

DOI: 10.22620/agrisci.2011.07.002

ИЗТОЧНИЦИ НА ЗАРОДИШНА ПЛАЗМА ПРИ ФУРАЖНИТЕ ВИДОВЕ *FESTUCA PRATENSIS* HUDS.
И *FESTUCA ARUNDINACEA* SCHREBER ЗА СЕЛЕКЦИЯ И СЪХРАНЕНИЕ *EX SITU/IN SITU*
SOURCES OF GERMLASM OF *FESTUCA PRATENSIS* HUDS. AND *FESTUCA ARUNDINACEA* SCHREBER FOR
BREEDING AND CONSERVATION *EX SITU/IN SITU*

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Резюме

Два тревни вида с широк потенциал за фуражното производство в Европа - *Festuca pratensis* Huds. (ливадна власатка) и *Festuca arundinacea* Schreber (тръстиковидна власатка), са избрани като модел за проучване на условията, които въздействат върху функционирането на естествените агроecosистеми (ливади и пасища) като консервационни местообитания на генетичните ресурси. В резултат на експедиции за колекциониране през летния период на 2006-2008 г. са събрани нови семенни проби от избраните видове. Общо 36 образци от *Festuca pratensis* и 10 образци от *Festuca arundinacea*, колекционирани от 43 местообитания (ливади и пасища), които принадлежат към 10 биогеографски района на България, са характеризирани по дескриптора на UPOV. Получените резултати от морфологичната оценка показват значителни различия между референтните сортове и ливадните екоципове основно по характеристиките, свързани с жизнения статус, растежния хабитус, височината на растенията, фазите на изметляване и цъфтеж. В резултат на електрофоретичен анализ, извършен по стандартния метод на ISTA, е установен нов белтъчен компонент в 6 образци с произход от България, който е с висока плътност и не се наблюдава в селекционните материали и екоципове с произход от Швейцария.

Целта на настоящото проучване е разширяване на генетичната основа на колекцията от фуражни житни треви от двата вида (*Festuca pratensis* Huds., *Festuca arundinacea* Schreber) с нова зародишна плазма – широко разнообразие от форми и популации, чрез колекциониране в широк обхват от различното екологично богатство: биогеографски региони, надморска височина, ливадни типове, начини на използване на териториите (пасища, ливади, земеделски и неземеделски земи).

Abstract

Two grass species with wide potential for forage production in Europe - *Festuca pratensis* Huds. and *Festuca arundinacea* Schreber are chosen as a model to investigate conditions affecting the functioning of natural agro ecosystems (meadows and pastures) as conservation sites for genetic resources. As a result of collecting expeditions in the summer of 2006 to 2008 new seed samples of target species were collected. A total of 36 accessions of *Festuca pratensis* and 10 accessions of *F. arundinacea* sampled in 43 sites (meadows and pastures), belonging to 10 bio geographic regions of Bulgaria were evaluated in accordance with the UPOV descriptor list. The results of the morphological data indicated significant differences between the referent cultivars and the grassland ecotypes mainly in features relating to plant vigor, growth habit, height of the plants, ear inflorescence and flowering. As a results of electrophoresis, applied in compliance with the standard method of ISTA, a new protein component was detected in 6 accessions of Bulgarian origin as a band with high density which was not observed in the breeding materials and ecotypes originating from Switzerland.

The aim of the investigation is to enlarge the genetic basis of the collections from forage grasses of the two species (*Festuca pratensis* Huds., *Festuca arundinacea* Schreber) with new germplasm – a large diversity of forms and populations by collecting from a wide range of distinct ecological niches: different bio-geographic regions, altitudes, grassland types, land use (pastures, meadows; agricultural and non-agricultural land).

Ключови думи: фуражни тревы, *Festuca pratensis*, *Festuca arundinacea*, зародишна плазма, електрофореза.
Key words: forage grasses, *Festuca pratensis*, *Festuca arundinacea*, germplasm, electrophoresis.

INTRODUCTION

Bulgarian forage grass collection is held in the National genebank located in the IPGR-Sadovo. A total of 5367 accessions are preserved under long term and medium term storage conditions (Guteva et al., 2007). Recently collecting priority is given to crop wild relatives of forage grasses as essential components of natural and semi-natural grasslands.

Two grass species with wide potential for forage production in Europe - *Festuca pratensis* Huds. and *Festuca arundinacea* Schreber are chosen in the present study as a model to investigate conditions affecting natural agro ecosystems. *Festuca pratensis* is an important component of permanent grassland in higher altitudes due to its good winter hardiness. During the last decades was observed decline of abundance of this species in intensively managed meadows (Carlen, 1994; Carlen et al., 2002; Peter-Schmid et al., 2008). It is suggested that a better knowledge of ecotype populations derived from well-defined habitats could assist for selecting adapted genotypes for further use in forage grass breeding and in defining valuable sites for *ex-situ* and *in-situ* conservation (Peter-Schmid et al., 2008). The large diversity of forms and populations should be achieved by collecting in a wide range of distinct ecological niches: different bio-geographic regions, altitudes, grassland types land use (pastures, meadows, agricultural and non-agricultural management).

On the other hand detection of similarity or genetic diversity between genotypes and populations of grasses on the basis of methods independent of environmental factors could assist both to the breeding process and biodiversity protection. As reported seed protein electrophoresis is known to be used for evaluation of grass varieties and presented clear-cut protein polymorphism (Krishnan and Slepser, 1997; Gilliland et al., 2000).

Two main objectives of this research are outlined: widening of genetic basis of collections from both grass species (*Festuca pratensis* Huds., *Festuca arundinacea* Schreber) with new germplasm and evaluation of diversity by phenotypic and genetic characterization.

MATERIALS AND METHODS

Over the period of three years (2006-2008) was carried out characterization of collecting sites and grassland habitats. In each one of the visited sites were recorded location characteristics, land use and management by the interview with farmers for grassland cultivation history. The botanical composition was determined according to Dietsl, 1995 within a square of 25 m² the abundance and yield of all species were estimated.

Itinerary: Central South Bulgaria (Tracian plain, Rhodopi); South West Bulgaria (Rila, Pirin, Osogovo, Vitosha); Eastern Bulgaria (Strandza, East Balkan); North Bulgaria (Forebalkan, Central Balkan, Danube plain hilly).

Single tillers from 60 separated plants were randomly sampled (at least 3 m between the sampled plants) at all collecting sites. Collected accessions were multiplied in the field with 120 spaced plants in isolation of triticale (50 m distance between the accessions). The multiplied seeds were used for further plot experiments for agronomical and laboratory evaluation. Seeds of all accessions were conserved in the gene bank and supplied with passport information (multi-crop-passport descriptors - EURISCO).

A field nursery with 60 plants per accession (6 replications of 10 plants each) were planted in the field of the Institute of Plant Genetic Resources, Sadovo in springtime of 2006 and of 2007. Observations were made according to UPOV guidelines by 11 morphological characters assessed for each individual plant (UPOV'2002).

Acid-PAGE (acid polyacrilamide gel electrophoresis) is carried out according to known standard reference method of ISTA (Drapper, 1987; Anonymous, 2003). Proteins are extracted from bulk sample of 40mg grinded and fine powdered seeds with 400µl extracting solution (0.05 g Pyronin G; 25 ml 2-chloroethanol) stained for a night at room temperature and centrifuged 20 min at 17000g. 20µl of extracts are loaded into wells. Gel medium of 10% acid-PAG (pH=3.1) is prepared in 1.5 mm thin gel, using vertical unit Consort E835 (with gel cassette 200 X 200 mm). Electrophoresis is carried out at 120 V for 3 hours at 19-20°C. Staining of gels is performed in solution of Coomassie Brilliant Blue G250:Coomassie 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.

RESULTS AND DISCUSSION

New entries involved in this study were collected during 2006-2008 in 43 sites belonging to 10 bio-geographic regions of Bulgaria. 46 ecotype populations include *Festuca pratensis* (36 acc.) and *F. arundinacea* (10acc.) (Table 1).

In situ conservation approaches are used to describe the ecogeographic surveys of original habitats. The visited permanent grasslands are divided into two parts: (1) lowlands and hilly areas and (2) mid mountain and high-mountain areas. Collecting sites are selected with regard to variation in geographic region, altitude and management intensity. All habitats are comprehensive documented in



Table 1. Collected accessions by regions and sites

Bio-geographic regions	Sites	Species <i>Festuca</i>		Total
		FES-PRA	FES-AR	
Central South Bulgaria				
Tracian plain	4	4	2	6
Rhodopi	15	13	2	15
South West Bulgaria				
Rila	6	6	-	6
Pirin	2	2	1	3
Osogovo	1	1	-	1
Vitosha	3	3	-	3
South Eastern Bulgaria				
Strandza	1	1	-	1
North Bulgaria				
Forebalkan	7	4	3	7
Central Balkan	2	2	-	2
Danube plain hilly	2	-	2	2
Всичко / Total	43	36	10	46

**FES-PRA - *Festuca pratensis* Huds.; FES-AR - *Festuca arundinacea* Schreber

terms of: geographic locations, relief, botanical composition and management intensity (Table 2 and Table 3).

Semi-natural grasslands have a relatively natural character because of significant richness in species thanks to low fertilizer input and continuous use as grasslands over a long period. Permanent grasslands in Bulgaria cover 18.7% of total agriculture area. Semi-natural grasslands cover 38.2% of permanent grasslands and 7.2% of total agriculture area.

The visited permanent grasslands include meadows and pastures in lowlands, hilly, mid mountain and high-mountain areas. Their utilization is not intensive especially during the last 10-15 years. There should be pointed that usually there is carried out not more than one cut of meadows per year. The grazing in summer covers April to November.

According to the statements of the farmers about the cultivation history of the sites all of them are with long use history and extensive type of utilization: traditionally for hay – only one and in rare cases two mowing and for grazing. In nine of the sites was observed reduced to no use (or casual use) because of decreasing stocking rate and the lack of improvement lead to grassland degradation, with a return to woody shrubs and expansion of forest over former grassland areas: Forebalkan - site No 2; Osogovo - site No 1; Rhodopi Mts. – sites No 3,5,13; Pirin - site No 3; Vitosha - sites No 1,2,3 (Table 2 and Table 3). Application of manure, irrigation on meadows and pastures has not been traditionally practiced in the country. Only in the sites No 2 and 3 in Rila Mts and in Pirin Mts – sites No 1 and 2 was carried out organic fertilization periodically and irrigation (grassland type *Lolietum multiflorii*; *Trifolio-lolion perennis*) (Table 2 and Table 3). None of the sites had been amended

by over-sowing with cultivars ever. According to the frequency of utilization and fertilization most of habitats could be classified into two groups: more of them are extensive and only four intermediate.

The botanical composition of visited habitats illustrates the significant species diversity, where the higher rate is observed of 40-50 species in grassland types of: *Lolii perennis* - *Arrhenatherum elatius* and *Holcus lanatus* - *Festuca pratensis* (Table 2 and Table 3). There should be pointed that *Festuca pratensis* is in abundance in mid and high mountain grassland habitats, where in contrast *F. arundinacea* is in abundance in low altitudes – plains and hilly areas. Only three ecotypes of tall fescue are collected on higher altitudes between 810-1219 m a.s.l. in Rhodopi and Pirin mountains: A6E0042 A6E0037 A7E0011. The higher percentage part of *Festuca pratensis* in the composed grass yield is observed in the sites of Rhodopi Mts. predominantly meadows types *Lolii-perennis*-*Arrhenatherum elatius* and *Holcus lanatus*-*Festuca pratensis* – 15 to 40% (Table 2 and Table 3).

A total 11 morpho-physiological characters were assessed on each spaced plant of *F.pratensis* and *F. arundinacea*. The growth habit before first hibernation and tendency to form inflorescences before first hibernation were already scored in autumn while all remaining characters were assessed in spring after the first hibernation (Table 4).

The results of the morphological data indicated differences between the cultivars (Preval and Cosmolit) and the grassland accessions of *F. pratensis* by characters: growth habit, natural plant height before vernalization and at ear emergence, length of longest stem, date of ear emergence, number of culms. One of the both Swiss

Table 2. Information for collecting sites and species for grasslands in mountain areas – Central and West South Bulgaria

Mountain's name	Rhodopi (1)										Rila (3)					Pirin (4)			Vitosha (5)			Balkan (2)							
	1	2	3	4	5	6	7	8	9	1	1	1	1	1	1	1	1	2	3	4	5	6	1	2	3	1	2	3	1
No of sites	990	679	1323	1366	1450	1360	1188	1509	1219	1140	1384	1134	1013	1412	1252	910	650	1090	1144	1800	810	810	1752	1338	1330	1378	1079	596	
Altitude m a.s.l.	990	679	1323	1366	1450	1360	1188	1509	1219	1140	1384	1134	1013	1412	1252	910	650	1090	1144	1800	810	810	1752	1338	1330	1378	1079	596	
Exposition	W	W	W	SE	W	W	W	SE	W	SE	SE	SE	N	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	
Number of species	42	40	43	37	42	40	44	40	47	42	36	40	39	42	43	28	38	35	33	26	30	22	25	28	36	36	40	42	
Management:																													
Number of cuts	1	1	no	2	no	1	1	1	1	2	1	1	no	1	2	1	2-3	1	1	1	1	2	2	no	no	no	no	1	
Irrigation																													
Fertilization (organic)																													
Type of grassland																													
<i>Lolium perennis</i> - <i>Arrhenatherum elatius</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
<i>Holcus lanatus</i> - <i>Festuca pratensis</i>																													
<i>Lolietum multiflori</i>																													
<i>Trifolium lolion perennis</i>																													
<i>Agrostis capillaris</i> - <i>Festuca falax</i>																													
Grasses %	68	65	72	80	60	75	60	70	75	75	75	70	75	65	75	80	78	78	75	80	85	85	65	60	65	55	72	73	
Legumes %	15	10	22	8	20	15	18	10	20	5	8	5	10	8	10	12	10	8	7	3	8	8	6	10	8	10	8	5	
Others %	17	25	6	12	20	10	22	20	10	20	17	20	25	15	27	15	8	12	14	18	17	7	19	30	21	35	20	22	
Yield classes of:																													
<i>Festuca pratensis</i>	6	6	5	3	7	5	5	6	6	5	5	3	5	6	6	5	2	3	3	3	3	5	5	5	6	5	5	6	
<i>Festuca arundinacea</i>																													
<i>Arrhenatherum elatius</i>	6	7	7	5	2	7	6	6	7	6	5	5	6	5	3	6	1	1	2	2	1	1	1	3	5	5	3	5	
<i>Trisetum flavescens</i>	3	2	4	3	2	7	3	5	4	2																			

Yields classes: 1= <1%, 2= 1-2%, 3= 2-5%, 4= 5-9%, 5=9-15%, 6= 15-25%, 7=25-40%, 8=40-60%, 9= >60%

Type of grasslands: *Lolium perennis*-*Arrhenatherum elatius*; *Holcus lanatus*-*Festuca pratensis*; *Lolietum multiflori*; *Trifolium multiflori*; *Trifolium lolion perennis*; *Agrostis capillaris*-*Festuca falax*;
Target species: *Festuca pratensis* and *Festuca arundinacea* *. Only 3 accessions of *Festuca arundinacea* (tall fescue) were collected in visited sites in middle and lower mountain grasslands – 1219 m a.s.l. is the highest point where was collected.

*exposition: (-) flat



Table 3. Information for collecting sites for grasslands in lowlands and hilly regions

Region names	Tracian plain (6)			Forebalkan (N) & Balkan (NE) (7)			Danube plain (10)			Strandza (8)	Osoгово (9)						
No of sites	1	2	3	4	5	6	1	2	3	4	5	6	7	1	2	1	1
Altitude m a.s.l.	230	173	149	149	148	330	65	420	402	443	488	422	400	85	55	250	639
Exposition	-	-	-	-	-	E	N	SW	SW	NW	NW	W	S	-	NW	E	S
Number of species	16	36	42	24	27	35	38	32	40	42	23	20	22	33	35	37	35
Management:																	
Number of cuts	1	grazing	1	1	1-2	1-2	no	1	1	1	1	1	1	2	grazing	1	no
Irrigation	Without irrigation																
Fertilization (organic)	Without fertilization																
Type of grassland																	
<i>Lolium perenne</i> - <i>Arrhenatherum elatius</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Chrysopogon grillus</i>																	
<i>Cynodon dactylon</i> - <i>lolium perenne</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grasses %	84	65	82	70	75	67	51	70	70	73	82	75	80	72	80	68	60
Legumes %	2	8	6	17	8	15	20	22	14	17	9	4	5	8	8	9	15
Others %	4	27	12	13	17	18	19	8	16	10	9	10	15	10	12	13	25
Yield classes of:																	
<i>Festuca pratensis</i>	2	1	4	6	3	4	4	4	5	6	2	2	2	2	2	2	4
<i>Festuca arundinacea</i>	9	3	1	2					8	3	8	3	8	5	5		
<i>Arrhenatherum elatius</i>	2	1	1	1	1	2	1	2	1	3	3	2					1

Yields classes: 1= <1%, 2= 1-2%, 3= 2-5%, 4= 5-9%, 5=9-15%, 6= 15-25%, 7=25-40%, 8=40-60%, 9= >60%. **Type of grasslands:** *Lolium perenne*-*Arrhenatherum elatius*: *Chrysopogon grillus*; *Cynodon dactylon* - *Lolium perenne*; **Target species** (of the project): *Festuca pratensis* is in low abundance in plain areas in contrast of *Festuca arundinacea* because of dry climatic conditions in low altitudes
**exposition: (+) flat

Table 4. Description of morphological characters measured on *F. pratensis* and *F. arundinacea* ecotype populations and reference cultivars

Characters	Description	Method
GH _{AU}	Growth habit before hibernation	Scored in autumn of the sowing year: 1=erect, 9=prostrate
GH _E	Growth habit during vegetative stage	Scored in autumn of the sowing year: 1=erect, 9=prostrate
NH _{AU}	Natural plant height before vernalization	Measured in autumn of the sowing year (cm)
TI _{AU}	Tendency to form inflorescences before vernalization	Scored in autumn of the sowing year 1=absent, 9=very strong
NH _E	Natural plant height at ear emergence	Measured at ear emergence (cm)
DE	Date of ear emergence	Determined as number of days after 1 st March (days)
LC	Length of longest stem	Measured 30 days after ear emergence (cm)
WF	Width of flag leaf	Measured 30 days after ear emergence (cm)
LF	Length of flag leaf	Measured 30 days after ear emergence (cm)
LE	Length of ear	Measured 30 days after ear emergence (cm)
NC	Number of culms	Counted 30 days after ear emergence (cm)

ecotypes (No 8014) is earlier – time of inflorescence emergence is in average 30 days (after 1st April). Growth habit before first hibernation at the majority of Bulgarian grassland accessions is intermediate to semi-prostrate. Medium to strong is the tendency to form inflorescences before hibernation of *F. arundinacea* accessions and absent or weak for *F. pratensis* accessions. An exception of this tendency is determined for one accession of *F. arundinacea* – A7E0010, which was estimated as weak (class 1-3). On the opposite, one accession of *F. pratensis* was estimated as medium (A5E0002- collected in Tracian lowland - Popovitz). Two accessions of *F. arundinacea*, originated from Danube plain hilly - A7E0010 and 92E0045 were late with more 18 and 15 days after the first of May (Table 5).

The correlations between presented ten morphological traits of *F. pratensis* are not sufficiently expressed. Poor positive correlation is detected between plant high and higher culm and poor negative correlation between heading date and plant high (Table 6).

Acid-PAGE: Seventeen populations of *F. pratensis* collected in Bulgaria were used for evaluation by acid-PAGE. For comparison two ecotypes from Switzerland and cv. Cosmolit are included in this accession set. The typical spectra of alcohol-soluble proteins (prolamins) extracted from *F. pratensis* seeds is shown in Fig 1. The polymorphism of protein spectra was described by repeated analyses of extracting procedures and separation in acid-PAGE. On the basis of component mobility and band density was created database (Table 7). A new protein component was

detected in 6 accessions as a band with high density which was not observed in the breeding material nor yet in the two ecotypes from Switzerland. However further analyses are needed in comparison with a larger number of Swiss ecotypes that will clarify the originality of this component.

CONCLUSIONS

Typical sources of both species *Festuca pratensis* and *Festuca arundinacea* are established within pastures and meadows from 10 different eco-geographic regions of Bulgaria. Data base is created for accessions and habitats. As result the national collection of genus *Festuca* is enriched with 46 new entries collected in 43 sites.

The permanent meadows in Rhodopi Mountains and in Balkan Mountains (Forebalkan) could be described as a rich complex of multi species plant communities. There dominated various grass species that compose 65-80% of the yield and that is the place of natural distribution of *Festuca pratensis*.

The results of field experiments is the basis for further improvement of existing information with new one concerning new collected germplasm. New valuable sources for breeding programs are detected possessing useful traits: growth habit, heading date, plant high.

By acid-PAGE is described the genetic variation between 17 populations from Bulgaria. This approach could be further used for identification of genotypes on the basis of biochemical molecular markers independent of environment pressure.



Table 5. *Festuca pratensis* collection nursery 2006-2007: Least Squares Means of 20 *Festuca pratensis* for morphological characters: two cultivars, two Swiss ecotype populations and 16 BG ecotype populations collected from 8 bio-geographic regions: Mid and high altitudes: Rhodopi Mts. 1; Rila 3; Pirin 4; Vitosha 5; Plain and hilly low altitudes: Tracian plain 6; Forebalkan (N) 7; Strandza Mts. 8; Osogovo Mts. 9; Danube plain hilly 10

Nr. acc	Origin	group	NH _{AU}	GH _{AU}	GHe	NHs	NHe	WF	LF	LC	NC	DE	
1 8014	Boppelsen	CH	43.1	3.6	4.3	76.3	106.6	0.73	13.6	117.1	191.3	30.5	
2 8015	Grandval	CH	38.5	4.3	4.8	72.7	97.1	0.68	9.7	113.9	171.6	41.1	
3 6738	Stojkite /Rhodopi Mts.	1	BG	40.6	3.6	4.4	61.2	90.6	0.61	8.8	115.5	106.5	48.1
4 98E0015	Velingrad	1	BG	34.8	5.2	4.7	82.1	106.3	0.63	11.1	120.4	129.6	39.4
5 98E0016	Yundola	1	BG	29.1	5.1	4.2	82.0	106.6	0.61	9.4	121.8	129.3	37.8
6 98E0008	Pamporovo	1	BG	20.2	5.5	4.9	78.9	105.7	0.72	13.8	116.1	119.9	40.3
7 6734	Slokucevo / Rila Mts.	3	BG	33.4	6.0	5.8	81.9	107.0	0.63	9.4	129.4	190.7	43.6
8 6977	Marica	3	BG	44.6	4.6	5.0	76.7	108.4	0.59	8.7	121.9	133.0	40.5
9 98E0012	Rila monastery 1	3	BG	40.5	4.9	4.1	78.3	107.0	0.61	10.3	121.5	126.2	41.6
10 98E0014	Rila monastery	3	BG	37.8	4.9	4.4	79.8	107.1	0.60	10.2	125.4	145.4	42.1
11 98E0021	Part.Poliana	3	BG	34.9	4.5	3.3	76.4	103.6	0.57	9.3	115.2	125.3	42.5
12 6982	Bansko / Pirim Mts.	4	BG	41.4	4.2	4.6	74.9	106.4	0.59	9.2	121.8	133.8	38.8
13 98E0013	Banderishki	4	BG	37.3	4.3	3.9	78.4	104.7	0.58	8.4	119.7	116.3	39.5
14 98E0018	Kopitoto 1/Vitosha	5	BG	32.9	5.1	4.3	78.6	106.9	0.62	10.8	123.0	127.2	43.2
15 6739	Dolnoslav / Trakia	6	BG	35.2	4.7	5.3	78.1	101.8	0.63	8.4	121.0	220.5	42.0
16 6733	Orizare / Forebalkan	7	BG	44.0	6.2	5.9	76.0	102.6	0.63	9.8	115.5	205.7	36.2
17 93E0028	Burgas / Strandza	8	BG	22.9	5.8	4.7	75.2	101.3	0.59	11.4	107.4	122.4	38.9
18 98E0020	Hisariaka / Osogovo	9	BG	38.0	5.5	4.7	82.4	110.3	0.66	10.5	122.6	133.7	39.8
19 Cosmolit	Cosmolit	St	35.4	3.9	4.0	84.2	111.8	0.66	9.7	123.8	146.0	37.2	
20 Preval	Preval	St	41.7	3.1	3.1	83.9	112.2	0.72	11.5	127.8	164.9	39.6	

NH_{AU} Natural plant height before vernalization, GH_{AU} Growth habit before hibernation, GHe Growth habit during vegetative stage, NH_s Natural plant height at spring, NH_E Natural plant height at ear, emergence, LC Length of longest stem, WF Width of longest stem, LF Length of flag leaf, LE Length of ear, DE Date of ear emergence (after 1st April).

Table 6. Correlation coefficients between morphological characters – *F. pratensis*

	NH _{AU}	GH _{AU}	GHe	NHe	LC	NC	WF	LF	LE	DE
NH _{AU}	1									
GH _{AU}	-0.23073	1								
GHe	-0.17352	0.392335	1							
NHe	0.206789	-0.24371	-0.22948	1						
LC	0.349954	-0.17815	-0.2739	0.673042	1					
NC	0.077206	0.091049	0.116293	0.217005	0.160634	1				
WF	0.195562	-0.10205	-0.10785	0.189211	0.220371	-0.02474	1			
LF	0.007691	-0.0382	-0.04326	0.00792	-0.06634	-0.02844	0.287504	1		
LE	0.036272	-0.04038	-0.05617	0.137772	0.18239	-0.03948	0.304043	0.279172	1	
DE	-0.09951	0.334063	0.247023	-0.64087	-0.27164	-0.146	-0.13148	-0.08055	-0.085	1

NH_{AU} Natural plant height before vernalization, GH_{AU} Growth habit before hibernation, GHe Growth habit during vegetative stage, NH_s Natural plant height in spring, NHe Natural plant height at ear, emergence, LC Length of longest stem, WF Width of flag leaf, LF Length of flag leaf, LE Length of ear, DE Date of ear emergence (after 1st April).

Table 7. ACID-PAGE of local populations *F. pratensis* from Bulgaria
 Rate of the band density: 0 – missing; 1- minor (traces); 2 – medium; 3 - dense

№	Accession	Position in the gel	Number of the component										
			1	2	B	3/3'	4	5	6	7	8	9	10
1.	Preval*	1,22	3	3	0	1	0	2	3	0	1	3	0
2.	93E028	2	3	1	0	1	0	0	1	0	2	3	0
3.	98E020	3	3	0	3	1	2	3	3	2	2	3	2
4.	98E012	4	3	1	3	1	1	1	0	0	3	3	1
5.	98E016	5	3	3	1	1	2	3	0	2	1	3	2
6.	98E015	6	3	1	2	1	0	0	0	2	2	3	1
7.	98E014	7	3	1	2	1	3	2	0	1	2	3	1
8.	98E021	8	3	0	0	1	1	0	1	2	1	1	1
9.	98E013	9	3	1	2	1	1	1	0	0	2	2	1
10.	98E008	10	3	1	0	0	1	1	0	3	2	3	0
11.	Cosmolit (St)*	11	3	2	0	1	0	1	0	1	2	2	1
12.	98E018	12	3	1	2	1	2	0	2	1	2	3	2
13.	A5E0001	13	3	3	0	0	3	2	0	2	1	3	1
14.	A7E0004	14	3	2	3	1	2	2	1	0	1	3	0
15.	A7E0008	15	3	1	1	0	1	1	2	0	1	3	0
16.	A7E0009	16	3	3	1	1	0	1	1	2	0	3	0
17.	A7E0006	17	3	2	1	0	1	1	1	3	0	3	0
18.	A7E0007	18	3	1	1	1	3	1	0	2	1	3	1
19.	A7E0005	19	3	1	0	0	3	2	0	2	1	3	2
20.	F.p.p.8015*	20	0	3	1	1	3	3	0	0	2	3	0
21.	F.p.p.8014*	21	0	3	1	1	2	3	0	0	2	3	0

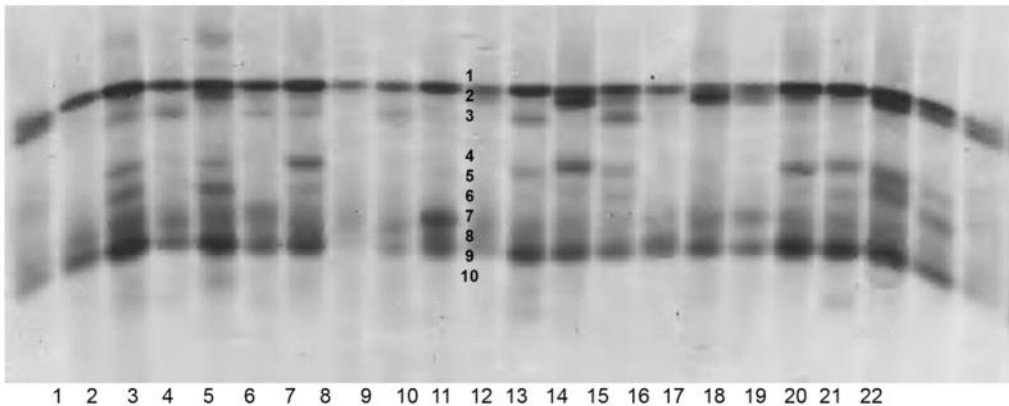


Fig. 1. A+CID-PAGE of seed proteins observed in local populations of *Festuca pratensis*

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Acknowledgements

This research was supported by SCOPES/Joint Research Projects No IB73A0-111010, Swiss National Science Foundation and The National Science Fund of Ministry of Education and Science, Bulgaria, project No CC – 1603 “Characterization of *Festuca* spp. (*Festuca pratensis* Huds. and *Festuca arundinacea* Schreber) from diverse habitats of Bulgaria”.

Статията е приета на 29.07.2011 г.
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