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Genetic variability of forage grass cultivars: A comparison of *Festuca pratensis* Huds., *Lolium perenne* L., and *Dactylis glomerata* L.

R. Kölliker, F.J. Stadelmann, B. Reidy & J. Nösberger*
*Institute of Plant Sciences, Swiss Federal Institute of Technology, CH–8092 Zurich, Switzerland; (*author for correspondence)*

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Summary

Three widely used cultivars of each of the species *Festuca pratensis* Huds., *Lolium perenne* L., and *Dactylis glomerata* L. were investigated by means of randomly amplified polymorphic DNA (RAPD) markers and vegetative growth traits in order to investigate genetic variability within each cultivar and to compare the level of diversity among cultivars and species. RAPD markers allowed a clear separation of the three species. Genetic variability based on RAPD markers was considerably lower for *F. pratensis* cultivars than for *L. perenne* and *D. glomerata* cultivars which showed similar levels of variability. The proportion of variability due to variation within cultivars, determined by an analysis of molecular variance, was lower in *F. pratensis* (64.6%) than in *L. perenne* (82.4%) and *D. glomerata* (85.1%). A comparison of *F. pratensis* and *L. perenne*, based on vegetative growth traits, confirmed the differences in genetic variability within cultivars. *F. pratensis* showed lower coefficients of genetic variation for eight of ten traits when compared to *L. perenne.* This study demonstrates considerable differences in genetic variability which may have consequences for the adaptability and persistency of individual cultivars.

Introduction

The pattern of genetic variability within the available germplasm substantially influences the choice of breeding material and with it the success of a plant breeding program. Variability among cultivars is required for a successful forage crop species in order to provide farmers with suitable cultivars for different environments and utilization systems. However, for some purposes such as the renovation or the overseeding of degenerated permanent pastures and meadows, single cultivars that are adapted to a broad range of environments may be more desirable: permanent grasslands are specially important in the uplands of central Europe, which often represent marginal habitats where environmental conditions vary greatly over time and space.

Within a cultivar, phenotypic plasticity may enable plants to adapt rapidly to a range of environments (Bradshaw, 1965). Phenotypic plasticity has been reported for many traits and species (MacDonald & Chinnappa, 1989; Brock et al., 1996; Petit et al., 1996), but the genetic basis of plasticity is very complex (Scheiner, 1993). Since genetic variability is crucial for adaptation (Silvertown & Lovett Doust, 1993), genetic variability within cultivars may be particularly important for long-term adaptability. There is only little information on the significance of variability within populations and these results are derived from studies with wild species (Dolan, 1994; Templeton, 1994). However, it was shown that genetic diversity can increase disease resistance in barley (Wolfe & McDermott, 1994). It was also suggested, that increasing the heterogeneity may enhance the adaptability of forage grass cultivars (Hayward, 1997). Molecular markers such as randomly amplified polymorphic DNA (RAPD) (Welsh & McClelland, 1990; Williams et al., 1990) allow an easy and rapid approach to genetic variability and have been used in various plants species (Schierenbeck et al., 1997). Although some

Table 1. Breeding history of nine cultivars of *Festuca pratensis*, *Lolium perenne* and *Dactylis glomerata* used for investigation

Cultivar	Origin and number (in parentheses) of parental clones
Festuca pratensis[a]	
Darimo[b]	German cultivar NFG (4) and Dutch cultivar Belimo (1)
Fure[c]	Norwegian ecotypes and older cultivars (84)
Préval[d]	Swiss cultivar Préfest (6), German cultivar Cosmos (1), and Swiss ecotypes (7)
Lolium perenne[a]	
Arion[e]	Swiss ecotypes (28; 26 of these clones originate from only 5 ecotypes)
Cavia[e]	Swiss ecotypes and older cultivars (total 12 clones)
Respect[f]	Cultivars Amigo (2), Melino (5), Talbot (2), and Dutch ecotypes (4)
Dactylis glomerata[g]	
Loke[h]	Swedish ecotypes
Prato[d]	Swiss variety Lara (11) and Dutch variety Baraula (2)
Reda[e]	Swiss ecotypes (9) and older cultivars (6)

[a] Investigated cultivars are diploid (2n = 2x = 14).
[b] Mommersteeg International, Vlijmen, The Netherlands.
[c] Norwegian Crop Research Institute, Ås, Norway.
[d] Swiss Federal Research Station for Plant Production, Changins, Switzerland.
[e] Swiss Federal Research Station for Agroecology and Agriculture, Zurich, Switzerland.
[f] Cebeco Zaden B.V., Vlijmen, The Netherlands.
[g] Investigated cultivars are tetraploid (2n = 4x = 28).
[h] Svalöf Weibull AB, Svalöv, Sweden.

of these studies focused on forage grasses (Charmet & Balfourier, 1994; Gunter et al., 1996), information on genetic variability within cultivars is available for only a few forage grass species (Loos, 1994; Xu et al., 1994; Huff, 1997). Therefore, our objective was to examine the genetic variability within cultivars of three important forage grass species in order to provide data which may help to better understand the genetic architecture of species and cultivars and which is important for plant breeding and for further investigations on the significance of genetic variability.

Festuca pratensis Huds. (meadow fescue) is a forage grass of high quality and yield potential, comparable in many respects to perennial ryegrass (*Lolium perenne* L.) Due to its winter-hardiness, it has a competitive advantage as a hay or as a silage crop in cooler regions (Aastveit & Aastveit, 1989). It is also a significant component of species-rich permanent pastures and hay fields in alpine regions and in eastern Europe. However, meadow fescue is only rarely found in intensively managed grasslands and shows low persistency when sown in mixture with other forage species. *L. perenne* is a highly productive species with a very good nutritive value and a high palatibility. It is one of the most important forage grasses of temperate regions, but its distribution in cooler regions and at higher altitudes is limited by a low tolerance to unfavourable climatic conditions and a high susceptibility to pink snow mould (*Fusarium nivale* (Fr.) Ces). *Dactylis glomerata* L. (orchardgrass) is a widespread species of good forage quality, well adapted to moderate fertility and low soil moisture. It is a companion species and strong competitor of *F. pratensis* in species-rich grasslands (Gügler, 1993).

We used RAPD markers as well as vegetative growth traits to assess genetic variability within three cultivars of each of the three species.

Material and methods

Plant material

Three cultivars of each of the species *Festuca pratensis* Huds., *Lolium perenne* L., and *Dactylis glomerata* L., currently recommended for cultivation in Switzerland (Lehmann et al., 1996), were used (Table 1). Care was taken to avoid tetraploid *L. perenne* cultivars and to select cultivars as distantly related as possi-

ble. Plants were grown from basic seed on autoclaved silica sand (particle size 0.8–1.2 mm). Two weeks after sowing, 28 single plants were randomly selected, transferred to hydroponics and kept in the growth chamber as individual plants; they were considered to be genotypes.

DNA extraction

Fresh leaf material of each genotype of *L. perenne* and *D. glomerata* cultivars was sampled seven weeks after sowing and stored at –80 °C. Genomic DNA was isolated by a modified hexadecyltrimethyl ammonium bromide (CTAB) extraction procedure (Doyle & Doyle, 1990). Frozen leaf tissue (approx. 100 mg) was ground in liquid nitrogen, transferred to a sterile Eppendorf tube, and lyophilized overnight (Hetovac VR-1, Heto Lab Equipment A/S, Birkerød, Denmark). Samples were incubated with 1 mL of CTAB buffer (10 g L^{-1} CTAB, 0.1 M Tris pH 7.5, 0.7 M NaCl, 0.01 M EDTA pH 8.0) for 90 min at 60 °C, treated with 10 μg mL^{-1} ribonuclease A (Boehringer Mannheim, Germany) at 37 °C for 30 min, extracted for 5 min with 450 μL chloroform/isoamyl alcohol (24:1 v:v), and centrifuged for 10 min at 7000 rpm. The aqueous phase was transferred to a new tube, and the DNA was precipitated with cold isopropanol (900 μL). DNA pellets were recovered by centrifugation (15 min at 5000 rpm), washed in 75% ethanol/10 mM ammonium acetate, dried under vacuum, and dissolved in 150 μL sterile H_2O. To remove compounds inhibiting PCR reactions, DNA of *D. glomerata* was cleaned using polyethylene glycol (13% w/v; PEG 8000). DNA concentration was estimated with a LS-2B filter fluorimeter (Perkin-Elmer Ltd., Buckinghamshire, England) as well as visually after electrophoresis in a 20 g L^{-1} agarose gel at 125 V for 1 h in TAE buffer (400 mM Tris, 20 mM EDTA, 200 mM sodium acetate) and staining with ethidium bromide.

DNA amplification and separation

Reactions were performed in 20 μL volumes containing: 1 × reaction buffer (10 mM Tris-HCl, 1.5 mM $mgCl_2$, 50 mM KCl, pH 8.3), 100 μM dATP, dGTP, dTTP, dCTP, 0.35 μM primer, 0.6 units EUROBIOTAQ® DNA polymerase (Eurobio, Les Ulis Cedex B, France) and 10 ng genomic DNA. Amplification was carried out in a Hybaid OmniGene temperature cycling system (Control Module with MicroBlock; Hybaid Ltd., Middlesex, UK) and was initiated by denaturation for 1 min at 94 °C, followed by 35 cycles of 30 sec at 35 °C, 2 min at 72 °C and 5 sec at 94 °C. The amplification was completed after 10 min at 72 °C. Reaction products were separated by electrophoresis in a 20 g L^{-1} agarose gel at 100 V for 2.48 h in TAE buffer, stained with ethidium bromide (0.5 μg mL^{-1}) and photographed under UV light with a Polaroid type 667 film (Polaroid Corp., Cambridge, MA, USA). Amplification products were considered RAPD markers and reproducibility was confirmed by running independent duplicate samples. To estimate the size of the RAPD markers, a 100 base pair (bp) marker was used as a standard.

For the selection of suitable primers, 140 decamer primers (Operon Technologies, Inc., Alameda, CA, USA) were initially screened using four unrelated ecotypes of *F. pratensis*, not associated with the three cultivars used in this study. According to the quality of the banding patterns and the number of polymorphic markers detected between the ecotypes, 12 primers (B01, B08, B11, B12, B15, H02, H19, Q5, R3, R11, R19, V16) were selected to investigate the 28 genotypes of the nine populations. For *F. pratensis* cultivars, RAPD profiles of previous investigations (Kölliker et al., 1998) were specifically evaluated in this comparison with *L. perenne* and *D. glomerata*.

RAPD marker analysis

RAPD markers were scored for presence (1) or absence (0) and entered into a binary vector representing the RAPD phenotype of each individual genotype. Only polymorphic markers that were reproducible and could be scored unequivocally in all genotypes were included in the analysis. The pair-wise distances between genotypes were estimated using the Euclidean distance of Excoffier et al. (1992), defined for RAPD markers by Huff et al. (1993) as

$$E_{ij} = \{\varepsilon_{ij}^2\} = n\left[1 - \frac{2n_{ij}}{2n}\right]$$

where n_{ij} is the number of bands shared by the two genotypes i and j and n the total number of polymorphic bands. Analysis of molecular variance (AMOVA) was performed using the WINAMOVA 1.55 program, kindly provided by L. Excoffier (http://anthropologie.unige.ch/~laurent/default.htm# Software programs). For principle coordinate analysis, the NTSYS-pc package (version 1.8) (Rohlf 1993) was used.

Variation in vegetative growth traits

Variation in vegetative growth traits was assessed in a growth chamber experiment using 28 genotypes of the *F. pratensis* cultivars Préval, Darimo, and Fure and the *L. perenne* cultivars Arion, Cavia, and Respect. The same genotypes as investigated by means of RAPD markers could be used for *L. perenne* but not for *F. pratensis.* Two clonal replicates per genotype were produced using two single tillers of similar weight. Replicated plants were cut to 5 cm tiller and root length, transferred into hydroponic containers ($0.30 \times 0.20 \times 0.22$ m; eight plants per container), arranged in two randomized blocks and grown in a complete nutrient solution modified according to Hammer et al. (1978) containing 1 mol m^{-3} NO_3^- and with a pH of 5.5. The medium was aerated continuously and replaced every seventh day. The pH was controlled daily (Sentron 1001 pH, Sentron Europe BV, Roden, The Netherlands) and adjusted if necessary (1 M H_2SO_4). The plants were cultivated in two growth chambers (PGV36, Conviron Instruments, Winnipeg, MB, Canada) at temperatures of 13/18 °C (day/night), 80% relative humidity, and a photoperiod of 16 h. Light (photosynthetic photon flux density 500 μmol $m^{-2}s^{-1}$) was provided by cool-white fluorescent lamps (Sylvania, CW/VHO, 215 W) and incandescent bulbs (100 W) at a ratio of 5:1. Seven days after cloning, the tip of the youngest leaf of the oldest tiller was marked with nail polish. Subsequently, the growth of the following three leaves was recorded and average leaf elongation duration (number of days between the lamina and the ligule appearance of one leaf) and average phyllochron (number of days between lamina emergence of two successive leaves) were calculated. Twenty-eight days after propagation, the growth habit of the plants was determined by visual scoring of the angle formed by the imaginary line through the region of the greatest leaf density and the vertical (1 = erect; 9 = prostrate). Plants were then harvested and separated into roots, leaf laminae, and tillers. The first two undamaged leaves of the tiller on which leaf growth was recorded were separated and used for the determination of single leaf area and single leaf length. The area of the leaf laminae was measured using a photoelectric meter (Model LI-3000A; Li-Cor, Lincoln, NE, USA), and leaves and tillers were counted. All fractions were dried at 65 °C for 48 h. The data were subjected to an analysis of variance using the GLM procedure of the SAS statistical package (Statistical Analyses System, Version 6.12, SAS Institute, Cary, NC, USA). Means of cultivars were compared by Duncan's multiple range test ($p < 0.05$). Variance components were estimated using the VARCOMP procedure of the SAS package. Coefficients of genetic variation were calculated according to Helgadottir and Snaydon (1986) as

$$\mathrm{CV_g} = \frac{\sqrt{\sigma_g^2}}{\bar{\mathrm{x}}} * 100$$

where σ_g^2 is the genotypic component of variance and $\bar{\mathrm{x}}$ the population mean of the character measured. For factor analysis, traits were averaged by genotype, and the values were transformed to standard deviates (Sokal & Rohlf, 1995). Factor analysis was applied using the varimax rotation method suggested by Kaiser (1958). Rotated factor values of 0.50 or greater were considered to be important in interpreting factor associations (Backhaus et al., 1996).

Table 2. Genetic diversity within cultivars and species of *Festuca pratensis*, *Lolium perenne* and *Dactylis glomerata*. Twenty-eight individual plants of three cultivars of each species were investigated

	Percentage of polymorphic markers[a]	Euclidean distance (E)[b]
Festuca pratensis		
Darimo	25	7.4
Fure	22	8.1
Préval	34	11.7
Within species	45	12.4
Lolium perenne		
Arion	52	17.1
Cavia	50	15.6
Respect	46	15.5
Within species	64	18.4
Dactylis glomerata		
Loke	49	17.1
Prato	56	19.4
Reda	50	18.0
Within species	64	20.3

[a] Total of markers scored: 104.
[b] Average distances for pair-wise comparisons of genotypes.

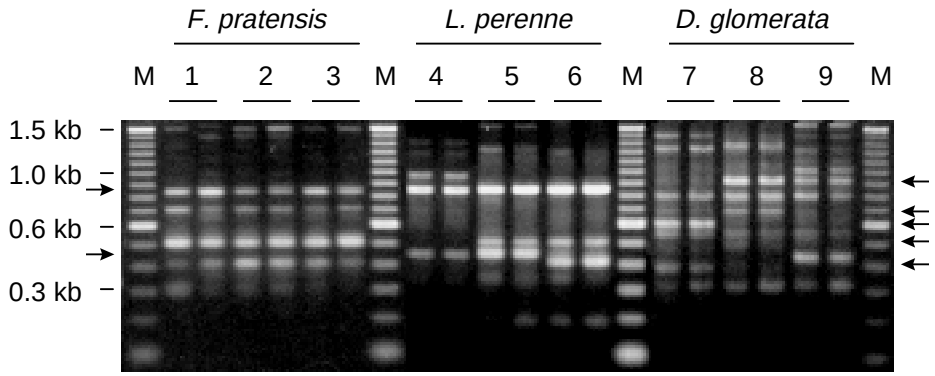

Figure 1. RAPD patterns of *F. pratensis*, *L. perenne*, and *D. glomerata* cultivars generated by primer OPB-12. Numbers indicate individual plants (two independent replicates). Arrows point to scorable polymorphic markers. M = 100 bp molecular marker.

Table 3. Analysis of molecular variance (AMOVA) for *Festuca pratensis*, *Lolium perenne*, and *Dactylis glomerata*, using 104 RAPD markers on three cultivars per species and 28 genotypes per cultivar

Source of variation	df	Sum of squares	Variance component[a]	% of total variance
Species	2	2711.6	15.4	63
Cultivar within species	6	368.0	1.9	8
Genotype within cultivar	243	1754.9	7.2	29
Within *Festuca pratensis*				
Cultivar	2	148.3	2.5	35
Genotype within cultivar	81	367.9	4.5	65
Within *Lolium perenne*				
Cultivar	2	112.5	1.7	18
Genotype within cultivar	81	651.1	8.0	82
Within *Dactylis glomerata*				
Cultivar	2	107.3	1.6	15
Genotype within cultivar	81	735.8	9.1	85

[a] All components were significant at $p < 0.001$, giving the probability of obtaining a more extreme random value computed from nonparametric procedures (1,000 data permutations).

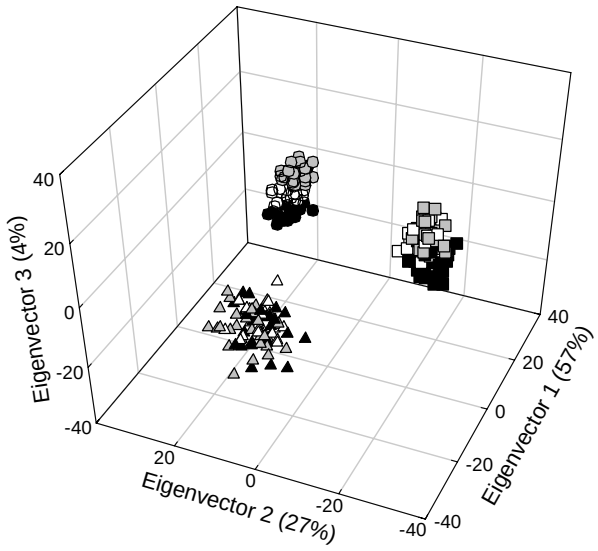

Figure 2. Principal coordinate analysis of 104 RAPD markers for three cultivars of *Festuca pratensis* (circles: black = Fure, grey = Préval, white = Darimo), *Lolium perenne* (squares: black = Arion, grey = Cavia, white = Respect) and *Dactylis glomerata* (triangles: black = Prato, grey = Reda, white = Loke).

Results

Characteristics of RAPD markers

The 12 primers generated 104 reproducible bands which were polymorphic and could be scored unequivocally across all genotypes (Figure 1). Fragment size ranged from 320 to 1500 bp. Each of the 252 genotypes was characterized through a unique RAPD phenotype. The percentage of polymorphic markers within each cultivar ranged from 22 to 56 (Table 2). There were four markers that occurred in all genotypes of *D. glomerata* but never in the other species. Such a fixed marker difference was also found for *F. pratensis* but not for *L. perenne*. While 41 markers were found in all three species, four were found only in *F. pratensis* and in *L. perenne* and 15 were only found in *D. glomerata*. Although marker frequency varied greatly across cultivars, no cultivar specific markers were identified.

Genetic variation based on RAPD markers

The first three eigenvectors of Principle Coordinate Analysis (PCO) extracted 88% of the total RAPD variation observed in the whole data set. All genotypes

Table 4. Mean values ($\bar{x}$) and coefficient of genotypic variation (CV_g) of five key vegetative growth traits of *F. pratensis* and *L. perenne* cultivars

Trait			*Festuca pratensis*			*Lolium perenne*		
			Darimo	Fure	Préval	Arion	Cavia	Respect
Tillers plant^{-1}	[no.]	$\bar{x}$	11.1[a]	10.7[a]	13.2[b]	21.9[e]	19.7[c]	17.7[d]
		CV_g	14.1	10.0	30.1***	26.2**	22.6***	27.6***
Leaf area plant^{-1}	[cm^2]	$\bar{x}$	214.6[a,b]	225.4[b]	225.7[b]	285.7[d]	250.3[c]	201.3[a]
		CV_g	5.9	19.4	30.3**	31.8***	26.1***	29.8***
Single leaf length[b]	[cm]	$\bar{x}$	21.9[d]	21.6[d]	21.7[d]	20.6[c]	17.6[b]	17.3[a]
		CV_g	7.5	9.3*	9.4	10.9***	13.6**	16.8***
Specific leaf area	[cm^2 g^{-1}]	$\bar{x}$	238.0[a]	249.5[b,c]	239.2[a,b]	234.1[a]	249.6[b,c]	257.5[c]
		CV_g	4.8	5.2	8.0*	6.0	0.8	6.4
Growth habit[c]	[no.]	$\bar{x}$	3.4[b]b	4.0[c]	2.6[a]	6.3[d]	6.4[d]	7.5[e]
		CV_g	26.8	36.5*	49.5**	17.9	20.7**	14.2

*, **, *** Significance of the mean square associated with the variance component at $p < 0.05$, 0.01 and 0.001.
[a] Means within rows followed by the same letter are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test.
[b] Average of two fully developed leaves.
[c] Visual scoring of the angle between the imaginary line through the region of the greatest leaf density and the vertical (1 = erect; 9 = prostrate).

were clearly separated into three groups according to species (Figure 2). While *D. glomerata* genotypes were clearly separated from the other genotypes by eigenvector 1, the distance between *F. pratensis* and *L. perenne* was due mainly to eigenvector 2. PCO resulted in a clear grouping of cultivars within *F. pratensis*, but grouping was poor within *L. perenne* and was not found in *D. glomerata*. However, PCO per species greatly improved the separation of cultivars within all three species (data not shown).

The three cultivars of *F. pratensis* showed the lowest variability within cultivars based on polymorphic markers and average Euclidean distance (Table 2), while Prato (*D. glomerata*) revealed the greatest diversity of all cultivars investigated. The genetic diversity within species was much lower for *F. pratensis* than for *L. perenne* and *D. glomerata*.

The variation observed in the whole data set was due mainly to variation among species (63%), while the variation among cultivars accounted for 8% and among genotypes for 29% (Table 3). Within species the sum of squares from analysis of molecular variance were lowest for *F. pratensis* (516.2) and highest for *D. glomerata* (843.1). Pair-wise comparison of variance heterogeneity within species (Bartlett's heteroscedasticity index) was significant only for the pairs *F. pratensis*/*D. glomerata* ($p < 0.05$) and *F. pratensis*/*L. perenne* ($p < 0.1$), but not for *D. glomerata*/*L. perenne* ($p < 0.7$). More than 80% of the variation within the species *L. perenne* and *D. glomerata* was due to variation between genotypes within cultivars (Table 3). This proportion dropped to 65% in *F. pratensis*, where 35% was due to variation between cultivars.

Variation in vegetative growth traits

Mean values of vegetative growth traits showed significant differences between cultivars and species. The number of tillers and the leaf area per plant were significantly lower for *F. pratensis* cultivars than for *L. perenne* cultivars with the exception of the low leaf area of Respect (Table 4). However, *F. pratensis* cultivars had significantly longer leaves and a more erect growth habit than cultivars of *L. perenne* (Table 4). Differences between the two species, found by comparing average values over all three cultivars were significant ($p < 0.05$) for all parameters except for specific leaf area.

Factor analysis explained 82.6% of the total variability (Table 5). Factor 1 included total leaf area, shoot dry weight, and number of tillers, factor 2 comprised leaf elongation duration and Phyllochron, and factor 3 contained growth habit. Single leaf parameters (single leaf area and length) were almost equally distributed between factors 2 and 3 (Table 5). While factor 1 did not separate the two species, factors 2 and 3 (single leaf parameters and growth habit) tend to separate *F. pratensis* plants from *L. perenne* plants (Figure 3). However, factor analysis did not enable the

Table 5. Varimax rotated scores for four factors of ten traits of *Festuca pratensis* and *Lolium perenne* from three cultivars per species and 28 genotypes per cultivar

Traits	Factors			
	1	2	3	4
Leaf area plant^{-1}	**0.97**	0.04	0.10	0.06
Shoot dry weight plant^{-1}	**0.94**	0.13	0.06	–0.21
Tillers plant^{-1}	**0.71**	–0.28	–0.48	0.00
Leaf elongation duration	0.15	**0.83**	0.22	–0.10
Phyllochron	–0.30	**0.71**	0.24	0.04
Single leaf area[a]	0.20	**0.50**	**0.75**	0.02
Single leaf length[a]	0.34	**0.59**	**0.61**	–0.05
Growth habit[b]	0.19	–0.14	**–0.80**	0.15
Specific leaf area	–0.03	–0.31	0.08	**0.88**
Shoot/root ratio	0.10	–0.34	0.32	**–0.75**
Variance explained by each factor	26.7%	21.3%	20.4%	14.2%

[a] Average of two fully developed leaves.
[b] Visual scoring of the angle between the imaginary line through the region of the greatest leaf density and the vertical (1 = erect; 9 = prostrate).

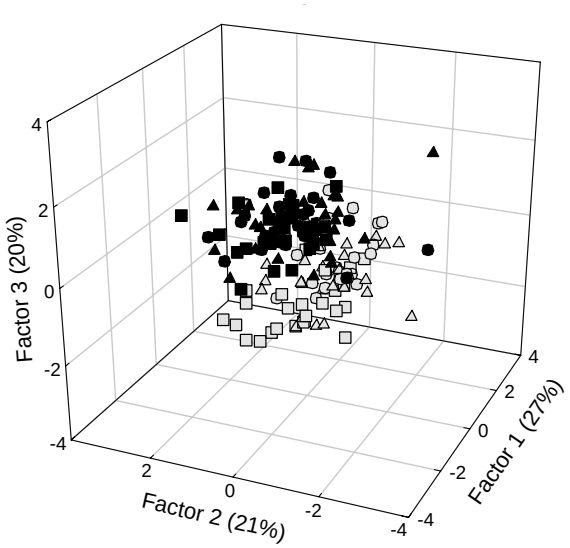

Figure 3. Factor analysis of ten key vegetative growth traits for three cultivars of *Festuca pratensis* (black: circles = Préval, squares = Darimo, triangles = Fure) and *Lolium perenne* (grey: circles = Arion, squares = Respect, triangles = Cavia).

separation of cultivars within species. This was also true for factor analysis within each species (data not shown).

Ranking of cultivars according to their genetic variability (expressed as coefficient of genotypic variation) depended greatly on the parameter investigated (Table 4). Except for Darimo, each cultivar showed the highest coefficient of genotypic variation for at least one parameter. Furthermore, Darimo showed very low values for most parameters investigated (Table 4). Average coefficients of variation were lower for *F. pratensis* than for *L. perenne* with the exception of specific leaf area and growth habit (Figure 4).

Discussion

Genetic variability within cultivars varied considerably depending on the species. This was true for the assessment of genetic variability by means of RAPD markers as well as by means of vegetative growth traits.

RAPD markers allowed a clear separation of the three species (Figure 2). *D. glomerata* plants were separated from *L. perenne* and *F. pratensis* plants primarily through eigenvector 1 which explained 57% of the variation, while the *L. perenne* and *F. pratensis* plants were separated by eigenvector 2, explaining only 27% of the variation. The close affinity of *L. perenne* and *F. pratensis* has also been reported in previous studies based on morphological traits (Bulinska-Radomska & Lester, 1988), isozymes (Charmet & Balfourier, 1994), and molecular markers (Stammers et al., 1995). Variability within *L. perenne* and *D. glomerata* cultivars was similar, while variability in *F. pratensis* cultivars was substantially lower (Table 2). The genetic variability found within *L. perenne* and *F. pratensis* cultivars is comparable to the findings of Huff (1997) and Kölliker et al. (1998); a comparable study for *D. glomerata* is not available. Genetic variability within natural populations of meadow fescue was found to be higher than that within cultivars (Kölliker et al., 1998), but is lower when compared to the variability within *L. perenne* and *D. glomerata* cultivars (Table 2).

Variability within species is due not only to variability within, but also between cultivars; thus, we used AMOVA to partition the sources of variation. The low genetic variability within *F. pratensis* was confirmed by AMOVA results per species (Table 3). While the total sum of squares was lowest for *F. pratensis*, the amount of variation due to cultivars was substantially higher when compared to *D. glomerata* and *L. perenne* (Table 3). The greater variation between cultivars could be explained in part by more diverse sources of parental genotypes (Table 1) or different breeding objectives for single cultivars. The genetic variability within individual cultivars may be

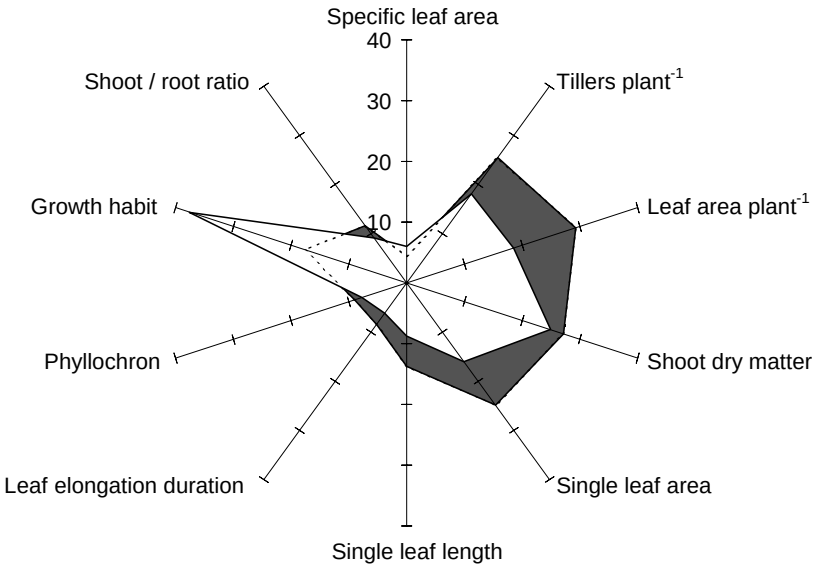

Figure 4. Average coefficient of genotypic variation of ten vegetative growth traits for *Festuca pratensis* (white) and *Lolium perenne* (black) based on three cultivars per species and 28 genotypes per cultivar.

influenced by the number of parental clones involved in breeding. This is a possible explanation for the low variability within Darimo which is based on only five parental clones. However, the other cultivars are all based on a comparable number of clones except for Fure which showed very low variability despite the 84 clones upon which it is based. Intense selection may also limit genetic variability within cultivars (Huff, 1997). It is difficult to estimate selection intensity based on information about the cultivars used in this study. However, it can be assumed that breeding methods and breeding intensity are comparable for all three species. Genetic variability is also influenced by the breeding system (Charlesworth & Charlesworth, 1995), and isozyme variation in the genus *Lolium* is known to decrease with a reduced level of cross-fertilization (Charmet & Balfourier, 1994). All three species investigated are true out-breeders with a high degree of self-incompatibility. Troll (1931) reported a lower self-fertilization rate for *D. glomerata* than for *F. pratensis* and *L. perenne*, but this may be due to the higher level of ploidy (Lundqvist, 1969). Moreover, genetic variability was lower in *F. pratensis*, although its self-fertilization rate is lower than that of *L. perenne* (Charmet & Balfourier, 1994). Genetic variability is usually higher in polyploid species (Xu & Sleper, 1991; Soltis & Soltis, 1993). This may play an important role in the high variability within *D. glomerata* (2n = 4x = 28) as compared to *F. pratensis* and *L. perenne* (2n = 2x = 14), although the difference between *D. glomerata* and *L. perenne* was small (Table 2). We selected the two diploid and closely related species *F. pratensis* and *L. perenne* to investigate genetic variability based on vegetative growth traits.

F. pratensis cultivars differed significantly from *L. perenne* cultivars for most vegetative growth traits investigated, while differences among cultivars of the same species were smaller. This is in good agreement with the RAPD results that showed clear differences between these two species but a less distinct separation of the cultivars within the species (Figure 2). *L. perenne* plants had more tillers and a more prostrate growth habit than *F. pratensis* plants (Table 4). This is an expression of the ability of *L. perenne* to withstand continuous and intensive grazing (Jung et al., 1996). The species-specific differences were also made evident by factor analysis (Figure 3); plants were mainly separated according to growth habit (factor 3) and single leaf parameters (factor 2) (Table 5). Although the separation was not as clear as with RAPD analysis, plants were grouped according to the respective species.

The low genetic variability within *F. pratensis* cultivars detected with RAPD markers was also reflected in morphological traits, although the ranking of the cultivars according to their coefficient of genotypic variation depended on the trait investigated (Table 4). This was also observed by Helgadottir & Snaydon (1986) who showed that variability depended not only on traits, but also on the site and the year of in-

vestigation. Different authors have reported a lack of congruence between variability estimates based on DNA markers, isozymes, and morphological traits (Beer et al., 1993; Fernando et al., 1997). Estimates of genetic variability based on morphological traits may be biased by phenotypic plasticity which can evolve independent from genetic variability (MacDonald and Chinnappa 1989). In our investigation, average variability within species was lower for *F. pratensis* for most vegetative growth traits (Figure 4), which is in good agreement with the RAPD data. Analysis of variance showed that the variation in growth habit was mainly due to variation between species (56%) and to variation between genotypes within cultivars (14%) rather than to variation between cultivars within species (4%) (data not shown). Therefore, the high variability in growth habit is unlikely to be a result of different breeding objectives for the individual cultivars (e.g. grazing types versus cutting), which would result in large differences between cultivars. Large variability in growth habit may be an advantage in competition for light because it ensures optimal positioning of leaf area in a multi-species sward. However, even if a high variability in some traits might be advantageous, the large differences in mean values between *F. pratensis* and *L. perenne* certainly remain responsible for the different adaptation of the two species.

To the best of our knowledge this is the first report to compare the genetic variability of widely used cultivars of three important forage grasses based on genetic markers and morphological traits. The genetic variability detected with RAPD markers was considerably lower for the three cultivars of *F. pratensis* when compared to the same number of cultivars of *L. perenne* and *D. glomerata*. Analysis of key vegetative growth traits confirmed the lower variability of *F. pratensis* as compared to *L. perenne*. Although plant adaptation is induced by environmental factors, it depends on genetic resources for a specific response (Bradshaw, 1984). Therefore, limited genetic variability could be one factor contributing to the decline of meadow fescue from intensively managed grassland. Further investigations are now needed to elucidate the significance of genetic variability for the adaptability and persistence of cultivars and species.

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