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Approaches and achievements in breeding of perennial grasses in Bulgaria (Review)

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Abstract: The issue of efficient forage production is gaining more and more economic importance and is directly related to the breeding of forage crops with high productivity and adaptability. The aim is to create new varieties of perennial grasses with high forage (hay, pasture and silage) and seed productivity, high forage quality, and/or for ornamental purposes, with high adaptive potential. During the period 1966 - 2024, through an ecologo-genetic approach and the application of classical and modern breeding methods, varieties of a wide range of grass fodder crops were created at the Institute of Forage Crops - Pleven, the only specialized breeding center for the country. A characterization of species and varieties of perennial grasses, for sown meadows and pastures and for decorative purposes, which are certified and entered in the list of varieties of Bulgaria, EU and OECD, including 8 varieties of 6 species: Cocksfoot Dabrava, Tall fescue Albena, Smooth brome Nika, the new varieties of perennial ryegrass - IFK Harmoniya, Tetrany and Tetramis, Crested wheatgrass Svezhina and Standard wheatgrass Morava. An flowcytometric analysis of the ploidy level, morphological description, biological requirements, phenological development and yield of forage and seeds, as well as forage quality, were performed on each variety. Original seeds of all varieties of perennial grasses of IFC - Pleven are in long-term storage in the National Genebank of Bulgaria in IPGR - Sadovo.

Key words: perennial grasses; breeding methods; criteria; species; variety

INTRODUCTION

The abundant grasslands in Europe and the world are facing unprecedented threats with urbanization, conversion to other crops and other factors leading to their gradual disappearance. Loss of these grasslands may also result in the loss of the benefits they provide (Ferris, 2007; Klootwijk et al., 2024). The issue of efficient forage production is gaining more and more economic importance and is directly related to the breeding of forage crops with high productivity and adaptability (Humphreys et al., 2006, Katova, 2023, Konkolewska et al., 2023). Research in this area is of particular importance for achieving sustainable agriculture. The main contribution of perennial grasses, including perennial ryegrass, cocksfoot, crested and standard wheatgrass, tall, meadow and red fescue, festulolium, etc. for adaptive forage production is based on their ability to create long-lasting grass stands with different directions of use - hay, pasture, phytoremediation and ornamental, pure and in mixtures with perennial legumes and to provide high-quality forage for ruminants (Katova, 2005, 2016, 2022, 2023; Vasilev, 2006; Vasileva, 2011; Vasileva and Vasilev. 2012; Petrova et al., 2022).

The need to create new high-yielding varieties and improve technologies for their seed production is of permanent importance and value for society, and in the case of perennial forage grasses, the period from the start of the breeding program to the registration and implementation of the variety is more than 15-20 years (Katova, 2023;

Katova et al., 2024). The study and efficient use of the gene pool of plant resources of perennial forage grasses is a priority (Katova, 2005, Boller and Green, 2010; Humphrays et al. 2010; Katova and Vulchinkov, 2019; Vulchinkov, 2022; Petkova, 2023). „Doing more with less" by using new knowledge and new developments is an important element of sustainable intensification. Growing the latest crop varieties in a suitable environment with good agricultural management offers an opportunity to optimize yields (Reheul et al., 2013, 2017**).**

A model plant and cosmopolitan representative of perennial grasses is perennial ryegrass (*Lolium perenne* L.), a high-quality pasture grass that provides year-round forage in Western Europe and is palatable to ruminants. It performs extremely well in areas with moderate temperatures and rainfall. It is one of the most important forage grasses worldwide. In Northern Ireland, a study was conducted at the variety trial station, Crossnacreevy, comparing dry matter yield and stand density of new cultivars introduced from 1973 to 2013 and biomass digestibility from 1980 to 2013. Dry matter yields show an overall significant mean annual increase of 0.52% under hay use and 0.35% under simulated grazing, with similar increases across maturity groups and ploidy levels (McDonagh et al., 2016).

For the Balkan Peninsula and neighboring Serbia, Sokolovic et al., (2010, 2017) made a historical review of perennial grass breeding with a 50-year tradition and 19 forage cultivars created. All varieties are characterized by high genetic potential for yield and dry matter quality. The most used breeding method for grasses in Serbia is phenotypic, recurrent selection modified to make the process shorter and more efficient, followed by polycross and synthetic breeding. The most important criteria are yield and quality, maturity, drought resistance, disease resistance and seed yield. In the future, in the breeding of grasses, special attention of breeders should be paid to selection in real production conditions, selection for improved resistance to drought (development and depth of roots) and maturity of different varieties, development of heterotic groups (populations) for hybrid selection, polyploid varieties, interspecific hybridization and introduction of new methods (DNA molecular markers) and new species of forage perennial grasses in the breeding process. For the USA, Quesenberry and Casler, (2001), Vogel and John, (2019). Casler and Vogel (2020), report progress in grass breeding, but the challenge for forage breeders remains to achieve „growing two blades of grass on a spot of land where only one used to grow," and thereby improve the supply of fodder and food for all mankind.

Main breeding characteristics:

Dry matter yield

One of the main goals of all breeding programs is to increase forage yield. Pasture harvesting involves approximately all mowed aboveground biomass with consequent loss of photosynthetic capacity, and a continuous, rhythmic, year-round supply of forage is required. For this reason, breeding for appropriate seasonal distribution must be considered in the total annual yield. Genetic improvement in annual dry matter yield of perennial ryegrass has been estimated in mowing trials in Europe and New Zealand to be about 4 to 5% per decade, but less than 1% in the USA, where there is little breeding activity with the species (Woodfield, 1999; Easton et al., 2002; Wilkins and Humphreys, 2003; van der Heijden and Roulund, 2010, Chapman et al., 2015, Teixeira et al., 2024). Annual grass forage dry matter production in Ireland ranges from 12.7 to 15.0 t/ha based on data from the Ministry of Agriculture and Food. More recent data from PastureBase Ireland shows that average annual grass forage production (2020) on efficient dairy and dry cows farms is 13.5 and 10.0 t DM/ha respectively (O'Donovan et al., 2022). Ireland is now one of the world leaders in pasture research, particularly in the areas of grazing utilization, decision support systems and variety selection indices. Future systems will require healthier grazing animals with stronger functional traits, more diverse grass stands supporting improved animal performance and requiring less fertilizer and chemicals, and will support more biodiversity and improved carbon storage. Genetic gain in annual dry matter yield of perennial ryegrass and white clover is estimated to be 4%–6% per decade (Brummer & Casler, 2014; Gilliland et al., 2021). This is modest compared to the genetic improvement of 10%–15% per decade in cereal yield. The lag in genetic improvement of forage species is due to several biological factors and limited investment in the seed industry, as forages are a minor species in terms of global seed sales (Annicchiarico et al., 2016). Genetic improvement in nutritional value in perennial ryegrass is modest, with dry matter digestibility (DMD) increasing by 5–10 g/kg dry matter (DM) (0.5%–1%) per decade (Gilliland et al., 2020). This may be due to limited breeding efforts as nutritive value is a relatively new addition to variety testing and was first added to the Ireland variety list in 2009. Grass and clover breeding systems are based on recurrent, phenotypic selection and selection between and within the family (Conaghan and Casler, 2011). High-throughput phenotyping using advances in instrumentation, optical sensors, and machine learning is needed to fully exploit genomic selection (GS). Phenotyping should continue on an annual basis to allow frequent updates and improvements to the GS model (Brummer and Casler, 2014). While theoretical models show a two- to threefold increase in genetic improvement with GS (Pembleton et al., 2018, Byrne, 2023), they are largely untested in forages for key traits such as dry matter yield and nutritional value (Arojju et al., 2020; Faville et al., 2020). Wilkins and Humphreys (2003) reported large regional variations in the genetic benefits of forage grass breeding. This variation is largely due to differences in climatic stress factors and different disease and pest pressures. In North Dakota, a mild humid climate with low disease incidence and few pests provides ideal conditions in which ryegrass can express its full genetic potential for forage dry matter production (Camlin, 1997). Perennial ryegrass breeders have achieved significant increases in dry mass yield over time and at reliable annual rates compared to cereals, given the greater challenge of increasing total shoot biomass in an allogamous species. The variety testing system is an important catalyst driving the speed and influencing the direction of progress made by breeders (Gilliland et al., 2020). There is documented evidence from North West Europe that breeders have successfully and consistently achieved rates of productivity growth of around +0.4–0.6% per year depending on the yield component and region (van Wijk and Reheul, 1991; Humphreys, 1999; Wilkins and Humphreys, 2003; Sampoux et al., 2016; Duller et al., 2019). Similar achievements have been reported in New Zealand (Easton et al., 2002). However, along with the other traditionally important traits for selection of longevity and disease resistance, the main goals for a selection differ little more than 45 years (Cooper and Breeze, 1971; Parsons et al., 2011). In this context, relatively modest genetic improvements in digestibility have been achieved, around 0.5–1.0 g/kg DM per year (Wilkins and Humphreys, 2003; McDonagh et al., 2016). Furthermore, Wilkins and Humphreys (2003) concluded that traits that influence nutritional value include crude protein (CP), water soluble carbohydrates (WSC), neutral detergent fiber (NDF) and organic matter digestibility (OMD). Selection for high WSC has been shown to improve CP metabolism of pasture grass (Miller et al., 2001) and silage (Merry et al., 2006) and reduce N excreted in urine. Evidence of varieties with improved animal performance and reduced potential for environmental impact will be highly valued by leading farmers and government regulators.

Over the past 100 years, considerable efforts have been made in Western Europe and elsewhere to improve perennial ryegrass for agriculture (Wilkins and Lovatt, 2011; Hofer et al., 2016; Duller et al., 2019). These increases in annual yield of approximately 9% per decade compares well with an increase of approximately 3% per decade from perennial ryegrass cultivation over the previous 40 years (Chaves et al., 2009; Sampoux et al. 2011). Furthermore, they are accompanied by a significant improvement in forage quality (Wilkins and Lovatt, 2011).

Breeding progress

The best way to assess the benefits of breeding is to directly compare the performance of the newest varieties of perennial ryegrass with the old ones. The only recently published data of this type are from Belgium, where the old variety Vigor (a late variety previously called Melle Pasture) was maintained and included as a control in VCU trials over a 40-year period (from 1963 to 2007, Chaves et al., 2009). The results were very similar in the intermediate and late maturity groups and in diploids and tetraploids. Compared to Vigor, total annual dry matter yield of candidate cultivars for 2-3 years of cultivation under infrequent mowing increased by 12.4%, fall soil cover by 21%, and crown rust resistance by 44%. In addition to in vitro dry matter yield and digestibility (DMD), the WSC and CP content of the grasses was determined at each cutting during the first three years of cultivation. The latest late maturing variety (the tetraploid Aberbite) produced 28% more DM yield than S23 and was 22 g kg-1 higher in DMD and 58 g kg-1 higher in WSC, although similar to S23 in the soil cover. These increases were not much lower than those from forage corn selection (14% per decade in DM yield and 5.4-6.1 gkg-1 per decade in DMD, respectively; (Lauer et al., 2001). In maize, however, it is possible to generate a selection every year, while in perennial ryegrass, where longevity is a key characteristic that must be balanced against other objectives, the minimum generation time is 4 years.

Future opportunities for breeding.

A wide range of traditional breeding methods are used by ryegrass breeders, including recurrent selection, mass selection of ecotypes, tetraploidy (Eeckhaut, 2004), hybridization between elite cultivars, introgression of ‚new‘ germplasm and hybrid production involving male sterility. Plant breeding is undoubtedly a highly specialized scientific discipline, requiring scientists with expertise in plant genetics, physiology, ecology, and also agronomy (Boller et al., 2010).

From the initial cross, through all the seed evaluation and propagation processes, to finally obtaining a product that can be delivered to the end user, it takes about 16 years (McDonagh et al., 2016). Increases in dry matter yield vary widely across Europe. Over the past 50 years, the increase in DM yield from important forage grass species such as perennial ryegrass has been 4-5% per decade in NW Europe, in southern France for Italian ryegrass and in Italy for tall fescue and cocksfoot. Dry matter (DM) production from the Northern Ireland recommended variety list (Gilliland, 2007) increased by 0.04 t DM/year in grass stands (frequent mowing) and 0.114 t DM/ha/year in silage grass stands (infrequent mowing), and a progressive increase average net increase of 5% yield of DM/ha/year is reported in the last decade.

Forage quality

The ability of a variety to influence animal performance is not only related to the amount of feed, but also to available metabolizable energy and any factor that can increase feed intake by animals. Forage quality varies greatly depending on flowering time, foliage, disease, growth rates, and many other plant-related factors. Although the genetic variation of many quality traits is small compared to the environmental component, any improvement in the quality of a variety is valuable. Varietal ploidy level also affects forage quality, with tetraploids having larger cells and a higher cell content to cell wall ratio resulting in lower dry matter content (Katova et al., 2008; Katova, 2009; Naydenova and Katova, 2013; Katova and Ilieva, 2017). In general, animals must consume more fresh mass of tetraploid cultivars to obtain the same DMI as from diploids (Peeters, 2004). There is almost universal agreement among agronomists, nutritionists and livestock producers that digestibility is the most important selection criterion for improving the nutritional value of grasses (Wheeler and Corbett, 1989; Smith et al., 1997). In vitro DM digestibility (IVDMD) can be increased by selection for IVDMD per se or other correlated traits such as water-soluble carbohydrates, neutral detergent fiber, acid detergent fiber, and lignin (Casler, 2001). Genetic changes in reproductive development can also lead to changes in whole-plant IVDMD. The IVDMD of the leaf lamina is generally higher than that of the generative stems consisting of leaf sheath, true stem and developing inflorescence (Buxton and Marten, 1989). Reproductive development or the leaf:stem ratio can be modified by selecting the timing and intensity of primary tillering and the frequency and intensity of subsequent tillering. However, when reducing the intensity of primary heading, the large contribution of stems to yield must be taken into account (Wilkins and Humphreys, 2003). The heritability (H2) of many laboratory estimates of forage nutritional value (eg, IVDMD, WSC, NDF, etc.) for perennial ryegrass is generally low to moderate (about 0.30 to 0.50) (Frandsen, 1986; Oliveira and Castro, 1994; Posselt, 1994). Digestibility is typically lowest mid-season (mid-April to July), making it a key target period for improvement. Differences between genotypes in digestibility are also greatest at mid-season when mean IVDMD is lowest (Wilkins, 1997; Gilliland et al., 2003). This suggests that mid-season will be the best time to sample grass to determine genotype ranking and differences in IVDMD (Wilkins, 1997) and will benefit most from improvement. Quality varies considerably between years (Walsh and Birrell 1987) and within-species variation in nutritional characteristics is large, indicating potential for improvement. Breeders require quality assessment techniques that are fast and accurate. Near-infrared reflectance spectroscopy (NIRS) is a rapid technique for predicting nutritional value with appropriate chemical analysis for calibration. The technique has been used to reliably predict DM digestibility, crude protein as well as Mg content (Smith et al. 1991). Humphries (1989) also showed genetic variation for water-soluble carbohydrate content (WSC), selection for high WSC, increased DM digestibility. Protein content has been improved by recurrent selection, but this leads to a decrease in yield, so that total protein yield is reduced (Arcioni et al., 1983).

Durability (Longevity)

In general, modern cultivars have greater longevity than older cultivars (Camlin, 1997). Cultivars are known to vary in resistance and one of the factors giving a ryegrass cultivar greater capacity to survive adverse conditions and better resistance is high crop density (Camlin and Stewart, 1978; Wilkins and Humphreys 2003), although this is often difficult for breeders to combine with high yield potential (Gilliland and Mann, 2001). Almost all germplasm used in breeding programs today originates from plants from old resistant pastures, although many may have been subject to a number of cycles of crossing and selection. Typically, persistence is determined by the stability of DM yield of a cultivar over several years, and in general, grasses that persist under frequent mowing persist well under grazing (Wilkins and Humphreys, 2003).

Varieties must have adequate tolerance to extreme stresses occurring on farms, such as winter cold, summer drought, intensive defoliation and trampling damage (Katova, 2024). In Europe, winter hardiness has long been critical for longevity, while in New Zealand summer drought tolerance is inextricably linked to endophyte-mediated pest tolerance (Caradus et al., 2021).

Most forage species used in New Zealand are cross-pollinating and therefore cultivars are populations of different but closely related genotypes. Plant breeding plays an important role in improving the productivity of grassland agriculture in New Zealand, while reducing the environmental footprint, in a more variable climate. Breeding for dry matter yield with reduced nitrogen fertilizer application, while understanding that nitrogen supply significantly affects yield, will require balancing yield and nitrogen use efficiency. Although the main objectives of plant breeding have not changed, the available knowledge and tools have developed rapidly in recent years, with genomic selection allowing faster and more accurate selection, the development of tools such as LiDAR for better trait evaluation of grasslands, metabolomics to provide a better understanding of linking plant function to genetics and more precise options for manipulating genes through gene editing technologies. Perennial ryegrass is native to Europe, temperate Asia and North Africa and now occurs both as a naturalized and extensively grown species in South Africa, North and South America, New Zealand and Australia (Cunninghame et al., 1994). It occurs as a diploid (2n = 14) obligate outbreeder and suffers from severe inbreeding depression. Globally, annual sales of agricultural seed are 50,000 t (British Seed Houses unpublished data). It is popular for its high digestibility (Frame, 1989), good forage yield, resistance, tolerance to grazing, ease of establishment, management and seed production (Wilkins, 1991). The area sown to pasture ryegrass in Australia is estimated to be more than six million ha. Pasture ryegrass is generally adapted to regions with more than 550 mm of annual rainfall with a growing season of more than 7 months in temperate to Mediterranean climates. Persistence is defined as the rate at which grass stand density declines over time and is important for nutritional value, as declining persistence allows for invasion by weed species. High plant growth rates during drought or late fall and winter will reduce longevity. The high ratio of vegetative to reproductive stems and the high rate of emergence of new stems help with grazing resistance. Drought and heat tolerance, disease and pest resistance, soil fertility and acidity, and grazing intensity are also factors that can have a significant impact on longevity. Both high yield and longevity can be simultaneously improved through selection and recombination (Katova et al., 2006, 2007; Katova, 2024).

Drought and heat resistance

Much of southern Australia experiences significant summer droughts lasting 2-4 months. These environments are similar to Mediterranean lowlands, where pasture ryegrass persists by avoiding drought stress by becoming dormant in summer and growing actively in winter and spring (Arcioni et al., 1980). The Algerian variety Medea (Silsbury, 1961) is an example of a summer passive variety that also shows good growth in autumn, winter and spring in southern Australia (Reed et al. 1987). Considerable variation in summer dormancy and drought tolerance occurs among species, and good sources have been identified in a wide range of materials originating from Mediterranean regions (Lee et al. 1993; Cunningham & Anderson - unpublished data).

Disease resistance

In general, perennial ryegrass has few major diseases that reduce forage yield, but resistance to crown rust (*Puccinia coronata*) and, in some regions, Drechslera leaf spot (*Drechslera siccans*), downy mildew and *Rhynchosporium* is beneficial (Connolly, 2001; Reheul et al., 2001). Likewise, resistance to seed production diseases such as stem rust (*Puccinia graminis*) is also important. Pasture ryegrass suffers from attack by at least 16 species of fungal pathogens, one species of bacterial pathogen (Wilkins, 1985), and at least three virus groups, each with a range of strains (Wilkins 1991). The main fungal pathogens in Australia are crown rust (*P. coronata*), stem rust (*P.graminus*), net spot (*Drechslera dictyoides*). Varieties need adequate levels of resistance to all these pathogens, especially in regions where disease outbreaks are severe. Crown rust has been reported to reduce yield by 37% and fresh grass weight by up to 94% in the ‚Victorian‘ countryside (Price, 1987).

Progress in the breeding of cool-climate perennial grasses through plant breeding has been significant in the 20th century, but much remains to be achieved in the 21st century. Key areas of focus should include:

1) Development of genetically diverse synthetic varieties using multiple recurrent selection with improved selection criteria,

2) Improving the regional adaptation of varieties, including selection for resistance to biotic and abiotic stress,

3) Improvement of feed quality through laboratory in vitro procedures,

4) Breeding for longevity in the early phases of breeding programs, and

5) Use of appropriate molecular tools in cultivar development.

For Bulgaria, the main breeding activity for perennial forage grasses is carried out at the Institute of Forage Crops - Pleven, specialized in research on forage legumes and grasses. In the NC - Sredets, a variety of perennial ryegrass Strandzha was created and registered, with a certificate from 2010, but it is not entered in the list of varieties (VL) of Bulgaria and is not maintained (Stoeva, 2010). The timothy "Troyan" and tall fescue "Elena" were created in the RIMSA - Troyan (An order n 1-437/28.04.1993), but they do not have certificates, they are not entered in the VL of Bulgaria and they are not maintained.

MATERIAL AND METHODS

The objects of study are from the family *Poaceae (Gramineae*), genera: *Lolium, Dactylis, Bromus, Festuca, Agropyron.* They are obligate cross-pollinating species with gametophytic self-incompatibility, anemophilic, with a basic chromosome set n= 7. Breeding programs involve recurrent selection of multiple parent clones. The selected clones are crossed and give rise to synthetic varieties, through polycross and progeny testing.

Approaches:

The purpose of breeding is to create and use methods to efficiently select the best genotypes to create improved varieties. The optimal selection system depends on the traits to be improved, the resources and the skills of the breeders.

The most important traits for breeding are: forage dry matter yield, longevity, resistance to drought, high temperatures, cold, diseases and pests, quality and nutritional value, seed yield. Special attention is paid to the expression of the trait under different modes of use in the breeding program and under real production conditions in a given region.

Recurrent breeding programs for in-population improvement are most appropriate for perennial forage grasses. Three different types of recurrent selection can be applied: (i) phenotypic recurrent selection, (ii) genotypic recurrent selection and (iii) marker-assisted selection.

Initial Source material

Plant genetic resources are invaluable to any breeding effort. The selection of starting material through complex research is crucial for the success of the breeding program, since breeding is a long-term process and many years of selection and recombination are conducted to arrive at the creation of a new variety (Boller et al., 2010, Katova, 2023).

Four categories of plant genetic resources are relevant to forage and ornamental grass species: wild relatives; ecotypes, local populations grown by farmers; varieties.

The following types of perennial grasses are the object of study:

- ♣ Perennial ryegrass
- ♣ Tall, meadow and red fescue
- ♣ Crested wheatgrass
- ♣ Standard wheatgrass
- ♣ Cocksfoot
- ♣ Smooth brome

Basic questions at the start

1. **What?** The aim is to create Bulgarian varieties of perennial grasses with high productivity and ecological stability.
2. **Why?** Weak adaptability of foreign varieties and wide distribution of species in the country. Bulgaria is located on the periphery of two centers of origin - Mediterranean and Caucasian, and is in 5th place in the world in terms of biodiversity.
3. **Where?** North Central Bulgaria, with two environmental limits (drought and cold) as a provocative background for adaptation of genetic resources.
4. **Who?** IFC - Pleven with one, consecutively, plus one breeder, in collaboration with ILVO and UG breeders in Belgium.

Breeding methods and schemes:

New genetic diversity is created by:

- Combinative and heterosis breeding,
- Application of polyploidization (induced tetraploidy),
- Hybridization, including remote

And a selection for:

- productivity,
- adaptability (temperature stress and drought),
- durability (longevity)
- leafiness
- forage quality.

In Fig. 1 and Fig. 2 shows an application of the recurrent selection and the polycross methods. The

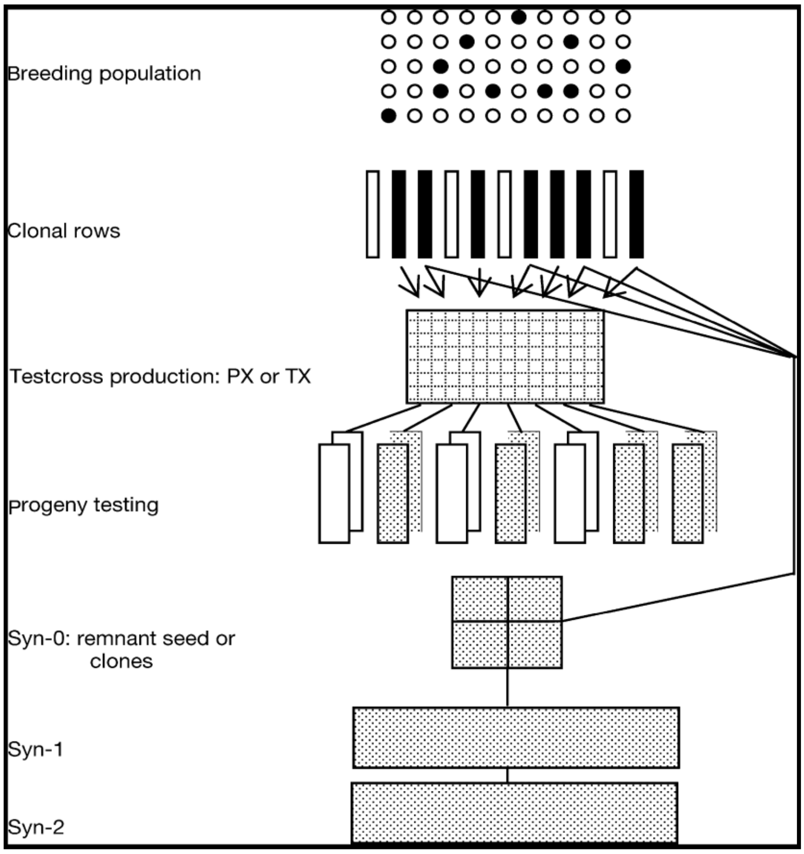


Figure 1. Breeding scheme for development of synthetic varieties

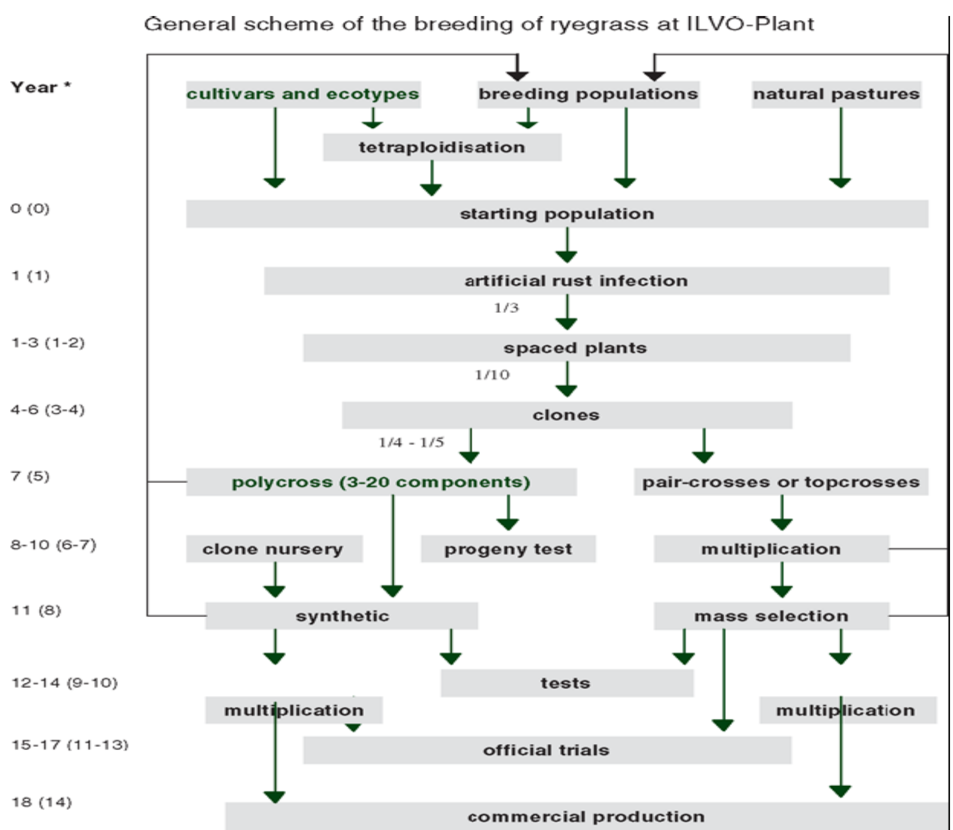


Figure 2. General scheme of the breeding of ryegrass at ILVO – Plant, Belgium

number of years is approximate and may refer to Italian ryegrass (in parentheses) and the others to perennial ryegrass Fig.2. The diagrams are based on Frandsen and Frandsen (1948), and adapted from Reheul and Baert (personal communication) and Van Bockstaele and Baert (2004). Figure 3 shows cells for hybridization of two parents in isolation.

Modern breeding process

Breeding programs were facilitated by the introduction of new technologies in the second half of the 20th century:

• Efficient parcel harvesters measure dry matter yield (Fig.4),

• cheap computers to record and quickly analyze data,

• NIRS for predicting feed quality indicators,

• and reliable flowcytometers to determine the ploidy level (Fig. 5). Doubling the chromosome set from a diploid to a tetraploid level through the use of colchicine widens the range of ryegrass types that are produced.

• However, none of the current cultivars are transgenic, as far as is known, and none of the

Figure 3. Crossing cells of two parent components. A series of pair wise crosses between ryegrass individuals with air circulation to ensure pollination of plants in isolation (Photo J. Baert).

Figure 4. The plot harvester for forage is equipped with a sampling device and with a built-in Near-Infrared Reflectance Spectrometer for ‚online' determination of the quality of forage (Photo U. Feuerstein)

cultivars have been created using marker-assisted selection.

Flowcytometry is the latest ploidy level determination technique that combines the advantages of microscopy and biochemical analysis to measure quantitative characteristics such as DNA and RNA content and total cellular protein content with high precision and rapid throughput (Eaton et al., 2004; Muirhead et al., 1985; Wang et al., 2009; Leus, 1998; Katova, 2015 b).

The history of forage crop breeding is less than 100 years old, and the breeding of perennial grasses has lasted less than 50 years. Breeding has been facilitated by increased assessment over the last three decades due to the invention of plot harvesting machines, data loggers, electronic systems, computer technology and more recently NIRS-online systems (Boller et al, 2010).

The creation of new varieties that meet the requirements of end users is the ultimate goal of any breeding program. In addition to choosing an effective breeding method, defining a prospective set of goals and choosing appropriate starting materials are of fundamental importance.

The global warming of the climate and the increased consumption of fodder from animal husbandry determine the need to use high-yielding, stress-tolerant to abiotic and biotic factors perennial forage grasses. In order for this to happen, original, authentic seeds from Bulgarian varieties are needed, possessing the greatest adaptability, productivity and quality under our soil and climate conditions. Some of them tolerate heavy, swampy, salty or acidic soils. They have a highly pronounced self-regulating and restorative ability (Katova, 2024).

During the period 1966 - 2024, a large amount of source breeding material (local populations and introduced varieties) of perennial forage grasses of cool and warm climates was collected and studied at the Institute of Forage Crops, Pleven (Tomov, 1979, 1980, 1983, 1987, 1989). Biodiversity of new plant forms and varieties has been created through the application of conventional and modern selection methods - targeted effective selection for productivity and adaptability, ecogenetic analysis of quantitative traits, polyploidization, hybridization, including interspecies. Bulgaria is a zone of adaptation of plant genetic resources. There are 5 soil-climatic areas, including vertical (mountainous) zonation. (Katova, 2005, Katova and Tomov, 2005, 2006). The following methodology was applied to determine selection characteristics: International classifier for study of collections of *Poacea* family (Buktheeva et al., 1985); Distinctiveness, uniformity and stability (DUS) test protocols for perennial ryegrass, tall and red fescue, cocksfoot as follows: (Community Plant Variety Office, *CPVO*): *CPVO Technical Protocol for DUS Tests for Ryegrass –TP/004/1 Final, English, Date: 23/06/2011*, (CPVO, 2011,a); *CPVO Technical Protocol for DUS Tests for Tall and Meadow Fescue – TP/039/1, Date: 01/10/2015*, (CPVO, 2015); *CPVO Technical Protocol for DUS Tests for Red Fescue – TP/067/1 Final, English Date:*

Figure 5. Flowcytometer for ploidy analysis and DNA quantity

23/06/2011, (CPVO, 2011,b). *CPVO Technical Protocol for DUS Tests for Cocksfoot – TP/031/1 Final, English Date: 25/03/* 2021).

The biochemical evaluation of the selection materials was carried out according to the following qualitative indicators and methods:

• Crude protein content (CP), % (Kjeldahl) (Sandev, 1979), CP=N x 6.25;

• Crude fibers (CF), % (Weende method) (Van Soest, 1964);

• Water soluble carbohydrates (WSC) – Ermakov et. al. (1987).

• Dry matter digestibility *in vitro* (IVDMD) (Aufrère, 1982).

The beginning of the breeding program to create tetraploid perennial ryegrass was set in 2000. The colchicine treatment was carried out according to the Perennial Ryegrass Protocol at DvP, Melle, Belgium during the specialization of the breeder from Bulgaria in the perennial grass breeder group J. Baert (Katova, 2015 a, b). Tetraploids have many advantages: better foliage, taller plants and upright habit, higher forage and seed productivity, higher digestibility than diploids, darker green leaf color; faster emergence and establishment of a grass stand; greater mass per 1000 seeds (TSW); higher content of WSC and lower cell walls; better palatability; higher level of intake of fresh mass by ruminants; better disease resistance, winter hardiness; higher nutritional value; better nitrogen uptake (Mayers, 1939; Paul, 1988; Baert and Reheul, 1997; Van Bockstaele, 1998; Lamote et al., 2002; Connoly, 2001; Reheul et al., 2003; Fulkerson et al., 2004; Halling et al., 2004; Smith and Elgersma, 2004; Eeckhaut, 2004; Edwards et al., 2007; Baert et al., 2014) and their creation is seen as a major advance in perennial ryegrass breeding (Camlin, 1997; Nair, 2004; Humphreys et al., 2010; Katova et al., 2016; Katova, 2017 a, b).

The results are in the form of histograms (Data Pool Application for Cell Analyzer - DPAC software, Münster, Germany). Standards were used for calibration - diploid Belgian cultivars Melvina and Vigor and tetraploid - Merlinda and Roy from perennial ryegrass and a database from (Bennett and Leitch, 2003, Bennett, 2004) (Royal Botanic Gardens, UK). The amount of DNA in a non-repeating haploid chromosome set of organisms is known as the C-value and is measured in picograms (pg). A certain amount of DNA base pairs corresponds to a peak with a certain height.

The results are presented as histograms for natural diploids in Fig.9. and for the induced tetraploids in Fig. 10 and Fig. 11. from 2 populations of perennial (*Lolium perenne* L.) NBG and SBG of IFK - 100% tetraploids from C3 and C4 generations. The obtained sample was analyzed with Partec Cell Analyzer CA-II (2000 and 2001) and Partec PAS III (2005 and 2007) devices, by reading the fluorescence intensity of the nuclear DNA.

RESULTS AND DISCUSSION

Breeding achievements in Bulgaria

The need for new varieties in farming practice, for sowing meadows and pastures or improving degraded ones, for feeding ruminants with the cheapest, high-quality forage also supports the green economy. The global warming of the climate and the increased consumption of forage require high-yielding, stress-tolerant to abiotic and biotic factors forage grasses. We need original, authentic seeds from the Bulgarian varieties, with the greatest adaptability, productivity and quality under our soil and climate conditions.

Historical overview

I. First stage - 32 years (1966 - 1998) – Hay Grass varieties

Table 1 shows species, varieties, breeding methods and photos of 3 hay varieties.

The first Bulgarian varieties of IFK - Pleven, authored by Prof. Petar Tomov are:

1. Cocksfoot (*Dactylis glomerata* L.) variety Dabrava. Tetraploid (Table 1, Fig. 6)

Author: Petar Tomov, Certificate No. 10066

Dabrava is a synthetic variety suitable for growing in mixtures with legumes, mostly with alfalfa. Plants have an upright habit (hay type) and reach 100 to 118 cm at full maturity. The variety is medium-late, with longevity (5 years and

Table 1. Used methods and photos of created hay varieties of perennial grasses in Bulgaria

1. Cocksfoot *(Dactylis glomerata* L.*)*	2. Smooth brome (*Bromus inermis* Leyss.)	3.Tall fescue (*Festuca arundinacea* Schreb.)
DABRAVA	NIKA	ALBENA
		
A selection of 8 clones of local origin and polycross, in isolation	A synthetic variety obtained by crossing Bulgarian naturally growing populations	Polycross of cloned stock from naturally growing populations

more), drought and cold resistance, leaf and stem rust tolerance and good forage quality. The beginning of sweeping (technical hay maturity for the first undergrowth) occurs around May 7-10. The plants are distinguished by great vigor in pure and mixed crops, growing very vigorously in spring and after mowing. In a mixture with alfalfa, the variety provides a dense and uniform grass stand. The Dabrava variety can be grown in all regions of the country under irrigated and non-irrigated conditions in mixtures with alfalfa, sainfoin and bird's foot trefoil. In pure stands, it provides 8-10 t/ha of dry mass and 0.5–0.6 t/ha of seeds. The forage has 13-19% crude protein, and digestibility of 60-65%. It is suitable for hay, pasture and silage.

2. Smooth brome (*Bromus inermis* Leyss.), Variety Nika. Oktoploid (Table 1, Fig. 7) Author: Petar Tomov, Certificate No 10776

Nika is distinguished by high resistance to adverse abiotic factors of the environment, extremely high summer and very low winter temperatures. The plants have a powerfully developed short-rooted root system and an upright habit. The height of the plants in the flowering phase is from 95 to 125 cm. The inflorescence is a single-crested, well-developed medium dense panicle with a length of 19-21 cm. The mass of

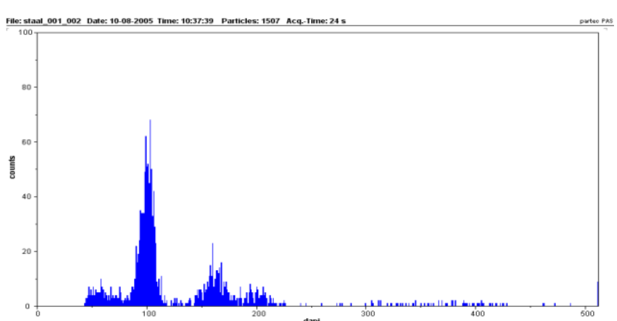


Figure 6. Tetraploid *Dactylis glomerata* – Dabrava

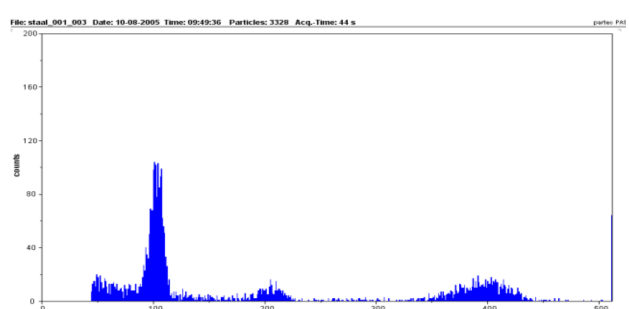


Figure 7. Octoploid *Bromus inermis – Nika*

1000 seeds is 4.1 g. The seeds have relatively low shattering ability. In pure stand cultivation, it provides 10 - 12 t/ha of dry mass and 0.7–0.8 t/ha of seeds. The forage has 17-19% crude protein, and digestibility of 65-68%. It is suitable for hay, pasture and silage. It is resistant to drought, cold and leaf diseases.

3. Tall fescue (*Festuca arundinacea* Schreb.) variety Albena. Hexaploid (Table 1, Fig.8) Author: Petar Tomov, Certificate No10676

The Albena variety is suitable for creating long-lasting hay or pasture grasses (9-10 years), for grassing areas subject to trampling and unsystematic grazing. Withstands dry and waterlogged soils, saline or contaminated with heavy metals. It can be used to create double mixes with alfalfa, sainfoin, perennial ryegrass. Individual crops are sown on swampy, saline or polluted soils. Suitable for areas at risk of erosion. The plants are evergreen, with a semi-erect habit, with leaves tucked around the stems. The variety is suitable for frequent mowing and grazing, resistant to leaf and stem rusts. The plants pollinate very well, and the seeds have low shattering. After mowing the plants, a powerful regrowths, and drought does not significantly affect the development of the regrowths. The variety is highly resistant to high summer temperatures. Plants grow until first frost. In a snowless winter, they can be grazed. When grown alone, it provides over 9 t/ha of dry mass and 0.6–0.7 t/ha of seeds. The feed has 13-14% crude protein, and digestibility of 60-63%. It is suitable for hay, pasture and silage. It is resistant to drought, cold, leaf diseases, acidic and saline soils.

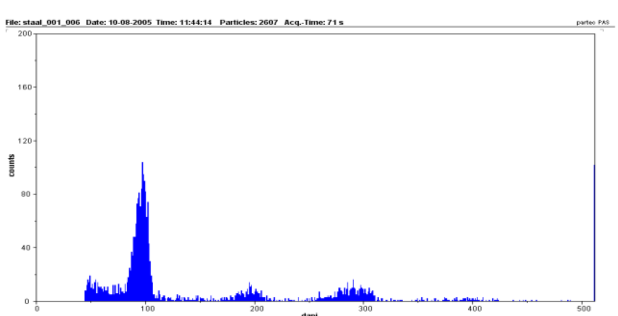


Figure 8. Hexaploid *Festuca arundinaceae*- Albena

II. Second stage - lead author: Prof. Dr. Aneliya Katova

The breeding process for 30 years (1995-2024) includes:

• collection of source material from expeditions and introduction to create working collections with donors to increase productive and adaptive potential

• application of modern selection methods:

• recurrent phenotypic selection,

• induced polyploidy, flow cytometric screening,

• polycross and testing of the generative offspring by complex traits - tandem selection „high productivity - ecological stability

• variety testing

• new variety

• proprietary and high category seeds.

Perennial grasses are major components of native and sown grasslands. Their use, as a source of fodder or for grassing sports and technical fields and landscaping, determines their multifunctional role and importance. During this stage, **new objects of breeding** are included and a new breeder starts his research activity.

Perennial ryegrass is the most economically important perennial grass grown in Europe, New Zealand, and the temperate regions of Japan, Australia, South Africa, and South America, used for forage, ornamental, and sport technical purposes. Intensive breeding activity has been carried out in the world for over 100 years and as a result the large number of varieties in the OECD list for 2024 (1775 pieces). Seed production after 2000 for the EU-27 countries averaged 83660 t of perennial ryegrass seed per year, and globally 209674 t per year.

Perennial ryegrass is preferred by farmers because of a number of advantages: tolerance to intensive grazing, trampling and frequent mowing, excellent nitrogen uptake and most importantly – higher nutritional value, compared to other perennial grasses (Katova, 2005, Naydenova et al ., 2014). World breeding has created many varieties of ryegrass characterized by specific eco-adaptability. The foreign varieties of perennial ryegrass tested in our country so far are in most cases highly productive, but with poor adaptability to our development conditions, not long-lasting and unsuitable for direct implementation in production (Tomov, 1987, Katova et al., 2006, 2007, 2008, 2016, Katova, 2023, 2024).

The multi-cutting and perenniality of perennial ryegrass are important prerequisites for the formation of the total production of vegetative mass for the entire period of use of the grassland. It is subjected year-round, and for years, to the influence of environmental factors, with frequent stressful situations. The resistance of the population to the adverse factors of local ecological conditions is of great importance for productivity and longevity (Katova, 2024).

One of the most interesting breeding opportunities for perennial ryegrass is the expansion of genetic variability through introgression of chromosomal segments or traits from the closely related *Lolium* and *Festuca* species. This additional genetic diversity opens up opportunities for greater breeding progress for some traits than is possible within the frame of perennial ryegrass species.

Most of these studies are led by Humphreys et al. (2006) and colleagues from IBERS in the UK. A similar but more general approach by crossing *Lolium* and *Festuca* species to create *Festulolium* has been adopted by many breeders at present (Nekrosas and Kemesyte, 2007, Zwierzykowski et al., 2011, Baert et al., 2020).

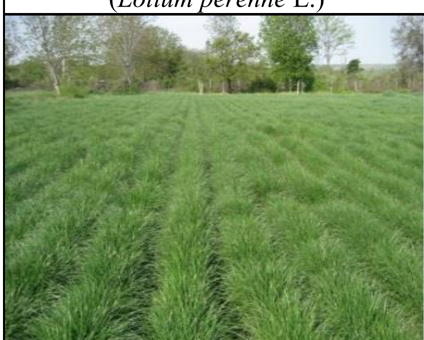
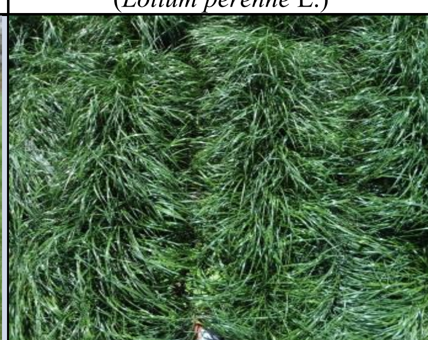
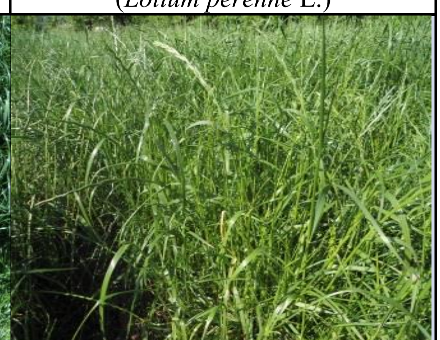
In IFC - Pleven, 3 new varieties of perennial ryegrass, the first for the country, were created (Katova, 2011, Katova et al, 2016, Katova, 2017 a, b).

1. Perennial ryegrass (Lolium perenne ***L.****),*
variety IFC Harmoniya ***– etalon (standard)***

Authors: Aneliya Katova, Petar Tomov, Georgi Georgiev, Anna Ilieva, Yordanka Naydenova (Table 2, fig. 9). Entered in NVL in 2009, Certificate No. 10846 from 2010.

Harmoniya is the first Bulgarian diploid, early variety of perennial ryegrass, suitable for creating pastures and landscaping. The plants are perennial with a well-developed bearded root system, intensive tillering, with a semi-erect to erect habit, forming numerous and well-leafed generative and vegetative shoots. The plants start developing in the spring when the soil warms up to 3-4°C and continue their vegetation until the first frosts.

Table 2. Used methods and photos of newly created perennial ryegrass varieties in Bulgaria

IFC Harmoniya (*Lolium perenne* L.)	Tetrany (*Lolium perenne* L.)	Tetramis (*Lolium perenne* L.)
		
Individual Phenotypic selection, Polycross of 91 Elite Diploid Genotypes of Native Origin	Polyploidization of a local breeding population, Flow cytometric screening and phenotypic selection followed by polycross of 45 tetraploid elite genotypes and breeding to the C4 generation.	Polyploidization of a local breeding population, Flow cytometric screening and phenotypic selection followed by polycross of 52 tetraploid elite genotypes and breeding to the C4 generation.

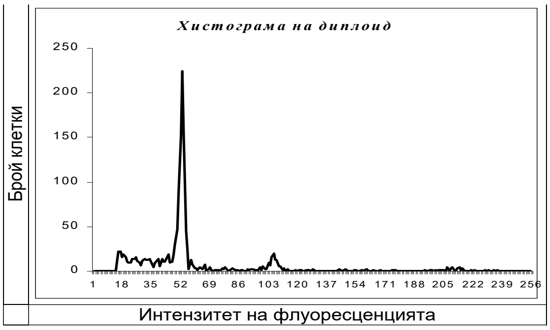


Figure 9. Histogram of diploid *Lolium perenne* –St - IFC Harmoniya

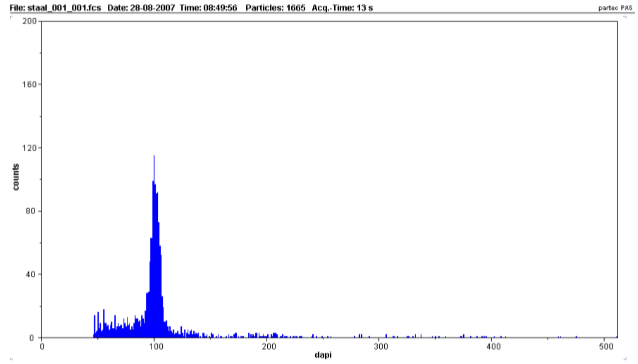


Figure 10. Histogram of tetraploid perennial ryegrass – NBG C4 =Tetrany variety

They form medium-tall stems (at the beginning of flowering 30-37 cm and at full flowering 47-57 cm). By April 15-20, they reach pasture maturity and the height of the grass stand 20-25 cm. The variety is suitable for frequent mowing and intensive grazing. It is characterized by greater durability, winter resistance and drought tolerance. Hay yield is on average 8000-9000 kg/ha, with high nutritional value: crude protein - 15-17%, water-soluble sugars - 6-8% and digestibility 67-72%, seed yield 500–600 kg ha^{-1}. It can participate as a component of pasture mixtures with alfalfa, white clover or bird's foot trefoil, and for decorative purposes in mixtures with red fescue.

The first tetraploid selection populations of perennial ryegrass were created based on Bulgarian germplasm by treatment with 0.2% colchicine solution. The number of living plants and their ploidy level at the age of two months was determined by flowcytometry.

Genotypic differences were found when treated with colchicine to induce polyploidy - IFC-1 has 37.5% live plants, 47 of them are tetraploids, i.e. 6.27%; IFC-2 has 45.0% live plants, 64 of them are tetraploids - 7.11%. An algorithm for the creation of Bulgarian tetraploids of perennial ryegrass has been approved. The resulting tetraploids are included in subsequent stages of the breeding process.

2. Perennial ryegrass (Lolium perenne ***L.****, variety Tetrany*

Author: Aneliya Katova (Table 2, fig. 10)

Registered in NVL in 2017, Certificate No. 11111

Tetrany is the first Bulgarian tetraploid variety of perennial ryegrass (*Lolium perennial* L.). The variety is early to medium early (heading stage occurs in the period from May 15-21), highly productive and long-lasting, ecologically stable (winter-resistant and tolerant of drought and high summer temperatures), tolerant of crown rust (Score 7-9). It has a high productivity of green and dry mass under irrigated and non-irrigated growing conditions. The variety is multifunctional, suitable for pasture, hay-pasture and decorative use, in pure stand or in mixtures with alfalfa and white clover for fodder, or with red fescue for decorative and sports-technical grass, with a high percentage of soil coverage. Plants grow early in spring when the soil warms up to 3-4 °C and continue their vegetation until the first frosts. By April 15-20, it reaches pasture maturity with a height of 25-30 cm. The yield of hay is the highest, compared to other types of perennial ryegrass, on average 9000-11000 kg/ha, with the highest nutritional value: crude protein - 17-19%, water-soluble sugars - 6-10% and digestibility 70-82%. Seed yield is 600-800 kg/ha.

3. Perennial ryegrass (Lolium perenne ***L.****) variety Tetramis*

Author: Aneliya Katova (Table 2, fig. 11).

Entered in NVL in 2017, Certificate No111112

Tetramis is a new tetraploid variety, very early (heading stage observed from April 26-30), high-

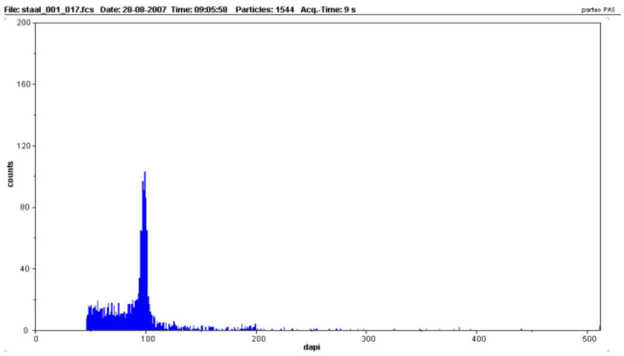


Figure 11. Histogram of tetraploid perennial ryegrass – SBG C4 =Tetramis variety

ly productive, ecologically stable (winter-hardy and tolerant of drought and high summer temperatures). It is distinguished by greater durability, tolerance to crown rust (7-9 score). It is suitable for creating meadow and pasture grasses, in pure stand or in mixtures with alfalfa, white clover and red fescue, as well as for decorative purposes. The variety tolerates frequent mowing and is resistant to trampling. Biomass has a high nutritional value. The plants have a well-developed bearded root system, intensive tillering, with an upright habit and are 65 cm high at the beginning of emergence and 88 cm at full emergence. They grow very early in spring when the soil warms up to 3-4 °C and continue their vegetation until the first frosts. By April 10, pasture maturity is reached with a height of 25-30 cm. Hay yield is on average 9000-10000 kg/ha, with high nutritional value: crude protein - 17%, water-soluble sugars - 6-8% and digestibility 70-75%. The seed yield is the highest compared to the other varieties of perennial ryegrass, averaging 700–900 kg/ha.

In NC - Sredets, with certificate No. 10864 from 2010, perennial ryegrass, variety Strandzha, was created and registered - a natural population, collected near the village of Bliznak, Malko Tarnovo municipality, through repeated mass selection. The variety is diploid. It is not registered in the NVL of Bulgaria and is not supported. Authors: Assoc., Dr. Kera Stoeva, Sredets and Prof., Dr. Miho Mihov - Dobrich.

In the RIMSA-Troyan, timothy variety Troyan and tall fescue Elena were created, approved by Order of the Minister of Agriculture in 1993, but they have not been issued certificates by the Patent Office of Bulgaria, are not entered in NVL and are not maintained as varieties. The author is Prof. Dr. Dimitar Mitev.

Genetic improvement of perennial grasses and creation of new cultivars with increased drought tolerance and water use efficiency is imperative. Crested wheatgrass (*Agropyron cristatum* (L) Gaertn.) is a xerophytic, perennial, tufted grass used for forage, ornamental and anti-erosion purposes. It grows naturally in the desert regions of southern Siberia and is adapted to the dry Canadian prairies. It was 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 cultivation began in the 1930s under a drought rescue program (Ogle, 2000). Breeding activity worldwide dates back to the early 20th century and in 1932 the first Fairway cultivar was registered in Canada.

There are no registered varieties of *Agropyron cristatum* in the EU countries, with the exception of Bulgaria and after that - Romania. Currently, there are a small number of varieties in the OECD lists (6 numbers - 4 from Canada: Fairway - 1932, Parkway - 1969, Kirk - 1987, AC Parkland - 1998; 1 - Bulgaria - Svezhina - 2010 (the first Bulgarian, in the Balkans and in Europe variety, and 1 - Romania - Flaviu).

Crested wheatgrass is preferred by farmers in Canada and the US because of a number of advantages: suitable for semi-arid and arid conditions, develops early in spring, long-lasting, with a deep root system, drought and winter hardy, easy to establish grass stands, preferred by ruminants and is of high nutritional value in spring and early summer as well as in autumn (Katova, 2012a).

Desert wheatgrass is more commonly used than crested wheatgrass throughout the West and especially in the more arid regions of the Great Basin and Southwest. Desert and crested wheatgrass seedings have been established on 10.4

million ha in North America (Asay *and* Knowels,1985). 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; Yancheva and Shamov, 1996; Ogle, 2000; Katova, 2012b).

The widespread adaptability of standard wheatgrass *A.desertorum* (Fisch.) Schult. (tetraploid type), has lead to its extensive use in the livestock industry of western Canada and the United States. Native to eastern Russia, western Siberia and central Asia, this species was first introduced to the University of Saskatchewan in 1911 and throughout western Canada in 1927.

There is no registered variety from *Agropyron desertorum*, in EU countries, except Bulgaria. There are small number of varieties included in OECD list for 2012 (varieties in total: 2 from USA: Nordan – 1953, Hycrest – 1984; 1 from Canada: AC Goliath – 2001 and 1 from Bulgaria – Morava – 2010 (The first Bulgarian, on the Balkans and in Europe and the newest in the world). Seed production over the world is concentrated mainly in Canada and USA, and in EU now there are only Breeder's seeds (Katova, 2012 b).

4. Crested wheatgrass (Agropyron cristatum L.) variety Svejina

Authors: Aneliya Katova, Petar Tomov, Anna Ilieva, Yordanka Naydenova (Table 3, fig. 12), Registered in the NVL in 2009, Certificate No. 10839

Svejina is the first Bulgarian diploid variety of crested wheatgrass, highly productive and ecologically stable. The plants are xeromesophytic, with an upright habit, a well-developed root system, grow in early spring when the soil warms up to 3-4 °C and continue their vegetation until the first frosts. Pasture maturity is reached by April 15-20 (grass height 20-25 cm). Svejina variety heading from May 18-23 and flowering June 6-10. It is distinguished by a very long durability of more than 10 years, high winter resistance and drought resistance, resistance to leaf diseases and tolerance to high summer temperatures. Hay yield is on average 8000-9000 kg/ha, with high nutritional value: crude protein - 17-19%, digestibility 60-68%. Seed yield is 400–500 kg/ha. It is suitable for creating pastures, anti-erosion turf, landscape maintenance and landscaping. It can participate as a component of pasture mixtures with alfalfa, white clover or bird's foot trefoil and also for decorative purposes

Table 3. Used methods and photos of newly created varieties of wheatgrass in Bulgaria

Svejina *(Agropyron cristatum* L.)	Morava *(Agropyron desertorum* Fisch.)
	
Two-fold individual phenotypic selection, clonal selection of 31 elite crested wheatgrass genotypes of local origin from Northeast Bulgaria and a population from Russia, and polycross.	Two-fold individual phenotypic selection, clonal selection of 15 elite genotypes of standard wheatgrass of local origin from North-Eastern Bulgaria, and polycross

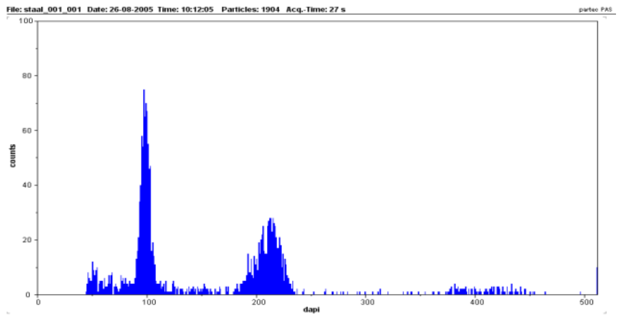


Figure 12. Diploid *Agropyron cristatum-Svejina*

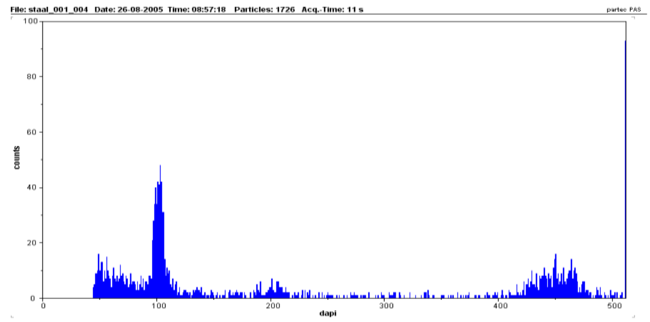


Figure 13. Tetraploid *Agropyron desertorum-Morava*

5. Standard wheatgrass (Agropyron desertorum Fisch.) variety Morava

Authors: Aneliya Katova, Petar Tomov, Anna Ilieva, Yordanka Naydenova (Table 3, Fig.13), Registered in the NVL in 2009, Certificate No10840

Morava is the first Bulgarian tetraploid variety of standard wheatgrass, highly productive and ecologically stable. The plants are xerophytic, perennial grasses with an erect habit, a well-developed root system with a very long life of more than 10 years, high winter hardiness and drought resistance and resistance to foliar diseases. The plants are 40-45 cm high at the beginning of flowering and 80-90 cm at full flowering, grow in early spring when the soil warms to 3-4 °C and continue their vegetation until the first frosts. The Morava variety heading from May 18-23 and flowering June 6-10. Hay yield is on average 9000-10000 kg/ha, with high nutritional value: crude protein - 17-19%, digestibility 60-68%. Seed yield is 500-600 kg/ha. It is suitable for hay and hay-pasture use, creation of anti-erosion turf, landscape maintenance and landscaping and as a component of hay mixtures with alfalfa or sainfoin.

Since its foundation in 1954, IFC - Pleven has created a number of varieties. Some of them have been irretrievably lost over time, one of the reasons being that there was no intellectual property protection.

Currently, 8 varieties of perennial grasses are certified, establishing a lengthy certification process:

• the Dabrava cocksfoot variety was entered in the variety list first in 1978, then after 20 years, a certificate was issued in 1998;

• variety smooth brome Nika was entered in the official variety list of our country in 1993, and after 15 years, in 2008 it received a certificate;

• tall fescue variety Albena was entered in the variety list in 1993, and after 12 years it has a certificate from 2005.

• The new varieties of grassland ryegrass IFK Harmoniya, crested wheatgrass Svejina and standard wheatgrass Morava are in official variety testing in the period 2006-2009. The varieties are registered in the Official Varietal List of the Republic of Bulgaria for the years 2010-2024, in the OECD lists for 2010-2024, and have Certificates from the Patent Office (PO) of the Republic of Bulgaria since 2010. The newest are the tetraploid perennial ryegrass varieties Tetrany and Tetramis, which successfully passed official variety testing in the period 2014 - 2016, registered in the VL of the Republic of Bulgaria for 2017 - 2024, in the OECD List for 2017 - 2024, and are with Certificates from the PO of the Republic of Bulgaria since 2017.

CONCLUSIONS

During the 58 – year period at the Institute of Forage Crops – Pleven, 8 varieties from 6 species of perennial grasses were developed as follows:

In hay and hay-pasture direction:

• cocksfoot (*Dactylis glomerata* L.) Dabrava – tetraploid;

• smooth brome (*Bromus inermis* Leyss.) Nika - octoploid;

• tall fescue (*Festuca arundinacea* Schreb.) Albena – hexaploid;

In pasture, hay-pasture and decorative, anti-erosion direction:

• perennial ryegrass (*Lolium perenne* L.) IFK Harmony - diploid, Tetrany - tetraploid and Tetramis - tetraploid;

• crested wheatgrass (*Agropyron cristatum* Gaerth.) Svejina - diploid and

• standard wheatgrass (*Agropyron desertorum* (Fich.) Schultes.) Morava - tetraploid.

Varieties have valuable characteristics such as high forage and seed productivity, longevity, leafiness, different maturity groups, stress tolerance (drought, high temperatures, cold), resistance to foliar diseases, high forage quality, suitable for various areas of use and different ploidy level, for mixtures with suitable forage legumes.

The Institute of Forage Crops carries out variety maintenance and seed production of breeder seeds and pre-basic seeds.

It was approved as an innovative scientific product by PKIT to Agricultural Academy -Sofia, Technology for seed production of perennial forage grasses (2024).

In the National Genebank of Bulgaria in IRGR - Sadovo, long-term, original seeds of the varieties of perennial grasses of IFC - Pleven are stored.

Acknowledgements

The present research was reported at an jubilee scientific conference with international participation “Sustainable and competitive agriculture under the conditions of global climate change“, held in September, 03-04 2024, in Maize Research Institute, Knezha, Bulgaria.

REFERANCES

Annicchiarico, P., Boller, B., Brummer, E. C. & Reheul, D. (2016). *Improving the focus of forage breeding research.* In: “Breeding in a World of Scarcity: *Proceedings of the 2015 Meeting of the Section Forage Crops and Amenity Grasses of Eucarpia*, Ghent, Belgium, 13–17 September 2015” (eds. I. Roldán-Ruiz, J. Baert and D. Reheul), Springer International Publishing, Switzerland,pages 251–268.

Arcioni, S., Mariotti, D., Falcinelli, M., & Cecarelli, S. (1980). Relationship between ecological adaptation and carbohydrate reserve evolution for two different varieties of *Lolium perenne* L. *Ann. Amelior Plantes* 30: 309–319.

Arcioni, S., Veronesi, F., Mariotti, D., & Falcinelli, M. (1983). Evaluation of the possibility of improving protein yield in *Lolium perenne* L. Z. *Pflanzenzüchtg.* 91: 203–210.

Arojju, S. K., Cao, M., Jahufer, M. Z. Z., Barrett, B. A., & Faville, M. J. (2020). Genomic predictive ability for foliar nutritive traits in perennial ryegrass. *G3 Genes Genomes Genetics* **10**: 695–708.

Asay, K. H., & Knowles, R. P. (1985). Current status and future of introduced wheatgrasses and wildrye for rangeland improvement. In: Carlson, J. R.; McArthur, E. Durant, Range plant improvement in western North America: *Proceedings of a symposium at the annual meeting of the Society for Range Manag*ement; 1985 February 14; Salt Lake City, UT. Denver, CO: Society for Range Management: 1-8.

Aufrere, J. (1982). Etude de la prévision de la digestibilité des fourrages par une méthode enzymatique**,** *Annales de Zootechnie* 31(2) DOI:10.1051/animres:19820202

Baert, J., & Reheul, D. (1997) The persistency of 4n perennial ryegrass in mixtures with 2n perennial ryegrass. In: Weddell, J.R.(ed.) *Seeds of Progress*, BGS occasional symposium N 31: 63 – 65.

Baert, J., Van Laere, K. , Van Waes, C., Ghesquiere, A., & Aper, J. (2020). Breeding and genetics of two new amphiploid *Festulolium* synthetics with improved yield and digestibility, Biologia plantarum 64:789-797, 2020 | DOI: 10.32615/bp.2020.138.

Baert, J., Ghesquiere, A., & Van Waees, C. (2014) Variation of cell wall digestibility in fodder grasses with particular focus on a perennial breeding pool, In: D. Sokolovic, C. uyghe and J. Radovic (Eds.) *Quantitative Traits breeding for multifunctional grasslands and turf,* Springer Science, 394., 261 – 265.

Bennett, M. D. (2004). Perspectives on polyploidy in plants – ancient and neo, *Biological Journal of the Linnean Society*, 82:411-423.

Bennett, M. D., & Leitch, I.J. (2003) Angiosperm DNA C-values database, release 4.0, available at website: http://www.rbgkew.org.uk/ cval/homepage.html.

Boller, B., & Green, S. (2010). Genetic resources, pp. 13-37. In: Boller B. et al (eds.) *Fodder crops and amenity grasses*. Handbook of plant breeding, Springer Science + Business Media, New York.

Boller, B., Posselt, U. K. & Veronesi, F. (2010). Handbook of Plant Breeding 5, *Fodder Crops and Amenity Grasses* , Springer Science+Business Media, LLC.

Brummer, E. C. & Casler, M. D. (2014). Cool-season forages. In: “Yield Gains in Major U.S. *Field Crops”*, CSSA Special Publication 33 (eds. S. Smith, B. Diers,

J. Specht and B. Carver), ASA, CSSA, and SSSA, Madison, WI, pages 33–51.

Bukhteeva, A.V., Shutova, Z. P., Korneichuk, V. A., Vatsek, V., Krcmar, B., Bares, I., Horvat, L., Osinsky, B., Kelner, E., & Neaggu, M. (1985). The International Comecon List of Descriptors for the family *Poacea*. USSR, Leningrad, 22-46.

Buxton, D. R., & Marten, G.C. (1989). Forage quality of plant parts of perennial grasses and relationship to phenology. *Crop Sci.* 29: 429-435.

Byrne, S. (2023). Genomic prediction of seasonal forage yield in perennial ryegrass. Grassland Research, 1–15. https://doi.org/10.1002/glr2.12058

Camlin, M. S. (1997) Grasses, In: Weddell, J.R.(ed.) *Seeds of Progress*, BGS Occasional Sympos., 31, 2 – 14. BSG, Reading

Camlin, M. S., & Stewart, R. H. (1975). Reaction of Italian ryegrass cultivars under grazing as compared with cutting. *Journal of the British Grassland Society* 30: 121–129.

Caradus, J. R., Bouton, J. H., Brumer, E. C., Favile, M. J., George, R. M., Hume, D. E, Inch, C. , Kerr, G. A., Montel, S., Stewart, A. V., & Woodfield, D. R. (2021). Plant breeding for resilient pastures, *Resilient Pastures – Grassland Research and Practice Series 17: 81-104*.

Casler, M. D., & Vogel, K. P. (2020). Forage Breeding. From Forages: *The Science of Grassland, Agriculture*, Volume II. Publications from USDA-ARS / UNL Faculty. 2482., https://digitalcommons.unl.edu/usdaarsfacpub/2482, 553 – 565.

Casler, M. D. (2001). Breeding perennial grasses for abiotic stress tolerance. In: P. Monjardino, A. da Camara Machdo, V. Carnide (eds.) Breeding for stress tolerance in fodder crops and amenity grasses. *Proceedings of the 23rd Meeting of the Fodder Crops and Amenity Grasses Section if Eucarpia*, Azores, Portugal, 1-4 October 200, ISBN 972-8612-05-2, Published: Department of Agricultural Sciences, University of Azores, Terceira Island, Azores, Portugal, 2001, 119-125.

Chapman, D. F., Muir, P. D., & Faville, M. J. (2015). Persistence of dry matter yield among New Zealand perennial ryegrass (*Lolium perenne* L.) cultivars: insights from a long-term data set. *Proceedings of the New Zealand Grassland Association, 77*, 177-184.

Chaves, B., De Vliegher, A., Van Waes, J., Carlier, L., & Marynissen, B. (2009). Change in agronomic performance of *Lolium perenne* and *Lolium multiflorum* varieties in the past 40 years based on data from Belgian VCU trials. *Plant Breeding* 128: 680-690.

Conaghan, P., & Casler, M. D. (2011). A theoretical and practical analysis of the optimum breeding system for perennial ryegrass. *Ir. J. Agric. Food Res., 50*, 47–63.

Connolly, V. (2001) Breeding improved varieties of perennial ryegrass. Teagasc Project report 3495, Crops Research Centre Oak Park, Carlow, ISBN 18411701998, 1-17.

Cooper J. P., & Breese E.L. (1971) Plant breeding: forage grasses and legumes. In: Wareing P.F. and Cooper J.P. (eds) *Potential crop production*, pp. 295–318. London: Heinemann Educational Books

CPVO Technical Protocol for Distinctness, Uniformity and Stability Tests for Meadow and Tall 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).

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 Cocksfoot – TP031/1 English, Date: 25./03/2021.

Cunningham, P. J., Blumenthal, M. J., Anderson, M. W., Prakash K.S. & Leonforte, A. (1994). Perennial ryegrass improvement in Australia, *New Zealand Journal of Agricultural Res*earch, 37:3, 295-310, DOI: 10.1080/00288233.1994.9513068

Duller, S., Lovatt, A., & Skot, L., (2019). One hundred years of forage grass breeding at Aberystwyth, In: Grassland Science in Europe, vol.24 – Improving sown grasslands through breeding and management, *Proceedings of the Joint 20th Symposium of the European Grassland Federation* and the 33rd Meeting of the EUCARPIA Section „Fodder Crops and Amenity Grasses", Zürich, Switzerland, 24-27 June 2019, 376-378.

Easton, H. S., Amyes, J. M., Cameron, N. E., Green, R. B., Kerr, G. A., Norross, M. G. & Stewart, A. V. (2002). Pasture plant breeding in New Zealand: where to from here? *Proceedings of the New Zealand Grassland Association* **64**: 173–179.

Eaton, T. D, Curley, J., Williamson, R. C., & Jung, G. (2004). Determination of the Level of Variation in Polyploidy among Kentucky Bluegrass Cultivars by Means of Flow Cytometry, *Crops Science*, Vol.44, 6, Pages 2168-2174.,https://doi.org/10.2135/cropsci2004.2168.

Edwards, G. R., Parsons, A. J., & Rasmussen, S. (2007). High sugar ryegrasses for dairy systems. pp. 307-334. In: *Proceedings of the 3rd Australasian Dairy Science Symposium*. Eds. Chapman, D.F.; Clark,D.A.; Macmillan, K.L.; Nation, D.P.

Eeckhaut, T. (2004) Ploidy breeding and interspecific hybridization in Spathiphyllum and woody ornamentals, PhD theses , Gent University, Belgium, 164.

Ermakov, A., Arasimovich, V., Yarosh, N., Peruanskii, Yu., Lukovnikova, G., & Ikonomova, M. (1987). Methods of biological study of plants, *Agropromizda*t, M., 134–135.

Faville, M., Cao, M., Schmidt, J., Ryan, D., Ganesh, S., Jahufer, M., Hong, S., George, R., & Barrett, B.

(2020). Divergent genomic selection for herbage accumulation and days-to-heading in perennial ryegrass. *Agronomy* **10**: 340.

Ferris, C. P. (2007). Sustainable pasture-based dairy systems – meeting the challenges. Can. J. Plant Sci. **87**: 723–738.

Frame, J. (1989). Herbage productivity of a range of grass species under silage cutting regime. *Grass & Forage Sci.* 44: 267–276.

Frandsen, H. N., & Frandsen, K.I. (1948). Polycross-methoden massekrydsnings-methode ved foraedling of fremmedbefrugtende planter. *Nordisk Jordfruksforskning*, pp: 239-261

Frandsen, K. J. (1986). Variability and inheritance of digestibility in perennial ryegrass (*Lolium perenne*), meadow fescue (*Festuca pratensis*) and cocksfoot (*Dactylis glomerata*). II. F1 and F2 progeny. *Acta Agriculturae Scandinavica* 36: 241–263.

Fulkerson, W. J. Slack, K., Bryant, R., & Wilson, F. (2004) Selection for more persistent perennial ryegrass (Lolium perenne L.) cultivars for subtropical/ warm temperate dairy regions of Australia, CSIRO Publishing, *Australian Journal of Experimental Agriculture* 43 (9), 1083 - 1091.

Gilliland, T., & Mann, R (2001). The silage yield potential of hybrid ryegrass over a 5-year period in Northern Ireland relative to other ryegrasses and timothy. *Grass and Forage Science* 56, 305–310.CrossRefGoogle Scholar

Gilliland, T. J., Ball, T., & Hennessy, D. (2021). Opportunities and challenges for breeding perennial ryegrass cultivars with improved livestock production potential, Irish *Journal of Agricultural and Food Research*, Perspective Paper • DOI: 10.15212/ ijafr-2020-0111 IJAFR, *59*(2), 233-245.

Gilliland, T. J., Johnston, J., & Connolly, C. P. (2007). A Review of Forage Grass and Clover Seed Use in Northern Ireland. *Grass and Forage Science* 62: p 239-254.

Gilliland, T.J., Annicchiarico, P., Julier, B., & Ghesquière, M. (2020). A proposal for enhanced EU herbage VCU and DUS testing procedures. *Grass and Forage Science* **75**: 227–241.

Gilliland, T. J., Barrett, P. D., Agnew, R. E., & Fearon, A.M. (2003). Variation in herbage quality traits and grazing value indicators among perennial ryegrass (*Lolium perenne* L.) varieties. *Vortrage fur Plfanzensuchtung* 59: 11-19.

Halling, M. A., Longland, A. C., Martens, S., Nesheim, L., & O'Kiely, P. (2004). Accumulation of WSC in two perennial ryegrass cultivars at nine European sites, Vol.9, *Grassland Science in Europe*, Switzerland, EGF 2004, Land Use Systems in Grassland Dominated regions,(Eds Luscher, A.; Jeangros, B.; Kessler, W.; Huguenin, O.; Lobsiger, M., Millar, N. and Suter, D.), 954 -956.

Hofer, D., Suter, M., Haughey, E., Finn, J. A., Hoekstra, N. J., Buchmann, N., & Lüscher, A. (2016). Yield of temperate forage grassland species is either largely resistant or resistant to experimental summer drought. *Journal of Applied Ecology 53*, 1023–1034.

Humphreys, M. W., Feurstein, U., Vandewalle, M., & Baert, J. (2010). Raygrasses, In: B. Boller et al. (eds.) *Fodder Crops and Amenity Grasses*, Handbook of Plant breeding 5, Springer Science Business Media, 211–260.

Humphreys, M. O. (1999). The contribution of conventional plant breeding to forage crop improvement. In: Proc. XVIII Intl. Grassl. Congr.1: 4-71 - 4-78. 8-19 June 1997. Winnipeg,Manitoba and Saskatoon, Saskatchewan, Canada

Humphreys, M. W. (1989). The controlled introgression of *Festuca arundinacea* into *Lolium multiflorum*. *Euphytica* 42: 105–116.

Humphreys, M. W., Yadav, R. S., Cairns, A. J., Turner, L. B., Humphreys, J., & Skot, L. (2006). A changing climate for grassland research. *In New Phytologist*, 169, 9-26.

Katova A., & Ilieva, A., (2017). Biochemical characteristics of tetraploid perennial ryegrass, In: Modern Aviation Training Trends", Proceedings of scientific conference , May 18 - 19, 2017 The Faculty of Aviation "V. Levski "- Dolna Mitropolia Bulgarian First edition, E -edition in an electronic form of CD ISBN 978-954-713-110-1 © Faculty of Aviation at the National University "V. Levski ", pp. 279 -285.

Katova, A. & Tomov, P. (2005) Study of new untraditional forage grass species tolerant in a marginal ecological conditions, I. Biological properties, Proceedings of the Union of Scientists – Rousse, Book 3. "*Agrarians and Veterinary Medicines Sciences*, *5*, 65-69.

Katova, A. & Tomov, P. (2006) Study of new untraditional forage grass species tolerant to marginal ecological conditions, II. Morphological traits, *Journal of Mountain Agriculture on the Balkans, 9*(1), 149-166.

Katova, A. & Vulchinkov, J. (2019). Variability of morphological characters of collection accessions of perennial ryegrass (*Lolium perenne* L.). *Bulgarian Journal of Agricultural Science, 25*(5), 1015–1023.

Katova, A. (2005). Evaluation of Perennial Ryegrass (*Lolium perenne* L.) Local Populations: Forage and Seed Productivity, *Plant Science*, *42*(1), 80– 85.

Katova, A. (2005a). Study of morphological traits, biological properties and agricultural value of plant germplasm of perennial ryegrass (*Lolium perenne* L.) with a view to breeding, *Abstract of Thesis for Doctor* (PhD), Pleven, 44p.

Katova, A. (2009) Polyploidy and polyploidization of perennial forage grasses, *Journal of Mountain Agriculture on the Balkans, (JMAB)*, 12(3), 523 – 535.

Katova, A. (2011). New perennial ryegrass variety (*Lolium perenne* L.) IFK Harmoniya. *Journal of Mountain Agriculture on the Balkans, 14*(4), 721-739.

Katova, A. (2012a). Svejina – the first Bulgarian Crested wheatgrass variety [*Agropyron cristatum* (L.) Gaertn.], *Journal of Mountain Agriculture on the Balkans, 15*(5), 1034-1056. ISNN: 1311-0489.

Katova, A. (2012b). Morava – the first Bulgarian Standard wheatgrass variety [*Agropyron desertorum* (Fisch.) Schultes]. *Banat's Journal of Biotechnology*, *3*(5), 58-66. Available on-line at http://dse.usab-tm.ro (SJIF 3,904), ISSN: 2068-4673, ISSN / eISSN 2068-4673.

Katova, A. (2015a). Development of tetraploid perennial ryegrass varieties I. Colchicine treatment, Proceedings of the Union of Scientist – Russe. *Agrarian and Veterinary Sciences, 7*, 83-89.

Katova, A. (2015b). Development of tetraploid perennial ryegrass varieties II. Flow cytometric analyses, Proceedings of the Union of ScientistRusse, *Agrarian and Veterinary Sciences, 7*, 90-95.

Katova, A. (2016). Study of growth and development of perennial ryegrass in pure stand and in mixtures with alfalfa. *Journal of Mountain Agriculture on the Balkans, 19*(2), 111–135.

Katova, A. (2017a). Tetrany – The first Bulgarian tetraploid perennial ryegrass variety (*Lolium perenne* L.). *Journal of Mountain Agriculture on the Balkans, 20*(1), 110-122.

Katova, A. (2022). Study of Species, Varieties and Breeding Populations of Perennial Grasses for Ornamental Purposes. In *Journal of Mountain Agriculture on the Balkans*, *25*(2), 128–158. ISSN 1311-0489 (Print) *Research Institute of Mountain Stockbreeding and Agriculture, Troyan.* ISSN 2367-8364 (Online).

Katova, A. (2023). Bulgarian varieties of perennial forage grasses developed at the Institute of Forage crops – Pleven, *Bulgarian Journal of Agricultural Science, 29,No 5, 2023, 841–853.*

Katova, A. (2024). Vitality and longevity of perennial ryegrass (*Lolium perenne L.*) accessions in North Central Bulgaria. *Bulg. J. Agric. Sci., 30*(3), 431–444

Katova, A., (2017b). Tetramis – New tetraploid perennial ryegrass variety. *Journal of Mountain Agriculture on the Balkans, 20*(1), 123 – 134.

Katova, A., Baert, J. & Reheul, D. (2016). Comparative characteristics of newly developed perennial ryegrass varieties in Bulgaria. In: Roldán-Ruiz et al. (eds.). Breeding in a World of Scarcity, Proceedings of the meeting of the section "Fodder crops and amenity grasses" of Eucarpia, Ghent, 2015. *Springer, Switzerland*, 35-40.

Katova, A., Baert, J., Hristov, K. & Van Bockstaele, E. (2007). Ecologo-genetic approach to perennial ryegrass breeding, In: Proceedings of the 14th Symposium of the European Grassland Federation, Ghent, Belgium, 3 – 5 September 2007 *„Permanent and Temporary Grassland Plant, Environment and Economy",* Eds; De Vliegher, A. and L. Carlier, Grassland Science in Europe, Vol. 12 pp: 86 – 89., ISBN 9789081100731. https://www.europeangrassland.org/fileadmin/documents/Infos/Printed_Matter/Proceedings/EGF2007_GSE_vol12.pdf

Katova, A., Hristov, K., Baert, J. & Van Bockstaele, E. (2006) Ecologo-genetical estimation of perennial ryegrass (*Lolium perenne* L.) European cultivars and local populations in Bulgaria, In: *Sustainable Grassland Productivity,* Proc. of the 21st General Meeting of the EGF, Badajoz, Spain, 3-6 April 2006, Eds: Lloveras, J., Gonzalez-Rodriguez, A., Vasquez-Yanez, O., Pineiro, J., Santamaria, O., Olea, L.&Poblaciones, M.J., 158– 160.

Katova, A., Ilieva, A., Baert, J. Hristov, K. & Van Bockstaele, E. (2008) Importance of the polyploidy for forage and seed productivity and forage quality of perennial ryegrass, In: Proceedings of the 22nd General Meeting of EGF, 9-12 June 2008, Uppsala, Sweden, Eds. A. Hopkins, T. Gustafsson, G. Dalin, N. Nilsdotter – Linde, E. Spörndly, *Biodiversity and Animal Feed Future Challenges for Grassland Production, 13* Grassland Science in Europe, 254 – 256.

Katova, A., Serafimov, P., Golubinova, I., Nikolova, I., Kertikov, T., Vasilev, E. & Vasileva, V. (2024). Technology for seed production of perennial grasses, Certificate No. 09/22.12.2023, Agricultural Academy-Sofia, IFC – Pleven, 60.

Katova, A. (2008). Study of the perennial grass species and varieties for ornamental purposes. *Journal of Mountain Agriculture on the Balkans (JMAB), 11*(4), 744–757.

Klootwijk, C.W., Bruinenberg, M, Cougnonq M., Hoekstra, N.J., Ripoll-Bosch, R., Schelfhout, S., Schils, R.L.M., Vanden Nest, T. , van Eekeren, N., Voskamp-Harkema, W. & van den Pol-van Dasselaar, A. (Eds.) (2024). Proceedings 'Why grasslands?, *Grassland Science in Eu*rope, Volume 29, The Netherlands 9-13 June, 2024

Konkolewska, A., Phang, S., Conaghan, P., Milbourne, D., Lawlor, A., & Kopecký, D., Frei, I. & Vymyslický, T. *(eds.) (2023). Novel technologies, strategies and crops to sustain forage production in future climate, Abstracts of the 35th Meeting of the Eucarpia Fodder Crops and Amenity Grasses Section in cooperation with the Eucarpia Festulolium Working Group, Brno 10–14 September, 2023, 160.*

Lamote, V., Baert, J., Roldan – Ruiz, I., DeLoose, M., & Van Bockstaele, E. (2002) Tracing of 2n egg occurrence in perennial ryegrass (Lolium perenne L.) using interploidy crosses, *Euphytica*, 123: 159 -164.

Lauer, J. G. Coors, J. G., & Flannery, P.J. (2001). Forage yield and quality of corn cultivars developed in different eras. *Crop Science 41*: 1449-1455.

Lee, C. K.; Cunningham, P. J. & Anderson, M. W. (1993): Breeding temperate forages for winter growth and persistence. *Proceedings of the 10th Australian Plant Breeding Conference, Gold Coast:* 67-71

Leus, L. (1998). The use of flow cytometry for plant breeding and seed production. *VLAOESO* n 5335 97091616 project, 3-13.

McDonagh, J., O'Donovan, M., McEvoy. M., & Gilliland, T.J., (2016). Genetic gain in perennial ryegrass (*Lolium perenne*) varieties 1973 to 2013, *Euphytica* (2016) 212:187–199, DOI 10.1007/s10681-016-1754-7.

Merry, R. J., Lee, M. R. F., Davies, D. R,; Dewhurst, R. J., Moorby, J. M., Scollan, N. D., & Theodorou, M. K. (2006). Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. *In vitro* and *in vivo* studies of nitrogen utilisation. *Journal of Animal Science 84*: 3049-3060.

Miller L. A., Moorby J. M., Davies D. R., Humphreys M. O., Scollan N. D., Macrae J. C., & Theodorou M. K. (2001) Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass andForage Science*, 56, 383–394.

Muirhead, K .A., Horan, P. K., & Poste, G. (1985) Flow cytometry present and future. *Biotechnology* 3:337–355.

Myers, W. M. (1939) Colchicine induced tetraploidy in perennial ryegrass. *J. Heredity*, 30, 499-504.

Nair, R. M. (2004). Developing tetraploid perennial ryegrass (Lolium perenne L.) populations. *New Zealand Journal of Agricultural Research* 47: 45-49.

Naydenova, Y., & Katova, A. (2013). Forage quality evaluation of perennial grass species in breeding process. *Journal of Mountain Agriculture on the Balkans,13*(6), 1519-1538. ISNN 1311-0489.

Naydenova, Y., Katova, A., & Petrov, A. (2014). Forage plant cell walls fiber components content and digestibility of new varieties perennial grasses in the vegetation. *Journal of Animal Science, Sofia, 18*(1-2), 184-191. ISSN 1310-0351.

Nekrośas, S., & Kemeśyte, V. (2007) Breeding of ryegrass and festulolium in Lithuania, Žemdirbystė / Zemdirbyste / *Agriculture*, vol. 94, No. 4 (2007), p. 29–39. UDK [633.265+633.264:633.265]:631.527.

O'Donovan, M., Dillon, P. , Conaghan, P., & Hennessy, D. (2022) Irish Grassland Research — main achievements and advancements in the past 60 yrs and where to progress to next, *Irish Journal of Agricultural and Food Research,* Special Issue article • DOI: 10.15212/ijafr-2020-0152 IJAFR , 61(1) , 2022 , 1-11.

OECD list-of-varieties -grasses-and-legumes, (2024). http://www.oecd.org/agriculture/seeds/documents/codes-schemes-list-of-varieties -grasses-and-legumes.pdf

Ogle, D. G. (2000). Crested wheatgrass, *Plant guide*, USDA, NRCS, Idaho State office, http://plants.usda.gov.

Oliveira, J. A. & Castro, P. (1994). Variability and selection of genotypes of perennial ryegrass for nutritive value using NIIRS, 153 – 154. Breeding for quality, *Proceedings of the 19th Fodder Crops Section Meeting* held in Brugge, Belgium, 5-8 October, 1994, Eds.: Dirk Reheul and An Ghesquiere, 289.

Parsons, A.J., Edwards, G. R., Newton, P.C.D., Chapman, D. F., Caradus, J. R., Rasmussen, S., & Rowarth, J. S. (2011). Past lessons and future prospects: plant breeding for yield and persistence in cool temperate pastures. *Grass and Forage Science 66*: 153-172.

Paul, C. (1988) Sortenwahl qualitatsorientiert DLG – Mitt.103, 8: 398 – 399.

Peeters, A. (2004). Wild and sown grasses. Profiles of a temperate species selection: ecology, biodiversity and use. FAO and A. Peeters.

Pembleton, L.W., Inch, C., Baillie, R. C., Drayton, M.C., Thakur, P., Ogaji, Y. O., Spangenberg, G. C., Forster, J. W., Daetwyler, H. D., & Cogan, N. O. I. (2018). Exploitation of data from breeding programs supports rapid implementation of genomic selection for key agronomic traits in perennial ryegrass. *Theoretical and Applied Genetics* 131: 1891–1902.

Petkova, M. (2023). Bioproductive and quality characteristics of perennial ryegrass (*Lolium perenne* L.) in a technological aspect, 2023, 162 pp. PhD, RIMSA, Troyan.

Petrova, S., Nikolov, B., Velcheva, I., Angelov, N., Valcheva, E., Katova, A., Golubinova, I., & Marinov-Serafimov, P. (2022). Buffer Green Patches around Urban Road Network as a Tool for Sustainable Soil Management. *Land* 2022, *11*, 343. https://doi.org/10.3390/land11030343.

Posselt, U. K. (1994). Selection parameters of quality traits in perennial ryegrass. *Proc. Eucarpia Fodder Crops Section* for 1994: 129–135.

Price, T. (1987) Ryegrass rust in Victoria. *Plant Protection Quarterly* **2**, 189.

Quesenberry, K. H. & Casler, M. D. (2001). Achievements and perspectives in the breeding of temperate grasses and legumes (pp. 517–524). In: A. Gomide, J.A., Mattos, W.R.S., da Silva, S.C. (eds.), *Proc. Int. Grasslands Congress*, XIX, Sao Pedro, Sao Paulo, Brazil. 11–21 Feb. 2001. FEALQ, Piricicaba, Sao Paulo, Brazil.

Reed, K. F. M., Cunninghan, P. J., Barrie J. T., & Chin, J. F. (1987). Productivity and persistence of cultivars and Algerian introductions of perennial ryegrass (*Lolium perenne* L.). *Aust. J. Exp. Agric*. 27, 267–274.

Reheul, D, Cougnon, M, Kayser, M, J., Pannecoucque, J., Swanckaert, J., De Cauwer, B., van den Pol-van Dasselaar, A., & De Vliegher, A. (2017). Sustainable intensification in the production of grass and forage crops in the Low Countries of north-west Europe. *Grass Forage Sci* . 2017;00:1–13.

Reheul, D., De Cauwer, B., & Cougnon, M. (2010). The role of forage crops in multifunctional agriculture. In: Boller B., Posselt U., Veronesi F.(eds). *Fodder crops and amenity grasses*. Springer, 1-12.

Reheul, D., De Cauwer, B., Cougnon, M., & Aper, J. (2013). What Global and/or European AgricultureWill Need from Grasslands and Grassland Breeding over the Next 10–15 Years for a Sustainable Agricultue, In: S. Barth, D. Milbourne (eds.). *Breeding Strategies for sustainable Forage and turf grass improvement*, 3-18.

Reheul, D., Baert, J., Boller, B., Bourdon, P., Cagas, B., Eickmeyer, F., Feurstein, U., Gaue, I., Ghesquiere, A., Grass, M.C., Hoks, I., Katova, A., Lellbah, H., Matzk, F., Muylle, H., Oliveira, J.A., Pronszuk,M., Roldan-Ruiz, I., Thorogood, D., Vanbellinghen, C., van Hee, F., Van Wijk, A., Visscher, J., Vijn, R., & Wolters, L. (2001). Crown rust, *Puccinia coronata* Corda: recent developments, In: Proceedings of the 3th Conference of *Harmful and Beneficial Microorganisms in Grassland, Pastures and Turf* (26.09.2000) Paderborn, Edited by Volker H. Paul and Peter D. Dapprich, University Soest, Germany, 25-36. , ISBN 9783935807036.

Reheul, D., Baert, J., & Ghesquiere, A. (2003) Progress in Breeding Perennial Fodder grasses, 1.In search of tetraplouid riegrass with a higher dry matter content (DMC), *Czech J.Genet. Plant Breed*.,39: 54 - 56.

Sampoux J. P., Baudouin, P., Bayle, B., Beguier, V., Bourdon, P., Chosson, J. F., Deneufbourg, F., Galbrun, C., Ghesquiere, M.,, Noel, D., Pietraszek, W., Tharel, B., & Viguie, A., (2011). Breeding perennial grasses for forage usage: An experimental ssessment of trait changes in diploid perennial ryegrass (*Lolium perenne* L.) cultivars released in the last four decades. *Field Crops Research* 123 (2011) 117–129.

Sandev, S. (1979). Chemical methods for analyses of forages. *Zemizdat,* Sofia (Bg).

Silsbury J. H. (1961). A study of dormancy, survival, and other characteristics in Lolium perenne L. at Adelaide, S.A. *Australian Journal of Agricultural Research* 12: 1–9.

Smith, H. J., & Elgersma, A. (2004) Durnal fluctuations in vertical distribution of chemical composition in a perennial ryegrass (*Lolium perenne* L.) sward during the season, Vol.9, *Grassland Science in Europe*, Switzerland, EGF 2004, Land Use Systems in Grassland Dominated regions,(Eds Luscher, A.; Jeangros, B.; Kessler, W.; Huguenin, O.; Lobsiger, M., Millar, N. and Suter, D.), 951-953.

Smith, K. F., Reed, K. F. M. & Foot, J. Z. (1997). An assessment of the relative importance of specific traits for the genetic improvement of nutritive value in dairy pasture. *Grass and Forage Science*, 52 (2), 167 – 175.

Sokolović, D., Babić, S., Radović, J., Lugić, Z., Simić, A., Zornić, V., & Petrović, M. (2017). Genetic resources of perennial forage grasses in Serbia: Current state, broadening and evaluation. *Plant Breeding and Seed Production*, 23, (1): 69-82.

Sokolović, D., Tomić, Z., & Babić, S. (2010). Perennial forage grasses breeding in Serbia: Achievements limits and prospects. *Biotechnology in Animal Husbandry*, 26, spec. issue, 91-106.

Stoeva, K. (2010). Strandja – new perennial ryegrass, *Plant Science*, 47(2), 190-192 (Bg).

Teixeira, C.S.P., Olykan, S.T. & Moot, D. J. (2024): A review of grass species yields and growth rates in Northland, New Zealand, *New Zealand Journal of Agricultural Research*, DOI: 10.1080/00288233.2024.2351614.

Tomov, P. (1979). New orchard grass variety Dabrava, *Plant Science*, *6,* 33-40.

Tomov, P. (1980). Comparative testing of local and introduced orchard grass varieties in mixture with alfalfa under irrigation, *Plant Science*, *4*, 49–57.

Tomov, P. (1983). Importance of local perennial grass populations for forage production improvement in Bulgaria, *Proc. of the Meeting of groups of researchers from member countries of CMEA*, Pieschany , Czechoslovakia, 82–87.

Tomov, P. (1987). Study on breeding and seed production of orchard grass *(Dactylis glomerata* L.), PhD – Doctor of Science, Pleven, 273.

Tomov, P. (1989). Diversity of *F. arundinacea* clones originated from Central North Bulgaria in respect of morphological characters with a breeding importance, *Genetics and breeding*, *5*, 424 – 427.

Van Bockstaele, E., & Baert, J. (2004). Improvement of perennial ryegrass (*Lolium perenne* L.), Jubilee conference “50 years IFC-Pleven”, *Plant Sciences*, Sofia, 41, 483-488.

Van Bockstaele, E. (1998). Aspects of breeding perennial ryegrass (*Lolium perenne* L.), *Plant Sciences*, Sofia, 9, 690 – 694.

Van der Heijden, S. A. G., & Roulund, N. (2010). Genetic Gain in Agronomic Value of Forage Crops and Turf: A Review, *In Sustainable Use of Genetic Diversity in Forage and Turf Breeding*, p 247-260.

Van Soest, P. J., & Wine, R. H. (1967) Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Journal of the Association of Official Analytical Chemists* 50, 50-55.

Van Soest, P. J. (1964). Symposium on Nutrition and Forage and Pastures: New Chemical Procedures for Evaluating Forages, *Journal of Animal Science*, *23*(3), 838–845.

Van Wijk, A. J. P. & Reheul, D. (1991). Achievements in fodder crops breeding in maritime Europe. In: “Fodder Crops Breeding: Achievements, Novel Strategies and Biotechnology: *Proceedings of the 16th Meeting of the Fodder Crops Section of Eucarpia*, Wageningen, Netherlands, 18–22 November 1990” (eds. A.P.M. Den Nijs and A. Elgersma), Pudoc, Wageningen, pages 13–18.

Vasilev, E. (2006). Dry matter yield from sainfoin in binary mixtures with perennial ryegrass and cocksfoot, *Field Crops Studies*, 2006, Vol.III-4, 571 – 575.

Vasileva, V., & Vasilev. E. (2012). Dry matter yield in some pasture grass mixtures with participation of subterranean clover (*Trifolium subterraneum* L.). *Journal of Mountain Agriculture on the Balkans*, Research Institute of Mountain Stockbreeding and Agriculture, Troyan, ISSN 1311-0489, vol. 15, 5, 1024-1033.

Vasileva, V. (2011). Study of productivity of perennial legumes in grass mixtures. *Journal of Mountain Agriculture on the Balkans*, Agricultural Academy, ISSN 1311-0489, vol. 14, 2, 296-307.

Vogel, K. P., & John, H. (2019). History of Grass Breeding for Grazing Lands in the Northern Great Plains of the USA and Canada, *Agronomy & Horticulture* - Faculty Publications. 1282. https://digitalcommons.unl.edu/agronomyfacpub/1282

Vulchinkov, Zh. (2022). Evaluation of perennial grasses – perennial ryegrass, cocksfoot, tall and red fescue in relation to selection" PhD, Pleven, 235.

Walsh, G. L., & Birrell, H.A. (1987) . Seasonal variations in the chemical composition and nutritive value of five pasture species in south-western Victoria, CSIRO Publishing, *Aust. J. Exp. Agr. 27*(6): 807-816.

Walton, P.D. (1981). Production and management of cultivated forages, Alberta, USA.

Wang, Y., Bigelow, C. A., & Jiang, Y. (2009). Ploidy Level and DNA Content of Perennial Ryegrass Germplasm as Determined by Flow Cytometry, *Hortscience* 44(7):2049–2052.

Wheeler, J. L., & Corbett, J. L. (1989). Criteria for breeding forages of improved feeding value: Results of a Delphi survey, January 1989, *Grass and Forage Science* 44:77-83

Wilkins, P. W., & Lovatt, J. A. (2010). Gains in dry matter yield and herbage quality from breeding perennial ryegrass, In: *Grasses for the future*, International Conference, Teagasc, 43-50.

Wilkins, P. W. (1997). Useful variation in *in vitro* digestibility within perennial ryegrass. Euphytica 93, 249–255.CrossRefGoogle Scholar

Wilkins, P.W., & Humphreys, M.O., (2003). Progress in breeding perennial forage grasses for temperate agriculture. *J. Ag. Sci.* 140:129–150.

Wilkins, P.W. (1991). Breeding perennial ryegrass for agriculture. Euphytica 1991, 52, 201–204.

Wilkins, P.W. (1985). Amelioration of disease in grassland through resistance breeding. *Brit. Grassld. Soc. Occasional Symposium* No. 18: 213–218.

Woodfield, D. R. (1999). Genetic improvements in New Zealand forage cultivars, *Proceedings of the New Zealand Grassland Association* 61: 3–7.

Yancheva, Ch., & Shamov, D. (1996). Studies on possibilities for using crested wheatgrass in grazing mixtures with sainfoin, *Plant Science*, *33*(8), 19–21.

Zwierzykowski, Z., Zwierzykowska, E., Taciak, M., Kosmala, A., Jones, R. N., Zwierzykowski, W., Książczyk, T., & Krajewski, P. (2011). Genomic structure and fertility in advanced breeding populations derived from an allotetraploid *Festuca pratensis* × *Lolium perenne* cross. - *Plant Breed*. **130**: 476-480, 2011,

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