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ORIGINAL ARTICLE

Response of oilseed rape leaves to sulfur and boron foliar application

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Abstract

Oilseed rape (*Brassica napus* L.) reacts differently to foliar application of sulfur (S) and boron (B), and for that reason it is important to find an early indicator that would inform about the direction of this reaction. This study aimed at evaluating the early response of two double-low cultivars of winter oilseed rape: hybrid (Nelson) and open pollinated (Digger) on the foliar fertilization with S and B in two terms: fall and spring, based on the rate of leaf greenness index (SPAD) and seven indicators of chlorophyll *a* fluorescence (FL). On 7th or 9th day after the application of liquid fertilizers, the selected parameters of FL and SPAD were determined on the leaves of rape. As a result, a significant effect of foliar B and S supplementation on the yield of oilseed rape was found. Principal component analysis (PCA) allowed for a separation for each of the cultivars the two parameters of FL, namely Tfm and Fv/F0, which are sensitive indicators of a physiological state of the rape plants shortly after foliar S and B dressing.

Keywords Leaf greenness index · Physiological state of rape · Principal component analysis · Yield

Introduction

Oilseed rape (*Brassica napus* L.) is one of the major oilseed crops worldwide (Shahzadi et al. 2015). According to a vast literature, oilseed rape has a high demand for sulfur (S) and boron (B) fertilization (Asad et al. 2002; Ahmad et al. 2012; Sienkiewicz-Cholewa and Kieloch 2015). This is because B and S are necessary for a synthesis of both glucosinolates and sulfur amino acids (Zhao et al. 1993). Many authors showed a very good reaction of oilseed rape to the application of both nutrients, but the method of B and S fertilization gives contrary results. Application of B fertilizers can be the most effective if incorporated into the soil (Malhi et al. 2003), seed and band placement may have toxic effects, and foliar application may be very effective to supply B to plants mostly when deficiency is noted during the growing season (Varga et al. 2014; Ma et al. 2015). Foliar fertilization is also an effective way to supply B to plants when root activity is restricted by dry soil (Mortvedt 1994).

Comparing the effect of the sulfur application method, Figas (2009) did not find significant differences in the seed yield, although growth trends were demonstrated on sulfur-fertilized soil facilities compared to objects on which sulfur was applied in a foliar manner. Higher yield of rape seeds fertilized with sulfur before sowing with top dressing and foliar fertilization was also obtained by Krauze and Bowszys (2001). Depending on the genotype and anthropogenic factors, beneficial effects were also obtained by sulfur fertilization of the winter in the phase of beginning of vegetation and budding (Rotkiewicz et al. 1996) or fertilizing spring rape in the elongation phase of shoots (Hocking et al. 1995).

Rape reacts differently to a foliar application of S and B, and for that reason it is important to find an early indicator that would inform about the direction of this reaction. For such use, the measurements describing the efficiency of the photosynthetic apparatus, i.e., fluorescence of chlorophyll *a* (Rohacek et al. 2008) and a leaf greenness index (SPAD) (Kulig et al. 2010) are suitable. The techniques

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of chlorophyll fluorescence measurements are easy, non-invasive and of high sensitivity (Kalaji et al. 2014). There are significant relationships existing between the values of SPAD and the crop yield, the selected physiological indicators and the biometric characteristics of plants (Müller et al. 2008; Wójtowicz and Wójtowicz 2008). Chlorophyll content is correlated with the accumulation of nitrogen in the plant and the availability of this nutrient in soil (Argenta et al. 2001). Studies have shown that there is a positive correlation between the amount of chlorophyll in the leaves and the rate of photosynthesis (Rumasz-Rudnicka 2010). Hossain et al. (2015) showed, moreover, an influence of boron fertilization on the improvement of rate of photosynthesis and its related gas exchange traits. Müller et al. (2005) found a non-linear relationship between the nitrogen content in the leaves and the value of the SPAD index. The difference in values of the SPAD index for rape during the growing season was confirmed by Ostrowska et al. (2008). In B-deficient *B. napus*, a drop of chlorophyll and carotenoid concentrations in the leaves is observed, which causes slight and non-significant changes in the process of photosynthesis, observed at the level of chlorophyll fluorescence (Hajiboland and Farhanghi 2010). Excess of B is also harmful to plants and it can lead to lower values of chlorophyll fluorescence parameters (Fv/Fm, Fv/F0), as was observed for oranges (Papadakis et al. 2004) and tomatoes (Guidi et al. 2011).

With reference to the above results, aim of this study was to evaluate an early response of two cultivars of winter oilseed rape to the foliar fertilization with sulfur and boron, based on the rate of leaf greenness index (SPAD) and the selected indicators of chlorophyll *a* fluorescence.

Materials and methods

Description of field experiment

A field experiment was carried out in 2008–2010 in Prusy near Krakow (Poland) on Luvic Chernozem (pH 5.8; P_2O_5 108 (medium); K_2O 112 (low); MgO 108 (high) g kg^{-1} d.m. soil; total N 0.15%; C_{org} 1.15%, C:N 7.7; total S 0.0192%, B 1.1 mg kg^{-1}).

It was a two-factorial split-plot experiment, with four replications. The area of plots was 11.2 m^2, and a harvested area 10 m^2. The following combinations of fertilizations were used: (1) control–NPKS; (2) NPKS + S (Thiotrac 300; 2×5 l/ha); (3) NPKS + S + B (Thiotrac 300; 2×5 l/ha + Bortrac 150; 2×3 l/ha). Thiotrac 300 (producer Yara Ltd) is a liquid fertilizer containing 15.2% N and 22.8% SO_3; Bortrac 150 (producer Yara Ltd) is a liquid fertilizer containing 10.9% B.

Two winter oilseed rape cultivars were used in this experiment: Digger (double-low open-pollinated cultivar, breeder: KWS Sp z o.o. Poland) and Nelson F1 (double-low hybrid cultivar, breeder Syngenta). Winter wheat was a forecrop. Prior to rape sowing 1.5 t ha of 50% CaO and NPKS 30/90/250/12 kg/ha fertilizers were applied to the soil. The first dose of N (100 kg/ha) was applied before the start of vegetation, second dose of N (75 kg/ha) was applied in the phase of shoot elongation of oilseed rape. Seeds of oilseed rape (60 pcs/m) were sown in 28-cm interrows. Terms of fertilizing, sowing and harvesting are presented in Table 1.

Weeds were controlled using herbicides in fall and spring: metazachlor and chinomerak, chlopiralide and pikloram. Pests and insects were 3–4 times sprayed with fungicides and insecticides: metkonazole; flusilazole and karbendazim; boskalide and dimoxystrobine; chloropiryphos + cypermetrine; deltametrine; acetamipride. Oilseed rape was harvested with a Combine Seedmaster Universal (Wintersteiger). Seed moisture was measured using a multi-grain moisture meter (DICKEY-John Europe) and a seed yield was calculated as per 85% of dry matter.

Measurements of chlorophyll a fluorescence (FL) and leaf greenness index (SPAD)

The measurement of indices was made in the fall and spring in both growing seasons, on 7th or 9th day after the foliar fertilization (Table 1). To mark the leaf greenness a SPAD 502DL meter was used (Minolta). Chlorophyll *a* fluorescence (FL) was determined by the HandyPea fluorometer

Table 1 Terms of the main agricultural practices and the measurements of chlorophyll *a* fluorescence (FL) indices as well as the leaf greenness index (SPAD) in both the vegetative seasons

Agricultural practice	2008/2009	2009/2010
Rape sowing	28.08.2008	31.08.2009
Foliar fertilization S + B in the fall	18.10.2008	22.10.2009
Fall measurements of FL and SPAD*	27.10.2008 (after 9 days)	29.10.2010 (after 7 days)
I N spring fertilization	1.04.2009	20.03.2010
II N spring fertilization	20.04.2009	19.04.2010
Foliar fertilization S + B in the spring	16.04.2009	19.04.2010
Spring measurements FL and SPAD*	22.04.2009 (after 7 days)	26.04.2010 (after 7 days)
Rape harvest	31.07.2009	2.08.2010

(Hansatech) after 20 min of a total leaf covering using special clips. Duration of light was of 1 s and of 0.7 gain with actinic light of 3000 μmol/m^2/s. Each time the measurements of FL and the SPAD were performed on ten plants per three plots at the same two leaves per each plant ($n = 20$) and then the measurement results were averaged ($n = 3$). In fall, the measurement were made when the plants were in a leaf development stage (BBCH 16–18), and in the spring in a phase of inflorescence emergence (BBCH 53–55). Seven indicators of FL were chosen for the analysis, and they are described in Table 2. Three of the indicators were from the basic indicators group and four of them from the performance index group (Table 2).

Statistical analysis

The results were analyzed statistically based on a two-factorial ANOVA for the split-plot design. The significance of differences between means was tested using the Tukey test, at the significance level of $p \leq 0.05$. To determine the correlation between indicators and yield of oilseed rape, a principal component analysis (PCA) was used. For a well-explained factor, the level of cumulative percentage of variance > 80% was assumed. To assess the adequacy of the correlation matrix, for comparisons of the partial correlations, a Kaiser–Mayer–Olkin (KMO) coefficient was used. Indicator was considered as important when KMO value > 0.5. Bartlett test of sphericity was used to verify the significance level between the variables at $p \leq 0.01$. Analyses were performed using the statistical software PAST ver. 3.04 (Hamme et al. 2001).

Weather conditions

During the autumn vegetation in both the growing seasons 2008/09 and 2009/10, a weather course was favorable for oilseed rape. In March 2009, a significant increase in rainfall was recorded comparing to multi-year period, while in April there was a strong deficiency of rainfall accompanied with very high air temperatures (Fig. 1). In the corresponding spring period in 2010, the course of weather conditions was very similar to the long-term period. From May to June 2009 and 2010, a strong rainfall was noticed well above the long-term period. The most wet months were noted in June 2009 of the rainfall by as much as 208% higher than the average long-term rainfall. The 2010 season was particularly wet in May 2010, when rainfalls exceeded the long-term rainfall by about 6.4 times (Fig. 1). Excess of rainfall led to a significant lodging of plants, impairing their growth as well as the flowering and pollination.

Results and discussion

The yield of winter oilseed rape in the 2008/09 and 2009/10 seasons depended strongly on climatic conditions; hence, the average yield in each of the growing seasons was of 3.73 and 3.20 t/ha in 2008/09 and 2009/10, respectively. The hybrid cv. Nelson yielded significantly better – 3.69 t/ha, compared to an open-pollinated cv. Digger – 3.23 t/ha (Table 3).

The reaction of cultivars to the foliar fertilization with S and B was also significant. The seed yield of cv. Nelson increased significantly by 17.5% following the foliar S application in 2010. In the other seasons the yield increase following the foliar S application, it was in a range of 2.5–3.8% (Table 3). A simultaneous foliar application of S and B always resulted in the desired yield-forming effect. In 2009, a significant increase in the yield was by 9.4% and 5.4% for cv. Digger and Nelson, respectively. In 2010, the yield was similar to that of control (NPKS) (Table 3), most probably because of an adverse effect of intensive rainfalls in the late spring months.

Table 2 Characteristics of the measured chlorophyll *a* fluorescence parameters

Parameter	Optimal ranges[a]	Description
General parameters		
Fv/Fm	0.79–0.84	The maximum yield of photosystem II (PS II)
Tfm	500–800 ms	Time to reach the maximal fluorescence
Area	–	Area above the fluorescence transient
Performance index parameters		
RC/ABS	–	Reaction center (RC) concentration per antenna chlorophyll
Fv/F0	–	Value proportional to the activity of the water-splitting complex on the Donor side of the PS II stomatal conductance
(1-Vj)/Vj	–	Forward electron transport
PI	–	Performance Index

[a]Optimal ranges are given according to the available literature (Kalaji et al. 2011)

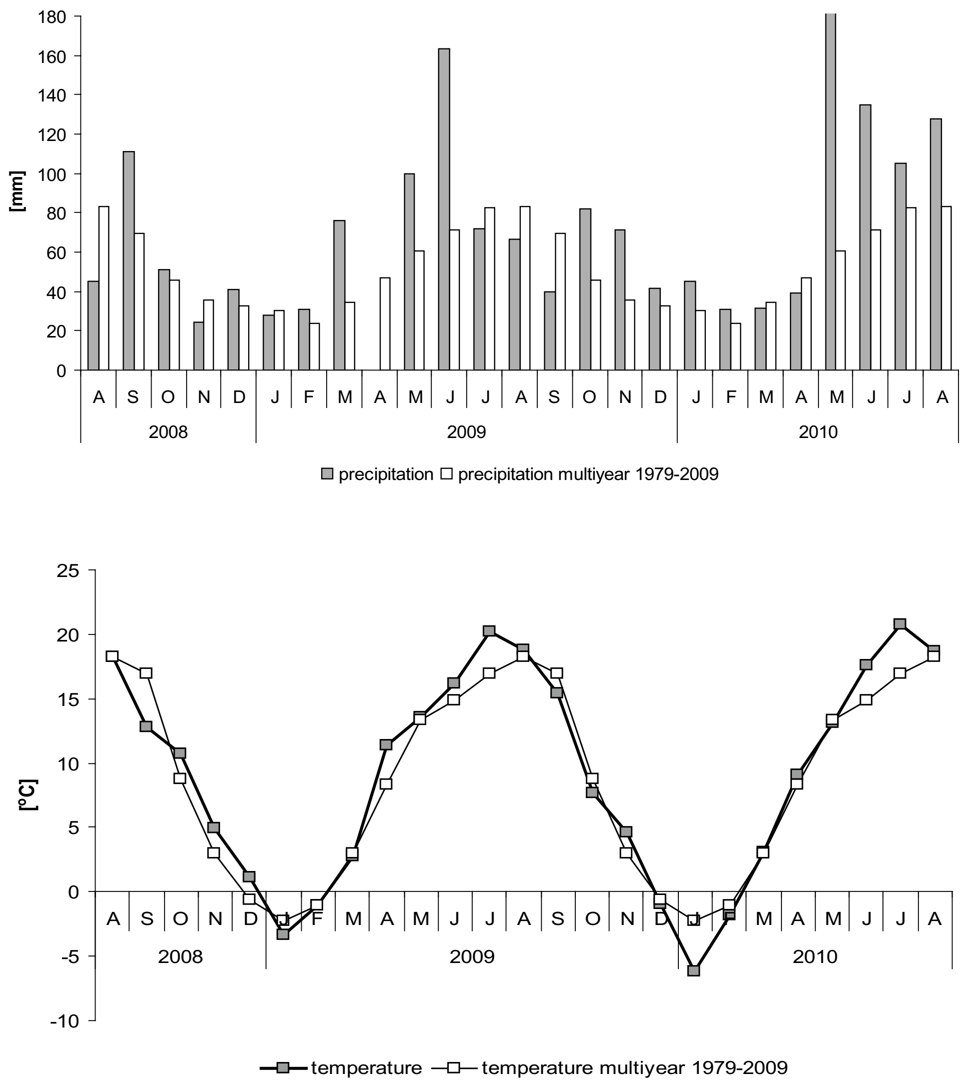

Fig. 1 Monthly precipitation and mean air temperatures during the vegetation of winter oilseed rape in 2008–2010, as compared to a multi-year period 1979–2009. *J* January, *F* February, *M* March, *A* April, *M* May, *J* June, *J* July, *A* August, *S* September, *O* October, *N* November, *D* December

A principal component analysis (PCA) allowed for a separation of diverse groups of terms (fall, spring) and seasons (2008/09, 2009/10) for each of the oilseed rape cultivars. KMO index for each of the two PCA analyses reached the required threshold value (> 0.5) in determining the adequacy of the correlation matrix. The groups terms–seasons were separated more clearly for cv. Digger (Fig. 2). For this cultivar a PCA explained 84.46% of a variability present in the sample. A set of four groups of terms–seasons for cv. Digger was mostly negative. At the same time, the group C: fall 2008 had more optimal values, comparing to the other groups (Fig. 2). Also, three FL indicators for cv. Digger, namely Fv/Fm and Fv/F0 (of very similar loads) and Tfm had better values, in comparison to the other indicators (Fig. 2). In the case of hybrid cv. Nelson, PCA explained 85.91% of the variation within the sample (Fig. 3). For this cultivar, a group B: spring 2010 showed more favorable values of indicators, compared to the other groups. Among the FL indicators three of them had better values, comparing to the others—Tfm, Fv/F0 and (Vj – 1)/Vj (Fig. 3).

To summarize, both PCA analysis showed the different groups of term–season for each of rape cultivars when the FL indicators displayed better values. In the case of cv. Digger, it was a group fall 2008, and in the case of hybrid cv. Nelson a group spring 2010. At the same time, for both cultivars the common FL indicators were found, i.e., Tfm and Fv/F0, that in each case reached better values than the other indicators. Therefore, the results of PCA indicated the desirability of precise measurements of these parameters in the early assessment of a physiological state of winter oilseed rape. At the same time, in both the PCA analyses, a leaf greenness index (SPAD) was revealed, but in each case it had less optimal values than the other indicators (Figs. 2, 3).

For the three indicators highlighted in the PCA, i.e., Fv/F0, Tfm and SPAD, a more detailed analysis of variance was performed, to compare their values in the early period after foliar application of fertilizers (Tables 4, 5).

Table 3 Seed yield (t ha^{-1}) of oilseed rape cv. Digger and Nelson as influenced by a fertilization and vegetative season

Fertilization	Year	Cultivars		
		Digger	Nelson	Mean
NPKS	2008/09	3.13	4.06	3.60
	2009/10	3.23	2.98	3.11
	Mean	3.18	3.52	3.35
NPKS + S	2008/09	3.25	4.22	3.73
	2009/10	3.31	3.50	3.41
	Mean	3.28	3.86	3.57
NPKS + S + B	2008/09	3.43	4.28	3.86
	2009/10	3.05	3.12	3.08
	Mean	3.24	3.70	3.47
Mean	2008/09	3.27	4.19	3.73
	2009/10	3.20	3.20	3.20
	Mean	3.23	3.69	3.46
$LSD_{0.05}$ fertilization		1.57		
$LSD_{0.05}$ cultivar		0.06		
$LSD_{0.05}$ year		0.58		
$LSD_{0.05}$ cultivar/year		0.09		

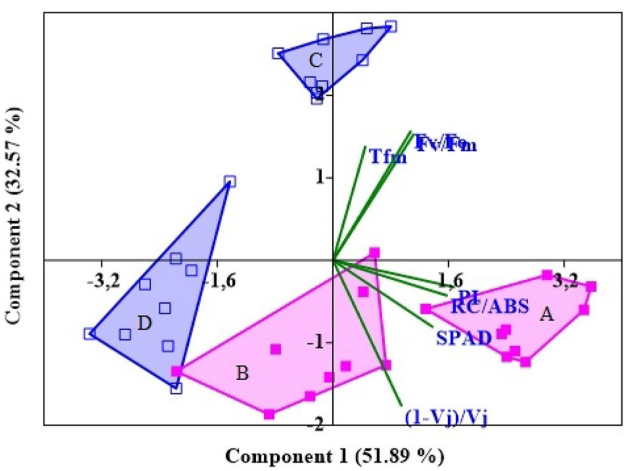

Fig. 2 Principal component analysis of chlorophyll a fluorescence and a leaf greenness index (SPAD) for open-pollinated oilseed rape cv. Digger in the different measurement terms (fall violet square and spring pink square) and growing seasons (2008/09 and 2009/10), for the fertilization combinations jointly. Indices in the upper right quadrant are of better values as compared to the other indices. Chi-square statistics 423.96; degrees of freedom 28; $p < 0.000001$, Keiser–Mayer–Olkin value 0.67. **a** Spring 2009, **b** spring 2010, **c** fall 2008, **d** fall 2009

There were no significant differences between the variants of fertilization in any of the terms and the cultivars of rape. For both rape cultivars, the distribution of values of particular indicators was similar (Tables 4, 5). Lower and more favorable value of Tfm indicator was each time recorded in the spring compared to autumn term and

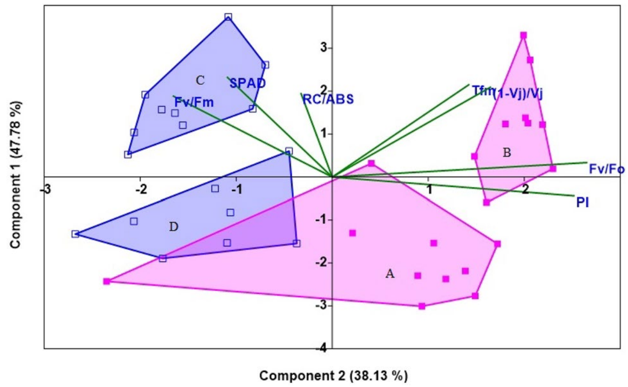

Fig. 3 Principal component analysis of chlorophyll a fluorescence and a leaf greenness index (SPAD) for hybrid oilseed rape cv. Nelson in the different measurement terms (fall violet square and spring pink square) and growing seasons (2008/09 and 2009/10), for the fertilization combinations jointly. Indices in the upper right quadrant are of better values as compared to the other indices. Chi-square statistics 166.24; degrees of freedom 21; $p < 0.000001$, Kaiser–Mayer–Olkin value 0.55. **a** Spring 2009, **b** spring 2010, **c** fall 2008, **d** fall 2009

especially, in the spring of 2009. In April 2009, a significant shortage of rainfall with high air temperatures was recorded, but March 2009 was wet much more then a multi-year norm (Fig. 1). In the spring period the values of Tfm were in a very narrow range of 336–361 for cv. Nelson and 376–431 for cv. Digger. On the other hand, significantly higher, so more favorable ratio of Fv/F0 indicator was each time recorded in the fall. A detailed analysis showed that there was a wide variation of Fv/F0 value in the fall between seasons, and more optimal values of Fv/F0 were noted in the fall 2009. In October 2009, there was a severe rainfall noted, but the temperature did not differ from a long-term period. The SPAD index was significantly different only between the terms—each time the higher values of SPAD were observed in spring, compared to autumn (Tables 4, 5).

Different authors showed different reactions of the photosynthetic apparatus to a fertilization of rape with sulfur and boron—lack of response (Weese et al. 2015), stimulation (Hossain et al. 2015) and even a reduction of values of some FL indicators (Ostrowska et al. 2008).

In our study, PCA analysis allowed a distinction of the two indicators of FL, namely Tfm and Fv/F0, each time of better values, in a short time after foliar application of sulfur and boron than the other indicators. Most probably, the correlation of adverse weather factors later in the growing season made the oilseed rape seed yield ultimately influenced by a foliar fertilization, as compared to the control.

Undoubtedly, the weather course in the month of indicators measurement as well as the development phase of the rape both influenced significantly the formation of the FL indicators (Tfm and Fv/F0). Especially, rainfall could

Table 4 Values of chlorophyll a fluorescence indicators Tfm and Fv/F0 and leaf greenness index SPAD for open-pollinated oilseed rape cv. Digger

Fertilization	Year	Tfm			Fv/F0			SPAD		
		Autumn	Spring	Mean	Autumn	Spring	Mean	Autumn	Spring	Mean
NPKS	2008/09	536	376	456	4.05	4.51	4.28	44.8	55.0	49.9
	2009/10	583	532	557	4.80	4.54	4.67	47.2	56.8	52.0
	Mean	559	454	507	4.42	4.53	4.47	46.0	55.9	50.9
NPKS + S	2008/09	525	431	478	3.99	4.32	4.16	42.6	51.3	47.0
	2009/10	578	580	579	5.03	4.64	4.84	48.7	55.4	52.0
	Mean	551	505	528	4.51	4.48	4.50	45.7	53.3	49.5
NPKS + S + B	2008/09	534	422	478	3.90	4.27	4.09	43.6	49.7	46.7
	2009/10	594	568	581	4.99	4.58	4.79	45.3	54.1	49.7
	Mean	564	495	530	4.45	4.43	4.44	44.5	51.9	48.2
Mean	2008/09	532	410	471	3.98	4.37	4.17	43.7	52.0	47.8
	2009/10	585	560	572	4.94	4.59	4.77	47.1	55.4	51.2
	Mean	558	485	522	4.46	4.48	4.47	45.4	53.7	49.5
$LSD_{0.05}$ fertilization		NS			NS			NS		
$LSD_{0.05}$ term		32.8			NS			1.18		
$LSD_{0.05}$ year		41.3			0.10			1.55		
$LSD_{0.05}$ year/fertilization		NS			0.17			NS		
$LSD_{0.05}$ term/year		52.7			0.15			NS		

NS non-significant differences

Table 5 Values of chlorophyll a fluorescence indicators Tfm and Fv/F0 and leaf greenness index SPAD for hybrid oilseed rape cv. Nelson

Fertilization	Year	Tfm			Fv/F0			SPAD		
		Autumn	Spring	Mean	Autumn	Spring	Mean	Autumn	Spring	Mean
NPKS	2008/09	498	336	417	4.08	4.32	4.20	43.6	53.0	48.3
	2009/10	613	482	547	4.95	4.31	4.63	45.6	51.7	48.6
	Mean	555	409	482	4.51	4.32	4.42	44.6	52.3	48.5
NPKS + S	2008/09	493	361	427	4.07	4.22	4.15	43.3	54.1	48.7
	2009/10	610	451	530	4.90	4.34	4.62	45.4	51.9	48.7
	Mean	552	406	479	4.49	4.28	4.38	44.4	53.0	48.7
NPKS + S + B	2008/09	566	338	452	3.75	4.27	4.01	42.0	53.6	47.8
	2009/10	562	550	556	4.96	4.51	4.73	44.0	51.8	47.9
	Mean	564	444	504	4.35	4.39	4.37	43.0	52.7	47.9
Mean	2008/09	519	345	432	3.97	4.27	4.12	43.0	53.6	48.3
	2009/10	595	494	544	4.94	4.39	4.66	45.0	51.8	48.4
	Mean	557	419	488	4.45	4.33	4.39	44.0	52.7	48.3
$LSD_{0.05}$ fertilization		NS			NS			NS		
$LSD_{0.05}$ term		18.3			0.12			1.30		
$LSD_{0.05}$ year		30.3			0.14			NS		
$LSD_{0.05}$ term/year		35.3			0.18			1.82		

NS non-significant differences

modify the level of absorption of foliar fertilizers by rape, thus affecting their physiological state.

In general, in autumn plants of rape that were in the early stages of development had a significantly higher value of Tfm, compared to the same plants analyzed in the spring, at a later stage of development. According to Reigosa and Weiss (2001) under stress conditions, Tfm value increases as a result of slowing down the transport of electrons from the reaction centers to plastochinons. In our research, during dry and warm April 2009 (the sum of rainfalls for that month was equal to 0.2 mm) a more favorable ratio of Tfm was recorded, compared to April 2010, when the temperature and precipitation did not deviate from the long-term period. Probably, a foliar fertilization with boron could affect the results. As was shown by Mortvedt (1994), a greater foliar assimilation of boron occurs in the conditions of soil drought. Perhaps, in the short term after the application of boron, the physiological condition of the rape plants was improved, as revealed by better values of Tfm index.

In turn, more favorable, significantly higher (cv. Digger) values of Fv/F0 indicator were recorded in the autumn, especially in October 2009 (that month was wet and of an average to a long-term temperature), as compared to October 2008, when the weather conditions were similar to the long-term period. Indicator Fv/F0, which is characterized by the efficiency of water splitting, is a very sensitive part of the photosynthetic electron transport chain (Pereira et al. 2000), much more sensitive than a commonly determined Fv/Fm indicator. As was shown by Hura et al. (2007) for various plant species, this Fv/F0 is reduced significantly in the dry conditions.

On the other hand, in the case of the leaf greenness index (SPAD), significant differences were noted only between the terms of measurement—each time higher SPAD values were observed in the spring terms, in the phase of rape inflorescence emergence, as compared with the fall. Undoubtedly, this result was affected by a two-time N fertilization in the spring, which most probably directly related to a higher content of chlorophyll in plants. Müller et al. (2005) showed a non-linear relationship between the nitrogen content and the SPAD index. A differentiation of values of the SPAD index during the growth of spring rape was also confirmed by Ostrowska et al. (2008). According to the other authors (Koohkan and Maftoun 2016), application of B decreases chlorophyll concentration in leaves, which may affect negatively the values of SPAD index, but this was not confirmed in our study.

Summing up, in this study, the additional foliar supplementation of S and B produced a higher seed yield of winter oilseed rape cultivars Nelson than Digger. In a short time (7th–9th day) after the foliar application of S and B, we showed varied values of FL and SPAD indicators for both cultivars. Generally, the better values of FL were recorded in fall 2008 and spring 2010, for cv. Digger and cv. Nelson, respectively. The two indicators of FL, namely Tfm (time required to reach the maximum fluorescence) and Fv/F0 (the activity of the water-splitting complex on the donor side of the PSII stomatal conductance) showed a differentiation, depending on the term of measurement (Fall or Spring) as well as the growing season (2008/09 or 2009/10), regardless of the fertilization of rape. A detailed statistical analysis showed that a better value of Tfm indicator was recorded in a warm and dry April, whereas the Fv/F0 indicator displayed better values during wet October. There was clear evidence that S and B fertilization affects the SPAD index, and the value of this index for each cultivar was higher in the spring, regardless of the growing season, following the N fertilization, when plants were in the phase of inflorescence emergence.

Author contribution statement KP carried out the experiment and wrote the manuscript; BK designed and carried out the experiment; BK and AL analyzed the data; WH and SP carried out statistical analysis.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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