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Full Length Research Paper

Comparison of protein extraction methods suitable for proteomics analysis in seedling roots of Jerusalem artichoke under salt (NaCl) stress

Qiang Chen[2,3,4†], Mei-De Zhang[2,3,4†] and Shi-Hua Shen[1*]

[1]The Research and Development Center for Energy Plant, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China.
[2]Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China.
[3]Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China.
[4]Graduate University of the Chinese Academy of Sciences, Beijing 100049, China.

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An efficient protein extraction method is a prerequisite for successful implementation of proteomics. In this study, seedling roots of Jerusalem artichoke were treated with the concentration of 250 mM NaCl for 36 h. Subsequently, six different protocols of protein extraction were applied to seedling roots of Jerusalem artichoke for comparing extraction efficiency by conducting two-dimensional electrophoresis (2-DE). The first-dimensional electrophoresis was differently performed by using tube gel and immobilized pH gradient (IPG) strips, respectively. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension was equally carried out. The results indicated that precipitation of ammonium acetate in methanol after phenol extraction (Method 6) provided the best effect. Less impurity was contained in extracted proteins and the 2-DE gel images showed less horizontal and vertical stripes, the most proteineous spots were extracted on the basis of the protocol, up to 903. Trichloroacetic acid (TCA) method (Method 1) exhibited relative less quality gel images. 248 protein spots were only obtained according to the protocol and were the least among six protocols. Although, Mg/Nonidet P-40 (NP-40) methods (Methods 3 and 4) could extract a large number of proteins, it exhibited the worst quality gel images among all protocols and the horizontal and vertical stripes on the gel images were the most severe. It might be caused by a high salt content in extracted proteins.

Key words: Proteomics, Jerusalem artichoke, roots, two-dimensional electrophoresis (2-DE), protocol, salt.

INTRODUCTION

One of the problems facing the whole world is shortage of energy and fossil fuel will be depleted. Many countries begin turning to search for new alternative energies. Bioenergy, clean and low-carbon emission, has caused people to pay more attentions (Tian et al., 2005; Wu et al., 2006; Jiang, 2008). Because different national conditions greatly vary, people have to adopt a way suited to their national conditions to produce bioenergy raw sources, such as the U.S. bioenergy strategic plan. In China, food security can not be broken because of its large numbers of population and limited arable land resources, but they have to assume the responsibility for energy-saving and emission reduction. Therefore, people have to be under the premise of keeping grain lands and not competing food with human to develop bioenergy in China (MAPRC, 2008). With these constraints in this context, the development prospect of bioenergy can only be turned to the lands not suitable for cultivation of grain, such as salinity, drought and other marginal lands to grow

*Corresponding author. E-mail: shshen@ibcas.ac.cn. Tel: +86 10 62836545. Fax: +86 10 62596594.

Abbreviations: 2-DE, 2-Dimensional electrophresis; **IPG,** immobilized pH gradient; **IEF,** isoelectric focusing; **PEG,** polyethylene glycol.

†These authors contributed equally to this work.

bioenergy raw materials (MAPRC, 2008).

Jerusalem artichoke originates in North America, Asteraceae *Helianthus*. It is classified as category of tuber and tuberous rooted vegetables in horticulture. It is a C_3 warm-season plant that could be cultivated at a relatively low cost with zero irrigation (Monti et al., 2005). It has also shown that Jerusalem artichoke is relatively resistant to salinity (Liu et al., 2003). It is an economically important crop species, which aerial parts as well as the tubers of plants are used as forage for cattle (Long et al., 2009). During recent years, Jerusalem artichoke has been recognized as a good source of fructose and inulin (Saengthongpinit and Sajjaanantakul, 2005) and it has also demonstrated that tuber of Jerusalem artichoke could be used for producing ethanol that can be employed as a fuel for vehicles after blending with petrol (Long et al., 2009). So it has a potential application in several industries (Xue and Liu, 2008).

Although, the work has partly been done to screen Jerusalem artichoke varieties that could grow normally on saline-alkali soils (Liu et al., 2003; Xue and Liu, 2008; Long et al., 2009), most of these targets are limited to the morphology biology, physiology as well as biochemistry and so on. However, the research has not been reported, in which Jerusalem artichoke roots under salt stress are investigated by using the method of proteomics analysis and Jerusalem artichoke roots how to make defenses in response to salt injury. Since no one has done the research, it is noteworthy to discover a proteome research method that is suitable for Jerusalem artichoke roots. In general, salt of protein samples can seriously interfere with 2-DE analysis, especially with high-salt treatment. Thus, salt removal and purification of protein samples are a very necessary step. In order to find out a suitable proteomic analysis method for Jerusalem artichoke roots treated with salt, we have done the part of this prophase research work, tried six different protein extraction protocols and partly compared glass tube gel with IPG strips in first dimension of 2-DE, hoping to find an ideal protein extraction protocol that could be suited to proteomic analysis of Jerusalem artichoke roots under salt stress. Sequentially providing a theoretical guidance to Jerusalem artichoke production on saline-alkali soils and expanding the feedstock supply of bioenergy to accelerate the industrialization process of bioenergy.

MATERIALS AND METHODS

Plant material and stress treatments

Jerusalem artichoke (*Helianthus tuberosus* L.) cultivar tuber was collected from Yulin of Shanxi Province, China. Before culture, cutting the tubers into small square pieces and the weight of each piece was about 1 g. Tuber slices with a single bud were surface sterilized with 75% ethanol (v/v) for 30 min, then washed with distilled water and immersed into 2.9×10^{-5} M Gibberellic acid (GA3) for 5 min to keep the future seedlings uniform. Subsequently, rinsed thoroughly with distilled water and germinated on moist sand in an incubator at 25°C until the buds' length was about 1 cm. The relative uniformly germinated slices were selected and cultured with half-strength Hoagland's nutrient solution in hydroponic system in the tissue culture room. Half-strength Hoagland's nutrient solution was renewed once every 2 days. The seedlings were subjected to 150 mM NaCl treatment by supplying salt into the nutrient solution after 21 days. On the second treatment day, replaced with new half-strength Hoagland's nutrient solution that contained 250 mM NaCl and kept this situation 36 h. Roots (5 cm section from root tips) treated with 250 mM NaCl were cut and rapidly frozen in liquid nitrogen and stored at -80°C for further proteomic analysis.

Protein extraction protocols of tube gel

In this part, we tried three different homogenate extract buffers and two kinds of protein precipitation methods. They constituted five kinds of extraction protocols, specific operational procedures are shown further:

Method 1 (M1), proteins were extracted using a modified protocol according to Shen et al. (2003). Seedling roots of Jerusalem artichoke were ground into fine powder in liquid nitrogen with a pre-cooled mortar and pestle. Exactly 2.0 g of each sample was homogenized in 8 ml homogenate buffer containing 20 mM Tris-HCl (pH 7.5), 250 mM sucrose, 10 mM ethylenedia-minetetraacetic acid (EDTA), 1 mM phenylmethanesulfonyl fluoride (PMSF), 1 mM dithiothreitol (DTT) and 1% TritonX-100. The homogenate was transferred into an Eppendorf tube and centrifuged at 15,000 × *g* for 15 min at 4°C, repeated twice. The supernatant was transferred to a new tube and proteins were precipitated using 1/4 volume 50% cold TCA in an icy bath for 30 min. The mixture was centrifuged at 15,000 × *g* for 15 min at 4°C and the supernatant was discarded. The pellet was washed with acetone containing 13 mM DTT three times, centrifuged and vacuum-dried. The dried powder was dissolved in sample buffer containing 7 M urea, 2 M thiourea, 4% (w/v) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS), 2% (v/v) ampholine, pH 3.5 to 10 (GE Healthcare Life Science), and 1% (w/v) DTT. Centrifuged at 15,000 × *g* for 15 min at 4°C, then the supernatant was used for the first-dimensional electrophoresis.

Method 2 (M2), extracted proteins in the light of Shen et al. (2003) and Hurkman and Tanaka (1986) with slightly modified seedling roots of Jerusalem artichoke were ground into fine powder in liquid nitrogen with a pre-cooled mortar and pestle. Exactly 2.0 g of each sample was homogenized in 8 ml homogenate buffer containing 20 mM Tris-HCl (pH 7.5), 250 mM sucrose, 10 mM EDTA, 1 mM PMSF, 1 mM DTT and 1% TritonX-100. The homogenate was transferred into an Eppendorf tube and then an equal volume of Tris-saturated phenol (pH 7.5) was added. Vortexed for 10 min at room temperature and placed in icy bath or at 4°C 1 h, centrifuged at 15,000 × *g* for 20 min at 4°C. The phenol phase was transferred to a new tube and added with an equal volume of extraction buffer. Shaking it well, centrifuged at 15,000 × *g* for 20 min at 4°C, repeated twice. Proteins were precipitated from the phenol phase by the addition of 3 volume of 0.1 M ammonium acetate in methanol and incubated at -20°C overnight. Next day, centrifuged at 15,000 × *g* for 20 min at 4°C, then discarded the supernatant, the pellet was washed with acetone containing 13 mM DTT three times, centrifuged at 15,000 × *g* 10 min at 4°C and vacuum-dried. The sample was dissolved according to M1 method for the first-dimensional electrophoresis.

Method 3 (M3) is Mg/Nonidet P-40 (NP-40) buffer and TCA precipitation; based on the method previously described by Ahsan et al. (2007) and Shen et al. (2003), with slight modifications. Briefly, samples were placed into a pre-cooled mortar and ground into fine powder in liquid nitrogen with a pestle. 2.0 g sample powder was resuspended in 8 ml Mg/NP-40 buffer containing 0.5 M Tris-HCl (pH 8.3), 2% (v/v) NP-40, 20 mM $MgCl_2$, 2% (v/v) 2-mercaptoethanol (2-ME), 1 mM PMSF and 1% (w/v) polyvinyl-

polypyrrolidone (PVPP), extensively homogenized. The method of TCA precipitation and the first-dimensional electrophoresis was identical to M1.

Method 4 (M4) is the Mg/NP-40 buffer, phenol extraction and precipitated with ammonium acetate in methanol was based on the method previously described by Ahsan et al. (2007) and Hurkman and Tanaka (1986), with some modifications. Briefly, the method of homogenate was the same to M3. The methods of extraction and precipitation as well as the first-dimensional electrophoresis were common with M2.

Method 5 (M5) is the phenol method according to Hurkman and Tanaka (1986), with some improvements. Roots were placed in nitrogen and ground into fine powder. 2.0 g powder was suspended in 8 ml extraction buffer (0.5 M Tris-HCl, pH 7.5, containing 0.7 M sucrose, 0.1 M KCl, 50 mM EDTA, 2 mM PMSF, and 2% v/v 2-ME) and fully homogenized. The mixture was incubated for 10 min at 4℃ and an equal volume of Tris-saturated phenol (pH 7.5) was added. After 10 min with shaking at room temperature, the phases were separated by centrifugation. The phenol phase was recovered and reextracted with an equal volume of extraction buffer. Proteins were precipitated from the phenol phase by the addition of 3 volume of 0.1 M ammonium acetate in methanol and incubated at -20℃ overnight. Next day, centrifuged at 15,000 × *g* for 20 min at 4℃, then discarded the supernatant, the pellet was washed with acetone containing 13 mM DTT three times, centrifuged at 15,000 × *g* for 10 min at 4℃ and vacuum-dried. The dried powder was dissolved on the basis of M1 for the first-dimensional electrophoresis.

Protein extraction protocols of IPG strips

The earlier mentioned tube gel researches were carried out in the first instance. However, the results showed that most protein spots concentrated in the pH 4 to 7 range and proteins could not be separated sufficiently in tube gel. Moreover, 2-DE results of the earlier mentioned extraction methods were not too ideal. So we tried a new improved protocol, given the stated tube gel results, we thought it was not necessary to perform the new protocol by using tube gel again. Thus, we directly carried it out by IPG strips and picked out M2 and M5 from slightly ideal extraction protocols of tube gel. Compared this new approach with M2 and M5 by using pH 4 to 7 IPG strips in first-dimensional electrophoresis, but the second-dimensional SDS-PAGE was equally applied.

Method 6 (M6), a new protein extraction buffer and proteins precipitated with a slight modification method described as Hurkman and Tanaka (1986). Seedling roots of Jerusalem artichoke were ground into fine powder in liquid nitrogen with a pre-cooled mortar and pestle. 2.0 g powder was homogenized in 8 ml homogenate buffer containing 50 mM Tris-HCl (pH 7.9), 0.5 mM EDTA, 0.7 M sucrose, 50 mM NaCl, 5% (v/v) glycerol, 2% (v/v) NP-40, 1 mM PMSF, 2% (v/v) 2-ME, 1% (w/v) PVPP. The homogenate was transferred into an Eppendorf tube and then an equal volume of Tris-saturated phenol (pH 7.5) was added. Vortexed for 10 min at room temperature and placed in icy bath or at 4℃ 1 h, centrifuged at 15,000 × *g* for 20 min at 4℃.

The phenol phase was transferred to a new tube and added with an equal volume of extraction buffer. Shaking it well, centrifuged at 15,000 × *g* for 20 min at 4℃, repeated twice. Proteins were precipitated from the phenol phase by the addition of 3 volume of 0.1 M ammonium acetate in methanol and incubated at -20℃ overnight. Next day, centrifuged at 15,000 × *g* for 20 min at 4℃ and then discarded the supernatant. The pellet was washed with acetone containing 13 mM DTT three times, centrifuged at 15,000 × *g* for 10 min at 4℃ and vacuum-dried. The dried powder was dissolved as the same as the earlier mentioned tube gels for the first-dimensional electrophoresis.

Protein separation by tube gel in the first-dimensional electrophoresis and followed by second-dimensional SDS-PAGE

2-DE was carried out according to Yang et al. (2007a) with some changes. The first-dimensional isoelectric focusing (IEF) was performed in 13 cm long glass tube with a 3 mm diameter. The gel contained 3.6% acrylamide, 8 M urea, 2% NP-40 and 2% ampholine (pH 3.5 to 10: pH 5 to 8 = 1:1). IEF was performed at 300 V for 0.5 h and then at 600 V for 15 h and 800 V for 1 h. Electrode solutions were 50 mM phosphoric acid at the anode and 50 mM sodium hydroxide at the cathode.

The pellet was suspended in 210 µl of a lysis buffer and then 80 µl protein suspension was loaded on the top of the tube gel (cathode). After 1-DE, IEF gels were equilibrated for 15 min twice in equilibration solution containing 62.5 mM Tris-HCl, pH 6.8, 2.5% SDS, 10% v/v glycerol and 5% 2-mercaptothanol. 2-DE was performed on vertical slab gels (138×87×1 mm); Laemmli buffer system was used to cast 5% stacking gel and 15% resolving gel. The tube gels were sealed on the top of the slab gels with 1% agarose and electrophoresis was carried out at 25 mA per gel for 3 to 4 h. After electrophoresis the gels were visualized by CBB R-250 staining.

Protein separation by IPG strips in the first-dimensional electrophoresis and followed by second-dimensional SDS-PAGE

2-DE was performed by Yang et al. (2007b) with some modifications. IEF was carried out using an IPGphor II electrophoresis system and immobiline dry-strips, linear pH gradient 4 to 7, length 11 cm (GE Healthcare Life Science). 200 µl of each sample from M2, M5 and M6 suspended in 210 µl lysis buffer was loaded during the rehydration step (12 h). IEF was performed by 1 h at 300 V, 1 h at 600 V, 1 h at 1000 V, 1 h at 8000 V, at last followed by 32 000 Vh, all at 50 µA/strip at 20℃ constant temperature. After IEF, the immobilized pH gradient (IPG) strips were incubated at room temperature for 15 min in 6 M urea, 30% (w/v) glycerol, 2.5% (w/v) SDS, 1% DTT, 50 mM Tris-HCl, pH 8.8. A second equilibration step was carried out for 15 min in the same buffer with the exception that DTT was replaced by 2.5% iodoacetamide. After equilibration, the strips were applied to vertical SDS-polyacrylamide gels (15% resolving and 5% stacking) and then sealed with 0.5% low-melting agarose in an SDS buffer. Then electrophoresis was performed in an SDS electrophoresis buffer (pH 8.3), containing 25 mM Tris base, 192 mM glycine and 0.1% SDS, for 3 to 4 h at 25 mA until the bromophenol blue reached the bottom of the gel. After electrophoresis, gels were stained with CBB R-250 for about 1 h followed by destaining. The material preparation and 2-DE were performed for three technical replicates to ensure the reliability of the results.

Image and data analysis

The stained gels were scanned using UMAX Power Look 2100XL scanner (UMAX, Inc., Taipei, China). The data and comparative analysis were performed using image master 2D-platinum version 5.0 software (GE Healthcare Life Science). Protein concentration was detected according to the Bradford method (Bradford, 1976). Three independent biological replicates were completed for each protein extraction procedure and 2-DE. All data were showed as means of three biological replicates with their standard deviation. The Duncan's new multiple range test ($P < 0.05$) was selected for data statistics.

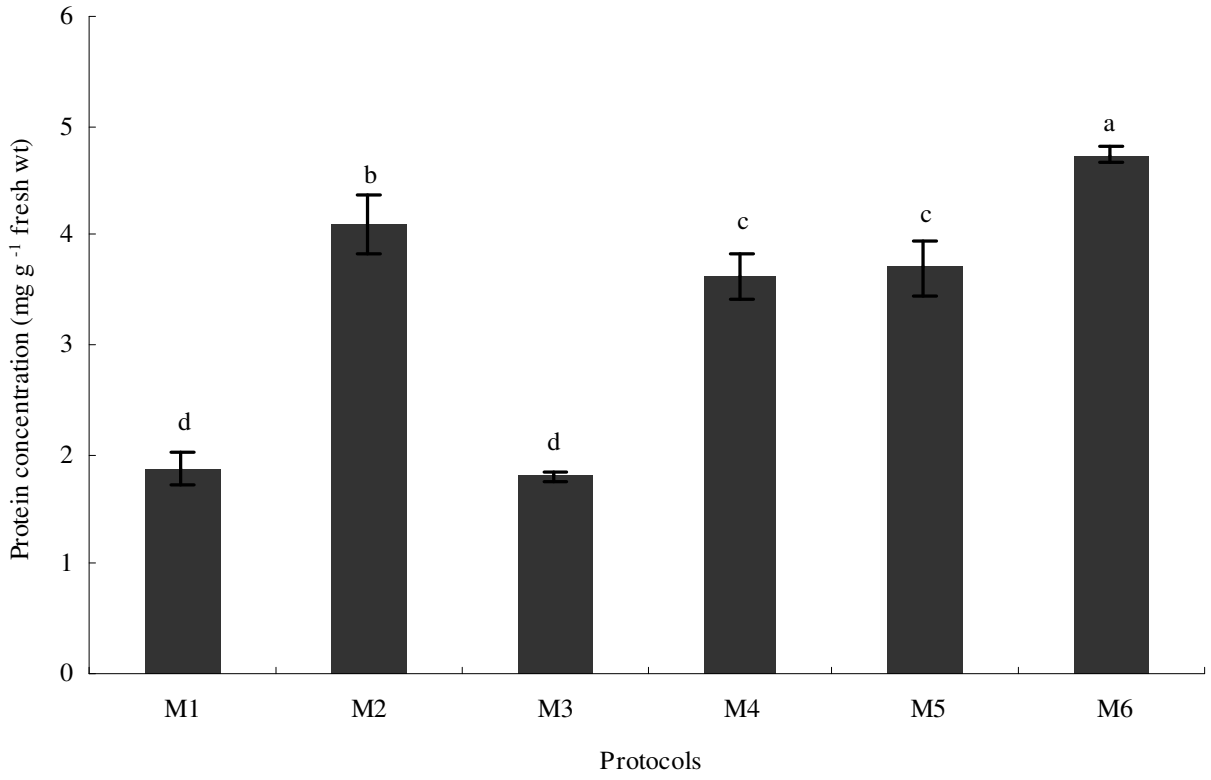

Figure 1. Protein concentration extracted with six different extraction protocols. Values are presented as mean ± standard deviation of three replicates. Low case letters a, b, c, d indicate significant differences using Duncan's new multiple range test ($P < 0.05$).

RESULTS

Effect of different protocols on protein concentration

From Figure 1, protein contents of the six different protocols greatly varied. Protein content extracted with M6 gave the greatest yields among all protocols, up to 4.73 mg g^{-1} FW. Following that was M2. However, M3 was in the lowest protein content, only 1.8 mg g^{-1} FW. Protein content of M1 was just more than M3. Protein contents extracted from M4 and M5 were between M1 and M2, no significant difference, but the protein content extracted with M5 was slightly larger than M4.

Effect of different protocols on gel images quality

The ImageMaster analysis results of protein with glass tube gel (Figure 2) showed that M2 gave the most spots during five kinds of protocols, up to 743, significantly higher than other methods. The protein spots that were obtained with the M5 were less than M2, but significantly more than other three extraction methods. The protein spots from M1 were the least, only 336 spots. There were less protein spots from M3 and M4, 385 and 419, respectively.

The gel analysis results from three kinds of protocols with IPG strips (Figure 3) indicated that the M6 gave the greatest number of protein spots among all protocols, reaching to 869 spots. Compared with M2-Tube gel and IPG strip, M6 got more protein spots, more than 129 and 127, respectively (Figures 2 and 3). M2-IPG strip obtained 740 protein spots, no difference with M2-Tube gel (Figure 2). Protein spots from M5-IPG strip were significantly smaller than M5-Tube gel, 479 and 574, respectively.

From Figure 4, M6 received the highest quality gel images. Followed by M2-IPG strip, if proteins were not concentrated in the pH 4 to 7 range and the tube gel was not very stable, the effect of M2-Tube gel was perhaps pretty good. The worst quality of 2-DE in all was from Mg/NP-40 protocols, M3 and M4 had heavier horizontal and vertical stripes, affecting protein analysis, although, the numbers of protein spots were higher than M1-Tube gel. The number of protein spots with M1 was the least, directly impacting on its image analysis. The quality of tube gel and IPG strip images of M5 was nearly the same, but weaker than M6 and M2.

DISCUSSION

In this study, six different protein extraction and precipitation protocols and two kinds of ways in the first

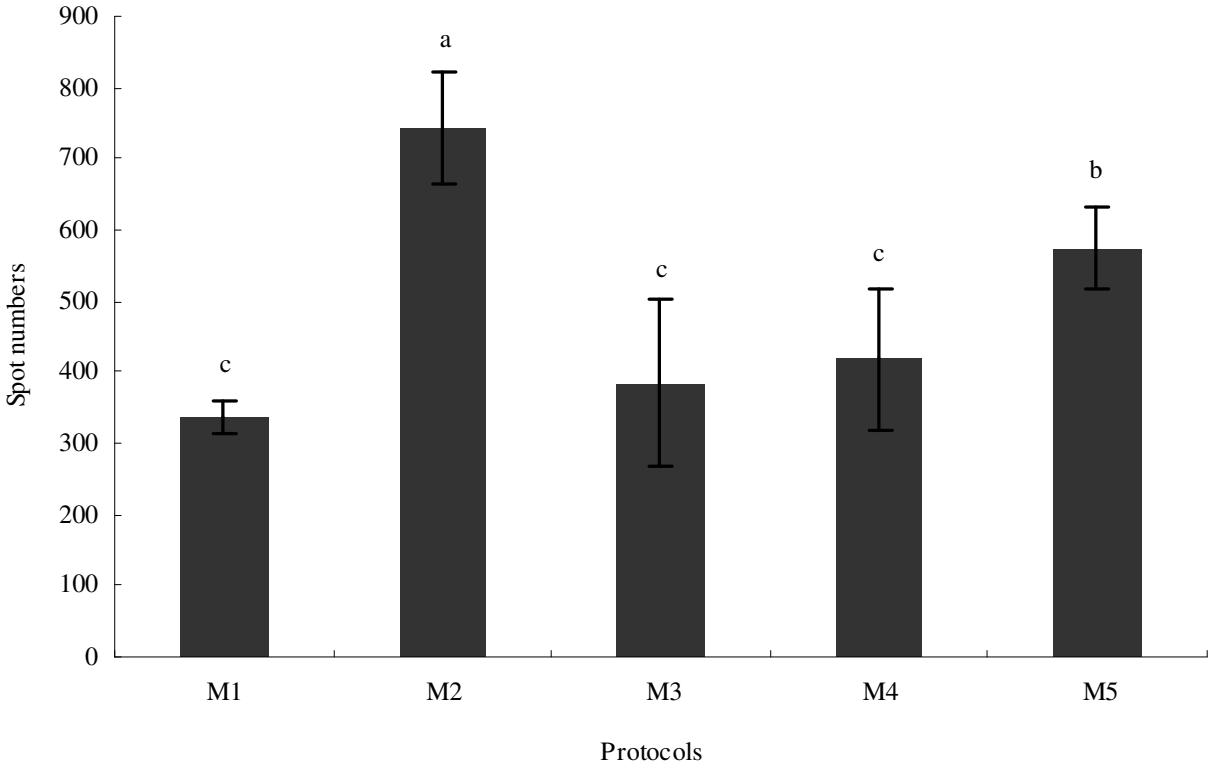

Figure 2. Numbers of protein spots separated by tube gel in the first dimension of 2-DE based on five different extraction protocols. Values are presented as mean ± standard deviation of three replicates. Low case letters a, b, c indicate significant differences using Duncan's new multiple range test ($P < 0.05$).

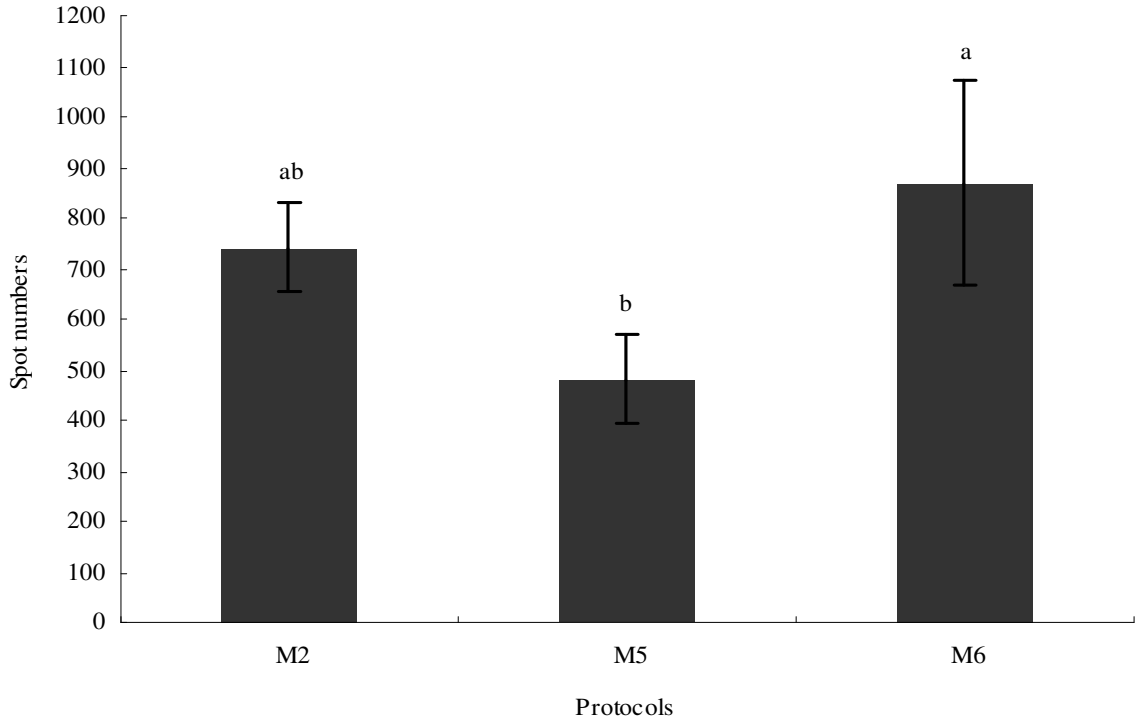

Figure 3. Numbers of protein spots separated by IPG strips in the first dimension of 2-DE based on three different extraction protocols. Values are presented as mean ± standard deviation of three replicates. Low case letters a, b indicate significant differences using Duncan's new multiple range test ($P < 0.05$).

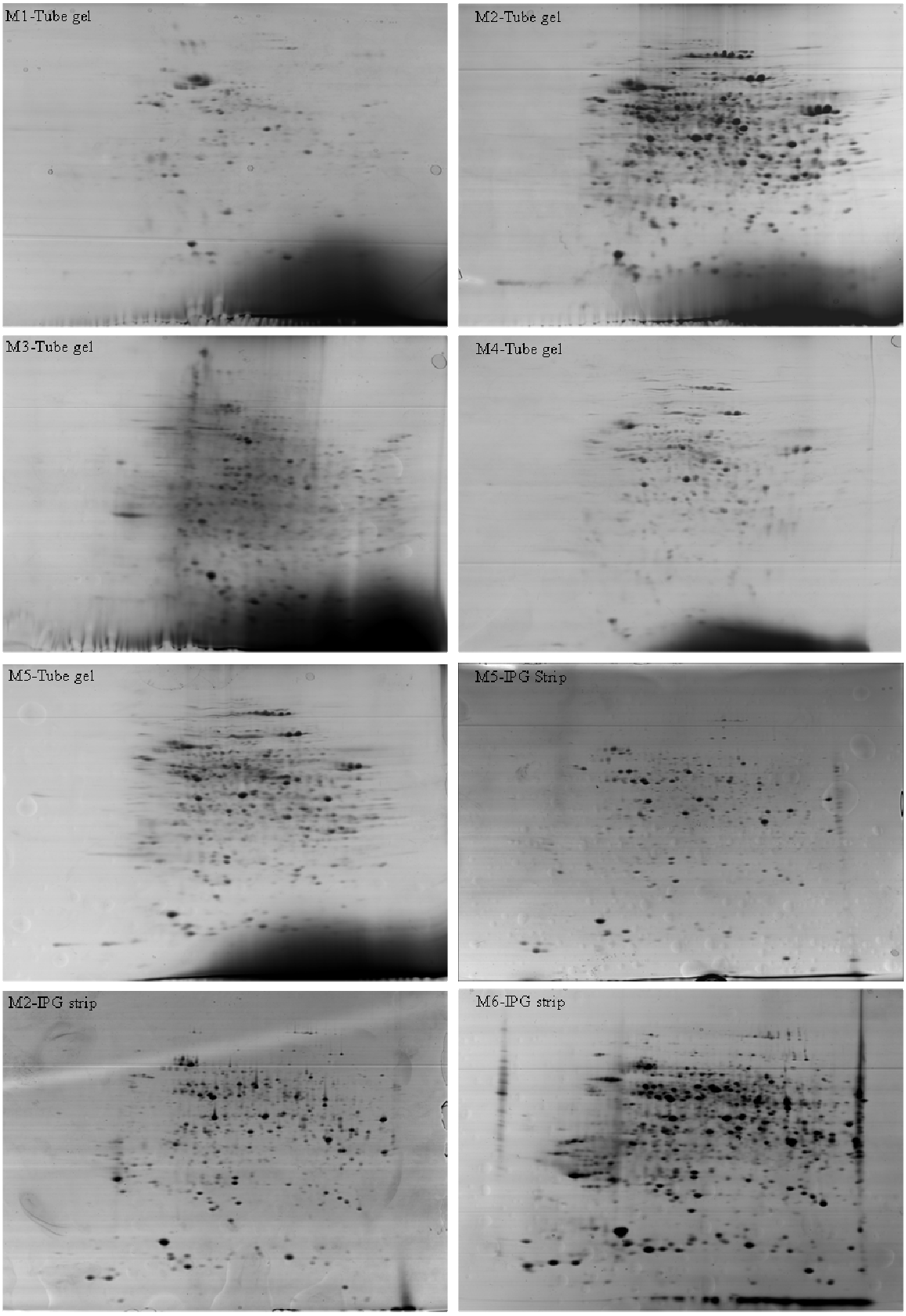

Figure 4. The 2-DE gel quality images partly obtained by tube gel and IPG strips in the first dimension of 2-DE based on six different extraction protocols. All the images are 138 × 87 × 1 mm.

dimension separation of 2-DE were compared to select the most suitable method for seedling roots of Jerusalem artichoke. Although, many different protocols were investigated (Saravanan and Rose, 2004; Carpentier et al., 2005; Natarajan et al., 2005; Maldonado et al., 2008; Sheorana et al., 2009; Xie et al., 2009), all works have been done only for some special tissue and organism, no any one protocol is universal for all plant tissues and organisms. Since no one has done experimental proteomics on seedling roots of Jerusalem artichoke, no any record is found to be referenced. Based on this reason, we carried out this study.

TCA precipitation is a common method used for proteomics analysis. From the results of Shen et al. (2003), TCA precipitation indicates that the method is a superior protocol for rice proteome analysis, especially in terms of two-dimensional gel electrophoresis (2-DE) separation and mass spectrometric analysis. But our results did not coincide with Shen's results. In all protocols, M1 showed the least protein spots. It exhibited that M1 was not highly efficient in extracting crude proteins from seedling roots of Jerusalem artichoke. It was maybe caused by the distinction of two kinds of plant tissues.

Mg/NP-40 buffer was mainly applied to plant leaves. Because Ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco) is the most abundant leaf protein. In general, elimination of Rubisco was used by a fractionation method based on 15% polyethylene glycol (PEG). This method is widely used as a potential protein extraction method to eliminate the abundant protein from samples for 2-DE analysis (Ahsan et al., 2007). There was no Rubisco in our study, so we did not add polyethylene glycol (PEG) into the buffer when proteins were extracted from the tissue sample by an Mg/NP-40 buffer. The images from M3 protocol were not ideal. In addition, there were more horizontal and vertical stripes. The M3 gained the worst quality image except for the protein spots. The effect of electrophoreses focus was also very bad. Although, protein contents and spots on gels of M4 were more than M3, the effect of electrophoreses focus was similar to M3. It showed that proteins extracted with M3 and M4 were not purity and it might be related to salt in tissue sample. Compared with other protocols, M3 and M4 did not adapt to proteomics analysis of seedling roots of Jerusalem artichoke.

Since Hurkman and Tanaka (1986) induced phenol into protein extraction process for enrichment and purification, many works have been done based on the method. The phenol method gives a higher protein yields and typically greater resolution and spot intensity, particularly with extracts from tissues containing high levels of soluble polysaccharides. The phenol protocol is highly effective with more recalcitrant tissues (Saravanan and Rose, 2004; Carpentier et al., 2005). Xie et al. (2009) reported that trichloroacetic acid/acetone precipitation combined with phenol extraction produces the purest sample and the most protein spots based on protein extraction method for cotton seedling. The TCA-acetone-phenol protocol provides the best results in terms of spot focusing, resolved spots, spot intensity, unique spots detected and reproducibility (Maldonado et al., 2008). From the mentioned works, we could be aware that phenol played a key in the removal of salt and enrichment of proteins. The highest protein yield was obtained according to M6 protocol. In this new extraction buffer, we added 5% glycerol to protect the protein not to be degraded and denaturalized. Because all detergents added in extraction buffer affect the absorption in 280 nm in the determination process of protein content, so we tried NP-40 instead of Triton X-100. From the last gel images and analysis results, M6 received the most number of protein spots and had less horizontal and vertical stripes. It exhibited that the gels were well on quality. Compared with other protocols, M6 was a relative suitable method for seedling roots of Jerusalem artichoke. We knew that proteins concentrated at pH 4 to 7 range from the previous tube gels, so M6 was only performed by IPG strips. M2 was the second efficient protocol that could be used to extract protein from seedling roots of Jerusalem artichoke. Compared tube gels with IPG strips; it was known that more proteins should be loaded on IPG strips. It meant more experimental materials would be needed. M5 originated in Hurkman and Tanaka (1986), it is a typical protocol which extracts protein by phenol. But there was the same problem such as M2 and that is to say more experimental materials would be prepared in the protocol. All the earlier mentioned protocols in this paragraph are based on phenol /ammonium acetate in methanol.

Results of this study showed that phenol/ammonium acetate in methanol was a relative proper protocol to extract protein from seedling roots of Jerusalem artichoke under salt stress and different homogenate buffers had significant difference, but M6 extract buffer was the most suitable buffer for seedling roots of Jerusalem artichoke under salt stress.

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REFERENCES

Ahsan N, Lee DG, Lee SH, Kang KY, Bahk JD, Choi MS, Lee IJ, Renaut J, Lee BH (2007). A comparative proteomic analysis of tomato leaves in response to waterlogging stress. Physiol. Planta, 131: 555-570.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Carpentier SC, Witters E, Laukens K, Deckers P, Swennen R, Panis B (2005). Preparation of protein extracts from recalcitrant plant tissues: an evaluation of different methods for two-dimensional gel electrophoresis analysis. Proteomics, 5: 2497-2507.

Hurkman WJ, Tanaka CK (1986). Solubilization of plant membrane proteins for analysis by two-dimensional gel electrophoresis. Plant Physiol. 81: 802-806.

Jiang ZM (2008). Reflections on energy issues in China (in Chinese). J. Shanghai Jiaotong Univ., 42: 345-359.

Liu ZP, Liu L, Chen MD, Deng LQ, Zhao GM, Tang QZ, Xia TX (2003). Study on the irrigation systems in agriculture by seawater (in Chinese). J. Nat. Resour. 18: 423-429.

Long XH, Chi JH, Liu L, Li Q, Liu ZP (2009). Effect of seawater stress on physiological and biochemical responses of five Jerusalem artichoke ecotypes. Pedosphere, 19: 208-216.

Maldonado AM, Echevarría-Zomeño S, Jean-Baptiste S, Hernández M, Jorrín-Novo JV (2008). Evaluation of three different protocols of protein extraction for Arabidopsis thaliana leaf proteome analysis by two-dimensional electrophoresis. J. Proteomics, 71: 461-472.

Ministry of Agriculture of the People's Republic of China (MAPRC) (2008). Agricultural biomass industry development plan (2007~2015) (in Chinese). http://www.agri.gov.cn/jhgb/t20080826-1168529.htm.

Monti A, Amaducci MT, Venturi G (2005). Growth response, leaf gas exchange and fructans accumulation of Jerusalem artichoke (*Helianthus tuberosus* L.) as affected by different water regimes. Eur. J. Agron. 23: 136-145.

Natarajan S, Xu CP, Caperna TJ, Garrett WM (2005). Comparison of protein solubilization methods suitable for proteomic analysis of soybean seed proteins. Anal. Biochem. 342: 214-220.

Saengthongpinit W, Sajjaanantakul T (2005). Influence of harvest time and storage temperature on characteristics of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. Postharvest Biol. Technol. 37: 93-100.

Saravanan RS, Rose JKC (2004). A critical evaluation of sample extraction techniques for enhanced proteomic analysis of recalcitrant plant tissues. Proteomics, 4: 2522-2532.

Shen SH, Jing YX, Kuang TY (2003). Proteomics approach to identify wound-response related proteins from rice leaf sheath. Proteomics, 3: 527-535.

Sheorana IS, Ross ARS, Olsonb DJH, Sawhney VK (2009). Compatibility of plant protein extraction methods with mass spectrometry for proteome analysis. Plant Sci. 176: 99-104.

Tian CL, Guo B, Liu CC (2005). Present situation and prospect of energy plants (in Chinese). Chin. J. Biopro. Eng. 3: 14-19.

Wu GJ, Liu J, Lou ZP, Kang L (2006). Development of energy plant: Progress and suggestions (in Chinese). Bull. Chin. Acad. Sci. 21: 53-57.

Xie CJ, Wang D, Yang XY (2009). Protein extraction methods compatible with proteomic analysis for the cotton seedling. Crop Sci. 49: 395-402.

Xue YF, Liu ZP (2008). Antioxidant enzymes and physiological characteristics in two Jerusalem artichoke cultivars under salt stress. Russ. J. Plant Physiol. 55: 776-781.

Yang PF, Chen H, Liang Y, Shen SH (2007a). Proteomic analysis of de-etiolated rice seedlings upon exposure to light. Proteomics 7: 2459-2468.

Yang PF, Li XJ, Wang XQ, Chen H, Chen F, Shen SH (2007b). Proteomic analysis of rice (*Oryza sativa*) seeds during germination. Proteomics, 7: 3358-3368.

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