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# Identification and purity testing of maize hybrids with one parent in common by ultrathin-layer isoelectric focusing of seed salt-soluble proteins

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**Abstract:** The objective of this study was to discriminate 11 maize hybrids with one parent in common using ultrathin-layer isoelectric focusing (UTLIEF) of seed salt-soluble proteins in 0.02% NaCl solution in the laboratory. Thirty-two protein bands were found in the pH range of 5.0-7.0, 62.5% of which were polymorphic. Six hybrids with the same male parent and 5 hybrids with the same female parent were differentiated by their patterns and numbers of polymorphic protein bands. The result showed that UTLIEF has the characteristics of high resolution to discriminate close genetic relationships. Meanwhile, the genetic purity of 11 hybrids were tested, and the purities for different hybrids were as follows: Shenyu10, 96%; Shenyu13, 96.7%; Shenyu16, 98%; Shenyu17, 99.3%; Shenyu18, 96%; Shenyu23, 96.7%; Shenyu21, 95.3%; Shenyu22, 97.3%; Shenyu29, 98.7%; Shenyu30, 100%; and Shenyu31, 98%.

**Key words:** Isoelectric focusing, maize, purity testing, salt-soluble proteins, ultrathin-layer

## Introduction

Maize (*Zea mays* L.) is one of the most extensively cultivated cereal crops on earth, and almost every country in the world cultivates maize commercially for a variety of uses. Maize has good heterosis potential for total yield, seed quality, disease resistance, and uniformity. Production and distribution of high-quality seeds is fundamental for potential crop yield. Genetic purity testing of seeds (i.e., the percentage of contamination by seeds or genetic material of other varieties or species) contributes to overall seed quality.

The conventional method of purity assessment is conducted in the field, based on morphological characters. However, replicated field observations are time-consuming and expensive. Moreover, the heavily genetically modified and globally abundant cultivation of maize has led to maize having a narrow genetic background. Morphology cannot provide information on the purity of specific genetic attributes that relate to the grain quality of varieties. There have been many reports on seed-lot purity testing in maize using biochemical assays, including Zein-IEF (Curtis et al. 1986), isozymes (Evola et al.

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1986; Orman et al. 1991), and protein polyacrylamide gel electrophoresis (PAGE) (Song et al. 1993). Assays involving polymerase chain reaction (PCR)-based DNA markers exhibit great potential for cultivar identification and seed quality control in various crops (Gethi et al. 2002; Mongkolporn et al. 2004; Dongre et al. 2005; Liu et al. 2007).

However, these procedures may be limited by factors such as their high data point cost, experimental conditions, and tissue types. When compared with PAGE, isozymes, and PCR-based DNA marker tests, ultrathin-layer isoelectric focusing (UTLIEF) has obvious advantages in terms of electrophoretogram resolution, a large sample size, the electrophoresis reagent, and electrophoretic velocity. Analysis of isozyme diversities has been used in a variety of identifications, but it proves difficult to find diversities when there are close genetic relationships, and there is the possibility of unstable results when using this method. Furthermore, the DNA technique based on PCR is characterized by high cost and a complex experimental procedure, which is currently hard to apply in large-scale and commercial seed quality testing.

This study was carried out to evaluate the ability of UTLIEF to distinguish maize hybrids with one parent in common, in order to apply the techniques to the evaluation of genetic purity in maize hybrids.

## Materials and methods

The seeds of 11 maize hybrids and their parents were kindly provided by the Maize Science Institute, Shenyang Academy of Agricultural Sciences, Shenyang, China (Table 1).

To identify the hybrids, 5 seeds of each hybrid were randomly chosen with 3 replications. For the genetic purity testing, 50 seeds of each hybrid were randomly chosen with 3 replications. Purity was defined formulas follows: (total number of tested seeds – different varieties of seeds) / total number of tested seeds ×100.

Individual whole seeds were crushed using a grinder and put into 1.5-mL centrifuge tubes. Each centrifuge tube was filled with 320 µL of 0.02% (w/v) NaCl solution and left at room temperature for 1-1.5 h for protein extraction, then centrifuged for 10 min at 10,000 rpm.

UTLIEF gels were cast on polyester support films (GEL-FIX, SERVA, Germany) as described by Radola (1980). Each gel, measuring 180 × 260 mm, was adjusted to a final concentration of 0.8 g of urea, 0.16 g of taurine, 5 mL of acrylamide, 0.22 mL of ampholytes (pH 5-7), 4 µL of N,N,N',N'-tetramethylethylenediamine, and 30 µL of 20% (w/v) ammonium peroxydisulfate. Adhesive tape (0.12 mm) was used as the spacer along the long

Table 1. List of maize hybrids and their parents.

| Hybrid | | Female parent | Male parent |
|---|---|---|---|
| Hybrids with the same male parent | Shenyu10 | Q1261 | Shen137 |
| | Shenyu13 | Shen7-004 | Shen137 |
| | Shenyu16 | K12 | Shen137 |
| | Shenyu17 | Shen151 | Shen137 |
| | Shenyu18 | Shen152 | Shen137 |
| | Shenyu23 | Shen501 | Shen137 |
| Hybrids with the same female parent | Shenyu21 | Shen3336 | Shen3265 |
| | Shenyu22 | Shen3336 | Shen3195 |
| | Shenyu29 | Shen3336 | Shen3267 |
| | Shenyu30 | Shen3336 | Dan340 |
| | Shenyu31 | Shen3336 | Shen3115 |

sides of the cover glass plates. An aliquot of 25 μL of supernatant from each centrifuge tube was pipetted onto the gels and electrophoresis was carried out on a horizontal electrophoresis unit connected to a cooling apparatus at 4 °C. Electrophoresis was first carried out at 200 V for 30 min, followed by up to 1000 V for 90 min. The composition of the positive buffer for the electrophoresis was 0.332% aspartic acid and 0.368% glutamate. The composition of the negative buffer for the electrophoresis was 0.472% arginine, 0.364% lysine, and 12% ethylenediamine. The gel was then stained for 50 min in a staining solution, the composition of which was 0.015% Coomassie Brilliant Blue R250, 0.045% Coomassie Brilliant Blue, 11% acetate, and 18% ethanol. Next, it was destained for 20 min in a decoloration solution, the composition of which was 5% acetate and 30% ethanol (Wang et al. 2000).

According to the characteristics of band color and band pattern, the polymorphic protein bands of UTLIEF were divided into 3 types of band pattern: band pattern A had the deepest colored and the widest bands; band pattern B had lighter colored, medium-width bands; and band pattern C only showed fuzzy traces of the bands.

## Results

### UTLIEF of seed salt-soluble proteins from 11 tested maize hybrids

Whole seeds were used to characterize polymorphic protein band differences among 11 maize hybrids. Different pH ranges of SERVALYT (SERVA) were tested, and pH 5.0-7.0 gave the best results (data not shown). The UTLIEF (pH 5.0-7.0) profiles of salt-soluble proteins from 11 tested maize hybrids are shown in Figures 1a and 1b. For each hybrid, 32 protein bands were identified, 20 (62.5%) of which were polymorphic. Almost all of the hybrids had an average of 30 bands, and most of the bands were of band pattern A.

Diagrammatic representations based on different migrations and the presence or absence of salt-soluble protein bands from the 11 tested maize hybrids are shown in Figures 2 and 3. In Figure 2, 6 hybrids with the same male parents were divided into 3 groups based on the number of bands of pattern A located in areas β, γ and δ. Group 1 contained Shenyu18 with 5 bands of pattern of A; group 2 contained Shenyu10, Shenyu13, and Shenyu17 with 4 bands of pattern A; and group 3 contained Shenyu16 and Shenyu23

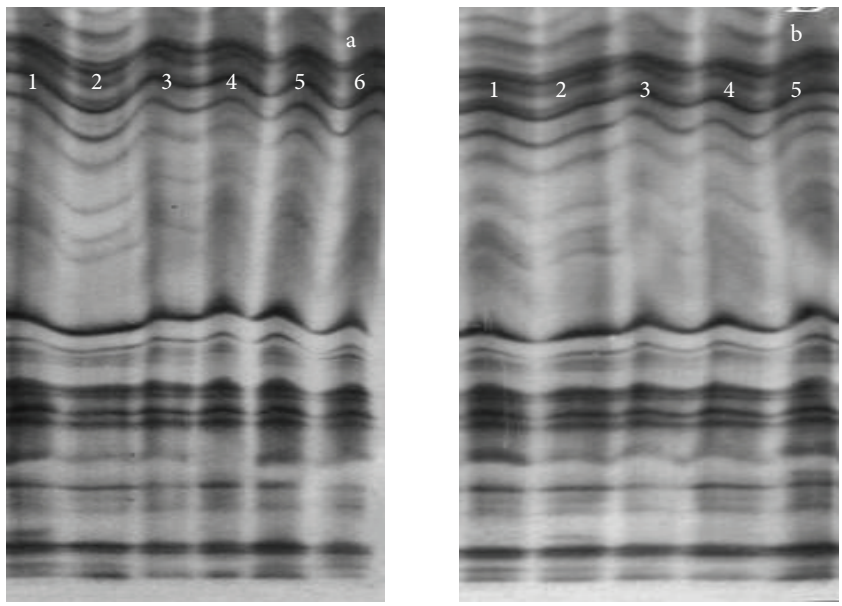


Figure 1. UTLIEF (pH 5-7) profiles of salt-soluble proteins from 11 tested maize hybrids: a) numbers 1-6 are Shenyu10, Shenyu13, Shenyu16, Shenyu17, Shenyu18 and Shenyu23, respectively; b) numbers 1-5 are Shenyu21, Shenyu22, Shenyu29, Shenyu30 and Shenyu31, respectively.

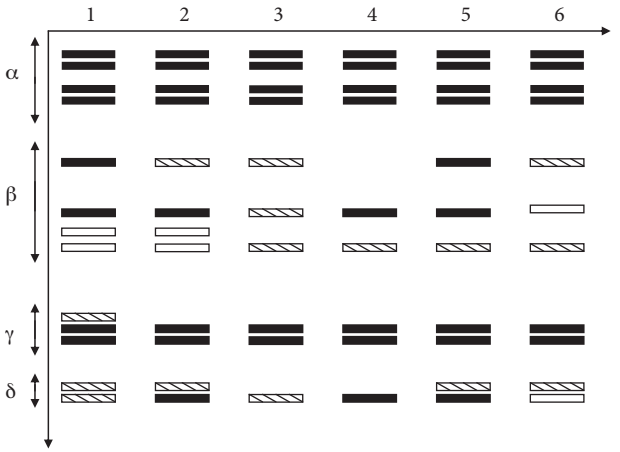


Figure 2. A diagrammatic representation of salt-soluble proteins from 6 maize hybrids with the same male parents. Numbers 1-6 are Shenyu10, Shenyu13, Shenyu16, Shenyu17, Shenyu18 and Shenyu23, respectively.

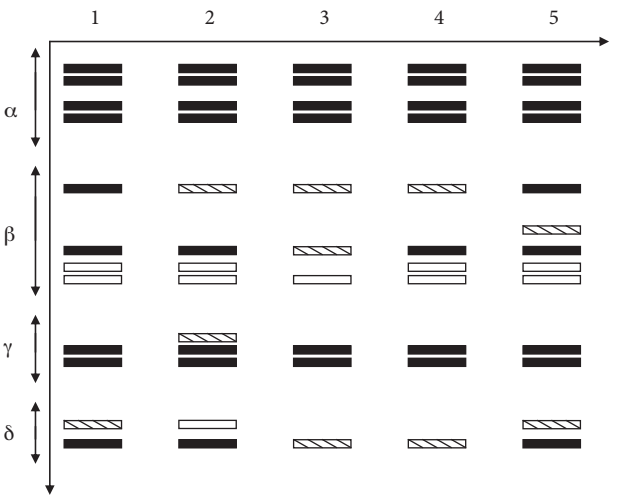


Figure 3. A diagrammatic representation of salt-soluble proteins from 5 maize hybrids with the same female parents. Numbers 1-5 are Shenyu21, Shenyu22, Shenyu29, Shenyu30, and Shenyu31 respectively.

with 2 bands of pattern A. Among these, Shenyu18 was clearly distinguishable from the other 5 hybrids solely based on the number of bands of pattern A. Shenyu10 had 2 bands of pattern A located in areas in β and γ, respectively, while both Shenyu13 and Shenyu17 had 4 bands of pattern A, 2 located in area γ, 1 located in area β, and 1 located in area δ, but they had different numbers of band pattern B. For Shenyu16 and Shenyu23, although both of them had 2 bands of pattern A in area γ, Shenyu16 had 4 bands of pattern B, 3 located in area β and 1 located in area δ, while Shenyu23 had 3 bands of pattern B, 2 located in area β and 1 located in area δ. Based on the above analysis, 6 hybrids could be individually identified. Five hybrids with the same female parents were divided into 4 groups based on the number of bands of pattern A located in areas β, γ and δ. Group 1 contained Shenyu21 and Shenyu31 with 5 bands of pattern A, group 2 contained Shenyu22 with 4 bands of pattern A, group 3 contained Shenyu30 with 3 bands of pattern A, and group 4 contained Shenyu29 with 2 bands of pattern A. Among these, Shenyu22, Shenyu29, and Shenyu30 can be easily distinguished from each other and from Shenyu21 and Shenyu31 solely based on the number of bands of pattern A. In regard to Shenyu21 and Shenyu31, Shenyu21 had only 1 band of pattern B in area δ, while Shenyu31 had 1 band of pattern B in area β and 1 in area δ. Therefore, 5 hybrids could be individually discriminated from each other. These results show that UTLIEF can be used to discriminate maize hybrids according to different migrations and the presence or absence of salt-soluble protein bands, because each hybrid has its own unique salt-soluble protein 'fingerprint'.

**Genetic purity of 11 tested maize hybrids determined by UTLIEF**

The 11 tested maize hybrids and their parents used for UTLIEF analysis are shown in Table 1. As examples, the UTLIEF of the salt-soluble proteins of single seeds of Shenyu16 and Shenyu23 are shown in Figures 4 and 5, respectively.

As shown in Figure 4, the band in the ninth panel lane is obviously different from the others, so it could have been a mixed seed, and the genetic purity of Shenyu16 was 98% for all replications (Table 2). Therefore, the final genetic purity of Shenyu16 was 98% (Table 2). In Figure 5, the 10th band was different from the others, and the genetic purity of Shenyu23 was 98% for 1 replication, but the other 2 replications were 96% (Table 2). The final genetic purity of Shenyu23 was 96.7% (Table 2). Using the same rule, the genetic purities of the other 9 hybrids were calculated and are listed in Table 2.

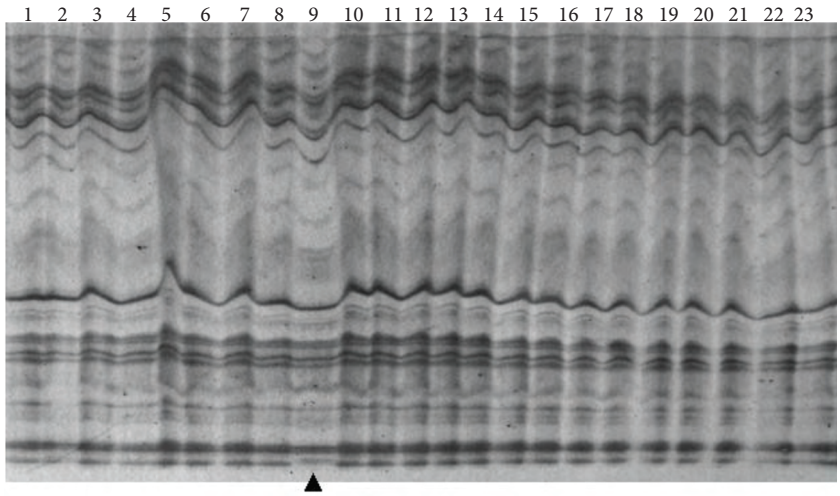


Figure 4. UTLIEF profile of the salt-soluble proteins from parts of Shenyu16 seeds. ▲ is mixed seeds, and 1-23 are parts of single Shenyu16 seeds.

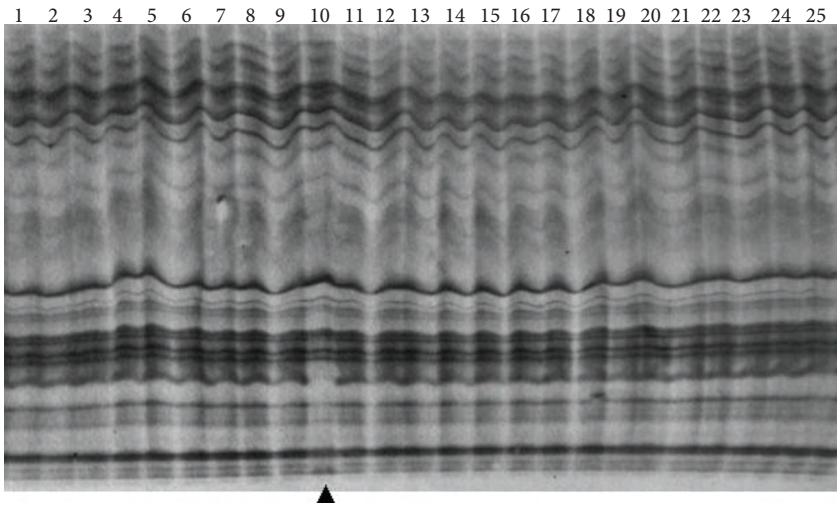


Figure 5. UTLIEF profile of the salt-soluble proteins from parts of Shenyu23 seeds. ▲ is mixed seeds, and 1-25 are parts of single Shenyu23 seeds.

## Discussion

Every year, public and private breeders in China release many improved maize varieties, which raises challenges in variety identification and the genetic purity testing of maize hybrids due to their having a narrow genetic background, especially for hybrids with close genetic relationships (i.e., one parent in common). It is necessary to set up a fast, economical, and effective system for testing the purity in order to prevent impure seeds from entering the market.

UTLIEF electrophoresis has been proposed as an additional method to certify genetic seed purity, and has also been applied in some crops (Lucchese et al. 1999; Wang et al. 2001; Zhao et al. 2005; Yan et al. 2006; Liu et al. 2007). In the present study, improved UTLIEF was employed to discriminate 11 maize hybrids cultivated in the northeast of China with 1 parent in common, and it delivered repeatable, stable results through replications. Among the 32 protein bands, 20 were polymorphic, and 11 tested maize

Table 2. The genetic purity of tested maize hybrids.

| Variety | Replication | Genetic purity (%) | Average genetic purity (%) |
|---|---|---|---|
| Shenyu10 | 1 | 98% | 96% |
| | 2 | 96% | |
| | 3 | 98% | |
| Shenyu13 | 1 | 98% | 96.7% |
| | 2 | 96% | |
| | 3 | 98% | |
| Shenyu16 | 1 | 98% | 98% |
| | 2 | 98% | |
| | 3 | 98% | |
| Shenyu17 | 1 | 98% | 99.3% |
| | 2 | 100% | |
| | 3 | 100% | |
| Shenyu18 | 1 | 98% | 96% |
| | 2 | 96% | |
| | 3 | 94% | |
| Shenyu23 | 1 | 98% | 96.7% |
| | 2 | 96% | |
| | 3 | 96% | |
| Shenyu21 | 1 | 96% | 95.3% |
| | 2 | 96% | |
| | 3 | 94% | |
| Shenyu22 | 1 | 98% | 97.3% |
| | 2 | 96% | |
| | 3 | 98% | |
| Shenyu29 | 1 | 98% | 98.7% |
| | 2 | 100% | |
| | 3 | 98% | |
| Shenyu30 | 1 | 100% | 100% |
| | 2 | 100% | |
| | 3 | 100% | |
| Shenyu31 | 1 | 98% | 98% |
| | 2 | 96% | |
| | 3 | 100% | |

hybrids exhibited specific protein bands. Most of the UTLIEF profiles of salt-soluble proteins of single seeds showed different variations at the protein level, which may distinguish maize hybrids. Some of the hybrids with no differences among the main protein bands, which were bands of pattern A, could be discriminated by observation of the secondary or auxiliary bands with band pattern B or band pattern C.

In conclusion, this study showed that UTLIEF profiles of salt-soluble proteins of single seeds are highly efficient and reproducible for the genetic purity testing of commercial maize hybrids.

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