Species and Varieties of Perennial Grasses for High Quality Forage in Bulgaria

Aneliya Ilieva Katova
(Institute of Forage Crops, Pleven, Bulgaria 5800)

Abstract: To obtain sustainable agriculture and adaptive forage production, it is necessary to develop new varieties of forage crops, combining high productivity and ecological stability. Perennial grass breeding in Bulgaria has now a strong 49 year-long tradition, especially in the Institute of Forage Crops in Pleven. The objective of breeding program was to develop new perennial grass varieties with high forage and seed productivity, high forage quality and high adaptive potential for pasture, hay and landscape improvement use. A great amount of initial breeding materials (local native populations and introduced varieties) of perennial forage grasses of cool and warm climate was collected and studied in the Institute of Forage Crops, Pleven during the period 1966-2015. Biodiversity of new plant forms and varieties was developed by applying conventional and modern breeding methods-purposeful efficient selection by productivity and adaptivity, ecologogenetic analysis of quantitative traits, polyploidization, hybridization, including interspecific one. The ploidy level was determined in the Institute of Genetics and Breeding, Merelbeke, Belgium in 2007. The samples were analyzed by Partec Cell Analyzer CA-II and software DPAC (Münster, Germany). The intensity of fluorescent emission correlated linearly with DNA quantity. The results were obtained as histograms. During these years six varieties of 6 perennial grass species were developed as follows: cocksfoot (*Dactylis glomerata* L.) Dabrava, smooth brome, (*Bromus inermis* Leyss.) Nika, tall fescue (*Festuca arundinacea* Schreb.) Albena, perennial ryegrass (*Lolium perenne* L.) IFK Harmoniya, crested wheatgrass (*Agropyron cristatum* Gaerth.) Svejina and standard wheatgrass (*Agropyron desertorum* (Fich.) Schultes.) Morava. Variety description was made according CPVO (2011) and UPOV (2006) technical guidelines. The perennial grass varieties have valuable characteristics, such as high forage and seed productivity, persistency, stress tolerance, forage quality, different direction of use and different ploidy level. The Institute of Forage Crops maintains the registered varieties and produces Prebasic and Basic seeds.

Keywords: perennial grasses; varieties; forage yield; forage quality; ploidy level
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To obtain sustainable agriculture and adaptive forage production, it is necessary to develop new varieties of forage crops, combining high productivity and ecological stability. Reestablishment and establishment of new grass areas intended for meadows and pastures, landscape maintenance and soil erosion control are required in connection with the taken measures for help to rural development in Bulgaria according to EU agreements (2014-2020) and for environment protection and sustainable land management. Under Bulgarian conditions, production of grass forage can be realized during the whole vegetation period with suitable species and varieties of cool and warm climate. Perennial grasses are the main components of native and sown swards in the lowlands and from hill to mountain belts [1]. Perennial grasslands occupy 40% of land used by the EU for agriculture, more than any other agricultural crop. *Lolium multiflorum* (Italian ryegrass) and *L. perenne* (Perennial ryegrass) are considered to provide the highest quality forage for animal production and provide safe animal products for human consumption. Unfortunately, these grasses are generally not adapted to grow in marginal and upland areas as they lack

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Biography of the first author: Aneliya Ilieva Katova (1966), Bulgarian, PhD Researcher, Associated Professor, Plant breeding, genetics and seed production of perennial grasses. E-mail: katova66@abv.bg.

the hardiness required. The recommended and grown in Bulgaria mesophytes and xeromesophytes perennial grasses (orchard grass, perennial and Italian ryegrass, timothy, smooth brome grass, meadow, red and tall fescue and meadow bluestem) form their main biomass during spring and an additional quantity-in late autumn, these are cool season grasses. The optimal temperature range for their development and growth is 20～25℃. In June, July, August and the first half of September under the influence of high temperatures and drought these species are in stress, they decrease or stop growing and have summer dormancy. The optimal temperature range for the development and growth of warm season grasses is 30～35℃. Recently, world ecological studies [2] show that the Earth climate is warming continuously. According to some authors for Bulgarian conditions [3-6] the warming will prolong the vegetation period. That gives us possibility for a new approach to searching and selecting perennial grasses with a view to grow and use them in changing ecological conditions of our country. Morphological and economical characteristics of some species of *Agropyron* genus in Southern Bulgaria was done [7], and results for biological properties of these species in Northern Bulgaria were presented [8-9]. We search and found new untraditional forage grass species, tolerant to marginal ecological conditions, which possess a complex of good characters and give enough vegetative mass during the warmest and the driest months of the year.

The objective was to develop new perennial grass varieties with high forage and seed productivity, high forage quality and high adaptive potential for pasture, hay and landscape improvement use.

1 Materials and methods

A great amount of initial breeding materials (local native populations and introduced varieties) of perennial forage grasses of cool and warm climate was collected and studied in the Institute of Forage Crops, Pleven during the period from 1966 (the beginning)-till 2015 nowdays [9-19]. Biodiversity of new plant forms and varieties was developed by applying conventional and modern breeding methods-purposeful efficient selection by productivity and adaptivity, ecologogenetic analysis of quantitative traits, polyploidization, hybridization, including interspecific one[15-16]. Main parameters for forage quality-crude protein content (CP) was determined after Kejldahl and *in vitro* dry matter digestibility (IVDMD) after Aufrere [17].

The ploidy level was determined by Katova[18-19] in the Institute of Genetics and Breeding, Melle, Belgium in 2007. The samples were analyzed by Partec Cell Analyzer CA-II and software DPAC (Münster, Germany). The intensity of fluorescent emission (after nucleus staining with specific fluorochroma) correlated linearly with DNA quantity. The results were obtained as histograms. The intensity of about 50 units corresponded to a diploid and that of about 100 units to a tetraploid.

Variety description was made according CPVO[9] and UPOV [20] technical guidelines.

The Institute of Forage Crops maintains the registered varieties and produces PB and B seeds. The region of the study combines to a great extent the climatic characteristics of Central North Bulgaria. For the 40-year period the average annual sum of rainfall was 578 mm, the average annual temperature －11.6℃. The average annual temperatures for the 10 years period were higher (12.3℃) than those for the previous 40-year period which confirmed the trend to global warming. The hottest months for the region of Pleven were June, July and August. Temperature maxima (46℃) were also recorded in these months. The months of December, January and February were coldest and temperatures often reached to －15℃/－28℃. The soil type was leached medium deep chernozem, poor to medium in humus, medium sandy clay one. The soil had slightly acidic to neutral reaction and low humus content.

2 Results and analysis

2.1 Values of cultivation and use (VCU) characteristics

During the 49-year period at the Institute of Forage Crops in Pleven six varieties of the following perennial grass species were developed: cocksfoot, smooth brome, tall fescue, perennial ryegrass, crested wheatgrass and standard wheatgrass. Main variety characteristics, such as ploidy level, direction of use, forage productivity with and without irrigation and in mixed swards with suitable legume components, persistency and tolerance to stress factors were presented in Table 1. Standard and crested wheatgrass were superior in dry matter yield to the species of cocksfoot, smooth brome and tall fescue in years with insufficient rainfall [7]. The mixed swards with legumes were higher productive than the pure grasses [21-31]. Weed-suppressive ability and productivity of mixed grass-legume swards and weed control of forage and seed production grass stands were studied [32-36]. Results were generalized using data from many comparative and competitive field trials and lab analysis.

Table 1 Some characteristics of Bulgarian perennial grass species and varieties developed at the Institute of Forage Crops in Pleven

Species	Cocksfoot (*Dactylis glomerata* L.)	Smooth brome (*Bromus inermis* Leyss.)	Tall fescue (*Festuca arundinacea* Schreb.)	Perennial ryegrass (*Lolium perenne* L.)	Crested wheatgrass (*Agropyron cristatum* Gaerth.)	Standard wheatgrass (*Agropyron desertorum* (Fish. &Schultes)
Varieties	DABRAVA	NIKA	ALBENA	IFK HARMONIYA	SVEJINA	MORAVA
Direction of use	Hay Grazing Silage Erosion control	Hay Grazing Silage Erosion control	Hay Grazing Silage Erosion control	Grazing Hay Silage Amenity Erosion control	Grazing Hay Erosion control	Hay Grazing Erosion control
Ploidy level	tetraploid	octoploid	hexaploid	diploid	diploid	tetraploid
	Dry matter yield/($t \cdot hm^{-2}$)-pure sward					
No Irrigation	8	9	9	8	9	10
Irrigation	12	13	11	10	-	-
	Dry matter yield/($t \cdot hm^{-2}$)-mixture with Legumes					
No Irrigation	16	13	13	11	11	12
Irrigation	19	15	14	12	-	-
Forage quality	Good	Very good	Good	Excellent	Very good	Good
Seed yield/($t \cdot hm^{-2}$)	0.5～0.6	0.6～0.7	0.6～0.7	0.5～0.6	0.4～0.5	0.5～0.7
Persistency, years	5～6	6～7	9～10	4～5	9～10	9～10
Stress tolerance to	Drought, Cold, Leaf diseases	Drought, Cold, Leaf diseases	Drought, Cold, Leaf diseases, Soil acidity, Soil salinity	Intensive grazing, Wear, Cold, Drought, Weed	Drought, Cold, Leaf diseases, Weed	Drought, Cold, Leaf diseases, Weed

2.2 Forage quality

Crude protein content and *in vitro* digestibility[37-41] of forage from perennial grass varieties depending on developmental stage and sward type are given in Table 2. Perennial ryegrass in pure stand or in mixture with white clover had the highest *in vitro* dry matter digestibility.

Variety Dabrava (*D. glomerata* L.) listed in

official variety list first in 1978, then in 1998; variety Nika (*B. inermis* Leyss.) listed in official variety list first in 1993; then 2008, variety Albena (*F. arundinacea* Schreb.) listed in official variety list first in 1993, 2005.

New varieties IFKHarmoniya (*L. perenne* L.), Svejina (*A. cristatum* Gaerth.) and Morava (*A. desertorum* (Fich.) Schultes.) were in official variety testing trials, 2006-2009. The varieties had been registered on the Official Variety List of the Republic of Bulgaria (OVL) for the years 2010-2015, on OECD list for the year 2010-2015, with Certificates from the Patent Office of the Republic of Bulgaria from 2010.

Bulgaria issituated on the borders of two gene centres (Mediterranean and Caucasian), where the biodiversity was the biggest [42] and was a zone of adaptation of plant genetic resources. There were 5 soil-climatic regions including vertical (mountain) zone. Perennial grasses: cocksfoot, smooth brome, tall fescue, perennial ryegrass, crested wheatgrass and standard wheatgrass grow and develop well from the lowlands to the high altitude, from hill to mountain belts [17,42-45].

Table 2 Crude protein content and *in vitro* dry matter digestibility of forage from perennial grass varieties

Species, Variety	Sward type	Crude protein/%			*In vitro* DMD/%		
		Tillering	Early heading	Aftermath	Tille-ring	Early heading	Aftermath
Cocksfoot DABRAVA	Pure	19～20	16～17	15	70	68	60
	Mixture(alfalfa)	20～21	17～18	16	75	70	65
Smooth brome NIKA	Pure	20～21	17～18	16	70	68	60
	Mixture(sainfoin)	21～22	18～19	17	75	70	65
Tall fescue ALBENA	Pure	18～19	15～16	14	70	65	59
	Mixture(alfalfa)	19～20	16～17	15	70	67	62
Perennial ryegrassI FK HARMONIYA	Pure	19～20	16～17	15	75	70	70
	Mixture(white clover)	20～21	17～18	16	75	72	72
Crested wheatgrass SVEJINA	Pure	18～19	15～16	14	70	65	62
	Mixture(alfalfa)	19～20	16～17	15	70	67	65
Standard wheatgrass MORAVA	Pure	18～19	15～16	14	70	65	62
	Mixture(sainfoin)	21～22	16～17	15	70	67	65

2.3 Ploidy level evaluation

The study was conducted to characterize theploidy level of registered Bulgarian varieties and breeding populations of perennial grasses in DvP, Melle, Belgium in 2007.

For the first time flowcytometry was used to determine nuclear DNA content expressed on a diploid basis (DNA pg $2C^{-1}$-values) for the following Poaceae species (Fig. 1): *Dactylis glomerata* L.-Dabrava (4n), *Festuca arundinacea* Schreb.-Albena (6n), *Bromus inermis* Leyss.-Nika (8n), *Agropyron cristatum* Gaerthn. (2n) and *Agropyron desertorum* Fich. Schultes (4n), and two induced polyploid breeding populations of *Lolium perenne* L. (Fig. 2 and Fig. 3), for which this first characteristic in DUS variety testing according to UPOV technical guides was missing. The first induced perennial ryegrass tetraploids were developed on the basis of Bulgarian germplasm and the analysis showed 100% 4n for C_3 generation. The flow cytometry was a precise, fast and valuable technique to measure nuclear DNA content and ploidy level of perennial forage grasses [46].

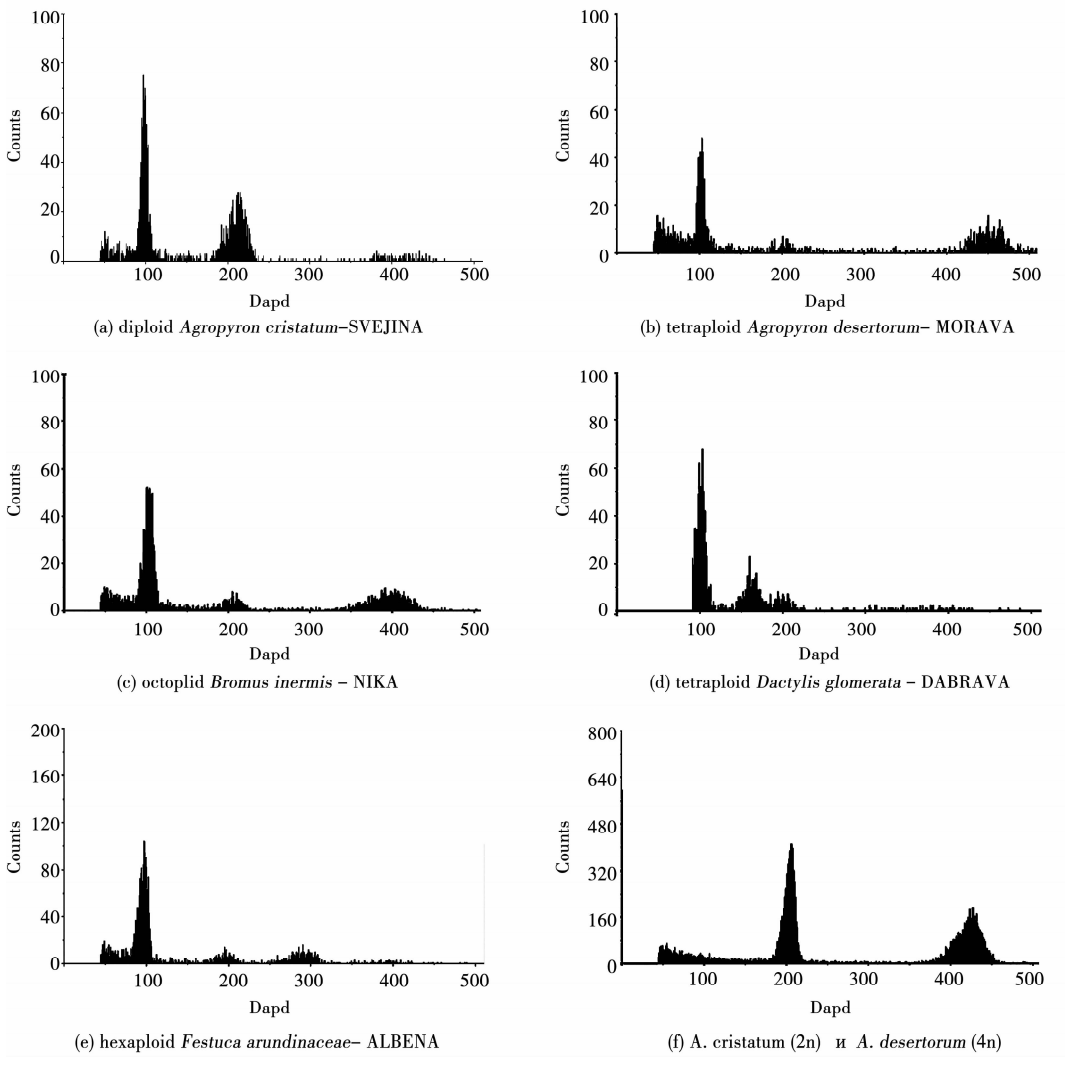

Fig. 1　Histograms as results from the flowcytometric analysis for the ploidy level of the Bulgarian varieties of perennial grasses

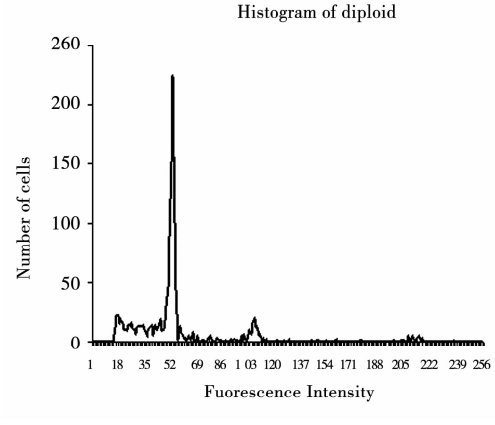

Fig. 2　Histogram of diploid *Lolium perenne*-St-IFK Harmoniya

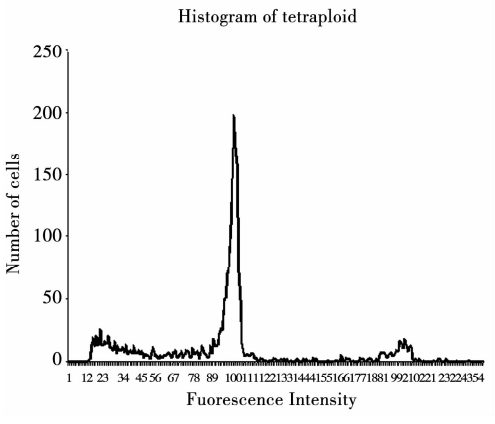

Fig. 3　Histogram of tetraploid *Lolium perenne*

3　Conclusions

Six varieties of 6 perennial grass species

were developed as follows: cocksfoot (*Dactylis glomerata* L.)Dabrava, smooth brome, (*Bromus inermis* Leyss.) Nika, tall fescue (*Festuca arundinacea* Schreb.) Albena, perennial ryegrass (*Lolium perenne* L.) IFK Harmoniya, crested wheatgrass(*Agropyron cristatum* Gaerth.) Svejina and standard wheatgrass(*Agropyron desertorum* (Fich.) Schultes.) Morava.

The perennial grass varieties of the Institute of Forage Crops have valuable characteristics, such as high forage and seed productivity, persistency, stress tolerance, forage quality, different direction of use and different ploidy level.

The Institute of Forage Crops maintains the registered varieties and produces Prebasic and Basic seeds.

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红三叶种子萌发及幼苗生长对混合盐胁迫的响应

袁国庆[1],申忠宝[2],张瑞博[2],李道明[2],潘多锋[2],王建丽[2],高　超[2]
(1.黑龙江省明水县种子管理站,黑龙江 明水 151700;2.黑龙江省农业科学院 草业研究所,黑龙江 哈尔滨 150086)

摘要:为了评价红三叶(*Trifolium pretense*)的耐盐碱能力,以红三叶新品系 Z1308 为对象,研究了该品系种子萌发及幼苗生长对不同浓度(0、40、80、120、160、200 mmol·L^{-1})碱性盐溶液(NaCl、Na_2SO_4、$NaHCO_3$和 Na_2CO_3)胁迫的响应规律。结果表明:红三叶在低浓度(≤ 40 mmol·L^{-1})胁迫时种子萌发能力和幼苗生长较强,而在高浓度(≥80 mmol·L^{-1})胁迫时则显著降低($P<0.05$)。红三叶发芽势、发芽率、发芽指数、种子活力指数与碱性盐溶液浓度均呈极显著的负相关($P<0.01$),其种子萌发的碱性盐溶液浓度的临界值为102.2 mmol·L^{-1}。红三叶根的生长对碱性盐胁迫更敏感。

关键词:红三叶,碱性盐,种子萌发,幼苗生长
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土地盐碱化是当前世界所关注的全球性重大生态问题,它不仅给农牧业生产带来严重的经济损失,而且还对生态环境安全构成巨大的威胁[1-2]。当今人类面临人口增加迅速,水土流失严重,淡水资源日益匮乏等严酷的现实,迫使人们开发利用盐碱荒地,已达到增加可耕地面积、缓解粮食危机、改善生态环境的目的。土壤改良是盐碱地开发利用的前提条件和首要任务,筛选和种植优良抗盐碱植物品种被公认为是盐碱地土壤改良最为有效的途径[3-6]。植物的耐盐碱能力与其自身的特性密切相关,同时在很大程度上也依赖于其所处的生长发育阶段[7-8]。因此,研究植物种子萌发及幼苗生长对盐碱胁迫的响应特征及规律,对耐盐碱植物的筛选与应用具有重要的现实意义。

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第一作者简介:袁国庆(1969-),男,黑龙江省明水县人,农艺师,从事种子管理及牧草育种方面的研究。E-mail:yuanguoqing1969@163.com。

保加利亚多年生优质牧草特种和品种

Aneliya Ilieva Katova
(饲料作物研究所,普列文 保加利亚 5800)

摘要:为了获得可持续性的农业饲料作物产量,发展多样的能够将高产的生态稳定性相结合的饲料作物是非常必要的。保加利亚的多年生草原育种工作,已经有 49 年之久,尤其是在普列文的饲料作物研究所。繁殖计划的目标是开发新的多年生草品种饲料和产量高的种子,高饲料质量和高适应性的牧场,提高干草和景观的利用率。于 1966-2015 期间,收集并研究了普列文大量的寒冷和温暖的气候常年饲料草饲料作物研究所的初始育种材料(当地人口和引进品种)。植物生物多样性的新形式和品种是应用传统和现代育种方法有目的地有效选择通过生产力和自适应性,生态基因分析量化特征,多倍化,以及种间杂交。2007 年,梅勒尔贝克在比利时遗传和育种研究所提出倍性水平,样本通过 Partec 细胞分析仪 CA-II 和软件 DPAC(德国明斯特)进行分析。荧光发射强度与 DNA 数量呈线性相关,此实验获得了直方图。在这几年当中,六种不同的多年地生草类物种已经演变成:鸭茅、无芒雀麦、尼卡、高羊茅、阿乐贝纳、多年生黑麦草、冰草和摩拉瓦酒,并根据 CPVO(2011)和 UPOV(2006)对新品种的技术准则进行了描述。这些多年生牧草品种有许多有价值的特性,例如有非常高的饲料价值和种子生产效率,具有持续性、胁迫耐受性、牧草优质,可以利用其不同的方向和倍性水平。饲料作物研究所保持注册品种和生产基础种子。

关键词:多年生草本植物;品种;产草量;牧草品质;倍性水平