-s1 from the trichokirin, but also carried out the analysis and research on the activity of the trichokirin-s1 (Shu *et al*., 2009) purified a novel ribosome-inactivating protein, designated Trichosanthrip, from mature seeds of *Trichosanthes kirilowii*.

A lot of literatures has reported the optimization of extraction method for *Trichosanthes kirilowii* protein. Ting-xia dong has used such as the species of the genus *Trichosanthes kirilowii* identification of protein electrophoresis (Dong *et al.*, 1990). Yan-mei song compared the 6 kinds of protein extraction method for *Trichosanthes kirilowii* seeds (Song *et al.*, 2011). A great deal of work has been carried on the *Trichosanthes kirilowii* protein comparative study (Li *et al.*, 2015; Song, 2015).

Seeds were collected *Trichosanthes kirilowii* from different places of production. The proteins were compared. The study established *Trichosanthes kirilowii* protein map. The differences protein bands and the common characteristic protein bands were analysed by LC-MS. It helps to provide reliable basis for the optimization of *Trichosanthes kirilowii* breeding research.

Materials and Methods

Plant materials and sample preparation: The *Trichosanthes kirilowii* seed were harvested from different places in China. The materials were weighed, leaved the seeds to dry naturally, then stored at 25°C.

Protein extraction (Tris-phenol extraction protocol): The *Trichosanthes kirilowii* seed (seed-coat, 0.2 g fresh weight) were ground to powder in a pre-cooled mortar by using liquid nitrogen. The sample powder was transferred into Eppendorf tubes for protein extraction. 0.6 ml extraction buffer (500 mM Tris, 50 mM EDTA, 700 mM sucrose, and 100 mM KCl, pH 8.0) was added to Eppendorf tubes for protein extraction. The tubes were vortexed for 30 min at 4°C. Then, one volume (v/v) phenol was added (Tris buffered to pH 7.9), and the solution was vortexed for 30 min at 4°C and centrifuged at 12,000 rpm for 30min. The upper phenol layer was transferred into another tube. The lower layer was re-extracted with one volume (v/v) each of phenol and extraction buffer, vortexed, and centrifuged, and the phenol layer was combined with the volume collected earlier. Next, 3–5 volumes (v/v) of 0.1 M ammonium acetate in methanol were added, and the mixture was maintained at -20°C overnight. The tube was centrifuged at 12,000 rpm for 15 min at 4°C. The pellet was washed twice with 0.1 M ammonium acetate in methanol and twice with acetone containing 0.07% 2-mercaptoethanol (ME). In each case, the pellet was completely suspended by vortexing and centrifuged at 12,000 rpm for 15 min at 4°C. Finally, the pellet was vacuum-dried.

Protein lysis: The pellets were dissolved in a lysis buffer (containing 8 M urea, 2 M Thio urea, 4% CHAPS, 65 mMDTT, and 2% Ampholyte), vortexed for 3 h, and centrifuged at 15,000 rpm for 30 min at 25°C. The protein concentration in the supernatant was quantified in accordance with Bradford (1976), using bovine serum albumin as the standard.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS- PAGE), 15% polyacrylamide gel (Laemmli *et al*., 1970) was utilized. Electrophoresis was carried out at 15 mA per gel for 30 min and 25 mA per gel until the dye reached the bottom of the gel. The gels were stained using Commassie Blue Staining Solution.

In-gel digestion: The gel of protein bands were cut with a scalpel. Each gel of protein bands was cut into 0.5mm3~1mm3, and mark them on the 1.5mL EP tube. Add 50mM DTT into the tube in 1h at 56°C. And 100mM IAM were added for 40min at 25°C. The proteins were digested with trypsin. The sample was incubated at 37°C overnight. The solution was extracted from the digestion tube and transferred to a new Eppendorf tube for collection. The sample was dried in a vacuum centrifuge and stored at -80°C.

Nano-LC-TOF analysis: A nano-LC system, equipped with Eksigent nano- LC 415 System (Eksigent, USA), and 3C18-CL-120 Column (3 μm, 120 Å, 75μm*150mm, Eksigent , USA), was used to separate mixed compounds. Triple TOF 5600 (Applied Biosystem, USA) was used to identify the target compounds. Data processing was performed in Analyst TF (Applied Biosystem, USA). The gradient elution used for the HPLC separation is shown in Table 1. The MS parameters were the following: MS scan range is from 350 to 1250 (m/z) for each 0.25s. MS_2 scan range is from 100 to 1500(m/z). From each scan was chosen 40 strongest parention for tandem scanning.

Table 1. Nano-LC gradient for sepatation of protein.

Time (min)	Flow rate (nL/min)	0. 1 % Formic acid (%)	Acetonitrile (%)
0	300	95	5
0.5	300	92	8
60	300	75	25
75	300	50	50
80	300	20	80
90	300	95	5

Results and Discussion

The SDS-PAGE gel of *Trichosanthes kirilowii* seed from different places of production: Representative Commassie Blus-stained gels for *Trichosanthes kirilowii* seed from different places of production are shown in Fig. 1.

From fig.1, the SDS-PAGE results showed that the protein bands in sample No.8 are different from those of other samples. At molecular weight of protein rang of 45kD to 66kD, there are two distinct bands in the sample No.8. From 20kD to 30kD, the sample No.8 has two different protein bands from other samples. And those protein bands were cut and identified by mass spectrometry. The protein bands to be detected were shown in Fig. 2.

The result of Nano-LC-TOF analysis: Five protein bands were digested with trypsin. And each bands prepared to nano-LC-TOF analysis system. The information of these protein bands were displayed in Tables 2, 3, 4, 5, 6.

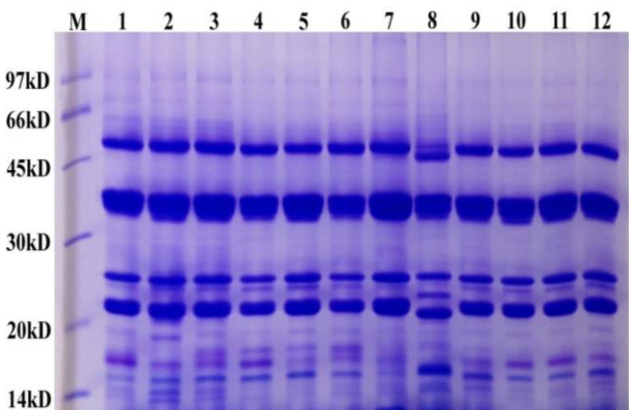

Fig. 1. Protein electrophoresis of *Trichosanthes kirilowii* seed from different places of production.

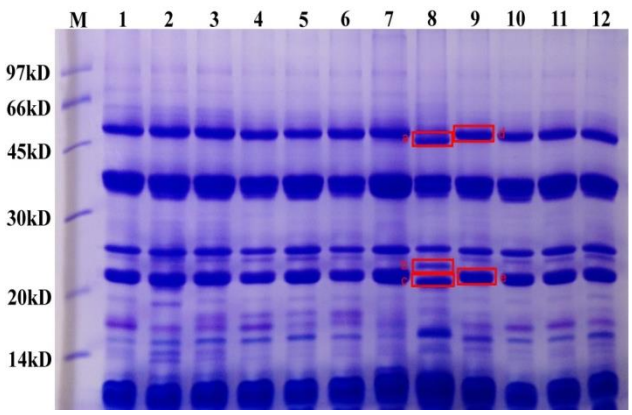

Fig. 2. The position of protein bands a,b,c,d,e are the protein bands which were cut and identified by mass spectrometry.

Table 2. Detailed information of protein band tabbed the a.

No.	Total	% Cov	Accession	Name	Species
1.	141.73	33.7	A0A0A0L4Q9	Uncharacterized protein	*Cucumis sativus*
2.	74.17	37.2	Q9ZWI3	PV100	*Cucurbita maxima*
3.	21.57	22.4	A0A0A0KKD9	Uncharacterized protein	*Cucumis sativus*
4.	19.32	44.6	A0A0A0K9L7	Uncharacterized protein	*Cucumis sativus*
5.	14.9	41.1	Q08375	Acetyl-CoA acyltransferase (3-ketoacyl-coa thiolase)	*Cucumis sativus*
6.	12	16.2	A0A0A0KC13	Citrate synthase	*Cucumis sativus*
7.	10.18	21.5	A0A0A0LRZ8	Aspartic proteinase	*Cucumis sativus*
8.	9.74	18.5	S8CHM2	Phosphoglycerate kinase	*Genlisea aurea*
9.	8.07	20.9	A0A199UHV7	Glutamate-glyoxylate aminotransferase 2 (Fragment)	*Ananas comosus*
10.	8	18.4	A0A067JHW3	Uncharacterized protein	*Jatropha curcas*
11.	7.05	19.3	A0A0A0KX30	Uncharacterized protein	*Cucumis sativus*
12.	6.18	19.5	A0A059CC25	Obg-like ATPase 1	*Eucalyptus grandis*
13.	6.16	29.3	A0A0A0LGI3	Fructose-bisphosphate aldolase	*Cucumis sativus*
14.	6.14	19	Q84VH4	Elongation factor 1-alpha (Fragment)	*Malva pusilla*
15.	30.1	81.8	F2YML9	Vicilin-like protein (Fragment)	*Citrullus lanatus*
16.	6.05	21.1	B9SR98	Isocitrate dehydrogenase [NADP]	*Ricinus communis*
17.	10	8.9	V4M1R7	Uncharacterized protein	*Eutrema salsugineum*
18.	6	8.3	A0A0A0LAB5	Uncharacterized protein	*Cucumis sativus*
19.	6	9.8	W0FVK2	Catalase (Fragment)	*Cucurbita maxima*
20.	8.24	17.4	O04057	Aspartic proteinase	*Cucurbita pepo*
21.	4.01	9.7	A0A0A0LSD8	Uncharacterized protein	*Cucumis sativus*
22.	8.87	20.7	Q42918	Acetyl-CoA C-acyltransferase (3-ketoacyl-coa thiolase b) (Fragment)	*Mangifera indica*
23.	8	9.3	A0A0Q3PKH8	Uncharacterized protein	*Brachypodium distachyon*
24.	6.14	12.9	W9R450	Fructose-bisphosphate aldolase	*Morus notabilis*
25.	4	16.1	A0A0A0K9P6	Uncharacterized protein	*Cucumis sativus*
26.	4	13.8	B1B626	rRNA N-glycosidase	*Trichosanthes kirilowii*
27.	4	5.9	A0A0A0LCR8	Uncharacterized protein	*Cucumis sativus*
28.	4	6	A0A0A0KXJ8	Uncharacterized protein	*Cucumis sativus*
29.	3	18.2	A0A0U4K3C1	Elongation factor 1-gamma	*Ziziphus jujuba*
30.	4.23	10	B9RHY3	Phosphoglycerate kinase	*Ricinus communis*
31.	4.2	10.1	Q9SEW4	Vicilin-like protein (Fragment)	*Citrus clementina*
32.	2.25	10.1	D5LMH3	Monodehydroascorbate reductase	*Lilium longiflorum*
33.	2.18	24.5	A0A0A0LHY6	Formate dehydrogenase, mitochondrial	*Cucumis sativus*
34.	8.21	27.3	A0A0A0LNN6	Uncharacterized protein	*Cucumis sativus*
35.	2.03	8.2	V4MMH1	Aspartate aminotransferase	*Eutrema salsugineum*
36.	2.03	4.3	V4UDH4	Uncharacterized protein	*Citrus clementina*
37.	2.02	6.3	A0A0A0KAV8	Aminoacylase	*Cucumis sativus*
38.	11.11	31.5	U5DIN0	Uncharacterized protein	*Amborella trichopoda*
39.	8	12.7	I1QGE3	Uncharacterized protein	*Oryza glaberrima*
40.	6.17	13.4	V4TK57	Uncharacterized protein	*Citrus clementina*
41.	6.01	25	A0A0A0LNB2	Uncharacterized protein	*Cucumis sativus*
42.	6	8.4	A0A0A0L7Y5	Uncharacterized protein	*Cucumis sativus*
43.	4.05	18.1	B9H3K3	Isocitrate dehydrogenase [NADP]	*Populus trichocarpa*
44.	4	11.9	A0A0K0MFZ6	ADH	*Stipa purpurea*
45.	2.02	18.7	B6VPZ9	Formate dehydrogenase, mitochondrial	*Lotus japonicus*
46.	2	15.4	M1BV50	Uncharacterized protein	*Solanum tuberosum*
47.	2	8	W9QZ41	6-phosphogluconate dehydrogenase, decarboxylating	*Morus notabilis*
48.	2	22.1	V7CEB9	Uncharacterized protein	*Phaseolus vulgaris*
49.	2	10	I3S6D2	Glyceraldehyde-3-phosphate dehydrogenase	*Medicago truncatula*
50.	2	5.7	M5XD26	Annexin	*Prunus persica*
51.	2	7.3	M1BNK9	Uncharacterized protein	*Solanum tuberosum*
52.	2	5.7	A0A0D2TX48	Uncharacterized protein	*Gossypium raimondii*
53.	2	5.2	A0A0A0L2W7	Uncharacterized protein	*Cucumis sativus*
54.	2	4.6	A0A0A0KYN6	Glutamate dehydrogenase	*Cucumis sativus*
55.	2	2.7	O48942	Beta-ketoacyl-ACP synthase I	*Perilla frutescens*
56.	2	2.3	J7MB60	Serine hydroxylmethyltransferase	*Polytomella* sp. *Pringsheim*
57.	2	2.4	B9MXY5	Isocitrate lyase	*Populus trichocarpa*
58.	2	4.6	B9GSQ3	Uncharacterized protein	*Populus trichocarpa*
59.	2	4.1	A0A0A0KT30	Uncharacterized protein	*Cucumis sativus*
60.	1.02	3.1	A0A0A0KAE2	Uncharacterized protein	*Cucumis sativus*
61.	0.81	2.6	D8SLC0	Putative uncharacterized protein	*Selaginella moellendorffii*
62.	0.37	1.4	W1NIP0	Uncharacterized protein	*Amborella trichopoda*
63.	4.59	12.7	A0A199UWG9	Isocitrate dehydrogenase [NADP]	*Ananas comosus*
64.	6.87	13.2	A0A0D6QU68	Uncharacterized protein	*Araucaria cunninghamii*
65.	6	21.4	M4CMT4	Uncharacterized protein	*Brassica rapa* subsp. *pekinensis*
66.	4.08	15.4	S8D2K5	Fructose-bisphosphate aldolase	*Genlisea aurea*
67.	4	6.4	A5BE40	Putative uncharacterized protein	*Vitis vinifera*

Table 3. Detailed information of protein band tabbed the b.

No.	Total	% Cov	Accession	Name	Species
1.	20.73	12.5	A0A0A0L4Q9	Uncharacterized protein	*Cucumis sativus*
2.	6	7.7	A0A0A0KM69	Ferritin	*Cucumis sativus*
3.	4.03	28.4	A0A0A0LBU6	Uncharacterized protein	*Cucumis sativus*
4.	4	5.4	A0A0A0L7Y5	Uncharacterized protein	*Cucumis sativus*
5.	4	11.9	H6TB40	HSP23.5	*Citrullus lanatus*
6.	4	5.1	A0A0A0KHQ1	Uncharacterized protein	*Cucumis sativus*
7.	2.21	16.3	E5F5W3	Glutathione peroxidase (Fragment)	*Picea sitchensis*
8.	2.19	11.1	A0A0A0LLE7	Serine hydroxymethyltransferase	*Cucumis sativus*
9.	2.14	14.9	A0A0A0L917	Uncharacterized protein	*Cucumis sativus*
10.	2.19	6.6	Q8GZP6	Allergen Ana o 2 (Fragment)	*Anacardium occidentale*
11.	2.1	16.5	M4R4G0	Hydroxyacyl-ACP dehydratase	*Camellia chekiangoleosa*
12.	2.06	8.8	A0A176VJ02	Uncharacterized protein	*Marchantia polymorpha* subsp. *polymorpha*
13.	2.02	9.3	Q40115	Ribonuclease (RNase LC1)	*Luffa aegyptiaca*
14.	4	20.5	A0A067G079	Ferritin (Fragment)	*Citrus sinensis*
15.	2.12	6.1	A0A090DLH8	Edestin 1	*Cannabis sativa*
16.	2	36.4	F2YML9	Vicilin-like protein (Fragment)	*Citrullus lanatus*
17.	2	6.3	B9RGD0	Annexin	*Ricinus communis*
18.	2	4	A0A0J8CCH0	Uncharacterized protein	*Beta vulgaris* subsp. *vulgaris*
19.	2	5.8	A0A0A0LXR3	Uncharacterized protein	*Cucumis sativus*
25.	2	5.3	Q38JD1	Predicted protein	*Physcomitrella patens* subsp. *patens*
26.	0.86	5.1	A0A0A0LC37	Uncharacterized protein	*Cucumis sativus*

Table 4. Detailed information of protein band tabbed the c.

No.	Total	% Cov	Accession	Name	Species
1.	67.6	45.5	A0A0A0L7E7	Uncharacterized protein	*Cucumis sativus*
2.	6.01	29.6	A0A0A0LBU6	Uncharacterized protein	*Cucumis sativus*
3.	6.04	9.1	A0A0J8CCH0	Uncharacterized protein	*Beta vulgaris* subsp. *vulgaris*
4.	4	3.9	V4SU04	Urease	*Citrus clementina*
5.	3.67	22.1	E5F5X2	Glutathione peroxidase (Fragment)	*Picea sitchensis*
6.	8.48	24.6	A0A0A0LNN6	Uncharacterized protein	*Cucumis sativus*
7.	2.01	10.9	V7CLI7	Uncharacterized protein	*Phaseolus vulgaris*
8.	3.26	21.4	M0RP81	Glutathione peroxidase	*Musa acuminata* subsp. *malaccensis*
9.	2	18.4	W9RSX6	60S ribosomal protein L11-2	*Morus notabilis*
10.	2	7	F4IMB5	ATPase, F1 complex, alpha subunit protein	*Arabidopsis thaliana*
11.	2	6.5	I0B569	Vicilin	*Vicia faba*
12.	2	11.2	W9RI49	40S ribosomal protein S15	*Morus notabilis*
13.	2	5.8	A0A0A0LTW2	Tumor-related protein	*Cucumis sativus*
14.	1	9.3	Q40115	Ribonuclease (RNase LC1)	*Luffa aegyptiaca*
15.	2.55	11.9	A0A199URK7	Glutelin type-A 1	*Ananas comosus*

Table 5. Detailed information of protein band tabbed the d.

No.	Total	% Cov	Accession	Name	Species
1.	81.48	30.5	A0A0A0L4Q9	Uncharacterized protein	*Cucumis sativus*
2.	76.11	37.8	Q9ZWI3	PV100	*Cucurbita maxima*
3.	11.09	25.2	M5XR34	Uncharacterized protein	*Prunus persica*
4.	9.16	26.6	Q3LUM1	Elongation factor 1-alpha	*Gossypium hirsutum*
5.	7.14	15.3	A0A0A0KAE2	Uncharacterized protein	*Cucumis sativus*
6.	6.6	11.4	A0A0A0KG56	Uncharacterized protein	*Cucumis sativus*
7.	6.02	12.5	O04057	Aspartic proteinase	*Cucurbita pepo*
8.	6	4.5	A0A067JF64	Uncharacterized protein	*Jatropha curcas*
9.	10.66	77.9	F2YML9	Vicilin-like protein (Fragment)	*Citrullus lanatus*
10.	4.03	10.6	J9PX40	Enolase	*Phytolacca americana*
11.	4.01	11.9	A0A0A0L818	Uncharacterized protein	*Cucumis sativus*
12.	4	6.1	A0A0A0LQE8	Beta-galactosidase	*Cucumis sativus*
13.	3.59	6.6	B9GSQ3	Uncharacterized protein	*Populus trichocarpa*
14.	3.18	12.1	W5I0B0	Uncharacterized protein	*Triticum aestivum*
15.	2.3	4.5	A0A0A0KY03	Uncharacterized protein	*Cucumis sativus*
16.	2.04	11.8	D7TFJ4	Putative uncharacterized protein	*Vitis vinifera*
17.	10.76	33.8	A0A0A0LQN5	Uncharacterized protein	*Cucumis sativus*
18.	4	5.8	A0A061DMF8	Aspartic proteinase A1 isoform 1	*Theobroma cacao*
19.	2	10.4	W9RJ43	Catalase	*Morus notabilis*
20.	2	4	I1MNX4	Uncharacterized protein	*Glycine max*
21.	1.89	2.2	W9RK76	Uncharacterized protein	*Morus notabilis*
22.	1.85	10.6	A0A0V0IPH7	Putative luminal-binding protein 5-like	*Solanum chacoense*
23.	8	22.5	A0A067JRR4	Uncharacterized protein	*Jatropha curcas*
24.	1.44	3.4	A0A0A0LAB5	Uncharacterized protein	*Cucumis sativus*
25.	1.34	15.5	R0IFZ3	DHAR class glutathione S-transferase	*Capsella rubella*
26.	5.88	11	V4U0A2	Uncharacterized protein	*Citrus clementina*
27.	2.68	8.2	V4RGK6	Uncharacterized protein	*Citrus clementina*
28.	0.94	7.5	V7AUN1	Uncharacterized protein	*Phaseolus vulgaris*

Table 6. Detailed information of protein band tabbed the e.

No.	Total	% Cov	Accession	Name	Species
1.	35.88	23.4	A0A0A0LNN6	Uncharacterized protein	*Cucumis sativus*
2.	5.82	14.1	Q9ZWI3	PV100	*Cucurbita maxima*
3.	5.08	6.1	A0A0J8CCH0	Uncharacterized protein	*Beta vulgaris* subsp. *vulgaris*
4.	3.6	6.9	A0A0A0L7Y5	Uncharacterized protein	*Cucumis sativus*
5.	2.61	6.1	Q8GZP6	Allergen Ana o 2 (Fragment)	*Anacardium occidentale*
6.	2.02	2.2	A0A072TII3	Uncharacterized protein	*Medicago truncatula*
7.	16.62	23.1	A0A0A0K9P5	Uncharacterized protein	*Cucumis sativus*
8.	2.01	13.3	H6TB40	HSP23.5	*Citrullus lanatus*
9.	2	20.3	A0A103Y8Z2	Mss4-like protein (Fragment)	*Cynara cardunculus* var. *scolymus*
10.	2	32.5	F2YML9	Vicilin-like protein (Fragment)	*Citrullus lanatus*
11.	2	5.8	A0A0A0LXR3	Uncharacterized protein	*Cucumis sativus*
12.	1.44	2.4	Q39651	PreproMP27-MP32	*Cucurbita cv. Kurokawa Amakuri*
13.	0.82	2	A0A0D2M6U4	Uncharacterized protein	*Gossypium raimondii*
14.	4.03	7	A0A199URK7	Glutelin type-A 1	*Ananas comosus*
15.	0.24	1.6	A0A151SD87	Retrovirus-related Pol polyprotein from transposon TNT 1-94	*Cajanus cajan*

Conclusions

In all, these proteins, rRNA N-glycosidase, Acetyl-CoA acyltransferase, Citrate synthase, Phosphoglycerate kinase, Isocitrate dehydrogenase, Fructose-bisphosphate aldolase, Ferritin, Urease, Hhydroxyacyl-ACP-dehydratase, Glutathione peroxidase were only indentified in the 8th sample. RRNA N-glycosylase, Citrate synthase and Isocitrate dehydrogenase are the key pace-making enzyme in the citric acid cycle. Acetyl-CoA acyltransferase is an enzyme that transformated Acetoacetyl-CoA to acetyl-CoA. Phosphoglycerate kinase is the key enzyme in the glycolytic pathway. Fructose-bisphosphate aldolase enhances the adaptation of plants to salt stress by promoting glycolysis and aerobic respiration (Theil, 2012) Hydroxyacyl ACP dehydrase is an enzyme involved in fatty acid synthesis. Glutathione peroxidase (GPx) is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. Ferritin is a kind of iron storage protein. The role of urease is to catalyze the hydrolysis of urea to ammonia and carbon dioxide. These are important enzymes in the growth and development of plants, thus it is possible to speculate that the No. 8 sample germination and growth process is superior to other samples. And there is another possibility that No. 8 sample and other samples did not belong to the same species.

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