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DIVERSIFICATION OF CHLOROPLAST PIGMENT CONTENTS DETERMINED BY DIFFERENTIAL SELECTION OF AGRICULTURE PRACTICES IN MAIZE CULTIVATION

Summary

The article presents the results of a 3-year field study aimed at evaluating the effect of sowing method and NP fertilizer application on chloroplast pigment contents in two different types of maize (Zea mays L.*) varieties. Cultivation method regardless of the years significantly shaping chloroplast pigment contents is the same factor that determines maize yield. Variety selection can but to a small extent correct the adverse effects of direct sowing systems on chlorophyll content. The lack of differences in the nitrogen nutritional status (SPAD) of plants at the BBCH 67 stage despite the considerable diversification of the experimental factor levels indicates good conditions for the uptake of this nutrient in the period up to flowering. Selection of the variety in combination with row NP fertilization is the factor supporting the nutritional status of maize at this stage. The "stay-green" type should be the more predisposed maize variety for direct sowing and row fertilization under these conditions due to the higher chlorophyll content of leaf blades.*
Key words*: maize (Zea mays* L.*), chlorophyll, critical stages*

DYWERSYFIKACJA ZAWARTOŚCI BARWNIKÓW CHLOROPLASTOWYCH WARUNKOWANA ZRÓŻNICOWANYM DOBOREM CZYNNIKÓW AGROTECHNICZNYCH W UPRAWIE KUKURYDZY

Streszczenie

Przedstawiono wyniki 3-letnich badań polowych, których celem była ocena wpływu sposobu siewu i aplikacji nawozu NP na zawartość barwników chloroplastowych przez dwa różne typy odmian kukurydzy (Zea mays L.*). Sposób uprawy roli istotnie, niezależnie od roku, kształtując zawartość barwników chloroplastowych jest czynnikiem warunkującym plonowanie kukurydzy. Dobór odmiany może, lecz w niewielkim stopniu, skorygować niekorzystny wpływ systemów siewu bezpośredniego na zawartość chlorofilu. Brak zróżnicowania stanu odżywienia roślin azotem (SPAD) w stadium BBCH 67, pomimo znacznej dywersyfikacji poziomów czynników doświadczalnych, wskazuje na dobre warunki pobierania tego składnika w okresie do kwitnienia. Czynnikiem wspomagającym stan odżywienia kukurydzy w tym stadium jest dobór odmiany w połączeniu z rzędowym nawożeniem NP. Bardziej predysponowaną odmianą kukurydzy do siewów bezpośrednich i nawożenia rzędowego ze względu na większą zawartość chlorofilu w blaszkach liściowych w tych warunkach siedliskowych powinna być odmiana w typie „stay-green".*
Słowa kluczowe: *kukurydza (Zea mays* L.*), chlorofil, fazy krytyczne*

1. Introduction

In addition to water, nitrogen fertilization is the basic agriculture factor that determines the quantity and quality of the obtained yield [1]. Application of high nitrogen doses in market economy must be economically justified and should take into account both the ecological and economic aspects [2]. For both of these reasons. it is important to diagnose the plant nutrition status with nitrogen during the growing season which allows to correct the applied doses [3]. Assuming that maize tends to take up excessive amounts of nitrogen it is essential to determine the minimum concentration of the component in the plant material that does not limit the maize productivity [4]. This concentration can be determined from the so-called nitrogen dilution curve i.e. a function describing the relationship between plant dry matter and nitrogen content [5]. The function equation coefficient for C3 and C4 plants have a constant value thus the above relationship can probably be treated as a biological law. According to Plenet and Cruz [6], the equation of the dilution curve for maize takes the following form: $\% N_{cr} = 3.40*DM^{-0.37}$, where N_{cr} is the critical nitrogen concentration in the dry matter of the above-ground plant parts and DM is the dry matter of these parts in the range from 1.0 t to 22 t ha^{-1}. Thus using the universal dilution curve. it is possible to determine the status of plant nitrogen nutrition in any maize canopy expressed in the form of the nitrogen nutrition index (NNI) [7]. Another method but an indirect one to determine the nitrogen content in plants is the measurement of chlorophyll content [8, 9]. Therefore the aim of the study was to determine chlorophyll content in the critical stages of maize growth in relation to the selected agricultural practices.

2. Material and Methods

2.1. Experimental field

The field experiment was carried out at the Department of Agronomy of Poznań University of Life Sciences in the years 2012-2014. It was carried out for three years in the same scheme in a split-split-plot design with three factors in 4 field replicates. The study involved the following factors: A - 1st order factor - two methods of maize sowing: A1 - sowing to the soil (traditional cultivation), A2 - direct sowing to the stubble after winter wheat (straw harvested); B - 2nd order factor - two types of varieties: B1 - traditional variety SY Cooky, B2 – stay-green variety Drim; C - 3rd order factor - 2 methods of supplying NP fertilizer: C1 - broadcast on the entire surface before seed sowing, C2 - in rows simultaneously with seed sowing. The same level of mineral fertilization (100 kg N·ha^{-1}, 30.8 kg P·ha^{-1} and 107.9 kg K·ha^{-1}) was applied on all experimental objects. Fertilization was balanced against phosphorus, which was applied at the whole required dose in the form of ammonium phosphate under the trade name of polidap NP. N and K fertilization was performed before maize sowing using urea and potassium salt (60%). The N dose was reduced by the amount of nitrogen present in the polidap. The assumed planting density in the years of research was 7.95 pcs·m^{-2}, with a spacing between rows of 70 cm and a sowing depth of 5-6 cm. The size of the plant for harvesting was 14 m^2.

2.2. Weather and soil conditions

Characteristics of climatic conditions during the research period were based on data from the meteorological station belonging to the Agronomy Department of the Poznań University of Life Sciences. located at the Agricultural Experimental Station Swadzim (52° 26′ N; 16° 45′ E). It should be noted that the sum of atmospheric precipitation in the period from sowing to the BBCH 15/16 stage was very diverse (Tab. 1). The significantly highest sum was recorded in 2013 while the precipitation in 2012 was lower by almost 50%. At the same time soil temperature at a depth of 10 cm was the lowest in 2012. while the highest in 2014 (Tab. 1). Thermal conditions during maize cultivation in the experimental years were similar to each other and amounted on average to 15.4°C in 2012, 15.6°C in 2013 and 16.1°C in the warmest year of 2014. Significantly greater differences between years occurred in the amount of precipitation. The highest sum of rainfall was recorded in 2012 (473.6 mm), which was 76.2 mm higher than the precipitation in 2013 and 121.8 mm higher from the rainfall in 2014 (Tab. 2).

According to the international FAO classification the soil of the experimental field was classified as *Albic Luvisols* while according to the American classification. it belonged to the order *Alfisols*. In terms of horizon it was defined as loamy sand underlined by loam according to the international classification. It was included in the 4th complex of agricultural usefulness (very good rye) and valuation class III b. Winter wheat was the forecrop for maize in each year of the field research.

2.3. Observations and measurements

Chloroplast pigment contents were measured at BBCH 15/16. The sample for chemical tests consisted of 8 randomly sampled plants from each plot. Chlorophyll content was determined using two methods: an indirect and direct one. For the direct method leaf weighed amounts were cut into 2-3 mm sections and immersed in 5 ml of DMSO (dimethyl sulfoxide). The samples were left for about 1 hour in the dark at room temperature and subsequently incubated at 65°C (water bath) for 30 minutes. The extract obtained after cooling was assayed spectrophotometrically for chlorophyll *a* and *b* contents. Chlorophyll pigment contents was determined using a spectrophotometer (Spekol type) at the appropriate wavelength. Extract absorbance for chlorophyll *a* was measured at 663 nm. for chlorophyll b at 645 nm and for carotenoids at 470 nm.

Table 1. Course of meteorological conditions in the period from sowing to the 5-6 leaf stage (BBCH 15/16) [10]
Tab. 1. Przebieg warunków meteorologicznych w okresie od siewu do fazy 5-6 liści (BBCH 15/16) [10]

Specification	Years		
	2012	2013	2014
Total rainfall during the sowing period – 5-6 leaf stage [mm]	43.9	86.8	81.5
Average air temperature during sowing – 5-6 leaf stage [°C]	12.6	13.2	14.6
Average soil temperature at a depth of 10 cm – 5-6 leaf stage [°C]	11.8	12.2	12.8

Table 2. The average monthly air temperature and the monthly sum of atmospheric precipitation in Swadzim for the growing season
Tab. 2. Średnia miesięczna temperatura powietrza i miesięczna suma opadów atmosferycznych w Swadzimiu dla okresu wegetacyjnego

Years	Temperature [°C]							
	IV	V	VI	VII	VIII	IX	X	Mean/Sum
2012	9.3	16.3	17.0	20.0	19.8	15.0	8.6	15.4
2013	8.9	15.6	18.4	22.0	20.2	13.2	10.8	15.6
2014	11.4	14.6	17.9	23.2	18.8	16.0	11.2	16.1
1957-2013	11.4	14.6	17.9	23.2	18.8	16.0	11.2	16.1
Years	Precipitation [mm]							
2012	17.4	84.4	118.1	136.2	52.7	28.4	36.4	473.6
2013	10.5	95.5	114.9	52.9	32.4	75.9	15.3	397.4
2014	50.3	80.7	44.6	51.5	56.5	39.2	29.0	351.8
1957-2013	31.4	54.1	59.0	76.0	57.8	43.8	37.3	359.4

Source: own study / Źródło: opracowanie własne

The amount of chlorophyll *a*, chlorophyll *b*, and the sum of chlorophyll *a+b* and carotenoids were calculated using the formulas from Arnon [11].

Chlorophyll $a = (12.7 \times A_{663} - 2.7 \times A_{645}) \times V \times (1000\ W)^{-1}$

Chlorophyll $b = (22.9 \times A_{645} - 4.7 \times A_{663}) \times V \times (1000\ W)^{-1}$

Chlorophyll $a+b = (20.2 \times A_{645} - 8.02 \times A_{663}) \times V \times (1000\ W)^{-1}$

Carotenoids $= (1000 \times A_{470} - 1.9 \times$ chlorophyll $a - 63.14 \times$ chlorophyll $b) / 214$

A – absorbance at a given wavelength,

V – total extract volume (cm^3),

W – sample weight (g).

The amount of individual pigments was given in $\mu g\ g^{-1}$ fresh weight and carotenoids in $mg\ g^{-1}$ fresh weight.

An optical device known in Europe as Hydro N-Tester, and in the USA as SPAD-502 was used in the indirect method of maize nutritional status determination [12]. This apparatus works by measuring the light absorption of the leaf at two wavelengths: 650 and 940 mm. The quotient of these differences is an indicator of chlorophyll content and is referred to as SPAD units (*Soil and Plant Analysis Development*). A high coefficient of determination (R^2) depending on the species. was shown between the instrument readings and the extracted quantity of chlorophyll [13].

2.4. Statistical analysis

One-year results were subjected to a univariate analysis of variance followed by a synthesis for multiple experiments. The significance of the differences was estimated at the level of $\alpha = 0.05$ using the Student's t-test. A polynomial curvilinear regression was determined for the means from the individual years [14].

3. Results

3.1. Chloroplast pigment contents in maize plants in the 5-6 leaf stage (BBCH 15/16)

3.1.1. Chlorophyll *a* content

Chlorophyll *a* content in the 5-6 leaves stage (BBCH 15/16) significantly depended on all three experimental factors. i.e. sowing method, variety and the method of NP fertilizer application (Tab. 3). Significantly higher values of this trait were demonstrated for sowing in cultivated soil. Drim "stay-green" variety and row application of NP fertilizer compared to maize sown directly into stubble, traditional variety SY Cooky and NP fertilizer broadcast application (Tab. 3). Chlorophyll *a* content in the conducted field experiment was also significantly affected by the interaction of the variety with NP fertilizer sowing method (Fig. 1). The method of fertilizer application had no significant effect on the content of chlorophyll *a* for the SY Cooky variety. In turn the Drim "stay-green" hybrid was characterized by a higher content of this pigment in row fertilization compared to the broadcast application (Fig. 1).

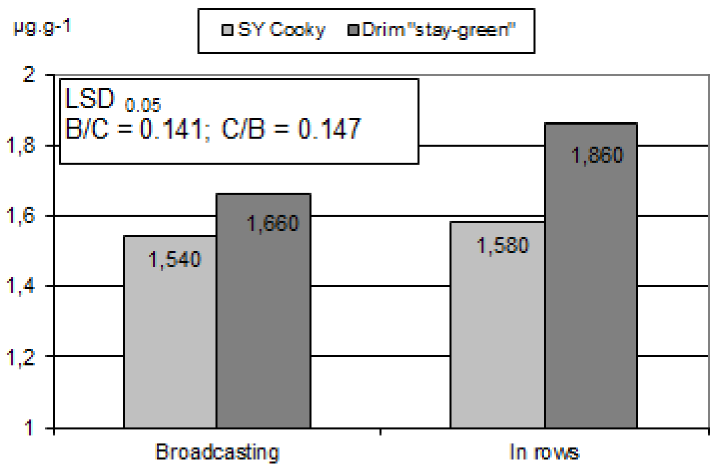
Table 3. Chlorophyll *a* content in the 5-6 leaf stage (BBCH 15/16) ($\mu g \cdot g^{-1}$)

Tab. 3. Zawartość chlorofilu a w fazie 5-6 liści (BBCH 15/16) ($\mu g \cdot g^{-1}$)

Experimental factors		Years			Average
		2012	2013	2014	
Maize sowing methods	sowing in traditionally cultivated soil	1.76	1.42	2.06	1.75
(A)	direct sowing	1.73	1.43	1.54	1.57
LSD 0.05		**n.s.**	**n.s.**	**0.379**	**0.180**
Varieties	SY Cooky	1.80	1.21	1.67	1.56
(B)	Drim „stay-green”	1.69	1.65	1.93	1.76
LSD 0.05		**n.s.**	**0.285**	**0.212**	**0.108**
NP fertilizer sowing methods	broadcasting	1.69	1.30	1.80	1.60
(C)	in rows	1.80	1.56	1.82	1.72
LSD 0.05		**n.s.**	**0.206**	**n.s.**	**0.100**
Average		1.74	1.43	1.80	1.66

n.s. – non-significant difference

Source: own study / Źródło: opracowanie własne



Source: own study / Źródło: opracowanie własne

Fig. 1. Chlorophyll *a* content in the 5-6 leaf stage (BBCH 15/16) depending on the interaction of the variety with NP fertilizer sowing method (2012-2014)

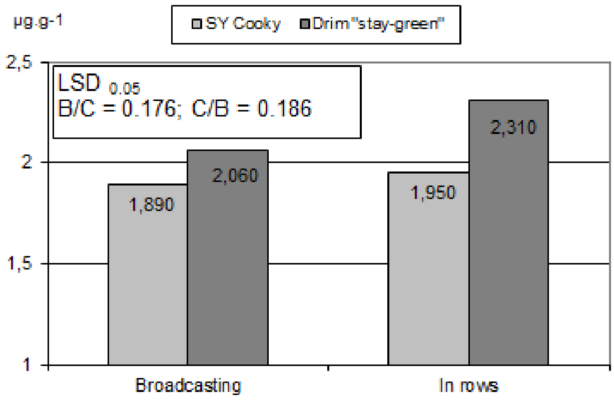
Rys. 1. Zawartość chlorofilu a w fazie 5-6 liści (BBCH 15/16) w zależności od współdziałania odmiany ze sposobem wysiewu nawozu NP (2012-2014)

3.1.2. Chlorophyll *b* content

Chlorophyll *b* content in the 5-6 leaves stage (BBCH 15/16) significantly depended on all three experimental factors. i.e. sowing method, variety and NP fertilizer application (Tab. 4). Significantly higher values of this trait were demonstrated for sowing in cultivated soil. Drim "stay-green" variety and row application of NP fertilizer compared to maize sown directly into stubble, traditional variety SY Cooky and NP fertilizer broadcast application (Tab. 4).

3.1.3. Chlorophyll *a+b* contents

Chlorophyll *a+b* contents in the 5-6 leaves stage (BBCH 15/16) significantly depended on all three experimental factors. i.e. sowing method, variety and the method of NP fertilizer application (Tab. 5). Significantly higher values of this trait were demonstrated for sowing in cultivated soil. Drim "stay-green" variety and row application of NP fertilizer compared to maize sown directly into stubble, traditional variety SY Cooky and NP fertilizer broadcast application (Tab. 5). Chlorophyll *a+b* contents in the conducted field experiment was also significantly affected by the interaction of the cultivar with NP fertilizer sowing method (Fig. 2). The method of fertilizer application had no significant effect on the content of chlorophyll *a* for the SY Cooky variety. In turn. the Drim "stay-green" hybrid was characterized by a higher content of this pigment in row fertilization compared to broadcast application (Fig. 2).



Source: own study / Źródło: opracowanie własne

Fig. 2. Chlorophyll *a+b* contents in the 5-6 leaf stage (BBCH 15/16) depending on the interaction of the variety with NP fertilizer sowing method (2012-2014)
Rys. 2. Zawartość chlorofilu a+b w fazie 5-6 liści (BBCH 15/16) w zależności od współdziałania odmiany ze sposobem wysiewu nawozu NP (2012-2014)

3.1.4. Carotenoid contents

Carotenoid contents in the 5-6 leaves stage (BBCH 15/16) significantly depended on all three experimental factors. i.e. sowing method, variety and NP fertilizer appliaction (Tab. 6). Significantly higher values of this trait were demonstrated for sowing in cultivated soil. Drim "stay-green" variety and row application of NP fertilizer compared to maize sown directly into stubble, traditional variety SY Cooky and NP fertilizer broadcast application (Tab. 6).

Table 4. Chlorophyll *b* content in the 5-6 leaf stage (BBCH 15/16) ($\mu g{\cdot}g^{-1}$)
Tab. 4. Zawartość chlorofilu b w fazie 5-6 liści (BBCH 15/16) ($\mu g{\cdot}g^{-1}$)

Experimental factors		Years 2012	2013	2014	Average
Maize sowing methods **(A)**	sowing in traditionally cultivated soil	0.41	0.33	0.49	0.41
	direct sowing	0.40	0.34	0.34	0.36
LSD 0.05		**n.s.**	**n.s.**	**0.120**	**0.048**
Varieties **(B)**	SY Cooky	0.42	0.27	0.37	0.35
	Drim „stay-green"	0.39	0.40	0.46	0.42
LSD 0.05		**n.s.**	**0.072**	**0.075**	**0.031**
NP fertilizer sowing methods **(C)**	broadcasting	0.39	0.31	0.42	0.37
	in rows	0.42	0.37	0.41	0.40
LSD 0.05		**n.s.**	**0.055**	**n.s.**	**0.026**
Average		0.41	0.34	0.41	0.39

n.s. – non-significant difference

Source: own study / Źródło: opracowanie własne

Table 5. Chlorophyll *a+b* contents in the 5-6 leaf stage (BBCH 15/16) ($\mu g{\cdot}g^{-1}$)
Tab. 5. Zawartość chlorofilu a+b w fazie 5-6 liści (BBCH 15/16) ($\mu g{\cdot}g^{-1}$)

Experimental factors		Years 2012	2013	2014	Average
Maize sowing methods **(A)**	sowing in traditionally cultivated soil	2.17	1.76	2.56	2.16
	direct sowing	2.15	1.78	1.89	1.94
LSD 0.05		**n.s.**	**n.s.**	**0.501**	**0.228**
Varieties **(B)**	SY Cooky	2.23	1.48	2.05	1.92
	Drim „stay-green"	2.09	2.06	2.39	2.18
LSD 0.05		**n.s.**	**0.356**	**0.287**	**0.138**
NP fertilizer sowing methods **(C)**	broadcasting	2.09	1.61	2.23	1.98
	in rows	2.23	1.93	2.22	2.13
LSD 0.05		**n.s.**	**0.256**	**n.s.**	**0.124**
Average		2.16	1.77	2.22	2.05

n.s. – non-significant difference

Source: own study / Źródło: opracowanie własne

3.2. Chlorophyll content expressed in SPAD units in the tassel flowering stage (BBCH 67)

None of the experimental factors, synthetically for the three study years, significantly influenced chlorophyll content expressed in SPAD units in the cob flowering stage (Tab. 7). Chlorophyll content expressed in SPAD units was significantly affected by the interaction between sowing method and variety in the discussed developmental stage (Fig. 3). The sowing method did not have a significant impact on the value of this trait for the SY Cooky variety. On the other hand, the "stay-green" Drim variety showed a significant increase in chlorophyll content expressed in SPAD units in case of direct sowing into stubble compared to sowing into cultivated soil (Fig. 3).

Table 6. Carotenoid contents in the 5-6 leaf stage (BBCH 15/16) ($\mu g \cdot g^{-1}$)
Tab. 6. Zawartość karotenoidów w fazie 5-6 liści (BBCH 15/16) ($\mu g \cdot g^{-1}$)

Experimental factors		Years			Average
		2012	2013	2014	
Maize sowing methods **(A)**	sowing in traditionally cultivated soil	8.02	4.79	6.57	6.46
	direct sowing	7.86	4.47	5.21	5.85
LSD 0.05		**n.s.**	**n.s.**	**0.412**	**0.240**
Varieties **(B)**	SY Cooky	8.21	4.21	5.38	5.93
	Drim „stay-green"	7.67	5.05	6.41	6.37
LSD 0.05		**n.s.**	**n.s.**	**n.s.**	**0.177**
NP fertilizer sowing methods **(C)**	broadcasting	7.96	4.53	5.91	6.13
	in rows	7.91	4.74	5.88	6.18
LSD 0.05		**n.s.**	**n.s.**	**n.s.**	**n.s.**
Average		7.94	4.63	5.89	6.15

n.s. – non-significant difference

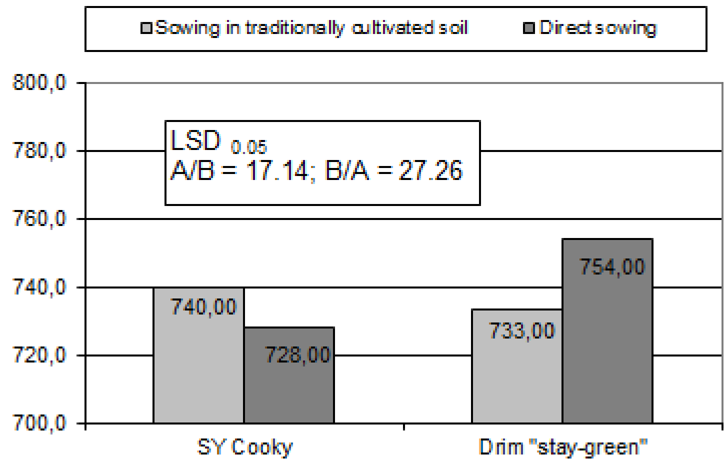
Source: own study / Źródło: opracowanie własne

Table 7. Chlorophyll content expressed in SPAD units (BBCH 67)
Tab. 7. Zawartość chlorofilu wyrażona w jednostkach SPAD (BBCH 67)

Experimental factors		Years			Average
		2012	2013	2014	
Maize sowing methods **(A)**	sowing in traditionally cultivated soil	736	752	722	737
	direct sowing	752	714	756	741
LSD 0.05		**n.s.**	**n.s.**	**n.s.**	**n.s.**
Varieties **(B)**	SY Cooky	738	724	739	734
	Drim „stay-green"	749	743	739	744
LSD 0.05		**n.s.**	**n.s.**	**n.s.**	**n.s.**
NP fertilizer sowing methods **(C)**	broadcasting	745	739	743	742
	in rows	743	727	736	735
LSD 0.05		**n.s.**	**n.s.**	**n.s.**	**n.s.**
Average		744	733	739	739

n.s. – non-significant difference

Source: own study / Źródło: opracowanie własne



Source: own study / Źródło: opracowanie własne

Fig. 3. Chlorophyll content expressed in SPAD units depending on the interaction of sowing method and variety (2012-2014)
Rys. 3. Zawartość chlorofilu wyrażona w jednostkach SPAD w zależności od współdziałania sposobu siewu z odmianą (2012-2014)

4. Discussion

Significantly higher chlorophyll content in leaf blades of the "stay-green" maize hybrid, as compared to the traditional variety is determined by the possibility of nitrogen accumulation until the end of plant growth [15]. According to this author, "stay-green" hybrids utilize two sources of nitrogen at the reproduction stage, remobilized from vegetative organs (leaves. shoots) and taken up from the soil. This allows the "stay-green" hybrids to take up mineral nitrogen from the soil at a rate that corresponds to the maize's demand for this macronutrient at the critical stage [15]. The author demonstrated a similar relationship in an earlier work [16] of higher chloroplast pigment contents in leaf blades in the "stay-green" hybrid compared to the traditional variety at the 5-6 leaf stage (BBCH 15-16). A higher chlorophyll content both in the juvenile stage (BBCH 15-16), which is a measure of better maize plant nutrition with nitrogen at the critical phase of maize nitrogen supply, resulted in a significantly higher grain yield obtained by this hybrid as compared to the conventional cultivar [17]. The obtained result, the relationship in the current study has confirmed previous literature reports that the productivity of maize cultivars depends on the content of chlorophyll $a+b$ in leaf blades [18]. Evaluation of the SPAD leaf greenness index values has already been studied in terms of using this parameter for arable crop yield estimation. Rostami et al. [19] reported in a study on maize that the yield of this species was significantly linearly correlated with the SPAD index values. This linear relationship indicates that the SPAD index can serve as a tool to estimate the yield of maize biomass for silage as well as grain yields [9].

5. Conclusions

1. Tillage method regardless of the years significantly shaping chloroplast pigment contents is the same factor that determines maize yield. Variety selection can although to a small extent correct the adverse effects of direct sowing systems.
2. The effects of direct sowing of the "stay-green" variety can be reduced, but not eliminated by a simultaneous application of phosphorus fertilizer in the BBCH 15/16 stage and in the subsequent stages of grain yield formation.
3. The nutritional status of maize in the nutrient-rich stands may only incidentally (depending on the years) show a reaction to variety selection and row application of NP-type fertilizers improving the values of chlorophyll content in leaf blades.
4. The lack of differences in the nitrogen nutritional status (SPAD) of plants at the BBCH 67 stage despite the considerable diversification of the experimental factor levels have indicated good conditions for the uptake of this nutrient in the period up to flowering. Selection of the variety in combination with row NP fertilization is the factor supporting the nutritional status of maize at this stage.
5. The "stay-green" type should be the more predisposed maize variety for direct sowing and row fertilization under these conditions due to the higher chlorophyll content of leaf blades.

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