Acta fytotechn. zootechn., 17, 2014(2): 58–64

Original Paper

Received 9. May 2014 | Accepted 10. June 2014 | Available online 23. June 2014

Content changes of assimilative pigments in leaves after fertilizer Mg-Titanit application

Peter Kováčik[1]*, Agnieszka Baran[2], Angelika Filová[1], Martin Vician[1], Jozef Hudec[1]

[1]*Slovak University of Agriculture in Nitra, Nitra, Slovakia*
[2]*Hugo Kołłątaj´s Agricultural University in Krakow, Poland*

The impact of the liquid fertilizer Mg-Titanit (MGT) containing titanium on the dynamics of the content changes of the total chlorophyll and carotenoids in the leaves of winter wheat and winter rape was studied in the field small-area trial carried out at the Haplic Chernozem. The trial involved 5 variants. 0 – control, MGT unfertilized variant; $2 \times Ti_{0.2}$ – spraying twice by MGT in the dose 0.2 l ha^{-1}; $3 \times Ti_{0.2}$ – spraying thrice by MGT in the dose 0.2 l ha^{-1}; $2 \times Ti_{0.4}$ – spraying twice by MGT in the dose 0.4 l ha^{-1}, $3 \times Ti_{0.4}$ – spraying thrice by MGT in the dose 0.4 l ha^{-1}. The fertilizer was used at two or three growth stages: wheat was fertilized at the stages BBCH 29, BBCH 32, BBCH 55 – 60; rape at the stages BBCH 50 – 52, BBCH 59, BBCH 66 – 67 during the years 2009/2010 – 2010/2011. The results proved that in the cultivation of winter wheat the content of the total chlorophyll was increased annually with two applied doses of Mg-Titanit if the spraying was carried out at the growth stage BBCH 32 (the second spraying). The winter rape reacted positively to Mg-Titanit spraying applied at the growth stage BBCH 50 – 52 (first spraying) by the chlorophyll formation in both years of the trial. The third spraying tended to decrease the content of total chlorophyll with both crops and both applied doses of Mg-Titanit in the most cases. In the overwhelming majority of cases the content of the total chlorophyll was higher in the plants treated with the dose of 0.2 l ha^{-1} than those treated by the dose 0.4 l ha^{-1}. The positive impact of Mg-Titanit on the content of the total chlorophyll was carried out by the increase of the amount of both assimilative pigments (chlorophyll *a* and chlorophyll *b*).

Keywords: chlorophyll, fertilizer, titanium, winter wheat, winter rape

1. Introduction

The photosynthesis plays the direct role in the production process of plants (Ducsay, 2011; Olšovská et al., 2013). It depends on the size of photosynthesizing surfaces and the efficiency of the photosynthetic apparatus which is determined by the quality of the pigmental system. The most important pigments are chlorophyll *a* and chlorophyll *b* which are worn out in the process of photosynthesis therefore they are renewed permanently (Baker, 1996). The mineral nutrition determines significantly the photosynthetic activity of plants. The leaves grow old with the insufficient nutrition and lose the ability of the photosynthetic activity. The effect of particular nutrients on the process of photosynthesis is very different (Larcher, 1988). The positive relation is usually recorded between the nutrients supply in a plant and the size of the leaf surface and the photosynthesis speed (Nátr and Lawlor, 2005; Namvar et al., 2013), or between the content of nitrogen, magnesium, sulphur and the content of the total chlorophyll in a plant (Filová and Krivosudská, 2013; Marschner, 2005; Tůma and Tůmová, 2006). The other authors as Ram et al. (1983) but also Carvajal and Alcaraz (1995), Kužel et al. (2003), Kováčik et al. (2012) recorded the increase of content of the total chlorophyll in the cultivated plants after the application of the fertilizer containing titanium. Similarly, Traetta-Mosca (1913), Pais (1983), Simon et al. (1988), Matuškovič (1995), Carvajal and Alcaraz, (1998) observed that the well-balanced titanium application increases not only the chlorophyll content but also the yield of the cultivated crops, in particular from 5 to 50 %. On the other hand, the excessive application of titanium decreases yield (Kužel et al. 2007). The negative effects of titanium are evident on the plants more frequently if the plants are cultivated on the soils with the insufficient magnesium content, with the content of soil organic carbon less than 0.7% and the pH less than 6.

The first evidence of the excessive plant nutrition with titanium is the decreased content of the total chlorophyll (Carvajal and Alcaraz, 1998; Hrubý et al., 2002). The numerical value of the reasonable and unreasonable dose of titanium is different depending on the particular crops in the different cultivating conditions. Therefore, the objective of these experiments was to determine the

*Correspondence: Peter Kováčik, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Sciences, Department of Agrochemistry and Plant Nutrition, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, e-mail: peter.kovacik@uniag.sk

DOI: 10.15414/afz.2014.17.02.58–64

impact of two doses of the fertilizer containing titanium, which were applied on two different crops (cereals and oil plants) during the growing season (in the first treatment – double application and in the second treatment – triple application) on total chlorophyll content in the leaves of winter wheat and winter rape.

2. Materials and methods

The dynamics of the content changes of the assimilative pigments after the application of the fertilizer Mg-Titanit (MGT) was studied in the field small-area trial carried out at the Haplic Chernozem (48° 42′ N, 17° 70′ E) during two years (2010 and 2011) with two crops (winter bread wheat and winter rape). The agrochemical parametres of the trial plots are given in the Table 1. They were determined by the following methods: N-NH_4^+ – colorimertically by Nessler`s agent, N-NO_3^- – colorimertically by the phenol 2.4 disulphonic acid, N_{an} = N-NH_4^+ + N-NO_3^-, P – colorimertically (Mehlich III – Mehlich, 1984), K and Ca – flame photometry (Mehlich III – Mehlich, 1984), Mg – AAS (Mehlich III – Mehlich, 1984), C_{ox} – oxidometrically (Ťjurin, 1966), N_t – by distilling (Kjeldahl – Bremner, 1960), pH/KCl –potentiometrically (1.0 mol.dm^{-3} KCl).

The wheat and rape trials involved 5 variants. Var. 1: 0Ti – control, MGT unfertilized variant; var. 2: 2 × $Ti_{0.2}$ – spraying twice by MGT in the dose 0.2 l ha^{-1}; var. 3: 3 × $Ti_{0.2}$ – spraying thrice by MGT in the dose 0.2 l ha^{-1}; var. 4: 2 × $Ti_{0.4}$ – spraying twice by MGT in the dose 0.4 l ha^{-1}, var. 5: 3 × $Ti_{0.4}$ – spraying thrice by MGT in the dose 0.4 l ha^{-1}. The fertilizer Mg-Titanit contained 3 % magnesium, 4 % sulphur, and 8.5 g of titanium in 1 litre, where titanium was in the form of titanium ascorbate and sulphur and magnesium in the form of magnesium sulfate ($MgSO_4$). The fertilizer Mg-Titanit, which has been categorized as a biostimulator since 2013, was applied at two or three growth stages: at wheat in the phenophases BBCH 29, BBCH 32, BBCH 55; at rape in the phases BBCH 50 – 52, BBCH 59, BBCH 66 – 67. 14 to 21 days after the fertilizer application by spraying the samples of the youngest, fully developed sound leaves were analysed from both crops and the pigment contents were determined. The spectrophotometric method was used to determine the content of the assimilative pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) in the acetonic extract. Extract was centrifuged 2 minutes at 2500 rpm. Absorbance (A) of the solution was measured by UV-VIS spectrophotometer (Jenway, UK), at 470 nm (carotenoids), 647 nm (chlorophyll *b*), and 663 nm (chlorophyll *a*), with correction for scattering at 750 nm; the measurements were done in three repetitions. The concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) in mg l^{-1} were determined by using the equations of Lichtenthaler (1987):

$$\text{Chl } a = 12.25 \times (A_{663} - A_{750}) - 2.79 \times (A_{647} - A_{750}) \times D$$

$$\text{Chl } b = 21.50 \times (A_{647} - A_{750}) - 5.10 \times (A_{663} - A_{750}) \times D$$

$$\text{Chl } a + b = 7.15 \times (_{A663} - A_{750}) + 18.71 \times (A_{647} - A_{750}) \times D$$

$$\text{Car} = [(1{,}000 \times (A_{470} - A_{750}) - 1.82 \times (\text{Chl } a) - 85.02 \times \times (\text{Chl } b)/198] \times D$$

The concentrations of the pigments were calculated in mg l^{-1}; A_n was the absorbance at given wavelengths (*n*) after correction for scattering at 750 nm; D was the optical thickness of cuvette; results were also recalculated in mg.m^{-2} using the volume of solution and the area of leaf segments: [mg m^{-2}] = V/1000 × 1/A, when V is volume of 80% acetone and A is area of leaf segments. The sampling was carried

Table 1 Agrochemical soil parameters before trial founding

Year	Plant	Depth	N_{an}	P	K	Ca	Mg	S	N_t	C_{ox}	pH_{KCl}
		m	mg kg^{-1}							%	
2009/2010	wheat	0.0–0.3	18.3	70	235	3600	317	13,8	2142	1.52	7.14
		0.3–0.6	12.7	33	200	7900	337	12,1	1233	1.31	7.32
		0.0–0.6	15.5	52	218	5750	327	13,0	1688	1.42	7.23
	rape	0.0–0.3	17.0	58	250	2600	415	14,5	1766	1.64	6.80
		0.3–0.6	7.1	21	191	4550	413	15,5	1651	1.34	6.98
		0.0–0.6	12.1	40	221	3575	414	15,0	1709	1.49	6.89
2010/2011	wheat	0.0–0.3	18.9	48	250	2450	390	41,9	1360	1.31	6.55
		0.3–0.6	27.3	10	155	9750	410	68,8	932	0.90	6.57
		0.0–0.6	23.1	29	203	6100	400	55,3	1146	1.11	6.56
	rape	0.0–0.3	15.4	38	210	3850	495	23,8	1360	1.60	6.92
		0.3–0.6	10.7	8.0	110	11450	515	45,1	838	0.90	6.98
		0.0–0.6	13.1	23	160	7650	505	34,5	1099	1.25	6.95

DOI: 10.15414/afz.2014.17.02.58–64

out at the following stages: wheat BBCH 32, BBCH 55, BBCH 65 – 67, rape BBCH 59, BBCH 66 – 67, BBCH 71.

Acquired results were processed by mathematical and statistical method, by analysis of variance (ANOVA) using Statgraphics PC program, version 5.0.

Results and discussion

The pigment biosynthesis in plants is influenced by a whole range of external and internal factors. Apart from the genetic factors also the light, temperature, water and last but not least nutrition rank among the important factors. The Figure 1 shows the fact that in the first year of the trial the wheat leaves treated with the fertilizer Mg-Titanit proved a higher content of the total chlorophyll (chlorophyll *a* + chlorophyll *b*) in each sampling than the wheat leaves cultivated in the control unfertilized variant. The highest and statistically significant increase of the contents of total chlorophyll was achieved at the growing stage BBCH 55, i.e. after the second spraying applied at the growing stage BBCH 32. The growth rates varied in the interval from 33.5 % to 37.3 %. A higher increase was recorded in the variant where a lower dose of fertilizer (0.2 l ha^{-1}) was applied. The first and third spraying (application at the growing stages BBCH 29 and BBCH 55) impacted on the content of the total chlorophyll insignificantly (Table 2). The first spraying tended to increase the contents of the total chlorophylls (crop samplings at BBCH 32) and the third spraying (var. 2 versus var. 3 and var. 4 versus var. 5) had the tendency to decrease the contents of the total chlorophylls (crop samplings at BBCH 67). Despite the recorded slightly negative impact of the third spraying on the content of the total chlorophylls their contents were higher compared with the contents of the total chlorophyll in the unfertilized variant.

The Table 2 shows that the increase of the total chlorophyll contents after spraying by Mg-Titanit was carried out through both chlorophyll *a* and chlorophyll *b*. The fertilizer Mg-Titanit also tended to increase the carotenoids content, except for the sampling after the first spraying.

The content changes of the total chlorophyll in the wheat leaves proved the single-apical progress (Figure 1) in the first year of the trial. At the stage of the massive vegetative growth and ear formation, i.e. from April to May the chlorophyll content was increased. It was decreased with the progressive senescence of the leaf system and the plants themselves. The differences related to the contents of the total chlorophyll between the plants sprayed by the fertilizer Mg-Titanit and unfertilized plants were gradually decreased.

In the second year of the trial, at the growth stage BBCH 32, i.e. 14 days after the first wheat spraying by the fertilizer Mg-Titanit, which was applied at the growth stage BBCH 29, unlike in the first year generally supposed (Kužel et al., 2003; Dobromilska, 2007) positive impact

Table 2 Impact of trial variants on dynamics of changes of pigment contents in leaves of winter wheat in 2010

Variant no.	Variant des.	Date	Growth stage	Chlor. *a* ($mg\ m^{-2}$)	Chlor. *b* ($mg\ m^{-2}$)	Chlorophyll *a* + *b* ($mg\ m^{-2}$)	Chlorophyll *a* + *b* (%)	Carotenoid ($mg\ m^{-2}$)
1	0Ti	19. 04. 2010	BBCH 32	302.29a	79.10a	381.39a	100.00	93.79a
2–3	$Ti_{0.2}$			307.10a	106.43b	413.53a	108.43	89.20a
4–5	$Ti_{0.4}$			284.23a	105.79b	390.01a	102.26	89.54a
$LSD_{0.05}$				23.528	22.131	33.079		14.143
$LSD_{0.01}$				27.739	28.046	41.664		21.428
1	0Ti	20. 05. 2010	BBCH 55	314.39a	95.77a	410.16a	100.00	90.22a
2–3	$Ti_{0.2}$			394.11b	169.16b	563.28b	137.33	112.80b
4–5	$Ti_{0.4}$			383.77b	163.89b	547.65b	133.52	107.39ab
$LSD_{0.05}$				25.592	22.201	36.264		18.972
$LSD_{0.01}$				29.926	38.637	55.246		28.744
1	0Ti	10. 06. 2010	BBCH 67	304.61a	81.72a	386.33a	100.00	33.96a
2	$2 \times Ti_{0.2}$			348.54a	82.58a	431.11a	111.59	36.36ab
3	$3 \times Ti_{0.2}$			331.21a	80.03a	411.25a	106.45	42.51b
4	$2 \times Ti_{0.4}$			330.08a	82.74a	412.83a	106.86	39.13ab
5	$3 \times Ti_{0.4}$			324.80a	82.76a	407.56a	105.50	35.48ab
$LSD_{0.05}$				47.307	17.513	51.285		8.090
$LSD_{0.01}$				60.944	24.551	66.941		11.342

Chlor. *a* – chlorophyll *a*, chlor. *b* – chlorophyll *b*, no. – number, des. – designation, $LSD_{0.05}$ – Least Significant Difference at the level $\alpha = 0.05$

DOI: 10.15414/afz.2014.17.02.58–64

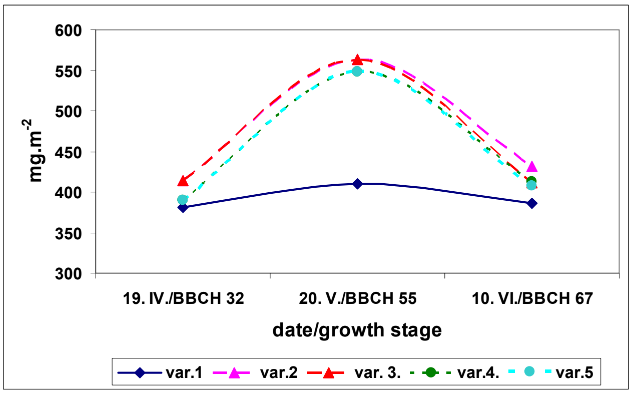

Figure 1 Dynamics of changes of total chlorophyll contents in leaves of winter wheat in 2010

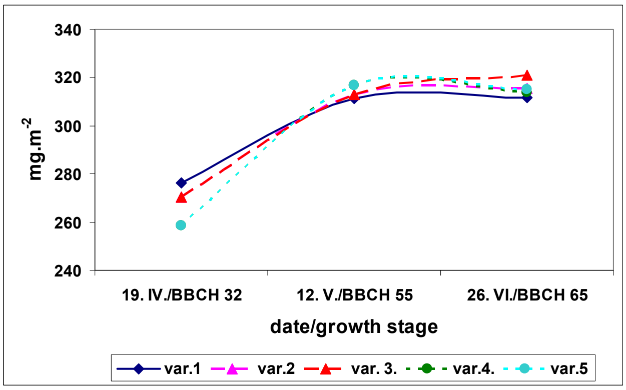

Figure 2 Dynamics of changes of total chlorophyll contents in leaves of winter wheat in 2011

of fertilizers containig titanium on the total chlorophyll content was not recorded (Figure 2). The following samplings of the variants treated by Mg-Titanit proved higher contents of the total chlorophyll compared with the unfertilized variant, however, the differences were not significant statistically (Figure 2 and Table 3). Higher contents of the total chlorophylls were detected in the variants where a higher dose of the tested fertilizer was applied. In the same year the application of Mg-Titanit had the insignificant impact on the content of the particular pigments (chlorophyll *a*, chlorophyll *b*, carotenoids). The similar findings about the conclusive and also inconclusive impact of the fertilizer containing titanium on the chlorophyll content in plants were detected by Vician et al. (2012).

The Figure 3 and Table 4 demonstrate that 5.4 % (var. 1 versus var. 2 and 3) and 11.4 % (var. 1 versus var. 4 and 5) content increase of the total chlorophylls was recorded at the growth stage of winter rape BBCH 59, i.e. 14 days after the first spraying. The highest content of chlorophylls after the first spraying, unlike wheat (year 2010 – Table 2), was detected in the rape leaves sampled from the variants where the highest dose of fertilizer was applied (0.4 l ha^{-1}). The differences in the content of total chlorophyll between the variants 4 and 5 and the other variants were statistically significant (Table 4).

Table 3 Impact of trial variants on dynamics of changes of pigment contents in leaves of winter wheat in 2011

Variant no.	Variant des.	Date	Growth stage	Chlor. *a* (mg m^{-2})	Chlor. *b* (mg m^{-2})	Chlorophyll *a* + *b* (mg m^{-2})	Chlorophyll *a* + *b* (%)	Carotenoid (mg m^{-2})
1	0Ti	19. 04. 2011	BBCH 32	185.58a	90.57b	276.15a	100.00	34.04a
2–3	$Ti_{0.2}$			182.85a	87.64ab	270.49a	97.95	34.61a
4–5	$Ti_{0.4}$			183.98a	74.64a	258.61a	93.65	33.87a
$LSD_{0.05}$				6.603	13.838	18.842		6.410
$LSD_{0.01}$				10.004	20.967	28.548		9.713
1	0Ti	12. 05. 2011	BBCH 55	205.17a	106.19a	311.35a	100.00	31.13a
2–3	$Ti_{0.2}$			209.49a	103.34a	312.82a	100.47	35.46b
4–5	$Ti_{0.4}$			208.96a	107.69a	316.65a	101.70	29.79a
$LSD_{0.05}$				8.042	12.767	17.416		2.932
$LSD_{0.01}$				12.184	19.344	26.387		4.443
1	0Ti	26. 05. 2011	BBCH 65	210.07a	101.62a	311.69a	100.00	35.44a
2	2 × $Ti_{0.2}$			212.76a	102.75a	315.50a	101.22	37.23a
3	3 × $Ti_{0.2}$			212.97a	108.19a	321.16a	103.04	35.85a
4	2 × $Ti_{0.4}$			210.70a	103.18a	313.88a	100.70	36.28a
5	3 × $Ti_{0.4}$			212.76a	102.37a	315.13a	101.10	34.40a
$LSD_{0.05}$				9.757	13.988	19.366		5.374
$LSD_{0.01}$				13.670	19.610	27.149		7.534

Chlor. *a* – chlorophyll *a*, chlor. *b* – chlorophyll *b*, no. – number, des. – designation, $LSD_{0.05}$ – Least Significant Difference at the level $\alpha = 0.05$

DOI: 10.15414/afz.2014.17.02.58–64

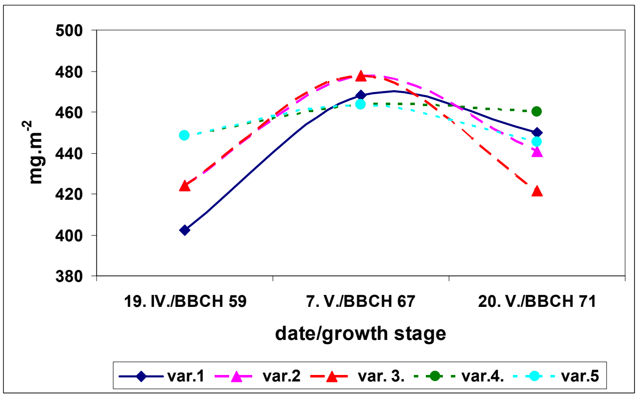

Figure 3 Dynamics of changes of total chlorophyll contents in leaves of winter rape in 2010

The second spraying had also the positive impact on the content of the total chlorophylls but only with the application dose 0.2 l ha^{-1} of the fertilizer Mg-Titanit. The dose 0.4 l.ha^{-1} did not influence their quantity (Table 4). The third spraying by Mg-Titanit (var. 3 versus var. 2 and var. 4. versus var. 5) decreased the total content of chlorophylls where their number was lower than the chlorophyll content on the unfertilized variant. The detected negative impact, still statistically insignificant, of the third spraying on the content of the total chlorophyll corresponds with the findings related to wheat which were recorded in the first year of the trial. The indicated findings prove that in the given year the foliar application of Mg-Titanit, regardless the model crop, did not lead to the increase of the chlorophyll contents at the later growth stages BBCH 55 (wheat) and BBCH 67 (rape).

In the second year of the winter rape trial the different development was recorded in the contents of the total chlorophyll in the rape leaves in comparison with the first year of the trial. However, the positive impact of the fertilizer Mg-Titanit on the formation of the total chlorophyll was proved (Figure 4). The given figure shows statistically significant differences in the content of the total chlorophyll immediately after the first spraying by the fertilizer (Table 5). The differences were decreased after the second spraying but the succession was retained. The lowest content was detected in the unfertilized variant and the highest one in the variants fertilized by the lower application dose (Figure 4). This trend was retained, apart from two exceptions, till the last plant sampling carried out after the third spraying by the fertilizer. The exceptions were the variants 4 and 5 where the highest dose of Mg-Titanit was used. In the variant 4 without the third spraying the highest content of the total chlorophyll was recorded. In the variant 5 with the third spraying the content of the total chlorophyll was nearly identical with the content of the variant 1. The comparison of the contents of the total chlorophylls between the variants 2 and 3 and the variants 4 and 5 proved again that the third application dose of the fertilizer Mg-Titanit tends to decrease the content of the total chlorophyll in the leaves of the cultivated crops. Taking into consideration

Table 4 Impact of trial variants on dynamics of changes of pigment contents in leaves of winter rape in 2010

Variant no.	Variant des.	Date	Growth stage	Chlor. *a*	Chlor. *b*	Chlor. *a* + *b*	Chlor. *a* + *b*	Carotenoid
				mg m^{-2}	mg m^{-2}	mg m^{-2}	%	mg m^{-2}
1	0Ti	19. 04. 2010	BBCH 59	310.02a	92.51a	402.52a	100.00	91.51ab
2–3	$Ti_{0.2}$			322.14a	102.13a	424.27a	105.40	89.50a
4–5	$Ti_{0.4}$			339.91a	108.62a	448.52b	111.43	95.25b
$LSD_{0.05}$				20.794	17.917	23.925		5.569
$LSD_{0.01}$				28.111	27.812	39.156		11.468
1	0Ti	7. 05. 2010	BBCH 67	342.71b	125.63a	468.34a	100.00	77.33a
2–3	$Ti_{0.2}$			336.61b	140.89a	477.49b	101.95	74.18a
4–5	$Ti_{0.4}$			325.85a	137.93a	463.78a	99.03	73.32a
$LSD_{0.05}$				10.302	17.939	8.502		8.727
$LSD_{0.01}$				21.030	26.906	15.363		13.404
1	0Ti	20. 05. 2010	BBCH 71	321.38c	128.50a	449.88b	100.00	76.35a
2	$2 \times Ti_{0.2}$			315.06bc	125.58a	440.64ab	97.95	76.38a
3	$3 \times Ti_{0.2}$			294.31a	127.21a	421.51a	93.69	70.71a
4	$2 \times Ti_{0.4}$			313.46bc	146.46b	459.92b	102.23	72.31a
5	$3 \times Ti_{0.4}$			302.54ab	142.75b	445.28b	98.98	70.61a
$LSD_{0.05}$				19.017	13.550	22.464		6.774
$LSD_{0.01}$				40.005	18.995	49.837		9.497

Chlor. *a* – chlorophyll *a*, chlor. *b* – chlorophyll *b*, no. – number, des. – designation, $LSD_{0.05}$ – Least Significant Difference at the level $\alpha = 0.05$

DOI: 10.15414/afz.2014.17.02.58–64

Table 5 Impact of trial variants on dynamics of changes of pigment contents in leaves of winter rape in 2011

Variant no.	Variant des.	Date	Growth stage	Chlor. *a* (mg m^{-2})	Chlor. *b* (mg m^{-2})	Chlor. *a* + *b* (mg m^{-2})	Chlor. *a* + *b* (%)	Carotenoid (mg m^{-2})
1	0Ti	11. 04. 2011	BBCH 59	347.49 a	128.00 a	475.48 a	100.00	92.73 a
2–3	$Ti_{0.2}$			380.76 b	140.40 b	521.15 c	109.61	97.80 a
4–5	$Ti_{0.4}$			367.14 b	138.07 ab	505.21 b	106.25	93.69 a
$LSD_{0.05}$				14.421	10.584	15.024		6.739
$LSD_{0.01}$				19.863	14.098	21.436		11.408
1	0Ti	5. 05. 2011	BBCH 66	321.68a	150.66a	472.34a	100.00	89.51 a
2–3	$Ti_{0.2}$			334.91a	179.17b	513.46b	108.70	89.51 a
4–5	$Ti_{0.4}$			330.29a	168.17ab	498.46ab	105.53	83.67 a
$LSD_{0.05}$				14.605	19.251	27.759		7.542
$LSD_{0.01}$				20.087	26.382	35.413		12.301
1	0Ti	19. 05. 2011	BBCH 71	349.25 a	170.05ab	519.30a	100.00	97.24 b
2	$2 \times Ti_{0.2}$			373.37 a	181.47bc	554.84b	106.84	92.71 b
3	$3 \times Ti_{0.2}$			358.33 a	186.07c	544.40b	104.83	86.63 a
4	$2 \times Ti_{0.4}$			375.29 a	189.66c	564.96b	108.79	93.85 b
5	$3 \times Ti_{0.4}$			349.88 a	169.36a	519.24a	99.99	93.19 b
$LSD_{0.05}$				27.584	12.487	21,842		5.864
$LSD_{0.01}$				34.917	19.036	32.325		10.047

Chlor. *a* – chlorophyll *a*, chlor. *b* – chlorophyll *b*, no. – number, des. – designation, $LSD_{0.05}$ – Least Significant Difference at the level $\alpha = 0.05$

the support of the chlorophyll formation in the leaves of winter rape and winter wheat these facts show that the spraying by Mg-Titanit should not be applied at the given growth stages or it should be carried out earlier.

The chlorophyll *a* and chlorophyll *b* did not participate by the same rate in the increase of the contents of the total chlorophylls. After the first spraying the contents increase was carried out predominantly by the chlorophyll *a*. After the second spraying it was mainly by the increase of the chlorophyll *b* content.

In general, the results of the quantitative changes of chlorophyll *a*, *b*, and *a* + *b* in the leaves of both model plants are identical with the findings of the author (Wang, 2009) who claimed that the leaves had a higher chlorophyll *a* content than *b* (Table 2). The average value of the chlorophylls *a* : *b* ratio was 2.73 : 1 in the wheat leaves and 2.45 : 1 in the rape leaves during the observed periods.

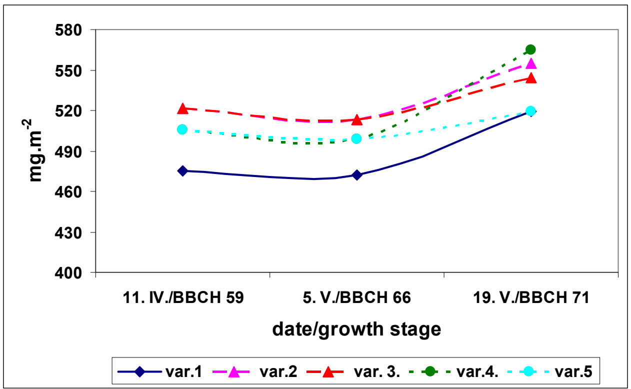

Figure 4 Dynamics of changes of total chlorophyll contents in leaves of winter rape in 2011

4. Conclusions

In the cultivation of winter wheat the content of the total chlorophyll was increased with both application doses of Mg-Titanit (0.2 and 0.4 l ha^{-1}) in each year if the spraying was applied at the growth stage BBCH 32 (the second spraying).

Winter rape reacted positively to the spraying by Mg-Titanit applied at the growth stage BBCH 50 – 52 (the first spraying) through the chlorophyll formation in both years of the trial.

The third spraying by both application doses Mg-Titanit tended mostly to decrease the content of the total chlorophyll in both crops.

The content of the total chlorophyll was predominantly higher in the plants fertilized by the dose 0.2 l.ha^{-1} than in those fertilized by the dose 0.4 l ha^{-1}.

The positive effect of Mg-Titanit on the content of the total chlorophyll was carried out by the increase of the amount of both assimilative pigments (chlorophyll *a* and chlorophyll *b*). In the most cases the application of the fertilizer Mg-Titanit determined more the content of chlorophyll *b* than chlorophyll *a*. The average value of chlorophyll ratio *a* : *b* in the wheat leaves was 2.73 : 1 and

DOI: 10.15414/afz.2014.17.02.58–64

in the rape leaves 2.45 : 1 related to the variants of the trial and the monitored years.

5. Acknowledgements

The study originated on the base of the project VEGA No. 1/0591/13 (Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences) and the financial grant of the company Intermag Sp. Z o. o. Olkusz, Poland

6. References

BAKER, N. (1996) *Photosynthesis and environment*. Boston: Kluwer Academic Publishers. 491 p.

BREMNER, J. M. (1960) Determination of nitrogen in soil by the Kjeldahl method. In *The journal of agricultural science,* vol. 55, no. 1, pp. 11–33.
DOI: http://dx.doi.org/10.1017/S0021859600021572.

CARVAJAL, M. and ALCARAZ, C. F. (1995) Effect of Ti(IV) on Fe activity in *Capsicum annuum*. In *Phytochemistry*, vol. 39, no. 5, pp. 977–980. DOI: http://dx.doi.org/10.1016/0031-9422(95)00095-O.

CARVAJAL, M. and ALCARAZ C. F. (1998) Why is titanium a beneficial element for plants? In *J. Plant Nutr*., vol. 21, no. 4, pp. 655–64. DOI: http://dx.doi.org/10.1080/01904169809365433.

DOBROMILSKA, R. (2007) Wpływ stosowania tytanitu na wzrost i plon pomidora drobnoowocowego. In *Roczniki akademii rolniczej w Poznaniu CCCLXXXIII*. 41, pp. 451–454.

DUCSAY, L. (2011) The chlorophyll, cadmium and zinc contents in sunflower leaves under cadmium and zinc stress condition. In *Chemické listy*, vol. 105, pp. 269–272 (in Slovak).

HRUBÝ, M., CÍGLER, P. and KUŽEL, S. (2002) Contribution to understanding the mechanism of titanum action in plant. In *J. Plant Nutr.,* vol. 25, no. 3, pp. 577–98.
DOI: http://dx.doi.org/10.1081/PLN-120003383.

FILOVÁ, A. and KRIVOSUDSKÁ, E. (2013) Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration. In: *Acta fytotechnica et zootechnica*, vol 16, no. 4, pp. 78–82.

KRIVOSUDSKÁ, E. and FILOVÁ, A. (2013) Evaluation of selected soybean genotypes (*Glycine max*. L.) by physiological responses during water deficit. In *Journal of Central European agriculture*,. vol. 14, no. 2, pp. 213–228.

KOVÁČIK, P., SKAWIŃSKI, M. and VICIAN, M. (2012) Growth and yield stimulation of winter wheat (*Triticum aestivum* L.) and winter oilseed rape (*Brassica napus* L.) by Mg- Titánit fertilizer In *Abstracts book for oral and poster presentations at The 1st world congress on the use of biostimulants in agriculture 26–29 November 2012, Strasbourg Congress centre.*

KUŽEL, S. et al. (2003) Mechanism of physiological effects of titanium leaf sprays on plants grown on soil. In *Biology of trace element research*, vol. 91, no. 2, pp. 179–189.
DOI: http://dx.doi.org/ 10.1385/BTER:91:2:179.

KUŽEL, S. et al. (2007) The effect of simultaneous magnesium application on the biological effects of titanium. In: *Plant Soil Environ*., vol. 53, no. 1, pp. 16–23.

LARCHER, W. (1988) *Physiological ecology of plants*. Prague: Academia, 1988, 361 p.

LICHTENTHALER, H. K. (1987) Chllorophylls and Carotenoides: Pigments of Photosynthetic Biomembranes. In *Methods Enzymology*, vol. 148c, pp. 350–382.
DOI: http://dx.doi.org/0.1016/0076-6879(87)48036-1.

MARSCHNER, H. (2005) *Mineral nutrition of higher plants*. 2nd ed. London: Elsevier Academic Press, 889 p.

MATUŠKOVIČ, J. (1995) Evaluation of yield and selected quality parameters interactions of strawberry variety „Senga Sengana" as influenced by utilization of „Titavin" preparator. In *Acta fytotechnica Universitatis agriculturae Nitra*, vol. 51, pp. 105–110.

MEHLICH, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. In *Commun. Soil Sci. Plant Anal.*, vol. 15, no. 12, pp. 1409–1416.
DOI: http://dx.doi.org/10.1080/00103628409367568.

NÁTR, L. and LAWLOR, D. W. (2005) Photosynthetic plant productivity. In Pessarakli M. (ed.) *Handbook of Photosynthesis*. Boca Raton: Taylor and Francis, pp. 501–524.

NAMVAR, A. et al. (2013). Organic and inorganic nitrogen fertilization effects on some physiological and agronomical traits of chickpea (*Cicer arietinum* L.) in irrigated condition. In *Journal of Central European agriculture*, vol. 14, no. 3, p. 881–893.

OLŠOVSKÁ, K. et al. (2013) Assessment of the photosynthesis-related traits and high temperature resistance in tetraploid wheat (*Triticum* l.) genotypes. In *Journal of Central European agriculture* (JCEA), vol. 14, no. 2, pp. 767–780.

PAIS, I. (1983) The biological importance of titanium. In *Journal of Plant Nutrition*, vol. 6, no. 1, pp. 3–131.
DOI: http://dx.doi.org/10.1080/01904168309363075.

RAM, N., VERLOO, M. and COTTENIE, A. (1983) Response of bean to foliar spray of titanium. In *Plant and Soil*, vol. 73, no. 2, pp. 285-290. DOI: http://dx.doi.org/10.1007/BF02197724.

SIMON, L. et al. (1988) Effect of titanium on growth and photosynthetic pigment composition of *Chlorella pyrenoidosa* (green alga). II. Effect of titanium ascorbate on pigment content and chlorophyll metabolism of Chlorella. In *New Results in the Research of Hardly Known Trace Elements and Their Role in the Food Chain*. Budapest: University of Horticultural and Food Science, pp. 87–101.

TŮMA, J. and TŮMOVÁ, L. (2006) Magnesium and its role in plants. In *The affect of abiotic and biotic stressful factor on plant´s property*. Prague: CZU, pp. 19–23 (in Czech).

TRAETTA-MOSCA, F. (1913) Titanium and the rare metals in the ash of the leaves of Kentucky tobacco cultivated in Italy. In *Gazzetta Chimica Italiana*, vol. 43, pp. 437–440.

ŤJURIN, I. V. (1966) The method of analyses of humus in soil. In *Voprosy genezisa i plodorodija počv*. Moskva: Nauka, 1966 (in Russia).

VICIAN, M. et al. (2012) The dynamics of changes of total chlorophylls contents after application of Mg-Titánit fertilizer. In *Scientific articles of Department of agrochemistry and plant nutrition and Department of soil science and geology.* Nitra: Slovak University of Agriculture, pp. 176–183 (in Slovak).

WANG, H., LIU, R. L. and JIN, J. Y. (2009) Effects of zinc and soil moisture on photosynthetic rate and chlorophyll fluorescence parameters of maize. In *Biol. Plantarum*, vol. 53, no. 1, pp.191–194. DOI: http://dx.doi.org/10.1007/s10535-009-0033-z.