

## **CONTENT OF CHLOROPLAST PIGMENTS IN THE PHASE OF MAIZE EAR BLOOMING DEPENDING ON NITROGEN AND MAGNESIUM FERTILIZATION**

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**Abstract.** Field experiment was carried out on the Didactic and Experimental Farm in Swadzim (52°26' N; 16°45' E) near Poznań, in the years 2004-2007. The three-factorial experiment was established in "split-plot" design with 4 replications. The reaction of two maize hybrid types on the application of 6 nitrogen doses and magnesium fertilization was studied. The influence of the studied factors on the content of chloroplast pigments in maize leaf blades in the phase of ear blooming (BBCH 67) was estimated. A greater content of chlorophyll a and a + b expressed in SPAD units was found in maize leaf blades of the stay-green type, as compared with leaf blades of the conventional Anjou 258 hybrid. Concentration of chlorophyll a and a + b was increasing in a linear way in the nitrogen range from 0 to 120 kg N·ha<sup>-1</sup>, while the amount of chlorophyll expressed in SPAD units ranged from 0 to 150 kg N·ha<sup>-1</sup>. The stay-green type hybrid showed to be better nourished with nitrogen, on the basis of chlorophyll content in maize leaf blades, in the phase of ear blooming (BBCH 67), in comparison with the conventional hybrid and, at the same time, a lower nitrogen fertilization was needed. A simultaneous fertilization with magnesium increased the content of chloroplast pigments, in comparison with the application of nitrogen only. With the increase in the assimilation area of a single maize plant and of a maize stand (LAI indicator), the chlorophyll concentration in leaf blades was decreasing in a linear way.

**Key words:** BBCH 67, chlorophyll, fertilizer application method, magnesium, maize hybrid types, nitrogen, SPAD

### **INTRODUCTION**

Nutritive components are supplied to plants in the form of mineral fertilizers. Mineral nutrition constitutes the basic agrotechnical treatment deciding about plant productivity, i.e. their intensity of organic substance accumulation which exerts an influence on the size of biomass creation [Costa et al. 2002, Subedi and Ma 2005]. In

order to realize the yield creating potential (i.e. to achieve the maximal biomass yield in the given environmental conditions), maize requires to be fully supplied with all nutritive components during the whole growing season. However, without any doubts, nitrogen is a component which distinctly limits the size of yield, both the vegetative and the generative one. Therefore, maize fertilization with nitrogen is not a simple problem, particularly for maize producers who believe that this species is able to bring high yields in all conditions, even in the extremely hard ones [Grzebisz 2008].

Therefore, the diagnosing of the nutritional status of maize plants with nitrogen is very important, permitting to use the optimal nitrogen doses in terms of justifying their application [Machul 2001]. The determination of plant requirement for nitrogen is done on the basis of quantitative or qualitative methods of plant nutrition with this element (in practice, they are called „plant tests”) [Zagórda and Walczyk 2007]. The present paper shows the possibility of the application of chlorophyll content tests in maize plant leaf blades, in the ear formation phase (BBCH 67) helping in the determination of the nitrogen nutritional status of plants.

The hypothesis of the presented experiment assumed that the nitrogen dose sizes and magnesium application and the maize hybrid types, as well as their mutual interaction, can modify the chloroplast content of pigments in maize leaf blades, being at the same time a measure reflecting maize nutrition with nitrogen.

In connection with this consideration, a 3-year field study was carried out with the purpose to estimate the nitrogen status of two types of maize hybrids, depending on the applied level of this component and on the method of magnesium dose application. This estimation was done on the basis of chlorophyll content in maize leaf blades in the ear blooming phase (BBCH 67) using the direct and indirect methods.

## MATERIAL AND METHODS

Detailed method of field experiment and the determination of the thermal and moisture conditions were described in an earlier paper by the author [Szulc et al. 2008], while the indirect and direct methods used for the estimation of chloroplast pigments in maize leaf blades can be found in the author’s recent paper [Szulc 2009].

## RESULTS AND DISCUSSION

In the period of the presented experiment, the content of chloroplast pigments a and a + b, chlorophyll (in SPAD units) and the a/b chlorophyll relation in the ear blooming phase (BBCH 67) – significantly depended on the hybrid type (Table 1). Definitely, a higher content of these pigments was found in the LG 2244 stay-green hybrid type, as compared with the Anjou 258 hybrid. These differences were: 0.08  $\mu\text{g}\cdot\text{g}^{-1}$  for chlorophyll a, 0.09  $\mu\text{g}\cdot\text{g}^{-1}$  for chlorophyll a + b, 33.8 for SPAD units and 0.47 for the a/b chlorophyll proportion, respectively (Table 1). The greater content of chloroplast pigments of the stay-green hybrid in comparison with the conventional Anjou 258 is conditioned by the possibility of the LG 2244 hybrid to accumulate nitrogen until the end of plant vegetation [Grzebisz 2008]. According to that author, the stay-green hybrids, during vegetation period, accumulate nitrogen from two sources, i.e. the nitrogen taken up from the soil and N remobilized from the vegetative organs (leaves,

shoots). Thanks to this possibility, the stay-green hybrids can take up mineral nitrogen with a speed which is adequate in the critical phase to the maize requirements for this microelement [Grzebisz 2008]. A similar, higher dependence on the chloroplast pigments, as compared with the traditional hybrid Anjou 258, in the phase of 5-6 leaves (BBCH 15-16), was shown in an earlier work by Szulc [2009]. A higher chlorophyll content, both in the juvenile phase (BBCH 15-16) as well as in the period of ear blooming (BBCH 67), which is a measure of a better maize nutritional status with nitrogen in these critical phases, consequently gave a significantly higher grain yield, in comparison with the traditional Anjou 258 hybrid [Szulc et al. 2008]. This result obtained in our own studies is a confirmation of earlier literature reports that productivity of hybrids depends on the content of chlorophyll a + b in maize leaf blades [Sulewska 1990].

Table 1. Content of chloroplast pigments and chlorophyll in SPAD units in the ear blooming phase (BBCH 67)

Tabela 1. Zawartość barwników chloroplastowych i chlorofilu w jednostkach SPAD w fazie kwitnienia kolb (BBCH 67)

Specification Wyszczególnienie	Chlorophyll – Chlorofil					a/b
	a	b	a + b	in units w jednostkach SPAD		
	µg·g <sup>-1</sup>					
Hybrid Odmiana	Anjou 258	1.91	0.40	2.31	694.6	5.62
	LG 2244	1.99	0.41	2.40	728.4	6.09
	LSD <sub>0,05</sub> – NIR <sub>0,05</sub>	0.030	ns – ni	0.050	15.732	0.298
Dose of N Dawka N kg·ha <sup>-1</sup>	0	1.67	0.35	2.04	647.5	5.85
	30	1.90	0.41	2.29	690.1	5.69
	60	2.01	0.42	2.44	702.5	5.80
	90	2.02	0.42	2.42	720.7	5.76
	120	2.05	0.42	2.48	749.4	6.26
	150	1.92	0.41	2.34	758.9	5.77
	LSD <sub>0,05</sub> – NIR <sub>0,05</sub>	0.191	ns – ni	0.139	17.184	0.219
Dose of Mg Dawka Mg kg·ha <sup>-1</sup>	0	1.94	0.41	2.35	705.1	5.77
	15 in rows – rzędowo	1.92	0.41	2.33	712.9	5.81
	15 broadcasting – rzutowo	1.92	0.41	2.32	716.6	5.99
	LSD <sub>0,05</sub> – NIR <sub>0,05</sub>	ns – ni	ns – ni	ns – ni	ns – ni	ns – ni

ns – ni – non significant differences – różnice nieistotne

Nitrogen fertilization level determined in a significant way the content of chloroplast pigments a, a + b, chlorophyll expressed in SPAD units and the a/b chlorophyll proportion in the phase of ear blooming (BBCH 67) – Table 1. Together with the increase of nitrogen fertilization level in the dose range from 0 to 120 kg N·ha<sup>-1</sup>, the content of chlorophyll a, a + b and chlorophyll a/b proportion increased in a linear way. The application of the highest dose level, i.e. a level of 150 kg N·ha<sup>-1</sup>, decreased the values of the mentioned features. In the case of chlorophyll expressed in SPAD units, the highest value was obtained for a dose of 150 kg N·ha<sup>-1</sup> (758.9), while the lowest value was shown by the treatment where no nitrogen was applied (647.5). Increased SPAD results in maize under the influence of the increasing nitrogen fertilization was widely discussed both in the Polish and in the foreign publications [Costa et al. 2001, Machul 2005, 2003, Scharf et al. 2006, Szulc 2009].

In the field experiment, the contents of chlorophyll a and a + b (BBCH 67 phase) significantly depended on the cooperation between nitrogen fertilization level and the maize hybrid type (Figs. 1 and 2). These dependences have been described by the second degree equation, whereby, for the LG 2244 stay green hybrid type, these dependences were running on a higher level, as compared with the Anjou 258 hybrids. For Anjou 258 hybrid, the maximal chlorophyll a content ( $2.01 \mu\text{g}\cdot\text{g}^{-1}$ ) was obtained with a nitrogen dose of  $97.5 \text{ kg}\cdot\text{ha}^{-1}$ , while for the chlorophyll a + b content ( $2.42 \mu\text{g}\cdot\text{g}^{-1}$ ), a N dose of  $96.2 \text{ kg}\cdot\text{ha}^{-1}$  has shown to be the best one. In the case of the hybrid LG 2244 stay-green type, the concentration of chloroplast pigments was higher, which was obtained at a lower N fertilization level. The corresponding values for LG 2244 were: a –  $2.06 \mu\text{g}\cdot\text{g}^{-1}$  with the N dose smaller by  $15 \text{ kg}\cdot\text{ha}^{-1}$ , chlorophyll a + b –  $2.56 \mu\text{g}\cdot\text{g}^{-1}$  with the N dose smaller by  $4.6 \text{ kg}\cdot\text{ha}^{-1}$  (Fig. 1 and Fig. 2). The result in our own experiment testified that the LG 2244 hybrid possesses higher possibilities to utilise the nitrogen supplied in mineral fertilizer, as at the same nitrogen fertilization its leaf blades showed a higher concentration of photosynthesizing pigments which decide about the yield size [Sulewska 1990, Neukirchen and Lammel 2002, Samborski and Rozbicki 2004].

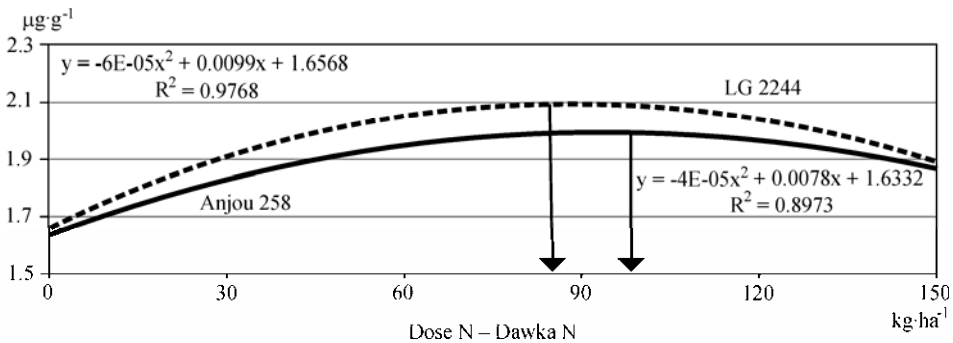


Fig. 1. Content of chlorophyll a in the ear blooming phase (BBCH 67), depending on nitrogen dose and hybrid type

Rys. 1. Zawartość chlorofilu a w fazie kwitnienia kolb (BBCH 67) w zależności od dawki azotu i typu odmiany

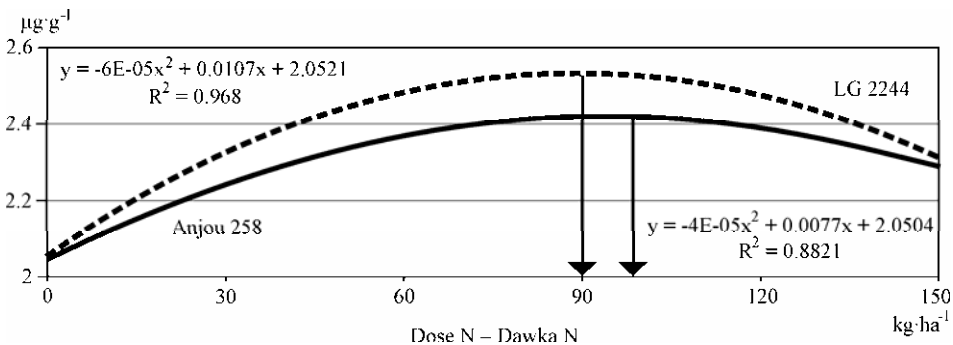


Fig. 2. Content of chlorophyll a + b in the ear blooming phase (BBCH 67), depending on nitrogen dose and hybrid type

Rys. 2. Zawartość chlorofilu a + b w fazie kwitnienia kolb (BBCH 67) w zależności od dawki azotu i typu odmiany

In the case of chlorophyll content in the phase of ear blooming (BBCH 67) expressed in SPAD units, its content depended on the interaction between the nitrogen dose and the hybrid type (Fig. 3). These dependences have been described by a linear function of the first degree, whereby, for the LG 2244 stay-green hybrid, the equation took place on a higher level, in comparison with Anjou 258 hybrid. Increased level in nitrogen fertilization caused a proportional increase in chlorophyll expressed in SPAD units. The obtained result of our own studies confirmed that the LG 2244 stay-green hybrid showed a higher chlorophyll content at the same nitrogen fertilization level (a higher potential productivity with smaller nitrogen doses). As reported by Machul [2005], maize hybrids show a relative differentiation of chlorophyll content, since it is a genetically conditioned feature and this explains the result obtained in the field experiment. In turn, Fotyma and Bezdusznik [2000] – quoted after Suderman et al. [1997] argued that maize hybrids differ in chlorophyll content to such a degree that SPAD read-outs should be standardized; it means that it should be expressed in relative values as a quotient of the read-out for the given fertilizer combination and the read-out for an object optimally nourished with nitrogen.

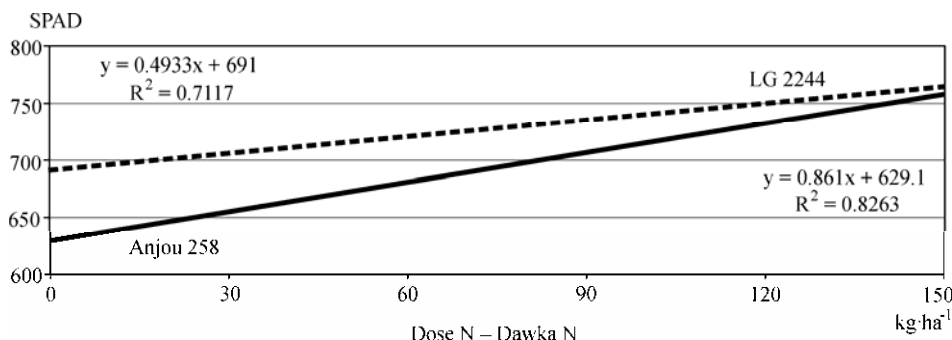


Fig. 3. Content of chlorophyll in SPAD units in the ear blooming phase (BBCH 67), depending on nitrogen dose and hybrid type

Rys. 3. Zawartość chlorofilu w jednostkach SPAD w fazie kwitnienia kolb (BBCH 67) w zależności od dawki azotu i typu odmiany

Content of chlorophyll a + b in the phase of ear blooming (BBCH 67) significantly depended on the cooperation of N dose with the dose of magnesium (Fig. 4). These dependences have been described by the equation of the second degree. Application of 15 kg Mg·ha<sup>-1</sup> by broadcasting or in rows decided about the fact that these dependences took place on a higher level, in relation to the course determined for nitrogen doses without magnesium (0 kg Mg·ha<sup>-1</sup>). Analysis of the magnesium application method showed that an application of 15 kg Mg·ha<sup>-1</sup> in rows, as compared with the broadcasting method, gave a higher effectiveness (Fig. 4). Content of chlorophyll depended on the plant nutritional status with nutritive components, particularly with nitrogen and sulphur and in a further sequence with magnesium and potassium [Neukirchen and Lammel 2002]. Furthermore, magnesium deficit decreases chlorophyll concentration and reduces the photosynthesis rate in plants, because it is the main component of this pigment and it plays the key function in the photosynthesis process [Grzebisz and Härdter 2006]. Then, there follows a decrease in photosynthesis which evokes some disturbances in the functioning of plant organism which, in consequence, limits the growth and the biomass

accumulated in the storage organs [Fischer 1997]. The main cause of such a situation is the impossibility of CO<sub>2</sub> binding with an organic acceptor which is regarded as the most important biological process permitting the inclusion of inorganic carbon into animated nature [Grzebisz and Hårdter 2006, Panak 1997].

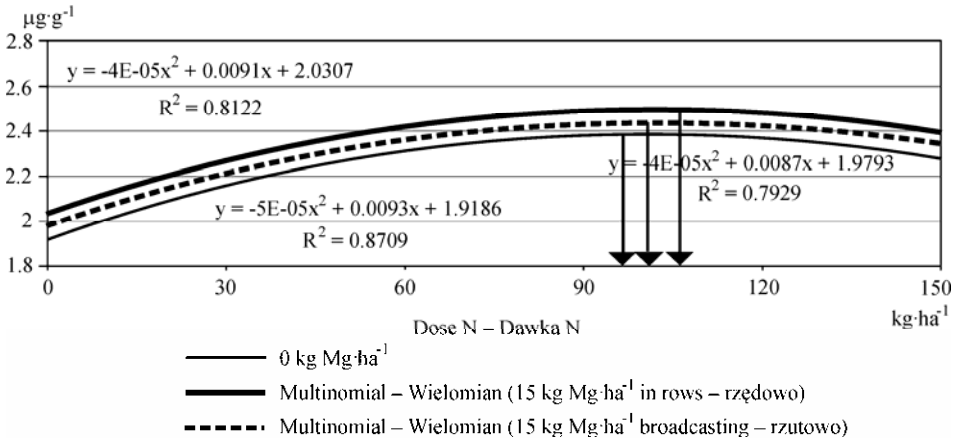


Fig. 4. Content of chlorophyll a + b in the ear blooming phase (BBCH 67), depending on nitrogen dose and on the method of magnesium application

Rys. 4. Zawartość chlorofilu a + b w fazie kwitnienia kolb (BBCH 67) w zależności od dawki azotu i sposobu wysiewu magnezu

Chlorophyll content closely depends on the plant assimilation area, which is indicated by straight line dependence between the content of chlorophyll a + b (in SPAD units) and the assimilation area of a single plant and the LAI indicator (Figs 5 and 6). With the growth of the assimilation area of a single plant and the assimilation area of a maize field (LAI indicator), the contents of chloroplast pigments a + b and chlorophyll expressed in SPAD units were subject to a uniform decrease. As reported by Wojcieszka-Wyskupajtyś [1996], an excessively large assimilation area of leaves leads to a mutual shading and decreases the photosynthesis of the shaded leaves with an unchanged and frequently increased respiration intensity, finally giving a decrease in the created biomass. This is one of the reasons why the potential photosynthetic possibilities of the total leaf area are only partially utilized. In order to define the dependences between chlorophyll content expressed in SPAD units and chlorophyll a, b and a + b, the coefficient of normal correlation has been applied (Figs 7 and 8). In the case of Anjou 258 hybrid, no significant dependence (of normal correlation) was found to be exerted by the particular chloroplast pigments a, b and a + b with the chlorophyll amount expressed in SPAD units. This effect was shown in the case of LG 2244 stay-green hybrid type (Fig. 7) for which all three chloroplast pigments a, b and a + b were significantly correlated with chlorophyll content expressed in SPAD units. Analysing the dependence between the chlorophyll content expressed in SPAD units and chlorophyll a, b and a + b, independent of the hybrid type and the nitrogen fertilization level, a significant correlation was found between these chloroplast pigments and the SPAD read-out (Fig. 8). Higher normal correlation coefficients between the chloroplast pigments and the chlorophyll expressed in SPAD units were found in the combination with 15 kg Mg·ha<sup>-1</sup> applied in rows.

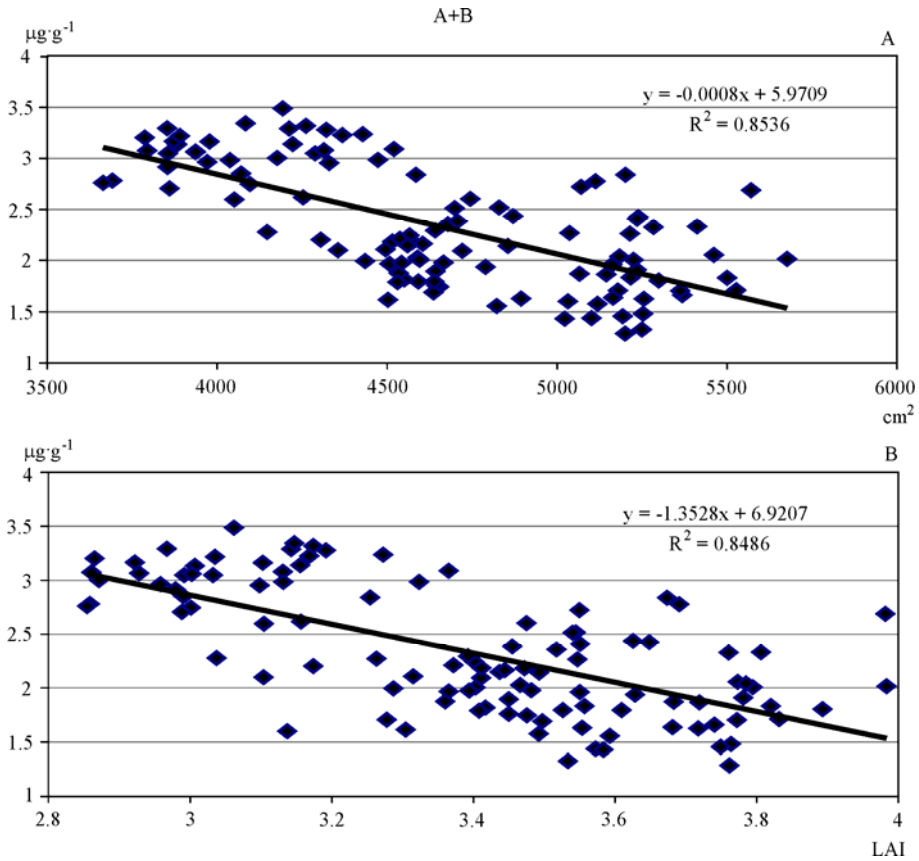
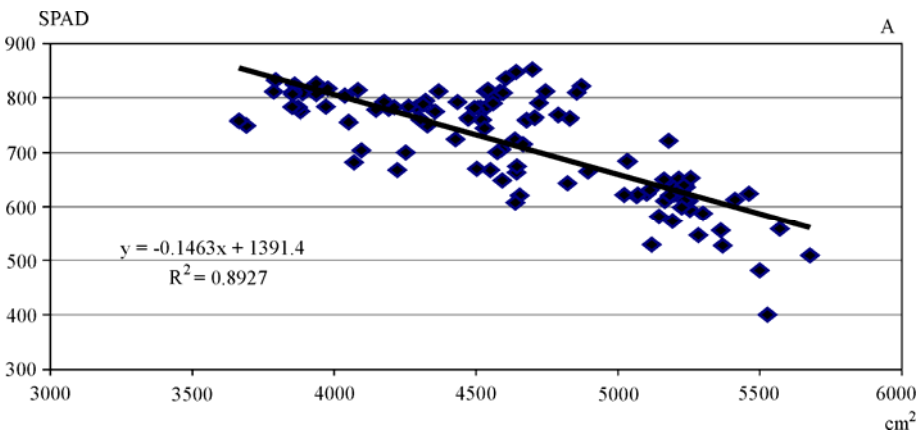


Fig. 5. Dependence of chlorophyll a + b content in the ear blooming phase (BBCH 67) on the size of the assimilation area of a single plant (A) and on LAI indicator (B)

Rys. 5. Zależność zawartości chlorofilu a + b w fazie kwitnienia kolb (BBCH 67) od wielkości powierzchni asymilacyjnej pojedynczej rośliny (A) oraz wskaźnika LAI (B)



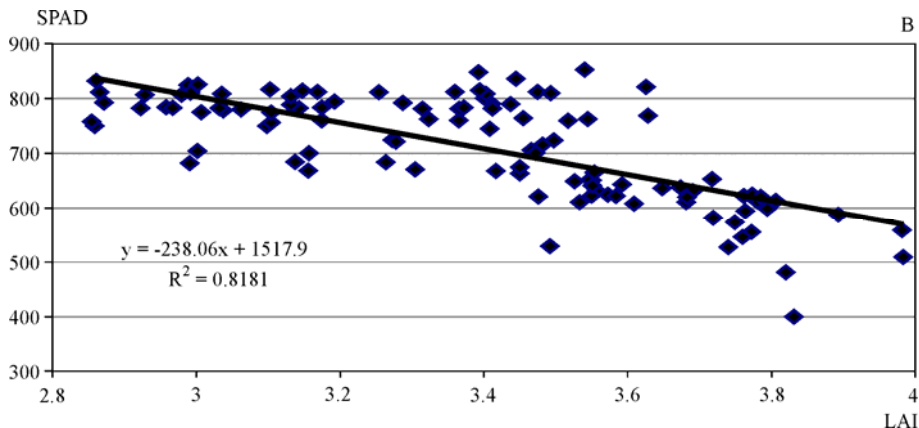


Fig. 6. Dependence of chlorophyll content in SPAD units in the ear blooming phase (BBCH 67) on the size of the assimilation area of a single plant (A) and on LAI indicator (B)

Rys. 6. Zależność zawartości chlorofilu w jednostkach SPAD w fazie kwitnienia kolb (BBCH 67) od wielkości powierzchni asymilacyjnej pojedynczej rośliny (A) oraz wskaźnika LAI (B)

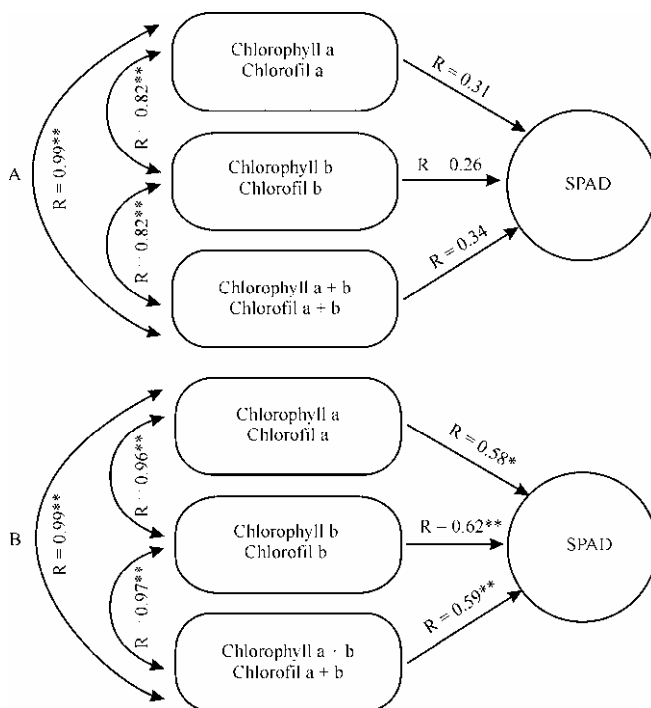


Fig. 7. Diagram of normal (simple) correlation coefficients for three chloroplast pigments of Anjou 258 (A) and LG 2244 stay-green (B) hybrids conditioning chlorophyll content in SPAD units, irrespective of nitrogen and magnesium doses

Rys. 7. Diagram współczynników korelacji prostej dla trzech barwników chloroplastowych odmiany Anjou 258 (A) i LG 2244 stay-green (B), warunkujących zawartość chlorofilu w jednostkach SPAD niezależnie od dawki azotu i magnezu



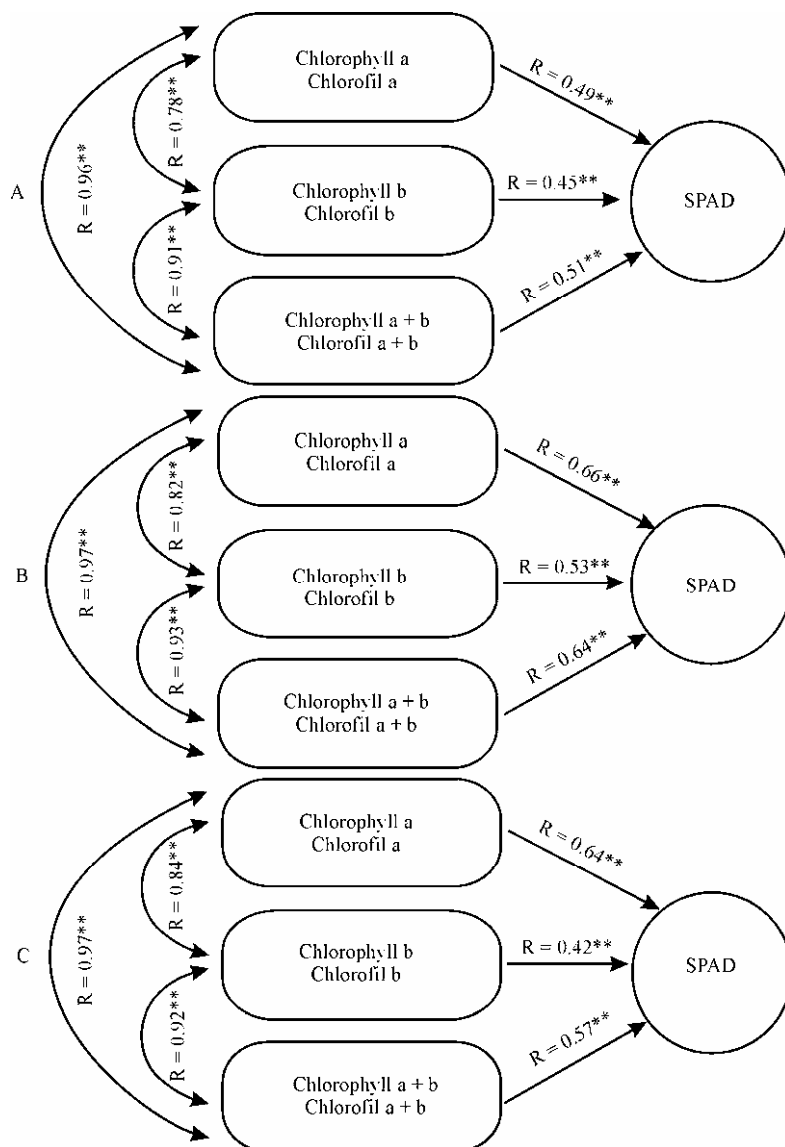


Fig. 8. Diagram of normal (simple) correlation coefficients for three chloroplast pigments of treatments with 0 kg Mg·ha<sup>-1</sup> (A), 15 kg Mg·ha<sup>-1</sup> applied in rows (B) and 15 kg Mg·ha<sup>-1</sup> by broadcast (C) conditioning chlorophyll content in SPAD units, irrespective of hybrid type and nitrogen dose

Rys. 8. Diagram współczynników korelacji prostej dla trzech barwników chloroplastowych obiektów z 0 kg Mg·ha<sup>-1</sup> (A), 15 kg Mg·ha<sup>-1</sup> – rzędowy wysiew (B) i 15 kg Mg·ha<sup>-1</sup> – rzutowy wysiew (C), warunkujących zawartość chlorofilu w jednostkach SPAD, niezależnie od typu odmiany i wielkości dawki azotu

## CONCLUSIONS

1. Hybrid stay-green type was characterized by a higher concentration of chlorophyll a, b and a + b expressed in SPAD units, as compared with the traditional hybrid in the phase of ear blooming (BBCH 67). Furthermore, also the proportion of the chloroplast a/b pigments was more favourable for this hybrid.

2. The highest chlorophyll a, a + b content and a/b proportion value were obtained for the nitrogen dose  $120 \text{ kg N}\cdot\text{ha}^{-1}$ , while the highest amount of chlorophyll expressed in SPAD units was obtained for a nitrogen dose of  $150 \text{ kg N}\cdot\text{ha}^{-1}$ .

3. LG 2244 stay-green type hybrid showed in its leaf blades a higher concentration of chlorophyll a and a + b, in comparison with the Anjou 258 hybrid. It was obtained with a smaller nitrogen fertilization.

4. Nitrogen and magnesium applied at the same time exerted a positive effect on chlorophyll a + b content in maize leaf blades (BBCH 67), in comparison with maize fertilization with nitrogen only.

5. With the increase in the single plant assimilation area and the increase in LAI indicator, the amount of a + b and the SPAD units decreased in a linear way.

6. In the case of the stay-green type hybrid, it was found that the content of chlorophyll expressed in SPAD units was significantly correlated with chlorophyll a, b and a + b.

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## ZAWARTOŚĆ BARWNIKÓW CHLOROPLASTOWYCH W FAZIE KWITNIENIA KOŁB KUKURYDZY W ZALEŻNOŚCI OD NAWOŻENIA AZOTEM I MAGNEZEM

**Streszczenie.** Doświadczenie polowe przeprowadzono w Zakładzie Dydaktyczno-Doświadczalnym w Swadzimiu (52°26' N; 16°45' E) koło Poznania w latach 2004-2007. Prowadzono je jako trzyczynnikowe w układzie „split-plot” w 4 powtórzeniach. Badano reakcję 2 typów odmian kukurydzy na stosowanie 6 dawek azotu oraz nawożenie magnezem. Oceniano wpływ tych czynników na zawartość barwników chloroplastowych w blaszkach liściowych kukurydzy w fazie kwitnienia kołb (BBCH 67). Większą zawartość chlorofilu a, a + b i wyrażonego w jednostkach SPAD stwierdzono w blaszkach liściowych kukurydzy odmiany typu stay-green niż w blaszkach liściowych mieszańca tradycyjnego. Koncentracja chlorofilu a i a + b wzrastała w sposób liniowy w przedziale dawek azotu od 0 do 120 kg N·ha<sup>-1</sup>, natomiast chlorofilu wyrażonego w jednostkach SPAD – w przedziale dawek azotu od 0 do 150 kg N·ha<sup>-1</sup>. Przy niższych poziomach nawożenia azotem mieszaniec typu stay-green zawierał więcej chlorofilu w blaszkach liściowych niż mieszaniec tradycyjny. Jednoczesne nawożenie azotem i magnezem zwiększało zawartość barwników chloroplastowych w porównaniu z wyłączeniem nawożenia kukurydzy azotem. Koncentracja chlorofilu w blaszkach liściowych

zmniejszała się w sposób liniowy wraz ze wzrostem powierzchni asymilacyjnej pojedynczej rośliny oraz łąnu kukurydzy (wskaźnik LAI).

**Słowa kluczowe:** azot, BBCH 67, chlorofil, magnez, SPAD, sposób aplikacji nawozu, typy odmian kukurydzy

Accepted for print – Zaakceptowano do druku: 17.08.2009