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Full Length Research Paper

Prolamin extraction from high polyphenol seeds of sorghum (*Sorghum* spp.) and species discrimination and varieties identification by ultrathin-layer isoelectric focusing

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The seeds of sorghum (*Sorghum* spp.) are rich in polyphenols, which cause protein precipitation and coloration of extracts. Because of this precipitation, proteins in seeds of sorghum cannot be effectively extracted using common methods. Here we report two modified methods for the seed protein extraction. The result showed that the method in which the samples were pretreated by polyvinylpyrrolidone (PVPP) before protein extraction (modified method 2) got more prolamine content and can be used for later ultrathin layer isoelectric focusing electrophoresis (UTLIEF) analysis. Seed prolamin variation between two sorghum (*Sorghum* spp.) species included twelve sorghum varieties and eight sudangrass varieties were analyzed by UTLIEF. The result showed that obvious polymorphisms within and among varieties can be revealed through electrophoresis of sorghum single seed sample. Meanwhile, polymorphisms within species and varieties are concealed when sample was bulk which contains more than 100 seeds. By means of visual evaluation of the presence or absence of the special region bands of electrophogram, sorghum and sudangrass can be discriminated from each other. Furthermore, 10 of 12 sorghum varieties or lines and six of eight sudangrass varieties can be identified successfully according to the unique banding patterns in the discriminated regions which Rf was 0.314 to 0.332 and 0.605 to 0.826, respectively.

Key word: Sorghum, species discrimination, variety identification, ultrathin layer isoelectric focusing electrophoresis.

INTRODUCTION

Ultrathin layer isoelectric focusing electrophoresis (UTLIEF) has been investigated and used as a valid tool for seed purity test, variety distinction and variety registration by many researchers (Van den Berg, 1990, 1991; Hahn and SchÖberlein, 1999a,b; Wang et al., 2001; Zhao et al., 2005; Pashkoulov et al., 2000). It also plays an important role in seed circulation and trade. UTLIEF method for variety identification has been standardized by ISTA for many cereal species such as rice and sunflower, etc (ISTA, 2005).

Genus sorghum (*Sorghum* spp.) is annual or perennial tropical and subtropical cereal grasses. It has many species and most of have high economic value, like grain sorghum (*Sorghum bicolor*), sudangrass (*S. sudanense*) and their hybrid (*Sorghum bicolor*, *S. sudanense*). Grain sorghum ranks fifth in worldwide production of cereal grains (Dendy, 1995) and it is not only used as human food but also as animal feed (Zhu et al., 2003). At the same time, much interest has been focused on the use of sorghum as a renewable resource for bioindustrial applications, such as ethanol production (Weng et al., 2007). Sudangrass and sorghum-sudangrass hybrid are all high quality forages and can be used as both a feed and an energy crop (Pedersen et al., 1998). Sorghum grows throughout the world and has abundant germplasm that

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Table1. Names, sources and physical descriptions of the tested samples.

Species	Variety/lines name	Polyphenol content (%)	Sources
Sudangrass	Xinsu no.2	3.024	Xinjiang Agricultural University
	Qitai	3.248	Qitai Grassland Station, Xingjiang
	Yanchi	1.832	Yanch i Experimental Station, N ingxia
	Wulate no.1	2.431	WulateQianqi SeedFarm,InnerMongolia
	Ningnong	1.564	Ningxia Agricultural College
	Piper	2.002	Forage Seed Laboratory of CAU
	Unknow1	1.917	Forage Seed Laboratory of CAU
	Unknow2	1.816	Forage Seed Laboratory of CAU
Sorghum	Liaoza no.11	1.465	Institute of Agricultural Science in Liaoning
	Liaoza no.12	1.554	Institute of Agricultural Science in Liaoning
	Liaoza no.15	0.527	Institute of Agricultural Science in Liaoning
	Liaoza no.14	0.947	Institute of Agricultural Science in Liaoning
	Jinliang no.5	1.229	Institute of Agricultural Science in Liaoning
	Jinza no.18	1.125	Institute of Agricultural Science in Shanxi
	623A	0.986	Institute of Agricultural Science in Shanxi
	Kang 4	0.730	Institute of Agricultural Science in Shanxi
	Changliang no.2	1.100	Institute of Agricultural Science in Gansu
	Super silezo	1.536	Forage Seed Laboratory of CAU
	Yuantian no.1	1.795	Forage Seed Laboratory of CAU
	AR3009	1.580	Forage Seed Laboratory of CAU

amounts to about a hundred thousand collections in the world (Cao et al., 2004). Thus, seed purity and variety reliable identification are very important for sorghum seed breeding, production and trading (Qian and Yang, 2003). However, there are only few reports on the use of electrophoretic procedure for distinguishing sorghum varieties and lines (Chauhan et al., 2002; Eswara-Reddy and Jacobs, 2002). In addition, the variety variability in kafirins which is prolamine fractions of sorghum which accounts for 50 - 60% of total endosperm storage protein is not studied. We suppose the major reason may be a reliable result can not be gotten due to the interference of the polyphenol in sorghum grains with seed storage protein extraction and their separation by electrophoresis. The aims of the present study were to establish a UTLIEF method and to test whether UTLIEF provides kafirin polymorphism useful for the identification of a wide range of *sorghum spp.* Samples, and to examine the potential of UTLIEF for variety identification of *sorghum spp.* by means of analyzing of bulked seed samples and single seeds.

MATERIALS AND METHODS

Materials

Eight sudangrass varieties/lines and twelve sorghum varieties/lines were chosen that represent the most widely cultivated varieties in China. The names, sources and physical descriptions of the tested materials are summarized in Table 1. The materials were collected and stored at -30°C for later analysis.

Sample preparation

Bulked seed samples were ground to a fine meal in an electric mill (produced by Amersham) until all material passed through a 20-mesh screen. Single seeds were cut into small pieces with a scalpel and placed in 1.5 ml Eppendorf centrifuge tubes.

Routine method for protein extraction

Seed storage protein was extracted by adding protein extraction buffer which is glycol solution contained 6% urea (1 mol/L) and 1% 2- mercaptoethanol (sample: extractions = 1 g/10 mL). The samples were mixed well and left for at least two hours at 4°C. After 30 s ultrasonic treatment of the centrifuge tubes, the sample were centrifuged for 10 min at 10000*g at 0 - 4°C. The supernatant was then stored at 4°C until used for isoelectric focusing.

Modified method for protein extraction

Certain amount of polyvinylpyrrolidone (PVPP) was added into the routine protein extraction (modified method 1) or the samples were pretreated by PVPP before protein extraction (modified method 2). Amount of PVPP was listed in Table 2. Other steps were same to the routine method.

Protein content assay

Coomassie G 250 method (Bradford, 1976) was chosen for protein

Table 2. The amount of PVPP added into different modified method.

Level	Amount of PVPP	
	Modified method 1 (PVPP: Solution, g/L)	Modified method 2 (PVPP: Sample, g/g)
1	1	0.5
2	5	1
3	10	2

content test in this experiment.

Isoelectric focusing

Ultrathin slab gels which are 0.15 mm thick were prepared between two glass plates using a flap technique and gels were polymerized onto a carrier sheet which name is Gel-Fix for PAGE. Prior to use, the gel side of the glass plate must be treated with Gel-Slick. Gels were 18 cm wide by 24 cm long. The polymerization solution for 10gels contained 16 g urea, 50 mL acrylamide (T = 6.8%, C = 2.5%), 4.4 mL ampholyte (Amersham) pH 2 - 9, 50 µL N N N'N'-tetramethylethyenediamine and 350 µL of 20% (w/v) ammonium peroxydisulfate. 6.5 mL of gel solution are required for one gel and poured it on the carrier sheet which put on the glass, the flap glass (the other glass) was covered with the carrier sheet. The gels were polymerized for at least 45 min at room temperature.

Isoelectric focusing was carried out by means of an electrophoresis chamber which has a cooling plate that holding a temperature of 10°C. The electrophoresis adopted the double focusing method, which means two anodic electrode wicks were soaked in anode buffer containing 0.33% (w/v) L-asparagin acid and 0.37% (w/v) L-glutamin acid and then placed at the top and at the bottom of the gel. One cathodic electrode wick was soaked in cathode buffer containing 0.47% (w/v) L-arginin, 0.36% (w/v) L-lysin and 12% (v/v) ethylene-diamine and placed in the middle of the gel. The application strips (52 wells) ware positioned 1.5 cm away from the anodes and samples of 15 µL were loaded into the wells of the application strips at each anodic end of the gel. Focusing was carried out for 75 min by means of a electrophoresis power supply (produced by Amersham, EPS3501) at increasing voltage from 200 V up to 2500 V at a maximum current of 28 mA and a maximum power of 100 W, at the same time, volt hours was set at 3000.

After focusing, the gel were fixed in 12% (w/v) trichloroacetic acid for 20 min, then stained in Coomassie Brilliant Blue solution containing 0.015% (w/v) Coomassie R 250 and 0.045% (w/v) Coomassie G 250, 11% (v/v) acetic acid, 18% (v/v) methanol and 71% (v/v) water for 30 - 50 min. Destain was carried out in a solution of 30% (v/v) methanol and 5% (v/v) glacial acetic acid until surplus color was removed and the background was clean. After rinsing with distilled water, the gels were dried at room temperature and can be stored for many years.

Evaluation of banding patterns

Banding patterns of bulked seed samples of the tested varieties were compared on the basis of differences in the presence/absence of bands and, in case of the same position of a certain band, also by means of their intensity. For banding pattern of single seeds, the frequencies of each band were determined for samples of 96 single seeds, to assess the variability within a variety.

RESULTS

Prolamin extraction and UTLIEF analysis of high polyphenol seeds of sudangrass and sorghum

623A, a sorghum variety which contain moderate polyphenol content was selected to evaluate validity of the modified methods. Protein contents were shown in Figure 1; only 1.246 mg/g protein was obtained by routine method. Two modified methods significantly enhanced protein content compared to routine method ($p < 0.05$). More amount of protein was extracted by modified method 2 than method 1 and levels 3 of modified method 2 could get highest amount of protein. Thus, modified method 2 in which the ratio of PVPP and sample was 2 (level 3) was selected for further analysis.

The effect of modified method on electrophoresis was shown in Figure 2. Few (two sorghum varieties) or no (one sudangrass variety) bands presented in the electrophogram for protein samples extracted by routine method. However, the electrophograms of the protein extracted by modified methods 1 and 2 were recognizable and distinct.

The comparison of electrophogram of bulked seed samples and single seed samples by UTLIEF

The sorghum variety of "623A" was selected to compare the electrophogram difference between bulked seed sample and single seed sample, and at the same time to research the banding pattern of single seeds to assess the variability within the variety.

For single seed analysis, 104 seeds of the variety were analyzed. The 10 of 104 seeds as an example was shown in Figure 3. Total of 14 differentiable bands were presented after electrophoresis and 11 bands were obvious polymorphisms between tested 104 seeds. The other 3 bands were presented in all tested caryopses and could be regard as the variety's characteristic bands.

The electrophogram of bulked seed samples were shown in Figure 4. Bulked seed sample which contain 10 seeds had variability between repetitions and could not character the variety's banding pattern. The variability between repetitions was decreased as the increase of the number of bulked seed samples. When the seed number of bulked samples was 200 caryopses, the variability between repetitions were disappeared. The result manifested that the seed bulked sample which contain 200 seeds can be selected as the identificated proof for variety identification.

Differentiation of the two *sorghum* species (sudangrass and sorghum) by UTLIEF

For species comparisons, all varieties (200 seeds/variety) of one species were pooled together and mixed well to

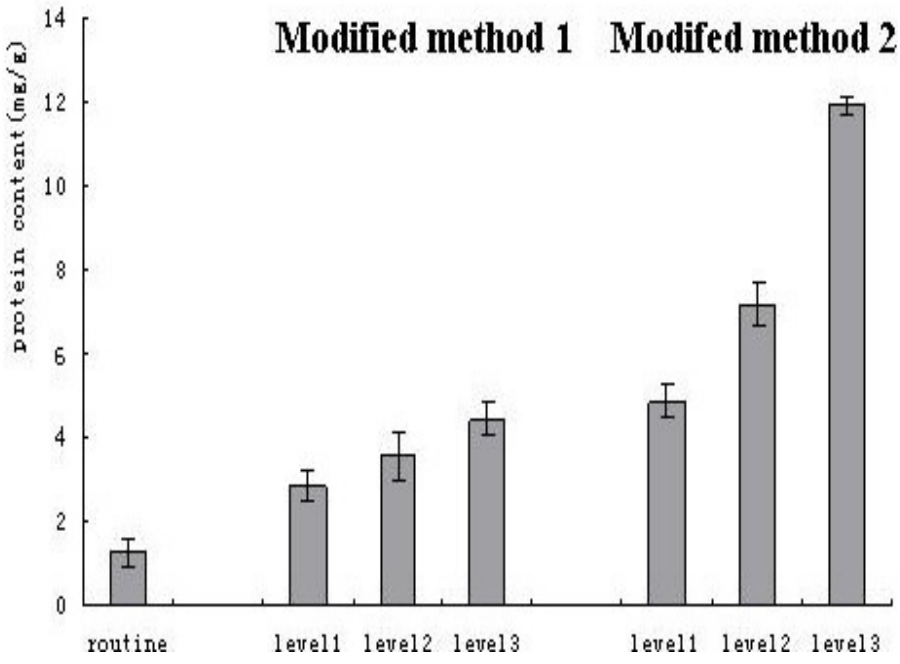

Figure 1. The comparison of protein contents which extracted by different methods.

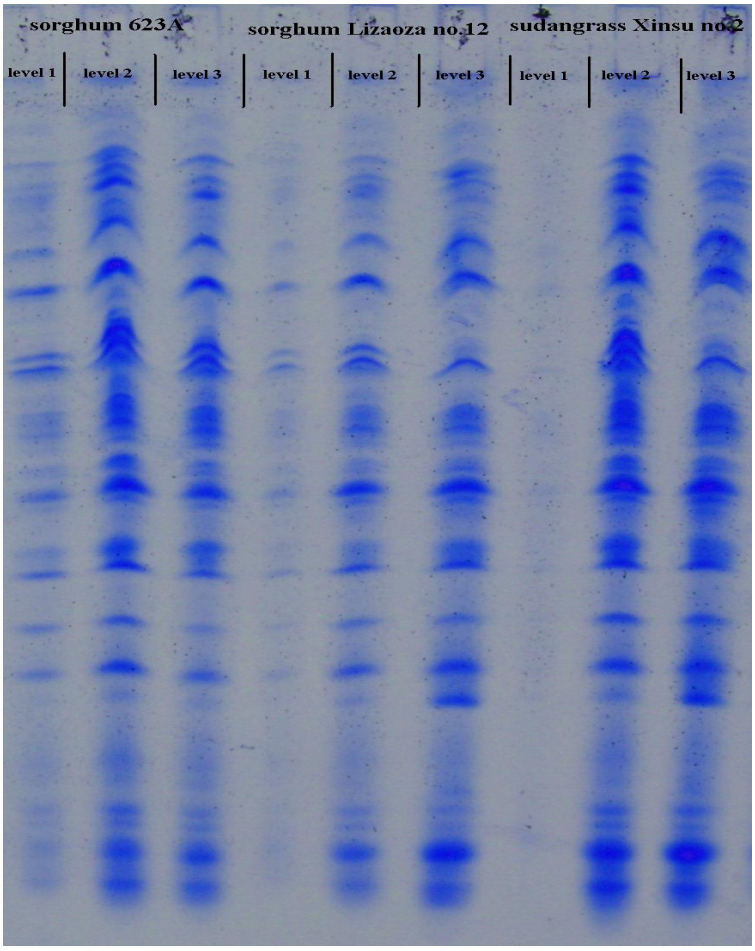

Figure 2. PVPP effect on ULTLIEF analysis.

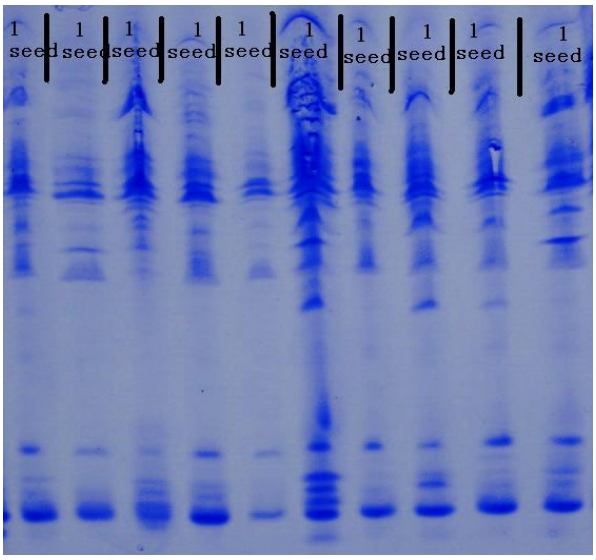

Figure 3. Electrophogram of single seed sample of "623A".

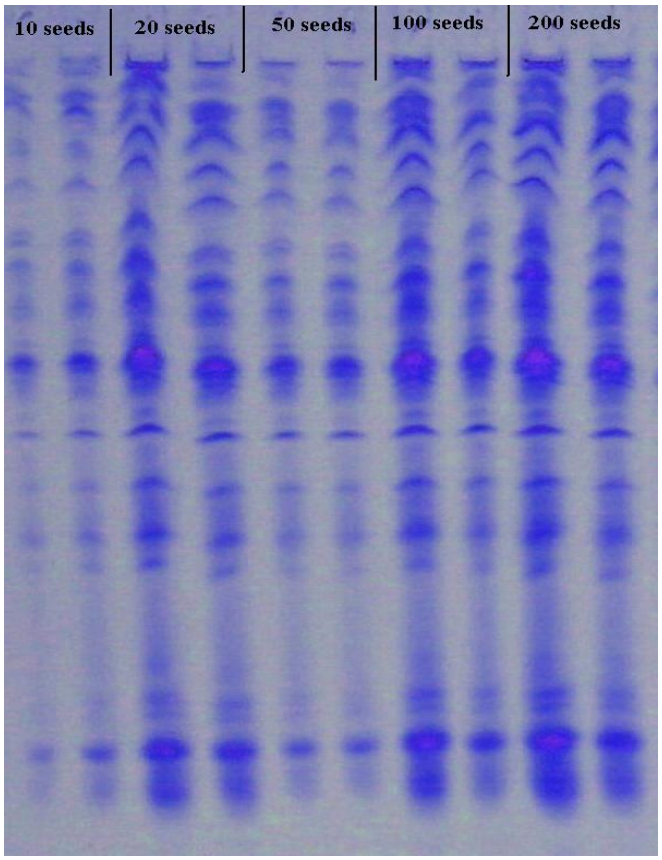

Figure 4. Electrophogram of bulked sample of "623A".

form bulk sample, thus, representative banding pattern of the species can be observed. Prolamin was extracted from triplicate of the bulk samples and stored at -30°C for later analysis.

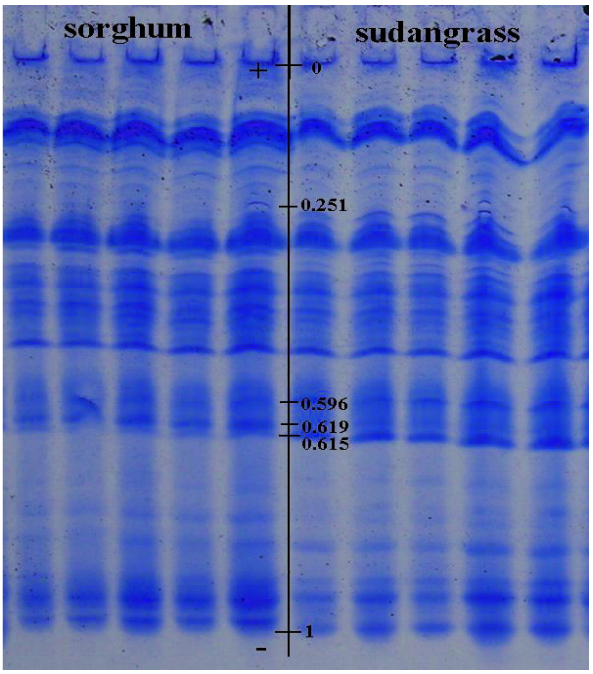

Figure 5. Species discrimination of sorghum (*Sorghum* spp.) by UTLIEF.

Figure 5 presents the UTLIEF binding patterns of two tested species. By means of visual evaluation of electrophogram, the presence or absence of the special region bands was criteria, sorghum and sudangrass can be differentiated form each other. For example, the two bands which Rf value was 0.307 and 0.619 were the characteristic bands of sorghum and absented in sudangrass. The two bands which Rf value was 0.596 and 0.628 were the characteristic bands for sudangrass and absented in sorghum.

Reliable discrimination and identification of twelve sorghum varieties and eight sudangrass varieties by UTLIEF

For varieties discrimination, the presence or absence of bands as well as band width and staining intensity were criteria. UTLIEF result of 12 sorghum varieties were shown in Figure 6. The region A with Rf was 0.314 to 0.332 and region B with Rf was 0.605 to 0.826 were main discriminated regions. According to double bands (arrow a and b in Figure 6), three different patterns appeared: (I) double bands in arrow a and double bands in arrow b (including Liaoza no.14, Liaoza no.15, Jinza no.18 and Changliang no.2); (II) double bands in arrow a and single band in arrow b (including Liaoza no.12, Jinliang no.5 and Yuantian no.1); (III) single band in arrow a and double band in arrow b (including Liaoza no.11, 623A, Kang4, Supersilezo and AR3009). Within each of these

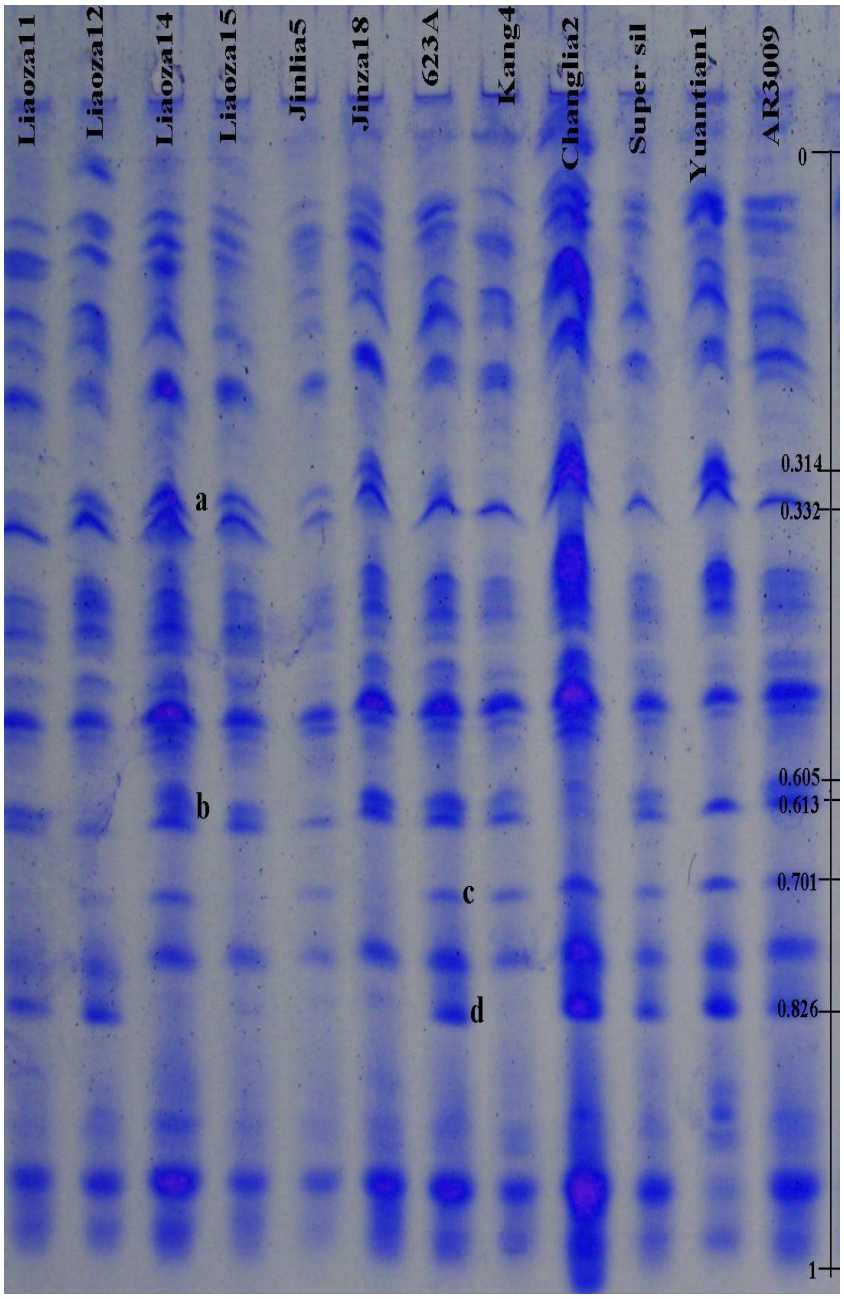

Figure 6. Varieties identification of grain sorghum (*Sorghum bicolor*) by UTLIEF.

three groups, visual evaluation was continued according to the bands in arrow c and d. 10 varieties and lines with unique banding patterns were detected. The remaining two varieties had same bands pattern.

Six of eight sudangrass varieties and lines had unique banding patterns according to discrimination criteria like arrow a, b, c and d in Figure 7. These six varieties/lines including: Xinsu no.2, Qitai, Wulate no.2, Ningnong, Piper and unknown 1. The other two varieties, Yanchi and Unknown 2 had similar banding pattern and could not be discriminated from each other.

DISCUSSION

All sudangrass varieties and mostly of sorghum varieties contain certain amount of polyphenol in grain. The protein extraction is very important in protein and enzyme analysis and is the basic step. However, owing to the limit of technology, the protein step is not perfect and need to be improved, especially for protein sample preparation (Lan et al., 2001). Polyphenols are secondary plant metabolites and existed extensively in nature. Sorghum is a good source of polyphenol which mainly present as

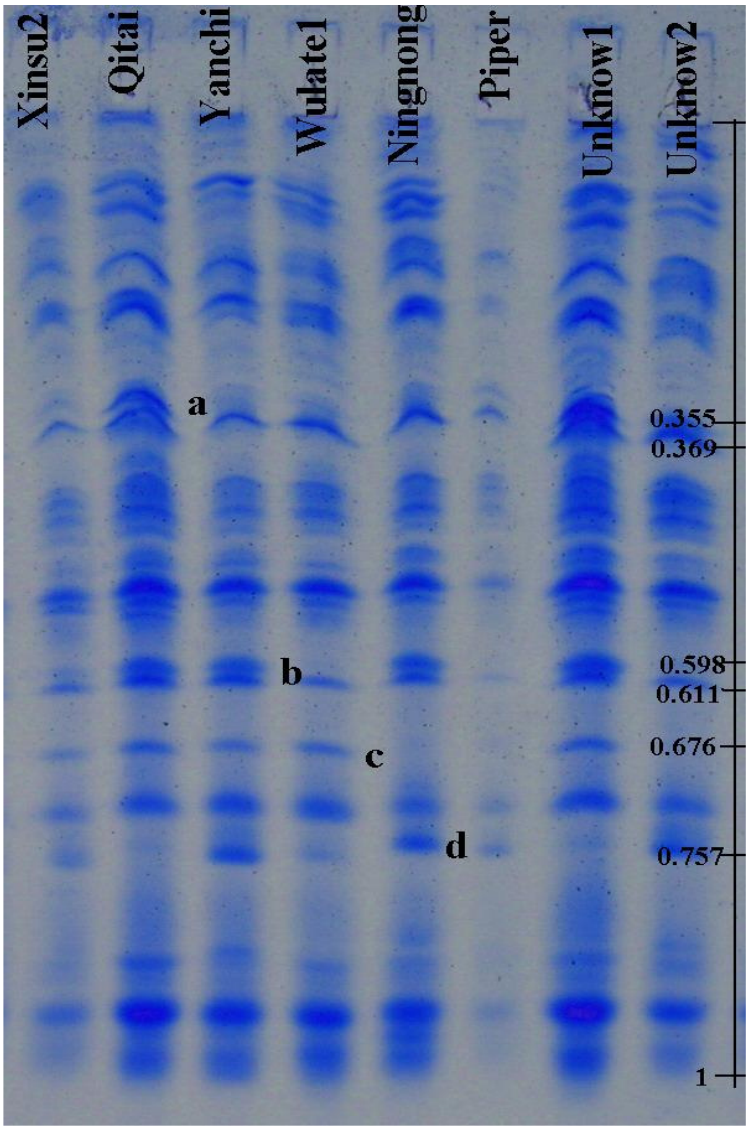

Figure 7. Varieties identification of sudangrass (*S. sudanese*) by UTLIEF.

phenolic acids, flavonoids and tannins. These polyphenols can react with biomacromolecule like protein and nucleic acid to form deposition, this bring big problem for protein extraction (Xin et al., 2007). On the other hand, polyphenol is easy oxidizes to quinone materials during the process of protein extraction; the latter can react with amido and mercapto of the protein. This often makes protein inactivation and deposition quickly, at the same time, make the extraction solution of tissue turn into brown (Borneman et al., 2001). The protein extraction of high polyphenol plants often is difficult by routine method and the extracted protein content is insufficient for electrophoresis analysis. The tannin-protein interaction can be dispelled by absorption agent, like formaldehyde (Jambunathan and Mertz, 1973) and polyvinylpyrrolidone (PVPP) (Borneman et al., 2001), the former is chemical absorption and the later is physical absorption. PVPP prefer reacting with polyphenols to other large molecule materials, so many researchers used it to absorb polyphenols in beer, juice and tea (Borneman et al., 2001; Waniska et al., 1992; Yi et al., 2001). Modified method like “add certain PVPP into routine protein extraction to solve the polyphenol problem” has been reported in seed of *Quercus acutissima* (Xin et al., 2007). Xin found that the optimal method for protein extraction from seeds of *Q. acutissima* was potassium phosphate buffer (50 mmolL^{-1}, pH 7.15), containing 1 mmol L^{-1} EDTA, 1 mmol L^{-1} AsA, 1 mmol L^{-1} DTT, 1 mmol L^{-1} GSH, 5 mmol L^{-1} $MgCl_2$, 0105 %Triton X-100 and 20% glycerol and equal amounts of PVPP to fresh weight of the material. In this study, two modified methods were adopted for sorghum prolamin extraction. The result shown that the method which the samples were pretreated by PVPP before protein extraction (modified

method 2) got more prolamine content than modified method 1 (certain amount of PVPP was added into the routine protein extraction). Thus, we suggest that the sorghum samples should be ground together with PVPP, thus, PVPP can adsorb and precipitate polyphenol preventing it from binding with protein as early as possible and PVPP can also help homogenize the material thoroughly.

Sorghum is an outcrossing plant and a sexually propagated cultivar of sorghum is a population of individuals that expresses a range of phenotypic characters. These individuals of a variety are also genetically distinct, so the discriminating pattern from electrophoresis is unlikely to occur in every plants of the cultivar. Due to these complexities in genetic structure, previous research about outcrossing species discrimination often adopted individual plant genotype analysis and bulk sample fingerprint analysis (Gilliland, 1989). Previous research about sorghum discrimination by electrophoresis often select seed bulked samples (Eswara-Reddy and Jacobs, 2002). Thus, how to decide the right seed numbers of the bulked sample for variety identification should be the first consideration. The bulked samples include different number seeds were analysis for UTLIEF and find that obvious polymorphisms within and among varieties can be revealed using electrophoresis of sorghum single seed sample. Meanwhile, polymorphisms within varieties are concealed when using bulk sample which contain more than 100 seeds.

Application of the research on cultivar identification will ultimately be applied by seed testing laboratories, so test method and procedure should be practical as well as effective. Morphologic identification may be reliable and exact than other methods, but long time for identification is a big question; on the other hand, many allogamous varieties are closely related and they are morphologically alike in the field. Using allelic frequencies, for example the PGI isoenzyme, to identify cultivars of sorghum is a method which focus much interest over the last 20 years (Yan and Huang, 1996). But for seed laboratories, there are usually not equipped to grow plant material needed for many of the enzyme analyses. At the same time, the enzyme may be varied with the change of environment and season. Seed storage proteins are direct products of gene translation and transcription and can therefore, be regarded as effective markers for the structural genes by which they are encoded. Because of the close genetic relatedness and the increasing number ofsorghum varieties, techniques for detecting biochemical differences as expressed by seed storage proteins are of great interest. Investigating a sufficient number of such seed protein markers allows the clucidation of the structure of the genome to a certain degree (Cooke, 1984). McDonald (1980) states that compared to mature field-grow plants, ungerminated seeds have a major advantage because they are of the same stage in their life cycle and are relatively stable physiologically.

Konarev et al., (1987) has shown that seed storage proteins have been less conserved in evolution and so may be more discriminating than other plant protein. The protein content of sorghum grain is in the range of 7.3 - 15.6% (dry matter basis) (Hulse et al., 1980) and mostly are storage proteins generally comprising 70 - 90% of the total protein (Lookhart and Bean, 2000). The prolamine fraction of sorghum called kafirin is poor in lysine content and accounts for 50 to 60 % of total endosperm storage proteins (Eswara-Reddy and Jacobs, 2002). So the seed storage protein was adopted in this paper for sorghum species discrimination and variety identification.

Some sorghum varieties can be quickly differentiated using the bleach (Chlorox) test, but the varieties which have close genetic relatedness can not be discriminated successfully. Despite the report of Eswara-Reddy and Jacobs who have described sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as a powerful tool for sorghum variety identification, the previous data show that this method is unsuitable for high polyphenol sorghum variety (unpublished data). The results of this study show that UTLIEF is a rapid, reproducible, easy to handle and cost-effective method and appeared to be the preferred method compared to SDS-PAGE because of its higher resolution power, higher number of analysed samples per gel and analytical advantages. Furthermore, UTLIEF fulfills the requirements of a method applicable for seed testing, that is, to distinguish between and identify varieties based on seed characters rather than morphological or physiological characters of plants. Electrophoresis methods, especially UTLIEF, can be regarded as a powerful and reliable instrument for variety description in seed testing as well as for genebank management and for the use in breeding programmes, example, for detection of hybrid and outcrossing rate.

REFERENCES

Borneman Z, Gokmen V, Nijhuis HH (2001). Selective removal of polyphenols and brown colour in apple juices using PESPPVP membranes in a single ultrafiltration process. Separation Purif. Technol. 22: 53-61.

Cao YX, Chen CG, Lu QS, Song RB (2004). Use and innovation of sorghum gerplasm. Liaoning Agric. Sci. 3: 23-25.

Chauhan P, Ram C, Mann A, Sangwan VP (2002). Molecular weight analysis of seed proteins of forage sorghum (*Sorghum bicolor* (L.) Moench). Seed Sci. Technol. 30: 11-16.

Cooke RJ (1984). The characterization and identification of crop cultivars by electrophoresis. Electrophoresis, 5: 59-72.

Dendy DAV (1995). Sorghum and the millets: Production and importance. In Sorghum and Millets, Chemistry and Technology, Dendy DAV, ED.; AACC: St. Paul, MN. pp. 11-26.

Eswara-Reddy NP, Jacobs M (2002). Sorghum lysine-rich cultivar verification by SDS-PAGE and southern blot. Acta Physiol. Plant. 24: 285-290.

Gilliland TJ (1989). Electrophoresis of sexually and vegetatively propagated cultivars ofallogamous species. Plant Varieties Seeds 2: 15-25.

Hahn H, SchÖberlein W (1999a). Characterization and identification of *Festulolium* hybrids by electrophoresis of seed proteins. Seed Sci.

Technol. 27: 525-542.
Hahn H, SchÖberlein W (1999b). Identification of *Festuca pratensis* varieties by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and ultrathin-layer isoelectric focusing (UTLIEF). Seed Sci. Technol. 27: 550-578.
Hulse JH, Liang EM, Pearson OE (1980). Sorghum and the millets: their Composition and Nutritive Value. Academic Press, London, pp. 81-91.
Jambunathan R, Mertz ET (1973). Relationship between tannin levels, rat growth, and distribution of proteins in sorghum. J. Agric. Food Chem. 21: 692-696.
Konarev VG, Gavriljuk IP, Gubareva NK (1987). Storage protein in the identification of species, cultivars and lines. Seed sci. Technol. 15: 675-678.
Lan Y, Qian XH, Wang G (2001). The Establishment of Sequential Extraction M ethod in the Proteome Analysis. Progressing Biochem. Biophys. 28: 415-418.
Lookhart GL, Bean SR (2000). Cereal protein: composition of their major fractions and methods for identification. In handbook of cereal science and technology; Kulp K, Ponte JG, Eds.; Dekker: N.Y. pp. 363-384.
Mcdonald JMB (1980). Oat cultivar characterization using electrophoresis. J. Seed Technol. 5: 88-101.
Pashkoulov DT, Tobutt KR, BoŠkoviĆ R (2000). Comparison of isoenzymes in Prunus avium separated by two different electrophoretic techniques. Plant breed. 119: 153-156.
Pedersen JF, Toy JJ, Johnson B (1998). Natural outcrossing of sorghum and sudangrass in the central great plains. Crop Sci. 38: 937-939.
Qian QH, Yang YW (2003). Study on identification technique of purity of sorghum seeds. J. Liaoning Agric. Vocation-Technol. College, 5: 3-5.
Van den Berg BM (1990). Inbred testing of tomato (*Lycopersicon esculentum* L.) F1 varieties by ultrathin-layer isoelectric focusing of seed protein. Electrophoresis, 11: 824-829.
Van den Berg BM (1991). A rapid and economical method for hybrid purity testing tomato (*Lycopersicon esculentum* L.) F1 varieties by ultrathin-layer isoelectric focusing of alcohol dehydrogenase varieties from seed. Electrophoresis, 12: 64-69.
Wang XF, Knoblaunch R, Leist N (2001). Identification of varieties and testing of hybrid purity of rice by ultrathin-layer isoelectric focusing of seed protein. Int. Rice Res. Note, 26: 18-19.
Waniska ED, Hugo LF, Rooney LW (1992). Practical methods to determine presence of tannins in sorghum. J. Appl. Poult. Res. 1: 122-128.
Weng XW, Liu GQ, Chen YS (2007). Investigation and analysis on vertical cooperation model in the industry of ethanol producing from sorgo stems. Renewable Energy Res. 25: 92-96.
Xin X, Jiang XM, Sun HM, Yu WW (2007). Protein Extraction from Polyphenol- Rich Seeds of Quercus acutissima. Scientia Silvae Sinicae, 43: 26-30.
Yan QC, Huang YJ (1996). The handbook of cereal varieties identification: China agricultural publishing, Beijing.
Yi GB, Cui YD, Liao LW, Li XM (2001). Study on PVPP adsorption behavior to polyphenol in green tea. Food Sci. 22: 14-16.
Zhao T, Yan M, Lu YP, Yang F, Huang J, Wang XF (2005). Genetic purity testing of two-line hybrid rice seeds by ultrathin-layer isoelectric focusing of protein. Seed Sci. Technol. 33: 45-52.
Zhu ZH, Li WX, Liu F (2003). Identification and evaluation of quality traits in sorghum germplasm. J. Plant Genet. Res. 4: 326-330.