ISSN 1392-3196
Žemdirbystė=Agriculture, vol. 99, No. 3 (2012), p. 247–254
UDK 633.15:631.84:631.576.331.2 631.165

# The effect of soil supplementation with nitrogen and elemental sulphur on chlorophyll content and grain yield of maize (*Zea mays* L.)

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## Abstract

Maize is a plant with a high productivity potential, which requires a much larger amount of nutrients during its growth and development compared to other cereal crops. Among the nutrients, nitrogen determines the size of the yield to the greatest extent. The purpose of this study was to evaluate the impact of nitrogen and elemental sulphur fertilization on chlorophyll content and grain yield of maize. Chlorophyll was determined at two development stages: at the 5–6 leaf stage and at the ear blooming stage. The field experiment was conducted at the Department of Agronomy, Poznań University of Life Sciences, on the fields of the Department of Teaching and Experimental Station in Swadzim during 2005–2008 in a random block design (split-plot) in four replications. The obtained results indicate that the content of chlorophyll *a* and *a* + *b* at the 5–6 leaf stage was significantly influenced by nitrogen fertilization in three out of four experimental years, while for the chlorophyll expressed in soil and plant analysis development (SPAD) units in two out of four experimental years. The grain yield of maize was significantly determined by the level of applied nitrogen and elemental sulphur. Greater effectiveness of the use of nitrogen applied together with elemental sulphur on grain yield was demonstrated. The curves describing this relationship for combined application of N and S were higher in comparison with the curve depicting only the influence of nitrogen dose on grain yield.

Key words: *Zea mays*, sulphur, nitrogen, chlorophyll, grain yield.

## Introduction

Maize is a plant with a high productivity potential, which requires a much larger amount of nutrients during its growth and development compared to other cereal crops. It is also characterized by the specific dynamics of nutrient absorption (accumulation), which requires the manufacturer to provide such a method of application of mineral fertilizers that completely meets the demand for particular nutrients according to the rhythm of maize growth. Among the nutrients, nitrogen determines the size of the resulting vegetative and grain yield to the greatest extent. It is the element which, when incorrectly used (too high doses, wrong form, wrong date of application), may lead to the excessive concentration of $N\text{-}NO_3$ in surface waters and groundwater causing their eutrophication (Szulc, 2010). The effective use of nitrogen depends among other things on forecrops, tillage and water availability (Timsina et al., 2001). Therefore, at present, solutions to agricultural practices which on the one hand increase nitrogen use efficiency and on the other hand improve economic profit from this treatment are being sought. This can be achieved by optimizing nitrogen fertilization of maize with other macroelements such as magnesium (Szulc et al., 2008 b) and by using elemental sulphur or sulfate (Szulc et al., 2008 c). According to Griffiths et al. (1995) and Jackson (2000), the supply of crops with sulphur has both economic and ecological importance as its deficiency decreases nitrogen use efficiency from mineral fertilizers, which in consequence threatens the environment by washing this macroelement into the soil profile. In addition, sulphur is an essential component of enzymes involved in the metabolism of nitrogen, i.e. nitrate and nitrite reductase. The foregoing finding is also confirmed by Janzen and Bettany (1984) who claim that sulphur is also necessary in order to assimilate nitrogen in plants. The literature on the subject is dominated by papers on fertilization with elemental sulphur or sulfate of crops with high demands in relation to this component, such as rape (Janzen, Bettany, 1984; Fotyma, 2003; Šiaudinis, 2010). However, there is a lack of papers and literature data which would describe the impact of this component on the growth, development and yield of plants characterized by the lower demand for sulphur in maize. Therefore, the purpose of the 4-year field studies was to evaluate the impact of the fertilization of maize cultivated in grain technology with elemental sulphur, depending on different levels of nitrogen supply (urea), on chlorophyll content and grain yield. Also a relationship between the application of elemental sulphur and nitrogen was analysed in order to limit the use of nitrogen delivered to soil in a mineral fertilizer. Furthermore, the impact of the tested factors on chlorophyll content at the two developmental stages, i.e. at the 5–6 leaf stage (BBCH 15–16) and at the ear blooming stage (BBCH 67) was determined. The extent to which the content of the tested concentration determines the size of the grain yield was also assessed.

## Materials and methods

*Field trial.* The field experiment was conducted at the Department of Agronomy, Poznań University of Life Sciences, on the fields of the Department of Teaching and Experimental Station in Swadzim during 2005–2008 in a random block design (split-plot, main plots – N, subplots – S) with two tested factors in four field replications. The field experiment was conducted on the podsolic soil, light clay sand grade, shallowly deposited on the light clay belonging to a good rye complex. According to the FAO international soil classification (FAO, 1977), the examined soils are classified as *Phaeozems* or as *Mollisols* – according to the US Soil Taxonomy (Soil Survey Staff, 1951). The abundance of basic macronutrients (P, K, Mg) in the soil in each year of the study influenced at the average level, while its acidity ranged from 5.8 in 2008 to 6.1 in 2006. Total sulphur ranged from 0.16 in 2006 to 0.19 g kg$^{-1}$ soil in 2008. Estimation of Mg in the soil was performed by the Schachtschabel method, while K and P were determined by the Egner-Riehm method (Lityński et al., 1976). The experiment investigated the effect of the six doses of urea $CO(NH_2)_2$ (with nitrogen: $N_1$ – 0, $N_2$ – 30, $N_3$ – 60, $N_4$ – 90, $N_5$ – 120 and $N_6$ – 150 kg ha$^{-1}$) and three doses of elemental sulphur ($S_1$ – 0, $S_2$ – 20 and $S_3$ – 40 kg ha$^{-1}$) on the chlorophyll content and grain yield of the maize variety 'Anjou 258' (FAO 260–270). Maize was sown in row spacing of 70 cm at a depth of 4–5 cm. The content of humus in the arable layer (0–25 cm) in the years of research ranged from 1.41% to 1.46%. Phosphorus at a rate of 80 kg ha$^{-1}$ $P_2O_5$ was used in the form of the granulated triple superphosphate and potassium was used at a rate of 120 kg ha$^{-1}$ $K_2O$ in the form of potassium salt.

*Meteorological conditions.* Thermal and humidity conditions during the growing season in the study years were diversified for the growth and development of maize (Table 1). Total precipitation from April to September was 305.4 mm in 2005, 295.8 mm in 2006, 332.9 mm in 2007 and 346.3 mm in 2008. Hydrothermal coefficients (Table 1) taking both air temperature and precipitation into account comprehensively revealed that during the field experiment period, especially in 2006, deficiencies of water in soil occurred in June and July (maize flowering period), which led to a significant reduction in the grain yield. In 2008, a drought occurred in May and June but during this period maize utilized water stored in soil in April (hydrothermal coefficient 2.92, Table 1).

***Table 1.*** Air temperature, atmospheric precipitation and values of hydrothermal water supply factor according to Sielianinov during vegetation seasons in the Experimental Station in Swadzim

| Year | Month | | | | | | | Vegetation season |
|---|---|---|---|---|---|---|---|---|
| | 04 | 05 | 06 | 07 | 08 | 09 | 10 | |
| | Monthly mean air temperature, °C | | | | | | | |
| 2005 | 9.4 | 13.3 | 16.5 | 19.9 | 17.3 | 16.0 | 10.5 | 14.7 |
| 2006 | 8.8 | 13.8 | 18.7 | 24.4 | 17.7 | 17.2 | 11.3 | 15.9 |
| 2007 | 10.8 | 15.2 | 19.3 | 18.9 | 19.2 | 13.7 | 8.5 | 15.1 |
| 2008 | 9.1 | 15.1 | 19.6 | 20.7 | 18.8 | 13.5 | 9.5 | 15.2 |
| | Sum of rainfall, mm | | | | | | | |
| 2005 | 14.5 | 74.3 | 19.1 | 97.4 | 60.7 | 34.4 | 5.0 | 305.4 |
| 2006 | 43.6 | 57.4 | 26.9 | 23.1 | 100.7 | 22.0 | 22.1 | 295.8 |
| 2007 | 9.3 | 77.0 | 59.6 | 87.0 | 48.1 | 33.4 | 18.5 | 332.9 |
| 2008 | 79.8 | 14.3 | 8.6 | 65.6 | 95.1 | 19.4 | 63.5 | 346.3 |
| | Values of hydrothermal water supply factor according to Sielianinov | | | | | | | |
| 2005 | 0.51 | 1.80 | 0.39 | 1.58 | 1.13 | 0.72 | 0.15 | 0.90 |
| 2006 | 1.65 | 1.34 | 0.48 | 0.31 | 1.84 | 0.43 | 0.63 | 0.95 |
| 2007 | 0.29 | 1.63 | 1.03 | 1.48 | 0.81 | 0.81 | 0.70 | 0.97 |
| 2008 | 2.92 | 0.30 | 0.15 | 1.02 | 1.63 | 0.48 | 2.32 | 1.26 |

*Chlorophyll.* The content of chlorophyll *a*, *b*, *a* + *b* and chlorophyll expressed in the units of the soil and plant analysis development (SPAD) was determined at the 5–6 leaf stage (BBCH 15–16) and during the ear blooming stage (BBCH 67). For a detailed description of the method for chlorophyll determination in maize leaf blades, please refer to the earlier paper (Szulc, 2009 a). Chlorophyll content was determined using a spectrophotometer (Spekol type) at the appropriate wavelength. For chlorophyll *a,* the measurement of absorbance of the extract was performed at the wavelength of 663 nm and for chlorophyll *b* at the wavelength of 645 nm. The content of chlorophyll *a*, chlorophyll *b* and total chlorophyll *a* + *b* was calculated using the formulas from the paper by Arnon (1949). The amount of particular pigments was given in μg g$^{-1}$ of the fresh weight: chlorophyll $a = (12.7 \cdot A_{663} - 2.7 \cdot A_{645}) \cdot V \cdot (1000\ W)^{-1}$; chlorophyll $b = (22.9 \cdot A_{645} - 4.7 \cdot A_{663}) \cdot V \cdot (1000\ W)^{-1}$; total $a + b = (20.2 \cdot A_{645} - 8.02 \cdot A_{663}) \cdot V \cdot (1000\ W)^{-1}$, where $A_w$ is the absorbance at a given wavelength *w*, V – the total volume of the extract (cm$^3$) and W – the weight of a sample (g). In the case of the indirect method to determine the state of nitrogen nutrition of maize, the optical instrument known in Europe as the "Hydro N-Tester" ("Minolta", Japan) (range from 0 to 800), was used. The "Hydro N-Tester" is a hand-held instrument measuring the light transmittance of a leaf at the two specific wavelengths, based on the spectral absorption features of chlorophyll. The first wavelength is located in the red range (650 nm), specifically absorbed by chlorophyll, while the second, located in the infrared range (940 nm), is absorbed by cell walls and water but very slightly by chlorophyll and living vegetation. The quotient of these differences is the indicator of chlorophyll content and is referred to as SPAD units. Assessment of the chlorophyll content expressed in SPAD units at the both analysed developmental stages of maize was performed in 30 randomly selected plants of each experimental plot.

*Statistical analysis*. Firstly, the normality of distribution of the studied traits was tested using Shapiro-Wilk's normality test (Shapiro, Wilk, 1965). A three-way analysis of variance was carried out to determine the effects of years (Y), nitrogen (N) doses, elemental sulphur (S) doses and years × nitrogen (Y × N) doses, years × elementary sulphur (Y × S) doses, nitrogen × elemental sulphur (N × S) doses and years × nitrogen × elemental sulphur (Y × N × S) doses interactions on the variability of grain yield, content of chlorophyll *a*, *b*, *a* + *b* and chlorophyll expressed in SPAD units at the stages of 5–6 leaves (BBCH 15–16) and ear blooming (BBCH 67). The Tuckey's least significant differences ($LSD_Y$, $LSD_N$, $LSD_S$, $LSD_{YN}$, $LSD_{YS}$, $LSD_{NS}$, $LSD_{YNS}$) for each trait were calculated. The relationship between traits was estimated using correlation coefficients at different level (Kozak et al., 2010). Dependence of grain yield on selected traits was analyzed by using multiple regression. Data analysis was performed using the statistical package *GenStat Release 10.1* (2007). We used the critical significant level equal to 0.05.

## Results

The influence of fertilization of maize cultivated for grain with elemental sulphur depending on varied level of plant supply with nitrogen was assessed in the study. The effect of nitrogen and elemental sulphur on chlorophyll content was assessed at two developmental stages and evaluation how their content determines height of grain yield was carried out.

The results of the analysis of variance indicated that the main effects of years were significant for all observed traits ($P < 0.001$). Doses of nitrogen determined all traits ($P < 0.05$) except grain yield ($F_{5,162} = 2.58$, $P = 0.522$). The effects of years × nitrogen doses were significant for chlorophyll *a* ($F_{15,162} = 3.43$, $P < 0.001$), *b* ($F_{15,162} = 3.10$, $P < 0.001$) and *a* + *b* ($F_{15,162} = 3.37$, $P < 0.001$) at ear blooming stage.

Such an arrangement of the results was obtained due to the fact that in 2008 the highest average daily air temperature accompanied by the lowest total precipitation was recorded at the discussed developmental stages in the analyzed periods of maize growth (Table 2).

***Table 2.*** Weather conditions during the studied development stages and number of days from sowing to 5–6 leaf stage and from sowing to ear blooming stage in the Experimental Station in Swadzim

| Specification | Years | | | |
|---|---|---|---|---|
| | 2005 | 2006 | 2007 | 2008 |
| Sowing date | 16 04 | 21 04 | 20 04 | 26 04 |
| 5–6 leaf stage | 29 05 | 26 05 | 23 05 | 25 05 |
| Number of days (sowing–5–6 leaves) | 43 | 35 | 33 | 29 |
| Sum of rainfall mm (sowing–5–6 leaves) | 81.7 | 65.2 | 74.8 | 14.1 |
| Mean air temperature °C (sowing–5–6 leaves) | 12.2 | 13.7 | 13.9 | 14.8 |
| Ear blooming stage | 23 07 | 14 07 | 10 07 | 14 07 |
| Number of days (sowing–ear blooming) | 98 | 84 | 81 | 79 |
| Sum of rainfall (sowing–ear blooming) | 179.4 | 139.2 | 185.5 | 88.6 |
| Mean air temperature °C (sowing–ear blooming) | 14.9 | 17.6 | 16.5 | 17.7 |
| Number of days (5–6 leaves–ear blooming) | 55 | 49 | 48 | 50 |
| Sum of rainfall (5–6 leaves–ear blooming) | 97.3 | 74.0 | 110.7 | 74.5 |
| Mean air temperature (5–6 leaves–ear blooming) | 13.5 | 15.6 | 15.2 | 16.2 |

Statistical analysis of the obtained results indicated the importance of years in values of the concentration of chlorophyll *a* ($P < 0.05$), *a* + *b* ($P < 0.001$) (Table 3) and chlorophyll expressed in SPAD units ($P < 0.05$) (Table 4) at the 5–6 leaf stage (BBCH 15–16) under the influence of nitrogen fertilization.

However, proving the significance of this factor impact on the value of the studied traits in testing for the interaction (year × dose of nitrogen) shows a strongly directed influence ($P < 0.001$) of this factor and allows one to generalize the reasoning for the entire climatic-soil district (Table 3). On average, for years the content of chlorophyll *a* and *a* + *b* in the discussed developmental phase increased in the range of nitrogen doses from $N_1$ – 0 to $N_5$ – 120 kg ha$^{-1}$ (respectively: from 1.41 to 1.68 µg g$^{-1}$ of the fresh weight for chlorophyll *a* and from 1.61 to 1.89 µg g$^{-1}$ of the fresh weight for the total content of *a* + *b*). However, the use of the highest level of nitrogen fertilization dose, i.e. $N_6$ – 150 kg ha$^{-1}$ caused a significant decrease in their concentration in the leaf blades compared to the $N_5$ – 120 kg ha$^{-1}$ rate. The effect of the nitrogen dose on the value of these traits was confirmed in 2005, 2006 and 2008. Only in 2007 no significant effect of the nitrogen dose on the chlorophyll *a* ($P = 0.238$) and *a* + *b* ($P = 0.387$) content was shown in the discussed stage of development. However, it should be noted that the highest average concentration of pigments in the leaf blades was obtained in that year (Table 3). Overall, in the case of chlorophyll *b* none of the factors tested in the experiment significantly modified the concentration of pigment at the 5–6 leaf stage (BBCH 15–16) within the four years of the study (Table 3). Only in one out of the four experimental years, i.e. in 2008, the dose of nitrogen significantly differentiated the value of this trait ($P = 0.027$). The significantly lowest chlorophyll concentration was found for the dose of $N_1$ – 0 kg ha$^{-1}$ N (0.16 µg g$^{-1}$ of the fresh weight) in relation to the doses of $N_4$ – 90 and $N_5$ – 120 kg ha$^{-1}$ (respectively: 0.20 µg g$^{-1}$ of the fresh weight and 0.21 µg g$^{-1}$ of the fresh weight), bearing in mind that this relationship must be regarded as accidental.

***Table 3.*** The content of chloroplast pigments (in µg g$^{-1}$ fresh matter) at the 5–6 leaf stage (BBCH 15–16)

| Chlorophyll | Year | Dose N kg ha$^{-1}$ | | | | | | $LSD_N$ | Dose S kg ha$^{-1}$ | | | $LSD_S$ | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 90 | 120 | 150 | | 0 | 20 | 40 | | |
| *a* | 2005 | 1.25 | 1.26 | 1.29 | 1.44 | 1.40 | 1.38 | 0.09 | 1.33 | 1.35 | 1.34 | n.s. | 1.34 |
| | 2006 | 1.02 | 1.07 | 1.17 | 1.33 | 1.31 | 1.23 | 0.16 | 1.21 | 1.20 | 1.16 | n.s. | 1.19 |
| | 2007 | 1.80 | 1.87 | 1.93 | 1.97 | 2.09 | 1.96 | n.s.* | 1.88 | 2.00 | 1.93 | n.s. | 1.94 |
| | 2008 | 1.55 | 1.70 | 1.71 | 1.94 | 1.91 | 1.74 | 0.22 | 1.82 | 1.72 | 1.74 | n.s. | 1.76 |
| | mean | 1.41 | 1.48 | 1.52 | 1.67 | 1.68 | 1.58 | 0.07 | 1.56 | 1.57 | 1.54 | n.s. | – |
| *b* | 2005 | 0.15 | 0.16 | 0.16 | 0.18 | 0.16 | 0.17 | n.s. | 0.16 | 0.17 | 0.16 | n.s. | 0.16 |
| | 2006 | 0.14 | 0.14 | 0.15 | 0.16 | 0.15 | 0.15 | n.s. | 0.15 | 0.16 | 0.15 | n.s. | 0.15 |
| | 2007 | 0.34 | 0.25 | 0.25 | 0.27 | 0.29 | 0.27 | n.s. | 0.30 | 0.27 | 0.26 | n.s. | 0.28 |
| | 2008 | 0.16 | 0.18 | 0.18 | 0.20 | 0.21 | 0.18 | 0.03 | 0.19 | 0.18 | 0.19 | n.s. | 0.18 |
| | mean | 0.20 | 0.18 | 0.18 | 0.20 | 0.20 | 0.19 | n.s. | 0.20 | 0.19 | 0.19 | n.s. | – |
| *a* + *b* | 2005 | 1.41 | 1.43 | 1.45 | 1.63 | 1.56 | 1.56 | 0.12 | 1.49 | 1.53 | 1.50 | n.s. | 1.51 |
| | 2006 | 1.14 | 1.20 | 1.31 | 1.49 | 1.47 | 1.38 | 0.18 | 1.36 | 1.34 | 1.30 | n.s. | 1.33 |
| | 2007 | 2.15 | 2.13 | 2.19 | 2.25 | 2.38 | 2.23 | n.s. | 2.20 | 2.28 | 2.20 | n.s. | 2.22 |
| | 2008 | 1.72 | 1.89 | 1.90 | 2.15 | 2.13 | 1.93 | 0.25 | 2.02 | 1.98 | 1.93 | n.s. | 1.95 |
| | mean | 1.61 | 1.66 | 1.76 | 1.88 | 1.89 | 1.77 | 0.09 | 1.77 | 1.76 | 1.73 | n.s. | – |

*Note.* *n.s. – not significant statistical difference; $LSD_{NS}$ is not significant, $LSD_{YNS}$ for chlorophyll *a* and *b* – n.s., $LSD_{YNS}$ for chlorophyll *a* + *b* – 0.27.

***Table 4.*** The content of chlorophyll expressed in soil and plant analysis development (SPAD) units

| Experimental factor | | 5–6 leaf stage (BBCH 15–16) | | | | | Ear blooming stage (BBCH 67) mean value for years 2005–2008 ± standard deviation |
|---|---|---|---|---|---|---|---|
| | | years | | | | | |
| | | 2005 | 2006 | 2007 | 2008 | years mean | |
| Dose N kg ha$^{-1}$ | 0 | 271.8 | 474.0 | 416.0 | 389.3 | 387.8 | 560.3 ± 14.6 |
| | 30 | 294.5 | 482.5 | 482.6 | 398.0 | 414.4 | 621.7 ± 21.4 |
| | 60 | 307.2 | 498.5 | 486.3 | 400.5 | 423.1 | 648.8 ± 19.9 |
| | 90 | 314.6 | 504.1 | 542.1 | 406.8 | 441.9 | 661.2 ± 23.7 |
| | 120 | 287.2 | 501.9 | 608.7 | 421.5 | 454.8 | 678.8 ± 38.9 |
| | 150 | 281.6 | 475.4 | 470.0 | 417.5 | 411.1 | 660.0 ± 12.7 |
| $LSD_N$ | | 22.35 | 32.12 | n.s.* | n.s. | 30.66 | 58.21 |
| Dose S kg ha$^{-1}$ | 0 | 295.9 | 487.4 | 511.5 | 408.0 | 425.7 | 643.9 ± 20.1 |
| | 20 | 293.4 | 492.0 | 489.8 | 414.6 | 422.4 | 643.0 ± 19.7 |
| | 40 | 289.2 | 488.9 | 501.5 | 394.0 | 418.4 | 646.1 ± 17.3 |
| $LSD_S$ | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Mean | | 292.8 | 489.4 | 501.0 | 405.6 | – | – |

*Note.* *n.s. – not significant statistical difference; $LSD_{NS}$ is not significant, $LSD_{YNS}$ for chlorophyll *a* and *b* – n.s., $LSD_{YNS}$ for chlorophyll *a* + *b* – 11.5.

In the case of chlorophyll content expressed in SPAD units, the value of this trait increased at the 5–6 leaf stage in the range of nitrogen doses from $N_1$ – 0 to $N_5$ – 120 kg ha$^{-1}$ (respectively: from 387.8 to 454.8) (Table 4). It should be noted, however, that the value of this trait for nitrogen doses in the range from $N_2$ – 30 to $N_6$ – 150 kg ha$^{-1}$ was statistically ($P > 0.05$) at the same level. The above generalization was confirmed in 2007 and 2008 but in 2005 and 2006 the highest concentration of chlorophyll expressed in SPAD units was found for the dose of nitrogen amounting to $N_4$ – 90 kg ha$^{-1}$ (Table 4). In the case of chlorophyll *a*, *b*, *a* + *b* (Table 5) and chlorophyll expressed in SPAD units (Table 4) at the ear blooming stage (BBCH 67), the conducted statistical analysis showed the interaction of years with nitrogen fertilization ($P < 0.001$, $P < 0.001$, $P < 0.001$ and $P < 0.001$ for, respectively, the contents of chlorophyll *a*, *b*, *a* + *b* and SPAD).

In contrast, for chlorophyll *b* also the interaction of years with elemental sulphur determined the development of this trait value ($P = 0.019$). The direction of these changes throughout all the years was similar. Statistically confirmed interaction resulted only from differences in factor strength in particular years of the experiment. Hence, aiming to present the relationships and formulate generalizations in a clearer form, the paper presents the influence of fertilization on the traits by using mean values from the years of the study. Content of chlorophyll *a*, *b*, *a* + *b* (Table 5) and chlorophyll expressed in SPAD units (Table 4) at the discussed maize development stage increased in the range of nitrogen doses from $N_1$ – 0 to $N_5$ – 120 kg ha$^{-1}$. However, the application of the highest level of nitrogen at the dose of $N_6$ – 150 kg ha$^{-1}$ resulted in a significant decrease of the pigments in maize leaves in relation to the dose of $N_5$ – 120 kg ha$^{-1}$, with the exception of chlorophyll expressed in SPAD units, for which the value of this trait for nitrogen doses ranging from $N_2$ – 30 to $N_6$ – 150 kg ha$^{-1}$ was statistically at the same level of significance ($P = 0.473$). For chlorophyll *b* at the ear blooming stage (BBCH 67), on average for the years of the study, its significantly highest level was found for the dose $S_2$ (0.27 µg g$^{-1}$ of the fresh weight), compared to the doses of $S_1$ and $S_3$, for which the value of this trait was statistically at the same level (Table 5). The obtained results indicate the significant ($P < 0.05$) influence of weather conditions, varied between the years of the study on the size of the resulting grain yield (Table 6).

***Table 5.*** The content of chlorophyll at the ear blooming stage (BBCH 67)

| Chlorophyll | Year | Dose N kg ha$^{-1}$ | | | | | | $LSD_N$ | Dose S kg ha$^{-1}$ | | | $LSD_S$ | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 90 | 120 | 150 | | 0 | 20 | 40 | | |
| *a* | 2005 | 1.19 | 1.24 | 1.46 | 1.55 | 1.68 | 1.58 | 0.23 | 1.41 | 1.48 | 1.45 | n.s. | 1.45 |
| | 2006 | 1.09 | 1.16 | 1.23 | 1.34 | 1.32 | 1.33 | 0.15 | 1.24 | 1.25 | 1.25 | n.s. | 1.24 |
| | 2007 | 1.90 | 1.94 | 1.94 | 2.51 | 2.22 | 1.51 | 0.18 | 1.95 | 2.05 | 1.83 | n.s. | 1.94 |
| | 2008 | 1.78 | 1.79 | 2.14 | 2.14 | 2.37 | 1.98 | 0.34 | 2.04 | 2.05 | 2.01 | n.s. | 2.03 |
| | mean | 1.48 | 1.53 | 1.69 | 1.79 | 1.89 | 1.59 | 0.22 | 1.65 | 1.70 | 1.63 | n.s. | – |
| *b* | 2005 | 0.21 | 0.21 | 0.25 | 0.24 | 0.26 | 0.25 | 0.04 | 0.22 | 0.25 | 0.24 | n.s. | 0.24 |
| | 2006 | 0.17 | 0.19 | 0.20 | 0.21 | 0.20 | 0.21 | n.s.* | 0.20 | 0.20 | 0.20 | n.s. | 0.20 |
| | 2007 | 0.27 | 0.29 | 0.29 | 0.30 | 0.32 | 0.20 | 0.06 | 0.28 | 0.30 | 0.26 | n.s. | 0.28 |
| | 2008 | 0.28 | 0.29 | 0.32 | 0.33 | 0.35 | 0.31 | 0.04 | 0.32 | 0.32 | 0.31 | n.s. | 0.31 |
| | mean | 0.23 | 0.24 | 0.26 | 0.27 | 0.28 | 0.24 | 0.04 | 0.25 | 0.27 | 0.25 | 0.017 | – |
| *a* + *b* | 2005 | 1.41 | 1.45 | 1.71 | 1.79 | 1.95 | 1.83 | 0.22 | 1.64 | 1.73 | 1.69 | n.s. | 1.69 |
| | 2006 | 1.27 | 1.35 | 1.43 | 1.55 | 1.53 | 1.54 | 0.14 | 1.44 | 1.45 | 1.45 | n.s. | 1.45 |
| | 2007 | 2.17 | 2.32 | 2.22 | 2.46 | 2.55 | 1.71 | 0.09 | 2.24 | 2.35 | 2.09 | 0.07 | 2.22 |
| | 2008 | 2.06 | 2.08 | 2.08 | 2.48 | 2.72 | 2.30 | 0.11 | 2.36 | 2.38 | 2.32 | n.s. | 2.35 |
| | mean | 1.72 | 1.77 | 1.95 | 2.06 | 2.18 | 1.84 | 0.25 | 1.91 | 1.97 | 1.88 | n.s. | – |

*n.s. – not significant statistical difference

***Table 6.*** Grain yield of maize

| Experimental factor | | Grain yield (dt ha$^{-1}$) 15% $H_2O$ | | | | |
|---|---|---|---|---|---|---|
| | | years | | | | |
| | | 2005 | 2006 | 2007 | 2008 | mean |
| Dose N kg ha$^{-1}$ | 0 | 66.7 | 9.8 | 98.0 | 97.1 | 67.9 |
| | 30 | 67.1 | 17.4 | 100.4 | 98.7 | 70.9 |
| | 60 | 70.3 | 19.6 | 103.1 | 99.4 | 73.1 |
| | 90 | 73.8 | 25.8 | 109.4 | 100.2 | 77.3 |
| | 120 | 71.5 | 32.8 | 107.5 | 99.5 | 77.8 |
| | 150 | 71.9 | 32.3 | 107.9 | 95.9 | 77.0 |
| $LSD_N$ | | n.s.* | 3.4 | 2.1 | n.s. | 2.4 |
| Dose S kg ha$^{-1}$ | 0 | 69.2 | 21.9 | 103.9 | 94.9 | 72.5 |
| | 20 | 70.2 | 23.4 | 104.7 | 99.9 | 74.6 |
| | 40 | 71.3 | 23.5 | 104.6 | 100.6 | 74.9 |
| $LSD_S$ | | n.s. | n.s. | n.s. | 2.7 | 2.0 |
| Mean | | 70.2 | 22.9 | 104.4 | 98.5 | – |

*n.s. – not significant statistical difference; $LSD_{NS}$ and $LSD_{YNS}$ are not significant

Maize grain yield was the lowest in 2006 (22.93 dt ha$^{-1}$), which was characterized by a period of drought occurring in June and July (Table 1). In turn, the highest maize yield was observed in 2007 (104.38 dt ha$^{-1}$), which was characterized by an optimal distribution of precipitation during the growth and development of this plant. The size of nitrogen dose only in 2007, while elemental sulphur in 2008 significantly modified the size of grain yield (Table 6).

In the years of the study, the same relationship as described above was observed but these differences could not be confirmed statistically. It should also be noted that the demonstrated effect of nitrogen doses ($P = 0.004$) and elemental sulphur ($P = 0.019$) on the size of the grain yield, although modified in the years, was so powerful that their influence was statistically proven in the synthetic configuration (Table 6). The significantly lowest grain yield was found for the nitrogen dose of $N_1$ – 0 kg ha$^{-1}$ (67.92 dt ha$^{-1}$), while the highest for the dose of $N_6$ – 120 kg ha$^{-1}$ (77.83 dt ha$^{-1}$). It should be noted, however, that the size of grain yield for nitrogen doses ranging from $N_4$ – 90 to $N_6$ – 150 kg ha$^{-1}$ was statistically at the same level. Regarding the impact of the dose of elemental sulphur on the value of this trait, the significantly lowest maize grain yield was obtained on the $S_1$ (72.46 dt ha$^{-1}$), compared to the two other levels of this micronutrient (respectively: $S_2$ – 74.58 and $S_3$ – 74.98 dt ha$^{-1}$). It should be noted, however, that the increase in the size of grain yield for the doses of elemental sulphur $S_2$ and $S_3$, compared to the $S_1$ dose did not differ significantly (Table 6). Analysis of grain moisture in grain at harvest did not confirm the significance of the effects of the tested factors on the value of this trait. Grain moisture ranged from 26.81% in 2007 to 31.71% in 2006. What is noteworthy is the highest moisture content of the maize grain in the dry year 2006 when maize yield was the lowest. Most pairs of the traits correlated statistically significantly. Only for the content of chlorophyll *b* at the BBCH 67 stage and chlorophyll expressed in SPAD units at the BBCH 15–16 stage and between chlorophyll expressed in SPAD units at the BBCH 15–16 stage and grain yield there were no significant linear correlations ($r = 0.090$ and $r = -0.029$, respectively). All statistically significant correlation coefficients were positive. Using multiple regression analysis the traits which determined the total grain yield were listed.

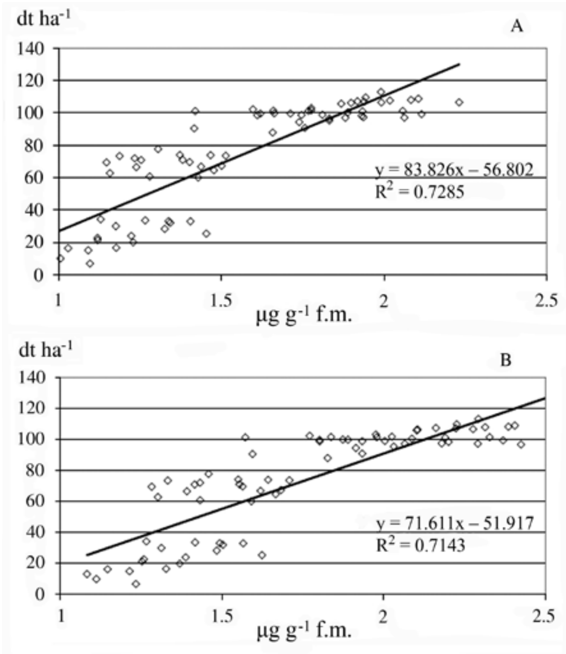
Among the traits analyzed in the paper, the content of chlorophyll *a* + *b* at the BBCH 15–16 phase ($x_2$), chlorophyll *a* + *b* at the BBCH 67 stage ($x_3$) and chlorophyll expressed in SPAD units at the BBCH 67 stage ($x_5$) had a directly proportional influence on grain yield, while the content of chlorophyll *b* ($x_1$), and chlorophyll expressed in SPAD units ($x_4$) – both at the BBCH 15–16 stage – had an inversely proportional influence:

$$gy = -57.5 - 112.2\,x_1 + 49.15x_2 + 16.87x_3 - 0.078x_4 + 0.105x_5.$$

The variability of these five traits explained 58.8% of the general grain yield variability.

## Discussion

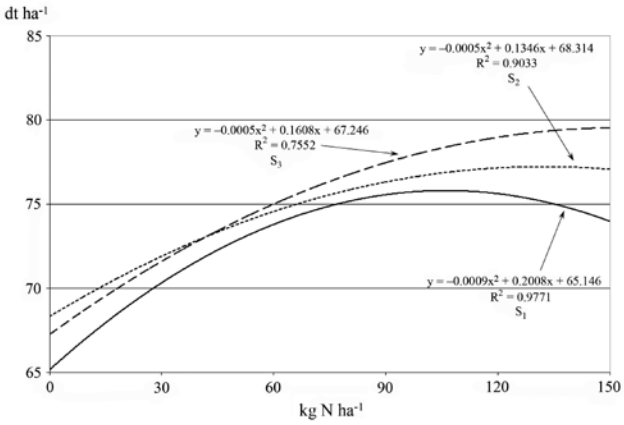
Maize is a very heliophilous and thermophilic plant. The assimilation surface (absorbing sunlight) of a single maize plant is large and amounts to approximately 4500 cm$^2$ (Szulc, 2009 b). In turn, the leaf area index (LAI), which describes the ratio of the leaf area to the ground surface where plants grow, is an important parameter describing the field of corn (Nieróbca, Faber, 1996). Nalborczyk et al. (1995) suggest that the size of the LAI determines photosynthetic efficiency and thus also indirectly the increase of the plant vegetative and grain yield. In addition, the productivity of maize hybrids depends on chlorophyll content in leaf blades. According to Szulc and Rybus-Zając (2009), chlorophyll content at the ear blooming stage (BBCH 67) depends strictly on the assimilation area of a single plant, as indicated by a rectilinear relationship between the content of chlorophyll *a* + *b*, as well chlorophyll expressed in SPAD units, and the assimilation surface of a single plant as well as the LAI indicator. As the assimilation area of a single plant and assimilation area of maize canopy (LAI) increased, the content of pigments declined steadily. According to Wojcieska-Wyskupajtys (1996), large leaf assimilation area leads to mutual shading and reduction of the photosynthetic rate of shaded leaves. This is one of the reasons for only partial utilization of the potential photosynthetic capabilities of the total leaf area. Chlorophyll content is a varietal characteristic; however, as numerous studies show, its concentration in leaf blades is further shaped by the agrotechnical interventions, e.g., fertilization, variety selection (Szulc et al., 2008 b; Szulc, Waligóra, 2010) and by the method of application of nitrogen fertilizer as well as by the inoculation of seeds with *Fusarium culmorum* fungus (Szulc et al., 2008 a). The development of maize is divided into a number of stages, each of which fulfils a specific role in its life cycle. The juvenile stage of maize growth shapes the size of the grain yield because the number of grain rows in the cob is determined. It is also a stage of maize growth when final adjustments to the prior (incorrect) use of mineral fertilizers can be made. Hence, in our study at the 5–6 leaf stage (BBCH 15–16) the content of chlorophyll and chlorophyll expressed in SPAD units was determined, as they are the methods indirectly characterizing the nutrition of the plant by nitrogen. According to Subedi and Ma (2005), nitrogen malnutrition of plants prior to the 8-leaf stage leads to an irreversible reduction in the number of cobs and potential kernels in the cob even up to about 30%. Binder et al. (2000) showed that the delay in nitrogen application until the stage of the 6th leaf reduced the grain yield by 12%. Our study demonstrated a significant impact of the dose of nitrogen (urea) on the amount of chlorophyll *a* ($P < 0.001$), *a* + *b* ($P < 0.001$) and chlorophyll expressed in SPAD units ($P = 0.047$) at the juvenile stage. The highest values of these traits were found for the nitrogen dose of $N_5$ – 120 kg ha$^{-1}$. Chlorophylls play an important role in biosynthetic processes occurring in green parts of the plant. Together with carotenoids they absorb light energy turning it into chemical energy which is used in the synthesis of organic compounds from simple substances (Młodzińska, 2009). The higher their concentration, the greater the potential productivity of crop plants is. Our study demonstrated the foregoing finding as with the increasing chlorophyll *a* and chlorophyll *a* + *b* content at the 5–6 leaf stage (BBCH 15–16) the yield of maize grain increased in a linear manner (Fig. 1).




*Note.* $R^2$ – coefficient of determination; both regression lines are statistically significant at the $\alpha = 0.05$ level.

***Figure 1.*** The dependence of grain yield (y) size on the content of chlorophylls *a* (A) and *a* + *b* (B) (x) in the stage of 5–6 leaves (BBCH 15–16)

Also Szulc and Waligóra (2010) and Szulc et al. (2011) showed in their field experiment that with the increasing chlorophyll content expressed in SPAD units at the 5–6 leaf stage the yield of maize grain also increased. This shows that good nitrogen nutrition of maize in the early phase of development determines final yield, as was confirmed by Binder et al. (2000) and Subedi and Ma (2005). In the presented study, a significant positive correlation between the contents of particular chlorophylls (except SPAD at the BBCH 15–16 stage) and grain yield was observed. However, considering the total impact of the traits on the yield, some of them (chlorophyll *b* and SPAD at the BBCH 15–16 stage) were characterized by an inversely proportional relationship. In addition to a typically nutritional aspect, the optimal supply of crops with sulphur has also an ecological significance as its deficiency leads to the reduced utilization of nitrogen fertilizer, which in consequence can lead to the eutrophication of soil by this macroelement (Jackson, 2000). In the absence of sulphur in the soil, nitrogen fertilizer does not show optimal performance. The introduction of additional (larger and thus economically unfounded) nitrogen doses in the situation of permanent deficiencies of this microelement in the soil increases these deficiencies, which in consequence leads to a further reduction in yields and deterioration of their quality (Jakubus, 2008). In the 4-year field experiment, the impact of fertilization with elemental sulphur on maize cultivated in grain technology, depending on the different levels of maize supply with nitrogen (urea), was analyzed. A significant impact of the dose of urea and elemental sulphur on the yield of grain was demonstrated (Table 6). The highest maize yield was obtained for the nitrogen dose of $N_5$ – 120 kg $ha^{-1}$ (77.83 dt $ha^{-1}$), while its size was statistically at the same level in the range of nitrogen doses from $N_4$ – 90 to $N_6$ – 150 kg $ha^{-1}$. The use of fertilization with elemental sulphur significantly increased maize grain yield by 2.12 dt $ha^{-1}$ (for the dose $S_2$ – 20 kg $ha^{-1}$) and by 2.52 dt $ha^{-1}$ (for the dose of $S_3$ – 40 kg $ha^{-1}$) in relation to the situation without the use of this microelement. The result obtained in our study corresponds with the results obtained by Szulc et al. (2008 c). They showed that due to the use of elemental sulphur with NPK fertilization the increase in the yield of maize grain ranged from 7.0% (for the dose of 40 kg $ha^{-1}$ S) to 8.4% (for 20 kg $ha^{-1}$ S) in relation to the exclusive use of NPK. Also Salvagiotti and Miralles (2008) showed an increase in the yield of wheat grain as a result of applying nitrogen with sulphur, which was reflected in the nitrogen utilization efficiency. Fotyma (2003) reported that the agricultural and physiological efficiency of fertilizing crops with nitrogen was higher after the application of fertilizers containing sulphur than without such treatment. According to this author, the utilization of nitrogen by rape and maize from the doses of the element above 100 kg $ha^{-1}$, after the application of fertilizers containing sulphur was higher by 10–15% than without such treatment, which is consistent with the literature on the subject. In our study, the yield of maize grain yield was also determined by the interaction of nitrogen doses with the doses of elemental sulphur, $P = 0.039$ (Fig. 2).




$R^2$ – coefficient of determination

***Figure 2.*** The size of maize grain yield depending on the relationship between the doses of nitrogen and elementary sulphur ($S_1$ – 0, $S_2$ – 20, $S_3$ – 40 kg $ha^{-1}$ S)

These relationships were described by the two equations, with the curves for $S_2$ and $S_3$ doses influencing at a higher level compared to the $S_1$ dose. This demonstrates the greater efficiency of using nitrogen when applied with elemental sulphur, as their interaction determines the assimilation of nitrogen in the plant, which was shown by Janzen and Bettany (1984).

## Conclusions

1. The content of chlorophyll *a* and *a* + *b* at the 5–6 leaf stage (BBCH 15–16) was significantly influenced by the nitrogen fertilization in three out of four years of the experiment, while for chlorophyll (in SPAD units) in two out of four years of the study; this also applied to the average value for the years. The increase in nitrogen fertilization resulted in increasing content of the pigments determined in maize leaf blades at the discussed developmental stage.

2. The content of chlorophyll *a*, *b* and *a* + *b* and chlorophyll (expressed in SPAD units) at the ear blooming stage (BBCH 67) increased in the range of nitrogen doses from $N_1$ – 0 to $N_5$ – 120 kg $ha^{-1}$. The use of the highest dose, i.e. $N_6$ – 150 kg $ha^{-1}$ decreased the value of these traits, compared to the level of $N_5$ – 120 kg $ha^{-1}$, with the exception of chlorophyll (in SPAD units) for which the value of this trait in the range of nitrogen doses from $N_2$ – 30 to $N_6$ – 150 kg $ha^{-1}$ was statistically at the same level. On average for the years of the study, the significantly highest content of chlorophyll *b* at the ear blooming stage (BBCH 67) was demonstrated for the dose of $S_2$ – 20 kg $ha^{-1}$, compared to the doses of $S_1$ – 0 and $S_3$ – 40 kg $ha^{-1}$, for which the value of this trait was statistically at the same level.

3. The size of the grain yield of maize was significantly determined by the level of applied nitrogen and elemental sulphur. The size of grain yield for nitrogen doses ranging from $N_4$ – 90 to $N_6$ – 150 kg $ha^{-1}$ did not differ significantly. The significantly lowest grain yield was found for the elemental sulphur dose of $S_1$ – 0 kg $ha^{-1}$ compared to the doses of $S_2$ – 20 and $S_3$ – 40 kg $ha^{-1}$.

4. Combined fertilization of maize with nitrogen and elemental sulphur increased the nitrogen use efficiency. The curves describing this relationship for combined application of N and S were higher in comparison with the curve depicting only the influence of nitrogen dose on grain yield.

5. The size of the grain yield of maize was determined predominantly by the content of chlorophyll *b*, *a* + *b* and chlorophyll expressed in SPAD units at the BBCH 15–16 stage and the content of chlorophyll *a* + *b* and chlorophyll expressed in SPAD units at the BBCH 67 stage.

Received 20 01 2012
Accepted 30 08 2012

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ISSN 1392-3196
Žemdirbystė=Agriculture, vol. 99, No. 3 (2012), p. 247–254
UDK 633.15:631.84:631.576.331.2 631.165

# Azoto ir elementinės sieros įtaka paprastojo kukurūzo (*Zea mays* L.) chlorofilo kiekiui ir grūdų derliui

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## Santrauka

Kukurūzai yra didelį produktyvumo potencialą turintys augalai, kuriems augimo ir vystymosi metu reikia žymiai didesnio kiekio maisto medžiagų nei kitiems grūdiniams augalams. Iš visų maisto medžiagų derliaus dydį labiausiai nulemia azoto kiekis. Tyrimo tikslas – įvertinti azoto ir elementinės sieros įtaką kukurūzų chlorofilo kiekiui ir grūdų derliui, juos auginant pagal javų auginimo technologiją. Chlorofilas nustatytas augalams esant 5–6 lapelių ir varpų žydėjimo tarpsnių. Lauko bandymas vykdytas 2005–2008 m. Poznanės gyvybės mokslų universiteto Agronomijos katedros Mokymo ir bandymų stoties laukuose Swadzime, taikant atsitiktinių imčių išskaidytų laukelių, išdėstytų 4 pakartojimais, metodą. Tyrimo rezultatai parodė, kad kukurūzų 5–6 lapelių augimo tarpsniu chlorofilo *a* ir *a* + *b* kiekiui esminės įtakos turėjo tręšimas azotu trejus iš ketverių tyrimo metų, o chlorofilui, išreikštam SPAD (angl. *soil and plant analysis development*) vienetais, – tręšimas azotu dvejus iš ketverių tyrimo metų. Kukurūzų grūdų derliui esminės įtakos turėjo tręšimo azotu ir elementine siera lygis. Didesnis azoto pasisavinimo efektyvumas grūdų derliui nustatytas jį panaudojus kartu su elementine siera. Kreivės, apibūdinančios azoto ir sieros naudojimo kartu ryšį kukurūzų grūdų derliui, buvo aukštesnės, lyginant su vien tik azoto įtaka.

Reikšminiai žodžiai: *Zea mays*, siera, azotas, chlorofilas, grūdų derlius.