DIFFERENTIATION OF *FESTUCA PRATENSIS* VARIETIES AND *FESTULOLIUM* HYBRIDS BY ELECTROPHORESIS

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ABSTRACT
In the present study seed proteins and isozymes of varieties and lines of *Festuca pratensis* and *Festulolium* were investigated with the aim of finding methods for the discrimination of meadow fescue varieties and the identification of *Festulolium* hybrids. The banding patterns of ultrathin-layer isoelectric focusing (UTLIEF) of bulked seed samples allowed a clear distinction of *Festulolium* hybrids of different crossing combinations from each other and in most cases also from their parental species. Despite the outcrossing character, identification of some hybrids and crossing parents was also achieved using protein patterns of single seeds. By means of isozyme electrophoresis of *Festuca pratensis* varieties the genotype and allele frequencies of 5 loci were detected. The 10 analysed varieties were successfully differentiated by comparisons of allele frequencies or genotype frequencies using Likelihood ratio test and c^2-test, respectively. Both methods, UTLIEF and isozyme electrophoresis, proved to be suitable for reliable distinguishing *of Festuca pratensis* and *Festulolium* varieties and lines.

KEYWORDS
Festuca pratensis, Festulolium hybrids, grasses, varieties, electrophoresis, isozymes, storage proteins, isoelectric focusing

INTRODUCTION
Forage and turf grasses are characterized by a great diversity. However, breeders, registration authorities, seed testing institutions and seed traders need characters for a reliable differentiation between varieties. Conventional morphologically based descriptions of varieties may be altered by environmental factors and is often not sufficient for unequivocal differentiation.

In the field of forage grass breeding intergeneric hybridization between *Festuca* and *Lolium* species has great importance. Varieties of *Festulolium braunii* are registered in Germany, and breeders' rights were granted for several other hybrids. But a clear separation of *Festulolium braunii* hybrids from their crossing parents, especially from *Lolium*, with the help of characters like ligula, auricle, number of chromosomes, inflorescence or caryopses morphology often proves to be difficult.

The purpose of the following study was to elucidate the suitability of electrophoretical methods for a fast and reliable identification of *Festuca pratensis* varieties as well as for the differentiation of *Festulolium* hybrids from each other and from their crossing parents.

MATERIAL AND METHODS
Material
Seed samples of varieties and lines of *Festuca pratensis*, *Festuca arundinacea, Lolium multiflorum* and *Lolium perenne* as well as of different Festulolium hybrids were obtained from various breeders and from the gene bank collection of the Institute of Plant Genetics and Crop Plant Research Gatersleben, Germany. The following hybrids were included in this study:
- *Festuca pratensis* Huds. x *Lolium multiflorum* Lam.: *Festulolium braunii* (K. Richt.) A. Camus
- *Festuca arundinacea* Schreb. x *Lolium multiflorum* Lam.: *Festulolium pabulare* nom. nov.
- *Festuca pratensis* Huds. x *Lolium perenne* L.: *Festulolium loliaceum* (Huds.) P. Fourn., Syn. *Festulolium adscendens* (Retz) A. et Gr.
- *Festuca arundinacea* Schreb. x *Lolium perenne* L.: *Festulolium holmbergi* (Dörfl.) P. Fourn.

For isozyme electrophoresis approx. 120 plantlets of 10 *Festuca pratensis* varieties were cultivated in single pots in the greenhouse until they reached the 6-8 leaf stage.

METHODS
Ultrathin-layer isoelectric focusing (UTLIEF)
The chloroethanol-soluble fraction of seed storage proteins was extracted with 30% 2-chloroethanol solution. For UTLIEF 0.12 mm thick polyacrylamide gels (2.56% Servalytes pH range 2-11) polymerized on Gel-Fix (SERVA) were used. Focusing took place at 10-C for 80 min at increasing voltage from 500 V up to 2500 V. The gels were fixed in 12% trichloroacetic acid and stained in Coomassie Blue solution (all chemicals are from SERVA, 69115 Heidelberg, Germany; the method was modified after Leist, 1988).

Isozyme electrophoresis
About 300 mg leaf material of *Festuca pratensis* plantlets were homogenized with 200 ml crushing solution by means of a cell sap press (E. Pollähne GmbH, 30974 Wennigsen, Germany) and centrifuged. The supernatants were used for electrophoretical separation. Malate dehydrogenase (MDH, EC 1.1.1.37) and phosphogluco-isomerase (PGI, EC 5.3.1.9) were separated by means of horizontal starch gel electrophoresis. Screening of glutamate-oxalacetate-transaminase (GOT, EC 2.6.1.1) and menadione-reductase (MEN, EC 1.6.99.2) was performed by vertical polyacrylamide gel electrophoresis.

GOT-2, GOT-3, MDH-2, MEN and PGI-2 genotypes and allele frequencies were determined for each variety. The 10 investigated varieties were distinguished from each other by paired comparisons. For the loci with genotype frequencies not deviating from Hardy-Weinberg (HW) proportions, comparisons were made by means of allele frequencies using the Likelihood ratio test (Hayward and Mc Adam, 1977; Nielsen et al., 1985; Weibull et al., 1991). In the other case, if HW proportions could not be found for certain loci, the possibility of comparing genotype distribution by c^2-test was applied (Gilliland et al., 1982).

RESULTS AND DISCUSSION
UTLIEF of seed storage proteins
For *Festulolium braunii* and *Festulolium loliaceum* the banding patterns allowed a clear differentiation of the hybrids from their parental species. In the patterns of *Festulolium braunii* a remarkable combination of characteristic bands of both parents occurred. Double or triple bands at pH 7.3 and a band at pH 7.7, both typical for *Festuca pratensis*, could be found with lower intensity at the same position in the *Festulolium braunii* hybrids. Furthermore, bands at pH 6.3 and pH 8.1 characteristical for *Lolium multiflorum* appeared in the *Festulolium braunii* patterns. In some cases the banding patterns of *Festulolium pabulare* differed from *Festuca arundinacea* only in the intensity of certain bands, but there are clear differences to *Lolium multiflorum*. Patterns of *Festulolium holmbergii* were not always clearly distinguishable from *Lolium perenne* patterns, whereas a distinction from the *Festuca* crossing parent was possible.
Banding patterns of bulked seed samples present only an average protein profile of genotypes included in a given variety, therefore also single seeds were investigated. Despite the high degree of genotypic variation within these outcrossing species, separation of *Festulolium loliaceum* from *Lolium perenne* and *Festuca pratensis* was possible. The patterns of *Festulolium loliaceum* showed bands resulting from the influence of *Festuca* as well as bands inherited by the *Lolium* parent.

Isozyme electrophoresis of *Festuca pratensis* varieties
A high degree of polymorphism was detected for the analysed loci especially for the PGI-2 locus with 5 alleles and for the MEN locus with 4 alleles. The genotype distributions appeared to be in HW proportions

for GOT-3 and PGI-2. Small deviations were revealed for MEN and MDH-2 and an excess of homozygotes was observed for GOT-2.
Table 1 shows the results of the paired comparisons by means of Likelihood ratio test for GOT-3 and PGI-2. On the basis of genotype frequencies, i.e. application of c^2-test (a=5%), 37, 35 and 28 out of 45 paired comparisons showed distinctness for MDH-2, MEN and GOT-2, respectively. Significance in all possible comparisons between the 10 varieties were revealed after combination of these 5 loci.

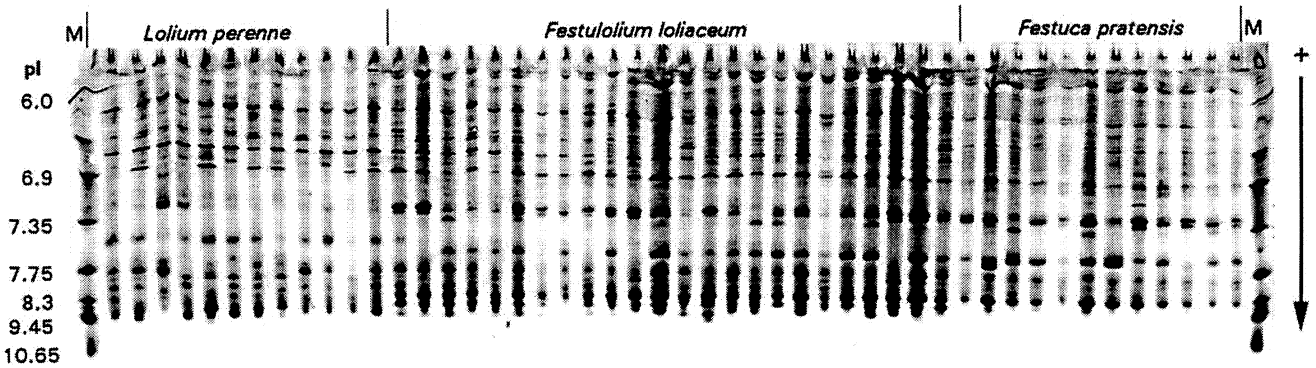
The present study shows that UTLIEF is a rapid and reliable method for the identification of *Festuca pratensis* varieties and for the differentiation of *Festulolium hybrids* from each other and from their parental species. The electrophoretical characters are not influenced by environmental conditions. Therefore seed protein banding patterns and isozyme electrophoresis represent a useful instrument in seed testing, plant breeding and gene bank management.

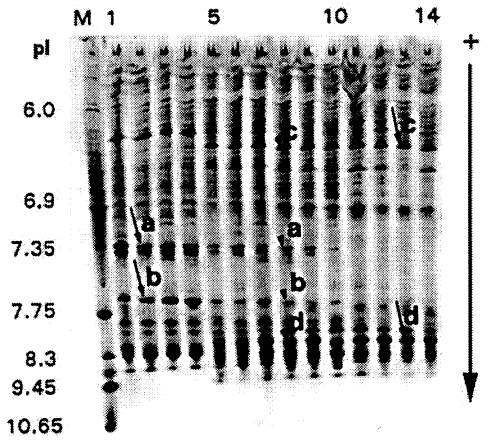
Figure 1

upper panel: banding patterns of the chloroethanol-soluble storage protein fraction of single seeds of *Festulolium loliaceum* in comparison with the parental species *Fastuca pratensis* and *Lolium perenne* following isoelectric focusing (pH 2-11)

left Panel: isoelectric focusing banding patterns (bulked seed sample) of varieties and lines of *Festulolium braunii* (lanes 5-10) and the crossign parents *Festuca pratensis* (lanes 1-4) and *Lolium multiflorum* (lanes (11-14).
(pH 2-11)

M - marker protein mixture for detection of isolelectric points.





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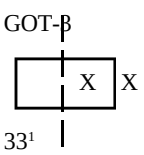

X - significant differences in the allele frequencies

[1] - 33 out of 45 paired comparisons are significant for GOT=3

[2] - 42 pit pf 45 [aored cp,[arospms are sogmofocamt fpr PGI-2

Table 1
Paired comparisons between the 10 tested varieties V 1 - V 10 by means of Likelihood ratio test (α = 5%)

	V 2	V 3	V 4	V 5	V 6	V 7	V 8	V 9	V 10
V 1	XX	XX	XX	XX		XX	XX	X	XX
V 2		XX		X		XX	X	XX	XX
V 3			XX	XX	XX	X	XX	XX	X
V4				X	XX	XX	X	XX	XX
V 5					XX	X	XX	X	XX
V 6						XX	XX	X	XX
V 7							XX	XX	X
V 8								XX	XX
V 9									XX

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