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# **IMPACT OF FOLIAR NITROGEN AND MAGNESIUM FERTILIZATION ON CONCENTRATION OF CHLOROPHYLL IN POTATO LEAVES**

## **ODDZIAŁYWANIE DOLISTNEGO NAWOŻENIA AZOTEM I MAGNEZEM** NA ZAWARTOŚĆ CHLOROFILU W LIŚCIACH ZIEMNIAKA

**Abstract:** The objective of the study has been to determine the effect of foliar nitrogen and magnesium fertilization on modifications in the concentration of chlorophyll in leaves of an medium-early edible potato cultivar called Zebra. Different rates of foliar magnesium fertilization were applied, from 0 % to 50 % of the full dose of this nutrient (80 kgN  $\cdot$  ha<sup>-1</sup>). The experiment was conducted in three series – without magnesium fertilization, with magnesium applied to soil  $(24 \text{ kgMg} \cdot \text{ha}^{-1})$  and with magnesium sprayed over leaves (12 kgMg  $\cdot$  ha<sup>-1</sup>). The highest concentration of chlorophyll *a* and *b* was obtained in 2005: 137.6 and 53.4 mg × 100 g of fresh mass of leaves. In the same year, the highest yield of potato tubers was produced: on average,  $27.86 \text{ Mg} \cdot \text{ha}^{-1}$ . This relationship, however, was not confirmed in the subsequent years. In 2006 and 2007, the two years with the weather conditions unfavourable to potato cultivation, the volume of yields was positively correlated with the concentration of chlorophyll in leaves, a relationship which was not detected in 2005, when the yield was the highest. Soil or foliar application of magnesium determined the synthesis of chlorophyll and accumulation of yield, especially in the years characterized by unfavourable weather conditions.

**Keywords:** chlorophyll, mineral fertilizers, magnesium, nitrogen, tuber yield, *Solanum tuberosum*, potato

#### **Introduction**

Natural pigments present in green plants are mainly magnesium-containing chlorophyll *a* (bluish green), which is the major photoreceptor, and chlorophyll *b* (greenish yellow), whose concentration in leaves is about 2- to 4-fold lower. The other pigments are pheophytin and carotenoids. Chlorophyll *a* and *b* differ in the substituent group at the third carbon atom (Chl *a* -CH<sup>3</sup> , Chl *b* -CHO) in the porphyrin ring, which has an atom of magnesium in its centre. Among the factors which affect the rate of chlorophyll synthesis are sunlight exposure and intensity [1, 2], and among such agronomic

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treatments the most important ones are nitrogen [3] and magnesium fertilization [4, 5]. By measuring the concentration of chlorophyll in leaves, it is possible to assess the plant's photosynthetic capability [6]. The potato is a plant which is capable of producing very high yields. The yields obtained in European countries (Belgium, the Netherlands, France) reaching  $40-50$  Mg tubers  $\cdot$  ha<sup>-1</sup> require high and adequately balanced fertilization, which will first of all affect the growth of aerial parts, including the assimilating leaf area, expressed by the *leaf green area* (LAI) or the *green area index* (GAI), as well as synthesis of the plant pigments in leaves responsible for the course of photosynthesis [5]. Concentrations of these pigments determine the rate of accumulation of assimilates in storage organs of plants [3]. The development of plant aerial mass responsible for photosynthesis is distinctly affected by high availability of nitrogen (N), which in turn ensures a high yield of tubers [7]. Potato plants respond very dynamically to nitrogen fertilization by raising the concentration of chlorophyll in leaves and the effects of nitrogen application are observable in just 36 hours afterwards – especially in the case of young and fast growing potato plants [8]. According to Wierzejska-Bujakowska [9], when growing edible potato, nitrogen should be introduced together with the other nutrients in the following ratio: N:P:K as 1.0:0.4:1.3. When nitrogen is not adequately balanced, the quality of harvested tubers suffers [10]. An excessively high share of nitrogen versus the other elements leads to the formation of a large aerial mass but the tubers are often not fully mature, have a low proper mass, contain low levels of complex compounds, such as storage starch, protein, polyphenolic substances, citric acid and vitamin C, but are too rich in simple sugars, free amino acids and mineral nitrogen [11, 12]. Under elevated N fertilization, the tuber formation process can be restricted [13, 14]. Apart from nitrogen, magnesium is another element which plays an important role in potato nutrition [5, 15]. Magnesium is taken up by plants during their later vegetative growth and therefore they need additional, foliar application of this nutrient. Recommended forms of magnesium for foliar treatments are magnesium sulphate heptahydrate in a concentration of 5 %, and magnesium sulphate monohydrate in a concentration of 2–3 %, which is recommended to be used together with an aqueous solution of urea [16–19].

In the presented experiment discussed the effect of foliar and soil nitrogen and magnesium fertilization has been tested with respect to concentrations of chlorophyll *a*, *b* and sum of chlorophyll *a+b* in leaves of cv. Zebra potato. The relationships between the applied fertilization variants and concentrations of particular forms of chlorophyll as well as potato yields have also been analyzed.

# **Material and methods**

The results originate from a three-year field experiment, set up at the Experimental Station in Tomaszkowo near Olsztyn  $(53°42'35"$  N,  $20°26'01"$  E) in 2005. The experiment was established on proper brown soil developed from weak loamy sand class IVb in the Polish soil valuation system, classified as good rye complex. According to the FAO/WRB (*World Reference Base for Soil Resources*) [20], this soil belonged to Cambisols – Brown Soils. The effect of foliar nitrogen fertilization combined with foliar and soil magnesium fertilization on concentrations of chlorophyll in leaves of a medium-early potato cultivar Zebra (*Plant Breeding Station in Szyldak, Ltd.*) was examined. The study involved a two-factor experiment in random blocks with four replications, including different nitrogen and magnesium fertilization variants, either applied to soil or sprayed over leaves. The experiment consisted of three series: in the first one, nitrogen fertilization alone was applied in a rate of 80 kgN  $\cdot$  ha<sup>-1</sup>, with a gradually increasing share of foliar nutrition  $(0, 10 \%, 20 \%, 30 \%, 40 \%, 50 \%)$  at the expense of soil fertilization, which equalled 80, 72, 64, 56, 48 and 40 kgN  $\cdot$  ha<sup>-1</sup>; the other two series included additional magnesium fertilization. In the second series, magnesium was introduced to soil in a rate of 24 kgMg  $\cdot$  ha<sup>-1</sup> and in the third one, it was sprayed over leaves in an amount of 12 kgMg  $\cdot$  ha<sup>-1</sup>. Phosphorus and potassium fertilization rates were constant in all the treatments and equalled 35 kgP and 100 kgK  $\cdot$  ha<sup>-1</sup>. The phosphorus fertilizer, granular triple superphosphate 20 % P ( $Ca(H_2PO_4)_2$ ) and the potassium one, potassium salt 50 % K (KCl) were applied in a single dose to soil before planting potatoes. Nitrogen was used as urea 46 % N ( $CO(NH<sub>2</sub>)<sub>2</sub>$ ), and magnesium in the form of magnesium sulphate  $(MgSO_4 \cdot 7H_2O)$ . Whole amounts of the fertilizers introduced to soil were applied before planting potatoes, and the ones used for foliar fertilization were sprayed in five doses during the plants' vegetative season. The first spraying treatment was performed after the rows of potato plants became compact and the first flower buds formed. The subsequent treatments were carried out in 7-day intervals. The working concentration of the solutions of fertilizers applied to leaves was 6.9 % of urea and 10.0 % of magnesium sulphate. The rows were spaced at 62.5 cm and the distance between planted potatoes in a row was 40 cm. Thus, the calculated plant density was 40 thousand plants per ha<sup>-1</sup>. The area of plots for harvest was 12.96 m<sup>2</sup>. The yields obtained from the plots after harvest were recalculated and expressed as Mg tubers  $\cdot$  ha<sup>-1</sup>.

Samples of leaves for analyses of the content of chlorophyll were taken from the 2nd and  $3<sup>rd</sup>$  tier in the apical part of a potato plant. The content of chlorophyll *a*, *b* and sum of chlorophyll  $a+b$  was determined with the colorimetric method using 80 % acetone solution as an extractant (POCh Gliwice) [21]. Leaf blades were cut into minute fragments and 1.0 g samples were taken from the green mass thus obtained. The samples were placed in tubes, to which 80 % acetone solution was poured. After 12-h maceration at  $4 \degree C$ , the content of each tube was homogenized, quantitatively transferred onto a Schott #G3 funnel and filtered under partial vacuum, rinsing with 80 % acetone. The filtrate was filled up to 100 cm<sup>3</sup> and thoroughly mixed, after which the absorbance was measured at the wavelengths of  $\lambda = 645$  and 663 nm versus 80 % acetone solution as a zero sample. The calculations included the equation proposed by Arnon [22], which is applied to colorimetric determination of concentrations of plant pigments [21, 23]. The coefficients specific for particular wavelengths were adopted from Mackinney's work [24].

- Chl  $a = 12.0 \cdot A_{663} 2.69 \cdot A_{645}$  [mgChl  $\cdot$  dm<sup>-1</sup>]
- Chl  $b = 22.90 \cdot A_{645} 4.68 \cdot A_{663}$
- Chl  $a+b = 20.20 \cdot A_{645} + 8.02 \cdot A_{663}$

The absorbance of the assayed chlorophyll solutions was determined in a 1 cm path length quartz cuvette flow spectrophotometer type Specol 220 (Carl Zeiss Jena). The content of chlorophyll expressed in mg  $dm^{-3}$  of extract was converted into mg 100  $g^{-1}$ of fresh mass of leaf tissue.

The results were processed statistically with ANOVA at the level of significance of  $\alpha$  = 0.05, using a Statistica v. 9.0 software package [25]. The correlation between the analyzed factors was established using a simple correlation coefficient, with the Microsoft Excel programme [26].

## **Results and discussion**

Chlorophyll is a plant pigment, whose content in a plant depends on several factors. They range from agronomic treatments, *eg* applied fertilization, to environmental conditions. The rate of chlorophyll synthesis can be limited, for instance, by water deficit in soil [27], high and low temperatures [28, 29], salinity [30] and light intensity [1, 2]. The results discussed in this paper concerning the content of chlorophyll in potato leaves demonstrate a high degree of variation between the years. Such high changeability in the content of chlorophyll under the influence of the weather occurring during potato cultivation has also been suggested by Wyszkowski [31]. In the three years of the experiment, the temperature, air humidity and soil moisture which prevailed in 2005 (Fig. 1) resulted in the highest concentration of chlorophyll *a* (Chl *a*) 137.6; chlorophyll b (Chl *b*) 53.4 and the sum of chlorophyll  $a+b$  (Chl  $a+b$ ) 191.0 mg  $\cdot$  100 g<sup>-1</sup> of *fresh mass* of leaves (FM) (Table 1). The high levels of chlorophyll in leaves sampled from potato plants grown in 2005 coincided with the highest tuber yields, on average 27.86 Mg tubers  $\cdot$  ha<sup>-1</sup>. The year 2005 proved to be very favourable for potato cultivation in terms of the temperatures and precipitation, which was reflected by high



Fig. 1. Temperatures and precipitation during the vegetative growth of potatoes at the Experimental Station in Tomaszkowo



Table 1





n.s. - non-significant differences. n.s. – non-significant differences.

Table 1 contd. Table 1 contd. yields Fig. 2. In 2006–2007, a considerable fall in yields produced by the tested potato cultivar was noted. In these years, the average yields were 22.43 and 15.75 Mg tubers  $\cdot$  ha<sup>-1</sup>. The contributing factor was the high total rainfall in August 2006 (173 mm) and July 2007 (165 mm), which exceeded the multiyear average for these months by 135 % and 143 %, respectively. Nonetheless, under such unfavourable conditions, it was found out that the volume of yields was significantly positively correlated with the content of chlorophyll in leaves, a relationship which did not occur in 2005, when the yields were very high (Table 2). In 2006, the correlation coefficient *r* – Chl  $a+b$  Yield was 0.82 in the series not fertilized with Mg; in the series with Mg introduced to soil, it was 0.91 and in the series with foliar application of Mg, it equalled 0.89. In 2007, significant correlation Chl  $a+b$  Yield was determined only in the series fertilized with magnesium added to soil  $r = 0.78$  and sprayed on leaves  $r = 0.79$ .

In the series without magnesium nutrition, the content of chlorophyll in leaves had no effect on the volume of yields. In the light of the above results, it seems reasonable to conclude that magnesium is a key element in the synthesis of chlorophyll and accumulation of yield, especially in years when the weather conditions are unfavourable.

Significant influence of magnesium fertilization on the content of chlorophyll in leaves has also been reported by Ciecko et al [3], Grzebisz et al [5] and Seidler and Mamzer [15]. This element participates directly in the synthesis of chlorophyll, having direct impact on assimilation. Improved magnesium nutrition of plants enables them to incorporate some of otherwise potentially unused resources of soil nitrogen to biomass, which enhances the useful plant yield [5]. In this research, the sum (Chl *a+b*) in the series with the soil fertilization of magnesium in the dose of 24 kgMg  $\cdot$  ha<sup>-1</sup> was 134.7 and in the series where  $12 \text{ kgMg} \cdot \text{ha}^{-1}$  of magnesium was sprayed on leaves, it equalled 121.1; in the series without magnesium fertilization, it was 120.3 mg  $\cdot$  100 g<sup>-1</sup> fresh mass (Table 1). Soil magnesium fertilization contributed to increasing the content of individual forms of chlorophyll by 11.5 mg Chl  $a \cdot 100 \text{ g}^{-1}$  fresh mass, *ie* by 13.7 %, and by 2.8 mg Chl  $b \cdot 100 \text{ g}^{-1}$  fresh mass, *ie* 7.6 % compared with the content of chlorophyll found in tubers of potato plants fertilized with nitrogen alone. Magnesium produced the most profound impact on chlorophyll concentration in leaves in 2006. In that year, the increase in the sum of Chl  $a+b$  in potato leaves owing to soil magnesium fertilization was 26.1 mg  $\cdot$  100 g<sup>-1</sup> fresh mass. Significant influence of magnesium fertilization was demonstrated in some earlier studies on the potato [3, 31]. However, the positive effect, *ie* increased concentration of chlorophyll in potato leaves, was achieved only when magnesium was applied to leaves. In the present study, soil application had a more beneficial influence, which may be indicative of differentiated action of this element, modified by agronomic and weather conditions.

Nitrogen foliar fertilization applied in amounts from 10 % to 50 % of the full dose of this nutrient contributed to a linear increase in the content of both forms of chlorophyll. In all the years, significant positive correlation was observed between the rising share of N applied to leaves in the total nitrogen dose and the content of chlorophyll (Table 2). Among the treatments without magnesium fertilization, the highest three-year average content of Chl *a*, Chl *b* and Chl *a+b* in potato leaves was found in leaves of potatoes growing on the plots where 40 kgN  $\cdot$  ha<sup>-1</sup>, *ie* 50 % of the full dose of nitrogen,





n.s. - non-significant correlation. n.s. – non-significant correlation.

Table 2

was applied as foliar spraying (Table 1). The increment in chlorophyll induced by this fertilization variant, compared with the treatments where all nitrogen was introduced to soil, was 14.9 mg Chl, mg Chl *a* × 100 g–1 fresh mass, *ie* 19.3 %, 4.8 mg Chl *b* × 100 g–1 fresh mass, *ie* 14 % and 19.7 mg Chl  $a+b \cdot 100 \text{ g}^{-1}$  fresh mass, *ie* 17.6 %. This increase was most noticeable in 2006, in which the rise in the content of Chl *a+b* caused by foliar application of nitrogen was 36.9 mg  $\cdot$  100 g<sup>-1</sup> fresh mass. In the other years, foliar nitrogen fertilization did not exert such strong influence on the leaf content of chlorophyll. Likewise, in the both series amended with magnesium, foliar nitrogen fertilization had a positive effect on the content of chlorophyll. The highest concentration of chlorophyll was found in the treatments fertilized with 24 kgN  $\cdot$  ha<sup>-1</sup>, which corresponded to 30 % of the full nitrogen dose. This fertilization variant, compared with the one where all nitrogen dose was applied to soil, induced an average increase in the content of Chl  $a+b$  of 6.7 mg  $\cdot$  100 g<sup>-1</sup> fresh mass, *ie* 5 % in the treatments with soil magnesium fertilization and 5.5 mg  $\cdot$  100 g<sup>-1</sup>, *ie* 4.6 % in the treatments with foliar magnesium application. Stronger impact of foliar than soil magnesium application on synthesis of chlorophyll pigments demonstrated in the present experiment find confirmation in literature [3, 16].

#### **Conclusions**

1. Under the influence of an increasing share of N applied to leaves in the total nitrogen rate of 80 kgN  $\cdot$  ha<sup>-1</sup>, within the range of 8 to 40 kgN  $\cdot$  ha<sup>-1</sup>, a linear increase in the content of Chl *a* and Chl *b* in potato leaves appeared. The maximum increase in the content of these pigments was 16.2 and 12.2 %, respectively.

2. The applied magnesium nutrition had a positive effect on the content of both forms of chlorophyll. The rate of 24 kgMg  $\cdot$  ha<sup>-1</sup> had a more beneficial effect (134.7 mg Chl  $a+b \cdot 100 \text{ g}^{-1}$  fresh mass) than the rate of 12 kgMg  $\cdot$  ha<sup>-1</sup> sprayed over leaves (121.1 mg Chl  $a+b \cdot 100 \text{ g}^{-1}$  fresh mass).

3. In 2006 and 2007, which were unfavourable to potato yields, concentrations of chlorophyll in leaves were positively correlated with the yields. The correlation coefficients were within the range of  $r = 0.78$  to  $r = 0.91$ , which may indicate a particularly important role of magnesium as an element directly affecting potato yields under the conditions that are not beneficial to potato cultivation.

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#### **ODDZIAŁYWANIE DOLISTNEGO NAWOŻENIA AZOTEM I MAGNEZEM** NA ZAWARTOŚĆ CHLOROFILU W LIŚCIACH ZIEMNIAKA

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Abstrakt: Celem badań było wyjaśnienie wpływu dolistnego nawożenia azotem i magnezem na kształtowanie się zawartości chlorofilu w liściach ziemniaka jadalnego średnio wczesnej odmiany Zebra. Zróżnicowane nawożenie dolistne azotem zastosowano w zakresie od 0 do 50 % pełnej dawki tego składnika (80 kgN · ha<sup>-1</sup>). Eksperyment przeprowadzono w trzech seriach – bez nawo¿enia magnezem, z magnezem stosowanym doglebowo (24 kgMg · ha<sup>-1</sup>) oraz z magnezem stosowanym dolistnie (12 kgMg · ha<sup>-1</sup>). Największą zawartość chlorofilu *a* i *b* uzyskano w 2005 r. – 137,6 mg i 53,4 mg na 100 g świeżej masy liści. W roku tym uzyskano jednocześnie najwyższy plon bulw – średnio 27,86 Mg · ha<sup>-1</sup>. W latach następnych nie potwierdzono tej zależności. Wzrastający udział N stosowanego dolistnie w ogólnej dawce azotu spowodował praktycznie liniowy przyrost ilości obu form chlorofilu. W latach niesprzyjających plonowaniu 2006 i 2007 stwierdzono, że ilość plonów była dodatnio skorelowana z zawartością chlorofilu w liściach, czego nie stwierdzono w roku 2005, w którym plon był najwiekszy. Zastosowany doglebowo, jak i dolistnie magnez miał decydujące znaczenie w syntezie chlorofilu i nagromadzaniu plonu szczególnie w latach o niesprzyjających warunkach pogodowych.

**S³owa kluczowe:** chlorofil, nawozy mineralne, magnez, azot, plon bulw, *Solanum tuberosum*, ziemniak