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The influence of inoculates on abundance of fungi in rhizosphere two cultivars of alfalfa

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SUMMARY

(*Medicago sativa* L.)
Sinorhizobium meliloti (*Sinorhizobium meliloti*)
Azotobacter chroococcum (*Azotobacter chroococcum*)
Colletotrichum trifolii - Coll-4 (*Colletotrichum trifolii*)
Colletotrichum destructivum - Coll 11 (*Colletotrichum destructivum*)
Coll 657)

(' +Z' ')).

Alfalfa (*Medicago sativa* L.) is a perennial legume characterized by high yield and good quality biomass. The rhizosphere of alfalfa abounds in different microorganisms which can have either a different effect on the plant development. A large number of microorganisms is introduced into the soil by application of microbial inoculation that brings changes in the abundance and composition of the indigenous population. The aim of the research was to investigate the effect of nitrogen-fixing bacteria (*Sinorhizobium meliloti* and *Azotobacter chroococcum*) and phytopathogenic fungi (*Colletotrichum trifolii* - isolate Coll-4 and two isolates of *Colletotrichum destructivum* – Coll 11 and Coll 657) on the number of fungi in the rhizospheric soil of alfalfa cultivars (Affinity+Z and Perry). In comparison to the control, the number of fungi in all inoculation treatments there were statistically significant decreases in the rhizosphere in both examined cultivars of alfalfa.

Key words: rhizosphere, fungi, alfalfa

INTRODUCTION

Alfalfa is one of the oldest and most important forage crops that, in addition to high yield potential and biomass quality, is characterized by the ability to fix nitrogen. This plant species can fix 100-400 kg N ha⁻¹ per year in association with *Sinorhizobium meliloti* (Peoples et al., 1995). The rhizosphere of alfalfa abounds in numerous microorganisms. Some of these microorganisms live on the roots or near it (azotobacter, actinomycetes etc.), while rhizobium enters the root tissue.

Also, they synthesize enzymes and suppress the activities of phytopathogens (Jemcev et al., 2004). Certain rhizospheric microorganisms such as *S. meliloti* provide alfalfa with nitrogen, produce polysaccharides (Ramos et al., 1987), vitamins B12, B1, B2 (Denison and Kiers, 2004). That way, by means of their metabolic products, rhizobia enhance the growth and development of plants (Stajkovi et al., 2011). This bacterium is often used in alfalfa production because of its favourable effects.

Another highly active rhizospheric bacterium is azotobacter, which fixes elementary nitrogen and produces biologically active substances: auxins, gibberellins, pyrodoxine, biotin and nicotinic acid (Dobbelaere et al., 2003). Azotobacter is used in the production of non-legumes but has given good results in the production of legumes (Andjelkovi et al., 2014). Certain microorganisms, in particular those interacting physically with plants in the rhizosphere, can also influence plant productivity negatively by causing disease or positively by enhancing plant growth (Ellouze et al., 2014).

Apart from useful microorganisms, rhizospheric soil contains phytopahtogenic microorganisms which cause different plant diseases. One of the most important alfalfa diseases is

kg N ha⁻¹

Sinorhizobium meliloti (Peoples et al., 1995).

(*azotobacter, actinomycetes* . . .),
(rhizobium)

(Jemcev et al., 2004).

S. meliloti,

(Ramos et al., 1987),

12, 1, 2 (Denison and Kiers, 2004).

(Stajkovi et al., 2011).

Azotobacter,

(Dobbelaere et al., 2003).

Azotobacter

(Andjelkovi et al., 2014).

(Ellouze et al., 2014).

(Vasi et al., 2009).
 (Vasi, 2013).
Colletotrichum trifolii Bain et Essary,
Colletotrichum destructivum O'Gara (Boland and Brochu, 1989).

Sinorhizobium meliloti
Azotobacter chroococcum
Colletotrichum (C.
trifolii C. *destructivum*)

() 2016 .
 : pH KCl 5,90; / ₂O
 6.44; 0.138%; 2.62%;
 P₂O₅ 6,6 mg/100 g; K₂O 24.05 mg/100 g.

: ' +Z'
 () ' ' (-
).
 5 ,

Sinorhizobium meliloti
Azotobacter chroococcum (10 ml -
 108 1
 ml). *S. meliloti* -
 YM Vincent
 (1970), . *Chroococcum*
 Feodorov (1949).

anthracnose (Vasi et al., 2009). Anthracnose causes significant losses in alfalfa crops in Serbia (Vasi, 2013). It is most commonly caused by *Colletotrichum trifolii* Bain et Essary but also by *Colletotrichum destructivum* O'Gara (Boland and Brochu, 1989). Phytopathogenic fungi have a negative effect on the plant growth and even cause plant death. Another possible consequences is a lower microbiological activity in the rhizosphere of the attacked plants.

The aim of the research was to investigate the effect of inoculating alfalfa with two nitrogen-fixing bacteria (*Sinorhizobium meliloti* and *Azotobacter chroococcum*) and two species of the phytopathogenic fungus *Colletotrichum* (C. *trifolii* and C. *destructivum*) on the number of fungi in the alfalfa rhizosphere.

MATERIAL AND METHODS

The experiment was carried out in vegetation pots in semi-controlled conditions at the Institute for Forage Crops in Kruševac during the spring (May and June) 2016. The soil chemical characteristics were the following: pH/KCl 5.90; pH/H₂O 6.44; total nitrogen 0.138 %; humus 2.62 %; P₂O₅ 6.6 mg/100g; K₂O 24.05 mg/100g. The alfalfa varieties used in the experiment were chosen upon their resistance to anthracnose and they were: Affinity+Z (highly resistant) and Perry (susceptible). The experiment was a two-factorial with 5 replicates, where the first factor was the isolate of phytopathogenic fungi and the second was the variant of microbial inoculation.

Before sowing, the seed was inoculated with *Sinorhizobium meliloti* and *Azotobacter chroococcum* (10 ml of inoculum per pot with 10⁸ cells in 1 ml). The *S. meliloti* cultures were grown on YM substrate by Vincent (1970), *A. Chroococcum* cultures were grown on the liquid substrate by Feodorov (1949). The plants were mown after six-seven weeks at the beginning of flowering and thereafter treated with *Colletotrichum*

Colletotrichum destructivum (Coll-11 CC 657) *Colletotrichum trifolii* (Coll-4).
4-6x10⁴/ml.

Tom.

1. *C. destructivum* (Coll-11) + *S. meliloti*;
2. *C. destructivum* (Coll-11) + *A. chroococcum*;
3. *C. destructivum* (Coll-11);
4. *C. destructivum* (CC 657) + *S. meliloti*;
5. *C. destructivum* (CC 657) + *A. chroococcum*;
6. *C. destructivum* (CC 657);
7. *C. trifolii* (Coll-4) + *S. meliloti*;
8. *C. trifolii* (Coll-4) + *A. chroococcum*;
9. *C. trifolii* (Coll-4);
10. .

(Wollum, 1982)

(Jarak and Djuri, 2006).
10-4)

STATISTICS 8.0.

, . . . LSD .

(Ellouze et al., 2014).

(Jarak et al., 2008).

destructivum (Coll-11 isolate and CC 657 isolate) and *Colletotrichum trifolii* (Coll-4 isolate) conidia. The number of conidia was 4-6x10⁴/ml. The number of conidia was determined by means of hemocytometer according to Tom.

The variants of the experiment were the following:

1. *C. destructivum* (Coll-11) + *S. meliloti*;
2. *C. destructivum* (Coll-11) + *A. chroococcum*;
3. *C. destructivum* (Coll-11);
4. *C. destructivum* (CC 657) + *S. meliloti*;
5. *C. destructivum* (CC 657) + *A. chroococcum*;
6. *C. destructivum* (CC 657);
7. *C. trifolii* (Coll-4) + *S. meliloti*;
8. *C. trifolii* (Coll-4) + *A. chroococcum*;
9. *C. trifolii* (Coll-4);
10. Control.

The effect of inoculation was determined at the end of the vegetation period. The number of microorganisms was determined by the method of agar plates (Wollum, 1982) by introducing a diluted soil suspension into proper media and counted per one gram of absolutely dry soil. The number of fungi was determined on Capek's agar medium (dilution 10⁻⁴) (Jarak and Djuri, 2006).

The results were processed by means of STATISTICS 8.0 programme. The significance of the difference between the investigated treatments was determined upon the analysis of variance, i.e. LSD test.

RESULTS AND DISCUSSION

Fungi are very widespread in the rhizosphere of plants. Soil fungi are a critical component of agroecosystems and provide ecological services that impact the production of food and bioproducts (Ellouze et al., 2014). For agricultural production most important are saprophytic fungi that act as decomposers (Jarak et al., 2008).

Our results indicate that the use of

Colletotrichum

- different species and different isolates of a phytopathogenic fungus *Colletotrichum*
- has different effects on the number of fungi in alfalfa rhizosphere soil.



1.

Picture 1. Colonies of fungi on agar (photo orig.)

destructivum (CC 657)
' +Z'

chroococcum

Coll-11 + A.
CC 657+ *S. meliloti*

- The application of microbial inoculants, especially those on the treatment with *C. destructivum* (CC 657)
- incultivar of alfalfa Affinity+Z had a positive effect which is confirmed by the data presented in the table (Table 1).
- Compared to the control only in the treatment this cultivar of alfalfa of co-inoculation with Coll-11+ *A. chroococcum* a statistically significant reduction is achieved and the treatment with CC 657+ *S. meliloti*. was not statistically different with the control. A large number of microorganisms is introduced into the soil by application of microbial inoculation that brings changes in the abundance and composition of the indigenous population.
- The effect of inoculation depends on the abundance of indigenous population, activity of the host plant, soil properties, etc. (Brockwell et al., 1995).

(Brockwell et al., 1995).

Table 1. The effect of inoculants on the number (log of number) of fungi in the rhizosphere of alfalfa

/Variant		/Cultivar of alfalfa	
		+Z/Affinity+Z	/Perry
1.	Coll-11+ <i>S. meliloti</i>	4.602 ^e	4.334 ^d
2.	Coll-11+ <i>A. chroococcum</i>	4.215 ^h	4.675 ^b
3.	Coll-11	4.755 ^b	4.316 ^d
4.	CC 657+ <i>S. meliloti</i>	4,382 ^g	4.771 ^a
5.	CC 657+ <i>A. chroococcum</i>	4.522 ^f	4,182 ^e
6.	CC 657	4.942 ^a	3.986 ^f
7.	Coll-4+ <i>S. meliloti</i>	4.680 ^c	4.486 ^c
8.	Coll-4+ <i>A. chroococcum</i>	4.517 ^f	3.986 ^f
9.	Coll-4	4.618 ^d	4.505 ^c
10.	Control	4.382 ^g	4.790 ^a

(p<0.05)

()

LSD

Note: Mean values with the same superscript(s) are not significantly different according to Fisher's LSD test (p<0.05)

CC 657
destructivum *S. meliloti*.
 Schwieger Tebbe (2000),
 (Gransee and Wittemnayer,
 2000).

In cultivar of alfalfa Perry, the largest number of microorganisms was recorded in the control variant (4.790 – log of number). In this cultivar in all variants of inoculation, there were statistically significant negative differences comparing to the control, except for the variation where the isolate CC 657 of *C. destructivum* and *S. meliloti* were applied. According to Schwieger and Tebbe (2000), introduced organisms may or may not have to have the influence on the existing structure of the microbial population.

Changes to the number of fungi in alfalfa cultivars – Affinity + Z and Perry to applied inoculation were different. Generally at Affinity + Z can be concluded increase in the number of fungi in almost all treatments, while in cultivar Perry the negative effect of the tested microorganisms recorded.

Rhizospheric microorganisms form a closed community with the root and are directly influenced by root secretions (Gransee and Wittemnayer, 2000) Root secretions are the source of carbon and energy for rhizospheric microorganisms

(Harsh et al., 2006). and their chemical composition and amount depend on the plant species (Harsh et al., 2006).

CONCLUSIONS

The obtained results showed that the application of microorganisms and their combination can different affect on abundance investigated microorganisms.

The application of microorganisms and combinations thereof can affect on the number of fungi varied depending on the variant of inoculation and cultivar of alfalfa. These are preliminary studies and to obtain complete information about presence of fungi or whether they are saprophytes or pathogens, it is necessary to continue research in this direction.

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Qualitative characterization of green forage from monoculture and mixture pea : oat swards

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SUMMARY

Field pea and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac - Serbia (21°19'35" E, 43°34'58" N). The experiment was designed with three replications according to a randomized complete block. Experiment was established in autumn in 2012, on October the 20th and the samples were taken in spring in 2013. The pea and oat were tested at five different mixture rates: A₁-100% pea; A₂-100% oat; A₃- 25% pea + 75% oat; A₄-50% pea + 50% oat and A₅- 75% pea + 25% oat. Green forage samples were assayed for DM (Dry Matter), CP (Crude Protein), CHO (Total Carbohydrates), NSC (Non-Structural Carbohydrates), Starch, NFC (Non-Fiber Carbohydrates), aNDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber), HCL (Hemicellulose), Lignin, DMD (Dry Matter Digestibility) and CHO fraction by CNCPS

)
CNCPS (Cornell Net Carbohydrate and Protein System).

(435.9 g kg⁻¹).

(CA),

(774.0 g kg⁻¹ DM).

:

1998).

(Adesogan and Salawu, 2002).

() *in vitro*

(Salawu et al., 1985),

(Faulkner, 1985).

(Salawu et al., 2001).

(Cornell Net Carbohydrate and Protein System) were calculated. It was realized that pea and oat can be planted successfully for herbage production. Monoculture pea had the lowest values of aNDF (435.9 g kg⁻¹ DM).

Pea monoculture and mixture with higher pea proportions contained higher CA fraction indicating that pea and mixture with higher pea proportions were better sources of fermentable CHO to ruminants.

The highest DMD was recorded for pea monoculture (774.0 g kg⁻¹ DM).

Key words: pea : oat mixtures, structural and nonstructural carbohydrates

INTRODUCTION

Bi-crops of various grain legumes and cereals have received much attention because of their high yields (Salawu et al., 2001). In particular, bi-crops with pea varieties or mixtures with high grain to straw ratios are considered to have a good balance of energy and protein contents (Anil et al., 1998). One anticipated advantage of feeding bi-crop mixtures of cereal and legumes is an improvement in the efficiency of nutrient utilization due to the possible synchronous supply of readily fermentable energy and protein in the rumen (Adesogan and Salawu, 2002).

Pea forages have higher CP (Crude Protein) and *in vitro* digestible organic matter, and lower neutral detergent fiber (NDF) and acid detergent fiber (ADF) than wheat (Salawu et al., 2001), and higher CP contents than oats (Faulkner, 1985).

However, adding pea to wheat, oat or barley increases forage CP concentration and decreases NDF and ADF (Salawu et al., 2001).

Knowledge of potential rumen

Cornell Net Carbohydrate and Protein System (CNCPS)

2007).

(21°19'35 "E, 43°34'58" N).

166 m

3.5% 6.5.

2012 . 20

2013 .

: 1-100%
; 2-100% ; 3-25% + 75%
; 4-50% + 50% 5- 75%
+ 25%

20 m².

, 300 kg ha⁻¹ NPK

(15:15:15)

2013 ..

2/3

(60 °)

48

(AOAC, 942.05),

(; AOAC 984.13)

(EE, AOAC 954.02)

degradability of feed fractions is key to assess their nutritive value and extent of utilization in ruminants. The Cornell Net Carbohydrate and Protein System (CNCPS) accounts for the effects of variation due to feed CHO (Carbohydrate) fractions, their relative ruminal degradation rates and ultimately their rate of passage through the intestine (Lanzas et al., 2007).

The objective of this experiment was to assess the effect of pea proportion on structural and nonstructural carbohydrate fractions in pea:oat mixture green forage.

MATERIAL AND METHODS

Field pea and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac - Serbia (21°19'35 E, 43°34'58 N). The study area was situated at altitude of 166 m above sea level in Central Serbia. Soil type was with an organic matter content of approximately 3.5% and a pH of 6.5.

The experiment was designed with three replication according to a randomized complete block. Experiment was established in autumn in 2012, on October the 20th and the samples were taken in spring in 2013. The pea and oat were tested at five different mixture rates: A₁-100% pea; A₂-100% oat; A₃- 25% pea + 75% oat; A₄-50% pea + 50% oat and A₅- 75% pea + 25% oat. All mixtures were sown on plots of 20 m². One level of fertilizer was applied, 300 kg ha⁻¹ NPK (15:15:15) before the seeding. Plant samples were taken in spring 2013, at forming the first pods on 2/3 plants of pea.

Green forage samples were assayed for DM (Dry Matter) by oven drying at 60° C for 48 h. Standard procedures described by the AOAC (1990) were used to determine ash (AOAC, 942.05), Crude Protein (CP; AOAC 984.13) and ether extract (EE, AOAC 954.02), but ash and EE were not

AOAC (1990),

$$[CP + EE] = 1000 - (NDF + ADF + HCL + lignin)$$

NRC (2001).

(),
()

Van Soest et al. (1991).

et al. (1999)

Hall (2000).

in vitro
(De Boevar et al., 1986).

F
(p< 0.05) (STATISTICA 6, Stat. Soft. 2006).

presented in this paper. Total carbohydrates [CHO = 1000 - (CP + Ash + EE)] and Non-Fiber carbohydrates [NFC = 1000 - (aNDF + CP + Ash + EE)] were calculated according to NRC (2001). NDF assayed with heat stable -amylase (aNDF), acid detergent fiber (ADF), hemicellulose (HCL) and lignin content were determined according to Van Soest et al. (1991). NSC (Non-Structural Carbohydrates - monosaccharides and disaccharides) were determined as total ethanol soluble carbohydrates according procedures described by Hall et al. (1999), and starch content was determined according enzymathic method by Hall (2000). Two stage pepsin-cellulase method was used for *in vitro* DM digestibility (De Boevar et al., 1986).

The experimental data were analyzed by a factorial analysis of variance for green forage samples in a completely randomized design using a model that accounted for the main effects of pea:oat mixtures. Effects were considered different based on significant (p< 0.05) F ratio (STATISTICA 6, Stat. Soft. 2006).

RESULTS AND DISCUSSION

The structural and nonstructural CHO fractions of the pea:oat mixture are presented in Table 1. The effects of the mixture rates were significant for the dry matter content. With regard to the mixture rates, the highest DM was obtained from the 100% oat plots (Table 1).

Several studies showed that the DM increased with the increasing rate of oat in mixtures of oat with pea (Uzun and Asik, 2012). Furthermore, oat physically supported the pea plants in such mixtures and provided most of the DM production.

An anlasys of variance found statistically significant differences among mixture rates for crude protein ratio (Table

1.
CNCPS

Table 1. Chemical composition and carbohydrate fractions by CNCPS of Pea:Oat mixture

/ Indicators	/ Treatments				
	A ₁	A ₂	A ₃	A ₄	A ₅
/DM, g kg ⁻¹	248.3 ^d	280.0 ^a	271.6 ^b	263.0 ^c	270.3 ^b
/CP, g kg ⁻¹ DM	190.3 ^a	114.5 ^e	126.8 ^d	152.8 ^c	167.3 ^b
/CHO, g kg ⁻¹ DM	685.3 ^d	753.6 ^a	736.2 ^b	705.6 ^c	697.4 ^{cd}
/NSC, g kg ⁻¹ DM	225.7 ^a	139.5 ^d	179.8 ^b	178.8 ^b	166.6 ^c
Starch, g kg ⁻¹ DM	66.7 ^a	67.7 ^a	65.2 ^a	67.4 ^a	56.5 ^b
/NFC, g kg ⁻¹ DM	279.1 ^a	106.5 ^e	148.5 ^d	165.2 ^c	200.4 ^b
/aNDF, g kg ⁻¹ DM	435.9 ^e	663.8 ^a	606.2 ^b	564.2 ^c	520.4 ^d
/ADF, g kg ⁻¹ DM	352.1 ^c	441.4 ^a	443.5 ^a	415.1 ^b	417.3 ^b
/HCL, g kg ⁻¹ DM	76.2 ^e	203.4 ^a	149.1 ^b	136.4 ^c	93.2 ^d
Lignin, g kg ⁻¹ DM	61.6 ^b	81.1 ^a	82.4 ^a	69.8 ^b	82.2 ^a
/DMD, g kg ⁻¹ DM	774.0 ^a	579.8 ^e	611.4 ^d	649.8 ^c	691.1 ^b
CA, g kg ⁻¹ CHO	329.4 ^a	185.2 ^d	244.3 ^c	253.4 ^b	238.9 ^c
CB ₁ , g kg ⁻¹ CHO	97.4 ^a	89.8 ^b	88.6 ^b	95.5 ^a	81.1 ^c
CB ₂ , g kg ⁻¹ CHO	166.8 ^a	77.9 ^d	79.2 ^d	110.7 ^c	147.9 ^b
CB ₃ , g kg ⁻¹ CHO	258.4 ^e	452.3 ^a	389.5 ^b	372.9 ^c	334.7 ^d
CC, g kg ⁻¹ CHO	148.0 ^b	194.7 ^a	197.9 ^a	167.4 ^b	197.2 ^a

A₁-100% pea + 0% oat; A₂-0% pea + 100% oat; A₃-25% pea + 75% oat; A₄-50% pea + 50% oat; A₅-75% pea + 25% oat
 - ; - ; a - ; - ; -
 (P < 0.05)

A₁-100% pea + 0% oat; A₂-0% pea + 100% oat; A₃-25% pea + 75% oat; A₄-50% pea + 50% oat; A₅-75% pea + 25% oat
 DM-Dry Matter; CP-Crude Protein; CHO-Total Carbohydrates; NSC-Non-Structural Carbohydrates; NFC-Non-Fiber Carbohydrates; aNDF-Neutral Detergent Fiber; ADF-Acid Detergent Fiber; HCL-Hemicellulose; DMD-Dry Matter Digestibility
 Different letters denote significantly different means (P < 0.05)

a (Assefa and Ledin, 2001).
 a
 a
 (1).
 a (435.9 g kg⁻¹ DM).
 25:75 :

Other important quality characteristics for forages are the concentrations of aNDF and ADF (Assefa and Ledin, 2001). The aNDF content differed significantly between treatments. aNDF content increased as the oat proportion increased in mixtures (Table 1). Monoculture pea had the lowest values of aNDF (435.9 g kg⁻¹ DM). In the case of ADF much smaller differences were observed. There were no significant differences between oat monoculture and 25:75 pea:oat mixture, and also between

50:50 75:25

a

(Velazquez-Beltran et al., 2002). Salawu et al. (2001)

Aesen et al. (2004)

Carr et al. (2004)

38.2%, 38.5%, 34.4% 36.5%. Strydhorst et al. (2008 .)

55.2% 41.8%. Lithourgidis et al. (2006)

50:50 a

CNCPS (1), CA,

50:50 and 75:25 pea:oat mixtures. The pea monoculture also had the lowest ADF content. The actual values for aNDF and ADF in this study and the lack of significant differences agreed with previous studies (Velazquez-Beltran et al., 2002). Salawu et al. (2001) indicated that adding pea to oat increased CP concentration and decreased NDF and ADF contents. Aesen et al. (2004) also reported that increasing the legume proportion resulted in decreased ADF and NDF concentrations for the legume-grass mixtures. Carr et al. (2004) found that pea, barley, oat, pea-barley and pea-oat mixtures of ADF values 38.2%, 38.5%, 34.4% and 36.5%, respectively. Strydhorst et al. (2008) reported that barley and pea-barley mixtures of NDF values were 55.2% and 41.8%, respectively.

Lithourgidis et al. (2006) reported lower values of CP, NDF, ADF and lignin for oat monoculture than results obtained in this study. This can be attributed to the different cultivar used in this study and possibly to the different growth stage of oat at harvest as compared with the other studies.

There were no significant differences between most of the treatments for lignin content; the only significant differences were between pea monoculture and 50:50 mixtures with other treatments (Table 1). As well as aNDF and ADF, the highest content of hemicellulose was obtained in DM of oat monoculture, whereas the lowest content was determined in DM of pea monoculture. Decreasing oat proportions in pea:oat mixtures also decreased content of hemicellulose.

When CNCPS CHO fractions of pea:oat mixtures (Table 1) were interpreted, it was observed that pea monoculture and mixture with higher pea proportions contained higher CA fraction indicating that pea and mixture with higher pea proportions were better

	1		
50:50			
1.			
2			
CB ₂			
CB ₃			
CC			
			CC
50:50			
Das et al. (2015 .)			
(774.0 g kg ⁻¹)			
(579.8 g kg ⁻¹)			
611.4	691.1 g kg ⁻¹		
75%	25%		

sources of fermentable CHO to ruminants. Amount of fraction CB₁ was comparable between pea:oat mixtures; pea monoculture and 50:50 pea:oat mixture had higher CB₁ content.

In typical ruminant diet, the amounts of fraction CB₂ and CB₃ are very important as these fractions represent the nonfiber CHO and available cell wall portion of ruminant feeds.

Pea monoculture had the highest CB₂ fraction and this fraction increased with increasing pea proportion in pea:oat mixtures, whereas pea monoculture contained the lowest CB₃ fraction and this fraction decreased with increasing pea proportion in pea:oat mixtures.

Fraction CC is the lignin bound cell wall content of a feed. Hence this fraction is indigestible both by ruminal microbes and the animal itself. Feeds with low CC fraction like pea monoculture and 50:50 pea:oat mixture in this investigation will be of superior quality in terms of CHO supply to ruminants.

Finding of Das et al. (2015) regarding various CHO fractions of forages though were different from our results, but they were highly comparable.

The DMD was significantly different among the experimental treatments. The highest DMD was recorded for pea monoculture (774.0 g kg⁻¹ DM). Despite the pea, oat monoculture had the lowest DMD (579.8 g kg⁻¹ DM). As a result of high digestibility, increasing pea proportion in pea:oat mixtures resulted in increasing of DMD from 611.4 to 691.1 g kg⁻¹ DM in mixtures contained 25% pea to 75% pea, respectively.

CONCLUSIONS

According to the data obtained from this research work, it was realized that pea and oat can be planted successfully for

75% + 25% 50% + 50%

herbage and hay production.

Moreover, pea and oat mixtures can be successfully ensiled and obtained high quality silages. Based on findings of the above study it was concluded that pea-oat mixtures generally were better feeds for ruminants from CHO supply point of view.

According to the results, cultivation of 50% pea + 50% oat and 75% pea + 25% oat mixtures for better structural and nonstructural carbohydrates ratio and DMD could be recommended.

ACKNOWLEDGEMENTS

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INTRODUCTION

- Field pea is one of the valuable sources of vegetable protein and along with other legumes is important to solve protein problem in the country (Kertikov, 2010). Pea plants contribute to maintaining soil fertility, which is very significant in terms of sharply reducing the importation of mineral fertilizers (Ryabtseva, 2009).
- It is an excellent precursor for most field and industrial crops (Hone cutt, 1998; u et al., 2003; Gramatikov and Koteva, 2006).
- Leading issue is the provision of free of chemical contamination of food, based on the health status of the population inhabiting all countries and continents (Nickel, 1982; Kuleva and Kuznetsov, 2004). In this connection the limited use of preparations of inorganic origin and treatment with bio preparations in the production of protein fodder crops, expands the opportunity for the development of organic farming in the country and there is no risk of contamination of the harvested produce.
- The aim of the experiment is to determine the impact of different technologies in growing spring field pea variety Kerpo on phenology, biometrics, the degree of lodging, grain yields and grain losses.

MATERIAL AND METHODS

- The experiment was conducted during the period 2011-2013 in the second experimental field in Institute of Forage Crops with pea cv. Kerpo. The variety has a high grain yield and earliness (Kertikova and Kertikov, 2013).
- The study was conducted on the soil subtype slightly leached chernozem, without irrigation. It used the split plot method with four repetitions of the variants and a size of 10 m² of harvest plot. Variants of the field experience: Variant 1 control – a conventional technology (Kertikov et al. 2003),

al., 2003),
 ; 2 –
 ; 3 –
 („ ”)
 ” ”
 3,5 l/da.
 -
 :
 (cm)
 ();
 (cm) (L);
 , % ();
 (kg/da);
 (kg/da).
 :
 (cm);
 (cm);
 ;
 (g); 1000
 (g).
 STATGRAPHYCS plus for
 Windows Version 2.1.

including fertilization and treatment with herbicides and insecticides; Variant 2 – without the use of preparations of inorganic origin; Variant 3 – treatment only with bio insecticide ("Ecofil P") of organic origin. Treatment with bio preparation "Ecofil P" is performed in phenophase full flowering at a dose of 3,5 l/da.

Agro-meteorological and phenological observations and readings were carried. The following indicators are recorded: stand height (cm) in its natural condition before harvest (H); stem length (cm) upright (L); lodging rate in % (C); economic and biological grain yield (kg/da); grain losses (kg/da). Grain harvest is done with miniature plot harvester. Structural analysis of grain yield include: stem length (cm); height of the first pod (cm); number of pods per plant; number of seeds per pod, weight of seeds per plant (g); mass of 1000 seeds (g). The data were processed with the software STATGRAPHYCS plus for Windows Version 2.1.

RESULTS AND DISCUSSION

2011-2013 . (1) -
 ,
 21 - 28 .
 (1) ,
 8,4
 9,8
 (7,1 7,6 mm/m²),
 - ,
 .
 02.04. 12.04.,
 - .

Presented by agro-meteorological conditions for the months of March to June 2011-2013 years (Figure 1) covering the vegetation period of spring forage peas period shows that a real possibility for the spring establishment of the field experiment exists between March 21st to 28th. In this regard made phenological observations and readings (Table 1) show that the sowing was done at present average daily temperatures 8,4 to 9,8 and insufficient rainfall (from 7,1 to 7,6 mm/m²), but good humid soil from winter rains.

In the early and later stages, agro-meteorological conditions are favorable for the conduct of vegetation processes. Phenophase germination is registered by 02.04. to 12.04. and stem formation and growth by about two weeks later. The period from phenophase germination to

- phenophase growth and stem formation is characterized by frequent rainfall, good moisture stockpiling and optimum temperatures for development of culture.

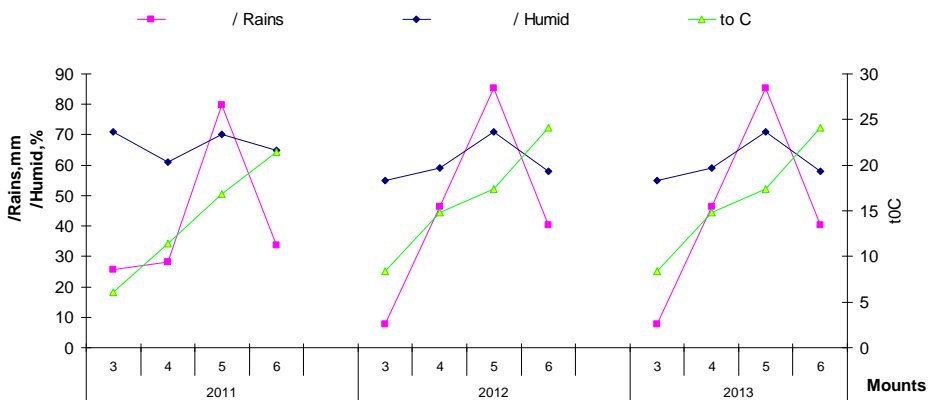


Fig. 1 Klimatogram for the vegetation period, 2011-2013

Depending on the difference in weather conditions in the particular years, this period covers 27-41 days. Until this moment there was no difference in duration of phenophase depending on the implementation of various agricultural maneuvers in the cultivation of the crop.

During the course of phenophase intensive growth – full budding, appeared the first differences around the measured height of the pea plants in various crops.

The smallest height (23,0-30,0 cm) was registered in plants by applying conventional technology (variant 1), and the highest in plants variant 2 (without preparations of inorganic origin). The plants from variant 3 (treatment only "Ecofil P") occupy an intermediate position.

1.
2011-2013

Table 1. Phenological observation of spring forage pea cv. Kerpo, 2011-2013

Treatment	/ Phenophase			
	sowing	emergency	growth (cm)	budding
1 –				
Variant 1 – control	21.03.-28.03.	02.04.-12.04.	26.04.-16.05.(23-30,0)	10.05.-19.05.
2 / Variant 2	21.03.-28.03.	02.04.-12.04.	26.04.-16.05.(27-28,8)	10.05.-23.05.
3 / Variant 3	21.03.-28.03.	02.04.-12.04.	26.04.-16.05.(25-31,0)	10.05.-23.05.

Treatment	full flowering	milk ripeness	technical maturity	() vegetation period (days)
1 –				
Variant 1 – control	18.05.-25.05.	04.06.-09.06.	24.06.-05.07.	89 – 108
2 / Variant 2	21.05.-30.05.	07.06.-15.06.	26.06.-06.07.	95 – 109
3 / Variant 3	22.05.-30.05.	07.06.-13.06.	26.07.-09.07.	93 – 112

3.

17,4 24,1
21,2 30,3

2 3

89-108

4 6

24 9

The differences in the heights of the plants in the different variants are mainly due to the strong weeding of crops in variant 2 and 3. From the data it is apparent also that the plants grown by standard technology entering about three to eight calendar days earlier in phase beginning of flowering compared with those of the other two variants. Under the influence of high air temperatures during the months of May and June (daily average of 17,4 to 24,1 and maximum of 21,2 to 30,3) phenophase full flowering and pod formation run intensive and accelerated. Phenological differences between variants in the spring pea also affect the onset and duration of the next phenophase. It was found to delay development of plants in the variant 2 and 3 in comparison with those of the control. The average growing season of pea grown in the conventional technology is 89-108 days. Its extension in the other variants is insignificant and is in the range from 4 days to 6 days. Perhaps extending the vegetation period is mainly due to the strong weeding, limited access to water plants, nutrients and less sunshine.

The grain harvesting, depending on the applied agronomic factors at different variants was made in technical maturity in the period from 24 June to 9 July.

2.
1,76 cm
(30,97 cm)

Climatic conditions in the years of study determine the variation in yield and formative structural elements. The data for the parameters from carrying biometric and structural analysis average three-year period are presented in Table 2. There were no differences in certain average length stems. The reported differences in the range of 1,76 cm are insignificant. The height of the first pod is smaller (30,97 cm) in plants grown in conventional technology (variant 1).

2. , 2011-2013 .
Table 2. Structural analysis of spring forage pea cv. Kerpo, 2011-2013

Treatment	/ Indicators		
	Length of the stems, (cm)	Height of the first pod (cm)	Number of pods per plant
1 –			
Variant 1 – control	52,15 ^a	30,97 ^b	6,0 ^a
2 / Variant 2	51,03 ^a	35,25 ^a	5,0 ^a
3 / Variant 3	52,79 ^a	34,43 ^a	5,0 ^a
Treatment	Number of seeds per pod	Seed weight per plant (g)	Weight of the 1000 seeds (g)
1 –			1000
Variant 1 – control	8,0 ^a	6,55 ^a	255,75 ^a
2 / Variant 2	8,0 ^a	5,18 ^b	207,84 ^c
3 / Variant 3	8,0 ^a	5,31 ^b	219,96 ^b

LSD 99.5% -

LSD 99.5% - means of the same column followed by the same letter was not significant different

3,46 cm 4,28 cm.

(5,18 g) (6,55 g)

Plants grown without treatment with preparations of inorganic origin and those treated with bio insecticide "Ecofil P" set first pods of greater height, average with 3,46 cm to 4,28 cm.

The results on the indicator number of seeds per pod show that three variants are the same values. In the case here stronger factor is characteristic of biological factor compared to technology of growing. Tracing back in the same table the weight of the seed of a plant can be seen that it is the greatest in the control variant (6,55 g) and the lowest (5,18 g) in plants grown without treatment with preparations of inorganic origin.

1000 g),
 (1 (255,75 g),
 35,79 47,91 g -
 (Skubisz, 2002; Zhang, 2004).
 3

The values of mass of 1000 seeds showed a similar trend. The greatest weight are the seeds of variant 1 (255,75 g), while the seeds of the other two variants (without treatment with preparations of inorganic origin and treated bio insecticide "Ecofil P") are from 35,79 to 47,91 g lighter.

For annual legumes including pea, of particular importance is the low degree of lodging of the plants. That same favors the significant reduction in losses of grain or green mass harvesting of culture (Skubisz, 2002; Zhang, 2004).

In this regard Table 3 reflects the degree of lodging of spring pea at the time of harvest for grain, the resulting yields and actual losses of seeds.

3. (kg/da)

, 2011-2013 .

Table 3. Grain yield (kg/da) and degree of lodging depending on the technology of cultivation of cv. Kerpo, 2011-2013

Treatment	/ Indicators		
	H cm	L cm	(%) Lodging (%)
1 –			
Variant 1 – control	25,50 ^b	55,13 ^a	52,67 ^a
2 / Variant 2	34,17 ^a	52,71 ^b	35,62 ^b
3 / Variant 3	36,07 ^a	55,43 ^a	34,33 ^b

LSD 99.5% -

LSD 99.5% - means of the same column followed by the same letter was not significant different

Treatment	Biological yield	Economic yield	Grain losses
1 –			
Variant 1 – control	384,93 ^a	296,10 ^a	88,83 ^a
2 / Variant 2	258,50 ^c	222,17 ^c	36,33 ^c
3 / Variant 3	301,17 ^b	255,13 ^b	46,03 ^b
LSD 99,5%	7,582 kg/da;	6,435kg/da;	3,013kg/da

Note: H - state; L -

/Average height of the stand in the natural /Average length of erect stems in the stand;

25,50 cm
 - 52,67%.
 34,17 cm 36,07 cm,

Data show that the crop grown on conventional technology at the time of harvesting is natural 25,50 cm height and degree of lodging – 52,67%. In crops of the other two variants, the height of plants at the time of harvesting are in the range from 34,17 cm to 36,07 cm, and as

	-	-	34,33%
35,62%	,		
	,		
			-
			-
			-
kg/da	296,10 kg/da.		384,93
	88,83 kg/da.		-
			-
	(- 258,50 kg/da	
	- 222,17 kg/da)		-
	,		
	"	"	

a result of the stronger the degree of weed infestation lodging is lower compared to the control, by 34,33% and 35,62%.

Results for grain yield of spring forage pea average for the period show that the differences between the three variants are significant. For cultivation and grain yield is the highest in the crop grown on conventional technology, respectively 384,93 kg/da and 296,10 kg/da. In this crop, however, sowing grain losses are the highest and reach 88,83 kg/da. The lowest are the values for the reported losses of seeds in crop grown without use of preparations of inorganic origin, but at the same time in it yields (biological – 258,50 kg/da and economic – 222,17 kg/da) are also the lowest. Grain yields and losses reported in the treated seed with bio preparation "Ecofil P" occupy an intermediate position.

CONCLUSIONS

The average growing season of pea grown in the conventional technology is 89-108 days. Its extension in the other variants is insignificant and is in the range from 4 days to 6 days.

In crop with applied standard technology, biometric and structural indicators of yields are the highest and are in the best combination to those of the other two variants.

The highest biological (384,93 kg/da) and economic (296,10 kg/da) yield is in crop with used standard technology of cultivation (control), but in it are also reported the highest losses of grain – 88,83 kg/da. The lowest biological and economic yield (258,50 kg/da and 222,17 kg/da) was harvested from the crop grown without the use of preparations of inorganic origin.

		89-108	
			4
6	.		-
	,		
			-
	.		
kg/da)	-	(384,93	
		(296,10 kg/da)	-
	(),	-
	- 88,83 kg/da.		-
		(258,50 kg/da	-
222,17 kg/da)			

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Changes in the content of plastid pigments in above-ground biomass of annual and perennial forage grasses after herbicides application

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SUMMARY

2014-2016 .
-
60 , 75 ,
(*Lolium perenne* L., *Dactylis glomerata* L.,
Festuca arundinacea Schreb *Agropyron*
desertorum (Fisch.) Schultes) 538
, 210 ,
75 ;
Sorghum sudanense Piper Stapf.,
BBCH 12-13
BBCH 12-14

During the period 2014-2016, at the
Institute of Forage Crops - Plevan was
conducted field trials to establish the
selectivity of herbicides Axial One, Akurat
60 WG, Pacifica, Alai Max, Eagle 75 WG
and Basis 75 DF at perennial forage
grasses *Lolium perenne* L., *Dactylis*
glomerata L., *Festuca arundinacea*
Schreb and *Agropyron desertorum*
(Fisch.) Schultes and Lumax 538 SC,
Laudis OD, Stellar 210 SL, Equip SK,
Pacifica, Axill One, Korello Duo, Corrida
75 VDG; Axial and Dikopur F in *Sorghum*
sudanense Piper Stapf. applied at growth
stage tillering for perennial grasses and
second leaves in Sudan grass.

It was found that the selectivity of
the herbicides and the plastid pigments
content in above-ground biomass of test

(74.7%),
(R) -1.0 -5.5.

21
60 1.0 g/da A.
desertorum 100.0
ml/da *D. glomerata* (15,9 18,2%)
(1,3 20,9%),

(EWRS)

: *L. perenne* -0,567; *D. glomerata* -0,701; *F. arundinaceae* -0,957; *A. desertorum* -0,703
S. sudanense -0,740.

21

(Liebman and Davis, 2000; Oerke, 2006; Meiss et al., 2010; Beckie and Tardif, 2012).

annual and perennial forage grasses showed a trend of reduction (up to 74.7%), the accumulation of photosynthetic pigments (chlorophyll a, b and carotenoids) at a degree of inhibition (R) ranges from -1.0 to -5.5.

The total chlorophyll content was increased 21 days after the administration of Acrust 60 WG at a dose of 1.0 g/da in *A. desertorum* and Axial One at a dose of 100.0 ml /da for *D. glomerata* (15.9 to 18.2%) and carotenoids (from 1.3 to 20.9%) versus control treatments.

Statistically significant negative correlations between the content of Photosynthetic plastid pigments and visual evaluation scores for phytotoxicity (EWRS) based on applied herbicides in annual and pereannual cereal forage crops as follows: *L. perenne* -0.567; *D. glomerata* -0.701; *F. arundinaceae* -0.957; *A. desertorum* -0.703 and *S. sudanense* -0.740 were established

The experimental results obtained showed that the determination of the photosynthetic pigments content on 21 days after treatment with herbicide can be used as a test for evaluation of the selectivity of post-emergent herbicides in the annual and perennial cereal forage crops tested.

Key words: selectivity, plastid pigments, herbicides, annual, perennial cereal forage grasses

INTRODUCTION

Weeds, diseases and pests are one of the major factors that decrease the yield and impair the quality of the production in forage crops (Liebman and Davis, 2000; Oerke, 2006; Meiss et al., 2010; Beckie and Tardif, 2012).

To obtain high yields from seed crops of annual and perennial forage crops, an important unit in their cultivation technology is the weed control.

(Nemat Alla and Younis, 1995; Rankova et al., 2008; Hrstova, 2009; Bazitov and Bazitov, 2010; Rankova, 2010; Rankova and Popov, 2011; Hristova et al, 2013; Georgiev et al., 2014; Prodanova-Marinova, 2014a 2014b).

(Rankova et al., 2013; El-Nahhal and Hamdona, 2015; da Costa et al., 2017) (Velcheva et al, 1997; Velcheva et al., 2012).

Bhatti et al. (1998); Radetski et al. (2000); Rankova (2004 2006); Rankova et al. (2006a, 2006b 2009) Anderson (2010) 2,4-

(Vecchia et al., 2001; Pechová et al., 2003; Rankova and Koumanov, 2005; 2006; Patra and Artenie, 2007; Nacheva et al., 2012; Vasileva, 2012; 2015; Goranovska et al, 2014; Ilieva and Vasileva, 2016).

Sorghum

(Kershner et al., 2012; Enchev and Georgieva-Andreeva, 2013; Yu et al., 2015), *Lolium perene* L., *Dactylis glomerata* L. *Agropyron desertorum*

- The herbicides with high selectivity and efficiency are a basic element in the modern technologies in cultivating a number of crops of plants (Nemat Alla and Younis, 1995; Rankova et al., 2008; Hrstova, 2009; Bazitov and Bazitov, 2010; Rankova, 2010; Rankova and Popov, 2011; Hristova et al, 2013; Georgiev et al., 2014, Prodanova-Marinova, 2014a 2014b).

- The selectivity of herbicides is their ability to kill weeds without phytotoxic effects on the growth and reproductive potential of crop plants (Rankova et al., 2013; El-Nahhal and Hamdona, 2015; da Costa et al., 2017) and soil biota (Velcheva et al, 1997; Velcheva et al., 2012).

According to studies by Bhatti et al. (1998); Radetski et al. (2000); Rankova (2004 2006); Rankova et al. (2006a, 2006b and 2009) and Anderson (2010) herbicides such as 2,4-D, glyphosate, chlorsulfuron, foramsulfuron and others can cause phytotoxic damage to crop plants, expressed in chlorosis, necrosis, leaf and stalk malformations, as well as functional disorders in the physiological processes - photosynthesis, breathing, water regime and others.

- The photosynthetic pigments content is an indicator of the sensitivity of plants to changes in the abiotic factors of the environment and as an indicator for determining the phytotoxicity of herbicides (Vecchia et al., 2001; Pechová et al., 2003; Rankova and Koumanov, 2005; 2006; Patra and Artenie, 2007; Nacheva et al., 2012; Vasileva, 2012; 2015; Goranovska et al, 2014; Ilieva and Vasileva, 2016).

- In species of the genus *Sorghum* found specific reactions to some of the used herbicides (Kershner et al., 2012; Enchev and Georgieva-Andreeva, 2013; Yu et al., 2015), while *Lolium perene* L., *Dactylis glomerata* L., and *Agropyron desertorum* (Fisch.) schultes are

(Fisch.) Schultes
 (Durán-Serantes et al., 2002; McCurdy, et al., 2008; Dimitrova and Katova, 2011; 2013; Kaspary, 2014).

()

2014-2016
 - ,
 .
 m² 3 m² - 5
 .
 : I. -
 - (*Sorghum sudanense* (Piper) Stapf.)
 II. - (*Lolium perenne* L., *Dactylis glomerata* L., *Festuca arundinaceae* Schre. *Agropyron desertorum* (Fresch.) Schultes.)
 1.
 „ptp 18“
 40 l/d
 P max 3 bar, V
 max 1.48, Q max 0.57 l/min,
 -
 (*S. sudanense* BBCH 12-13,
 -
 (*L. perenne*, *D. glomerata*, *F. arundinaceae* and *A. desertorum* BBCH 12-14
 .

extremely limited or missing (Durán-Serantes et al., 2002; McCurdy, et al., 2008; Dimitrova and Katova, 2011; 2013; Kaspary, 2014).

The aim of this present study was to investigate the selectivity of the group of herbicides and their effects on the accumulation of plastid pigments (chlorophylls and carotenoids) in above-ground biomass of annual and perennial grass forage grasses under field conditions.

MATERIAL AND METHODS

The studies were conducted during the period 2014-2016 in the experimental field of the Institute for Forage Crops - Pleven on leached black ground under non-irrigated conditions.

Field trials were conducted perpendicularly in three replicates with a test area of 5 m² per annual cereal and 3 m² of perennial cereal forage crops.

Two groups of forage crops are the subject of study: Group I. - Annual cereal - (*Sorghum sudanense* (Piper) Stapf.) and II. Group - Perennial cereal - (*Lolium perenne* L., *Dactylis glomerata* L., *Festuca arundinaceae* Schre and *Agropyron desertorum* (Fresch.) Schultes.). Trial treatments and herbicides characteristics are shown in Table 1.

The application of the herbicides was done with the ptp 18 knapsack sprayer at 40 l/da with a conical nozzle pressure Pmax 3 bar, Vmax 1.64 l and Qmax l/min for the annual cereal crops - (*S. sudanense* in growth stage BBCH 12-13, and in perennial forage crops (*L. perenne*, *D. glomerata*, *F. arundinaceae* and *A. desertorum* in growth stage BBCH 12-14 in the year of sowing.

1.

Table 1. Trial treatments and herbicides characteristics

Species	Active ingredients	Herbicides	/Dose g/(ml)/da
<i>L. perenne</i>	45 g/l + 5 g/l 45 g/l pnioksaden + 5 g/l florasulam	Axial One e	100.0
	600 g/kg - 600 g/kg Metsulfuron-methyl	60 ccurate 60 WG	1.0
<i>F. arundinaceae</i>	30 g/kg + 90 g/kg - + 10 g/kg - 30 g/kg mesosulfuron-methyl + 10 g/kg iodosulfuron-methyl sodium + 90 g/kg mefenpyr-diethyl	+ Pacifica + Biopower	35.0
<i>A. desertorum</i>	143 g/kg +143 g/kg 143 g/kg tribenuron methyl +143 g/kg metsulfuron methyl	Ally Max	3.5
	750 g/kg 750 g/kg chlorsulfuron	75 Eagle 75 WG	2.0
	500 g/kg + 250 g/kg 500 g/kg rimsulfuron + 250 g/kg thifensulfuron-methyl	75 + 90 Basis 75 DF + Trend 90	2.5
<i>D.glomerata</i>	45 g/l + 5 g/l 45 g/l pnioksaden + 5 g/l florasulam	Axial One e	100.0
	600 g/kg - 600 g/kg Metsulfuron-methyl	60 ccurate 60 WG	1.0
	30 g/kg + 90 g/kg - + 10 g/kg - 30 g/kg mesosulfuron-methyl + 10 g/kg iodosulfuron-methyl sodium + 90 g/kg mefenpyr-diethyl	+ Pacifica + Biopower	35.0
<i>S. sudanense</i>	375 g/l s- + 125 g/l + 37,5 g/l	538 Lumax 538 SC	300.0
	375 g/l s-metolachlor + 125 g/l terbuthylazine + 37.5 g/l mesotrione		
	44 g/l + 22 g/l - 44 g/l timmoturon + 22 g/l isoxadifen-ethyl	Laudis OD	200.0
	50 g/l + 160 g/l 50 g/l topramesone + 160 g/l dicamba	210 Stelar 210 SL	100.0
	22,5 g/l + 22,5 g/l 22.5 g/l foramsulfuron + 22.5 g/l antidote	Equip SK	250.0
	30 g/kg + 90 g/kg - + 10 g/kg - 30 g/kg mesosulfuron-methyl + 10 g/kg iodosulfuron-methyl sodium + 90 g/kg mefenpyr-diethyl	+ Pacifica + Biopower	35.0
	45 g/l + 5 g/l 45 g/l pnioksaden + 5 g/l florasulam	Axial One e	100.0
	70.8 g/kg + 14.2 kg/da 70.8 g/ g	+ Corello Duo + Cloquintocet	26.5
	750 g/kg 750 g/kg of tribenuron methyl	75 Corrida 75 DVG	1.5
	45 g/l / 45 g/l pnioksaden	/ Axial	90.0
	600 g/l 2,4 / 600 g/l 2,4 D amine salt	/ Dickopour F	120.0

(DAT) 21st day after treatment (DAT) the selectivity of the herbicides on the crop plants was visually determined using the EWRS (European Weed Research Society) – score from 1 to 9 (score 1 – without damages; score 9 – the crop is completely destroyed).

For the purposes of the study on the 21st day after treatment (DAT) the selectivity of the herbicides on the crop plants was visually determined using the EWRS (European Weed Research Society) score from 1 to 9 (score 1 – without damages; score 9 – the crop is completely destroyed).

21st day after treatment (DAT) to determine the content of plastid pigments (chlorophyll a, chlorophyll b, carotenoids, and total a+b). The content of photosynthetic pigments was determined spectrophotometrically by Zelenski and Mogileva (1980). Degree of inhibition (R_i) of plastid pigments (chlorophyll a + b and carotenoids) was determined by the equation (1):

$$R_i = \left\{ \frac{\ln N_1 - \ln N_0}{t} \right\} \cdot 100 \quad (1)$$

where $\ln N_1$ – characteristics in each treatment, $\ln N_0$ – characteristic in the control treatment, t – duration after treatment, days.

Major agro-meteorological characteristics of the period of study were recorded: rainfall amount (in mm) and average 24-hour air temperature on average for the growing season (in °C). The Marton's index was used to characterize the aridity (de Marton, 1926; Paltineanu et al., 2007). The collected data were analyzed using the software STATGRAPHICS Plus for Windows Version 2.1

Samples of fresh plant biomass were taken on the 21st day after treatment (DAT) to determine the content of plastid pigments (chlorophyll a, chlorophyll b, carotenoids, and total a+b). The content of photosynthetic pigments was determined spectrophotometrically by Zelenski and Mogileva (1980).

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RESULTS AND DISCUSSION

Estimating the complex effect of some major meteorological factors, rainfall amount and average 24-hour air temperatures, the studied years can be divided conventionally into two groups: 2014 and 2015 – conditions for the growth and development of the tested annual and perennial cereal forage crops at aridity (I_{ar-DM}) of the growing season (III-IX): 29.6 and 25.41, respectively and 2016 – unfavourable at aridity of 22.3 (Table 2).

The total rainfall amount during the period of study can be presented in the following ascending order: 2016<2015<2014 and in inverse relation 2014<2015<2016

2015 . – : 2014

(I_{ar-DM}) (III-IX)

: 29,6 25,4 2016 . – - 22.3 (2).

2016<2015<2014,

2014<2015<2016

(
2014 .)
-1.3 +3.4⁰
314,8%
(12,7
2).

- with regard to average 24-hour air
- temperatures. The agro-meteorological
- conditions during the years of study
(except for 2014) showed temperature
- deviations from -1.3 to +3.4⁰ and
- stronger variability in the rainfall amount
- from 12.7 to 314.8%, as compared to
- those for the many-year period (Table 2).

2.
(2014-2016 .)

Table 2. Meteorological indicators during the years and period average (2014-2016)

Period of study	/ Vegetation period							III - IX, t ⁰ C Average for III - IX, t ⁰ C
	The average monthly emperature of the air, t ⁰ C							
	III	IV	V	VI	VII	VIII	IX	
2014	9.7	14.9	16.7	20.6	23.1	23.7	17.9	18.1
<i>Deviation⁰</i>	3.3	2.9	-1.0	-0.6	-0.3	0.8	-0.4	0.0
2015	6.8	12.2	18.8	20.7	25.5	24.4	20	18.3
<i>Deviation⁰</i>	0.4	0.2	1.1	-0.5	2.1	1.5	1.7	0.2
2016	8.5	15.4	16.4	23	24.6	23.5	19.4	18.7
<i>Deviation⁰</i>	2.1	3.4	-1.3	1.8	1.2	0.6	1.1	0.6
<i>50 . (1964-2013) Average 50 years (1964-2013)</i>	6.4	12.0	17.7	21.2	23.4	22.9	18.3	18.1
Period of study	/ Vegetation period							III - IX, mm Amount for III - IX, mm
	The average monthly emperature of the air, t ⁰ C							
	III	IV	V	VI	VII	VIII	IX	
2014	39.7	32.3	83	54.3	71.8	23.9	142.6	447.6
<i>e, % / Deviation,%</i>	111.5	66.3	132.0	85.2	116.7	52.5	314.8	123.2
2015	76.9	43.6	30.6	95.9	21.5	29.9	130.3	428.7
<i>e, % / Deviation,%</i>	216.0	89.5	48.6	150.5	35.0	65.7	287.6	118.0
2016	68.4	72.5	77.2	46.1	7.8	31.2	61.8	365.0
<i>e, % / Deviation,%</i>	192.1	148.9	122.7	72.4	12.7	68.6	136.4	100.5
<i>50 . (1964-2013) Average 50 years (1964-2013)</i>	35.6	48.7	62.9	63.7	61.5	45.5	45.3	363.2
Period of study	De Martonne De Martonne aridity index, I _{ar-DM}							III - IX Average for III - IX
	III	IV	V	VI	VII	VIII	IX	
	2014	46.8	15.6	37.3	21.3	26	8.5	
2015	48.9	23.6	12.8	37.5	7.3	10.4	52.1	25.4
2016	49.9	34.3	35.1	16.8	2.7	11.2	25.2	22.3
<i>50 . (1964-2013) Average 50 years (1964-2013)</i>	26	26.6	27.3	24.5	22.1	16.6	19.2	22.7

.)
(3)
(2).

- The visual assessment of the
- selectivity of tested herbicides in annual
- and perennial cereal forage crops,
- expressed by different visible symptoms
- of phytotoxicity (chlorosis, necrosis, etc.),
- depends on the type of applied herbicide
- (Table 3) and the agrometeorological
- conditions during the study period (Table 2).

3.

2014-2016

Table 3. Plastid pigments content in some annual and perennial cereal forage crops for the period 2014-2016

Species	Years	Herbicides	Dose, g/ (ml)/da	, mg/100g FW / Plastid pigments mg/100g FW								
				/ Chlorophyll			Carote noids	Total	% Ct	R _i	EMPS	
				a	b	a+b						
<i>L. perenne</i>	2014	/Control		96.38c	67.26c	163.64c	31.26b	194.90b	100.0	0.0	1.0	
		e /Axial	100.0	52.67a	34.53a	87.20a	20.20a	107.4a	55.1	-2.8	3.0	
		60 ccurate 60WG	1.0	95.7b	63.50b	159.28b	37.36c	196.64c	100.9	0.0	6.0	
	2016	/Pacifica	35.0	*	*	*	*	*	*	*	*	8.5
		/Control		128.78d	101.70d	230.48d	50.08d	280.56d	100	0.0	1.0	
		/Ally Max	3.5	114.92c	94.94c	209.86c	36.90c	246.76c	88.0	-0.6	4.0	
	75 /Eagle75WG	2.0	95.56b	79.44b	175.00b	29.92b	204.92b	73.0	-1.5	7.0		
	75 /Basis	2.5	24.18a	30.44a	54.62a	14.47a	69.09a	24.6	-6.7	5.0		
	75DF											
<i>D. glomerata</i>	2014	/Control		88.84a	60.12a	148.96a	32.76a	181.72a	100.0	-0.3	1.0	
		e /Axial	100.0	115.95b	77.40b	193.35b	41.58b	234.93b	129.3	0.9	6.0	
		60 ccurate 60WG	1.0	*	*	*	*	*	*	*	*	8.5
	2016	/Pacifica	35.0	*	*	*	*	*	*	*	8.5	
		/Control		99.48	67.34	166.82	37.28	204.10	100.0	0.2	1.0	
		e /Axial	100.0	*	*	*	*	*	*	*	*	8.5
<i>F. arundinaceae</i>	2014	60 ccurate 60WG	1.0	*	*	*	*	*	*	*	8.5	
		/Pacifica	35.0	*	*	*	*	*	*	*	8.5	
		/Control		102.48d	76.52d	179.00d	30.98d	209.98d	100	-1.4	1.0	
	2016	/Ally Max	3.5	78.32c	66.40c	144.72c	24.88c	169.6c	80.8	-2.4	4.5	
		75 /Eagle75WG	2.0	64.27b	49.79b	114.06b	24.13b	138.19b	65.8	-3.4	3.5	
		75 /Basis	2.5	28.27a	29.30a	57.57a	13.49a	71.06a	33.8	-6.5	4.5	
	2014	75DF		107.74b	72.24b	179.98b	33.74b	213.72b	100.0	0.4	1.0	
		e /Axial	100.0	88.12a	62.34a	150.46a	29.72a	180.18a	84.3	-0.4	3.0	
		60 ccurate 60WG	1.0	127.74c	83.76c	211.50c	40.78c	252.28c	118.0	1.2	4.0	
<i>. desertorum</i>	2016	/Pacifica	35.0	*	*	*	*	*	*	*	8.5	
		/Control		123.02c	104.90c	227.92c	33.86c	261.78c	100	-0.3	1.0	
		/Ally Max	3.5	91.74a	76.14a	167.88a	30.22a	198.1a	75.7	-1.7	6.5	
	2014	75 /Eagle75WG	2.0	105.12b	81.84b	186.96b	34.22b	221.18b	84.5	-1.1	8.0	
		75 /Basis	2.5	*	*	*	*	*	*	*	9.0	
		75DF										
<i>S. sudanense</i>	2015-2016	/Control		92.71i	68.89ef	172.70d	32.54ab	205.24fg	100.0	0	1.0	
		538 Lumax 538SC	300.0	129.26j	84.50f	213.76e	37.27ab	251.03g	135.2	1.4	2.5	
		/Laudis	200.0	61.65e	48.08abcd	109.73bc	21.98ab	131.71bcd	71.0	-1.6	3.4	
		210 Stelar 210SL	100.0	49.04b	34.18ab	83.21ab	18.20ab	101.41abc	54.6	-2.9	5.2	
		/Equip SK	250.0	130.77k	43.41cd	108.80c	36.18ab	144.98de	78.1	-1.2	8.0	
		/Pacifica	35.0	51.26c	18.00a	43.63a	14.56ab	58.19a	31.3	-5.5	7.4	
		e /Axial	100.0	52.72d	36.20abc	88.91abc	19.30ab	108.21abcd	58.3	-2.6	6.9	
		One										
		Corello Duo	26.5	62.12f	43.30abc	105.42bc	22.24ab	127.66bcd	68.8	-1.8	7.5	
		75 Corrida 75DVG	1.5	77.55h	48.65bcd	126.20c	25.41ab	151.60cde	81.7	-1.0	6.0	
		/Axial	90.0	36.56a	27.64b	64.20a	11.99a	76.19ab	41.0	-4.2	8.0	
		/Dickopour F	120.0	76.52g	49.78bcd	126.30c	25.12b	151.42cde	81.6	-1.0	4.0	

: Ct% - ; EWRS (European Weed Research Society) - ; Ri - ; FW - ; a, b, c, d, j ; P = 0,05.

Legend: Ct% - Percentage compared to the control treatment, %; Ri - degree of inhibition; FW - fresh weight; EWRS (European Weed Research Society) score from 1 to 9 (score 1 - without damages; score 9 - the crop is completely destroyed); * - was not specified content of plastid pigments because of reported high phytotoxicity in the treatments; a, b, c, d, j statistically significant differences in P = 0.05

(1 3) 21 -
 (4.5 9.0).
 (3-4)
 L.
L. perenne, 60
A. desertorum, 75
D. glomerata,
 568 ,
S. sudanense

For all species of annual and perennial forage crops the tested herbicides (Table 1 and 3) on the 21st day after treatment had a strong phytotoxic effect (from 4.5 to 9.0 score).

A relatively weak phytotoxicity effect (score 3-4) was reported after application on Axial One and Alay Max in *L. perenne*, Axial One and Acurate 60 WG in *A. desertorum*, Eagle 75 WG after treatment with *D. glomerata*, and Lumax 568 SC, Laudis OD and Dicuplor F in *S. sudanense* in early stages of crop development.

The results of the analysis of the total content of plastid pigments and chlorophyll for all treatments of the experiments are presented in Table 3.

Low values of chlorophyll a were reported in all treatments of annual and perennial cereal forage crops (from 24.18 to 114.92 mg/100g FW) as compared to control treatments (88.48 to 128.78 mg/100g FW).

mg/100g FW) (24,18 114,92 -
 mg/100g FW). (88,48 128,78 -

From the analysis of the data shows that the content of chlorophyll b is relatively less in the compared to the established for chlorophyll a (Table 3). This dependence can be explained by the phytotoxic effect of the herbicides and the biological characteristics of the tested forage crops.

3).
 Kannangara & Hanson (1998); Nacheva et al. (2012)

According to the studies of Kannangara & Hanson (1998); Nacheva et al. (2012) at the stress, plants increase the synthesis of anthocyanins.

For the conditions of the study, it was found that decreasing the content of photosynthetic plastid pigments as an indicator of phytotoxicity of herbicides confirms the observed visible symptoms of chlorosis in annual and perennial

(Tonev, 2000; Kasparý, 2014).

○

(EWRS)

(r) : *L. perenne* -0,567; *D. glomerata* -0,701; *F. arundinaceae* -0,957; *A. desertorum* -0,703 *S. sudanense* -0,740.

(EWRS)

(1).

().

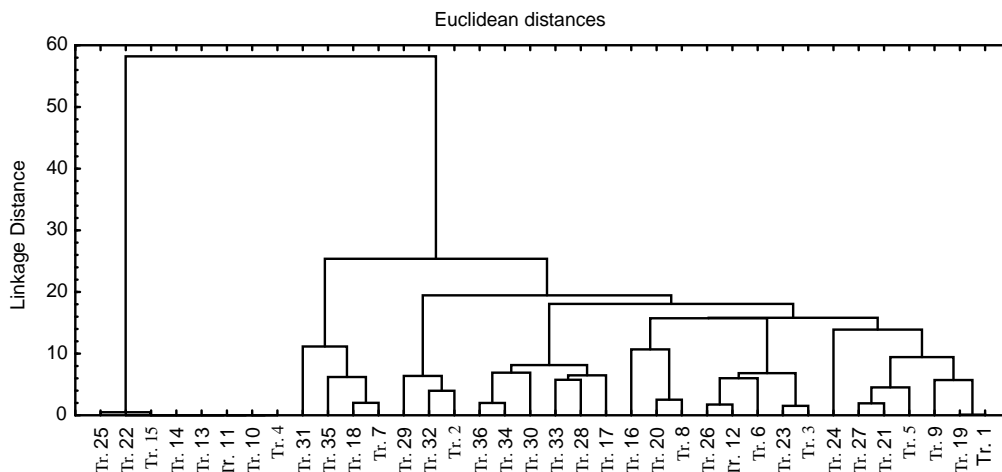
S. sudanense (69,2%)
L. perenne (15,4%) *F.*
arundinaceae (15,4%).
A.
desertorum; *F. arundinaceae*; *L. perenne*
S. sudanense,
L. perenne (37,5%).
A. desertorum; *D. glomerata*; *L. perenne*
S. sudanense,
A. desertorum – 42,9%,
L. perenne 28,5%
D. glomerata *S. sudanense* – 14,3% .

This dependence can be explained by the selectivity of the active substances of the tested herbicides and their ability to exert an inhibitory effect on the synthesis of photosynthetic plastid pigments (Tonev, 2000; Kasparý, 2014).

On the basis of the obtained results of the photosynthetic pigments content in above-ground biomass and the visual phytotoxicity assessment (EWRS) of the tested herbicides in annual and perennial cereals forage crops, correlation relations were found where the correlation coefficient (r) significant correlations as follows: *L. perenne* -0.567; *D. glomerata* -0.701; *F. arundinaceae* -0.957; *A. desertorum* -0.703 and *S. sudanense* -0.740.

Based on the values of the content of plastid pigments, carotenoids according to the scores assessment of phytotoxicity (EWRS) of the test herbicides were made hierarchical cluster analysis to group the test species of annual and perennial cereal forage crops. Data from the analysis are presented graphically in the form of a dendrogram (Figure 1).

There were 5 groups (clusters) as a result of the study. The first three clusters were formed with predominant involvement of *S. sudanense* (69.2%) and negligible of the species *L. perenne* (15.4%) and *F. arundinaceae* (15.4%). Four species of *A. desertorum* are combined; *F. arundinaceae*; *L. perenne* and *S. sudanense*. In the fourth cluster, *L. perenne* (37.5%) occupies the largest share. The fifth cluster is formed by the species *A. desertorum*; *D. glomerata*; *L. perenne* and *S. sudanense*, the prevalence of *A. desertorum* is 42.9% and the other three types are presented as follows *L. perenne* 28.5%, followed by *D. glomerata* and *S. sudanense* - 14.3%.



/Legend: *L. perenne* [Tr.1 - /Control untreated; Tr.2 - Axial One; Tr.3 - ccurate 60WG; Tr.4 - Pacifica; Tr.5 - Ally Max; Tr.6 - Eagle75WG; Tr.7 - Basis 75DF]. *D. glomerata* [Tr.8 - /Control untreated; Tr.9 - Axial One; Tr.10 - ccurate 60 WG; Tr.11 - Pacifica]. *F. arundinaceae* [Tr.12 - /Control untreated; Tr.13 - Axial One; Tr.14 - ccurate 60 WG; Tr.15 - Pacifica; Tr.16 - Ally Max; Tr.17 - Eagle75 WG; Tr.18 - Basis 75DF]; *A. desertorum* [Tr.19 - /Control untreated; Tr.20 - Axial One; Tr.21 - ccurate 60WG; Tr.22 - Pacifica; Tr.23 - Ally Max; Tr.24 - Eagle75WG; Tr.25 - Basis 75DF]. *S. sudanense* [Tr.26 - /Control untreated; Tr.27 - Lumax 538SC; Tr.28 - Laudis OD; Tr.29 - Stelar 210SL; Tr.30 - Equip SK; Tr.31 - Pacifica; Tr.32 - Axial One; Tr.33 - Corello Duo; Tr.34 - Corrida 75DVG; Tr.35 - Axial; Tr.36 - Dickopour F].

. 1.

(EWRS)

Fig. 1. The dendrogram of the clustering of the species of annual and perennial cereals forage crops, type distribution based on the content of photosynthetic plastid pigments and balls assessment of phytotoxicity (EWRS) of the herbicides applied post emergences of the crops

21

The experimental results obtained showed that the determination of the photosynthetic pigments content on 21 days after treatment with herbicide can be used as a test for evaluation of the selectivity of post-emergent herbicides in the annual and perennial cereal forage crops tested.

CONCLUSIONS

sudanense - BBCH 12-13
L. perenne, *D. glomerata*, *F. arundinaceae*
A. desertorum BBCH 12-14

74.7%,

S.

The application of herbicides on *S. sudanense* - in growth stage BBCH 12-13 and *L. perenne*, *D. glomerata*, *F. arundinaceae* and *A. desertorum* in growth stage BBCH 12-14 in the crop year reduced the accumulation of

(R_i) -1.0 -5.5

21

60 1.0 g/da A.

desertorum 100.0

ml/da *D. glomerata* (15,9

18,2%) (1,3 20,9%),

(EWRS)

: *L. perenne* -0,567; *D. glomerata* -0,701; *F. arundinaceae* -0,957; *desertorum* -0,703 *S. sudanense* -0,740.

photosynthetic pigments chlorophyll a, b and carotenoids) to 74.7%, with a degree of inhibition (R_i) of -1.0 to -5.5 in the particular agrometeorological conditions.

The total chlorophyll content was increased 21 days after the administration of Acrust 60 WG at a dose of 1.0 g/da in *A. desertorum* and Axial One at a dose of 100.0 ml/da for *D. glomerata* (15.9 to 18.2%) and carotenoids (from 1.3 to 20.9%) versus control treatments.

Statistically significant negative correlations between the content of Photosynthetic plastid pigments and visual evaluation scores for phytotoxicity (EWRS) based on applied herbicides in annual and perennial cereal forage crops as follows: *L. perenne* -0.567; *D. glomerata* -0.701; *F. arundinaceae* -0.957; *A. desertorum* -0.703 and *S. sudanense* -0.740 were established

The experimental results obtained showed that the determination of the photosynthetic pigments content on 21 days after treatment with herbicide can be used as a test for evaluation of the selectivity of post-emergent herbicides in the annual and perennial cereal forage crops tested.

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**Comparative biological and economic characteristics
of perspective forms Sudan grass (*Sorghum sudanense*
(Piper) Stapf.) II. For forage**

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SUMMARY

<p>2014-2016</p> <p style="text-align: center;">2</p> <p style="text-align: center;">(M 300/43 200/86).</p> <p>Kazitachi 1 (<i>Sorghum</i> <i>vulgare</i> var. <i>saccharatum</i> <i>Sorghum</i> <i>vulgare</i> var. <i>sudanense</i> (Piper) Stapf).</p> <p style="text-align: center;">BBCH-47),</p> <p>2015</p> <p style="text-align: center;">50%</p> <p>300/43, 16,9 16,0% Kazitachi, 1 - 10,7 12,4% (Kazitachi + 1) 13,7</p>	<p>During the period 2014-2016 year in the experimental field of the Institute of Forage Crops – Pleven in terms of competitive variety trials were studied 2 mutant forms Sudan grass (M 300/43 and 200/86). Sudan grass variety Kazitachi and Endje 1 (<i>Sorghum vulgare</i> var. <i>saccharatum</i> <i>Sorghum vulgare</i> var. <i>sudanense</i> (Piper) Stapf) are used as standards. Some biometric indicators have been studied with a direct relation to the productivity of fresh biomass. For forage (cutting in BBCH-47) three cuts were harvested, for growth stage period. Four cuts in 2015 were harvested. On average for the period, the share of the first cutting is highest in all variants, exceeding 50% of the total biomass. For the period mutant form M 300/43 formed the highest amount of fresh and dry biomass, which exceeds respectively by 16.9 and 16.0% variety Kazitachi, Endje 1 - 10.7 and 12.4% and the average standard (Kazitachi + Exceptions 1) respectively 13.7 and 9.1%. The mutant</p>
---	--

9,1%.

-

25,5 39,8%,
14,5 27,1%

Kazitachi
4,8%
6,5%.

: *Sorghum*
sudanense (Piper) Stapf.,

(*Sorghum sudanense* (Piper) Stapf.)
Sorghum

2-3
(Zamfir et al. 2001;
Kikindonov et al., 2016).

(Moyer et al., 2003;
Tahir et al., 2005; Kikindonov et al.,
2013).

(Glamoclija et al.
2010).

(Slanev et al., 2015).

(Enchev et al., 2016).

form produces a relatively higher share of fresh biomass during the summer droughts in years of the study reaching a second cut of 25.5 to 39.8% and in third cut from 14.5 to 27.1% of the total amount of fresh biomass for a vegetation period. The mutant form exceeds the original variety Kazitachi with content of crude protein 4.8% and water-soluble sugars by 6.5%.

Key words: *Sorghum sudanense* (Piper) Stapf., breeding, mutant forms, biomass yield

INTRODUCTION

Thipical of Sudan grass (*Sorghum sudanense* (Piper) Stapf.) is the rapid growth and productivity of fresh biomass during the warm months, providing 2-3 otters per vegetation period (Zamfir et al., 2001; Kikindonov et al., 2016).

Sudan grass is used mainly for grazing, hay and silage, thanks to its high productive potential and its valuable economic qualities, especially in drought conditions (Moyer et al., 2003; Tahir et al., 2005; Kikindonov et al., 2013).

Sudan grass plays an important role in solving the problem of shortage of quality forage and the introduction of a large number of forage crops in the production and green feed conveyor system (Glamoclija et al., 2010). Sudan grass productivity is heavily influenced by agrometeorological factors and growing conditions, and has exceptional plasticity with respect to self-regulation of seed density by braking (Slanev et al., 2015).

Priority in fodder crop breeding programs is to increase the productivity of green biomass, the height of the growing season and the quality of the raw material (Enchev et al., 2016).

2007).

(Rooney et al.,

(Assis et al., 2008).

Thanks to the selection programs and seed production technologies, productivity of biomass from Sudan grass is increased (Rooney et al., 2007). The main purpose in the selection of the forage species is to increase the fresh and dry matter and the quality of the forage (Assis et al., 2008).

The aim of the study is to evaluate the mutant forms of fresh biomass production under the conditions of a competitive variety experience.

MATERIAL AND METHODS

2014-2016
-
M 300/43 M 200/86
(*Sorghum vulgare* var. *sudanense* (Piper) Stapf.)

Kazitachi (*Sorghum vulgare* var. *sudanense* (Piper) Stapf.)

1,

(*Sorghum vulgare* var. *sudanense* (Piper) Stapf x *Sorghum vulgare* var. *saccharatum*) (Kikindonov and Slanev, 2011)
(1 Kazitachi).

10 m²

().

10

15 30

(Dechev et al., 1987).

2015

The experimental work was carried out during 2014-2016 on the Second Experimental Field of IFC - Pleven under non-irrigated conditions on soil subtype leached black earth. In three consecutive years, a commercial evaluation of two mutant forms Sudan grass M 300/43 and M 200/86 (*Sorghum vulgare* var. *sudanense* (Piper) Stapf.) was carried out in terms of the dynamics of fresh and dry biomass accumulation in a competitive variety experience. Variety Kazitachi (*Sorghum vulgare* var. *sudanense* (Piper) Stapf.) – the originating variety for the mutant forms and variety Endje 1, registered in the Bulgarian official catalogue of varieties (*Sorghum vulgare* var. *sudanense* (Piper) Stapf x *Sorghum vulgare* var. *saccharatum*) (Kikindonov and Slanev, 2011) and the average standard (Endje 1 and Kazitachi) were used for the standard variety. The experience is based on a block method in four replicates and the size of the experimental plot of 10 m² (according to IASAS methodology). Sowing is done manually with permanent warming of the topsoil above 10°C between 15th and 30th April. Events have been done depending on the biological requirements of the crop, as well as the technology for cultivation of sorghum for grain and silage (Dechev et al., 1987). In the years of study, three and in the year 2015 four cuts were formed and harvested, depending on

(BBCH 45-49) (Meier, 2001).

(N) = N x 6.25;
() - ;
() - (Sandev, 1979);
-
550 °C;
() - Weende
(2007)
() (Ermakov et al., 1987),
(I_{ar}-DM)

(1926).
De Martonne

STATGRAPHYCS plus for Windows
Statistica version 10.

2013 (1).

2014
(BBCH 09-11/13).

agrometeorological conditions in growth stage booting (BBCH 45-49) (Meier, 2001).

A biochemical analysis of all Sudan grass genotypes has been carried out, which includes indicators for the basic biochemical composition and nutritional value of the forage: Crude protein (CP) by the classical Keldahl method (Sandev, 1979), after determining the amount of total nitrogen (CP) = N x 6.25; Calcium (Ca) – complexometrically; Phosphorus (P) – colorimetric (Sandev, 1979); Ash – by burning in a muffle furnace at a temperature of 550 °C; Crude fiber (CF) (Webere method) (AOAS 2007) and Water-soluble sugars (WSS) (Ermakov et al., 1987) as a percentage of the dry matter.

The dryness index (I_{ar}-DM) for the vegetation period was determined by the classic method of De Martonne (1926).

The mathematical and statistical processing of the experimental data was performed with the software STATGRAPHYCS plus for Windows and Statistica version 10.

RESULTS AND DISCUSSION

In agrometeorological terms, the years of the study (2014-2016) differ significantly in the amount and distribution of rainfall and relatively less in relation to the average daily air temperature dynamics during the vegetation period of the Sudan compared to the average multiannual values for the 1964-2013 (Table 1).

Distributions of rainfall are relatively favourable and evenly in 2014, against the critical growth stages from culture development - germination/first-third leaf (BBCH 09-11/13).

In relation to temperature the vegetation period of study is characterized by sub-

-1.0°),
 (+ 0.8 +2.9°)
 1964-2013 .

normal mean monthly values (from -0.3 to -1.0°C) compared to the multiannual period (1964-2013), with the exception of April and August (from + 0.8 to +2.9°C).

1.
 (2014-2016 .)

Table 1. Meteorological indicators during the years and period average (2014-2016)

Period of study	/ Vegetation period						IV-IX, t°C Average for IV-IX, t°C
	The average monthly temperature of the air, t°C						
	IV	V	VI	VII	VIII	IX	
2014	14,9	16,7	20,6	23,1	23,7	17,9	19,5
<i>° /Deviation°</i>	2,9	-1	-0,6	-0,3	0,8	-0,4	0,2
2015	12,2	18,8	20,7	25,5	24,4	20,0	20,3
<i>° /Deviation°</i>	0,2	1,1	-0,5	2,1	1,5	1,7	1,0
2016	15,4	16,4	23,0	24,6	23,5	19,4	20,4
<i>° /Deviation°</i>	3,4	-1,3	1,8	1,2	0,6	1,1	1,1
2014-2016 Average 2014-2016	14,2	17,3	21,4	24,4	23,9	19,1	20,1
45 . (1964-2013) Average 45 years (1964-2013)	12,0	17,7	21,2	23,4	22,9	18,3	19,3
Period of study	, mm						IV-IX, mm Amount for IV-IX, mm
	Monthly rainfall, mm						
	IV	V	VI	VII	VIII	IX	
2014	32,3	83	54,3	71,8	23,9	142,6	407,9
<i>e, % / Deviation, %</i>	66,3	132,0	85,2	116,7	52,5	314,8	124,5
2015	43,6	30,6	95,9	21,5	29,9	130,3	351,8
<i>e, % / Deviation, %</i>	89,5	48,6	150,5	35,0	65,7	287,6	107,4
2016	72,5	77,2	46,1	7,8	31,2	61,8	296,6
<i>e, % / Deviation, %</i>	148,9	122,7	72,4	12,7	68,6	136,4	90,5
2014-2016/ Average 2014-2016	49,5	63,6	65,4	33,7	28,3	111,6	352,1
45 . (1964-2013) Average 45 years (1964-2013)	48,7	62,9	63,7	61,5	45,5	45,3	327,7
Period of study	De Martonne De Martonne aridity index, I _{ar-DM}						IV-IX Average for IV-IX
	IV	V	VI	VII	VIII	IX	
	2014	15,6	37,3	21,3	26,0	8,5	
2015	23,6	12,8	37,5	7,3	10,4	52,1	23,3
2016	34,3	35,1	16,8	2,7	11,2	25,2	19,5
45 . (1964-2013) Average 45 years (1964-2013)	26,6	27,3	24,5	22,1	16,6	19,2	22,4

2015
 (+0.2 +2.1°) -
 1964–2013.

In the 2015 vegetation period, higher values (from +0.2 to +2.1°) of the average monthly air temperature were reported except in June compared to the same for the average period of 1964-2013.
 -
 -
 -
 -
 - The quantity and distribution of precipitation is of significant unevenness, a pronounced tendency to drought is

observed in the most critical growth stages of the development of the first leaf - germination (BBCH 09-11).

In 2016 the vegetation period is the Sudan grass is relatively well secured with precipitation. The high amounts of vegetative precipitation (from 122.7 to 148.9%) and their relatively favorable distribution in the critical growth stages from the culture development have a corrective effect on the elevated values of the average monthly air temperatures (+ 3.4°C), which promotes uniform germination and garnishing of crops.

Assessing the integral impact of some of the major meteorological factors – rainfall and average monthly air temperatures during the growing season of crops (IV - IX) is determined by years as follows: 2014 semi-wet ($I_{(Marton)} = 27,7$) > 2015 moderately dry ($I_{(Marton)} = 23,3$) > 2016 semi-dry ($I_{(Marton)} = 19,5$) (Table 1).

The expressed agrometeorological differences during the period of study are a prerequisite for enhancing the deficiency between individual Sudan grass genotypes in the formation of fresh and dry biomass yields (Figure 1 and 2). The average yield of genotypes ranges from 3463.29 to 4270.91 kg/da fresh and dry biomass from 669.92 to 859.84 kg/da.

Relatively high yields were reported in 2014 and 2016 when rainfall was comparatively favorable compared to the biological requirements of culture and in the relatively unfavorable year 2015 were the lowest.

The tested genotypes differ significantly in productivity. Average for the period the largest amount of fresh biomass is formed by the mutant form M 300/43, which exceeds Kazitachi by 16,9%, Endje 1 - 10,7% and Kazitachi + Engje 1, respectively, by 13,9%.

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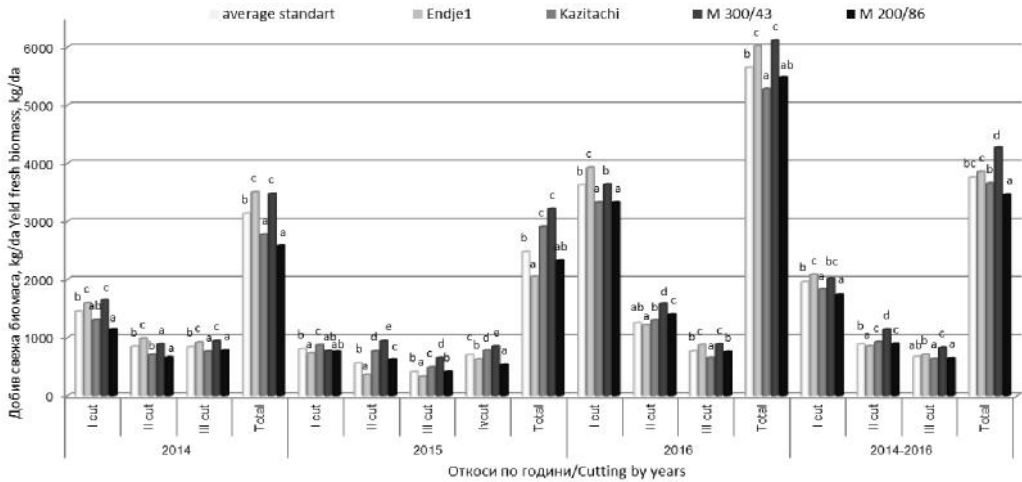
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13,7% (Kazitachi + 1)
 =0,05
 1,
 5,3 10,2% Kazitachi
 M 200/86 (1).

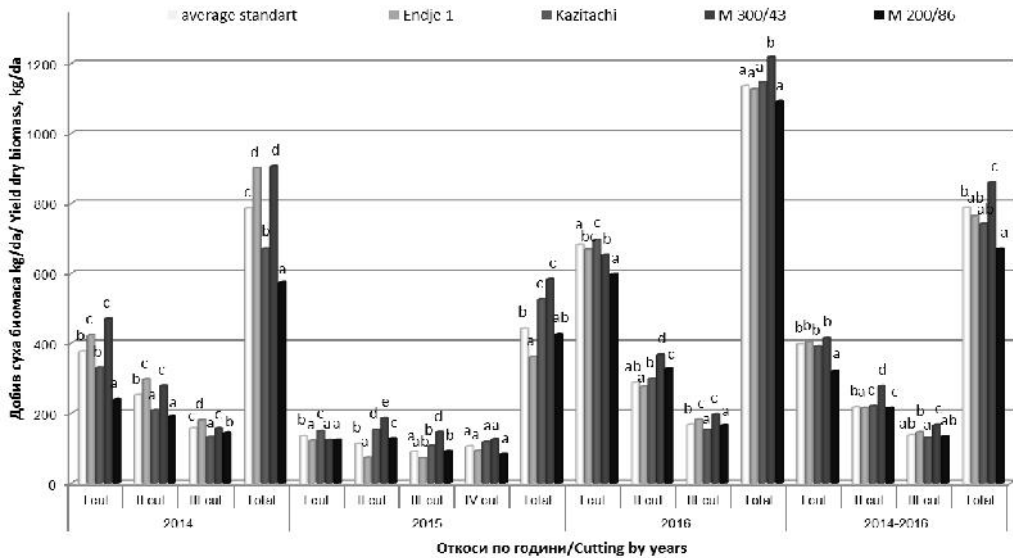
The results obtained were statistically proven at P = 0.05 and during the three years of the study, regardless of the agrometeorological conditions dynamics. High yield of fresh biomass is obtained from the variety Endje 1, which exceeds from 5.3 and 10.2% respectively Kazitachi variety and mutant M 200/86 (Figure 1).



1. 2014-2016
Fig. 1. Yield of fresh biomass by cuts, by year and average for the period 2014-2016

300/43,
 M 200/86,
 (=0,05)
 Kazitachi
 1,
 (Kazitachi+ 1) (2).

The results obtained with respect to the yield of dry biomass per unit area retain the differentiation between the individual Sudan grass genotypes. The highest yield of dry biomass by years and the study period was formed by the mutant form M 300/43 and relatively lower than the mutant form M 200/86, the differences being statistically proven (P = 0.05) compared with the reported values for the Kazitachi and Endje 1 varieties, as well as the average standart (Kazitachi + Endje 1) (Figure 2).



2.

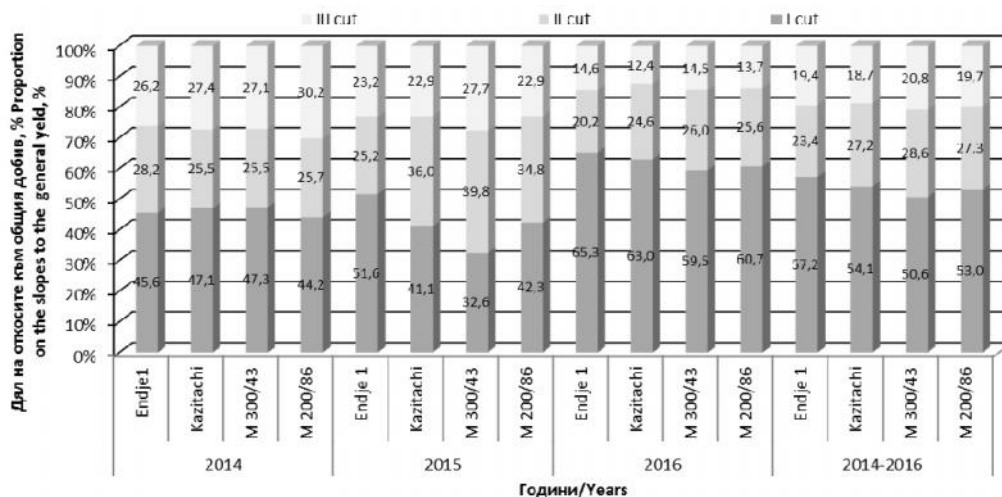
2014-2016

Fig. 2. Yield of dry biomass by cuts, by year and average for the period 2014-2016

Year	Cut	average standart	Endjc 1	Kazitachi	M 300/43	M 200/86
2014	I cut	320	350	380	450	250
	II cut	250	280	300	320	280
	III cut	180	200	220	250	150
	Total	750	830	900	1020	680
2015	I cut	150	180	200	220	120
	II cut	120	150	180	200	100
	III cut	100	120	150	180	80
	Total	370	450	530	600	300
2016	I cut	450	500	550	600	400
	II cut	300	350	400	450	300
	III cut	200	250	300	350	250
	Total	950	1100	1250	1400	950
2014-2016	I cut	350	400	450	500	350
	II cut	250	300	350	400	250
	III cut	180	220	280	320	180
	Total	780	920	1080	1220	780

The Sudangrass genotypes revealed that during the years of the study, the largest share of the total amount of fresh biomass per unit area occupied a first cut (from 32.6 to 65.3%), followed by a second (from 20.2 to 39.8%) and the smallest share occupies a third cut (from 12.4 to 30.2%) (Figure 3). Average for the period, the trend remains – the largest share of the first cut from 50.6 to 57.7%, followed by a second cut (from 23.4 to 28.6%) and a third cut (18.7 to 20.8%).

With a relatively higher proportion of fresh biomass formed during the summer droughts, the mutant form M 300/43 – In the second cut from 25.5 to 39.8% and in the third cut from 14.5 to 27.1%.



. 3.

2014-2016

Fig. 3. Shares of the cuts to the total biomass yield of fresh biomass at year and average for 2014-2016

(mm) (°C)
2).

Correlations between the formed fresh and dry biomass yields and the influence of some of the main meteorological factors - average daily air temperature (°C) and rainfall (mm) (Table 2) are established.

2.

2014-2016

Table 2. Correlation between the yield of fresh and dry biomass and average 24^{hour} air temperature and precipitation for the period 2014-2016

Genotypes		/ Biomass yield			
		/ Fresh		/ Dry	
		(°) / Average 24 ^{hour} air temperature (°)		(mm) Precipitation (mm)	
I / cut	Endje 1	-0,353	0,042	0,705	0,891
	Kazitachi	-0,444	-0,288	0,603	0,751
	M 300/43	-0,317	0,074	0,730	0,937
	M 200/86	-0,667	-0,379	0,610	0,684
II / cut	Endje 1	-0,693	-0,896	0,809	-0,346
	Kazitachi	0,112	-0,353	-0,764	-0,386
	M 300/43	0,092	-0,493	0,751	-0,240
	M 200/86	-0,022	-0,285	-0,671	-0,451
III / cut	Endje 1	-0,714	-0,383	0,818	0,130
	Kazitachi	-0,903	-0,294	0,818	0,130
	M 300/43	-0,804	0,106	0,692	0,272
	M 200/86	-0,725	-0,438	0,599	0,280

0.937), (r 0.610 -

18,4 20,6%. -

(r -0,671 0,809) -

23,8 25,2% -

0.818). -0.714 -0.903 (r e 0.599 -

(2). -

E 1 M 300/43 -

M 200/86 12,57% 12,25%, -

Kazitachi. 4,8, 2,2 1,3% -

300/43 M 200/86 -

23,4 27,96%. -

E 1 2,6 3,4%. -

2011). (Silungwe, -

The amount of precipitation is a determining factor in the production of fresh and dry biomass in the first cut (r ranges from 0.610 to 0.937), with no significant differences between the Sudan grass genotypes. The dry matter in the formed biomass is lower than 18.4 to 20.6%. The influence of the factor rainfall is significant in the accumulation of fresh biomass (r is in the range of -0.671 to 0.809) per unit area in a second cut, which coincides with the summer droughts for the region. In this period, the dry matter reaches from 23.8 to 25.2% of the biomass in the Sudan grass genotypes.

In the formation of the extraction of fresh biomass, the impact of air temperatures (r is in the range of -0.714 to -0.903) and the amount of precipitation (r e from 0.599 to 0.818) are influenced by a third cut.

The results of biochemical analysis show variation between individual Sudan grass genotypes over the years and averaged over the agro meteorological conditions (Table 2).

The relative highest percentage of crude protein in above-ground biomass averaged over the study period was reported in the variety Endje 1 and mutant M 300/43 and M 200/86 ranges from 12.57% to 12.25%, which exceeds Kazitachi variety respectively by 4.8, 2.2 and 1.3%. The raw fiber content for Mutant forms M 300/43 and M 200/86 ranged from 23.4 to 27.96% during years of the study. On average, over the same period, they exceeded the insignificant indicator variety Endje 1 of 2.6 to 3.4%. Macroelements Ca and P play a very important role in the metabolism of plants.

They are also important for metabolic processes in animals (Silungwe, 2011). The percentage of phosphorus in the vegetative biomass of Sudan grass

0,250 0,332% 2016
 1,3 1,9
 2014 2015
 0,440 0,680,
 0,487% (M 200/86) 0,575% (E 1).
 (Soetan et al., 2010).
 (Iliev and Naidenova, 2016).
 E 1 (6,21%) M 200/86 (5,97%), Kazitachi M 300/43
 - 6,01 6,05%.

genotypes during the years of study ranges from a narrow diapason of 0.250 to 0.332%, with the exception of 2016 when the values are reduced from 1.3 to 1.9 times compare to 2014 and 2015.

The calcium content of vegetative biomass varies from 0.440 to 0.680, with mean differences over time between genotypes being insignificant and ranging from 0.487% (M 200/86) to 0.575% (Endje 1). The deficiency of these macroelements in forage has a negative impact on the growth and reproduction characteristics of the animals (Soetan et al., 2010).

The content of water-soluble sugars contributes significantly to the uptake, digestion and use of feed (Iliev and Naidenova, 2016). With a higher content of water-soluble sugars in above-ground dry biomass, the average duration of the study was Endje 1 (6.21%) and relatively less in mutant form M 200/86 (5.97%), whereas Kazitachi and mutant form M 300/43 occupy an intermediate position respectively of 6,01 and 6,05%.

3.

Table 3. Biochemical characterization of sudangrass

Years	Treatment	CP, %	CF, %	, %	, %	WSS, %	Ash, %
2014	Endje 1	10,03	26,23	0,250	0,550	6,10	8,45
	Kazitachi	11,00	27,25	0,310	0,490	5,63	8,04
	M 300/43	11,67	26,23	0,300	0,500	6,17	8,20
	M 200/86	11,94	26,34	0,300	0,480	5,67	8,14
2015	Endje 1	16,22	22,63	0,332	0,636	5,62	9,00
	Kazitachi	16,25	23,08	0,314	0,584	5,00	8,47
	M 300/43	16,28	23,40	0,328	0,680	5,17	8,90
	M 200/86	15,50	24,24	0,303	0,540	5,35	8,43
2016	Endje 1	10,18	26,74	0,193	0,540	6,90	8,46
	Kazitachi	8,71	28,01	0,213	0,450	6,40	8,23
	M 300/43	9,76	27,96	0,175	0,470	6,80	8,65
	M 200/86	9,30	27,63	0,176	0,440	6,90	8,23
2014-2016	Endje 1	12,14	25,20	0,258	0,575	6,21	8,64
	Kazitachi	11,99	26,11	0,279	0,508	5,68	8,25
	M 300/43	12,57	25,86	0,268	0,550	6,05	8,58
	M 200/86	12,25	26,07	0,260	0,487	5,97	8,27

3463,29 4270,91 kg/da
669,92 859,84 kg/da

0,809), 0.610 0.937 -0,671

-0.714 -0.903)
(r e 0.599 0.818).

(50,6 54,1%),
(23,4 28,6%)
(18,7 20,8%).

Kazitachi 10,7 12,4%
300/43,
16,0 16,7%

14,5 25,5 39,8%,
27,1%

Kazitachi
4,8%
6,5%.

CONCLUSIONS

The average yield of the studied Sudan grass genotypes ranges from 3463.29 to 4270.91 kg/da fresh and dry biomass from 669.92 to 859.84 kg/da.

- There are marked differences between genotypes and agrometeorological conditions during the study period. The determining factor in the formation of fresh biomass yields in the first and second cuts is the amount of rainfall (r varies from 0.610 to 0.937 and from -0.671 to 0.809), while in third cut the integral impact of air temperatures (r is from -0.714 to -0.903) and rainfall (r is from 0.599 to 0.818). The yield of fresh biomass for the vegetation period of the Sudan grass was formed with the largest proportion of the first cut (from 50.6 to 54.1%), followed by a second (from 23.4 to 28.6%), and the lowest is the share of third cut (from 18.7 to 20.8%).

The highest yield of fresh and dry biomass is M 300/43, which exceeds Kazitachi variety respectively with 16.0 and 16.7% and 10.7 and 12.4% variety Endje 1. The highest share of fresh biomass in the summer droughts, reaching a second cut of 25.5 to 39.8%, and in third cut from 14.5 to 27.1% of the total amount of fresh biomass for a vegetation period. The mutant form exceeds the original Kazitachi variety with raw protein content of 4.8% and water-soluble sugars by 6.5%.

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