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Nitrogen, phosphorus and potassium content in maize dry biomass under the effect of different levels of mineral fertilization

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Abstract. *The aim of the study was to assess the influence of different fertilizer doses on the content of macroelements (nitrogen, phosphorus and potassium) in dry biomass and grain of maize during the 2016 – 2018 period. A field experiment with fertilization of maize was carried out on Alluvial-meadow soil (Fluvisol) in the region of Tsalapitsa village, near Plovdiv. Three variants of mineral fertilization were studied V2 ($N_{15}P_{10}K_0$), V3 ($N_{20}P_{15}K_0$) and V4 ($N_{25}P_{20}K_0$), and a control variant V1 ($N_0P_0K_0$) – without fertilization. It was established that N% content in maize dry biomass was affected significantly by the variants of fertilization (18% of the variance). Significant differences ($P \leq 0.05$) between the control variant and all the variants of fertilization were established. Increasing the fertilizer dose, nitrogen content in dry biomass increased, too. The highest was the average content of nitrogen in maize leaves (0.94%), followed by the cobs (0.71%) and the lowest was the content in the stems (0.58%). Phosphorus and potassium content of dry biomass were affected significantly by the year of the study (10% and 9% of the variance, respectively). At the 7-8th leaf growth stage of maize, the highest nutrients content (N, P, K) in dry biomass were reported. With aging of plants the nutrient content in their biomass decreased. Nitrogen, ph*osphorus and potassium content in maize grain was significantly affected by the year of the experiment. Mineral fertilization had impact mostly on the nitrogen content of the grain, which was the highest in V3 variant, accepted as optimal – 0.66% on average.

Keywords: maize, fertilizer doses, plant biomass, grain, two-factor Anova

Introduction

Maize is one of the main agricultural crops in Bulgaria. Its biological plasticity allows growing in many regions of the country (Stoyanov and Donov, 1996a). Nutrient content, and especially that of basic macroelements such as nitrogen, phosphorus and potassium, determines the biological value of the production obtained (Wylupek et al., 2014).

Genotype characteristics of hybrids, climatic conditions during the vegetation, available nutrients, soil moisture and aeration, the level of groundwater, etc. have significant influence on the element composition of plants (Stoyanov and Donov, 1996b; Donov, 2001; Skowronska and Filipek, 2010). Nutrients content of plant tissues depends not only on their concentration in soil but also on the meteorological conditions during the growing season (Aleksandrova and Donov, 2003). The optimization of mineral fertilization is very important for obtaining high quality production. Plant nutrients never act independently, they often have synergistic and sometimes antagonistic effect (Bak et al., 2016).

Nitrogen is one of the main plant nutrients and its deficiency leads to reduction of their growth and development. It performs different functions playing a key role in plant metabolism. Nitrogen participates in various metabolic pathways of great importance for plants including protein synthesis (Bak et al., 2016). Nitrogen is the most mobile from all the nutrients, therefore, it is subjected to loss from leaching and volatilization (Stoyanov and Donov, 2003b). Potassium and phosphorus play an important role in the physiological and biochemical functions of plants. They increase the ability of plants to resist various abiotic and biotic stress factors (Mengel and Kirkbi, 2001).

The aim of our study was to assess the effect of different fertilizer doses on the macroelement content (nitrogen, phosphorus and potassium) of the plant biomass and grain of maize, during the three-year field experiment.

Material and methods

During the 2016-2018 period, on a plot of Alluvial-meadow soil (Fluvisol), in a long-term experiment, at the experimental field of Tsalapitsa village near Plovdiv, a field trial with fertilization of maize (PR35F38, 520 according to FAO) was carried out. The soil was characterized by light texture and adequate water permeability (Stoicheva et al., 2006), the organic matter content of soil was low – 1.07% and the pH (H_2O) ~ 6.7. Under the effect of long-term mineral fertilization, considerable changes in the values of strongly acid and weakly acid cation exchange of the Fluvisol were observed (Atanassova et al., 2013). An increased buffering was observed with the Fluvisol in the acid pH interval (5.3-4) within the variants of the highest fertilization rate.

The content of available nutrients (N, P, K) in soil before the set-up of the experiment (in autumn 2015) were the following: mineral N - 18.8 mg kg^{-1}, P_2O_2 - 4.8 mg $100g^{-1}$ and K_2O - 26.9 mg $100g^{-1}$.

Three levels of fertilization with nitrogen and phosphorus were assessed. Due to the good soil supply with potassium, fertilization with this element was not applied. When determining the fertilizer

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doses, the balance approach was used. Variant V1 ($N_0P_0K_0$) was the control variant (no fertilization). The V3 ($N_{20}P_{15}K_0$) variant was calculated to compensate 100% of nitrogen uptake by the production obtained – optimal variant. Variants V2 ($N_{15}P_{10}K_0$) and V4 ($N_{25}P_{20}K_0$) had nitrogen fertilizer doses calculated to compensate 75% and 125% of nitrogen uptake by plants, thus simulating the shortage and the luxury consumption of this element. Phosphorus fertilizer rates were determined to compensate completely the uptake of this element by plants. Nitrogen was applied as NH_4NO_3 once a year, before plant seeding and phosphorus - as triple superphosphate, during the previous autumn for each of the experimental years. A randomized block design with 3 replicates was used, each experimental plot was 100.8m^2.

The maize was sown in crop-optimal conditions, from 10th-20th of April with a seed density of 55000 plants/ha. It was grown as monoculture with full compensation of water consumption, drip irrigation installed. All necessary agro-technical measures for the cultivated crop were maintained.

Plant samples were taken at three growth stages of the maize development: 7-8th leaf, tasseling and milk-dough stage. Grain samples were taken at maturity stage of maize.

Nitrogen content of plant samples was determined by wet digestion according Ginsburg method and Kjeldahl distillation (Peterburgskii, 1986). Phosphorus was determined colorimetrically by molybdenum blue, and potassium by flame photometer (Peterburgskii, 1986). Soil mineral nitrogen content was determined by the Kjeldahl (Methods of soil analysis, 1982), mobile forms of phosphorus and potassium, by the method of Ivanov (1984), pH (H_2O) values were determined potentiometrically in a soil: water ratio 1: 2.5.

Statistical processing of the data was performed by using One-way Anova and Multifactor Anova methods from the package Statgraphics Centurion 18.

Results and discussion

Meteorological parameters (temperature, precipitation)

Air temperature and soil humidity are factors that strongly influence the content and uptake of basic macroelements independently of the soil type and fertilization applied (Aleksandrova and Donov, 2003). Data for the mean daily temperature and precipitation, obtained from the automatic weather station on the experimental field of Tsalapitsa village, for the period of investigation were compared with a long-term period (1961-1990), accepted as a norm and are presented in Table 1.

Table 1. Meteorological data for the period of the investigation (2016-2018), compared with a long-term period (1961 - 1990)

Parameters	Year	Months								
		I	II	III	IV	V	VI	VII	VIII	IX
t °C	2016	-0.3	8.0	9.4	15.5	16.8	23.2	25.6	24.3	20.0
	2017	-3.9	2.7	10.1	12.5	17.2	23.3	24.9	24.8	20.2
	2018	3.4	4.2	7.5	16.8	19.8	22.0	23.8	24.7	20.0
	1961-1990	0.3	2.7	6.6	12.0	17.0	20.7	22.5	21.8	18.2
Precipitation, mm	2016	42.6	18.8	52.6	45.8	55.5	26.2	4.6	5.6	1.6
	2017	38.8	28.7	3.8	38.4	62.1	29.2	41.8	5.8	61.2
	2018	13.0	50.5	75.0	21.0	42.0	86.0	44.4	41.8	5.4
	1961-1990	40.5	32.0	41.0	43.6	59.9	48.2	45.7	33.1	28.8

During the experimental period (2016-2018) the sum of winter and spring precipitations (January - March) was close to the climatic norm, which gave a good moisture content at the beginning of maize growth. In March 2017, the rainfall was significantly lower than that reported for the long-term period, but it was compensated during the next two months. In 2016 and 2017, the spring months April and May were characterized by favorable climatic conditions - high precipitation and optimal temperatures, very close to the long-term period (Table 1). For the same period, lower humidity and higher temperatures characterized the year 2018.

Summer months (June - September) defined the year 2016 as very hot. Average monthly and maximum temperatures were higher than the norm, especially during the months June, July and August. Summer rainfalls were insufficient, especially in the maize-critical periods (at the end of July the rainfall sums were nearly 10 times lower than the climatic norm, in August was almost the same). Similar tendencies were observed in the summer of 2017, August was particularly dry and hot with an average monthly temperature of 24.8°C and a sum of precipitation only 5.8mm. Heavy rain (approximately 90mm) fell in June 2018, as compared to the climatic norm of 55mm. Although maize needs sufficient soil moisture, the intensive rainfall affected negatively its development. It creates conditions for leaching of nutrients, especially nitrogen, through the soil profile.

Nitrogen, phosphorus and potassium content of dry biomass

Average nitrogen content in the dry biomass of maize, for the three-year experimental period is presented in Figure 1. Average data vary between 0.33% and 1.22%, as the highest was nitrogen content in maize leaves and the lowest in maize stems. Data differences depend on the year of the experiment, the growth stage of the crop, the vegetative organs of maize and the variants of fertilization. To assess the combined effect of the factors fertilization and year of the experiment on the nitrogen, phosphorus and potassium content of maize biomass, two-factor Anova was used (Table 2).

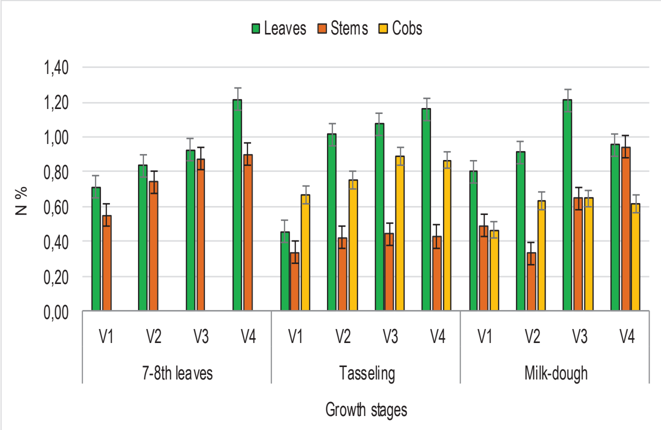

Figure 1. Average nitrogen content (%) of maize biomass, depending on the growth stage, vegetative organs and variants of fertilization of the crop, for the period (2016-2018)

From the two factors of the investigation, significant effect (P≥95%) on the nitrogen content in maize biomass had factor B, i.e. variants of fertilization (Table 2). Due to the high variation of the data within the three-year period, significant influence of factor A – year of the experiment - was not established. The variability of the data, due to the growth stage of plants and their vegetative organs, was also the reason for the high percentage of residual in the Two-factor analysis. Although significant variations of the data for nitrogen content regarding the years were not reported in 2018, N% content of plants was generally lower than in the previous two years. In June 2018, the weather was characterized by heavy rainfall, almost twice as high as the long-term period (Table 1). Unfavorable climatic conditions during this period had a negative influence on the nitrogen uptake and the yield formation in 2018. It is known that nitrogen is an element with high water migration, which depends on the soil physical properties, the fertilization, the regime of humidification (rainfall and irrigation), etc. (Stoicheva et al., 2006; Zotarelli et al., 2007; Simeonova, 2016). According to Mitchell (2011), not only the intense rainfall but also the seasonal fluctuations of the temperature are the reason for substantial losses of nitrate nitrogen. Nitrate nitrogen migration is one of the main pathways to exclude this element from the biological cycle.

Table 2. Two-factor Anova of the data for N, P and K (%) contents in maize biomass

Source	Sum of squares	N, %	P-value	Sum of squares	P, %	P-value	Sum of squares	K, %	P-value
Main effects									
A: Year	0.190	3	0.277	0.077	10	0.010	9.9	9	0.026
B: Fertilization	1.218	18	0.002	0.057	8	0.072	4.9	4	0.290
Residual	5.459	79		0.602	82		98.9	87	
Total (corrected)	6.839			0.735			114.0		

Mineral fertilization had a significant effect on the nitrogen content of maize biomass (18% of the variance), Table 2. Significant differences (P≥95%) were established between the control variant V1 ($N_0P_0K_0$) and all the variants of fertilization – V2 ($N_{15}P_{10}K_0$), V3 ($N_{20}P_{15}K_0$) and V4 ($N_{25}P_{20}K_0$) (Figure 2). With the increase of the fertilization norm, the plant nitrogen content increased, too. Significant differences between variants V2 and V4, which simulate a deficiency and excess of nitrogen, were also established. Maize is an agricultural crop that responds sensitively to the applied mineral fertilization (Bak et al., 2016; George et al., 2016), which affects both the obtained yields and nitrogen accumulation in plant biomass.

Regarding the plant growth stages, the highest nitrogen content was established in the 7-8th leaf stage, and lower at the next two phases, tasseling and milk-dough stage (Figure 1). In the early periods of their development, the plants were more sensitive to the applied fertilization and they accumulated rapidly the nutrients from the soil. In the next two phases, the nutrient content of the biomass decreased, due to the dilution effect and the process of remobilization of N and P from the vegetative organs (mainly the leaves) to the plant's generative organs (Skowronska and Filipek, 2010). There was high variation of nitrogen content depending on the maize vegetative organs (Figure 1.) Maize leaves were characterized by the highest N% content - 0.94% on average, followed by 0.71% for the maize cobs, and 0.58% for the maize stems. Similar results for the nitrogen content in plant vegetative organs were obtained by Bak et al. (2016).

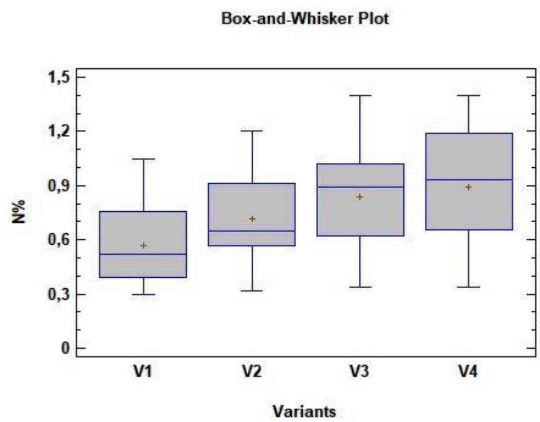

Figure 2. One-way Anova of N% content in maize biomass, depending on the variants of fertilization, average for the period 2016-2018

For the period of the investigation the average phosphorus content (% from the dry weight) in maize varied within narrow limits. The lowest was P% in the stems of variant V2 ($N_{15}P_{10}K_0$), 0.15% on average during the milk-dough stage, the highest was in the leaves of the control variant V1 ($N_0P_0K_0$) - 0.49% during the 7-8th leaf stage (Figure 3).

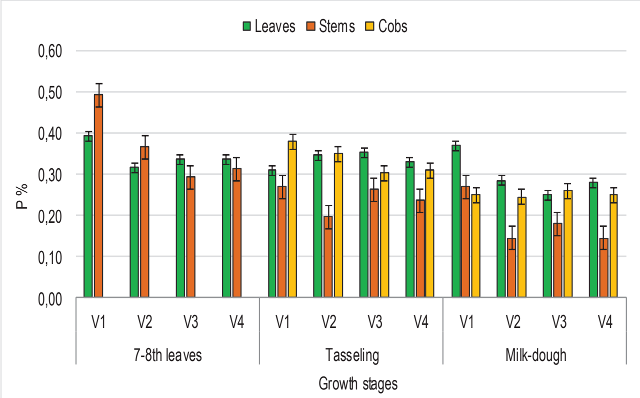

Figure 3. Average phosphorus content (%) of maize biomass, depending on the growth stage, vegetative organs and variants of fertilization of the crop, for the period (2016-2018)

Two-factor Anova analysis of the data for phosphorus in plant biomass showed that from both factors, only the experimental year had significant impact (P≥95%), (Table 2). The variability during the years was the reason for 10% of the variance in the experiment. Unlike the results for nitrogen, which was the lowest in 2018, the data for phosphorus were the lowest in 2016 and they are significantly different from 2017 and 2018. The reason for these results could be different weather and soil conditions during the three-year period of the experiment. The summer in 2016 was very hot and this could be a precondition for the weaker uptake of phosphorus by plants. Phosphorus availability to plants depends on a number of factors such as soil acidity, soil temperature and humidity, the presence of different ions in the solution, the organic matter content, etc. In the investigation of Sheppard and Racz (1984), it was found that with increasing of the soil temperature the extractability of available phosphorus decreased due to its stronger fixation and inclusion in specific sorption reactions.

Regarding the growth stages of maize, phosphorus content visibly decreased during the growing season (Figure 3). It was the highest, 0.37% on average, in the initial period of maize development. During the 7-8th leaf growth stage the plants were in a period of intensified growth and they accumulated higher amounts of nutrients in their biomass. The trend was the same as for the nitrogen. During the next two stages, tasseling and milk-dough stage, the average P% content decreased to 0.30% and 0.20%, respectively. The decrease of phosphorus and other nutrient contents with aging of plants is associated with the increase of dry matter, which is known as „dilution effect“ (Fageria et al., 2013).

The variants of fertilization had no significant influence on the phosphorus content in maize biomass (Table 2). However, unlike nitrogen, the highest phosphorus content was reported in the control variant, 0.34% on average. The variants of fertilization had average P% content between 0.27 and 0.28%. This could be also explained by the „dilution effect“. Regarding plant vegetative organs, the lowest was P% content in maize stems (Figure 3) and it decreased with aging of plants.

The content of potassium in dry biomass of maize varied the most from the three studied macroelements. It was 5.4% on average in the stems of the control variant during 7-8th leaf growth stage and 0.7% in the stems and also in the fertilized variants (V2, V3 and V4) during the milk-dough stage (Figure 4). Significant influence had the year of study, with 9% of the variance (Table. 2). Similar to nitrogen, potassium content was the lowest in 2018, which was statistically different (P≤0.05) from the previous two years.

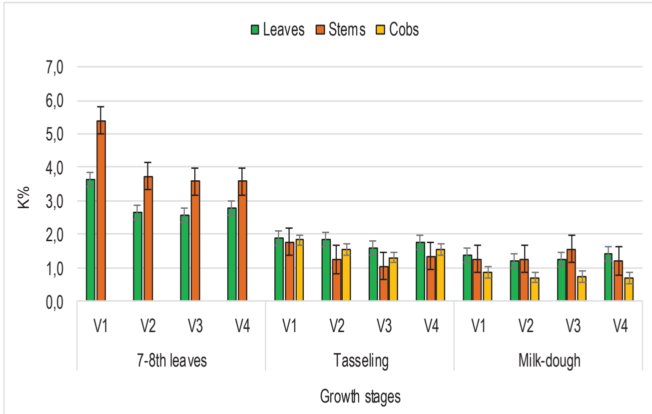

Figure 4. Average potassium content (%) of maize biomass, depending on the growth stage, vegetative organs and variants of fertilization of the crop, for the period (2016-2018)

The phase of maize development had the strongest influence on the potassium content in the dry biomass (Figure 4). The tendency was the same as observed for nitrogen and phosphorus content, the potassium content was the highest in the leaves and stems in the 7-8th leaf growth stage. During this first stage of the observation potassium was accumulated 2-3 times more compared to the next two growth stages – tasseling and milk-dough stages. Depending on the vegetative organs, potassium was the highest in the stems of maize (2.3% on average), which was the opposite compared to that observed in nitrogen and phosphorus. The average content of K% in leaves and cobs was 2.0% and 1.9%, respectively. In the investigation of Bak et al. (2016) potassium content was also higher in the stems of maize (between 1.5% and 2.7%) depending on the fertilization applied.

Similar to the phosphorus content, potassium had higher average values in the control variant and lower in the variants of fertilization. This trend could again be associated with the accumulation of a larger amount of dry biomass in the variants with applied fertilization as compared to the control.

Nitrogen, phosphorus and potassium content of maize grain

The average nitrogen content of maize grain varied within relatively narrow range, between 0.43 and 0.84%. Two-factor Anova analysis showed that both factors, A: year of the experiment and B: variants of fertilization, had significant influence (Table 3).

Table 3. Two-factor Anova of the data for N, P and K (%) contents in maize grain

Source	Sum of squares	N, %	P-Value	Sum of Squares	P, %	P-Value	Sum of Squares	K, %	P-Value
Main effects									
A: Year	0.143	43	0.000	0.018	41	0.000	0.702	95	0.000
B: Fertilization	0.075	22	0.003	0.006	14	0.043	0.003	0.5	0.464
Residual	0.119	35		0.020	45		0.033	4.5	
Total (corrected)	0.335			0.043			0.738		

The change of N% content of maize grain was stronger depending on the year of the experiment (43% of the variance), (Table 3). The grain had also accumulated lower amounts of nitrogen in 2018, similar to the vegetative biomass, but here it was statistically significant. This could be explained with the nitrogen losses during 2018 as a result of the intensive rains. The variants of fertilization had their significant impact, with 22% of the variance.

When considering N% content of the grain under the effect of different fertilizer doses (Figure 5), it was noticeable that at the highest fertilizer dose - variant V4 ($N_{25}P_{20}K_0$), the nitrogen content slightly decreased compared to V2 ($N_{15}P_{10}K_0$) and V3 ($N_{20}P_{15}K_0$) variants where the nitrogen values of the grain were higher and they were significantly different from those of the control variant. The average nitrogen content of maize grain was considerably influenced by the fertilizer dose. Similar results were obtained by Bak et al. (2016) when fertilizing with phosphorus and potassium. They found that N and P content were accumulated to 60-70% in the grain of maize, and the average nitrogen content of the grain of the variants with fertilization was about 1.51% and 1.53%.

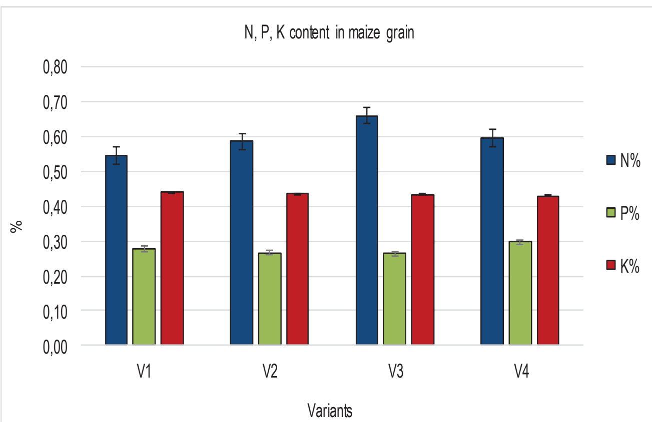

Figure 5. Average N, P and K content (%) of maize grain, depending on the variants of fertilization of the crop, for the period (2016-2018)

Phosphorus content of grain was significantly influenced by the year of the experiment and the fertilization applied (Table 3). Although the values varied within very narrow range - from 0.20% to 0.33%, in the different years the accumulation of phosphorus in maize grain had significant differences. The highest was P% content in the grain of V4 variant – the highest fertilizer dose, 0.30% on average, similar was the content of the control variant, while in another group were V3 and V2 variants with average values of 0.26%.

Potassium content of grain varied between 0.29% and 0.60%. The highest was in 2018, and the lowest in 2016. Although it was not significant, potassium content was the highest in the grain of the control variant, and lower in the variants of fertilization.

Conclusion

Nitrogen content (N%) of maize biomass in the conducted experiment was significantly affected by the variants of fertilization (18% of the variance). Significant differences were established between the control variant V1 ($N_0P_0K_0$) and all the variants with applied fertilization. With increasing the fertilization rate, the nitrogen content of the dry biomass increased, too. There were differences between average nitrogen content of leaves (0.94% N), stems (0.58% N) and cobs (0.71% N). Phosphorus and potassium content in the vegetative biomass of maize significantly differ under the effect of the year of investigation (10% and 9% of the variance, respectively). Unlike nitrogen, phosphorus and potassium were accumulated more in the biomass of the control variant compared with the variants with fertilization. Higher levels of N, P and K (%) were established in 7-8th leaf growth stage of maize. While nitrogen and phosphorus predominated in the maize leaves, potassium was accumulated mainly in the stems. Nitrogen, phosphorus and potassium content of maize grain were significantly affected by the year of the experiment. N% content was lower in 2018, and P% and K% contents – in 2016. Mineral fertilization influenced mostly the nitrogen content of the grain and it was the highest in V3 variant (accepted as optimal in the investigation).

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