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Original article

Photosynthetic activity and chlorophyll pigment concentration in *Medicago* x *varia* T. Martyn leaves treated with the Tytanit growth regulator

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ABSTRACT

The purpose of the research was to determine the effect of the foliar use of a growth regulator with the trade name of Tytanit, containing titanium ascorbate, on photosynthetic activity and chlorophyll content in *Medicago* × *varia* T. Martyn leaves. There were two kinds of plots: C – control series; Ti – plants treated with Tytanit, containing 8.5 g of titanium in 1 dm^3. The following parameters were determined: maximum photosystem II efficiency (F_v/F_m) in a dark-adapted state, actual photosystem II efficiency ($\Delta F/F_{m'}$) in a light-adapted state, photochemical quenching factor (QP), non-photochemical quenching factor (QN), and chlorophyll *a* and b content. The Fisher-Snedecor test was used to determine whether the impact of experimental factors was significant, and the HSD 0.05 value was calculated using Tukey's test. Compared to control, the photosynthetic apparatus performance of alfalfa was positively affected by the regulator compared to control. Tytanit applied to plant leaves increased their photosynthetic activity as a result of an increase in the content of chlorophyll pigments. It was also found that periods of rainfall deficiency did not affect the beneficial effects of the regulator.

1. Introduction

As a chemical element Titanium is not the main or complementary nutrient for plants, but, according to Brown and Saa (2015) and Kocira et al. (2017), it stimulates plant growth and development. Its content in plants is low, ranging from 0.1 mg kg^{-1} to 10 mg kg^{-1}DM (Tlustošet al., 2005), and it is not excessively accumulated in plant tissues (Asli and Neumann, 2009). Products containing titanium can be applied to soil or to leaves. The reaction of plants to this chemical element is species specific (Kováčik et al., 2014; Tlustošet al., 2005). Moreover, Wadas and Kalinowski (2017a) have found that in certain aspects it is variety specific. Kleiber (2017) reported that plant age and concentrations of other minerals in tissues were important for the effects of titanium. It can stimulate the uptake of certain nutrients by plants and increase the activity of enzymes such as catalase, peroxidase, lipoxygenase and nitrate reductase. In addition, as evidenced by numerous studies (Tan and Wang, 2011; Wadas and Kalinowski, 2017a, 2017b), titanium increases chlorophyll content in plant leaves.

Titanium is found in soil mainly in the form of minerals such as ilmenite, consisting of titanium-iron oxide and titanium oxide (IV), but in this form it is not bioavailable. Titanium has a significant biological effect on plants, being beneficial in low concentrations and toxic in higher concentrations (Kuželet al., 2003). Optimal doses and time of titanium application to many crops have not yet been determined. In the present experiment titanium was applied to plant leaves in the form of a growth regulator called Tytanit. Manufactured by Intermag Ltd., this product contains 8.5 g Ti per dm^3 (0.8% m/m), in the form of Ti-ascorbate (Wadas and Kalinowski, 2017a). There are many studies in the available literature on the impact of the Tytanit growth regulator on the yield of agricultural plants, vegetables and fruits (Du et al. 2010; Grajkowski and Ochmian, 2007; Radkowski and Radkowska, 2010; Wadas and Kalinowski, 2017b). However, there is a lack of studies directly relating to the effect of Tytanit on individual parameters determining the photosynthetic activity of plant leaves. Therefore, the studies presented in this paper are

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innovative, as they complement the lack of knowledge in this area. Used in the experiment the method of evaluating photosynthesis based on chlorophyll fluorescence (CF) in large part replaces conventional measurements of its intensity. CF is a highly sensitive attempt at assessing photosynthetic capacity, being completely non-invasive and allowing studying photosynthesis in vivo. It is particularly useful in situations of the impact of various environmental factors on plants. In the production of plant biomass, the size of photosynthetically active radiation (PAR), with its effect on dry matter production, is crucial. Energy absorbed in the photosynthesis process can be used for carbon assimilation, or can be lost when radiated as fluorescent radiation. Thus, there is a close relationship between the amount of fluorescence and the intensity of photosynthesis. Therefore, chlorophyll fluorescence is a measure of the state and condition of the photosynthetic apparatus (Zhanga et al., 2000).

The species of the plant that was selected in the present studies was hybrid alfalfa (*Medicago* × *varia* T. Martyn) var. Comet. In numerous literature sources (Ilieva and Vasileva, 2013; Pietrzak and Grela, 2013; Vasileva and Ilieva, 2011), alfalfa is treated as a valuable crop due to its beneficial chemical composition (protein, fibre and carotenoid content). Pietrzak and Grela, (2013) indicate that the nutritional value of the plant depends on a number of factors, including, but not limited to, the type of soil on which it is grown, the amount of precipitation and on fertilizer treatment. Alfalfa is mainly used as animal feed, an additive with high content of easily digested protein. According to Pietrzak and Grela (2013), it is also used in medicine due to its antifungal, detoxifying, diuretic and anticancer properties. It slows down the aging process of cells, relieves rheumatic pain and strengthens the immune system. Additionally, alfalfa contains many components necessary for the proper functioning of the human body, e.g. vitamins and enzymes. In addition to being used as a dietary supplement, alfalfa causes better absorption of Ca, Fe and other nutrients such as sugars and protein (Gaweł and Grzelak, 2013). It is also a species that reacts very positively to the foliar application of growth regulators (Godlewska and Ciepiela, 2018; Sosnowski et al., 2017; Sosnowski et al., 2019).

The aim of the experiment was to determine the effect of the foliar use of titanium ascorbate growth regulator (Tytanit) on photosynthetic activity and chlorophyll content in *Medicago* × *varia* T. Martyn leaves. The studies were intended to demonstrate its effect on photosynthetic activity determined by measuring the induction of chlorophyll fluorescence and chlorophyll *a* and b content, treating plants twice during each growth cycle over three years.

2. Material and methods

2.1. Characteristics of the conditions for conducting the experiment

The research was carried out as a field experiment conducted at the experimental facility of University of Natural Sciences and Humanities in Siedlce (52°10′03″N; 22°17′24″E) between 2015 and 2017. In the autumn of 2014, experimental plots of 6 m^2 (2 m × 3 m) were established. Between plots paths of 1.5 m were kept as herbicide fallow. The soil on which the experiment was founded had a granulometric composition of loamy sand. Organic carbon content (C_{org}) in the soil was 13.5 gkg^{-1}DM, with total nitrogen of 1.30 gkg^{-1}DM and the C:N ratio of 10.4:1. Soil pH of 6.8 was close to neutral. In addition, soil contained high amounts of forms of available phosphorus and magnesium, but forms of available potassium were within the limits of moderate content. Due to relatively high content of soil nutrients, neither pre-sowing nor post-sowing mineral fertilizer treatment was applied to *Medicago* × *varia* T. Martyn. Testing of the Tytanit growth regulator, the experiment was conducted in a split-plot design with three replicates.

The regulator was used in the form of two sprays during each of the three growth cycles (altogether six sprays in the growing season). The application time was based on the growth and development stages of *Medicago* × *varia* T. Martyn, adopted according to the European BBCH scale, with following characteristics of the BBCH growth stages:

- the first spray when the first internode was visible (BBCH 31),
- the second spray when the first flower buds were visible outside leaves (BBCH 51).

The spraying liquid was made by dissolving 0.4 dm^3 $ha^{-1}$of Tytanit in 200 $dm^3 \cdot ha^{-1}$ of water, with the control plants treated with the same amount of water. The dose of the regulator was measured according to the manufacturer's recommendations.

Plots were marked as follows:

- C – control plants sprayed with water,
- Ti – plants sprayed with the Tytanit regulator.

In the years of its full use (2015–2017), alfalfa was harvested three times, when 40–50% of inflorescences were in bloom:

- H1 - spring harvest in the second half of June,
- H2 - summer harvest in the second half of August,
- H3 – summer harvest in the first half of October.

All cultivation treatments were applied according to good agricultural practices. Plant pests did not exceed the threshold of harmfulness, and therefore no pesticides were used, with undesirable plants removed manually.

2.2. Determination of photosynthetic activity

Measurement of photosynthetic activity of plant leaves was carried out in each growth cycle. For this purpose, on the seventh day after the second spray of alfalfa with the regulator (Table 1), leaves were collected from 10 randomly selected plants on each plot. Photosynthetic activity was determined by measuring the induction of chlorophyll fluorescence by means of the fluorometer (PAM 2000, Heinz Walz GmbH, Effeltrich, Germany). The following parameters were determined (Bolhàr-Nordenkampf and Öquist, 1993):

- in a dark-adapted state: maximum photosystem II efficiency (F_v/F_m),
- in a light-adapted state: actual photosystem II efficiency ($\Delta F/F_{m'}$), photochemical quenching factor (QP) and non-photochemical quenching factor (QN).

All measurements were made during the growing season with six replicates, using well-developed leaves. The 2030-B clip, a light emitting diode at 650 nm and a standard intensity of 0.15 μmol m-2 s-1 PAR were used. During the dark-adapted stage leaves were kept in darkness for 15 min.

Table 1
Dates of measurements of fluorescence and chlorophyll pigments.

Harvest	Measurement dates		
	2015	2016	2017
H1	17.06	15.06	19.06
H2	22.07	24.07	25.07
H3	24.09	23.09	28.09

2.3. Chlorophyll content determination

Chlorophyll *a* and b content was determined according to Khaleghi et al. (2012). The dates of material collection for the determination of pigment content in each growth cycle are presented in Table 1. The optical density of supernatants was determined with the Bio-RADSmartSpect TM Plus Spectrophotometer (equipment and instruments used in experimental work should be mentioned by their common name and between parentheses model, brand, city, state, and country of the manufacturer) at 440, 465, and 663 nm. Next, the results were calculated according to the following formulas:

$$\text{Chlorophyll a content} = [12.7(E663) - 2.69(E645)]w/v, \quad (1)$$

$$\text{Chlorophyll b content} = [22.9(E645) - 4.68(E663)]w/v, \quad (2)$$

where E is extinction at a particular wavelength; v is the amount of 80% acetone (cm^3) used for extraction; w is the sample weight (g).

2.4. Weather conditions

Sielianinov's hydrothermal coefficient was calculated in order to determine temporal variation of meteorological conditions and their effects on plant growth and development. The hydrothermal coefficient (*K*) was calculated on the basis of monthly precipitation (*P*) and monthly sums of daily air temperatures (*t*), using the following formula (Radzka et al., 2015):

$$K = \frac{P}{0.1}\sum t, \quad (3)$$

The K values are presented in Table 2. According to Radzka et al. (2015), extreme conditions occur when the value of the K-factor is below 0.7 and above 2.5. Thus, according to Table 2 optimal temperature and humidity conditions were only in April 2017 and September 2015. Throughout the experiment, the best conditions were at the beginning of each growing season. The most difficult situation for plants was in 2015 and 2017, when apart from May and the end of the growing season, the weather ranged from moderately dry to very dry. There was a lack of periods with extreme droughts in the growing season of 2016, when wet and quite wet spells prevailed.

2.5. Statistical analysis

The results of the research were processes statistically using ANOVA for repeated (three years), multi-factor, and recurrent measurements (three harvest in a growing season). The Fisher-Snedecor test was used to determine whether the effect of Tytanit was significant, while the value of the $HSD_{0.05}$ was calculated with Tukey's test. The Statistica program version 12.0 (Dell Inc., Tulsa, Oklahoma, USA) was applied for all other calculations. Means in the tables marked with the same letters in lines/columns do not differ significantly.

3. Results

The results of the studies indicate (Tables 3 to 8) a multidirectional effect of the growth regulator Tytanit, compared to the control series, on the photosynthetic activity of *Medicago* × *varia* T. Martyn leaves, as evidenced by the values of chlorophyll content and fluorescence induction parameters.

3.1. Photosynthetic activity

Fluorescent measurements made using plant leaves showed that maximum photosystem II efficiency (F_v/F_m) in a dark-adapted state varied across the growing periods (2015–2017) and harvests (H1- spring, H2 – summer, H3 - autumn) in a statistically significant way as an effect of the Tytanit growth regulator (Table 3).

The smallest value of the maximum photosystem II efficiency with the F_v/F_m ratio of 0.555 was recorded for control plants in 2015 during the summer growth cycle and in 2016 during the spring growth cycle. In turn, the highest value of the Fv/Fm (0.669) was in plants treated with the regulator in the autumn of 2015. Furthermore, the statistical analysis of the data showed a significant increase in the maximum photosystem II efficiency in plants treated with Tytanit; its average increase relative to control was 12.2%. A similar trend was noted in the case of the actual photosystem II efficiency (Table 4). For plants treated with the regulator, there was a 19.5% increase in the actual photosystem II efficiency ($\Delta F/F_{m'}$). In plants treated with Tytanit its higher values were recorded during the spring growth cycle. In turn, for all growing seasons, the highest value of the $\Delta F/F_{m'}$ was recorded in 2016.

It can therefore be assumed that the treatment of *Medicago* × *varia* T. Martyn plants with the Tytanit regulator may have caused better nutrition of plant cells with nitrogen, as evidenced by the increases in photosynthetic parameters such as the maximum (F_v/F_m) and actual ($\Delta F/F_{m'}$) photosystem efficiency of alfalfa leaves (Tables 3 and 4).

The findings indicated that none of the regulator doses significantly affected the photochemical quenching coefficient (QP), the values of which ranged from 0.537 to 0.556 (Table 5). However, the values of certain parameters of photosynthetic activity were dependent on weather conditions. The photochemical quenching coefficient (QP) and the non-photochemical quenching coefficient (QN) across the doses and growing seasons assumed the highest values in plants of the summer harvest with QN of 0.135 and QP of 0.592 (Tables 5 and 6). As the meteorological data indicate (Table 2), dry periods alternated with humid ones in each summer season. A very dry summer in 2015 was exceptional, with very large water deficits in both July and August.

It is therefore worth noting that the periodic absence of precipitation, with warm sunny weather, did not result in a decrease in the efficiency of the primary photosynthesis reactions, that is to say, non-cyclic transport of electrons proceeded in these conditions smoothly.

Table 2
The value of Sielianinov's hydrothermal coefficient (K) in the growing season.

Years	Months						
	April	May	June	July	August	September	October
2015	1.22 (md)	2.63 (sw)	0.87 (d)	1.08 (md)	0.18 (ed)	1.46 (o)	1.94 (mw)
2016	1.89(mw)	0.82 (d)	1.02(md)	2.15 (w)	1.05 (md)	0.86 (d)	3.65 (ew)
2017	1.38 (o)	1.81 (mw)	1.74 (mw)	0.49 (sd)	1.92 (mw)	0.64 (sd)	0.12 (ed)

$K \leq 0.4$ extreme drought (ed). $0.4 < K \leq 0.7$ severe drought (sd). $0.7 < K \leq 1.0$ drought (d). $1.0 < K \leq 1.3$ moderate drought (md). $1.3 < K \leq 1.6$ optimal (o). $1.6 < K \leq 2.0$ moderately wet (mw). $2.0 < K \leq 2.5$ wet (w). $2.5 < K \leq 3.0$ severely wet (sw). $K > 3.0$ extremely wet (ew)

Table 3
The effect of Tytanit (Ti) on maximum photosystem II efficiency (F_v/F_m) of alfalfa leaves.

Extract	Harvest	Study year			Mean
		2015	2016	2017	
C	H1	0.611Aa	0.560Aa	0.612Aa	0.594A
	H2	0.555Ba	0.561Aa	0.561Ba	0.559A
	H3	0.557Ba	0.555Aa	0.608ABa	0.573A
Ti	H1	0.632Aa	0.630Aa	0.645Aa	0.639A
	H2	0.650Aab	0.637Ab	0.678Aa	0.644A
	H3	0.669Aa	0.631Ab	0.643Aa	0.648A
Mean Tytanit effect					
C		0.574Ba	0.559Ba	0.594Ba	0.576B
Ti		0.650Aa	0.633Aa	0.655Aa	0.646A
Mean harvest effect					
H1		0.622Aa	0.595Aa	0.629Aa	0.615A
H2		0.603Aa	0.599Aa	0.620Aa	0.607A
H3		0.613Aa	0.593Aa	0.626Aa	0.611A
Mean		0.613a	0.596a	0.625a	

Means in lines with the same lower-case letter do not differ significantly.
Means in columns with the same upper-case letter do not differ significantly.

Table 4
The effect of Tytanit (Ti) on actual photosystem II efficiency ($\Delta F/F_{m'}$) of alfalfa leaves.

Extract	Harvest	Study year			Mean
		2015	2016	2017	
C	H1	0.404Aab	0.430Aa	0.393Ab	0.409A
	H2	0.393Ab	0.444Aa	0.410Ab	0.416A
	H3	0.393Ab	0.442Aa	0.403Ab	0.413A
Ti	H1	0.583Aa	0.549Aa	0.470Ab	0.543A
	H2	0.447Bb	0.553Aa	0.422Ab	0.474B
	H3	0.419Bb	0.584Aa	0.447Ab	0.482B
Mean Tytanit effect					
C		0.397Ba	0.449Ba	0.402Ba	0.416B
Ti		0.483Aab	0.562Aa	0.446Ab	0.497A
Mean harvest effect					
H1		0.494Aa	0.490Aa	0.432Ab	0.472A
H2		0.420Ab	0.499Aa	0.416Ab	0.445B
H3		0.406Bb	0.513Aa	0.425Ab	0.448B
Mean		0.440b	0.501a	0.424b	

Means in lines with the same lower-case letter do not differ significantly.
Means in columns marked with the same upper-case letter do not differ significantly.

Table 5
The effect of Tytanit (Ti) on the non-photochemical quenching coefficient (QN) of alfalfa leaves.

Extract	Harvest	Study year			Mean
		2015	2016	2017	
C	H1	0.108Aa	0.106Aa	0.109ABa	0.108A
	H2	0.115Aa	0.112Aa	0.118Aa	0.115A
	H3	0.111Aa	0.105Aa	0.106Ba	0.109A
Ti	H1	0.139Ba	0.125Ab	0.142ABa	0.135AB
	H2	0.166Aa	0.135Ab	0.160Aa	0.154A
	H3	0.120Ba	0.123Aa	0.130Ba	0.124B
Mean Tytanit effect					
C		0.111Ba	0.108Ba	0.111Ba	0.110B
Ti		0.142Aa	0.128Ab	0.144Aa	0.138A
Mean harvest effect					
H1		0.124ABa	0.116Ab	0.126ABa	0.122B
H2		0.141Aa	0.124Ab	0.139Aa	0.135A
H3		0.116Ba	0.114Aa	0.118Ba	0.115B
Mean		0.127a	0.118b	0.128a	

Means in lines with the same lower-case letter do not differ significantly.
Means in columns with the same upper-case letter do not differ significantly.

3.2. Chlorophyll pigments

The Tytanit growth regulator increased chlorophyll *a* and b content in *Medicago* × *varia* T. Martyn (Tables 7 and 8) in a statistically significant way. This increase was 11.7% for chlorophyll *a* and 15.1% for chlorophyll *b*.

The smallest concentration of chlorophyll *a* and b in plants was in 2015 and 2017, when, according to the distribution of Seliani-

Table 6
The effect of Tytanit (Ti) on the photochemical quenching coefficient (QP) of alfalfa leaves.

Extract	Harvest	Study year			Mean
		2015	2016	2017	
C	H1	0.520ABa	0.555ABa	0.523ABa	0.533AB
	H2	0.605Aa	0.556Ab	0.628Aa	0.596A
	H3	0.456Ba	0.503Ba	0.461Ba	0.473B
Ti	H1	0.613Aa	0.556Ab	0.605Aa	0.592A
	H2	0.596Aa	0.566Ab	0.632Aa	0.598A
	H3	0.456Ba	0.513Aa	0.492Ba	0.487B
Mean Tytanit effect					
C		0.527Aa	0.538Aa	0.537Aa	0.534A
Ti		0.555Aa	0.545Aa	0.577Aa	0.559A
Mean harvest effect					
H1		0.567Aa	0.551Aa	0.565Ba	0.558AB
H2		0.601Aa	0.556Ab	0.630Aa	0.592A
H3		0.456Ba	0.503Aa	0.477Ca	0.475B
Mean		0.541a	0.542a	0.557a	

Means in lines with the same lower-case letter do not differ significantly.
Means in columns marked with the same upper-case letter do not differ significantly.

Table 7
The effect of Tytanit (Ti) on chlorophyll *a* content (mg 100 g^{-1} fresh weight) in alfalfa leaves.

Extract	Harvest	Study year			Mean
		2015	2016	2017	
C	H1	215Ba	214Ba	196Ba	208B
	H2	242Aa	263Aa	169Bb	225A
	H3	174Cc	253Aa	203Ab	210B
Ti	H1	239Ba	236Aa	226Ba	234B
	H2	265Aa	254Aab	199Cb	240A
	H3	193Cb	262Aa	273Aa	243A
Mean Tytanit effect					
C		210Bb	243Aa	189Bc	214B
Ti		232Ab	251Aa	233Ab	239A
Mean harvest effect					
H1		227Ba	225Ba	211Bb	221A
H2		254Aa	259Aa	184Cb	232A
H3		184Cc	258Aa	238Ab	227A
Mean		222b	247a	211b	

Means in lines marked with the same lower-case letter do not differ significantly.
Means in columns marked with the same upper-case letter do not differ significantly.

Table 8
The effect of Tytanit (Ti) on chlorophyll *b* content (mg 100 g^{-1} fresh weight) in alfalfa leaves.

Extract	Harvest	Study year			Mean
		2015	2016	2017	
C	H1	98ABa	107Bb	96Ba	100B
	H2	109Ab	116Aa	91Bb	105AB
	H3	90Bb	124Aa	121Aa	112A
Ti	H1	107Bb	121Ba	119Ba	116A
	H2	130Aa	137Aa	125Aa	121A
	H3	100Bb	129ABa	128Aa	119A
Mean Tytanit effect					
C		99Bb	116Ba	103Bb	106B
Ti		112Ab	129Aa	124Aa	122A
Mean harvest effect					
H1		103Bb	114Ba	108Bb	108A
H2		120Aa	127Aa	108Bb	118A
H3		95Cb	127Aa	125Aa	115A
Mean		106b	123a	114ab	

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Means in columns with the same upper-case letter do not differ significantly.

nov's coefficient (Table 2), there was a drought in the summer and autumn seasons. Then, there were extremely dry periods in October 2017 and August 2015, with very dry July and September in 2017. Drought stress during those months caused a significant decrease in the content of chlorophyll pigments in leaves. The smallest chlorophyll *a* content (211 mg 100 g^{-1}FM) was recorded in 2017, 14.6% lower than in 2016. In turn, the concentration of chlorophyll *b* was reduced most by the drought in 2015, when it was 13.8% lower than in 2016. However, studying the interaction between growing seasons and regulator effects, it turned out that

the content of chlorophyll pigments across harvests was significantly higher in years with extremely dry periods.

4. Discussion

The increase in the F_v/F_m parameter increased the plant demand for photosynthetic products and it lowered plant stress in the growth and development process. Furthermore, as Demmig-Adams and Adams (1992) indicate, increasing maximum photosystem II efficiency means activating the photosystem in a dark-adapted state resulting from the absence of photo inhibition in nitrogen-deficient plant cells. Thus, according to Khaleghi et al. (2012) and Laisk et al.(2014), the energy consumed for the transport of electrons is not reduced. At the same time, according to Nishiyama et al.(2006), an increase in the activity of reaction centres of PSII cells in a dark-adapted state is the effect of supplying them with the right amount of nitrogen, which translates into high activity of the photosynthetic apparatus and into increased efficiency of light energy conversion.

According to Michałek and Sawicka(2002), genetic conditions have a very strong impact on the formation of fluorescence parameters, which may explain a lack of variation in the value of the photochemical quenching coefficient (QP) as a response to Tytanit regulator application. Studying the effects of Tytanit on the photochemical indicators of *Dactylis glomerata* leaves Sosnowski et al. (2020) recorded similar results. According to the authors, none of the Tytanit doses significantly affected this parameter.

In addition to the present experiment, effects of drought stress on chlorophyll fluorescence rates were studied by other researchers. Kianiet al. (2008) observed that increasing water stress did not result in a long-term decrease in the photosynthesis indicators of *Helianthus annuus* L. plants, but it reduced the actual transport efficiency of PSII electrons. In addition, the authors' analysis of QTL (the Quantitative Trait Loci) showed that several genomic areas were involved in the complete variability of chlorophyll fluorescence parameters during drought stress. Most QTL were specific to a given stress condition. This shows that photosynthetic control of gene expression varied under changing water conditions.

In the present experiment the Tytanit growth regulator positively affected chlorophyll *a* and b content in *Medicago* × *varia* T. Martyn. As reported by Yokoya et al. (2007) and Zhao et al. (2016), these photosynthetic pigments are responsible for collecting and transmitting absorbed light to photosynthetic reaction centres, and their concentration is linked to the effectiveness of photosynthesis. In addition, according to Zhao et al. (2016), increased content of these pigments may be one of the factors increasing photosynthetic activity. Thus, as evidenced in the present experiment, the Tytanit regulator increased the concentration of chlorophyll pigments in *Medicago* × *varia* T. Martyn leaves, which in turn increased their photosynthetic activity (Tables 7 and 8). Similar results were reported by Wadas and Kalinowski (2017b), who examined the effect of Tytanit on the leaf assimilation surface and on chlorophyll content in very early varieties of potato. They found that foliar application of the Tytanit growth regulator resulted in a stimulating effect of titanium ions on the leaf assimilation surface and chlorophyll content in potato plants. As a response to Tytanit application, the plants formed a larger leaf assimilation surface area, also under stressful conditions. In addition, according to studies conducted in China (Tan and Wang, 2011), after triple use of foliar fertilizer containing titanium, leaves were dark green, shiny and dense, which was also confirmed after application of Tytanit to *Medicago* × *varia* T. Martyn crops.

Other authors (Kováčik et al., 2014; Radkowski, 2013) found that Tytanit stimulated chlorophyll content in *Phleum pratense* L., winter wheat and winter rape leaves. In addition, the authors reported that varied doses and times of Tytanit application only slightly affected chlorophyll content in the leaves of crop species. Kováčiket al. (2014) recorded a positive effect of Mg-Tytanit double application on chlorophyll content in winter wheat and winter rape leaves. Chlorophyll content was higher in plants treated with a Mg-Tytanit dose of 0.2 dm^3 ha^{-1} than in plants treated with a dose of 0.4 dm^3 ha^{-1}. The third spray of both Mg-Tytanit doses tended to reduce the content of chlorophyll in leaves. In turn, the present studies indicated that the content of chlorophyll pigments in *Medicago* × *varia* T. Martyn leaves was also dependent on weather conditions.

The literature confirmed the effect of drought stress on chlorophyll *a* and b content in crops. Kiani et al. (2008) observed that chlorophyll *a* and b content in sunflower leaves decreased as the water deficit increased. Reductions in chlorophyll content in cotton leaves during drought conditions were also noted by Massacci et al. (2008). Similar results were recorded by Arji and Arzani (2008) in their research on the effects of drought stress on selected *Olea europaea* physiological parameters.

5. Conclusions

1. Foliar application of the Tytanit growth regulator to *Medicago* × *varia* T. Martyn improved the functioning of the photosynthetic apparatus. This included, but was not limited to, the maximum and actual photosystem II efficiency and the non-photochemical quenching coefficient.
2. The Tytanit growth regulator used on hybrid alfalfa leaves increased their photosynthetic activity, which resulted from higher content of chlorophyll pigments. It increased the concentration of chlorophyll *a* and b by 11.7% and 15%, compared to control.
3. It was found that periods with water deficiencies during the hybrid alfalfa growing period did not inhibit the effects of the Tytanit growth regulator on an increase in chlorophyll *a* and b content during dry and extremely dry months.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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