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Genetic Analysis of Ginseng Germplasm by Lactate Polyacrylamide Gel Electrophoresis of Seed Protein

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Abstract : Systematic electrophoretic analysis of alcohol-soluble proteins and salt-soluble proteins of 247 *Panax ginseng* (*P.g*) and *Panax quinquefolium* (*P.q*) germplasms seed was carried out on an improved lactate-polyacrylamide gel electrophoresis, a method with high resolving power, good reproducibility and stability. The electrophoregrams of proteins, according to their migration rate, were classified into four groups such as α, β, γ and ω for the alcohol-soluble proteins and three such as I, II and III for the salt-soluble ones. *Panax ginseng* or *Panax quinquefolium* had their own unique band pattern distinguishable from each other, regarding as their specific"fingerprint". In this study, 3 of 168 (1.8%) *P.g* germplasms and 1 of 79 (1.3%) *P.q* germplasms had their own unique band pattern, showing that *P.g* and *P.q* germplasms have poor genetic diversity in species. The band patterns of dry seed and stratified seed (embryo rate=60%) were basically the same. The band number of the F_1 hybrid of *P.g*×*P.q* was exactly equivalent to the number of the common bands plus the specific bands of the two parents, indicating that the difference of band patterns was a genetic trait controlled by the nuclear genes. The electrophoregram of F_1 of *P.g*×*P.q* could be predicted by that of the two parents and the band pattern of the F_1 hybrids could be demnonstrated by that of the mixed seed extract from the two parents.

Key words : *Panax ginseng*, *Panax quinquefolium*, seed protein, genetic diversity, lactate-PAGE.

Introduction

Panax ginseng and *Panax quinquefolium* are valuable medical plants, and have become a superior industrial in the northeast of China. For perennial plant, traditional method of identification and evalution of ginseng germplasm in field with wasting time, high cost and large amount of work has restricted the development of ginseng breeding. It is badly in need of a technology with good ability, relative simplicity and time-saving in operation to utilize in identifying germplasm of ginseng.[1,2] Cultivated *P.g* and *P.q* today are the offsprings of wild ginseng that have been mixcultured for long time and their hereditary background are unknown. It has theoretically important significance to the study on genetic diversity and relationship of inter-species or intra-species, and hybridization characteristics, to promote the genetic breeding of ginseng.

Seed proteins consist of a number of polypeptides that have similar molecular weight but considerable heterogeneity in isoelectric point,[3] so electrophoretic analysis of seed proteins has been extensively used for crop cultivar identification, genetic diversity analysis and germplasm resources genetic analysis, etc..[4,5] The electrophoregram of seed proteins of many crop cultivars has been regarded as a genetic marker and also used in mapping genetic codes. An acid lactate-PAGE procedure has been developed as a basic method of the electrophoretic identification of wheat or corn. This procedure has been preliminarily proved to be suitable for separating wheat gliadin or corn

albumin and globulin fractions.[6,7] The aim of this study is to evaluate the feasibility of this procedure in distinguishing different genotypes and genetic diversity of *P.g* and *P.q* including its rsolving power, stability, reproducibility and the genetic expression of parent band patterns in their F_1 hybrids and to provide a method for the application to ginseng germplasm resources identification and evaluation, classification, etc..

Materials and Methods

1. Sample preparation

A total of 247 germplasms and 36 hybrids of *P. g* and *P.q* were studied. *Panax ginseng* samples were collected from Ji'an, Fusong and Zuojia in Jilin province and Kuandian in Liaoning province. The *Panax quinquefolium* samples were collected from Zuojia. All the hybrids of *P.g*×*P.q* were bred by our laboratory.

A granule of dry seed was ground with a single seed mill. The ground powder was put into 25 m*l* centrifuge tube with addition of equal-volume extract of proteins (alcohol-soluble protein: 25% 2-ethylene chlorhydrin, 18% urea, 0.05% methyl green; salt-soluble protein: 0.5 mol/L NaCl, containing 15% sucrose and 0.05% methyl green), mixed thoroughly and extracted for 1h at room temperature, then centrifuged at 4000 rpm/min for 5 min. The supernatants were used for electrophoresis.

2. Preparation of the working solutions

The stock solution, tank buffer, separation gel and concentration gel were prepared according to the proportion and volume as shown in Table 1.

3. Electrophoresis

The vertical plate electrophoretic apparatus was used with 145 mm×100 mm×1.0 mm gel slab. Electrophoresis was carried out at 500 V, 30 mA for 1.5 h. The gel was stained with Coomassie Brilliant Blue R250 (40 m*l* 0.14% Coomassie Brilliant Blue R250 alcohol solution dissolved in 160 m*l* 12.5% trichloroacetic acid).

Results and Discussion

1. Resolving power

This electrophoretic procedure showed a high resolving power to the alcohol-soluble proteins and salt-soluble proteins of *P.g* and *P.q* seeds. In general, the electrophoregrams showed 30 bands or so, among which the clearest resolving bands might reach 20 in the alcohol-soluble proteins. Among all the bands, only one which was heavily stained and stable in location seemed to be common to all germplasms. Its relative migration rate was 0.48 (Fig. 1, arrow 1). Another two bands also existed in most cases, with relative migration rates of 0.79 and 0.33, respectively (Fig. 1, arrow 1 and 3). For convenient description, the whole lot of the bands were classified into four groups designated as α, β, γ and ω in relation to the above-mentioned three common or nearly common bands as markers. The α band group was fast moving with migration rates from 0.57 to 1.0, containing 8~10 bands. All the bands in this group stained lighter with unclear boundary, so it is difficult to use as identification markers.

Table 1. Recipes for stock, extraction and buffer solutions

Stock solutions	Mixed proportion	
	Separation gel	Concentration gel
1 Acrylamide 95 g, Bisacrylamide 3.8 g, distilled water 500 m*l*.	14 m*l*	–
2 Sodium lactate 2.81 m*l*+lactic acid to pH 3.2, H_2O 100 m*l*.	2 m*l*	–
3 Ascorbic acid 0.48 g, ferrous sulfate · $7H_2O$ 8 mg, H_2O 100 m*l*.	2 m*l*	1 m*l*
4 Sodium lactate 3 m*l*+lactic acid to pH 5.6, H_2O 100 m*l*.	–	1 m*l*
5 Acrylamide 26 g, bissacrylamide 5.2 g, H_2O 100 m*l*.	–	2 m*l*
6 Ammonium persulphate 11.41 g, H_2O 100 m*l*.	80 μ*l*	80 m*l*
7 Distilled water.	20 m*l*	–

Tank buffer: Glycine 4 g+lactic acid to pH 3.4, H_2O 2000 m*l*

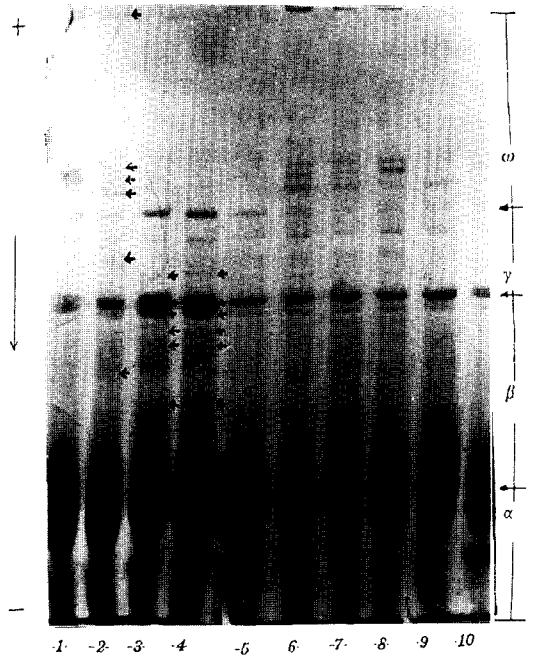

Fig. 1. Lactate-PAGE eletrophoregrams of alcohol-soluble seed protein for major *Panax ginseng* and *P. quinquefolium* germplasm. 1. *P.g* (092); 2. *P.g* (089); 3. *P.q* (521); 4. *P.q* (503); 5. *P.g* (116); 6. *P.g* (100); 7. *P.g* (008); 8. *P.g* (043); 9. *P.g* (034); 10. *P.g* (056).

The β band group was mid-fast moving, including the bands with migration rates from 0.36~0.57, 8~10 bands could be identified, among which 4~5 bands had heavier stains and clear boundary, so they could be used as identification markers. The γ band group was the moderate moving with migration rates of 0.26~0.36, containing 4~5 bands. All of them were stained heavily and clearly, so this group was the main distinguishing region for germplasms identification. 8~10 slow moving bands with migration rates of less than 0.26 were designated as ω group, most of which were narrow but heavily stained and distinctive bands. Thus this group is also a major region for germplasms identification.

In salt-soluble proteins, the electrophoregrams showed 20 bands or so, among which the clearest resolving bands might reach 15. Among all the bands, two bands existed in most cases, with relative migration rates, the whole lot of the bands were classified into three groups designated I, II and III. The band group I was fast moving with migration rates from 0.76 to 1.0, containing 6~8 bands, among which 3~4 bands had heavier stains and clearer boundary, they could be used as identification markers. The band group II was the moderate moving with migration rates of 0.17~0.76, containing 8~10 bands, among which 5~6 bands had heavier stains and clearer boundary, showing a marked difference among species. Thus this group is also a major region for germplasms identification. 3~5 slow moving bands with migration rates of less than 0.16 were designated as group III. All the bands in this group with unclear boundary were difficult to be used as identification markers.

2. Unique band pattern of *Panax ginseng* and *Panax quinquefolium*

Fig. 1 shows the electrophoregrams of the alcohol-soluble protein of *P.g* and *P.q*. It shows very clearly that *P.g* or *P.q* has its own specific pattern in band number, staining intensity, migration position, etc. and is easily distinguishable from each other in β, γ or ω regions (Fig. 1, arrow). The electrophoregram of the salt-soluble protein of *P.g* and *P.q* shows that it also has its own specific pattern in band number, staining intensity, migration position, and is easily distinguishable from the other in the regions II too (Fig. 2, arrow).

3. Genetic diversity of *Panax ginseng* and *Panax quinquefolium* germplasms

In our studies, only 3 of 168 (1.8%) *P.g* germplasms and 1 of 79 (1.3%) *P.q* germplasms were distinguished by their own unique band pattern or "fingerprint" (Fig. 3, arrow, Fig. 4, arrow), indicating that *P.g* and *P.q* have poor genetic diversity in cultivated population, and there is not closely related to collection sites. This method was also found to be difficult to distinguish some of the fruit colour strains or stem colour strains among species.

4. Electrophoregraphic comparison of dry seed and stratified seed of *Panax ginseng*

Fig. 5 shows the electrophoregrams of dry

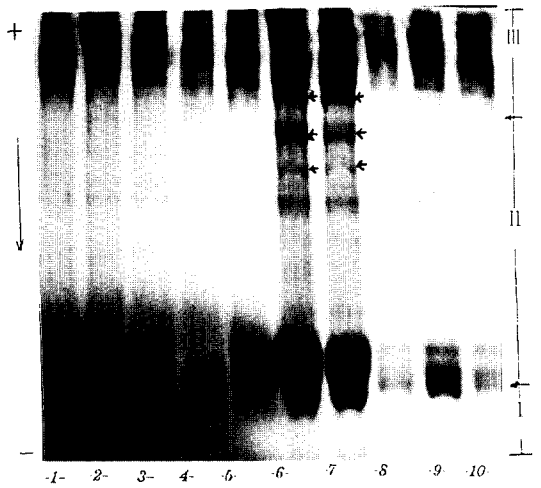

Fig. 2. Lactate-PAGE eletrophoregrams of salt-soluble seed protein for major *Panax ginseng* and *P. quinquefolium* germplasm. 1. *P.g* (085); 2. *P.g* (089); 3. *P.g* (114); 4. *P.g* (071); 5. *P.g* (120); 6. *P.q* (529); 7. *P.q* (510); 8. *P.g* (001); 9. *P.g* (061); 10. *P.g* (112).

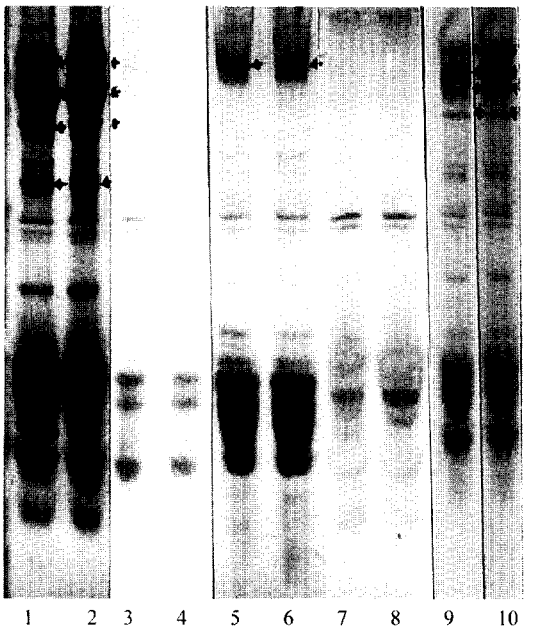

Fig. 3. Lactate-PAGE eletrophoregrams difference of alcohal-soluble seed protein for major *Panax ginseng* germplasm. 1, 2. *P.g* (041); 3, 4. *P.g* (043); 5, 6. *P.g* (068); 7, 8. *P.g* (034); 9, 10. *P.g* (137).

seed (no stratification) and stratified seed (whole kernel and seed embryo, embryo rate=60%) of *P.*

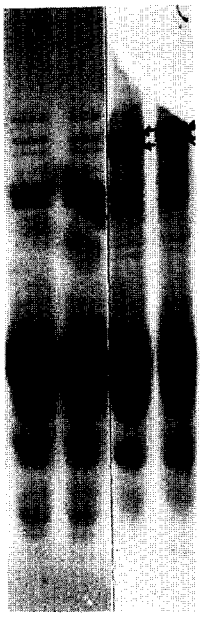

Fig. 4. Lactate-PAGE eletrophoregrams difference of alcohal-soluble seed protein for *P. quinquefolium* germplasm. 1, 2. *P.q* (053); 3, 4. *P.q* (533).

g. It shows that the band patterns of dry seed and stratified seed are basically the same in band number, mobility, width and staining intensity. This indicates that the electrophoregrams of both dry seed and the stratified seed (embryo or whole kernel) could be used as "fingerprint" for germplasm identification. Considering that seed stratification is time consuming and difficult, electophoresis for dry seed is quick and convenient, the latter seem to be more favorable for most cases.

5. Prediction and genetic expression of the band patterns of F_1 hybrid of *Panax ginseng*× *Panax quinquefolium*

Fig. 6 shows the electrophoregrams of *P.g* (017), *P.g* (017)×*P.q* (512), *P.q* (512)×*P.g* (071), *P.q* (512) and the electrophoregram from mixed extraction solutions of *P.g* (017)+*P.q* (512). The bands number of the F_1 is exactly equivalent to that of the common bands plus the specific bands of the two parents. The band patterns of reciprocal crosses are also exactly the same. It indicates that the number of electrophoretic bands

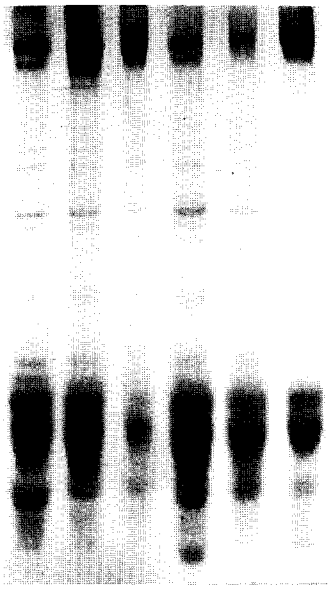

Fig. 5. Lactate-PAGE eletrophoregrams for dry seed and stratified seed of *P. ginseng*. 1, 4. Dry seed; 2, 5. Stratified seed embryo; 3, 6. Stratified whole kernel.

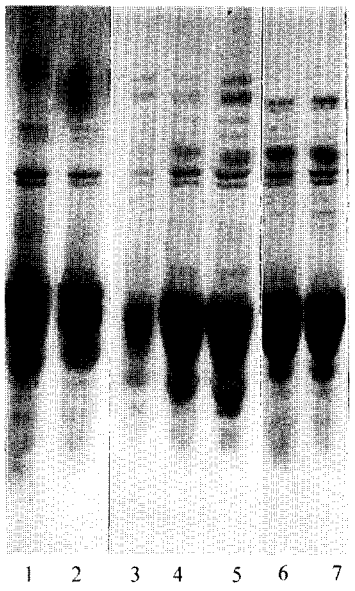

Fig. 6. Lactate-PAGE eletrophoregrams from *Panax ginseng* (071)×*P. quinquefolium* (512) hybrid F_1. 1, 2. *P.g* (071); 3. *P.g* (071)×*P.q* (5 12); 4. *P.q* (512)×*P.g* (071); 5. *P.g* (071)×*P.q* (512); 6,7. *P.q* (512).

could be an expression of genetic trait controlled by nuclear genes. It is also interesting that the electrophoregram of mechanically mixed extraction solutions of the two species were almost equal to that of their F_1 hybrid. Therefore, we could easily predict the F_1 electrophoregram from that of their two parents, and could also easily demonstrate the F_1 electrophoregram from that of the mixed extraction solutions of their parents.

Because of the high resolving power, relative good ability of genotype distinguishment and reproducibility, the inexpensive equipment and chemicals required, the relative simplicity, and time-saving in operation, the improved lactate-PAGE provides a new method for ginseng germplasm identification and probably be used in other research fields such as ginseng classification, evolution, etc..

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