

The dynamics of photosynthetic parameters of *Phaseolus vulgaris* and *Vicia fabo* under strong cadmium stress

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One of the most toxic metals is cadmium, which inputs include those from commercial fertilizers, sewage sludge and other wastes used as soil amendments and also atmospheric deposition. The aim of this experiment was to study the dynamics of photosynthetic parameters of bean (*Phaseolus vulgaris* L.) and broad bean (*Vicia fabo* L.) under strong cadmium stress effect. Plants were sown in a neutral (pH 6.0–6.5) peat substrate, when 2nd true leaf unfolded, growth substrate was watered with 6 mM concentration CdSO₄ solution. Gas exchange parameters (photosynthetic rate (Pn), transpiration rate (Tn), intercellular CO₂ concentration (Ci) and water use efficiency (WUE)) were measured every day with portable photosynthesis system LI-6400. Content of photosynthetic pigments was analyzed in acetone extract using a spectrophotometer on the last 5th day of the experiment. On the first day of treatment the Pn of Cd treated *P. vulgaris* plants decreased only by 4.3% and statistically insignificant, while Pn of *V. fabo* decreased by 35.9% ($p < 0.05$). Pn of *P. vulgaris* was decreasing till 3rd day after treatment, when it was 96% ($p < 0.05$) lower compared to reference treatment. On the contrary, the lowest Pn of Cd treated *V. fabo* plants was on the first day and it started to increase from the second day till the last one. WUE of Cd treated *P. vulgaris* was decreasing till the third day too and it improved a little on the last one, but still it was 54% ($p < 0.05$) lower compared to the reference. While WUE of *V. fabo* increased by 23% ($p < 0.05$) and 16% ($p > 0.05$) on the first and the last day after Cd treatment, respectively compared to the reference. The changes, detected on the last day of the experiment, of chlorophyll *a* and *b* ratio were different too, i. e. for *P. vulgaris* it decreased by 5.7% ($p > 0.05$), and for *V. fabo* – increased by 1.4% ($p > 0.05$) compared to the reference. Also decrease of dry biomass of *P. vulgaris* was greater than that of *V. fabo*.

Key words: stress dynamics, Cd, net photosynthesis, intercellular CO₂ concentration, transpiration, water use efficiency, photosynthetic pigments, dry biomass, *Phaseolus vulgaris*, *Vicia fabo*

INTRODUCTION

Cadmium (Cd) is a toxic trace pollutant for humans, animals and plants, which enters the

environment mainly from anthropogenic processes and is then transferred to the food chain (Templeton and Liu, 2010). It is easily taken up by roots and transported to other parts of the plant (Gallego et al., 2012; Saidi et al., 2014). When soil Cd concentrations exceed 0.5 mg/kg,

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it is generally considered evidence of soil pollution. Cd is translocated into plant tissues due to an interference with essential elements, such as calcium (Ca) and potassium (K) (Uraguchi et al., 2009), and it becomes toxic when it accumulates at high concentrations in plant tissues (Hassan et al., 2013).

Cd is an effective inhibitor of photosynthesis (Mohamed et al., 2012). A linear relationship between photosynthesis and inhibition of transpiration was observed in clover, lucerne, and soybean that suggest Cd inhibited stomatal opening (Barcelo, Poschenrieder, 1990). Cd damages the photosynthetic apparatus, in particular the light harvesting complex II and photosystems I and II (Siedlecka et al., 1997). The inhibition of root Fe(III) reductase induced by Cd leads to Fe(II) deficiency which seriously affects photosynthesis (Alkantara et al., 1994). Cd also causes alteration in leaf gas exchange (López-Climent et al., 2011) stomatal closure in higher plants (Poschenrieder et al., 1989) and an overall inhibition of photosynthesis (Mohamed et al., 2012). In *A. thaliana* Cd altered the activity of photosynthetic apparatus (Mohamed et al., 2012) while decreasing the potential quantum yield of PSII (Maksymiec et al., 2007). Similarly, the synthesis and level of pigments are decreased in other plant species under the influence of Cd (Mohamed et al., 2012; Irfan et al., 2013).

Cadmium has no biological function and is not even essential for plant growth. Being water soluble, Cd²⁺ ion can be easily absorbed in tissues and can cause various phytotoxic visible symptoms (Hu et al., 2009; Valentovicova et al., 2010). Despite cadmium toxicity, plants have evolved a complex array of mechanisms to maintain optimal cadmium levels and avoid the detrimental effects of excessively high concentrations. Among the mechanisms, the activity of ROS-scavenging enzymes, including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), is considered as the most important protective mechanism to minimise the metal-induced oxidative damage in several plants (Parlak, Yilmaz, 2013; Li et al., 2014).

Different plant species and varieties show a wide range of plasticity in Cd tolerance, from

a high degree of sensitivity to a hyper-accumulating phenotype of some tolerant plants (Saidi et al., 2014). Legume plants are less tolerant to Cd toxicity than cereals and grasses. Although there have been many reports on the photochemical and biochemical events occurring in photosynthesis during Cd toxicity, a lot of contradictory data can be found in the literature (Popova et al., 2009). For instance, in leaves of *Brassica napus* Cd leads to a reduction of mesophyll cell size, while leaf thickness and cell size increase in *Pisum sativum* exposed to this heavy metal (Sandalio et al., 2001). Thus, this experiment was focused on the study the dynamics of photosynthetic parameters of bean (*Phaseolus vulgaris* L.) and broad bean (*Vicia fabo* L.) under strong cadmium stress effect.

MATERIALS AND METHODS

Bean (*Phaseolus vulgaris* L.) and broad bean (*Vicia fabo* L.) were chosen for the investigation. Experiments were carried out in a vegetation room with controlled environment: photoperiod – 14 h, average temperature of 20–25 °C, relative humidity – 60%. “Philips Master Green Power CG T” 600 W lamps, light intensity at the level of plants 14000 Lx provided light.

P. vulgaris (10 seeds per pot) and *V. fabo* (5 seeds per pot) were sown in a neutral (pH 6.0–6.5) peat substrate in 5 L pots (21 cm in diameter). 10 days after germination, when 2nd true leaf unfolded (at leaf development stage (BBCH-12)) (Growth stages..., 2001), growth substrate of *P. vulgaris* and *V. fabo* plants was watered with 6 mM concentration Cd₂SO₄ solution. Reference treatment groups were watered with distilled water all the time. In each treatment there were three pots of replication. The treatment variants were chosen according to earlier made experiments at the Environmental Department of the VMU (Januškaitienė, 2012). Duration of the experiment was five days.

The investigated indices: photosynthetic rate, intercellular CO₂ concentration, transpiration rate and water use efficiency were measured every day of the experiment. Photosynthetic pigments and dry biomass of shoot

(foliage) of plants were measured at the end of the experiment.

Gas exchange parameters were measured with portable photosynthesis system LI-6400 (LI-COR, USA). Photosynthetic rate (Pn) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), intercellular CO_2 concentration (Ci) ($\mu\text{mol CO}_2 \text{ mol air}^{-1}$), transpiration rate (Tn) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and water use efficiency (WUE) ($\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) of second fully expanded leaves were registered every 3 seconds for 10 minutes; from these data a mean of day of measured indices was calculated. Environment conditions during experiments were as follows: air flow rate – $400 \mu\text{mol s}^{-1}$; block and leaf temperature – 25°C ; CO_2 concentration in sample cell – $380\text{--}400 \mu\text{mol CO}_2 \text{ mol}^{-1}$; relative humidity in sample cell – 30%; lightness in quant – $190\text{--}210 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

The second fully expanded leaves were harvested and photosynthetic pigments were analysed using a spectrophotometer (Genesys 6, ThermoSpectronic, USA) in 100% acetone extracts prepared according to Wettstein's method (Wettstein, 1957). Photosynthetic pigments were expressed in mg g^{-1} of fresh weight.

At the end of the experiment the plants were harvested and dried in an oven at 60°C until constant dry foliage biomass was obtained. The biomasses were expressed in mg plant^{-1} .

ANOVA was used to determine the effects of cadmium and plant species. And for independent variables comparison Student's t and U tests were used. All analyses were performed by STATISTICA and the results were expressed as mean values and their confidence intervals (CI) ($p < 0.05$).

RESULTS

The response of *P. vulgaris* and *V. fabo* to cadmium stress was rather different. On the first day of treatment the photosynthetic rate of Cd treated *P. vulgaris* plants decreased only by 4.3% and statistically insignificant ($p > 0.05$), while photosynthetic rate of *V. fabo* decreased by 35.9% ($p < 0.05$) (Fig. 1). The photosynthetic rate of *P. vulgaris* was decreasing till 3rd day after treatment, when it was 95.9% ($p < 0.05$) lower compared to reference treatment. On the contrary, the lowest (36%; $p < 0.05$), compared to reference treatment, photosynthetic rate of Cd treated *V. fabo* plants was on the first day and it started to increase from the second day till the last one, when photosynthetic rate in Cd treated *V. fabo* leaves was only 17.1% ($p < 0.05$) less compared to reference treatment.

The highest changes of intercellular CO_2 concentration of *P. vulgaris* plants were also detect-

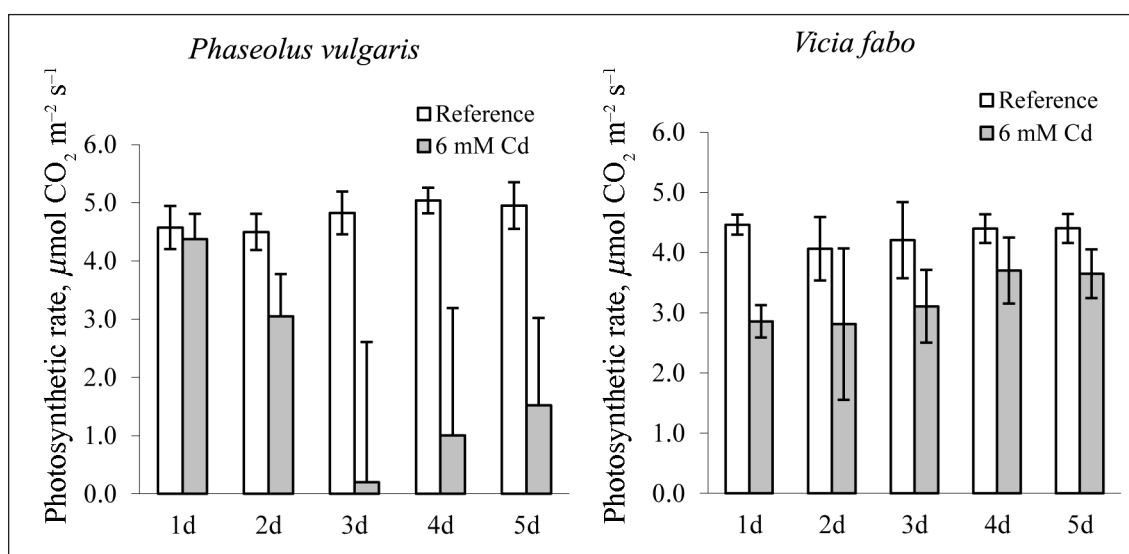


Fig. 1. The dynamics of photosynthetic rate in *Phaseolus vulgaris* and *Vicia fabo* leaves after 6 mM Cd treatment. The values are means \pm CI_{0.05}

ed on the third day, when it increased by 70.1% ($p < 0.05$) compared to reference treatment (Fig. 2). Higher, compared to reference treatment, intercellular CO_2 concentration of *P. vulgaris* was detected on the last day of experiment too, when C_i increased by 25.8% ($p > 0.05$). While the changes of intercellular CO_2 concentration of *V. fabo* plants were different, i. e. it decreased by 11.7% ($p < 0.05$) and 10.6% ($p < 0.05$)

on the first and last day, respectively, compared to reference treatment.

The changes of transpiration rate of the investigated plants were different, too. On the first day transpiration rate of *P. vulgaris* increased by 5.4% ($p > 0.05$), while transpiration rate of *V. fabo* decreased by 47.6% ($p < 0.05$) compared to plants of reference treatment (Fig. 3). The highest decrease of transpira-

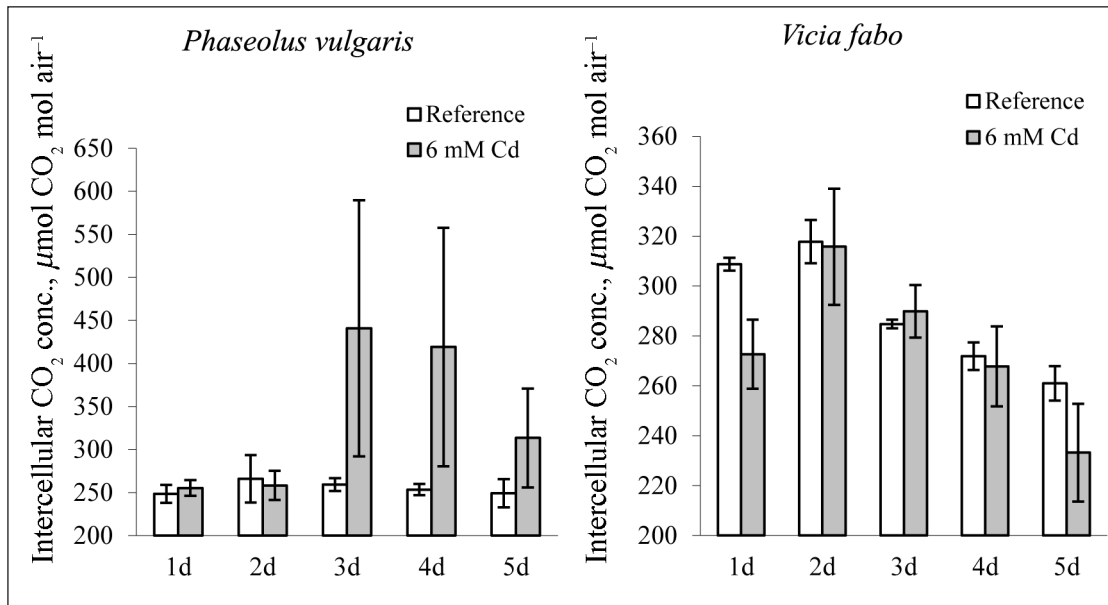


Fig. 2. The dynamics of intercellular CO_2 concentration in *Phaseolus vulgaris* and *Vicia fabo* leaves after 6 mM Cd treatment. The values are means $\pm \text{CI}_{0.05}$

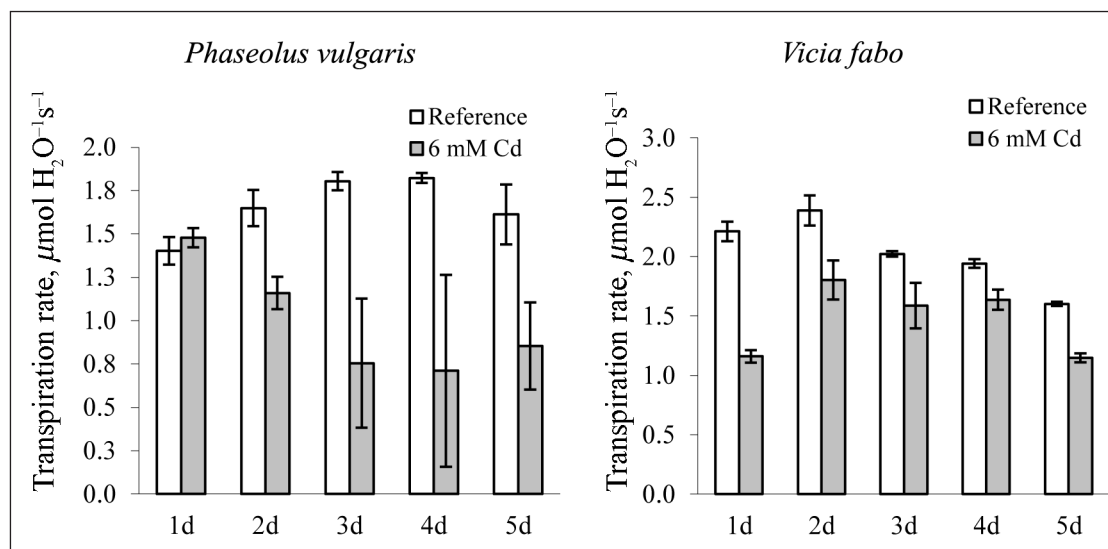


Fig. 3. The dynamics of transpiration rate in *Phaseolus vulgaris* and *Vicia fabo* leaves after 6 mM Cd treatment. The values are means $\pm \text{CI}_{0.05}$

tion rate of *P. vulgaris* was detected on the 4th day of experiment, when it decreased by 61.0% ($p < 0.05$) compared to reference. Transpiration rate of Cd treated *V. fabo* plants was lower, too, on the fourth day, but only 15.7% ($p < 0.05$) compared to reference treatment. On the last day of experiment transpiration rate of Cd treated plants was lower than that of non-treated, and higher decreases were typical for *P. vulgaris* plants.

Water use efficiency of Cd treated *P. vulgaris* plants was decreasing till third day, when WUE reached negative values and was more than 2 times lower than in reference plants. WUE of *P. vulgaris* slightly improved on the last one, but still it was 54% ($p < 0.05$) lower compared to reference treatment (Fig. 4). While WUE of *V. fabo* plants increased by 23% ($p < 0.05$) and 16% ($p > 0.05$) on the first and the last day after treatment, respectively, compared to reference.

The content of photosynthetic pigments detected on the last day of the experiment is presented in Fig. 5. Concentration of chlorophylls (*a* and *b*) and carotenoids increased in all Cd treated plant leaves, but statistically insignificant ($p > 0.05$). The changes of chlorophyll *a* and *b* ratio of the investigated plants were dif-

ferent, i. e. for *P. vulgaris* it decreased by 5.7% ($p > 0.05$), and for *V. fabo* – increased by 1.4% ($p > 0.05$) compared to reference treatment.

The decreases of dry biomass of Cd treated *P. vulgaris* were higher than *V. fabo* (Fig. 6). On the fifth day after Cd treatment, the dry biomass of *P. vulgaris* was 44.9% ($p < 0.05$) lower compared to reference, and decrease of dry biomass of *V. fabo* was only 0.5% and statistically insignificant ($p > 0.05$) compared to reference treatment.

DISCUSSION

As one of the most toxic environmental pollutants cadmium (Cd) has a strong influence on metabolic activities of plants by inducing a number of physiological changes, such as growth inhibition, changes in water and ion metabolism, photosynthesis inhibition, enzyme activity changes, and free radical formation (Jia et al., 2014). Plants use various mechanisms to cope with cadmium, which depends not only on the intensity of stress but also on plant individual characteristics. In this research plants tolerance to Cd stress was different. *V. fabo* was more tolerant to cadmium stress than *P. vulgaris*, but this was seen only on the third day, when photosynthetic

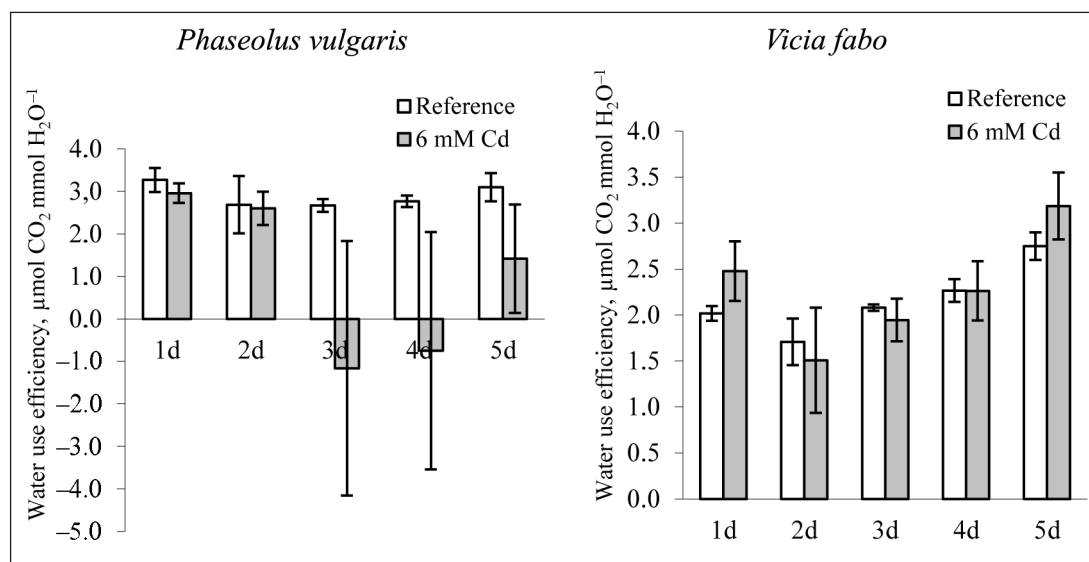


Fig. 4. The dynamics of water use efficiency of *Phaseolus vulgaris* and *Vicia fabo* after 6 mM Cd treatment. The values are means \pm CI_{0.05}

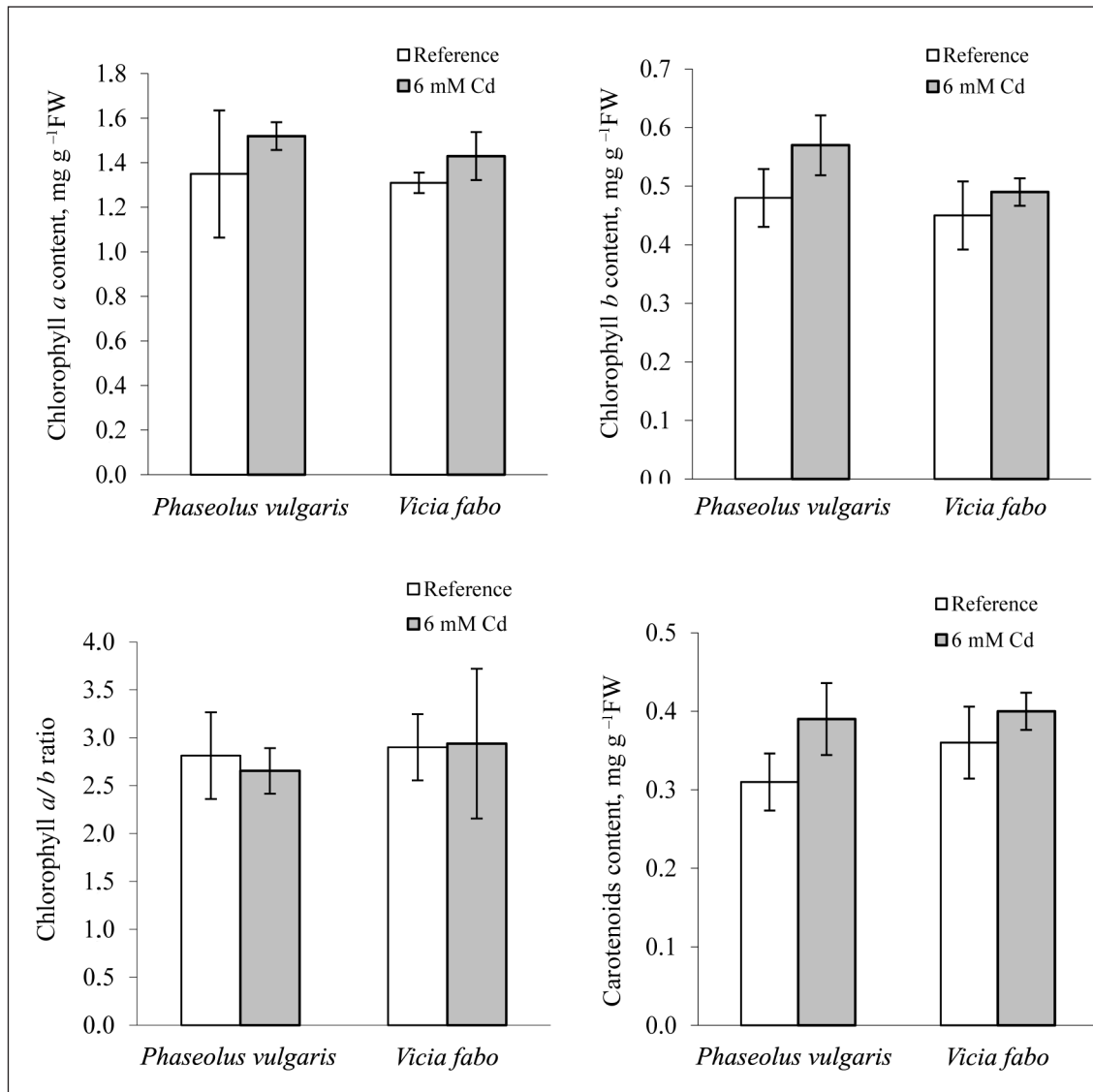


Fig. 5. The changes of chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b* ratio and carotenoids in *Phaseolus vulgaris* and *Vicia fabo* leaves under 6 mM Cd treatment. The values are means \pm CI_{0.05}, FW – fresh weight. The values are means \pm CI_{0.05}

rate of *P. vulgaris* and *V. fabo* plants decreased by 95% ($p < 0.05$) and 26% ($p < 0.05$) respectively, compared to reference treatment (Fig. 1). Higher negative effect of cadmium on photosynthetic rate of *P. vulgaris* plants stayed on the 5th day after treatment, too. Damages of the photosynthetic apparatus are related to different direct and indirect mechanisms induced by Cd. As a consequence of its capacity to replace essential metals in metal binding proteins, Cd can induce inhibition of chlorophyll (Chl) synthesis and also disturb PSII

function (Bertrand, Poirier, 2005; Faller et al., 2005; Kucera et al., 2008). A direct inhibition of O₂ evolution by Cd is also possible (Pagliano et al., 2006). Due to its stable binding to single bondSH groups of proteins, Cd may interfere directly with enzymes related to Chl biosynthesis and C assimilation, and also with the correct assembly of the pigment-protein complexes of both photosystems (Barylka et al., 2001; Basa et al., 2014). Strong effect on enzymatic reaction of photosynthesis also shows a very high intracellular CO₂ concentration

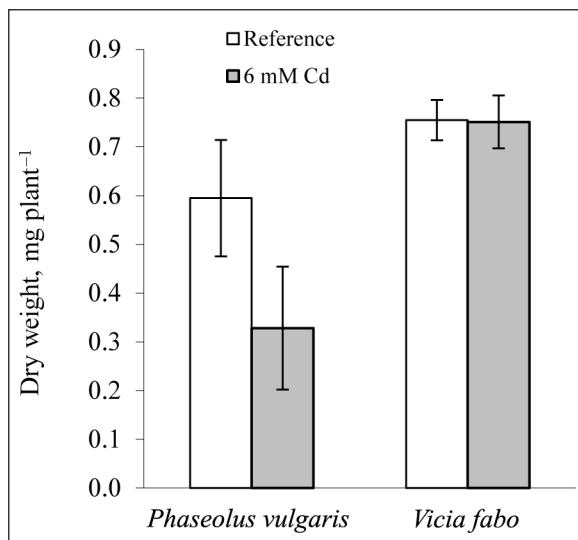


Fig. 6. The changes of dry weight of *Phaseolus vulgaris* and *Vicia fabo* under 6 mM Cd treatment. The values are means \pm CI_{0.05}

(Wahid et al., 2007) in *P. vulgaris* leaves on the third and fourth day after treatment (Fig. 2). While on these days the changes of intracellular CO₂ concentration in *V. fabo* leaves were statistically insignificant.

Toxicity of Cd in plants is associated with reduced water and nutrient uptake (Hassan et al., 2013) and thus reduction in water use efficiency was observed with Cd treatment in *P. vulgaris* leaves (Fig. 4). This might be due to the inhibition of absorption and translocation of water (Poschenrieder, Barcelo, 1999). Similar results were presented by other researchers, too (Rascio et al., 2008). Also, the water use efficiency of Cd treated *V. fabo* plants increased. Cadmium may affect stomatal closure in leaves (Wang et al., 2009) and more reduce transpiration rate than photosynthesis, so this caused an increase in WUE of cadmium treated *V. fabo* plants (Fig. 4).

Chlorophylls *a* and *b* are important factors of photosynthesis inhibition, and their concentration in plant tissues decreases under abiotic stress environment. It is known that the decrease of chlorophyll content inhibits plant ability to absorb and utilize light energy and, consequently, leads to reduced photosynthesis (Jia et al., 2004).

The content of chlorophylls and carotenoids was postulated as a simple and reliable indicator of heavy metal toxicity for higher plants (Goncalves et al., 2009). But the effects of cadmium on the photosynthetic apparatus are different. In one part of the studies it is presented that Cd is a potent inhibitor of the photochemical activity of the chloroplasts (Mobin, Khan, 2007), but in another that it is not sensitive to Cd. In this research, the content of chlorophylls and carotenoids increased in all Cd treated plant leaves, but statistically insignificant ($p > 0.05$) (Fig. 5).

Cadmium competes for plant nutrient status and subsequently alters its physiology. High concentrations of Cd decreased the cell growth as well as the plant yield (Irfan et al., 2013). In this research, dry weight of cadmium treated plants decreased, too, but statistically significant only for *P. vulgaris* (Fig. 6). As has been mentioned above, cadmium damages the photosynthetic apparatus. The data of the current research confirms it too, and a higher negative effect was detected for photosynthetic parameters of *P. vulgaris* plants than of *V. fabo* (Figs. 1–4). So, a contact of plants with Cd leads to the reduction of total photosynthetic area and plant biomass (Lopez-Millan et al., 2009), that is why the changes of dry biomass accumulation were also higher for *P. vulgaris* plants.

CONCLUSIONS

On the first day of treatment photosynthetic rate of Cd treated *P. vulgaris* plants decreased only by 4.3% and was statistically insignificant, while photosynthetic rate of *V. fabo* decreased by 35.9% ($p < 0.05$). Photosynthetic rate of *P. vulgaris* was decreasing till 3rd day after treatment, when it was 96% ($p < 0.05$) lower compared to reference treatment. On the contrary, the lowest photosynthetic rate of Cd treated *V. fabo* was on the first day and it started to increase from the second day till the last one.

Water use efficiency of Cd treated *P. vulgaris* was decreasing till third day and it slightly improved on the last one, but still it was 54% ($p < 0.05$) lower compared to reference. While

water use efficiency of *V. fabo* increased by 23% ($p < 0.05$) on the first day, and the changes from the second till fifth day were statistically insignificant.

The changes, detected on the last day of the experiment, of chlorophyll *a / b* ratio were different too, i. e. for *P. vulgaris* it decreased by 5.7% ($p > 0.05$), and for *V. fabo* – increased by 1.4% ($p > 0.05$) compared to reference.

The decreases of dry biomass of *P. vulgaris* were higher than *V. fabo*. On the fifth day after Cd treatment, the dry biomass of *P. vulgaris* was 44.9% ($p < 0.05$) lower compared to reference, while the decrease of dry biomass of *V. fabo* was only 0.5% ($p > 0.05$) compared to reference treatment.

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PHASEOLUS VULGARIS IR VICIA FABO FOTOSINTEZĖS RODIKLIŲ DINAMIKA ESANT STIPRIAM KADMIO STRESUI

Santrauka

Kadmis yra vienas toksiškiausių metalų, kurio šaltiniai – cheminės trąšos, dirvožemio tręšimas nuotekų dumbliu bei kitomis atliekomis ir emisijos iš atmosferos. Tyrimo tikslas – išsiaiškinti pupelės (*Phaseolus vulgaris* L.) ir pupos (*Vicia fabo* L.) fotosintezės rodiklių dinamikos pokyčius esant stipriam kadmio stresui. Augalai sėti į neutralaus rūgštumo (pH 6,0–6,5) substratą, išleidę du tikrusius lapelius buvo paveikti 6 mM CdSO₄ koncentracijos tirpalu. Eksperimento trukmė – penkios dienos. Fotosintezės rodikliai (fotosintezės intensyvumas (Pn), transpiracijos intensyvumas (Tn), viduląstelinis CO₂ kiekis (Ci) ir vandens naudojimo efektyvumas (WUE)) matuoti kiekvie-

ną dieną nešiojama fotosintezės sistema LI-6400. Pigmentų kiekiai lapuose nustatyti acetono ekstrakte spektrofotometriškai paskutinę (5-ą) eksperimento dieną. Pirmą eksperimento dieną kadmiumu paveiktų *Phaseolus vulgaris* fotosintezės intensyvumas sumažėjo statistiškai nereikšmingai – tik 4,3 %, o *Vicia fabo* Pn – 35,9 % (p < 0,05). *Phaseolus vulgaris* Pn mažėjimas tęsėsi iki trečios dienos (po poveikio), kai fotosintezės intensyvumas buvo net 96 % (p < 0,05) mažesnis, palyginti su kontroliniais augalais. Priešingai, kadmiumu paveiktų *Vicia fabo* mažiausias fotosintezės intensyvumas nustatytas pirmą eksperimento dieną, kuris toliau gerėjo iki paskutinės dienos. Kadmiumu paveiktų *Phaseolus vulgaris* vandens naudojimo efektyvumas taip pat mažėjo iki trečios dienos, šiek tiek pagerėjo paskutinę dieną, bet vis tiek buvo 54 % (p < 0,05) mažesnis nei kontrolinių augalų. *Vicia fabo* WUE padidėjo tiek pirmą (23 %; p < 0,05), tiek ir paskutinę (16 %; p > 0,05) dieną, palyginti su kontroliniais augalais. Skirtingi chlorofilų *a* ir *b* santykio pokyčiai nustatyti paskutinę eksperimento dieną, t. y. *Phaseolus vulgaris chl a/b* – mažėjo (5,7 %; p > 0,05), o *Vicia fabo* – didėjo (1,4 %; p > 0,05), palyginti su kontroliniais augalais. Sausos biomasės pokyčiai taip pat buvo didesni *Phaseolus vulgaris*, o *Vicia fabo* biomasė kito statistiškai nereikšmingai.

Raktažodžiai: streso dinamika, Cd, fotosintezės intensyvumas, viduląstelinis CO₂ kiekis, transpiracija, vandens naudojimo efektyvumas, fotosintetiniai pigmentai, sausa biomasė, *Phaseolus vulgaris*, *Vicia fabo*