

GRAIN PROTEIN OF SORGHUM DEPENDING ON NITROGEN RATES

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Abstract

*The response of grain protein concentration and yield of sorghum to nitrogen fertilization in rates 0, 60, 120, 180, 240 and 300 kg N.ha⁻¹ was studied in the experimental field of Agricultural University of Plovdiv, Bulgaria in 2017-2018 under non-irrigated conditions. Without nitrogen fertilization sorghum hybrid EC Alize formed grain with 10.98%-11.10% protein. Rate N₃₀₀ significantly increased concentration of grain protein by 15.0% and 21.9%, respectively in 2017 and 2018, compared to N₀ plants. Fertilization N₆₀ - N₃₀₀ proven increased grain protein yield over the N₀. Rate N₁₈₀ provided higher grain protein yield of 708 kg.ha⁻¹ in 2017 and higher N₂₄₀ and N₃₀₀ rates showed a downward trend in protein yield within limits 677-708 kg.ha⁻¹. In more favourable in terms of rainfall 2018, the highest grain protein yield 907 kg.ha⁻¹ was obtained at N₂₄₀. Application of N₃₀₀ proven reduced by 80 kg.ha⁻¹ the protein yield, compared to N₂₄₀. Rates 0-300 kg N.ha⁻¹ highly positively correlated with grain protein concentration (0.864**,-0.962**) and protein yield (0.839**,-0.874**) of sorghum.*

Key words: protein, concentration, yield, grain.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth leading cereal grain produced worldwide after wheat, corn, rice, and barley (FAO, 2013). In Bulgaria the production of grain sorghum has increased in recent years and sorghum is one of the top ten grown crops in the country. The modern varieties are hybrids with high productivity potential appropriate for application of intensive forage grain production technologies (Kertikov, 2007; Kikindonov et al., 2008). This crop has better ability to tolerate drought stress compared with other crops and is known as an index for drought resistance of agronomic crops (Kebede, 2001; Wenzel, 2001). Sorghum is mainly grown under non-irrigated fields where stressful conditions during grain filling can limit productivity and increase the dependence of the yield of spare assimilations (Kaye et al., 2007). It is a multipurpose crop belonging to the Poaceae family, which are C₄ carbon cycle plants with high photosynthetic efficiency and productivity (Tari et al., 2012). Soil fertility management is important to facilitate the rapid development of sorghum plants, but many agricultural soils have a limited ability to supply available nitrogen for target yields and nitrogen is the most limiting

nutrient for cereal crops including for sorghum production (Gerik et al., 2014). Nitrogen is the main nutrient for C₄ plant productivity (Hao et al., 2014). It plays a critical role in cell division during the plant growth (Stals & Inzé, 2001) and the deficit of soil nitrogen leads to lower sorghum biomass due to reductions in leaf area, chlorophyll index, and photosynthetic rate (Zhao et al., 2005; Hirel et al., 2007; Mahama et al., 2014). Nitrogen fertilizer is known to boost the aboveground biomass yield (Amaducci et al., 2004; Anderson et al., 2013). Nitrogen fertilization had a significant impact on the concentration of protein in the grain and grain protein yield (Anfinrud et al., 2013). Application of nitrogen in rates 120-240 kg N.ha⁻¹ increase crude protein content to higher levels to support rapid weight gains and milk yields (Hoffman et al., 2001; Kaufman et al., 2013). The nitrogen doses 50-200 kg.ha⁻¹ contributed to an increase in the crude protein together with an increase in dry matter and/or protein concentration and crude protein increased 59.5-312.9% (Melo et al., 2017). The phosphorus and potassium fertilization slightly affected grain protein yield of sorghum grown under good phosphorus and potassium soil availability (Franco et al., 2017). Improper nitrogen fertilization and excess nitrogen resulted in environmental impacts,

such as the pollution by nitrate (NO₃-N) leaching and nitrous oxide emissions (Miller & Cramer, 2004; Ramu et al., 2012), as well as increasing production costs (Marsalis et al., 2010). Thus, coordination of sorghum N demand with N supply is critically important to maximize economic efficiency, optimize biomass quality, and minimize loss of soil NO₃-N and environmental pollution (Schroder et al., 2000; Zhu et al., 2000; Rooney et al., 2007; Cui et al., 2008; Meki et al., 2017). The objective of this study was to determine the effect of nitrogen fertilization rates on the concentration of protein in the grain and grain protein yield of sorghum grown under non-irrigated conditions.

MATERIALS AND METHODS

The investigation was carried out during the period 2017-2018 on the experimental field of Agricultural University of Plovdiv, Bulgaria, under non-irrigated conditions after wheat as predecessor. The effect of nitrogen fertilization in rates 0, 60, 120, 180, 240 and 300 kg N.ha⁻¹ on the concentration of protein in the grain and grain protein yield was studied at grain sorghum hybrid EC Alize. The experimental design consisted of a randomized, complete block design with four replications. The size of individual trial plots was 20 m². Total nitrogen as NH₄NO₃ was applied as pre-sowing fertilization on the background P₅₀K₅₀ fertilization as triple superphosphate and potassium chloride, respectively. Standard farming practices for the region of Southern Bulgaria were applied.

Table 1. Content of available nitrogen, phosphorus and potassium in the soil

Year	Soil depth, cm	N min, mg.kg ⁻¹	P ₂ O ₅ , mg.100 g ⁻¹	K ₂ O, mg.100 g ⁻¹
2017	0-30	27.6	15.8	21.0
	30-60	22.1	13.9	24.0
2018	0-30	33.8	17.3	23.1
	30-60	20.4	14.1	22.9

The soil type of the experimental field is alluvial-meadow *Mollic Fluvisols* (FAO, 2006) with slightly alkaline reaction pH_{H2O} = 7.80. The content of available nutrients in the soil before sowing of the sorghum was determined in soil layers 0-30 and 30-60 cm and pointed

out in Table 1. The soil had low content of mineral nitrogen and it was good supplied with available phosphorus (Egner-Ream method) and exchangeable potassium (extracted by 2N HCL).

Meteorological conditions during vegetation period of sorghum were recorded daily in the experimental area and are given in Table 2, together with the long-term average of temperature and precipitations.

Table 2. Hydro-thermal conditions during sorghum vegetation period

Year	April	May	June	July	August
	Temperature (°C)				
2017	12.7	17.6	23.7	25.1	25.4
2018	16.4	19.2	28.8	30.5	24.2
Long-term norm	12.2	17.2	20.9	23.2	22.7
	Precipitation (l.m ⁻¹)				
	2017	26.1	52.7	15.4	29.8
2018	25	112.3	118.9	94.7	35.1
Long-term norm	45	65	63	49	31

The values of temperature and precipitations during the vegetation period of sorghum characterized hydro-thermal conditions of 2017 as warm and dry. In contrast, the months of May, June and July of 2018 were characterized as extremely humid. The amount of precipitation exceeded nearly twice the values of long-term norm for the region.

The total nitrogen concentration of sorghum grain in maturity was analyzed by Kjeldahl method after wet digestion by H₂SO₄ and H₂O₂ as a catalyst (Tomov et al., 2009). The grain protein concentration was calculated from the percentage of nitrogen in the grain multiplied by a coefficient of 5.6 (Mariotti et al., 2008). Grain protein yield in kg.ha⁻¹ was calculated according to the formula:

(Grain yield in kg.ha⁻¹ multiplied by the percentage of protein in the grain)/100.

An overall analysis of variance (ANOVA) was performed to evaluate the effect of the experimental treatments on the referred variables. In order to establish the difference among the means Duncan's multiple range test at level of significance $p \leq 0.05$ was used. The regression analysis was done for assessment of the effect of nitrogen fertilization rates on the grain yield, grain protein concentration and grain protein yield of sorghum. Correlation test

with significance level reported ($p < 0.05$ or $p < 0.01$) was based on Pearson's correlation coefficient.

RESULTS AND DISCUSSIONS

During the comparatively dry 2017 experimental year, the average grain yield was 5234 kg.ha⁻¹, and under the meteorologically favourable year of 2018, average grain yield was 6803 kg.ha⁻¹ (Table 3). Regarding the grain yield of sorghum, the strong proven effect of the nitrogen fertilization of dose N₁₈₀ was established in 2017. The yield increase was by 25.8% above the control plants. In 2017, the application of N₁₂₀, N₂₄₀ and N₃₀₀ resulted in similar grain yields 5355-5455 kg.ha⁻¹. Higher grain yields of sorghum in 2018 were found in rates N₂₄₀ and N₁₈₀, which exceeded the N₀ by 38.4 and 35.7%, respectively.

Table 3. Grain yield of sorghum depending on nitrogen fertilization rates, kg.ha⁻¹

Rates	2017 year	% to N ₀	2018 year	% to N ₀
N ₀	4572 ^{d*}	100	5023 ^d	100
N ₆₀	4897 ^c	107.2	5441 ^c	108.4
N ₁₂₀	5378 ^b	117.7	6090 ^b	121.3
N ₁₈₀	5750 ^a	125.8	6812 ^a	135.7
N ₂₄₀	5455 ^b	119.3	6951 ^a	138.4
N ₃₀₀	5355 ^b	117.3	6180 ^b	123.1
<i>Average</i>	<i>5234</i>		<i>6803</i>	

*Values with identical letters within each column are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

According to various authors, the total nitrogen content in the sorghum grain usually changed from 1% N to 3% N (Singh & Axtell, 1973). The concentration of grain protein ranged from 6% to 18% and the average content was 11% (Lasztity, 1996; de Mesa-Stonestreet et al., 2010). Our results showed the similar values of grain protein in the range of 10.98-13.38% (Table 4). In experimental 2017, the grain protein concentration changed from 11.10% (N₀ control) to 12.77% (N₃₀₀). The concentration of grain protein in experimental 2018 varied from 10.98% (N₀ control) to 13.38% (N₃₀₀). The higher average grain protein concentration of all studied nitrogen rates was established in 2018, which characterized as more favourable on precipitation compared to the dry conditions

during sorghum vegetation of harvested 2017. In each of the two experimental years, the protein concentration of the grain enhanced with the increase of applied mineral nitrogen. No proven differences of grain protein concentration were found between the control plants and the plants cultivated at low N₆₀ rate. A tendency was indicated an increase of the protein concentration of the sorghum grain between the variant of high N₃₀₀ fertilization and N₁₈₀ and N₂₄₀ rates. Application of 300 kg N.ha⁻¹ showed a significant higher protein concentration of grain compared to the grain protein percentage of plants cultivated at N₆₀ and N₁₂₀ rates. This was observed during both experimental years. Sorghum formed a grain which contained an average of 11.04 % protein when the plants grown without nitrogen fertilization. As the nitrogen fertilization increased to the N₃₀₀ rate, the concentration of grain protein significantly increased up to 12.77% (in 2017) and 13.38% (in 2018). These values were higher by 15.0% and by 21.9%, respectively, compared to N₀ control plants of the two experimental years. The present results confirm the main effect of nitrogen fertilization on the protein concentration in cereals grain and they correspond to the results for grain sorghum of the other researchers (Assefa et al., 2010; Ciampitti et al., 2016; Van Oosterom et al., 2010).

Table 4. Protein concentration of grain of sorghum depending on nitrogen fertilization, %

Rates	2017 year	% to N ₀	2018 year	% to N ₀
N ₀	11.10 ^{c*}	100	10.98 ^c	100
N ₆₀	11.34 ^c	102.2	11.82 ^{bc}	107.7
N ₁₂₀	12.11 ^b	109.1	12.38 ^b	112.8
N ₁₈₀	12.32 ^{ab}	111.0	12.82 ^{ab}	116.8
N ₂₄₀	12.42 ^{ab}	111.9	13.05 ^a	118.9
N ₃₀₀	12.77 ^a	115.0	13.38 ^a	121.9
<i>Average</i>	<i>12.01</i>		<i>12.40</i>	

*Values with identical letters within each column are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

The results of grain protein yield of sorghum presented in Table 5 indicated that nitrogen application in N₆₀ - N₃₀₀ rates significantly increased grain protein yield over the unfertilized control. This was observed in both experimental years. The hydro-thermal conditions during sorghum vegetation affected

the grain and grain protein yields. The sorghum vegetation period of experimental 2018 was characterized with more rainfall. As a result of that, the average grain protein yield of all studied treatments was 759 kg.ha⁻¹ or by 127.8 kg.ha⁻¹ higher compared to the obtained average protein yield in harvested 2017. Sorghum had the lowest yields of grain protein when cultivated without nitrogen fertilization. In 2017, the obtained grain protein yield from N₀ variant was 508 kg.ha⁻¹.

The protein yield of the control plants in harvested 2018 was 551 kg.ha⁻¹. Nitrogen fertilization in 60, 120, 180, 240 and 300 kg N.ha⁻¹ proven increased yield of grain protein of sorghum in comparison of unfertilized control in both experimental years. The grain protein yields of nitrogen received plants exceeded the yield of N₀ grown plants by 9.3%-39.4% and by 16.7% to 64.6%, respectively, in the harvested 2017 and in 2018 years.

In 2017, grain protein yield increased in a parallel with the levels of applied mineral nitrogen up to the N₁₈₀. The fertilization rate of 180 kg of N.ha⁻¹ provided a high yield of grain protein and the increase was by 39.4% relative to the protein yield of unfertilized control. The results showed a tendency for lower grain protein productivity of sorghum within limits 677-708 kg.ha⁻¹ at application of higher N₂₄₀ и N₃₀₀ rates. In 2017, the effect of the higher mineral nitrogen 240 kg N.ha⁻¹ and 300 kg N.ha⁻¹ on the grain protein yield was mathematically unproven with regard to the obtained grain protein yields of sorghum fertilized with N₁₈₀ and N₁₂₀ rates.

Table 5. Grain protein yield of sorghum depending on nitrogen fertilization, kg.ha⁻¹

Rates	2017 year	% to N ₀	2018 year	% to N ₀
N ₀	508 ^{d*}	100	551 ^c	100
N ₆₀	555 ^c	109.3	643 ^d	116.7
N ₁₂₀	651 ^b	128.2	754 ^c	136.8
N ₁₈₀	708 ^a	139.4	873 ^{ab}	158.5
N ₂₄₀	677 ^{ab}	133.3	907 ^a	164.6
N ₃₀₀	684 ^{ab}	134.6	827 ^b	150.1
Average	631.2		759	

*Values with identical letters within each column are not significantly different at p<0.05 according to Duncan's multiple range test.

In 2018 harvested year, which was more favorable in terms of rainfall, the positive effect

of applied nitrogen on the protein yield was observed up to the rate N₂₄₀. The highest grain protein yield (907 kg grain protein.ha⁻¹) was obtained with 240 kg N.ha⁻¹ application, but the difference with the yield at N₁₈₀ fertilization (873 kg grain protein.ha⁻¹) was not proven. Application of the higher N₃₀₀ rate significantly reduced the grain protein yield of sorghum plants by 80 kg.ha⁻¹ in comparison with the obtained yield in variant N₂₄₀.

Table 6. Correlation coefficients of nitrogen fertilization rates with grain yield, grain protein concentration and grain protein yield of sorghum

Year	Grain yield	Grain protein concentration	Grain protein yield
2017	0.724*	0.864**	0.839**
2018	0.770*	0.962**	0.874**

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

Positive correlations between examined parameters of sorghum were recorded in the study (Table 6). The results indicated that nitrogen fertilization in rates 0-300 kg N.ha⁻¹ was highly positively correlated with the grain yield, grain protein concentration (r = 0.864**) and protein yield (r = 0.839**) in experimental 2017. Also, a positive significant correlation was achieved between the nitrogen fertilization rates and yields of grain and protein, and protein concentration of grain (r = 0.962**) in harvested 2018.

Table 7. Regression models of sorghum grain yield, grain protein concentration and grain protein yield depending on nitrogen fertilization rates.

Parameter	Equation	R ²
2017		
Grain yield	y = -0.026x ² + 10.6x + 4497	0.923
Protein concentration	y = -1E-05x ² + 0.01x + 11.0	0.959
Grain protein yield	y = -0.004x ² + 1.68x + 494	0.940
2018		
Grain yield	y = -0.04x ² + 17.1x + 4816	0.884
Protein concentration	y = -2E-05x ² + 0.014x + 11.0	0.995
Grain protein yield	y = -0.006x ² + 2.83x + 525	0.947

Regression analysis for the dependencies between the resulting parameters (grain yield, grain protein concentration, and grain protein yield) and the factor nitrogen fertilization on sorghum hybrid EC Alize indicated that

correlations were represented by equations of the second degree (Table 7). High values of coefficients of determination ($R^2 > 0.850$) were found for the studied parameters of sorghum productivity in dependence of nitrogen fertilization. The regression model indicated that the grain yield increased with the raise of applied nitrogen rate up to N_{180} and the grain protein concentration increased up to N_{300} fertilization of in both experimental years.

CONCLUSIONS

Without nitrogen fertilization sorghum hybrid EC Alize formed grain with 10.98%-11.10% protein. Applied mineral nitrogen enhanced the protein concentration of sorghum grain, but effect of N_{60} was not proven compared to the N_0 control. Rate 300 kg N.ha⁻¹ significantly increased concentration of grain protein by 15.0% and 21.9%, respectively in 2017 and 2018, compared to N_0 plants.

Fertilization N_{60} - N_{300} significantly increased grain protein yield over the unfertilized control. The N_{180} rate provided higher grain protein yield of 708 kg.ha⁻¹ in 2017. The increase was by 39.4% relative to the protein yield of N_0 control. Application of higher N_{240} and N_{300} rates showed a downward trend in protein yield within limits 677-708 kg.ha⁻¹. In more favourable in terms of rainfall 2018 harvested year, the highest grain protein yield 907 kg.ha⁻¹ was obtained at fertilization 240 kg N.ha⁻¹. Application of N_{300} significantly reduced by 80 kg.ha⁻¹ the grain protein yield of sorghum, compared to N_{240} . The fertilization in rates 0–300 kg N.ha⁻¹ highly positively correlated with grain protein concentration (0.864** - 0.962**) and protein yield (0.839** - 0.874**).

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