Seed Protein Electrophoresis for Assessment of Genetic Variation within Genotypes of Meadow Fescue (*Festuca pratensis* Huds)

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Abstract: The protein diversity of 45 genotypes (forty genotypes selected within two gene pools and five varieties) of meadow fescue was examined. Genetic variation was described using ISTA/UPOV methods for crop variety identification. Modifications of acid-PAGE of alcohol-soluble proteins (prolamins), and SDS-PAGE of salt-soluble proteins (globulins) were elaborated for seed analyses of *Festuca pratensis* Huds. The results of this study indicated that the genotypes of meadow fescue could effectively be differentiated on the basis of polymorphism, detected between protein patterns. SDS-PAGE presented a higher differentiation power and better repeatability; thus could be used as a rapid and reliable method for the identification of *F. pratensis* genotypes in breeding programmes and the seed industry.

Keywords: acid-PAGE; genetic diversity; meadow fescue; SDS-PAGE

Grassland husbandry has always played an important role in the agriculture of European countries, especially in the mountains where pastures and meadows predominate. Meadow fescue (*Festuca pratensis* Huds.) is one of the major grassland species that is in use as local populations, or as cultivars released through plant breeding. Often, the determination of differences between grass varieties is difficult because of the similarity of phenotypic characters and/or the close relationship in pedigree (Krishnan & Sleper 1997).

Meadow fescue is a diploid ($2n = 2x = 14$) outbreeder with a gametophytic self-incompatibility system, controlled by two genes designated S and Z (Lundqvist 1961, 1962; Bauman *et al.* 2000). As a result, a large genetic diversity could be expected within populations. Measurements of genetic diversity, present in populations and cultivars, could assist in selecting genotypes for future development of commercial varieties. Kolliker *et al.* (1999) in a study of *F. pratensis*, *Lolium perenne* and *Dactylis glomerata* illustrated that genetic variability may have consequences for the adaptability and persistence of individual cultivars. Determination of similarity, or genetic diversity, between genotypes and populations of grasses on the basis of methods independent of environmental factors could assist both in the breeding process and biodiversity protection. Recently, on the basis of DNA molecular markers technology, this approach seems to have been achieved. However, it can be mentioned that methods like biochemical markers often can be easier for the identification of grass cultivars, from the practical point of view, because they are cheaper, independent of environment, and they present clear-cut protein polymorphism (Krishnan & Sleper 1997; Gilliland *et al.* 2000). That is the reason seed protein electrophoresis should be used as an international method for the assessment if one variety is distinct,

Table 1. List of genotypes evaluated for genetic variation by seed protein

Accession code	Origin of progenies	Method of obtaining/note	Accession code	Origin of progenies	Method of obtaining/note
M8233/01	Schmidt 1*	isolation within gene pool	M8217/02	Schmidt 2*	isolation within gene pool
M8366/02			M8218/02		
M8328/02			M8208/02		
M8347/02			M8210/02		
M8360/02			M8228/02		
M8330/02			M8233/02		
M8309/02			M8236/02		
M8358/02			M8204/02		
M8374/02			M8240/02		
M8376/02			M8241/02		
M8302/02			M8251/02		
M8329/02			M8262/02		
M8331/02			M8281/02		
M8352/02			M8290/02		
M8364/02			M8294/02		
M8361/02			M8297/02		
M8378/02			M8282/02		
M8327/02			M8283/02		
M8388/02			M8216/02		
M8390/02			M8206/02		
FP0005	polycross progenies of Schmidt 1 and 2	candidate for registration	Preval	commercial variety used as a standard in field	breeding
FP0015					
FP0025					
FP0035					

*Schmidt 1 and Schmidt 2 are local populations collected in Switzerland, and used further as breeding materials

uniform, and stable (DUS). The same DUS criteria are also used world-wide for the granting of Plant Breeders' Rights (PBR); an intellectual property protection system (Cooke & Reeves 2003). As reported, seed protein electrophoresis is known to be used for the evaluation of grass varieties; however the methods have not been equilibrated with international standards (Krishan & Sleper 1997; Hahn & Schöberlein 1999).

The aim of this study is to evaluate the applicability of ISTA/UPOV methods by seed protein electrophoresis as tools for the determination of genetic diversity within genotypes of *Festuca pratensis* Huds.

MATERIALS AND METHODS

Seeds derived from isolated plants of 45 genotypes of meadow fescue accessions, harvested in ART-Reckenholz, were used (Table 1). Forty genotypes belonged to two gene pools designated Schmidt 1 and Schmidt 2. Five of the genotypes are breeding varieties: cv. Preval as well as FP0005, FP0015, FP0025, and FP0035 (candidate varieties created in ART). The candidate varieties consist of polycross progenies belonging to the two populations mentioned above.

The genetic variation is described, using a modification of the methods applied by ISTA/UPOV for cereals (Drapper 1987; Anonymous 2003):

Acid-PAGE (acid polyacrilamyde gel electrophoresis) is carried out according to the known standard reference method of ISTA (Draper 1987; Anonymous 2003). Proteins are extracted from a bulk sample of 40mg finely ground powdered seeds with 400 µl extracting solution (0.05 g Pyronin G; 25 ml 2-chloroethanol), stained overnight at room temperature, and centrifuged for 20 min at 17 000 g. Then, 20 µl of the extracts are loaded into wells. Gel

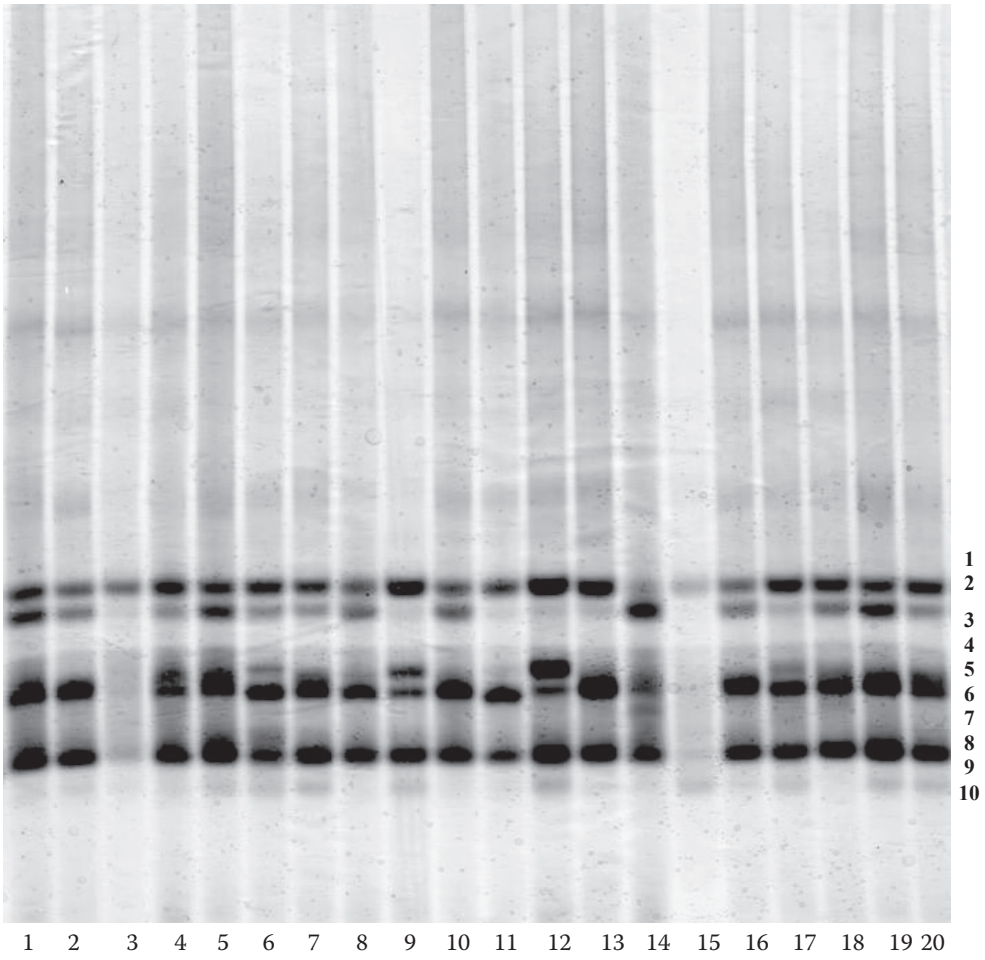

Figure 1. Example of acid-PAGE protein spectra observed in *F. pratensis* accessions (computer assisted laser densitometer scan)

1 – M8388; 2 – M8390; 3 – FP0005; 4 – FP0015; 5 – FP0025; 6 – M8360; 7 – FP0035; 8 – M8327; 9 – M8328; 10 – M8388; 11 – M8217; 12 – M8236; 13 – M8262; 14 – M8283; 15 – M8206; 16 – M8390; 17 – FP0005; 18 – FP0015; 19 – FP0025; 20 – FP0035

medium of 10% acid-PAGE (pH = 3.1) is prepared in a 1.5 mm thick gel, using a Consort E835 vertical unit (with gel cassette 200 × 200 mm). Electrophoresis is carried out at 120 V for 3 h at 19°C–20°C. Staining of gels is performed in a solution of Coomasie Brilliant Blue G250:Coomasie Brilliant Blue R-250 (1:3), dissolved in trichloroacetic acid/methanol. Gels are stored in polyethylene sleeves at +10°C for further evaluation and documentation.

SDS-PAGE (sodium-dodecilsulfat polyacrilamyde gel electrophoresis) is carried out using a modification of the methods used for barley identification, according to UPOV (Anonymous 2003). The progress in this analysis is the specification of protocols according to the purposes of the present study – the evaluation of *Festuca pratensis* seed proteins. The modification mainly concerns the protein extracting procedure as follows:

Stock extraction buffer (according ISTA/UPOV) for barley:

6.25 ml 1M tris/HCl (pH 6.8), 12.05 ml dist. H_2O, 2 g SDS, 10 g pyronin, 10 g glycerol.

Extracting solution for *F. pratensis* seeds:

5.66 ml stock extraction buffer, 3.00 ml DMF (dimethylformamide), 1.58 ml mercaptoethanol, filled up to 20 ml with dist. H_2O. This must be prepared fresh.

Extracting procedure:

A bulked sample of 0.5 g seeds is milled in a rotary type electric coffee grinder; then passed through a mortar and pestle for better separation of the seed groats from seed coats.

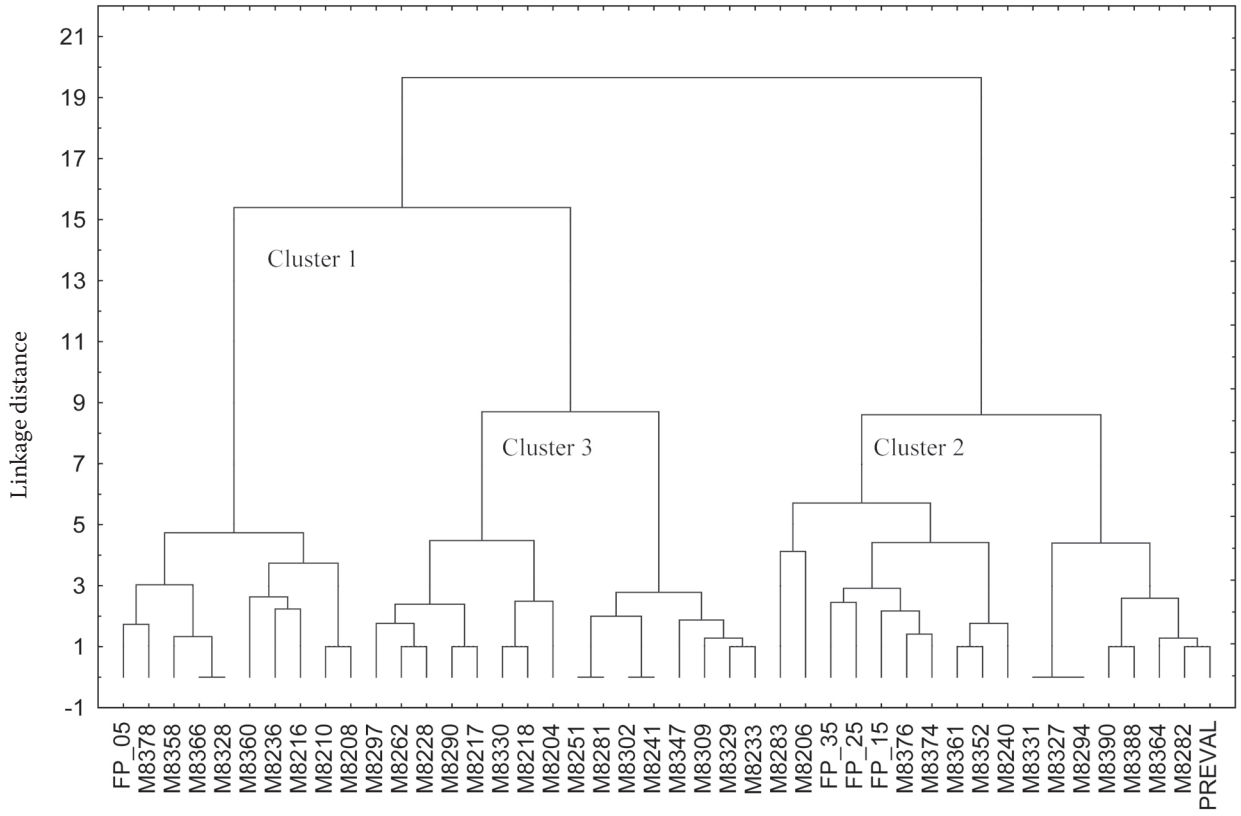

Figure 2. Cluster analyses of acid-PAGE patterns observed in *Festuca pratensis* accessions (Ward's method, Euclidean distances)

Seed meal (40 mg) is extracted with 500 µl of freshly prepared extracting solution. The next procedures include: 1h extraction at room temperature, heating in boiled water for 10 min, and cooling and centrifugation at 18 000 g for 5 min. Protein extracts are stored at –18°C for further electrophoretic separation and repeated analyses.

Electrophoresis:

Twenty µl of extracts are loaded into the wells. The gel (1.5 mm thick) consists of two parts: resolving (main gel, 10% acrylamide, pH = 8.8) and stacking gel (upper gel, 3.5% acrylamide, pH = 6.8). Vertical unit Consort E835 is used for the gel preparation and electrophoresis. Protein separation is carried out for about 3 h (1 h at 150 V, and 2 h at 300 V) at 19°C–20°C. Gels are fixed in 15% (w/v) trichloroacetic acid for 30 min, and then stained as described above for the acid-PAGE procedure.

RESULTS AND DISCUSSION

Acid-PAGE of alcohol-soluble seed proteins (prolamins), extracted from *F. pratensis* seeds, presents a typical spectra of this plant species (Figure 1). As shown, polymorphism is observed in fast moving protein components, where ten clear distinct bands are detected. The polymorphism of the protein spectra is described by repeated analyses of extracting procedures and separation in acid-PAGE. Three accessions were used as standards: cv. Preval, M8360, and M8206. They were disposed in permanent positions in all gels for comparison with the analyzed genotypes. A database was created, on the basis of component mobility and band density, which was further used for clustering of the accessions.

The tree-cluster analysis (Ward's method, Euclidean distances) illustrates the distribution of genotypes in three main clusters, where Cluster 3 could be suggested to be a sub-cluster of Cluster 1 (Figure 2). Separation by a diverse rate of distances (1–20) within each sub-cluster was detected for a better understanding of the genetic variability between the analysed genotypes. According to these analyses, the following accessions were determined as identical (distance 0): M8366 and M8328; M8251 and M8281; M8302 and M8241; M8331, M8327, and

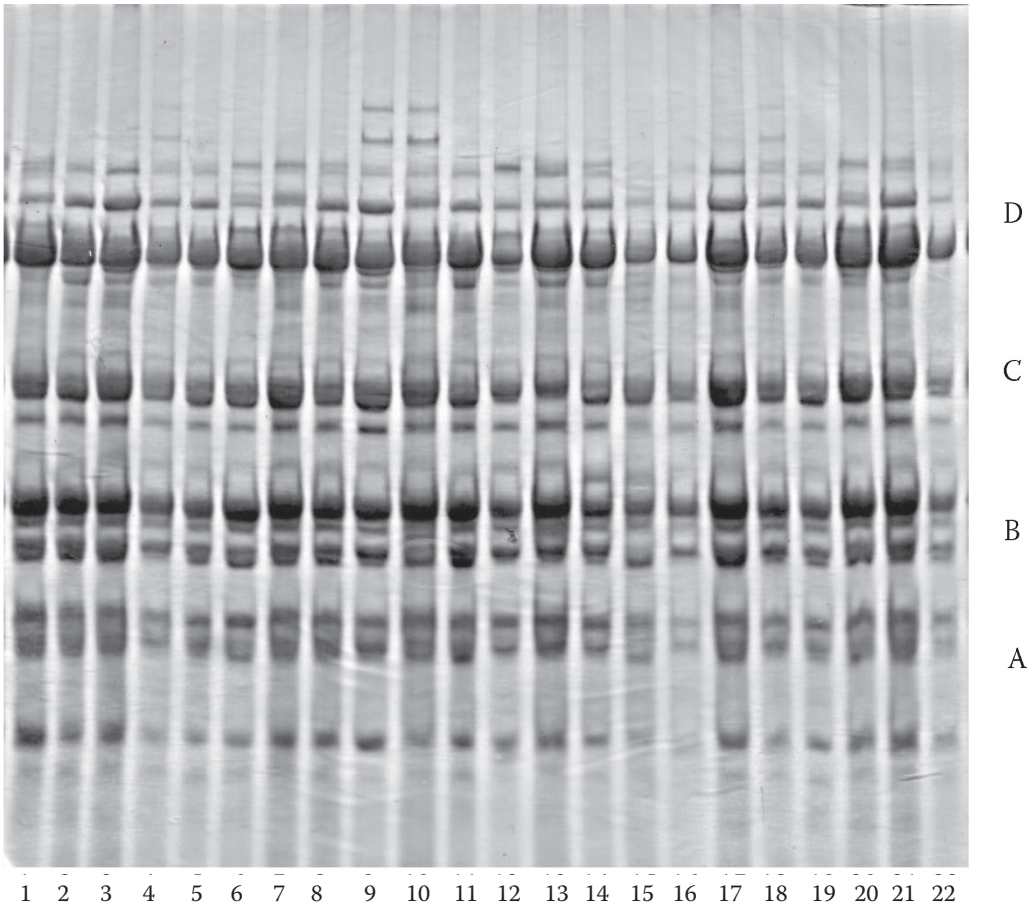

Figure 3. Sample analysis of SDS-PAGE protein electrophoresis carried out with *F. pratensis* seed accessions (computer assisted laser densitometric scan)

1 – Preval; 2 (11)– M8388; 3 – M8390; 4 – FP0005; 5 – FP0015; 6 – FP0025; 7 – M8360; 8 – FP0035; 9 – M8240; 10 – M8241; 11 (2) – M8388; 12 – M8217; 13 – M8236; 14 – M8262; 15 – M8283; 16 – M8206; 17 – M8290; 18 – FP0005; 19 – FP0015; 20 – FP0025; 21 – FP0035; 22 – Preval

M8294. Two of the groups of identical accessions are composed of members of one and the same population: M8366 and M8328 – Schmidt 1; M8251 and M8281 – Schmidt 2. The third group consists of two members of Schmidt 1 (M8331, M8327) and one member of Schmidt 2 (M8294). The candidate varieties FP0015, FP0025, and FP0035 form into one sub-cluster in the framework of Cluster 2; whereas FP0005 falls into Cluster 1. Two accessions (M8283 and M8206), originating in Schmidt 2, are divided into a separate group. On the basis of the results, it can be suggested that the genotypes were described as more or less close by patterns of alcohol-soluble proteins. However, there should be pointed that the repeatability of analyses is in question because of a/the limited number of prolamin bands and their fluctuation (by mobility and by density) from one gel to another.

SDS-PAGE electrophoresis of salt-soluble proteins (globulins) of *F. pratensis* seeds reveals a higher distinguishing power than acid-PAGE does. The number of developed protein components varies from 22 to 31 between the evaluated genotypes (Figure 3). The control samples (cv. Preval, M8360 and M8206) are fixed in permanent positions of all gels, as mentioned before. Four groups of components are described where a higher polymorphism is observed in group D (slow moving proteins). Polymorphism in group B is also clearer than in A and C. The accuracy of the test is proven by comparison in one gel (M8388, position 2 and 11, Figure 3), and comparison of accessions described as 'similar' in separated gels. Two pairs: M8376 and M8290; and M8378 and M8294, are described as similar by visual evaluation.

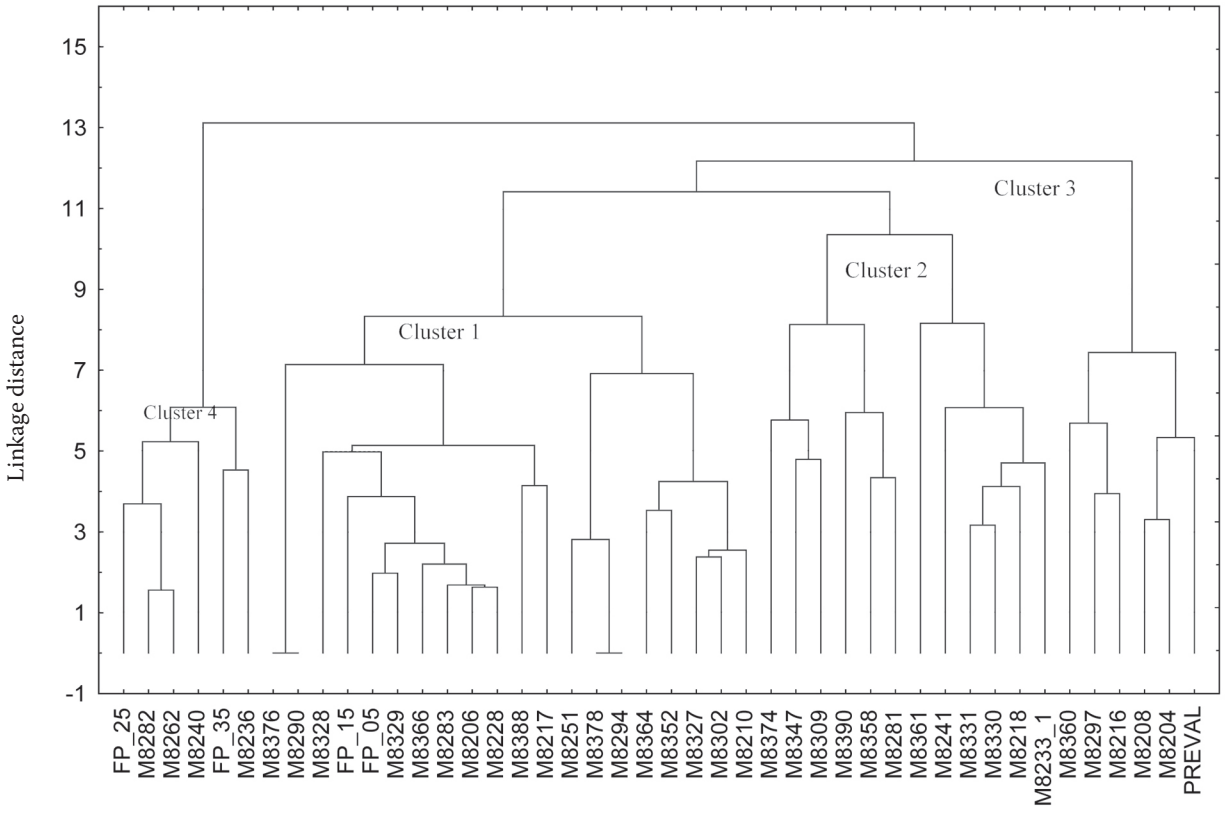

Figure 4. Cluster analyses of SDS-PAGE patterns observed in *Festuca pratensis* accessions (Ward's method, Euclidean distances)

Tree-cluster analysis (Ward's method, Euclidean distances) was carried out using the matrix created on the basis of protein components mobility and bands density (Figure 4). Four main clusters are determined in the rate of distances (1–14). Cluster 1 and Cluster 2 are sub-clustered to Cluster 3, by joining another group of more distinct genotypes (M8208, M8204, Preval, M8297, M8216, and M8360). The most diverse group of genotypes gets put into Cluster 4, including the candidate varieties FP0025 and FP0035. The accessions in this group belong to gene pool Schmidt 2. The other two candidate varieties (FP0005 and FP0015) come into one sub-cluster of Cluster 1. This sub-cluster is composed of close relating genotypes of the two investigated gene pools – Schmidt 1 and Schmidt 2. The results presented show that this approach could be used for identification of similar genotypes. Probably, because of large variations and overlapping within the examined accessions, it is difficult to create a relationship between the selected genotypes and their origin (Schmidt 1 and Schmidt 2). However, it should be pointed out those genotypes with similar globulin spectra were detected in both populations.

SDS-PAGE presents differences between *Festuca pratensis* genotypes more clearly, and possesses a higher repeatability than acid-PAGE. This is our reason for confirming the success in application of SDS-PAGE for genotype identification and genetic diversity assessment of *Festuca pratensis*.

CONCLUSIONS

Seed protein electrophoresis of *Festuca pratensis* seeds by the ISTA/UPOV methods describes variations between genotypes on the basis of the polymorphism of protein patterns with different solubility, band mobility, and density. SDS-PAGE of salt-soluble proteins illustrates a higher distinguishing power than the acid-PAGE of alcohol-soluble proteins, and possesses clear repeatability of the results. There should be indicated both, that *F. pratensis* is an outcrossing plant species, and how difficult it is to distinguish grass lines

phenotypically. Furthermore keeping in mind the unpredictable effect of environmental factors, such as site and year of harvest, we suggest that SDS-PAGE be used as a rapid and reliable method for routine identification of meadow fescue genotypes in breeding programs. Precise differentiation in protein banding patterns is possible on the basis of the presence or absence of unique polypeptides, and the creation of matrices for statistical analyses. Their clustering allows the ranging of genotypes in either closer or distinct groups, which could also be used for the breeder's needs, as well as in the seed industry.

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