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Preparation of Protein Extraction from Flower Buds of *Solanum lycopersicum* for Two-Dimensional Gel Electrophoresis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NC and TL designed the study. Authors XZ and JR performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors XZ and GY managed the analyses of the study. Author HF managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Find a suitable method for the protein extraction from flower buds of *Solanum lycopersicum*.
Study Design: Compare some kinds of protein extraction methods and find the best one among them suitable to tomato flower buds.
Place and Duration of Study: Biological Science and Technology College, between June 2010 and July 2011.
Methodology: The proteins for electrophoresis were extracted using different methods, such as trichloroacetic acid /acetone (TCA/acetone), Sodium dodecyl sulfate (SDS), Tris-saturated phenol (Tris-Phen), Phenol/SDS and Direct lysis method. After silver staining, different patterns of protein spots were observed in the gels.
Results: Few spots were found by SDS and Phenol/SDS extractions, more spots by

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immediate dissolution but the most impurities, less protein productivity though more spots by Tris-Phen extractions, and more protein productivity and better apart effect by TCA/acetone. The 2-DE image background was the clear and the protein spots were the most by TCA/acetone method.
Conclusion: TCA/acetone method is much more suitable as extraction method for protein two-dimensional electrophoresis of tomato flower buds.

Keywords: Flower buds; protein extraction; Solanum lycopersicum; two-dimensional gel electrophoresis.

1. INTRODUCTION

Tomato is one of the most common fruits and vegetables in the world. There are many problems in the tomato's growth along with the greenhouse area increasing year by year. It is badly needed to be solved that how to increase the number of tomato flower buds and improve the quality of flower buds to increase the economic benefits. Key proteins related to flower bud differentiation and development might be analyzed and identified by the use of two-dimensional electrophoresis methods. And the key proteins may provide evidence for being clear the mechanism of flower bud differentiation.

There are various non-proteins which can have a large impact on the process of protein extraction and two-dimensional electrophoresis in plant samples, such as salts, organic acids, pigments, polyphenols, polysaccharides, and other secondary metabolites. Sample preparation is the first and the most important step in two-dimensional electrophoresis, and its success determines whether the two-dimensional electrophoresis' success or not. The organs in tomatoes which used proteomics study mainly focus on leaves [1,2], seeds [3], fruits [4,5], pollens [6,7], cotyledons [8], roots [9,10] and seedlings [11], etc., while the flower buds have not been reported yet.

There has not a general method for all samples preparation in two-dimensional electrophoresis analysis, because of the diversity kinds of the analysis samples [12]. The young tomato flower bud tissues also contain amount of pigments, phenols, polysaccharides and other secondary metabolites, which could impact on the two-dimensional electrophoresis analysis. Tomato flower buds are small and young, highly vulnerable and destructionable in the process of the protein extraction. Finding a suitable method of protein sample preparation from tomato flower buds is particularly important. In this study, the protein sample preparation method from tomato flower buds was established for further research and the proteomics provide the basis for regulation of tomato flower bud differentiation.

2. MATERIALS AND METHODS

2.1 Plant Materials

Tomato (*Solanum lycopersicum* cv. Liaoyuanduoli) seeds were sown in the soil with peat:vermiculite (v/v) = 1:2, put in the solar greenhouse. The size of plot was 15×25 cm (up caliber was 15cm, height was 25cm). Flower buds about 0.3~0.5mm cut from plants under stereoscopic microscope were collected in the liquid nitrogen frozen when the first inflorescence going to open well. Then flower buds were stored at – 80ºC until use.

2.2 Protein Extraction

2.2.1 TCA / acetone method

Frozen tomato flower buds about 1g were finely powdered with 0.1g polyvinylpyrrolidone (PVPP) in liquid N_2 using a mortar, and suspended in 5mL of cold acetone solution (10% TCA and 0.07% β-mercaptoethanol) for 12 hours at -20℃, then centrifuged at 12000 g 30min at 4℃. Precipitate was washed with 5mL of cold acetone solution (containing 0.07% β-mercaptoethanol), and then placed at -20℃ for 1h, the supernatant was discarded after centrifugation 30min at 12000g. Repeat washing until precipitate's colour is white [13].

Protein pellets were dried and 20mg dry powder were solved in 1mL lysis buffer (7 mol/L urea, 2mol/L thiourea, 4% CHAPS, 1.0% TBP, 1.0% DTT, 1.0% Cock tail, 2.0% IPG Buffer), shaked 3-4 hours at 30℃. The supernatant centrifuged at 12000 r/min 10min, was placed into a 1.5mL centrifuge tube and stored at -20℃ for use. Experiments were repeated 3 times.

2.2.2 SDS method

Frozen tomato flower buds about 1g were finely powdered with 0.1g PVPP in liquid N_2 using a mortar, and suspended in 5mL extract buffer [0.175 mol/L Tris-HCl (pH8.8), 5% SDS, 0.03mol/L DTT, 15% glycerol]. Add 4-fold volume of cold acetone to vortex mix and -20℃ overnight. The supernatant was discarded after centrifuged at 12000 g 20min (4℃). Washing precipitate repeat with 80% cold acetone until precipitate's colour is white [14]. Protein pellets were dried and 20mg dry powders were solved in 1mL lysis buffer. Experiments were repeated 3 times.

2.2.3 Phenol method

Frozen tomato flower buds about 1g were finely powdered with 0.1g PVPP in liquid N_2 using a mortar, and suspended in 5mL extract buffer (1% PVP, 0.7mol/L sucrose, 0.1mol/L KCl, 0.5mol/L pH7.5 Tris-HCl, 0.5mol/L EDTA, 1mmol/L PMSF, 2% β-mercaptoethanol). The supernatant added an equal volume of Tris phenol vortexing with 30min at 4℃ after centrifuged at 1000r/min 30min (4℃). This process repeat 3 times. Then collect the 3 phenol phases, mixed with 5 volumes of 0.1mol/L ammonium acetate cold ethanol overnight at -20℃. Centrifugating the tube at 12000g 30min, washing the precipitate with 0.1mol/L ammonium acetate cold ethanol three times and cold acetone once [15]. Protein pellets were dried and 20mg dry powders were solved in 1mL lysis buffer. Experiments were repeated 3 times.

2.2.4 Phenol/SDS method

Frozen tomato flower buds about 1g were finely powdered with 0.1g PVPP in liquid N_2 using a mortar, and then resuspended in 15mL cold acetone. After vortexing thoroughly for 30s, the tubes were centrifuged at 10000 g for 3min (4℃). The resultant pellet was washed once more with cold acetone. After the initial two washes, the pellet was transferred into a mortar and allowed to dry at room temperature. The dried powder was further ground to a finer powder by the aid of quartz sand. The fine powder was sequentially rinsed with cold 10% TCA in acetone until the supernatant was colorless, then with cold aqueous 10% TCA twice and finally with cold 80% acetone twice. The final pellet was dried at room temperature and resuspended in 0.8mL phenol (pH 8.0) and 0.8 mL dense SDS buffer (30% sucrose, 2%

SDS, 0.1M Tris-HCl, pH8.0, 5% β-mercaptoethanol). The mixture was vortexed thoroughly and the phenol phase was separated by centrifugation at 10000g for 10min. The upper phenol phase was collected. 5 volumes of cold methanol plus 0.1M ammonium acetate was added to the phenol phase and the mixture was stored at -20℃ for 30min. Precipitated proteins were recovered at 10000g for 5min, and then washed with cold methanolic ammonium acetate twice and cold 80% acetone once[16]. Protein pellets were dried and 20mg dry powders were solved in 1mL lysis buffer. Experiments were repeated 3 times.

2.2.5 Direct lysis method

Frozen tomato flower buds about 1g were finely powdered with 0.1g PVPP in liquid N_2 using a mortar, and then resuspended in 1mL lysis buffer. Experiments were repeated 3 times.

2.3 Protein Quantification

Protein was quantified by the Modified Bradford [17] with bovine serum albumin as standard. After protein quantification, protein solutions were packed with 35μg proteins in 125μL buffer.

2.4 Two-dimensional Gel Electrophoresis

The first isoelectric focusing (IEF) gel electrophoresis using 7cm pH3-10 IPG strip. Focused strips were equilibrated using dithiothreitol solutions(50mmol/L Tris-HCl, 6mol/L urea, 30% glycerol, 2% SDS, 1% DTT) and iodoacetamide solutions (50mmol/L Tris-HCl, 6mol/L urea, 30% glycerol, 2% SDS, 4% IAA) [18]. IEF parameters were set according to Table 1. IPG strips were transferred to 11% SDS-PAGE, electrophoresis conditions as follows: 5mA/gel, 0.5h; 10mA/gel. The gels were stained by silver [19].

Table 1. IEF parameters

Step	Voltage	Time
1	50v	1h
2	100v	1h
3	250v	1h
4	500v	1h
5	1000v	1h
6	4000v	3h
7	4000v	6h
8	500v	

3. RESULTS AND DISCUSSION

3.1 Comparisons of Different Effect of Different Methods for Protein Extraction

By comparing the proteins extracted of different methods，the protein powder production and protein purity were very significant difference. The protein powder production and protein purity were similar by TCA/acetone method and SDS method, which dry powder percent was 7.8% and 6.6%, protein yield was 0.14% and 0.19%, respectively. Both of them were higher than those of getting by phenol (1.0% of dry poweder percent, 0.032% of protein yield) and phenol/SDS(0.36% of dry poweder percent, 0.040% of protein yield) methods. But

all of them were much lower than the direct lysis method (Table 2). Phenol and phenol/SDS method were not suitable for the whole protein extract from tomato flower buds in the level of protein yield.

Table 2. Comparisons of the proteins extracted by different methods

Method	Dry powder percent/ %	Protein yield /%
TCA/acetone	7.8 Aa	0.14 Cc
SDS	6.6 Bb	0.19 Bb
Phenol	1.0 Cc	0.032 Ee
Phenol/SDS	0.36 Dd	0.040 Dd
Direct lysis method	——	0.306 Aa

Date：After the data in the same column, significant differences was shown by Lower case letters（α=0.05）,Uppercase letters mean that the difference was extremely significant（α=0.01).

3.2 Comparisons of the 2-DE Maps from Different Protein Sample Obtained by the Different Methods for Protein Extraction

A few spots were displayed by SDS and phenol/SDS methods, indicating that those methods could bring a mount of protein losses. Black background was shown by direct lysis method, indicating that there were many impurities, which covered a part of the proteins. A good resolution and more spots were observed by TCA/acetone and phenol methods (Fig. 1). Compared with the pattern of maps and the protein yield, TCA/acetone method was much more suitable for protein extraction of tomato flower buds.

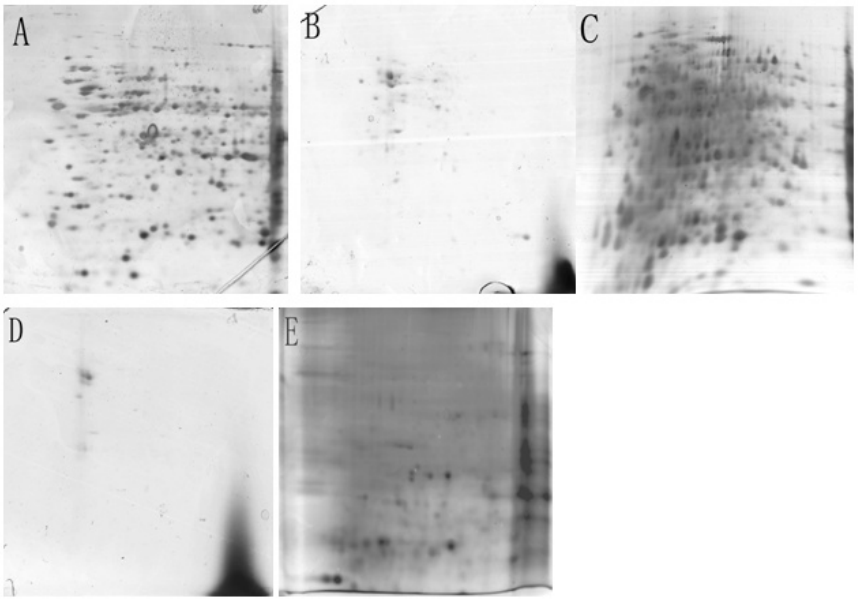

Fig. 1. Comparisons of the 2-DE maps obtained by the different methods from tomato flower buds (A) TCA/acetone (B) SDS (C) Phenol (D) Phenol/SDS (E) Direct lysis method

Sample preparation is one of the most crucial steps for high-quality resolution of proteins in 2-DE. Most problems can be traced to co-extraction of non-protein cellular components that can affect protein migrations [20]. The methods of sample preparation include dissolving the sample directly and precipitating the proteins in order to get the full protein without the impurities. Selecting the suitable method for tomato flower buds of protein extraction is necessary to a good two-dimensional electrophoresis map.

We tried to use the direct lysis method to obtain protein from the young tomato flower buds. However, Fig. 1E showed that much more impurities resulted in a black background, and the impurities seriously impacted on isoelectric focusing and the quality of the map. The bad map showed that the removing the impurities in the protein precipitation of tomato flower buds was necessary.

TCA/acetone, phenol and SDS methods were the traditional methods. In addition, there was the phenol/SDS method. Low protein yield (0.04%) was obtained by Phenol/SDS method, and a few protein spots observed might be too frequently operate to get more useful proteins. The number of spots in Fig. 1B was relatively less, possibly because of SDS with negative charge in the isoelectric focusing process [21].

TCA/acetone and phenol methods are most commonly used in plant sample preparation. TCA/acetone can remove the polysaccharides and small molecules, reducing the interference of secondary metabolites and proteins degradation [22]. Phenol method can effectively remove many organic impurities by different phase's dissolution. While Phenol method has more steps and lower protein yield, but TCA/acetone method is simple and quick with high protein yield. So the TCA/acetone method was the most suitable for the tomato flower buds to extract the proteins because the samples of tomato flower buds were difficult to obtain.

4. CONCLUSION

In summary, five kinds of protein extraction methods were compared in this study. The results showed that TCA/acetone method was the most suitable for protein extraction of tomato flower buds, and got the ideal 2-DE map. The protein extracted from TCA/acetone method might be used for further research and provided the basis for regulation of tomato flower bud differentiation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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