



ORIGINAL PAPER

EFFECT OF TITANIUM APPLICATION ON LETTUCE GROWTH UNDER Mn STRESS

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ABSTRACT

Titanium (Ti) is classified as a beneficial element for plants. The aim of this study was to determine the effect of various Ti concentrations in nutrient solution on the alleviation of Mn-stress in hydroponically grown lettuce (*Lactuca sativa* L. cv. Zeralda). Plants were fertigated with nutrient solution of the following chemical composition (mg dm⁻³): N-NH₄ < 10, N-NO₃ 150, P-PO₄ 50, K 150, Ca 150, Mg 50, Fe 3.00, Mn 19.2, Zn 0.44, Cu 0.03, B 0.01, pH 5.50, EC 1.8 mS cm⁻¹. The following Ti levels were tested (in mg Ti dm⁻³ of nutrient solution): 0 (control), 0.36; 0.72; 1.09 (denoted as Ti-I, Ti-II, Ti-III). The Ti source was Ti⁴⁺ ascorbate (Tytanit®). Ti had a positive effect on plant yielding: significantly the highest mean weight of a lettuce head and also the highest percentage of dry matter in leaves were found at Ti-II (+24.1% and 36.6%, respectively when compared with the control). Increasing Ti nutrition positively influenced the chlorophyll content (which was the highest at Ti-II and Ti-III), while it did not modify the Relative Water Content (RWC) in leaves. Despite the improved plant yielding, there were no differences in concentrations of macroelements (N, P, K, Ca, Mg) and some metallic microelements (Fe and Mn) in leaves. In turn, Ti nutrition significantly modified the nutrient uptake by aerial parts of plants, which increased up to Ti-II, while decreasing at Ti-III. The highest uptake of nutrients was recorded at Ti-II. Given the significant improvement in yield, Ti nutrition could be considered as an effective method to alleviate Mn-stress in lettuce.

Keywords: titanium, fertigation, manganese toxicity, macroelement, microelement, nutrient uptake, *Lactuca sativa* L.

INTRODUCTION

Titanium (Ti) is classified as a beneficial element for plants, which improves their growth and development (MILLS, JONES 1996, CARVAJAL, ALCARAZ 1998, KRÓL 1999, KLEIBER, MARKIEWICZ 2013). Plants treated with Ti are characterized by a higher content of chlorophyll and more intensive photosynthesis (GRENDA 2003, RADKOWSKI 2013). Ti also has an effect on the nutrient uptake and enzymatic activity (MARSCHNER 1995, DAOOD 1998, MICHALSKI 2008), MALINOWSKA, KALEMBASA 2012). LESKÓ et al. (2002) reported a positive effect of Ti, which limited the plant damage caused by heavy metals.

Manganese (Mn) is an essential trace element for plants. However, its excessive nutrition may lead to severe stress in plants, significantly reducing the biomass production and photosynthesis (KLEIBER et al. 2014) and causing biochemical disorders (MILLALEO et al. 2010, KLEIBER 2014a). Among the elements which alleviate Mn stress of plants there are silicon (MAKSIMOVIĆ et al. 2007, ZANÃO JÚNIOR et al. 2010, KLEIBER 2014b, KLEIBER et al. 2015) and selenium (KLEIBER et al. 2016, unpublished), which are also considered to be beneficial elements for plants. The aim of this study was to determine the effect of titanium application (up to 1.09 mg Ti dm⁻³ of nutrient solution) on the alleviation of Mn-stress of hydroponically grown lettuce.

MATERIALS AND METHOD

Plant cultivation

Vegetation experiments (2 independent spring cycles) were conducted in a greenhouse located at the Experimental Station of the Departments of the Faculty of Horticulture and Landscape Architecture, the Poznań University of Life Sciences (Poland). Every experiment was established in a systematic design with 8 replications (a replication was one single plant). The greenhouse was equipped with a modern climate control system. The following ambient conditions were maintained throughout the experiments: temperature 18-20°C; RH 65% to 75%. The research objective was to assess the influence of varied titanium application levels into nutrient solution on the plants' response described by the nutrient status and yielding of hydroponically grown lettuce (*Lactuca sativa* L. cv. Zeralda) under strong Mn-stress (19.2 mg Mn dm⁻³ of nutrient solution). In the case of lettuce grown under hydroponic conditions, this effect was found when the concentration of Mn in nutrient solution increased from 0.5 mg dm⁻³ to 19.2 mg dm⁻³ (KLEIBER 2014a), resulting in the significantly highest reduction of plant yielding and disorders in nutrient leaf concentrations under the highest Mn concentration.

Seedlings were prepared 3 weeks before the each vegetation experiment. The seeds were sown individually to rockwool fingers, which 48h before were

watered up with nutrient solution (NS). Later, the seedlings (in 3-4 leaves phase) were put in rockwool blocks (Grodan; 10 x 10 x 10 cm). After another 1 week, they were transferred to a special hydroponic system with recirculation of NS.

Plants were fertigated with NS of the following chemical composition (mg dm^{-3}): N-NH₄<10, N-NO₃150, P-PO₄ 50, K 150, Ca 150, Mg 50, Fe 3.00, Mn 19.2, Zn 0.44, Cu 0.03, B 0.01, pH 5.50, EC 1.8 mS cm^{-1} . The following Ti concentrations in NS were tested (in mg dm^{-3}): 0 (control); 0.36; 0.72; 1.09 (described as Ti-I, Ti-II and Ti-III). In this experiment, the Ti source was Ti⁴⁺ ascorbate (Tytanit®, Intermag, Olkusz, Poland) containing 8.5 g Ti dm^{-3} . Nutrient solution was dosed 6 times daily per 5 min in each watering cycle.

Biometrical measurements and chlorophyll measurement

The following were determined: the weight of a lettuce head (g) dry matter content (%) and the Relative Water Content (%) (GONZÁLES, GONZÁLES-VILAR 2001). On the last day of each experiment, the chlorophyll content index was also calculated, using a Minolta camera soil plant analysis development-502 (SPAD-502, Konica Minolta, Japan), and expressed in SPAD units. The measurements were made on the outside and the most developed leaves.

Chemical analysis

On the last day of every cycle, the aerial parts of the plants were collected, dried at 45-50°C and ground. In order to assay the total forms of N, P, K, Ca and Mg, the plant material was digested in concentrated (96%, analytically pure) sulphuric acid with the addition of hydrogen peroxide (30%, analytically pure). For analyses of total Fe, Mn, Zn and Cu, the plant material was digested in a mixture of concentrated nitric (ultra pure) and perchloric acids (analytically pure) at a 3:1 ratio. After digestion of the plant material, the following determinations were performed: N-total using the distillation method according to Kjeldahl in a Parnas Wagner apparatus; P, colorimetrically with ammonium molybdate, and K, Ca, Mg, Fe, Mn, Zn and Cu using flame atomic absorption (on a AAS, on a Carl Zeiss Jena apparatus).

Statistical analysis

The data were analyzed using Statistica 12 (StatSoft Inc., Tulsa, OK, USA). Results of chemical analyses and plant yielding measurements were processed by Anova and the Duncan test ($\alpha = 0.05$). The coefficient of variation (% CV) of the nutrient concentration in leaves and the uptake by aerial parts of plants were also calculated.

RESULTS AND DISCUSSION

Titanium nutrition had a positive effect on plant yielding expressed as the mean weight of a lettuce head – significantly the highest one was found in the case of Ti-II (0.72 mg Ti dm³), while the lowest one was obtained in the other treatments (control, Ti-I and Ti-III) – Table 1. A similar effect was

Table 1

Effect of titanium nutrition on plant yield (g head⁻¹), RWC (Relative Water Content), dry matter (% DM) and chlorophyll content (express in SPAD units)

Parameter	Ti level in nutrient solution (mg dm ⁻³)			
	control	0.36	0.72	1.09
Plant yield (g head ⁻¹)	96.3a	101.2a	119.5b	98.2a
RWC	0.821a	0.821a	0.796a	0.822a
DM (%)	2.51ab	3.01bc	3.43c	2.25a
Chlorophyll content (SPAD units)	23.1a	23.6a	25.7b	25.2b

Key for Tables 1-3: means in rows marked with different letters differ significantly at $p = 0.05$

observed for the content of dry matter in leaves – the highest value was recorded for Ti-II, while the lowest one was found in the case of Ti-III (3.43% and 2.25%, respectively).

Abiotic stress (e.g. caused by metal toxicity) leads to an increase in Reactive Oxygen Species (ROS): peroxide hydrogen, superoxide radical and hydroxyl radicals (SAMADI et al. 2015), which are highly toxic and cause damage, for example to the photosynthetic or respiratory electron transport. Increasing Mn-stress (comparing 0.5 mg Mn dm⁻³, which is a hydroponic Mn concentration and the tested concentration: 19.2 mg Mn dm⁻³) in lettuce caused oxidative stress and reduced its yielding by approx. 13.1% (KLEIBER 2014a). In the present study, titanium nutrition (at level Ti-II) improved plant yielding by about 24.1% comparing with the control (without Ti treatment). A similar and positive response of plants to titanium ascorbate application has been found in other research, where negative effects of cadmium damage in wheat seedlings were reduced (LESKÓ, SIMON-SARKADI 2002).

Photosynthetic pigments (chlorophylls and carotenoids) are very important primary metabolites, as they play an important role in antioxidant activities (SHARAFZADEH, ALIZADEH 2011). Titanium nutrition (at level Ti-II and Ti-III) had a positive effect on the chlorophyll content (SPAD) in lettuce leaves, but it did not modify the Relative Water Content (RWC) – Table 1. Lettuce typically shows a close relationships between the SPAD measurement result and the chlorophyll content (LEÓN et al. 2007). RADKOWSKI (2013) and WHITTED-HAAG et al. (2014) also found that Ti significantly affected the chlorophyll content in plant tissues. In turn, MOAVENI et al. (2011) reported that nanoparticles (NP) of TiO₂ could increase the levels of chlorophyll pig-

ments and the RWC. Under Ti treatment, chlorophyll concentrations in blue-green algae and *Anacystis nidulans* increased, possibly owing to Ti participation as a redox catalyst in electron transport from the PSII to the PSI (KISS et al. 1985). The cited authors claim that the same process may occur in plants, in which Ti might catalyse activities of some metallic elements involved in the photosynthetic process. The results of SAMADI et al. (2015) indicated that different TiO₂ and NP-TiO₂ treatments had a positive effect on root and shoot length, as well as levels of photosynthetic pigments (chlorophylls and carotenoids). NP-TiO₂ enhanced catalase (CAT) activity. The ROS stress produced by NPs can stimulate antioxidant enzymes in plants.

The role of Ti in plant metabolism has not been thoroughly clarified to date (TLUSTOŠ et al. 2005). Ti influences enzymatic activities in plants (DUMON, ERNST 1988, DAOOD 1998), but the toxicity limit could be relatively distinct (MAROTI et al. 1984). Also KUŽEL et al. (2003) claim that Ti has significant biological effects on plants, being beneficial at low and toxic at higher concentrations. HRUBÝ et al. (2002) proposed a hypothesis to explain the beneficial effects of Ti as a result of the phenomenon called “hormesis”: some poisons at low doses can cause stimulation by inducing counter effects, thus eliminating the toxic effects of these poisons. In the present study, the increasing Ti concentration up to 0.72 mg dm⁻³ stimulated plant yielding, whereas at 1.09 mg dm⁻³ plant yielding decreased significantly.

Titanium nutrition did not modify significantly the concentration of either macroelements (N, P, K, Ca, Mg) or Fe and Mn in leaves; significant differences were found only in the case of Zn and Cu (Table 2). The lowest concentration of the mentioned microelements was found for Ti-II (both for Zn and Cu), while the highest one occurred in the case of Ti-III (for Zn) and Ti-I (for Cu). Plant response to Ti application could be varied depending on species: in tomato leaves a significant effect was found for N, P, Ca and Mg (KLEIBER, MARKIEWICZ 2013), as well as Fe and Mn concentrations (MARKIE-

Table 2
Effect of titanium nutrition on macro- (g kg⁻¹ DM) and microelement (mg kg⁻¹ DM) concentration in lettuce leaves

Nutrient	Ti level in nutrient solution (mg dm ⁻³)			
	control	0.36	0.72	1.09
N	48.75a	48.00a	51.50a	52.00a
P	14.77a	13.94a	12.97a	14.37a
K	112.93a	113.43a	110.93a	114.55a
Ca	23.68a	22.57a	21.75a	23.30a
Mg	9.60a	9.41a	9.07a	9.62a
Fe	143.4a	133.1a	122.3a	108.2a
Mn	338.4a	336.7a	337.8a	339.0a
Zn	119.4ab	123.5ab	114.5a	132.5b
Cu	13.28ab	15.23b	10.77a	12.16ab

WICZ, KLEIBER 2014). Also KALEMBASA et al. (2015) stated that foliar application of Ti (in the form of Tytanit fertilizer) may significantly diversify the concentrations of Zn, Li, Ni, and Pb in petioles and leaf blades of celery. ALCARAZ-LOPEZ et al. (2004) found that Ti led to significant increases in Ca, Fe, Cu and Zn concentrations in plants. The above authors attributed the improvement in Ca assimilation to the beneficial Ti effect on absorption, translocation and assimilation processes.

Despite the lack of significant changes in the concentrations of N, P, K, Ca, Mg, Fe and Mn in leaves, Ti nutrition significantly modified the uptake of macro- and micronutrients by aerial parts of plants (Table 3). The highest nutrient uptake was found in the case of Ti-II, which also resulted in the highest yielding of plants. The uptake of all the nutrients decreased in response to the most intensive Ti nutrition, for which significant deterioration of plant yielding was also found. The determined coefficients of variation (CV) for nutrient concentrations in lettuce leaves under Ti treatment were clearly lower (from 0.28% to 19.39% for Mn and Fe, respectively), while being higher for nutrient uptake (from 20.67% to 31.74%, respectively, for P and Fe) – Table 4.

Among the elements which alleviate Mn stress of hydroponically grown lettuce there are silicon (KLEIBER 2014b, KLEIBER et al. 2015) and selenium

Table 3
Effect of titanium nutrition on macro- (g plant⁻¹) and microelement (mg plant⁻¹) uptake by aerial parts of lettuce

Nutrient	Ti level in nutrient solution (mg dm ⁻³)			
	Control	0.36	0.72	1.09
N	0.118a	0.146a	0.211b	0.115a
P	0.036b	0.042b	0.053c	0.032a
K	0.273b	0.345c	0.455d	0.253a
Ca	0.057ab	0.069bc	0.089c	0.051a
Mg	0.023a	0.029a	0.037b	0.021a
Fe	3.464ab	4.055ab	5.011b	2.390a
Mn	8.174b	10.255c	13.847d	7.488a
Zn	2.883a	3.761b	4.695c	2.928a
Cu	0.321a	0.464b	0.441b	0.269a

Table 4
Effect of titanium nutrition on the coefficient of variation (% CV) of nutrient concentration in leaves and uptake by aerial parts of plants

CV (%)	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Concentration in leaves	9.49	7.05	1.79	12.59	8.56	19.39	0.28	7.95	17.88
Uptake by aerial parts	27.88	20.67	23.87	26.80	23.80	31.74	24.89	21.52	25.22

(KLEIBER et al. 2016 unpublished). The effect of Si on Mn concentrations could be varied, e.g. decreasing (ZANAÓ JÚNIOR et al. 2010) or causing no reduction (MAKSIMOVIĆ et al. 2007, KLEIBER et al. 2015). Selenium influences Mn concentration in lettuce leaves, but the response is not linear (KLEIBER et al. 2016 unpublished). In the current study, Ti nutrition did not modify Mn concentration in leaves, while having a significant effect on the uptake of that element by aerial parts of plants. Important and significant result of the presented study, apart from the previously known positive effect of titanium on yielding in different plant species (MILLS, JONES 1996, CARVAJAL, ALCARAZ 1998, KRÓL 1999, KUŽEL et al. 2003, GRAJKOWSKI, OCHMIAN 2007, RADKOWSKI, RADKOWSKA 2013), is that Ti nutrition may positively influence yielding of plants grown under strong Mn-stress.

CONCLUSIONS

Based on the conducted study, it can be posited that titanium nutrition had a significant effect on plant yielding (expressed as the mean weight of a lettuce head) and chlorophyll content with the simultaneous lack of influence on the Relative Water Content (RWC) in plant tissues. Despite the significant increase in plant yielding, there were no differences in the concentrations of N, P, K, Ca, Mg, Fe and Mn in leaves, whereas the concentrations of Zn and Cu were influenced significantly. Ti nutrition significantly modified the nutrient uptake by aerial parts of plants in comparison to the control. Concluding, application of Ti (at a concentration of 0.72 mg dm^{-3}) in nutrient solution used for plant fertigation was an effective method to alleviate Mn-stress.

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