

**(*Lolium perenne* L.)**

, 5800  
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**Tetrany – the first Bulgarian tetraploid perennial  
 ryegrass variety (*Lolium perenne* L.)**

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**SUMMARY**

2000-2014 . -  
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 , ( ) :  
 , ( )  
 ) 45 ( 4  
 ,  
 ,  
 - ,  
 . ( )  
 (2007-2009 .) 22172.0 kg ha<sup>-1</sup>  
 kg ha<sup>-1</sup>, 7390.7  
 ( ) ( )

During the period 2000-2014, at the Institute of Forage Crops - Pleven the first for Bulgaria tetraploid perennial ryegrass variety Tetrany – early to medium early, highly productive, environmentally stable (winter hardy and tolerant to drought) and persistent was created. It is based on methods, induced polyploidy of the local breeding population, flow cytometric screening and phenotypic selection of tetraploids followed by polycross (multiple hybridization) of 45 elite genotypes perennial ryegrass and reproduction to C 4 generation. The variety is multifunctional, suitable for pasture, hay-pasture and decorative direction of use, alone or in mixtures with alfalfa and white clover to feed or red fescue for ornamental and sport-technical swards with a high percentage of ground cover. The results of a successful competitive variety trial (CVT) in Pleven (2007-2009) a total of 22172.0 kg ha<sup>-1</sup> dry matter yield and annual average 7390.7 kg ha<sup>-1</sup>, and official state variety testing for cultivation and use (VCU) in three points (Chepinci, Plovdiv and Selanovtsi) with an average yield of dry mass of 12557.9 kg ha<sup>-1</sup>, and distinctness, uniformity and stability (DUS)

12557.9 kg ha<sup>-1</sup>,  
 ( ) 1 ( )  
 ( ) (2014-  
 2016 ),  
 .  
 2018 . 2018 ., OECD ( )  
 -  
 2018 .,  
 -  
 2017 .  
 :  
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 , ,

at 1 point (Chepinci) Executive Agency for Variety and Seed (IASAS) (2014-2016) and a description of the variety Tetryny are presented.

The variety will be registered in the Official Variety List (OVL) of Bulgaria for 2018, in the OECD list for 2018 and in the common European catalog of varieties of agricultural crops for 2018, is expected certificate from the Patent Office of the Republic of Bulgaria in 2017.

**Keywords:** perennial ryegrass, the first tetraploid variety in Bulgaria, description, VCU, DUS

(*Lolium perenne*  
 L.) -  
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 100  
 OECD  
 2017 . (1558 ).  
 2000 . - 27  
 83660 t  
 ,  
 209674 t (Humphreys et  
 al., 2010).  
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 (Frame, 1991; Peeters, 2004).  
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 (Katova, 2005; 2016).

## INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.), is the economically main forage grass sown in Europe, New Zealand, and in the temperate regions of Japan, Australia, South Africa and South America. Perennial ryegrass is also widely used in amenity grassland including sports turf. This is reflected in a 100 years period of effort in plant breeding activity and high number of varieties on the OECD list and in 2017 (1558) and seed production since 2000 the EU-27 countries have produced, on average, 83,660 t perennial ryegrass seed per year and in global scale 209674 t per year (Humphreys et al., 2010). Perennial ryegrass is preferred by farmers owing to a number of advantages: tolerance to intensive grazing, trampling and frequent cutting, excellent nitrogen assimilation and the most important quality – higher nutritive value as compared to the other grasses (Frame, 1991; Peeters, 2004). The world breeding developed many ryegrass varieties characterized by specific eco-adaptability. The foreign varieties tested in our country in most cases were high productive but have slight adaptability to our agro-climatic conditions, weren't persistent and not suitable for direct introduction in agricultural practice (Katova, 2005; 2016).

1889 .  
( ),  
1930 .  
Aberystwith S.23 (Jenkin,  
1949; Stapledon and Davies, 1941; Wilkins  
and Lovatt, 2010).

(Camlin, 1997; Casler et al., 1997; Jensen  
et al., 2001; Jung et al., 1996).

7000-8000 kg  
ha<sup>-1</sup> (Sokolovi et al., 2010);  
9000-10000 kg ha<sup>-1</sup>  
(Shpakov et al., 2002; Bumanne and  
Berzins, 2008); 15000 kg ha<sup>-1</sup>  
(Jeangros and Thomet, 2004),  
17500 kg ha<sup>-1</sup>  
(Connolly, 2001).

0,5-0,6 %  
(Humphreys et al., 2006; Boller et  
al., 2010).

1995 .

2010 .

(Katova, 2011).

(Stoeva,  
2010).

(Humphreys et al.,  
2010; Burns et al., 2012; Katova et al.,  
2016).

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2000 . -

(2000-2014

In the world first breeding activity started  
in 1889 . in Welsh experimental station  
(Grate Britain), where during 1930 the first  
perennial ryegrass variety Aberystwith  
S.23 was developed (Jenkin, 1949;  
Stapledon and Davies, 1941; Wilkins and  
Lovatt, 2010). The breeding is directed to  
development of high productive varieties.  
For comparatively short time the high  
forage and seed yielding varieties were  
developed (Camlin, 1997; Casler et al.,  
1997; Jensen et al., 2001; Jung et al.,  
1996). Average annual forage yield  
strongly depends from geographical  
region of growing and its between 7000-  
8000 kg ha<sup>-1</sup> in Serbia (Sokolovi et  
al., 2010); 9000-10000 kg ha<sup>-1</sup> in  
Russia and Latvia (Chpakov et al., 2002;  
Bumanne and Berzins, 2008); 15000 kg  
ha<sup>-1</sup> in Switzerland (Jeangros and  
Thomet, 2004), up to 17500 kg ha<sup>-1</sup> for  
Northern Ireland (Connolly, 2001). In  
Great Britain yield increase for newly  
registered variety by 0,5-0,6 % yearly  
(Humphreys et al., 2006; Boller et al.,  
2010).

In 1995 a breeding program with  
perennial ryegrass was started in the IFC-  
Pleven and in 2010 the first Bulgarian  
diploid variety IFK Harmonia (Katova,  
2011) was registered and protected with a  
certificate of the Patent Office. A new  
variety of perennial ryegrass Strandja,  
originated from a local adapted population  
(Stoeva, 2010) was created in the ESA -  
Sredets. The tetraploid varieties are highly  
productive on fodder and seed, with high  
feed quality and leaf diseases resistance  
(Humphreys et al., 2010; Burns et al.,  
2012; Katova et al., 2016).

The aim is to create a new  
tetraploid variety of perennial ryegrass, to  
carry out variety tests (VCU and DUS)  
and description.

## MATERIAL AND METHODS

Breeding process (2000-2014) by  
years included:

2000 – Treatment by colchicine of  
breeding population with local origin (2000

(2000- ) – 1-2 - seeds) – seedlings for 1-2 months; Survival - % alive plants – from 10 to 50 % and screening for 4 plants by Flowcytometer – from them 5-10 % - tetraploids; progenies of 45 parental plants (genotypes) - 1 – 190 individual plants – 4 , (Katova, 2015 a,b).  
 2001 . - ;  
 2002 . - ;  
 316,58 g. - 1 - NBG - ;  
 2003 . - ;  
 2004 . - 2 ;  
 2005 . - ;  
 3 - 100% - ;  
 2007 . - 4 ;  
 - 100% - ;  
 2007 . - 2009 . - ;  
 2011 . - ( ) ;  
 ( ) - 2012 . 2013 . - ;  
 ( ) ;  
 - " ) →  
 → →  
 ( ), UPOV.  
 e -  
 ANOVA,  
 Excel.  
 :  
 Poacea (Buktheeva et al., 1985);  
 UPOV - TG/4/7/ (UPOV, 1990), TG/35/13/ (UPOV, 1999), TG/4/8 (UPOV, 2006), CPVO-TP/004/1 Final English Date: 23/06/2011.

seeds) – seedlings for 1-2 months; Survival - % alive plants – from 10 to 50 % and screening for 4 plants by Flowcytometer – from them 5-10 % - tetraploids; progenies of 45 parental plants (genotypes) - 1 – 190 individual plants – 4 , (Katova, 2015 a,b).

2001 – field establishment – in polycrosses under isolation with winter rye;

2002 – first seeds from tetraploid plants - 1 – NBG - 316,58 g. Screening for ploidy level;

2003 – seedlings – multiplication under isolation;

2004 – 2 seeds;

2005 – screening for ploidy level - 3 – 100% - tetraploids;

2007 – 4 ploidy check – 100% - tetraploids;

2007 till 2009 – competitive variety testing (CVT);

2011 – sowing of prenatal multiplication (PM) – 2012 and 2013 – first Breeder's seeds for application and Official testing.

Breeding process carried out by application of modern breeding methods (induced poliploidization, polycross and progeny testing for complex characteristics – tandem selection „high productivity – ecological stability) → variety testing → new variety → Breeder's seeds and high category seeds.

The experimental plant material was studied for many traits (quantitative and qualitative), characters and properties, according to UPOV methodical guides. For data processing the statistical method ANOVA was applied, modern programmes and calculation tables in Excel were used. International classifier for studying collections from *Poacea* for complex estimation was used (Buktheeva et al., 1985); technical questioners for DUS and VCU according to UPOV - TG/4/7/ (UPOV, 1990), TG/35/13/ (UPOV, 1999), TG/4/8 (UPOV, 2006), CPVO-TP/004/1 Final English Date: 23/06/2011.

The region of the study – Pleven, where the competitive variety trial for

1964–2014  
 550 mm.  
 2007-2009  
 (13,3°)  
 50- (11,9°),  
 (46°)  
 -15° -28°  
 03 2007 ..  
 2007 . - 1 , 2008 . - 3  
 , 2009 . - 3  
 2  
 - 7  
 5  
 5 m<sup>2</sup>, 3  
 (NBG (Tetryny), Roy, Pandora), 4  
 ; 200 kg ha<sup>-1</sup>,  
 N  
 a. . 60 kg ha<sup>-1</sup>, -  
 50 kg ha<sup>-1</sup>, 12 cm,  
 1,5 cm,  
 :  
 - 168 m,  
 m<sup>2</sup>, 2 , 4  
 - 200 kg ha<sup>-1</sup>,  
 - 80 kg ha<sup>-1</sup>, - 24.03.2014  
 „ « »,  
 25 kg ha<sup>-1</sup>, 11,5 cm,  
 1,5 cm,  
 2015 . 2

forage was conducted, combined climatic peculiarities in North Central Bulgaria-temperate continental climate area. Average annual precipitation sum was 550 mm during the period of 1964–2014. Average annual temperatures for the period 2007-2009 were higher (13,3°) to that for previous 50 years period (11,9°), which confirmed the tendency of global warming. During the last decade the temperature maximums for July and August (46°), and minimums for January and February reached to -15 -28° were recorded. The soil type is leached chernozem, poor to medium for humus, with slightly acid to neutral pH reaction. These soil climatic conditions are provoke background for selection and adaptation of genetic resources to drought and cold.

Competitive Variety Trial (CVT) was established on 3<sup>rd</sup> April 2007 and measurement of dry biomass productivity started in 2007 year – 1 cut, 2008 – 3 cut for the Bulgarian and 2 cuts Belgian varieties, 2009 – 3 cut for the Bulgarian and 2 cuts Belgian varieties, and in total for the period – 7 cuts for forage for the Bulgarian and 5 cuts Belgian varieties as standard the average between two Belgian varieties used. Plot size was 5 m<sup>2</sup>, 3 variants (NBG (Tetryny), Roy, Pandora), 4 replicates; fertilizing: P<sub>2</sub>O<sub>5</sub> – 200 kg ha<sup>-1</sup>, K<sub>2</sub>O - 150 kg ha<sup>-1</sup>, N a.s. – 60 kg ha<sup>-1</sup>, sowing – manual, sowing rate 50 kg ha<sup>-1</sup>, inter row distance 12 cm, depth 1,5 cm, without irrigation.

Official variety testing for VCU was conducted in three locations as follows:

Location Selanovci – soil type carbonate chernozem, above sea level height – 168 m, precursor – set aside, plot size was – 10 m<sup>2</sup>, 2 variants, 4 replicates; fertilizing: P<sub>2</sub>O<sub>5</sub> – 200 kg ha<sup>-1</sup>, N – 80 kg ha<sup>-1</sup>, sowing – 24.03.2014, sowing machine «Saxonia», sowing rate 25 kg ha<sup>-1</sup>, inter row distance 11,5cm, depth 1,5 cm. Reporting of dry mass production in 2015 is at 2 cuts and in 2016 also 2

2016 . 2, 4  
 - . -  
 166 m, -  
 m<sup>2</sup>, 2 - 10  
 , 4  
 - 300 kg ha<sup>-1</sup>,  
 150 kg ha<sup>-1</sup>,  
 80 kg ha<sup>-1</sup>, - 04.04.2014 .,  
 25 kg ha<sup>-1</sup>,  
 12 cm, 1,5 cm,  
 4  
 2015 . 4 - 2016 ., 8  
 - .  
 - 515 m, -  
 , 2 , 4 - 10 m<sup>2</sup>  
 - 250 kg ha<sup>-1</sup>,  
 130 kg ha<sup>-1</sup>,  
 80 kg ha<sup>-1</sup>, - 04.04.2014 .,  
 25 kg ha<sup>-1</sup>,  
 13,7 cm, 1,5 cm,  
 4  
 2015 . 4 - 2016 ., 8  
 ( )  
 2015 2016 .,  
 2014-2016 .

cuts, a total of 4 cuts under non-irrigating conditions.

Location Plovdiv - soil type alluvial lawn, above sea level height – 166 m, precursor – wheat, plot size – 10 m<sup>2</sup>, 2 variants, 4 replicates, fertilizing: P<sub>2</sub>O<sub>5</sub> – 300 kg ha<sup>-1</sup>, K<sub>2</sub>O 150 kg ha<sup>-1</sup>, N – 80 kg ha<sup>-1</sup>, sowing – manual in 04.04.2014, sowing rate 25 kg<sup>1</sup>, inter row distance 12 cm, depth 1,5 cm. Reporting of dry mass production in 2015 is at 4 cuts and in 2016 also 4, a total of 8 cuts under irrigating conditions.

Location Chepinci – soil type alluvial lawn, above sea level height - 515 m, precursor – forage pea, plot size – 10 m<sup>2</sup>, 2 variants, 4 replicates, fertilizing: P<sub>2</sub>O<sub>5</sub> – 250 kg ha<sup>-1</sup>, K<sub>2</sub>O 130 kg ha<sup>-1</sup>, N – 80 kg ha<sup>-1</sup>, sowing – 04.04.2014., manual, sowing rate 25 kg ha<sup>-1</sup>, inter row distance 13,7 cm, depth 1,5 cm. Reporting of dry mass production in 2015 is at 4 cuts and in 2016 also 4, a total of 8 cuts under irrigating conditions.

VCU (dry matter yield) was measured in the three locations during years 2015 and 2016, and for DUS – 2014-2016, as a standard variety IFK Harmoniya is included.

## RESULTS AND DISCUSSION

Description of perennial ryegrass variety *Tetryny*

Variety *Tetryny* is tetraploid and had been developed by the methods: poliploidization of local breeding population, three-fold flow cytometric screening and phenotypic selection of tetraploids followed by polycross (multiple hybridization) of 45 elite genotypes perennial ryegrass and reproduction to C 4 generation.

*Tetryny's* plants are perennial, with well developed tuft root system, intensive tillering, semi-erect to intermediate habit (score 3-5). Variety *Tetryny* formed many and very leafy generative and vegetative stems.

Generative shoots are 2,8 mm width of

C 4 –

( 3-5).

2,8 mm

	4-5	53-66%	stems, smooth surface, comparatively lodging resistant, with 4-5 number of leaves. The plant leafness is 53-66%. Plants are medium in height at the beginning of heading 50-58 cm and in full heading 78 cm.
78 cm.	50-58 cm		
	(3-4 mm)	(17 m)	Leaves are linear flat or slightly curve, very dark green, smooth and have brightness in lower surface. The tongue is membranous, medium in height with sharp edge. Auricles are short and they don't blank the stem. The length (17 m) and width of the leaves (3-4 mm) are medium to long in size. Inflorescence is spike with length 19-25 cm. the number of spikelets per spike are 21-25. Seeds are elongate, boat-like. Thousand seed weight (TSW) is 2,38-3,71 g.
25 cm		19-21-25.	
2,38-3,71 g.		1000	
	3-4 °		Plants grow up early in spring when soil temperature is 3-4 ° and continued vegetation till the first frosts. Pasture stage (maturity) reached till 15-20 April (height of the stand 25-30 cm). The variety persist mani cuttings and grazing. Tetrany is early to medium type – heading is between 15-21 May.
15-20		25 -30 cm).	
(			
15-21			This variety differs to introduced foreign varieties with higher persistency, winter hardiness and tolerance to drought and high summer temperatures, tolerance to leaf diseases – crown rust (score 7-9).
			Average dry matter yield is 8000-11000 kg. ha <sup>-1</sup> , and seed yield 600-800 kg. ha <sup>-1</sup> . Biomass has the highest nutritive value in comparison to the other perennial grass species – crud protein content – 17-19 %, water soluble carbohydrates content – 6-10 % and in vitro dry matter digestibility 70-82 %.
kg da <sup>-1</sup> ,	( 7-9).	800-1100	
	60-80 kg da <sup>-1</sup> .		
	– 17-19%,	– 6-10%	
	70-82 %.		
			The variety is multifunctional, suitable for pasture, hay-pasture and amenity direction of use, in pure stands or in mixtures with white clover, and also with diploid perennial ryegrass for forage, or with red fescue for ornamental and sports fields, with high percent of density.
			Results from Competitive Variety trial (CVT) with perennial ryegrass, including Tetrany (NBG), IFC - Pleven, 2007-2009
	(NBG),		
, 2007-2009 .			The variants of the study are one

NBG 2  
 (Roy  
 (2007-  
 1  
 Pandora).  
 2009 .)  
 .  
 NBG (22172,0 kg ha<sup>-1</sup>),  
 7390.7 kg ha<sup>-1</sup>.  
 NBG.  
 , %  
 100%  
 NBG, 16,2%  
 27,4% Roy.  
 ,  
 53,8 % NBG 69,5%  
 Pandora.  
 NBG 30 %, 11,1%  
 Pandora 12,2% Roy.  
 NBG  
 .  
 ,  
 ,  
 ,  
 ( , 2017).  
 ,  
 .  
 (Katova, 2005 ; Katova et al., 2016).

candidate variety NBG, and two Belgian reference – standard varieties average from them (Roy and Pandora). For the three-year period (2007-2009) according to the VCU methodology in Table 1 are presented the data on the production of dry biomass total and average for the period and as a percentage relative to the average standard.

The highest total yield is the NBG (22172.0 kg ha<sup>-1</sup>) and the annual average is 7390.7 kg ha<sup>-1</sup>. The excess over the specified average is 62% for the NBG.

The distribution of dry mass production per year, per share, of the total yield for the survey period, accepted as 100%, is as follows: in the year of establishment of the swards, the share of the yield is 16.2% for perennial ryegrass NBG, up to 27,4% for Roy variety.

In the second year, maximum productivity is achieved for all variants, with a share of total productivity ranging from 53.8% for NBG to 69.5% for Pandora grassland. The yield as a share of total productivity in the third year is highest for NBG 30% and for Belgian the lowest for the whole period 11.1% for Pandora and 12,2% for Roy. Varieties have a different multi-year strategy and differ in the number of cuts and distribution of yields. The highest yield of fodder is NBG as a result of the largest number of cuts and the most even distribution over seasons over the years. Belgian varieties have less longevity under our conditions, lower forage yields, a smaller number of cuts, as feed productivity is strongly influenced by environmental conditions and they are selected under conditions characterized by different values of temperatures and precipitations (Katova, 2017). The tendency that local forms are higher productive and more persistent in comparison with introduced varieties was confirmed (Katova, 2005 ; Katova et al., 2016).



1. , NBG ( ),  
 - , 2007-2009 .  
**Table 1. Results from Competitive Varietal Trail, including NBG (Tetryn), IFC - Pleven, 2007-2009**

Variant	/ Dry matter yield, kg.ha <sup>-1</sup>			/ Total	Annual average	, % against St (2+3)
	2007	2008	2009			
1. NBG	3628.6	11928.9	6614.5	<b>22172.0</b>	<b>7390.7</b>	<b>162</b>
2. Roy	4142.0	9150.3	1849.9	15142.2	5047.4	111
3. Pandora	2371.0	8477.0	1353.6	12201.6	4067.2	89
Average	3380.5	9852.1	3272.7	16505.3	5501.8	
STDEV	911.2	1829.8	2904.7	5123.1	1707.7	
P 0.1%	865.3	1737.7	2758.5	4865.1	1621.7	
P 0.01%	1355.1	2721.2	4319.8	7618.8	2539.6	<b>100</b>

kg.ha<sup>-1</sup> (2015 – 2016)

2

3

( )

( )

2016 . 34,6 %

2015 . – 3,6 %.

12557.9 kg.ha<sup>-1</sup>  
 14,6%,

-11

10760 kg.ha<sup>-1</sup> (Sokolovic et al., 2010; 2011).

Results from Official national variety testing for Value of Cultivation and Use (VCU) for dry matter yield kg.ha<sup>-1</sup> (2015 – 2016)

After accelerated multiplication of original Breeder's seeds of the variety IFK Harmoniya and fulfill technical questioners the application for certification and variety testing was done. Results from VCU test are presented on Table 2 for locations, years and average from three locations and two years (in ecological net) and comparison with standard (IFK Harmoniya). The highest dry matter yield was obtained in location Selanovci, soil type carbonate chernozem where for 2016 year DMY of Tetryn exceeded standard by 34,6 % and for 2015 by 3,6%. Average value of dry matter yield of Tetryn for the period and locations was 12557.9 kg.ha<sup>-1</sup> and exceeded standard by 14%, which determined the variety as high productive for this geographical region, without irrigation. For comparison the new Serbian variety of perennial ryegrass - 11 has annual average dry matter yield 10760 kg.ha<sup>-1</sup> (Sokolovic et al., 2010; 2011).

2.

( )

kg.ha<sup>-1</sup>

(2015-2016)

**Table 2. Results from the National Official varietal testing for VCU, dry matter yield kg.ha<sup>-1</sup> (2015-2016)**

Location	Chepinci		Plovdiv		Selanovci		Average from 3 locations <sup>3</sup>		
	2015	2016	2015	2016	2015	2016	2015	2016	Average 2015-2016
Variety									
Years									
1.	17095.0	12462.5	6082.5	4305.0	7977.3	17640.5	10384.9	11469.3	<b>10927.1</b>
IFK Harmoniya - St	100%	100%	100%	100%	100%	100%	100%	100%	<b>100%</b>
2.	19167.5	13065.0	6435.0	4670.0	8261.3	23748.3	11287.9	13827.8	<b>12557.9</b>
Tetryny	112.1%	104.8%	105.8%	108.5%	103.6%	134.6%	108.7%	120.6%	<b>114.6%</b>
GD 5%	320.6	363.9	287.1	96.4	154.9	864.1			
GD 1%	460.7	522.9	417.5	138.4	222.6	1241.6			
GD 0.1%	677.7	769.2	606.8	203.6	327.5	1826.5			
, %	0.54	0.84	1.44	0.67	0.53	1.38			
Accuracy of trial,%									

. 3.

( )

2014-2016 .

**Table 3. Results of national official varietal testing for distinctness, uniformity and stability (DUS) for the period 2014-2016**

	/ Variety		/ Tetryny	
	/ Characteristics		/ Expression	Score /
1.	/ Ploidy		/ Tetraploid	4
2.	:	/ Plant: habitus in the autumn	/ Semi-erect	3 - 5
3.			/ Absent	1
4.	/ Tendency to form inflorescence in sowing year			-
5.	Time of inflorescence appearance in the year of sowing			9
6.	/ Leave: color		Very dark green	3 - 5
7.	Plant: habitus in spring		/ Semi erect to intermedium	7
8.	Plant: natural height in spring		/ High	3 - 5
9.	Time of heading in the second year		early to intermediate	7
10.	Plant: natural height during inflorescences appearance		/ High	7
11.	/ Flag leaf: length		/ Very long	9
12.	/ Flag leaf: width		/ Wide	7
13.	( ) / Stem: length of the longest stem (including inflorescence when is fully expended)		/ Long	7
14.	Inflorescence: length (when is fully developed)		/ Many	7
15.	Inflorescence: number of spikelets			7

V. Results from Official national variety testing for Distinctness, Uniformity and Stability (DUS) for 2014-2016 period (Table 3.) were obtained from one location – Chepinci in 3 years period and they were unidirectional, corresponding the author's description. Ploidy level was determined by the author using flowcytometric method in Institute of Genetics and Plant breeding, Melle, Belgium in 2000, 2002, 2005 and 2007. The variety Tetrany is new, original, uniform and stable and differs from reference varieties. Main characters are measured and presented in Table 3 with expression and score.

The first for Bulgaria tetraploid perennial ryegrass variety Tetrany - early to medium early, highly productive, environmentally stable (winter hardy and tolerant to drought) and persistent was created. It is based on methods, induced polyploidy of the local breeding population, flow cytometric screening and phenotypic selection of tetraploids followed by polycross (multiple hybridization) of 45 elite genotypes perennial ryegrass and reproduction to C 4 generation. The variety is multifunctional, suitable for pasture, hay-pasture and decorative direction of use, alone or in mixtures with alfalfa and white clover to feed or red fescue for ornamental and sport-technical swards with a high percentage of ground cover. The results of a successful competitive variety trial (CVT) in Pleven (2007-2009) a total of 22172.0 kg ha<sup>-1</sup> dry matter yield and annual average 7390.7 kg ha<sup>-1</sup>, and official state variety testing for cultivation and use (VCU) in three points (Chepinci, Plovdiv and Selanovtsi) with an average yield of dry mass of 12557.9 kg ha<sup>-1</sup>, and distinctness, uniformity and stability (DUS) at 1 point (Chepinci) Executive Agency for Variety and Seed (IASAS) (2014-2016) and a description of the variety Tetrany are presented.

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## CONCLUSIONS

The first for Bulgaria tetraploid perennial ryegrass variety Tetrany - early to medium early, highly productive, environmentally stable (winter hardy and tolerant to drought) and persistent was created. It is based on methods, induced polyploidy of the local breeding population, flow cytometric screening and phenotypic selection of tetraploids followed by polycross (multiple hybridization) of 45 elite genotypes perennial ryegrass and reproduction to C 4 generation. The variety is multifunctional, suitable for pasture, hay-pasture and decorative direction of use, alone or in mixtures with alfalfa and white clover to feed or red fescue for ornamental and sport-technical swards with a high percentage of ground cover. The results of a successful competitive variety trial (CVT) in Pleven (2007-2009) a total of 22172.0 kg ha<sup>-1</sup> dry matter yield and annual average 7390.7 kg ha<sup>-1</sup>, and official state variety testing for cultivation and use (VCU) in three points (Chepinci, Plovdiv and Selanovtsi) with an average yield of dry mass of 12557.9 kg ha<sup>-1</sup>, and distinctness, uniformity and stability (DUS) at 1 point (Chepinci) Executive Agency for Variety and Seed (IASAS) (2014-2016) and a description of the variety Tetrany are presented.

2018 .	2018 .	OECD ( )	The variety will be registered in the Official Variety List (OVL) of Bulgaria for 2018, in the OECD list for 2018 and in the common European catalog of varieties of agricultural crops for 2018, is expected certificate from the Patent Office of the Republic of Bulgaria in 2017.
2017 .			<ul style="list-style-type: none"> <li>- At this stage after Certification there</li> <li>- is necessary quantity of original breeder's seed to set up seed production system. In</li> <li>- Institute of Forage Crops the high</li> <li>- category PB and B seeds are producing. There are high consumer's demands –</li> <li>- forthcoming implementation and realization.</li> </ul>

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**(*Lolium perenne* L.)**

, 5800  
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**Tetramis – new tetraploid perennial ryegrass variety  
(*Lolium perenne* L.)**

Aneliya Katova

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**SUMMARY**

2000-2014 . -  
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)  
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(  
) 52  
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,  
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( )  
(2007-2009 ) 19769,7 kg ha<sup>-1</sup>  
6589.9 kg ha<sup>-1</sup>,  
( )

- During the period 2000 - 2014, at the Institute of Forage Crops - Pleven the new tetraploid perennial ryegrass variety Tetramis – very early, highly productive, environmentally stable (winter hardy and tolerant to drought) and persistent was created. It is based on methods, induced polyploidy of the local breeding population, flow cytometric screening and phenotypic selection of tetraploids followed by polycross (multiple hybridization) of 52 elite genotypes perennial ryegrass and reproduction to C 4 generation.

- The variety is multifunctional, suitable for pasture, hay-pasture and decorative direction of use, alone or in mixtures with alfalfa and white clover to feed or red fescue for ornamental and sport-technical swards.

- The results of a successful competitive variety trial (CVT) in Pleven (2007-2009) a total of 19769,7 kg ha<sup>-1</sup> dry matter yield and annual average 6589.9 kg ha<sup>-1</sup>, and official state variety testing for cultivation and use (VCU) in three points (Chepinci, Plovdiv and Selanovtsi) with an average

( , )  
 13549.7 kg ha<sup>-1</sup>,  
 ( )  
 ( ) (2014-2016 ),  
 2018 ., OECD  
 2018 .,  
 2017 .  
 :

yield of dry mass of 13549.7 kg ha<sup>-1</sup>, and distinctness, uniformity and stability (DUS) at 1 point (Chepinci) Executive Agency for Variety and Seed (IASAS) (2014-2016) and a description of the variety Tetramis are presented.

The variety will be registered in the Official Variety List (OVL) of Bulgaria for 2018, in the OECD list for 2018 and in the common European catalog of varieties of agricultural crops for 2018, is expected certificate from the Patent Office of the Republic of Bulgaria in 2017.

**Key words:** perennial ryegrass, new Bulgarian tetraploid variety, description, VCU, DUS

## INTRODUCTION

*perenne* L.) - (*Lolium*  
 ,  
 .  
 ,  
 .  
 (Wilkins, 1991, Humphreys et al. 2010).  
 ,  
 ,  
 (Katova, 2005; 2016; 2017).

Perennial ryegrass (*Lolium perenne* L.) is the most common perennial grass in the world in moderate areas because of the combination of high digestibility and tolerance of grazing. The main objective of the breeding for its agricultural use is to increase the total and seasonal yield of dry biomass, durability, tolerance of drought and cold, leaf diseases and adequate seed yield.

Except for fodder it is widely used for decorative and sporting technical purpose (Wilkins, 1991, Humphreys et al. 2010). The foreign varieties tested in our country in most cases were high productive but have slight adaptability to our agro-climatic conditions, weren't persistent and not suitable for direct introduction in agricultural practice (Katova, 2005; 2016; 2017).

Multi-cutting and perenniality of English ryegrass are important prerequisites for accumulation of the total production of vegetative mass for whole period of using the sward. Perennial ryegrass is under the influence of the environmental factors often with stressful

(Casler, 2001).

(Connolly, 2001; Humphreys, 2006; Boller et al., 2010; Katova, 2011; 2016).

situation during the year, for many years. (Casler, 2001). The population resistance to unfavorable factors of local ecological conditions has great importance for productivity and persistency. Bulgaria is situated in Mediterranean gene center where the species was originated. Here, in natural populations there are genotypes, which combined resistance to unfavorable environmental conditions with high potential for productivity and yielding. Creating new varieties is achieved mainly by hybridization, recurrent selection, polycross and application of induced poliploidization (Connolly, 2001; Humphreys, 2006; Boller et al., 2010; Katova, 2011; 2016).

2000 .. (15-17 .)

(Katova, 2016; 2017; Katova et al., 2016).

The tetraploidization is widely applied in modern varieties worldwide, and in IFC - Pleven this method is applied in the breeding programs since 2000, and after a long selection process (15-17 years) the first tetraploid varieties of perennial ryegrass in Bulgaria are tested (Katova, 2016; 2017; Katova et al., 2016).

(

The aim is to create a new tetraploid variety of perennial ryegrass, to carry out variety tests (VCU and DUS) and description.

**MATERIAL AND METHODS**

(2000-2014

2000 . -

2000 - 1-2

- 10 50 % - % 4

5-10 % - , 52

1 - 265 - 4 ,

(Katova, 2015 a; b).

2001 . -

2002 . -

1 - SBG -

560,62g,

2003 . - -

Breeding process (2000-2014) by years included:

2000 – Treatment by colchicine of breeding population with local origin (2000 seeds) – seedlings for 1-2 months. Survival % alive plants – from 10 to 50 % and screening for 4 plants by Flowcytometer – from them 5-10 % – tetraploids; progenies of 52 parental plants (genotypes) - 1 – 265 individual plants – 4 , (Katova, 2015 a; b).

2001 – field establishment – in polycrosses under isolation with winter rye;

2002 – first seeds from tetraploid plants - 1 – SBG – 560,62 g. Screening for ploidy level;

2003 – seedlings – multiplication under isolation;



2004 .- 2 ;  
 2005 .- ;  
 3-100% - ;  
 2007 . - 4 ;  
 -100% - ;  
 2007 . 2009 .-  
 ( ) ;  
 2011 . - - 2012 . 2013 . -  
 2014 . -  
 ( )  
 , ,  
 -  
 " ) → →  
 →  
 ( ) ,  
 UPOV. e -  
 ANOVA,  
 Excel. :  
*Poacea* (Buktheeva *et al.*,  
 1985);  
 UPOV - TG/4/7/ (UPOV, 1990),  
 TG/35/13/ (UPOV, 1999), TG/4/8 (UPOV,  
 2006), CPVO-TP/004/1 Final English Date:  
 23/06/2011.  
 2014 . 1964-  
 578 mm.  
 15 .  
 (12,3 ° )  
 50- . (11,6 ° ),  
 (46 ° )  
 -15  
 -28 ° .

2004 - 2 seeds;  
 2005 - screening for ploidy level -  
 3-100% - tetraploids;  
 2007 - 4 ploidy check - 100% -  
 tetraploids;  
 2007 till 2009 - competitive variety  
 testing (CVT);  
 2011 - sowing of prenatal  
 multiplication (PM) - 2012 . 2013 . - first  
 Breeder's seeds for application and Official  
 testing.

Breeding process carried out by  
 application of modern breeding methods  
 (induced poliploidization, polycross and  
 progeny testing for complex characteristics  
 - tandem selection „high productivity -  
 ecological stability) → variety testing → new  
 variety → Breeder's seeds and high  
 category seeds.

The experimental plant material  
 was studied for many traits (quantitative  
 and qualitative), characters and  
 properties, according to UPOV methodical  
 guides. For data processing the statistical  
 method ANOVA was applied, modern  
 programmes and calculation tables in  
 Excel were used. International classifier  
 for studying collections from *Poacea* for  
 complex estimation was used (Buktheeva  
*et al.*, 1985); technical questioners for  
 DUS and VCU according to UPOV -  
 TG/4/7/ (UPOV, 1990), TG/35/13/ (UPOV,  
 1999), TG/4/8 (UPOV, 2006), CPVO-  
 TP/004/1 Final English Date: 23/06/2011.

The region of the study - Pleven,  
 where the competitive variety trial for  
 forage was conducted, combined climatic  
 peculiarities in North Central Bulgaria-  
 temperate continental climate area.  
 Average annual precipitation sum was  
 550 mm during the period of 1964-2014.  
 Average annual temperatures for the  
 period 2007-2009 were higher (13,3 ° )  
 to that for previous 50 years period (11,9  
 ° ), which confirmed the tendency of  
 global warming. During the last decade  
 the temperature maximums for July and  
 August (46 ° ), and minimums for  
 January and February reached to -15 -28  
 ° were recorded. The soil type is leached

03 2007 ..  
 2007 . - 1 , 2008 - 3  
 2009 . - 3 2 - 7  
 5  
 5  
 m<sup>2</sup> , 3 : SBG ( ), Roy  
 Pandora, 4 ; 200  
 kg ha<sup>-1</sup>,  
 N a. . 60 kg ha<sup>-1</sup>,  
 50 kg ha<sup>-1</sup>,  
 12 cm, 1,5 cm,  
 :  
 - 168 m, -  
 - 10 m<sup>2</sup>  
 , 2 , 4  
 - 200 kg ha<sup>-1</sup>,  
 - 80 kg ha<sup>-1</sup>, - 24.03.2014  
 „ «Saxonia» ,  
 25 kg ha<sup>-1</sup>, 11,5 cm,  
 1,5 cm,  
 2015 . 2  
 2016 . 2  
 4  
 -  
 166 m, -  
 - 10 m<sup>2</sup>, 2  
 , 4  
 - 300 kg ha<sup>-1</sup>, 150  
 kg ha<sup>-1</sup>, - 80 kg ha<sup>-1</sup>,  
 - 04.04.2014 ..  
 25 kg ha<sup>-1</sup>, 12 cm,  
 1,5 cm,  
 4  
 4 - 2016 .. 8

chernozem, poor to medium for humus, with slightly acid to neutral pH reaction. These soil climatic conditions are provoke background for selection and adaptation of genetic resources to drought and cold.

Competitive Variety Trail (CVT) was established on 3 April 2007 and measurement of dry biomass productivity started in 2007 year – 1 cut, 2008 – 3 cut for the Bulgarian and 2 cuts Belgian varieties, 2009 – 3 cut for the Bulgarian and 2 cuts Belgian varieties, and in total for the period - 7 cuts for forage for the Bulgarian and 5 cuts Belgian varieties as standard the average between two Belgian varieties used. Plot size was 5 m<sup>2</sup>, 3 variants (SBG (Tetramis), Roy, Pandora), 4 replicates; fertilizing: P<sub>2</sub>O<sub>5</sub> - 200 kg ha<sup>-1</sup>, K<sub>2</sub>O - 150 kg ha<sup>-1</sup>, N a.s. – 60 kg ha<sup>-1</sup>, sowing – manual, sowing rate 50 kg ha<sup>-1</sup>, inter row distance 12 cm, depth 1,5 cm, without irrigation.

Official variety testing for VCU was conducted in three locations as follows:

Location Selanovci – soil type carbonate chernozem, above sea level height – 168 m, precursor – set aside, Plot size was – 10 m<sup>2</sup>, 2 variants, 4 replicates; fertilizing: P<sub>2</sub>O<sub>5</sub> – 200 kg ha<sup>-1</sup>, N – 80 kg ha<sup>-1</sup>, sowing – 24.03.2014, sowing machine «Saxonia», sowing rate 25 kg ha<sup>-1</sup>, inter row distance 11,5cm, depth 1,5 cm. Reporting of dry mass production in 2015 is at 2 cuts and in 2016 also 2, a total of 4 cuts under non-irrigating conditions.

Location Plovdiv – soil type alluvial lawn, above sea level height – 166 m, precursor – wheat, plot size – 10 m<sup>2</sup>, 2 variants, 4 replicates, fertilizing: P<sub>2</sub>O<sub>5</sub> – 300 kg ha<sup>-1</sup>, K<sub>2</sub>O 150 kg ha<sup>-1</sup>, N – 80 kg ha<sup>-1</sup>, sowing – manual in 04.04.2014, sowing rate 25 kg<sup>1</sup>, inter row distance 12 cm, depth 1,5 cm. Reporting of dry mass production in 2015 is at 4 cuts and in 2016 also 4, a total of 8 cuts under irrigating conditions.

515 m, -  
 m<sup>2</sup>, 2 , 4 - 10  
 80 kg ha<sup>-1</sup>, 130 kg ha<sup>-1</sup>, - 250 kg ha<sup>-1</sup>,  
 13,7 cm, 25 kg ha<sup>-1</sup>, - 04.04.2014 .,  
 2015 . 4 - 2016 ., 4  
 2016 .,  
 2014-2016 .

Location Chepinci - soil type  
 alluvial lawn, above sea level height - 515  
 m, precursor - forage pea, plot size - 10  
 m<sup>2</sup>, 2 variants, 4 replicates, fertilizing:  
 P<sub>2</sub>O<sub>5</sub> - 250 kg ha<sup>-1</sup>, K<sub>2</sub>O 130 kg ha<sup>-1</sup>, N -  
 80 kg ha<sup>-1</sup>, sowing - 04.04.2014.,  
 manual, sowing rate 25 kg ha<sup>-1</sup>, inter row  
 distance 13,7 cm, depth 1,5 cm.  
 Reporting of dry mass production in 2015  
 is at 4 cuts and in 2016 also 4, a total of 8  
 cuts under irrigating conditions.

VCU (dry matter yield) was  
 measured in the three locations during  
 years 2015 and 2016, and for DUS -  
 2014-2016, as a standard variety IFK  
 Harmoniya is included.

## RESULTS AND DISCUSSION

### Description of perennial ryegrass variety Tetramis

Variety Tetramis is tetraploid and had been developed by the methods: poliploidization of local breeding population, three-fold flow cytometric screening and phenotypic selection of tetraploids followed by polycross (multiple hybridization) of 52 elite genotypes perennial ryegrass and reproduction to C 4 generation.

Tetramis's plants are perennial, with well developed tuft root system, intensive tillering, erect habit (score 1). Variety Tetramis formed many and very leafy generative and vegetative stems. Generative shoots are 2,9 mm width of stems, smooth surface, comparatively lodging resistant, with 4-5 number of leaves.

The plant leafness is 35-43%. Plants are tall in height at the beginning of heading 65 cm and in full heading 88 cm. Leaves are linear flat or slightly curve, light green, smooth and have brightness in lower surface. The tongue is membranous, medium in height with sharp edge. Auricles are short and they do not blank the stem. The length (15-17 m) and width of the leaves (3-4 mm) are

1000	22-29 cm - 21-23.	3,71-4,89 g.	3-4 °	10 ( 25-30 cm).	26-30	( 7-9).	8000-10000 kg ha <sup>-1</sup> , 900 kg ha <sup>-1</sup> .	700-	- 17%, - 6-8 %	70 – 75 %.	( ) , SBG ( ) 2 (Roy Pandora). (2007-2009 ) 1	<p>medium to long in size. Inflorescence is spike with length 22-29 cm and number of spikelets per spike are 21-23. Seeds are elongate, boat-like. Thousand seed weight (TSW) is 3,71-4,89 g.</p> <p>Plants grow up very early in spring when soil temperature is 3-4 ° and continued vegetation till the first frosts. Pasture stage (maturity) reached till 10 April (height of the stand 25-30 cm).</p> <p>The variety persist mani cuttings and grazing. Tetramis is very early heading is between 26-30 April. This variety differs to introduced foreign varieties with higher persistency, winter hardiness and tolerance to drought and high summer temperatures, tolerance to leaf diseases – crown rust (score 7-9).</p> <p>Average dry matter yield is 8000-10000 kg. ha<sup>-1</sup>, and seed yield 700-900 kg. ha<sup>-1</sup>.</p> <p>Biomass has the highest nutritive value in comparison to the other perennial grass species – crude protein content – 17 %, water soluble carbohydrates content – 6-8 % and in vitro dry matter digestibility 70-75 %.</p> <p>The variety Tetramis is multifunctional, suitable for pasture, hay-pasture and amenity direction of use, in pure stands or in mixtures with legumes, and also with diploid perennial ryegrass for forage, or with red fescue for ornamental and sports fields, with high percent of density.</p> <p><b>Results from Competitive Variety trial (CVT) with perennial ryegrass, including SBG Tetramis (Tetramis), IFC - Plevén, 2007-2009</b></p> <p>The variants of the study are one candidate variety SBG (Tetramis), and two Belgian reference – standard varieties average from them (Roy and Pandora). For the three-year period (2007-2009) according to the VCU methodology in Table 1 are presented the data on the production of dry biomass total and average for the period and as a percentage relative to the average standard.</p>
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SBG – 19769,7 kg ha<sup>-1</sup>  
 6589,9 kg ha<sup>-1</sup>.

45 % SBG.

, %

100% :

17,5%  
 SBG, 27,4% Roy.

, 61,3 % SBG 69,5%  
 Pandora.

SBG 21.2 %, 11,1%  
 Pandora 12, 2% Roy.

The highest total yield is the SBG (19769,7 kg ha<sup>-1</sup>) and the annual average is 6589,9 kg ha<sup>-1</sup>. The excess over the specified average is 45% for the SBG.

The distribution of dry mass production per year, per share, of the total yield for the survey period, accepted as 100%, is as follows: in the year of establishment of the swards, the share of the yield is 17.5% for perennial ryegrass SBG, up to 27,4% for Roy variety. In the second year, maximum productivity is achieved for all variants, with a share of total productivity ranging from 61.3% for SBG to 69.5% for Pandora perennial ryegrass.

The yield as a share of total productivity in the third year is highest for SBG 21,2% and for Belgian the lowest for the whole period 11.1% for Pandora and 12,2% for Roy. Varieties have a different multi-year strategy and differ in the number of cuts and distribution of yields.

1. , . SBG ( ),  
 - , 2007-2009 .

**Table 1. Results from Competitive Varietal Trail, including SBG (Tetramis), IFC - Pleven, 2007-2009**

Variant	/ Dry matter yield kg.ha <sup>-1</sup>					
	2007	2008	2009	Total	Annual average	% against St (2+3)
1. SBG (Tetramis)	3464.5	12121.6	4183.6	<b>19769.7</b>	<b>6589.9</b>	<b>145</b>
2. Roy	4142.0	9150.3	1849.9	15142.2	5047.4	111
3. Pandora	2371.0	8477.0	1353.6	12201.6	4067.2	89
Average	3325.8	9916.3	2462.4	15704.5	5234.8	
STDEV	893.6	1939.3	1511.2	3815.3	1271.8	
P 0.1%	848.6	1841.7	1435.1	3623.2	1207.7	
P 0.01%	1328.9	2884.0	2247.3	5673.9	1891.3	

SBG -

The highest yield of fodder is SBG as a result of the largest number of cuts and the most even distribution over seasons over the years. Belgian varieties have less longevity under our conditions, lower forage yields, a smaller number of cuts, as feed productivity is strongly influenced by environmental conditions and they are selected under conditions characterized by different values of temperatures and precipitations (Katova, 2017). The tendency

2017). (Katova, , -

(Katova, 2005; Katova et al., 2016).

( )  
kg.ha<sup>-1</sup> (2015-2016)

100%).

T 2.

that local forms are higher productive and more persistent in comparison with introduced varieties was confirmed (Katova, 2005; Katova et al., 2016).

. Results from Official national variety testing for Value of Cultivation and Use (VCU) for dry matter yield kg.ha<sup>-1</sup> (2015-2016)

After accelerated multiplication of original Breeder's seeds of the variety IFK Harmoniya and fulfill technical questioners the application for certification and variety testing was done. Results from VCU test are presented on Table 2 for locations, years and average from three locations and two years (in ecological net) and comparison with standard (IFK Harmoniya).

( ) kg ha<sup>-1</sup>

(2015-2016)

**Table 2. Results from the National Official varietal testing for VCU, dry matter yield kg.ha<sup>-1</sup> (2015-2016)**

Location	Chepinci		Plovdiv		Selanovci		Average from 3 locations		Average 2015-2016
	2015	2016	2015	2016	2015	2016	2015	2016	
Variety									Average
1.									2015-2016
IFK Harmoniya	17095.0	12462.5	6082.5	4305.0	7977.3	17640.5	10384.9	11469,3	<b>10927.1</b>
- St	100%	100%	100%	100%	100%	100%	100%	100%	<b>100%</b>
2.									<b>13549.7</b>
Tetramis	20117.5	15947.5	61425.0	4590.0	10643.0	23157.8	12534.4	14565,0	<b>123.9%</b>
GD 5%	122%	128%	101%	106.6%	133%	131.3%	120.7%	127%	
GD 1%	320.6	363.9	287.1	96.4	154.9	864.1			
GD 0.1%	460.7	522.9	417.5	138.4	222.6	1241.6			
	677.7	769.2	606.8	203.6	327.5	1826.5			
, %	0.54	0.84	1.44	0.67	0.53	1.38			
Accuracy of trail,%									

2015 . 31,3 %

2016– 33,0 %.

22% 2015 28%

2016 . 1 7 %.

The highest dry matter yield was obtained in location Selanovci, soil type carbonate chernozem where for 2015 year DMY of Tetramis exceeded standard by 31,3 % and for 2016 by 33,0%. In Chepintsi, high yields of the new variety have been obtained, with years exceeding the standard by 22% in 2015 and by 28% in 2016. In the third place Plovdiv exceeds the yield from 1 to 7%. Average value of dry matter yield of Tetramis for the period and

13549.7 kg.ha<sup>-1</sup>  
(10927,1 kg.ha<sup>-1</sup>) 23,9%,

-11 -  
10760 kg.ha<sup>-1</sup>

(Sokolovic et al., 2010; 2011).

V.

2014-2016 .

1 -

3

2000, 2002, 2005 2007 .

3

3.

locations was 13549.7 kg.ha<sup>-1</sup> and exceeded standard (10927,1 kg.ha<sup>-1</sup>) by 23,9%, which determined the variety as high productive for this geographical region, without irrigation. For comparison the new Serbian variety of perennial ryegrass -11 has annual average dry matter yield 10760 kg.ha<sup>-1</sup> (Sokolovic et al., 2010, 2011).

**V. Results from Official national variety testing for Distinctness, Uniformity and Stability (DUS) for 2014-2016 period**

Results for DUS (Table 3.) were obtained from one location – Chepinci in 3 years period and they were unidirectional, corresponding the author's description. Ploidy level was determined by the author using flowcytometric method in Institute of Genetics and Plant breeding, Melle, Belgium in 2000, 2002, 2005 and 2007. The variety Tetramis is new, original, uniform and stable and differs from reference varieties. Main characters are measured and presented in Table 3 with expression and score.

**Table 3. Results of national official varietal testing for distinctness, uniformity and stability (DUS) for the period 2014 -2016**

/ Variety		/ Tetramis	
/ Characteristics		/ Expression	/ score
1.	/ Ploidy	/ Tetraploid	4
2.	: / Plant: habitus in the autumn	/ Erect	1
3.		/ Absent	1
4.	Tendency to form inflorescence in sowing year	-	-
5.	: / Leave: color	/ Green	5
6.	: / Plant: habitus in spring	/ Erect	1
7.	: / Plant: natural height in spring	/ Very tall	9
8.	: / Time of heading in the second year	/ Very early	1
9.	: / Plant: natural height during inflorescences appearance	/ Very tall	9
10.	: / Flag leave: length	/ Long	7
11.	: / Flag leave: width	/ Wide	7
12.	: / Stem: length of the longest stem (including inflorescence when is fully expended)	/ very long	9
13.	: / Inflorescence: length (when is fully developed)	/ Long	7
14.	: / Inflorescence: number of spikelets	/ Many	7

## CONCLUSIONS

New Bulgarian tetraploid perennial ryegrass variety Tetramis – very early, highly productive, environmentally stable (winter hardy and tolerant to drought) and persistent was created. It is based on methods, induced polyploidy of the local breeding population, flow cytometric screening and phenotypic selection of tetraploids followed by polycross (multiple hybridization) of 52 elite genotypes perennial ryegrass and reproduction to C 4 generation. The variety is multifunctional, suitable for pasture, hay-pasture and decorative direction of use, alone or in mixtures with alfalfa and white clover to feed or red fescue for ornamental and sport-technical swards. The results of a successful competitive variety trial (CVT) in Pleven (2007-2009) a total of 19769,7 kg ha<sup>-1</sup> kg ha<sup>-1</sup> dry matter yield and annual average 6589.9 kg ha<sup>-1</sup>, and official state variety testing for cultivation and use (VCU) in three points (Chepinci, Plovdiv and Selanovtsi) with an average yield of dry mass of 13549.7 kg ha<sup>-1</sup>, and distinctness, uniformity and stability (DUS) at 1 point (Chepinci) Executive Agency for Variety and Seed (IASAS) (2014 - 2016) and a description of the variety Tetramis are presented.

The variety will be registered in the Official Variety List (OVL) of Bulgaria for 2018, in the OECD list for 2018 and in the common European catalog of varieties of agricultural crops for 2018, is expected certificate from the Patent Office of the Republic of Bulgaria in 2017.

At this stage after Certification there are necessary quantities of original breeder's seed to set up seed production system.

In Institute of Forage Crops the high category PB and B seeds are producing. There is high consumer's demands – forthcoming implementation and realization.



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## Chemical composition, digestibility and feeding value of perennial ryegrass

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### SUMMARY

<p>21</p> <p>2 (L.),</p> <p>(Van Soest),</p> <p>(Aufreere),</p> <p>17,92%, 1,83%</p> <p>42,56%</p> <p>28,37%</p> <p>78,00%</p> <p>.13,</p> <p>.10, 11, 12,</p> <p>14, 17</p> <p>1.</p> <p>(RFV) 108</p> <p>.13 – 146</p>	<p>- composition, digestibility, energy and protein feeding value of 21 accessions perennial ryegrass (<i>Lolium, perenne</i> L.) from collection nurseries KN1 and KN2 – Bulgarian and introduced ecotypes and varieties in spring, summer and autumn growths are evaluated in field trial in the Institute of Forage Crops, Pleven. The parameters of general chemical composition (Weende analysis), plant cell walls fiber components content by detergent analysis (Van Soest), enzyme <i>in vitro</i> digestibility of dry and organic matter (method d'Aufreere), potential energy and potential protein feeding value by different systems are determined. In the <b>first spring growth</b> the highest protein content 20,88%, the lowest of fiber components CF 17,92%, NDF 42,56%, ADF 28,37%, ADL 1,83% and the highest digestibility of dry matter 78,00% belongs to Var.13, followed by <b>tetraploids</b> of variants 10, 11, 12, 14, 17 in KN1.</p> <p>The mean Relative Feeding Value (RFV) is 108 rel.% and the highest is in Var.13 – 146 rel.%. Energy feeding value is also</p>
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.13.   
 .13 – 160   
 .14 – 166 g   
 g kg<sup>-1</sup>   
 kg<sup>-1</sup>.   
 -   
 .12,   
 .13.   
 101 .%,   
 10,11,12,13,14 1   
 .3 5 2   
 RFV .13 –130   
 %   
 - .12 .13,   
 UFL-UFV 0,854-0,782 .12   
 0,815-0,717 .13.   
 .12 PBD-PDIN-PDIE:174,137,110 g   
 kg<sup>-1</sup>.   
 -   
 RFV   
 111 .%, -   
 .10 – 136 .%,   
 -   
 162 g kg<sup>-1</sup>   
 125 g kg<sup>-1</sup>.   
 :   
 (*Lolium perenne* L.), ,   
 ,

- the highest in Var.13 as well the same   
 - variant shows the highest energy feeding   
 value. The maximal protein feeding value   
 demonstrates Var.13 – 160 g kg<sup>-1</sup> dry   
 matter and Var.14 – 166 g kg<sup>-1</sup>. In the   
**second summer growth** the better   
 forage quality – high protein content and   
 low fiber components content belong to   
 Var.12, followed by Var.13. The mean   
 RFV is 101 rel.% as Variants   
 10,11,12,13,14 from 1 and Var.3 and 5   
 from 2 exceed the mean. The maximal   
 RFV belong to Var.13 – 130 rel.%. Energy   
 net feeding value is the highest for Var.12   
 and Var.13, UFL-UFV 0.854-0.782   
 respectively for Var.12 and 0.815-0.717   
 for Var. 13. The maximal protein feeding   
 value belongs to Var.12: PBD-PDIN-   
 PDIE: 174, 137, 110g kg<sup>-1</sup> respectively.   
 The changes in perennial ryegrass forage   
 digestibility from high to low are   
 consequently from first toward third and   
 second growths. The mean RFV of the   
 accessions from **third autumn growth** is   
 111 rel.% and the highest is for Var.10 –   
 136 rel.%, which demonstrates the   
 highest net energy and protein feeding   
 value – Total Digestible Protein 162 g kg<sup>-1</sup>   
 dry matter at mean value 125 g kg<sup>-1</sup>.

**Key words:** perennial ryegrass   
 (*Lolium perenne* L.), breeding,   
 digestibility, energy and protein feeding   
 value

## INTRODUCTION

Perennial ryegrass is one of the   
 most used grass species for sowing   
 grasslands in temperate areas, and it has   
 received significant breeding efforts   
 throughout the world. It is especially used   
 in Europe where it represents as far as   
 50% of the marketed grass seed   
 (Humphreys et al., 2010).

It is a leafy and highly tillering grass that   
 can produce high herbage yields of good   
 feeding value for ruminant animals during   
 several years.

(Humphreys et al., 2010).

20

3

(Katova et al., 2016).

NIRS.

10

(Sampoux et al., 2010).

(*Lolium perenne* L.)

- Varieties combining improved quality with excellent agronomic performance are our focus. Because the breeding process of perennial ryegrass is significantly longer than other arable plants, work of more than a decade usually precedes cultivar release (Humphreys et al. 2010).

Perennial ryegrass breeding in Bulgaria has a strong 20 year-long tradition, especially in the Institute of Forage Crops in Pleven. During these 20 years, three cultivars from local origin were created and release has started on the Bulgarian market (Katova et al., 2016).

- Most breeding programmes had long not included bio-chemical traits related to feeding value as selection criteria.

- However, progress in the understanding of herbage feeding value has helped to define relevant bio-chemical criteria that can now be easily predicted by NIRS methods.

Consequently, the use of these biochemical traits has become increasingly familiar in the last 10 years both to assess cultivar feeding value and to breed for improved feeding value (Sampoux et al., 2010).

- The aim of the study is to evaluate forage quality by chemical composition, digestibility, energy and protein feeding value of perennial ryegrass (*Lolium perenne* L.) accessions from collection nurseries as the initial breeding resource with a view to the breeding.

## MATERIAL AND METHODS

During the autumn of 2015, in the experimental field of the Institute of forage Crops - Pleven a collection nursery is based consisting of 21 samples (13 species and 2 candidates and 6 ecotypes of origin – Bulgarian – 9, Belgian – 9, Romanian – 2 and Chinese – 1; by ploidy

2015 .

-

21

2

;

- 9, - 2

1;

- 10 -

50/50 cm, 50  
2016  
65°  
20 min 105°  
1.0 mm  
QC 136 QB 114, Labor Mim,  
(Goering and Van Soest, 1970) (AOAC, 2010) (EN ISO13906 2008)  
/Neutral-detergent fiber ( /NDF)/,  
/Acid-detergent fiber ( /ADF)/  
detergent lignin ( /ADL)/.  
= - ; =  
(Akin and Chesson, 1990).  
*in vitro*  
( /IVDMD) ( /IVOMD)  
et al., 2010).  
Aufreere (1982) (Todorov

10 – Diploid and 11 – tetraploid) grassland populations, with plants being individually arranged, through seedings at a distance of 50/50 cm. Each sample is represented by 50 individual plants. In 2016 simultaneously to the assessment of the production potential of each growth and number, samples are taken for chemical analysis. The forage is obtained from three growths – spring, summer and autumn and its principal chemical composition and digestibility of dry matter are determined. Plant sample preparation from the above ground part of the plants is effectuate by air ventilation at 65° till crumbly at previous fixing for 20 min at 105° and grinding till particle size 1,0 mm consequently at laboratory mill QC 136 and QB 114, Labor Mim, Hungary, and obligatory screening.

The forage principal chemical composition is determined by Weende systematic analytic procedure (2010) and the parameters are established: Dry matter; Crude ash (Mineral matter, MM); Crude protein (CP) by Kjeldhal method; Crude fiber (CF) - by Heneberg&Stoman method. Detergent analysis of Goering&Van Soest (1970) (EN ISO13906 2008) was performed as a standard systematic chemical analysis of plant cell walls fiber components. The following fiber fractions: Neutral-detergent fiber (NDF); Acid-detergent fiber (ADF), Acid-detergent lignin (ADL) are determined. Polyosides hemicellulose and cellulose as a cell walls components, contained in fiber fraction are presented emperically: Hemicellulose = NDF – ADF; Cellulose = ADF – ADL. The degree of lignification is presented as relation of ADL and NDF/100 (Akin&Chesson 1989). Enzyme *in vitro* digestibility of dry (IVDMD) and organic (IVOMD) matter is determined by two stage pepsin-cellulase enzyme method of Aufreere (Todorov et al. 2010). Evaluation of feeding value on the basis of fiber components is performed as Relative feeding value RFV; potential intake of digestible dry matter

RFV; (Linn&Martin, 1991).  
 (0,779xADF%); (DDM%=88,9-  
 (DMI /% body weight/=120/NDF%)  
 (RFV=DDMxDMI/1,29)  
 : 1.  
 UFL-UFV (INRA,  
 1988), ( -  
 /FUM-FUG),  
 Todorov (1997).  
 ( ) ;  
 (AOAC 2010)  
 dMO<sub>in vivo</sub>  
 Andrieu Demarquilly (1989)  
 in vitro  
 (UFL-UFV), ( - )  
 (VEM-VEVI)  
 2. (PDIN=PDIA+PDIMN  
 PDIE=PDIA+PDIME)  
 (INRA 1988)  
 : TDP/PBD –  
 Total Digestible Protein/Protein Brute  
 Digestible, PDIN,  
 PDIE –

(Linn&Martin, 1991). The Digestible Dry Matter (DDM%=88,9-(0,779xADF%); Dry Matter Intake (DMI /% body weight/=120/NDF%) and Relative Feeding Value (RFV=DDMxDMI/1,29) are estimated.

*The feeding value estimation – energy and protein is performed as: 1. The Energy feeding value is calculated by French system: UFL-UFV (INRA, 1988), recalculated in Bulgarian by coefficients, followed by Todorov (1997). The following parameters are estimated: Gross energy /GE/, Metabolic energy /ME/ on the basis of equations according to experimental values of CP, CF (AOAC 2010) and IVOMD. The coefficient of digestibility of organic matter dMO<sub>in vivo</sub> (Andrieu and Demarquilly, 1989) is received by relationship on the basis of *in vitro* organic matter digestibility, determined experimentally. Net energy is determined according French (UFL-UFV), Bulgarian – Feed units for milk and Feed units for growth (FUM-FUG) and Dutch (VEM-VEVI) systems.*

*2. The potential Protein feeding value is performed by French system (INRA 1988). The parameters: TDP/PBD - Total Digestible Protein/Protein Brute Digestible and a really digestible protein in ruminant small intestine – PDIN (Protein digestible in intestine, depending on nitrogen) PDIN=PDIA+PDIMN and PDIE (Protein digestible in intestine depending on energy) PDIE=PDIA+PDIMN in g kg<sup>-1</sup> dry matter are established.*

*In comparative analysis in two vegetative stages and organic active products the individual and mean values and coefficients of variation of the parameters of forage feeding value are evaluated.*

## RESULTS AND DISCUSSION

The forage quality of 21 collection samples of pasture ryegrass of collection nursery CN1 in the first growth is studied

( 1).  
 15,75%,  
 20,88% ( . 3)  
 10.  
 12 – 17,32%  
 .13– 17,92%.  
 . 13 – 42,56%  
 1 54,69%.  
 . 13 – 1,83%,  
 10, 11, 12, 13, 14 17.  
 1.

(Table 1). The mean value of crude protein in dry matter is 15,75% and the maximal is 20,88% (Var.13). The number of samples with a crude protein content above the mean value is 10.  
 The crude fiber content is lowest in Var.12– 17,32% and Var.13 – 17,92%. With the lowest values of total fiber components, NDF is also Var.13 – 42,56% at the mean value of NDF for all samples of CN1 54,69%. ADL is also the lowest for Var.13 – 1,83%, followed by Variants 10, 11, 12, 14 and 17.

**Table 1. Principal composition, fiber components content and digestibility of perennial ryegrass in collection nurseries, first growth**

Variant	DM	Ash	CP	CF	NDF	ADF	ADL	HEMI	CELLU	LIGNIF	IVDMD	IVOMD
1 1/	91,27	8,49	13,65	25,22	59,46	34,46	3,47	25,00	31,00	5,8	64,48	67,32
2 CN1	92,42	8,55	12,96	22,94	58,38	33,24	3,23	25,14	30,01	5,5	65,04	67,55
3	91,54	10,67	13,88	25,84	59,42	38,69	4,61	20,73	34,08	7,8	55,55	58,97
4	91,97	9,09	11,22	27,61	56,80	40,44	5,72	16,36	34,72	10,1	59,07	62,16
5	92,25	11,82	14,68	22,66	52,88	33,89	3,42	18,99	30,47	6,5	64,00	67,08
6	91,84	11,28	15,16	23,00	56,85	34,08	3,44	22,77	30,64	6,1	65,19	37,17
7	90,56	15,90	18,76	21,04	54,93	29,18	2,54	25,75	26,64	4,6	69,27	75,54
8	91,77	10,38	14,41	23,05	60,58	34,26	3,56	26,32	30,70	5,9	61,82	64,08
9	92,14	11,87	16,85	21,13	58,26	34,56	3,38	23,70	31,18	5,8	66,26	68,49
10	90,66	14,63	18,18	19,68	45,58	29,14	2,28	16,44	26,86	5,0	71,21	76,15
11	92,66	12,56	17,88	19,84	46,32	29,32	2,28	17,02	27,04	4,9	71,17	75,11
12	90,63	14,61	20,20	17,32	46,39	28,78	2,15	17,61	26,63	4,6	77,93	79,68
13	90,92	14,87	20,88	17,97	42,56	28,37	1,83	14,19	26,54	4,3	78,00	81,94
14	92,02	12,60	19,51	20,81	53,26	31,11	2,45	22,15	28,66	4,6	71,29	72,53
15	91,94	10,28	15,36	21,21	56,79	29,86	4,16	26,93	25,70	7,3	69,07	71,34
16	91,07	11,80	19,41	20,80	49,30	29,28	2,50	20,02	26,78	5,1	75,07	66,09
17	93,02	9,54	9,46	30,56	58,42	42,62	6,29	15,89	36,33	10,8	53,92	55,70
3 2/	91,92	10,65	14,61	27,06	58,49	37,09	3,97	21,40	33,12	6,8	61,55	64,52
4 CN2	92,59	9,04	12,47	27,21	61,93	37,47	4,24	24,46	33,23	6,8	60,01	62,88
5	91,40	10,68	13,70	25,92	55,83	36,39	3,90	19,44	32,49	7,0	61,89	64,04
6-1	91,43	10,75	17,38	23,85	53,50	34,47	3,27	19,03	31,20	6,1	65,56	67,46
6-2	92,21	11,78	16,06	25,29	57,36	37,80	3,40	19,56	34,40	5,9	62,96	65,00
Min	90,56	8,49	9,46	17,32	42,56	28,37	1,83	14,19	25,70	4,3	53,92	55,70
Max	93,02	15,90	20,88	30,56	61,73	42,62	6,29	26,93	36,33	10,8	78,00	81,94
Mean	<b>91,74</b>	<b>11,45</b>	<b>15,75</b>	<b>23,18</b>	<b>54,69</b>	<b>33,84</b>	<b>3,46</b>	<b>20,63</b>	<b>30,38</b>	<b>6,2</b>	<b>65,92</b>	<b>67,76</b>
SD	0,68	2,09	3,02	3,37	5,41	4,12	1,11	3,70	3,16	1,68	6,48	6,91
CV	<b>0,7</b>	<b>18,2</b>	<b>19,2</b>	<b>14,5</b>	<b>9,9</b>	<b>12,2</b>	<b>32,2</b>	<b>17,9</b>	<b>10,4</b>	<b>27,2</b>	<b>9,8</b>	<b>10,2</b>

65,92%.  
 1  
 - These accessions are tetraploids. The mean digestibility of the forage dry matter of CN1 accessions is high – 65,92%.  
 - Nine of the accessions exceeded the mean digestibility, the highest being the

specimen of a perennial ryegrass Var.13–78,00% and Var.12 – 77,93%.

Digestibility of organic matter of perennial ryegrass corresponds to that of the dry matter. Var.13 of CN1 in the first growth has the highest forage quality.

The potential energy and the potential protein feeding value of the perennial ryegrass accessions from CN1, the first growth is presented in Table 2. The mean value of the relative feeding value (RFV) is 108 rel.%. The highest is of Var.13 - 146 rel.%. Energy feeding value is also the highest for Var.13. Maximum of protein feeding value has Var.13 - 160 g kg<sup>-1</sup> of dry matter and Var.14 - 166 g kg<sup>-1</sup>.

specimen of a perennial ryegrass Var.13–78,00% and Var.12 – 77,93%.

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**Table 2. Energy and protein feeding value of perennial ryegrass in collection nurseries, first growth**

					UFL	UFV				VEM	VEVI	PBD	PDIN	PDIE	
		DDM	DMI	RFV	GE	ME	UFL	UFV	FUM	FUG	VEM	VEVI	PBD	PDIN	PDIE
1	1	62,06	2,02	97	11,42	6,05	0,768	0,671	0,637	0,548	893	1873	94	85	87
2	CN1	63,01	2,06	100	11,39	6,03	0,772	0,676	0,640	0,553	892	1871	88	81	86
	3	58,76	2,02	92	11,45	5,59	0,664	0,553	0,551	0,452	814	1750	97	87	82
	4	57,40	2,11	94	11,32	5,72	0,706	0,602	0,586	0,492	836	1784	71	70	79
	5	62,50	2,27	110	11,49	5,89	0,736	0,632	0,610	0,517	871	1838	106	92	89
	6	62,35	2,11	102	11,51	5,94	0,742	0,639	0,615	0,522	878	1850	110	95	90
	7	66,17	2,18	112	11,73	6,13	0,777	0,676	0,644	0,552	921	1915	146	118	103
	8	62,11	1,98	96	11,47	5,83	0,720	0,615	0,597	0,502	858	1819	102	90	86
	9	61,98	2,06	99	11,60	6,01	0,752	0,649	0,623	0,530	893	1872	126	106	94
	10	66,20	2,63	135	11,72	6,23	0,800	0,701	0,663	0,573	938	1942	147	118	103
	11	66,06	2,59	132	11,65	6,28	0,812	0,717	0,673	0,586	943	1950	137	112	101
	12	66,48	2,59	133	11,78	6,42	0,838	0,745	0,695	0,609	972	1996	136	127	108
	13	66,80	2,82	146	11,82	6,52	0,857	0,766	0,710	0,626	990	2023	160	131	111
	14	64,67	2,25	113	11,73	6,22	0,786	0,685	0,651	0,560	931	1931	166	122	102
	15	65,64	2,11	108	11,51	6,17	0,796	0,702	0,660	0,573	920	1915	111	96	93
	16	66,09	2,43	125	11,72	6,37	0,820	0,725	0,680	0,592	956	1971	152	122	104
	17	55,70	2,05	89	11,24	5,29	0,619	0,506	0,513	0,413	761	1668	71	59	70
3	2	60,01	2,05	95	11,48	5,84	0,717	0,611	0,594	0,500	859	1820	104	92	87
	4CN2	59,71	1,94	90	11,37	5,79	0,715	0,611	0,593	0,499	848	1803	83	78	82
	5	60,55	2,15	101	11,44	5,79	0,713	0,608	0,591	0,496	851	1807	96	86	85
	6-1	62,05	2,24	108	11,61	6,05	0,750	0,647	0,622	0,529	895	1877	131	109	97
	6-2	59,45	2,09	96	11,56	5,85	0,713	0,606	0,591	0,495	862	1824	119	101	90
	Min	55,70	1,94	89	11,24	5,29	0,619	0,506	0,513	0,413	761	1668	71	59	70
	Max	66,80	2,82	146	11,82	6,52	0,857	0,766	0,710	0,626	990	2023	166	131	111
	<b>Mean</b>	<b>62,53</b>	<b>2,22</b>	<b>108</b>	<b>11,54</b>	<b>6,00</b>	<b>0,753</b>	<b>0,652</b>	<b>0,624</b>	<b>0,533</b>	<b>890</b>	<b>1868</b>	<b>120</b>	<b>99</b>	<b>92</b>
	SD	3,21	0,24	16	0,16	0,29	0,057	0,062	0,047	0,051	54	84	27	19	10
	CV	5,1	10,9	15,2	1,4	4,8	7,5	9,6	7,5	9,6	6,1	4,5	22,5	19,5	11,1



1  
15,88%  
3).  
12 – 21,86% 13 – 20,99%  
-  
-  
-  
.12 45,26%  
.13 – 49,22%.  
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.12,  
.13.  
3.

In a second growth, perennial ryegrass accessions of CN1 have a mean protein content of 15,88% (Table 3). Also here Var.12 – 21,86% and Var.13 – 20,99% have the highest protein content and the lowest content of corresponding fiber components. Var.12 contains 45,26% of NDF, and Var.13 – 49,22%.  
The best forage quality - high protein and low in fiber belong to Var.12, followed by Var.13.

**Table 3. Principal composition, fiber components content and digestibility of perennial ryegrass in collection nurseries, second growth**

Variant	DM	Ash	CP	CF	NDF	ADF	ADL	HEMI	CELLU	LIGNIF	IVDMD	IVOMD
1 1	92,63	9,15	13,24	28,19	63,93	39,79	5,02	24,14	34,77	9,4	50,6	52,61
2 CN1	92,61	10,36	14,70	26,53	61,33	36,60	4,84	24,73	31,76	7,9	54,71	55,85
3	92,74	10,75	15,18	27,18	58,66	36,35	5,12	22,31	31,23	8,7	54,65	56,80
4	93,0	10,21	14,41	28,46	60,75	37,18	5,19	23,57	31,99	8,5	51,28	52,46
5	93,40	9,59	14,61	28,13	60,88	37,74	5,28	23,14	32,46	8,7	52,40	54,42
6	92,67	9,41	14,66	27,51	62,48	36,69	4,77	25,79	31,92	7,6	56,07	57,96
7	91,74	10,53	15,45	26,17	59,80	35,02	5,30	24,78	29,72	8,8	59,43	61,69
8	92,76	8,94	13,34	26,78	62,69	39,07	5,61	23,62	33,46	8,9	53,24	54,76
9	92,82	10,51	15,66	27,05	59,03	35,68	4,83	23,35	30,85	8,2	56,86	58,41
10	93,26	11,58	17,02	23,63	53,83	33,42	4,91	20,41	28,51	9,1	60,15	62,26
16	93,89	9,8	15,64	25,18	61,67	35,75	4,42	25,92	31,33	7,2	52,57	55,64
17	94,20	9,62	12,65	28,47	60,38	39,27	5,74	21,11	33,53	9,5	49,74	51,28
11 1	94,04	10,05	17,09	25,33	53,09	30,19	3,71	22,90	26,48	7,0	65,61	65,80
12 CN2	94,61	11,49	21,86	18,66	45,26	33,49	3,27	11,77	30,22	7,2	76,64	77,64
13	94,61	11,30	20,99	20,85	49,22	26,02	3,36	23,20	22,66	6,8	73,54	73,90
14	94,20	11,30	19,82	21,41	50,32	27,31	3,85	23,01	23,46	7,6	67,80	68,44
3 2	93,76	10,0	14,87	25,63	57,60	32,22	3,70	25,38	28,52	6,4	63,00	64,08
4 CN2	93,18	9,30	12,43	24,99	61,78	33,44	3,71	28,34	29,73	6,0	58,76	60,70
5	94,00	10,86	16,57	22,50	53,71	26,91	3,32	26,80	23,52	6,2	67,75	69,03
6	91,94	10,66	17,53	22,76	64,40	30,08	3,21	34,32	26,87	4,3	69,74	71,37
Min	91,74	8,94	12,43	18,66	45,26	26,02	3,21	11,77	22,66	4,3	49,74	51,28
Max	94,61	11,58	21,86	28,47	64,40	39,79	5,74	34,32	34,77	9,5	76,64	77,64
<b>Mean</b>	<b>93,30</b>	<b>10,24</b>	<b>15,88</b>	<b>25,27</b>	<b>58,04</b>	<b>34,41</b>	<b>4,50</b>	<b>24,43</b>	<b>29,65</b>	<b>7,7</b>	<b>59,73</b>	<b>61,26</b>
SD	0,83	0,83	2,59	2,80	5,34	4,17	0,85	4,59	3,48	1,33	8,04	7,63
CV	0,9	8,1	16,3	11,1	9,2	12,2	19,0	18,8	11,8	17,3	13,4	12,4

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. 4,3  
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At CN2, in the first growth, Var.6 has the lowest degree of lignification - coefficient 4,3 at a mean value of 7,7. In a second growth, the degree of lignification of the accessions of perennial ryegrass is higher than the first, with the same protein content (Table 3). The digestibility of tetraploid perennial

- : .12 – 76,64%  
 .13 – 73,54%,  
 59,73%.

101  
 %, 10,11, 12, 13  
 14 1 3 5 2  
 ( 4).  
 RFV .13 – 130 %.

. 12 .  
 13, UFL-UFV 0,854-0,782  
 . 12 0,815-0,717 .13. -

.12, PBD-PDIN-PDIE:  
 174, 137, 110 g kg<sup>-1</sup>.

ryegrass accessions is the highest:  
 Var.12 – 76,64% and Var.13 – 73,54%, at  
 an average of 59,73%. Accession  
 digestibility exceeds the mean value in  
 eight of the variants, all of which are  
 tetraploid forms of perennial ryegrass.

The mean relative feeding value  
 which is 101 rel.%, is exceeded in  
 variants 10, 11, 12, 13 and 14 of CN1  
 and Var.3 and 5 of CN2 (Table 4).  
 Maximal RFV has Var.13 – 130 rel.%.  
 The net energy feeding value is also the  
 highest in Var.12 and Var.13, UFL-UFV,  
 respectively, of 0,854-0,782 Var.12 and  
 0,815-0,717 Var.13. Maximal protein  
 feeding value of the forage has Var.12,  
 PBD-PDIN-PDIE: 174, 137, 110 g kg<sup>-1</sup>.

#### 4.

**Table 4. Energy and protein feeding value of perennial ryegrass in collection nurseries, second growth**

						UFL	UFV			VEM	VEVI	PBD	PDIN	PDIE
Variant	DDM	DMI	RFV	GE	ME	UFL	UFV	FUM	FUG	VEM	VEVI	PBD	PDIN	PDIE
1 1	57,90	1,88	84	11,41	5,37	0,614	0,498	0,509	0,406	774	1687	91	83	76
2 CN1	60,39	1,96	92	11,48	5,50	0,637	0,522	0,528	0,426	797	1724	105	92	81
3	60,58	2,05	96	11,51	5,53	0,642	0,527	0,532	0,431	803	1734	110	95	83
4	59,94	1,98	92	11,46	5,35	0,604	0,486	0,501	0,397	771	1683	102	90	79
5	59,50	1,97	91	11,47	5,47	0,628	0,512	0,521	0,419	791	1714	104	92	80
6	60,32	1,92	90	11,47	5,63	0,664	0,552	0,551	0,451	819	1758	104	92	83
7	61,62	2,01	96	11,52	5,76	0,692	0,583	0,574	0,476	843	1795	112	97	87
8	58,46	1,91	87	11,41	5,48	0,638	0,524	0,529	0,428	792	1716	92	84	78
9	61,11	2,03	96	11,53	5,63	0,660	0,547	0,547	0,447	820	1759	114	98	85
10	62,87	2,23	109	11,60	5,78	0,692	0,581	0,573	0,474	848	1803	128	107	90
16	61,05	1,95	92	11,52	5,58	0,647	0,532	0,536	0,435	809	1742	114	98	83
17	58,31	1,99	90	11,38	5,27	0,597	0,479	0,495	0,392	758	1662	85	79	74
11 1	65,38	2,26	115	11,59	6,00	0,739	0,634	0,613	0,518	886	1863	128	107	93
12 CN1	62,61	2,65	129	11,83	6,58	0,854	0,762	0,708	0,623	892	2028	174	137	110
13	68,63	2,44	130	11,78	6,40	0,815	0,717	0,675	0,586	960	1977	166	132	106
14	67,63	2,38	125	11,73	6,14	0,759	0,654	0,629	0,535	912	1902	155	124	100
3 2	63,80	2,08	103	11,49	5,87	0,721	0,616	0,598	0,503	962	1825	107	93	87
4 CN2	62,85	1,94	95	11,37	5,68	0,694	0,587	0,575	0,480	930	1775	86	78	80
5	67,94	2,23	118	11,57	6,08	0,766	0,666	0,635	0,544	903	1888	123	104	94
6	65,47	1,61	82	11,62	6,20	0,792	0,694	0,656	0,567	927	1926	132	110	98
Min	57,90	1,61	82	11,37	5,27	0,597	0,479	0,495	0,392	758	1662	85	79	74
Max	68,63	2,65	130	11,83	6,58	0,854	0,762	0,708	0,623	962	2028	174	137	110
Mean	<b>62,32</b>	<b>2,07</b>	<b>101</b>	<b>11,54</b>	<b>5,74</b>	<b>0,695</b>	<b>0,580</b>	<b>0,577</b>	<b>0,473</b>	<b>845</b>	<b>1798</b>	<b>116</b>	<b>100</b>	<b>87</b>
SD	3,24	0,23	15	0,13	0,47	0,070	0,081	0,061	0,067	66	102	25	16	10
CV	<b>5,2</b>	<b>11,2</b>	<b>14,9</b>	<b>1,1</b>	<b>8,2</b>	<b>10,1</b>	<b>14,0</b>	<b>10,5</b>	<b>14,2</b>	<b>7,8</b>	<b>5,7</b>	<b>21,6</b>	<b>16,3</b>	<b>11,5</b>

5  
 16,72%,  
 . 10 – 20,47%.  
 -  
 18,42%,  
 21,82%.  
 - 46,49%  
 54,71%.  
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 . 3 – . 3,7  
 7,4. -  
 .10 –  
 71,13%.  
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 5.

Table 5 shows the composition and digestibility of the perennial ryegrass accessions in a third growth. The mean protein content is 16,72% and the maximal value is in Var.10 – 20,47%. It also has the lowest fiber content of 18,42%, at mean value 21,82%. The content of the NDF is corresponding – 46,49% at an average of 54,71%.

The contents of ADF, ADL and degree of lignification are also corresponding. The degree of lignification is lowest in Var.3 – coeff. 3,7 at an mean value of coeff. 7,4.

The highest digestibility is in perennial ryegrass Var.10 – 71,13%. The changes in digestibility of perennial ryegrass from high to low are as follows: from first to third and second growths.

**Table 5. Principal composition, fiber components content and digestibility of perennial ryegrass in collection nurseries, third growth**

Variant	DM	Ash	CP	CF	NDF	ADF	ADL	HEMI	CELLU	LIGNIF	IVDMD	IVOMD
1 1	94,23	8,90	13,14	24,37	61,02	33,21	4,60	27,86	28,61	7,5	58,05	58,81
2 CN1	94,65	11,19	15,16	22,81	56,40	29,57	4,20	26,83	25,57	7,4	63,09	64,80
3	94,18	11,19	16,64	23,16	53,64	30,15	2,00	23,49	28,15	3,7	70,43	71,26
4	94,40	11,94	17,65	21,54	53,71	29,55	4,13	24,16	25,42	7,7	63,43	64,57
5	94,42	10,65	15,99	23,69	57,44	31,60	4,43	25,84	27,17	7,7	59,66	59,73
6	94,61	11,49	18,76	18,99	53,53	27,34	4,11	26,19	23,23	7,6	66,93	67,65
7	94,29	12,01	16,27	20,62	56,28	30,14	4,12	26,14	26,02	7,3	65,91	67,80
8	94,32	11,37	14,87	22,39	56,78	32,02	4,62	24,76	27,40	8,1	60,40	62,15
9	93,92	11,54	18,84	22,64	51,87	29,24	4,14	22,63	25,10	8,0	67,80	69,56
10	93,83	13,71	20,47	18,42	46,49	26,56	3,95	19,93	22,61	8,5	71,13	73,76
16	93,63	11,76	16,96	21,20	54,48	29,50	3,77	24,98	25,73	6,9	67,07	69,07
17	94,26	11,04	15,85	22,10	54,81	34,72	4,93	20,09	29,79	9,0	62,40	63,68
Min	93,63	8,90	13,14	18,42	46,49	26,56	2,00	19,93	22,61	3,7	58,05	58,81
Max	94,65	13,71	20,47	24,37	61,07	34,72	4,93	27,86	29,69	9,0	71,13	73,76
<b>Mean</b>	<b>94,23</b>	<b>11,40</b>	<b>16,72</b>	<b>21,82</b>	<b>54,71</b>	<b>30,30</b>	<b>4,08</b>	<b>24,41</b>	<b>26,23</b>	<b>7,4</b>	<b>64,69</b>	<b>66,07</b>
<i>SD</i>	0,30	1,09	2,00	1,79	3,52	2,30	0,73	2,51	2,11	1,31	4,19	4,58
<b>CV</b>	<b>0,3</b>	<b>9,6</b>	<b>11,9</b>	<b>8,2</b>	<b>6,4</b>	<b>7,6</b>	<b>17,9</b>	<b>10,3</b>	<b>8,0</b>	<b>17,6</b>	<b>6,5</b>	<b>6,9</b>

( 6).  
 -  
 111 .%,  
 . 10 – 136 .%

The mean relative feeding value of the accessions of perennial ryegrass from the third growth is 111 rel.% and the highest is for Var.10 - 136 rel.% (Table 6). This variant also demonstrates the highest net energy feeding value and

– protein feeding value - total digestible protein 162 g kg<sup>-1</sup> dry matter at a mean value of 125 g kg<sup>-1</sup>.

6.

**Table 6. Energy and protein feeding value of perennial ryegrass in collection nurseries, third growth**

		UFL		UFV		VEM		VEVI		PBD		PDIN		PDIE	
Variant	DDM	DMI	RFV	GE	ME	UFL	UFV	FUM	FUG	VEM	VEVI	PBD	PDIN	PDIE	
1	63,03	1,96	96	11,40	5,64	0,680	0,571	0,564	0,466	823	1763	90	82	80	
2	65,86	2,13	109	11,51	5,84	0,720	0,614	0,597	0,502	861	1823	110	95	88	
3	65,41	2,24	113	11,58	6,16	0,784	0,686	0,650	0,560	917	1911	124	104	96	
4	65,88	2,23	114	11,63	5,87	0,713	0,604	0,591	0,494	867	1832	134	111	93	
5	64,28	2,09	104	11,54	5,69	0,675	0,563	0,560	0,460	831	1777	118	100	87	
6	67,80	2,24	118	11,68	6,06	0,751	0,647	0,623	0,529	900	1884	144	118	97	
7	65,42	2,13	108	11,57	5,96	0,744	0,641	0,617	0,523	884	1858	121	102	93	
8	63,96	2,11	105	11,50	5,72	0,693	0,584	0,574	0,477	839	1788	107	93	86	
9	66,12	2,31	118	11,69	6,14	0,765	0,663	0,634	0,541	913	1904	145	118	99	
10	66,21	2,58	136	11,79	6,24	0,789	0,688	0,654	0,562	937	1940	162	128	105	
16	65,92	2,20	112	11,60	6,05	0,759	0,657	0,629	0,537	898	1881	127	106	95	
17	61,85	2,19	105	11,54	5,83	0,712	0,604	0,590	0,494	858	1818	116	100	89	
Min	61,85	1,96	96	11,40	5,64	0,675	0,563	0,560	0,460	823	1763	90	82	80	
Max	67,80	2,58	136	11,79	6,24	0,789	0,688	0,654	0,562	937	1940	162	128	105	
<b>Mean</b>	<b>65,13</b>	<b>2,20</b>	<b>112</b>	<b>11,58</b>	<b>5,93</b>	<b>0,734</b>	<b>0,627</b>	<b>0,603</b>	<b>0,512</b>	<b>877</b>	<b>1848</b>	<b>125</b>	<b>105</b>	<b>92</b>	
SD	1,58	0,15	10	0,10	0,20	0,038	0,043	0,033	0,035	36	57	19	15	7	
<b>CV</b>	<b>2,4</b>	<b>6,8</b>	<b>8,9</b>	<b>0,9</b>	<b>3,3</b>	<b>5,2</b>	<b>6,8</b>	<b>5,5</b>	<b>6,8</b>	<b>4,2</b>	<b>3,1</b>	<b>15,5</b>	<b>14,0</b>	<b>7,3</b>	

**CONCLUSIONS**

⇒ The forage quality evaluation accompanies the evaluation by productive potential in the breeding programs with perennial ryegrass in IFC – Pleven since the initial material in the collections.

⇒ There are significant variations in the parameters determining the forage quality and feeding value of the collection accessions.

⇒ The first breeding cycle has been performed and the accessions exceeded the mean value for the collection, Top 5, as follows:

⇒ By Crude protein content: 13 (20,88%), 12 (20,20%), 14 (19,51%), 16 (19,41%), 7(18,76%), 15,75%;

⇒ By degree of lignification: 13, 12, 14, 11, 7, (4,3 4,9) 6,2;

⇒	:	13,12,14,16,10 71,07% 65,92%;	-	13,12,14,16,10 with the highest values of 71,07% to 78,0%, at an average of 65,92%;
⇒	,		-	⇒ By energy and protein feeding value, the results correspond to the principal biomass composition of the selected accessions.
⇒	-		-	⇒ Accessions with the highest complex forage quality evaluation are tetraploid varieties, which proves induced polyploidy as a suitable breeding method for perennial ryegrass.

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## Plastid pigments and water soluble carbohydrates content in perennial ryegrass, grown in pure stand and in mixtures with alfalfa

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### SUMMARY

The aim is to study the content of plastid pigments and water-soluble carbohydrates of the first Bulgarian perennial ryegrass variety *Harmoniya* and the first tetraploid candidate variety *NBG*, grown alone and in mixed crops with alfalfa – the most common varieties of IFC - Pleven 6 and *Dara*. During the period 2012-2014 in the IFC-Pleven the field experiment on leached black soil in non-irrigated conditions, by block method in 3 repetitions was carried on. Experimental variants are 8: 1 to 4 – alone single crops, and 5 to 8 – mixed - binary. The content of water-soluble carbohydrates is determined by the method of Ermakov et al. (1987) and plastid pigments (chlorophyll a, chlorophyll b and carotenoids) by the method of Zelenskii and Mogileva (1980). Data on plastid pigments and water-soluble carbohydrates (average, minimum, maximum, standard deviations) are presented by regrowths and total





(Jolaosho et al., 2009; Luscher et al., 2014).

(Nurmakova, 2013; Smirnova et al., 2013).

b, “ ”

(Gilmore and Govindjee, 1999).

(Vasileva, 2011).

( ) (Vasileva, 2015).

ha<sup>-1</sup>, 10

8-9 t

(Jolaosho et al., 2009; Luscher et al., 2014).

One of the most important factors determining crop productivity is the process of photosynthesis by which green plants accumulate organic matter and energy. Photosynthesis is an indicator of the reaction of plants to the changes in the external environment factors and the degree of adaptation to the new conditions (Nurmakova, 2013; Smirnova et al., 2013). The absorption and transformation of solar energy is achieved through photosynthetic pigments – chlorophyll a and b, and carotenoids.

The base chlorophyll is "a" that provides greater efficiency in the process of converting carbon dioxide and water into organic compounds. Carotenoids, in addition to light-collecting, also have a protective function against photo-oxidation of chlorophylls and prevent the destructive photocoervation of organic protoplasmic compounds in the presence of free oxygen (Gilmore and Govindjee, 1999).

The Productivity and durability of grass mixtures depend to a large extent on the compatibility of species (Vasileva, 2011). Therefore, it is important to select the components of blends and their research on different indicators. Interestingly, alfalfa mixtures are a major grass-fodder crop with a perennial ryegrass, with higher productivity and nutritional value than other grasses.

As a result of the better physiological status of the plants, expressed by the content of plastid pigments, is also the higher proportion of leaves/stems of perennial ryegrass mixed with different legumes (birdfoot's trefoil, sainfoin, subterranean clover) (Vasileva, 2015).

Under suitable conditions, in Latvia, the yield of dry matter from perennial ryegrass and alfalfa is 8-9 t ha<sup>-1</sup>, and for a long period of up to 10 years – an average of

6,5 t ha<sup>-1</sup>, .  
 (Kadziulienė et al., 2011).

6.5 t ha<sup>-1</sup>, is a high-yield and persistent mixture (Kadziulienė et al., 2011). There is no data in Bulgaria about mixed cultivation of perennial ryegrass and alfalfa, and there are registered new varieties of both crops, which motivates us to conduct the survey.

The aim is to study the content of plastid pigments and water-soluble carbohydrates of the first Bulgarian perennial ryegrass variety Harmoniya and the first tetraploid candidate variety NBG, grown alone and in mixed crops with alfalfa – the most common varieties of IFC - Pleven 6 and Dara.

## MATERIAL AND METHODS

2012 . -  
 kg da<sup>-1</sup>, 20 kg da<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> N – 5  
 :  
 3  
 – 5m<sup>2</sup>.  
 8: 1 4 –  
 , 5 8 –  
 ; 2.  
 ; 5.  
 6 (50:50); 6.  
 +  
 (50:50); 7. NBG +  
 6 (50:50); 8.  
 NBG + (50:50).  
 26.03.2012,  
 1 cm  
 : – 3 kg da<sup>-1</sup>  
 1,5 kg da<sup>-1</sup>  
 ;  
 kg da<sup>-1</sup> - 3  
 da<sup>-1</sup> 1,5 kg  
 4 kg da<sup>-1</sup>  
 2 kg da<sup>-1</sup>

The experiment is established in the spring of 2012 in IFC - Pleven on black soil, without irrigation. Before sowing is imported 200 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and N - 50 kg ha<sup>-1</sup> and in coming years the same amount of ammonium nitrate in early spring as soon as possible. Scheme: Block method in three replications, size of the experimental plots - 5m<sup>2</sup>. Variants of the experiment are 8: 1 to 4 - single species, individual crops, and from 5 to 8 - mixed – binary, as follows: 1. Perennial ryegrass variety IFC - Harmoniya - diploid; 2. tetraploid perennial ryegrass breeding population - NBG; 3. Alfalfa variety Plevin 6; 4. Alfalfa variety Dara; 5. Harmoniya ryegrass + alfalfa Plevin 6 (50:50); 6. ryegrass Harmoniya + alfalfa Dara (50:50); 7. ryegrass NBG + alfalfa Plevin 6 (50:50); 8. ryegrass NBG + alfalfa Dara (50:50). Sowing was carried out on 26.03.2012, by hand to a depth of 1 cm sowing the following rates: lucerne – 30 kg ha<sup>-1</sup> in separate sowing and 15 kg ha<sup>-1</sup> for mixed farming; ryegrass - 30 kg ha<sup>-1</sup> in separate sowing and 15 kg ha<sup>-1</sup> for mixed cultivation of diploid variety and 40 kg ha<sup>-1</sup> in separate sowing and 20 kg ha<sup>-1</sup> for mixed cultivation of tetraploid breeding population. The experiment is harvested for forage at the beginning of ear formation of the grass component in the first growth and early bloom alfalfa in next growths.

Ermakov et al. (1987),  
 Zelenskii Mogileva (1980).  
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 ( )  
 1).  
 -  
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 -  
 343.44 mg/100 g fr. wt,  
 215.34 mg/100 g fr. wt.,  
 6 – 286.56 mg/100g fr. wt,  
 – 342.87 mg/100 g fr. wt.  
 -  
 -  
 -  
 NBG  
 (243,40 mg/100 g fr. wt)  
 (205,20 mg/100 g fr. wt).  
 -  
 -  
 -  
 (211,88 mg/100g fr wt.)  
 6  
 (337.50 mg/100g fr wt.).

The content of water-soluble carbohydrates (WSC) is determined by the method of Ermakov et al. (1987) and plastid pigments (chlorophyll a, chlorophyll b and carotenoids) by the method of Zelenskii and Mogileva (1980). Data on plastid pigments and water-soluble carbohydrates (average, minimum, maximum, standard deviations) are presented by regrowths and total average for the period.

## RESULTS AND DISCUSSION

The obtained data show that the total content of plastid pigments in ryegrass and lucerne varieties in individual crops and their mixtures varies according to the regrowths (Table 1). In the case of pure stand cultivation of the two crops the larger differences in the values of this indicator depending on the variety were found in the first growth. In perennial ryegrass variety *Harmoniya* the total content of plastid pigments is 343.44 mg/100 g fr. wt, and for the candidate variety *NBG* is 215.34 mg/100 g fr. wt., for alfalfa variety *Pleven 6* – 286.56 mg / 100g fr. wt, and in *Dara* variety – 342.87 mg/100 g fr. wt. In the following regrowths, the values of the total content of plastid pigments in perennial ryegrass are closely related. Only in the fifth regrowth the total content of plastid pigments in the *NBG* variety (243.40 mg/100 g fr. Wt) exceeds this content in the *Harmoniya* variety (205.20 mg/100 g fr. wt).

For alfalfa varieties, there are also no significant differences in this indicator in the second, third and fifth growth. In the fourth growth, a significantly lower content of *Dara* variety plastid pigments was found (211.88 mg/100g fr wt) compared to *Pleven 6* (337.50 mg 100g fr wt).

In the mixtures of perennial ryegrass with alfalfa, the obtained data show a variation of the total content of plastid pigments as

( 1).  
1.

compared to the pure stands of these crops in different regrowths (Table 1).

**Table 1. Total plastid pigments content in perennial ryegrass and Lucerne in pure stands and in mixtures**

Variant	Plastid pigments, mg/100 g fr. wt.					
	1 growth	2 growth	3 growth	4 growth	5 growth	Average
1 L.p. Harmoniya	343,44	325,77	386,22	306,51	205,2	313,42
2 L.p. NBG	215,34	314,88	392,72	294,54	243,4	292,18
3 M.s. Pleven 6	286,56	314,85	370,64	337,5	268,36	315,59
4 M.s. Dara	343,87	314,28	390,6	211,88	284,12	308,75
5 Harmoniya+Pleven 6	293,19	312,78	<b>420,64</b>	322,2	273,2	<b>324,4</b>
6 Harmoniya + Dara	304,8	356,2	369,72	280,89	314,96	315,41
7 NBG + Pleven 6	263,04	335,34	310,1	225,6	308,4	304,63
8 NBG + Dara	257,97	313,41	<b>517,48</b>	287,58	315,08	<b>347,07</b>
average	288,53	323,44	394,77	283,34	276,59	315,18
min	215,34	312,78	310,10	211,88	205,20	292,18
max	343,87	356,20	517,48	337,50	315,08	347,07
SD	40,79	14,43	55,00	41,19	35,88	14,95
P 0,05	28,26	10,00	38,11	28,54	24,86	10,36

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3  
+ 6 (420,64 mg/100g fr.  
wt) NBG + (517.48 mg/100g fr wt.)  
-  
-  
-  
-  
Vasileva  
(2015), Ilieva and Vasileva (2016)  
Vasileva et al. (2017).

This variation is probably related to complex relationships in mixed crops, including different plant height, different light absorption, competitive relations.

The highest total content of plastid pigments, both in pure stands of both crops and in their mixtures, was found in third regrowth.

Mixtures Harmoniya + Pleven 6 (420.64 mg/100g fr.wt) and NBG + Dara (517.48 mg/100g fr wt.) are highest in comparison to the components of these mixtures in pure stands. In order to increase the content of perennial ryegrass in the plastid pigments in legumes, birdfoot's trefoil, sainfoin, subterranean clover in comparison with the pure stands reported (2015), Ilieva and Vasileva (2016) and Vasileva et al. (2017). In the fourth and fifth regrowths the content of plastid pigments in all variants is reduced.

b,

+b  
247,87  
mg/100g fr. wt. 299,71 mg/100g fr. wt.,  
44, 07 mg/100g  
fr. wt. 47,36 mg/100g fr. wt.,  
292.18 mg/100g fr. wt.  
347.07 mg/100g fr. wt. ( 2).

The mean data for five regrowths show that the content of chlorophyll a and b, carotenoids and, respectively, the total content of plastid pigments are close to both the pure stands of perennial ryegrass and alfalfa varieties and their mixtures.

The total content of chlorophylls a + b ranges between 247.87 mg / 100g fr. wt. and 299.71 mg/100g fr. wt., of carotenoids between 44, 07 mg/100g fr. wt. and 47.36 mg/100g fr. wt. and the total content of plastid pigments between 292.18 mg/100g fr. wt. and 347.07 mg / 100g fr. wt. (Table 2).

2.

**Table 2. Plastid pigments in perennial ryegrass and Lucerne in pure stands and in mixtures (average for the period by regrowths)**

Variant	/ Plastid pigments, mg/100 g fr. wt.				
	chl. a	chl. b	chl. a+b	carotenoids	total
1 L.p. Harmoniya	152,02	114,68	266,70	46,72	313,42
2 L.p. NBG	140,60	107,27	247,87	44,31	292,18
3 M.s. Pleven 6	157,64	111,38	269,02	46,57	315,59
4 M.s. Dara	155,81	108,87	264,68	44,07	308,75
5 Harmoniya + Pleven 6	164,01	113,68	277,69	46,71	324,40
6 Harmoniya + Dara	163,21	104,86	268,07	47,34	315,41
7 NBG + Pleven 6	155,54	104,86	260,40	44,23	304,63
8 NBG + Dara	175,99	123,72	299,71	47,36	347,07
average	158,10	111,17	269,27	45,91	315,18
min	140,60	104,86	247,87	44,07	292,18
max	175,99	123,72	299,71	47,36	347,07
SD	10,26	6,28	14,94	1,45	15,98
P 0,05	7,11	4,35	10,36	1,00	11,07

NBG +  
– 347.07 mg/100g fr wt.

The NBG + Dara mixture has the highest total content of plaster pigments – 347.07 mg/100g fr wt. The data obtained show that, when perennial ryegrass and alfalfa are mixed, they do not adversely affect each other as components of the mixtures, they do not cause shading and do intensive photosynthesis.

	-			
	(	3).		
		6,56%		
	7,26%		-	
NBG.			6	
1,96%,		- 1,68%.		
	.			
	,			
Lawson and Kelly (2014)				
(2005).	,			
	.			
	-	NBG		
	-	9,50%	8,60%	
	.			
	,			
			6	
			6	
	-	- 3,50%	0,90%	
		2,40%	0, 20%	
	.			
	.			
	+	6	1,80%,	
	+	- 1,86%,	NBG +	
6	1,84%	NBG +	- 1,64%.	

The content of water-soluble carbohydrates in perennial ryegrass is significantly higher than alfalfa (Table 3).

On average, for five regrowths, it is 6.56% for the Harmoniya variety and 7.26% for the NBG variety candidate. In the alfalfa variety Plevan 6 is 1.96% and in the variety Dara - 1.68%. The content of water-soluble carbohydrates varies with regrowths. In perennial ryegrass varieties, it is the highest in the second and third growth, decreases in the fourth and increases in autumn growth. Lawson and Kelly (2014) and Katova (2005) reported such a trend. In the first, third and fourth regrowths the values of the water-soluble carbohydrates in the perennial ryegrass varieties studied are very close. In the second and fifth growth NBG perennial ryegrass candidate has the highest content - 9.50% and 8.60% respectively. A similar tendency to reduce the water-soluble carbohydrates in the summer months and increase in spring growths is observed in alfalfa.

In the first, third and fifth growth alfalfa varieties Plevan 6 and Dara show close values of the content of water-soluble carbohydrates. In the second and fourth growth Plevan 6 variety has a higher content of 3.50% and 0.90%, respectively, against 2.40% and 0.20% respectively in the Dara variety.

In alfalfa ryegrass mixtures, the water soluble sugar content is close to that of alfalfa cultivars grown alone. Average from five growths WSC content in the mixture Harmony + Plevan 6 is equal to 1.80%, Harmony + Dara - 1.86%, NBG + Plevan 6 1.84% and NBG + Dara - 1.64%.

## 3.

**Table 3. Water soluble carbohydrates content in in perennial ryegrass and Lucerne in pure stands and in mixtures (average for the period by regrowths)**

Variant	Water Soluble Carbohydrates, %					Average
	1	2	3	4	5	
	growth	growth	growth	growth	growth	
1 L.p. Harmoniya	6,4	<b>7,8</b>	<b>7,7</b>	3,8	<b>7,1</b>	<b>6,56</b>
2 L.p. NBG	6,4	<b>9,5</b>	<b>7,7</b>	4,1	<b>8,6</b>	<b>7,26</b>
3 M.s. Pleven 6	2,4	3,5	0,6	0,9	2,4	1,96
4 M.s. Dara	3	2,4	0,6	0,2	2,2	1,68
5 Harmoniya + Pleven 6	2,4	3,2	0,6	0,6	2,2	1,8
6 Harmoniya + Dara	2,7	3,2	0,5	0,4	2,5	1,86
7 NBG + Pleven 6	3	2,7	0,9	0,6	2	1,84
8 NBG + Dara	2,4	2,6	0,6	0,9	1,7	1,64
average	3,59	4,36	2,40	1,44	3,59	3,08
min	2,40	2,40	0,50	0,20	1,70	1,64
max	6,40	9,50	7,70	4,10	8,60	7,26
SD	1,64	2,53	3,06	1,47	2,50	2,22
P 0,05	1,14	1,76	2,12	1,02	1,73	1,54

**CONCLUSIONS**

- The average data for the study period of five regrowths show differences in the content of plastid pigments (chlorophyll a and b, carotenoids and, respectively, the total content) both between the studied perennial ryegrass and alfalfa in pure stands and between their Mixtures.
- The NBG + Dara mixture has the highest total content of plastid pigments - 347.07 mg / 100g fr wt, followed by Harmonia + Pleven 6 - 324.40 mg / 100g fr wt.
- The content of water-soluble carbohydrates in perennial ryegrass is significantly (three times) higher than alfalfa. On average, for five regrowths, it is 6.56% for the Harmoniya variety and 7.26% for the candidate variety NBG, which is tetraploid, in the alfalfa variety Pleven 6 is 1.96% and in the Dara variety— 1.68%.
- In mixtures of perennial ryegrass with alfalfa, the water soluble carbohydrates content is similar to that of alfalfa varieties grown in pure stands.

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## Under sowing of degraded seed production stands with subterranean clover

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### SUMMARY

The possibility for under sowing of degraded white clover seed production stands with subterranean clover and thereafter used for forage was studied in a field trial in the Institute of Forage Crops, Pleven, Bulgaria.

Three subterranean clover subspecies, i.e. *Trifolium subterraneum* ssp. *brachycalycinum* (cv. "Antas"), *Trifolium subterraneum* ssp. *yaninicum* (cv. "Trikkala") and *Trifolium subterraneum* ssp. *subterraneum* (cv. "Denmark") were used. The under sowing was performed during the autumn of the fourth year of using of white clover stands with 400 germinated seeds/m<sup>2</sup>.

The stands were used for forage for three years after under sowing and every year two cuts were harvested. It was found that subterranean clover did not negatively effect the white clover development, increased dry mass productivity and reduced the weed infestation.

*Trifolium subterraneum* ssp. *brachycalycinum* showed the best development. Dry mass yield from under

*Trifolium subterraneum* ssp. *brachycalicinum*

11.70%<sup>e</sup> -

sowed degraded white clover seed production stands with *Trifolium subterraneum* ssp. *brachycalicinum* on average for the period was found 11.70% higher. Under sowing of degraded seed production white clover stands with subterranean clover is possible agrotechnical measurement and the stands thereafter could be used for forage.

**Key words:** under sowing, degraded seed production stands, subterranean clover, white clover

## INTRODUCTION

Legumes are becoming increasingly important in the system of sustainable agriculture (Nacheva and Georgieva, 2007; De Vliegher et al., 2011; Luscher et al., 2014). However, their productivity with advancing age of the crop (for perennial legumes) decreased due to loss of plants, the swards diluted and free spaces are occupied by weeds (Dimitrova, 1986; Vasilev, 2004, 2008; Sulas et al., 2006; Petkova et al., 2015).

Direct under sowing is one of measures which allow the improvement of botanical composition of degraded stands. It is using for the recovering and/or prolonging the persistence of the degraded pasture stands. Under sowing is applying in degraded stands, where the legume components are dropped due to the short duration, less adaptability to over use, adverse soil and climatic conditions or other factors. Through under sowing expect that it prolongs the durability of the stands and enhance the productivity and quality of the forage (Springer, 1997).

For areas with a temperate climate most used crops for under sowing are species of the genera clover (*Trifolium* spp.), trefoil (*Lotus* spp.) and alfalfa (*Medicago* spp.) (Entz et al., 2007; Escaray et al., 2012). Very suitable for this purpose are self-seeding crops due to

(Nacheva and Georgieva, 2007; De Vliegher et al., 2011; Luscher et al., 2014).

(Dimitrova, 1986; Vasilev, 2004, 2008; Sulas et al., 2006; Petkova et al., 2015).

(Springer, 1997).

(*Trifolium* spp.), (*Lotus* spp.) and (*Medicago* spp.) (Entz et al., 2007; Escaray et al., 2012).

their possibility of prolonged presence in the sward. In one season they could provide productivity of forage as well seeds for propagation (Carneiro, 1999; Naydenova et al., 2013; Bartholomew, 2014).

Subterranean clover (*Trifolium subterraneum* L.) is species with self-seeding ability (Yakimova and Yancheva, 1986; Piano et al., 1996). It has a low widespread habitat and occupies open spaces between other plants from the lowest floor of the sward, as well coexists well with perennial grasses and legumes. It grows up early in the spring and forms a dense sward (Porqueddu et al., 2003). Reproductive organs are formed in early May and the seeds ripen before the end of the spring in hedgehog-shaped heads that remain on the soil surface (Frame et al., 1998). Substantial part of the formed seeds is hard and germinates after two-three years. This biological specificity turns the superficial soil layer into an original seed bank (Pecetti and Piano, 1994). The precipitations during the late summer contribute to emergence of new self-sown plants (Vasilev, 2006, 2009). For areas with a Mediterranean climate the selection of subterranean clover for hardiness of the seeds is leading, which allows its gradual emergence and thus self-supporting in the stands for several years, although it is an annual type (Loi et al., 2005).

Subterranean clover is a widespread component in the pastures and other grasslands of the temperate areas of Central and Northern Europe and America (Nichols et al., 2012; Lucas et al., 2015). For Bulgaria it is relatively new crop and is found in open dry grasslands in the plains and lowlands (Assyov et al., 2012). The studies with subterranean clover in recent years showed that it has practical applicability for the climatic conditions of Bulgaria (Ilieva and Vasileva, 2011; Vasileva et al., 2011;

(Carneiro, 1999; Naydenova et al., 2013; Bartholomew, 2014).  
 (*Trifolium subterraneum* L.)  
 (Yakimova and Yancheva, 1986; Piano et al., 1996).  
 (Porqueddu et al., 2003).  
 (Frame et al., 1998).  
 (Pecetti and Piano, 1994).  
 (Vasilev, 2006, 2009).  
 (Loi et al., 2005).  
 (Nichols et al., 2012; Lucas et al., 2015).  
 (Assyov et al., 2012).  
 (Ilieva and Vasileva, 2011;

Vasileva et al., 2011; Ilieva et al., 2015; Naydenova and Vasileva, 2015, 2016; Kirilov and Vasileva, 2016; Vasileva et al., 2016).

(Vasileva, 2015; Vasileva et al., 2015).

et al., 2005).

(2010)

– *Trifolium subterraneum* ssp. *brachycalycinum* ( “Antas”), *Trifolium subterraneum* ssp. *yananicum* ( “Trikkala”) *Trifolium subterraneum* ssp. *subterraneum* ( “Denmark”).

400 /m<sup>2</sup>  
12 cm.  
: ( )  
- ; + *Trifolium subterraneum* ssp. *brachycalycinum*;  
+ *Trifolium subterraneum* ssp. *yananicum*;  
+ *Trifolium subterraneum* ssp. *subterraneum*.

Ilieva et al., 2015; Naydenova and Vasileva, 2015, 2016; Kirilov and Vasileva, 2016; Vasileva et al., 2016). Some of studies involved the use of the species for direct under sowing of degraded seed production stands. They were performed in alfalfa (Vasileva, 2015; Vasileva et al., 2015). The results showed that this measure improves botanical composition, weed infestation decreased two- and threefold and increased dry mass yield.

In this study we aim to investigate the possibility for under sowing of degraded white clover seed production stands with subterranean clover and thereafter use for forage.

## MATERIAL AND METHODS

The experimental work was carried out on the experimental field of Institute of Forage Crops, Pleven, Bulgaria on slight leached chernozem soil subtype without irrigation. Seed production white clover stands was sown in 2007 and cared according with accepted technology (Chakarov et al., 2005).

Long plots method was used, plot size of 5 m<sup>2</sup> and 4 replications of the treatments. During the fourth year (2010) from the use of the swards, across the rows, under sowing with three subterranean clover subspecies was performed, i.e. *Trifolium subterraneum* ssp. *brachycalycinum* (cv. Antas), *Trifolium subterraneum* ssp. *yananicum* (cv. Trikkala) and *Trifolium subterraneum* ssp. *subterraneum* (cv. Denmark). Under sowing was done with 400 germinated seeds/m<sup>2</sup> and between rows spacing of 12 cm. Treatments were as follows: white clover (without under sowing) – control; white clover + *Trifolium subterraneum* ssp. *brachycalycinum*; white clover + *Trifolium subterraneum* ssp. *yananicum*; white clover + *Trifolium subterraneum* ssp. *subterraneum*.

9.05. 15.06. ( ; 08.05.  
19.06. , 26.04. 19.06.  
)  
0.25 m<sup>2</sup>  
(%)  
( )  
SPSS 2012.

- The swards was used in the  
- direction for forage three years after under  
- sowing and each year two cuts were  
harvested (on 9.05. and 15.06. in the first;  
on 08.05. and 19.06. in the second, and  
on 26.04. and 19.06. in the third year).  
Immediately before each cutting samples  
from 0.25 m<sup>2</sup> was taken and botanical  
analysis performed. Dry mass yield was  
recorded (calculated from fresh mass  
yield and dry matter content in percent)  
(drying to the constant weight).  
Experimental data were processed  
statistically using a software product  
SPSS 2012,

## RESULTS AND DISCUSSION

Agrometeorological conditions  
during the period of study could be  
determined as unfavourable (Table 1). In  
the year after under sowing long dry  
period with extremely high temperatures  
occurs. Unevenly distributed rainfall and  
long drought (duration of 82 days) in the  
summer characterized the second  
experimental year.

### 1.

**Table 1. Agro meteorological conditions for the period of study**

/ Months/years	/First year		/Second		/Third year	
	after under sowing		year after under sowing		after under sowing	
	temperature °C	rainfall l/m <sup>2</sup>	temperature °C	rainfall l/m <sup>2</sup>	temperature °C	rainfall l/m <sup>2</sup>
I	-1.0	32.8	-0.1	44.2	0.5	19.0
II	0.2	27.2	-5.2	17.8	3.9	60.9
III	6.1	25.7	8.5	7.6	6.3	39.6
IV	11.4	28.2	14.8	46.3	14.0	50.8
V	16.8	79.8	17.4	85.2	19.5	63.7
VI	21.4	33.6	24.1	40.3	21.3	112.4
VII	23.5	50.2	27.8	1.4	22.7	105.8
VIII	23.6	41.3	25.8	35.6	24.9	20.2
IX	22.0	0.0	21.1	21.0	18.4	15.8
X	11.1	50.2	15.0	56.0	12.2	59.2
XI	7.6	0.4	8.1	4.0	8.7	29.1
XII	3.9	28.6	-1.0	56.7	0.2	2.3
Average, sum	12.2	398.0	13.0	416.1	12.7	578.8

( 2).  
90%. -  
73.79% ,  
*Trifolium subterraneum* ssp. *brachycalycinum*  
78.03% , *Trifolium*  
*subterraneum* ssp. *subterraneum*.  
-  
- *Trifolium*  
*subterraneum* ssp. *brachycalycinum*  
(16.89%) vs. (9.32%)  
*Trifolium subterraneum* ssp. *yananicum*  
(14.05%) vs. (9.72%).

Botanical composition  
Botanical composition of the  
swards in the first year after under sowing  
showed high weed infestation in the first  
cut (Table 2). In the control variant the  
weed infestation reached 90%. In the  
variants with under sowing the weed  
infestation varied from 73.79% in the  
swards under sowed with *Trifolium*  
*subterraneum* ssp. *brachycalycinum* to  
78.03% in the swards under sowed with  
*Trifolium subterraneum* ssp. *subterraneum*.  
In these swards the highest part of  
subterranean clovers was observed -  
*Trifolium subterraneum* ssp. *brachycalycinum*  
(16.89%) vs. white clover (9.32%) and  
*Trifolium subterraneum* ssp. *yananicum*  
(14.05%) vs. white clover (9.72%).

## 2.

Table 2. Botanical composition of under sowed degraded white clover seed production stands with subterranean clover, %

Treatments	White clover		Tr. subterraneum		Weeds	
	First cut	Second cut	First cut	Second cut	First cut	Second cut
/First year after under sowing						
/Control	8.79	16.23	-	-	91.21	83.77
+ brachycalycinum	9.32	15.90	16.89	6.57	73.79	77.53
+ yananicum	9.72	13.71	14.05	5.47	76.23	80.82
+ subterraneum	13.95	16.07	8.02	3.33	78.03	80.60
SE (P=0.05)	1.18	0.59	2.61	0.95	3.89	1.27
/Second year after under sowing						
/Control	35.82	50.00	-	-	64.18	50.00
+ brachycalycinum	37.91	38.46	30.46	15.38	31.63	46.15
+ yananicum	31.59	43.53	27.25	10.59	41.16	45.88
+ subterraneum	21.39	44.44	18.20	11.11	60.41	44.44
SE (P=0.05)	3.67	2.36	3.67	1.51	7.76	1.18
/Third year after under sowing						
/Control	24.84	30.00	-	-	75.16	70.00
+ brachycalycinum	27.60	28.30	15.34	8.38	57.06	63.32
+ yananicum	23.13	23.34	13.76	7.28	63.11	69.38
+ subterraneum	18.63	20.41	10.10	6.02	71.27	73.57
SE (P=0.05)	1.88	2.21	1.55	0.68	4.06	2.12

„ ( ) (Mihovsky, 1993).

*Trifolium subterraneum* ssp. *brachycalycinum* -

*Trifolium subterraneum* ssp. *brachycalycinum* -

*Trifolium subterraneum* ssp. *subterraneum* 64.18% 60.41% -

(Lucas et al., 2015). -

*Trifolium subterraneum* ssp. *brachycalycinum* *Trifolium subterraneum* ssp. *yananicum* (31.63 41.16%). -

50%. -

( 44.44% 46.15%). -

10.10% 15.34%, 6.02% 8.38%.

The part of subterranean clover in the second cut decreased almost threefold compared to the first cut, while the part of white clover increased. White clover has the ability to "colonize" any free space in the neighborhood (Mihovsky, 1993). No noticeable difference in the participation of subterranean clover depending on the subspecies was found, but weed infestation in under sowed swards with *Trifolium subterraneum* ssp. *brachycalycinum* was less compared to others. Thus, *Trifolium subterraneum* ssp. *brachycalycinum* prevent weed infestation in the year after under sowing the degraded seed production white clover stands.

In the second year after under sowing the weed infestation was relatively weak. A larger part of weeds was observed in the control variant and those under sowed with *Trifolium subterraneum* ssp. *subterraneum* in the first cut, 64.18% and 60.41%, respectively. This subterranean clover subspecies has small size of leafes and is less competitive (Lucas et al., 2015). Significantly lower was the weed infestation in variants with under sowing with *Trifolium subterraneum* ssp. *brachycalycinum* and *Trifolium subterraneum* ssp. *yananicum* (31.63% and 41.16%).

In the second cut the part of weeds was 50%. Subterranean clover contributes to reducing weed infestation in the swards, but very weak. No noticeable difference for the subspecies tested and the part of weeds varied in a narrow range (from 44.44% to 46.15%).

During the third year after under sowing, the advanced development of white clover prevent the development of subterranean clover and the possibility for its development was significantly reduced. Botanical composition showed low participation of subterranean clovers in the two cuts. In the first cut the part of subterranean clover was from 10.10% to 15.34%, while in the second - from 6.02% to 8.38%.



*Trifolium subterraneum ssp. brachycalicinum* -  
 -  
 -  
 -  
*Trifolium subterraneum ssp. brachycalicinum*  
*Trifolium subterraneum ssp. yaninicum*,  
 13.48%  
 9.44% -  
 ( 3). -  
*Trifolium subterraneum ssp. brachycalicinum*.  
*Trifolium subterraneum ssp. brachycalicinum*  
 -  
 , 12.03% -  
 -  
 13.43% 14.18%,  
*Trifolium subterraneum ssp. yaninicum* *Trifolium subterraneum ssp. brachycalicinum*.  
 12.82%  
*Trifolium subterraneum ssp. brachycalicinum*  
 8.97%  
 -  
 (128.00 kg/da).  
*Trifolium subterraneum ssp. brachycalicinum*  
*Trifolium subterraneum ssp. yaninicum*  
 -  
 13.50% 11.22%,  
 17.29 kg/da 14.36 kg/da  
 -  
*Trifolium subterraneum ssp. brachycalicinum*. -

There is a trend, *Trifolium subterraneum ssp. brachycalicinum* (from the three subterranean clover subspecies) to have the best development and biggest part in the swards.

Dry mass yield  
 Regarding the dry mass productivity, in the first cut in the first year after under sowing from the swards under sowed with *Trifolium subterraneum ssp. brachycalicinum* and *Trifolium subterraneum ssp. yaninicum*, it was found dry mass yield being higher as compared to non under sowed by 13.48% and 9.44%, respectively (Table 3). In the second cut a similar trend was observed for *Trifolium subterraneum ssp. brachycalicinum* only. On average for the two cuts, dry mass yield obtained from under sowed degraded white clover seed production stands with *Trifolium subterraneum ssp. brachycalicinum* was by 12.03% higher.

Data for dry mass yield in the second year corresponded with these for botanical composition. The presence of subterranean clover increased dry mass yield in the first cut with 13.43% and 14.18% when under sowing was done with *Trifolium subterraneum ssp. yaninicum* and *Trifolium subterraneum ssp. brachycalicinum*, respectively. In the second cut the exceeding in dry mass yield as compared to the control was by 12.82% for *Trifolium subterraneum ssp. brachycalicinum* and by 8.97% for the other two subspecies. The average dry mass yield for the year was found be lowest in the control (128.00 kg/da). In swards under sowed with *Trifolium subterraneum ssp. brachycalicinum* and *Trifolium subterraneum ssp. yaninicum* dry mass yield was by 13.50% and 11.22% higher. From these swards there were 17.29 kg/da and 14.36 kg/da more dry mass obtained compared to non under sowed ones.

During the third year dry mass yield was low and on average from the two cuts it increased significantly only for under sowing with *Trifolium subterraneum*

kg/da) - (9.46 | *ssp. brachycalycinum*. The additional amount of dry mass (9.46 kg/da) was also less than in previous years.

3.

**Table 3. Dry mass yield from under sowed degraded white clover seed production stands with subterranean clover, kg/da**

Treatments	First cut	Second cut	Average	+, kg/da
/First year after under sowing				
/Control	159.59	137.02	148.30	-
+ brachycalycinum	181.11	151.16	166.14	17.84
+ yaninicum	174.65	142.38	158.52	10.22
+ subterraneum	161.13	138.12	149.63	1.32
SE (P=0.05)	5.23	3.21	4.16	
/Second year after under sowing				
/Control	128.80	127.20	128.00	-
+ brachycalycinum	147.06	143.51	145.29	17.29
+ yaninicum	146.10	138.62	142.36	14.36
+ subterraneum	137.45	138.62	138.03	10.03
SE (P=0.05)	4.27	3.46	3.77	
/Third year after under sowing				
/Control	108.82	100.20	104.51	-
+ brachycalycinum	119.30	108.63	113.97	9.46
+ yaninicum	115.77	103.27	109.52	5.01
+ subterraneum	111.23	100.00	105.62	1.11
SE (P=0.05)	2.33	2.01	2.14	

*Trifolium subterraneum ssp. brachycalycinum*

11.70% - (1).  
14.86 kg/da

e

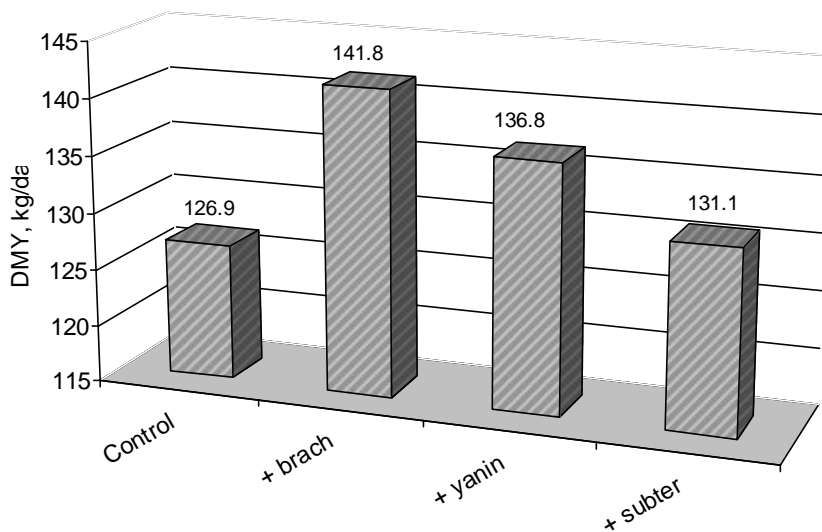
(Mannetje, 2006; Gornall et al., 2010; Aranjueloa et al., 2014).

ú

Dry mass yield on average for three years when under sowing was done with *Trifolium subterraneum ssp. brachycalycinum* was by 11.70% higher than that of non under sowed swards (Figure 1). There was 14.86 kg/da additionally amount of dry mass.

The permanent changes having occurred in the last ten-year periods in the climate, present a serious risk to the perennial swards because they relate to their capacity for several years (Gornall et al., 2010; Aranjueloa et al., 2014). Subterranean clover is an annual leguminous species, but because of the biological ability for self-seeding with the presence in the sward at the beginning and end of vegetation, behaves as a perennial type and increases the

- persistence of the swards. In our study we found this crop could be used for under sowing of degraded stands.



. 1.

(SE=0.05, 3.25)

**Fig. 1. Dry mass yield from under sowed Degraded white clover seed production stands with subterranean clover, average for three years after under sowing (SE=0.05, 3.25)**

## CONCLUSIONS

Subterranean clover can be used for under sowing of degraded white clover seed production stands. It did not negatively effect the white clover development, increased dry mass productivity and reduced the weed infestation, thereafter the stands could be used for forage. From the three subspecies studied (*Trifolium subterraneum ssp. brachycalicinum*, *Trifolium subterraneum ssp. yaninicum* and *Trifolium subterraneum ssp. subterraneum*) *Trifolium subterraneum ssp. brachycalicinum* showed the best development and took the biggest part in the swards.

(*Trifolium subterraneum ssp. brachycalicinum*, *Trifolium subterraneum ssp. yaninicum* *Trifolium subterraneum ssp. subterraneum*) -  
*Trifolium subterraneum ssp. brachycalicinum*.

		After under sowing with <i>Trifolium</i>
<i>subterraneum</i>	<i>ssp. brachycalycinum</i>	<i>subterraneum ssp. brachycalycinum</i> dry mass
	11.70%	yield on average for the period was found
	14.86 kg/da	to increase by 11.70% and 14.86 kg/da
		additional dry mass yield was obtained.

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