Middle-East Journal of Scientific Research 6 (4): 382-385, 2010
ISSN 1990-9233

Identification of Seed Purity of Maize Hybrids by Ultrathin-Layer Isoelectric Focusing Electrophoresis of Seed Salt-Soluble Proteins

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Abstract: Ultrathin-layer isoelectric focusing electrophoresis (UTLIEF) technique was used to identify seed purity of 5 maize hybrids, widely grown in Northeast China. The electropherogram of seed salt-soluble proteins showed high resolving power, good repeatability and 66.7% of 10-dozens of bands were polymorphic. Based on analysis of seeds, the 5 tested maize hybrids owned the feature bands of two parents, suggested that they were all true hybrid seeds. The purity of Shenyu 17: 96.7%, Shenyu 20: 95.3%, Shenyu 24: 97.3%, Danyu 39: 96.7% and Zhengdan 958: 98.7%, respectively.

Key words: Maize · Seed purity · Ultrathin-layer isoelectric focusing · Salt-soluble protein

INTRODUCTION

Electrophoresis is used to separate and identify seed proteins based on differences of crop genotypes. Ultrathin-layer isoelectric focusing electrophoresis (UTLFE) technology, was developed by Radola [1] and improved by some other foreign laboratories. Comparing with traditional electrophoresis technology, UTLIEF has obvious advantages in map resolution, electrophoresis duration, reagent consumption and number of detected samples one time, which has been used in many seed testing laboratories and was written in the 'International Rules for Seed Testing'. UTLIEF has used in rice (*Oryza sativa*) [2-4], barley (*Hordeum vulgare* L.) [5], pepper (*Capsicum frutescen*) [6] and oat (*Avena sativa* L.) [7].

Maize (*Zea mays* L.) is one of the most important cereal crops in China, of which production and quality levels play a crucial role in national economic development. Hybrid maize accounts for 90% in Chinese maize production, but seed purity is only about 90%. For this reason, the national maize production reduces 4 billion kilograms annually. Meanwhile, the controversial cases involving crop variety ownership has increased. It is urgent need to establish a quick, effective, simple and economical system to identify maize varieties and genetic purity for standardizing maize seed market and promoting the development of maize industry. In China, the identification of maize variety and genetic purity was focused on isozyme polyacrylamide gel electrophoresis or seed salt-soluble proteins polyacrylamide gel electrophoresis, there has been no reports on maize seed salt-soluble proteins by UTLIEF. Therefore, the feasibility and effectiveness of variety identification and genetic purity testing on 5 Northeast China maize hybrids using seed salt-soluble protein by UTLIEF were studied in this paper for providing methods of hybrid maize varieties identification and reference of evaluation and utilization of maize germplasm resources.

MATERIALS AND METHODS

Plant Materials: The seeds of 5 maize hybrids and their parents were kindly provided by Maize Science Institute, Shenyang Academy of Agricultural Sciences, Shenyang, China (Table 1).

Ultrathin-layer Isoelectric Focusing: Seeds of five hybrids were crushed and put in 1.5 mL centrifuge tube. Each centrifuge tube was filled with 320μl 0.02% (w/v) NaCl solution to extract proteins. After 1-1.5 h at room

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Table 1: Five tested maize hybrids and parents

Hybrid	Parent	Validation time
Shenyu17	Shen151/Shen137	2001
Shenyu20	Shen151/Shen139	2004
Shenyu24	Shen502/Shen503	2007
Danyu39	C8605-2/Dan598	2001
Zhengdan958	Zheng58/Chang72	2000

temperature, the centrifuge tube was vibrated for about 1 min and then centrifuged for 10 min at 10000 r/min. For the identification of varieties, 5 seeds of each hybrid were randomly chosen with two replications. For the genetic purity testing, 50 seeds of each hybrid were randomly chosen with three replications.

UTLIEF gels were cast on polyester support films (Gel-Fix, GE) as described by Radola [1]. Each gel was made to a final concentration of 0.8 g urea, 0.16 g taurine, 5 ml acrylamide (T=6.8%, C=2.5%), 0.22 ml of pH 5-7 ampholytes, 4 µl N N N'N'-tetramethylethylenediamine and 30 µl of 20% (w/v) ammonium peroxydisulphate. Adhesive tape (0.12mm) was used as the spacer along the long sides of a cover glass plate. 25µl 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□ following Zhao *et al* [4].

Statistic Analysis of UTLIEF Results: Purity = (total number of tested seeds–the number of different variety seeds) /total number of tested seeds¡Á100%.

RESULTS AND DISCUSSION

Ampholyte Selection: The key of variety identification by UTLIEF is to choose pH range of ampholytes to detect different materials. Each protein has its own charge and a group of proteins may have positive charges or negative charges, which lead to come together at certain range of pH values. If charges come together too much, the protein bands may focus densely and not easy to distinguish, if too little, the differences between protein bands cannot be fully demonstrated. In this experiment, broad range of pH3-10 was chosen and the differences of the bands were found mainly in the range of pH 5-7.

Variety Identification of 5 Maize Hybrids: UTLIEF profile of salt-soluble proteins from seeds of 5 tested maize hybrids and 9 inbred lines was shown in Figure 1. Salt-soluble protein bands separated by improved UTLIEF have high resolution, inbred lines and hybrids all have their own unique salt-soluble protein bands and can be discriminated from each other by á, â and ã areas. In this study, the specific bands of Shenyu24, Danyu39 and Zhengdan958 (parents and F_1 as one group) are mainly located in ã area. Shenyu 17 and Shenyu 20 have the same female-parent and the main differences are located in â area.

Based on the UTLIEF profile, a real hybrid seed should have the characteristic protein bands of both parents. Seeds with female marker band (FMB) but no male marker bands (MMB) are considered to be 'false hybrids', whereas seeds with neither MMB nor FMB present are considered to be mixed varieties. For Zhengdan958 cross (Zheng58×Chang72), as shown in Figure 1, the male parent, Chang72, had a characteristic band MMB1, the female parent, Zheng58, had two characteristic bands FMB1 and FMB2 and MMB1, FMB1 and FMB2 were all present in Zhengdan958 (the F_1 generation), so they were real hybrid seeds. For Danyu39 cross, MMB1 was found to exist in the male parent, Dan598, FMB1 was found to exist in the female parent, c8605-2 and MMB1 and FMB1 were both present in Danyu39 and thus they were considered as real hybrids. For the same reason, Shenyu24 with the male parent three characteristic bands MMB1, MMB2, MMB3 and female parent characteristic band FMB1, Shenyu20 with MMB1, MMB2 and FMB1, Shenyu17 with MMB1 and FMB1, so they were also considered real hybrid seeds.

Genetic Purity Testing of 5 Maize Hybrids: Average genetic purity was obtained by calculating the average of 3 repeated purity following the genetic purity test. For examples, the salt-soluble protein UTLIEF profiles of Shenyu 17 and Shenyu 20 genetic purity testing were shown in Figure 2 and Figure 3, respectively. As shown in Figure 2, the band in the twenty-eighth panel lane was obviously different from others without the male parent characteristic band MMB1, it could be a mixed seed or self-pollination seed and the genetic purity of Shenyu17 was 98% for replication (Table 2), the other two replications were 96% and 96%, respectively. So the final genetic purity of Shenyu17 was 96.7% (Table 2). In Figure 3, the tenth and twenty-second panel lane had neither MMB1, MMB2 nor FMB1£¬and thus were different from others, it could be mixed seeds, the genetic purity of Shenyu20 was 96% for replication□, the other two replications were 96% and 94%, respectively (Table 2). The final genetic purity of Shenyu20 was 95.3% (Table 2). By the same rule, the genetic purity of the other three hybrids were calculated and listed in Table 2.

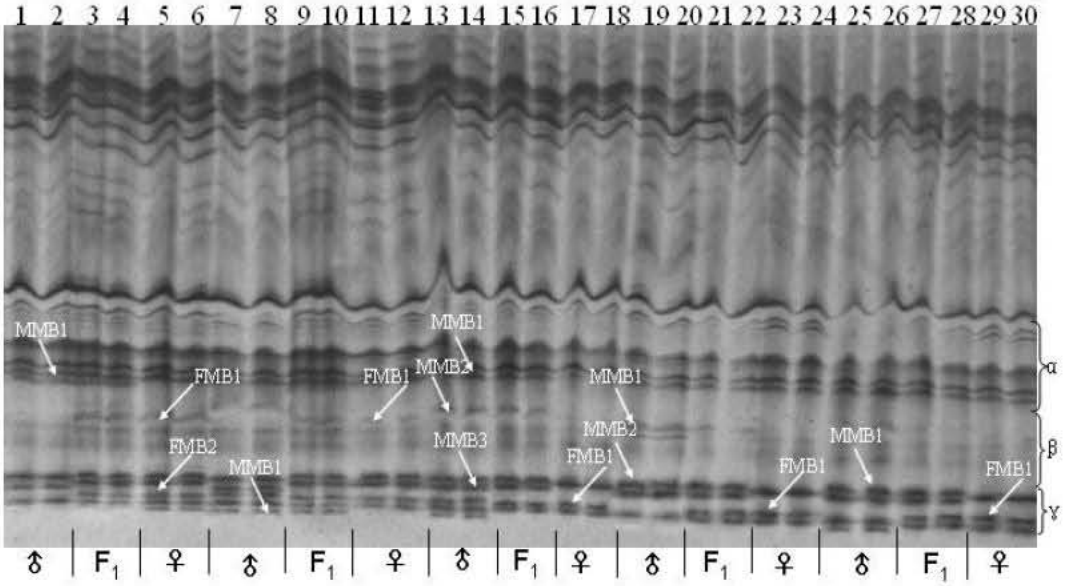

Fig. 1: The UTLIEF profile of 5 maize hybrids and their parents. Lane 1 and 2, Chang72 (two replications); lane 3 and 4, zhengdan958; lane 5 and 6, Zheng58; lane 7 and 8, Dan598; lane 9 and 10, Danyu39; lane 11 and 12, c8605-2; lane 13 and 14, Shen503; lane 11 and 12, Shenyu24; lane 13 and 14, Shen502; lane 15 and 16, Shen139; lane 17 and 18, Shenyu20; lane 19 and 20, Shen151; lane 20 and 21, Shen137; lane 21 and 22, Shenyu17; lane 23 and 24, Shen151; FMB represents female marker band, MMB represents male marker band

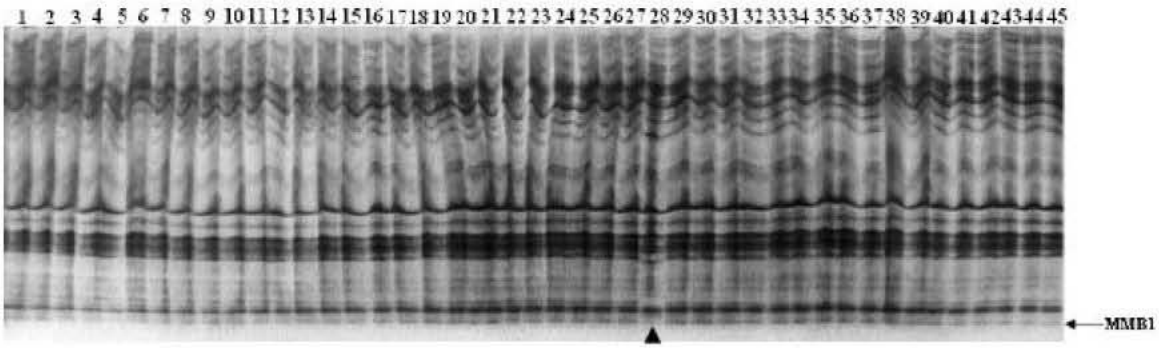

Fig. 2: The salt-soluble protein UTLIEF profile of genetic purity testing of Shenyu17. MMB1 represents male marker band, ▲ is the mixed seed or self-pollinated seed

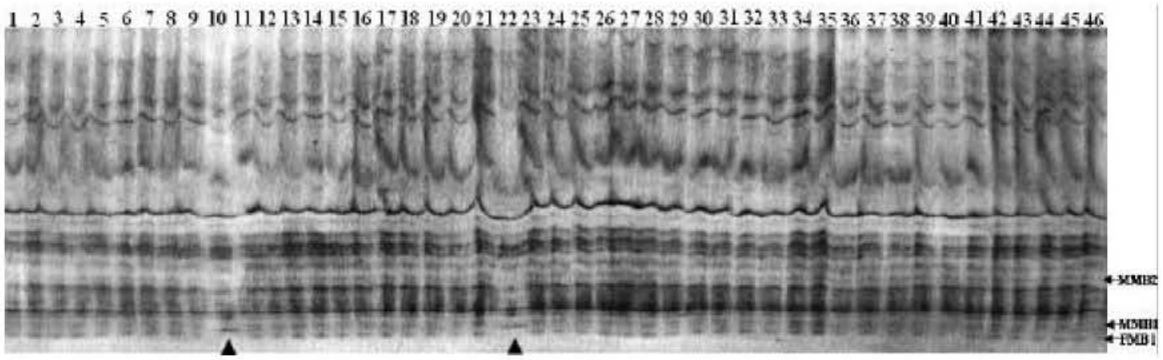

Fig. 3: The salt-soluble protein UTLIEF profile of genetic purity testing of Shenyu20. MMB1 and MMB2 represent male marker band, FMB1 represent female marker band, ▲ is the mixed seed

Table 2: The purity of 5 tested maize hybrids

Variety	Shenyu 17			Shenyu 20			Shenyu 24			Danyu 39			Zhengdan958		
Replication	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Genetic purity (%)	98	96	96	96	96	94	98	96	98	96	96	98	98	100	98
Average genetic purity (%)		96.7			95.3			97.3			96.7			98.7	

Genetic purity of maize hybrid seeds has been an important influence on seed quality. On the one hand, with the market competition increasing, the best way for seed companies to occupy the market and seek development is to own high quality seeds. On the other hand, farmers can not distinguish the real hybrid seeds from the false ones, neither the poor quality ones. If the false or poor quality seeds were put into production, the farmers' interests would be severe damaged due to maize yield reduction. Therefore, the rapid purity identification of maize hybrid seed has been received much concern.

In this study, an improved UTLIEF was used to identify 5 maize hybrids from Northeast China. The characteristic protein bands of 5 tested maize hybrids were clear enough to discriminate varieties accurately and effectively and the results of repeated tests were reliable. Five tested maize hybrids had characteristic bands from the parents based on analyzing the result of electrophoresis and were considered to be the real hybrid seeds. With the help of UTLIEF profiles, seed purity of 5 tested maize hybrids was also calculated. UTLIEF required neither strict conditions like isozyme electrophoresis, nor complex operations like molecular marker technique, which can be applied in most seed companies and laboratories in China for variety identification and genetic purity testing.

ACKNOWLEGEMENTS

This project was ?nancially supported by grants of Liaoning provincial educational department project (2009.1-2012.12), Shenyang scientific instrument project (2009.1-2012.12) and Shenyang basic research for application project (F10-205-1-24).

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