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ACCURACY OF AC AND AT METHODS IN MILK RECORDING IN THE BALKAN GOATS BREED IN MACEDONIA

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Abstract

Pacinovski, N., V. Dzabirski, G. Dimov, K. Porcu, G. Cilev, E. Eftimova, N. Nikolova, N. Mateva, B. Palasevski, G. Trajkovski and A. Palasevska, 2018. Accuracy of ac and at methods in milk recording in the Balkan goats breed in Macedonia, *Bulg. J. Agric. Sci.*, 24 (Suppl. 2): 3-9

The aim of this research was to compare the AC and AT methods (as alternative methods) with A4 method (as referent method), for determination of milk production, on the day of recording of the indigenous Balkan goat, in the period of 2014-2016 with milking of goats in the morning and evening. Simultaneously, the influence of the following factors has been tested: year (year of kidding), lactation, month of kidding, milk yield level. It was determined that the largest deviation in the prediction of milk yield according to the AT method during morning milking was determined in 2016 with milk yield underestimated for -5.3 L, whereas the smallest deviation was registered in 2015 with milk yield underestimated for -1.8 L. The largest deviation in the prediction of milk yield according to the AC method during morning milking was determined in goats in first lactation, with milk yield overestimated for +3.8 L, whereas the smallest deviation was determined in goats in fourth lactation with milk yield underestimated for -0.3 L.

With the analysis of all abovementioned factors, it was determined that the AT method is more suitable as alternative and cheaper method for determination of accurate amount of milk.

Key words: Balkan goat, daily lactation, method AT, method AC, predicted daily milk yield

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Introduction

According to the ICAR regulations, there are few methods (A4, A5, AC and AT) that provide an opportunity for milk recording in goats, during the lactation period. The first two methods (A4 and A5) are used for measurement of milk yield during morning and evening milking on the day of milk recording. If there is third milking in the afternoon, these two methods cover the same.

The AC method is used for measurement of the amount of morning or evening milk, after which a correction coefficient is used for determination of milk yield on the day of test.

The AT method is used for measurement of the amount of morning and evening milk, alternatively (with measurement of morning milk for one month and evening milk the other month), to the end of the milking period, whereby during the calculation of the obtained milk in the day of test, the amount of milk from each milking is doubled.

The aim of this study was to determine the accuracy of two methods for milk recording (AC and AT), and prediction of the actual milking milk yield in the Balkan goats, measured twice a day (according to the standard A4 method), (ICAR, 2009, ICAR, 2012), in the morning and evening. Also we measured different level of milk production of goats.

Regarding the deficit of such testing of goat milk, the comparison and discussion of the results was performed with similar testing, realized in sheep.

Material and Methods

We used a flock of Balkan Goat breed as basic experimental material, located in the South-East part of Republic of Macedonia.

The testing included individual records for measured milk (morning and evening milk) on the day of test of 242 goats, for a period of 3 years (2014-2016), or a total of 726 lactation records, in age from first to ninth lactation (Table 1).

A total of 4598 individual lactation tests were realized during the three years of milk production or according to the age range: 588 tests in first lactation, 882 tests in second lactation, 1318 tests in third lactation, 919 tests in fourth lactation, 598 tests in fifth lactation, 195 tests in sixth lactation, 56 tests in seventh lactation, 30 tests in eighth lactation and 12 tests in ninth lactation (Table 2).

Mainly combined (barn-pasture) system of breeding was used on the farm, which means usage of the available vegetation during almost the entire year, whereas in a certain period of the year, especially in winter, goats were fed additionally with meadow hay (November-February) and concentrate (January-April). Kids stayed with their mothers depending on the purpose. Kids intended for slaughter stayed with their mothers until they were sold for meat (2-2,5 months age), whereas those intended for reproduction, stayed little longer, up to 3 months. The milking period started after the weaning of the kids.

Table 1. Distribution of tested goats by parity per year of kidding

Year	Lactation									Total
	I	II	III	IV	V	VI	VII	VIII	IX	
2014	84	53	70	26	4	3	2	0	0	242
2015	0	84	53	70	26	4	3	2	0	242
2016	0	0	84	53	70	26	4	3	2	242
Total	84	137	207	149	100	33	9	5	2	726

Table 2. Individual lactation tests in goats

Year	Lactation									Total
	I	II	III	IV	V	VI	VII	VIII	IX	
2014-2016	588	882	1318	919	598	195	56	30	12	4598

The A4 method was used as an reference method, which means it was used for measurment of daily milk production per goat (morning and evening milk), in the interval from 28 to 34 days.

The testing of milk started after the weaned of the kids and lasted until the moment of drying (between end of October to the middle of November). There were realized a total of 7 milk tests per animal in 2014, 6 milk tests in 2015 and 6 milk tests in 2016.

In order to determine milk yield using the AC method, the total obtained milk was calculated for each day of testing (in the morning and evening), whereby the total amount of milk was obtained (in the morning and evening). In this way the correction coefficient for morning and evening milking was formed and the individual milk yield for the day of test was predicted. The predicted milk yield using the AC and AT method was compared with the actual milk yield (method A4).

In order to determine the influence of the level of milk yield on the accuracy of measurement of total milk per day per goat, the lactations were divided in three groups with equal number: low-productive (1.12-3.60 L), middle-productive (3.60-5.87 L) and highly productive (5.90-18.27 L). Each group consisted of 242 lactations.

With this method, the lactations of certain goats may be included in different groups during certain years of monitoring of milk yield.

The traits of testing were marked as it follows:

• Total – the milk yield measured according to A4 method;

• Predml (Prediction by morning milking) – (milking milk yield by AC method with individual test in the morning);

• Predel (Prediction by evening milking) – (milking milk yield according to the AC method with individual test in the evening);

• Difml (Diference with morning milk) – (difference between AC at morning milking and A4 method);

• Difel (Diference with evening milk) – (difference between AC method at evening milking and A4 method);

• Atfml – (lactation milk yield according to the AT method with first month of test at the morning milking);

• Atfel – (lactation milk yield according to the AT method with first month of test at the evening milking);

• Atdifml – (difference between AT method with first month of test in morning milking and A4 method);

Regarding the abovementioned traits, the influence of the following factors has been tested:

- Year (Year of kidding), (2014, 2015, 2016);
- Lactation (1, 2, 3, 4, 5, 6, 7, 8, 9),
- Month of kidding (December, January, February),
- Milk yield level – groups with level of milk yield according to the A4 method: low – 1/3 of the lactations with lowest milk yield, average – 1/3 of the lactations with average milk yield and high - 1/3 of the lactations with highest milk yield;
- The analyses were performed using the package programs SPSS (SPSS, 1994).

Results and Discussion

The influence of studied factors (year, lactation, month of kidding and level of milk yield) on the actual, predicted milk yield and the differences between the actual and predicted milk yield are presented in Table 3.

According to the data in the same table, the factor -Level of milk yield had highly significant influence ($P < 0.001$) on almost all traits. This factor had no influence ($P > 0.05$) on the difference between the AT and A4 method during morning milking in the first month of testing (Atdifml).

The year is significant factor on a part of the tested traits and non-significant factor on the remaining traits. For example, this factor had highly significant influence ($P < 0.001$) on milking milk yield according to the A4 method (Total L – milking milk yield according to the A4 method), milking milk yield according to the AT method during evening milking in the first month of testing (Atfel) and on the difference between AT and A4 method during morning milking in the first month of testing (Atdifml).

Lower influence ($P < 0.01$) the year had on the milking milk yield according to the AC method during evening milking (Predel) as well as on the milking milk yield according to the AT method with morning milking in the first month of testing (Atfml).

Significant influence of the year, on the determined and expected daily milk yield (using A4 and AC methods) has been determined in East-Frisian breed of sheep in Macedonia (Pacinovski et al., 2015).

Similar results about the influence of lactation on daily milk production were obtained in Awassi and East-Friesian sheep in Macedonia, (Dimov et al., 2005 and Djabirski et al., 2006).

The factor – year had no influence on the remaining traits (Predml, Difml and Difel). The remaining two factors -Lactation and Month of kidding did not manifest any influence ($P > 0.05$) on any of the tested traits.

Coefficient of determination (R2), showed that the complex of the studied factors determine from 70.3% to 74.1% of the variation of the measured milk yield, and from 8.6% to 9.4% of variation of the difference between the predicted and total milk yield for the milking only period by the AC method and considerably lower for AT method 1.57% (Table 3). This is maybe the too great variation due to the method of calculation. This indicates, that AT method is more precise in prediction (for Atfml and Atfel) in terms of influence of the environmental factors and so also because of the close values of R2 with Total. Therefore AT method could be proposed for the practice.

The average milk yield measured according to the AC method during morning milking is 157 L, whereas during evening milk is 155.2 L (Table 4). The highest amount of milk yield determined according to the AC method during morning milking was measured in 2014 (162.5 L) whereas the lowest amount was measured in 2016 (151.1 L). The same ratio highest-lowest milk yield per year was determined in milk yield measured according to the AC method, during evening milking. There was no tendency in the year effect on AC prediction; in 2014 the evening test gave higher prediction, while in 2015 and 2016 morning test gave higher prediction. In all measurements the AC method gave somewhat lower measurement vs A4 method.

The difference between the measured and predicted milk yield according to the AC method during morning milking is 0.9 L, whereas the difference between the predicted and measured milk during evening milking is -0.73 L.

Analyzing by year, the largest deviation in the prediction of amount of milk yield according to the AC method during morning milking was determined in 2016 (+2.1 L.), whereas the smallest deviation was registered in 2014 with milk yield was underestimated for – 0.9 L. In the prediction of milk yield according to the AC method during evening milking, the largest deviation was registered in 2016, with milk yield underestimated for -1.6 L, whereas the smallest deviation was also registered in 2014, with milk yield overestimated for +0.6 L.

For example, the results obtained at Bulgarian Synthetic Dairy Sheep, show that the total amount of milking milk is slightly decreasing with AC method for 120 days (Ivanova, 2013). Also some results from Awassi sheep in Macedonia show that the correlation between the two methods (A4 and AC) is high, with maximal variations in prediction from 1.9 to 3.4 L, (Gievski et al., 2006).

By using the AC method for prediction of milking milk yield during morning milking in the first month of the testing, the milk yield was 151.9 L, whereas by using the AT method for prediction of milking milk yield during evening milking in the first month of the testing, the milk yield was 160.1 L. The difference between the measured and predicted milk yield using the AC and AT method during morning milking in the first month of the testing was -4.1 L, whereas the difference during evening milking in the first month of the testing was alternatively +4.1 L.

The largest deviation in the prediction of milk yield according to the AT method during morning milking was determined in 2016 with milk yield underestimated for -5.3 L, whereas the smallest deviation was registered in 2015 with milk yield underestimated for -1.8 L.

Analyzing according to the age i.e. number of lactation, the highest amount of lactation milk yield was determined in goats in seventh lactation (166.8 L), whereas the lowest amount was determined in goats in first lactation (149 L).

The largest deviation in the prediction of milk yield according to the AC method during morning milking was determined in goats in first lactation, with milk yield overestimated for +3.8 L, whereas the smallest deviation was determined in goats in fourth lactation with milk yield underestimated for -0.3 L. The same ratio largest-smallest deviation in milk yield according to the AC method during evening milking was determined in goats in same age (first and fourth lactation).

The largest deviation in the prediction of milk yield according to the AT method during morning milking was determined in goats in fifth lactation (-3.2 L) whereas the smallest deviation was registered in goats in ninth lactation with milk yield underestimated for -0.4 L.

Having in mind that goat kidding is mainly in a period of three months (January-February), the highest amount of milk yield was determined in goats that kidding in February (156.2 L) whereas the lowest amount of milk yield was determined in goats that gave birth in January (155.6 L).

The largest deviation in the prediction of milk yield according to the AC method during morning milking was determined in goats that gave birth in December, with milk yield overestimated for +4.7 L, whereas the smallest deviation was determined in goats that gave birth in January, with milk yield underestimated for 0.7 L.

In relation to the milk yield predicted according to the AC method during evening milking, the largest deviation was registered in goats that gave birth in December (+3.6 L) and the smallest deviation was registered in goats that gave birth in January, with milk yield underestimated for -0.5 L.

In the prediction of milk yield according to the AT method during morning milking, the largest deviation was determined in goats that gave birth in January and February (-3.5 L), whereas the smallest deviation was determined in goats that gave birth in December, with milk yield underestimated for -2.4 L.

Having in mind that we divided goats in three groups according to the amount of milk yield: goats with low, average and high amount of milk yield, the average milk yield in goats with low amount of milk yield is 80.7 L, 143.5 L is the

Table 3. Influence of factors on the actual, predicted lactation milk yield and the difference between actual and predicted yield of goats

Source	df	Totall	Predml	Predel	Difml	Difel	Atfml	Atfel	Atdifml
Year	2	***	NS	**	NS	NS	**	***	***
Lactation	8	NS	NS	NS	NS	NS	NS	NS	NS
Month of kidding	2	NS	NS	NS	NS	NS	NS	NS	NS
Milk yield level	2	***	***	***	***	***	***	***	NS
R Squared, %		74.1	70.3	73.8	8.6	9.4	73.9	74.0	1.57

Significance: *** P<0.001; ** P<0.01; * P<0.05; NS P>0.05

Table 4. Effects of factors on the actual, predicted lactation milk yield and the difference between actual and predicted yield of goats, Lit.

Effect	N	Totall	Predml	Predel	Difml	Difel	Atfml	Atfel	Atdifml
Year									
2014	242	173.8	162.5	164.0	-0.9	0.6	158.3	168.5	-5.1
2015	242	165.6	157.0	154.3	1.5	-1.2	153.7	157.4	-1.8
2016	242	158.7	151.1	147.4	2.1	-1.6	143.8	154.3	-5.3
Average, L		166	157	155.2	0.90	-0.73	142	160.1	-4.10
Lactation									
1	84	149.0	152.8	146.1	3.8	-2.9	146.5	151.4	-1.5
2	138	152.0	153.1	151.1	1.2	-0.8	148.6	155.4	-2.7
3	208	154.8	156.3	153.7	1.5	-1.1	151.3	158.2	-2.9
4	149	159.6	159.3	159.8	-0.3	0.2	155.9	163.2	-3.0
5	99	155.4	155.8	155.0	0.4	-0.4	151.4	159.4	-3.2
6	32	157.5	160.3	155.5	2.8	-2.1	154.0	161.1	-2.2
7	9	166.8	165.7	167.5	-1.0	0.7	161.6	171.9	-2.7
8	5	149.7	150.5	148.9	0.8	-0.7	144.5	154.9	-1.9
9	2	159.1	158.0	159.5	-1.0	0.5	153.4	164.7	-0.4

Table 4. Continued

Average, L		156	157	155.2	0.91	-6.6	152	160	-2.28
Month of kidding									
12	74	156.1	160.9	152.5	4.7	-3.6	152.6	159.6	-2.4
1	490	155.6	154.8	156.1	-0.7	0.5	151.3	159.9	-3.5
2	162	156.2	154.9	157.1	-1.3	0.9	151.8	160.6	-3.5
Average, L		156	157	155.2	2.70	-2.20	152	160	-3.13
Milk yield level									
Low	242	80.7	79.0	82.2	-1.7	1.5	76.8	84.6	-3.0
Middle	242	143.5	140.9	145.4	-2.5	1.9	139.4	147.5	-3.1
High	242	243.7	250.7	238.1	7.0	-5.7	239.5	248.0	-3.3
Average, L		156	157	155.2	2.80	-2.3	152	160	-3.13

average amount of milk yield in goats with average amount of milk yield and 243.7 L is the average amount of milk yield in goats with high amount of milk yield.

Analogous to the level of milk yield (low, average and high), the largest i.e. smallest deviation in the prediction of milk yield according to the AC method was determined during morning milking (+4.7 and -0.7 L) but also during evening milking (+3.6 and -0.5 L).

In the prediction of milk yield according to the AT method during morning milking the largest deviation was determined in goats with low level of milk yield, with milk yield underestimated for -3.0 L.

Conclusions

Based on the conducted tests, the following can be concluded:

• AT method gives slightly better prediction of the milk yield for milking only period and is less influenced by the year, age and production level,

• It was determined that the largest deviation in the prediction of milk yield according to the AT method during morning milking was determined in 2016 with milk yield underestimated for -5.3 L, whereas the smallest deviation was registered in 2015 with milk yield underestimated for -1.8 L.

• The largest deviation in the prediction of milk yield according to the AC method during morning milking was determined in goats in first lactation, with milk yield overestimated for +3.8 L, whereas the smallest deviation was determined in goats in fourth lactation with milk yield underestimated for -0.3 L.

• Therefore, the general conclusion is that the AT method is more suitable as alternative and cheaper method for determination of accurate amount of milk.

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RELATIONSHIPS BETWEEN SOIL WATER REPELLENCY, PHYSICAL AND CHEMICAL PROPERTIES IN HYDROPHOBIC TECHNOGENIC SOILS FROM THE REGION OF MARITSA-IZTOK COAL MINE IN BULGARIA

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Abstract

Atanasova, I. M. Banov, T. Shishkov, Z. Petkova, B. Hristov, P. Ivanov, E. Markov, I. Kirilov and M. Harizanova, 2018 relationships between soil water repellency, physical and chemical properties in hydrophobic technogenic soils from the region of Maritsa-Iztok coal mine in *Bulgaria. Bulg. J. Agric. Sci.*, 24 (Suppl. 2): 10-17

Soil water repellency (soil hydrophobicity) is considered a key mechanism for sequestration of organic carbon. Technogenic soils from mine areas containing clays and irregularly distributed lignitic particles are heterogeneous materials exhibiting small-scale spatial variability of water repellency. Non-vegetated and pine-afforested spoils from the area of Maritsa-Iztok lignite coal basin in Bulgaria were studied. The technogenic soils were characterized by severe to extreme hydrophobicity and heavier texture at the pine-vegetated site, as well as extreme acidity (pH 3-4). Principle Component Analysis (PCA) and cluster analysis were carried out, in order to study the simultaneous interaction of soil characteristics and properties with the aim to assess their role in the overall data variability in the process of data reduction to several unrelated components. The PCA was based on twelve factors: WDPT, sand, silt and clay contents, hygroscopic moisture, cation exchange capacity (CEC), organic carbon (total organic carbon TOC, humic organic carbon HOC and fulvic organic carbon, FOC), total nitrogen (N) and mineral nitrogen (MN) and electrical conductivity (EC). Three principle components were identified with eigenvalue > 1, describing 79% of the total variability. There was a significant positive correlation between WDPT and TOC, HOC, FOC, MN and a negative correlation with the % of hygroscopic moisture. The results obtained indicate that TOC comprised mainly of particulate organic carbon (POC) containing coal particles was significantly correlated with the sand fraction, CEC and MN, and was the main driver of soil water repellency in the studied mine soils.

Key words: soil water repellency, technogenic soil, mine, soil properties, principal component analysis
Abbreviations: WDPT – Water Drop Penetration Time, OC – Organic Carbon, CEC – Cation Exchange Capacity
SWR – Soil Water Repellency, SOM – Soil Organic Matter, TOC – Total Organic Carbon, HOC – Humic Organic Carbon, FOC – Fulvic Organic Carbon, EC – Electrical Conductivity, MN – Mineral Nitrogen, PCA – Principle Component Analysis, CA – Cluster Analyses, PC – Principal Components

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Introduction

It has been widely accepted that restoration of disturbed mine lands should become an imperative practice in many countries following mining activities for ecological, social and economic reasons (Frouz, 2013). The reclamation of land disturbed by coal mining activities involves converting the infertile and often toxic spoils into self-sustaining lands. A widely accepted practice in reclamation activities requires the removal and reinstallment of topsoil from the pre-mining environment to ensure return of productivity of the affected land. A widely accepted reclamation method is through forestation (Haigh and Gentcheva-Kostadinova, 2007, Krümmelbein et al. 2012).

In the Maritsa-Iztok coal producing region reclamation into forestry dominated landscapes has been applied as a common practice since 1970s (Treykyashki, 1987) by depositing clay strata in landfills and reinstallment of humus rich soil layer on top of the spoils. Another way of land reclamation has been to mix coal ash, a waste product from the thermal power plant with geological overburden layers containing yellow, green, blue and black clays deposited in depth of the geological profile over the coal seams that have been excavated in the process of coal production. Tree planting (*Pinus nigra* L.) and ash treatment has been shown to increase substrate pH and improve soil chemical and physical properties (Zheleva et al. 2004). The effects of excavated overburden mine wastes can be multiple, e.g. soil erosion and air and water pollution, metal toxicity, loss of biodiversity, and eventually loss of economic wealth (Wong, 2003; Sheoran et al., 2010). Lignitic technogenic soils exhibit spatial variability of water repellency (hydrophobicity) due to partial mixing of different overburden sediments and irregular distribution of lignitic particles, lignite-coated sand, clay particles and influence water and solute movement in reclaimed soils (Gerke et al. 2001). Water repellency in soils is associated with the formation of hydrophobic layers of organic compounds on soil particle surfaces and/or interstitial hydrophobic particulate organic matter (Morley et al., 2005). These compounds are thought to originate from plant leaf waxes, decomposing organic matter, root exudates, and their biodegradation products or from microbial activity (Hallet et al. 2006). The effect of high soil temperature on water repellency has also been investigated in laboratory-based studies and is well established (Atanassova and Doerr, 2011). Organic compound classes thought to be associated with soil water repellency include alkanes, fatty acids, fatty alcohols, aldehydes, ketones and ω-hydroxy fatty acids and α,ω-dicarboxylic acids characteristic of roots (Morley et al., 2005; Atanassova and Doerr, 2010, 2011; Mao et al. 2015). The degree of water repellency varies with moisture content and temperature (Dekker and Ritsema, 1994; De Jonge et al., 1999).

The major aims of the present study are: (i) to assess various chemical and physical soil properties of vegetated and non-vegetated water repellent (hydrophobic) technogenic soils in the vicinity of the biggest coal producing complex in Bulgaria, and (ii) to get information about the interrelationships between the persistence of soil water repellency and various soil characteristics, therefore outline groups of characteristics of similar sources or origin, as well as those specifically related to soil hydrophobicity.

Materials and Methods

The study was carried out on two plots separated by a distance of 30 m from each other at the village of Obruchishte, Maritsa-Iztok coal mines. The experimental plots were located in a ~ 1 ha large area, which was afforested with *P. nigra*. The spoils were created in 1970s and consisted of loam-textured Pliocene overburden sediments from the nearby open-cast lignite mine Troyanovo 1, GPS coordinates (UTM-system): Site 1: N 42.16434, E 25.94285, and Site 2: N 42.16452, E 25.94318. A lack of plant density and water repellency was observed in plots of ~ 200 m^2 amongst a uniformly pine vegetated area. Grids Δ 2 m, ~ 40 m^2 were constructed at a bare non-vegetated and another pine vegetated sites, ~ 40 m apart from each other. Soil cores were taken to a depth of 0-10 (0-5) and 10-20 cm and at two points to a depth of 0-50 cm using a 3 cm wide and 25 cm long core sampler. The layers at the non-vegetated site (0-20 cm) have sandy loam texture of degraded finely dispersed lignite, coal ash particles and black, greyish-green and yellow clays. At the pine-vegetated site the substrate was of heavier clay texture at the 10-20 cm depth. At both sites, the parent mine sediments contained irregularly distributed lignitic particles of sand grain size, and coarser fragments. Layers of black clays of heavy clay texture were located to a lower depth of > 20 cm. The substrate 0-50 cm was found to be water repellent since water drop penetration time (WDPT) which is an indicator of the persistence of water repellency was > than several minutes. Soil samples were classified as strongly (60 s < WDPT< 600 s) and severely > 600 s water repellent according to the scale of De Bano (1981). The soil samples were weighed to determine the field moisture content and subsequently equilibrated at the ambient air humidity before measuring water drop penetration time (WDPT) in the laboratory at controlled humidity and temperature. After sampling, soils were transferred to the laboratory, air-dried at room temperature and milled to < 2 mm fraction.

All chemical analyses were performed on < 2 mm material without further grinding, except for the organic carbon analysis (sample size < 250 μm). For chemical analysis a 100 g sub-sample of the < 2 mm fraction was obtained by the coning and quartering method to reduce sample variability. Organic carbon (OC) in the studied spoils was determined with the modified Tjurin's method (oxidation with $K_2Cr_2O_7$/H_2SO_4) and organic carbon fractionation by the method of Kononova, 1963, (modified by Filcheva & Tsadilas, 2002), cation exchange capacity was determined as sum of titratable acidity (pH 8.2) and extractable Ca, by saturation with K malate at pH 8.2 (Ganev & Arsova 1980). Sample water-repellency (soil hydrophobicity) was measured by the water drop penetration time (WDPT) method (Doerr et al. 2002). Three droplets of distilled water (80 µl) were placed on the soil surface and the time recorded for droplet penetration. Total nitrogen was analysed by the Kjeldahl procedure and mineral NH_4^+-N and NO_3^--N after Bremner (1965). Soil texture analysis was performed according to the method of Kachinski (Kachinskii, 1965) Statistical analysis (principal component PCA and cluster analysis, CA) was performed by SPSS 22 for MS Windows.

Results and Discussion

We suspect that not only water repellency is the cause of lack of vegetation, but also the low pH and metal toxicity (mostly AI, ~ 33% of total CEC) are the other possible factors (Table 1a), Atanassova et al. unpublished data). The reason for the very low pH encountered is due to the oxidation of sulfide to sulfate which solubilizes Fe (II), subsequently oxidized to Fe (III). Soil water repellency was higher in the surface 0-5; 0-10 cm layers, than in the sub-surface layers (10-20 cm) which correlated with the higher organic matter content. We further investigated potential correlations between persistence (WDPT) of SWR and other soil properties such as total and humified organic carbon, and total and mineral nitrogen, pH, hygroscopic moisture, texture, cation exchange capacity and electrical conductivity.

The organic matter of the more water repellent soil samples was generally higher than that of the less water repellent lower layers (Table 1b).

The correlation matrix is presented in Table 2. The degree of SWR, measured through WDPT was positively correlated with the total and fractionated (humic and fulvic) soil organic carbon (R_{TOC} = 0.699*, R_{HOC} = 0.499* and R_{FOC}= 0.442*), Table 2.

Some authors (Sepehrnia et al., 2016) reported that soil organic matter (SOM) was associated with water repellency. It has been also speculated that SOM enhances soil water repellency, and vice-versa water repellency protects organic matter against microbial decomposition. A positive correlation between SOM and both the degree and persistence of water repellency was found by Leelamanie (2014) and Jeyakumar et al. (2014). However, Doerr et al., (2000) found a lack of correlation. The explanation for this inconsistency may be that that it is the quality of SOM rather than total quantity that causes water repellency, therefore a certain amount of specific hydrophobic compounds might be needed to cause SWR. Values of organic carbon in the technogenic soils, as determined by the Tjurin's method varied with depth and between plots in the range of 1.7%-11%. These values reflect the resistance of the degraded lignitic particles to microbial hydrolysis and oxidation. The mine soil material in the depth of 0-10 cm, contained lignitic particles of sand grain size. The colour of the technogenic soil is predominantly black (upper layer) with a yellow-green-grayish appearance deeper in the soil profile at > 20 cm depth. We suspect that hydrophobic compounds from the lignite which cause the luster or greasiness of the clays are the most probable source of water repellency. In the water repellent spoils under pine vegetation, soil hydrophobicity might have a miscellaneous source, both lignitic particles and waxes from the pine vegetation. Total organic carbon (TOC) containing lignitic particulate organic matter as a source of water repellency is strongly correlated with the sand fraction of the soil (R_{TOC} = 0.446* and R_{HOC}= 0.579* and R_{FOC} = 0.462*).

Relationships between soil chemical properties (e.g. increase of repellency with decreasing pH) and soil water repellency are scarce (Krueger et al. 2016). A positive significant relationship was obtained between WDPT and mineral nitrogen (R = 0.580*) and a negative (R = -492*) with hygroscopic moisture (Table 2). Water repellency is a transient property depending ultimately on soil moisture content. SWR is usually highest in dry soils and disappears when the soil water content exceeds a critical threshold (Doerr et al. 2000). The variability of the critical water content depends on the wetting history of the soil or the heterogeneous distribution of water in and around the soil aggregates (Dekker et al., 2001). Soil water repellency is more commonly encountered in dry soils and has serious consequences for carbon and nutrient cycling, therefore is significantly affecting microbial activity, as well.

Table 1a. Physical and physico-chemical soil properties

Site	Depth cm	WDPT (s)	Sand %		Silt %		Clay %		Hygroscopic moisture %		CEC	
		Range median	Mean ±	SD	Mean ±	SD	Mean ±	SD	Mean ±	SD	Mean ±	SD
Pine vegetation	0-5	14-9589 1200	47.5	7.21	22.4	0.07	28.0	7.21	8.2	0.69	41.27	3.89
	10-20	2-128 10	25.5	20.45	22.04	0.03	55.1	21.72	8.4	0.54	44.60	3.52
non-vegetated	0-10	76-14440 1282	59.0	1.89	21.8	0.08	17.8	3.86	8.2	0.63	67.77	6.16
	10-20	202-2470 614	62.8	4.84	21.3	0.10	17.0	2.37	7.8	1.9	69.90	8.70

* Range and median (underlined).

Table 1b. Soil chemical properties

Site	Depth cm	pH		EC		TOC %		HOC %		FOC %		N total %		ΣN-NH_4^+ and NO_3^- mg/kg	
		Mean ±	SD	Mean ±	SD	Mean ±	SD	Mean ±	SD	Mean ±	SD	Mean ±	SD	Mean ±	SD
Pine vegetation	0-5	4.58	0.20	0.19	0.14	5.09	0.42	1.87	0.52	0.66	0.31	0.22	0.03	15.17	2.4
	10-20	4.18	0.43	0.63	0.14	3.00	1.67	1.03	0.89	0.29	0.42	0.10	0.04	11.40	1.7
non-vegetated	0-10	3.18	0.11	1.72	0.76	6.43	3.98	2.49	1.23	0.79	0.45	0.11	0.02	40.33	19.5
	10-20	3.13	0.10	1.61	0.69	6.11	0.96	2.96	0.51	0.92	0.56	0.12	0.01	42.87	18.5

WDPT-mineral nitrogen relationship observed is in line with the studies of Elbl et al. (2014) who have found that changes of soil water content have an impact on microbial activity, hydraulic conductivity, soil hydrophobicity and loss of mineral nitrogen from soil.

A positive, although insignificant correlation was also observed between WDPT and CEC (R= 0.408) and significant correlation with TOC, HOC and FOC (R = 0.466*; R = 0.472*; R = 0.638*) which sustains the hypothesis of the intimate association of the hydrophobicity causing compounds with soil colloids of organic, and/or organo-mineral nature. Cation exchange capacity (CEC) as a source of ions in solution is significantly correlated with EC (R = 0.939*) and mineral nitrogen (MN, R = 0.804*), but negatively correlated with clay (R = -0.603*) and positively with the sand fraction R = 0.636*. Such a trend is plausible if organic matter is assumed to be the main source of cation exchange in the studied soils, while a reduction in the CEC of the clay fraction might have occurred through blocking by organic matter of the negative charge of the clay minerals or by inter-lamellar amorphous gibbsite or brucite layers balancing the permanent charge of the clay minerals in the acid pH = 3-4 leading to a decrease or lack of reactive cation exchange sites. A significant correlation was also observed between EC and FOC, containing the most reactive organic carbon, including low-molecular weight organic carbon, as well mineral nitrogen providing NO_3^- and NH_4^+ ions in solution.

We suspect that the small-scale variability of water repellency may be correlated with the spatial distribution of particulate lignitic particles and lignite-coated minerals grains. We are further studying the effect the spatial pattern of water repellency has on water and contaminant movement in the technogenic soils (unpublished data).

To get more information about the inter-relation between the different soil characteristics and properties, principal component (PCA) and cluster analyses (CA) were conducted with the twelve measured parameters at the different sampling sites and layers. The PCA identified three principal components (PCs) (Table 3) explaining 46.7%, 17.8% and 14.4% of the total variance (79%). The components matrix (Table 3) distinguishes between components that are as different from each other as possible, and helps interpretation by allocating each variable primarily on one of the components. The 1st principal component is mainly loaded by WDPT, CEC, EC, % sand, TOC, HOC, FOC, mineral N (MN) and through a strong inverse relationship with the % clay. Total nitrogen (N) and contents of silt are mainly related to component 2, and % hygroscopic moisture to component 3.

Table 2. Correlation matrix between the measured parameters

* Significant at p< 0.05

	WDPT	CEC	SAND	SILT	CLAY	TOC	HOC	FOC	MN	EC	M	N
WDPT	1.000	0.408	0.137	0.066	-0.116	0.699*	0.499*	0.442*	0.580*	0.326	-0.492*	-0.017
CEC	0.408	1.000	0.636*	0.100	-0.603*	0.466*	0.472*	0.638*	0.804*	0.939*	-0.207	-0.375
SAND	0.137	0.636*	1.000	0.263	-0.985*	0.446*	0.579*	0.462*	0.528*	0.441*	-0.112	0.236
SILT	0.066	0.100	0.263	1.000	-0.419	0.182	0.117	0.260	0.029	0.072	0.100	0.408
CLAY	-0.116	-0.603*	-0.985*	-0.419	1.000	-0.442*	-0.553*	-0.463*	-0.498*	-0.413	0.097	-0.297
TOC	0.699*	0.466*	0.446*	0.182	-0.442	1.000	0.862*	0.714*	0.666*	0.284	-.0466*	0.338
HOC	0.499*	0.472*	0.579*	0.117	-0.553	0.862*	1.000	0.645*	0.526*	0.262	-0.238	0.285
FOC	0.442*	0.638*	0.462*	0.260	-0.463*	0.714*	0.645*	1.000	0.612*	0.609*	-0.157	0.212
MN	0.580*	0.804*	0.528*	0.029	-0.498*	0.666*	0.526*	0.612*	1.000	0.733*	-0.706*	-0.120
EC	0.326	0.939*	0.441*	0.072	-0.413	0.284	0.262	0.609*	0.733*	1.000	-0.171	-0.518*
M	-0.492*	-0.207	-0.112	0.100	0.097	-0.466*	-0.238	-0.157	-0.706*	-0.171	1.000	-0.076
N	-0.017	-0.375	0.236	0.408	-.0297	0.338	0.285	0.212	-0.120	-0.518	-0.076	1.000

The dendrogram of Fig. 1 supports represents three major branches (groups). The first major group can be sub-divided into two sub-groups. The first sub-group is represented by CEC, EC, MN and % sand content and the second sub-group by TOC, HOC, FOC and WDPT. The second main group is represented by the % silt and total N and the third main group by the % hygroscopic moisture and the % clay content. The organic carbon parameters TOC, FOC and HOC are intermediately placed in the cluster tree, confirming their linkage to components 1 and 2. The cluster analysis supports the relationships obtained from the PCA analysis for grouping of various soil parameters.

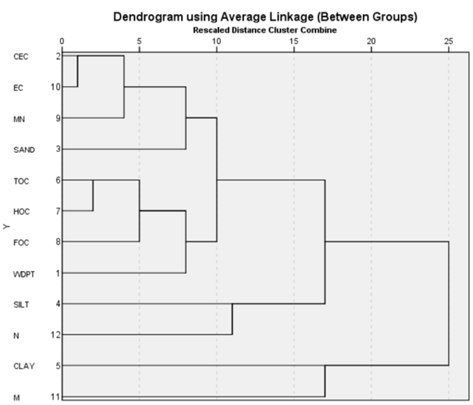

Fig. 1. Cluster analysis of the twelve variables analyzed

Table 3. Components matrix. Three components extracted

	Component		
	1	2	3
WDPT	.611	-.143	-.558
CEC	.840	-.411	.325
SAND	.744	.279	.447
SILT	.248	.541	.288
CLAY	-.732	-.362	-.472
TOC	.820	.239	-.425
HOC	.773	.292	-.189
FOC	.801	.089	.007
MN	.879	-.308	-.164
EC	.702	-.572	.351
M	-.443	.169	.625
N	.087	.922	-.214

Conclusions

Technogenic soils from the area of Maritsa-Iztok lignite coal basin in Bulgaria were studied at two non-vegetated and pine-afforested plots. The soils were characterized by severe to extreme hydrophobicity and heavier texture at the pine-vegetated site, as well as extreme acidity (pH 3-4). Principle Component Analysis (PCA) and cluster analysis were performed with the aim to assess the interrelation between the measured parameters and the persistence of soil water repellency, as revealed by the water drop penetration time (WDPT). Three principle components were identified describing 79% of the total variability. The WDPT was significantly positively correlated with TOC, as well as humic and fulvic organic carbon (HOC and FOC), mineral nitrogen (MN) and negatively correlated with the % hygroscopic moisture. The results obtained indicate that total organic carbon (TOC) containing irregularly distributed coal and ash particles, as well as humic and fulvic organic carbon (HOC and FOC) were significantly correlated with the mineral nitrogen (MN), and were the main drivers of soil water repellency in the studied technogenic soils.

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GEOCHEMICAL ASSOCIATIONS IN TECHNOGENIC SOILS (TECHNOSOLS) OF CONTRASTING HYDROLOGICAL CHARACTERISTICS FROM THE REGION OF MARITSA-IZTOK COAL MINE IN BULGARIA

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Abstract

Atanassova I., M. Benkova, M. Banov, T. Simeonova, L. Nenova and M. Harizanova, 2018. Geochemical associations in technogenic soils (technosols) of contrasting hydrological characteristics from the region of maritsa-iztok coal mine in Bulgaria, *Bulg. J. Agric. Sci.*,(Suppl. 2):18-26

Studied were metal associations and contamination status of hydrophilic and hydrophobic technogenic soils from the area of Maritsa-Iztok coal mine region in Bulgaria. Four sites with contrasting hydrologic and acidic properties were chosen for investigation. At two of the sites following humus and non-humus reclamation under grass vegetation, soils were hydrophilic. At the other two sites (non-vegetated and pine-vegetated), soils were extremly hydrophobic (water drop penetration time WDPT 10802s - 14440s). The Principle Component (PCA) and cluster analyses performed on the hydrophobic technogenic soils revealed that four principle components were distinguished explaining 80.5% of the total variance of the thirteen variables tested. The first component was loaded by Zn, Co, Ni, Mg and % clay content, implying affiliation of the aforementioned metals to overburden clays, the second by WDPT, Cu, Cr, Fe, organic carbon (OC) and cation exchange capacity (CEC) implying affiliation of metals to the organic matrix of the coal and ash, the third, by Mn and Fe, and the fourth by Pb related to other anthropogenic sources. For the hydrophilic Technosols only two principle components were distinguished containing 91% of the total variance of the twelve original variables. Most of the heavy metals and Mg loaded the 1st component as well as the cation exchange capacity (CEC), % clay and OC. Manganese and lead loaded the 2nd component and were not related with rest of the metals, which indicates a different source. In some samples of the hydrophobic Technosols the measured contents of Cu and Pb exceeded the national guidelines for agricultural arable lands. The hydrophobicity of the Technosols seems to be related to the contents of Cu and Cr present in the soil organic matrix.

Key words: heavy metals, technogenic soil, hydrophobicity, Principal Component Analysis, geochemistry

Abbreviations:WDPT – Water Drop Penetration Time, OC – Organic Carbon, CEC – Cation Exchange Capacity, SOM – Soil Organic Matter, PCA – Principle Component Analysis

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Introduction

Mining activities cause land alteration and may lead to drastic changes in the ecological conditions. The waste piles often pose extreme threats to environmental restoration. Mining activities lead to disruption of the morphology of the soil profile, soil horizons, disturbance of soil microbial populations and the ecosystem in general (Sheoran et al. 2010). The overburden dumps are often characterized by elevated bioavailability of metals, lack of enough moisture, increased compaction and relatively low organic matter content. They may be acidic, due to pyrite metal sulphides and, which generate acid-mine drainage. A common practice in land reclamation is to apply surface soil humus layer, which assists in the prevention of further oxidation of the overburden layers of black clays consisting of a mix of sandstone, pyrites and waste coal, therefore facilitating the subsequent revegetation of the site (Barnhisel and Hower, 1997). Forestation, or biological reclamation initiates soil-forming processes in surface coal mine spoils (Krümmelbein et al., 2012).

Garbuchev et al. (1975) have conducted complex long-term studies of the Maritsa-Iztok coal basin for reclamation of lands for agricultural purposes. Pliocene sediments located above the coal layer are used as a main substrate for reclamation purposes. Hristov and Banov (1996) studied several profiles reclaimed without spreading of humus horizon on the surface, with different land use type and different period of development for establishment of changes that occur in sthe ubstrates. Banov and Marinkina (2002) studied the conditions for biological restoration of reclaimed soils from Maritsa-Iztok Mines through spreading a humus layer of ~ 40 cm thickness. The authors found that the reclaimed soils were of heavy texture which determines unfavorable water physical properties. In spite of the low humus content (1.52 to 2.35%), the pH is slightly alkaline (pH H2O 7.3 – 7.7) which prevents mobilization of heavy metals. The yellow and green clays could be a suitable substrate for development of vegetation, however the high acidity of the black clays may cause serious environmental consequences. Magnesium exhibits higher contents in primary and secondary minerals in the yellow clays comprising the uppermost layer of the stratigraphic profile, immediately underlying the soil layer and comprising a major component of the reclaimed soils (Garbuchev et al., 1975). A useful amendment and ameliorant of the overburden substrate is the coal ash (pH 7-7.3) a waste product from the nearby coal incineration thermal power plant. Heavy metals in coal may be classified according to their association with the organic fraction, the mineral fraction or with both.

The elements including As, Cd, Hg, Pb and Zn are mostly concentrated in the organic fraction of the coal, while Cr, Cu and Se were present in both the mineral and organic matter (Chadwick et al. 1987). Coal ash is characterized by higher concentration of metals (Schwab et al., 1991) with arsenic pollution being directly related to trace element production from the combustion of coal (Huggins et al. 1993). The trace elements may be enriched to 10 times during coal combustion (Fernandez-Turiel et al., 1994).

The objective of the present study was to evaluate the local geochemical and contamination associations of eight heavy metals i.e. Cu, Zn, Co, Cr, Ni, Mn, Fe and Pb ($\rho > 5$ g/cm^3, Duffus, 2002), and the alkaline earth metal Mg (ρ 1.7 g/cm^3) and relate these patterns with the hydrophobicity and/or hydrophilicity of the studied soils.

Materials and Methods

The study was carried out on four plots, two of them near Mednikarovo village, subject to humus and non-humus reclamation > 20 years ago, and the other two at Obruchishte village, between Troyanovo 1 and Troyanovo 3 mines of Maritsa-Iztok coal mine in Bulgaria. The experimental plots near Obruchishte were located on several hectares of large area, afforested with *P. nigra*. These spoils were created > 30 years ago and consisted of loam-textured Pliocene overburden sediments from the nearby open-cast lignite mines. The GPS coordinates (UTM-system) of the sites are: Obruchishte, Site 1: N 42.16434, E 25.94285, and N 42.16452, E 25.94318 and Mednikarovo, Site 2: N 42.11007, 42.11445 and E 26.03877, 26.02697. A lack of plant density and water repellency (hydrophobicity) was observed at spots of~200 m^2 amongst a uniformly pine vegetated area at the Obruchishte site.

At the four sites, grids Δ 2 m, ~ 40 m^2 were constructed and sampling was at two depths where water repellency was demonstrated on the field 0-5 (10) cm and at 10-20 cm. At the non-vegetated site of Obruchishte, soils were of sandy loam texture mixed with degraded finely dispersed lignitic particles and coal ash, and of sandy clay (0-5 cm) and clay texture at 10-20 cm at the pine vegetated site. Layers of greyish-green and yellow clays intermixed with coal and ash were located at surface depths of 0-10 cm and prevailing black clays at depths > 20 cm. At the non-vegetated site, the substrate 0-50 cm was found to be water repellent since water drop penetration time (WDPT) which is an indicator of the persistence of potential water repellency was > than several minutes. Soil cores were taken to a depth of 0-10 and 10-20 cm and at two points to a depth of 0-50 cm using a 3 cm wide and 25 cm long core sampler. Soil samples in the field were classified as non-repellent (WDPT < 5s), strongly (60 s < WDPT< 600 s) and severely > 600s water repellent according to the scale of De Bano (1981). The soil samples were equilibrated at the ambient air humidity for four days before measuring water drop penetration time (WDPT) in the laboratory at controlled humidity and temperature.

Soils at the Mednikarovo site were non-water repellent (hydrophilic), since WDPT was < 5s. At Mednikarovo site the investigated soils were: (i) humus layer-reclaimed soil of clay loam texture and non-humus reclaimed soil of sandy loam texture. The surface horizon with ~ 40 cm depth is a translocated humus horizon of natural Vertisol occupying the territory prior to mining. The sub-layers of ~ 2 m are composed of yellow and green clays comprising the overburden sediments of the stratigraphic profile and possessing suitable physico-chemical characteristics (pH ~ 7).

Total organic carbon (TOC) in the studied spoils was determined by oxidation with $K_2Cr_2O_7/H_2SO_4$ and organic carbon fractionation by the method of Kononova (1963), modified by Filcheva and Tsadilas (2002), cation exchange capacity (CEC) was assessed as sum of titratable acidity (pH 8.2) and extractable Ca, by saturation with K malate at pH 8.2 (Ganev and Arsova, 1980). Soil water-repellency (soil hydrophobicity) was measured by the water drop penetration time (WDPT) method (Doerr et al. 2002). Three droplets of distilled water (80 μl) were placed on the soil surface and the time was recorded for droplet penetration. Total (pseudo-total) content of metals were assessed according to ISO 11466:1995 aqua-regia method.

Statistical analysis (principal component PCA and cluster CA) was performed by SPSS 22 for MS Windows.

Results and Discussion

General soil characteristics

The mobility of heavy metals in coal mine spoils-turned soils depends strongly on the properties of the technogenic soils. In the process of coal mine spoils-water interaction, sulfides release metals, which are then adsorbed and complexed by organic matter and iron (oxy)hydroxides resulting from pyrite oxidization (Dang et al., 2002). During the natural weathering of coal mine spoils in acid conditions, a considerable fraction of these metals becomes mobile and is released to the environment. Texture is another factor influencing heavy metal adsorption. The soils at Obruchishte under pine vegetation contain between 21% and 36% clay (< 0.001 mm) in the 0-5 cm layer and 30-70% in the 10-20 cm layer, and those at the non-vegetated plot had a lighter texture, i.e. 14-21.8% clay at a depth of 0-20 cm. The Technosols under humus and non-humus reclamation at Mednikarovo site were heavier in texture ranging from 23-44% clay for the 0-10 cm layer and from 26-51% clay for 10-20 cm layer. The soil reaction was near neutral (Table 1) thus preventing most of the heavy metals from being mobilized to considerable amounts. At the Obruchishte site, however, soil samples were extremely acidic (Table 1), although having been mixed with coal ash (coal ash from the cinder embankment has a pH 7-7.3, Zheleva and Tsolova, 2004). The high acidity of the non-vegetated site is a result of the weathering of black clays present in the overburden layers releasing amphoteric metals such as Fe and Al. The electrical conductivity of the hydrophobic technogenic soils was an order of magnitude higher than in the hydrophilic soils from Mednikarovo (Table 1), due the higher ionic strength (mostly sulfate) of overburden clays containing coal and ash impurities. Total organic carbon (TOC) of Mednikarovo soils was low ~ 1% and was higher for the humus reclaimed soils, than in the non-humus reclaimed ones. The higher TOC of the hydrophobic technogenic sols than in the hydrophilic ones was due to admixtures of coal and ash during the reclamation process (Table 1).

Total metal contents and geochemical associations

Total metal contents at the four sites of the reclaimed soils studied (Table 2 a,b) were below the maximum permissible loads designated by the national regulation standards (Regulation No 3, 2008). However, at the very acid pH values of the Obruchishte Technosols contents of Cu and Pb for some samples (Table 2) exceeded the national regulation standards. In addition, the soluble metal concentrations e.g. Al, Fe, Cu, Zn and Co, provoked by the extremely acid pH ~ 3 were higher than maximum permissible levels for surface waters (Regulation No 12, 2002, unpublished data, Project DN 06/1 NSF, Ministry of Education and Science).

Statistical analysis

Hydrophobic Technosols (Obruchishte)

The Principal Component Analysis (PCA) for the water repellent Technosols at Obruchishte was used in the simultaneous study of thirteen factors and explained the data variability in the process of factor reduction to several unrelated components. Four factors with eigenvalue > 1 were extracted when analyzing the data. The Principal component analysis was based on the thirteen variables: Cu, Cr, Co, Ni, Pb, Zn, Fe, Ni, Mn, Mg, % clay, % TOC and cation exchange capacity

Table 1. Main soil properties of the experimental soils. (From Atanassova et al., 2017, submitted to BJAS) Atanassova et al. (2018)

Site	Depth cm	WDPT * (s)	pH		EC (mS/cm)		TOC %		$CEC_{8.2}$ (cmol.kg-1)		Clay %	
			Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Mednikarovo humus reclamation	0-10	1	6.9	0.01	0.04	0.01	1.50	0.02	56.3	0.2	38.6	5.21
	10-20	2	6.9	0.02	0.05	0.01	1.53	0.03	56.3	0.1	45.4	4.48
Mednikarovo, non-humus reclamation	0-10	2	7.2	0.01	0.04	0.005	1.04	0.02	29.6	0.2	24.4	1.25
	10-20	1	7.2	0.01	0.04	0.005	0.37	0.01	29.8	0.1	26.6	0.96
Obruchishte, pine vegetation	0-5	14-9589 1200	4.6	0.20	0.19	0.14	5.09	0.42	41.27	3.89	28.0	7.21
	10-20	2 – 128 10	4.2	0.43	0.63	0.14	3.00	1.67	44.60	3.52	55.1	21.7
Obruchishte, non-vegetated	0-10	76-14440 1282	3.2	0.11	1.72	0.76	6.43	3.98	67.77	6.16	17.8	3.86
	10-20	202-2470 614	3.1	0.10	1.61	0.69	6.11	0.96	69.90	8.70	17.0	2.37

* Range and median (underlined)

Table 2a. Total (pseudo-total) contents (aqua-regia) of metals, mg.kg^{-1} in Technosols.

Site	Depth, cm	Cu		Zn		Co		Cr		Ni	
		Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Mednikarovo humus reclamation	0-10	22.83	1.26	36.3	0.76	12.8	0.58	26.7	1.44	26.5	0.87
	10-20	22.33	1.26	36.5	4.33	12.8	0.76	26.5	3.97	27.0	1.0
Mednikarovo, non-humus reclamation	0-10	7.83	0.58	18.8	3.18	4.3	0.76	4.5	1.32	5.8	1.61
	10-20	6.33	1.26	19.8	5.01	3.8	0.29	4.5	0.87	5.0	1.0
Obruchishte, pine vegetation	0-5	63.67	4.25	57.7	10.0	6.0	0.87	9.3	0.29	12.7	0.76
	10-20	62.83	7.15	54.0	9.85	6.2	2.25	9.0	2.29	15.5	4.27
Obruchishte, non-vegetated	0-10	73.83	9.29	39.8	3.75	4.8	0.29	11.5	2.5	10.8	0.58
	10-20	69.83	7.97	41.7	7.22	4.7	0.58	12.5	1.73	10.0	3.61

(CEC), as it has been proved that relationship exists between litho(geo)genic metals and some soil parameters, such as organic carbon and % clay contents (Micó et al., 2006). When analyzing data for the hydrophobic soils, four components have been identified with eigenvectors (eigenvalue) > 1, explaining 43,5%, 17,1%, 11,1% and 8,8 of the total variance 80,7%. The correlation matrix and the component matrix are presented in Table 3 and Table 4. The first three components explaining 70.5% of the variance (80.5%) were rotated in space and the respective distribution is presented in Figure 1. The 1st component was loaded by Zn, Co, Ni, Mg, % clay and Mn (by a lower coefficient). The 2nd component was loaded by WDPT, Cu, Cr, OC, CEC and Fe, the 3rd component, by Mn and Fe, therefore these metals are influenced by two components, and the 4th component was loaded by Pb. Manganese readily substitutes for Fe^{2+} and Mg^{2+} in minerals (Ure and Berrow, 1982). The negatively charged MnO_2 is responsible for the high degree of association of Mn oxides with some transition metals, in particular with Co, Ni, Cu, Zn, Pb, etc. The distribution of MnO_2 in soil has been found to be closely related to the contents of Fe_2O_3 and a close correlation exists between Mn and ferrous iron in igneous rocks, with Mn:Fe ratios in the range 0.015-0.02 (Mielke, 1979).

In our study a positive, although insignificant correlation existed between Mn and Fe (R= 0.408) (Table 3). The dual origin of Mn, as observed by the statistical analysis may be due to the fact that Mn may in addition have a biogenic source, since it is involved in electron transport in photosynthesis and enzymatic reactions. This assumption is supported by the higher values observed for manganese in the vegetated Technosols (Table 2), than the non-vegetated site.

Cluster analysis for grouping variables in a cluster containing members of similar characteristics and/or origin was performed on the water repellent soils (Figure 2). Two main groups were distinguished in the dendrogram. The first main group was subdivided into two sub-groups: one consisted of the geogenic metals Co, Ni, Zn, Mg, related with the clay fraction and the other by Mn, Fe and Pb. The second main group consisted of the metals Cr and Cu closely related with TOC, WDPT and CEC. This branch of the cluster tree contains the metals related with organic matter causing water repellency, most probably present in the black clays, containing coal and ash having being added as an ameliorant to the substrates.

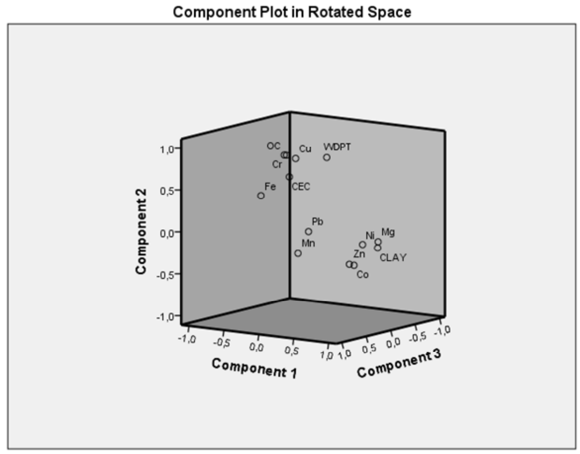

Fig. 1. Three components in rotated space (hydrophobic Technosols, Obruchishte site).

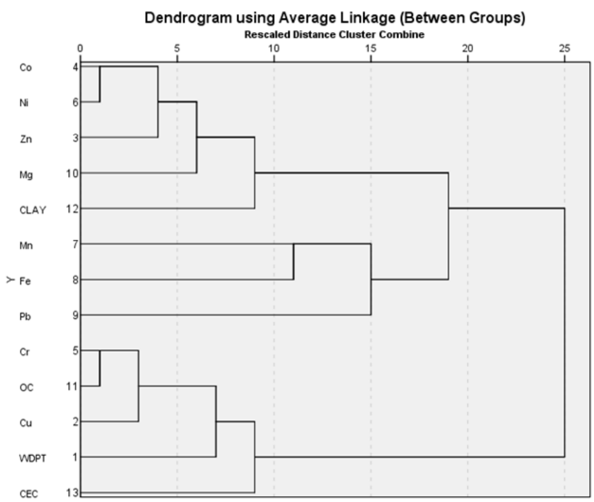

Fig. 2. Cluster analysis with dendrogram for the hydrophobic Technosols from Obruchishte site.

Table 2b (continued). Total (pseudo-total) contents (aqua-regia) of metals, mg.kg^{-1} in Technosols (continued).

Site	Depth, cm	Mn		Fe		Pb		Mg	
		Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Mednikarovo humus reclamation	0-10	950	229.13	21183	3286	17.3	4.3	68.8	0.76
	10-20	1000	229.13	24217	1767	40.6	35.4	68.3	0.76
Mednikarovo, non-humus reclamation	0-10	900	785.8	8500	6106	25.2	34.9	61.8	0.76
	10-20	566.7	275.4	5317	1955	24.7	23.7	61.7	0.76
Obruchishte, pine vegetation	0-5	800	217.95	9800	985	30.0	9.9	61.5	3.12
	10-20	833	711.22	13133	10069	43.7	16.2	62.2	1.76
Obruchishte, non-vegetated	0-10	400	50	12583	3761	27.0	13.3	59.5	0.87
	10-20	366.7	76.4	12917	5.07	38.0	5.1	60.0	1.80

Table 3. Correlation matrix for heavy metals and major soil properties of the hydrophobic technogenic soils at Obruchishte site (coal and coal ash treated).

	WDPT	Cu	Zn	Co	Cr	Ni	Mn	Fe	Pb	Mg	TOC	CLAY	CEC
WDPT	1.000	.487*	-.278	-.177	.549*	-.087	-.135	.247	.203	-.063	.699*	-.116	.408
Cu	.487*	1.000	-.483*	-.659	.706*	-.352	-.261	.354	-.217	-.303	.757*	-.240	.394
Zn	-.278	-.483	1.000	.674*	-.365	.690*	.623*	-.289	-.126	.648*	-.401	.434	-.533*
Co	-.177	-.659*	.674*	1.000	-.378	.852*	.465*	-.185	.147	.577*	-.491*	.398	-.501*
Cr	.549*	.706*	-.365	-.378	1.000	-.161	-.123	.615	-.002	-.262	.811*	-.462*	.623*
Ni	-.087	-.352	.690*	.852*	-.161	1.000	.475*	-.016	.041	.691*	-.396	.587*	-.446*
Mn	-.135	-.261	.623*	.465*	-.123	.475	1.000	.403	.124	.267	-.116	.146	-.503
Fe	.247	.354	-.289	-.185	.615	-.016	.403	1.000	.307	-.270	.442*	-.359	.222
Pb	.203	-.217	-.126	.147	-.002	.041	.124	.307	1.000	.085	-.124	-.064	-.050
Mg	-.063	-.303	.648	.577*	-.262	.691*	.267	-.270	.085	1.000	-.446*	.615*	-.402
TOC	.699*	.757*	-.401	-.491	.811*	-.396	-.116	.442*	-.124	-.446*	1.000	-.442*	.466*
CLAY	-.116	-.240	.434	.398	-.462*	.587*	.146	-.359	-.064	.615*	-.442	1.000	-.603*
CEC	.408	.394	-.533*	-.501*	.623*	-.446	-.503	.222	-.050	-.402	.466*	-.603*	1.000

Table 4. Rotated component matrix for the hydrophobic soils at Obruchishte: varimax with Kaiser normalization

	Component			
	1	2	3	4
WDPT	.080	.825	-.164	.283
Cu	-.288	.792	-.053	-.301
Zn	.742	-.315	.318	-.213
Co	.747	-.339	.230	.219
Cr	-.238	.873	.203	.022
Ni	.883	-.074	.248	.072
Mn	.383	-.143	.856	.004
Fe	-.256	.458	.701	.289
Pb	.006	-.029	.102	.940
Mg	.863	-.093	-.097	.077
OC	-.324	,856	.128	-.142
CLAY	.754	-.199	-.244	-.121
CEC	-.511	.521	-.244	.120

Table 6. Rotated component matrix for the hydrophilic soils at Mednikarovo: varimax with Kaiser normalization. for the hydrophilic soils from the Mednikarovo site.

	Component	
	1	2
Cu	.993	.074
Zn	.969	-.040
Co	.992	.102
Cr	.993	.028
Ni	.987	.129
Mn	.269	.805
Fe	.900	.405
Pb	-.068	.846
Mg	.983	.110
TOC	.852	.281
CLAY	.905	.147
CEC	.983	.113

Table 5. Correlation matrix for heavy metals and major soil properties of the hydrophilic technogenic soils at Mednikarovo site (humus and non-humus reclamation).

	WDPT	Cu	Zn	Co	Cr	Ni	Mn	Fe	Pb	Mg	TOC	CLAY	CEC
WDPT	1.000	.949*	.993*	.990*	.992*	.317	.919*	.003	.993*	.878*	.889*	.990*	1.000
Cu	.949*	1.000	.956*	.966*	.955*	.232	.853*	-.084	.946*	.777*	.860*	.943*	.949*
Zn	.993*	.956*	1.000	.992*	.998*	.335	.935*	.035	.984*	.861*	.906*	.992*	.993*
Co	.990*	.966*	.992*	1.000	.990*	.251	.909*	-.003	.983*	.829*	.889*	.987*	.990*
Cr	.992*	.955*	.998*	.990*	1.000	.339	.939*	.075	.984*	.860*	.904*	.996*	.992*
Ni	.317	.232	.335	.251	.339	1.000	.585*	.391	.363	.504*	.342	.299	.317
Mn	.919*	.853*	.935*	.909*	.939*	.585*	1.000	.259	.924*	.869*	.881*	.918*	.919*
Fe	.003	-.084	.035	-.003	.075	.391	.259	1.000	.023	.092	.080	.088	.003
Pb	.993*	.946*	.984*	.983*	.984*	.363	.924*	.023	1.000	.856*	.881*	.982*	.993*
Mg	.878*	.777*	.861*	.829*	.860*	.504*	.869*	.092	.856*	1.000	.740*	.848*	.878*
TOC	.889*	.860*	.906*	.889*	.904*	.342	.881*	.080	.881*	.740*	1.000	.907*	.889*
CLAY	.990*	.943*	.992*	.987*	.996*	.299	.918*	.088	.982*	.848*	.907*	1.000	.990*
CEC	1.000	.949*	.993*	.990*	.992*	.317	.919*	.003	.993*	.878*	.889*	.990*	1.000

*Significant at $p < 0.05$

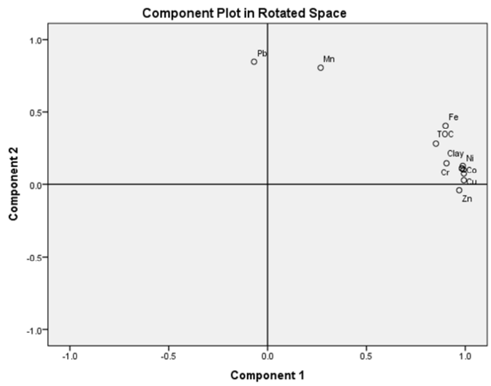

Fig. 3. Component plot in rotated space for the hydrophilic Technosols from the Mednikarovo site.

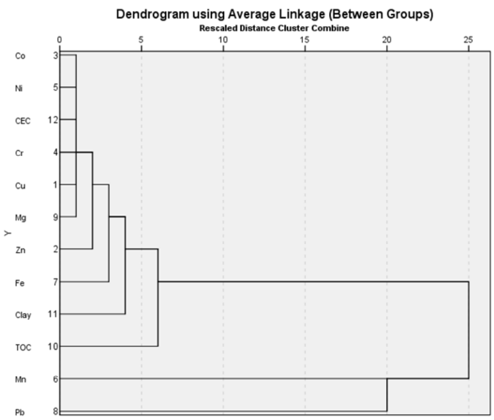

Fig. 4. Cluster analysis for the hydrophilic Technosols from the Mednikarovo site.

Hydrophilic Technosols (Mednikarovo)

A completely different pattern was obtained when performing the PCA and Cluster analyses for the hydrophilic soils from Mednikarovo. Only two principle components were identified explaining 79% and 12% of the total variance (91%, Table 6, Figure 3). The correlation matrix (Table 5) revealed strong statistically significant correlations between most of the heavy metals and their close association and strong correlation with the first component (Table 6). The second component was loaded by the elements Mn and Pb, implying a different source for these metals. The cluster analysis supports the PCA data (Figure 4). The dendrogram includes two main groups. All the metals Co, Ni, Cr, Cu, Mg, Zn and Fe were related with the clay and organic carbon (OC) content from the first main group. The fact that for the water repellent Technosols only Cu and Cr were associated closely with OC implies more or less association with different type and/or source of organic carbon compared with the organic carbon of the hydrophilic soils. The second main group consists of Mn and Pb of different origin and/or source. Manganese may have biogenic sources and Pb predominantly anthropogenic, e.g. from vehicle emissions during transportation of overburden sediments.

Conclusions

Assessed were metal associations of hydrophilic and hydrophobic Technosols possessing contrasting hydrologic and acidic characteristics from the region of Maritsa-Iztok coal mine region in Bulgaria. The PCA and cluster analyses performed on the water repellent Technosols revealed that four principle components were distinguished explaining 80.5% of the total variance of the thirteen variables tested. Zinc, Co, Ni, Mg contents were related with the % clay, implying affiliation of the aforementioned metals to overburden clays. Water repellency (WDPT), Cu, Cr, Fe, organic carbon (OC) and cation exchange capacity (CEC) were closely associated, indicating links of these metals to the organic matrix of the coal and ash. Manganese and Fe had a dual source, while Pb is related to different anthropogenic sources, e.g. motor vehicle emissions.

For the hydrophilic soils most of the heavy metals and Mg were present in organo-mineral associations with the clay fraction and organic carbon. Manganese and Pb were not related with the rest of the metals, and therefore originate from a different source. The measured total contents of Cu and Pb for some hydrophobic soils exceeded the national guidelines for agricultural arable lands at the acid pH~3. The hydrophobicity of the soils seems to be related to the concentrations of Cu and Cr present in the soil organic matrix.

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EFFECT OF BAYKAL EM-1 ON GROWTH DEVELOPMENT AND MICROBIOLOGICAL STATUS OF SUCKLING PIGS

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Abstract

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In an experiment with 15 equalized by piglet age, origin and size litters, of which 8 litters in the trial and 7 litterts in the control group, the effect of Baikal EM-1 feed additive, a multi-purpose microbiological product containing a complex of living, useful microorganisms, on the weight development and the microbiological status of the gastrointestinal tract of the pigs, was studied. The additive was included in the feed of the sows and pigs at a dosage of 10 ml / kg of feed, from the day 8th after birth to the day 35th at weaning of the pigs. On the day 21st and day 35th the body weights of the pigs were recorded, and on the day 35th, 10 rectal tampon samples (RTS) from the two groups were taken and microbiologically examined.

It was found that the data on weight development, total growth and average daily gain did not show any significant differences in the benefit of the trial group, which contradicts the hitherto established for many other feed additives. Microbiological tests showed that 40% of the RTS of both groups were positive for E. coli, of which in the trial group 75% were nonpathogenic and 25% pathogenic (O139:F4) in and in the control group 75% pathogenic (O139:F4) and 25% non-pathogenic, which represents the product as a good prophylactic remedy.

Key words: pigs, feed additives, probiotics, “Baykal EM-1”, average daily gain, effectiveness, E. coli.

Introduction

Feed additives are a group of substances with a wide range of action, which are classified as: nutritional additives defined as “separate components” and „additives” and non-nutritional additives, including “nutritional antibiotics” and “alternatives to nutritional antibiotics” (Angelova, 2000; Angelova and Tenchev, 2008). In pig holdings, stationary for colibacteriosis and other gastroenteritis diseases in pigs, besides etiotropic and symptomatic therapy, a number of immunoprophylaxis and metaphylaxis measures are applied (Yordanov, 2008, 2014; Dimitrova, 2009, 2010; Lyutskanov, 2013; Dimitrova et al., 2015).

In the intestinal forms of colibacteriosis with good therapeutic and metaphylactic effect, the antibiotics streptomycin, neomycin, apraplan and spectinomycin have recently been applied. In recent years as a result of the acquired resistance of some coli-strains to them, the aminoglycosides are current – gentamycin, kanamycin and amikacin; amphenicoles-florfenicol and thiamphenicol, some quinolones such as flumectin (Plamb, 2002; Popova, 2009; Vestič, 2012) and fluoroquinolones-ciprofloxacin, pefloxacin, enrofloxacin and

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marbofloxacin (Dimitrova, 2010; Popova,2013; Fraile, 2013; Dimitrova et al., 2014; Yordanov et al., 2016; Petkova, 2017).

The emergence of multiple resistance to antimicrobials focuses on the attention of both human and veterinary doctors on their use. Therefore, each case requires estimation for necessity the need for antibiotics (Close, 2000; Drumev, 2001; FVE, 2003; Dimitrova et al., 2014). In connection with the problem of resistance, the Committee for veterinary medical products of European Union has stopped using some antibiotics and has completely prohibit use of nutritional antibiotics as growth promoters (Yordanov and Dimitrova, 2014).

Various remedies, alternatives to nutritional antibiotics, such as acidifiers, oligosaccharides, enzymes, prebiotics, probiotics and other biologically active substances of plant origin could be successfully applied in the prosperous and relatively prosperous herds for bacterial intestinal infections (Dimitrova et al., 2003; Virtanen, 2004; Yordanov et al., 2006; 2009; Mateva, 2010; Popova, 2013). According to Petkova (2017), the feed additives Tariben and Periben, representing combinations of tannin or pectin with essential oil of oregano and benzoic acid, reduce the carrying of pathogenic and conditionally pathogenic bacteria to 50% and stimulate the improvement of the general and clinical condition of the pigs. Parvulov and Markov (1996) and Murgov and Denkov (2007) pay spetial attention to probiotics and probiotic foods, especially lactic acid products. It is known that there are about 100 bacterial species of in the gastrointestinal tract of humans and animals that can be useful (lactic acids) and harmful (decaying) and are found among themselves in symbiotic or antagonistic relationships. The fact that the gastrointestinal flora is closely related to the health of the host shows that its balance is of great importance for the health (Slavchev, 2004).

Baykal EM-1 is a multi-purpose microbiological product developed by Russian microbiologists, a complex of living, useful microorganisms, used to restore the soil and enhance the immunity of plants, animals and humans. Manufacturer is a company “EM-Corporation”, Russia, Ukraine. Used in Canada, Japan, the Netherlands, Austria, Denmark and many other countries. His composition includes nearly 60 strains of microorganisms from the groups of photosynthetic bacteria, lactic acid bacteria, actinomycetes, yeasts and fermenting fungi. The mechanism of action is that the animals received a complex of living, useful microorganisms who form colonies in the animal’s body, displace the pathogenic microflora from the active growth zone and enrich the environment with the products of its vital activity. Preliminary studies of Ukrainian farms (INDUSTAR, Kharkov region) show that the product helps balancing the microflora of the digestive tract in animals, and this has a positive effect on the reduction of enterocolitis, dysbacteriosis, diarrhea, etc. In addition, the product has a positive effect on the animal’s live weight gain (***, 2005). The aim of this study was to determine the effect of Baykal EM-1 on the weight development and microbiological status of the gastrointestinal tract in suckling pigs.

Materials and Methods

The experiment was carried out in Experimental farm of Agricultural Institute - Shumen. A total of of 154 suckling pigs of 15 litters, of which 7 litters (72 pigs) included in the control group and 8 litters (82 pigs) included in the trial group, equalized by age, origine, sequence of farrowing and number of pigs in the litters, were studied. Combined feed for sows and pigs (Tables 1 and 2) in the trial group was treated with Baikal EM-1, at a dose of 10 ml/kg of fodder, and the feed of the control group was fed without any additives. The trial and control groups received the specified diets from 8th to 35th day of birth.

On the day 1st, 21st and 35th after birth, weights of the pigs of both groups were recorded. Total and daily gains were determined for periods from day 1st to day 21st and from day 22nd to day 35th and generally from day 1 to day 35 days after birth. The data were statistically processed with Microsoft Excel for statistically significant differences between groups using t-test.

At day 35th, 10 numbers rectal tampon samples (RTS) from each group were taken and tested microbiologically for pathogenic bacteria. Generally accepted selective and differentiating feed media for isolation and determination of the fermentation activity of the isolates were used. The occurrences of α- and β-hemolysis or the absence of these were recorded on blood agar. To determine the serologic groups of Escherichia coli (E. coli) isolates and to determine pathogenicity factors, we applied the corresponding serological tests with specific agglutinating sera (O8, O20, O25, O74, O78, O101, O136, O138, O139, O141, O147, O148, O149, O157 and for F4 and F5) and ready diagnostic kits.

Results

During the entire trial period, no sickness in pigs and no mortality in both groups were recorded. No difference in the body status of the pigs in the trial and in the control group during the whole experimental period was mentioned. Data on weight development, total and average daily gain are shown in Table 3. Pigs from both groups had one and the same live weight at birth (1.579 kg and 1.582 kg) and on the day 21st day a difference of 0.255 kg in advantage of the control group is registered. This differentiation in the weight development of the pigs continued in the next period from the day 22nd to the day 35th day, with control animals exceeding those in the experimental group by 0.367 kg. Expressed in total gain this difference was 0.258 kg in the favor of the control group for the first period and 0.112 kg for the second period, also for the control group. Calculated for the entire trial period, from day 1st to day 35th day after birth, the difference in total gain was 0.369 kg in the favor of the control group or 5.1% less total gain for the trial group.

The estimated average daily gain for the first period in the control group was 0.233 kg, and in the trial group 0.225 kg or 8 g lower, and in the second period, respectively, 0.204 kg and 0.191 kg or 13 g lower than that in the control group. For the entire trial period, the average daily gain was 0.215 kg and 0.205 kg respectively, or 10 g (4.7%) lower in the trial group.

In the microbiological study of RTS taken at the end of the experiment, it was found that from the experimental group 4 RTS (40% of the 10 tested) were positive for E. coli, of which 3 samples were non-hemolytic, non-O-serotype non-having F4 and F5 fimbrial factors and one sample positive for hemolytic E. coli, serogroup O139 positive for F4. From the control group, 4 RTS (40% of the 10 tested) were positive for E. coli, of which 3 isolates were haemolytic, serogroup O139, F4 positive and one is non-hemolytic, O-serotype-non-typing and negative for F4 and F5.

Table 1. Content of energy and nutrients in 1 kg feed for suckling pigs

Components	Unit
Metabolizable energy	10.59 MJ
Crute protein	22.10%
Fiber	3.70%
Crute Fat	2.20%
Lysine	1.40%
Methionine	0.45%
Calcium	1.25%
Phosphorus	0.85%
Sodium	0.03%

Table 2. Component composition, energy and nutrients content in 1 kg of feed of lactating sows

Components	%
Wheat	30.0
Barley	18.5
Maize	14.0
Wheat brain	12.0
Sorghum	10.0
Lysine	0.2
Vitamin-Mineral Premix -15%	0.2
Bioconcentrate X-16	14.0
Limestone	0.5
Dicalcium Phosphate	0.4
NaCl	0.2
Total:	**100.0**
1kg of feed contains:	
Metabolizable energy, MJ	12.13
Crute protein, %	14.48
Crute fat,g	22.22
Fiber, g	46.46
Lysine, %	0.83
Methionine + Cystine, %	0.51
Calcium, %	0.93
Phosphorus, %	0.60

Discussion

According to defined of Working Group by the World Health Organization and the International Scientific Association for Probiotics and Prebiotics (Reid and Friendship, 2003), “oral probiotics are living microorganisms, which have a healthy effect on the recipient when are given in adequate quantities”. The results of the clinical examinations of the trial and control pigs during the whole experiment did not show any visible differences. This circumstance hindered extra-laboratory assessment of the prophylactic efficacy in animals receiving Baykal EM-1 additive at a dose of 10 ml / kg of feed. In contrast, publications for other supplements reported by a number of authors (Dimitrova, 2009; Slavchev, 2004; Yordanov et al., 2006, 2009; Mateva, 2010, Yordanov and Dimitrova, 2014; Dimitrova et al., 2015; Petkova, 2017) have shown positive effect of the additives.

The reported data of the weight development of the suck-

Table 3. Growth development of suckling pigs with and without microbiological additive Baykal EM-1

Groups / Indexes	Control n=72			Experimental n=82		
	$\bar{x} \pm S\,\bar{x}$	C	E	$\bar{x} \pm S\,\bar{x}$	C	E
Live weight at birth, kg	1.579	18.73	2.19	1.582	15.74	1.73
Live weight at day 21, kg	5.859 ± 0.11	16.18	1.90	5.604 ± 0.10	15.74	1.73
Live weight at day 35- weaning, kg	9.121 ± 0.20	19.07	2.23	8.754 ± 0.18	18.24	2.00
Total gain 1-21 day, kg	4.279 ± 0.10	19.66	2.30	4.022 ± 0.09	19.90	2.18
Total gain 22-35 day, kg	3,262 ± 0,15	38.54	4.51	3.151 ± 0.13	38.62	4.24
Total gain 1-35 day, kg	7.541 ± 0.19	21.89	2.56	7.172 ± 0.17	21.24	2.33
Average daily gain- day 1-21, kg	0.233	38.52	4.51	0.225	38.64	4.24
Average daily gain- day 22-35, kg	0.204 ± 0.01	19.67	2.30	0.191 ± 0.01	19.90	2.18
Average daily gain- day 1-35, kg	0.215 ± 0.01	21.86	2.56	0.205 ± 0.01	21.27	2.33

ling pigs included in the trial and control group, such as the total and the average daily gain, for the period from the day 1st to day 21st day and for the period from the day 22nd to the day 35th day after birth and for the whole experimental period, show statistically significant differences in favor of the control group. This is in contradiction with the opinions of a lot of authors such as Angelova (2000), Virtanen (2004), Angelova and Tenchev (2008), Parvulov and Markov (1996) and Slavchev (2004), investigated the effect of feeding lactic acid products as additives for feed diets. In the same line are findings of Yordanov et al. (2009), who established a good result with the Tanacid and Carbovet supplements, studies of Matteva (2010), investigated the effect of administering the Nadstim immunomodulator to suckling and growing pigs and studies of Virtanen (2004) and Popova (2013), which tested herbal remedies for the decontamination of organic livestock wastes. Our results are also not in agreement with the results from an experiment conducted with fermented feed and application of Baykal EM-1 in Russia, showed 11% increase of the total gain in the trial group of growing pigs.

The established imbalance between the total gain, respectively the average daily gain, in the pigs of the two groups during the first period (up to the day 21st) and the second period (from the day 22nd to the day 35th day) is difficult to be explained only with the physiological condition of the mother sows with manifestations of estrus and reduced milk secretion about 20 days after birth. The question remains, however, why the product Baykal EM-1, in which a large number of useful microorganisms are included, according to the product characteristics – lactic acid bacteria, actinomycetes, yeasts and fermenting mushrooms, has not contributed to the most important effects of the use of probiotics, such as improvement of the body and health condition and improvement of the productive indicators (Angelova, 2000; Slavchev, 2004; Murgov and Denkov, 2007; Angelova and Tenchev, 2008). It is likely that different factors from the external environment have contributed to this. Moreover, it is fairly to be outlined that in the two-month study conducted in Russia with weaned pigs was used fermented with Baykal EM-1 fodder and especially its variety Baykal EM-1-Y. The probiotic product "Baykal EM-1-Y" is a collection of bacterial cells and metabolic products of the bacteria Lactobacillus casei 21, Lactococcus lactis 47, Saccharomyces cerevisiae 76 and Photopseudomonas palistris 108, a clear, light-to dark solution, having pH 2.8-3.5, with a nice smell of kefir silage. Bacteriological studies of gastric content, wall fragments of the stomach and small intestine of pigs at 2-2.5 months with the addition of a product in animal diets indicate, that Baykal EM-1-Y in combination with the microflora of the gastrointestinal tract in pigs has a certain capacity to inhibit the growth of Proteus, E. coli, coccobacillus and promotes the growth of lactic acid bacteria and yeasts. In addition, an increase in the total aminoacid content in the blood of the pigs from 32.25 mg/100g to 39.95 mg/100g (P<0.05) was found. This is due to the fact that during the fermentation process, feeds were enriched with amino acids through the EM-1 additive. Once milled, they are included in the bloodstream and used for protein biosynthesis. Ultimately, this helps to improve the growth of experimental young animals that at 4 months of age had a higher average live weight compared to control ones with almost 11% (P <0.05) (***, 2005).

In contrast to the absence of a positive result in the clinical and weight development of the pigs in our experiment, the results of microbiological testing of RTS at the end of the trial period are clear enough and highlight the positive effect of Baykal EM-1 application on sows and pigs of the trial group. Predominantly (75%) non-hemolytic E. coli are isolated from RTS of the experimental pigs, which are not serotyped to the most common in the pigs "O" groups and have no pathogenic factors F4 and F5, therefore are not pathogenic. This fact is an evidence of a change in the intestinal microflora of the pigs, as seen by the presence of only one pathogenic E. coli strain of O139: F4, which corresponds to the findings of Dragoycheva et al. (2011), Lyutskanov (2013), Dimitrova et al. (2014), Yordanov and Dimitrova (2014), Petkova (2017). The positive effect of the product is confirmed by the microbiological data of RTS taken from the pigs in the control group. It is clear that isolated hemolytic E. coli (75%) are in all cases typified as O139 and positive for F4, which determines them as highly pathogenic and specific in pigs before and after weaning. These results are in line with the established by Dragoycheva et al. (2011), Lyutskanov (2013), Dimitrova et al. (2014), Yordanov (2014), Petkova and kol. (2014), Petkova (2017) that the virulence determinants of enterotoxigenic Escherichia coli (ETEC) are dependent on their serotype and pig age. It is also found that probiotics including lactic acid bacteria contribute to the replacement of the pathogenic microflora, a change in the pH of the stomach and gut and have a healthy effect. It is desirable to continue the studies for greater reliability, proof of the efficacy of Baykal EM-1 in pigs and its subsequent application in practice.

Conclusions

The analysis of the weight development of suckling pigs fed with and without addition of Baykal EM-1 from the day 8th to the weaning of the pigs at day 35th did not reveal any significant differences.

The microbiological tests of the rectal tampon samples from suckling pigs receiving Baykal EM-1 feed additive from the day 8th to the weaning of the pigs at day 35th at a dosage of 10 ml/ kg feed presents the product as an effective prophylactic remedy.

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OPTIMUM REGIONAL IRRIGATION REQUIREMENTS UNDER CHANGING CLIMATE IN BULGARIA

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Abstract

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Net irrigation requirements (NIR, mm) that fully satisfy crop development and yield formation are basic in irrigation systems' design and management. Bulgarian practice usually adopts the irrigation scheduling developed by Zahariev et al. (1986) that provide information on 31 crops and 97 irrigation regions (IR). Years, having probability of occurrence of an irrigation depth P_I=10, P_I=25 and P_I=50%, were considered. To cope with climate uncertainties and drought aggravation, simulations were performed for past (1950-1980) and present (1951-2004) weather conditions of unified Agro-Climatic AC regions. In former studies the irrigation scheduling simulation WinISAREG model was calibrated for maize using data from long-term experiments carried out in fields of diverse soil, climate and management conditions. Optimal AC regions were defined on the grounds of average reference evapotranspiration totals for July-August relative to the period 1951-2004 $ET_{oJul-Aug}$. Thus, $ET_{oJul-Aug}$ served as an indicator of regional NIR and IR unification into AC regions. The impacts of soil properties were characterised by total available soil water TAW, being "small" if TAW=116, "average" if 136<TAW<157 and "large" when 173<TAW<180 mm m^{-1}. NIR were computed by model application to soils of small and large TAW in each AC region and 1951-2004 period. Results indicate that when EToJul-Aug increases from 260 to 330 mm, NIR in "average" demand year (P_I=50%) increase from 160 to 310 mm for soils of "small" TAW. Relative to 1951-1980, unified conventional irrigation demands were compared to those simulated. Results showed that the former were mostly in the range of those derived by model simulations. It was concluded that the model took better into account the impact of climate change and different TAW. Maps illustrate findings of the study over country territory in "an average", "a moderately dry" and "a very dry" season.

Key words: Regional irrigation requirements, ETo, WinISAREG model, Climate Change

Introduction

Net irrigation requirement that fully satisfies crop water requirements for development and yield formation is basic in designs and management of irrigation systems. Completely different is the problem of irrigation demand when a maximum economical return is aimed at. Conventional Bulgarian irrigation practice usually adopts the irrigation scheduling and demands developed by Zahariev et al. (1986) that are based on experimental data relative to the period 1950-1980 and

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application of Delibaltov's equition of crop evapotranspiration (1959):

$$ET=Z. \Sigma t^o \quad (1)$$

where Z – a coefficient that takes into account crop variety and development stage; Σt^0 – a total average daily air temperature over a decade.

The book of Zahariev et al. consists predominantly of tables that provide information on the timing of a conventional application depth of 60 mm and the respective seasonal irrigation demand (ID) relative to 31 crops and 97 irrigation regions (IR). Three particular years having probability of occurrence of an ID P_I=10%, P_I=25% and P_I=50% are considered respectively. The huge volume of the book however hampers its practical application that makes advisable to reduce the number of regions.

Climate change and drought aggravation detected during the last 35 years have created uncertainties for irrigation management in this country (Alexandrov, 2011; Popova et al., 2014; Moteva et al., 2015). Undoubtedly, they have influenced crop evapotranspiration, yield decrease due to water stress and corresponding net irrigation requirements to overcome these losses (Popova and Pereira, 2008; Popova, Ivanova and Doneva, 2014; Popova and Ivanova 2015; 2016a; 2016b). In 1998 FAO published a new methodology on reference evapotranspiration calculation using the equation of Penman-Monteith (Allen et al, 1998). Numerous climate parameters, as maximum and minimum air temperature and others are involved in the suggested relationship. Independent studies, carried out in different parts of the world, have shown that the validated ETo-PM-FAO56 equation takes better into account the impact of variable microclimatic factors on reference and actual crop evapotranspiration (Liu et al., 1998; Liu and Pereira, 2001; Popova, Kercheva, Pereira, 2006; Popova, 2008; Pereira et al., 2015). The objective of the present study is to provide a practically oriented methodology on regional irrigation requirements optimization under the conditions of climate uncertainties in Bulgaria by applying the previously validated water balance and irrigation scheduling WINISAREG simulation model to maize crop (Pereira et al., 2003; Popova, Eneva, Pereira, 2006; Ivanova and Popova, 2011; Popova and Pereira 2011). The unification of irrigation regions is based on the average reference evapotranspiration totals for July and August $ET_{0\ July-Aug}$.

Developed methodology

Variability of soil characteristics is really substantial in this country (Boneva, 2012). Regarding irrigation scheduling and net irrigation requirement NIR, mm, however the impact of soil has been taken directly into account by Total Available Soil Water TAW mm m^{-1}. The latter is computed as a difference between soil water storage at Field Capacity (FC) and Wilting Point (WP). In Popova (ed.) 2012 the impact of soil characteristics on net irrigation requirement is taken into account by the difference mentioned above. The characteristic of "small" available soil water is related to the group of soil varieties having TAW=116 mm m^{-1}. The soils of "medium" water holding capacity are those of TAW within the range 135-157mm m^{-1}, while those of "large" TAW have a 173-180mm m^{-1} difference between FC and WP.

Climate conditions are the main factor when computing crop water requirement for irrigation. In the present study it is characterized by average reference evapotranspiration ETo estimated by the Penman-Monteith equation according to the methodology of FAO 56 (Allen et al., 1998). In 2008 Moteva, Kazandjiev, Georgieva determined ETo using required climate data monitored on a daily basis in 30 representative Agro-Meteorological (MS) stations of the country over the period 1971-2000. Average cumulative totals of $ET_{0\ July-Aug}$ were computed for three typical periods of crop development: March-October, April–June and July-August. The example developed below deals with grain maize. The main part of irrigation for this and other summer crops usually takes part in "July-August". Thus, it is accepted that the impact of climate on soil water balance and crop development under irrigated maize could be characterized precisely by using the average total of reference evapotranspiration over the period "July-August". For example average total $ET_{0\ July-Aug}$ is 220 mm in the station of Dragoman, while it is 320mm in the station of Sandanski, i. e. the difference is about 100 mm. Thus, it makes sense to divide the plain country territory into five Agro-Climatic (AC) regions. The average values of $ET_{0\ July-Aug}$ relative to each of the unified regions over the period 1971-2000 are respectively: 230, 250, 270, 290, и 310 mm (Table 1).

The average totals of $ET_{o\ July-Aug}$ presented within parentheses in the same table 1, namely 260, 275, 285, 310 и 330, refer to the longer 1951-2004 period. They were delivered during our previous studies (Popova (ed.), 2012) using climate data on maximum and minimum air temperature observed in the Agro-Meteorological Stations (MS) of Sofia, Silistra, Lom/Varna, Pleven/Plovdiv and Sandanski respectively by National Institute of Meteorology and Hydrology. The ET_o calculation procedures are those recommended by FAO56, applied after respective validation as described in Popova, Kercheva, Pereira, 2006; Popova, 2008 and Popova (ed.)

2012. Thus, each of the

estimated $ET_{o\ July\text{-}Aug}$ value would differ by less than 10 mm that is 4.5% from the average regional values given in Table 1. Presuming the requirements of irrigation practice, such deviations are completely acceptable.

Undoubtedly, when considering another crop like wheat, vegetables and others, the period of substantial climate impact on NIR should be completely different. It should be point out as well that when using experimental data as a basis of validation for each evapotranspiration calculation method, errors are unavoidable and not less than 10%.

Respective NIR were computed by application of the validated water balance and irrigation scheduling WinISAREG model to soil groups and climate stations representing the main agro-climatic regions of this country (Popova (Ed.) 2012; Popova et al., 2014; 2015). In table 1, in addition to average reference evapotranspiration sum $ET_{o\ July\text{-}Aug}$, net irrigation requirement NIR relative to different levels of probability P_I [%] of a NIR occurrence 10, 25, 50, 75 and 90% is presented as well. The latter takes into account the possible range of NIR variability for maize crop over more that 90% of the years within the period 1951-2004. In each cell of the table, the upper number refers to the soil group of "small" TAW (116 mm m^{-1}) while the lower number is valid for the group of "large" TAW (173-180 mm m^{-1}). NIR relative to the soil group of "average" TAW is about 20 mm less than that simulated for soils of "small" water holding capacity TAW=116 mm m^{-1}. Meteorological station (MS) that provide the required climate data to each Agro-Climatic (AC) region are listed in the second column of table 1 (Moteva et al., 2008; Popova (ed.) 2012).

Referring to III and IV AC Regions of average total $ET_{0\ July-Aug}$ 285 and 310 mm respectively (Table 1), it is observed that NIR values relative to Varna and Pleven are separated from the others in the group since they differ from them by up to 50 mm from them.

When average total $ET_{oJul\text{-}Aug}$ increases from 260 to 330 mm, NIR in "the average" demand year (P_I=50%) increase from 160 to 310 mm when soils of "small" TAW are considered. Such range of deviation is substantial and reflects the impact of climate uncertainty on maize irrigation in this country. When net irrigation requirements NIR relative to soils of large water holding capacity are less than 40 mm in the very wet years (P_I >90%), it is admissible not to irrigate.

Result and Discussions

1. Estimation of net irrigation requirement by using WINISAREG model and experimental data.

Net irrigation requirement NIR, mm, for maize crop

computed by using the validated WinISAREG model (Popova and Pereira, 2011) is compared with that estimated on the grounds of a 9-year irrigation experiment carried out in Tsalapitsa field, Plovdiv region (Varlev, Kolev, Kirkova, 1994). The probability curve of occurrence of a NIR shown in Fig.1 is built upon simulations over a 54-year period (1951-2004).

In that case monthly precipitation data observed at Tsalapitsa field (1970-2004) were extended to a longer period (1951-2004) by using a previously derived statistically significant correlation between available data for Plovdiv and Tsalapitsa (R^2=0.74) with a regression coefficient b=0.89 (Popova et al., 2011). Correlations for extending average monthly maximum T_{max} and minimum air temperature T_{min} were derived in a similar way, producing quite significant correlation (R^2=0.96-0.997) with a regression coefficient b=0.926-0.974.

In the same Fig. 1 respective net irrigation requirements for maximum yield during each experimental season over 1983-1991 are plotted in open symbols (o) as well. It is observed that, except for the NIR in two of the wet seasons in 1989 (P_I=70%) and 1983 (P_I=85%), model simulation results practically coincide with experimentally based ones (Fig. 1). Thus, computed NIR relative to the period mentioned above, are acceptably precise and could be used in the irrigation practice. Observed deviation in the two wet years having probability P_I=70% and P_I=85% (about 40mm) is logic since the 54-year period is much more representative that that of a 9 - year field experiment (Fig. 1). The results also indicate that extreme values of NIR are registered only during the longer 54-year period and do not occur during the period of field experiments.

Results in Fig. 2 refer to the empirical probability curves of occurrence of a net irrigation requirement NIR mm under fully irrigated maize in Plovdiv, computed by WinISAREG simulation model when the impact of three soil groups of different TAW is considered. The highest curve refers to a soil of "small" TAW of 116 mm m^{-1}, as the soil in Tsalapitsa is. The results are based again upon the 1951-2004 period (54-year).

The figure also shows that maize cultivation in soil of "large" TAW (180 mm m^{-1}) leads to much less NIR than that relative to soil of "small" TAW.

2. Comparing irrigation requirements by "Zahariev et al." and those computed by using WinISAREG model

It is well deserved to find out the difference between the experimentally based seasonal irrigation depths derived by Zahariev et al.(1986) and those got after unification of relevant model simulations results (Popova (Ed.) 2012) within

Table 1. Net Irrigation Requirements of maize NIR [mm] depending on probability P_I of occurrence of a NIR in the Unified Agro-Climatic (AC) regions of Bulgaria, 1951-2004.

Average Reference Evapotranspiration $ET_{0\ July-Aug}$ [mm] for the periods 1971-2000 and (1951-2004)	Agro Meteoro-logical station (MS)	Probability P_I [%] of occurrence of a NIR				
		10	25	50	75	90
230 (**260**) **AC region I**	**Sofia**, Dragoman	280/230	230/180	**160**/110	120/70	80/30
250 (**275**) **AC region II**	Knezha, Pavlik-eni, Targovishte, V.Tarnovo, G. Delchev, Do-brich, **Silistra**	300/240	240/190	180/130	140/90	90/40
270 (**285**) **AC region III**	Vidin, **Lom**, Obraztsov chif-lik, Kyustendil, Rila, Kazanlak, Ivanova, Kar-nobat	320/260	260/210	200/150	160/100	90/40
	Varna	300/240	240/190	210/160	140/130	130/50
290 (**310**) **AC region IV**	**Pleven**	330/270	280/210	210/140	130/80	80/20
	Yambol, Sadovo, Plovdiv, Elhovo, Chirpan, Sliven, Burgas	370/310	310/260	250/200	190/135	100/40
310 (**330**) **AC region V**	Haskovo, Svi-lengrad, Petrich, **Sandanski**	380/310	360/300	**310**/270	280/210	240/180

five Agro-Climatic regions (AC). For that purpose Table 2 and Maps of Net Irrigation Requirement relative to different climatic years are composed (Figs. 3a 3b 3c) that also mark the location of MS, IR and unified AC regions.

In contrast to the results object of our study in Table 1 that are based upon model simulations over the 1951-2004 period, table 2 is related to the shorter 1951-1980 period that represents the "past" weather conditions. Table 2 consists of data on net irrigation depth estimated by Zahariev et al. (1986) in 30 Irrigation Regions (IR) during the years of probability P_I of a NIR occurrence 10, 25, 50%. Table 2 presents also the simulated net irrigation requirement when the impact of soils of "small" (116) and "large" (173-180) total available water TAW mm m^{-1} is taken into account.

It is observed that in AC region IV of average total $ET_{oJul-Aug}$=292 mm irrigation depth of "Zahariev" is 240 mm at IR Plovdiv, Elhovo, Sliven and Yambol in "average" irrigation demand year (P_I=50%), while simulated NIR by WinISAREG model application to soils of diverse water holding capacity is within the range 230-170 mm. Regarding the dry year (P_I=10%), experimentally based irrigation depth of "Zahariev" is 300 mm at all compared IR, while in model simulation it is within the range 320-260 mm.

Similar results are found in the remaining AC regions of average total $ET_{oJul-Aug}$ 258, 272, 281, 286 and 315-326 mm (Table 2). Diversity of irrigation depth in some of the examined

AC regions, for instance those around Varna, is

due to the remoteness of IR from the MS (o, Fig.3) or spatial variability of precipitation. In most of the cases however the irrigation depth by "Zahariev-1986", after IR unification into five AC regions, is within the range of NIR found by the application of validated WinISAREG model.

Considering the AC regions I and II of 258<$ET_{oJul-Aug}$<272 mm, the seasonal irrigation depths of 300, 240 and 180 mm by "Zahariev-1986" are valid in eight of ten IR and quite close to simulated NIR at Sofia and Silistra (Table 2, Fig.3).

Referring to AC region III of 272<$ET_{oJul-Aug}$ <281 mm, irrigation depth combinations rise to five in totally eight irrigation regions IR while NIR increases from 240, 240 and 180 mm at IR86Kazanlak to 300, 300 and 240 mm at IR1Vidin and IR47 Varna-Goren chiflik. The maps also show that IR46-48Markovo, Provadia and Goren chiflik (o, Fig. 3) are far off the coastal zone by 50-70 km. As a result the irrigation depth by "Zahariev-1986" surpasses the simulated one when using Northern-Black Sea climate data observed in the Varna MS (Table 2). Only in the dry year (P_I=10) and in soils of "large" TAW the "Zahariev" irrigation depth surpasses the simulated one by 110 mm. The difference however becomes smaller (20-50 mm) in the case of soils of "small" TAW.

Regarding AC region IV of 286<$ET_{oJul-Aug}$ <292 mm, the combinations of irrigation depth are four at the IR17-22Pleven (Table 2, Fig.3). Table 2 shows that "Zahariev" seasonal irrigation depths of 300, 300 and 240 mm at probability level P_I=10, P_I=25 and P_I=50% occur in half of the IR of AC region IV.

Irrigation depth relative to AC region V of $ET_{oJul-Aug}$=326 mm increases by 60 mm during the dry years when compared with those relative to AC region IV. Irrigation depth in the average demand year however increases only in the southernmost IR66 Petrich, 81 Svilengrad and 65 Sandanski (Table 2)

Finally, it is concluded that net irrigation requirement NIR simulated by the validated WinISAREG model varies in a larger range than that published by Zahariev et al. (1986). Thus, the model takes better into account the impact of variable water holding capacity of the soil and climate uncertainties in this country.

3. Mapping and analyses of irrigation requirements

It is of interest to follow the dynamics of "wet" and "dry" areas of drought intensity on maps that represent distribution of Net Irrigation Requirement in the scale of the country in "an average"1970, "a moderately dry"1981 and "the extremely dry"2000 over the period 1951-2004 (Figs. 3a 3b 3c). Symbols and names of MS, numbers of IR by "Zahariev" unified in Agro-Climatic AC regions according to $ET_{oJul-Aug}$ are plotted

as well (Figs. 3a 3b 3c).

Spatial distribution of NIR in Fig. 3a shows that 1970 is really "average" in terms of irrigation requirements at AC regions II, IV and V in South Bulgaria but "wet" at AC regions I (Sofia), II, III and IV (Central North and North-West Bulgaria). The same characteristics could be indicated by the probability curves of occurrence of a NIR at different locations showing that PI is within the range 45-60% at Tsalapitsa, Stara Zagora, Sandanski and Sofia when P_I=25 at Plovdiv in 1970 (Figs. 1 and 2; Popova (Ed.) 2012). It is observed also that in 1970 the dominant code in South Bulgaria is "brown" of NIR=250 mm in the Thrace and "orange" of NIR=300 mm, indicating a higher drought intensity around Haskovo, Yambol and Rila. "Yellow" code of NIR=220/230 mm spreads over IR of Elhovo and Stara Zagora, while a "green" one of NIR=180 mm pervades along the Black Sea coastal area.

In 1970 the "blue" code of 110<NIR<120 mm prevails in IR52 Sofia and IR53 Elin Pelin (AC region I), over Central and North-West Bulgaria (AC regions II, III, IV) and also around IR43Silistra and IR37 Targovishte. Logically, such low irrigation requirement has a high level of probability of occurrence in IR17-22 Pleven, IR5 Lom (P_I=90-95%) and IR43 Silistra (P_I=70%) (Popova (Ed.), 2012). Net irrigation requirement increase to 150-180 mm at probability level P_I=85% in IR46-48 around Varna.

During the "moderately dry" 1981 (Fig. 3b) the whole territory of AC regions IV (Plovdiv, Sliven, Yambol, Elhovo) and V (Stara Zagora, Haskovo and Svilengrad) in South Bulgaria is caught by a high intensive drought of "orange" code for NIR=350 mm. On the contrary, the "blue" wet zone of 110<NIR <120 mm in North Bulgaria shrinks substantially to IR17, 18, 19 and 20 around Pleven and IR37 Targovishte. The IR of Knezha, Belene, Levski and Pavlikeni in AC regions II and IV pass over to the zone of a higher NIR=190 mm. A typical feature of 1981 is that dryness sweeps the zones around Vidin, between Ivanovo and Belene (NIR=250 mm) and near Shabla (NIR=210 mm) in North Bulgaria.

During the "extremely dry"2000 the "blue" zone of "small" NIR disappears, while drought intensity increases all over the country (Fig. 3c). As a result, Net Irrigation Requirement reaches the record 490 mm in AC regions IV (Sliven) and V (Stara Zagora), 440 mm in Kazanlak, Yambol and Svilengrad, 410 mm in Plovdiv, Elhovo and Rila and 390-340 mm in AC region I (Sofia). The "yellow" code of NIR=240-290 mm dominates the extreme East and West regions of North Bulgaria. "Brown" zone of NIR=410 mm appears in IR15Knezha, 28Veliko Tarnovo, 30Gorna Oryahovitza and Obraztsov chiflik, while the "green" code relative to IR18-22Pleven, 67Targovishte, 43Silistra and 46-48 Varna points to NIR=310-330 mm.

Table 2. Comparing irrigation demands by "Zahariev – 1986" and WINISAREG model, 1951-1980

Average seasonal $ET_{oJul-Aug}$ (mm), 1951-1980	Source	Agro-Meteorological station / [MS]and Irrigation region (IR)	Net irrigation requirements NIR, mm, with probability P_I [%]		
			P_I =10%	P_I =25%	P_I =50%
258 AC region I ( )	Mathematical model	Sofia	300/250	230/180	160/110
	„Zahariev "	Elin Pelin IR 53	240	180	180
		Sofia IR **52**	**300**	**240**	**180**
272 AC region II ( )	Mathematical model	Silistra	285/230	235/175	190/140
	„Zahariev "	Pavlikeni IR **29**, Targovishte IR **37**, Knezha IR **15**,V. Tarnovo(Karsisen IR **28** and G. Oryahovitsa IR **30**), G. Delchev IR **67**;	**300**	**240**	**180**
		Silistra IR 43	300	300	240
281 AC region III ( )	Mathematical model	Lom	290/230	240/190	190/140
		Varna	250/**190**	220/170	205/150
	„Zahariev "	**Kazanlak IR 86**;	**240**	**240**	**180**
		Kyustendil IR60,Rila IR 62, Varna–Markovo IR 46;	300	240	180
		Varna–Provadia IR 48,	300	240	240
		Vidin IR 1, Varna-Goren Chiflik IR 47	**300**	**300**	**240**
272		Lom IR 5	360	300	240
286 AC region IV ( )	Mathematical model	Pleven	325/270	245/190	190/130
	„Zahariev "	IR 17-22:Sadovec (17);	300	240	180
		Levski (22);	300	300	180
		Dolna Mitropolia (18), Novachene (20);	**300**	**300**	**240**
		Gulyantsi (19), Belene (21)	360	300	240
	Mathematical model	Plovdiv	320/260	280/220	230/170
292	„Zahariev "	Sliven IR 89, Yambol IR91;	300	240	240
		Plovdiv IR 72, Elhovo IR92	**300**	**300**	**240**
315	Mathematical model	Stara Zagora (IR 85)	320/280	300/250	250/200
	„Zahariev "	Stara Zagora	300	240	180
326 AC region V ( )	Mathematical model	Sandanski IR 65	380/320	350/300	300/240
	„Zahariev"	Haskovo IR 83;	**360**	300	240
		Petrich IR **66**, Svilengrad IR **81**;	**360**	300	**300**
		Sandanski IR **65**	**360**	**360**	**300**

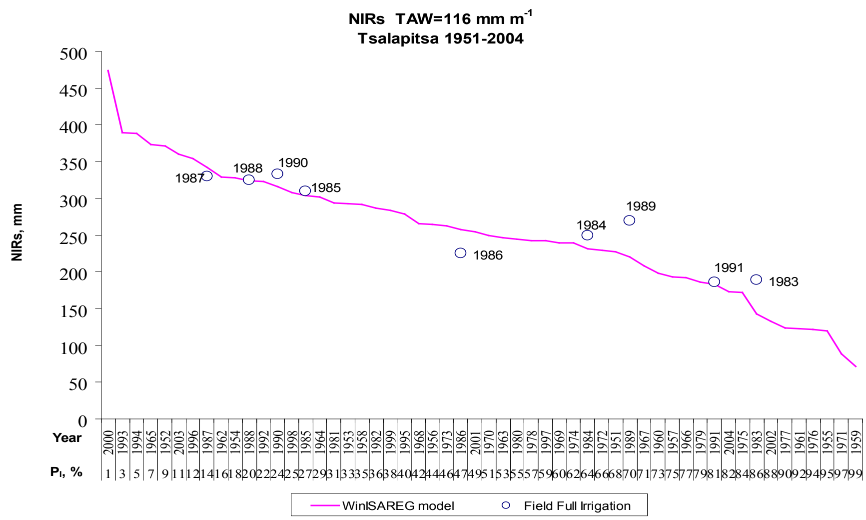


Fig. 1. Probability curve of occurrence of a Net irrigation requirement (NIR, mm) for maize crop at Tsalapitsa experimental field, an alluvial soil of small total available water TAW=116 mm m-1, 1951-2004.

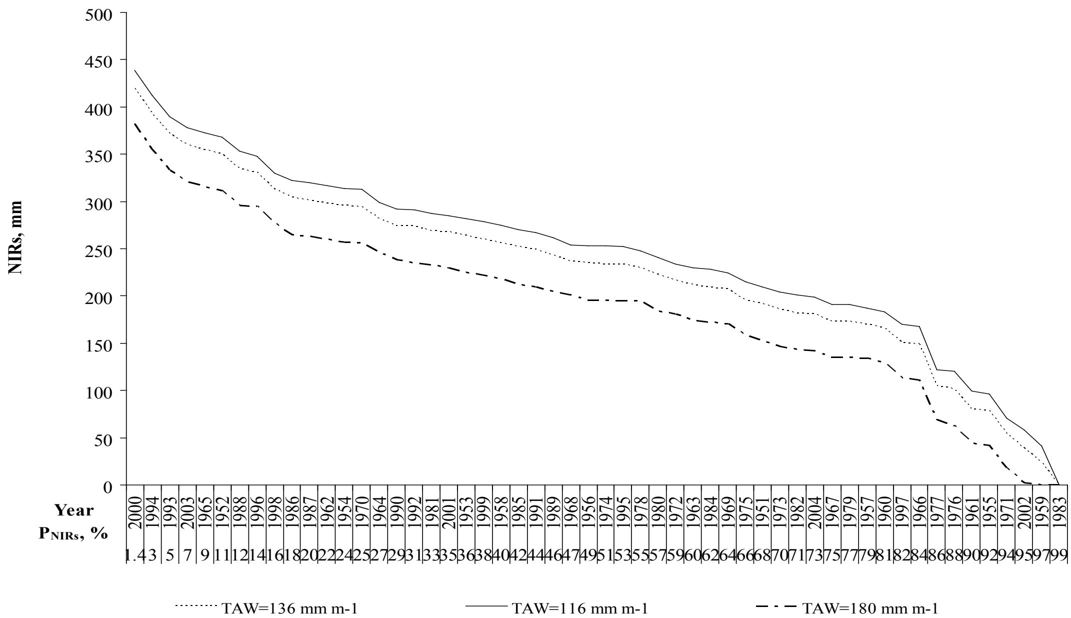
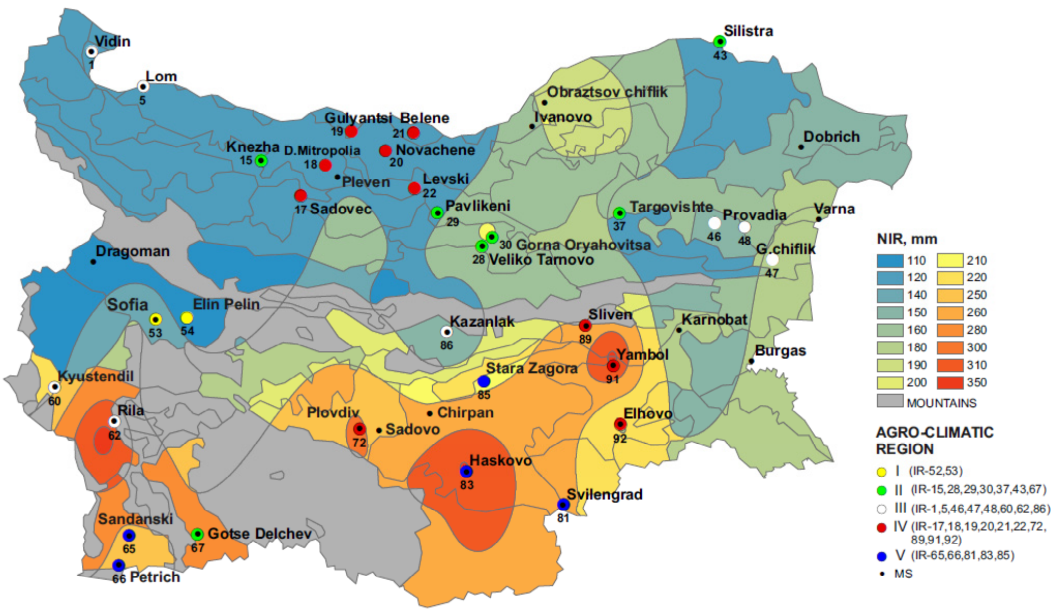
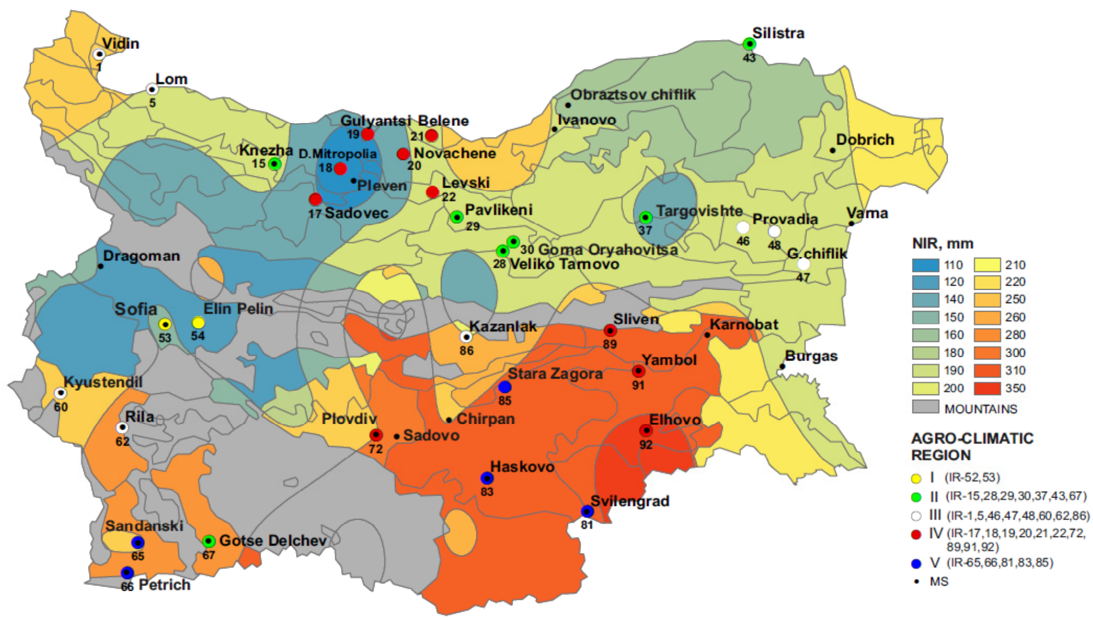


Figure 2. Probability curves of occurrence of a Net Irrigation Requirement (NIR, mm) for maize crop as influenced by small, average and large Total Available Soil Water TAW (mm m-1), Plovdiv, 1951-2004.



a) an "average" 1970



b) a "moderately dry" 1981

Fig. 3. Map of Meteorological Stations MS (.), Unified Irrigation IR (Zahariev et al., 1986) and Agro-Climatic AC Regions (, , , ,) (Tables 1 and 2) and Net Irrigation Requirements of maize (NIR,mm) relative to: a) an "average" 1970; b) a "moderately dry" 1981 and c) the "extremely dry" 2000, 1951-2004.

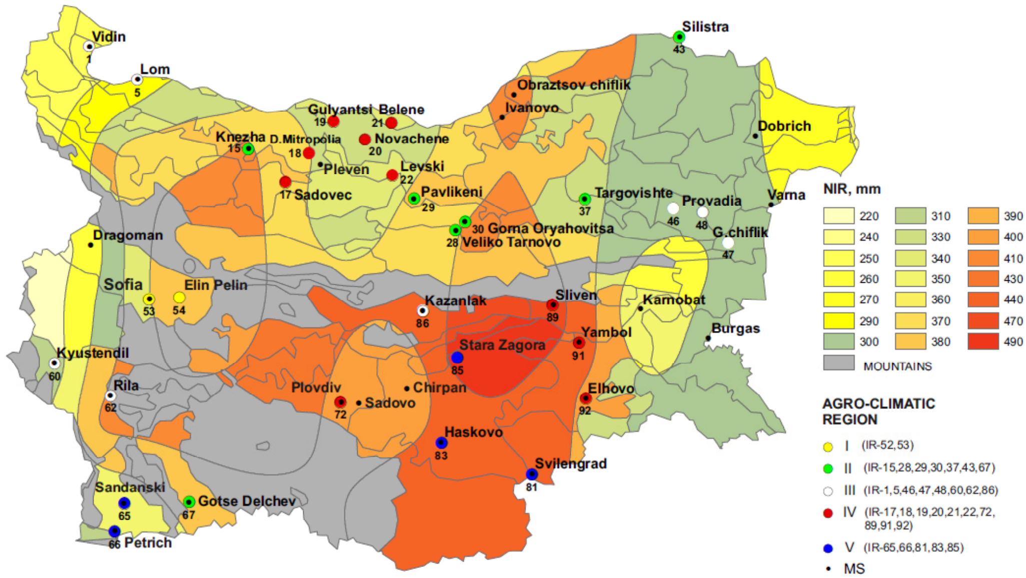


Fig. 3 c) the "extremely dry" 2000, 1951-2004.

The main difference between methodology presently developed and that of Zahariev et al. (1986) is the number of irrigation regions, for which NIR has been defined for. Regarding "Zahariev", their number is 97 while our results are related to only five unified Agro-Climatic regions and three soil groups in terms of soil water holding capacity. In this way, the information derived by the proposed methodology consists of only one page per crop (Table 1). Thus, if 31 crops are considered, including the Methodology section, 35 pages will be required instead of more than 640 p. in the "Zahariev" book. Such a multiple reduction of information on net irrigation requirements will facilitate considerably its use and practical application.

An advantage of the developed methodology is also the built probability curves of occurrence of a NIR within the range 2 %< P_I <98 %, such as the book of "Zahariev" does not comprise. That sort of NIRs provides an opportunity to evaluate the economical income of irrigation all over the range of climate variability and change in this country (Popova et al., 2014; 2015). In addition, maps of spatial NIR distribution and unified Agro-Climatic regions relative to maize crop have been worked out.

Conclusions

• The book of Zahariev et al. (1986) provides information on seasonal irrigation depth relative to 31 crops and 97 Irrigation Regions (IR) at three levels of probability of occurrence of a depth, namely: 10, 25 and 50%. Data are based on empirical results relative to the period 1950-1980 and had been used in design and exploitation of national irrigation systems till 1990.

• On the basis of data on present climate (1951-2004) and three groups of soil, Net irrigation requirements NIR relative to maize crop are determined by using the previously validated WinISAREG simulation model. Climate variability and change during the specified period have been accounted for.

• A methodology that defines net irrigation requirements for maize relative to five unified Agro-Climatic regions of this country and three levels of total available soil water is developed. Irrigation regions' unification is based on average reference evapotranspiration totals $ET_{0\ July-Aug}$ computed by the Penman-Monteith-FAO56 equation.

• Regarding the remaining irrigated crops, the number and cover of the specific Agro-Climatic regions as well as value of the respective average ET_0 totals will be different.

• Net Irrigation Requirement in table 1 is presented within the range 10 %< P_I <90 % of probability of occurrence of a NIR. That makes possible to build probability of exceedance curves for NIR in the whole range of present climate variability and change in Bulgarian plains.

• Created maps of Net Irrigation Requirement and unified Agro-Climatic regions visualize finding of the study.

• Considering the imposed objectives, the accuracy of net irrigation requirements found by the developed methodology is completely satisfactory. At the same time, it reflects the impact of present climate uncertainties. Multiple reduction of the volume of information facilitates its use in design and exploitation of the national irrigation systems.

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CHEMICAL AND TECHNOLOGICAL CHARACTERISTIC OF PLUM CULTIVARS OF *Prunus domestica* L.

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Abstract

Dimkova S., D. Ivanova, B. Stefanova, N. Marinova and S. Todorova, 2018 Chemical and technological characteristic of plum cultivars of Prunus domestica L., *Bulg. J. Agric. Sci.*, 24 (Suppl. 2): 43-47

During the period 2015-2016 the chemical composition was investigated and some biometric parameters were studied in fresh fruit of 8 plum cultivars in an experimental plantation of RIMSA, in the vicinity of the town of Dryanovo. 'Stanley' cultivar was used as a standard. The other cultivars included in this study are: 'Malvazinka', 'Green Renclode', 'Tegera', 'Hanita', 'Jojo', 'Čačanska Najbolja' and 'Čačanska rodna'. 'Malvazinka' is distinguished by the largest and most beautiful fruits – 48.88 g (2015), while the smallest fruit is found in 'Čačanska rodna' (16.06 g – 2015). For most cultivars, the relative share of the stone from the full weight of the fruit is higher in 2016, which we believe is due to the soil and climate conditions in the area. For 'Hanita' and 'Stanley' the stone is over 6% of the weight of the fruit. The fruits of the early cultivar 'Tegera' are distinguished with high content of dry matter – 24.3%/ 2015/, followed by 'Jojo' – 23.9%. The highest total sum of sugars is found in 'Stanley' (14.46%), followed by 'Tegera' – 14.01%. Regarding the content of organic acids, the fruits of 'Stanley' have the lowest content in both years. Tanning substance content ranges from 0.093% for 'Malvasinka to 0.451% for 'Stanley'.

'Stanley' cultivar shows the best chemical and technological indicators of fresh fruit in the conditions of Dryanovo, followed by 'Tegera'.

Key words: Prunus domestica; fresh fruits; chemical composition

Introduction

The economic importance of plums of genus Prunus is great due to the wide use of their fruits in the food industry. The greatest economic significance has cultivars of Prunus domestica L. – home plum (Zhuvinov et al., 2012). They are used for fresh consumption, for drying and processing in compotes, nectars, jams, preserves, for baby foods; for freezing, etc. (Velkov et al., 1970; Zhuvinov et al., 2012; Iliev 1988)

The chemical composition of fresh plums determines to a great extent their taste and technological qualities. It changes and depends on the conditions of the environment, the cultivar characteristics and the soil and climate conditions (Velkov et al., 1970; Frayman et al., 1969). Plum fruits have a dry matter content of 9.6% to 31%. The total amount of sugar in plums grown in Moldova is on average about 11% and the content of acids, mainly malic, ranges from 0.50% to 1.43% (Frayman et al. 1969).

It is found from our previous research (Dimkova, 1996; 2003 and Vitanova et al., 2010) conducted in Dryanovo with other plum cultivars, that 'Mirabelle de Nancy' contains the highest dry matter (23.13%) and the highest total sugar content (15.36%). Iliev and Shtarkova (1995) studied 25 plum cultivars and found that the dry matter was in the range of 15.76% ('Opal') to 24.50% ('Izobilie'). 'Green Renclode' cultivar showed the following results: dry matter – 23.79%; organic acids – 0.97% and total sugars – 12.91%. Stefanova (2010), has found a high content of organic acids in 'Hanita' and 'Jojo'

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cultivars. These cultivars have been included in this study to establish the chemical composition of fresh fruit under the conditions of Dryanovo.

Regarding the possibilities for processing of plums, Velkov et al., (1970) consider that the cultivars with high-quality fresh fruits have also good quality processed products. The most suitable for processing into dried fruit are cultivars with high content of dry matter and high sugar content. An important indicator of cultivars for processing is the colour of fresh fruit, as fruits with dark blue color are preferred. Also, the degree of technological maturity of the fruit has its influence in processing as plums are not harvested in full maturity, but in technological – for processing in compotes and jam. Iliev (1988) found that the best fruit-filled compotes are obtained from 'Stanley', 'Green Renclode', and 'Pozhegacha' cultivars under the conditions of the Dryanovo region.

The purpose of the present study is to compare some biometric indicators of 8 plum fruit cultivars and to study their content of dry matter, sugars, acids and tanning substances under the conditions of the Fore-Balkan as a guide for the direction of their use.

Material and Methods

The surveys were conducted in the period 2015-2016 in the Branch of the Research Institute of Mountain Stockbreeding and Agriculture in the town of Dryanovo, at an altitude of 308 m. The experimental trees were planted in the spring of 2008 on pseudopodzolic gray forest soil, at distances of 5x4 m and rootstock of yellow cherry plum in 4 replications. The plantation is grown under non-irrigating conditions.

In this study are included the following eight cultivars of home plum: 'Stanley' (control), 'Malvazinka', 'Green Renclode', 'Tegera', 'Hanita', 'Jojo', 'Čačanska Najbolja', 'Čačanska rodna'. All trees are grown on the same agricultural background.

Average fresh fruit samples of 3kg for chemical analysis were randomized during the fruit's maturity.

The observation of the chemical composition of fresh fruits includes:

• dry matter, determined refracometrically;

• sugar content - according to the method of Bertran and Kolthoff (Stanchev et al., 1968);

• organic acids as malic, titrimetric method with 0.1 n NaOH;

• tanning and colouring substances – according to Neubauer – Löwenthal (Ermakov et al., 1972).

The biometric measurements of fruits are carried out according to the Methods for Studying Plant Resources in Fruit Orchard Cultivars (Nedev et al., 1979).

Statistical data processing was performed using the ANOVA program with LSD test at $\alpha = 0.05$.

Results and Discussion

The climate conditions during the period of plum vegetation (from March to October), in different years, are presented in Fig. 1. Temperatures and precipitation have a significant impact on fruit growth, vegetative growth and the chemical composition of fruit. With regard to the monthly rainfall, it can be seen that they are unevenly distributed during vegetation, especially pronounced in 2016. The rainfall sum was over 130 l/m^2 in May 2016 and in July of the same year when the fruits were growing intensively, it was minimal – 7.4 l/m^2. In 2015, the rainfall amount per month is more evenly distributed, but there was the lowest rainfall amount in July – 34.7 l/m^2.

With regard to the average monthly temperatures, it can be seen from Fig. 1 that there are no significant differences in the two years of the survey. The highest temperatures in the region were found in July – 24.6°C (2015) and the lowest in March, when was the beginning of the vegetation – 5.9°C (2015).

We found significant differences in this study, between cultivars in terms of sizes and weight of fruit and stone. These differences are due to the cultivar peculiarities and the response of the trees to the growing conditions during the two years of the survey. The results of the biometric measurements of fresh fruit are presented in Table 1.

It is clear from data in Table 1 that 'Stanley' and 'Malvazinka' have the highest height of the fruit (44.05 mm) and 'Green Renclode' has the lowest height – 26.99 mm (2016). The fruit width varies from 28.38 mm (2015) for 'Čačanska rodna' to 43.46 mm for 'Malvazinka' (2016). The fruit thickness is more than 30 mm, as only 'Čačanska rodna' is below these parameters (2015). The largest thickness of fruit we found for 'Malvazinka' – 44.46 mm (2015).

As regards the fruit weight, the cultivars differ substantially. 'Malvazinka' is the cultivar with the largest fruit size (48.88 g - 2015). Next to it is 'Green Renclode' with 36.54 g, and 'Čačanska rodna' has the smallest fruit weight (Table 1).

The fruit stone weight is an important indicator, especially in the processing of plum fruits. In the studied cultivars, the highest stone mass – 1.86 g is found in Stanley, followed by 'Jojo' (1.69 g). Fruits of 'Čačanska rodna' has the smallest stone weight and in both years of the present study. Differences were demonstrated at $\alpha = 0.05$.

The relative share of the stone from the fruit weight is a very important indicator in the processing of plums. According to Velkov et al. (1970) this share is from 2.36% to 5.79% for individual cultivars. In the present study we found that the share of the stone was over 6% in 'Hanita' and 'Stanley' (2016). The lowest share of the stone was found in 'Malvazinka' – 2.33% (2015).

The taste of plums and their technological qualities depend on the beneficial substances contained in them - sugars, acids, tannins and colouring substances, vitamins and others. The results of the chemical composition of fresh fruit in 8 cultivars are presented in Table 2.

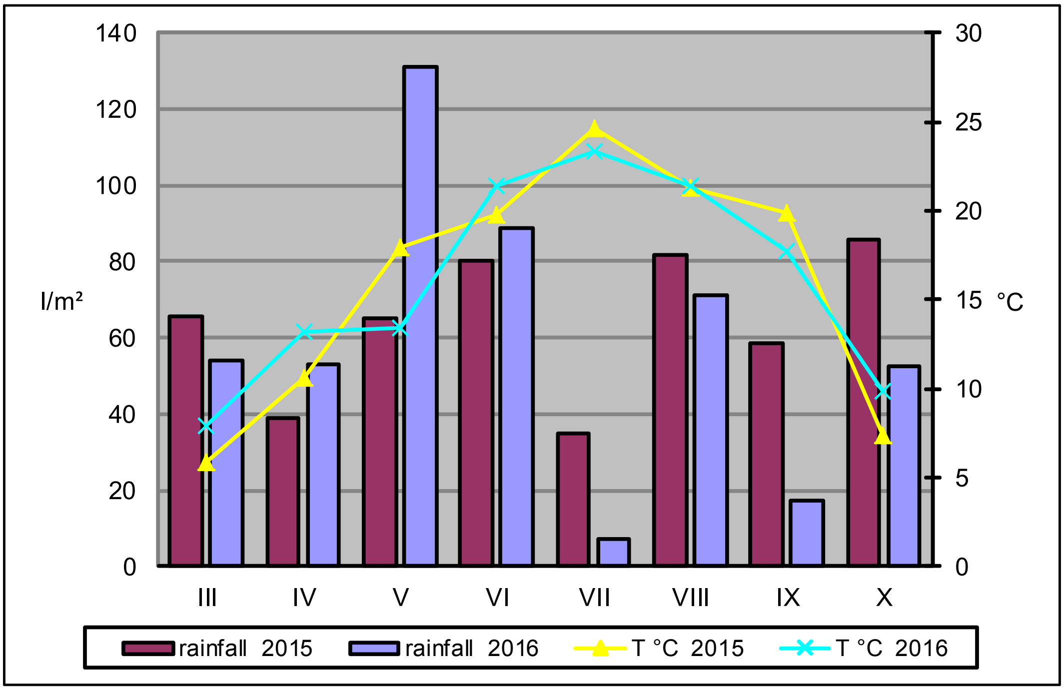

Fig. 1 Meteorological data for the region of Dryanovo – 2015-2016

It can be seen in Table. 2 that the dry matter of the cultivars tested is in the range of 18.9% for 'Čačanska Najbolja' (2016) up to 24.3% (2015) for 'Tegera'. 'Jojo', 'Čačanska rodna', 'Hanita', 'Malvazinka', 'Green Renclode' showed dry matter with more than 20%, which exceeded the control – 'Stanley'.

The content of total sugars (glucose, fructose and sucrose) is of great importance for the taste properties of the fruits. The high sugar content in plums is the basis for obtaining a higher yield, both in drying and in other types of processing. Table 2 shows that 'Stanley' ranks first according to this indicator (14.46%, 2016), followed by 'Tegera' – 14.01% (2015). During the two-year study, 'Čačanska Najbolja' and 'Čačanska rodna' are distinguished by low-sugar content (10.08%-10.48%). From the above results, it can be assumed that 'Stanley' and 'Tegera' will be suitable for the production of dried fruit.

The lowest content of organic acids, such as malic, in plum fruits was found for 'Stanley', followed by 'Green Renclode' (0.81% and 0.90%, respectively). It is generally known that the very high and very low acid content in plum fruits degrades the quality of their products. The highest acid content is found in 'Hanita' (1.63%), which confirms the results of Stefanova (2010).

Miloshevic et al. (2012) also found the highest content of soluble solids, titratable acids and sugars in fruits of 'Hanita', while the highest values of the ratio of total sugars and acidity were observed in 'Katinka'. From the results obtained, it can be assumed that 'Hanita' is not a suitable cultivar for dried fruit.

A very important indicator of the taste properties of the fruit is the ratio of total sugars to acids. This ratio ranges in plums from 3 to 35, and the average is about 12 -Velkov at all.

Table 1. Biometric measurements of fresh fruit in 2015 and 2016.

Sort / Year	Stanley	Malva zinka	Green Renclode	Tegera	Hanita	Jojo	Cacanska najbolja	Cacanska rodna	LSD α=0,05
Height (mm)									
2015	41.66	44.05	39.18	42.6	38.83	46.78	41.21	37.58	2.41
2016	44.05	41.86	26.99	40.75	38.18	42.98	42.56	40.96	1.97
Width (mm)									
2015	31.88	42.92	39.51	34.05	31.08	33.88	35.21	28.38	2.17
2016	35.37	43.46	29.77	32.56	32.46	35.32	38.25	32.07	2.02
Thickness (mm)									
2015	32.52	44.46	38.99	36.15	31.36	37.4	36.49	29.26	1.95
2016	34,64	40.27	30.64	31.18	30.67	33.07	35.54	31.49	2.01
Fruit weight (g)									
2015	24.43	48.88	36.42	27.64	21.88	33.16	33.06	16.06	3.79
2016	29.45	41.91	36.54	23.76	23.51	28.74	31.84	26.04	4,23
Stone weight (g)									
2015	1.34	1.14	1.45	1.46	1.2	1.69	1.23	0.86	0.14
2016	1,86	1.17	1.32	1.37	1.61	1.64	1.58	1.02	0.13
Share of the stone (%)									
2015	5.48	2.33	3.98	5.28	5.48	5.09	3.72	5.35	
2016	6.32	2.79	3.61	5.76	6.85	5.71	4.96	3.92	

Table 2. Chemical composition of fresh fruits

Sort / Year	Stanley	Malva zinka	Green Renclode	Tegera	Hanita	Jojo	Cacanska najbolja	Cacanska rodna
Soluble solids (%)								
2015	19.7	21.2	19.3	24.3	19.8	23.9	19.5	22.3
2016	20.1	19.4	22.5	20.6	21.0	20.2	18.9	21.2
Total sugar (%)								
2015	11.15	11.50	10.85	14.01	10.16	12.46	10.08	10.48
2016	14.46	10.80	13.44	13.80	12.10	11.83	11.18	10.56
Organic acids (%)								
2015	0.81	1.14	1.05	1.03	1.63	1.16	1.05	1,38
2016	0.85	1.14	0.90	1.09	1.34	1.07	0.99	1.30
Sugar-acid ratio								
2015	13.76	10.09	10.33	13.60	6.23	10.74	9.60	7.59
2016	17.01	9.47	14.93	12.66	9.03	11.06	11.29	8.12
Tanning and colouring substances (%)								
2015	0.451	0.145	0.118	0.145	0.145	0.262	0.162	0.145
2016	0.440	0.093	0.104	0.158	0.118	0.207	0.138	0.135

(1970). In the present study, this indicator is first in 'Stanley', followed by the 'Green Renclode' and 'Tegera' (2016). For most cultivars, this ratio is lower in 2015 compared to 2016 (Table 2). The lowest sugar/acid ratio is found in 'Hanita' /6.23 – 9.03/.

Concerning the content of tannins and colouring substances, the results of Table 2 show that their highest content was in the case of 'Stanley' during both years of the study (0.440% - 0.451%) and at the second place was 'Jojo' (0.207% - 0.262%). The lowest results of that indicator are for 'Malvazinka' – 0.093%.

Conclusions

From the study we can draw the following more important conclusions:

• The cv. 'Malvazinka' has the largest and most impressive fruits;

• The fruit weight in 'Čačanska rodna' is not satisfactory and is not suitable for processing into dried fruit;

• Cultivars with the best chemical composition are 'Stanley', 'Tagera' and 'Green Renclode', which have a good combination of sugars and acids;

• Fruits of cv. 'Hanita' have a high acid content in both years of the study, indicating that they will not be suitable for drying.

• Fruits of cv. 'Stanley', concerning their weight, dark color and a combination of sugars and acids is best suited for processing - drying and compote.

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COLOUR PARAMETERS OF FRESH AND DRIED PLUM FRUIT OF CULTIVAR 'TEGERA', AFTER APPLICATION OF SOME CONVENTIONAL AND ORGANIC FERTILIZERS

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Abstract

Hristova D., D. Georgiev, B. Brashlyanova and P. Ivanova, 2018. Colour parameters of fresh and dried plum fruit of cultivar 'Tegera', after application of some conventional and organic fertilizers, *Bulg. J. Agric. Sci.*, 24 (Suppl. 2): 48-51

The influence of some fertilizers with conventional and biological application on the color parameters of fresh and dried plum fruits of 'Tegera' cultivar is studied. The trees are cultivated in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture - Troyan. The indicators were reported by the CIE Lab system in three colour coordinates L - color brightness, + a - red color; + b - yellow color. It was found that in fresh fruit plums after the application of bio fertilizers the quantitative value was increased for two indicators: brightness of the colour and yellow colour tone compared to the control. Red and yellow colour values in dried fruit were higher for conventional fertilization.

Key words: plum, ferilization, brightness of colour, colour parameters

Introduction

Plum (P. domestica L.), along with apple and pear, are among the most wide spread fruits in temperate regions of the world (Zohary et al., 2012). Due to the abundance of bioactive compounds, such as anthocyanins, pectin, carotenoids, plums represent a valuable component of our diet in terms of their nutritional and dietary value (Ionica et al., 2012). Plums can be eaten fresh, dried or processed into various products (juice, jam, fruit preserve, pestil, etc.). Unfortunately, the consumption of fresh plums is seasonal. Their processing offers an alternative that allows their availability and consumption throughout the year. The method of drying increase the period of duration of their storage and consumption. It is one of the oldest and most important thermal treatment techniques aimed at inactivating enzymes, reducing water activity and limiting microbial growth (Krokida et al., 2001). The application of different processing techniques can lead to significant losses of natural bioactive compounds. Colour is the most important maturity indicator for many fruit species (Drake et al., 1982). It is also used to determine the quality in many applications (Blasco, Aleixos and Molto, 2003; Cubero, Aleixos, Molto, Gomez-Sanchis end Blasco, 2010; Quevedo, Aguilera, and Pedreschi, 2008; Rocha and Morais, 2003). It is not only an important sensory attribute that provides basic information about the quality of human perception, but also has a close connection with quality factors, such as freshness, maturity, diversity, attractiveness and preservation of food. Therefore, color is an important classification factor for most food products (Wu and Sun, 2012), because consumers first rate the food by color, then by taste and aroma. It is mainly influenced by the concentration and distribution of various anthocyanins in

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the fruit skin (Gao and Mazza, 1995; Fanning et al., 2014), as well as by other factors such as light, temperature, method of treatment and others (Lancaster et al.,1997). Wherefore soil properties and fertilization are an important element of soil fertility management because they are one of the main factors influencing the processes in the soil, the activity of the root system and the absorption of nutrients from the plants and, as a result, the production quality (Stockdale et al., 2002; WWOOF, 2011).

The aim of the present study is to investigate the influence of some fertilizers with conventional and biological application on the colour parameters of fresh and dried fruits of 'Tegera' cultivar.

Material and Methods

The experiment was carried out in 2016 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture - Troyan.

The object of the study are fruits of plum cultivar 'Tegera'. The fruit species is cultivated according its agrotechnical requirements. The experiment is set up in the following variants:

I variant – Bio fertilizers - including the following fertilizers: Agriful (soil application) - 5 l/da, Tecamin Flower (foliar application) - 0.3%, Teknokel Amino Ca (foliar application) – 0.4%;

II variant – Conventional - Yara Mila Complex (soil application) – 0.500 kg/tree, YaraVita Frutrel (foliar application) - 0.500 ml/da, Yara Vita Universal Bio (foliar application) – 0.500 ml/da;

III variant – Granulation of chicken manure – 0.500 kg/tree;

IV variant – Control.

Fertilization schedule:

Agriful – applied five times from the beginning of vegetation over a period of 15-20 days;

Tecamin Flower - imported twice. Applied before flowering and during the formation of a fruit-set;

Tecnokel Amino Ca – imported twice. Applied after flowering and a month before harvesting;

Yara Mila Complex – imported once in the intra row spacing;

YaraVita Frutrel – four-fold application. First in the phase of winter buds, in a phase of white button, during the formation of fruit-set and a month before the harvest;

Yara Vita Universal Bio – three-fold application. Applied before and after blossoming and after harvest.

Granulation of chicken manure - one application in the intra row spacing.

The drying process of fruits was carried out in the FRDI-Plovdiv, by means of a heat pump.

Drying took place at temperatures up to 45°C, which preserved high quality and native properties of the product. The process runs in a closed cycle using the same air and eliminates the additional microbial visitation from outside air.

The colour characteristics of different variants of fresh and dried plum fruit of 'Tegera' cultivar is reported in the laboratory of Food Research and Development Institute - Plovdiv.

The colour is determined according to Gardner Colour Scale – by a labaratory apparatus "GOLORGRAD2000" of BYK-GARDNER INC. USA. Plum samples are milled in a laboratory apparatus "МРІЯ" –2M with a mesh diameter of 4 mm. The sample was deaerated in a vacuum chamber at a vacuum of 0.85 kPa for 10 minutes. The indicators are reported according CIE Lab system. The color coordinates L, a and b were taken during the measurement: L – color brightness; + a – red colour; + b – yellow colour.

The value of the colour tone or the dominant wavelength is represented by the ratio a / b.

Results and Discussion

Determining the degree of influence of applied fertilizers after agrotechnical measures on fruit colour characteristics is an important element characterizing their quality. In this connection is the opinion of Maskan et al., 2002 on fruit colour parameters that can be used to describe colour changes and provide useful data on quality control of vegetables and fruits. Data from the tests on fresh and dried fruits of 'Tegera' cultivar are presented in Tables 1 and 2.

The highest value of colour brightness of fresh fruit is found in the variant with bio fertilizer – 25.10 (Table 1), followed by the control and the other two fertilization variant. According to this indicator the variability in the values is very low. As regards the red colour tone, the highest values are found in the fruits of the control – 31.08 and the bio-fertilization variant – 29.21. Significant difference in the results of this indicator is reported between the conventional fertilization and control. The fertilization has the greatest impact on yellow colour component of fruits in the bio-fertilizer variants – 15.81 and the lowest in the conventional fertilization – 9.20. The quality indicator of colour tone has the highest value in conventional fertilization, followed by control, chicken manure and bio-fertilization. The impact of organic fertilizers on the brightness and yellow colour tone can be taken into account as an analysis of the results for fresh fruit.

Table 1. Colour characteristics of fresh plums of 'Tegera' cultivar

Variant \ Colour characteristics	L	a	b	a/b
I	25.10±0.78	29.21±0.51	15.81±0.42	1.85
II	21.34±0.12	21.57±0.35	9.20±0.19	2.34
III	22.45±0.25	27.85±0.68	13.01 ±0.05	2.14
IV	24.04±0.11	31.08±0.82	13.70±0.78	2.27
CV %	7.17	15.03	21.32	10.07

Table 2. Colour characteristics of dried plums of 'Tegera' cultivar

Variant \ Colour characteristics	L	a	b	a/b
I	18.65±0.79	5.07±0.72	0.73±0.13	6.94
II	17.32±0.73	6.69±0.62	4.71±0.51	1.42
III	19.50±0.22	4.96±0.66	2.38±0.85	2.08
IV	17.16±0.46	4.67±0.22	1.18±0.33	3.96
CV %	6.15	17.03	79.19	68.69

The colour brightness of dried fruit has lower value in comparison with fresh. The reported results are in the range of 17.16 (control) to 19.50 (chicken manure) (Table 2). Compared to fresh, dried fruit, there are no significant differences in the values between the variants of the red color component. The largest amount is recorded in the conventional fertilization – 6.69, and the lowest in the nontreated control – 4.67. After the drying process, the colour component has higher values for nontreated variants. A high variation coefficient was established for the yellow colour tone as a result of its significant variation between the experiments. The highest amount is found in the conventional fertilization – 4.71, followed by the chicken fertilizer – 2.38, the control – 1.18 and the bio fertilization – 0.73. The significant change can be noted in the values of the organic fertilizer component in dried fruits compared to the same variant of fresh ones. In the same context, the lowest reduction in yellow colour tone is recorded in conventional fertilizers – 4.71.

The same tendency for significant differences between the variants is also observed in the colour characteristic of colour tone. In dried plum fruits, the first bio-fertilizer variant is distinguished by its high value of 6.94, which is a sign of deterioration between the fruit's color parameters compared to the fresh ones.

The reported control quantity is almost twice lower - 3.96, followed by the chicken fertilizer variant – 2.08 and the conventional fertilization – 1.42. The different values found between the variants set a high variation coefficient of 68.69%. From the analysis of the quality characteristics, the least change is recorded in the variants of fresh and dried fruit with the application of chicken manure.

Conclusions

It was found that in fresh fruit plums after the application of bio fertilizers the quantitative value was increased for two indicators: brightness of the colour and yellow colour tone compared to the control. Red and yellow colour tone values in dried fruit were higher for conventional fertilizer variant.

The determination of the colour tone characterizing the quality indicator of the fruit is most consistent with the chicken manure variant.

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COMPARATIVE STUDY ON AGE OF CONCEPTION OF BUFFALO HEIFERS IN DIFFERENT FARMING SYSTEMS

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Abstract

Ilieva Y., V. Planski, K. Hristov and P. Penchev, 2018. Comparative study on age of conception of buffalo heifers in different farming systems, Bulg. J. Agric. Sci., 24 (Suppl. 2): 52-56

With the aim to assess the effect of different farming systems on age of first conception, a comparative study was initiated on buffalo heifers from three farms for the period 2010-2015. All three farms (SIC, SIB and IMR) practice immediate separation of newborn from dam, 7-day colostrum and 3-month weaning period, live weight (LW) being monitored on Fm-3 only. Number of heifers assigned (n), farming system (FS), feeding in suckling period (SP) and use of natural-service bull (NS) were as follows: SIC – [n = 38, FS – free-stall plus pasture, SP – cow milk, NS – full time]; SIB – [n = 42, FS – tie-stall plus pasture, SP – buffalo milk, NS – by day]; IMR – [n = 69, FS – tie-stall plus yard, SP – milk replacer after first month, NS – by day, after heifers attain 380 kg LW]. The data were processed using the conventional statistical procedure. The results demonstrate the favourable effect of buffalo milk used in pre-weaning period, expressed in earlier age of conception in the heifers from SIB (729 days), as opposed to suckling cow milk on SIC – by 86 days ($P < 0.05$). Differences in farming system are implied by the considerably lower age of first conception established on SIB, in comparison to IMR (by 147 days, $P < 0.001$) where no pasture is used and breeding to bull is afforded after reaching a threshold live weight. The study found pronounced seasonality of reproduction, expressed in large percentage of heifers (75%) breeding in the period August-December. A tendency was also observed the heifers born in spring to conceive youngest.

Key words: buffalo heifers, age of first conception, breeding season

Introduction

Age of first conception has considerable impact on the economic and genetic efficiency of buffalo farming. The low reproductive capacity of the Bubalus bubalis species, expressed in delayed first pregnancy, the low conception rates and the low growth rates account for the relatively large portion of young (non-lactating) animals in the herds. Young buffaloes require greater resources for longer period of time with no apparent financial returns which affects end profitability (Peeva, 2000; Khan et al., 2008).

Due to hampered ovulation detection in buffaloes, age of puberty is difficult to establish (Barile, 2005b). According to Kanchev (1988) and Terzano (2010), puberty – with the relevant specific endocrine changes in the organism leading to estrus, first ovulation and hence to conception – is genetically predetermined (species specific). Despites, age of first conception has wide ranges of variation (El'Ashry 1992; Пеева 2000; Sule et al., 2001) and, in the same time, relatively low additive genetic variance (Penchev, 1999; Bashir, 2006; Naz and Ahmad, 2006). Namely the low heritability, besides rendering selection a hard and slow process, indicates that the phenotypic variability is explained mainly by non-genetic factors. Such factors, except the climatic conditions and forage resources, include components of farming technology and management (Hafez, 1955; Zicarelli, 2007).

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The objective of the present study was to comparatively evaluate age of first conception in buffalo heifers from different farming systems.

Material and methods

The study assigned 149 buffalo heifers from three farms with different systems of farming. As they are situated in close vicinity in the North-East of Bulgaria, they are considered under one and the same climatic conditions.

All three farms practice immediate separation of newborn from dam, 7-day colostrum and 3-month weaning period. After day 20 the calves are fed alfalfa hay ad libitum and concentrate feed prepared according to the standard norms for this category.

On the farms with semi-intensive system (pasture farming) the animals are supplemented concentrate feed, roughage and fodder, according to the norms for this category.

Each farm uses a natural-service bull.

The number of animals assigned and the management conditions on each farm are as follows:

Farm SIC. The observation is on 38 heifers for the period 2010-2014 bred in semi-intensive (SI) system – free-stall and pasture farming. The growing heifers are admitted to pasture from 8 months of age, for 8 hours a day in summer and for 7 hours in winter. After the colostrum period, the calves are fed cow (C) milk twice daily, 6 L per head per day. The presence of the bull in the herd is full time (day and night).

Farm SIB. The observation is on 42 heifers for the period 2010-2015 bred in semi-intensive (SI) system – tie-stall and pasture farming. The heifers are admitted to pasture from 12 months of age, for 8 hours in summer and for 7 hours in winter. In the pre-weaning period, the calves are fed buffalo (B) milk twice daily, 4 L/d. The presence of the bull in the herd is during the day.

Farm IMR. The observation is on 69 heifers for the period 2010-2015 bred in intensive (I) system – tie-stall and exercise yard. The calves are fed 4 L/d buffalo milk until one month of age and the same quantity of milk replacer after that. The heifers are exposed to the bull after reaching live weight of 380 kg, and the bull is present in the herd during the day.

Live weight is monitored on IMR only.

The information about the matings for SIC and SIB was taken from the breeding record books, and for IMR – derived mathematically from the data about age at first calving.

The data were processed using the Conventional Statistical Procedure.

Results and Discussion

The results, presented in Table 1, show significant differences in age of first conception among the studied farms. As a result of feeding cow milk to the heifer calves during their suckling period on farm SIC, the age of their conception is by 86 days, or nearly by 3 months ($P< 0.05$), higher in comparison to the other farm with pasture system (SIB). Using the natural buffalo milk during the complete pre-weaning period on SIB had favourable effect on heifers' conditional development and hence on their puberty and age of first breeding (729 days). In our opinion, the late age of conception on IMR (by 147 days compared to SIB, $P< 0.001$) is due to the shift to milk replacer after one month of age. Our results are in agreement with those of El'Ashry (1992) showing lowering the age of conception to 27 months in heifers fed buffalo milk in the suckling period and confirm the close association between level of feeding and reproductive development (Terzano et al., 1996, 1997; Borghese et al., 1997; Nanda et al., 2003).

Determinative condition for the later breeding of the heifers on farms SIC and IMR is the alteration of their diet from buffalo milk to cow milk or to milk replacer that had taken place in the suckling period. The numerous trials searching for an alternative of the buffalo milk for suckling buffalo calves in the past have resulted in considerable decrease in growth rate (Abou-Hussein and Raafat, 1962; Arora et al., 1974). It should be born in mind that cow milk (also milk replacers for bovine calves) has different composition from buffalo milk – chiefly concerning the Ca/P ratio, but also Ca/protein and content of colloidal Ca (Ferrara and Itrieri, 1974; Kapadiya et al., 2016) – which renders it inappropriate for suckling buffalo calves and their further body development, skeletal system in particular (Zicarelli, 2000; Gonzalez, 2011).

Except by the intensive technology providing unnatural suckling in younger age of the female calves and no natural grazing in their further development, the late age of first conception on IMR is predetermined by the target live weight (380 kg) that is set for the heifers to be exposed to the bull. The monthly weight measurement of the grower heifers resulted in average daily gain of 600 g from birth to 18 months, which is close to the previously observed development in the Bulgarian Murrah (Peeva, 2000), Italian Mediterranean (Terzano et al., 1996) and Nili-Ravi (Bhatti et al., 2007) breeds.

Body weight is definitely strongly linked to age of conception, according to El'Ashry (1992) and Gupta (2016) being even more important than age. That is why, through live weight or body condition score, control on growth and level of feeding is prerequisite since the consequences of malnutri tion of grower animals are hardly compensated by a following abundant feeding (Campanile, G. et al., 2001; Barile, 2005a), and that is highly dependent on system of farming.

In accordance, farming experience in Italy has shown the favour of semi-intensive systems, as heifers raised on mixed diet have earlier onset of puberty than those exclusively on pasture (Borghese et al., 1997); on the other hand, too fast growth (intensive system) results in obesity and hence in hampered conception (Terzano et al., 1996; Borghese et al., 1996), and in addition to disproportional skeletal development (pelvis in particular) associated with reproductive disorders in breeding age (Zicarelli, 2000).

In addition, practice teaches that excessively early conception (at insufficiently high body weight) of buffalo heifers can affect their further productive and reproductive (calving interval) performance and longevity. Hence, age of first conception is not to be considered alone but in combination with the other reproductive and economic traits.

The data about season of birth (Table 1, differences non-significant) indicate that greatest is the portion of heifers born in summer – 47%. These animals have comparatively late age of conception (836 days), while latest breeders are those born in winter (851 days). The earliest age of first conception belongs to the heifers born in spring (787 days) – the results commensurate with our previous study (Penchev et al., 2014). Bearing in mind that selection of heifers based on season of birth can afford most favourable season of reproduction, accompanied with control over growth and live weight to attain breeding age (Bhatti et al., 2007), it can be recommend replacement selection to be focused on animals born in spring.

The seasonal incidence of conceptions is presented with Figure 1. There is a pronounced seasonality of breeding, starting from August and continuing to December – a period in which three thirds of the heifers have become pregnant. Our study is in compliance with those of Parmeggiani et al. (1993, 1994) and Barile (2005a) that the percentage of heifers coming in heat in the period of short day length is considerably higher. According to Kanchev et al. (1993) and Perera (2011) this is due to seasonal ovarian activity which in the temperate zones is associated with photo period and melatonin secretion.

The results regarding season of birth and season of conception herein confirm the observed seasonality of calving of the buffaloes in Bulgaria (Penchev, 1999; Peeva, 2000; Penchev et al., 2014). Accordingly, advanced preparatory measures for adequate level of nutrition of the heifers during the breeding season can contribute for increasing the conception rates. This is essential for the economy of the farm because of the imminent risk an animal with missed estrus in the main breeding season to enter a prolonged anestrus.

To summarize, management of reproduction in a buffalo herd is to be focused on maintaining optimal age of first conception based on harmonic system of farming, with special attention on feeding of all the categories from suckling heifer calves to breeding heifers, and in balance with the other reproductive and economic traits. The results herein and elsewhere imply recommendable semi-intensive pasture technology for grower and reproductively advanced heifers. This, in combination with adequate control of the breeding process in respect to seasonality and other peculiarities of reproduction of the water buffalo species, is investment in the future for the farmer.

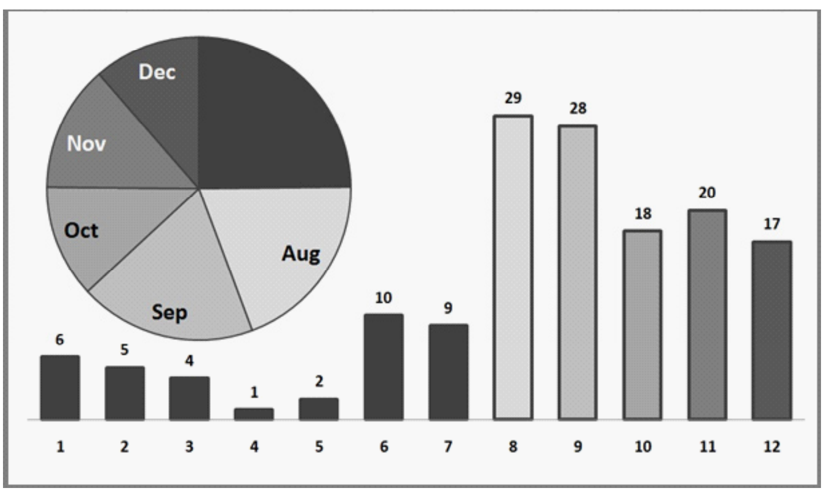

Fig. 1. Seasonality of first conception – numbers (bars chart) and percentage (pie chart)

Table 1. Age of first conception by farm, season of birth, and season of calving

Variables	n	(x)̅± Sx̅	CV	t-test
Farm				
SIC	38	815±24,1	18,2	2-1*
SIB	42	729±24,3	21,6	
IMR	69	876±19,3	18,3	
Season of birth				
Winter	12	851±39.7	16,2	
Spring	30	787±30.5	21,2	n.s.
Summer	70	836±19.9	19,9	
Autumn	37	803±29.4	22,3	
Season of calving				
Winter	28	792±30.1	20,1	
Spring	7	812±81.7	26,6	
Summer	48	800±22.7	19,6	n.s.
Autumn	66	846±21.3	20,4	
Mean	149	819±13.7	20,3	

Significance by t-test: ** – $P \leq 0.001$; * – $P \leq 0.05$; n.s. – $P > 0.05$

Conclusions

The study established significantly lower age of first conception on the buffalo farm with technology practicing feeding buffalo milk to suckling calves and pasture for grower heifers (729 days) – by 86 days lower than the farm with cow milk and pasture ($P < 0.05$) and by 147 days than that with milk replacer and no pasture available ($P < 0.001$).

Management of reproduction is to be in accordance also with the specificity of breeding seasonality, expressed in large portion of buffalo heifers conceiving in the period August to September.

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EARLY FORECASTING CORN YIELD USING GROUND TRUTH DATA AND VEGETATION HEALTH INDICES IN BULGARIA

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Abstract

Kogan F., Z. Popova, R. Singh and P. Alexandrova, 2018. Early forecasting corn yield using ground truth data and vegetation health indices in Bulgaria, *Bulg. J. Agric Sci.*, 24 (Suppl. 2): 57-67

Weather-related maize crop yield losses due to the transition from a planned state to market economy and the increasing climate uncertainties and drought aggravation have been a concern for farmers and policy-makers in Bulgaria since 1990. This paper discusses the possibilities to use operational satellite-based vegetation health (VH) indices for modelling maize crop yield relative to semi-early A1 and late A2 cultivar technology for early warning of drought-related grain losses. The indices were tested in Pleven oblast (Gorni Dabnik) and Burgas oblast (Sadievo) that represent main grain productive regions of North-West and South-East Bulgaria. Correlation and regression analysis were applied to model maize gain yield observed in the experimental fields of Gorni Dabnik and Sadievo from VH indices during 1982-1991. Strong correlations between Pleven maize grain yield relative to semi-early A1 maize varieties and VH indices were found during the critical period of maize development, which starts in May (week 16) and ends in June (week 23) for technology A1B1. For the late cultivar technology A2B1, the critical period of maize starts in June (week 22) and ends much latter in August-Sept (weeks 32 and 41). Relative to Burgas, for corn late cultivar A2, strong correlations of yield deviations from the trend produced by the A2B1 technology dYi with VH indices occur during week 27 and week 28 (July). Several models were constructed where VH indices could serve as independent variables (predictors). Thus, drought-related corn yield losses relative to semi-early and late cultivars could be predicted in Pleven oblast and Burgas oblast in advance of harvest and official grain production statistic is released.

Key words: Corn yield forecasting, long-term field experiments, satellite – base vegetation health indices, Correlation and Regression analysis

Introduction

During the first 15 years of the twenty-first century and last two decades of the previous century, South East Europe including Bulgaria, like most of the other regions of the world, experienced the impact of increased climate variability, rising temperatures and increased frequency of droughts (Gregoric (Ed.) 2012; Kogan and Guo, 2016; Popova et al., 2012; 2014; 2015). Droughts of severe-to-exceptional intensity covered 7-16% of world land (Kogan et al., 2013). These droughts had adverse consequences for societal sustainability worldwide since they reduced agricultural production, caused shortage of food and much related harm. In Bulgaria, due to the combined impacts of transition from a planned state to a market economy with increasing climate uncertainties and droughts frequency, maize crop yield losses have been a concern for

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farmers and policy-makers since 1990.

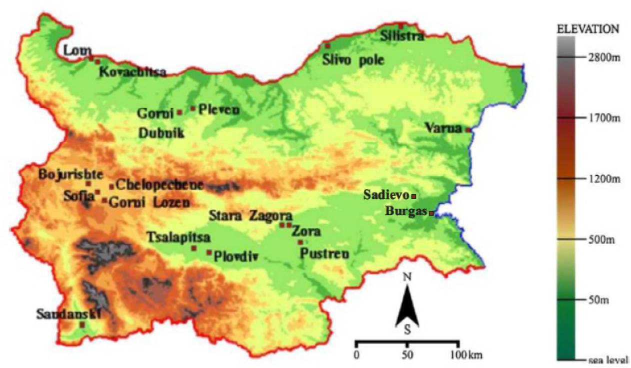
The climate in the most plains of Bulgaria is moderate to transitional continental with semi-arid features. Plovdiv (La 42°09', Lg 24°45', Alt 160 m) and Stara Zagora (La 42°25', Lg 25°39', Alt 169 m) in the Thracian Lowland and Sandanski (La 41°34', Lg 23°17', Alt 206 m) experience the warmest and driest climate, while Sofia (La 42°15', Lg25°45', Alt 555 m) is one of the coolest and wettest agricultural region in this country (Map 1).

The summer is wetter in the Danube plain than in the Thrace. However some territories around Pleven (La 43°25', Lg 24°36', Alt 134 m), Silistra (La 44°07', Lg 27°16', Alt 16 m) and Varna (La 43°12', Lg 27°55', Alt 39 m) are drought prone (Alexandrov (Ed.), 2011; Popova, (Ed.) 2012).

To cope with the situation of crop yield losses, in our previous studies a vulnerability assessment to agricultural drought was carried out by using climate data trend test analyses, simulations with the soil water balance, irrigation scheduling and crop yield evaluation WINISAREG model (Pereira et al., 2003; Stewart et al., 1997) and application of standard precipitation index SPI (McKee, 1993; Pereira et al., 2010) over the period 1951-2004 (Popova et al., 2012; 2014; 2015). The model was validated using independent data sets relative to long term experiment with late and semi-early maize hybrids and soils of different total available water (TAW) in various locations (Popova, Eneva, Pereira, 2006; Popova, 2008; Popova and Pereira, 2011; Ivanova and Popova, 2011). Simulations were performed for the eight regions (called Oblasti in Bg), representing the varieties of climate and soil in Bulgarian plains, as referred above (Popova (Ed) 2012). Results have shown that rainfed maize is associated with great yield variability (29<Cv<72%) in this country. The most variable yields were found for southern locations (Sandanski, Stara Zagora and Plovdiv) when rainfed maize was grown on soils of low total available water TAW. The variability of respective yields in the Danube Plain (Pleven, Silistra, Varna and Lom) proved to be much lower (30<Cv<55%) than that in the Thracian Lowland (40<Cv<70%). Considering an economical relative yield decrease threshold of potential maize productivity, resulted in 30 % years of risk in Plovdiv, 20% in Sofia and 63% in Sandanski. Results for North Bulgaria have shown lower impacts, where only 10% of the years are risky in Pleven and Silistra. It has been observed that risky years increase when TAW decreases.

It has been also found that a version of seasonal SPI2 for "July-Aug", that is an average of the index during the period of high crop sensitivity to water stress, could be a good indicator of maize vulnerability to drought. For South Bulgaria and soils of large TAW (180 mm m^{-1}), economical losses are produced when SPI2"July-Aug" < 0.2 in Sandanski, < -0.50 in Plovdiv and Stara Zagora and < -0.90 in Sofia. In North Bulgaria, the threshold "July-Aug" SPI2 ranges between -0.75 (Lom) and -1.5 (Pleven). The results proved that rainfed maize is significantly less vulnerable to drought in North than in South Bulgaria. However, if TAW=116 mm m^{-1}, rainfed agriculture is related to high economical losses also along the Black Sea coast (Varna) and in Lom during normal years of SPI2 "July-Aug" less than +0.20.

This paper, in addition to our previous studies on vulnerability assessment of agricultural drought referred above (Popova et al, 2012; 2014; 2015), discusses the possibilities to use the cost effective operational satellite-based vegetation health (VH) indices for modelling maize crop yield well in advance for early warning of drought related risk of grain losses. The study is carried out by using datasets from long-term field experiments in Gorni Dabnik, Pleven oblast, (43°21'La; 24°21'Lg; 149 m Alt) and Sadievo, Burgas Oblast, (42°32'La; 26°03' Lg; 154 m Alt) (Map ; Stoyanov, 2008) and NOAA1 operational space technology of satellite – base vegetation health indices (Kidwell et al., 1997). The expected output is: (a) finding whether experimental corn yields could correlate strongly with VH indices during the critical period of crop development; (b) investigating whether on such basis VH indices can be used as indicators of corn yields; and (c) building statistical models and studding their performance.



Map 1. Experimental fields of ISSNP and meteorological stations of NIMH in Bulgaria.

Material and Methods

1. Ground Truth Data: Crop data are obtained during the so called "balance" long-term experiment of Agro-ecology department of the "N. Poushkarov" Institute of Soil Science. The experiment was carried out with two typical corn varieties in eight representative agro-climatic regions of Bulgaria over the period 1975-1991 (Stoyanov, 2008). Observations were performed in fully irrigated plots at five levels of fertilization supply ranging from 0 to 125% of the optimum rate in fertilization treatments, named B1 (0), B2 (125%), B3 (100%), B4 (75%) and B5 (50%). The experiments provided crop data time series on annual grain yield relative to semi early A1 (Px-20 and P-37-37) and late A2 (H708) corn cultivars.

2. Satellite data: The satellite data represent solar radiation reflected or emitted from the land surface measured by the Advanced Very High Resolution Radiometer AVHRR. These data, named global vegetation index (GVI), are collected by NOAA[1] . They are available from 1981 through present. The data set used in this study was developed by sampling the 4-km^2 global area coverage data and compositing from the daily afternoon observations to seven-day composite (Kidwell 1997). The Global Vegetation Indices (GVI) digital counts in the visible (VIS, 0.58–0.68 μm, Ch1), near-infrared (NIR, 0.72–1.1 μm, Ch2) and infrared (IR, 10.3–11.3 μm, Ch4) spectral regions are used. The VIS and NIR counts were converted into reflectance using pre-launch calibration coefficients, and the resulting values were post-launch calibrated. The normalized difference vegetation index (NDVI) was calculated from the corrected VIS and NIR values as:

$$NDVI=(NIR-VIS)/(NIR+VIS) \quad (1)$$

NDVI index becomes widely used for environmental monitoring because it matches well with vegetation biomass, leaf area index and crop yield (Kogan et al., 2012).The Ch4 counts were converted into brightness (radiative) temperature (BT) following Kidwell (1997).

3. Satellite-based VH indexes

The VH indices were calculated from the NDVI and the BT (equations (2), (3) and (4)) as described by Kogan (1997).

$$VCI=100\times((NDVI) - (NDVI)_{min})x((NDVI)_{max}-(NDVI)_{min})^{-1} \quad (2)$$

[1] NOAA=National Oceanic and Atmospheric Administration

$$TCI=100\times((BT)_{max}-(BT))x((BT)_{max}-(BT)_{min})^{-1} \quad (3)$$

$$VHI= \alpha xVCI+(1-\alpha)xTCI \quad (4)$$

where NDVI, $NDVI_{max}$, $NDVI_{min}$, BT, BT_{max} and BT_{min} are the smoothed weekly NDVI or BT and their 1982–1991 absolute maximum (A_{max}) and absolute minimum (A_{min}).

The vegetation condition index (VCI) characterizes greenness and vigour, and through them, the chlorophyll and moisture contents of the vegetation canopy. The temperature condition index (TCI) characterizes how hot the land surface and the conopy are. Moreover the TCI characterizes the moisture availability through the near-surface radiation and aerodynamic conditions (Jensen, 2000; Kogan et al., 2011). The vegetation health index (VHI) combines both VCI and TCI. VH indices change from 0, quantifying severe vegetation stress, to 100, quantifying favorable conditions. The average spatial values of VH indices for each week during 1982–1991 were calculated for the area of selected experimental fields at Gorni Dabnik (43^{o}21'La; 24^{o}21'Lg; 149 m Alt) and Sadievo (42^{o}32'La; 26^{o}03' Lg; 154 m Alt), representing typical corn production conditions in North-West and South-East Bulgaria.

4. Methodology consists of: (a) choosing locations (experimental fields) representing important maize productive regions and real agricultural technologies (experimental treatments) that have produced a trend to the yield time series; (b) extracting the weather component from the values of the selected yield series and from the weekly NDVI and BT series and (c) to correlate the weather-related components of crop yield with NDVI and BT components. It is an adaptation of the methodology aiming at forecasting field crops production from satellite-based vegetation health indices (Kogan et al., 2003, 2005; 2011; 2015). However instead of the national statistic data, corn yield data series from long-term field experiments (1975-1991) are used (Stoyanov, 2008).

A relationship between the ground data and the satellite data, characterizing weather component has been searched. The data were expressed as a deviation from a standard: for yield (expressed as dY) – from the trend produced by agricultural technology B1 (unfertilized fully irrigated corn) on productivity of A1 (semi-early) and A2 (late) corn cultivars and for VH (VCI, TCI and VHI) – from normalized difference vegetation index NDVI and BT climatology. Both correlation and regression analysis of these deviations were performed to study the association of actual deviations of yield dY with VCI, TCI and VHI indices. Thus the dY were correlated to each week's VCI and TCI during 1982–1991 applying the "one-in one-out" technique (Jack Knife test) to investigate whether the deviation dY produced by agricultural technologies

A1B1 and A2B1 correlate strongly with VH indices during a ‘critical’ period of strong corn response to changes in weather conditions. For Bulgarian lowlands such critical period normally covers “July-August” but it fluctuates according to regional climate characteristics that influence the date limiting corn flowering, yield formation and irrigation scheduling to manage droughts.

5. Combining ground observation data with Satellites data

Actual corn yield deviation from the trend dY was correlated with each week’s VCI, TCI and VHI during 1982-1991 to study how dY correlates with VH - indices during the period of strong crop response to weather conditions. Two types of dY models could be applied: (a) With the independent variables for the week with the highest Pearson correlation coefficient (eq.5) and (b) Several weeks indices with the Pearson correlation coefficient greater than 0.5. In this case the mean values for the selected weeks were used as independent variables (eq.6)

$$dY=a_o+b_1(VCI)_i+b_2(TCI)_j+b_3(VHI)_k+e \qquad (5)$$

$$dY=a_0+b_1\Sigma(VCI)_i/n+b_2\Sigma(TCI)_j/m+b_3\Sigma(VHI)_k/p+e \qquad (6)$$

where i, j and k is the week number for VCI, TCI and VHI, respectively; n, m and p is the number of weeks for which the mean VCI, the mean TCI and the mean VHI, respectively, are calculated; and e is the error.

The approach of cross-validation (‘leave-one-out’) is used. In this ”Jack Knife Test” a single year was left out one by one from the data set, a model was built and prediction was made for the eliminated year (Kogan et al., 2015). As a result, 9 independent comparisons between the model predictions and ground observations were made.

To estimate the reliability of independent predictions, the corresponding verification model statistics were performed. Summary measures and difference measures test criteria have been applied: The first criteria includes the mean of the observed (O_i) and predicted (P_i) values, while the second criteria describes the quality of simulation by using the mean bias error (MBE, eq. (7)) and the root mean square error (RMSE, eq.(8)). They all are based on the term of (P_i–O_i) and calculated according to Willmott (1982):

(A) Mean bias error (MBE): $MBE=\Sigma((P_i)-(O_i))/n, \qquad (7)$

(B) Root mean squared error (RMSE): $RMSE=\Sigma((P_i)-(O_i))^2/n \qquad (8)$

The summation is done from case 1 (i = 1) to case n (i = n).

Results and Discussions

1. Experimentally-based yield time series analyses: As referred above, the ground truth data collected during the “balance” experiment (1975–1991, Stoyanov, 2008) were used after some graphical and statistical tests, as shown in figures 1, 2 and 3. Regarding time series graph, it could be concluded that fertilization technologies B2, B3 and B4 combined with a semi-early cultivar A1 (Px-20/P-3737), an appropriate irrigation and crop protection, have not practically produced any yield trends over the sixteen-year period. Contrarily, the agricultural technology A1B1 (same cultivar but unfertilised), produced a negative trend of yield decrease of -11 kg da^{-1} yr^{-1}. These yields were approximated by equation (9) (Brockwell and Davis, 2000):

$$Y_i = T_i + dY_i \qquad (9),$$

where T is a slowly changing function representing the deterministic component (trend) regulated by agricultural technology A1B1, dY is a random component regulated by weather fluctuations and i is the year or coded year number.

Fig.2 compares the end-of-season yield time series 1975-1991 relative to two fully irrigated corn cultivars, a semi-early (A1B1, shown in a dashed line) versus a late one (A2B1, shown in a full line), grown without fertilization at four locations, representing the agro-climate potential for summer crops of southern and northern Bulgarian plains (Map). As it is seen in the figure, when comparing northern (Figs.2a and 2b) to southern selected locations (Figs. 2c and 2d), the role of corn cultivar was enhanced in the fertile Gorni Dabnik and Slivo Pole ,The Danube plain, during severe droughts in the in the 80-ies (Slavov, Koleva, Alexandrov, 2004; Koleva and Alexandrov, 2008; Stoyanov, 2008; Popova et al., 2012; 2015).

A trend of the form $T_i = a_o+a_1t_1$ was fitted to field data relative to the period 1982-1991, for which satellite – based data are available (Fig. 3).

Parameters ao (intercept) and a1 (slope) have been derived by minimizing the differences $\Sigma(Y_i - T_i)^2$. Slopes were estimated for the fields of Sredetz (42^{o}16’La; 25^{o}40’ Lg; 173 m Alt), Sadievo (42^{o}32’La; 26^{o}03’ Lg;154 m Alt) and Gorni Dabnic(43^{o}21’La; 24^{o}21’Lg; 149 m Alt), as -24.95, -14.95 and -16.85 kg da-^{1} year-^{1} for the technology A_2 (a high demanding late cultivar H708) versus -7.21, 5.69 and 12.76 kg da^{-1} year^{-1} for the technology A_1 (a semi-early cultivar P-3737) respectively (Figs. 3a 3b and 3c). Since the slopes are not

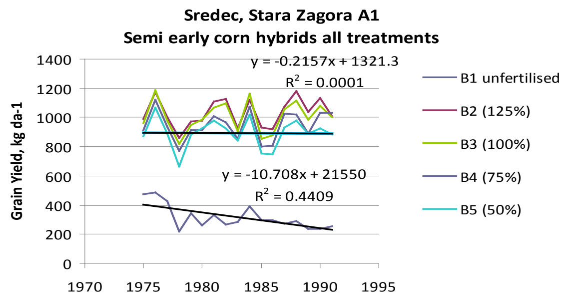


Fig. 1. Trend analyses of annual corn yield time series relative to different fertilization technologies (B1 to B5) combined with semi-early cultivar technology (A1), 1975-1991.

large, the random component of the yield dYi (eq.9) that is regulated by the weather conditions can be approximated by the difference $dY_i = Y_i - T_i$ (Kogan et al., 2015).

2. Combining ground observation data with Satellites data

Figure 4 illustrates the dynamics of correlation coefficients for the actual yield deviation dYi from the trend produced by two corn cultivar technologies consisting of: (a) a semi-early A1B1 and (b) a late A2B1 cultivar grown at Gorny Dabnik experimental field during 1982–1991 with each week's VCI, TCI and VHI respectively. During mid July-September, when the corn flowering and yield formation is taking place (Allen

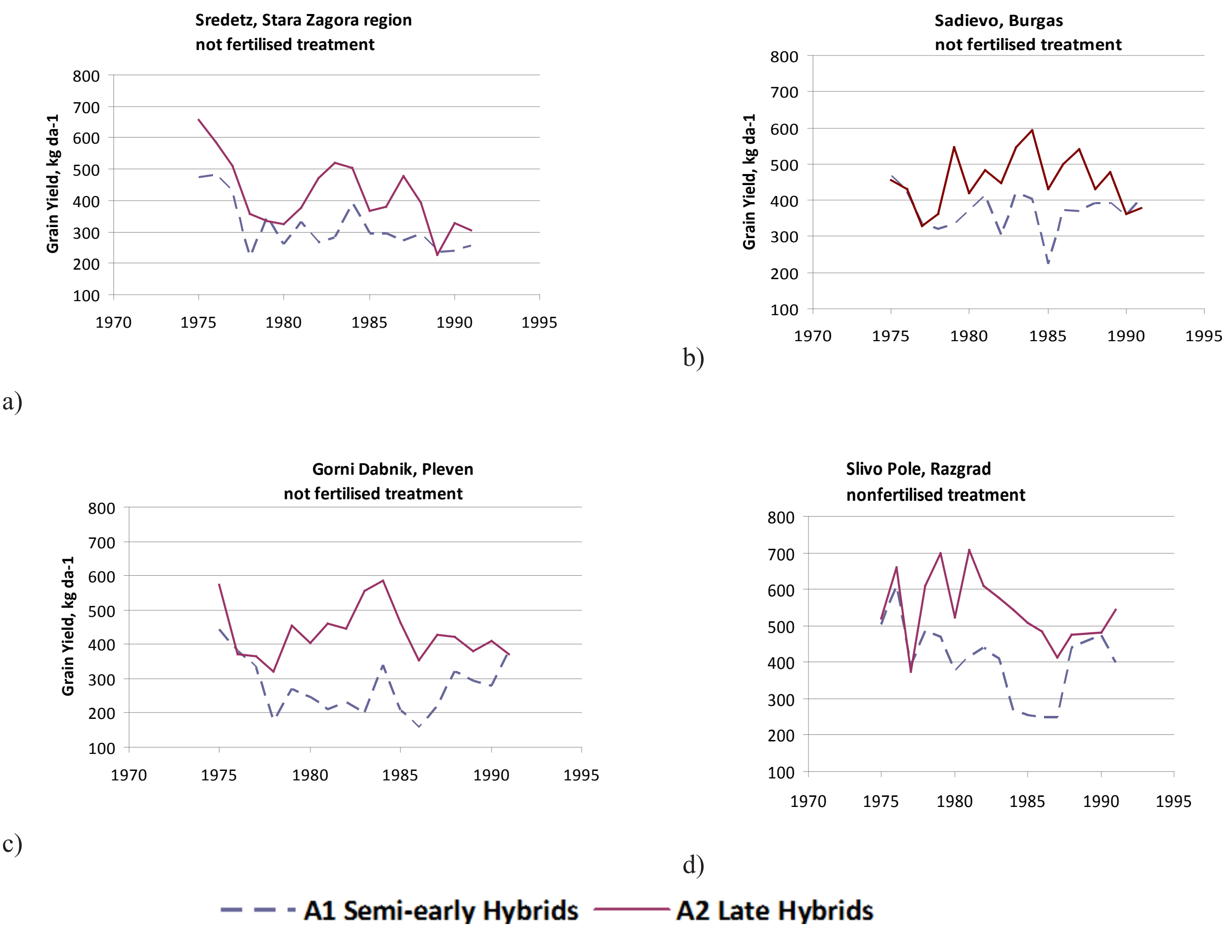
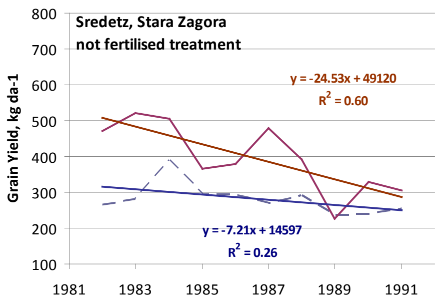
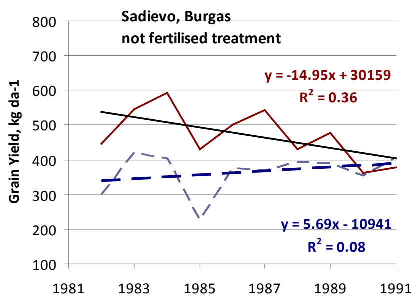


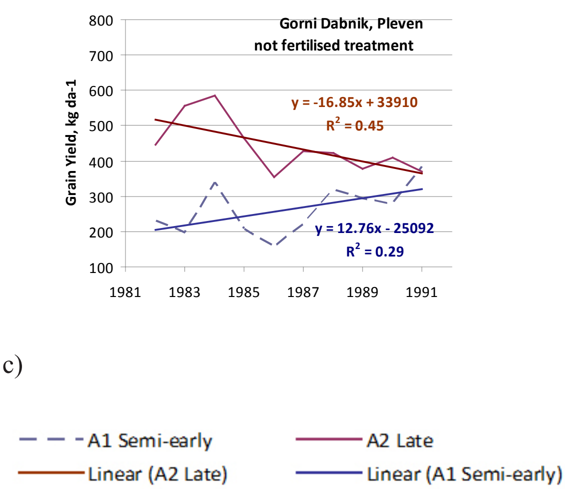
Fig. 2. Comparing the annual crop yield time series relative to two corn cultivars A1 (a semi-early P-3737) and A2 (a late H708) combined with fertilization treatment B1 at the experimental fields of: a) Sredetz (42°16'La; 25°40' Lg; 173 m Alt) and b) Sadievo (42°32'La; 26°03' Lg;154 m Alt), South Bulgaria, and c) Gorni Dabnic (43°21'La; 24°21'Lg;149 m Alt) and d) Slivo pole (43°55' La; 24°21' Lg;25 m Alt), North Bulgaria, 1975-1991.



a)



b)



c)

Figure 3. Trends of the annual yield time series relative to corn cultivars A1 (semi-early P-3737) and A2 (late H708) relative to: a) Sredetz and b) Sadievo, Southeast Bulgaria and c) Gorni Dabnic experimental field, Northwest Bulgaria, 1982-1991.

et al., 1998), correlations of dY with VCI, TCI and VHI show two picks of significant correlations.

For the semi-early cultivar technology A1B1 (Fig.4a) strong correlations of dY however are found earlier in May and June: with VHI (CC=0.60; Partial CC =0.89) that occurs during week 16 (May) and during week 21 (end of June) with TCI (CC=0.594; Partial CC =-0.036). The highest correlation coefficient with VCI is practically 0.5 during week 23 (CC=0.47; but Partial CC=-0.286) and below 0.5 during week 17(CC=-0.34; Partial CC=-0.9) (Fig. 4a).

Thus the Regression summary of the tests performed for technology A1B1 at Gorni Dabnic, Pleven region (Fig. 5) indicates the four calculated variables VCI17, VCI23, VHI16 and TCI21 (the week's number given in subscript), the intercept ao and the four slope coefficients ai of linear regression: dYi=0.439973 0.010243 VCI17 0.00134397 VCI23 + 0.0223175 VHI16 0.000153034 TCI21(eq.10). The relationship between the actual (dY) and estimated (EdY) deviation from the trend is very strong with correlation coefficient CC=0.95 while the Yield Independent test results in CC=0.84 (Fig. 5).

For the corn late cultivar technology A2B1 (Figs. 4b and 6), differently to the semi-early cultivar technology A1B1 (Figs. 4a and 5), strong correlations of dY with VCI (CC=-0.53 and CC=0.57; Partial CC=-0.70 and Partial CC=0.73) occur latter during week 32 and during week 41 (August–September), and much earlier with TCI (CC=0.60; Partial CC=-0.475) in week 17 (May).

Regression summary of the tests carried out for the late corn cultivar technology A2B1 at Gorni Dabnic field shows the four calculated variables for the respective weeks VCI_{22}, VCI_{32}, VCI_{41} and TCI_{17}, the slope coefficients ai and the intercept a_o = 0.933 of linear regression eq.11:

$$dY_i=0.932589 + 0.00477279\ VCI_{22} - 0.00333117\ VCI_{32} + 0.00704330\ VCI_{41} - 0.00272812\ TCI_{17} \qquad (11)$$

The relationship between the actual (dY) and estimated (EdY) deviation from the trend of A2B1 is still strong with CC=0.87 while the Yield Independent test results in CC =0.63 (Fig. 6) that is slightly lower than those of technology A1B1 (Fig. 6).

Figure 7 shows the correlation coefficient of dYi for agricultural technology A2B1 (a late cultivar H708, unfertilized) with each week's VCI, TCI and VHI computed for Sadievo experimental field, Burgas region, where the climate is influenced by the southern Black Sea.

For corn late cultivar (Figure 7), the correlation of dYi with VCI (CC=0.33 and CC=0.41; Partial CC=-0.75 and Partial CC=0.77) occurs only during week 27 and week 28 (July), while with TCI (CC=0.52 and CC=0.47; Partial CC=0.52

and Partial CC=-0.39) correlation is found during week 5 and week 6 (January).

Regression summary of the tests carried out for the technology late corn cultivar A2B1 at Sadievo field, Burgas Region (Figure 8) let to four calculated variables for the respective weeks VCI_{27}, VCI_{28}, TCI_5 and TCI_6, the intercept $a_o = 1.045$ and the slope coefficients a_i of linear regression eq.10:

$$dY=1.04511 - 0.01926VCI_{27} + 0.020634VCI_{28} + 0.0064522TCI_{05} - 0.0043098TCI_{06} \quad (12)$$

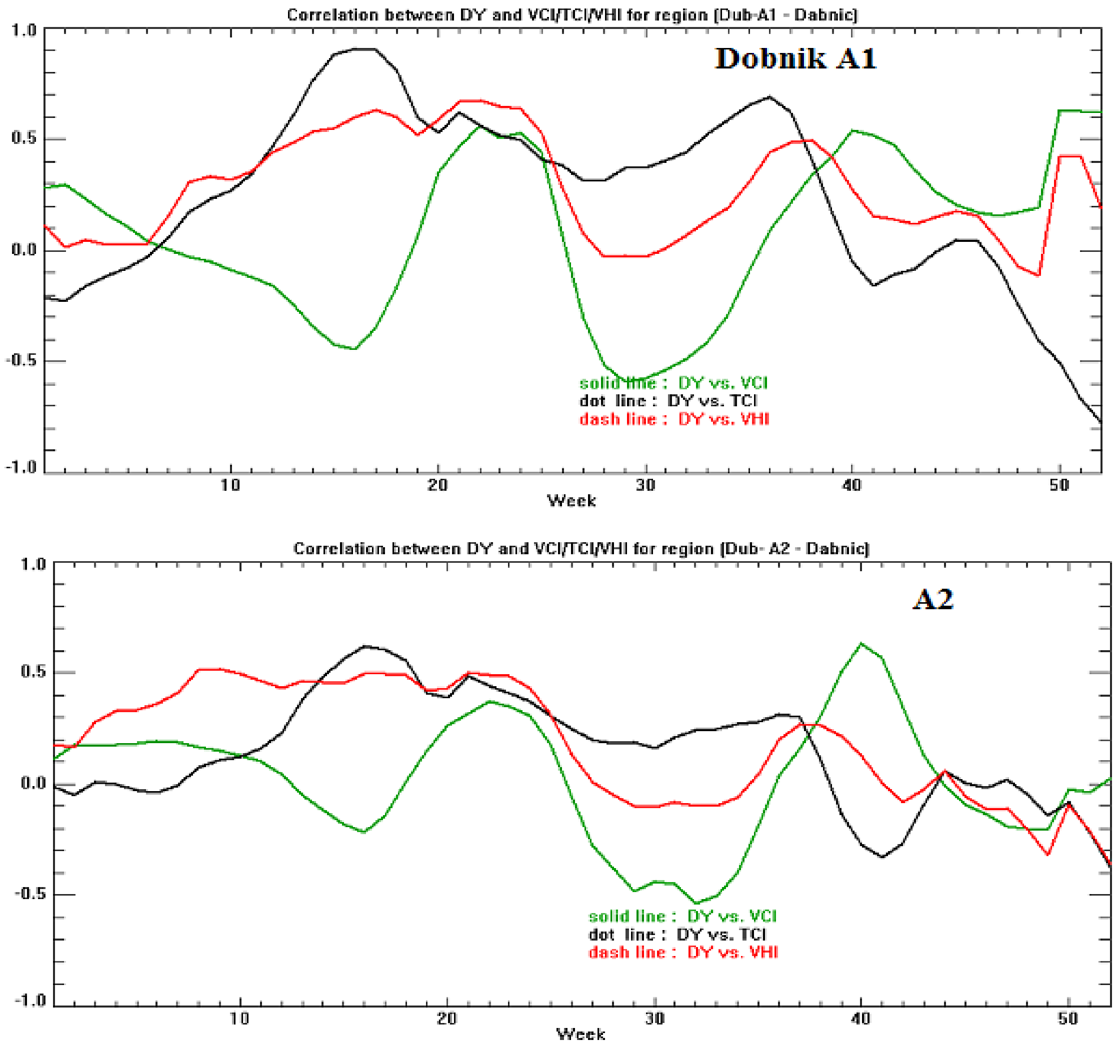
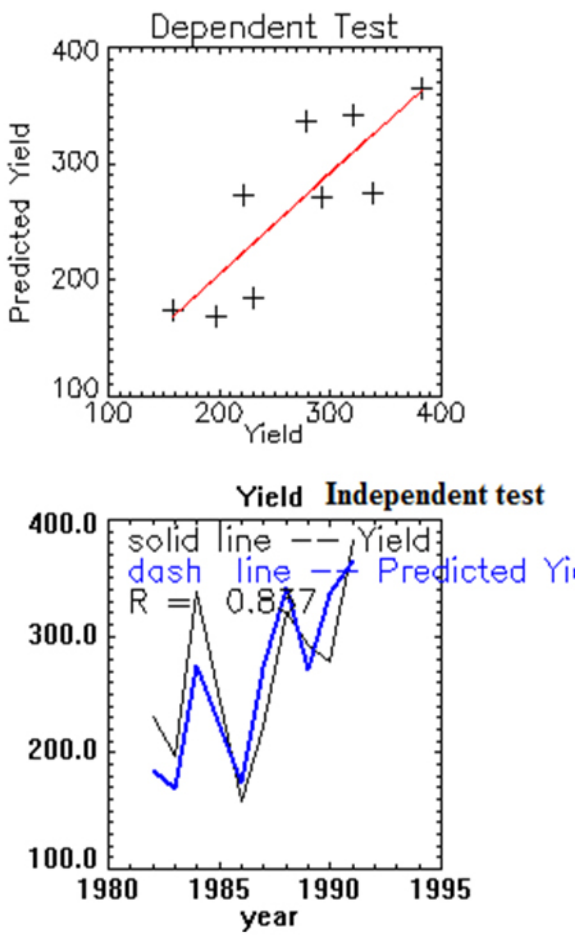


Fig. 4. Dynamics of the Pearson Correlation coefficient between the actual deviations of yield dY relative to agricultural technology: a) A1B1 (a semi-early cultivar P-3737) and b) A2B1 (a late cultivar H708) and the vegetation health indexes VCI, TCI and VHI, unfertilized corn, Gorni Dabnik, Pleven region.



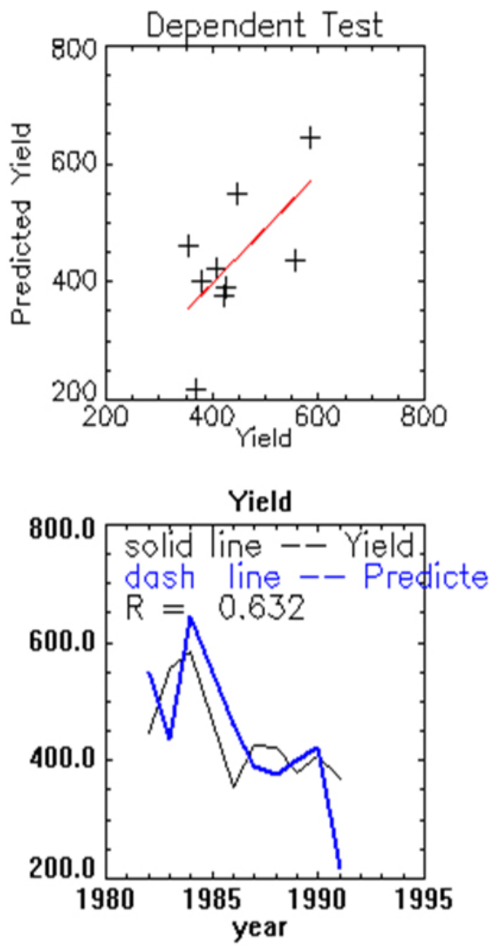


Fig. 5. Regression summary and graphs of the tests performed for agricultural technology A1B1 (a semi-early cultivar, P-3737, unfertilized corn), Gorni Dabnic, Pleven region, 1982-1991.

Fig. 6. Regression summary of the tests performed for agricultural technology A2B1 (a late cultivar H708, unfertilized corn), Gorni Dabnic, Pleven, 1982-1991.

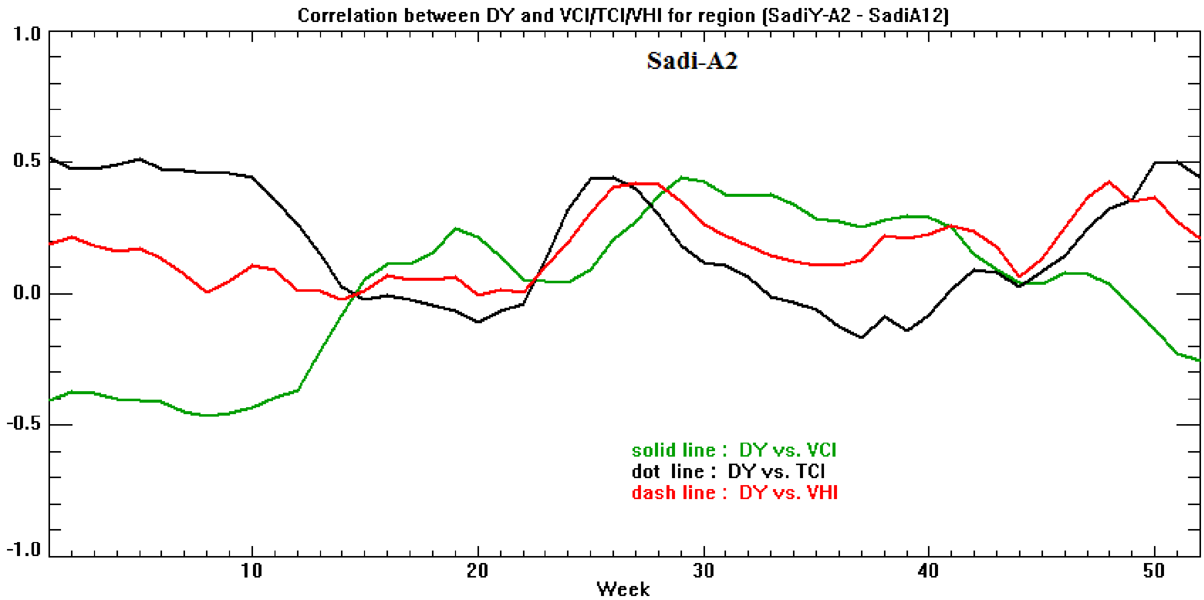


Fig. 7. Dynamics of the Pearson Correlation coefficient between the actual deviation of yield dY and the vegetation health indexes VCI, TCI and VHI relative to agricultural technology A2B1 (a late cultivar H708, unfertilized), Sadievo, Burgas Region, 1982-1991.

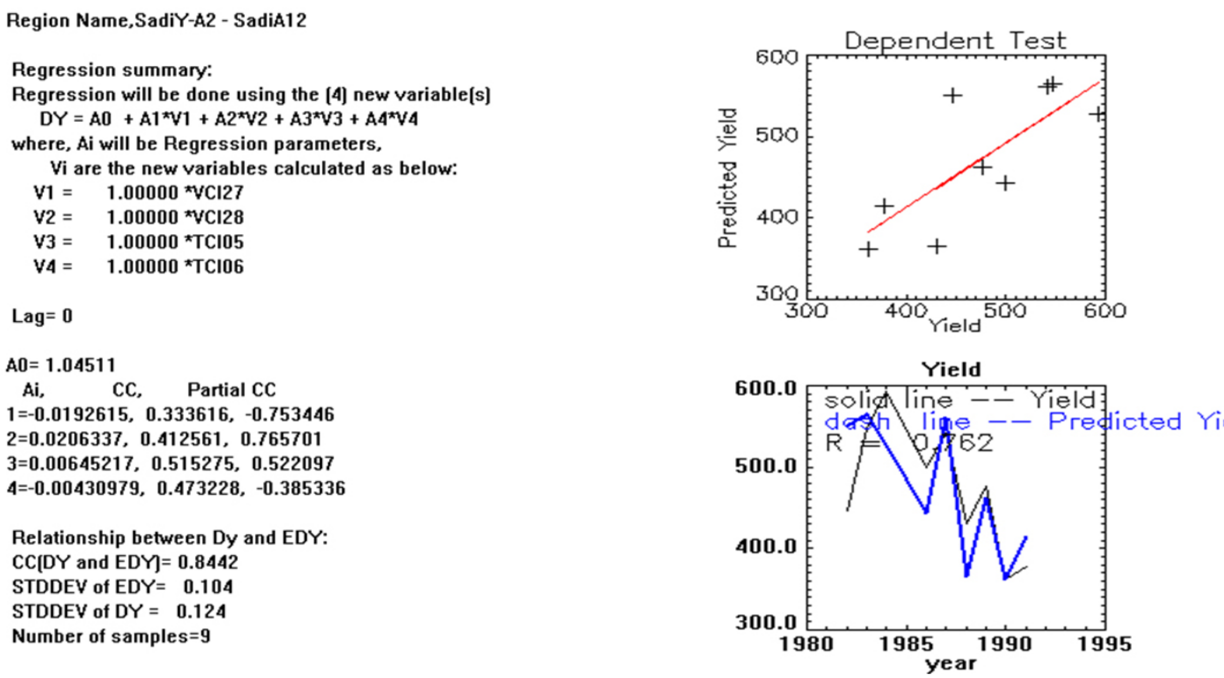


Fig. 8. Regression summary of the tests performed for agricultural technology A2B1 (a late cultivar), Sadievo, Burgas region, 1982-1991.

Conclusions

In this paper, three satellite-based globally universal VH indices characterising vegetation greenness and vigour (VCI), moisture and thermal conditions (TCI) and vegetation health (VHI) were used as yield predictors of two corn cultivars (a semi-early and a late one) in the experimental fields of Gorni Dabnik, North-West Bulgaria, and Sadievo, South-East Bulgaria. The regions were Pleven and Burgas respectively and the first one is a major grain producer in this country. Previously this technique was applied and showed good results to model different crops (wheat, corn, sorghum, rice, etc.) in USA, Russia, Kasahstan, China and other countries. In this case study, the VH proxy was limited to the case of statistical modelling of crop yield relative to unfertilised corn that used to be a common agricultural technology during the transition from a state-planned to a market economy in this country. The study has shown very good results of dependent validation test (CC of 87 and 95%) and good results of independent validation (CC of 63 - 84%). The developed models were quite accurate and reliable in prediction of corn grain yield before official statistics of grain harvest is released. From the three indices characterizing moisture (VCI), thermal (TCI) and vegetation health (VHI) conditions, the first and the third were the best in the study but all three were good predictors of corn yield. The article also showed that there is potential for VH application in modelling corn yield in a larger regional and country scale. Further investigation of yield losses predictors might include combining satellite data with national statistics harvested maize yield data. The VH indices and data are delivered every week to http://www.star.nesdis.noaa.gov/smcd/emb/vci/VH/index.php.

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EFFECT OF THE HYBRIDIZATION ON THE GREEN MASS PRODUCTIVITY IN MILKY-WAX STAGE OF SORGHUM x SUDANGRASS HYBRIDS++

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Abstract

Enchev S., K. Slanev, G. Kikindonov and Tz. Kikindonov, 2018. Effect of the hybridization on the green mass productivity in milky-wax stage of sorghum x sudangrass hybrids++. *Bulg. J. Agric. Sci.*, 24 (Suppl. 2):68-72

In recent years Sorghum x Sudangrass hybrids are becoming a promising forage crop for production of green mass especially in conditions of extreme drought during vegetation. The results from tests for productivity heterosis in Sorghum x Sudangrass hybrids in the period 2015-2016 are presented. The registered heterosis effects towards pollinators and the standard varieties enables the selection of hybrids with high green mass yield potential. Perspective hybrids have been selected with proven higher green and dry mass productivity than the standard commercial varieties.

Key words: Sorghum, Sudangrass, hybrids, heterosis effect

Introduction

The implementation of Sorghum and Sudangrass in practice widens the possibilities for the design of schemes for sustainable agriculture. These crops are popular with their resistance to droughts, especially for regions with high summer temperatures and lower and unevenly distributed rainfalls during the vegetation (Fribourg et al., 1995; Moyer et al., 2004; Kertikov, 2007; Marinov-Serafimov and Golubinova, 2015). The CMS application in Sorghum has given the possibility for use of sterile lines as maternal components (House, 1995) and lines and varieties of Sudangrass as pollinators for the receipt of F1 hybrids. Thus the breeding potential of the hybrids is seriously increased. The hybrids between Sorghum (Sorghum bicolor (L) Moench) and Sudangrass (Sorghum sudanense (Piper) Stapf) distinguish themselves with high green mass yield for the production of high quality silages. The productivity of these hybrids is affected by the agro-climatic factors and the conditions of growth, but they are extremely plastic, adaptive, and manifest their high productive potential even in the warmest and dry summer months (Uzun et al., 2009; Golubinova et al., 2016; Golubinova and Marinov-Serafimov, 2016). They differ with big stems and leaves and satisfactory yield of forage with twofold or multifold cutting in flowering stage, but are most useful with a single cutting in late milky-wax maturity phase for silage production (Snyman and Youbert, 1996). The breeding of new hybrid varieties is focused on productivity, dry matter content, crude protein, fibers, low content of durine, resistance to falling down and foliar diseases (Kalton, 1988).

The aim of the present study is, on the basis of two-year test results, to analyze the heterosis performances of Sorghum x Sudangrass hybrids of our breeding program regarding their green mass productivity in milky-wax stage.

Material and Methods

The study is carried out in the experimental fields of the Agricultural Institute – Shumen during 2015-2016. The soil type of the region is a carbonate black-earth with a good mechanical structure and weakly alkaline reaction of the soil solution. The forerunner crop is beet. The mechanized sowing is made on 25-30th of April, at 45 cm space between the rows, with a high sowing rate – 300000 seeds per ha. The long parcels method has been used for randomization of the

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field tests, with 4 repetitions for each variant, the area of the experimental plot is 10.8 m2, and of the harvest plot – 5.4 m^2. The Group Standard includes the used in practice Sudangrass varieties Vercor (France) and Yantar (a sweet form from Russia). F1 Sorghum x Sudangrass hybrids have been tested, of two selected Sudangrass pollinators with eight male sterile (MS) grain sorghum lines of the AI-Shumen breeding program. The pollinator SV is an elite typical Sudangrass origin, while the SZ pollinator is a stabilized population from the hybridization of Sudangrass with Sweet Sorghum.

The meteorological conditions (vegetation rainfalls, relative air humidity and daily temperatures) during the first year of the test are favorable for the plants' development, while 2016 could be characterized with a too lately coming spring and the insufficient quantities of the vegetation rainfalls. The cutting of the experimental plots is made when the plants have reached milky-wax stage – in 2015 – on 20-22nd of August, and at the end of the same month in 2016. The green mass yield for silage production has been registered. The effect of heterosis is calculated according Abramova (1985) – the real heterosis (HP) is the exceedance in the green mass yield of the hybrids towards the yield of the pollinators (the more productive parents), and the competitive heterosis is the exceedance of the green mass yield of the hybrids towards that of the Standard varieties. The dispersion analysis of the obtained results is according to Lidanski (1988).

Results and Discussion

In the years of tests the typical Sudangrass forms fall down the sweet forms of Sudangrass. The Sudangrass variety Verkor forms significantly lower green mass yield in milky-wax stage than the yield of the sweet form Yantar. The same is valid for the Sudangrass pollinators in the study. The Sudangrass population SV falls significantly down the stabilized population from the hybridization of Sudangrass with Sweet Sorghum SZ. In 2015 (Table 1) the average green mass yield of the tested hybrids of SV is 81.8 t/ha (144.2% of the Standard), and the yield of the SZ hybrids is 84.2 t/ha (148.5% of the Standard). The real heterosis of the SV hybrids is with significantly higher values than the hybridization effect of the SZ hybrids. The SV hybrids realize 143.4% of the pollinator's green mass yield, while the SZ hybrids' yield is on average of 103.3% of the SZ pollinator's yield. The majority of the SV hybrids exceed the green mass yield of the SV pollinator with proved differences. Only the hybrid combination S8 x SV has a negative value of HP, i.e. it forms a lower green mass yield than the yield of the relevant pollinator..At the same time the hybrid of the same MS line (S8) with the SZ pollinator in 2015 forms the highest green mass yield in milky-wax stage – 102.2 t/ha, and is the only one hybrid in the group of SZ hybrids with a proved real heterosis value. All the other tested hybrids of SZ don't show a proved real heterosis, either falling down, or exceeding insignificantly the green mass yield of the productive pollinator SZ. The heterosis effect towards the parents could not give an exact idea about the breeding value of the hybrids. The competitive heterosis (CHE) is the real measurement of the hybrid's value. And the 2015 test results show really high values of CHE for almost all of the Sorghum x Sudangrass hybrids. The exceptions are the hybrids S15 x SZ (with proved negative value of CHE) and the hybrid S8 x SV. By the way, both of these hybrids fall back the relevant pollinator in their green mass productivity. The highest CHE is calculated for the hybrid of the MS line S10 with the Sudangrass pollinator SV (with a green mass yield of 120 t/ha, which is more than double the Standard's yield). The majority of the hybrids with the pollinator SZ also have proved CHE. The results of the tests in 2015 show very good effect of the hybridization of Grain Sorghum lines with Sudangrass pollinators regarding the yield of green mass in milky-wax stage. We could outline the MS lines SC, SA, S9, S10, S13 and S14, which show a very good combining ability with both pollinator types.

The late spring in 2016 brought to low intensity of the initial growth of the plants in the experiment. This, together with the insufficient vegetation rainfalls in the same year were preconditions for the lower green mass productivity of the Sudangrass pollinators and their hybrids with Grain Sorghum MS lines. The presented results of the productivity test and the calculated effects of heterosis (Table 2) show that the yield of SV is 32.6 t/ha (24.4 t/ha lower than the yield of the same pollinator in 2015), and the yield of the SZ pollinator is 52.6 t/ha (28.9 t/ha lower than the yield it formed in 2015). Almost the same is the situation with the tested Sorghum x Sudangrass hybrids – the average yield of the SV hybrids is 61.9 t/ha, and of the SZ hybrids – 64.7 t/ha – significantly lower than the yields they formed in the previous year. But such unfavorable for the crops meteorological conditions are welcome for breeders, who could take the advantage to receive a better idea of the materials they test. In conditions of water deficiency the Sudangrass pollinator SZ again forms much higher yield of green mass than the typical sudangrass population SV. The highest green mass yield forms its hybrid with the SA male-sterile grain sorghum line. – 90.3 t/ha (153.3% of the Standard's yield). The hybrids S14 x SV, SA x SV and S13 x SZ also show very good green mass productivity. The results of the test confirm that higher HP values should be expected for the hybrids of pollinators with lower green mass productivity. The positive effect of the hybridization is much better expressed for the hybrids of SV – all of them exceed the pollinator's yield with proved differences, i.e. the hybrids of SV have proved HP values. And this is normal if the average green mass yield of the SV hybrids is 189.9% of the pollinator SV's yield. Such a proved heterosis is shown by the hybrids of SZ with the MS lines SA and S13. The highest competitive heterosis effect is shown by the hybrid SA x SZ (+53.3 - proved positive CHE value) and S13 x SZ. The hybrids of the MS lines SA and S14 with the pollinator SV are also with high positive values of CHE.

The results of our two years tests confirm the green mass productivity potential of the Sorghum x Sudangrass hybrids, established in our previous studies. We could add also the yield components as a great advantage of the hybrids as a source for high quality silages.. The hybrids are with much higher part of the broom in the total green mass yield during the milky-wax stage, which is of great effect for the fermentation processes during the silage production.

Table 1. Heterosis effect in Sorghum x Sudangrass hybrids in milky-wax stage, 2015

Origin	t/ha	Отн.%	HP (F1 – P)	CHE (F1 – St)
Verkor	45.0	79.4		
Yantar	68.3	120.6		
Standard	56.7	100.0		
SV (P1)	57.0	100.5		
SA x SV	71.1	125.4	24.9	25.4
SC x SV	94.8	167.2	66.7	67.2
S8 x SV	48.1	84.8	- 15.7	- 15.2
S9 x SV	87.4	154.1	53.6	54.1
S10 x SV	120.0	211.6	111.1	111.6
S13 x SV	69.6	121.0	20.5	21.0
S14 x SV	83.7	147.6	47.1	47.6
S15 x SV	79.3	139.9	39.4	39.9
SZ(P2)	81.5	143.7		
SA x SZ	74.4	131.2	- 12.5	31.2
SC x SZ	85.9	151.5	7.8	51.5
S8 x SZ	102.2	180.2	36.5	80.2
S9 x SZ	85.9	151.5	7.8	51.5
S10 x SZ	69.6	122.8	- 20.9	22.8
S13 x SZ	91.1	160.7	17.0	60.7
S14 x SZ	88.9	156.8	13.1	56.8
S15 x SZ	75.6	133.3	- 10.4	-33.3
GD 1%	16.6	24.5		
P%	6.32			

Table 2. Heterosis effect in Sorghum x Sudangrass hybrids in milky-wax stage, 2016

Origin	t/ha	Отн.%	HP (F1 – P)	CHE (F1 – St)
Verkor	42.2	71.6		
Yantar	75.6	128.4		
Standard	58.9	100.0		
SV (P1)	32.6	55.3		
SA x SV	77.7	131.9	76.6	31.9
SC x SV	57.8	98.1	42.8	- 1.9
S8 x SV	59.3	100.7	45.4	0.7
S9 x SV	60.0	101.9	46.6	1.9
S10 x SV	58.5	99.3	44.0	- 0.7
S13 x SV	40.7	69.1	13.8	- 30.9
S14 x SV	77.8	132.1	76.8	32.1
S15 x SV	63.7	108.1	52.8	8.1
SZ(P2)	52.6	89.3		
SA x SZ	90.3	153.3	64.0	53.3
SC x SZ	64.4	109.3	20.0	9.3
S8 x SZ	46.7	79.3	- 10.0	- 20.7
S9 x SZ	54.1	91.9	2.6	- 8.1
S10 x SZ	66.7	113.2	23.9	13.2
S13 x SZ	74.8	127.0	37.7	27.0
S14 x SZ	66.7	113.2	23.9	13.2
S15 x SZ	54.1	91.9	2.6	- 8.1
GD 1%	19.7	33.4		
P%	6.27			

Conclusions

The differences of the characteristics of the pollinators used in hybridization and in the vegetation rainfalls quantities determine different manifestations of heterosis in the green mass productivity of Sorghum x Sudangrass hybrids. In conditions of normal vegetation rainfalls quantities significant part of the tested Sorghum x Sudangrass hybrids exceed the Standard Sudangrass varieties in their yield of green mass in milky-wax stage.

The hybrids SA x SV, S14 x SV, SA x SZ and S13 x SZ have the highest competitive heterosis values in the two years of tests. They could be suggested for testing in the system of State Variety Trials.

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CHEMICAL COMPOSITION AND FATTY ACID PROFILE OF LIPIDS IN CARP (*CYPRINUS CARPIO* L.) MEAT AS AFFECTED BY COOKING METHODS

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Abstract

Ivanova A. S., M. J. Angelova-Romova, Z. Y. Petkova, G. A. Antova and T. A. Hubenova, 2018. Chemical composition and fatty acid profile of lipids in carp (*Cyprinus carpio* L.) meat as affected by cooking methods. *Bulg. J. Agric. Sci.*, 24 (Suppl. 2): 73-80

The aim of the present study is to analyze the chemical composition of the meat and fatty acid profile of carp lipids (Cyprinus carpio L.) before and after heat treatment (baking and frying). The water content (weight method), proteins (Kjeldahl procedure), fat (arbitrage method), ash (weight method) and fatty acid composition (GC) are determined for carp from aquaculture – pond – and cage reared and for free-living carp from Zhrebchevo dam lake. It has been established that the level of water in the baked and fried carp decreases as a result of the heat treatment. Increases in protein, fat and ash content in heat treated meat samples compared to fresh carp meat are reported. The fatty acid profile of baked and fried carp showed a slight decrease in the level of saturated and monounsaturated fatty acids. An increase in the percentage of polyunsaturated fatty acids as a result of heat treatment is reported. The omega-3 fatty acid group is characterized by minor changes and relatively stable levels in baked and fried carp.

The values of the biologically important ω-6: ω-3 ratio in the fresh carp meat from all tested rearing groups ranged from 1.69 to 2.63%. For baked and fried meat samples, higher values are reported, respectively 4.83-6.28% for baked and 4.28-7.60% for fried ones. As a result of the thermal treatment, the values increase in both processing methods and exceed the recommended 5:1 ratio. The fried carp meat are characterized by higher ω-6:ω-3 ratios compared to baked, indicating that carp lipids in baked carp are more balanced and biologically more complete and have a lower risk factor for human health.

Key words: Cyprinus carpio L., cooking method, chemical composition, lipids, fatty acid composition

Introduction

The carp (*Cyprinus carpio* L.) is one of the most cultivated fish species in the world and the major species of the freshwater aquaculture in Bulgaria, mainly due to the existing tradition of its consumption and its relatively constant demand on the market. This raises the issue of its quality and safety in both fresh and processed (after heat treatment) state in connection with the healthy human nutrition.

Techniques for the heat treatment of the meat are widely used to improve its quality and safety. Foods like meat and fish become edible and more acceptable after heat treatment. The various cooking methods affect the nutritive value of fish and especially vitamins, flavor compounds and polyunsaturated fatty acids (Marimuthu et al., 2012).The fish and fish products are thermally treated in various ways to improve their hygienic quality by inactivating pathogenic microorganisms and to emphasize their flavor and taste (Bognar 1998; Ghelichpour et al., 2012; Talab, 2014). During their processing chemical and physical reactions occur, resulting in improved

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or lowered nutritive value. For example, the digestibility increases due to protein denaturation, but the content of thermolabile compounds, fat-soluble vitamins or polyunsaturated fatty acids often decreases (Garcia-Arias et al., 2003; Alizade et al., 2009). The cooking methods and the processing temperatures have a direct impact on the preservation of the food quality.

Most of the studies so far have been focused on determining the chemical composition and fatty acid profile of lipids in fresh carp meat. Data on the carp meat quality after heat treatment are fragmented. In this relation the aim of this work is to determine the chemical composition of carp meat and the fatty acid profile of its lipids before and after heat treatment (baking and frying).

Material and Methods

For the purpose of the study, a chemical characterization of the carp (*Cyprinus carpio* L.) meat and fatty acid profile of its lipid is made. The carp are raised in different fish farms in the country applying different rearing systems – semi-intensive polycultural rearing in ponds (Tri Voditsi Experimental base of IFA, Plovdiv) and intensive cage rearing in Zhrebchevo dam lake. Parallel study has been made also with free-living carp in the same dam lake (Table 1).

The carp in the ponds are fed with cold-pressed sunflower meal and wheat. The fish in the cages are fed with extruded feed (Bulmix, Sliven, BG) containing 24% protein and 4% fat in ratio 6:1. A representative number of fish weighing between 1511-2205 g (October, 2016) was selected for investigation at random choice.

For analysis individual samples are prepared from fish musculature (the lateral muscle) taken from one and the same position after removal of the skin and subsequent homogenization of the meat.

The carp cutlets are baked in a conventional oven according to a standardized method for the preparation of seafood (Association of Official Analytical chemists, 1980). The carp cutlets divided into two parts of roughly equal size are smeared with sunflower oil, placed in aluminum trays (individually for each sample) and baked for 40 minutes at 180°C. The fried samples are prepared from carp cutlets divided into two parts of roughly equal size and fried in sunflower oil at 180°C in a ceramic-coated pan.

The chemical composition of the fresh and thermal treated carp meat (n=5) was individually analyzed for each sample to determine: water content, % (through drying at 105°C, 24h; ISO 5984); protein content, % (by the method of Kjeldahl, ISO 5983, using a semi-automated DK 6 digester unit and UDK 132 distillation system, Velp Scientifica); fat content, % (by the method of Smidt-Boudzynski-Ratzlaff); mineral content, % (by burning in a muffle furnace at 550°C, ISO 6496).

The fatty acid composition of lipids is determined by applying gas chromatography (GC) after transmethylation of the respective sample with 2% H_2SO_4 in absolute CH_3OH at 50°C (ISO 5509: 2000). Fatty acid methyl esters (FAME) are purified by thin-layer chromatography (TLC) on 20x20 cm plates covered with 0.2 mm silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane: diethyl ether (97:3, v/v). GC is performed on a HP 5890 series II (Hewlett Packard GmbH, Vienna, Austria) gas chromatographer equipped with a 75 m x 0.25 mm (I.D.) x 18 µm (film thickness) capillary SP – 2560 column (Supelco, Sigma-Aldrich, St. Louis, MO, USA) and a flame ionization detector. The column temperature is programmed from 140°C (5 min), at 4°C/min to 240°C (7 min); injector and detector temperatures are kept at 250°C. Hydrogen is the carrier gas at a flow rate 0.8 ml/min; split was 50:1. Identification of fatty acids is performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions (ISO 5508: 2004). The analytical standard of fatty acid methyl esters (SUPELCO F.A.M.E. Mix C4-C24, purity ~ 99%) is from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All solvents and reagents are of analytical grade from Merck (Darmstadt, Germany) and are used without additional purification.

The data are processed statistically, taking into account the average (x), its error (Sx) and the coefficient of variation (Cv, %) with the application of statistical program MSOffice 2010. The statistical significance of differences between two samples is determined by the Student's t-test at a level of $P<0.05$.

Results and Discussion

Chemical composition of carp meat before and after heat treatment

The values for water, protein, fat and ash content in fresh carp meat and after heat treatment are shown in Table 2. Considering the effect of the rearing system on the water content in the carp meat in the fresh samples, it is highest in the meat of the free living carp – 79.93%; followed by this from the semi-intensive rearing system (in ponds) – 76.25%, and the lowest – 75.28% is for the intensively reared carp (in cages).

This trend persists also after the thermal treatment of the meat. The water content in the baked and fried samples from the pond- and cage-reared fish is significantly lower ($P<0.01$,

Table 1. Characteristics of the studied carp rearing systems

Production systems	Pond-reared carp	Free-living carp	Cage-reared carp
Geographical	42°7′58.8″ N	42°37′16.6″ N	42°37′16.6″ N
coordinates	24°28′1.2″ E	25°51′45.9″ E	25°51′45.9″ E
Area, ha	1.3	2500	5
Depth, m	1.3	3.5	4.0
Average weight, g	1511	1947	2205
Degree of intensification	Semi-intensive		Intensive -
	(different grain feed and natural feed -zooplankton and benthos)	(natural feed - mostly zooplankton and benthos)	(only balanced pellets)

Table 2. Chemical composition of carp meat before and after heat treatments

Parameter	Heat treatment	Free-living carp	Pond-reared carp	Cage-reared carp
% Water	Fresh meat	79.93 ± 0.39	76.25 ± 0.88	75.28 ± 0.71
		(0.85)	(1.99)	(1.64)
	Oven baked fish steak	71.67 ± 0.07	67.61 ± 1.22	64.87 ± 1.42
		(0.17)	(3.12)	(3.78)
	Fish steak fried in oil	67.80 ± 1.56	66.40 ± 0.38	64.20 ± 0.07
		(3.96)	(1.00)	(0.20)
% Protein	Fresh meat	17.76 ± 0.38	17.08 ± 0.10	17.23 ± 0.36
		(3.75)	(0.98)	(3.61)
	Oven baked fish steak	24.78 ± 0.55	23.49 ± 0.03	24.60 ± 1.59
		(3.85)	(0.22)	11.21
	Fish steak fried in oil	24.12 ± 0.70	21.71 ± 0.39	21.64 ± 0.17
		(4.99)	(3.08)	(1.33)
% Fat	Fresh meat	1.48 ± 0.50	5.86 ± 0.98	6.68 ± 0.98
		(58.36)	(29.06)	(25.50)
	Oven baked fish steak	2.50 ± 0.55	7.91 ± 1.19	9.50 ± 0.30
		(38.13)	(26.07)	(5.54)
	Fish steak fried in oil	6.76 ± 0.92	10.91 ± 0.81	13.14 ± 0.17
		(23.63)	(12.92)	(2.19)
% Ash	Fresh meat	0.83 ± 0.01	0.82 ± 0.04	0.82 ± 0.02
		(2.68)	(7.66)	(3.65)
	Oven baked fish steak	1.05 ± 0.07	0.99 ± 0.01	1.03 ± 0.02
		(11.25)	(2.33)	(3.90)
	Fish steak fried in oil	1.13 ± 0.03	0.99 ± 0.05	1.02 ± 0.06
		(4.04)	(8.03)	(9.64)

*Mean values±Standard errors of means of 4 different determinations (coefficient of variation), (n=4)

P <0.001) than in the free-living carp. In the case of the baked fish it is 71.67% for the free-living carp and 64.87% for the cage-reared carp. In the fried carp, the water decreases even further and reaches values of 67.8% for free-living carp and 64.2% for the cage-reared carp. The water loss in the baked samples is 10-11%, while for the fried ones this percentage is higher and is in the range of 13-15%.

The data show a greater loss of water at baking for cage-reared fish (with higher fat content in the meat) compared to the pond-reared carp and to the free-living carp from the in Zhrebchevo dam lake. The baked carp meat is characterized by significantly lower water content compared to fresh carp meat (P<0.01, P<0.001). The heat treatment of carp meat influences the water content, as the quantity of water in baked and fried meat is significantly lower.

The protein content of the fresh meat in the free-living carp and in the carp from aquaculture is close – 17.76%-17.08%. After heat treatment (baking and frying) the protein content increases, respectively, in the baked samples to 24.78-23.49% and in the fried to 24.12-21.64%. A higher increase in the protein content is observed in the baked carp, with the highest values being from the free-living carp.

According to Kiosev and Dragoev (2009), frying mainly changes the proteins in fish meat. The frying causes complete destruction of tissue enzymes as well as destruction of microorganisms. Best results are obtained when a temperature between 160-180°C is used. At temperatures above 180°C, the meat is blackened, becomes bitter, dehydrated and very dry. Regardless of how it is baked - in a baking cabinet (radiant convection method), hot-baking (contact heating) and infra-roasting without the presence of fat or a minimum of fat, baking is done at 170-200°C until the temperature inside meat reaches 75-80°C.

The baked carp meat from all the studied rearing groups were characterized by significantly higher protein levels of 24.60 ± 1.59% (cage-reared carp) to 24.78 ± 0.55% (free-living carp), (P<0.01, P<0.001). The increased protein content in baked and fried fish compared to the fresh meat is at the expense of decreasing the amount of water in the heat treated meat. The results indicate that the level of the proteins in the heat treated samples is influenced by the type of the thermal treatment method used.

The heat treatment processes also affect the fat content in the carp meat. Considering the influence of the rearing system on the fat content in the fresh carp samples it is the lowest in the meat of the free-living carp - 1.48%; followed by the pond-reared carp – 5.86%, the highest being in intensive cage-reared carp – 6.68%. It is significantly lower than the fat content in the baked and fried samples. After thermal treatment, the fat content in carp from all three rearing systems increases to 2.50%, 7.91% and 9.5% respectively for the baked and to 6.76%, 10.91% and 13.14% respectively for the fried fish. Greater fat increase is found in the fried carp and lower in the baked carp; in the intensive cage-reared carp it is more pronounced (almost 50%). According to Ninan et al. (2008) and Rathod and Pagarkar (2013), the increased fat level is due to the reduction of water content in the heat-treated meat.

The thermal treatment of carp meat (baking and frying) also affects the ash content. The ash content of the carp muscle tissue in the thermal untreated samples is close to 0.82-0.83% for both the free-living and aquaculture-carp. The heating resulted in a significant increase of the ash level to 0.82-0.83% in the fresh meat; to 0.99-1.05% in the baked and to 0.99-1.13% in the fried carp (P<0.05, P<0.01).

Fatty acid profile in carp lipids in fresh meat and after heat treatment

The everage values for the fatty acid composition of the carp lipids in fresh state and after heat treatment are shown in Table 3.

The level of the saturated fatty acid group as a percentage of the total fatty acids in the fresh carp meat ranges from 27.4% (in the free-living carp) to 32.1% (in the cage-reared carp) of the total fatty acids; for the baked meat from 24.6% to 27.1% and for the fried carp respectively from 24.4% to 25.0%. A slight decrease in the level of saturated fatty acids is reported after administration of the processes of baking and frying. The values obtained for the saturated fatty acids in fresh carp meat are close to those obtained by Hadjinikolova (2008).

The level of the monounsaturated fatty acids group in the meat from the free-living carp and from aquaculture, regardless of its thermal treatment, is very close – 68.0-75.6%. The content of monounsaturated fatty acids for the fresh carp meat is from 57.7% (free-living carp) to 61.5% (pond-reared carp); for baked carp from 38.8% (free-living carp) to 56.9% (pond-reared carp); and for fried carp from 46.6% (free-living carp) to 56.6% (cage-reared carp).

The analysis of the results shows a decrease in the level of monounsaturated fatty acids resulting from the thermal treatment. They are most represented by the oleic acid (C18:1). For the fresh carp meat, the values for the oleic acid are in the range of 50.5% (cage-reared carp) to 54.1% (pond-reared carp) of the total fatty acid content. The second of the monounsaturated fatty acids is the palmitooleic (C16:1), with values of 5.3% (free-living carp) to 7.7% (cage-reared carp). Low concentrations of less than 0.5% are registered for the other MUFA. The results obtained show that the oleic acid determines the level of the monounsaturated fatty acids and its level is in line with the values for fresh water fish cited in the literature

Table 3. Fatty acid lipid composition of carp meat before and after heat treatments, n=3

Fatty acids, %	Free-living carp			Pond-reared carp			Cage-reared carp		
	Fresh meat	Oven baked fish steak	Fish steak fried in oil	Fresh meat	Oven baked fish steak	Fish steak fried in oil	Fresh meat	Oven baked fish steak	Fish steak fried in oil
C 8:0	0.1	-	-	-	-	0.1	-	-	0.1
C 10:0	-*	-	-	-	-	-	0.1	-	-
C 12:0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1
C 14:0	1.1	1.0	1.0	1.2	1.0	1.0	1.3	1.1	1.1
C 14:1	0.1	-	0.1	0.1	0.1	0.1	-	0.1	0.1
C 15:0	0.2	0.5	0.4	0.4	0.3	0.3	0.2	0.1	0.1
C 16:0	19.9	17.3	16.6	21.9	18.3	17.1	24.1	17.9	17.7
C 16:1	5.3	3.4	4.2	7.0	5.5	5.4	7.7	5.6	6.3
C 17:0	0.2	0.7	0.5	0.2	0.2	0.2	0.3	0.2	0.2
C 17:1	0.2	0.4	0.2	0.3	0.3	0.2	0.3	0.2	0.1
C 18:0	5.2	6.0	5.2	5.8	5.4	5.1	5.7	4.5	4.4
C 18:1	51.6	34.4	41.9	54.1	50.7	49.9	50.5	48.3	49.6
C 18:2 (ω-6)	9.2	26.4	23.4	4.4	13.1	16.3	5.1	15.9	14.2
C 18:3 (ω-6)	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2
C 18:3 (ω-3)	3.9	3.8	3.1	2.9	2.4	2.2	3.4	3.1	3.2
C 20:0	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2
C 20:2 (ω-6)	0.3	0.6	0.4	0.2	0.2	0.2	0.2	0.3	0.2
C 20:3 (ω-6)	0.3	0.5	0.2	0.3	0.3	0.2	0.2	0.3	0.2
C 20:4 (ω-6)	0.5	1.5	0.6	0.5	0.6	0.4	0.4	0.6	0.3
C 20:5 (ω-3)	-	0.2	0.3	-	0.1	-	0.1	0.1	0.2
C 22:0	0.1	0.5	0.4	0.1	0.2	0.2	0.2	0.2	0.2
C 22:1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2
C 22:2 (ω-6)	0.3	0.9	0.2	-	0.1	0.1	0.2	0.1	0.2
C 24:0	0.3	0.8	0.6	0.4	0.4	0.4	0.2	0.5	0.5
C 24:1	0.3	0.5	0.1	0.2	0.3	0.2	0.2	0.4	0.4
C 22:6 (ω-3)	0.2	0.8	0.2	0.3	0.3	0.2	0.2	0.5	0.2
Saturated FA	27.4	27.1	25.0	30.0	25.9	24.4	32.1	24.6	24.4
Unsaturated FA	72.6	72.9	75.0	70.0	74.1	75.6	68.0	75.5	75.6
Monounsaturated FA	57.7	38.8	46.6	61.5	56.9	55.9	58.6	54.5	56.6
Poly unsaturated FA	14.9	34.2	28.4	8.6	17.2	19.7	9.4	21.0	19.0
∑ ω-6	10.8	29.5	24.8	5.4	14.4	17.3	5.9	17.4	15.4
∑ ω-3	4.1	4.7	3.6	3.2	2.8	2.4	3.5	3.6	3.6

Table 3. Continued

Ratio ∑ ω-6: ∑ ω-3	2.63	6.28	7.50	1.69	5.50	7.60	1.69	4.83	4.28

*- Not identified

(Hadjinikolova, 2004; Guler et al., 2008).

As a result of the thermal treatment, the level of polyunsaturated fatty acids increased. The baked carp meat is characterized by higher values in an interval of 17.2% (pond-reared carp) to 34.2% (free-living carp) of the total fatty acids content compared to the fried carp meat. For the fried carp, the values are 19.0% (cage-reared carp) to 28.4% (free-living carp). The highest rate of increase is reported for the free-living carp, where the values are approximately 19% higher for the baked samples and 14% for the fried ones. For the other two carp groups, the increase in the polyunsaturated fatty acids is 8-11%.

The changes in the polyunsaturated fatty acids are mainly due to an increase in the level of ω-6 fatty acids. Their average value in the baked carp are from 14.4% (pond-reared carp) to 29.5% (free-living carp), and in the fried carp from 15.4% (cage-reared carp) to 24.8% (free-living carp). ω-3 FA are characterized by minor changes and relative stabile level in the baked and fried meat samples.

There is a significant increase in the level of linoleic acid (C18:2 ω-6) in the baked and fried fish, which has a large share in the ω-6 group. This was observed in the carp of all three rearing groups studied. The higher values of the linoleic acid (C18:2 ω-6) and arachidonic (C20:4 ω-6) acids in the baked carp are a good indicator for better quality and nutritive value of the fish lipids in baked carp, compared to the fried ones.

The results show that the application of heat treatment leads to a decrease in the level of oleic acid (C18: 1) in the carp muscle tissue. According to Marichamy et al. (2009) the composition of fatty acids in fish muscle tissue changes after heat treatment depending on temperature, contact surface width, fish size and initial fat content. Our results show that this is most evident in samples of baked free-living carp from Zhrebchevo dam lake, which have the lowest fat level in the fresh fish fillet (1.48%).

The heat treatment also affects the level of the docosahexaenoic fatty acid (C22:6 ω-3). Its amount increases from 0.2% to 0.8% in baked free-living carp, and from 0.2% to 0.5% in baked cage-reared carp. Such influence is not taken into account for the pond-reared carp. The two essential fatty acids, eicosapentaenoic and docosahexaenoic, are considered as susceptible to oxidation as a result of thermal heating processes (Sant'Ana and Mancini-Filho, 2000).

The values of the biologically important ω-6:ω-3 ratio in the studied fresh carp meat ranged from 1.69-2.63. Lower values are observed in the carp from aquaculture – cage- and pond-reared. The application of both heat treatment methods leads to an increase in the ω-6:ω-3 ratio, respectively 4.83-6.28 for the baked and 4.28-7.60 for the fried carp. According to the World Health Organization guidelines (WHO, 2008) it is assumed that with a ω-6:ω-3 ratio of less than 5:1, raw materials and natural products have a low risk factor for human health.

According to Steffens and Wirth (2005) the ω-6:ω-3 ratio in carp ranges to a great extent (0.8 and 2.4) and is most affected by the type of feed used. Knowing the need to dietary inclusion of omega-3 essential fatty acids and their health-enhancing effects, nutritionists recommend a diet rich in omega-3 fatty acids as well as a decrease in ω-6: ω-3 ratio of 15-20: 1 to 1-4: 1 in the modern diet (Gebauer et al., 2006, Raes et al., 2004).

In our study, the fresh carp meat has a ratio of less than 5:1. The application of both heat treatment methods leads to an increase in the ω-6:ω-3 ratio, respectively to 6.28 for the baked and to 7.60 for the fried carp. Fried carp meat has higher values than those of baked ones. This gives reason to believe that carp lipids in baked carp are more balanced and biologically complete and have a lower risk factor for the human health.

Samples of the baked and fried carp reared in cages by using extruded pellets retain the biologically important ω-6:ω-3 ratio below the recommended level (5:1) as opposed to the free-living and pond-reared carp. This shows that the use of balanced pellets in the aquaculture leads to a product with a balanced fatty acid distribution and good nutritive value.

Piggot and Tucker (1990) in their studies indicate that the ω-6:ω-3 ratio can be used as an indicator for comparing the relative nutritive value of fish lipids. According to Arts et al. (2001) omega-3 fatty acids with C20 and C22 hydrocarbon atoms have more valuable nutritive properties than those with C18. In the cases of their dominance, especially with regard to eicosapentaenoic (C20:5 ω-3) and docosahexaenoic (C22:6 ω-3) acids, they are responsible for changes in the ω-6:ω-3 ratio. The same authors note that when using the omega-6:

ω-3 ratio in determining the nutritive value, the quantitative values of the individual polyunsaturated fatty acids should also be taken into account.

Conclusions

The method of the thermal treatment of carp meat has a significant effect on the content of the main components of its chemical composition. A greater change in the level of proteins and minerals is recorded in the baking process, while during the frying the fat content is more affected.

The fatty acid profile showed that the major group of fatty acids in the carp lipids in the fresh and processed meat is the monounsaturated fatty acid group. It was found that the application of the heat treatment led to a negligible decrease in the level of unsaturated fatty acids (UFA) and increase of the polyunsaturated fatty acids (PUFA). The increase of polyunsaturated fatty acids is a result of the thermal treatment and impacts to a greater extent the ω-6 fatty acids group compared to the ω-3 fatty acids group.

The heat treatment results in a significant increase in the level of linoleic (C18:2 ω-6), oleic (C18:1), palmitoleic (C16:1) and arachidonic (C20:4 ω-6) fatty acids in the baked carp meat which is a good indicator for better quality and nutritive value of fish lipids in baked carp meat compared to the fried ones.

It is established that the baked carp meat is characterized by a lower ω-6:ω-3 ratio, making it more balanced as food than the fried ones. It is recommended from both types of thermal treatment of carp meat the applying of the baking method more often than the fried, which preserves better the nutritive value of fish fat and the processed product has a lower risk factor for the human health.

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MICROBIOLOGICAL CHARACTERISTICS OF BIOCHAR AMENDED ALLUVIAL MEADOW SOIL

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Abstract

Petkova, G., K. Nedyalkova, A. Mikova and Irena Atanassova, 2018. Microbiological characteristics of biochar amended alluvial meadow soil, *Bulg. J. Agric. Sci.*, 24 (Suppl. 2): 81-84

Biochar (BC), produced by the pyrolysis of biomass under limited oxygen is highly stable and resistant to microbial decay and can serve as a long-term sink of carbon. During the last years biochar from different origin is studied as a soil amendment in many countries. The ecological importance of biochar addition is connected with decreasing of greenhouse gasses emissions and associated with them climate changes, and improvement of soil structure and fertility. The effect of biochar amendment on microbial amount and activity of Alluvial meadow soil was studied in field experiments with wheat and maize cultivated in crop rotation. The number of the main groups of soil microorganisms, CO_2-production and microbial biomass were determined. A stimulating effect of biochar amendment on the soil microflora was established in the study. The CO_2-production and the bacterial amounts were positively affected to the greatest extent. The results obtained confirm the importance of biochar addition as a promising method for soil fertility conservation.

Key words: biochar, soil microorganism, CO_2-production, microbial biomass, Alluvial- meadow soil

Introduction

The irrational use of soil resources in the last decades has lead to reduction of soil organic matter in many countries. This negatively affects not only soil fertility but also the associated global environmental problems, climate changes, biodiversity loss, desertification of large areas. In order to counteract the processes of soil organic matter decrease and maintain a positive balance of humic substances, it is required to utilize variable sources of organic matter. Recently, biochar has been of great interest in this regard. It is obtained by pyrolysis (incomplete combustion) of biomass and is a subject of research in many countries (Great Britain, New Zealand, Japan, USA, etc.) as a means of improving soil structure and fertility (Ogawa, 1994; Steiner et al., 2004; Lehmann, 2007). The presence of polycyclic and aromatic structures in BC makes it very resistant to decomposition in the soil. During its slow mineralization, compounds involved in the humus substances synthesis are formed. At the same time, BC has a high capacity to adsorb chemical substances, preventing the nutrient elements leaching by surface water. In addition, the decomposition of agricultural wastes, due to their rapid mineralization is connected with emission of large amounts of CO_2 in the atmosphere. Carbon in BC is in a stable form, therefore during its decomposion smaller amounts of CO_2 are released leading to reduced emissions of CO_2 in the atmosphere.

Most of the studies on the use of BC in agriculture are from agronomic standpoint. Less research has been conducted on the hydro-physical and water-retention properties

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of biochar (Kinney et al. 2012; Gray et al. 2014). Often the hydrophobic character in biochar treated soil was attributed to fungal colonisation (Abel et al. 2013). In recent years, data were obtained proving its positive effect on particular groups of soil microorganisms, nodule-forming bacteria, (Mia et al., 2015), mycorrhizal fungi (Ishii and Kadoya, 1994; Warnock et al., 2007) and also on general indicators of soil biological activity (Kolb et al., 2008; Steiner et al., 2008). Other authors establish no impact of BC addition on soil microflora (Ameloot, 2014; Sun et al., 2014). Sun et al. (2014) reported that soil respiration, nitrification and arylsulphatase activity of two soils were not affected by biochar amendment. Obviously, the effect of BC on soil microorganisms varies depending on its composition, conditions of production, its physical properties, soil type, rates of application and duration of BC degradation in soil. Until now, there is no evidence of a negative impact of BC on plant roots.

The aim of the present study is to determine the influence of BC addition on the microbiota of Alluvial meadow soil depending on the duration of the period following BC application in soil.

Materials and Methods

The study was conducted in April 2017 in the experimental field of Nikola Poushkarov Institute of Soil Science, Agrotechnologies and Plant Protection in the village of Tsalapitza. The samples from a field experiment with wheat "Sadovo 1" variety on Alluvial meadow soil (Fluvisol) were analyzed. The following fertilization was applied: N10 (in the form of urea urea), P12 (as triple superphosphate) - and K10 (applied as potassium sulphate). The P and K fertilizers were introduced prior to the main soil tillage and N fertilizer before the pre-sowing treatment. There was also spring nourishment with ammonium nitrate in March. General soil characteristics were the following: total organic C $\%_{2012,\ 2016,\ 2017}$ (0.8%-1%), total $N2_{012,\ 2016,\ 2017}$(0.09-0.1%) $pH_{soil\ 2012}$ (5.7-6.0); $pH_{soil\ 2016}$ (6.0-6.3); $pH_{soil\ 2017}$ (6.0-6.1). Grids Δ 2 m, ~ 40 m^2 were constructed at the four plots and samples for microbiological analysis were taken at six points at 0-10 cm depth. Sample water-repellency (soil hydrophobicity) was measured by the water drop penetration time (WDPT) method (Doer et al. 2002). The soil samples were weighed and subsequently equilibrated at the ambient air humidity before measuring water drop penetration time in the laboratory at recorded humidity and temperature. The WDPT measured at the laboratory for all soils treated with biochar including the control variant, and the biochar used in the field trial showed that sample were hydrophilic (non-water repellent, WDPT < 5s).

The following treatments were included in the study: 1. Soil without biochar amendment; 2.Soil amended in 2012 with BC (200 kg/da), produced by pyrolysis of maize residues at 500^0C; 3. Soil amended in 2016 with BC (230 kg/da), produced by pyrolysis of rice straw at the same conditions; 4. Soil amended with biochar, produced by pyrolysis of oak residues at the same conditions (applied at rate of 300 kg/da in 2017). The samples of treatments 2 and 3 were taken from parcels where the wheat was cultivated with maize in crop rotation. All samples were collected from a soil depth 0-10 cm in the phase tillaring and analyzed for the following microbiological parameters: 1. Colony-forming units (CFU) number of the main groups of soil microorganisms by dilution plate method (Gushterov et al., 1977). The following nutrient media were utilized: meat-peptone agar for ammonifying bacteria; stach-ammonium agar for actinomycetes and Chapek agar for microscopic fungi; 2. CO_2-production (total biological activity) (Alef, Nannipieri, 1998); 3. Microbial biomass quantity was assessed by the method of Anderson-Domsch (Anderson-Domsch,1978).

All analyses were done using three replicates per treatment. The least significant differences (LSD) were determined at $P \leq 0.05$ using ANOVA (the test of Duncan).

Results and Discussion

The data presented in Table 1 show that the number of ammonifying bacteria was significantly higher than that of the control soil (up to two times) in all biochar amended treatments regardless of the year of its application to the soil. Unlike the mentioned group of microorganisms, the numbers of actinomycetes and microscopic fungi were clearly dependent on the duration of the period following biochar addition. For example, in treatment 4 (BC applied in the 2017 year), the amount of actinomycetes was close to that of the control. Unlike actinomycetes, the number of microscopic fungi was higher, however the difference with the control is not statistically proven. In treatment 3 (BC applied in 2016) the amounts of these groups of microorganisms were significantly higher than treatment 1. Higher values for the number of main groups of soil microorganisms in the treatment with five-year long period following BC addition to the soil prove its slow mineralization. This is probably connected with a gradual release of substances used as nutrient sources by the soil microorganisms. The results on the amounts of major groups of microorganisms are in accordance with the data of Prayogo et al. (2008) and Petkova et al. (2015), which established that biochar addition stimulated the development of soil bacterial populations.

The values obtained for CO_2-production and quantity of microbial biomass (Figures 2 and 3) are in agreement with the data related to the number of soil microorganisms and prove the positive effect of the biochar addition on the soil microflora. The CO_2 production values for treatments with BC amendment applied in 2017 and 2016 were significantly higher than that of the control. In treatment 2 the CO_2-production was close the control, probably due to the delayed biochar mineralization. Relatively easily assimilated by the soil microorganisms chemical compounds in BC are likely to be depleted and mineralization processes occur with low intensity. The amount of microbial biomass is higher in all biochar amended treatments compared to the control, and the differences are statistically proven for treatments with BC applied in the present year and a year ago. The results obtained confirmed the data of Kobl et al. (2008), which reported for increase of microbial biomass and total biological activity after BC addition to soil.

The presented results show a long-term positive impact of the BC amendment on the soil microorganisms. The mechanisms of impact of BC on the size and composition of soil biota have not been elucidated yet. Keech et al. (2005) suggest that BC adsorbs substances with allelopathic activity, phenolic compounds and others, and reduce the concentration or deactivating micro-toxic compounds in the soil. According to Ameloot (2013), local BC particles in the soil create areas with increased nutrient concentration and amounts of microorganisms. Other authors (Zackrisson, 1996; Warnock et al., 2007) consider that the high porosity of BC may provide favoraruble habitats for microorganisms, altering predation rates by soil microfauna.

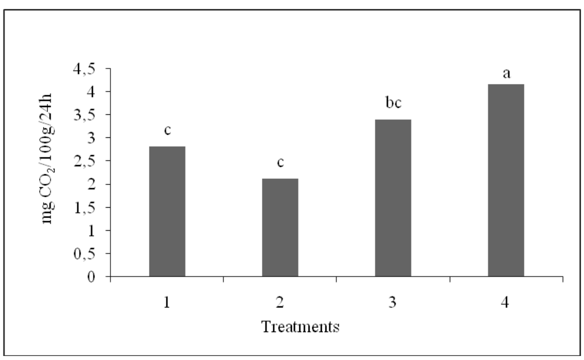

Fig. 1. CO_2-production of Alluvial Meadow Soil Amended with Biochar
1.Control; 2. + BC (applied in 2012); 3.+ BC (applied in 2016); 4.+ BC (2017);

Table 1. Number of the microorganisms (CFU/g) of Alluvial meadow soil amended with BC

Treatments	Ammonifying bacteria	Actinomycetes	Microscopic fungi
	1.10^5	1.10^5	1.10^3
1. Control	3.33^{c}	0.5^{b}	0.073^{b}
2.+ BC applied in 2012	7.53a	0.93^{ab}	0.14^{a}
3.+ BC applied in 2016	6.81^{ab}	1.4^{a}	0.15^{a}
4.+ BC applied in 2017	5.63^{b}	0.58^{b}	0.13^{ab}
LSD $P \leq 0.05$	1.56	0.54	0.061

*Values in the same column, which are followed by different letters are different at $P \leq 0.05$

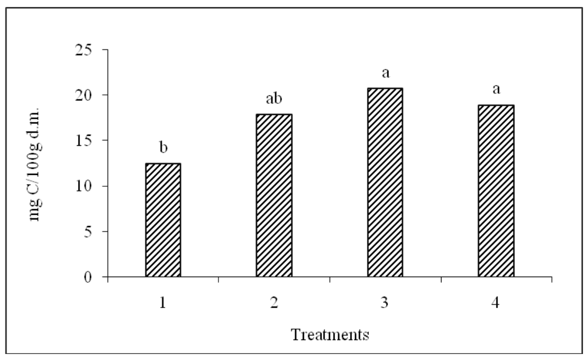

Fig. 2. Microbial Biomass of Biochar Amended Alluvial Meadow Soil
1.Control; 2. + BC (applied in 2012); 3.+ BC (applied in 2016); 4.+ BC (2017);
*Values in the same column, which are followed by different letters are different at $P \leq 0.05$

Conclusion

Key outcomes of the study are the following: (i) A long-term beneficial effect of biochar amendment in Aluvial-meadow soil on microbial biomass and the growth of soil microorganisms has been established. The bacterial populations and CO_2-production were stimulated to the greatest extent; (ii) the results obtained confirm the importance of biochar addition as a promising method for soil fertility conservation.

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SOURCES OF SPECIFIC VARIANCE AND HERITABILITY OF FREE JUMP QUALITIES IN 2-YEARS OLD HORSES FROM THE EAST BULGARIAN BREED

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Abstract

Sabeva, I., M. Popova, Sv. Kastchiev †,2018. Sources of specific variance and heritability of free jump qualities in 2-years old horses from the East Bulgarian breed. Bulg. J. Agric. Sci., 24 (Suppl. 2): 85-89

The influence of different sources of variability on the phenotypic variance of free jump qualities, the rate of the additive variance and the correlative relationships with traits characterizing movement have been studied. The study was carried out on the basis of data from the complex assessment by its own productivity of 191 East Bulgarian horses at the age of 2 years old, tested during the period of 2006-2016. Performance tests for 2 years old horses have been conducted in two consecutive days with pre-adaptive period for one week. No preliminary selection has been applied for the participants. There was a relatively constant structure of judging committee. Qualities of free jump (without rider) and allures were evaluated with a 10-score system with accuracy to 0.5. Registration of animals and traits was made by Association East Bulgarian Horse. Analyses of variance, estimations of different sources of variability, heritability and correlations were made by mixed model methodology.

Family belonging of horses, lineal belonging of dams and sex were statistically proven sources of phenotypic variance. The progeny of mares from Ohota, Likuiushta and Longuza families, and those originated from world famous in sport horse lines of Devis Own (through stallions Da Kapo and Don Primero), Ramzes (through Raskalino), Alme Z (through Kuidams Rubin) and Ladykiller (through Limnos) was characterized with very good jumping abilities.

Favorable genetic correlations were established between qualities of free jump and: correctness (0.59); overall gaits (0.39); qualities of free gallop (0.35). Phenotypic and genetic correlations between qualities of free jump and evaluation of walk and trot were low. There were moderate values of free jump heritability.

Key words: free jump, heritability, correlation, performance test, 2-years old sport horse.

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Introduction

East Bulgarian horse breed is a small population with limited genetic pool. During the last two generation intervals the purpose of the selection was to improve jumping abilities. The assessment of heredity determined variance was important for establishing the breeding goals and methods for their achievement. According to Koenen & Aldridge (2002) in Germany and Netherlands genetic analyses were based on data from station performance tests and competitions; in Denmark and Sweden – on station performance tests; and in Belgium, France and Ireland – only on competitions. In Bulgaria short-term breeding tests have been carried out since 2006.

In a study on the results of stallions' performance test Olsson et al. (2000) have established average to high heredity determination of free jump (0.47±0.13), high repeatability of trait (0.58) and high genetic correlation with the results of assessment under rider. According to Olsson et al. (2000), Viklund et al. (2008), Posta et al. (2010b) and etc. there were some favorable genetic correlations ranged from 0.26 to 0.70 between assessments of gallop and jump. Evaluation of heritability of free jump in Oldenburg mares varied from 0.541 to 0.564 depending on their age at the time of test (Becker et al., 2011). Investigations of Hanni Luehrs-Behnke (2002) based on mares' performance tests made of all German Warmblood horses indicated lower heritability – 0.32. Posta et al. (2010a) have established heritability from 0.39 to 0.49 and high genetic correlations between assessments of free jump, walk, trot and gallop without a rider in mares from Hungarian sport horse. There are low and rarely moderate values of the additive variance of traits characterizing jumping abilities of different ages in disciplines of equestrian sport (Koenen et al. 1995, Ricard & Chanu 2000, Posta et al. 2010, Prochniak et al. 2015, Rovere et al. 2015, etc.). Summarizing the results from 17 scientific works Thoren et al. (2006) concluded that analyses from tests' data of younger horses and younger stallions indicated higher heritability values and higher genetic correlations with the successive sport results.

The first complex assessment by its own productivity of East Bulgarian horses was made at the age of 2 years old and the weight of free jump assessment is 20% from the complex one. That's why we made the present study to investigate the influence of different sources of variability on the phenotypic variance of free jump qualities, the value of the additive variance and the correlative relationships with traits characterizing movement qualities.

Material and Methods

The study was worked out on the basis of data from the complex assessment by its own productivity of 191 East Bulgarian horses at the age of 2 years old, tested during the period of 2006-2016. Performance tests for 2 years old horses have been conducted in two consecutive days with pre-adaptive period for one week. No preliminary selection has been applied for the participants. There was a relatively constant structure of judging committee. Qualities of free jump (without rider) and allures were evaluated with a 10-score system with accuracy to 0.5. Registration of animals and traits was made by Association East Bulgarian Horse.

Analyses of variance, estimations of different sources of variability, heritability and correlations were made by mixed model methodology. It was established with preliminary studies that factors year of test and month of birth of horses didn't influence significantly on the phenotypic variability. The structure of the used operational model had the following linear expression:

$$Y_{ijksnlop}=\mu+S*L_i+F_j+LM_k+YB_s+G_n+e_{ijksnlop}$$

where: $Y_{ijklmop}$ – observation vector; μ- population average; $S*L_i$, F_j, LM_k, YB_s and G_n, are: random effect of interaction sire*line (i=37) and: fixed effects of family (j=16); dam's line (к=21); birth year (s=14); sex (n=2); $e_{ijksnlop}$ – residual variance.

The value of the additive variance was calculated through the sire's half-sib analyses including effects of linear belonging of horses, years of birth and sex. Without having any essential differences of the phenotypic variation, MINQUE estimation of the additive component of variance increased when the size of offspring groups grew bigger (more than 2, more than 3 and more than 5) and the correlations between classes decreased. The above mentioned statements were based on the information from preliminary investigations on the parameters of the used data files.

Results and Discussion

Judges estimated the qualities of jump through the basic criteria technique, strength and natural ability to map out the distance before and after the jump. Scores of free jump were from 5.40 to 8.83 score, average 6.79 at the standard deviation from 0.50 and coefficient of variation from 7.43%. The results from the analyses of variance for jump and correctness (without a rider) only are presented in Table 1, because of the favorable genetic correlations between correctness of movement and trot, free gallop and totally for gaits determined in our previous study (Popova and Sabeva, 2017).

The influence of family belonging of horses, lineal belonging of dams and sex were statistically proved in both of the given traits. Differences between sexes of free jump were in favor of males (BLUE = + 0. 012). At two years of age colts are often more athletic than fillies and showed better strength potentialities.

East Bulgarian horse was acknowledged as a breed in 1951 on the basis of formed 16 genealogical lines and more than 27 families. In addition to pure breeding, a grading with thoroughbreds and stallions from the recognized sport warmblood breeds has been periodically carried out. At the present stage of breed development, the breeding work was carried out with 15 family nests differing by degree of expression of certain exterior, constitutional and productive characteristics. The families of Ohota, Likuiushta and Longuza possessed productivity over the average for the population regarding the qualities of the free jump, having in mind the bulk of the progeny groups, (table 2).

During the last three generation intervals the selection was oriented towards changing the constitutionally-productive type - from racing to suitable one for the equestrian sport disciplines. Results from the present and earlier studies (Sabeva, 1990) indicated that the improvement of jumping abilities was the slowest for the families of Nerazdelna, Kilia and Leila which representatives have surpassed their coevals in regards to their racing time. The achievements of horses from Krastanka and Vodka families were poorer in the racing disciplines and surpassed significantly the average of the free jumping qualities. Mares from Ohota, Nagaika, Malta, Longuza, Likuyushta and Slavyanka families had wide genealogical matched couples and their progeny surpassed all the others in both productive directions.

Progeny of dams belonging to old lines of Tihany, Zenger, Vustershire and Edelknabe got negative constants (table 2). Progeny of mares originated from thoroughtbreeds Makar, Grapholog, Dracedion, and Galego showed productivity above the average for the population. Grapholog, Dracedion, and Galego are representatives of the Tedy line, and Makar – of Bayardo through branching of Gey Krusader. Mares originating from world famous in equestrian sport lines of Devis Own (through stallions Da Kapo and Don Primero), Ramzes (through Raskalino) Alme Z (through Kuidams Rubin) and Ladykiller (through Limnos) gave birth of horses with better jumping abilities. The positive effect of these lines varied in the range of 0.08 to 1.33 scores.

Favorable genetic correlations were established between qualities of free jump and: correctness (0.59); overall gaits (0.39); qualities of free gallop (0.35). There were lower phenotypic and genetic correlations between qualities of free jump and estimations of walk and trot (table 3). The value of heredity determined variance was 0.43 in model without interactions and 0.55 in model with nested effects of sires in lines. Effects of genealogical groups in long term selected breed with formed structure are result from different interlineal and lineal-family crosses and their use as nested effects may lead to overestimation or underestimation of the heritability. In this case the use of nested effect caused 12% calculative difference. The range of such differences provides useful information to breeders, especially when there are low and moderate values of phenotypic and genetic correlations, and the differences between them for the same trait are not big (Table 3). Due to the small set of data and low threshold of the offspring groups (2-29 animals), the value of the additive variance may be considered as overestimated. However, it can be assumed that the inheritance of the free jump in East Bulgarian breed is within the range of moderate values.

Table 1. Anova

Sources of variability	df	Free jump F- test	Correctness F- test
Sire*line	37	random	random
Family	15	+	+
Dam's line	20	+	+
Year of birth	13	n.s.	n.s.
Sex	1	+	+
R^2 of model		0.50	0.51

Table 3. Heritability, phenotypic and genetic correlations

Traits	Free jump h^2 = 0.43	
	Rp	Rg
Gaits overall	0.37	0.39
Walk	0.21	0.29
Trot	0.18	0.21
Free gallop	0.32	0.35
Correctness	0.51	0.59

Table 2. Effect of genealogical groups

Family	n	Free jump		Dam's line	n	Free jump	
		BLUE ± SE	BLUE-constsnt			BLUE ± SE	BLUE-constsnt
Krastanka	2	7.22 ± 0.43	0.31	Tihany	14	6.65 ± 0.18	-0.26
Hana	8	6.41 ± 0.22	-0.50	Zenger	11	6.45 ± 0.20	-0,45
Hodeida	4	6.55 ± 0.29	-0.36	Vustershire	33	6.73 ± 0.13	-0.17
Kilia	5	6.80 ± 0.26	-0.25	Edelknabe	2	6.18 ± 0.41	-0.73
Longuza	10	6.94 ± 0.22	0.03	With origin from purebred			
Slavyanka	5	6.93 ± 0.28	0.02	Makar	7	7.05 ± 0.23	0.13
Likuiushta	17	6.97 ± 0.15	0.06	Kajus	9	6.66 ± 0.21	-0.25
Leila	22	6.82 ± 0.15	-0.09	Graphology	4	7.04 ± 0.29	0.13
Nerazdelna	13	6.58 ± 0.17	-0.33	Dracedion	2	7.14 ± 0.40	0.23
Ohota	27	7.09 ± 0.15	0.18	Giacint	5	6.53 ± 0.27	-0.37
Genoveva	3	6.91 ± 0.36	-0.01	Galego	6	7.27 ± 0.24	0.36
Malta	3	7.05 ± 0.33	0.14	Other	11	6.63 ± 0.20	-0.27
Nagaika	3	8.51 ± 0.34	1.59	With origin from			
Vodka	3	7.02 ± 0.39	0.11	Devis Own	20	7.17 ± 0.15	0.26
others	66	6.72 ± 0.10	-0.19	Dampfross	15	6.90 ± 0.17	-0.01
μ ± SE	191	6.91±0.10		Adeptus XX	21	6.87 ± 0.16	-0.04
				Ramzes	4	7.02 ± 0.29	0.11
				Alme Z	2	8.24 ± 0.58	1.33
				Cor de la Bry-ere	4	6.61 ± 0.30	-0.30
				Ladykiller	4	6.99 ± 0.34	0.08
				Gagne Si Pen	7	6.54 ± 0.25	-0.36
				Tempelhuter	2	7.22 ± 0.55	0.31
				others	8	7.20 ± 0.19	0.29
μ ± SE	191	6.91±0.10			191	6.91±0.10	

Conclusions

Family belonging of horses, lineal belonging of dams and sex were statistically proven sources of phenotypic variance. The progeny of mares from Ohota, Likuiushta and Longuza families, and those originated from world famous in sport horse lines of Devis Own (through stallions Da Kapo and Don Primero), Ramzes (through Raskalino), Alme Z (through Kuidams Rubin) and Ladykiller (through Limnos) was characterized with very good jumping abilities.

Favorable genetic correlations were established between qualities of free jump and correctness, qualities of free jump and overall gaits, qualities of free jump and qualities of free gallop. Phenotypic and genetic correlations between qualities of free jump and evaluation of walk and trot were low value. There were moderate values of free jump heritability.

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REACTION OF *SORGHUM VULGARE* VAR. *TECHNICUM* [KÖRN.] IN THE EARLY GROWTH STAGES OF DEVELOPMENT IN DROUGHT AND WATER DEFICIENCY IN LABORATORY CONDITIONS

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Abstract

Marinov-Serafimov Pl., I. Golubinova and St. Enchev, 2018. Reaction of *Sorghum vulgare* var. *technicum* [Körn.] In the early growth stages of development in drought and water deficiency in laboratory conditions, *Bulg. J. Agric. Sci*., 24 (Suppl. 2): 90-99

The effect of polyethylene glycol PEG – 20000 on the induction of water stress on germination and the initial development of five genotypes (varieties and local populations) *Sorghum vulgare* var. *technicum* [Körn.] was determined under laboratory conditions at the Institute of Forage Crops – Pleven. In order to simulate the water deficit induced by osmotic stress, different concentrations (1.25%; 2.5%; 5.0%; 7.5%; 10.0%; 12.5%; 15.0%; 17.5% and 20.0%) of non-ionic water soluble polymer polyethylene glycol of molecular weight 20000 (PEG – 20000) were used in the study.

It was found that: Osmotic stress induced by the addition of PEG inhibits root growth and shoot IR% from 15.03 to 72.29% at higher applied concentrations (from 10.0 to 20.0%). Lower applied concentrations (1.25 to 7.5%) had a stimulating effect IR% from 11.71 to 135.77%. according to control treatments for all tested genotypes. There was a specific variety reaction with regard to the effect of PEG on seedling growth (cm) and formation of fresh weight on seedlings (g) in the tested genotypes *Sorghum vulgare* var. *technicum* (Körn.). It was found that with relatively good tolerance to osmotic stress it is possible to determine Szegedi 1023 variety and the AS17P local variety (TI average varied from 2.28 to 2.55). With low coefficients of tolerance, i.e. the high sensitivity of drought in the early growth stages of development (BBCH – 09-10) were the G16V and MI16N local varieties (TI average varied from 1.29 to 1.36) while GL15A local variety occupies an intermediate position – TI – 1.62. In the Szegedi 1023 variety and AS17P local variety, unlike the growth of the seedlings, the formed fresh biomass in g for one seedlings increases with the increasing level of PEG 20000 treatments, except for the highest applied concentrations (17.5 and 20.0%), while the GL15A, G16V and MI16N local varieties formed fresh biomass of the roots, shoots and seedlings decreased IR% varied from 22.22 to 100.0% and the differences were statistically significant ($p = 0.05$). which can be explained by their sensitivity to drought in the early growth stages of development (BBCH-09-10). The G16V and MI16N local varieties found superior and might be productive in further breeding programmes for drought tolerance.

Key words: *Sorghum vulgare* var. *technicum* [Körn.], polyethylene glycol (PEG), water stress, seedling.

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Introduction

Drought is one of the major abiotic factors that suppress the growth and development of cultivated plants and considerably reduce their productivity. Water deficit is its basic component that causes a number of morphological and physiological changes in the plants растенията (Arora et al., 2002; Ali et al., 2011a; Zapryanova and Nencheva, 2013; Zapryanova et al., 2016).

The research on the physiological mechanisms of plant resistance in laboratory conditions gives an opportunity to the determine specific response and tolerance to drought in the early growth stages of development in a number of agricultural crops (Alexieva, 2003; Borrell et al., 2014; Tsago et al., 2014; Rauf et al., 2016; Zapryanova et al., 2016)

The drought simulation in laboratory conditions at Sorghum species was accomplished with a nonionic water-soluble polyethylene glycol with high molecular weight (>6000). The use of PEG allows to recreate the necessary osmotic potential and prevents water absorption roots, i.e. the to simulate soil drought (Bibi et al., 2010; O'Donnell et al., 2013; Tsago et al., 2014; Zhang et al., 2015; Donchev et al. 2016; Fadoul et al., 2016).

According to Munns (2002), Bozhanova et al. (2005); Bibi et al. (2012); O'Donnell et al. (2013); Zhou et al., 2013; Basha et al. (2015); Chipilski et al. (2017) degree of inhibition of growth processes in the shoot under the influence of osmotic stress correlated with drought tolerance of a species or genotype.

The *Sorghum* species are a multipurpose crop grown for food, animal feed and industrial purposes. According to studies a number of authors (Ali et al. 2011b) It is considered more tolerant to many stresses, including heat, drought, salinity and flooding as compared to other cereal crops, however, the Sorghum species in the early growth stages and development effected by drought stress – reduction of yield and plant growth (Bibi et al., 2012; Calvino and Messing, 2012; O'Donnell et al., 2013).

In the literaure there is data on evidence of differences between *Sorghum* species in terms of their sensitivity to drought in the their early growth stages of development (Zhou et al., 2013). Identification of *Sorghum vulgare* var. *technicum* (Körn.) (broom corn) genotypes that can withstand drought in the early stages of growth and development BBCH 09-10 is important to increase the production of fodder and seeds QI et al. (2016). Tolerant genotypes of broom corn to drought in the early stages of development may be recommended for cultivation in drought-affected areas.

The purpose of the study is to identify the reaction to water deficit in local varieties of *Sorghum vulgare* var. *technicum* (Körn.) (broom corn) in the early stages of their development under laboratory conditions by using a non-ionic water-soluble polyethylene glycol (PEG) having a molecular weight of 20000.

Materials and Methods

In order to establish the influence of water deficit in laboratory conditions on the genotypes. *Sorghum vulgare* var. *technicum* (Körn.) (broom corn) at the initial growth stages and development were used adapted method Chaniago et al. (2017).

Two factors have been studied: Factor A genotypes of *Sorghum vulgare* var. *technicum* (Körn.) (broom corn), local varieties from the region of Central Northern Bulgaria and a Hungarian variety: a1 – Szegedi 1023 (Hungarian variety); a_2 – AS17P; a_3 – GL15A; a_4 – G16V; and a_5 – MI16N (local varieties) Factor B – polyethylene glycol concentration (PEG 20000): b_1 – 0.0% (control); b_2 – 1.25%; b_3 – 2.5%; b_4 – 5.0% and b_5 – 7.5%; b_6 – 10%; b_7 – 12.5%; b_8 – 15.0%; b_9 – 17.5% and b_{10} – 20.0% (w/v).

Technique of bioassay. Two hundred seeds from tested genotypes broom corn were germinated on two layers of filter paper Filtrak 388 in 140 mm Petri dishes were pipetted distilled water at a ratio of 1:2.5 to the mass of the seeds. The prepared samples were placed in an incubator at 48 h at 23^{o}C ± 2^{o}C. Seeds were considered as germinated if they exhibited radicle extension by more than ≥ 3.0 mm.

Successively, twenty germinated seeds of each genotypes *Sorghum vulgare* var. *technicum* (Körn.) (according factor A) were placed between filter paper Filtrak 388, in the Petri dishes for all tested concentrations polyethylene glycol (PEG 20000). From all concentrations of polyethylene glycol (PEG 20000) (Factor B) in the petri dishes, 5 ml of solution was pipetted. The prepared samples were placed in an incubator at 22 ± 2^{o}C in the dark for five days. Each treatment consisted of six replicates, including the control treatment.

Effect assessment. For assessing experimental results, the following parameters were used:. Biometric parameters: root, shoot and seedling length, cm; fresh biomass weight per seedling, g. Length was measured using graph paper and weight on an analytical balance; Mathematical-statistical evaluation and calculated formulas:

Percent inhibition (IR) was determined by the equation (1) (Ahn et al., 2005):

$$IR\% = [(C-T)/C].100 \quad (1)$$

where C – characteristic in the control treatment; T – characteristics in each treatment. Positive values “+” show inhibition effect, while negative “-” values show stimulation effect.

Tolerance index was determined by the equation (2) (Chaniago et al., 2017):

$$TI = \frac{Y_d}{Y_n} \cdot \frac{Y_d}{H_{yd}} \quad (2)$$

where Y_d and Y_n respectively represent observed variable under drought and normal condition. Hyd is the highest observed variable under drought condition.

All experimental data were statistically processed using the software STATGRAPHICS Plus for Windows Version 2.1.

Results and Discussion

The effect of osmotic stress induced by the Poly Ethylene Glycol (PEG 20000) on the seedling length (cm) on the tested genotypes *Sorghum vulgare* var. *technicum* (Körn.) (broom corn) varieties is presented in Table 1. Seedling development in lab conditions have been recognized as testing procedure in sorghum it was found that with the increase in PEG concentrations, the rate and growth of seedlings decreased IR% from 2.6 to 96.4.

For all tested genotypes had a reduction in seedling length at higher concentrations of PEG (17.0 and 20.0%), while at lower applied concentrations (IR% from 1.25 to 15.0%) was established a stimulating effect, according the control treatments, the differences are significant at the p = 0.05 (Table 1).

The maximum value for the seedling length was observed in concentration 1.25% and minimum value was observed in 20.0% PEG for all genotypes. The differences among genotypes were also highly significant for the studied indicator (Table 1).

The growth of seedling indicated that all genotypes in early growth stage (BBCH – 09-10) (Hess, et al, 1997) after treatments in the 17.5 and 20.0% PEG suffered significant physiological stress despite the proven drought resistance of the tested species.

Similar results were reported by O’Donnell et al. (2013) and Damame et al., (2014); Chaniago et al., (2017) according to whom applied of 20.0% PEG in early growth stage of *Sorghum* species provoked significant physiological stress.

There was a specific variety reaction with regard to the effect of PEG on seedling growth in the tested *Sorghum vulgare* var. *technicum* (Körn.) genotypes.

Average seedlings length at broom corn local variety GL15A (8.99 cm) was established that grows intensively when exposed to 15.0% PEG and formed the longest seedling average for the treatment. Relatively long seedling formed local varieties GL15A (14.42 cm) and G16V (13.36 cm). Seedlings length at the Szegedi 1023 varieties and local variety MI16N and AS17P was significantly different to other local varieties and ranges from 8.95 to 8.80 cm. Two local varieties G16V and SMI16N conditionally can be identified as susceptible to drought stress.

The reduction on the seedling length (IR%) according control treatments at the two local varieties G16V and SMI16N and was ranging from 0.95 to 57.7% conditionally can be identified as susceptible to drought stress. It was found that the average length of the root and shoot in the tested genotypes of broom corn, irrigated with distilled water, ranges over a broad range of roots: from 4.44 to 7.94 cm and from 2.33 to 7.97 cm at shoots.

From the biometric measurements, it was found that the GL15A and G16B local varieties form the relatively longest root and shoot, respectively, from 7.94 and 7.84 cm and from 5.69 to 7.97 cm respectively. Smallest root length and shoot were recorded at AS17P and Sz16 local varieties respectively: from 4.44 and 4.80 cm at the root and from 2.33 to 4.13 cm. With regard to the studied indicators the local variety MI16N – has an intermediate position (Table 1). Therefore, the observed differences in the studied genotypes broom corn with regard to the osmotic stress of PEG could be also explained by genetic differences, because the comparisons between them were conducted at the same concentrations of the applied of PEG.

Osmotic stress induced by the addition of PEG inhibits root growth and shoot IR % from 15.03 to 72.29% at higher applied concentrations (from 10.0 to 20.0%). Lower applied concentrations (1.25 to 7.5%) had a stimulating effect IR % from 11.71 to 135.77%, according to control treatments for all tested varieties. PEG indicates a stronger depressant effect on the shoot growth (on average from 24.3 to 72.3%) according to compared to root growth (on average from 15.03 to 35.56%), that indicating the increase in shoot is affects more strongly the osmotic stress, compared to the growth of the root. Similar results were reported by Bozhanova et al. (2005); Bibi et al. (2010); O’Donnell et al. (2013) Donchev et al. (2016). According to the studied on these authors the observed dependence can be explained by a protective reaction of plants to prevent dehydration, given the important role that roots play in supplying the plant with water and nutrients.

A high negative correlation was detected between germination and the root growth (r of -0.755 to -0.931) and shoot growth (r of -0.668 to -0.985), and the seedling length (r of -0.944 to -0.985) for all tested genotypes of broom corn.

With relatively good tolerance to osmotic stress it is possible to determine Szegedi 1023 variety and the local variety AS17P (TI average varied from 2.28 to 2.55). With low coefficients of tolerance, i.e., the high sensitivity of drought in the early growth stages of development (BBCH -09-10) were the G16V and MI16N local varieties (TI average varied from 1.29 to 1.36) while GL15A local variety occupies an intermediate position – TI – 1.62 (Table 1 and 2, Figure 1).

Reducing fresh biomass of root, shoot and seedling is common the response of crop plants when subjected to moisture deficiency (Sharp and Davies, 1979, Santamaria et al., 1990; Ambika et al., 2011 Song et al., 2013).

Formation of fresh biomass (g per seedling) was genotype dependent.

Szegedi 1023 variety and AS17P local variety, unlike the growth of the seedlings, the formed fresh biomass (g for seedlings) increases with the increasing level of PEG 20000 treatments, except for the highest applied concentrations (17.5 and 20.0%), and the differences were statistically significant (p = 0.05) (Table 2).

The water stress induced by the application of PEG 20000 also decreases IR% from 22.22 to 100.0% the formed fresh biomass of the primary root, leaf and and seedligs at the GL15A, G16V and MI16N local variety, and the differences were statistically significant (p = 0.05), which can be explained by their sensitivity to drought in the early growth stages of development (BBCH – 09-10).

Dhanda et al. (2004), Hajime (1999) Bibi et al. (2010), Sabadin et al. (2012) also observed significant differences for various seedling traits contributing to drought in Sorghum species, respectively.

Data from dispersion analysis (Table 3) showing hierarchical allocation of variations among factors determing the osmotic stress induced by the addition of different PEG 20000 concentrations (% w/v) on the tested species howed that factors A (η^2 varied from 9.5 to 13.2) interaction AxB (η^2 from 3.8 to 5.9) had statistically significant action but factor B (concentration of PEG 20000 % w/v) (η^2 from 19.1 to 21.5) had the strongest effect for the root, shoot and seedlings lenght. Regarding weight parameters root, shoot and seedlings weight, factor B also to had a statistical significance (η2 from 19.6 to 40.2). followed by factor A genotipes (η2 from 4.10 to 25.9). Interaction of the genotypes factor A applied concentration – factor B had a relatively high proportion of total variation of η2 from 25.5 to 47.5 had statistical significance regarding formed fresh biomass of root, shoot and seedlings.

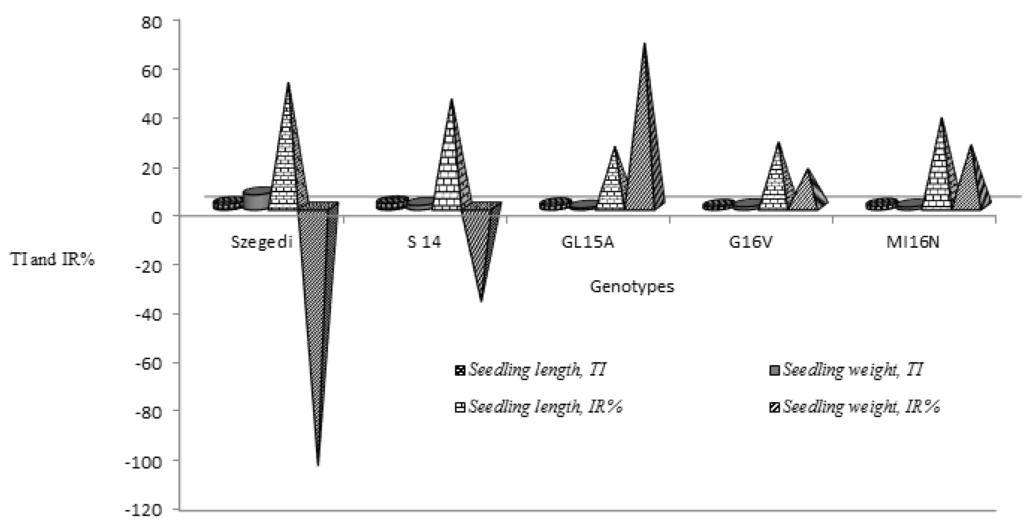

Fig. 1. Sensitivity to drought in *Sorghum vulgare* var. *technicum* (Körn.) genotypes
Legend: IR% - Percent inhibition. Positive values "+" show inhibition effect, while negative "-" values show stimulation effect; TI – Tolerance index, coefficient average for all tested concentrations of PEG 20000

Table 1. Growth of seedlings in *Sorghum vulgare* var. *technicum* (Körn.) genotypes under different concentrations of PEG 20000

Genotypes	Indicators	PEG concentrations,%									
		0.0	1.25	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0
Szegedi 1023	Shoot, cm	4.80c	7.07e	5.60cd	5.56cd	7.20e	6.73cd	5.78cd	5.24cd	2.36b	0.30a
	IR%	0.00	-47.29	-16.67	-15.83	-50.00	-40.21	-20.42	-9.17	50.83	93.75
	TI	1.00	4.41	2.77	2.73	4.58	4.00	2.95	2.42	0.49	0.06
	Root, cm	4.13cd	5.12de	6.20e	5.86e	3.64c	3.73c	3.55c	3.47c	1.88b	0.00a
	IR%	0.00	-23.97	-50.12	-41.89	11.86	9.69	14.04	15.98	54.48	92.74
	TI	1.00	3.38	4.95	4.42	1.71	1.79	1.62	1.55	0.46	0.07
	Seedling, cm	8.93cd	12.1e	11.80de	11.42cde	10.84cde	10.46cde	9.33cde	8.70c	4.24b	0.30a
	IR%	0.00	-35.50	-32.14	-27.88	-21.39	-17.13	-4.48	2.58	52.52	96.64
	TI	1.00	3.87	3.68	3.44	3.10	2.89	2.30	2.00	0.47	0.03
AS17P	Shoot, cm	4.44bc	8.24e	7.53de	6.78de	5.58cd	5.88cd	4.16bc	3.34b	3.49b	0.30a
	IR%	0.00	-85.59	-69.59	-52.70	-25.68	-32.43	6.31	24.77	21.40	93.24
	TI	1.00	4.38	3.66	2.97	2.01	2.23	1.12	0.72	0.79	0.07
	Root, cm	2.33b	7.68d	5.63c	5.34c	5.21c	4.47c	4.63c	2.13b	2.16b	0.30a
	IR%	0.00	-229.61	-141.63	-129.18	-123.61	-91.85	-98.71	8.58	7.30	87.12
	TI	1.00	11.72	6.30	5.67	5.39	3.97	4.26	0.90	0.93	0.13
	Seedling, cm	6.78b	15.92f	13.16ef	12.13cd	10.79cd	10.35cd	8.79bc	5.47b	5.65b	0.60a
	IR%	0.00	-134.81	-94.10	-78.91	-59.14	-52.65	-29.65	19.32	16.67	91.15
	TI	1.00	6.62	4.52	3.84	3.04	2.80	2.02	0.78	0.83	0.09
GL15A	Shoot, cm	7.94cd	10.07e	9.25e	8.09cd	8.07cd	6.79bc	7.73cd	6.58abc	4.78ab	4.19a
	IR%	0.00	-26.83	-16.50	-1.89	-1.64	14.48	2.64	17.13	39.80	47.23
	TI	1.00	2.67	2.25	1.72	1.72	1.21	1.57	1.14	0.60	0.53
	Root, cm	5.69abc	8.83e	9.31e	7.50bcde	7.59cde	6.66bcd	6.10abc	8.55de	4.22a	5.45a
	IR%	0.00	-55.18	-63.62	-31.81	-33.39	-17.05	-7.21	-50.26	25.83	4.22
	TI	1.00	3.25	3.61	2.34	2.40	1.85	1.55	3.04	0.74	0.96
	Seedling, cm	13.62bc	18.91d	18.57d	15.59cd	15.67cd	13.45cd	13.84bc	15.12bc	8.99a	9.64ab
	IR%	0.00	-38.84	-36.34	-14.46	-15.05	1.25	-1.62	-11.01	33.99	29.22
	TI	1.00	2.92	2.82	1.98	2.01	1.48	1.56	1.87	0.66	0.71
G16V	Shoot, cm	7.84bcd	10.28ef	10.68f	8.46de	8.03cd	6.27abc	6.04ab	5.58a	5.85a	4.71a
	IR%	0.00	-31.12	-36.22	-7.91	-2.42	20.03	22.96	28.83	25.38	39.92
	TI	1.00	2.30	2.49	1.56	1.41	0.86	0.80	0.68	0.75	0.60

Table 1. Continued

	Root, cm	7.97d	9.55e	7.89d	6.96cd	6.11bc	5.19ab	5.20ab	4.72ab	4.45a	4.46a
	IR%	0.00	-19.82	1.00	12.67	23.34	34.88	34.76	40.78	44.17	44.04
	TI	1.00	2.57	1.76	1.37	1.05	0.76	0.76	0.63	0.56	0.56
	Seedling, cm	15.81cd	19.83e	18.58de	15.43cd	14.14bc	11.46ab	11.24ab	10.30a	10.30a	8.93a
	IR%	0.00	-25.43	-17.52	2.40	10.56	27.51	28.91	34.85	34.85	43.52
	TI	1.00	2.41	2.12	1.46	1.23	0.81	0.78	0.65	0.65	0.56
MI16N	Shoot, cm	6.32ef	8.34f	5.46de	5.25cde	4.94bcde	3.21abc	2.41a	3.52abcd	2.82ab	3.09ab
	IR%	0.00	-31.96	13.61	16.93	21.84	49.21	61.87	44.30	55.38	51.11
	TI	1.00	3.90	1.67	1.55	1.37	0.58	0.33	0.70	0.45	0.49
	Root, cm	5.31cdef	7.34ef	7.36e	6.28def	4.84bcd	5.26cde	3.24abc	2.24a	2.10a	2.85ab
	IR%	0.00	-38.23	-38.61	-18.27	8.85	0.94	38.98	57.82	60.45	46.33
	TI	1.00	4.83	4.86	3.54	2.10	2.48	0.94	0.45	0.40	0.54
	Seedling, cm	11.63cde	15.67e	12.83de	11.52cd	9.77bcde	8.47abc	5.66a	5.76ab	4.92a	5.94ab
	IR%	0.00	-34.74	-10.32	0.95	15.99	27.17	51.33	50.47	57.70	48.93
	TI	1.00	4.29	2.88	2.32	1.67	1.25	0.56	0.58	0.42	0.51

Legend: IR% - Percent inhibition. Positive values "+" show inhibition effect, while negative "-" values show stimulation effect; TI – Tolerance index; Different letters in columns indicate significant differences by the LSD test at p=0.05 probability

Table 2. Accumulation of fresh biomass g for one seedlings in *Sorghum vulgare* var. *technicum* (Körn.) genotypes under different concentrations of PEG 20000

Genotypes	Indicators	PEG concentrations,%									
		0.0	1.25	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0
Szegedi 1023	Shoot, g	0.005a	0.018c	0.019c	0.010b	0.014b	0.013b	0.012b	0.014b	0.006b	0.005a
	IR%	0.00	-260.0	-280.0	-100.0	-180.0	-160.00	-140.0	-180.0	-20.0	0.00
	TI	1.00	12.96	14.44	4.00	6.53	6.76	5.76	6.53	1.20	1.00
	Root, g	0.02b	0.06e	0.05d	0.05d	0.05d	0.05d	0.04c	0.04c	0.01a	0.02b
	IR%	0.00	-47.29	-16.67	-15.83	-50.00	-40.21	-20.42	-9.17	50.83	93.75
	TI	1.00	4.41	2.77	2.73	4.58	4.00	2.95	2.42	0.49	0.06
	Seedling, g	0.03b	0.08f	0.07e	0.06d	0.06d	0.06d	0.06d	0.05d	0.02c	0.03a
	IR%	0.00	-35.50	-32.14	-27.88	-21.39	-17.13	-4.48	2.58	52.52	96.64
	TI	1.00	3.87	3.68	3.44	3.10	2.89	2.30	2.00	0.47	0.03
AS17P	Shoot, g	0.01a	0.02b	0.01a	0.02b	0.01a	0.01a	0.01a	0.02b	0.01a	0.01a
	IR%	0.00	-100.0	0.00	-100.0	0.00	0.00	0.00	-100.0	0.0	0.0
	TI	1.00	4.00	1.00	4.00	1.00	1.00	1.00	4.00	1.00	1.00

Table 2. Continued

	Root, g	0.05a	0.08d	0.09e	0.08d	0.08d	0.07c	0.06b	0.06b	0.06b	0.05a
	IR%	0.00	-60.00	-80.00	-60.00	-60.00	-40.00	-20.00	-20.00	-20.00	0.00
	TI	1.00	2.56	3.24	2.56	2.13	1.96	1.44	1.20	1.20	1.00
	Seedling, g	0.06a	0.10e	0.10e	0.09d	0.09d	0.08c	0.08c	0.08c	0.07b	0.06a
	IR%	0.00	-66.67	-66.67	-50.00	-50.00	-33.33	-33.33	-33.33	-16.67	0.00
	TI	1.00	2.78	2.78	2.25	1.93	1.78	1.78	1.52	1.17	1.00
GL15A	Shoot, g	0.04d	0.01b	0.02c	0.01b	0.01b	0.01b	0.01b	0.01b	0.01b	0.00b
	IR%	0.00	75.00	50.00	75.00	75.00	75.00	75.00	75.00	75.00	100.00
	TI	1.00	0.25	1.00	0.25	0.25	0.25	0.25	0.25	0.25	-
	Root, g	0.12g	0.07f	0.06e	0.05d	0.05d	0.05d	0.04c	0.04c	0.03b	0.00a
	IR%	0.00	41.67	50.00	58.33	58.33	58.33	66.67	66.67	75.00	100.00
	TI	1.00	1.36	1.00	0.69	0.69	0.69	0.44	0.44	0.25	-
	Seedling, g	0.16g	0.08f	0.08f	0.07e	0.06d	0.06d	0.05c	0.05c	0.03b	0.00a
	IR%	0.00	50.00	50.00	56.25	62.50	62.50	68.75	68.75	81.25	100.00
	TI	1.00	1.33	1.33	1.02	0.75	0.75	0.52	0.52	0.19	-
G16V	Shoot, g	0.01a	0.02b	0.01a	0.02b	0.02b	0.01a	0.01a	0.01a	0.01a	0.01a
	IR%	0.00	-100.00	0.00	-100.00	-100.00	0.00	0.00	0.00	0.00	0.00
	TI	1.00	4.00	1.00	4.00	4.00	1.00	1.00	1.00	1.00	1.00
	Root, g	0.09d	0.12e	0.09d	0.07c	0.07c	0.06b	0.06b	0.06b	0.05a	0.05a
	IR%	0.00	-33.33	0.00	22.22	22.22	33.33	33.33	33.33	44.44	44.44
	TI	1.00	3.20	1.80	1.09	1.09	0.80	0.80	0.80	1.00	0.56
	Seedling, g	0.10d	0.14f	0.11e	0.09c	0.09c	0.07b	0.07b	0.07b	0.06a	0.06a
	IR%	0.00	-40.00	-10.00	10.00	10.00	30.00	30.00	30.00	40.00	40.00
	TI	1.00	3.27	2.02	1.35	1.35	0.82	0.82	0.82	1.00	0.60
MI16N	Shoot, g	0.01a	0.01a	0.01a	0.01a	0.01a	0.01a	0.01a	0.01a	0.01a	0.01a
	IR%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	TI	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Root, g	0.07f	0.06d	0.08e	0.06d	0.06d	0.05c	0.04b	0.04b	0.03a	0.04b
	IR%	0.00	14.29	-14.29	14.29	14.29	28.57	42.86	42.86	57.14	42.86
	TI	1.00	1.29	2.29	1.29	1.71	0.89	0.57	0.76	0.75	0.57
	Seedling, g	0.08d	0.07c	0.09e	0.08d	0.07c	0.05b	0.05b	0.05b	0.04a	0.04a
	IR%	0.00	12.50	-12.50	0.00	12.50	37.50	37.50	37.50	50.00	37.50
	TI	1.00	1.23	2.03	1.60	1.53	0.63	0.63	0.78	0.80	0.63

Legend: IR% - Percent inhibition. Positive values "+" show inhibition effect. while negative "-" values show stimulation effect; TI – Tolerance index; Different letters in columns indicate significant differences by the LSD test at p=0.05 probability

Table 3. Main effects of the factors tested

Indicators	Causes of variation	Intercept	Factor A - genotypes	Factor B – concentracion	Interaction AxB	Error	Total
Root length	Degrees of freedom	1	4	9	36	5920	5969
	Sum of squares	156937.4	9755.8	15258.9	4342	44692.6	73994.5
	Mean square	156937.4	2439	1695.4	120.6	7.5	
	Influence of factors, η2		13.2	20.6	5.9		
Shoot lenght	Degrees of freedom	1	4	9	36	5914	5963
	Sum of squares	204287.8	9227.5	18480.8	4580.5	64366	96753.5
	Mean square	204287.8	2306.9	2053.4	127.2	10.9	
	Influence of factors, η2		9.5	19.1	4.7		
Seedling length	Degrees of freedom	1	4	9	36	5914	5963
	Sum of squares	718366	36079.2	66809.1	11712.7	197274.3	311403.2
	Mean square	718366	9019.8	7423.2	325.4	33.4	
	Influence of factors, η2		11.6	21.5	3.8		
Root weight	Degrees of freedom	1	4	9	36	250	299
	Sum of squares	0.044068	0.000425	0.002031	0.004932	0.002998	0.010388
	Mean square	0.044068	0.000106	0.000226	0.00022	0.000013	
	Influence of factors, η2		4.1	19.6	47.5		
Shoot weight	Degrees of freedom	1	4	9	36	250	299
	Sum of squares	0.961068	0.041272	0.063292	0.040568	0.01405	0.159132
	Mean square	0.961068	0.010818	0.007255	0.001405	0.000012	
	Influence of factors, η2		25.9	39.8	25.5		
Seedling weight	Degrees of freedom	1	4	9	36	250	299
	Sum of squares	1.4283	0.03676	0.09026	0.08058	0.01705	0.2247
	Mean square	1.4283	0.01194	0.01067	0.00225	0.00014	
	Influence of factors, η2		16.4	40.2	35.9		

LSD at the 0.05 probability level

Conclusion

The genetic potential of five sorghum genotypes (one variety and four local variety) was evaluated through artificially created water stress by PEG of molecular weight 20000 in laboratory conditions to drought tolerance in the early growth stages and development BBCH 09-10.

Osmotic stress induced by the addition of PEG inhibits root growth and shoot IR% from 15.03 to 72.29% at higher applied concentrations (from 10.0 to 20.0%). Lower applied concentrations (1.25 to 7.5%) had a stimulating effect IR% from 11.71 to 135.77%, according to control treatments for all tested genotypes.

There was a specific variety reaction with regard to the effect of PEG on seedling growth (cm) and formation of fresh weight on seedlings (g) in the tested genotypes *Sorghum vulgare* var. *technicum* (Körn.).

It was found that with relatively good tolerance to osmotic stress it is possible to determine Szegedi 1023 variety and the AS17P local variety (TI average varied from 2.28 to 2.55). With low coefficients of tolerance, i.e. the high sensitivity of drought in the early growth stages of development (BBCH – 09-10) were the G16V and MI16N local varieties (TI average varied from 1.29 to 1.36) while GL15A local variety occupies an intermediate position – TI – 1.62.

In the Szegedi 1023 variety and AS17P local variety, unlike the growth of the seedlings, the formed fresh biomass in g for one seedlings increases with the increasing level of PEG 20000 treatments, except for the highest applied concentrations (17.5 and 20.0%), while the GL15A, G16V and MI16N local varieties formed fresh biomass of the roots, shoots and seedlings decreased IR% varied from 22:22 to 100.0% and the differences were statistically significant (p = 0.05). which can be explained by their sensitivity to drought in the early growth stages of development (BBCH-09-10).

The G16V and MI16N local varieties found superior and might be productive in further breeding programmes for drought tolerance. Selection can be made on the basis of these characters at early growth stage to screen a broom corn genotypes for drought stress or water deficit.

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EFFECT OF MINERAL AND ORGANIC FERTILIZATION ON THE PRODUCTIVITY OF STEVIA (*Stevia rebaudiana* B.)

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Abstract

Enchev S., A. Mehmed, G. Kikidonov, 2018. Effect of mineral and organic fertilization on the production of Stevia (*Stevia rebauduana* B.), *Bulg. J. Agric. Sci.*, 24 (Suppl. 2): 100-103

An experiment has been made in the fields of the Agricultural Institute – Shumen during 2013-2015 to study the effect of three organic and mineral products: Bioactive (100ml/da), Humustim (40 ml/da) and mineral nitrogen (20 kg/da), on the productive qualities of stevia (Stevia rebaudiana B.) For that aim I year seedlings were used. The parameters fresh and dry mass of a single plant, and fresh and dry mass yield from one da, were measured.

It was established, that the highest fresh mass yield from a single plant is formed by the variant, treated with mineral Nitrogen (20 kg/da) – the average yield of fresh mass is with 90 g (75.8%) higher than that of the untreated control. The tested organic and mineral products have positive influence on the dry mass yield index for the three years of testing.

Key words: stevia, organic and mineral products, productivity, fresh mass, dry mass

Introduction

Stevia (Stevia rebaudiana Bertoni) is a perennial bushy cross-pollinating plant, with natural area of distribution the humid regions of Paraguay, Columbia and Brasil. Numerous researches for finding new natural, non-caloric sweeteners indicate that stevia is an excellent substituent of sugar. Its sweetness is due to the diterpenic glucosides Rebaudiside A and Stevioside, which are up to 300 times sweeter than sugar (Geuns, 2004).

The adaptability of the plant allows its introduction in different countries (Sumida, 1968; Lewis, 1992). In Bulgaria stevia is cultivated since 1984 in the former Sugar beet Institute – Shumen (Kikindonov, 2013). The positive effects of different biostimulators for increasing the productive capacities of stevia is proved in the reports of some researchers (Das et al., 2007; Mamta et al., 2010; Lei and Yan, 2011; Špicnagel et al, 2011; Jing Wu et al., 2013; Inugraha et al., 2014). There are also data for preparations, increasing the stevia's resistance to different stress factors, low and high temperatures, diseases (Jain et al, 2009; Ren, et al, 2011; Raziye et al., 2015; Mehdi et al, 2017). The content of the active components of the plant – stevioside and rebaudiside A depends in a great extent on the applied agro-technology and the use of organic-mineral products.

The aim of our study is to establish the efficiency of some organic-mineral products' application on the productivity of stevia.

Material and Methods

The experiment has been carried out on the experimental fields of Agricultural Institute – Shumen, during the period 2013-2015. It has been studied the influence of the organic-mineral products Bioactiv (100 ml/da), Humustim (40 ml/da) and mineral Nitrogen (20 kg/da), applied once in the stage of 2-4 branch of the plants. The seedlings used are of the stevia

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variety Stela, bred in Agricultural Institute – Shumen, produced in the Tissue Cultures Lab of the Institute and adapted for field conditions in a green house. The forerunner crop is sugar beet. The soil type is carbonate black soil with 3.3% humus, very high content of $CaCO_3$, and slightly alkaline reaction of the soil solution (pH 7.4-7.8). The planting is in the beginning of May, when the soil temperature is over 100C. Each experimental plot has been planted with 20 plants in 4 repetitions. The treatment has been done with a manual sprayer. Three hillings have been made by hand during the vegetation, and the soils humidity has been kept at 70-75% of the marginal soils humidity by drip irrigation.

The following variants were tested:

1. Treated with organic fertilizers

* Bioactiv – 100% activated epsomit ($MgSO_4$ x 7 H_2O).

* Humustim – 41.05% ashes and mineral substances and 58.95% organic matter, including humine acids, fulvic acids, potassium, nitrogen, P, Ca, Mg.

* Mineral Nitrogen – Amonium nitrate (NH_4NO_3)

2. Control variant – non-treated.

The studied parameters are fresh and dry mass of a single plant, and fresh and dry mass yield per da. Dispersion analysis (Lidanski, 1988) was used for determination of the statistical significance of the differences between the tested variants.

Results and Discussion

The values of the measured weights of fresh and dry mass of a single plant during the period of study are presented on Figures 1 and 2. The tested organic-mineral products have affected positively the harvested fresh and dry mass of a single plant. The biostimulators increase the adaptive capacities of stevia plants in conditions of hot summer vegetation. The comparatively low yields in 2013 are due to the shorten period of adaptation in the green house and the development of fungal diseases. The increase of the fresh mass yield of a plant by the application of organic-mineral products in comparison with the non-treated variant is 18-21%.

The most important indication of the efficiency of a certain agro-technology measure is its effect on the productivity. All the tested applications of organic-mineral products bring to increase of the fresh mass yield per decar, compared to the yield of the non-treated control (Table 1). In 2013, when the time for adaptation in green house was not enough the stevia plants on the field did not react so categorically to the organic-mineral products treatments. The fresh mass yields of the treated variants do not differ substantially from the control variant. But for the next years of tests, in optimum adaptation conditions, the fresh mass yield of all the variants treated with organic-mineral products is higher than the yield of the non-treated control variant. This exceedance is proved statistically for the fresh mass yields from the variants treated with Bioactiv and mineral Nitrogen in 2014 and 2015, as well as for their average values for the entire period of our study. The yields of fresh mass per da of harvested area reach values of 1026.6-1063.2 kg/da for the variants treated with Bioactiv, and 1101.9-1119.2 kg/da for the variants fertilized with mineral Nitrogen. It is obvious, that the highest is the positive effect on the fresh mass yield from one year old seedlings of the treatment with mineral Nitrogen – the plants treated with this mineral fertilizer gave 26.0% higher fresh mash mass yield than the non-treated control of stevia.

The dry mass yield is a significant parameter for the economical value of stevia, because this is the product, which is realized on the market. From the data given on Table 2 we could accept as normal the lower values of dry mass yield in 2013 (from 82.5 to 114.5 kg/da). The interesting fact noted here is, that the strongest is the effect of the treatment with Humustim – the dry mass yield of the treated with Humustim variant is proved higher than the yield of the non-treated control and the variant treated with Bioactive. Similar results were obtained by Vasileva (2015) in pea and vetch. In the next year of tests the yield of dry mass for all the variants is much, almost three times higher than in the previous year (234.6 - 289.3 kg/da). All the variants treated with an organic-mineral product gave proven higher dry mass yield than that of the non-treated control. In 2015 the variant fertilized with mineral Nitrogen gave the highest yield of dry mass – 318.6 kg/ha. And this yield exceeds the yield of the non-treated control, and that of the variants, treated with Bioactiv and Humustim. The average values of the dry mass yield for the three years of testing are 195.0 to 237.7 kg/da. The treatment with organic-mineral products proves to be efficient for the higher dry mass yields of the relevant variants. The variants treated with Bioactiv, and especially the one fertilized with mineral Nitrogen, gave proven higher dry mass yields.

Table 1. Fresh mass yield (kg/da) of one-year seedlings of stevia, variety Stela, treated with organic-mineral products

Variant	2013	2014	2015	Mean	Relative %
Control	577.1	858.6	873.2	769.6	100.0
Bioactive	697.0	1063.2 +	1026.6 +	928.9 +	120.7 +
Humustim	679.3	918.6	942.6	846.8	110.0
Mineral nitrogen	688.2	1119.2 +	1101.9 +	969.8 +	126.0 +
GD 5 %				134.9	19.0

Table. 2. Dry mass yield (kg/da) of one-year seedlings of stevia, variety Stela, treated with organic-mineral products

Variant	2013	2014	2015	Mean	Relative %
Control	82.5	234.6	268.0	195.0	100.0
Bioactive	89.7	278.6 +	273.3	213.9 +	109.7
Humustim	114.5 +	260.6 +	255.3	210.1	107.7
Mineral nitrogen	105.2	289.3 +	318.6 +	237.7 +	121.9 +
GD 5 %				16.2	18.6

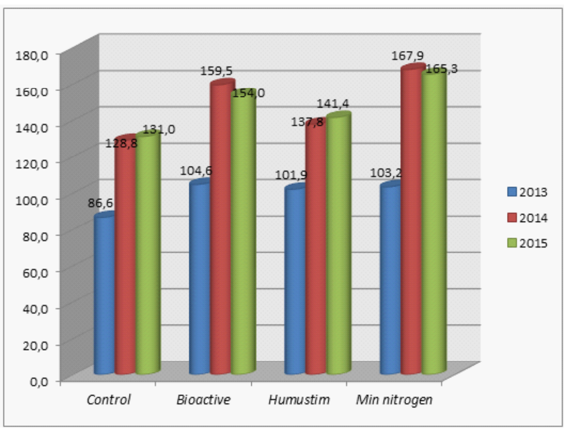

Fig. 1. Fresh mass of a plant, g

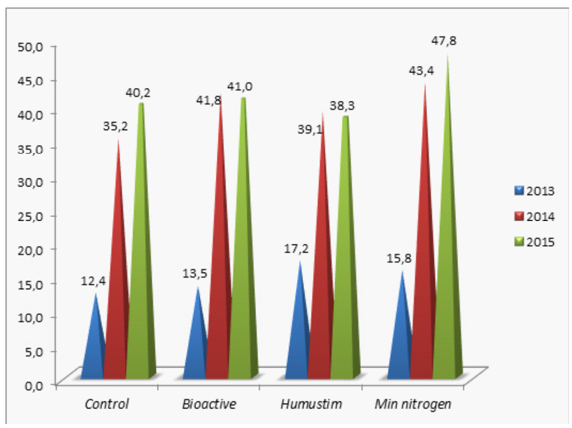

Fig. 2. Dry mass of a plant, g

Conclusions

The treatments of one-year old seedlings of stevia with the organic-mineral products Bioactiv, Humustim and mineral Nitrogen have positive effect on the development of stevia plants, resulting in much higher biomass yields.

The fertilization with mineral Nitrogen and the treatment with Bioactive increase significantly the fresh and dry mass yields of stevia plants.

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SELF-SEEDING OF SUBTERRANEAN CLOVER IN DEGRADED BIRDSFOOT TREFOIL SEED PRODUCTION STANDS

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Abstract

Vasileva V. and S. Enchev, 2018. Self-seeding of subterranean clover in degraded birdsfoot trefoil seed production stands, *Bulg. J. Agric. Sci.*, 24 (Suppl. 2):104-108

In connection with the dilution of seed production birdsfoot trefoil stands the possibility of under-sowing them with self-seeding crops was studied. The trial was carried out on the experimental field of Institute of Forage Crops, Pleven, Bulgaria with birdsfoot trefoil (*Lotus corniculatus* L.) Targovishte 1 variety. In autumn after the fourth year of use of the stands (for seed production direction) an under-sowing with three subterranean clover subspecies was done as follows: T*rifolium subterraneum ssp. brachycalicinum (Antas variety), Trifolium subterraneum ssp. yaninicum (Trikkala variety)* and *Trifolium subterraneum ssp. subterraneum (Denmark variety)*. The capacity of self-sowing of subterranean clover in birdsfoot trefoil stands was assessed. It was found that subterranean clover effectively used the autumn-winter soil moisture, formed a sufficient number of seeds for self-seeding and germinated plants occupied the sites of the dropped birdsfoot trefoil plants. *Trifolium subterraneum ssp. brachycalicinum* showed the best self-seeding ability. Thus, after under-sowing with subterranean clover, degraded seed production birdsfoot trefoil stands could be used for forage.

Key words: birdsfoot trefoil, degraded seed production stands, self-seeding, subterranean clover

Introduction

The productivity of the perennial leguminous forage crops which have an important place in the sustainable agriculture systems decreased with the age of sowing. The swards are going to dilute and the free spaces are occupied by weeds (Vasilev, 2004; Sulas et al., 2006; Petkova et al., 2015).

Birdsfoot trefoil is a valuable forage crops, suitable for cultivation, both alone and in mixtures (Vuckovic, 2004; Chourkova, 2011; Zekić et al., 2012).

In relation to more effectively usage of the resources self-seeding crops are becoming more important because of the possibility of longer-lasting presence in the swards (Bartholomew, 2014). In one season they could provide productivity of forage as well seeds for propagation (Carneiro, 1999; Naydenova et al., 2013).

Subterranean clover (*Trifolium subterraneum* L.) is speices with self-seeding ability (Yakimova and Yancheva, 1986; Piano et al., 1996; Howieson et al., 2008). It has a low widespread habitat and occupies open spaces between other plants from the lowest floor of the sward, as well coexists well with perennial grasses and legumes (McCaskill et al., 2016). It grows up early in the spring and forms a dense sward (Porqueddu et al., 2003). Reproductive organs are formed in early May and the seeds ripen before the end of the spring in hedgehog-shaped heads that remain on the soil surface (Frame et al., 1998). Substantial part of the formed seeds is hard and germinates after two-three years. This biological specificity turns the

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superficial soil layer into an original seed bank (Pecetti and Piano, 1994). The precipitations during the late summer contribute to emergence of new self-sown plants (Vasilev, 2006).

Subterranean clover is relatively new crop for Bulgaria and is found in open dry grasslands in the plains and lowlands (Assyov et al., 2012). The studies with subterranean clover in recent years showed that it has practical applicability for the climatic conditions of Bulgaria (Vasileva and Vasilev, 2017; Naydenova and Vasileva, 2015, 2016; Kirilov and Vasileva, 2016; Vasileva et al., 2016). Some of studies involved the use of the species for direct under sowing of degraded perennial seed production stands and the self-seeding capacity of subclover in these stands. Such studies were done for alfalfa and white clover where the best self-seeding capacity showed *Trifolium subterraneum ssp. brachycalicinum* (Vasileva, 2015, 2017).

In this study we aim to investigate the self-seeding capacity of three subclover subspecies through under sowing of birdsfoot trefoil seed production stands.

Materials and Methods

The experimental work was carried out on the experimental field of Institute of Forage Crops, Pleven, Bulgaria on slight leached chernozem soil subtype without irrigation. Seed production birdsfoot trefoil stands (Targovishte 1 variety) was sown in 2007 and cared according with accepted technology. Long plots method was used, plot size of 5 m^2 and 4 replications of the treatments. During the autumn of fourth year from the use of the swards, across the rows, under sowing with three subterranean clover subspecies was performed, i.e. *Trifolium subterraneum ssp. brachycalicinum (Antas variety), Trifolium subterraneum ssp. yaninicum (Trikkala variety) and Trifolium subterraneum ssp. subterraneum (Denmark variety)*.

Under sowing was done with 400 germinated seeds/m2 and between rows spacing of 12 cm. Treatments were as follows: birdsfoot trefoil (without under sowing) – control; birdsfoot trefoil + *Trifolium subterraneum ssp. brachycalicinum*; birdsfoot trefoil + *Trifolium subterraneum ssp. yaninicum*; birdsfoot trefoil + *Trifolium subterraneum ssp. subterraneum*.

In the year after under sowing the number of germinated self-seeded subclover plants was recorded from 0.25 m^2 and equated to the number of m^2. They were done at the beginning of August and in early November, respectively in cotyledons, the first not true leaves and first true leaves stages. Total number of germinated self-seeded plants was calculated. Experimental data were processed statistically using a software product SPSS 2012.

Results and Discussion

Agrometeorological conditions particularly the amount and distribution of rainfall are important factor for self-seeding of subterranean clover. They could be defined as unfavourable during the period of study (Table 1). In the year after under sowing long dry period with extremely high temperatures occurs. Rainfall during the second half of the growing season (after mid-July) was below the average norm and not conducived to germination of self-seeded plants. Subterranean clover was found successfully self-seeded by mid to late growing season despite the unfavorable conditions.

The number of germinated self-seeded plants in the first true leaves stage was found to ranges within relatively narrow limits (Table 2) and was 28 plants/m^2 for *Trifolium subterraneum ssp. brachycalicinum*, 22 plants/m^2 for *Trifolium subterraneum ssp. yaninicum* and less, 15 plants/m^2 for *Trifolium subterraneum ssp. subterraneum*. Fewer number of germinated plants of *Trifolium subterraneum ssp. subterraneum* is related to the weaker competitiveness of this subspecies recognized by Lucas et al. (2015).

With the advancing of vegetation agrometeorological conditions were exeptionally bad. In September, the average daily temperature was high (22^{o}C) and rainfall lacked (0.0 l/m^2), which depress the development of subterranean clover. After the precipitations (50.2 l/m^2) fallen in the second ten days of October new self-seeded and germinated plants was recorded at the beginning of next month. The data are shown in Figure 1. The number of germinated plants in stage cotyledons for *Trifolium subterraneum ssp. yaninicum* (54 number of plants/m^2) was the highest, followed by *Trifolium subterraneum ssp. brachycalicinum* (46 number of plants/m^2) and *Trifolium subterraneum ssp. subterraneum* (38 number of plants/m^2). The number of germinated plants in first not true leaves stage varied from 10 to 25 number of plants/m^2 and in the first true leaves stage from 15 to 28 number of plants/m^2, respectively.

The total number of germinated self-seeded plants in early November was highest for Trifolium subterraneum ssp. brachycalicinum (99 number of plants/m^2), followed by *Trifolium subterraneum ssp. yaninicum* (83 number of plants/m^2) and *Trifolium subterraneum ssp. subterraneum* (63 number of plants/m^2). We found that T*rifolium subterraneum ssp. brachycalicinum* showed best potential for self-seeding when was used for under sowing of degraded birdsfoot trefoil seed production stands. We assume that this is probably due to the higher germination of seeds of *Trifolium subterraneum ssp. brachycalicinum*. In the Mediterranean area high germi nation rate and low hard seeds of subterranean clover is not seen as a good characteristics. Species and varieties with a higher percentage of hard seeds are appreciated due to the possibility of their later activation and greater reliability of the stands (Pecetti and Piano, 1994; Carneiro, 1999; Lemus, 2013). For areas with a Mediterranean climate the selection of subterranean clover for hardiness of the seeds is leading, which allows its gradual emergence and thus self-supporting in the stands for several years, although it is an annual type (Loi et al., 2005).

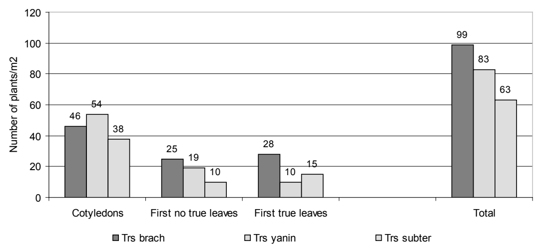

Fig. 1. Number of germinated self-seeded subterranean plants in under sowed degraded seed production birdsfoot trefoil stands (early November first year after under sowing) (SE=0.05) - cotyledons stage (4.6); first no true leaves stage (4.3); first true leaves stage (5.3), total (10.4)

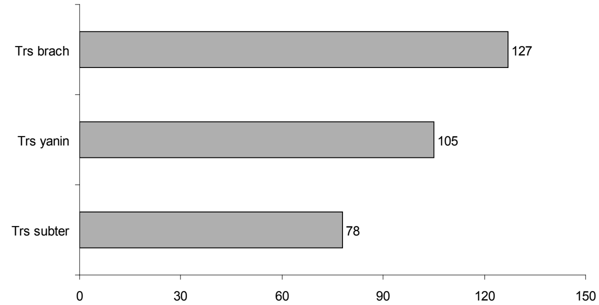

Fig. 2. Total number of germinated self-seeded subterranean plants in under sowed degraded seed production birdsfoot trefoil stands in the year after under sowing (SE=0.05, 14.1)

For the conditions of the experiment *Trifolium subterraneum ssp. subterraneum* showed weaker ability for self-seeding and germination in degraded stands. Analogous results we received when alfalfa seed production stands were under sowed with this subterranean clover subspecies (Vasileva, 2015).

The trend *Trifolium subterraneum ssp. brachycalicinum* to show the best self-seeding ability and emergence was confirmed by the total number of germinated self-seeded plants, i.e. *Trifolium subterraneum ssp. brachycalicinum* (127 number of plants/m^2) vs. *Trifolium subterraneum ssp. yaninicum* (105 number of plants/m^2) and *Trifolium subterraneum ssp. subterraneum* (78 number of plants m^2) (Figure 2). After botanical

Table 1. Agrometeorological conditions in the first year after under sowing

Months	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Av/sum
Temp, ^oC	-1.0	0.2	6.1	11.4	16.8	21.4	23.5	23.6	22.0	11.1	7.6	3.9	12.2
Rains, l/m^2	32.8	27.2	25.7	28.2	79.8	33.6	50.2	41.3	0.0	50.2	0.4	28.6	398.0

Table 2. Number of germinated self-seeded subterranean plants in under sowed degraded seed production birdsfoot trefoil stands (early August, first year after under sowing)

Treatments	Number of self-seeded plants/m^2
Birdsfoot trefoil + Tr. subterraneum ssp. brachycalicinum	28±3.0
Birdsfoot trefoil + Tr. subterraneum ssp. yaninicum	22±2.1
Birdsfoot trefoil + Tr. subterraneum ssp. subterraneum	15±1.5
Average	22±0.9
SE (P=0.05)	3.7

±, STDEV

composition analyses high percent of participation of this subspecies was found (Vasileva, 2017).

Experimental data showed that subterranean clover used effectively autumn-winter soil moisture and formed seeds successfully. In the conditions of our study subterranean clover formed sufficient number of seeds which in case of favorable conditions are self-seeded. This confirms the high ecological plasticity of the species found by other authors (Pecetti and Piano, 1994).

So, due to the self-seeding capacity subterranean clover could be successfully used for under sowing of degraded seed production stands. The degradation may result from various factors - short duration of the species, less adaptability to over use, adverse soil and climatic conditions, ect. The germinated plants occupied the sites of the dropped plants. Under sowing with subterranean clover prolongs the durability of the stands. In addition, self-seeding ability of subterranean clover and possibility degraded perennial stands be under sowed with subterranean clover allows more efficient use of resources – one of the challenges of modern sustainable agriculture.

Conclusions

When birdsfoot trefoil seed production stands were under sowed with subclover, Trifolium subterraneum ssp. brachycalicinum showed the best potential for self-seeding and the total number of germinated self-seeded plants was found 127 number of plants/m^2 vs. *Trifolium subterraneum ssp. yaninicum* (105 number of plants/m^2) and *Trifolium subterraneum ssp. subterraneum* (78 number of plants/m^2). The self-seeding ability allows subterranean clover be used for under sowing of degraded seed production birdsfoot trefoil stands, thus to prolonge their durability and the stands could be used for forage.

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THE EFFECT OF GROWTH RATE IN PURE-BRED AND CROSS-BRED BOARS ON THEIR SEMEN CHARACTERISTICS

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Abstract

Szostak, B., Ł. Przykaza and A. Apostolov,2018. The effect of growth rate in pure-bred and cross-bred boars on their semen characteristics, Bulg. J. Agric. Sci., 24 (Suppl.2): 109-114

The aim of the study was to examine the effect of daily growth rate on the semen characteristics of purebred boars (Polish Large White and Polish Landrace) and crossbred boars (Duroc x Pietrain and Pietrain x Duroc). The research was carried out on 29 purebred boars (2024 ejaculates) and 22 crossbred boars (1156 ejaculates). The following characteristics were analysed: ejaculate volume, sperm concentration, percentage and number of live spermatozoa, and number of insemination doses obtained per ejaculate. A high rate of growth in the boars was found to have no negative effect on the quality of their ejaculates. In both purebred and crossbred boars, the largest ejaculate volume, the highest percentage of live sperm and the most insemination doses per ejaculate were obtained for the boars with the highest daily weight gain (over 851 g).

The semen of crossbred boars with the same intensity of growth as purebred boars (801-850 g and > 851 g) was characterized by significantly greater ejaculate volume, a higher sperm concentration, a higher live sperm count in the ejaculate, and a higher average number of insemination doses prepared from one ejaculate. The results indicate that the level of the selection index currently used in the breeding of boars has no negative impact on the characteristics of their ejaculates.

Key words: growth rate, boars, ejaculates

Introduction

The dynamic growth and increasing importance of artificial insemination in pig breeding is linked to the measurable benefits of its use. Artificial insemination can significantly accelerate genetic gain and reduce the risk of spreading infectious diseases. It also substantially reduces breeding costs and enables improvement of the spatial organization of pig breeding. To obtain the desired results from the use of insemination, particular attention should be paid to the proper selection and proper use of boars kept at sow insemination stations involved in the production and distribution of semen. Apart from proper development of the reproductive organs, sufficient libido and high-quality semen, males should also have very good fattening and carcass performance. In work on the improvement of pigs, special attention is paid to growth rate and lean meat content. However, intensive selection in this direction not only has benefits, but may have negative effects as well, manifested as poorer development of the reproductive, digestive and musculoskeletal systems, an increase in cardiac insufficiency, and lower resistance to stress (Radhamer 1993; Kawęcka 2002). Studies by many authors have shown significant negative correlations between some fattening and reproductive characteristics of boars (Weeb et al. 1998; Milewska 2007; Udała et al. 2015). Fiałkowska et al. (2000), in a study of the influence of the growth parameters of Duroc

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boars during the rearing period on their semen characteristics, concluded that this factor had no effect on semen traits. Many authors express the view that the correlations between growth rate and semen characteristics in boars are generally low and insignificant (Dziadek and Kamyczek 1991; Oh, et al. 2006). The inconsistent results published in the scientific literature by different authors may result from genetic differences in the animals or the scale of the factors studied. Boars of different breeds or crossbreds may produce ejaculates that differ significantly in the physical characteristics of the semen (Bertoni et al. 2002; Kondracki 2003; Wysokińska and Kondracki 2005]. Crossbred boars generally produce ejaculates with more favourable characteristics than purebred boars.

In view of the lack of unambiguous and consistent opinions on the possible effect of the growth rate of boars on their breeding value, the aim of the research was to determine the effect of weight gains in purebred boars (Polish Large White and Polish Landrace) and crossbred boars (Duroc x Pietrain and Pietrain x Duroc) during the period rearing on their semen parameters.

Materials and Methods

The present study aims to assess the influence of the daily weight gain in pure-bred (Polish Large White, Polish Landrace – pulled in one group) and cross-bred (Pietrain x Duroc, Duroc x Pietrain – pulled in one group) boars on their semen characteristics in conditions of the insemination station. Additionally, the differences of semen features between pure- and cross-bred boars were evaluated. The material for the study consisted of 29 pure-bred (2024 ejaculates) and 22 cross-bred (1156 ejaculates) boars used at the Sow Insemination Station in Białka (Poland). On the basis of the results of performance testing carried out on the boars' 180th day of life, taken from breeding documentation, the boars were divided into three groups according to the daily weight gain (I – 700 – 800 g, II – 801 – 850 g, III – > 851 g). During the growth period the boars were kept in groups and fed complete mixed rations according to Nutrient Requirements of Pigs (1993). From the start of their exploitation for breeding the boars were kept in identical environmental conditions, in individual pens with litter.

Semen was collected from the boars by the manual method, using a phantom. A detailed quantitative and qualitative evaluation of the ejaculates was performed using common methods and based on the following characteristics: volume, sperm concentration, percentage of sperm with progressive movement, number of sperm with progressive movement per ejaculate and per insemination dose, number of insemination doses obtained per ejaculate. Statistical analyses were performed using Statistica software. All results were expressed as the means ± standard error of the mean (SEM). The influence of the growth rate was evaluated using a one-way ANOVA, followed by the Tukey's post hoc test. The differences between pure- and cross-bred boars were analyzed with unpaired t-test. A value of $P < 0.05$ was considered to be statistically significant.

Results and Discussion

Tables 1 and 2 show the values of semen parameters measured in pure-bred and hybrid boars depending on the daily weight gain. Our studies have indicated that the volume of semen of the both pure- and cross-bred boars was significantly higher in the groups II (268.458 ± 1.541 ml, $P < 0.01$; 287.034 ± 2.408 ml, $P < 0.001$) and III (270.258 ± 3.992 ml, $P < 0.05$; 294.855 ± 4.817 ml, $P < 0.001$) than in the group I (255.090 ± 3.136 ml; 260.673 ± 4.133 ml). Similarly, the percentage of sperm with progressive movement, number of sperm with progressive movement per ejaculate and number of insemination doses obtained per ejaculate significantly ($P < 0.05$ – $P < 0.001$) increased simultaneously with the range of the daily weight gain. The concentration of spermatozoa was significantly higher in groups II and III only in cross-bred boars ($P < 0.05$). However, there were no significant differences of number of spermatozoa per insemination dose between groups in both breeds.

Both the purebred boars (Polish Large White and Polish Landrace) and crossbreds (Duroc x Pietrain and Pietrain x Duroc) used in the study had very good daily weight gains, ranging from 751 to 851 grams. Analysis of features characterizing the ejaculates of boars with different growth rates showed that boars with daily gains of over 851 g produced ejaculates with the most favourable parameters. These results confirm our earlier research (Szostak et al. 2016) carried out on Duroc x Pietrain x Pietrain x Duroc crossbreds, where significantly the largest average ejaculate volume was obtained for boars with daily weight gains in the range of 851-900 g. Falkenberg et al. (1989) reported a stronger libido in faster growing boars, which according to the authors may indicate earlier sexual maturity in faster growing individuals. In a study on selected fattening and meat performance traits and semen quality in young purebred and crossbred boars, Udala et al. (2015) found that ejaculate volume and total ejaculate sperm count were positively correlated with daily weight gains, while sperm concentration and growth rate were negatively correlated. A significant effect of daily weight gains on ejaculate volume in boars of the breeds Swedish Landrace, Large White and Duroc has also been reported by Savić et al. (2014). According to the authors, selection aimed at increasing carcass meat content and weight gains in growing boars has no negative impact on their future reproductive capacity. Despite the great economic importance of production traits (growth and carcass quality), they should not be the only selection criterion; assessment of the breeding value of boars should be based on evaluation of their libido and ejaculate characteristics.

A study by Petz (2004) found negligible correlations between semen characteristics and the performance parameters of young boars, which according to the author means that meatiness and growth rate have no negative effect or an insignificant effect on subsequent reproductive results. The author states that one of the reasons for the insignificant correlations between these traits may be the measurement of phenotypic values in different age groups of animals. A study by Wolf (2009) found that the genetic correlations between the growth rate and meatiness of young boars and ejaculate volume and sperm concentration were close to zero. Oh et al. (2006), on the other hand, reported a low, negative phenotypic correlation between average daily weight gains in young boars and the volume of their ejaculates ($r = -0.02$) and a low but positive relationship between daily gains and the ejaculate sperm concentration.

Table 1. The influence of the daily weight gain on the semen characteristics of pure-bred boars

Traits of semen	Daily weight gain [g]						Significance of differences
	751 - 800 (I)		801 - 850 (II)		> 851 (III)		
	X	SEM	X	SEM	X	SEM	
Ejaculate volume [ml]	255.090	3.136	268.458	1.541	270.258	3.992	I-II**; I-III*
Concentration of spermatozoa [thous./mm3]	252.848	2.591	258.166	1.325	257.271	3.537	n.s.
Percent of live spermatozoa [%]	76.254	0.303	76.704	0.127	79.208	0.175	I-III***; II-III***
Number of live spermatozoa [bln]	50.219	0.970	54.118	0.499	56.720	1.415	I-II*; I-III***
Number of spermatozoa per insemination dose [bln]	2.567	0.010	2.568	0.003	2.554	0.007	n.s.
Number of insemination doses	19.713	0.399	21.218	0.201	22.321	0.566	I-II*; I-III***

*P < 0.05, **P < 0.01, ***P < 0.001, n.s. – no significances

Table 2. The influence of the daily weight gain on the semen characteristics of cross-bred boars

Traits of semen	Daily weight gain [g]						Significance of differences
	751 - 800 (I)		801 - 850 (II)		> 851 (III)		
	X	SEM	X	SEM	X	SEM	
Ejaculate volume [ml]	260.673	4.133	287.034	2.408	294.855	4.817	I-II***; I-III***
Concentration of spermatozoa [thous./mm3]	251.623	4.333	270.320	1.830	271.590	4.686	I-II**; I-III**
Percent of live spermatozoa [%]	78.141	0.294	76.684	0.175	85.202	5.202	I-III*; II-III**
Number of live spermatozoa [bln]	53.041	1.538	59.854	0.719	65.869	1.860	I-II**; I-III***; II-III*
Number of spermatozoa per insemination dose [bln]	2.565	0.009	2.547	0.005	2.539	0.008	n.s.
Number of insemination doses	20.889	0.638	23.680	0.292	26.179	0.744	I-II**; I-III***; II-III*

*P < 0.05, **P < 0.01, ***P < 0.001, n.s. – no significances

Table 3 shows the levels of significance of differences in semen characteristics between pure-bred and cross-bred boars within the ranges of the daily weight gain. We have observed significant differences of studied semen parameters between pure- and cross-bred boars. The cross-bred boars have significantly higher: volume of semen (P < 0.001), concentration of spermatozoa (P < 0.001), number of sperm with progressive movement per ejaculate (P < 0.001) and number of insemination doses obtained per ejaculate (P < 0.001), observed in the range II (801 – 850 g) of daily weight gain. Only number of spermatozoa per insemination dose was significantly higher in pure-bred boars (P < 0.001). In the range III (> 851 g) were similar differences with exception of the percent of live spermatozoa and the number of spermatozoa per insemination dose – that did not differ. In the range I (700 – 800 g) only percent of live spermatozoa was significantly higher in cross-bred boars (P < 0.001), there were no differences in the other parameters in this range of the daily weight gain.

In general, the manifestation and variability of the ejaculate characteristics of boars during their use for breeding are influenced by a variety of genetic (Bertani et al. 2002, Szostak 2003, Borucka-Jastrzębska et al., 2008) and non-genetic factors (Knecht et al. 2013, Szostak et al. 2015). In our study, differences were noted between purebred boars (Polish Large White and Polish Landrace) and crossbreds (Duroc x Pietrain and Pietrain x Duroc). The crossbreds were characterized by better semen characteristics. Our results are in agreement with those reported by other authors (Muczyńska et al., 2010, Udała et al., 2015). The superior semen parameters of crossbred boars are due to heterosis, which occurs when pigs of different breeds are crossbred. Particularly high ejaculate parameters were noted for the crossbred boars with daily weight gains of 801-850 g (group II) and above 851 g (group III). Our results confirm that a high rate of daily growth does not negatively affect the quantity and quality of boar semen, which may indicate that faster growing boars attain sexual maturity and stabilization of the spermatogenesis process earlier.

Table 3. The differences between pure- and cross-bread boars in semen characteristics within the ranges of the daily weight gain

Traits of semen	Daily weight gain [g]		
	751 - 800 (I)	801 - 850 (II)	> 851 (III)
Ejaculate volume [ml]	n.s.	P < 0.001	P < 0.001
Concentration of spermatozoa [thous./mm3]	n.s.	P < 0.001	P < 0.05
Percent of live spermatozoa [%]	P < 0.001	n.s.	n.s.
Number of live spermatozoa [bln]	n.s.	P < 0.001	P < 0.001
Number of spermatozoa per insemination dose [bln]	n.s.	P < 0.001	n.s.
Number of insemination doses	n.s.	P < 0.001	P < 0.001

n.s. – no significances

Conclusion

The high rate of growth of the boars studied had no negative effect on the quality of their ejaculates. In both the purebred and crossbred boars, the largest ejaculate volume, the highest percentage of live sperm and the most insemination doses per ejaculate were obtained for the boars with the highest daily weight gains (over 851 g).

There were also significant differences between the semen characteristics of the purebred and crossbred boars. These differences were particularly pronounced in boars with higher daily weight gains (801-850 and > 851 g).

The results obtained indicate that the intensity of current selection in breeding of boars of the breeds analysed does not adversely affect their future reproductive performance.

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