

## THE BIOSTIMULANT ASAHI SL PROTECTS THE GROWTH OF *Arabidopsis thaliana* L. PLANTS WHEN CADMIUM IS PRESENT

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**Abstract.** Biostimulants are compounds of diverse formulations, that improve quantity and quality of yield. Their positive effect on plants is more evident in stressful conditions. Biostimulants increase a plant's tolerance to stresses and enable the repair of damage caused by unfavourable conditions. In this study the effect of Asahi SL on *Arabidopsis thaliana* L. grown in the presence of cadmium ( $\text{Cd}^{2+}$ ) was evaluated.  $\text{Cd}^{2+}$  negatively affected examined parameters and processes, leading to disturbances in plant growth and performance. *A. thaliana* treated with Asahi SL were higher and advanced in development. Biomass accumulation was stimulated by Asahi SL due to a higher efficiency of the photosynthetic apparatus, manifested by higher: (i) leaf area, (ii) chlorophyll content and (iii) intensity of photosynthesis. Despite higher intensity of transpiration and lower stomatal resistance, the relative water content was unchanged in Asahi SL – treated plants due to the stimulation of root development. Therefore, it can be concluded that Asahi SL protects plants against  $\text{Cd}^{2+}$  stress.

**Keywords:** stress conditions, biomass accumulation, efficiency of photosynthetic apparatus, water status

### INTRODUCTION

Plants are constantly exposed to environmental stresses such as heavy metals (HM), e.g. cadmium ( $\text{Cd}^{2+}$ ). Although it is not essential for plants,  $\text{Cd}^{2+}$  is readily taken up by roots and translocated to aboveground parts [Połec-Pawlak et al. 2005]. The amount of  $\text{Cd}^{2+}$  transported to leaves is greater than 10% of the total amount taken up by plants,

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and depends on plant species, the concentration of  $\text{Cd}^{2+}$  and its form [Połec-Pawlak et al. 2005].  $\text{Cd}^{2+}$  causes disruption to cellular structures, negatively affects physiological processes and induces oxidative stress [Gallego et al. 2012, Dias et al. 2013, Tran and Popova 2013, Nováková et al. 2015]. As a result, the growth and development of plants exposed to  $\text{Cd}^{2+}$  is inhibited and aging accelerated [Gallego et al. 2012, Dias et al. 2013, Tran and Popova 2013].

Defence mechanisms in plants are insufficient to mitigate the negative effects of  $\text{Cd}^{2+}$  [Scebba et al. 2006]. Nazar et al. [2012] suggest changes in fertilization as a strategy for reducing  $\text{Cd}^{2+}$  toxicity. Alleviation of  $\text{Cd}^{2+}$  toxicity may be also obtained by application of calcium [Ahmad et al. 2015], endophytes [Khan et al. 2014], selenium [Saidi et al. 2014], silicon [Farooq et al. 2013] and plant growth regulators, including phenolic compounds [Asgher et al. 2015]. However, these technologies are not cheap and/or simple to be applied in the agriculture. A promising alternative could be the use of biostimulants, which positively affect a plant's vital processes and improve a plant's tolerance to stresses [du Jardin 2015]. Asahi SL, also known as Atonik, is used around the world as a biostimulant affecting plant's vital processes at each level of its biological organisation, starting from the molecular, biochemical and physiological processes, to plant cells and organs, all the way to canopy [Gawrońska et al. 2008, Przybysz et al. 2014]. Its positive effect on yield is well proven [Djanaguiraman et al. 2009, Serrano et al. 2010, Grabowska et al. 2015, Kwiatkowski et al. 2015, Kocira et al. 2015]. Asahi SL – treated plants are more advanced in their growth and development, and accumulate more biomass [Djanaguiraman et al. 2005, Haroun et al. 2011, Przybysz et al. 2014], mainly due to greater efficiency of their photosynthetic apparatus [Gawrońska et al. 2008, Djanaguiraman et al. 2009, Borowski 2010, Przybysz et al. 2014, Pokluda et al. 2016] and improved water status [Gawrońska et al. 2008, Borowski 2010, Przybysz et al. 2014]. The positive effects of Asahi SL are more evident when plants are grown under adverse conditions. It has been found that biostimulants play a protective role against abiotic stresses, such as low or high temperatures, drought, noble metals and salinity [Gawrońska et al. 2008, Borowski 2010, Przybysz et al. 2014, Pokluda et al. 2016]. To the best of the authors' knowledge, works published so far have not studied effect of Asahi SL on HM stress in plants. Therefore, in this study the effect of Asahi SL application on *Arabidopsis thaliana* L. grown under HM stress generated by  $\text{Cd}^{2+}$  is evaluated.

## MATERIALS AND METHODS

**Plant material and growing conditions.** *A. thaliana* Col 4 seeds (Lehle Seeds, TX, USA) were kept for three days at 4°C and sown onto multi-plates filled with a mixture of substrate and sand in the proportion of 2:1 v/v. Uniform, six-week-old seedlings at growth stage 1.12 [Boyes et al. 2001] were transplanted to hydroponics filled with 0.3 dm<sup>3</sup> of Hoagland solution in modification of Siedlecka and Krupa [2002]. The nutrient solution was continuously aerated and exchanged weekly. Plants were grown in growth chambers (Simez Control s.r.o. Vsetin, Czech Republic) with a photoperiod of

8/16 h day/night, temperature of 20/18°C, irradiance of 280  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PAR, pH of 6.6 and relative humidity of 70%.

During the first week the growing solution was used at half strength, and thereafter the complete composition of nutrients was applied. Two weeks later, plants were transplanted to hydroponics and during the nutrient solution change,  $\text{Cd}^{2+}$  and Asahi SL were added.  $\text{Cd}^{2+}$  was added in the form of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  at concentrations of 25 and 50  $\mu\text{M}$ . Asahi SL, which is composed of sodium ortho-nitrophenolate (0.2%), sodium para-nitrophenolate (0.3%) and sodium 5-nitroguaiacolate (0.1%) was added at a concentration of 0.005% v/v. After treatment, the plants grew for a further three weeks. The control plants were grown in both,  $\text{Cd}^{2+}$  and Asahi SL free medium.

**Measured parameters and methods used.** Two weeks after exposure to  $\text{Cd}^{2+}$  and Asahi SL, the following parameters were measured: (i) gas exchange: intensity of photosynthesis and transpiration, stomatal resistance (LICOR 6200 Photosynthesis System, Lincoln, Nebraska, USA); (ii) chlorophyll content (CCM-200, Opti-Sciences, USA) and (iii) chlorophyll *a* fluorescence (Handy PEA, Hansatech, UK). Measurements were performed on fully developed leaves that were free from injury and any symptoms of senescence.

At harvest, the plants were divided into roots and aboveground parts. The roots were rinsed with distilled and redistilled water to remove  $\text{Cd}^{2+}$  from their surface. Samples were collected to determine (i) the relative water content (RWC, expressed as ratio of tissue fresh weight to tissue turgid weight) and (ii) the membrane injury (conductometrically, MultiLevel 1, WTW, Germany) and data were recorded on (iii) the height of plants, (iv) the number of pods, (v) the number and area of leaves, (vi) the length of roots (Leaf Area and Analysis System/Root Length Measurement System, Skye, UK) and (vii) fresh weight (FW) and dry matter (DM), after drying of particular organs for 24 hours at 105°C followed by a subsequent three days at 75°C.

**Statistical data analysis.** Data were subjected to analysis of one factorial ANOVA using Statgraphics Plus 4.1. (Statpoint Technologies Inc., Warrenton, VA, USA). Differences between means of plants treated and untreated with Asahi SL were evaluated by post-hoc Tukey's honestly significant difference test (HSD). Means were considered to be significantly different at  $P < 0.05$ .

In total two experiments were carried out. Since the results obtained in both showed similar trends, data presented here are from the experiment with a wider range of parameters measured. There were five biological replications (one plant per container). The number of replications (separate sampling) for a given parameter ranged between three and nine, and is indicated in the specific tables or figures. The data presented are mean  $\pm$ SE.

## RESULTS

**Effect of  $\text{Cd}^{2+}$  application.** Treatment with  $\text{Cd}^{2+}$  caused yellowing of leaf blades and reduced leaf area, but there was slightly greater number of leaves on plants treated with examined metal (tab. 1). Plants exposed to  $\text{Cd}^{2+}$  were smaller by 11% and 17%

respectively for 25 and 50  $\mu\text{M}$  of  $\text{Cd}^{2+}$ , and produced 28% fewer pods than the untreated ones. The length of roots was slightly increased when plants were treated with  $\text{Cd}^{2+}$  at 25  $\mu\text{M}$ , while it was not affected at 50  $\mu\text{M}$  (tab. 1). The colour of roots changed from white to brown.

Table 1. Effect of Asahi SL application on selected morphological parameters of *A. thaliana* plants grown in the presence of  $\text{Cd}^{2+}$ . Presented values are mean  $\pm$ SE, n = 5

$\text{Cd}^{2+}$ ( $\mu\text{M}$ )	Plant height (cm)		Roots length (cm)		Number of pods		Number of leaves		Leaf area ( $\text{cm}^2$ )	
	Asahi SL									
	-	+	-	+	-	+	-	+	-	+
Control (0)	37.1 $\pm 2.45$	41.4 $\pm 3.42$	16.9 $\pm 0.59$	17.2 $\pm 0.42$	41.2 $\pm 10.2$	42.5 $\pm 5.60$	143.0 $\pm 3.22$	103.5* $\pm 6.96$	207.8 $\pm 0.88$	271.3* $\pm 4.34$
25	33.0 $\pm 1.41$	36.8 $\pm 1.26$	18.3 $\pm 1.43$	19.5 $\pm 1.93$	29.6 $\pm 5.84$	44.0* $\pm 4.57$	148.0 $\pm 17.4$	133.5 $\pm 2.78$	164.7 $\pm 19.0$	179.5 $\pm 6.04$
50	30.7 $\pm 3.56$	31.2 $\pm 4.81$	16.9 $\pm 1.87$	17.3 $\pm 0.78$	29.8 $\pm 8.67$	52.2 $\pm 37.2$	145.5 $\pm 14.3$	141.0 $\pm 3.62$	143.5 $\pm 18.0$	201.5* $\pm 4.98$

\* – values differ significantly at  $\alpha = 0.05$  as determined by HSD of Tukey's test

Table 2. Effect of Asahi SL on fresh weight (FW) and dry matter (DM) of *A. thaliana* plants grown in the presence of  $\text{Cd}^{2+}$ . Presented values are mean  $\pm$ SE, n = 5

$\text{Cd}^{2+}$ ( $\mu\text{M}$ )		Whole plant		Aboveground part		Roots	
		Asahi SL					
		-	+	-	+	-	+
Control (0)	fresh	12.4 $\pm 0.10$	15.1 $\pm 0.38$	9.86 $\pm 0.07$	12.4 $\pm 0.36$	2.59 $\pm 0.06$	2.72 $\pm 0.02$
25	weight	8.91 $\pm 0.06$	9.36 $\pm 0.44$	7.16 $\pm 0.05$	7.31 $\pm 0.33$	1.65 $\pm 0.01$	2.05 $\pm 0.14$
50	(g)	8.07 $\pm 0.61$	9.99 $\pm 0.06$	6.37 $\pm 0.45$	8.05 $\pm 0.06$	1.70 $\pm 0.16$	1.94 $\pm 0.02$
Control (0)	dry	1.08 $\pm 0.01$	1.17 $\pm 0.03$	0.93 $\pm 0.00$	1.03 $\pm 0.03$	0.15 $\pm 0.00$	0.14 $\pm 0.00$
25	matter	0.75 $\pm 0.04$	0.85 $\pm 0.03$	0.66 $\pm 0.04$	0.74 $\pm 0.03$	0.09 $\pm 0.00$	0.11 $\pm 0.00$
50	(g)	0.65 $\pm 0.04$	0.83 $\pm 0.01$	0.57 $\pm 0.04$	0.72 $\pm 0.00$	0.08 $\pm 0.00$	0.11 $\pm 0.00$

The total FW of  $\text{Cd}^{2+}$ -treated plants reduced by 28 and 35%, while DM by 31 and 40% for 25 and 50  $\mu\text{M}$  of  $\text{Cd}^{2+}$  respectively (tab. 2). Biomass accumulation in above-ground parts decreased by 27–35% and 29–39% for FW and DM respectively. Application of  $\text{Cd}^{2+}$  also reduced the FW (by 34–36%) and DM (by 40–47%) of roots (tab. 2).

Plants treated with  $\text{Cd}^{2+}$  had decreased efficiency of photosynthetic apparatus, which was manifested by reduced chlorophyll content (by 26–28%, tab. 3) and intensity of photosynthesis (by 22%, fig 1a). On the other hand  $\text{Cd}^{2+}$  had a positive effect on stomatal resistance, which decreased by 14–16% (fig. 1b). The maximum efficiency of PSII (Fv/Fm) irrespectively of  $\text{Cd}^{2+}$  treatment was always at an optimal level, while performance index (P.I.) decreased by 3–11% compared to the control (tab. 3).

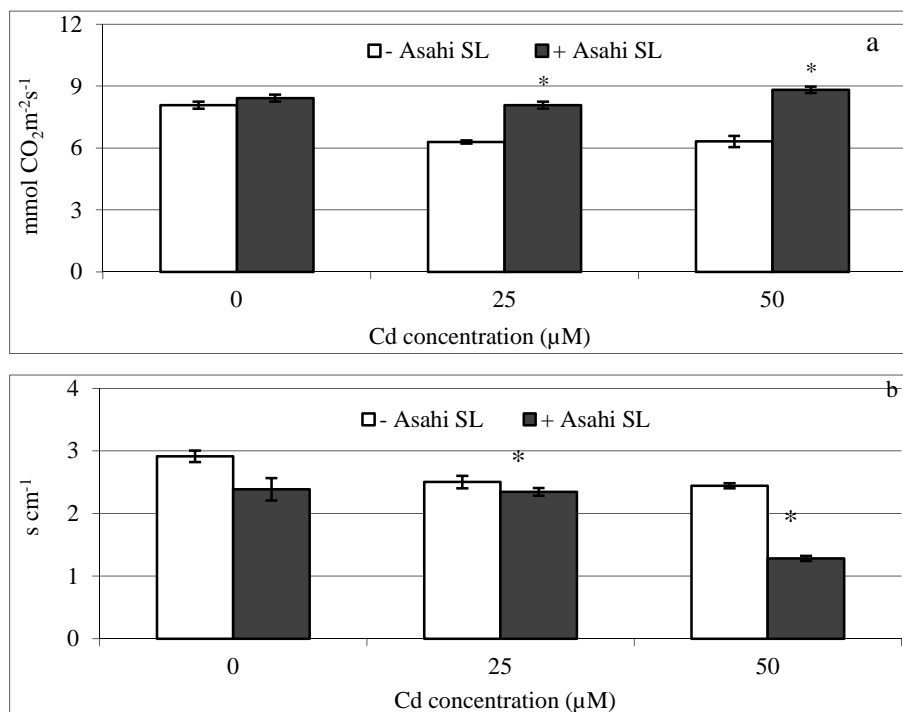


Fig. 1. Effect of Asahi SL on (a) intensity of photosynthesis and (b) stomatal resistance in *A. thaliana* plants grown in the presence of Cd. Presented values are mean  $\pm$ SE, n = 9 (3 biological replicates  $\times$  3 measurements for each)

Table 3. Chlorophyll content, chlorophyll *a* fluorescence (Fv/Fm and P.I.) in *A. thaliana* plants grown in the presence of Cd<sup>2+</sup>. The values presented are mean  $\pm$ SE, n = 6 (3 biological replicates  $\times$  2 measurements, chlorophyll content and chlorophyll *a* fluorescence) or 9 (3 biological replicates  $\times$  3 measurements, gas exchange)

Cd <sup>2+</sup> (μM)	Chlorophyll content(relative values)		Fv/Fm		P.I.	
	-	+	-	+	-	+
Control (0)	27.7 $\pm$ 0.98	26.5 $\pm$ 0.95	0.86 $\pm$ 0.00	0.85 $\pm$ 0.00	3.30 $\pm$ 0.03	3.16 $\pm$ 0.05
25	20.5 $\pm$ 1.14	23.3 $\pm$ 0.80	0.86 $\pm$ 0.00	0.85 $\pm$ 0.00	3.20 $\pm$ 0.07	3.18 $\pm$ 0.05
50	20.0 $\pm$ 1.11	22.4 $\pm$ 1.20	0.85 $\pm$ 0.00	0.84 $\pm$ 0.00	2.93 $\pm$ 0.05	2.86 $\pm$ 0.04

\* – values differ significantly at  $\alpha = 0.05$  as determined by HSD of Tukeys test

The intensity of transpiration in plants treated with Cd<sup>2+</sup> increased, while RWC slightly reduced (tab. 4). Cd<sup>2+</sup> negatively affected membrane injuries, both in leaves and roots (fig. 2).

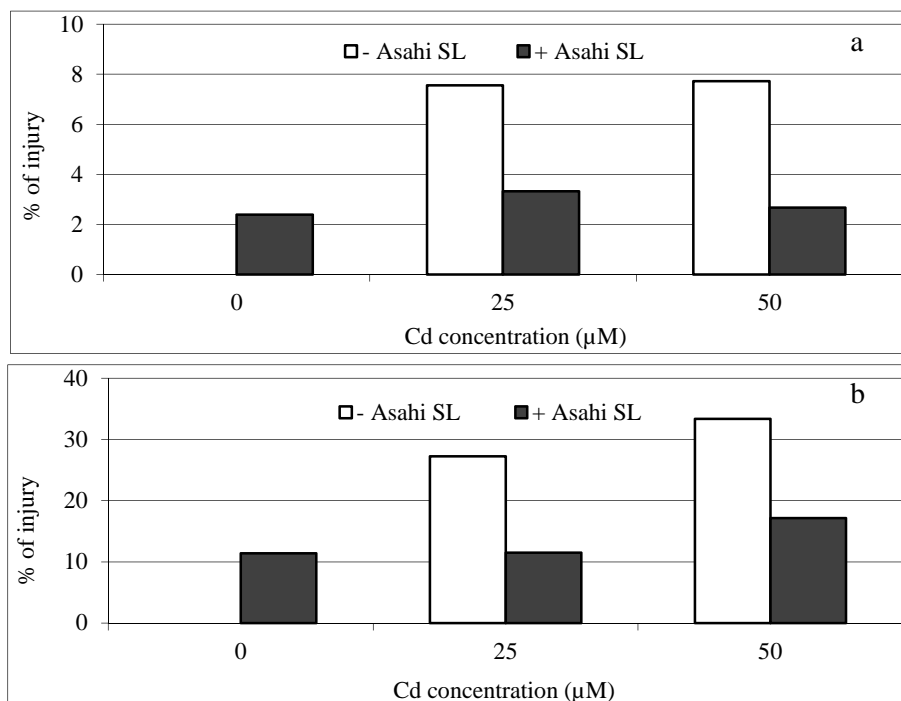


Fig. 2. Membrane injury in (a) leaves and (b) roots of *A. thaliana* plants grown in the presence of Cd. The values presented are mean  $\pm$ SE, n = 3

Table 4. Intensity of transpiration and relative water content (RWC) in leaves of *A. thaliana* plants grown in the presence of Cd<sup>2+</sup>. The values presented are mean  $\pm$ SE, n = 9 transpiration rate (3 biological replicates  $\times$  3 measurements for each) or n = 3 for RWC

Cd <sup>2+</sup> (μM)	Intensity of transpiration (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )		RWC (%)	
	Asahi SL			
	-	+	-	+
Control	3.21 $\pm$ 0.09	3.42 $\pm$ 0.21	93.7 $\pm$ 0.29	91.9 $\pm$ 0.38
25	3.42 $\pm$ 0.13	3.92* $\pm$ 0.06	88.3 $\pm$ 0.97	89.5 $\pm$ 0.73
50	3.70 $\pm$ 0.06	5.63* $\pm$ 0.18	91.0 $\pm$ 0.28	90.0 $\pm$ 0.43

**Effect of Asahi SL application on plants treated with Cd<sup>2+</sup>.** Asahi SL usually had a positive effect on the growth and development of *A. thaliana* plants grown in the presence of Cd<sup>2+</sup> (tab. 1). Plants treated with the biostimulant were taller (by 2–11%) and developed more pods (by 49–75%, significantly at 25 μM Cd<sup>2+</sup>). Leaf area was greater in Asahi SL-treated plants (by 9–40%), and this result was significant at 50 μM of Cd<sup>2+</sup>, while the number of leaves decreased after biostimulant application (by 3–10%).

Asahi SL positively influenced root growth, as they were 2–6% longer in the biostimulant-treated plants (tab. 1).

Plants treated with Asahi SL accumulated more biomass (tab. 2). Total FW was higher by 5–24% and DM by 13–28% compared to plants treated only with  $\text{Cd}^{2+}$ . The positive effect of the biostimulant on FW of aboveground parts and roots separately was recorded as 2–26% (rosette) and 14–24% (roots). DM accumulation in both organs was also stimulated, by 12–26% in the case of aboveground parts and 22–37% in the roots. The greater increase in FW and DM was always recorded in plants exposed to 50  $\mu\text{M}$  of  $\text{Cd}^{2+}$  (tab. 2).

The chlorophyll content was greater after biostimulant application by 12–14% (tab. 3). Treatment with Asahi SL in all cases significantly increased the intensity of photosynthesis (by 28–39%, fig. 1a) and decreased stomatal resistance (by 6–47%, fig. 1b) in plants exposed to  $\text{Cd}^{2+}$ . The effect of Asahi SL on values of Fv/Fm and P.I. was only marginal (tab. 3).

The application of Asahi SL significantly increased the intensity of transpiration, which was 15–52% higher (tab. 4). RWC remained practically unchanged (tab. 4).

The level of membrane injuries considerably decreased (by 57–65% in leaves and 48–58% in roots) in plants exposed to  $\text{Cd}^{2+}$  and treated with Asahi SL (fig. 2).

## DISCUSSION

Protecting crops against unfavourable growing conditions is the main challenge facing modern agriculture nowadays. Despite employing the most advanced agrotechnologies, the crop potential is still not fully exploited. Therefore this study attempted to evaluate the effect of the Asahi SL on *A. thaliana* plants exposed to HM stress with  $\text{Cd}^{2+}$  co-treatment as an example.

The application of  $\text{Cd}^{2+}$  had negative effects on *A. thaliana* plants. The growth of  $\text{Cd}^{2+}$  – treated plants was impaired (tab. 1) and they accumulated less biomass (tab. 2), which has previously been demonstrated by Dias et al. [2013], Gallego et al. [2012] and Tran and Popova [2013]. Reduced biomass accumulation recorded in this study was the result of decreased efficiency in photosynthetic apparatus (tab. 3, fig. 1). Negative effect of  $\text{Cd}^{2+}$  on photosynthetic apparatus was also shown by Dias et al. [2013] and Tran and Popova [2013]. The influence of  $\text{Cd}^{2+}$  on water relations in this work was ambiguous (tab. 4). In  $\text{Cd}^{2+}$  – treated plants the intensity of transpiration increased (tab. 4) due to reduced stomatal resistance (fig. 1b). In contrast to the above, Dias et al. [2013] record a reduction in intensity of transpiration as an effect of  $\text{Cd}^{2+}$  treatment. Greater water losses via transpiration led to a reduction in RWC (tab. 4). The effect of  $\text{Cd}^{2+}$  on plasma membranes was negative, causing an injuries, the level of which was higher in roots than in leaves (fig. 2), what has also been shown by Nováková et al. [2015].

This study showed that the Asahi SL positively affected *A. thaliana* plants exposed to  $\text{Cd}^{2+}$ . Biostimulant-treated plants were more vigorous and grew taller (tab. 1). Stimulation of plant growth might be attributed to the higher concentration and/or activity of

auxins caused by nitrophenolates, the active compounds of Asahi SL [Djanaguiraman et al. 2005]. The leaf area of plants treated with the biostimulant increased, and this was more pronounced at the higher  $\text{Cd}^{2+}$  concentration (tab. 1). These results confirmed studies by Djanaguiraman et al. [2005], Gawrońska et al. [2008] and Przybysz et al. [2010, 2014], who also recorded an increased leaf area in Asahi SL-treated plants. However, despite the fact that the biostimulant favoured a larger leaf area, their number was lower (tab. 1). In contrast to the above, Haroun et al. [2011] demonstrate an increased number of leaves in Asahi SL-treated *Solanum lycopersicum*.

In parallel to accelerated growth and development, Asahi SL-treated plants accumulated more biomass (tab. 2). A favourable biostimulant effect on biomass accumulation has also been demonstrated by other researchers on different plant species grown under various conditions [Gawrońska et al. 2008, Djanaguiraman et al. 2009, Przybysz et al. 2010, 2014, Haroun et al. 2011, Kwiatkowski 2015]. Higher biomass production was attributed to increased efficiency of photosynthetic apparatus (tab. 3, fig. 1). Asahi SL-treated plants had higher chlorophyll content (tab. 3), increased intensity of photosynthesis (fig. 1a) and reduced stomatal resistance (fig. 1b), which ensured more efficient gas exchange. These results were in line with studies carried out by Gawrońska et al. [2008], Borowski [2010], Djanaguiraman et al. [2009] and Przybysz et al. [2010, 2014] who have also recorded a higher chlorophyll content in biostimulant-treated plants. Increased intensity of photosynthesis has previously been reported in *A. thaliana* [Gawrońska et al. 2008, Przybysz et al. 2010, 2014], cucumber [Borowski 2010], winter oilseed rape [Przybysz et al. 2014, Kazda et al. 2015] and cotton [Djanaguiraman et al. 2009]. According to Przybysz et al. [2014] and Kazda et al. [2015], the positive effect of Asahi SL on the intensity of photosynthesis is maintained for a period of seven weeks after treatment. In this study the examined biostimulant did not affect chlorophyll *a* fluorescence parameters (tab. 3), which has been demonstrated also by Przybysz et al. [2010] on *A. thaliana* plants grown in optimal conditions. On the other hand, Djanaguiraman et al. [2009] and Pokluda et al. [2016] record an increase of Fv/Fm in cotton and coriander treated with Asahi SL. Przybysz et al. [2014] demonstrated that Fv/Fm and P.I. of field-grown oilseed rape plants did not differ between the control and biostimulant-treated plants until there was a spring frost, but after the frost these parameters were higher in the biostimulant-sprayed plants.

Asahi SL also affected the water status of plants grown in the presence of  $\text{Cd}^{2+}$  (tab. 4). After biostimulant application, the intensity of transpiration increased (tab. 4). Similar results have been obtained for cucumber [Borowski 2010] and *A. thaliana* [Gawrońska et al. 2008, Przybysz et al. 2010, 2014]. Despite the higher intensity of transpiration, differences in the values of RWC between plants treated and untreated with Asahi SL were slight (tab. 4), which suggest that the biostimulant improved water uptake. Greater water uptake after Asahi SL application has been reported by Przybysz et al. [2010, 2014] in *A. thaliana* plants grown in drought conditions. Improved water uptake is related to a better-developed root system, both in terms of length and biomass (tab. 1), as presented in this work and some others studies [Haroun et al. 2011, Przybysz



et al. 2010, 2014]. Higher values of RWC as a result of biostimulant spray have also been recorded by Gawrońska et al. [2008] and Przybysz et al. [2014].

This study proved that the application of the biostimulant diminished the negative impact of HM stresses in *A. thaliana*. Plants grown in the presence of  $\text{Cd}^{2+}$  and treated with Asahi SL had accelerated growth and development and accumulated more biomass. Mitigation of  $\text{Cd}^{2+}$  stress by Asahi SL can be explained by the fact, that phenolic compounds are increasing antioxidant activity in plants exposed to HM [Michalak 2006]. Serrano et al. [2010] and Pokluda et al. [2016] showed that biostimulant significantly increased activity of the antioxidant enzymes, total antioxidant activity and content of total phenolic compounds. Another direct evidence that Asahi SL protects plants against the negative effects of stress was provided in this work by the decreased level of plasma membranes from injuries caused by  $\text{Cd}^{2+}$ , both in roots and leaves (fig. 2). The reduction in membrane injuries was more pronounced in roots, which were in direct contact with the  $\text{Cd}^{2+}$  and also Asahi SL (fig. 2). A decrease in plasma membrane injuries was also found in biostimulant-treated cotton [Djanaguiraman et al. 2009] and *A. thaliana* [Gawrońska et al. 2008, Przybysz et al. 2014].

Since many defence mechanisms against different unfavourable conditions, especially of abiotic origin, are similar, it can be assumed that Asahi SL probably also decreased the negative effects of other stresses not mentioned in this work. The protective role of Asahi SL against stresses, which was shown in this study on the example of HM stress, has also been reported for cotton, maize, oil seed rape, *A. thaliana* and *Amaranthus* sp. grown under drought, spring frost, drought, high salinity and low temperature [Przybysz et al. 2014, Kazda et al. 2015, Pokluda et al. 2016]. However, the possible protective effect of the examined biostimulant also depends on many other factors not discussed here, mostly the level and duration of stresses and the moment of Asahi SL application.

## CONCLUSIONS

1. Application of Asahi SL under HM stress conditions had a positive effect on nearly all the parameters/processes examined.

2. *A. thaliana* plants treated with the biostimulant had accelerated growth and development, increased biomass accumulation, greater efficiency of photosynthetic apparatus and improved water status.

3. Asahi SL plays a protective role against HM stress and could be used by plant producers as an efficient tool mitigating the effects of stresses.

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## REFERENCES

- Ahmad, P., Sarwat, M., Bhat, N.A., Wani, M.R., Kazi, A.G., Tran, L.-S.P. (2015). Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. PLoS ONE, 10(1), e0114571.
- Asgher, M., Khan, M.I.R., Anjum, N.A., Khan, N.A. (2015). Minimising toxicity of cadmium in plants – role of plant growth regulators. Protoplasma, 252, 399–413.
- Borowski, E. (2010). The effect of the method of application and concentration of Asahi SL on the response of cucumber plants to chilling stress. ActaAgrobot., 63(2), 161–169.
- Boyes, D.C., Zayed, A.M., Ascenzi, R., McCaskill, A.J., Hoffman, N.E., Davis, K.R., Görlach, J. (2001). Growth stage-based phenotypic analysis of Arabidopsis: a model for high throughput functional genomics in plants. Plant Cell, 13(7), 1499–1510.
- Dias, M.C., Monteiro, C., Moutinho-Pereira, J., Correia, C., Gonçalves, B., Santos, C. (2013). Cadmium toxicity affects photosynthesis and plant growth at different levels. Acta Physiol. Plant., 35(4), 1281–1289.
- Djanaguiraman, M., Pandiyan, M., Devi, D.D. (2005). Response of cotton to Atonik and TIBA for growth, enzymes and yield. J. Biol. Sci., 5(2), 158–162.
- Djanaguiraman, M., Sheeba, J.A., Devi, D.D., Bangarusamy, U. (2009). Cotton leaf senescence can be delayed by nitrophenolate spray through enhanced antioxidant defence system. J. Agron. Crop. Sci., 195(3), 213–224.
- du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. Sci. Hortic., 196, 3–14.
- Farooq, M.A., Ali, S., Hameed, A., Ishaque, W., Mahmood, K., Iqbal, Z. (2013). Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. Ecotox. Environ. Saf. 96, 242–249.
- Gallego, S.M., Pena, L.B., Barcia, R.A., Azpilicueta, C.E., Iannone, M.F., Rosales, E.P., Zawoznik, M.S., Groppa, M.D., Benavides, M.P. (2012). Unravelling cadmium toxicity and tolerance in plants: Insight into regulatory mechanisms. Environ. Exp. Bot. 83, 33–46.
- Gawrońska, H., Przybysz, A., Szalacha, E., Słowiński, A. (2008). Physiological and molecular mode of action of Asahi SLbiostimulator under optimal and stress conditions. Monographs series: Biostimulators in modern agriculture: General Aspects, Gawrońska, H. (ed.). Editorial House Wieś Jutra, Warsaw, 54–76.
- Grabowska, A., Kunicki, E., Sękara, A., Kalisz, A., Jezdinsky, A., Gintrowicz, K. (2015). The effect of biostimulants on the quality parameters of tomato grown for the processing industry. Agrochimica, 59(3), 203–217.
- Haroun, S.A., Shukry, W.M., Abbas, M.A., Mowafy, A.M. (2011). Growth and physiological responses of *Solanum lycopersicum* to atonik and benzyl adenine under vernalized conditions. J. Ecol. Nat. Environ., 3(9), 319–331.
- Khan, A.L., Waqas, M., Hussain, J., Al-Harrasi, A., Lee, I.-J. (2014). Fungal endophyte *Penicilliumjanthinellum* LK5 can reduce cadmium toxicity in *Solanum lycopersicum* (*Sitens* and *Rhe*). Biol. Fertil. Soils, 50, 75–85.
- Kocira, A., Kocira, S., Stryjecka, M. (2015). Effect of Asahi SL application on common bean yield. Agric. Agric. Sci. Proc., 7, 103–107.
- Kazda, J., Herda, G., Spitzer, T., Řičařová, V., Przybysz, A., Gawrońska, H. (2015). Effect of nitrophenolates on pod damage caused by the brassica pod midge on the photosynthetic apparatus and yield of winter oilseed rape. J. Pest Sci., 88(2), 235–247.
- Kwiatkowski, C.A. (2015). Yield and quality of chamomile (*Chamomillarecutita* (L.) Rausch.) raw material depending on selected foliar sprays and plant spacing. Acta Sci. Pol. Hortorum Cultus, 14(1), 143–156.

- Kwiatkowski, C.A., Haliniarz, M., Kołodziej, B., Harasim, E., Tomczyńska-Mleko, M. (2015). Content of some chemical components in carrot (*Daucus carota* L.) roots depending on growth stimulators and stubble crops. *J. Elem.*, 20(4), 933–943.
- Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol. J. Environ. Stud.*, 15(4), 523–530
- Nazar, R., Iqbal, N., Masood, A., Khan, M.I.R., Syeed, S., Khan, N.A. (2012). Cadmium toxicity in plants and role of mineral nutrients in its alleviation. *Am. J. Plant Sci.*, 3(10), 1476–1489.
- Nováková, K., Navrátil, T., Šestáková, I., Le, M.P., Vodičková, H., Zámečnicková, B., Sokolová, R., Bulíčková, J., Gál, M. (2015). Characterization of cadmium ion transport across model and real biomembranes and indication of induced damage of plant tissues. *Monatsh. Chem.*, 146, 819–829.
- Pokluda, R., Sękara, A., Jezdinský, A., Kalisz, A., Neugebauerová, J., Grabowska, A. (2016). The physiological status and stress biomarker concentration of *Coriandrum sativum* L. plants subjected to chilling are modified by biostimulant application. *Biol. Agric. Horticult.*, 32(4). doi: 10.1080/01448765.2016.1172344
- Poleć-Pawlak, K., Ruzik, R., Abramski, K., Czurzyńska, M., Gawrońska, H. (2005). Cadmium speciation in *Arabidopsis thaliana* as a strategy to study metal accumulation system in plants. *Anal. Chim. Acta*, 540(1), 61–70.
- Przybysz, A., Gawrońska, H., Gajc-Wolska, J. (2014). Biological mode of action of a nitrophenolates-based biostimulant: case study. *Front. Plant Sci.*, 5(713). doi: 10.3389/fpls.2014.00713
- Przybysz, A., Wrochna, M., Slowinski, A., Gawronska, H. (2010). Stimulatory effect of Asahi SL on selected plant species. *Acta Sci. Pol. Hortorum Cultus*, 2(09), 53–64.
- Saidi, I., Chtourou, Y., Djebali, W. (2014). Selenium alleviates cadmium toxicity by preventing oxidative stress in sunflower (*Helianthus annuus*) seedlings. *J. Plant Physiol.*, 171, 85–91.
- Scebba, F., Arduini, I., Ercoli, L., Sebastiani, L. (2006). Cadmium effects on growth and antioxidant enzymes activities in *Miscanthus sinensis*. *Biol. Plant.*, 50(4), 688–692.
- Serrano, M., Zapata, P.J., Castillo, S., Guillén, F., Martínez-Romero, D., Valero, D. (2010). Antioxidant and nutritive constituents during sweet pepper development and ripening are enhanced by nitrophenolate treatments. *Food Chem.*, 118, 497–503.
- Siedlecka, A., Krupa, Z. (2002). Simple method of *Arabidopsis thaliana* cultivation in liquid nutrient medium. *Acta Physiol. Plant.*, 24(2), 163–166.
- Tran, T.A., Popova, L.P. (2013). Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turk. J. Bot.*, 37, 1–13.

## BIOSTYMYLATOR ASAHI SL CHRONI ROŚLINY *Arabidopsis thaliana* L. ROSNĄCE W OBECNOŚCI KADMU

**Streszczenie.** Biostymulatory poprawiają ilość i jakość plonu. Preparaty te pozytywnie wpływają na procesy życiowe rośliny, zazwyczaj wyraźniej w warunkach stresu. Stosowanie biostymulatorów zwiększa tolerancję roślin na stresy i przyspiesza procesy naprawcze uszkodzeń wywołanych przez niekorzystne warunki. W pracy zbadano wpływ Asahi SL na rośliny *Arabidopsis thaliana* L. rosnące w obecności kadmu ( $Cd^{2+}$ ).  $Cd^{2+}$  negatywnie wpływał na mierzone parametry i procesy, prowadząc do zaburzeń wzrostu i osłabienia kondycji roślin. Rośliny *A. thaliana* traktowane Asahi SL były wyższe, a ich rozwój był przyspieszony. Akumulacja biomasy zwiększyła się u roślin rosnących w obecności Asahi SL w wyniku lepszej wydajności aparatu fotosyntetycznego, wyrazo-

nej zwiększaną (i) powierzchnią liści, (ii) zawartością chlorofilu i (iii) intensywnością fotosyntezy. Pomimo wyższej transpiracji i obniżonych oporów aparatów szparkowych, względna zawartość wody roślin traktowanych Asahi SL nie zmieniała się, co związane było z stymulacją rozwoju systemu korzeniowego. Można zatem wnioskować, że Asahi SL chroni rośliny rosnące w warunkach stresu wywołanego  $\text{Cd}^{2+}$ .

**Słowa kluczowe:** warunki stresowe, akumulacja biomasy, wydajność aparatu fotosyntetycznego, gospodarka wodna

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