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EFFECT OF NITROGEN FERTILIZATION AND METHODS OF MAGNESIUM APPLICATION ON CHLOROPHYLL CONTENT, ACCUMULATION OF MINERAL COMPONENTS, AND MORPHOLOGY OF TWO MAIZE HYBRID TYPES IN THE INITIAL GROWTH PERIOD PART I. CONTENT OF CHLOROPHYLL AND MINERAL COMPONENTS

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Abstract. Field experiment was carried out in the Didactic and Experimental Department in Swadzim (52°26' N; 16°45' E) near Poznań in years 2004-2007. The experiment was established in a „split-plot" design with 3 factors and 4 field replications. Reactions of two maize hybrid types to differentiated nitrogen and magnesium fertilization and to the method of magnesium application were studied. The influence of these factors on chlorophyll content and mineral components in the dry matter of maize plants in the phase of 5-6 leaves (BBCH 15-16) was estimated. On the basis of chloroplast pigments content, it was found that the hybrid LG 2244 stay-green type was better nourished with nitrogen, in comparison with the traditional hybrid Anjou 258. The highest nitrogen content in dry plant matter was found after the application of a 90 kg $N \cdot ha^{-1}$ nitrogen dose, while for magnesium of a 150 kg $N \cdot ha^{-1}$ dose. A better method of magnesium application has been shown to be the sowing in row method, in comparison with the broadcasting method. As a result of magnesium sown in rows, the Mg content in plant dry matter was higher compared with the broadcasting method and with a zero dose of magnesium.

Key words: BBCH 15-16, fertilizer application method, magnesium, maize hybrid types, nitrogen, stay-green

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INTRODUCTION

Maize (*Zea mays* L.) uptakes great amounts of nutritive components from the soil. They exert an influence not only on the size of the obtained yields, but also on their fodder and consumption values [Costa et al. 2002, Scharf et al. 2002]. In order to achieve full utilization of the yield-creating possibilities of maize, the farmer must apply nutritive components so that the plant can uptake them according to the rhythm of plant growth. In maize development, a series of phases occurs where each of them plays a definite role in maize life cycle. In the phase of 6-12 leaves, maize builds its potential yield structure because at that time the ear setting takes place and the number of grain rows in the ears is determined [Grzebisz 2008]. This feature is genetically determined. Nevertheless, as a result of the action of environmental and agronomic stresses, there comes a decrease in the number of rows in the ears. According to Subedi and Ma [2005], nitrogen malnutrition of plants, before the phase of the 8th leaf, leads to irreversible reduction (even up to 30%) in the number of cobs and in the potential grains in the cob. Binder et al. [2000] showed that the delay in nitrogen application until the phase of the 6th leaf reduced the grain yield by 12%. The above results were obtained on the basis of field studies with the application of conventional hybrids only.

The hypothesis of the present experiment assumed that the cultivars of stay-green type, because of more abundant leafage and big biomass [Andrews et al. 2000], may have other requirements for nutritive components, in comparison with the traditional hybrid. Furthermore, in case of maize in the phase of 5-10 leaves, on the basis of the content of the particular mineral components in dry matter, one can make a final correction of the faulty fertilization method chosen earlier.

In this connection, studies were undertaken whose objective was to define the nutritional status of two maize hybrids in the phase of 5-6 leaves, depending on different nitrogen fertilization levels and on the magnesium dose application method.

MATERIAL AND METHODS

Field studies were carried out at the Didactic and Experimental Farm in Swadzim (52°26' N; 16°45' E), near Poznań in years 2004-2007. The results of the studies carried out in 2006 were disqualified because of prevailing drought in the period of maize growth and development. The experiment was carried out in a „split-plot" design with 3 factors in 4 field replications. Two hybrids were studied: Anjou 258 and LG 2244 stay-green type. Six nitrogen doses were used: 0, 30, 60, 90, 120, and 150 kg $N \cdot ha^{-1}$, and the doses of magnesium of: 0 kg $Mg \cdot ha^{-1}$, 15 kg $Mg \cdot ha^{-1}$ (in rows), and 15 kg $Mg \cdot ha^{-1}$ (broadcasting), in the form of kieserite were applied. A detailed method of the experiment and the description of thermal and moisture conditions are both contained in an earlier work by the authors [Szulc et al. 2008b].

Measurement of the content of chloroplast pigments and of mineral components was carried out in the phase of 5-6 leaves (BBCH 15-16). Samples for the chemical determinations consisted of 8 randomly taken plants from each plot. The content of chlorophyll was determined by two methods: the direct and the indirect one. In the case of the direct method, the leaf weighed portion was cut into 2-3 mm sections, which were poured with 5 ml DMSO (dimethyl sulfoxide). The samples were kept in the dark at room temperature and then incubated at 65°C (water bath) for 30 minutes. In the

obtained extract, after cooling down, the a and b chlorophyll contents were spectrophotometrically determined.

The content of chlorophyll pigments was spectrophotometrically determined using Spekol spectrophotometer at an adequate wave length. For chlorophyll a, the measurement of extract absorbance was carried out at the wave length of 663 nm, while for chlorophyll b, the same was done at the wave length of 656 nm. For carotenoids, 470 nm wave length was used. The amount of a and b chlorophyll and the amount of the sum of a + b chlorophyll, as well as the number of carotenoids were calculated with the use of the formulae contained in the paper of Arnon [1949]:

$$\text{Chlorophyll a} = (12.7 \cdot A_{663} - 2.7 \cdot A_{645}) \cdot V \cdot (1000\ W)^{-1}$$
$$\text{Chlorophyll b} = (22.9 \cdot A_{645} - 4.7 \cdot A_{663}) \cdot V \cdot (1000\ W)^{-1}$$
$$\text{Chlorophyll a + b sum} = (20.2 \cdot A_{645} + 8.02 \cdot A_{663}) \cdot V \cdot (1000\ W)^{-1}$$
$$\text{Carotenoids} = (1000 \cdot A_{470} - 1.9 \cdot \text{chlorophyll a} - 63.14 \cdot \text{chlorophyll b})/214$$

where:

A – absorbance at a given wave length,
V – total volume of the extract, cm^3,
W – sample weight, g.

The number of the particular pigments is quoted in $\mu g \cdot g^{-1}$ of fresh matter, while the weight of carotenoids is given in $mg \cdot g^{-1}$ of fresh weight.

In case of the indirect method, maize nutritional status with nitrogen was defined using an optical apparatus known in Europe as Hydro N-Tester, while in the USA as SPAD-502 apparatus. This apparatus operates by measuring light absorption by a leaf at the wave lengths of 650 and 940 nm. The quotient of these differences indicates the chlorophyll content, and it is defined in SPAD units [Soil and Plant Analysis Development]. A high determination coefficient (R^2) was shown, depending on the species, between the readings of the apparatus and the extracted amount of chlorophyll [Piekielek et al. 1995, Scharf et al. 2006].

The analysis of the content of mineral components (N, P, K, Ca, Mg, Na) in dry matter in the phase of 5-6 leaves (BBCH 15-16) was carried out in the laboratory of the Department of Soil and Plant Cultivation, University of Life Sciences in Poznań, according to the methods described by Gawęcki [1994]. Furthermore, potassium and calcium were determined in flame spectrophotometer Flapho 40, while phosphorus and magnesium were identified in Spekol 11 colorimeter. Nitrogen and sodium were determined after previous mineralization of plant material and the determination of mineralizates concentration.

RESULTS AND DISCUSSION

In the phase of 5-6 leaves (BBCH 15-16), the content of chloroplast pigments, chlorophyll a, chlorophyll a + b, chlorophyll expressed in SPAD units, and carotenoids depended exclusively on the type of hybrid (Table 1). Significantly higher amounts of chlorophyll a, chlorophyll a + b, and SPAD units were found for the hybrid LG 2244 stay-green type, in comparison with the traditional Anjou 258 hybrid. The respective differences were: 0.1 $\mu g \cdot g^{-1}$, 0.11 $\mu g \cdot g^{-1}$, and 15. In the case of carotenoids, the LG 2244

hybrid showed a significantly smaller amount of these pigments in leaf blades (smaller by 0.29 mg·g^{-1} of dry matter), in comparison with Anjou 258 hybrid. The obtained results were confirmed by the author in his earlier studies [Szulc et al. 2008a]. He found that the hybrid of stay-green type, on the basis of chlorophyll content in the phase of 5-6 leaves, was better nourished with nitrogen in comparison with the traditional hybrid. Thomas and Smart [1993] explained the feature of the permanent green colour (stay-green) i.e. the phenotypes showing a delayed senescence – by the fact that they possess a higher content of water and chlorophyll in leaf blades in comparison with traditional maize hybrids. The identified smaller amount of carotenoids in LG 2244 hybrid testifies to a higher photooxidation of chlorophyll (a smaller protection barrier) in comparison with the traditional hybrid Anjou 258.

Table 1. Content of chloroplast pigments and chlorophyll in SPAD units in the phase of 5-6 leaves (BBCH 15-16)

Tabela 1. Zawartość barwników chloroplastowych i chlorofilu w jednostkach SPAD w fazie 5-6 liści (BBCH 15-16)

Specification Wyszczególnienie		Chlorophyll – Chlorofil				Carotenoids Karetonoidy
		a	b	a + b	in SPAD units w jednostkach SPAD	
		µg·g^{-1}				mg·g^{-1} f.m
Hybrid Odmiana	Anjou 258	1.58	0.29	1.88	283	7.11
	LG 2244	1.68	0.31	1.99	298	6.82
	$LSD_{0.05}$ – $NIR_{0.05}$	0.070	ns	0.090	9.9	0.117
Dose of N Dawka N kg·ha^{-1}	0	1.58	0.31	1.89	283	7.63
	30	1.52	0.28	1.81	287	6.67
	60	1.56	0.28	1.85	294	6.83
	90	1.62	0.30	1.92	297	7.06
	120	1.65	0.29	1.95	296	6.86
	150	1.63	0.32	1.96	285	6.72
	$LSD_{0.05}$ – $NIR_{0.05}$	ns	ns	ns	ns	ns
Dose of Mg Dawka Mg kg·ha^{-1}	0	1.61	0.30	1.92	290	6.91
	15 in rows – rzędowo	1.61	0.29	1.91	293	7.16
	15 broadcasting – rzutowo	1.60	0.30	1.91	289	6.83
	$LSD_{0.05}$ – $NIR_{0.05}$	ns	ns	ns	ns	ns

ns – non-significant differences – różnice nieistotne

In the performed experiment, no effect of any of the studied experimental factors was exerted on the contents of phosphorus and potassium in the dry matter of plants in the phase of 5-6 leaves (BBCH 15-16). Hybrid type modified merely the contents of calcium and sodium in plant dry matter (Table 2). A significantly higher amount of these mineral components was found in LG 2244 hybrid compared with Anjou 258. These differences showed: 0.93 g·kg^{-1} of dry matter for calcium and 0.43 g·kg^{-1} of dry matter for sodium. Nitrogen dose exerted a significant influence on the contents of nitrogen and magnesium in the dry matter of the aboveground parts of maize in the phase of 5-6 leaves (BBCH 15-16). Under the influence of increased fertilization, the N content increased from 36.0 g·kg^{-1} dry matter for the dose of 0 kg N·ha^{-1} to 37.8 g·kg^{-1} d.m. for the dose of 90 kg N·ha^{-1}. In the discussed developmental phase, the application of a higher fertilization level with this microelement caused a drop in the nitrogen content in the dry matter of plants.

Table 2. Content of mineral components in the aboveground maize parts in the phase of 5-6 leaves (BBCH 15-16)
Tabela 2. Zawartość składników mineralnych w częściach nadziemnych kukurydzy w fazie 5-6 liści (BBCH 15-16)

Specification Wyszczególnienie		Content of nutrients, g·kg^{-1} d.m. Zawartość składników, g·kg^{-1} s.m.					
		N	P	K	Mg	Ca	Na
Hybrid Odmiana	Anjou 258	37.0	3.21	42.29	2.60	4.90	1.07
	LG 2244	36.4	3.08	41.31	2.61	5.83	1.50
	$LSD_{0.05}$ – $NIR_{0.05}$	ns	ns	ns	ns	0.932	0.236
Dose of N Dawka N kg·ha^{-1}	0	36.0	3.27	42.48	2.51	5.41	1.32
	30	36.4	3.10	41.04	2.52	5.46	1.28
	60	36.9	3.04	41.33	2.51	5.28	1.23
	90	37.8	3.09	41.51	2.59	5.17	1.26
	120	36.9	3.17	41.65	2.67	5.40	1.33
	150	36.2	3.19	42.80	2.80	5.48	1.29
	$LSD_{0.05}$ – $NIR_{0.05}$	1.70	ns	ns	0.220	ns	ns
Dose of Mg Dawka Mg kg·ha^{-1}	0	36.5	3.13	41.91	2.56	5.42	1.30
	15 in rows – rzędowo	37.0	3.14	42.18	2.65	5.21	1.30
	15 broadcasting – rzutowo	36.7	3.16	41.31	2.58	5.46	1.26
	$LSD_{0.05}$ – $NIR_{0.05}$	ns	ns	ns	0.061	ns	ns

ns –non-significant differences – różnice nieistotne

Nitrogen content in dry matter of maize depended also on the interaction of the nitrogen dose size and the hybrid type. The plotted curves have been described by equations of the 2nd degree, whereby for Anjou 258 cultivar, the curve course was on a higher level in relation to that of the LG 2244 stay-green type. The maximal nitrogen content (38.1 g·kg^{-1} d.m.) for Anjou 258 hybrid was obtained with the dose of 71 kg N·ha^{-1}, while for LG 2244 hybrid, the maximal content was smaller by 1.5 g·kg^{-1} d.m., with the nitrogen dose higher by 4 kg N·ha^{-1} (Fig. 1).

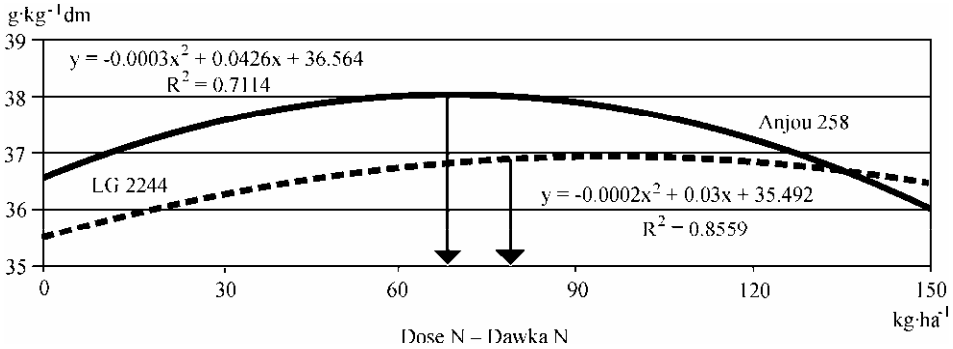

Fig. 1. Content of nitrogen in maize dry matter in the phase of 5-6 leaves (BBCH 15-16)
Rys. 1. Zawartość azotu w suchej masie kukurydzy w fazie 5-6 liści (BBCH 15-16)

Magnesium content in dry matter increased in a linear way together with the nitrogen dose increasing from 2.51 g·kg^{-1} d.m. (0 kg N·ha^{-1}) to 2.80 g·kg^{-1} d.m. (150 kg N·ha^{-1}). Furthermore, the magnesium content in the dry matter of maize depended also

on the size of magnesium dose and on the method of its application. The greatest amount of Mg was accumulated by maize fertilized with 15 kg Mg·ha^{-1} applied in rows (2.65 g·kg^{-1} d.m.), in comparison with 0 kg and 15 kg Mg·ha^{-1} (by broadcasting), between which no significant difference was found (Table 2).

From soil solution, plants uptake Mg^{2+} cations, which reach the root surface in two ways, i.e. by way of direct contact (12%) and by way of water transpiration current (88%). In a soil abundant in assimilable magnesium, the number of ions reaching the root surface exceeds almost three-fold the metabolic requirements of plants in relation to this element [Grzebisz and Härdter 2006]. This fact explicitly stresses the importance of magnesium concentration in soil solution, which is a necessary condition for Mg accumulation in the plant. This fact explains the higher content of magnesium in the dry matter of the plant, which occurs as a result of the Mg fertilization in rows. This happens because in the case of fertilization in rows, the fertilizer is placed below the seed into a moist, not loosened soil layer. Subsequently, because of water ascension, magnesium contained in the fertilizer is available to the plants from the very beginning of plant vegetation. In turn, the broadcasting method causes the fertilizer to be spread on the surface of the loosened soil layer which, in case of rain shortage, becomes quickly dry and the uptake of magnesium is limited.

The content of magnesium in maize plant dry matter depends also on the interaction of the nitrogen dose with the hybrid type. The plotted curves have been described by equations of the 1st degree, whereby, for Anjou 258 hybrid, the increment of magnesium content under the influence of the increased nitrogen dose was higher in relation to the hybrid LG 2244 stay-green type – Fig. 2.

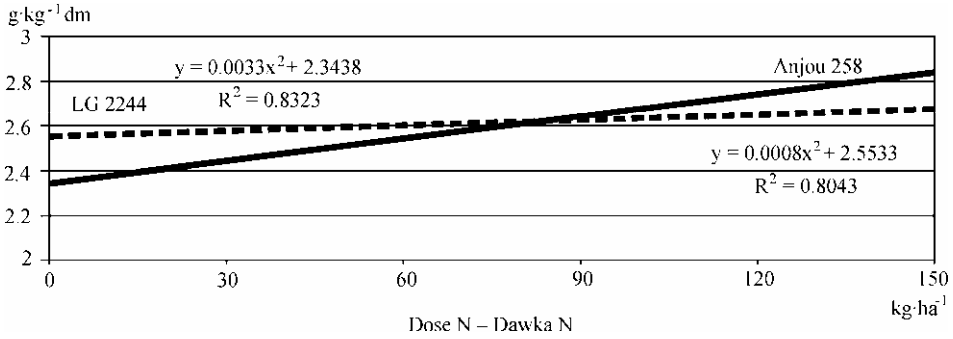

Fig. 2. Content of magnesium in maize dry matter in the phase of 5-6 leaves (BBCH 15-16)
Rys. 2. Zawartość magnezu w suchejmasie kukurydzy w fazie 5-6 liści (BBCH 15-16)

CONCLUSIONS

1. LG 2244 stay-green type hybrid, in the phase of 5-6 leaves (BBCH 15-16), contained a greater amount of chloroplast pigments a, chloroplast pigments a + b, and SPAD units but a smaller amount of carotenoids in comparison with the traditional hybrid Anjou 258.

2. LG 2244 stay-green type in the phase of 5-6 leaves (BBCH 15-16) in dry matter contained a higher content of calcium and sodium in comparison with the traditional hybrid Anjou 258.

3. The lowest amount of nitrogen in maize dry matter, in the phase of 5-6 leaves, was found for the dose of 0 kg $N \cdot ha^{-1}$, while the highest content was found for the dose of 90 kg $N \cdot ha^{-1}$.

4. Magnesium content in dry matter increased together with the increase of nitrogen dose from 0 to 150 kg $N \cdot ha^{-1}$.

5. The greatest content of magnesium was found for the dose of 15 kg $Mg \cdot ha^{-1}$ (sown in rows), in comparison with the doses of 0 and 15 kg $Mg \cdot ha^{-1}$ (by broadcasting).

6. Anjou 258 hybrid accumulated a greater content of nitrogen in dry matter in comparison with the hybrid LG 2244 stay-green type.

7. Magnesium content in both studied hybrids increased in a linear way together with the increase of the nitrogen fertilization size, whereby the increase of Mg in Anjou 258 hybrid was higher than in LG 2244 stay-green type.

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WPŁYW NAWOŻENIA AZOTOWEGO I METODY STOSOWANIA MAGNEZU NA ZAWARTOŚĆ CHLOROFILU, GROMADZENIE SKŁADNIKÓW MINERALNYCH I NA MORFOLOGIĘ DWÓCH TYPÓW ODMIAN KUKURYDZY W POCZĄTKOWYM OKRESIE WZROSTU CZ. I. ZAWARTOŚĆ CHLOROFILU I SKŁADNIKÓW MINERALNYCH

Streszczenie. Doświadczenie polowe przeprowadzono w Zakładzie Dydaktyczno-Doświadczalnym w Swadzimiu koło Poznania w latach 2004-2007 (52°26' N; 16°45' E). Doświadczenie prowadzono w układzie „split-plot" z 3 czynnikami w 4 powtórzeniach polowych. Badano reakcję dwóch typów odmian kukurydzy na zróżnicowane nawożenie azotem oraz magnezem (sposób aplikacji magnezu). Oceniano wpływ tych czynników na zawartość chlorofilu oraz składników mineralnych w suchej masie roślin kukurydzy w fazie 5-6 liści (BBCH 15-16). Na podstawie zawartości barwników chloroplastowych stwierdzono, że odmiana LG 2244 typ stay-green była lepiej odżywiona azotem w stosunku do tradycyjnego mieszańca Anjou 258. Największą zawartość azotu w suchej masie roślin stwierdzono po zastosowaniu dawki azotu 90 kg $N \cdot ha^{-1}$, natomiast magnezu – 150 kg $N \cdot ha^{-1}$. Korzystniejszym sposobem aplikacji magnezu była metoda rzędowa niż rzutowa. W wyniku zastosowania takiej metody wysiewu magnezu w suchej masie roślin stwierdzono istotnie większą zawartość tego składnika mineralnego w porównaniu z metodą rzutową oraz obiektem z zerową dawką magnezu.

Słowa kluczowe: azot, BBCH 15-16, magnez, sposób aplikacji nawozu, stay-green, typy odmian kukurydzy

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