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Vitality and longevity of perennial ryegrass (*Lolium perenne* L.) accessions in North Central Bulgaria

Aneliya Katova

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

Abstract

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Perennial ryegrass is the most widely grown temperate grass species globally as a source of forage (grazing, hay, silage) or for amenity use. It is a part of landscape, protects soil from water and wind erosion, enriches it with organic substances and improves its fertility. During the period 2015–2020 in the Institute of Forage crops (IFC) – Pleven a collection nursery was studied in field non-irrigated conditions on leached black soil, in block duplicate method, in 2 replications with a total of 19 accessions of perennial ryegrass, with 50 individual plants each (25 plants for forage and 25 plants – for seeds), including 14 varieties and 5 ecotypes, 10 tetraploid and 9 diploid, from Bulgaria (BG), Belgium (BE), and Romania (RO). The aim is to determine the vitality per year and longevity (after many years, more than 3–4) of perennial ryegrass collection accessions and to make a selection of the most vital and long – lasting genotypes. Scale of survival according to the International Classifier for the family *Poaceae* (Bukhteeva et al., 1985) was used – limiting factors of the environment are cold – winter, drought and high temperatures – summer. An autumn inventory of the individual plants no./% was carried out annually, as 25 plants per sample = 100%., and vitality classification as follows: *Very Low* – less than 1 to 20% of plants are left alive; *Low* – more than 21 to 50%; *Medium* – more than 51% to 70%; *High* – more than 71 to 90%; *Very High* – more than 90% of the plants are still alive. Computer statistical processing of the data (via Excel, at P = 0.05) included variance analyses. Data on vitality and longevity are characterized by: marginal values (min and max), arithmetic mean (x), standard deviation (SD) and coefficient of variation (CV,%). Variation was considered weak, moderate, or strong at CV values, respectively: up to 10%; > 10–20%, and > 20% (Dimova and Marinkov, 1999). When grown for fodder, the local ecotypes Sokolare, Topolovgrad and Bekovi skali are the most viable by years and the longest surviving up to 5 years, with the percentage of plants alive in the fifth year being the highest for the Sokolare ecotype. Ecotype Sokolare is strongly influenced by the method of use, which is the longest-lasting and most viable when grown for fodder – 5 years and the shortest – when grown for seeds – 3 years. All diploid varieties live up to 4 years, and only the Harmoniya variety up to 5 years, with the Bulgarian varieties Harmoniya and Strandzha being the most viable and long-lasting when grown for fodder. Harvesting once a year for seeds extends the life cycle of diploid varieties up to 5 years and for the Harmoniya variety – up to 6. The Bulgarian tetraploid varieties Tetrany, Tetramis and the Belgian varieties Floris, Melpetra and Melverde are the most durable and long-lasting. One-time harvesting per year for seeds extends the life cycle of the tetraploid varieties up to 5 years, and for the Bulgarian varieties Tetrany, Tetramis and the Belgian varieties Floris, Melpetra and Melverde – up to 6.

Keywords: perennial ryegrass; vitality; longevity; ecotype; diploid; tetraploid; variety

Introduction

Knowing the performance characteristics of grass is immensely useful for grassland producers. It allows appropriate selection of varieties that will perform well under a particular system. The grasses are drawn up after rigorous testing for attributes such as yield, persistency, quality and disease resistance. Individual varieties differ in performance characteristics depending on maturity group and ploidy (Peeters, 2004, Boller et al, 2010, Humphreys et al., 2010). These differences may be further exaggerated by factors such as climate, soil type and system of farming (Franzluebbers et al., 2020, Anderson, 2023). Sustainability is a hot topic now, and it's great to see that there is a growing awareness of the impact that agriculture can have on the environment (Katova et al., 2007). However, improving such aspects as yield, fiber digestibility and disease resistance have always been important goals for farmers and researchers. Advancements in technology and breeding programs have led to improvements in the longevity and persistence of many forage species (Connolly, 2001, Katova, 2005, Kertikova, 2014, Kertikova and Kertikov, 2021, Katova, 2023, Katova et al., 2024). Perennial ryegrass (*Lolium perenne*) is the most widely grown temperate grass species globally. It is used as a source of forage (grazing, hay, silage) or for grassing of sports and technical terrains and layingout off lawns, parks and gardens (Petrova et al, 2022, Katova, 2023). It is a part of landscape, protects soil from water and wind erosion, enriches it with organic substances, maintains and improves its fertility (Katova, 2005, Boller and Greene, 2010; Humphreys et al., 2010; Katova, 2016; Petkova et al., 2021). In the world, the first breeding activity with perennial ryegrass began in 1889 at the Welsh Experimental Station (Great Britain) (Duller et al., 2019). Now the selection of perennial ryegrass is carried out in many countries with different ecological features. Morphological signs, biological properties and economic qualities are improved (Katova, 2005). In the past, the main goal of selection was to increase longevity (Jordan, 1988, Ingram, 1994). The longevity and productivity of perennial ryegrass varieties depend on their genetic backgrounds and the complex impact of environmental conditions and resources (including diseases). The most long-lasting are the varieties created in the regions of the genetic origin of the species (Tomov, 1983). Drought is the main environmental factor hampering world agriculture production. In the face of warming climate and reduced fresh water resources it become obvious that search for any factors decreasing water use is strongly recommended (Keep et al., 2021). Drought, high salinity, and extreme temperatures are major adverse environmental stresses that plants often encounter, which is further complicated by the potential impact of climate change (Miao et al., 2022). Cool-season turf grasses often suffer from extended periods of drought during the summer but water supplies used to irrigate turf are limited and are in competition for use by agriculture, recreation etc. (Żurek et al., 2018, Katova, 2022). From the very name perennial grass, it is understood that perennial ryegrass completes its ontogenetic development not in one, but in many (3-4 and more) years. Unlike annuals, where the life cycle is completed in less than one calendar year, perennial ryegrass is exposed to environmental factors year-round, and for many years, with frequent stress situations (Casler, 2001, Bachvarova et al., 2008). That is why the resistance of the population to adverse factors of the local ecological conditions is of great importance for the productivity and longevity of the grassland. Bulgaria is located on the border between the Mediterranean and Caucasian gene centers, where the species originated and the diversity is greatest (Hristov, 2004, Katova, 2007, Katova, 2016, Katova, et al., 2016). There is a correlation between polyploidy and perenniality (Ramsey and Ramsey, 2014). Polyploids are looking generally different from their progenitors in morphological, ecological, physiological, and ytological characteristics that can contribute both to exploitation of a new niche and to reproductive isolation. Therefore, polyploidy is a major mechanism for adaptation and speciation in plants. The development of new crops and the interspecific gene transfer and also the origin of new crops can be traced with the help of polyploidy breeding (Dar et al., 2017). Local populations and varieties of perennial ryegrass are more winter-resistant and long-lasting than all introduced specimens, in the conditions of Bulgaria (Katova, 2005; Petkova, 2023). Perennial ryegrass has very positive attributes: easy to establish, productive in fertile soils, digestible, palatable, easy to ensile (high sugar content), wide range of varieties available, suitable for wide range of management circumstances (cutting, grazing, short-term and long-term swards); and some shortcomings: less productive in dry, infertile soils, some varieties susceptible to winter kill, lower mineral content compared with legumes and forage herbs. Perennial ryegrass can stay productive for up to 4 years and stands can be maintained for about 3-4 years (Koleva, 2002). It lowers production costs, and in a dry environment, it grows as soon as soil moisture allows. A little bit of soil moisture is all it needs to get growing. During autumn or summer rains, a sward of perennial grass will grow almost immediately. Perennial grasses are major components of native and sown grasslands. 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. World plant breeding has created many varieties of perennial ryegrass with specific eco-adaptability. The OECD World Trade List (2024) includes 1,775 varieties of perennial ryegrass. Foreign varieties are in most cases highly productive, but with poor adaptability to our conditions, not long-lasting and unsuitable for direct implementation in production. In IFC – Pleven, the first Bulgarian variety of diploid perennial ryegrass IFK Harmoniya was created and registered in the country‘s variety list in 2010, a standard in Executive agency for variety testing, approbation and seed control (EAVTASC) (Katova, 2011), and in 2017 two new tetraploid perennial ryegrass varieties Tetrany and Tetramis were recognized (Katova, 2017 a, 2017 b). In Agricultural experiment station (AES) – Sredets, a new variety of perennial ryegrass Strandzha, from a local population, was created (Stoeva, 2010). From an economic point of view, pastures are the cheapest forage, but the key is the availability of a sufficient amount of biomass with high digestibility and nutritional value. The multi-cutting and perenniality of perennial ryegrass are important prerequisites for the formation of the total biomass production 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. Resistance to the adverse factors of local environmental conditions is of great importance for productivity and longevity. Tetraploids have been found to have many positive qualities: better foliage, taller plants, higher forage and seed productivity, higher water and sugar content, higher digestibility than diploids; faster germination; greater mass per 1000 seeds; better palatability; better disease resistance; winter hardiness; higher nutritional value; ruminants have a preference for them, and their creation is considered a major advance in perennial ryegrass breeding (Katova et al., 2008, Katova, 2015 a, 2015 b, Marinov-Serafimov et al, 2021). All this determines the need for a preliminary study of perennial ryegrass varieties suitable for the soil-climatic conditions in the Middle Balkan region, and clarification of the technologies for cultivation in a separate crop or in mixtures with other grasses. In the foothills of Bulgaria, tetraploid varieties of perennial ryegrass Tetramis and Tetrany have the best adaptability, resistance and productivity. The essential importance of the genotype (variety) in independent hay cultivation is proven, and the results confirm the value of the Bulgarian breeding and, accordingly, the adaptive potential in improving the species. As a result of the comparative testing of Bulgarian and foreign varieties of perennial ryegrass, it was established that the Bulgarian tetraploid varieties Tetramis and Tetrany show the highest resistance and productivity in the conditions of the Central Balkan. In the foothills of Bulgaria, tetraploid varieties of perennial ryegrass Tetramis and Tetrany have the best adaptability, resistance and productivity (Petkova et al, 2021, Bozhanska, 2023, Petkova, 2023). Persistence can best be defined as the continuity of forage yield relative to a cultivar's potential. Though there is limited evidence of differences in persistence between cultivars, there is interest in including persistence in the DairyNZ Forage Value Index (FVI). (Dodd et al., 2018). It has been proposed that persistence be included as a trait in the Forage Value Index (FVI), by Chapman et al. (2017) to enable farmers to make better choices between cultivars and to give plant breeders direction for germplasm selection. A fundamental requirement for this development is the identification of a suitable measure of cultivar performance values for persistence. This is challenging since, by definition, persistence is a long-term outcome which is expensive to measure directly. An additional challenge is to define what it is about pastures that should persist, or conversely what is not persisting. Operationally, this can be either an entity (the grass itself) or a characteristic of the entity (e.g. a yield or forage quality trait, Parsons et al., 2011). Indeed, in New Zealand there are few agronomic field assessments of forage grasses longer than 3 years. Most studies of greater than 3 years have compared different grass species (e.g. Barker et al., 1993), and only four have incorporated comparisons of multiple genotypes/cultivars within species (Allan and Keoghan. 1994; Black and Moir 2015, Chapman et al., 2015; Lee et al., 2017). Except for Allan and Keoghan (1994), who used a plant survival score, these studies have used DM yield and/or botanical composition as the main measure of persistence. Lee et al. (2017) observed no difference in the persistence of four perennial ryegrass cultivars under substantial climate and pest stress in the Waikato, based on DM yields, and this is the only study to have reported long-term measurements of tiller density (over 6 years). The design and implementation of relevant data collection over periods much greater than 3 years is therefore a vital step in resolving this situation, and generating performance values for persistence in the FVI. "A proficient and sustained delivery of highly utilisable, high yielding herbage" is the way (Gilliland et al, 2021).

The aim is to determine the vitality per year and longevity (after many years, more than 3 -4) of perennial ryegrass collection accessions and to make a selection of the most vital and long – lasting genotypes.

Material and Methods

During the period 2015-2020, in the Institute of Forage crops (IFC) – Pleven, a collection nursery was studied in field non-irrigated conditions on leached black soil, in block duplicate method, in 2 replications with a total of 19 accessions of perennial ryegrass, with 50 individual plants each (25 plants for forage and 25 plants – for seeds), including 14 varieties and 5 ecotypes, 10 tetraploid and 9 diploid, from Bulgaria (BG), Belgium (BE), and Romania (RO)) Table 1. Research began in 2015 with field experiments – 1 collection nursery with a total of 19 accessions x 25 plants (475): one trial for fodder and one for seeds or total 950 genotypes on an area of 500 m^2, planting in autumn 2015 by seedlings (Table 1). Spring and autumn fertilization of plants with 60kg Nha^{-1} in the form of ammonium nitrate (NH4NO3) was carried out annually during the vegetation. The trial were performed under non-irrigated conditions. Watering was done only to the young seedlings in the first year. Protocols for testing the distinctness, homogeneity and stability of perennial ryegrass, of the Community Plant Variety Office (Community Plant Variety Office, *CPVO*): – *CPVO Technical Protocol for Distinctness, Uniformity and Stability Tests for Ryegrass – TP/004/1 Final, English, Date: 23/06/2011*, (CPVO, 2011,a) was used.

Fodder productivity (dry mass) per plant – Harvesting of the green mass is done individually by manual mowing with a sickle at a height of 5-7 cm. The first cut is done in the beginning of anthesis phase, according to the groups of maturity, and the next cuttings – approximately at 4 week intervals or more, depending on the environmental conditions during the growing season. Dry matter content was determined by weight after fixing at 105 °C and drying at 60 °C to constant weight. After evalueting the productivity of green mass, a pooled sample was formed for each accession. Medium samples representative of the accession were taken from it to determine: dry matter content – fodder (100–120 g); and for the chemical composition of dry biomass (250–300 g). Results are published (Vulchinkov and Katova, 2019 a)

Seed productivity per plant – Twenty-five (25) plants from a plot of seed testing trail are harvested separately in envelopes, with their ears cut, trimmed, cleaned and weighed – SWP, g. Results are published (Vulchinkov and Katova, 2019 b).

Individual plants according to the Diversity, Uniformity and Stability Tests (DUS) laid down in the Protocols of the Common Plant Variety Office in the methodologies of the Union for the Protection of Plant Varieties (UPOV TG/4/8: 2006-04-05) were count early in spring after growth starts. Experimental data on alive plants – number and %, where 25 plants are equal to 100 % are characterized by: marginal values (min and max), arithmetic mean (x), standard deviation (SD) and coefficient of variation (CV, %) for 2016, 2017, 2018, 2019 and 2020. for each accession by individual phenotypic assessment, average for a group (ecotypes, varieties, diploid and tetraploid) and for the collection. Variation is considered weak, moderate or strong at CV values, respectively: up to 10%, > 10–20%, and > 20% (Dimova & Marinkov, 1999). The samples are grouped and differentiated for different groups by vitality (each year) and longevity (after 3–4 years). Scale of survival according to the International Classifier for the family *Poaceae* (Bukhteeva et al., 1985) was used – limiting factors of the environment are cold – winter, drought and high temperatures – summer: An autumn inventory of the individual plants no./% was carried out annually, as 25 plants per sample = 100%, and vitality classification as follows:

Very Low – less than 1 to 20% of plants are left alive;
Low – more than 21 to 50% of plants remain alive;
Medium – more than 51% to 70% of plants remain alive;
High – more than 71 to 90% of the plants remain alive;
Very High – more than 90% of the plants are still alive.

In table 1 number of cuts for forage by years and in total for the period per each accession is shown.

During 2020 in small number of accessions the plants are alive and only seed productivity was measured in two trials: Harmoniya, Tetramis, Melpetra and Floris.

Methods for statistical data processing

Computer statistical processing of the data (via Excel, at P = 0.05) included variance analyses. Data on vitality (% live plants within a year) and longevity (% live plants after 3–4 years) are characterized by: marginal values (min and max), arithmetic mean (x), standard deviation (SD) and coefficient of variation (CV, %). Variation was considered weak, moderate, or strong at CV values, respectively: up to 10%; > 10–20%, and > 20% (Dimova and Marinkov, 1999). The samples are grouped and differentiated for different groups by vitality (each year) and longevity (after 3–4 years).

Agro-climatic and soil characteristics

The geographic coordinates of Pleven are 43° and 35“ north latitude, and 24° and 35“ east longitude at an average altitude above sea level of 163 m. The experiment was carried out at the Institute of Forage Crops – Pleven under the conditions of Central Northern Bulgaria, at the transition between the Northern and Middle climatic regions of the Danube Plain, and falls into the moderately continental climatic sub-region. In terms of mechanical composition, the soil in the area is of the leached chernozem type with a heavy sandy clay profile, without significant changes at a depth of up to 40 cm, and as a subtype they are slightly and moderately leached.

Over a period of fifty years (1964–2013), the average annual rainfall is 546.6 mm; average annual air temperature is

Table 1. Collection nursery of perennial ryegrass accessions established in 2015 and number of cuts for forage by years and total

Accession	Type	Origin	Ploidy	Number of cuts / year for forage				Total
				2016	2017	2018	2019	sum
Sokolare	ecotype	BG	*2n*	*4*	*4*	*3*	*1*	12
Ravnogor 1	ecotype	BG	*2n*	*4*	*4*	*3*	*0*	11
Topolovgrad	ecotype	BG	*2n*	*4*	*5*	*4*	*0*	13
Bekovi skali	ecotype	BG	*2n*	*4*	*5*	*3*	*1*	13
Ravnogor 2	ecotype	BG	*2n*	*3*	*4*	*3*	*0*	10
Harmonya	variety	BG	*2n*	*4*	*5*	*4*	*0*	13
Strandzha	variety	BG	*2n*	*4*	*5*	*4*	*0*	13
Mara	variety	RO	*2n*	*4*	*4*	*3*	*0*	11
Iljo	variety	BE	*2n*	*2*	*4*	*2*	*0*	8
Tetramis	new variety	BG	*4n*	*4*	*5*	*4*	*0*	13
Tetrany	new variety	BG	*4n*	*4*	*4*	*4*	*1*	13
Merlinda	variety	BE	*4n*	*4*	*5*	*4*	*0*	13
Roy	variety	BE	*4n*	*4*	*5*	*3*	*0*	12
Meltador	variety	BE	*4n*	*3*	*5*	*3*	*0*	11
Meracoli	variety	BE	*4n*	*3*	*5*	*3*	*0*	11
Melpetra	variety	BE	*4n*	*3*	*5*	*3*	*0*	11
Floris	variety	BE	*4n*	*3*	*5*	*3*	*2*	13
Magura	variety	RO	*4n*	*3*	*4*	*3*	*0*	10
Melverde	variety	BE	*4n*	*3*	*4*	*3*	*0*	10

11.9°C; (Tables 2 and 3). The amounts of precipitation for the study period (2015–2020) are respectively for 2015 – 707.51 mm; for 2016 – 636.84 mm; for 2017 – 755, 1 mm, for 2018 – 688.4 mm, for 2019 – 552.3 mm and for 2020 – 550.3 mm, .i.e. for the first 4 consecutive years (2015-2018) it is observed excess of the amount of precipitation compared to the fifty-year period, and 2019 and 2020 are equal to it, the last year having the least precipitation. The meteorological conditions in 2015, 2016, 2017, 2018, 2019 and 2020 for the region of Pleven differ significantly from the average multi-year values (climatic norms) for the period from 1964–2013 – (table 2, 3).

Mean annual temperatures (Table 2) for the study period ranged from 12.8°C for 2017; 12.9°C for 2018; 13.2°C for 2016; 13.7°C for 2015 and 2019, and 13.8°C for 2020. All of them are higher than the average for 1964–2013 (11.9°C), which is more than 1.9°C compared to the previous fifty-year period (Katova, 2005).The trend of global warming, which was also reported by Slavov and Aleksandrov (1996; 1996,a), is even better expressed for the period 2015–2020, compared to the above-mentioned one. The continental climate characteristic of the Pleven region is expressed in large temperature amplitudes. For example, for the survey years 2017, there is an absolute minimum of -4.4°C for the month of January and a maximum in August of 24.4°C. In 2020, the temperature range is in the smaller range of 3.0–25.0°C (January-August). For 2019, the January-August amplitude is also large from 0.5°C to 24.9°C. The year 2020. and 2019 have the lowest annual rainfall (550.3 mm and 552.3 mm) and emerged as the driest for the study period. There is a distinct rainfall maximum in April (116.8 mm); relatively high values for May (82.8 mm) and June (89.6 mm); as well as a pronounced drought in the second half of the year. The better distribution of rainfall in March, April, May and June has a favorable influence on spring tillering, early heading, earing and seeding in perennial ryegrass (Katova, 2005). Rainfall in September and October is important for the assimilation of nitrogen fertilizers and better autumn germination. Heavy rains have an adverse effect at the end of May, when it flowers, as well as in the first half of July, when its seeds are harvested, causing a high percentage of seed falling.

In terms of monthly rainfall distribution, 2015–2018 are more favorable than 2019 and 2020.

These soil climatic conditions are provoke background for selection and adaptation of genetic resources to drought and cold (Bolller and Green, 2010).

Table 2. Average monthly and average annual air temperature °C

Years – months	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average for the year, t°C
2015	1.9	2.3	6.8	12.2	18.8	20.7	25.5	24.4	20.0	11.2	9.8	11.0	13.7
2016	0.5	8.7	8.5	15.4	16.4	23.0	24.6	23.5	19.4	10.8	6.1	1.1	13.2
2017	*-4.4*	2.9	10.3	12.2	17.0	23.0	24.0	24.4	19.5	12.6	7.2	5.0	12.8
2018	2.0	2.0	5.3	16.9	19.6	21.8	22.9	24.0	18.9	15.4	5.1	1.2	12.9
2019	0.5	4.4	10.1	12.2	17.0	22.3	23.5	24.9	20.0	14.4	10.7	4.4	13.7
2020	3.0	7.1	8.3	12.7	17.4	21.4	23.9	25.0	21.4	14.7	5.8	4.6	13.8
1964–2013	-0.6	1.9	6.4	12.0	17.7	21.2	23.4	22.9	18.3	12.1	6.2	1.0	11.9

The aridity index of De Martonne could be calculated for the year period – (12 months) and could satisfy Formula 1.

$$I_{DMY} = \frac{P_Y}{T_Y + 10} \quad (1)$$

where:

IDMY – De Martonne index of the year period
PY – Sum of precipitation of the year period
TY – Mean air temperature of the year period.

One of the most important stages in determining the effect of drought on a given trait is evaluating the intensity and duration of drought for each specific period of the investigation. This is necessary because drought as a factor is not possible to control under natural conditions. One of the most common methods is using the aridity index of De Martonne, which is based on the ratio of the sum of precipitation of the period to the mean temperature of the period (Formula 1) (Croitoru et al., 2012, Stoyanov, 2023). The original index gives an idea about drought during the entire calendar or growing period (12 months).

The aridity index (de Martonne according to Kuzmova 2003; Paltineanu et al., 2007) represents a relationship between the annual amount of precipitation and the average annual temperature + 10°C. With relatively little variation of the second indicator (denominator), the higher value of this index (in a wet year) depends on the annual amount of precipitation (numerator).

The complex influence of increased average monthly air temperatures combined with abundant precipitation in 2015–2018 for the Pleven region classified them as semi-humid and humid (IDM (de Martonne) = 27.49 to 33.69) (Table 3 and 4). The meteorological conditions in 2019 and 2020 for the region of Pleven are characterized by higher air temperatures and smaller amounts of precipitation (abundant or absent) compared to the multi-year average values (climatic norms) for the period from 1964–2013 (Tables 2 and 3). In the radically different situation in the years 2019 and 2020, the values of the Aridity Index (IDM (de Martonne) are 23.30 and 15.27, which defines the climate then as moderately dry to semi-arid, respectively Tables 4 and 5. In 2020, for the months of January, March, April, August, September and November, the values of the aridity index (IDM (de Martonne)) are less than 10, i.e. dry, and the annual amount of precipitation is 550.3 mm close to the climatic norm of 546.6 mm, but the distribution is highly uneven (Bachvarova et al., 2008).

Table 3. Monthly and annual amount of precipitation, mm

Years – months	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual amount, mm
2015	12.4	39.2	68.4	43.6	30.6	95.9	21.5	29.9	130.3	92.9	50.1	92.7	707.5
2016	98	46	77	72.5	77.2	46.1	7.8	31.2	61.8	53.8	40.2	25.2	636.8
2017	41.3	16.2	61.5	37.6	154.3	1.9	155.9	28.5	37.4	108.9	55.4	56.2	755.1
2018	30.9	72.0	98.1	20.2	47.5	155.2	118.9	22.2	15.4	16.1	61.9	30.0	688.4
2019	17.7	22.6	19.0	116.8	82.8	89.6	51.3	39.0	1.3	12.3	78.6	21.3	552.3
2020	4.6	56.8	8.5	14.5	39.4	36.7	39.4	14.1	20.8	94.1	12.9	21.3	550.3
1964–2013	38.0	33.3	36.4	48.4	63.3	63.6	61.7	45.0	47.2	35.5	37.1	37.1	546.6

Table 4. De Marton aridity index for months. years and multi-year period (1964 – 2013)

Years	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual IDM
2015	12.50	38.24	48.86	23.57	12.75	37.49	7.27	10.43	52.12	52.58	30.36	52.97	33.69
2016	112	29.52	49.95	34.25	35.09	16.76	2.71	11.18	25.22	31.04	29.96	27.24	27.49
2017	88.5	15.07	36.35	20.32	68.58	0.69	55.02	9.94	15.21	57.82	38.65	44.96	33.11
2018	30.9	72	76.94	9.01	19.26	58.57	43.37	7.84	6.39	7.61	49.19	32.14	30.03
2019	20.23	18.83	11.34	63.14	36.80	33.29	18.38	13.41	0.52	6.05	45.57	17.75	23.30
2020	4.25	39.86	5.57	7.67	17.26	14.03	13.95	4.83	7.95	45.72	9.80	17.51	15.27
1964–2013	48.51	33.58	26.63	26.40	27.42	24.46	22.17	16.41	20.01	19.28	27.48	40.47	25.0

Table 5. Boundary values of the De Marton Index. determining the type of climate

Type of climate	Climate type De Marton index values
Dry	IDM < 10
Semi-dry	10.0 <= IDM < 20.0
Moderately dry	20.0 <= IDM < 24.0
Semi-humid	24.0 <= IDM < 28.0
Humid	28.0 <= IDM < 35.0
Very humid	35.0 <= IDM < 55.0
Extremely humid	IDM > 55.0

Results and Discussions

Evaluation of vitality and longevity of perennial ryegrass accessions is done acording genetic type, origin, ploidy level, year, environmental conditions and system of use for 2015–2020 period. Perennial ryegrass is a medium-lived plant species (Koleva, 2002).

The ecotypes are local from regions of Bulgaria with different vertical zonation – Northwest Danube Plain – Sokolare, Ravnogor and Bekovi skali – Rodopi Mountain and Topolovgrad – Strandzha Mountain. The average annual assessment of vitality according to the scale of Bukteeva et al. (1985) for perennial ryegrass ecotypes grown for fodder is very high – 100% in the year of establishment of the nursery 2015, it remains very high in 2016 – 92.8%, in 2017 d is high 70.4%, in 2018 it is average –58.4%, and in 2019 – low – 19.2%, in 2020 – there are no living plants (Table 6). Variability in terms of vitality is from low – 10.73% to high 40.7%, 50.7% to 112.58%. The local ecotypes Sokolare, Topolovgrad and Bekovi skali are the longest-lived by years and the longest surviving up to 5 years, with the percentage of living plants in the fifth year being the highest for the Sokolare ecotype. Ecotypes Ravnogor 1 and Ravnogor 2 have proven lower vitality and have poor longevity up to 4 years (Table 6).

Vitality and longevity data for native perennial ryegrass ecotypes grown for seed are presented in Table 7. From 2015 to 2017, similar mean annual values for very high and high vigor were observed – 100%, 88.8% and 71.2%, respectively, as when growing the forage pasture ryegrass ecotypes. In

Table 6. Vitality and longevity of perennial ryegrass ecotypes grown for forage

Collection number	% alive plants – forage use					
year	2015	2016	2017	2018	2019	2020
Sokolare	100	100	88	80	56	0
Ravnogor 1	100	84	56	32	4	0
Topolovgrad	100	100	80	64	20	0
Bekovi skali	100	100	100	92	12	0
Ravnogor 2	100	80	28	24	4	0
average	100	92.8	70.4	58.4	19.2	0
min	100	80	28	24	4	0
max	100	100	100	92	56	0
stdev	0	9.96	28.65	29.61	21.61	0
cv.%	0	10.73	40.7	50.7	112.58	0
Confidence 0.01		11.47	33.00	34.11	24.89	

Table 7. Vitality and longevity of perennial ryegrass ecotypes grown for seeds

Collection number	% alive plants – seeds harvesting					
year	2015	2016	2017	2018	2019	2020
Sokolare	100	88	88	0	0	0
Ravnogor 1	100	96	72	28	12	0
Topolovgrad	100	100	92	88	48	0
Bekovi skali	100	80	80	36	20	0
Ravnogor 2	100	80	28	4	4	0
average	100	88.80	71.20	32	16.8	0
min	100	80.00	28.00	0	0	0
max	100	100.00	88.00	92	48	0
stdev	0	9.12	25.04	36.88	19.06	0
cv.%	0	10.27	35.17	115.24	113.44	0
Confidence 0.01		10.51	28.85	42.48	21.96	

2018 and 2019, the average vitality was lower, compared to that in fodder cultivation, as the Sokolare ecotype died after the third year, and the most vibrant and long-lasting were the Topolovgrad, Bekovi skali and Radnogor 1 ecotypes, as the most a high % of living plants for the 5th year is observed for the Topolovgrad ecotype – 48/%, in 2020 – there are no living plants of any ecotype (Table 7). Ecotype Sokolare is strongly influenced by the method of use, which is the longest-lasting and most viable when grown for fodder 5 years and the shortest-lived – when grown for seeds – 3 years. Variability in the sign of vitality is from low – 10.27% in the second year to high 35.17%, 113.44% to 115.24, in the following years.

The Table 8 presents the vitality by year of diploid varieties of perennial ryegrass for the period 2015 – 2020, grown for forage. The diploid varieties are of origin as follows: Harmoniya and Strandzha – Bulgarian, Mara – Romanian, Ilio – Belgian.

Average vitality by year is very high in 2015 – 100%, high – 88% in 2016, medium in 2017 – 53% and low in 2018 – 38%, to very low in 2019 – 2%, as in 2020 , no live plants in any of the diploid varieties, and in 2019 only the Harmoniya variety had 8% live plants in the fifth year of forage cultivation. The rest of the varieties finish their development by the fourth year, with Harmoniya having the relatively highest % of living plants – 44%, followed by Strandja, and Mara and Ilio varieties – 24%-20%.

The variability of the vitality trait is from a medium – 16.18% in the second year to a high of 32.83%, 36.80% to 200%, in the following years.

When grown for seeds of diploid varieties of perennial ryegrass, different vitality was observed by year: very high in 2015 – 100%, high – 82% in 2016, high in 2017 – 72% and low in 2018 – 44%, low in 2019 – 31%, to very low in 2020 – 1% on average, with only the Harmoniya variety having 4% live plants in the sixth year of growing for seed.

In both growing regimes, the Harmoniya variety is the longest-lasting. The variability of the vitality trait is strong – 40.42% in the second year and even higher 59.49%, 83.65%, 90.25% to 200%, in the following years (Table 9).

The table 10 presents the vitality by year of tetraploid perennial ryegrass varieties for the period 2015 – 2020, grown for forage. The tetraploid varieties are of origin as follows: Tetrany and Tetramis – Bulgarian, Magura – Romanian, and the remaining 7 varieties – Belgian.

Average vitality by year is very high in 2015 – 100%, very high – 91.2% in 2016, average in 2017 – 67.60%, average in 2018 – 58.80%, to very low in 2019 –6.80%, and in 2020 there are no living plants for any of the tetraploid varieties, and in 2019 only the Tetrany variety and the Tetramis variety from the Bulgarian ones have, respectively 24% and 4%, and from the Belgian varieties Floris, Melpetra Meracoli, had 32%, 4% and 4% live plants respectively in the fifth year of forage cultivation. The rest of the varieties finish their development by the fourth year, with Floris having the relatively highest % of live plants – 32%, followed by Tetrany – 24%, and Tetramis, Melpetra and Meracoli varieties – 4%. The variability of the sign of vitality is from weak – 5.77% in the second year to high 30.36%, 41.32%, to 168.79%, in the following years.

When grown for seeds of the tetraploid varieties of perennial ryegrass, different vigor was observed by year: very high in 2015 – 100%, very high – 94% in 2016, high in 2017 – 75.20% and medium in 2018 – 49.60%, low in 2019 – 28%, to very low in 2020 – 5.60% on average, with only 5 varieties having alive plants in the sixth year of cultivation for seeds: Bulgarian -Tetramis – 12%, Tetrany – 8 %, and the Belgian – Melpetra – 20%, Floris-12% and Melverde – 4%. In both modes of cultivation, the Bulgarian varieties: Tetramis and Tetrany, and the Belgian varieties – Melpetra

Table 8. Vitality and longevity of diploid perennial ryegrass varieties grown for forage

Collection number	% alive plants – forage					
Years	2015	2016	2017	2018	2019	2020
Harmoniya	100	80	56	44	8	0
Strandja	100	100	68	40	0	0
Mara	100	100	60	24	0	0
Iljo	100	72	28	20	0	0
average	100	88.00	53.00	32.00	2.00	0.00
min	100	72.00	28.00	20.00	0.00	0.00
max	100	100.00	68.00	44.00	8.00	0.00
stdev	0	14.24	17.40	11.78	4.00	0.00
cv.%	0	16.18	32.83	36.80	200.00	0
Confidence 0.01		18.33	22.41	15.17	5.15	

Table 9. Vitality and longevity of diploid perennial ryegrass varieties grown for seeds

Collection number	% alive plants – seeds					
Years	2015	2016	2017	2018	2019	2020
Harmoniya	100	100	88	32	16	4
Strandja	100	96	96	92	68	0
Mara	100	100	96	48	36	0
Iljo	100	32	8	4	4	0
average	100	82.00	72.00	44.00	31.00	1.00
min	100	32.00	8.00	4.00	4.00	0.00
max	100	100.00	96.00	92.00	68.00	4.00
stdev	0	33.39	42.83	36.81	27.98	2.00
cv.%	0	40.72	59.49	83.65	90.25	200.00
Confidence 0.01		43.00	55.17	47.40	36.03	2.58

and Floris, are the longest-lasting. The variability of the vitality trait is low – 6.42% in the second year and high 21.10%, 51.32%, 75.59% to 126.88%, in the following years (Table 11).

Table 10. Vitality and longevity of tetraploid perennial ryegrass varieties grown for forage

Collection number	% alive plants – forage					
Years	2015	2016	2017	2018	2019	2020
Tetramis	100	96	72	64	4	0
Tetrany	100	96	80	80	24	0
Merlinda	100	88	60	60	0	0
Roy	100	88	80	76	0	0
Meltador	100	88	72	68	0	0
Meracoli	100	92	72	68	4	0
Melpetra	100	80	56	48	4	0
Floris	100	92	88	88	32	0
Magura	100	96	16	12	0	0
Melverde	100	96	80	24	0	0
average	100	91.20	67.60	58.80	6.80	0.00
min	100	80.00	16.00	12.00	0.00	0.00
max	100	96.00	88.00	88.00	32.00	0.00
stdev	0	5.27	20.52	24.30	11.48	0.00
cv.%	0	5.77	30.36	41.32	168.79	0
Confidence 0.01		4.29	16.72	19.79	9.35	

Table 11. Vitality and longevity of tetraploid perennial ryegrass varieties grown for seeds

Collection number	% alive plants – seeds					
Years	2015	2016	2017	2018	2019	2020
Tetramis	100	96	96	36	16	12
Tetrany	100	96	96	92	68	8
Merlinda	100	92	60	4	4	0
Roy	100	100	72	48	16	0
Meltador	100	96	68	60	20	0
Meracoli	100	100	84	68	28	0
Melpetra	100	80	80	56	56	20
Floris	100	88	72	56	44	12
Magura	100	96	44	16	8	0
Melverde	100	96	80	60	20	4
average	100	94.00	75.20	49.60	28.00	5.60
min	100	80.00	44.00	4.00	4.00	0.00
max	100	100.00	96.00	92.00	68.00	20.00
stdev	0	6.04	15.87	25.45	21.17	7.11
cv.%	0	6.42	21.10	51.32	75.59	126.88
Confidence 0.01		4.92	12.92	20.73	17.24	5.79

Figure 1. clearly shows that all local ecotypes survive up to 4 years, with the most viable and long-lasting being the Sokolare, Topolovgrad and Bekovi skali ecotypes when grown for fodder, and in Ravnogor 1 and 2 the vitality sharply decreases after the second – third year of growing and harvesting for fodder.

In Figure 2, local seed-growing ecotypes have different vigour. Ecotype Sokolare survives only 3 years under this regime, while the other ecotypes survive 5 years, but with varying vitality. The most viable and long-lasting are the Topolovgrad and Bekovi skali ecotypes. Harvesting once a year for seeds extends the life cycle of ecotypes up to 5 years.

Figure 3. clearly shows that all diploid varieties survive up to 4 years, and only the Harmoniya variety up to 5 years, with the Bulgarian varieties Harmony and Strandzha being the most viable and long-lasting when grown for fodder, and in the case of Mara and Ilio, the vitality sharply decreases after second – third year of growing and harvesting for fodder.

In Figure 4, diploid varieties when grown for seed have different vigor. The Harmony cultivar survives up to 6 years under this regime, while the other ecotypes survive 5 years, but with varying vitality. The Bulgarian varieties Harmony and Strandzha and the Romanian variety Mara are the most vital and long-lasting. A single harvest per year for seeds extends the life cycle of diploid varieties up to 5 years, and for the Harmony variety – up to 6.

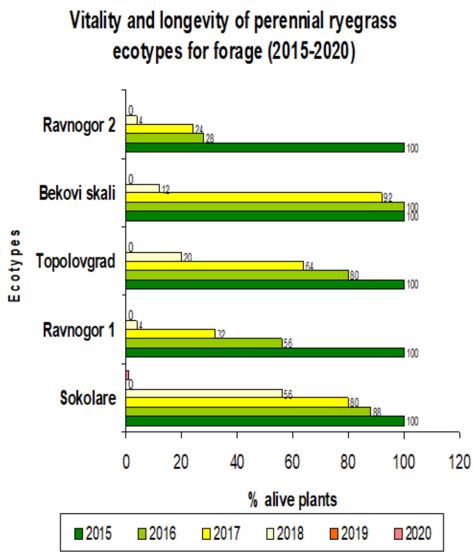

Fig. 1. Vitality and longevity of perennial ryegrass ecotypes grown for forage (2015–2020)

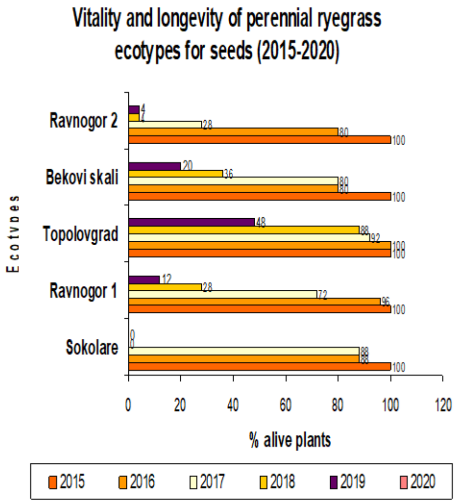

Fig. 2. Vitality and longevity of perennial ryegrass ecotypes grown for seeds (2015–2020)

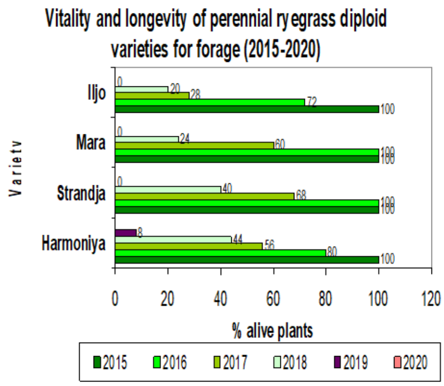

Fig. 3. Vitality and longevity of perennial ryegrass diploid varieties grown for forage (2015 – 2020)

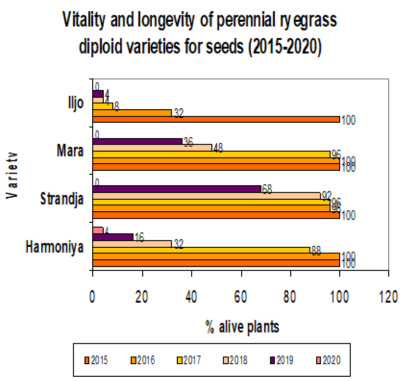

Fig. 4. Vitality and longevity of perennial ryegrass diploid varieties grown for seeds (2015–2020)

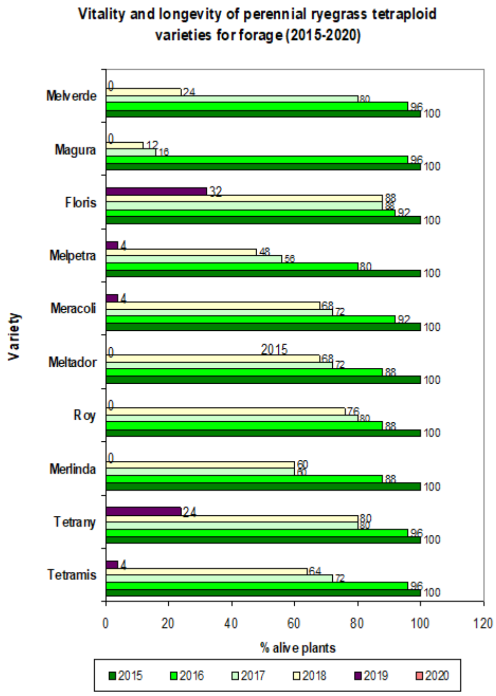

Fig. 5. Vitality and longevity of perennial ryegrass tetraploid varieties grown for forage (2015–2020)

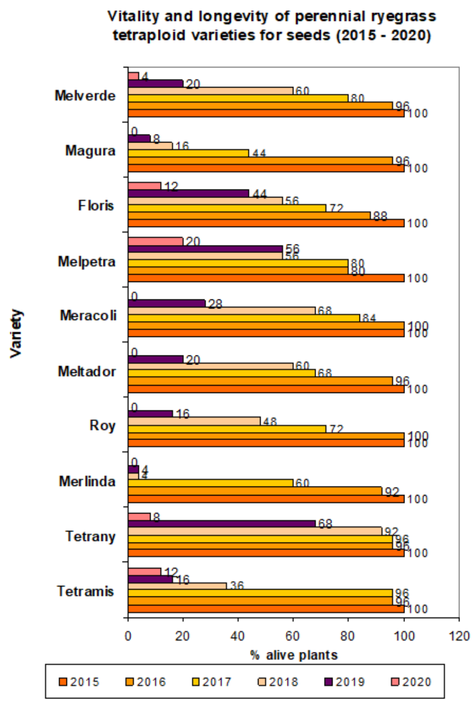

Fig. 6. Vitality and longevity of perennial ryegrass tetraploid varieties grown for seeds (2015–2020)

In Figure 5, it is clearly seen that 5 of the tetraploid varieties survive up to 4 years, and the remaining 5 – up to 5 years, with the Bulgarian varieties Tetrany and Tetramis and the Belgian Floris, Melpetra Meracoli being the most viable and long-lived when grown for fodder, and Magura and Melverde vigor declines sharply after the third-fourth year of growing and harvesting for fodder. Merlinda, Roy and Meltador have high to medium vitality in the fourth year, but complete their life cycle by 4 years.

In Figure 6, tetraploid varieties when grown for seed have different vigor. Five cultivars survive up to 6 years under this regime, while the remaining 5 survive 5 years, but with varying vigour. The Bulgarian varieties Tetrany, Tetramis and the Belgian varieties Floris, Melpetra and Melverde are the most durable and long-lasting. One-time harvesting per year for seeds extends the life cycle of the tetraloid varieties up to 5 years, and for the Bulgarian varieties Tetrany, Tetramis and the Belgian varieties Floris, Melpetra and Melverde – up to 6.

Concusions

Evaluation of vitality and longevity of perennial ryegrass accessions is done acording genetic type, origin, ploidy level, year, environmental conditions and system of use for 2015–2020 period.

When grown for fodder, the local ecotypes Sokolare, Topolovgrad and Bekovi skali are the most viable by years and the longest surviving up to 5 years, with the percentage of live plants in the fifth year being the highest for the Sokolare ecotype. Ecotypes Ravnogor 1 and Ravnogor 2 have a proven lower vitality and have a lower longevity up to 4 years.

Vitality and longevity of local perennial ryegrass ecotypes grown for seed from 2015 to 2017 had similar annual average values for very high and high vigor of 100%, 88.8%, and 71.2%, respectively, as growing ecotypes pasture ryegrass for forage. In 2018 and 2019, the average vitality was lower, compared to that in fodder cultivation, as the Sokolare ecotype died after the third year, and the most vital and long-lasting were the Topolovgrad, Bekovi skali and Radnogor 1 ecotypes, as the most a high % of living plants for the 5th year is observed for the Topolovgrad ecotype – 48%, in 2020 – there are no living plants of any ecotype.

Ecotype Sokolare is strongly influenced by the method of use, which is the longest-lasting and most viable when grown for fodder – 5 years and the shortest – when grown for seeds – 3 years. Variability in the sign of vitality is from low – 10.27% in the second year to high 35.17%, 113.44% to 115.24, in the following years.

All diploid varieties live up to 4 years, and only the Harmoniya variety up to 5 years, with the Bulgarian varieties Harmoniya and Strandzha being the most viable and long-lasting when grown for fodder, and in the case of the Mara and Ilio varieties, vitality sharply decreases after the second – third year of cultivation and harvesting for fodder.

Diploid varieties when grown for seed have different vigor. The Harmoniya cultivar survives up to 6 years under this regime, while the other ecotypes survive 5 years, but with varying vitality. The Bulgarian varieties Harmoniya and Strandzha and the Romanian variety Mara are the most vital and long-lasting. Harvesting once a year for seeds extends the life cycle of diploid varieties up to 5 years, and for the Harmoniya variety – up to 6.

Viability per year of tetraploid perennial ryegrass cultivars for the period 2015–2020 grown for forage is strongly influenced by origin and method of cultivation. The tetraploid varieties are of origin as follows: Tetrany and Tetramis – Bulgarian, Magura – Romanian, and the remaining 7 varieties – Belgian. Average vitality by year is very high in 2015 – 100%, very high – 91.2% in 2016, average in 2017 – 67.60%, average in 2018 – 58.80%, to very low in 2019 – 6.80%, and in 2020 there are no living plants for any of the tetraploid varieties, and in 2019 only the Tetrany variety and the Tetramis variety from the Bulgarian ones have, respectively 24% and 4%, and from the Belgian varieties Floris, Melpetra Meracoli , had 32%, 4% and 4% live plants respectively in the fifth year of forage cultivation. The rest of the varieties finish their development by the fourth year, with Floris having the relatively highest % of live plants – 32%, followed by Tetrany – 24%, and Tetramis, Melpetra and Meracoli varieties – 4%. The variability of the sign of vitality is from weak – 5.77% in the second year to high 30.36%, 41.32%, to 168.79%, in the following years.

The Bulgarian varieties Tetrany, Tetramis and the Belgian varieties Floris, Melpetra and Melverde are the most durable and long-lasting. One-time harvesting per year for seeds extends the life cycle of the tetraploid varieties up to 5 years, and for the Bulgarian varieties Tetrani, Tetramis and the Belgian varieties Floris, Melpetra and Melverde – up to 6.

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