Adam RADKOWSKI[1]

LEAF GREENNESS (SPAD) INDEX IN TIMOTHY-GRASS SEED PLANTATION AT DIFFERENT DOSES OF TITANIUM FOLIAR FERTILIZATION

INDEKS ZAZIELENIENIA LIŚCI (SPAD) TYMOTKI ŁĄKOWEJ W UPRAWIE NASIENNEJ W ZALEŻNOŚCI OD NAWOŻENIA DOLISTNEGO TYTANEM

Abstract: One-factor field experiment in randomized block design with four replications (plot size for harvest was 10 m^2). The experimental field covered degraded black earth soil formed from loess, class I, wheat group of very good quality, by the agricultural soil classification system. The experiment was conducted at the Plant Breeding Station of Malopolska Plant Growing Company – HBP LLC in Skrzeszowice near Krakow (200 m a.s.l.) from 2006 to 2009. The experimental factor was spraying with titanium fertilizer labelled Tytanit at three different concentrations: 0.02, 0.04 and 0.08 %. During growing periods chlorophyll content was measured by means of SPAD readings. Relative chlorophyll concentration indices were growing from shoot elongation stage to anthesis stage, when the highest value was obtained, whereas at milky ripe stage SPAD readings diminished to the level prior to shoot elongation. Foliar fertilization with Tytanit applied at 0.08 % concentration resulted in the highest increase in SPAD value (the difference averaged 6 % over plants from the control object). Again, plants form the objects treated with the most diluted preparation (0.02 %) had the least chlorophyll content over the control one (difference of about 2 %).

Keywords: timothy-grass, Skald cultivar, titanium, SPAD

Introduction

In modern agricultural production the use of growth stimulants alone, or as additives in solutions of foliar fertilizers, is a factor which enable to reduce detrimental effect of frost, ground chills or drought [1–3]. Such a task can be fulfilled by titanium. This is one of the most important microelements, which exerts positive effect on the biochemistry of plants as regards processes leading to earlier and highest crop production [4]. Titanium feeding stimulates activity in many enzymes, *eg* catalase, peroxydase, lipoxygenase or nitrate reductase. Additionally, it increases some metabolic

[1] Department of Grassland, Agricultural University of Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 43 59, fax: +48 12 633 62 45, email: rrradkow@cyf-kr.edu.pl

processes, encourages pollination, fecundation and development of seed and fruit. Titanium leads to higher chlorophyll content in leaves, augments their growth and development. This element also enhances growth and development processes in plants, make them less vulnerable to adverse environmental conditions, improves their resistance against fungal and bacterial diseases. Titanium feeding has shown beneficial influence on the uptake of other nutrients, both from soil and from fertilizers [2, 5, 6]. Investigation into the effects of titanium on plant yielding has been conducted mainly in vegetables and field crops, and their results has confirmed the positive outcomes of titanium feeding. However, there is hardly any reports addressing the consequences of titanium fertilization for pasturable grass yield in a seed plantation. Timothy-grass, when grown for seeds, is a species that requires special attendance in terms of nitrogen fertilization in early development. The species gives a positive response to higher nitrogen concentration in soil and produces numerous vegetative shoots at the expense of generative organs [7]. Consequently, it is recommended to divide nitrogen supply into smaller doses. Due to the use of a SPAD meter one can immediately notice any undesirable changes in plant condition. Measurements proceeded by the chlorophyll content meter are non-destructive; the head of the instrument is placed over the sample of plant tissue and approximately by 2 seconds one can obtain an estimated value of chlorophyll concentration. Currently an increasing number of foliar fertilizers offered on the market contains slight amounts of titanium.

The field studies aimed at finding changes in chlorophyll content in timothy-grass of Skald cultivar grown for seed as influenced by foliar titanium feeding with commercial fertilizer labelled Tytanit at three different Tytanit concentrations: 0.02, 0.04 and 0.08 %.

Materials and methods

A field experiment was conducted at the Plant Breeding Station in Skrzeszowice, which belongs to Malopolska Plant Growing Company – HBP LLC, Krakow, from 2006 to 2009. The experiment was established according to randomized block design with four replications and located over degraded black earth formed from loess; according to the agricultural soil classification system the soil was assigned to class I, wheat group of very good quality. Its chemical properties were as follows: pH_{KCl} – 7.32, P_2O_5 – 190 mg, K_2O – 155 mg and Mg – 69 mg of available nutrient per 1 kg of soil. The soil was moderately rich in available manganese and zinc, while available forms of copper were at low concentration.

Water and temperature conditions in years from 2006 to 2009 against multi-year averages (1991–2004) are presented in Table 1. As can be seen from these data each constellation of meteorological circumstances in the individual experimental years was different.

During the growing seasons (April to September) in year 2006, 2007, 2008 and 2009 total precipitation amounted to 325.0, 465.0, 377.7, and 359.6 mm respectively, while respective mean air temperatures were 13.9. 12.5. 11.7 and 16.1 ^{o}C. More specifically we can describe that in 2006 rainfall occurrence was irregular over growing season. From the average values of Sielianinov coefficient one may say that fairly wet

Table 1

Hydrologic and thermal conditions for years 2006 to 2009 compared with multi-year averages (1991–2004) for Plant Breeding Station in Skrzeszowice

Month	Year				
	2006	2007	2008	2009	1991–2004
Precipitation [mm]					
IV	41.5	27.0	47.0	0.4	51.8
V	65.0	67.0	32.0	94.5	68.0
VI	64.0	84.0	53.5	123.1	74.9
VII	31.0	60.0	144.5	69.9	92.4
VIII	101.5	64.0	39.0	49.0	65.2
IX	22.0	163.0	61.7	22.7	56.3
X	14.0	54.0	46.4	65.0	49.6
XI	57.0	34.0	15.5	79.2	30.4
Total	396.0	553.0	439.6	503.8	488.6
Average air temperature [^{o}C]					
IV	7.8	5.4	4.6	11.4	8.6
V	12.5	11.5	9.8	14.2	14.3
VI	15.4	16.6	15.2	16.8	17.2
VII	19.0	16.6	15.5	20.4	19.1
VIII	15.9	15.5	14.8	18.8	18.6
IX	13.0	9.4	10.0	15.1	13.2
X	6.4	4.7	8.1	7.9	8.6
XI	5.1	0.5	3.9	5.2	3.3
Mean	11.9	10.0	10.2	13.7	12.9
Sielianinov hydrothermal coefficient					
IV	1.8	1.7	3.4	0.0	2.0
V	1.7	1.9	1.1	2.1	1.5
VI	1.4	1.7	1.2	2.4	1.5
VII	0.5	1.2	3.0	1.1	1.6
VIII	2.1	1.3	0.9	0.8	1.1
IX	0.6	5.8	2.1	0.5	1.4
X	0.7	3.7	1.8	2.7	1.9

conditions prevailed in April and May, optimum ones in June, then July was very dry, August wet, while September very dry again. Total precipitation in August amounted 101.5 mm and it was 36.3 mm higher in relation to multi-year average. In 2007 meteorological circumstances during growing season were characterized by lower air temperature (by 2.9 ^{o}C) and higher precipitation (by 64.4 mm) than in years 1991–2004. Relatively wet conditions occurred in April, May and June, fairly dry ones in July and August, whereas extremely wet in September and October. The highest amounts of rainfall were noted in September (163.0 mm), high precipitation was observed in June (84.0 mm), May (67.0 mm) and August (64.0 mm), while the lowest one in April (27.0 mm). In 2008 air temperature was lower (by 2.7 ^{o}C) than multi-year average, the same was true for precipitation (by 49.0 mm). From calculated values of Sielianinov hydrothermal coefficient it is possible to conclude that April and July were extremely wet months. In 2009 meteorological circumstances during growing season were characterized by higher air temperature (by 0.8 ^{o}C) and higher precipitation (by 15.2 mm) in relation to multi-year average. The values of hydrothermal coefficient indicate at wet conditions in May and June, although extremely dry in April.

The seeds were sown in rows, distance between the rows being 40 cm, and amount of seeds required was 4 kg $\cdot$ ha^{-1}, without protection of companion plants.

Soil mineral fertilization was dosed as follows. In the year of sowing pre-seed fertilizers were broadcast, *eg* 50 kgN $\cdot$ ha^{-1} as ammonium nitrate, 60 kgP_2O_5 $\cdot$ ha^{-1} as triple superphosphate, and 80 kgK_2O $\cdot$ ha^{-1} as 57 % potassium chloride. Additionally in autumn phosphorus and potassium fertilizers were top-dressed in the same quantities as applied in spring. For each year of usage phosphorus-and-potassium application in spring was the same (60 kgP_2O_5 $\cdot$ ha^{-1} as triple superphosphate and 80 kgK_2O $\cdot$ ha^{-1} as 57 % potassium chloride), whereas nitrogen application rate was 100 kgN $\cdot$ ha^{-1}.

Foliar feeding was proceeded with the use of a fertilizer named Tytanit at three different concentrations: 0.02, 0.04 and 0.08 %. Tytanium content in the fertilizer is 8.5 g per litre. The first foliar spray was applied at shoot elongation stage, and the next took place 2 weeks afterward. In case of massive weed expansion Chwastox Extra 330 SL, Aminopielik D, Starane 250 SL and Gold 450 EC at the doses of 1.5, 1.5, 0.6, and 1.5 dm^3 $\cdot$ ha^{-1} respectively were employed. Additionally, the experimental field was treated with pre-emergent spray against pests with the aid of Owadofos 50 solution (1–2 dm^3 $\cdot$ ha^{-1}). The first spray was done in early springtime, when the plants started growing, and the second took place after ear emergency, but still before anthesis.

For each year of the study the influence of foliar titanium fertilization on chlorophyll content was analyzed. Leaf greenness (SPAD) indices were measured with a Minolta SPAD 502 DL chlorophyll meter in upper leaves, at four stages of development, *eg* shoot growth, ear formation, flowering and milky ripeness. Measurements were done using thirty fully developed leaves from each plot.

Obtained data underwent statistical analysis with the use of the ANOVA procedure. The values of confidence limits were found by means of Tukey's test at the confidence level of α = 0.05. Statistical description covered the values of SPAD index.

Results and discussion

Leaf greenness indices (SPAD values) in examined timothy-grass plants of Skald cultivar ranged between 38.75 and 45.53 (Table 2) depending on measuring time. The study demonstrated that foliar application of Tytanit fertilizer at different concentrations (0.02, 0.04 and 0.08 %) had significant impact on leaf greenness index (SPAD) values over head formation, anthesis and milky ripe kernel stages. Relative chlorophyll concentration was growing with plant development up to the anthesis stage. Similar tendency was observed for the respective developmental stages, albeit in winter triticale of Woltario cultivar, by Jaskiewicz [8] and to a certain extent in rape by Kulig et al [9].

Table 2

Leaf greenness (SPAD) index in timothy-grass of Skald cultivar over stages of development and with different levels of Tytanit fertilizer (mean for four years)

Tytanit concentration	Developmental stage			
	Shoot elongation	Head emergency	Anthesis	Milky ripe kernels
Control	38.75	39.64	41.45	39.03
0.02 %	39.38	39.79	43.27	39.64
0.04 %	39.49	40.49	44.48	40.05
0.08 %	39.93	41.25	45.53	41.10
$LSD_{0.05}$	ns	0.42	1.19	0.59

Leaf greenness index reached the highest values at the anthesis stage, while at the milky ripe kernel stage it diminished again to the level prior to head emergency.

From the descriptive statistics of our data it can be seen that over the analyzed developmental stages SPAD values showed low coefficients of variations (Table 3). Average SPAD values ranged between 39.39 and 43.68, which corresponds with good growing conditions in this experiment.

Table 3

Descriptive statistics of SPAD index values in timothy-grass of Skald cultivar over stages of development (mean for four years)

Developmental stage	Range		Mean	Standard deviation	Coefficient of variation
	min	max			
Shoot elongation	38.78	41.33	39.39	2.21	5.61
Head emergency	39.25	42.02	40.29	1.62	4.01
Anthesis	40.28	45.32	43.68	1.49	3.40
Milky ripe kernels	39.02	41.94	39.96	1.24	3.10

The plants fertilized with Tytanit at the 0.02 % concentration appeared to have the lowest SPAD values. Conversely, at the highest Tytanit concentration (0.08 %) leaf greenness index reached maximum. Positive influence of foliar feeding with certain fertilizer on crop yield in plants was described by some authors including Faber et al [10], Czuba et al [11], Szewczuk [12] as well as Kocon and Grenda [13]. These works has demonstrated that after treating plants with a mixture containing titanium ions chlorophyll content in leaves was 16 to 65 % higher compared with control plants. Beneficial effect of titanium ions in plants is attributable to at least four mechanisms: augmented activity of oxidation-reduction reactions, accelerated nutrient uptake, more vigorous pollen grains and enhanced resistance against certain fungal pathogens. It has been demonstrated in literature that foliar feeding with titanium led to an increase in plant chlorophyll content; on the other hand chlorophyll synthesis is stimulated by iron ions. One may come to conclusion that it is due to improved bioactivity of iron, which is responsible for enzyme synthesis, that plant photosynthesis will be encouraged [4, 14].

Differentiated SPAD readings among distinct stages of plant development was shown by Fox et al [15] for winter wheat, Jaskiewicz [8] for winter triticale or Kulig et al [9] for rape. In the environment with light soils Rozbicki and Samborski [16] found optimal SPAD values for winter triticale to be lower. An investigation conducted by Samborski [17] suggests that the most accurate evaluation concerning nitrogen feeding in plants can be performed at the beginning of shoot elongation stage and boot stage (initial head formation).

SPAD readings obtained in this study were high, in particular for plants receiving application of the highest titanium concentration. Soil acidity/alkalinity measure was neutral (pH_{KCl} = 7.32), which improved the efficiency of spraying. According to relevant literature, natural titanium availability and uptake by plants is determined mainly by soil pH value as well as by the amount of organic substance in soil. With growing pH value and organic matter content the availability of titanium ions decreases [13].

On the basis of calculated regression equations it was possible to confirm linear correlation between seed production and leaf greenness index (Table 4). Coefficient of determination R^2 would indicate rather strong relationship between seed production and SPAD value, especially at anthesis and milky ripe stages.

Table 4

Regressive relationship between timothy-grass yield and SPAD index over stages of development

Developmental stage	Regression equation	R^2
Shoot elongation	y = 0.9536x + 32.34	0.69
Head emergency	y = 1.2434x + 31.097	0.63
Anthesis	y = 1.2867x + 34.172	0.85
Milky ripe kernels	y = 1.4547x + 29.209	0.87

According to Faber et al [10] positive outcomes of foliar plant feeding can be accounted for by the effect of stimulated plant metabolism, so that nutrient uptake in the root system is enhanced. Moreover, these findings are supported with the reports from many authors [5, 10, 13, 18–20], which showed that foliar feeding in wheat, rape and other plants had positive impact on photosynthesis and effective nitrogen utilization, and this finally led to higher crop of these plants. Obtained positive effect of titanium on leaf greenness index (or chlorophyll content) substantiates foliar feeding of timothy--grass seed plantation as advisable, even in such tillage conditions, where one may believe that the availability of nutrients must be satisfactory. The specific conditions here, as it was presented previously in the methodological section, included degraded black earth formed from loess; by the agricultural soil classification system it was class I, or wheat group of very good quality.

Conclusions

1. Application of Tytanit fertilizer in three different concentrations had a significant influence on leaf greenness (SPAD) index. SPAD values were increasing with Tytanit concentration.

2. Relative chlorophyll concentration index was growing with the stages of plant development. The highest levels were demonstrated at the anthesis stage, while at the milky ripe kernels stage they diminished to those prior to head emergency.

3. Higher concentration of the solution (0.08 %) led to utmost increase in SPAD readings (averaged 6 %) in relation to the control object.

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Instytut Produkcji Roślinnej, Zakład Łąkarstwa
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Jednoczynnikowe doświadczenie polowe założono metodą losowanych bloków, w czterech powtórzeniach (powierzchnia poletka do zbioru wynosiła 10 m^2). Na polu doświadczalnym występował czarnoziem zdegradowany wytworzony z lessu, należący do I klasy bonitacyjnej, kompleksu przydatności rolniczej pszennego bardzo dobrego. Doświadczenie prowadzono w latach 2006–2009 w Stacji Hodowli Roślin należącej do Małopolskiej Hodowli Roślin – HBP Kraków w Skrzeszowicach pod Krakowem (200 m n.p.m.). Czynnikiem doświadczenia był oprysk tytanem w formie nawozu Tytanit w trzech stężeniach: 0,02, 0,04 i 0,08 %. W okresie wegetacji dokonano pomiaru zawartości chlorofilu w postaci odczytów SPAD. Wskaźnik względnej zawartości chlorofilu wzrastał od fazy strzelania w źdźbło do fazy kwitnienia, gdzie uzyskano największą wartość, natomiast w fazie dojrzałości mlecznej wartość odczytu SPAD zmalała do poziomu sprzed kłoszenia. Zastosowane nawożenie dolistne w stężeniu 0,08 % Tytanitu spowodowało najwyższy wzrost wartości SPAD (różnica wynosiła średnio 6 % w stosunku do roślin z obiektu kontrolnego). Z kolei rośliny z obiektów, gdzie wykonano oprysk o najmniejszym stężeniu (0,02 %) miały poza obiektem kontrolnym najniższą zawartość chlorofilu (różnica na poziomie 2 %).

Słowa kluczowe: tymotka łąkowa, odmiana Skald, tytan, SPAD