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Evaluation of Romanian alfalfa varieties under the agro–environmental conditions in northern Bulgaria

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Abstract. The aim of the present study was to determine forage productivity, crude protein content, grass stand height and regrowth rate of Romanian alfalfa varieties in the specific agro–environmental conditions in Northern Bulgaria during four growing seasons. Six Romanian alfalfa varieties–Catinca, Magnat, Madalina, Sandra, Roxana and Daniela were included in the experiment. Bulgarian Prista 3 variety was used as a standard. The analysis of results for studied traits showed that with good performance concerning both forage productivity and rapid recovery after cutting Sandra variety was distinguished. Regarding dry matter yield and crude protein content, for four years of study no significant differences were detected among the alfalfa varieties. The yields of Romanian varieties were satisfactory and less below (2.22 %) the average yield produced by Prista 3 local variety. With respect to the crude protein (CP) content, as the most important quality parameter, the studied varieties fall into the medium CP content group, excepting Prista 3 and Roxana varieties falling into the high CP content group. There were found statistically significant differences among the varieties regarding grass stand height and regrowth rate after cutting. The studied Romanian varieties are characterized by high forage productivity and high crude protein content in dry matter. They could be evaluated as the varieties suitable for cultivation in the conditions of the Northern Bulgaria.

***Keyword**:* alfalfa, varieties, crude protein, dry matter yield, grass stand height, regrowth.

Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops worldwide due to its high yield, forage quality and adaptability to different climatic conditions.

It can be used directly for grazing or conserved as silage or hay and is a reliable forage species that could represent a significant contribution to the livestock sector [BORREANI *and* TABACCO, 2006].

Alfalfa also survive long periods of water stress by impeding its vegetative growth [ANNICCHIARICO *et al.,* 2010, [BAGIU *et al., 2012*; BUTNARIU and CORADINI, 2012] and accessing water from deep layers through its long root system [VOLAIRE, 2008].

Alfalfa is cultivated in more than 80 countries in an area exceeding 35 million hectare [RADOVIC *et al.,*2009, VARDANIAN *et al.,* 2018; STOLERU *et al.,* 2018, CAUNII *et al.,* 2015; IANCULOV *et al.,* 2004].

World production of alfalfa was around 436 million tons [FAO, 2006].

Numerous varieties have been created worldwide, by using highly divergent genetic materials and different breeding methods.

Varieties and their genetic characteristics crucially determine the volume and stability of yield, as well as the quality of alfalfa forage [STANISAVLJEVIĆ, 2008, SAMFIRA *et al.,* 2014; BUTNARIU; 2012; BUTU *et al.,* 2014a, STOLERU *et al.,* 2016].

Growth stage, cut number, stand density, moisture conditions at harvest and processing method are the most important causes of variation for yield of alfalfa [VERONESI *et al.,* 2010; VASILEVA, 2013, PETRACHE *et al.,* 2014, BUTNARIU *et al.,* 2014, BARBAT 2013, BUTU *et al.,* 2015].

Other authors reported that the environmental factors of year and location had a profound effect on the interpretation of forage yield differences among cultivars [HILL *and* BAYLOR, 1983; LAMB *et al.,* 2006, PUTNOKY *et al.,* 2013, BUTNARIU *et al.,* 2014; BUTNARIU and GIUCHICI, 2011].

The year x location x cultivar interaction demonstrated that forage yields among the cultivars differed in each year within each location.

A sequence of cause and effect relationships exists between the environment, agronomic practices, plant response, and nutritive value.

The best trade–off between quantity and quality in forage crops is one the most important targets of the research, since these traits are generally inversely correlated and depend on many factors [MUELLER *and* ORLOFF, 1994; TESTA *et al.,* 2011; VASILEVA *and* KOSTOV, 2015].

Factors affecting alfalfa hay quality are: soil fertility, variety, the presence of other species, the use of pesticides, climatic conditions, harvesting (season, time of day and stage of development at harvest) and the method of preservation [STANCHEVA *et al.,* 2008; BEKOVIĆ *et al.,* 2010, BUTNARIU *et al.,* 2012].

Protein content in alfalfa dry matter varies from 18 to 25 % depending on the growth stage (cutting cycle), cultivar difference and other factors [NIKOLOVA *et al.,* 2016, PENTEA *et al.,* 2016, BUTU *et al.,* 2014c. SAMFIRA *et al.,* 2015, BUTNARIU *et al.,* 2015b; BUTU *et al.,* 2014b].

Alfalfa varieties in the world, created with one or several positive characteristics, but they often have different adaptability to environmental conditions [STRBANOVIC *et al.,* 2017, DIMITRIU *et al.,* 2016; GEORGIEVA *et al.,* 2018; BUTNARIU and CAUNII, 2013].

There are many studies about alfalfa varieties performance in different agro–ecological conditions of the world [SVIRSKIS, 2007; HAYEK *et al., 2008;* MARINOVA *and* IVANOVA, 2009; ALTINOK *and* KARAKYA, 2002; GELETI *et al.,* 2014].

The purpose of most commercial alfalfa breeding programs is to create high quality varieties and stable yield in as many various environments as possible [SHEAFFER *et al.,* 1988, GROZEA et al., 2017].

The aim of present study was to determine forage productivity, crude protein content, grass stand height and regrowth rate of six Romanian alfalfa varieties under specific agro–environmental conditions in Northern Bulgaria.

Material and methods

The experiment was carried out from 2012 to 2015 at the Experimental field of Institute of Agriculture and Seed Science „Obraztsov chiflik"–Rousse.

The Experimental field of Institute is located in the northern climatic region of Danube plane, (43°48' N, 26°03 W and altitude 152 m).

The soil type was strongly leached chernozem, with low humus content.

It varied from 2.03 % to 2.17 % average for layer 0–40 cm.

Soil reaction is slightly acid (5.84–5.94).

There is a continental climate. Winter in this region is colder in comparison with the other non–mountain regions of the country.

The summer is droughty with low relative humidity and high daily temperatures mainly during July and August.

Six Romanian alfalfa varieties–Catinca, Magnat, Madalina, Sandra, Roxana and Daniela were included in the experiment.

Bulgarian Prista 3 variety, developed at the Institute of Agriculture and Seed Science „*Obraztsov chiflik*" was used as a standard.

The experiment was sown on 17 March 2012.

The experimental design was a randomized block with four replications.

The harvesting plot size was 10 m^2. Seeding rate was 25 kg ha^{-1} for all varieties.

Alfalfa was grown without irrigation and green mass harvesting was carried out in early flowering stage, when approximately 10–20 % of the plants were flowering.

During the period of investigation 13 cutting were made (first year–2 cuttings, II^{-nd} year–4, III^{-rd} year–4 and IV^{-th} year–3).

Conventional analyses of traits dry matter yield, crude protein content, grass stand height and regrowth rate were made.

Before every cutting the grass, stand height was recorded by measuring the height in 5 places in each harvesting plot from the soil surface to the top of the majority normally developed stems.

Green yield was determined by weight. A random sample (400–g) of forage was taken from each plot and dried for dray matter (DM) determination.

The crude protein content (CPC, %) was determined using the Kjeldahl method.

The regrowth rate (cm) was recorded by measuring the height of the grass stand on studied varieties (in 5 places in each harvesting plot) ten days after each harvest.

The obtained experimental data were analysed by the One–way analysis of variance (ANOVA) method.

Significance of differences by Duncan's multiple range test (DMRT) was tested.

The SPSS Statistics 19 software product was used.

Results and discussion

Weather conditions during 2012–2015 were relatively favorable for alfalfa stand development.

Table 1.

Characteristics of the studied Romanian alfalfa varieties during 2012–2015

Varieties	2012	2013	2014	2015
	Grass stand height, cm			
Prista 3–st	92	79	78	61
Catinca	86	76	70	59
Magnat	88	75	77	59
Madalina	96	77	76	58
Sandra	91	78	72	57
Roxana	92	75	72	62
Daniela	84	77	75	60
Mean	89.86	76.71	74.29	59.43
	Regrowth rate, cm			
Prista 3–st	15.50	18.32	17.88	16.56
Catinca	13.70	18.12	17.46	14.84
Magnat	13.30	17.70	16.66	14.66
Madalina	14.40	17.66	16.98	13.98
Sandra	15.60	18.24	17.32	14.56
Roxana	14.70	18.00	17.78	16.66
Daniela	13.20	17.22	15.44	13,88
Mean	14.34	17.89	17.07	15.02
	Dry matter yield, t ha^{-1}			
Prista 3–st	2.11	23.00	7.73	5.91
Catinca	2.07	23.80	6.91	5.37
Magnat	1.58	23.50	7.91	5.01
Madalina	1.93	23.40	7.52	5.10
Sandra	1.84	23.50	7.60	5.72
Roxana	2.08	23.00	6.67	5.67
Daniela	1.94	23.10	6.85	5.39
Mean	1.94	23.30	7.32	5.45
	Crude protein content in dry matter, %			
Prista 3–st	20.88	20.44	20.19	19.75
Catinca	20.38	19.50	19.38	19.59
Magnat	18.31	20.38	21.02	19.66
Madalina	20.06	20.25	18.25	18.98
Sandra	20.31	20.13	19.75	18.68
Roxana	20.76	19.94	20.25	19.25
Daniela	19.75	20.15	18.69	19.17
Mean	20.06	20.11	19.65	19.30

Total rainfall (221.4 mm) for the autumn–winter (October 2011–March 2012) was close to the long–term norm of 235.1 mm for the region.

In the alfalfa sowing year precipitation for April and May were below average norm, respectively of 25 % and 45 %.

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Sustained drought after sowing resulted in suppressed growth and development of the alfalfa stands in the beginning of the first growing season.

Autumn–winter soil moisture supply during October 2012–March 2013 was sufficient for vigorous first regrowth.

The next months were characterized by temperatures and precipitations close to the long–term norms, which ensured normal development of alfalfa varieties and provided formation of four regrowths.

The total rainfall and temperature course during third and fourth alfalfa growing season were similar to the long–term norms and tend to good development of the alfalfa stands.

The meteorological condition during period of study allowed alfalfa varieties to manifest their yield potential.

The plant height morphological trait is an important yield component and it is often used as a criterion when choosing superior genotypes in an early stage of selection [TUCKAK *et al*., 2008].

Variation in grass stand height is genotypic character and therefore, expressed in the form of better adaptability to environmental conditions.

According to first year of the stand life grass stand height varied widely among the alfalfa varieties (Table 1).

The average values showed that maximum grass stand height was recorded for Madalina (96 cm) followed by Prista 3 and Roxana (92.00 cm) while the minimum plant height for Daniela (84 cm) and Catinca (86 cm) was recorded.

In second growing season Prista 3 and Sandra varieties ranked first [PETRACHE *et al.,* 2014, BUTNARIU *et al.,* 2014, BARBAT 2013, BUTU *et al.,* 2015].

The results showed that for third and fourth year of stand life the highest grass stand height for Prista 3 (79 cm) and Roxana (62 cm) varieties was recorded [BUTNARIU *et al.,* 2014, BARBAT 2013, BUTU *et al.,* 2015].

For four–year period of study, average grass stand height ranged between 77.50 cm and 72.75 cm in Prista 3 and Catinca, respectively (Table 2).

Table 2.

Mean values of investigated agronomic traits of alfalfa varieties

Varieties	Dry matter yield, $t\ ha^{-1}$	Crude protein content, %	Grass stand height, cm	Regrowth rate, cm
Prista 3–st	9.69 ^{a}	20.32 ^{a}	77.50 ^{a}	17.07 ^{a}
Catinca	9.54 ^{a}	19.71 ^{a}	72.75 ^{b}	16.03 ^{ab}
Magnat	9.50 ^{a}	19.84 ^{a}	74.75 ^{a}	15.58 ^{ab}
Madalina	9.49 ^{a}	19.39 ^{a}	76.75 ^{a}	15.76 ^{ab}
Sandra	9.67 ^{a}	19.72 ^{a}	74.50 ^{ab}	17.07 ^{a}
Roxana	9.36 ^{a}	20.05 ^{a}	75.25 ^{a}	16.15 ^{ab}
Daniela	9.32 ^{a}	19.44 ^{a}	74.00 ^{ab}	14.94 ^{b}
Mean	9.5	19.78	75.07	16.09

^{a,b} Values in the columns followed by the same letter are not significantly different according to Duncan's Multiple Range Test.

The regrowth rate after cutting for the first growing season was ranged from 13.20 cm (Daniela variety) to 15.60 cm (Sandra), followed by Prista 3 – 15.50 cm.

Sandra variety maintained the first place in the second year with 18.24 cm regrowth rate.

The results showed that for third and fourth year of stand life the fastest regrowth for Roxana and Prista 3 varieties was recorded. Regarding the mean values for period of study, high potential for rapid recovery after mowing in Sandra and Prista 3 was found.

During first growing season under the agro–ecological conditions of Northern Bulgaria the highest dry matter yield (2.11 $t\ ha^{-1}$) Prista 3 local variety was achieved, followed by Roxana (2.08 $t\ ha^{-1}$) and Catinca (2.07 $t\ ha^{-1}$).

The lowest dry matter yields from Magnat variety (1.58 t ha^{-1}) were obtained. Varieties started to show their actual performances beginning with the second growing season.

The highest dry matter yield in 2013 was produced by Catinca (23.8 t ha^{-1}), being 2.6% above the mean yield produced during the year.

Relatively lower forage yield for Prista 3 variety was reported, as compared to the mean yield.

The data indicated that there were no considerable differences in phenotypic expression of investigated trait.

The yield decline in third and fourth year and may have been associated with the alfalfa stand age.

The dry matter yields of the Romanian varieties were insignificantly lower than yields by Prista 3 were established.

The results obtained suggest that the forage productivity of Romanian varieties was satisfactory and less below (2.22 %) the average yields produced by Prista 3 local variety.

There were no found statistically significant (P≤0.05) differences among the varieties regarding alfalfa dry matter yield.

Similar results were obtained by Altinok and Karakya, who ascertained that the forage yields of the local varieties were slightly higher (2.6 %) than introduced varieties under Ankara conditions [ALTINOK *and* KARAKYA, 2002].

Svirskis and collab. evaluated 12 alfalfa varieties of different origin and they found that the domestic cultivars Žydrune and Birute yielded more than the Canadian variety Alfagraze [SVIRSKIS *et al.*, 2003, BUTU *et al.*, 2015a, BUTU *et al.*, 2015; STOLERU *et al.*, 2018].

Regarding the crude protein content, as the most important quality parameter, in the first and second production years the highest concentrations in dry matter were ascertained.

The data showed that the crude protein concentration varied widely in years–from 21.02 % (Magnat) to 18.25 % (Madalina) in the third year.

The highest crude protein content for Prista 3 variety was recorded during first growing season, which was above the average for Romanian varieties with 0.95%. [PETKOVA *et al.*, 2005, BUTNARIU and SAMFIRA, 2012] also reported that the four–year average CP yield of seven alfalfa genotypes and five varieties of diverse origin ranged from 18.85 % (NS Banat ZMS II) to 21.79 % (Bulgarian Ax 93/3).

The data presented in table 1 showed that in coming three years of stand life the highest crude protein content for Prista 3 and Magnat Romanian variety was obtained.

The mean values for study period regarding crude protein content showed that maximum concentration for Prista 3 was recorded followed by Roxana.

There were no found statistically significant (P≤0.05) differences among the varieties regarding crude protein content. [IVANOV, 1980, IANCULOV *et al.*, 2005, STOLERU *et al.*, 2018] classified alfalfa varieties according to crude protein content into three groups, being as follows: varieties having a high CP content (20 % and above), a medium content of CP 18–20 % and those having a low CP content (below 18 %).

According to the classification, the studied varieties fall into the medium CP content group, excepting Prista 3 and Roxana varieties falling into the high CP content group.

Conclusions

The values of the investigated traits, which determine the economic significance of Romanian alfalfa varieties, are a sign for their adaptability to soil and meteorological conditions for the region of study.

The analysis of results for studied traits showed that with good performance concerning both forage productivity and rapid recovery after cutting Sandra variety was distinguished.

Regarding dry matter yield and crude protein content, for four years of study no significant differences were found among the alfalfa varieties.

With respect to the crude protein content, as the most important quality parameter, the studied varieties fall into the medium CP content group, except Prista 3 and Roxana varieties falling into high CP content group.

There were found statistically significant differences among the varieties regarding grass stand height and regrowth rate after cutting.

The studied Romanian varieties, are characterized by high forage productivity and high crude protein content in dry matter.

They could be evaluated as the varieties suitable for cultivation in the conditions of the Northern Bulgaria.

Declaration of conflict of interests

The authors report that they have no other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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