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Bulgarian varieties of perennial forage grasses developed at the Institute of Forage crops – Pleven

Aneliya Katova

Agricultural Academy, Institute of Forage Crops – Pleven
Corresponding author: katova@hotmail.com

Abstract

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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. The indirect benefit of genetic improvement from plant breeding is substantial through yield and quality improvements and increased resilience to changing climatic conditions. Plant breeding is a particularly dynamic and research intensive activity with long lag times between investment and returns, which emerge in the form of varietal seed stock with particular traits or characteristics that are attractive to Bulgarian cropping and forage producers. Commercial plant breeding follows a number of stages and producing a marketable variety can be the product of 20 years development. Perennial grass breeding in Bulgaria has now a strong 56 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 1966–2022. Biodiversity of new plant forms and varieties was developed by complex 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, Melle, 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 eight varieties of 6 perennial grass species were developed as follows: cocksfoot (*Dactylis glomerata* L.) Dabrava – tetraploid, smooth brome, (*Bromus inermis* Leyss.) Nika – octoploid, tall fescue (*Festuca arundinacea* Schreb.) Albena – hexaploid, perennial ryegrass (*Lolium perenne* L.) IFK Harmoniya – dipolid, Tetrany and Tetramis – tetraploids, crested wheatgrass (*Agropyron cristatum* Gaerth.) Svejina – diploid and standard wheatgrass (*Agropyron desertorum* (Fich.) Schultes.) Morava – tetraploid. Variety description was made according CPVO (2011, 2015, 2021) and UPOV (2006) technical guidelines for different species. The perennial grass varieties have valuable characteristics, such as high forage and seed productivity, persistency, stress tolerance, forage quality, different direction of use (hay, pasture, silage, erosion control, amenity, phytoremediation of polluted urban soils), different ploidy level and earliness. In last years study they confirmed useful characteristics and stability. They are registered on the National List, and entered on the EU Common Catalogue and becomes freely marketable across the EU, and also in OECD list. The Institute of Forage Crops maintains the registered varieties and produces Breeder's, Prebasic and Basic seeds. Original seed samples from each perennial grass variety are presented and entered long storage at the Bulgarian National Plant Genetic Bank in Sadovo.

Keywords: perennial grasses; Bulgarian varieties; forage yield; forage quality; ploidy level

Introduction

Forage plant breeding is expected to play a greater role in improving grassland performance in the future. Increases in performance will no longer be achieved by further increases in inputs, but by increases in efficiency that enable the maintenance of performance with reduced inputs (Parsons et al. 2011).The potential performance of the sward is shaped by its vegetation composition. In general, sward improvement can be addressed in two ways: (i) species selection and composition, and (ii) varietal selection within a forage species. Breeding work starts with species selection, the actual genetic improvement is then done by developing varieties within the species. (Isselstein & Komainda, 2021). The range of forage plants cultivated by breeding is small compared to the range of species found in semi-natural or permanent grassland. By expanding the range and adding new species to the sward, the traits of the sward can be significantly changed in the short term. Examples of this are hitherto little-noticed forage plant species that contain specific secondary metabolites. These can exert positive effects on the nutrient use efficiency in the digestive tract of the livestock (Kapp-Bitter et al., 2021). This refers to a reduced excretion of nitrogen in urine, or the reduction of enteric methane emission. The same applies to the drought tolerance of swards. The variability within common grass species such as perennial ryegrass is not sufficient to adequately maintain forage production under increasing incidence of periodic drought (Hofer et al., 2016) making the introduction of new species with an inherently higher drought tolerance meaningful. From a grassland management perspective, it would be desirable to broaden the range of species to be sown and to improve these new species in important forage production properties through breeding. For the future of forage plant breeding there is a need to better consider traits that support the non-production functions of grasslands, in addition to the production function. These include, above all, the reduction of emissions of greenhouse gases, the reduction of environmentally harmful nutrient losses or the increased accumulation of carbon in the soil. In the course of forage plant breeding, the traits of single species are improved. Thereby, it is not clear to what extent improved traits become visible in species mixtures that are common in the farming practice. In mixtures, different plant species interact, there is competition for growth factors and there are also complementary effects. The expression of species-specific traits can thus be more or less effective. In recent years, there have been increasing efforts to specifically combine forage plant mixtures in order to maximise the complementarity of different species. It is necessary to examine to what extent breeding of individual species can take into account the suitability of a new variety for such 'designer mixtures'. Intercropping can increase the biomass of plants and reduce the accumulation of heavy metals in plants. However, the mechanisms of intercropping increasing plant biomass and resistance to heavy metals are still unclear. (Cui et al., 2018).

To obtain sustainable agriculture and adaptive forage production, it is necessary to develop new varieties of forage crops, combining high productivity and ecological stability. The indirect benefit of genetic improvement from plant breeding is substantial through yield and quality improvements and increased resilience to changing climatic conditions. Plant breeding is a particularly dynamic and research intensive activity with long lag times between investment and returns, which emerge in the form of varietal seed stock with particular traits or characteristics that are attractive to Bulgarian cropping and forage producers. Commercial plant breeding follows a number of stages and producing a marketable variety can be the product of 20 years development. Perennial grass breeding in Bulgaria has now a strong 56 year-long tradition, especially in the Institute of Forage Crops in Pleven (IFC – Pleven). Importance of perennial grasses is their role in preserving biodiversity in natural meadows and pastures and artificial agrocenoses for present and future generations. English ryegrass (*Lolium perenne* L.), cocksfoot (*Dactylis glomerata* L.), broadleaf and narrow-leaved fescues (*Festuca* ssp.) are valuable perennial grasses with many uses. Apart from being a source of fodder (grazing, hay and / or silage) for domestic ruminants, they are used for decorative, landscape and anti-erosion purposes. Importance of perennial grasses is the ability to create long-lasting grass stands with different directions of usehay, pasture and ornamental, pure and in mixtures with perennial leguminous crops and to provide high-quality fodder for ruminants. The need of new high-yielding varieties and improve the technologies for their seed production is of permanent importance and value for society. In the case of perennial forage grasses, the period from the start of the breeding program to the registration and implementation of the variety passes more than 15–20 years. Research and efficient use of the gene pool of plant resources of perennial forage grasses is a priority.

Drought is one of the critical abiotic stresses that significantly affect agricultural production, and current models predict an increase in its severity and intensity in the future. Generally, polyploidy has been found to improve the resistance of plants to abiotic stress. Understanding the role of ploidy in resistance to drought was achieved by comparing the response between diploids and their respective induced autotetraploids of Westerwolths ryegrass (*Lolium multiflorum* ssp. *multiflorum*). High dry matter yield and quality associated with resistance to drought is one of the most import-

ant criteria in Westerwolths ryegrass breeding. Field trials clearly demonstrated an inhibition of dry matter production in the diploid cultivars and the respective induced tetraploids in response to drought. However, the induced tetraploids produced more dry matter yield than their diploid progenitors, indicating that the increase in ploidy level affected tolerance to drought. (Akinroluyo et al., 2020.)

Global warming demands new varieties with increase tolerance to drought and water use efficiency. Crested wheatgrass (*Agropyron cristatum* (L) Gaertn.) and standard wheatgrass (*Agropyron desertorum* Fish. Schultes) are xerophytic, perennial, tufted grasses used for forage, ornamental and anti-erosion purposes. Grow naturally in the desert regions of southern Siberia and is adapted to the dry Canadian prairies. They are introduced to the Great Plains of North America from Eastern Europe and Central Asia, parts of the former USSR, China, Afghanistan, Turkey and Iran in 1898, and its cultivation began in the 1930s under a drought rescue program. Breeding activity worldwide dates back to the early 20th century and in 1932 the first Fairway variety was registered in Canada. There are no registered varieties of *Agropyron cristatum* in the EU countries, with the exception of Bulgaria and after thatRomania. Crested wheatgrass is preferred by farmers in Canada and the USA, due to a number of advantages: suitable for semi-arid and arid conditions, develops earliest in spring, long-lasting, with a deep root system, drought and winter hardy, easy to establish grass stands, preferred by ruminants and high nutritional value in spring and early summer as well as in autumn (Walton, 1981, Yancheva &Shamov, 1996). The breeding aim is to produce varieties with greater winter hardiness and better performance under dry conditions. *Agropyron desertorum* is more drought tolerant than crested wheatgrass and more productive (Walton, 1981; Ogle, 2000).

Perennial ryegrass (*Lolium perenne* L.) is one of the most economically and environmentally important grass species for the temperate zone (Humphreys et al., 2010, Sokolovic et al. 2010, 2011, Wilkins & Lovatt, 2010). Perennial ryegrass (*Lolium perenne* L.) is used as a source of forage (grazing, hay, silage) or for grassing of sports and technical terrains and laying-out off lawns, parks and gardens. It is a part of landscape, protects soil from water and wind erosion, enriches it with organic substances, maintains and improves its fertility. Perennial ryegrass is preferred by farmers owing to a number of advantages: good tillering during sward establishment, quick regrowth, and excellent nitrogen assimilation, tolerance to intensive grazing and trampling or frequent cuts and higher nutritive value than the other grasses. There is no universal variety equally suitable for all ecological conditions (Katova, 2005, Katova & Vulchinkov, 2019). The problem of perennial ryegrass adaptation and development of varieties with maximum yield stability and forage quality for given regional ecological conditions occupies a central place in contemporary breeding programs. World breeding has developed many ryegrass varieties with specific ecological adaptability. However foreign varieties have low adaptability to our agro-climatic conditions. The wide spread of perennial ryegrass in natural swards of Bulgaria suggests that local ecotypes are adapted to environmental conditions. This valuable adaptive potential could be used in the development of Bulgarian varieties of perennial ryegrass.

Basic questions at the beginning of breeding program for perennial grasses in Bulgaria are:

1. *What*? The aim is to develop Bulgarian varieties of perennial forage grasses with high productivity and ecological stability.

2. *Why*? Low adaptation of foreign varieties and wide spreading of the species in our country. Bulgaria is in periphery of 2 gene centers of origin – Mediterranean and Caucasian, rich of biodiversity – 5th in the world.

3. *Where*? North Central Bulgaria with two environmental limits (cold and drought) as the selective background for adaptation of genetic resources.

4. *Who?* IFC – Pleven, only grass breeder, in collaboration with Belgian and Chinese scientists.

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.

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 IFC – Pleven during the period 1966 – 2022 (Tomov, 1973, 1976, 1979, 1980, 1983, 1987, 1989, Katova & Tomov, 2005, 2006). Biodiversity of new plant forms and varieties was developed by complex 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, Melle, 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. The following was used for determination of the characteristics: International classifier for study of collections of Poacea family *Lolium genus* (Buktheeva et al., 1985); Protocols for testing the distinct-

ness, homogeneity and stability of perennial ryegrass, tall fescue and red fescue of the Community Plant Variety Office (Community Plant Variety Office, *CPVO*):*CPVO Technical Protocol for Distinctness, Uniformity and Stability Tests for Ryegrass –TP/004/1 Final, English, Date: 23/06/2011*, (CPVO, 2011,a);*CPVO Technical Protocol for Distinctness, Uniformity and Stability Tests for Tall and Meadow Fescue – TP/039/1, Date: 01/10/2015*, (CPVO, 2015);*CPVO Technical Protocol for Distinctness, Uniformity and Stability Tests for Red Fescue – TP/067/1 Final, English Date: 23/06/2011*, (CPVO, 2011,b). Methodology for conducting tests for distinctness, homogeneity and stability of ryegrass of the International Union for the Protection of New Varieties of Plants (*International Union for the Protection of New Varieties of Plants, UPOV*):*UPOV Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability of Raygrass) – TG/4/8*,26 p. (UPOV, 2006),

CPVO Technical Protocol for Distinctness, Uniformity and Stability Tests for Cocksfoot – TP/031/1 Final, English Date: 25/03/ 2021).

Chemical analyses in laboratory conditions, as the content of fodder quality indicators were determined as follows:- *content of dry matter (DM), %* – by the weight method, after fixing at 105 °C and full drying at 60 °C to constant weight

– *crude protein (CP), % DM* – by the method of Kjeldahl, CP=N x 6,25; (Sandev, 1979),

– *crude fibre (CF), % DM* – Weende analysis; (Van Soest, 1964);

– *water soluble carbohydrates (WSC), % DM* – after Ermakov *et al.* (1987)

– *dry matter digestibility (DMD), % DM* – after Jones u Hayward (1975).

Determination of ploidy level by flowcytometric analysis: The ploidy level of perennial grasses colection accessions and varieties was determined by the author in the Institute of Genetics and Breeding, Merelbeke, Belgium. 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 fluorochromes) 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 (Katova, 2009).

Breeding process (1995–2022) included:

- collecting of starting materials from expeditions and introduction for development of working collections with donors for increasing productive and adaptive potential →
- application of modern breeding methods: recurrent phenotypic selection, induction of the polyploidy, polycross and progeny testing for complex characteristics – tandem selection „high productivity – ecological stability) →
- variety testing → new variety → Breeder's seeds and high category seeds.

New genetic diversity is develop through: Combinative and heterosis breeding, Application of polyploidization, Hybridization including remote and selection for: productivity, adaptability (temperature stress and drought), persistency, longevity, forage quality.

Results and Discussions

During these 56 years eight varieties of 6 perennial grass species were developed as follows: cocksfoot (*Dactylis glomerata* L.) Dabrava – tetraploid, smooth brome, (*Bromus inermis* Leyss.) Nika – octoploid, tall fescue (*Festuca arundinacea* Schreb.) Albena – hexaploid, perennial ryegrass (*Lolium perenne* L.) IFK Harmoniya – dipolid, Tetrany and Tetramis – tetraploids, crested wheatgrass (*Agropyron cristatum* Gaerth.) Svejina – diploid and standard wheatgrass (*Agropyron desertorum* (Fich.) Schultes.) Morava – tetraploid.

Breeding achievements

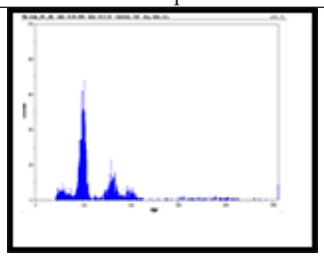
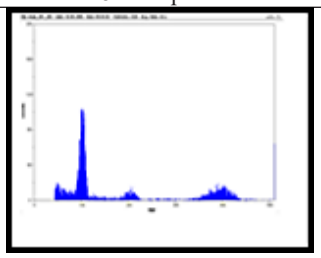
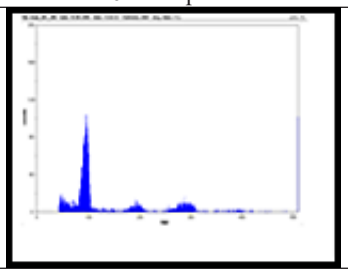
*First stage (1966 – 1995)*Hay Varieties , Author Prof. Peter Tomov, Dsc

First Bulgarian cocksfoot variety is Dabrava is tetraploid (4n). in pure stand dry matter yield **(**DMY) is 8 t/ha, in mixture with alfalfa exceeded 12 t/ha, and under irrigation -1820 t/ha. Seed yield (SY) is 0,5–0,6 t/ha. Dabrava is suitable for hay, grazing, silage, erosion control and is stress toleranant to drought, cold and leaf diseases. (Table 1)

First Bulgarian smooth brome variety Nika is octoploid (8n). DMY is 9 t/ha and SY 0,6–0,7 t/ha. Nika is suitable for hay, grazing, silage, erosion control and is stress tolerant to drought, cold and leaf diseases. (Table 1)

First Bulgarian tall fescue variety Albena is hexaploid (6n). DMY is 9 t/ha and SY 0,6–0,7 t/ha. Albena is suitable for hay, grazing, silage, erosion control and is stress tolerant to drought, cold, leaf diseases, acidic and saline soils. (Table 1). The nodule formation process of bird's foot trefoil and subterranean clover was positively affected in mixtures with tall fescue (Vasileva and Tariq, 2018). Cocksfoot cv. Dabrava, tall fescue cv. Albena perennial ryegrass cv. Harmoniya IFC, all of them Bulgarian cultivars created in IFC – Pleven are found suitable components for mixtures with widely used legumes and contributed to higher productivity and persistence of the pasture systems (Vasilev, 2008, Vasileva, 2015, Vasileva & Tariq, 2018, Vasileva & Vasilev, 2020).

Table 1. Bulgarian varieties of perennial grasses for hay, meadows and pastures

Species	Cocksfoot *(Dactylis glomerata* L.*)*	Smooth brome (*Bromus inermis* Leyss.)	Tall fescue (*Festuca arundinacea* Schreb.)
Varieties	DABRAVA	NIKA	ALBENA
Picture			
Certificate			
Direction of use	Hay Grazing Silage Erosion control	Hay Grazing Silage Erosion control	Hay Grazing Silage Erosion control
Ploidy level	4n tetraploid	8n octoploid	6n hexaploid
Histogram			
Dry matter yield/(t ha^{-1})pure sward			
No Irrigation	8	9	9
Irrigation	12	13	11
Dry matter yield /(t ha^{-1}) mixture with Legumes			
No Irrigation	16	13	13
Irrigation	19	15	14
Forage quality	Good	Very good	Good
Seed Yield/(t ha^{-1})	0.5 ~0.6	0.6~ 0.7	0.6~0.7
Persistency, years	5~6	6~7	9~10
Stress tolerance to	Drought Cold Leaf diseases	Drought Cold Leaf diseases	Drought Cold, Leaf diseases Soil acidity Soil salinity

Second stage (1995–2022) – Pasture and amenity varieties – New Breeding Objects.

Crested wheatgrass (*Agropyron cristatum* (L) Gaertn.) and standard wheatgrass (*Agropyron desertorum* Fish. Schultes) are xerophytic, perennial, tufted grasses used for forage, ornamental and anti-erosion purposes. We developed two new varieties from each of this species (Table 2).

The first Bulgarian crested wheatgrass variety Svejina – diploid, high productive, ecologically stable (winter hardy and drought resistant), resistant to leaves diseases and exceptionally long-lived and persistent had been developed during the period 1995–2010 in the IFC – Pleven. Recurrent phenotypic selection, vegetative propagation (clones selection) of 31 elite genotypes with local origin from Northeastern Bulgaria and Russian population and polycross methods were applied to its creation (Katova, 2012a). The variety is multifunctional, suitable for pasture, hay-pasture and amenity direction of use, for erosion control and landscape maintaining. It can be component of pasture mixtures with white clover or bird's foot trefoil. The results from successful competitive varietal test trial (CVT) in Pleven (2001–2004). average DMY 8709 kg ha^{-1}) and from National official varieties testing trials for Value of Cultivation and Use (VCU average DMY 9410 kg ha^{-1}) from three locations (Chepinci, Plovdiv and Selanovci) and for Distinctness, Uniformity and Stability (DUS) in one location (Chepinci) of the Executive Agency for Variety Testing, Field Inspection and Seed Control (EAVTFISC) (2006–2008) are presented for the variety Svejina. Crested wheatgrass can successfully use as a component of perennial swards for combine use in dual mixtures with sainfoin, bird's foot trefoil and Lucerne or in triple mixtures with sainfoin and bird's foot trefoil (Chakurov and Dimitrova, 2003). Dry matter yield average for six years increased till 153,0% compare to pure stand of crested wheatgrass. Wheatgrass is a highly aggressive species and alone or in mixtures with sainfoin, bird's foot trefoil or alfalfa suppresses the development of weeds, and in an ecological aspect it maintains and leaves clean weeds for the following crops.

The first Bulgarian and European standard wheatgrass variety Morava – tetraploid, high productive, ecologically stable (winter hardy and drought resistant), resistant to leaves diseases and exceptionally long-lived and persistent had been developed during the period 1995–2010 in the IFC – Pleven. Recurrent phenotypic selection, vegetative propagation (clones selection) of 15 elite genotypes with local origin from Northeastern Bulgaria and polycross methods were applied to its creation. The variety is multifunctional, suitable for hay, hay-pasture use, for erosion control and landscape maintaining. It can be component of hay mixtures with alfalfa and sainfoin. The results from successful competitive varietal test trial (CVT) in Pleven (2001–2004 average DMY 9615 kg ha^{-1}) and from National official varieties testing trials for Value of Cultivation and Use (VCU average DMY 10021 kg ha^{-1}) from three locations (Chepinci, Plovdiv and Selanovci) and for Distinctness, Uniformity and Stability (DUS) in one location (Chepinci) of the Executive Agency for Variety Testing, Field Inspection and Seed Control (EAVTFISC) (2006–2008) are presented for the variety Morava. (Katova, 2012b).

Perennial ryegrass is economicaly most important grass species in the world; Cosmopolitan, in Europe, New Zealand, temperate regions of Japan, Australia, South Africa and South America. It is used for fodder, ornamental and sport technical purposes (Boller et al., 2010, Connolly, 2001, Reheul et al., 2010, 2013, Conagan & Casler, 2011, Casler & Vogel, 2020, Petkova et al., 2021). Intensive breeding activity has been carried out in the world for over 100 years and the large number of varieties in the OECD list more than 1600 (Katova, 2005, 2005 a, Humphreys et al., 2010, Gilliland et al., 2021). Perennial ryegrass has many advantages: tolerance to intensive grazing, trampling and frequent mowing, excellent nitrogen uptake and most importantly – higher nutritional value compared to other grasses. World selection has created many varieties with specific eco-adaptability. The foreign varieties tested in Bulgaria are in most cases highly productive, but with poor adaptability to our conditions, and unsuitable for direct implementation in production (Katova et al., 2006). Three new varieties of perennial ryegrass were developed during the period 1995–2017 in IFC – Pleven, Bulgaria (Katova, 2011, 2017 a,b). Different breeding methods were applied to increase breeding efficiency. Twofold individual phenotypic selection, clonal selection of 91 elite diploid genotypes with local origin and polycross were applied for creation of variety IFK Harmoniya. Polyploidization of local breeding population, threefold flowcytometric screening and selection of tetraploid genotypes, polycross of 45 tetraploid elite genotypes with local origin and reproduction to C_4 generation were used to develop variety Tetrany. The same methods were followed for developing Tetramis variety but other 52 tetraploid elite genotypes with local origin were involved in polycross of and reproduction to C_4 generation.

IFK Harmoniya is the first Bulgarian early diploid, high productive, ecologically stable (winter hardy and drought tolerant), persistent, multifunctional, suitable for pasture, hay-pasture and amenity direction of use, in pure stands or in mixtures with white clover, alfalfa, bird's foot trefoil for forage, or with red fescue for ornamental and sports fields, with high percent of density (Katova, 2011). Tetrany is the

Table 2. Bulgarian varieties xeromezophytes species from genus *Agropyron*

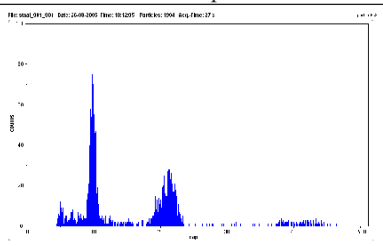
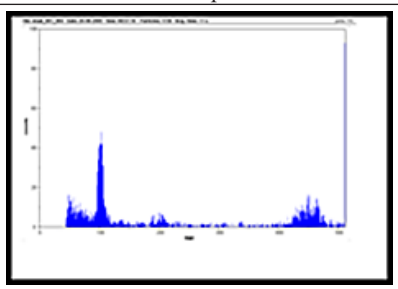
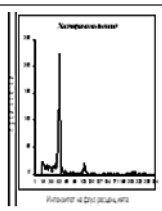
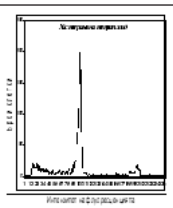
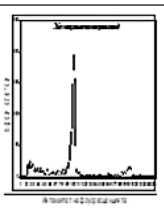
Species	Crested wheatgrass (*Agropyron cristatum* Gaerth.)	Standard wheatgrass (*Agropyron desertorum* (Fish.&Schultes)
Varieties	SVEJINA	MORAVA
Picture		
Certificate		
Direction of use	Grazing, Hay Erosion control Phytoremediation of polluted urban soils	Hay, Grazing Erosion control Phytoremediation of polluted urban soils
Ploidy level	2n diploid	4n tetraploid
Histogram		
Dry matter yield/(t ha^{-1})pure sward		
No Irrigation	9	10
Irrigation	–	–
Dry matter yield /(t ha^{-1})mixture with Legumes		
No Irrigation	11	12
Irrigation	–	–
Forage quality	Very good	Good
Seed Yield/(t ha^{-1})	0.4~0.5	0.5~0.7
Persistency, years	9~10	9~10
Stress tolerance to	Drought, Cold, Leaf diseases Weed	Drought, Cold Leaf diseases Weed

Table 3. Bulgarian variety of perennial ryegrass (*Lolium perenne* L.)

Species	**Perennial ryegrass (*Lolium perenne* L.)**		
Varieties	IFK HARMONIYA	TETRANY	TETRAMIS
Picture			
Certificate			
Direction of use	Grazing, Hay Silage, Amenity Erosion control Phytoremediation of polluted urban soils	Grazing, Hay Silage, Amenity Erosion control Phytoremediation of polluted urban soils	Hay, Grazing Silage, Amenity Erosion control Phytoremediation of polluted urban soils
Maturity	Early	Early to intermedium	Very early
Ploidy	2n diploid	4n tetraploid	4n tetraploid
Histogram			
Dry matter yield/(t ha^{-1})pure sward			
No Irrigation	8	9	9
Irrigation	10	11	10
Dry matter yield /(t ha^{-1})mixture with Legumes			
No Irrigation	11	11	12
Irrigation	12	–	–
Forage quality	Excellent	Excellent	Excellent
Seed Yield/(t ha^{-1})	0.5~0.6	0.4~0.5	0.5~0.7
Persistency, years	4~6	4~6	4~6
Stress Tolerance to	Intensive grazing Wear, Cold Drought, Weed	Intensive grazing Wear, Cold, Drought, Leaf diseases, Weed	Intensive grazing Wear, Cold, Drought, Leaf diseases, Weed

first Bulgarian tetraploid variety, early to intermedium, persistent, winter hardy, drought tolerant and resistant to crown rust, high productive and leafy with the highest nutritive values forage. Tetramis is new tetraploid, very early, persistent, winter hardy, drought tolerant and resistant to crown rust, high productive for forage and seed.

Harmoniya is early type – heading is between 3–10 May. This variety differs to introduced foreign varieties with higher persistency, winter hardiness and tolerance to drought and high summer temperatures. Average dry matter yield is 8000– 9000 kg ha^{-1}, and seed yield 500–600 kg ha^{-1}. Biomass has the highest nutritive value in comparison to the other perennial grass species – crud protein content 15–17%, water soluble carbohydrates content 6–8% and in vitro dry matter digestibility 67–72 % (Naydenova et al., 2014). (Table 3 and Table 4).

Tetrany is the first Bulgarian tetraploid, early to intermedium variety of perennial ryegrass. It is very persistent, winter hardy, drought tolerant, tolerant to high summer temperatures and leaf disease – crown rust (score 7–9). Average dry matter yield is 8000–11,000 kg ha^{-1}, and seed yield is 600–800 kg ha^{-1}. Biomass has the highest nutritive value in comparison to the other perennial grass species – crud protein content1719%, water soluble carbohydrates content – 6–10% and in vitro dry matter digestibility 70–82% (Katova and Ilieva, 2004). Tetrany suitable for pasture, hay-pasture and amenity direction of use, in pure stands or in mixtures with white clover, alfalfa (Katova, 2016) or bird's foot trefoil for forage, or with red fescue for ornamental and sports fields, with high percent of density (Katova, 2017 a). (Table 3 and Table 4).

Tetramis is new tetraploid Bulgarian very early perennial ryegrass variety. It is very persistent, winter hardy, drought tolerant, tolerant to high summer temperatures and leaf disease – crown rust (score 7–9). Average dry matter yield is 8000–10,000 kg ha^{-1}, and seed yield is700–900 kg ha^{-1}. Biomass has high nutritive value: crud protein content17 %, water soluble carbohydrates content – 6–8 % and in vitro dry matter digestibility 70–75% (Naydenova & Katova, 2013). Tetramis is suitable for pasture, hay-pasture and amenity direction of use, in pure stands or in mixtures with legumes, or diploid ryegrass for hay and with red fescue for ornamental and sports fields Katova (2017b). (Table 3 and Table 4).

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. (Table 5).

New varieties IFK Harmoniya (*L. perenne* L.), Svejina (*A. cristatum* Gaerth.) and Morava (*A. desertorum* (Fich.) Schultes.) were in official variety testing trials, 20062009. The varieties have been registered on the Official Variety List of the Republic of Bulgaria (OVL) for the years 2010–2022, on OECD list for the year 2010–2022, with Certificates from the Patent Office of the Republic of Bulgaria from 2010. The newest variety of perennial ryegrass Tetrany and Tetramis been registered on the Official Variety List of the Republic of Bulgaria (OVL) for the years 2017–2022, on OECD list for the year 2017v2022, with Certificates from the Patent Office of the Republic of Bulgaria from 2017 (Table 5).

Bulgaria is situated on the borders of two gene centres (Mediterranean and Caucasian), where the biodiversity is the biggest (Katova et. al, 2016) and is a zone of adaptation of plant genetic resources. There are 5 soil-climatic regions including vertical (mountain) zone. Perennial grasses: cocksfoot, smooth brome, tall fescue, perennial ryegrass, crested

Table 4. Comparative results for VCU characters between new perennial ryegrass varieties by the breeder

№	Character	IFK Harmoniya	Tetrany	Tetramis
1.**	Crude protein, gkg-1 DM	150–170	170–190	170
2.**	Water soluble carbohydrates WSC, %	6–8	6–10	6–8
3.***	IVDMD, %	67–72	70–82	70–75
4.**	Rust resistance	5	7–9	7-9
5.**	Leafiness, %	55-65	53–66	35–43
6.***	Group of maturity	early	early to intermedium	very early
7.***	Heading date	3–10 May	15–21 May	26–30 April
8.***	Stem thickness, mm	1.8	2.8	2.9
9.***	Ear length, cm	17–19	19–25	22–29
10.***	Number of spikelets in ear	21–23	21–25	21–23
11. ***	Dry matter yield, kg ha^{-1}	8000–9000	8000–11,000	8000–10,000
12.***	Seed yield, kg ha^{-1}	500–600	600–800	700–900
13.***	Thousand seed weight, g	1.31 – 1.51 g	2.38–3.71	3.71–4.89

** differences between 2 varieties; *** differences between 3 varieties

Table 5. List of Bulgarian forage grass species, varieties and they certificates

№	Plant species	Variety name	Certificate №	Date of issue
1.	Cocksfoot	Dabrava	№ 10066	21.10.1998
2.	Tall fescue	Albena	№ 10676	30.12.2005
3.	Smooth brome	Nika	№ 10776	29.08.2008
4.	Crested wheatgrass	Svejina	№ 10839	26.02.2010
5.	Standard wheatgrass	Morava	№ 10840	26.02.2010
6.	Perennial ryegrass	IFK Harmoniya	№ 10846	26.02.2010
		Tetrany	№ 11111	26.09.2017
		Tetramis	№ 11112	26.09.2017

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wheatgrass and standard wheatgrass grow and develop well from the lowlands to the high altitude, from hill to mountain belts (Peeters, 2004, Bojanska & Churkova, 2020, Vasileva & Vasilev, 2020).

The perennial grass varieties have valuable characteristics, such as high forage and seed productivity, persistency, stress tolerance, forage quality, different direction of use (hay, pasture, silage, erosion control, amenity (Katova, 2008, 2022), phytoremediation of polluted urban soils (Petrova et al. 2022), different ploidy level (Katova et al., 2008, Katova, 2009, 2015 a, b) and earliness. In last years study they confirmed useful characteristics and stability (Vulchinkov, 2022). The new Bulgarian tetraploid perennial ryegrass varieties *Tetrany* and *Tetramis* prove their productive capabilities, the first being of the stable type in terms of fodder and seed productivity, and the scond of the responsive type, respectively. The cocksfoot varieties *Dabrava* and tall fescue *Albena* also confirm their biological and economic qualities. (Figures 1, 2, 3).

They are registered on the National List, and entered on the EU Common Catalogue and becomes freely marketable

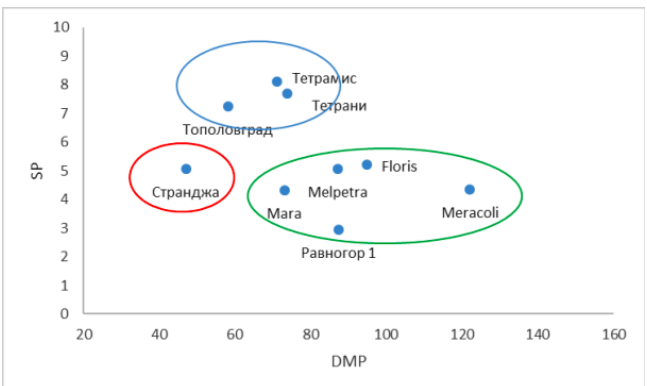

Fig 1. Cluster analysis (nesting design) of 9 perennial ryegrass accessions for the characteristics of seed and dry mass productivity at two-year averages (2017–2018) (according to Vulchinkov, 2022)

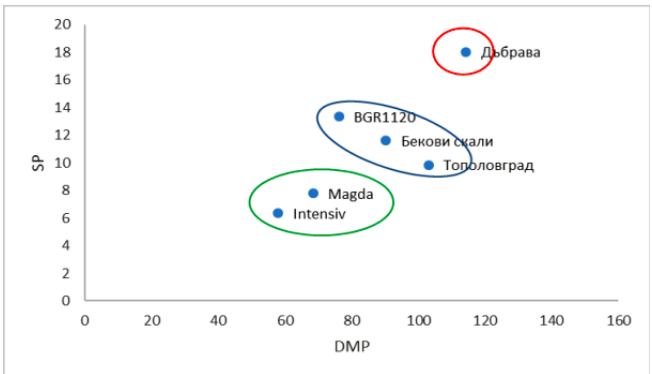

Fig. 2. Cluster analysis (nesting design) of 6 cocksfoot accessions for the characteristics of seed and dry mass productivity at two-year averages (2017–2019) (according to Vulchinkov, 2022)

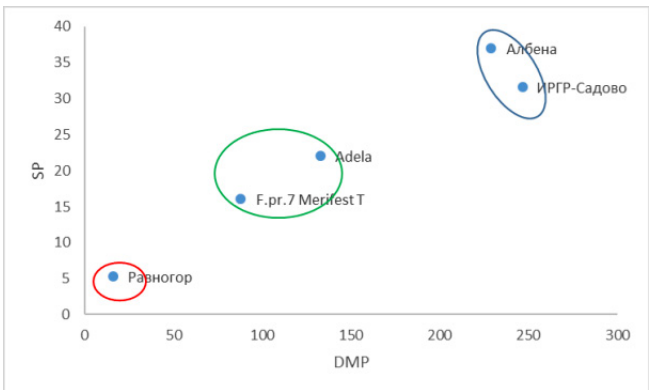

Fig. 3. Cluster analysis (nesting design) of 5 festuca accessions for the characteristics of seed and dry massproductivity at two-year averages (2017–2019) (according to Vulchinkov, 2022)

across the EU, and also in OECD list. The Institute of Forage Crops maintains the registered varieties and produces Breeder's, Prebasic and Basic seeds. Original seed samples from each perennial grass variety are presented and entered long storage at the Bulgarian National Plant Genetic Bank in Sadovo.

Conclusions

⇒ Eight varieties of 6 perennial grass species were developed for 56 years period as follows:

1 – cocksfoot (*Dactylis glomerata* L.) Dabrava,

1 – smooth brome, (*Bromus inermis* Leyss.) Nika,

1 – tall fescue (*Festuca arundinacea* Schreb.) Albena,

3 – perennial ryegrass (*Lolium perenne* L.): IFK Harmoniya, Tetrany, Tetramis

1 – crested wheatgrass (*Agropyron cristatum* Gaerth.) Svejina and

1 – standard wheatgrass (*Agropyron desertorum* (Fich.) Schul.) 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. Original seed samples from each perennial grass variety are presented and entered long storage at the Bulgarian National Plant Genetic Bank in Sadovo.

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